ecoli_clusters

April 21, 2023

1 E coli clustering

The purpose of this notebook is to develop the methods for clustering analysis of membrane transporters, with the example of *E coli*.

1.1 Sequence dataset generation

1.1.1 Reading data

Reading pre-processed Uniprot data for all organisms:

[ ]:

```
from subpred.util import load_df

sequences = load_df("uniprot")
sequences
```

[ ]:

```
                                       gene_names  \
Uniprot
A0A0C5B5G6                                 MT-RNR1
A0A1B0GTW7                            CIROP LMLN2
A0JNW5      BLTP3B KIAA0701 SHIP164 UHRF1BP1L
A0JP26                                      POTEB3
A0PK11                                      CLRN2
…                                                 …
X5L4R4                                      NOD-2
X5MBL2                                      GT34D
X5MFI4                                      GT34D
X5MI49                                      GT34A
X5MPI5                                      GT34C

                                                 protein_names  reviewed  \
Uniprot
A0A0C5B5G6  Mitochondrial-derived peptide MOTS-c (Mitochon…      True
A0A1B0GTW7  Ciliated left-right organizer metallopeptidase…      True
A0JNW5      Bridge-like lipid transfer protein family memb…      True
A0JP26                   POTE ankyrin domain family member B3      True
A0PK11                                               Clarin-2      True
…                                                           …       …
X5L4R4      Nucleotide-binding oligomerization domain-cont…     False
```

```
X5MBL2      Putative galacto(Gluco)mannan alpha-1,6-galact…    False
X5MFI4      Putative galacto(Gluco)mannan alpha-1,6-galact…    False
X5MI49      Putative galacto(Gluco)mannan alpha-1,6-galact…    False
X5MPI5                Xyloglucan alpha-1,6-xylosyltransferase     False

            protein_existence  \
Uniprot
A0A0C5B5G6                  1
A0A1B0GTW7                  1
A0JNW5                      1
A0JP26                      1
A0PK11                      1
…                       …
X5L4R4                      2
X5MBL2                      2
X5MFI4                      2
X5MI49                      2
X5MPI5                      2

                                                     sequence  organism_id
Uniprot
A0A0C5B5G6                                   MRWQEMGYIFYPRKLR         9606
A0A1B0GTW7  MLLLLLLLLLLPPLVLRVAASRCLHDETQKSVSLLRPPFSQLPSKS…        9606
A0JNW5      MAGIIKKQILKHLSRFTKNLSPDKINLSTLKGEGELKNLELDEEVL…        9606
A0JP26      MVAEVCSMPAASAVKKPFDLRSKMGKWCHHRFPCCRGSGKSNMGTS…        9606
A0PK11      MPGWFKKAWYGLASLLSFSSFILIIVALVVPHWLSGKILCQTGVDL…        9606
…                                                        …          …
X5L4R4      MSPGCYKGWPFNCHLSHEEDKRRNETLLQEAETSNLQITASFVSGL…      586796
X5MBL2      KVLYDRAFNSSDDQSALVYLLLKEKDKWADRIFIEHKYYLNGYWLD…        3352
X5MFI4      MDEDVLCKGPLHGGSARSLKGSLKRLKRIMESLNDGLIFMGGAVSA…        3352
X5MI49      MVNDSKLETISGNMVQKRKSFDGLPFWTVSIAGGLLLCWSLWRICF…        3352
X5MPI5      MRESVKRGLPSVRGPANGRSLALPKARSIQKTFNNLKITILCGFVT…        3352

[1021957 rows x 6 columns]
```

1.1.2 Selecting organism

Filtering for *E. Coli* Strain K12:

[ ]:
```
sequences = sequences[sequences.organism_id == 83333]
sequences
```

[ ]:
```
                        gene_names  \
Uniprot
P00509           aspC b0928 JW0911
P00803           lepB b2568 JW2552
P00804       lspA lsp b0027 JW0025
```

```
P00861           lysA b2838 JW2806
P00946       manA pmi b1613 JW1605
…                            …
P76154           ydfK b1544 JW1537
P0AEG8           dsrB b1952 JW1936
P33668           ybbC b0498 JW0487
A0A7H2C7B0     speFL ECK4660 b4803
A0A0A6YVN8  D-tagatose 3-epimerase

                                                protein_names  reviewed  \
Uniprot
P00509      Aspartate aminotransferase (AspAT) (EC 2.6.1.1…      True
P00803      Signal peptidase I (SPase I) (EC 3.4.21.89) (L…      True
P00804      Lipoprotein signal peptidase (EC 3.4.23.36) (P…      True
P00861      Diaminopimelate decarboxylase (DAP decarboxyla…      True
P00946      Mannose-6-phosphate isomerase (EC 5.3.1.8) (Ph…      True
…                                                   …       …
P76154                            Cold shock protein YdfK      True
P0AEG8                                       Protein DsrB      True
P33668                       Uncharacterized protein YbbC      True
A0A7H2C7B0        Leader peptide SpeFL (Arrest peptide SpeFL)     False
A0A0A6YVN8                             D-tagatose 3-epimerase     False

            protein_existence  \
Uniprot
P00509                      1
P00803                      1
P00804                      1
P00861                      1
P00946                      1
…                       …
P76154                      2
P0AEG8                      2
P33668                      2
A0A7H2C7B0                  2
A0A0A6YVN8                  1

                                                     sequence  organism_id
Uniprot
P00509      MFENITAAPADPILGLADLFRADERPGKINLGIGVYKDETGKTPVL…        83333
P00803      MANMFALILVIATLVTGILWCVDKFFFAPKRRERQAAAQAAAGDSL…        83333
P00804      MSQSICSTGLRWLWLVVVVLIIDLGSKYLILQNFALGDTVPLFPSL…        83333
P00861      MPHSLFSTDTDLTAENLLRLPAEFGCPVWVYDAQIIRRQIAALKQF…        83333
P00946      MQKLINSVQNYAWGSKTALTELYGMENPSSQPMAELWMGAHPKSSS…        83333
…                                                   …          …
P76154      MKSKDTLKWFPAQLPEVRIILGDAVVEVAKQGRPINTRTLLDYIEG…        83333
P0AEG8      MKVNDRVTVKTDGGPRRPGVVLAVEEFSEGTMYLVSLEDYPLGIWF…        83333
```

```
P33668      MKYSSIFSMLSFFILFACNETAVYGSDENIIFMRYVEKLHLDKYSV…        83333
A0A7H2C7B0                 MENNSRTMPHIRRTTHIMKFAHRNSFDFHFFNAR        83333
A0A0A6YVN8  MNKVGMFYTYWSTEWMVDFPATAKRIAGLGFDLMEISLGEFHNLSD…        83333

[3284 rows x 6 columns]
```

1.1.3 Filtering

Keeping proteins that have been manually reviewed, or have sequence evidence at protein level:

[ ]:

```
sequences = sequences[(sequences.protein_existence == 1) | (sequences.reviewed)]
sequences
```

[ ]:

```
                        gene_names  \
Uniprot
P00509           aspC b0928 JW0911
P00803           lepB b2568 JW2552
P00804       lspA lsp b0027 JW0025
P00861           lysA b2838 JW2806
P00946       manA pmi b1613 JW1605
…                            …
P76157           ynfN b1551 JW5254
P76154           ydfK b1544 JW1537
P0AEG8           dsrB b1952 JW1936
P33668           ybbC b0498 JW0487
A0A0A6YVN8  D-tagatose 3-epimerase

                                                    protein_names  reviewed  \
Uniprot
P00509      Aspartate aminotransferase (AspAT) (EC 2.6.1.1…      True
P00803      Signal peptidase I (SPase I) (EC 3.4.21.89) (L…      True
P00804      Lipoprotein signal peptidase (EC 3.4.23.36) (P…      True
P00861      Diaminopimelate decarboxylase (DAP decarboxyla…      True
P00946      Mannose-6-phosphate isomerase (EC 5.3.1.8) (Ph…      True
…                                                       …       …
P76157                          Uncharacterized protein YnfN      True
P76154                               Cold shock protein YdfK      True
P0AEG8                                          Protein DsrB      True
P33668                          Uncharacterized protein YbbC      True
A0A0A6YVN8                            D-tagatose 3-epimerase     False

            protein_existence  \
Uniprot
P00509                      1
P00803                      1
P00804                      1
P00861                      1
```

```
P00946                      1
…                       …
P76157                      2
P76154                      2
P0AEG8                      2
P33668                      2
A0A0A6YVN8                  1

                                                     sequence  organism_id
Uniprot
P00509      MFENITAAPADPILGLADLFRADERPGKINLGIGVYKDETGKTPVL…        83333
P00803      MANMFALILVIATLVTGILWCVDKFFFAPKRRERQAAAQAAAGDSL…        83333
P00804      MSQSICSTGLRWLWLVVVVLIIDLGSKYLILQNFALGDTVPLFPSL…        83333
P00861      MPHSLFSTDTDLTAENLLRLPAEFGCPVWVYDAQIIRRQIAALKQF…        83333
P00946      MQKLINSVQNYAWGSKTALTELYGMENPSSQPMAELWMGAHPKSSS…        83333
…                                                      …          …
P76157      MREYPNGEKTHLTVMAAGFPSLTGDHKVIYVAADRHVTSEEILEAA…        83333
P76154      MKSKDTLKWFPAQLPEVRIILGDAVVEVAKQGRPINTRTLLDYIEG…        83333
P0AEG8      MKVNDRVTVKTDGGPRRPGVVLAVEEFSEGTMYLVSLEDYPLGIWF…        83333
P33668      MKYSSIFSMLSFFILFACNETAVYGSDENIIFMRYVEKLHLDKYSV…        83333
A0A0A6YVN8  MNKVGMFYTYWSTEWMVDFPATAKRIAGLGFDLMEISLGEFHNLSD…        83333

[3283 rows x 6 columns]
```

1.1.4 Sequence clustering the proteins:

The clustering at 70% sequence identity does not remove a lot of proteins for *E. coli*. This is different for other organisms.

[ ]:

```
from subpred.cdhit import cd_hit

clusters = cd_hit(sequences=sequences.sequence, identity_threshold=70)
sequences = sequences.loc[clusters]
sequences
```

```
cd-hit: clustered 3277 sequences into 3236 clusters at threshold 70
```

[ ]:

```
                        gene_names  \
Uniprot
P00509           aspC b0928 JW0911
P00803           lepB b2568 JW2552
P00804       lspA lsp b0027 JW0025
P00861           lysA b2838 JW2806
P00946       manA pmi b1613 JW1605
…                            …
P77564           ydhW b1672 JW1662
P76157           ynfN b1551 JW5254
```

```
P0AEG8          dsrB b1952 JW1936
P33668          ybbC b0498 JW0487
A0A0A6YVN8  D-tagatose 3-epimerase

                                                 protein_names  reviewed  \
Uniprot
P00509      Aspartate aminotransferase (AspAT) (EC 2.6.1.1…      True
P00803      Signal peptidase I (SPase I) (EC 3.4.21.89) (L…      True
P00804      Lipoprotein signal peptidase (EC 3.4.23.36) (P…      True
P00861      Diaminopimelate decarboxylase (DAP decarboxyla…      True
P00946      Mannose-6-phosphate isomerase (EC 5.3.1.8) (Ph…      True
…                                                        …        …
P77564                           Uncharacterized protein YdhW      True
P76157                           Uncharacterized protein YnfN      True
P0AEG8                                          Protein DsrB      True
P33668                           Uncharacterized protein YbbC      True
A0A0A6YVN8                             D-tagatose 3-epimerase     False

            protein_existence  \
Uniprot
P00509                      1
P00803                      1
P00804                      1
P00861                      1
P00946                      1
…                         …
P77564                      2
P76157                      2
P0AEG8                      2
P33668                      2
A0A0A6YVN8                  1

                                                      sequence  organism_id
Uniprot
P00509      MFENITAAPADPILGLADLFRADERPGKINLGIGVYKDETGKTPVL…        83333
P00803      MANMFALILVIATLVTGILWCVDKFFFAPKRRERQAAAQAAAGDSL…        83333
P00804      MSQSICSTGLRWLWLVVVVLIIDLGSKYLILQNFALGDTVPLFPSL…        83333
P00861      MPHSLFSTDTDLTAENLLRLPAEFGCPVWVYDAQIIRRQIAALKQF…        83333
P00946      MQKLINSVQNYAWGSKTALTELYGMENPSSQPMAELWMGAHPKSSS…        83333
…                                                        …          …
P77564      MGKMNHQDELPLAKVSEVDEAKRQWLQGMRHPVDTVTEPEPAEILA…        83333
P76157      MREYPNGEKTHLTVMAAGFPSLTGDHKVIYVAADRHVTSEEILEAA…        83333
P0AEG8      MKVNDRVTVKTDGGPRRPGVVLAVEEFSEGTMYLVSLEDYPLGIWF…        83333
P33668      MKYSSIFSMLSFFILFACNETAVYGSDENIIFMRYVEKLHLDKYSV…        83333
A0A0A6YVN8  MNKVGMFYTYWSTEWMVDFPATAKRIAGLGFDLMEISLGEFHNLSD…        83333

[3236 rows x 6 columns]
```

1.1.5 Filtering for transmembrane transporters using GO

Loading all GO Uniprot annotations Read pre-processed Uniprot GO annotations:

[ ]:
```
go_annotations_uniprot = load_df("go")
go_annotations_uniprot
```

[ ]:
```
              Uniprot    qualifier       go_id evidence_code aspect
0          A0A009FND8      enables  GO:0000166           IEA      F
1          A0A009FND8      enables  GO:0005524           IEA      F
2          A0A009FND8      enables  GO:0051082           IEA      F
3          A0A009FND8      enables  GO:0140662           IEA      F
4          A0A009FND8  involved_in  GO:0006457           IEA      P
…                 …          …          …            …    …
7452013        Z9JND5      enables  GO:0000166           IEA      F
7452014        Z9JND5      enables  GO:0005524           IEA      F
7452015        Z9JND5      enables  GO:0051082           IEA      F
7452016        Z9JND5      enables  GO:0140662           IEA      F
7452017        Z9JND5  involved_in  GO:0006457           IEA      P

[7452018 rows x 5 columns]
```

Dataset of transmembrane transport GO terms in E Coli

- Used to filter proteins for transmembrane transporters

Creating dataset of transporter GO term annotations

Filter for GO terms that are descendants of ”transmembrane transporter activity

GO annotations for the clustered sequence dataset from *E Coli*.

[ ]:
```
from subpred.ontology import GeneOntology, GO_FILE

go_owl = GeneOntology(GO_FILE)
transmembrane_transport_go_terms = go_owl.get_descendants(
    go_owl.get_identifier("transmembrane transporter activity")
)
# filter GO annotations for descendants of transmembrane transporter activity
go_annotations_tmtp = go_annotations_uniprot[
    go_annotations_uniprot.go_id.isin(transmembrane_transport_go_terms)
].reset_index(drop=True)
go_annotations_tmtp
```

[ ]:
```
            Uniprot qualifier       go_id evidence_code aspect
0        A0A014M993   enables  GO:0015288           IEA      F
1        A0A014M993   enables  GO:0015481           IEA      F
2        A0A014M993   enables  GO:0042958           IEA      F
3        A0A022PEV8   enables  GO:0015288           IEA      F
4        A0A022PEV8   enables  GO:0015481           IEA      F
```

```
…          …      …        …                …    …
94155      X5M917  enables  GO:0022857            IBA      F
94156      Z4YKJ7  enables  GO:0015172            IEA      F
94157      Z4YKJ7  enables  GO:0015179            IEA      F
94158      Z4YKJ7  enables  GO:0015293            IEA      F
94159      Z4YKJ7  enables  GO:0015501            IEA      F

[94160 rows x 5 columns]
```

```
[ ]: # add ancestors of all remaining terms
     from subpred.ontology import add_go_ancestors
     go_annotations_tmtp = add_go_ancestors(go_annotations_tmtp, go_owl)
     go_annotations_tmtp
```

```
[ ]:           Uniprot qualifier evidence_code aspect       go_id
     0       A0A014M993   enables           IEA      F  GO:0022829
     1       A0A014M993   enables           IEA      F  GO:0005215
     2       A0A014M993   enables           IEA      F  GO:0015288
     3       A0A014M993   enables           IEA      F  GO:0003674
     4       A0A014M993   enables           IEA      F  GO:0022803
     …            …         …          …     …          …
     741880      Z4YKJ7   enables           IEA      F  GO:0015075
     741881      Z4YKJ7   enables           IEA      F  GO:0015294
     741882      Z4YKJ7   enables           IEA      F  GO:0022853
     741883      Z4YKJ7   enables           IEA      F  GO:0005416
     741884      Z4YKJ7   enables           IEA      F  GO:0022890

     [741885 rows x 5 columns]
```

```
[ ]: # filter GO annotations and their ancestors for descendants of transmembrane␣
       ↪transporter activity again
     go_annotations_tmtp = go_annotations_tmtp[
         go_annotations_tmtp.go_id.isin(transmembrane_transport_go_terms)
     ].reset_index(drop=True)
     go_annotations_tmtp = go_annotations_tmtp.drop_duplicates().
       ↪reset_index(drop=True)
     go_annotations_tmtp
```

```
[ ]:           Uniprot qualifier evidence_code aspect       go_id
     0       A0A014M993   enables           IEA      F  GO:0022829
     1       A0A014M993   enables           IEA      F  GO:0015288
     2       A0A014M993   enables           IEA      F  GO:0022803
     3       A0A014M993   enables           IEA      F  GO:0015267
     4       A0A014M993   enables           IEA      F  GO:0022857
     …            …         …          …     …          …
     581847      Z4YKJ7   enables           IEA      F  GO:0015075
     581848      Z4YKJ7   enables           IEA      F  GO:0015294
```

```
581849      Z4YKJ7   enables           IEA      F  GO:0022853
581850      Z4YKJ7   enables           IEA      F  GO:0005416
581851      Z4YKJ7   enables           IEA      F  GO:0022890

[581852 rows x 5 columns]
```

```
[ ]: # annotate with labels
     go_annotations_tmtp = go_annotations_tmtp.assign(
         go_term=go_annotations_tmtp.go_id.transform(go_owl.get_label)
     )
     go_annotations_tmtp
```

```
[ ]:            Uniprot qualifier evidence_code aspect       go_id  \
     0       A0A014M993   enables           IEA      F  GO:0022829
     1       A0A014M993   enables           IEA      F  GO:0015288
     2       A0A014M993   enables           IEA      F  GO:0022803
     3       A0A014M993   enables           IEA      F  GO:0015267
     4       A0A014M993   enables           IEA      F  GO:0022857
     …              …         …           …     …           …
     581847      Z4YKJ7   enables           IEA      F  GO:0015075
     581848      Z4YKJ7   enables           IEA      F  GO:0015294
     581849      Z4YKJ7   enables           IEA      F  GO:0022853
     581850      Z4YKJ7   enables           IEA      F  GO:0005416
     581851      Z4YKJ7   enables           IEA      F  GO:0022890

                                                   go_term
     0                          wide pore channel activity
     1                                     porin activity
     2            passive transmembrane transporter activity
     3                                   channel activity
     4                    transmembrane transporter activity
     …                                                    …
     581847           ion transmembrane transporter activity
     581848              solute:cation symporter activity
     581849    active ion transmembrane transporter activity
     581850             amino acid:cation symporter activity
     581851  inorganic cation transmembrane transporter act…

     [581852 rows x 6 columns]
```

```
[ ]: # Filtering for qualifier. How many samples do we lose?
     print(
         f"Percentage of transmembrane transporter annotations with enables␣
      ↪qualifier: {go_annotations_tmtp[go_annotations_tmtp.qualifier == 'enables'].
      ↪shape[0] / go_annotations_tmtp.shape[0] * 100:.3f}%"
     )
```

```
go_annotations_tmtp = go_annotations_tmtp[go_annotations_tmtp.qualifier == "enables"]
go_annotations_tmtp
```

```
Percentage of transmembrane transporter annotations with enables qualifier:
97.408%
```

[ ]:

```
           Uniprot qualifier evidence_code aspect       go_id  \
0       A0A014M993   enables           IEA      F  GO:0022829
1       A0A014M993   enables           IEA      F  GO:0015288
2       A0A014M993   enables           IEA      F  GO:0022803
3       A0A014M993   enables           IEA      F  GO:0015267
4       A0A014M993   enables           IEA      F  GO:0022857
…            …       …           …    …         …
581847      Z4YKJ7   enables           IEA      F  GO:0015075
581848      Z4YKJ7   enables           IEA      F  GO:0015294
581849      Z4YKJ7   enables           IEA      F  GO:0022853
581850      Z4YKJ7   enables           IEA      F  GO:0005416
581851      Z4YKJ7   enables           IEA      F  GO:0022890

                                                  go_term
0                              wide pore channel activity
1                                          porin activity
2              passive transmembrane transporter activity
3                                        channel activity
4                      transmembrane transporter activity
…                                                   …
581847             ion transmembrane transporter activity
581848                   solute:cation symporter activity
581849      active ion transmembrane transporter activity
581850               amino acid:cation symporter activity
581851  inorganic cation transmembrane transporter act…

[566772 rows x 6 columns]
```

Before filtering for the electronically inferred IEA annotations, we will only keep annotations for the clustered *E. coli* sequence dataset above, to see what the actual difference in sample count is. *E. coli* is a model organism, and therefore there should be more experimentally verified annotations compared to the thousands of other organisms in the dataset with similar proteins.

The percentage of IEA annotations in *E. coli* should be much lower than for the average Uniprot organism.

[ ]:

```
assert not sequences.duplicated().any()
f"% of e coli proteins with transmembrane transport annotation after preprocessing and clustering: {sequences[sequences.index.isin(go_annotations_tmtp.Uniprot)].shape[0]/sequences.shape[0]*100:.2f}%"
```

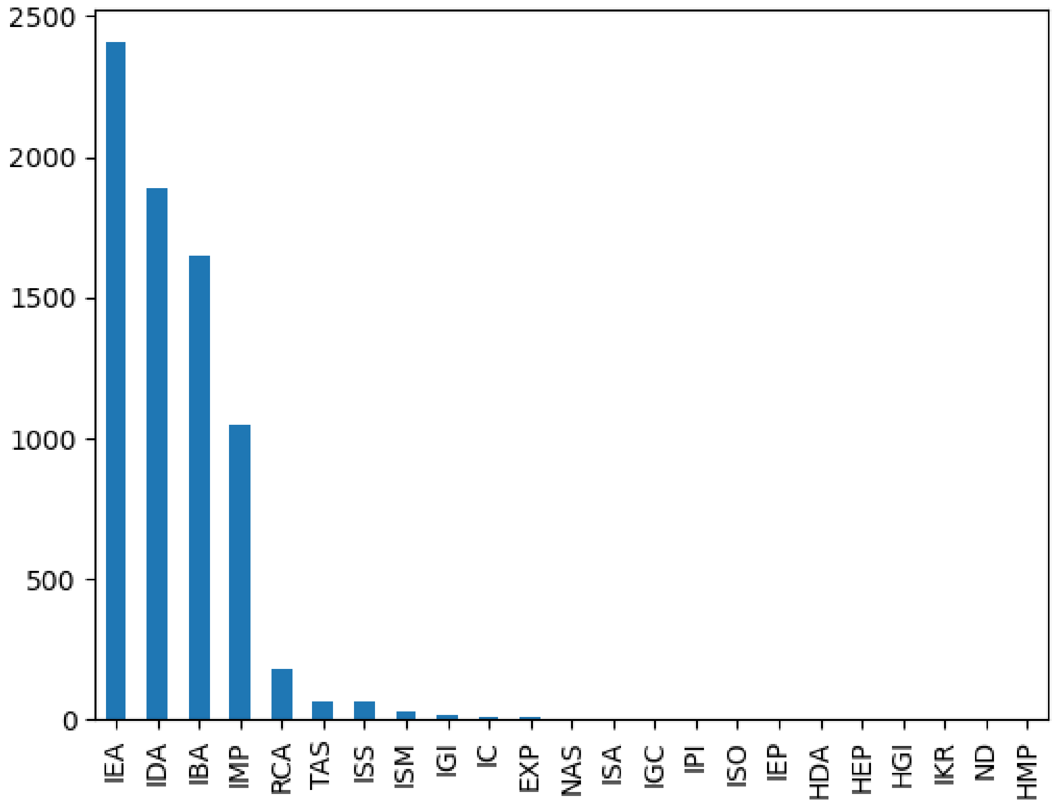
```
[ ]: '% of e coli proteins with transmembrane transport annotation after
     preprocessing and clustering: 14.52%'
```

```
[ ]: go_annotations_tmtp = go_annotations_tmtp[
         go_annotations_tmtp.Uniprot.isin(sequences.index)
     ].reset_index(drop=True)
     go_annotations_tmtp
```

```
[ ]:      Uniprot qualifier evidence_code aspect       go_id  \
     0     A5A627   enables           IDA      F  GO:0022857
     1     A5A627   enables           IDA      F  GO:0015075
     2     A5A627   enables           IDA      F  GO:0008509
     3     A5A627   enables           IDA      F  GO:0015103
     4     A5A627   enables           IDA      F  GO:0022803
     …       …         …            …     …           …
     7374  Q6BEX0   enables           IEA      F  GO:0051119
     7375  Q6BEX0   enables           IEA      F  GO:0005354
     7376  Q6BEX0   enables           IEA      F  GO:0015145
     7377  Q6BEX0   enables           IEA      F  GO:0103116
     7378  Q6BEX0   enables           IEA      F  GO:0015399

                                                 go_term
     0                  transmembrane transporter activity
     1              ion transmembrane transporter activity
     2            anion transmembrane transporter activity
     3     inorganic anion transmembrane transporter acti…
     4          passive transmembrane transporter activity
     …                                                 …
     7374         sugar transmembrane transporter activity
     7375     galactose transmembrane transporter activity
     7376  monosaccharide transmembrane transporter activity
     7377           ABC-type D-galactofuranose transporter
     7378  primary active transmembrane transporter activity

     [7379 rows x 6 columns]
```

```
[ ]: # filter for evidence code. how many samples do we lose?
     go_annotations_tmtp.evidence_code.value_counts().plot.bar()
```

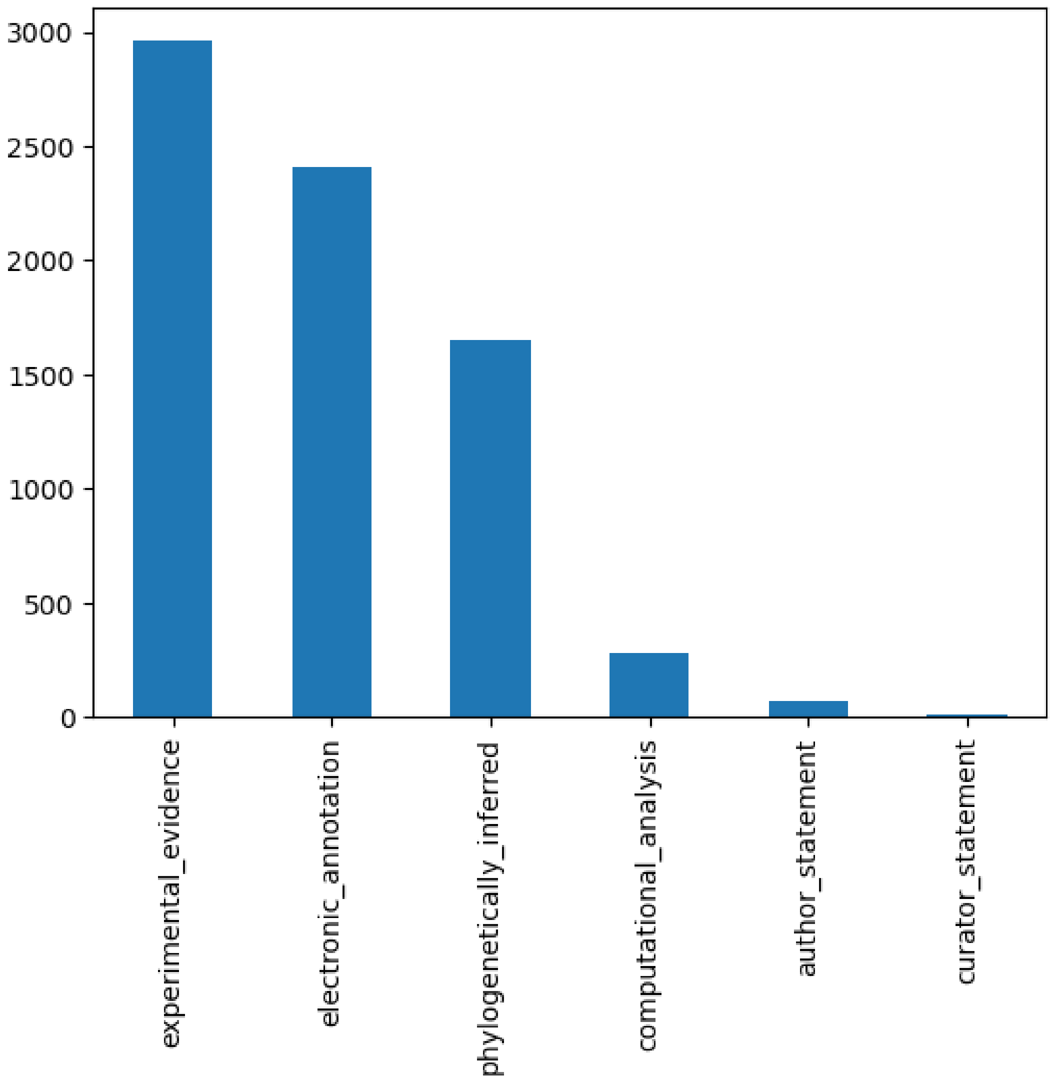
```
[ ]: <AxesSubplot:>
```



Most annotations have experimental evidence!

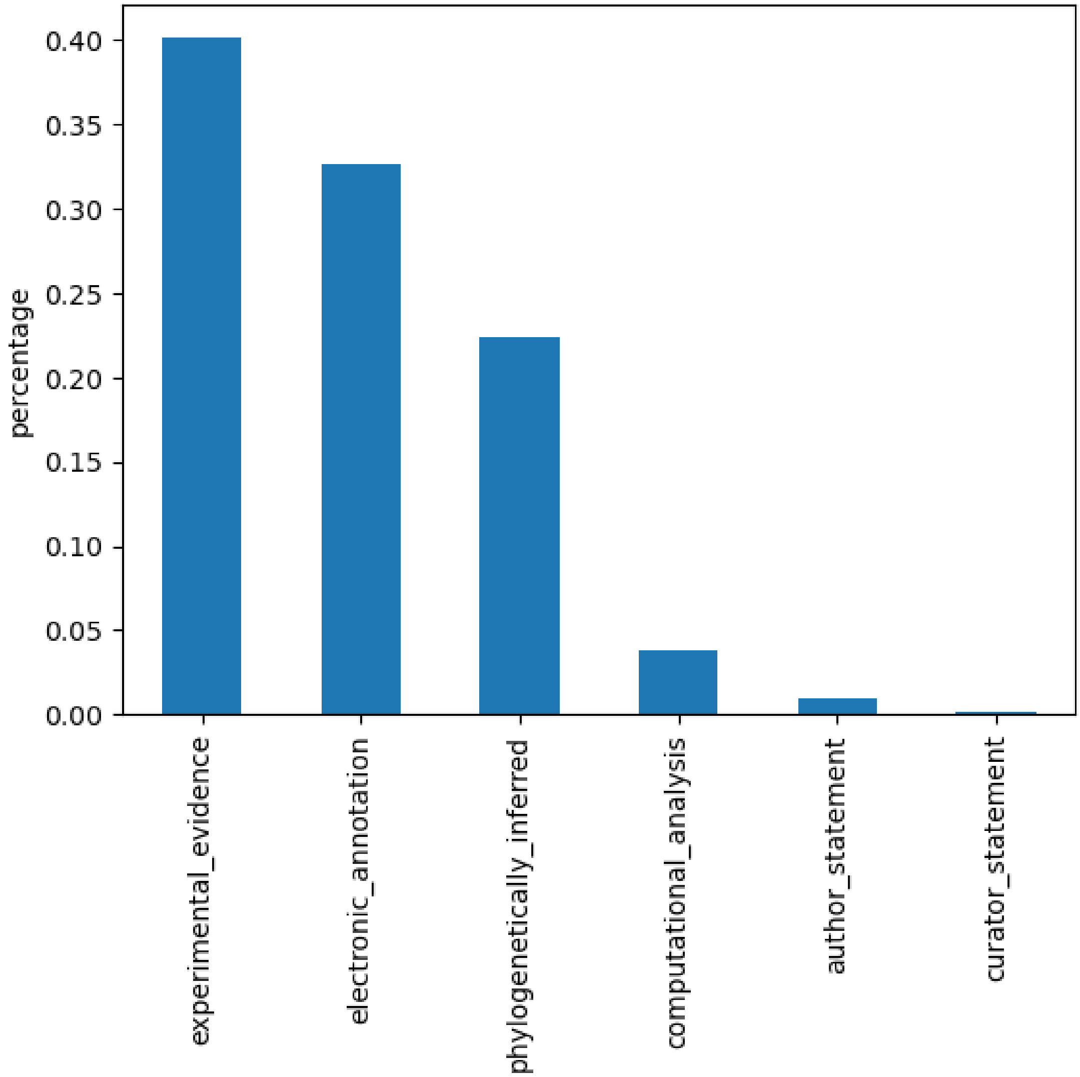
```
[ ]: from subpred.ontology import EVIDENCE_CODE_TO_DESCRIPTION

     go_annotations_tmtp.evidence_code.map(
         EVIDENCE_CODE_TO_DESCRIPTION
     ).value_counts().plot.bar()
```

```
[ ]: <AxesSubplot:>
```



```
[ ]: g = go_annotations_tmtp.evidence_code.map(
         EVIDENCE_CODE_TO_DESCRIPTION
     ).value_counts(normalize=True).plot.bar()
     g.set_ylabel("percentage")
```

```
[ ]: Text(0, 0.5, 'percentage')
```



Another point is that the GO annotation dataset also contains Uniprot annotations assigned by InterPro. There can be cases where the same annotation is in the dataset twice, where one of those annotations has an IEA annotation while the other one does not. What is the actual difference?

```
[ ]: tmp = (
         go_annotations_tmtp[go_annotations_tmtp.evidence_code != "IEA"]
         .drop(["qualifier", "evidence_code", "aspect"], axis=1)
         .drop_duplicates()
         .reset_index(drop=True)
     )
     print(
         f"Without IEA terms: {tmp.shape[0]} annotations in {tmp.Uniprot.unique().
       ↪shape[0]} proteins."
     )
```

```
tmp = (
    go_annotations_tmtp.drop(["qualifier", "evidence_code", "aspect"], axis=1)
    .drop_duplicates()
    .reset_index(drop=True)
)
print(
    f"With IEA terms: {tmp.shape[0]} annotations in {tmp.Uniprot.unique().
  ↪shape[0]} proteins."
)
```

```
Without IEA terms: 3348 annotations in 357 proteins.
With IEA terms: 4172 annotations in 470 proteins.
```

For now, we will filter out the IEA terms and only keep the verified data.

[ ]:
```
# filter for evidence codes:
go_annotations_tmtp = go_annotations_tmtp[
    go_annotations_tmtp.evidence_code != "IEA"
].reset_index(drop=True)
go_annotations_tmtp
```

[ ]:
```
     Uniprot qualifier evidence_code aspect       go_id  \
0     A5A627   enables           IDA      F  GO:0022857
1     A5A627   enables           IDA      F  GO:0015075
2     A5A627   enables           IDA      F  GO:0008509
3     A5A627   enables           IDA      F  GO:0015103
4     A5A627   enables           IDA      F  GO:0022803
…       …        …           …    …        …
4969  Q59385   enables           IBA      F  GO:0005375
4970  Q59385   enables           IBA      F  GO:0046915
4971  Q59385   enables           IBA      F  GO:0022804
4972  Q59385   enables           IBA      F  GO:0042626
4973  Q59385   enables           IBA      F  GO:0022890

                                                go_term
0                         transmembrane transporter activity
1                     ion transmembrane transporter activity
2                   anion transmembrane transporter activity
3     inorganic anion transmembrane transporter acti…
4               passive transmembrane transporter activity
…                                                    …
4969         copper ion transmembrane transporter activity
4970  transition metal ion transmembrane transporter…
4971             active transmembrane transporter activity
4972  ATPase-coupled transmembrane transporter activity
4973  inorganic cation transmembrane transporter act…

[4974 rows x 6 columns]
```

Now, there are still some annotations left with identical uniprot accession and go term, but different evidence codes. This happens because the GO dataset contains annotations made both by Uniprot and by InterPro, and they can assign different evidence codes. The next step is to remove the columns that we no longer need, and delete duplicates:

```
[ ]: go_annotations_tmtp = (
         go_annotations_tmtp.drop(["qualifier", "evidence_code", "aspect"], axis=1)
         .drop_duplicates()
         .reset_index(drop=True)
     )
     go_annotations_tmtp
```

```
[ ]:      Uniprot       go_id                                           go_term
     0     A5A627  GO:0022857                 transmembrane transporter activity
     1     A5A627  GO:0015075             ion transmembrane transporter activity
     2     A5A627  GO:0008509           anion transmembrane transporter activity
     3     A5A627  GO:0015103  inorganic anion transmembrane transporter acti…
     4     A5A627  GO:0022803         passive transmembrane transporter activity
     …        …         …                                                 …
     3343  Q59385  GO:0022853      active ion transmembrane transporter activity
     3344  Q59385  GO:0015399  primary active transmembrane transporter activity
     3345  Q59385  GO:0043682         P-type divalent copper transporter activity
     3346  Q59385  GO:0019829  ATPase-coupled cation transmembrane transporte…
     3347  Q59385  GO:0005375      copper ion transmembrane transporter activity

     [3348 rows x 3 columns]
```

```
[ ]: # make backup of entire (filtered) genome, since we need a reference set later␣
      ↪for the annotations
     sequences_all = sequences.copy()
     sequences = sequences[sequences.index.isin(go_annotations_tmtp.Uniprot)]
     sequences
```

```
[ ]:                          gene_names  \
     Uniprot
     P02916            malF b4033 JW3993
     P03959            kdpA b0698 JW0686
     P05825   fepA fep feuB b0584 JW5086
     P0AA78            exuT b3093 JW3064
     P0AAD6       sdaC dcrA b2796 JW2767
     …                               …
     P77328      ybbY glxB4 b0513 JW0501
     P76198            ydiN b1691 JW5274
     P33011            yeeA b2008 JW1990
     P37327            yfdC b2347 JW2344
     P39282            yjeM b4156 JW5739
```

```
                                             protein_names  reviewed  \
Uniprot
P02916   Maltose/maltodextrin transport system permease…      True
P03959   Potassium-transporting ATPase potassium-bindin…      True
P05825   Ferrienterobactin receptor (Enterobactin outer…      True
P0AA78   Hexuronate transporter (Aldohexuronate transpo…      True
P0AAD6   Serine transporter SdaC (H(+)/L-serine symport…      True
…                                                    …       …
P77328                       Putative purine permease YbbY      True
P76198                Inner membrane transport protein YdiN      True
P33011                         Inner membrane protein YeeA      True
P37327                         Inner membrane protein YfdC      True
P39282                     Inner membrane transporter YjeM      True

         protein_existence                                           sequence  \
Uniprot
P02916                   1  MDVIKKKHWWQSDALKWSVLGLLGLLVGYLVVLMYAQGEYLFAITT…
P03959                   1  MAAQGFLLIATFLLVLMVLARPLGSGLARLINDIPLPGTTGVERVL…
P05825                   1  MNKKIHSLALLVNLGIYGVAQAQEPTDTPVSHDDTIVVTAAEQNLQ…
P0AA78                   1  MRKIKGLRWYMIALVTLGTVLGYLTRNTVAAAAPTLMEELNISTQQ…
P0AAD6                   1  METTQTSTIASKDSRSAWRKTDTMWMLGLYGTAIGAGVLFLPINAG…
…                    …                                                …
P77328                   1  MFNFAVSRESLLSGFQWFFFIFCNTVVVPPTLLSAFQLPQSSLLTL…
P76198                   1  MSQNKAFSTPFILAVLCIYFSYFLHGISVITLAQNMSSLAEKFSTD…
P33011                   1  MRADKSLSPFEIRVYRHYRIVHGTRVALAFLLTFLIIRLFTIPEST…
P37327                   1  MDNDKIDQHSDEIEVESEEKERGKKIEIDEDRLPSRAMAIHEHIRQ…
P39282                   1  MPHTIKKMSLIGLILMIFTSVFGFANSPSAYYLMGYSAIPFYIFSA…

         organism_id
Uniprot
P02916         83333
P03959         83333
P05825         83333
P0AA78         83333
P0AAD6         83333
…              …
P77328         83333
P76198         83333
P33011         83333
P37327         83333
P39282         83333

[357 rows x 6 columns]
```

1.2 Feature generation

Here, we will use PSSM and protein embeddings features to encode the protein sequences into vectors of length n.

1.2.1 ProtNLM

One of the most reliable features at the moment seems to be ProtNLM, which provides NLP embeddings for individual proteins. Swissprot has pre-calculated embeddings for all *E. Coli* proteins available for download.

There is also ProtT5, which can be calcualted for new proteins.

[ ]:

```
import h5py
import pandas as pd
import numpy as np


def read_uniprot_embedding(h5_path: str):
    with h5py.File(h5_path, "r") as file:
        embeddings_dict = {
            sequence_id: np.array(embedding) for sequence_id, embedding in file.
 ↪items()
        }
        return pd.DataFrame.from_dict(embeddings_dict, orient="index")


feature_protnlm = read_uniprot_embedding("/home/ad/protnlm_test/
 ↪ecoli-per-protein.h5")
feature_protnlm
```

[ ]:

```
                   0         1         2         3         4         5    \
A0A385XJ53  0.061157  0.082031  0.001612  0.039703 -0.020401  0.034180
A0A385XJE6  0.047943  0.122864  0.007145  0.014442  0.024536  0.034729
A0A385XJK5  0.050293  0.093811 -0.043182 -0.043671 -0.029007 -0.071594
A0A385XJL2  0.039551  0.090271 -0.019913  0.009315 -0.047882 -0.038605
A0A385XJL4  0.008354  0.159546  0.024719  0.047485  0.031525  0.043854
…                …         …         …         …         …         …
Q93K97      0.043854  0.006367 -0.031769  0.036469  0.020828  0.040802
Q9XB42     -0.001819  0.088806 -0.038422  0.023849 -0.067871  0.024200
Q9Z3A0      0.006672  0.109619 -0.061584  0.024643 -0.006783 -0.079468
U3PVA8      0.036987 -0.017639 -0.028152 -0.019852  0.025330  0.019958
V9HVX0      0.085876 -0.004467  0.137939 -0.072998 -0.004997  0.049927

                   6         7         8         9    …      1014      1015  \
A0A385XJ53 -0.004898 -0.057495  0.023514 -0.089600  …  0.032349 -0.059021
A0A385XJE6 -0.040802 -0.031281  0.009224 -0.065063  … -0.004765  0.001526
A0A385XJK5  0.019318 -0.126831  0.006989 -0.072693  …  0.045349 -0.014725
A0A385XJL2  0.059692 -0.034180  0.008598 -0.089661  …  0.049866 -0.025406
```

```
A0A385XJL4 -0.046173 -0.039490  0.017197 -0.031097  … -0.040344 -0.025070
…                …        …         …        …   …        …         …
Q93K97     -0.007046 -0.109863  0.037109 -0.007458  … -0.020798  0.034088
Q9XB42      0.062622 -0.184937  0.071777  0.039307  …  0.045532 -0.050568
Q9Z3A0     -0.015144 -0.142212  0.071228  0.038208  …  0.029312  0.003822
U3PVA8      0.012833 -0.129395 -0.018860 -0.003590  … -0.029663  0.032928
V9HVX0      0.000803 -0.106567 -0.032684  0.104553  …  0.026123  0.034149

                1016      1017      1018      1019      1020      1021  \
A0A385XJ53 -0.021179 -0.051971  0.054962 -0.005638 -0.088074 -0.094727
A0A385XJE6  0.021637 -0.024689  0.042328 -0.009949 -0.060638 -0.045258
A0A385XJK5  0.029755 -0.141113 -0.025711  0.098572 -0.029022  0.077271
A0A385XJL2  0.007912 -0.066223  0.031174  0.077637  0.030319  0.118652
A0A385XJL4  0.058197 -0.053284  0.074280 -0.032410 -0.082153 -0.038849
…                …        …         …        …         …         …
Q93K97      0.026230 -0.078735  0.013145  0.006065 -0.043488 -0.061035
Q9XB42     -0.017609 -0.083496 -0.017700  0.008072 -0.038635  0.016205
Q9Z3A0      0.022324 -0.174927  0.006115  0.036255 -0.054504 -0.008636
U3PVA8      0.026566 -0.147827 -0.055145 -0.061310  0.017197  0.147217
V9HVX0     -0.021530 -0.145874 -0.035492 -0.009529 -0.033844 -0.008926

                1022      1023
A0A385XJ53 -0.043701 -0.071533
A0A385XJE6 -0.067078  0.024902
A0A385XJK5 -0.000576 -0.016922
A0A385XJL2  0.055847 -0.060638
A0A385XJL4 -0.109070 -0.011330
…                …        …
Q93K97     -0.018387 -0.012543
Q9XB42      0.038605  0.015205
Q9Z3A0     -0.030502  0.061249
U3PVA8      0.019318 -0.080444
V9HVX0      0.000296  0.009964

[4402 rows x 1024 columns]
```

How many proteins in the dataset have embeddings available in the dataset?

[ ]:

```
print(
    f"embeddings are available for {len(set(sequences.index) &␣
 ↪set(feature_protnlm.index))}/{len(set(sequences.index))} of proteins in the␣
 ↪dataset"
)
```

```
embeddings are available for 357/357 of proteins in the dataset
```

Final feature dataset:

```
[ ]: feature_protnlm_all = feature_protnlm.loc[
         list(set(sequences_all.index) & set(feature_protnlm.index))
     ]
     feature_protnlm_all
```

```
[ ]:               0         1         2         3         4         5         6  \
     P38101 -0.027802  0.033447  0.030701  0.040771  0.011818  0.023941 -0.016602
     P0AC02  0.044037  0.015450  0.007996  0.016800 -0.003355 -0.037872  0.003719
     P0AA60  0.030121  0.077637  0.030197  0.041077  0.006519  0.006325 -0.006943
     P69776  0.028473  0.003372  0.038666  0.031403  0.026398 -0.087524  0.056580
     P0AE12  0.027039  0.050659  0.057678  0.009445  0.006168  0.052002 -0.076233
     …          …       …       …       …       …       …       …
     P0ACE3 -0.003746 -0.071350 -0.020996  0.015190 -0.008286 -0.007133  0.046509
     P76236  0.008453 -0.034546  0.000566  0.002733 -0.019348  0.036591 -0.030899
     P0AEK4  0.026413  0.042297  0.001595 -0.000384  0.037476  0.049805 -0.076172
     P76373  0.033051  0.056915  0.013344  0.044830 -0.004059  0.058685 -0.058868
     P0AE08  0.013206  0.053284 -0.011551  0.028427 -0.006275  0.055023 -0.027374

                   7         8         9  …      1014      1015      1016  \
     P38101 -0.064209 -0.029434  0.004803  … -0.012726  0.009651  0.004013
     P0AC02 -0.033508  0.005539  0.003214  … -0.033936  0.012589 -0.010529
     P0AA60 -0.070923  0.021500 -0.017624  … -0.019196  0.029724  0.041687
     P69776 -0.087891 -0.048065  0.049683  … -0.076538 -0.067139 -0.068909
     P0AE12 -0.061188  0.033142  0.001889  … -0.010094 -0.011948 -0.000252
     …          …       …       …  …       …       …       …
     P0ACE3 -0.132080  0.032837  0.001653  … -0.018478 -0.030243  0.028198
     P76236 -0.048431  0.038055  0.027405  … -0.002909 -0.030655 -0.042725
     P0AEK4 -0.022064 -0.007061 -0.049011  … -0.025711 -0.020782 -0.055725
     P76373 -0.067505  0.030151 -0.033356  … -0.042572  0.008202 -0.025146
     P0AE08 -0.049927  0.035461 -0.020432  … -0.026077 -0.002666  0.036804

                1017      1018      1019      1020      1021      1022      1023
     P38101 -0.082092 -0.009453 -0.036499  0.006760 -0.032410  0.005623  0.008682
     P0AC02 -0.009094  0.045654  0.025299 -0.039673 -0.059143 -0.011368  0.039551
     P0AA60 -0.076660  0.028290 -0.019257  0.002077 -0.041016 -0.059875  0.028793
     P69776 -0.040680  0.003717 -0.085754 -0.095520  0.024826  0.015671  0.025879
     P0AE12 -0.027695  0.060059  0.020416 -0.033905  0.020676 -0.019073  0.067139
     …          …       …       …       …       …       …       …
     P0ACE3 -0.071106 -0.011383  0.051392 -0.013016  0.014565  0.034515 -0.011024
     P76236 -0.145264  0.036438  0.050964  0.005383 -0.040466  0.007732  0.060089
     P0AEK4 -0.055847  0.021713 -0.023041  0.003216 -0.044861 -0.002573  0.000482
     P76373 -0.048035  0.057159  0.018311  0.018906  0.028046  0.022675  0.023788
     P0AE08 -0.053772  0.030731 -0.021637 -0.009789 -0.035645 -0.003216  0.055878

     [3210 rows x 1024 columns]
```

[ ]:

```
feature_protnlm = feature_protnlm.loc[
    list(set(sequences.index) & set(feature_protnlm.index))
]
feature_protnlm
```

[ ]:

```
            0         1         2         3         4         5         6     \
P32715  0.007694 -0.009727 -0.010185  0.034180  0.016388  0.037506 -0.010513
P0ABT8  0.019699  0.009666  0.019333  0.056458 -0.009315 -0.009865  0.000576
P38101 -0.027802  0.033447  0.030701  0.040771  0.011818  0.023941 -0.016602
P0AA60  0.030121  0.077637  0.030197  0.041077  0.006519  0.006325 -0.006943
P0AFF0  0.033051  0.088989  0.023438  0.066101 -0.052155 -0.021606 -0.011993
…          …       …       …       …       …       …       …
P02930  0.044159  0.014931  0.003866  0.034851  0.013206 -0.014984 -0.041687
P09348  0.050934  0.016052  0.006355  0.029160 -0.036224  0.045929 -0.026138
P23200  0.003391  0.094299  0.035431  0.071594 -0.003857 -0.024323 -0.024643
P25737  0.040405  0.171997  0.026505  0.028519 -0.011833  0.009850 -0.025375
P77308  0.028152  0.034607 -0.001821  0.026489  0.019714 -0.019623  0.001829

            7         8         9     …      1014      1015      1016  \
P32715 -0.070007  0.063110 -0.026749  … -0.036743 -0.016571  0.001406
P0ABT8 -0.048920  0.041473  0.011383  … -0.032471 -0.003183 -0.018677
P38101 -0.064209 -0.029434  0.004803  … -0.012726  0.009651  0.004013
P0AA60 -0.070923  0.021500 -0.017624  … -0.019196  0.029724  0.041687
P0AFF0 -0.019547  0.028503 -0.070984  … -0.023438  0.004807  0.022018
…          …       …       …  …       …       …       …
P02930  0.018982  0.000612  0.002163  … -0.020538  0.006428 -0.030411
P09348 -0.069824  0.030792 -0.058014  … -0.047424  0.025681  0.002359
P23200 -0.069031  0.037415 -0.062927  … -0.006245 -0.019119 -0.011192
P25737 -0.015388  0.007412 -0.057922  … -0.003839 -0.015144 -0.016495
P77308 -0.024872  0.023071 -0.010658  … -0.005348  0.005089 -0.004429

            1017      1018      1019      1020      1021      1022      1023
P32715 -0.016296  0.038147  0.023193 -0.027542 -0.037598 -0.023773  0.009583
P0ABT8 -0.074036 -0.029434 -0.017426 -0.011108 -0.062988  0.029266 -0.023651
P38101 -0.082092 -0.009453 -0.036499  0.006760 -0.032410  0.005623  0.008682
P0AA60 -0.076660  0.028290 -0.019257  0.002077 -0.041016 -0.059875  0.028793
P0AFF0 -0.049438 -0.001275 -0.014069 -0.005836  0.032166 -0.041382  0.030121
…          …       …       …       …       …       …       …
P02930 -0.004768  0.082092  0.004097 -0.035370 -0.028091 -0.062561 -0.037720
P09348 -0.044037  0.018661 -0.004982 -0.008614 -0.009727 -0.029449  0.008087
P23200 -0.050079  0.026276 -0.031708 -0.025757  0.001470 -0.014984 -0.019226
P25737 -0.019714  0.043549 -0.058014 -0.032440 -0.008308 -0.009323  0.002542
P77308 -0.070557  0.034973 -0.040710 -0.026428 -0.043945  0.012451 -0.015884

[357 rows x 1024 columns]
```

1.2.2 PSSM

At the moment, the PSSMs for Uniref90 with three iterations are still being calculated. We will use the Uniref50 PSSMs with three iterations as an example. We have only calculated PSSMs for proteins that are annotated with the GO term *transmembrane transporter activity* or one of its descendant GO terms, therefore we first have to filter for that.

[ ]:

```
from subpred.pssm import calculate_pssm_feature

feature_pssm = calculate_pssm_feature(
    sequences=sequences.sequence,
    tmp_folder="../data/intermediate/blast/pssm_uniref50_3it/",
    blast_db="../data/raw/uniref/uniref50/uniref50.fasta",
    iterations=3,
    verbose=False,
)
feature_pssm
```

[ ]:

```
              AA        AR        AN        AD        AC        AQ        AE  \
P02916  0.819672  0.370902  0.387295  0.325820  0.336066  0.397541  0.350410
P03959  0.936430  0.342298  0.420538  0.322738  0.586797  0.386308  0.344743
P05825  0.724662  0.339527  0.407095  0.371622  0.234797  0.385135  0.337838
P0AA78  1.000000  0.050773  0.185430  0.000000  0.423841  0.161148  0.070640
P0AAD6  0.787425  0.281437  0.419162  0.215569  0.541916  0.299401  0.251497
…          …       …       …       …       …       …       …
P77328  0.739726  0.469178  0.493151  0.452055  0.559932  0.503425  0.460616
P76198  0.817708  0.338542  0.361979  0.192708  0.447917  0.375000  0.257812
P33011  0.790368  0.456091  0.368272  0.373938  0.456091  0.433428  0.424929
P37327  0.878543  0.129555  0.178138  0.161943  0.364372  0.251012  0.194332
P39282  0.887671  0.216438  0.356164  0.112329  0.309589  0.263014  0.178082

              AG        AH        AI  …        VL        VK        VM  \
P02916  0.530738  0.319672  0.497951  …  0.665984  0.415984  0.633197
P03959  0.616137  0.371638  0.501222  …  0.660147  0.337408  0.745721
P05825  0.530405  0.315878  0.378378  …  0.592905  0.290541  0.500000
P0AA78  0.724062  0.030905  0.596026  …  0.790287  0.384106  0.735099
P0AAD6  0.565868  0.323353  0.661677  …  0.703593  0.338323  0.706587
…          …       …       …  …       …       …       …
P77328  0.695205  0.457192  0.635274  …  0.691781  0.460616  0.698630
P76198  0.726562  0.291667  0.643229  …  0.768229  0.354167  0.789062
P33011  0.504249  0.416431  0.563739  …  0.708215  0.359773  0.594901
P37327  0.603239  0.165992  0.429150  …  0.647773  0.344130  0.639676
P39282  0.706849  0.167123  0.501370  …  0.734247  0.230137  0.728767

              VF        VP        VS        VT        VW        VY        VV
P02916  0.594262  0.411885  0.510246  0.606557  0.446721  0.508197  0.725410
P03959  0.647922  0.418093  0.559902  0.603912  0.518337  0.518337  0.792176
P05825  0.530405  0.300676  0.398649  0.496622  0.427365  0.423986  0.717905
```

```
P0AA78  0.688742  0.419426  0.551876  0.622517  0.571744  0.474614  0.805740
P0AAD6  0.682635  0.356287  0.535928  0.568862  0.544910  0.592814  0.781437
…            …        …        …        …         …        …        …
P77328  0.655822  0.503425  0.635274  0.667808  0.479452  0.515411  0.789384
P76198  0.783854  0.403646  0.598958  0.679687  0.554688  0.549479  0.807292
P33011  0.558074  0.362606  0.481586  0.572238  0.475921  0.475921  0.682720
P37327  0.554656  0.348178  0.465587  0.599190  0.469636  0.461538  0.821862
P39282  0.676712  0.276712  0.473973  0.531507  0.476712  0.410959  0.791781

[357 rows x 400 columns]
```

1.3 Annotation datasets

Here, we create gene/protein annotation datasets. Later, these annotations will be used for calculating enrichment in clusters.

1.3.1 GO

Preprocessing:

```
[ ]: go_annotations_uniprot = load_df("go")
         # filter for proteins in our e coli dataset
     go_annotations_ecoli = go_annotations_uniprot[
         go_annotations_uniprot.Uniprot.isin(set(sequences_all.index.tolist()))
     ].reset_index(drop=True)
     go_annotations_ecoli
```

```
[ ]:          Uniprot     qualifier       go_id evidence_code aspect
     0      A0A0A6YVN8       enables  GO:0046872           IEA      F
     1          A5A615    located_in  GO:0005886           IDA      C
     2          A5A615    located_in  GO:0005886           IEA      C
     3          A5A615    located_in  GO:0005886           RCA      C
     4          A5A615    located_in  GO:0016020           IEA      C
     …            …            …          …           …     …
     39610      Q93K97   involved_in  GO:0006753           IBA      P
     39611      Q93K97   involved_in  GO:0019693           IBA      P
     39612      Q93K97  is_active_in  GO:0005829           IBA      C
     39613      Q93K97    located_in  GO:0005829           HDA      C
     39614      Q93K97    located_in  GO:0005829           IDA      C

     [39615 rows x 5 columns]
```

```
[ ]: go_annotations_ecoli = add_go_ancestors(go_annotations_ecoli, go_owl=go_owl)
     go_annotations_ecoli
```

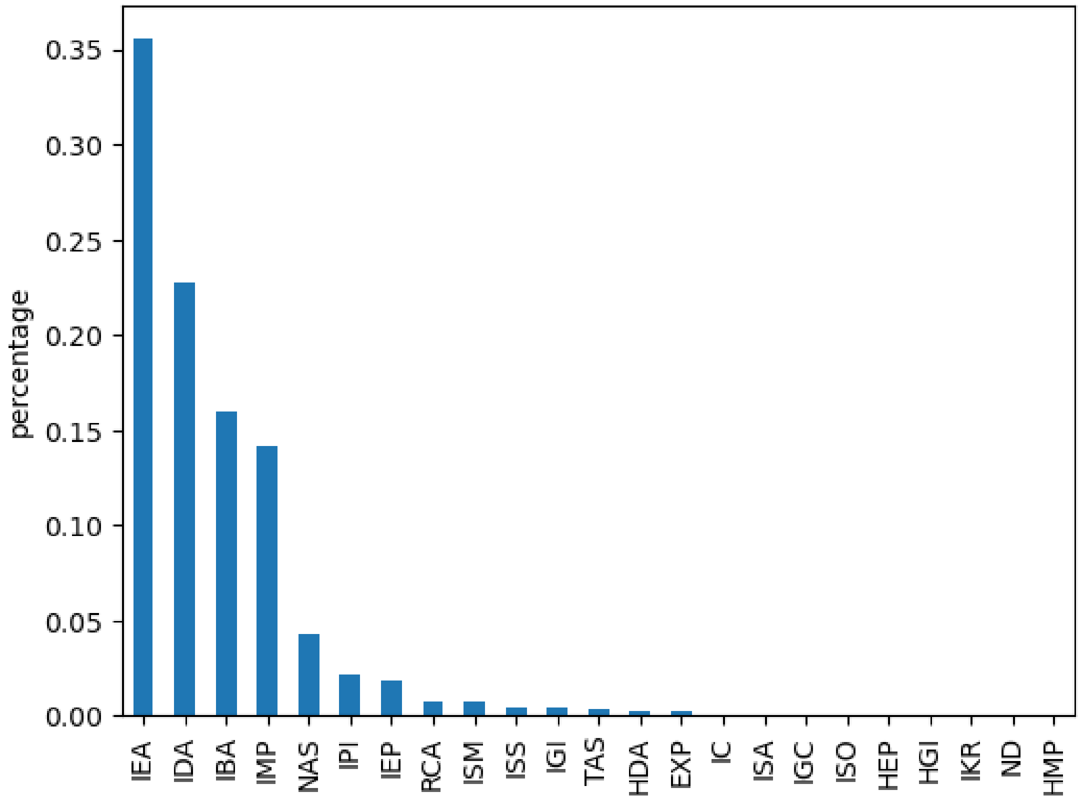
```
[ ]:           Uniprot   qualifier evidence_code aspect       go_id
     0       A0A0A6YVN8     enables           IEA      F  GO:0046872
     1       A0A0A6YVN8     enables           IEA      F  GO:0005488
```

```
2        A0A0A6YVN8     enables          IEA      F  GO:0043169
3        A0A0A6YVN8     enables          IEA      F  GO:0003674
4        A0A0A6YVN8     enables          IEA      F  GO:0043167
…            …             …         …    …       …
270350       Q93K97  located_in          HDA      C  GO:0005829
270351       Q93K97  located_in          HDA      C  GO:0005575
270352       Q93K97  located_in          IDA      C  GO:0110165
270353       Q93K97  located_in          IDA      C  GO:0005829
270354       Q93K97  located_in          IDA      C  GO:0005575

[270355 rows x 5 columns]
```

Evidence codes: Only about 35% of annotations have evidence code IEA, and some of those might even have non-IEA duplicates in the dataset, since the annotations stem from multiple sources. We can remove the IEA terms.

```
[ ]: # filter/analyze evidence codes
     g = go_annotations_ecoli.evidence_code.value_counts(normalize=True).plot.bar()
     g.set_ylabel("percentage")
```

```
[ ]: Text(0, 0.5, 'percentage')
```



[ ]:

```
go_annotations_ecoli = (
    go_annotations_ecoli[go_annotations_ecoli.evidence_code != "IEA"]
    .drop("evidence_code", axis=1)
    .drop_duplicates()
    .reset_index(drop=True)
)
go_annotations_ecoli
```

[ ]:

```
        Uniprot                   qualifier aspect       go_id
0        A5A615                  located_in      C  GO:0110165
1        A5A615                  located_in      C  GO:0005886
2        A5A615                  located_in      C  GO:0016020
3        A5A615                  located_in      C  GO:0005575
4        A5A616  acts_upstream_of_or_within      P  GO:0007154
…           …                           …    …         …
138480   Q93K97                is_active_in      C  GO:0005829
138481   Q93K97                is_active_in      C  GO:0005575
138482   Q93K97                  located_in      C  GO:0110165
138483   Q93K97                  located_in      C  GO:0005829
138484   Q93K97                  located_in      C  GO:0005575

[138485 rows x 4 columns]
```

Qualifiers

- Contains some "NOT" qualifiers that have to be removed, otherwise okay.
- Some qualifiers represent inaccurate relations between genes and terms, they should be removed.
    - The most significant removed term is *acts_upstream_of_or_within*, which makes up more than 50% of biological process annotations.

Explanations of inaccurate relations:

- **acts_upstream_of_or_within**: The acts upstream of or within annotation relation when the mechanism relating the gene product's activity to the Biological Process is not known. Annotations using this relation often come from mutant phenotypes for which further characterization has not been performed, but the authors wish to report the effect that the gene has on a process.
- **colocalizes_with**: where the resolution of an assay is not accurate enough to say that the gene product is a bona fide component member
- **contributes_to**: A gene product is associated with a GO Molecular Function term using the qualifier 'contributes to' when it is a member of a complex that is defined as an "irreducible molecular machine" - where a particular Molecular Function cannot be ascribed to an individual subunit or small set of subunits of a complex.
- **acts_upstream_of**: The acts upstream of gene product to GO term relation is used when the mechanism relating a gene product's activity to a Biological Process is known and the activity occurs before the Biological Process but is neither an integral part of the process, nor an integral part of a process that regulates it. Curators should always strive to use one

of the more specific child relations that capture the directionality of the effect, either positive or negative.

```
[ ]: for aspect in ["C", "P", "F"]:
         print("###", aspect)
         print(
             go_annotations_ecoli[go_annotations_ecoli.aspect == aspect]
             .qualifier.astype("str")
             .value_counts()
             .to_string()
         )
```

```
### C
located_in          11217
is_active_in         3364
part_of              2683
colocalizes_with        7
### P
acts_upstream_of_or_within        49569
involved_in                       41647
NOT|involved_in                      45
NOT|acts_upstream_of_or_within       17
acts_upstream_of                      8
### F
enables            29318
contributes_to       553
NOT|enables           57
```

```
[ ]: go_annotations_ecoli = go_annotations_ecoli[
         ~go_annotations_ecoli.qualifier.str.startswith("NOT")
     ]
     go_annotations_ecoli = go_annotations_ecoli[
         ~go_annotations_ecoli.qualifier.isin(
             [
                 "contributes_to",
                 "colocalizes_with",
                 "acts_upstream_of",
                 "acts_upstream_of_or_within",
             ]
         )
     ]
     go_annotations_ecoli = go_annotations_ecoli.reset_index(drop=True)
     go_annotations_ecoli.qualifier.astype("str").value_counts()
```

```
[ ]: involved_in     41647
     enables         29318
     located_in      11217
     is_active_in     3364
```

```
part_of           2683
Name: qualifier, dtype: int64
```

```
[ ]: for aspect in ["C", "P", "F"]:
         print("###", aspect)
         print(
             go_annotations_ecoli[go_annotations_ecoli.aspect == aspect]
             .qualifier.astype("str")
             .value_counts()
             .to_string()
         )
```

```
### C
located_in      11217
is_active_in     3364
part_of          2683
### P
involved_in    41647
### F
enables    29318
```

There are two additional qualifiers for the cellular component annotations:

- **is_active_in**: The 'is active in' relation is used to relate a gene or gene product to the GO Cellular Component in which the gene or gene product enables its Molecular Function, e.g. ced-3 'is active in' cytosol. This indicates that the ced-3 gene product executes its molecular function in the cytosol.
- **part_of**: 'part of' is used to link gene products to protein-containing complexes when the gene product is an integral component of the protein-containing complex

The former could be interesting, we could filter the "transmembrane transporter activity" for genes that are actually active in the membrane, at least if enough proteins have that annotation. We should look at some examples for the latter, to get a picture of what that relation looks like in practice.

Are all proteins in E coli that are annotated with "transmembrane transporter activity" also "active_in" the membrane?

```
[ ]: # TODO
     proteins_transmembrane_transporter_activity_ecoli = go_annotations_ecoli[
         (go_annotations_ecoli.aspect == "F") & (go_annotations_ecoli.go_id == "GO:
      ↪0022857")
     ].Uniprot.tolist()
     print(
         "total number of transmembrane transporters: ",
         len(set(proteins_transmembrane_transporter_activity_ecoli)),
     )
     tmp = go_annotations_ecoli[
         go_annotations_ecoli.Uniprot.
      ↪isin(proteins_transmembrane_transporter_activity_ecoli)
         & (go_annotations_ecoli.aspect == "C")
         & (go_annotations_ecoli.qualifier == "is_active_in")
     ]
     tmp = tmp.assign(go_term=tmp.go_id.transform(go_owl.get_label))
     print("transmembrane transporters with 'is_active_in' annotation:", tmp.Uniprot.
      ↪unique().shape[0])
     print("distribution of 'is_active_in' annotations:")
     tmp.go_term.value_counts()
```

```
total number of transmembrane transporters:  357
transmembrane transporters with 'is_active_in' annotation: 120
distribution of 'is_active_in' annotations:
```

```
[ ]: cellular anatomical entity                    120
     cellular_component                            120
     membrane                                      104
     plasma membrane                                80
     outer membrane-bounded periplasmic space       12
     periplasmic space                              12
     cell outer membrane                             9
     outer membrane                                  9
     cell division site                              2
     intracellular organelle                         1
     organelle                                       1
     membrane-bounded organelle                      1
     intracellular membrane-bounded organelle        1
     cytosol                                         1
     Name: go_term, dtype: int64
```

It could be the case that this qualifier is simply not used in every instance where the relation would be most appropriate, or that the remaining terms are electronically annotated and not experimentally known (we removed IEA annotations). Or the majority of our transporters do not actively transport any substrates across the membrane and are instead part of protein complexes, or are actually upstream of the transport.

What are some examples of transmembrane transport proteins without the “is_active_in” annotation or the “part_of” annotation?

```
[ ]: tmp = go_annotations_ecoli[
         go_annotations_ecoli.Uniprot.
      ↪isin(proteins_transmembrane_transporter_activity_ecoli)
         & (go_annotations_ecoli.aspect == "C")
         & (go_annotations_ecoli.qualifier == "located_in")
     ]
     tmp = tmp.assign(go_term = tmp.go_id.transform(go_owl.get_label))
```

```python
#TODO some weird proteins in there, maybe find some go term filters? or tcdb filter?
# display(tmp.Uniprot.unique())
# # tmp.Uniprot.unique()

# sequences_all.loc[tmp.Uniprot.unique()]

tmp.go_term.value_counts()
```

[ ]:

```
cellular_component
349
cellular anatomical entity
327
membrane
309
plasma membrane
282
protein-containing complex
143
membrane protein complex
136
transporter complex
125
transmembrane transporter complex
122
plasma membrane protein complex
81
ATP-binding cassette (ABC) transporter complex
73
ATPase dependent transmembrane transport complex
73
ATP-binding cassette (ABC) transporter complex, substrate-binding subunit-
containing     57
cell outer membrane
23
outer membrane
23
outer membrane-bounded periplasmic space
14
periplasmic space
14
catalytic complex
11
oxidoreductase complex
10
cytosol
8
```

```
proton-transporting ATP synthase complex
7
proton-transporting two-sector ATPase complex
7
efflux pump complex
7
respiratory chain complex
6
NADH dehydrogenase complex
6
respiratory chain complex I
6
potassium:proton antiporter complex
5
cytochrome complex
4
TAT protein transport complex
4
extrinsic component of membrane
4
cytochrome o ubiquinol oxidase complex
4
maltose transport complex
3
glycerol-3-phosphate-transporting ATPase complex
3
macrolide transmembrane transporter complex
3
cell envelope Sec protein transport complex
3
intracellular protein-containing complex
3
cytoplasm
3
enzyme IIA-maltose transporter complex
3
MacAB-TolC complex
3
ProVWX complex
2
methionine-importing ABC transporter complex
2
methionine-importing complex
2
divisome complex
2
cobalamin transport complex
```

```
2
division septum
2
organelle
2
cell projection
2
non-membrane-bounded organelle
2
bacterial-type flagellum
2
bacterial-type flagellum stator complex
2
cell septum
2
extrinsic component of plasma membrane
2
cell division site
2
ATPase complex
1
extrinsic component of cytoplasmic side of plasma membrane
1
EmrE multidrug transporter complex
1
external side of plasma membrane
1
side of membrane
1
tripartite ATP-independent periplasmic transporter complex
1
MsbA transporter complex
1
Name: go_term, dtype: int64
```

Interestingly, there are about 125 proteins that seem to be part of transporter protein complexes. If they do not show up during the clustering, we should look for ways to filter or classify them.

What are some examples of the part_of relation wrt cellular component annotations?

[ ]:

```
tmp = go_annotations_ecoli[
    go_annotations_ecoli.Uniprot.
 ↪isin(proteins_transmembrane_transporter_activity_ecoli)
    & (go_annotations_ecoli.aspect == "C")
    & (go_annotations_ecoli.qualifier == "part_of")
]
tmp = tmp.assign(go_term = tmp.go_id.transform(go_owl.get_label)).
 ↪reset_index(drop=True)
```

```
tmp.go_term.value_counts()
```

```
[ ]: cellular_component
     91
     protein-containing complex
     89
     membrane protein complex
     84
     transmembrane transporter complex
     66
     transporter complex
     66
     plasma membrane protein complex
     58
     ATPase dependent transmembrane transport complex
     51
     ATP-binding cassette (ABC) transporter complex
     47
     ATP-binding cassette (ABC) transporter complex, substrate-binding subunit-
     containing    14
     catalytic complex
     11
     oxidoreductase complex
     11
     NADH dehydrogenase complex
     7
     respiratory chain complex
     6
     plasma membrane respiratory chain complex I
     6
     respiratory chain complex I
     6
     pore complex
     5
     cytochrome o ubiquinol oxidase complex
     4
     cation-transporting ATPase complex
     4
     TAT protein transport complex
     4
     proton-transporting two-sector ATPase complex, catalytic domain
     4
     proton-transporting ATP synthase complex, catalytic core F(1)
     4
     cytochrome complex
     4
     intracellular protein-containing complex
```

```
4
cell envelope Sec protein transport complex
4
cellular anatomical entity
4
potassium ion-transporting ATPase complex
4
efflux pump complex
3
membrane
3
MacAB-TolC complex
3
maltose transport complex
3
proton-transporting ATP synthase complex, coupling factor F(o)
3
proton-transporting two-sector ATPase complex, proton-transporting domain
3
macrolide transmembrane transporter complex
3
plasma membrane
2
outer membrane protein complex
1
outer membrane
1
cell outer membrane
1
envelope
1
ion channel complex
1
cell envelope
1
Name: go_term, dtype: int64
```

The relation part_of seems to be used for proteins that are part of complexes. Only one proteins in “part_of” “outer membrane”, the others seem to be located_in outer membrane. On the other hand, the located_in annotations contain 125 protein complex annotations, so it could just be the case that qualifier and complex membership are unrelated.

We will keep the “is_active_in” and “part_of” cellular component annotations in the dataset, there is no clear reason to exlude them.

Labels Annotating the dataset with labels/names of GO terms:

```
[ ]: go_annotations_ecoli = go_annotations_ecoli.assign(
         go_term=go_annotations_ecoli.go_id.map(go_owl.get_label)
     )
     go_annotations_ecoli
```

```
[ ]:       Uniprot     qualifier aspect       go_id                     go_term
     0      A5A615    located_in      C  GO:0110165  cellular anatomical entity
     1      A5A615    located_in      C  GO:0005886             plasma membrane
     2      A5A615    located_in      C  GO:0016020                    membrane
     3      A5A615    located_in      C  GO:0005575          cellular_component
     4      A5A616    located_in      C  GO:0110165  cellular anatomical entity
     …       …           …    …         …                         …
     88224  Q93K97  is_active_in      C  GO:0005829                     cytosol
     88225  Q93K97  is_active_in      C  GO:0005575          cellular_component
     88226  Q93K97    located_in      C  GO:0110165  cellular anatomical entity
     88227  Q93K97    located_in      C  GO:0005829                     cytosol
     88228  Q93K97    located_in      C  GO:0005575          cellular_component

     [88229 rows x 5 columns]
```

1.3.2 Keywords

```
[ ]: keywords_uniprot = load_df("keywords")
     keywords_ecoli = (
         keywords_uniprot[keywords_uniprot.Uniprot.isin(sequences_all.index)]
         .drop_duplicates()
         .reset_index(drop=True)
     )
     keywords_ecoli
```

```
[ ]:           Uniprot                    keyword
     0           P00509               3D-structure
     1           P00509           Aminotransferase
     2           P00509                  Cytoplasm
     3           P00509  Direct protein sequencing
     4           P00509        Pyridoxal phosphate
     …           …                        …
     23130       P0AEG8         Reference proteome
     23131       P33668         Reference proteome
     23132       P33668                     Signal
     23133   A0A0A6YVN8               3D-structure
     23134   A0A0A6YVN8              Metal-binding

     [23135 rows x 2 columns]
```

1.3.3 Interpro

```
[ ]: interpro_all = load_df("interpro")
     interpro_ecoli = interpro_all[
         interpro_all.Uniprot.isin(sequences_all.index)
     ].reset_index(drop=True)
     interpro_ecoli
```

```
[ ]:          Uniprot interpro_id                   type  \
     0         P00509   IPR004839                 Domain
     1         P00509   IPR000796                 Family
     2         P00509   IPR004838           Binding_site
     3         P00509   IPR015424  Homologous_superfamily
     4         P00509   IPR015421  Homologous_superfamily
     …            …          …                      …
     12755     P77296   IPR011990  Homologous_superfamily
     12756     P0AEG8   IPR019717                 Family
     12757     P33668   IPR028921                 Domain
     12758 A0A0A6YVN8   IPR036237  Homologous_superfamily
     12759 A0A0A6YVN8   IPR013022                 Domain

                                                          name
     0                        Aminotransferase, class I/classII
     1                         Aspartate/other aminotransferase
     2       Aminotransferases, class-I, pyridoxal-phosphat…
     3               Pyridoxal phosphate-dependent transferase
     4       Pyridoxal phosphate-dependent transferase, maj…
     …                                                      …
     12755  Tetratricopeptide-like helical domain superfamily
     12756                                   Dextransucrase DSRB
     12757                                      NTF2 fold domain
     12758                        Xylose isomerase-like superfamily
     12759               Xylose isomerase-like, TIM barrel domain

     [12760 rows x 4 columns]
```

Interpro annotation types and names:

We can drop the type column, since the type can be derived from the name. Only keeping the name, since that makes the results easier to interpret:

```
[ ]: interpro_ecoli = interpro_ecoli.drop(["interpro_id", "type"],axis=1).
      ↪rename(columns={"name":"interpro"})
     assert not interpro_ecoli.duplicated().any()
     interpro_ecoli
```

```
[ ]:          Uniprot                                     interpro
     0         P00509                  Aminotransferase, class I/classII
```

```
1          P00509                    Aspartate/other aminotransferase
2          P00509  Aminotransferases, class-I, pyridoxal-phosphat…
3          P00509          Pyridoxal phosphate-dependent transferase
4          P00509  Pyridoxal phosphate-dependent transferase, maj…
…            …                                                   …
12755      P77296  Tetratricopeptide-like helical domain superfamily
12756      P0AEG8                                 Dextransucrase DSRB
12757      P33668                                     NTF2 fold domain
12758  A0A0A6YVN8                   Xylose isomerase-like superfamily
12759  A0A0A6YVN8          Xylose isomerase-like, TIM barrel domain

[12760 rows x 2 columns]
```

1.3.4 TCDB

```
[ ]: tcdb_all = load_df("tcdb_substrates")
     tcdb_ecoli = (
         tcdb_all[
             (tcdb_all.variable == "Uniprot") & (tcdb_all.value.isin(sequences_all.
      ↪index))
         ]
         .drop_duplicates()
         .reset_index(drop=True)
         .drop("variable", axis=1)
         .rename(columns={"value": "Uniprot"})[["Uniprot", "tcdb_id"]]
     )
     tcdb_ecoli
```

```
[ ]:     Uniprot      tcdb_id
     0    P00803  9.B.391.1.3
     1    P02916    3.A.1.1.1
     2    P02925    3.A.1.2.1
     3    P02929    2.C.1.1.1
     4    P03959    3.A.3.7.1
     ..      …          …
     702  Q46909   2.A.1.1.95
     703  P28303   2.A.66.1.4
     704  C1P611  1.C.135.1.1
     705  P23481    3.D.1.9.1
     706  P31827  1.B.14.19.1

     [707 rows x 2 columns]
```

1.3.5 Records

Finally, we transform the dataframes into lists of tuples, so they work with the refactored enrichment analysis method

[ ]:

```
records_kw = keywords_ecoli.to_records(index=False).tolist()

records_go_cc = (
    go_annotations_ecoli[go_annotations_ecoli.aspect == "C"]
    .drop(["qualifier", "go_id", "aspect"], axis=1)
    .drop_duplicates()
    .to_records(index=False)
    .tolist()
)
records_go_mf = (
    go_annotations_ecoli[go_annotations_ecoli.aspect == "F"]
    .drop(["qualifier", "go_id", "aspect"], axis=1)
    .drop_duplicates()
    .to_records(index=False)
    .tolist()
)
records_go_bp = (
    go_annotations_ecoli[go_annotations_ecoli.aspect == "P"]
    .drop(["qualifier", "go_id", "aspect"], axis=1)
    .drop_duplicates()
    .to_records(index=False)
    .tolist()
)
records_go_all = (
    go_annotations_ecoli.drop(["qualifier", "go_id", "aspect"], axis=1)
    .drop_duplicates()
    .to_records(index=False)
    .tolist()
)

records_tcdb_class = (
    tcdb_ecoli.assign(tcdb_class=tcdb_ecoli.tcdb_id.transform(lambda x: x[:3]))[
        ["Uniprot", "tcdb_class"]
    ]
    .drop_duplicates()
    .to_records(index=False)
    .tolist()
)
records_tcdb_mechanism = (
    tcdb_ecoli.assign(tcdb_class=tcdb_ecoli.tcdb_id.transform(lambda x: x[:1]))[
        ["Uniprot", "tcdb_class"]
    ]
    .drop_duplicates()
    .to_records(index=False)
    .tolist()
)
```

```
records_interpro = interpro_ecoli.drop_duplicates().to_records(index=False).
 ↪tolist()
```

Dictionary of all annotation lists:

[ ]:
```
records_all_dict = {
    "Keywords": records_kw,
    "GO Cellular Component": records_go_cc,
    "GO Molecular Function": records_go_mf,
    "GO Biological Process": records_go_bp,
    "TCDB Class": records_tcdb_class,
    "TCDB Mechanism": records_tcdb_mechanism,
    "Interpro Domains": records_interpro,
}
```

1.4 Kmeans Clustering

First, we will try to cluster the entire dataset with k-means clustering.

What is the optimal number *k* of clusters? There are four metrics that are commonly used to estimate clustering quality for different *k*:

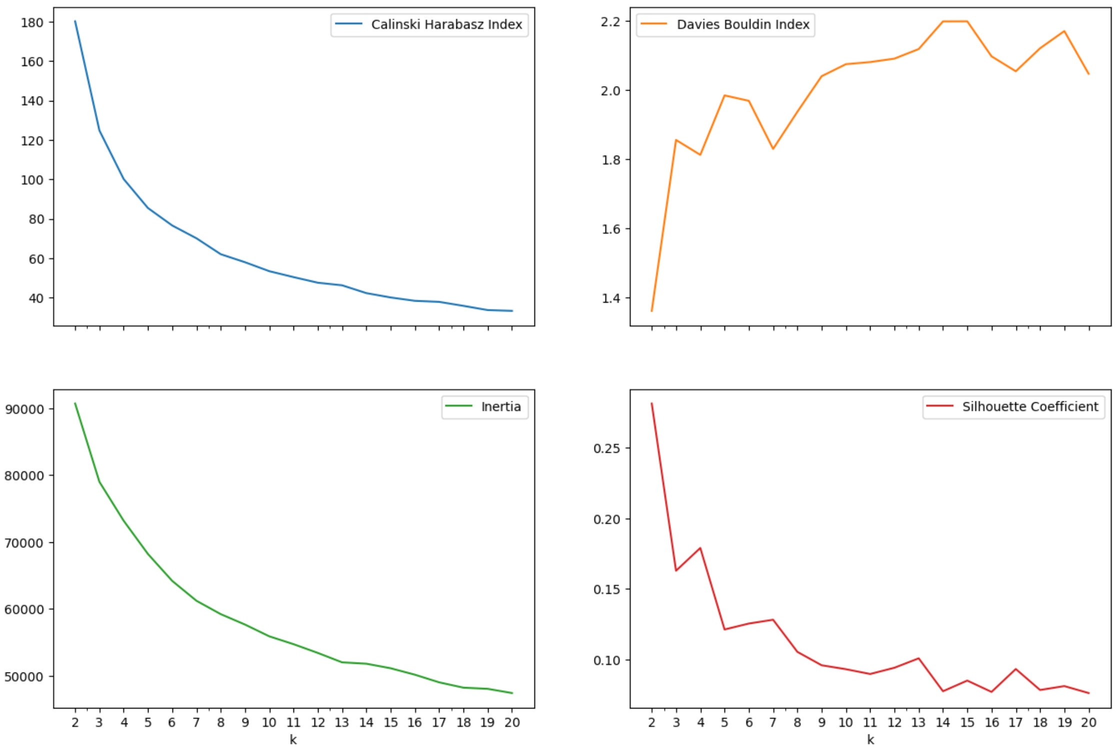
- **Elbow plot/Inertia**: the more it looks like an elbow, the better. the optimal number of clusters is at the "joint of the arm"
- **Silhouette**: Number between -1 and 1. The higher the better. Measures tightness/overlap of clusters
- **Calinsky Harabasz**: Higher score is better
- **Davies Boldin**: The closer to 0 the better. Average similarity between any cluster and its closest cluster.

[ ]:
```
from subpred.clustering import clustering_quality_plots
```

For PSSM Feature: Here, we are using PSSMs generated from Uniref50, with 3 iterations of Psiblast. These PSSMs often yielded the best results in Manuscript 1.

[ ]:
```
clustering_quality_plots(feature_pssm)
```

[ ]:
```
array([[<AxesSubplot:xlabel='k'>, <AxesSubplot:xlabel='k'>],
       [<AxesSubplot:xlabel='k'>, <AxesSubplot:xlabel='k'>]], dtype=object)
```

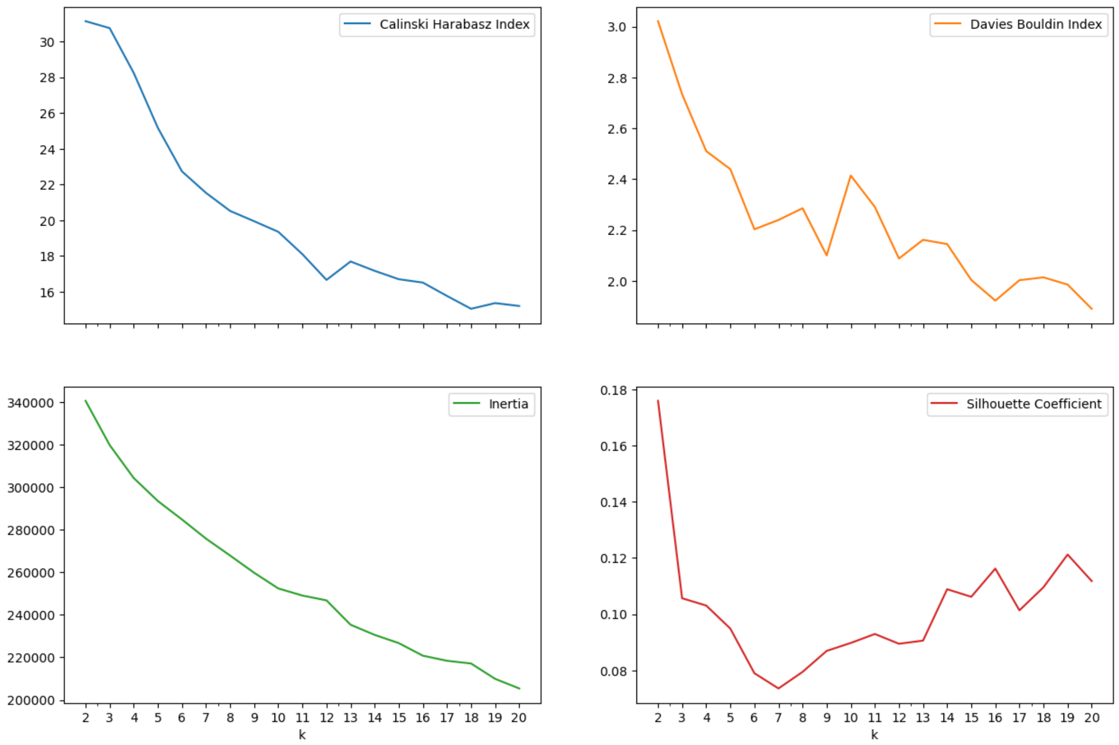


The elbow plot could look better, and the other scores point towards k=2.

ProtNLM feature: This feature was provided by Uniprot, and calculated in collaboration with Google Research. Each protein sequence is transformed into a word embedding, a vector of length 1024.

```
[ ]: clustering_quality_plots(feature_protnlm)
```

```
[ ]: array([[<AxesSubplot:xlabel='k'>, <AxesSubplot:xlabel='k'>],
            [<AxesSubplot:xlabel='k'>, <AxesSubplot:xlabel='k'>]], dtype=object)
```



It actually looks like PSSM feature works better for clustering. We should look at a dendrogram to see what the data looks like.

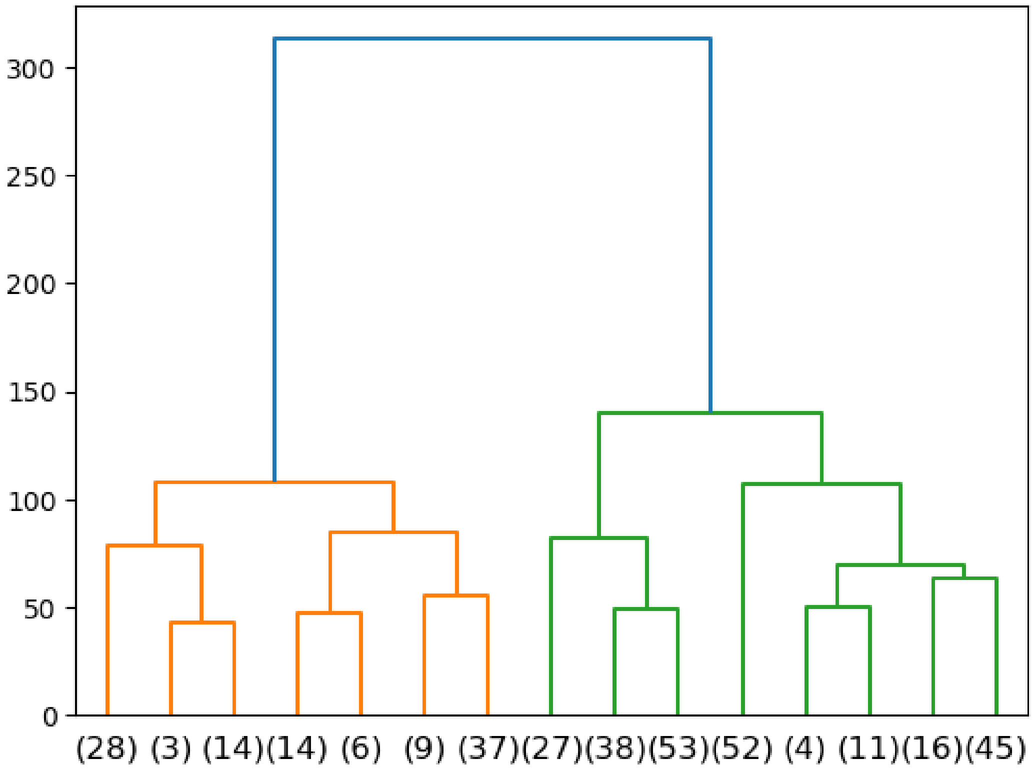
1.5 Hierarchical clustering using PSSMs

We will try hierarchical clustering first in order to find good annotations to create clusters, then try KMeans again. The clustering looks better when using PSSM instead of embeddings.

Here, we are using Ward clustering. Other methods, like the "single" or "centroid" methods, could be useful later for removing outliers from the individual clusters.

[ ]:

```
from subpred.clustering import get_linkage, dendrogram_plot, get_cluster_labels

linkage = get_linkage(feature_pssm)
dendrogram = dendrogram_plot(linkage, max_leaves=15)
```



There seem to be two cluster that can be easily distinguished with Ward-Clustering and PSSM feature. What are they?

Analyzing the first two clusters

```
[ ]: labels_twoclusters = get_cluster_labels(linkage, n_clusters=2,␣
       ↪index=feature_pssm.index.tolist())
     labels_twoclusters.value_counts()
```

```
[ ]: 2    246
     1    111
     dtype: int64
```

Cluster 1 contains roughly half as many proteins as cluster 2.

Enrichment analysis For each cluster, we use a hypergeometric test to calculate p-values. The log fold change is calculated as well, and the percentage of proteins annotated with the annotation that are part of in the cluster.

Only entries with FDR-adjusted p-value < 0.05 are shown here.

We used the set of transmembrane transport proteins as the reference set for the hypergeometric test, instead of the all the proteins in the genome. If we would use the entire genome instead, then the most significiantly enriched annotations would be related to transmembrane transport, since that is what we filtered the subset for.

[ ]:
```
from subpred.enrichment_analysis import cluster_enrichment_analysis

reference_set_transmembrane_transporters = set(sequences.index.unique().
 ↪to_list())
reference_set_whole_genome = set(sequences_all.index.unique().to_list())

cluster_enrichment_analysis(
    cluster_labels=labels_twoclusters,␣
 ↪reference_set=reference_set_transmembrane_transporters,␣
 ↪annotations_dict=records_all_dict, p_cutoff=0.05
)
```

```
============================================================
CLUSTER 1
============================================================
Keywords
                   annotation  total_reference  annotated_reference  \
4                3D-structure              357                  116
0                      Signal              357                   46
1   Direct protein sequencing              357                   55
2         Cell outer membrane              357                   27
3   Transmembrane beta strand              357                   27
11                ATP-binding              357                   42
9                 Translocase              357                   37
5                   Periplasm              357                   10
6              Disulfide bond              357                   10
7                    TonB box              357                    6
8         Fimbrium biogenesis              357                    6
10                   Receptor              357                    7

    total_subset  annotated_subset   expected  percentage_of_annotated  \
4            111                58  36.067227                    50.00
0            111                38  14.302521                    82.61
1            111                37  17.100840                    67.27
2            111                23   8.394958                    85.19
3            111                23   8.394958                    85.19
11           111                21  13.058824                    50.00
9            111                20  11.504202                    54.05
5            111                 9   3.109244                    90.00
6            111                 9   3.109244                    90.00
7            111                 6   1.865546                   100.00
8            111                 6   1.865546                   100.00
10           111                 6   2.176471                    85.71
```

```
         lfc             p         p_fdr  p_bonferroni
4   0.685364  1.242733e-07  2.386048e-06  1.193024e-05
0   1.409730  1.026404e-14  9.853483e-13  9.853483e-13
1   1.113458  1.681339e-09  8.070427e-08  1.614085e-07
2   1.454039  2.078635e-09  5.701398e-08  1.995489e-07
3   1.454039  2.078635e-09  5.701398e-08  1.995489e-07
11  0.685364  5.081834e-03  4.065467e-02  4.878561e-01
9   0.797839  1.844908e-03  1.771112e-02  1.771112e-01
5   1.533361  1.586527e-04  2.343179e-03  1.523066e-02
6   1.533361  1.586527e-04  2.343179e-03  1.523066e-02
7   1.685364  8.214637e-04  9.277707e-03  7.886051e-02
8   1.685364  8.214637e-04  9.277707e-03  7.886051e-02
10  1.462972  4.275824e-03  3.731628e-02  4.104791e-01

GO Cellular Component

                                 annotation  total_reference  \
4                protein-containing complex              357
5                  membrane protein complex              357
0                       cell outer membrane              357
1                            outer membrane              357
2  outer membrane-bounded periplasmic space              357
3                         periplasmic space              357

   annotated_reference  total_subset  annotated_subset   expected  \
4                  155           111                63  48.193277
5                  147           111                60  45.705882
0                   27           111                23   8.394958
1                   27           111                23   8.394958
2                   16           111                14   4.974790
3                   16           111                14   4.974790

   percentage_of_annotated       lfc             p         p_fdr  p_bonferroni
4                    40.65  0.386520  4.930142e-04  6.507788e-03  3.253894e-02
5                    40.82  0.392583  7.039776e-04  7.743754e-03  4.646252e-02
0                    85.19  1.454039  2.078635e-09  9.145993e-08  1.371899e-07
1                    85.19  1.454039  2.078635e-09  9.145993e-08  1.371899e-07
2                    87.50  1.492719  2.822946e-06  5.323269e-05  1.863144e-04
3                    87.50  1.492719  2.822946e-06  5.323269e-05  1.863144e-04

GO Molecular Function

                                    annotation  total_reference  \
0                                      binding              357
5                              protein binding              357
3   passive transmembrane transporter activity              357
4                             channel activity              357
11                                 ion binding              357
9                heterocyclic compound binding              357
10             organic cyclic compound binding              357
```

```
12                    carbohydrate derivative binding              357
6                           identical protein binding              357
1                          wide pore channel activity              357
2                                      porin activity              357
7   siderophore uptake transmembrane transporter a…              357
8                       fimbrial usher porin activity              357

    annotated_reference  total_subset  annotated_subset   expected  \
0                   119           111                59  37.000000
5                    77           111                38  23.941176
3                    44           111                26  13.680672
4                    44           111                26  13.680672
11                   47           111                24  14.613445
9                    38           111                21  11.815126
10                   38           111                21  11.815126
12                   37           111                20  11.504202
6                    27           111                17   8.394958
1                    19           111                15   5.907563
2                    19           111                15   5.907563
7                     6           111                 6   1.865546
8                     6           111                 6   1.865546

    percentage_of_annotated       lfc             p     p_fdr  p_bonferroni
0                     49.58  0.673190  1.321832e-07  0.000037      0.000037
5                     49.35  0.666505  1.158074e-04  0.005443      0.032658
3                     59.09  0.926372  3.727963e-05  0.002336      0.010513
4                     59.09  0.926372  3.727963e-05  0.002336      0.010513
11                    51.06  0.715738  1.748256e-03  0.041084      0.493008
9                     55.26  0.829754  9.280026e-04  0.024923      0.261697
10                    55.26  0.829754  9.280026e-04  0.024923      0.261697
12                    54.05  0.797839  1.844908e-03  0.040020      0.520264
6                     62.96  1.017940  3.837330e-04  0.015459      0.108213
1                     78.95  1.344327  1.399789e-05  0.001579      0.003947
2                     78.95  1.344327  1.399789e-05  0.001579      0.003947
7                    100.00  1.685364  8.214637e-04  0.027253      0.231653
8                    100.00  1.685364  8.214637e-04  0.027253      0.231653
GO Biological Process

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        1.B              357                   25           111
```

```
   annotated_subset  expected  percentage_of_annotated       lfc  \
0                21  7.773109                     84.0  1.433826

              p         p_fdr  p_bonferroni
0  2.070293e-08  1.863264e-07  1.863264e-07
```

TCDB Mechanism

```
  annotation  total_reference  annotated_reference  total_subset  \
1          3              357                  103           111
0          1              357                   42           111

   annotated_subset   expected  percentage_of_annotated       lfc  \
1                43  32.025210                    41.75  0.425129
0                30  13.058824                    71.43  1.199938

              p         p_fdr  p_bonferroni
1  4.484665e-03  1.345399e-02  2.690799e-02
0  1.093920e-08  6.563519e-08  6.563519e-08
```

Interpro Domains

```
                                           annotation  total_reference  \
0          Peptide/nickel binding protein, MppA-type              357
2             Solute-binding protein family 5 domain              357
1                    Solute-binding protein family 5              357
9            Fimbrial membrane usher, conserved site              357
14              PapC, N-terminal domain superfamily              357
13                          PapC, N-terminal domain              357
12          PapC-like, C-terminal domain superfamily              357
11                     PapC-like, C-terminal domain              357
10     Outer membrane usher protein FimD, plug domain              357
8                      Outer membrane usher protein              357
7     Solute-binding protein family 5, conserved site              357
6   TonB-dependent receptor-like, beta-barrel doma…            357
5           TonB-dependent receptor-like, beta-barrel              357
4    TonB-dependent receptor, plug domain superfamily              357
3                TonB-dependent receptor, plug domain              357
15                          TonB box, conserved site              357
16              TonB-dependent siderophore receptor              357

    annotated_reference  total_subset  annotated_subset  expected  \
0                     8           111                 8  2.487395
2                     9           111                 8  2.798319
1                     9           111                 8  2.798319
9                     6           111                 6  1.865546
14                    6           111                 6  1.865546
13                    6           111                 6  1.865546
12                    6           111                 6  1.865546
```

```
11                    6           111                 6  1.865546
10                    6           111                 6  1.865546
8                     6           111                 6  1.865546
7                     6           111                 6  1.865546
6                     6           111                 6  1.865546
5                     6           111                 6  1.865546
4                     6           111                 6  1.865546
3                     6           111                 6  1.865546
15                    5           111                 5  1.554622
16                    5           111                 5  1.554622

    percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
0                    100.00  1.685364  0.000073  0.018401      0.018401
2                     88.89  1.515439  0.000485  0.048865      0.122162
1                     88.89  1.515439  0.000485  0.048865      0.122162
9                    100.00  1.685364  0.000821  0.021790      0.207009
14                   100.00  1.685364  0.000821  0.021790      0.207009
13                   100.00  1.685364  0.000821  0.021790      0.207009
12                   100.00  1.685364  0.000821  0.021790      0.207009
11                   100.00  1.685364  0.000821  0.021790      0.207009
10                   100.00  1.685364  0.000821  0.021790      0.207009
8                    100.00  1.685364  0.000821  0.021790      0.207009
7                    100.00  1.685364  0.000821  0.021790      0.207009
6                    100.00  1.685364  0.000821  0.021790      0.207009
5                    100.00  1.685364  0.000821  0.021790      0.207009
4                    100.00  1.685364  0.000821  0.021790      0.207009
3                    100.00  1.685364  0.000821  0.021790      0.207009
15                   100.00  1.685364  0.002728  0.041662      0.687426
16                   100.00  1.685364  0.002728  0.041662      0.687426

============================================================
CLUSTER 2
============================================================
Keywords
            annotation  total_reference  annotated_reference  total_subset  \
1        Cell membrane              357                  304           246
2  Cell inner membrane              357                  302           246
3        Transmembrane              357                  295           246
0  Transmembrane helix              357                  268           246
4              Symport              357                   51           246
5             Antiport              357                   19           246

   annotated_subset    expected  percentage_of_annotated       lfc  \
1               228  209.478992                    75.00  0.122228
2               226  208.100840                    74.83  0.119040
3               219  203.277311                    74.24  0.107482
0               215  184.672269                    80.22  0.219369
4                45   35.142857                    88.24  0.356694
```

```
5               19   13.092437                   100.00  0.537266

              p         p_fdr  p_bonferroni
1  1.236558e-08  6.986555e-07  1.397311e-06
2  5.559409e-08  2.094044e-06  6.282133e-06
3  4.322393e-06  1.221076e-04  4.884304e-04
0  7.861795e-15  8.883828e-13  8.883828e-13
4  5.692928e-04  1.286602e-02  6.433009e-02
5  6.745755e-04  1.270451e-02  7.622703e-02

GO Cellular Component

        annotation  total_reference  annotated_reference  total_subset  \
1         membrane              357                  316           246
0  plasma membrane              357                  285           246

   annotated_subset    expected  percentage_of_annotated       lfc  \
1               228  217.747899                    72.15  0.066375
0               222  196.386555                    77.89  0.176864

              p         p_fdr  p_bonferroni
1  3.653903e-04  1.187518e-02  2.375037e-02
0  2.190972e-12  1.424132e-10  1.424132e-10

GO Molecular Function

                                          annotation  total_reference  \
0  secondary active transmembrane transporter act…              357

   annotated_reference  total_subset  annotated_subset   expected  \
0                   88           246                76  60.638655

   percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
0                    86.36  0.325762  0.000017  0.006927      0.006927

GO Biological Process

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        2.A              357                  152           246

   annotated_subset    expected  percentage_of_annotated       lfc  \
0               133  104.739496                     87.5  0.344621
```

```
              p         p_fdr  p_bonferroni
0  1.484410e-11  1.187528e-10  1.187528e-10
```

```
TCDB Mechanism
```

```
  annotation  total_reference  annotated_reference  total_subset  \
0          2              357                  152           246

   annotated_subset    expected  percentage_of_annotated       lfc  \
0               133  104.739496                     87.5  0.344621

              p         p_fdr  p_bonferroni
0  1.484410e-11  7.422050e-11  7.422050e-11
```

```
Interpro Domains
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset, annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr, p_bonferroni]
Index: []
```

It looks like the first cluster contains channels and pores, some of which are located in the outer membrane. It also contains active proteins (TCDB mechanism 3), and proteins that are part of membrane protein complexes. The TCDB class 1.B (beta barrel channels) is enriched as well. Also, there are some strongly enriched domains in cluster 1 that mostly correspond to "binding" and "beta-barrel" and "outer membrane".

Cluster 2 contains 86% of the secondary active transporters, and 87% of the total proteins in TCDB class 2.A . The majority of the proteins is located on the inner membrane, and has helical structure. It contains every antiporter, and 88% of the symporters.

So far, the two clusters are clearly distinguishable, but the separation is not 100% perfect for any annotation. Cluster 1 sill contains about 15% of the inner membrane proteins, and cluster 2 contains 15% (i.e. four) of the outer membrane proteins.

There seem to be some receptor complex domains in cluster 1, which proteins contain those? Let's look at one example:

[ ]:

```
interpro_ecoli[
    (interpro_ecoli.interpro == "TonB-dependent receptor, plug domain")
    & (
        interpro_ecoli.Uniprot.isin(
            labels_twoclusters[labels_twoclusters == 1].index.tolist()
        )
    )
].merge(sequences.reset_index()[["Uniprot", "protein_names"]], how="left", on="Uniprot")
```

[ ]:

```
  Uniprot                              interpro  \
0  P05825  TonB-dependent receptor, plug domain
```

```
1  P06971  TonB-dependent receptor, plug domain
2  P16869  TonB-dependent receptor, plug domain
3  P75780  TonB-dependent receptor, plug domain
4  P17315  TonB-dependent receptor, plug domain
5  P76115  TonB-dependent receptor, plug domain

                                       protein_names
0  Ferrienterobactin receptor (Enterobactin outer…
1  Ferrichrome outer membrane transporter/phage r…
2  FhuE receptor (Outer-membrane receptor for Fe(…
3  Catecholate siderophore receptor Fiu (Ferric i…
4                                 Colicin I receptor
5               Probable TonB-dependent receptor YncD
```

These proteins seem to be outer-membrane beta barrel proteins that are responsible for siderophore uptake, a large molecule that bacteria use for iron uptake. Therefore they are classified as iron transporters.

The first cluster also contains many proteins with the “binding” keyword. What are some examples for that?

```
[ ]: interpro_ecoli[
         (interpro_ecoli.interpro == "Solute-binding protein family 5")
         & (
             interpro_ecoli.Uniprot.isin(
                 labels_twoclusters[labels_twoclusters == 1].index.tolist()
             )
         )
     ].merge(sequences.reset_index()[["Uniprot", "protein_names"]], how="left",␣
      ↪on="Uniprot")
```

```
[ ]:   Uniprot                         interpro  \
     0  P23847  Solute-binding protein family 5
     1  P77348  Solute-binding protein family 5
     2  P33590  Solute-binding protein family 5
     3  P23843  Solute-binding protein family 5
     4  P75797  Solute-binding protein family 5
     5  Q46863  Solute-binding protein family 5
     6  P76128  Solute-binding protein family 5
     7  P33913  Solute-binding protein family 5

                                            protein_names
     0  Dipeptide-binding protein (DBP) (Periplasmic d…
     1          Periplasmic murein peptide-binding protein
     2                  Nickel-binding periplasmic protein
     3            Periplasmic oligopeptide-binding protein
     4                    Glutathione-binding protein GsiB
     5  Probable deoxycholate-binding periplasmic prot…
```

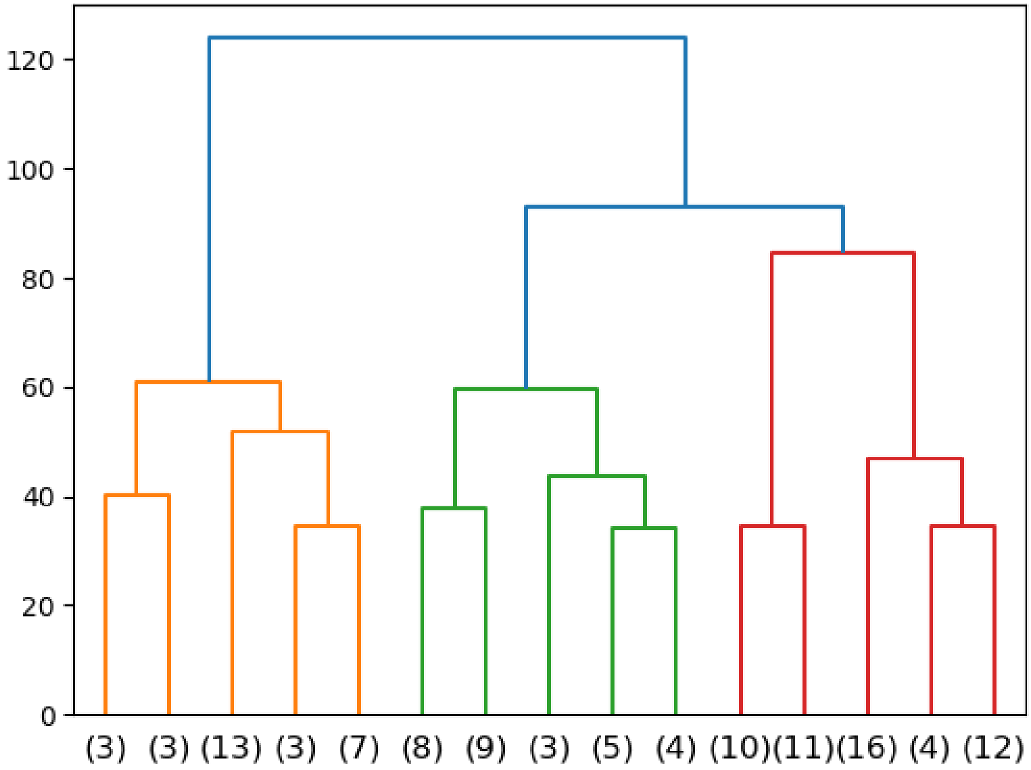
```
6   Probable D,D-dipeptide-binding periplasmic pro…
7                           Uncharacterized protein YejA
```

This protein family seems to be involved in outer membrane peptide transport. The “binding” proteins are the parts of a protein complex that actually interacts with the molecule. Most of them are annotated with the GO term “ATP-binding cassette (ABC) transporter complex, substrate-binding subunit-containing”

Sub-clusters What happens when we perform an enrichment analysis on just the left or the right cluster?

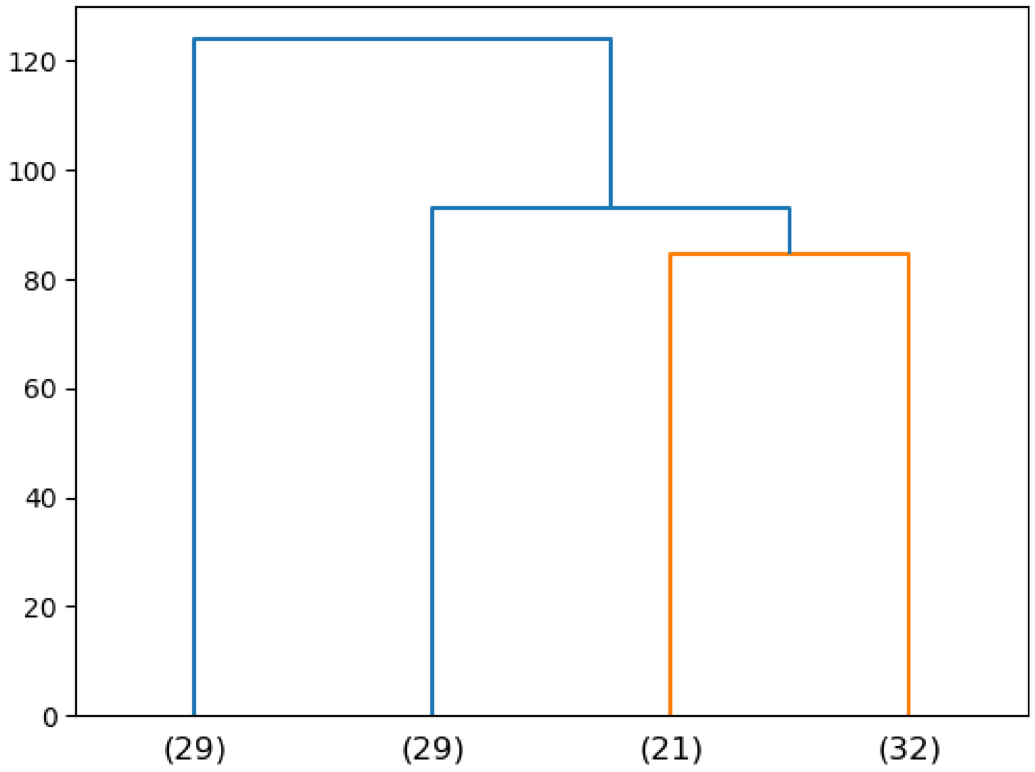
Left cluster: We previously found that cluster 1 contains active transport complex members, outer membrane proteins and beta barrels channels. Can we divide that cluster further?

```
[ ]: reference_set_leftcluster = labels_twoclusters[labels_twoclusters == 1].index.
       ↪tolist()
     feature_pssm_leftcluster = feature_pssm.loc[reference_set_leftcluster]
     linkage_leftcluster = get_linkage(feature_data=feature_pssm_leftcluster)
     dendrogram_leftcluster = dendrogram_plot(linkage_leftcluster)
```



From the dendrogram, we can see that we get four clusters at a minimum distance of ~70 in the dendrogram (i.e. the y axis). This division leads to a adequate sample sizes for machine learning, especially when including electronically annotated data etc.

```
[ ]: dendrogram_leftcluster = dendrogram_plot(linkage_leftcluster,max_leaves=4)
     labels_leftcluster = get_cluster_labels(linkage_leftcluster, n_clusters=4,␣
      ↪index = feature_pssm_leftcluster.index)
```



What do the four clusters represent?

```
[ ]: cluster_enrichment_analysis(cluster_labels=labels_leftcluster,␣
      ↪reference_set=reference_set_leftcluster, annotations_dict=records_all_dict)
```

```
============================================================
CLUSTER 1
============================================================
Keywords
            annotation  total_reference  annotated_reference  total_subset  \
0  Transmembrane helix              111                   53            29

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                22  13.846847                    41.51  0.667946  0.000401

      p_fdr  p_bonferroni
0  0.023672      0.023672
```

GO Cellular Component

```
               annotation  total_reference  annotated_reference  total_subset  \
1                membrane              111                   88            29   
0         plasma membrane              111                   63            29   
2  oxidoreductase complex              111                    7            29   
3        catalytic complex             111                    8            29   

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
1                29  22.990991                    32.95  0.334984  0.000361   
0                25  16.459459                    39.68  0.603011  0.000129   
2                 6   1.828829                    85.71  1.714042  0.001193   
3                 6   2.090090                    75.00  1.521397  0.003873   

      p_fdr  p_bonferroni  
1  0.006131      0.012262  
0  0.004403      0.004403  
2  0.013519      0.040556  
3  0.032916      0.131665  
```

GO Molecular Function

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

GO Biological Process

```
                                    annotation  total_reference  \
3                     cellular metabolic process              111   
0  generation of precursor metabolites and energy             111   
1                        electron transport chain             111   
2             respiratory electron transport chain             111   

   annotated_reference  total_subset  annotated_subset  expected  \
3                    9            29                 7  2.351351   
0                    6            29                 6  1.567568   
1                    6            29                 6  1.567568   
2                    6            29                 6  1.567568   

   percentage_of_annotated       lfc         p     p_fdr  p_bonferroni  
3                    77.78  1.573865  0.001097  0.046092      0.184367  
0                   100.00  1.936435  0.000210  0.017623      0.035245  
1                   100.00  1.936435  0.000210  0.017623      0.035245  
2                   100.00  1.936435  0.000210  0.017623      0.035245  
```

TCDB Class

```
  annotation  total_reference  annotated_reference  total_subset  \
0        3.D              111                     6            29

   annotated_subset  expected  percentage_of_annotated       lfc        p  \
0                 6  1.567568                    100.0  1.936435  0.00021

      p_fdr  p_bonferroni
0  0.001888      0.001888

TCDB Mechanism

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

Interpro Domains

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

============================================================
CLUSTER 2
============================================================
Keywords

                  annotation  total_reference  annotated_reference  \
0                     Signal              111                   38
1        Cell outer membrane              111                   23
2  Transmembrane beta strand              111                   23
3              Disulfide bond             111                    9
4                   Periplasm             111                    9
5                    Receptor             111                    6
6                    TonB box             111                    6
7          Fimbrium biogenesis            111                    6
8              Iron transport             111                    5
9                        Iron             111                    7

   total_subset  annotated_subset  expected  percentage_of_annotated  \
0            29                29  9.927928                    76.32
1            29                20  6.009009                    86.96
2            29                20  6.009009                    86.96
3            29                 9  2.351351                   100.00
4            29                 8  2.351351                    88.89
5            29                 6  1.567568                   100.00
6            29                 6  1.567568                   100.00
7            29                 6  1.567568                   100.00
```

```
8           29                 5  1.306306                   100.00
9           29                 5  1.828829                    71.43

       lfc             p         p_fdr  p_bonferroni
0  1.546488  3.886363e-19  1.360227e-17  1.360227e-17
1  1.734801  2.451501e-12  3.432101e-11  8.580252e-11
2  1.734801  2.451501e-12  3.432101e-11  8.580252e-11
3  1.936435  1.981978e-06  1.734231e-05  6.936922e-05
4  1.766510  7.163434e-05  5.014404e-04  2.507202e-03
5  1.936435  2.097920e-04  1.048960e-03  7.342718e-03
6  1.936435  2.097920e-04  1.048960e-03  7.342718e-03
7  1.936435  2.097920e-04  1.048960e-03  7.342718e-03
8  1.936435  9.265811e-04  3.603371e-03  3.243034e-02
9  1.451008  1.280480e-02  4.481681e-02  4.481681e-01
```

```
GO Cellular Component
                                 annotation  total_reference  \
0                       cell outer membrane              111
1                            outer membrane              111
2  outer membrane-bounded periplasmic space              111
3                          periplasmic space              111

   annotated_reference  total_subset  annotated_subset  expected  \
0                   23            29                19  6.009009
1                   23            29                19  6.009009
2                   14            29                11  3.657658
3                   14            29                11  3.657658

   percentage_of_annotated       lfc             p         p_fdr  p_bonferroni
0                    82.61  1.660800  9.774054e-11  1.433528e-09  2.150292e-09
1                    82.61  1.660800  9.774054e-11  1.433528e-09  2.150292e-09
2                    78.57  1.588512  1.542102e-05  9.693211e-05  3.392624e-04
3                    78.57  1.588512  1.542102e-05  9.693211e-05  3.392624e-04
```

```
GO Molecular Function
                                          annotation  total_reference  \
7                                   channel activity              111
8         passive transmembrane transporter activity              111
0                         wide pore channel activity              111
1                                     porin activity              111
2         peptide transmembrane transporter activity              111
3           amide transmembrane transporter activity              111
4  siderophore-iron transmembrane transporter act…              111
5  siderophore uptake transmembrane transporter a…              111
6                       fimbrial usher porin activity              111

   annotated_reference  total_subset  annotated_subset  expected  \
7                   26            29                13  6.792793
```

```
8                   26            29                13  6.792793
0                   15            29                12  3.918919
1                   15            29                12  3.918919
2                   13            29                10  3.396396
3                   14            29                10  3.657658
4                    6            29                 6  1.567568
5                    6            29                 6  1.567568
6                    6            29                 6  1.567568

   percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
7                    50.00  0.936435  0.002446  0.023304      0.198086
8                    50.00  0.936435  0.002446  0.023304      0.198086
0                    80.00  1.614507  0.000004  0.000192      0.000288
1                    80.00  1.614507  0.000004  0.000192      0.000288
2                    76.92  1.557923  0.000063  0.001702      0.005106
3                    71.43  1.451008  0.000182  0.003687      0.014747
4                   100.00  1.936435  0.000210  0.002832      0.016993
5                   100.00  1.936435  0.000210  0.002832      0.016993
6                   100.00  1.936435  0.000210  0.002832      0.016993
```

```
GO Biological Process
```

```
                                      annotation  total_reference  \
4                                amide transport              111
5                              peptide transport              111
6                   cellular component assembly               111
7                cellular component organization              111
8  cellular component organization or biogenesis              111
0                       cell projection assembly              111
1                                 pilus assembly              111
2                             pilus organization              111
3                   cell projection organization              111

   annotated_reference  total_subset  annotated_subset  expected  \
4                   11            29                 8  2.873874
5                   11            29                 8  2.873874
6                    9            29                 7  2.351351
7                   10            29                 7  2.612613
8                   10            29                 7  2.612613
0                    6            29                 6  1.567568
1                    6            29                 6  1.567568
2                    6            29                 6  1.567568
3                    6            29                 6  1.567568

   percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
4                    72.73  1.477003  0.000877  0.016265      0.089458
5                    72.73  1.477003  0.000877  0.016265      0.089458
6                    77.78  1.573865  0.001097  0.015991      0.111937
7                    70.00  1.421862  0.002975  0.035696      0.303418
```

```
8                    70.00  1.421862  0.002975  0.035696      0.303418
0                   100.00  1.936435  0.000210  0.008560      0.021399
1                   100.00  1.936435  0.000210  0.008560      0.021399
2                   100.00  1.936435  0.000210  0.008560      0.021399
3                   100.00  1.936435  0.000210  0.008560      0.021399

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        1.B              111                   21            29

   annotated_subset  expected  percentage_of_annotated       lfc  \
0                18  5.486486                    85.71  1.714042

              p         p_fdr  p_bonferroni
0  1.348221e-10  2.696442e-10  2.696442e-10

TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          1              111                   30            29

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                18  7.837838                     60.0  1.199469  0.000003

      p_fdr  p_bonferroni
0  0.000006      0.000006

Interpro Domains

                                           annotation  total_reference  \
0          Peptide/nickel binding protein, MppA-type              111
2             Solute-binding protein family 5 domain              111
1                    Solute-binding protein family 5              111
9            Fimbrial membrane usher, conserved site              111
14               PapC, N-terminal domain superfamily              111
13                           PapC, N-terminal domain              111
12          PapC-like, C-terminal domain superfamily              111
11                      PapC-like, C-terminal domain              111
10    Outer membrane usher protein FimD, plug domain              111
8                       Outer membrane usher protein              111
7    Solute-binding protein family 5, conserved site              111
6   TonB-dependent receptor-like, beta-barrel doma…              111
5          TonB-dependent receptor-like, beta-barrel              111
4   TonB-dependent receptor, plug domain superfamily              111
3                TonB-dependent receptor, plug domain              111
15                          TonB box, conserved site              111
16               TonB-dependent siderophore receptor              111
17           TonB-dependent receptor, conserved site              111
18                 Vitamin B12 transporter BtuB-like              111
19                      TonB-dependent receptor-like              111
```

```
    annotated_reference  total_subset  annotated_subset  expected  \
0                     8            29                 8  2.090090
2                     8            29                 8  2.090090
1                     8            29                 8  2.090090
9                     6            29                 6  1.567568
14                    6            29                 6  1.567568
13                    6            29                 6  1.567568
12                    6            29                 6  1.567568
11                    6            29                 6  1.567568
10                    6            29                 6  1.567568
8                     6            29                 6  1.567568
7                     6            29                 6  1.567568
6                     6            29                 6  1.567568
5                     6            29                 6  1.567568
4                     6            29                 6  1.567568
3                     6            29                 6  1.567568
15                    5            29                 5  1.306306
16                    5            29                 5  1.306306
17                    4            29                 4  1.045045
18                    3            29                 3  0.783784
19                    3            29                 3  0.783784

    percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
0                     100.0  1.936435  0.000010  0.000228      0.000457
2                     100.0  1.936435  0.000010  0.000228      0.000457
1                     100.0  1.936435  0.000010  0.000228      0.000457
9                     100.0  1.936435  0.000210  0.001038      0.009860
14                    100.0  1.936435  0.000210  0.001038      0.009860
13                    100.0  1.936435  0.000210  0.001038      0.009860
12                    100.0  1.936435  0.000210  0.001038      0.009860
11                    100.0  1.936435  0.000210  0.001038      0.009860
10                    100.0  1.936435  0.000210  0.001038      0.009860
8                     100.0  1.936435  0.000210  0.001038      0.009860
7                     100.0  1.936435  0.000210  0.001038      0.009860
6                     100.0  1.936435  0.000210  0.001038      0.009860
5                     100.0  1.936435  0.000210  0.001038      0.009860
4                     100.0  1.936435  0.000210  0.001038      0.009860
3                     100.0  1.936435  0.000210  0.001038      0.009860
15                    100.0  1.936435  0.000927  0.002639      0.043549
16                    100.0  1.936435  0.000927  0.002639      0.043549
17                    100.0  1.936435  0.003966  0.010355      0.186391
18                    100.0  1.936435  0.016473  0.039705      0.774240
19                    100.0  1.936435  0.016473  0.039705      0.774240

============================================================
CLUSTER 3
============================================================
Keywords

            annotation  total_reference  annotated_reference  total_subset  \
0  Transmembrane helix              111                   53            21
1  Cell inner membrane              111                   76            21
2        Cell membrane              111                   76            21
3        Transmembrane              111                   76            21

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                21  10.027027                    39.62  1.066495
1                21  14.378378                    27.63  0.546488
2                21  14.378378                    27.63  0.546488
3                21  14.378378                    27.63  0.546488

              p         p_fdr  p_bonferroni
0  1.369139e-08  3.696676e-07  3.696676e-07
1  1.251526e-04  1.126373e-03  3.379119e-03
2  1.251526e-04  1.126373e-03  3.379119e-03
3  1.251526e-04  1.126373e-03  3.379119e-03
```

```
GO Cellular Component

        annotation  total_reference  annotated_reference  total_subset  \
0  plasma membrane              111                   63            21

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                20  11.918919                    31.75  0.746747  0.000029

      p_fdr  p_bonferroni
0  0.000465      0.000465
```

```
GO Molecular Function

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
GO Biological Process

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        2.A              111                   19            21
```

```
   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                10  3.594595                    52.63  1.476099  0.000239

      p_fdr  p_bonferroni
0  0.000957      0.000957

TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          2              111                   19            21

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                10  3.594595                    52.63  1.476099  0.000239

      p_fdr  p_bonferroni
0  0.000957      0.000957

Interpro Domains

                             annotation  total_reference  annotated_reference  \
0           MFS transporter superfamily              111                    6
1  Major facilitator superfamily domain              111                    5
2         Major facilitator superfamily              111                    5
3    Amino acid permease/ SLC12A domain              111                    4
4   Amino acid permease, conserved site              111                    4
5             ABC transporter, permease              111                    4

   total_subset  annotated_subset  expected  percentage_of_annotated  \
0            21                 6  1.135135                    100.0
1            21                 5  0.945946                    100.0
2            21                 5  0.945946                    100.0
3            21                 4  0.756757                    100.0
4            21                 4  0.756757                    100.0
5            21                 4  0.756757                    100.0

        lfc         p     p_fdr  p_bonferroni
0  2.402098  0.000024  0.000935      0.000935
1  2.402098  0.000159  0.002477      0.006192
2  2.402098  0.000159  0.002477      0.006192
3  2.402098  0.000999  0.007795      0.038974
4  2.402098  0.000999  0.007795      0.038974
5  2.402098  0.000999  0.007795      0.038974

============================================================
CLUSTER 4
============================================================
Keywords

            annotation  total_reference  annotated_reference  total_subset  \
3  Cell inner membrane              111                   76            32
4        Cell membrane              111                   76            32
```

```
0          ATP-binding              111                   21            32
1   Nucleotide-binding              111                   21            32
2          Translocase              111                   20            32

   annotated_subset   expected  percentage_of_annotated       lfc  \
3                29  21.909910                    38.16  0.404469
4                29  21.909910                    38.16  0.404469
0                19   6.054054                    90.48  1.650026
1                19   6.054054                    90.48  1.650026
2                14   5.765766                    70.00  1.279843

              p         p_fdr  p_bonferroni
3  8.325553e-04  1.073071e-02  4.828821e-02
4  8.325553e-04  1.073071e-02  4.828821e-02
0  4.685519e-11  1.811734e-09  2.717601e-09
1  4.685519e-11  1.811734e-09  2.717601e-09
2  2.700550e-05  5.221063e-04  1.566319e-03
GO Cellular Component
                                          annotation  total_reference  \
3                         protein-containing complex              111
4                           membrane protein complex              111
6                                transporter complex              111
7                  transmembrane transporter complex              111
2                    plasma membrane protein complex              111
0   ATPase dependent transmembrane transport complex              111
1     ATP-binding cassette (ABC) transporter complex              111
5  ATP-binding cassette (ABC) transporter complex…               111

   annotated_reference  total_subset  annotated_subset   expected  \
3                   63            32                26  18.162162
4                   60            32                25  17.297297
6                   51            32                22  14.702703
7                   50            32                21  14.414414
2                   34            32                18   9.801802
0                   30            32                17   8.648649
1                   28            32                16   8.072072
5                   26            32                14   7.495495

   percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
3                    41.27  0.517576  0.000715  0.008406      0.033624
4                    41.67  0.531381  0.001002  0.009419      0.047095
6                    43.14  0.581422  0.002053  0.013783      0.096480
7                    42.00  0.542877  0.005134  0.030160      0.241282
2                    52.94  0.876878  0.000300  0.004708      0.014123
0                    56.67  0.974988  0.000154  0.007254      0.007254
1                    57.14  0.987061  0.000250  0.005877      0.011754
5                    53.85  0.901331  0.001949  0.015271      0.091624
```

```
GO Molecular Function

                                           annotation  total_reference  \
15                                            binding              111
3                                         ion binding              111
0                                       anion binding              111
2                                  nucleotide binding              111
14                    organic cyclic compound binding              111
13                      heterocyclic compound binding              111
12                    carbohydrate derivative binding              111
11                             small molecule binding              111
1                        nucleoside phosphate binding              111
10                          adenyl nucleotide binding              111
9                           purine nucleotide binding              111
8          purine ribonucleoside triphosphate binding              111
7                              ribonucleotide binding              111
6                       adenyl ribonucleotide binding              111
5                       purine ribonucleotide binding              111
4                                         ATP binding              111
16  ATPase-coupled transmembrane transporter activity              111
17                             ATP-dependent activity              111
18                      ABC-type transporter activity              111

    annotated_reference  total_subset  annotated_subset   expected  \
15                   59            32                29  17.009009
3                    24            32                20   6.918919
0                    19            32                18   5.477477
2                    18            32                17   5.189189
14                   21            32                17   6.054054
13                   21            32                17   6.054054
12                   20            32                17   5.765766
11                   19            32                17   5.477477
1                    18            32                17   5.189189
10                   17            32                16   4.900901
9                    17            32                16   4.900901
8                    17            32                16   4.900901
7                    17            32                16   4.900901
6                    17            32                16   4.900901
5                    17            32                16   4.900901
4                    17            32                16   4.900901
16                   15            32                11   4.324324
17                   15            32                11   4.324324
18                    9            32                 8   2.594595

    percentage_of_annotated       lfc             p         p_fdr  \
15                    49.15  0.769754  1.853565e-07  1.795641e-06
3                     83.33  1.531381  2.593757e-10  1.005081e-08
0                     94.74  1.716413  3.226268e-11  5.000715e-09
```

```
2                     94.44  1.711954  1.897301e-10  1.176327e-08
14                    80.95  1.489561  3.825004e-08  4.088797e-07
13                    80.95  1.489561  3.825004e-08  4.088797e-07
12                    85.00  1.559951  8.623081e-09  1.028137e-07
11                    89.47  1.633951  1.528203e-09  1.973929e-08
1                     94.44  1.711954  1.897301e-10  1.176327e-08
10                    94.12  1.706953  1.054161e-09  2.042436e-08
9                     94.12  1.706953  1.054161e-09  2.042436e-08
8                     94.12  1.706953  1.054161e-09  2.042436e-08
7                     94.12  1.706953  1.054161e-09  2.042436e-08
6                     94.12  1.706953  1.054161e-09  2.042436e-08
5                     94.12  1.706953  1.054161e-09  2.042436e-08
4                     94.12  1.706953  1.054161e-09  2.042436e-08
16                    73.33  1.346957  1.565482e-04  1.386570e-03
17                    73.33  1.346957  1.565482e-04  1.386570e-03
18                    88.89  1.624491  1.699957e-04  1.386807e-03

    p_bonferroni
15  2.873025e-05
3   4.020323e-08
0   5.000715e-09
2   2.940817e-08
14  5.928756e-06
13  5.928756e-06
12  1.336577e-06
11  2.368715e-07
1   2.940817e-08
10  1.633949e-07
9   1.633949e-07
8   1.633949e-07
7   1.633949e-07
6   1.633949e-07
5   1.633949e-07
4   1.633949e-07
16  2.426497e-02
17  2.426497e-02
18  2.634934e-02
```

```
GO Biological Process
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
TCDB Class
```

```
  annotation  total_reference  annotated_reference  total_subset  \
0        3.A             111                   37            32

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                20  10.666667                    54.05  0.906891  0.000055

      p_fdr  p_bonferroni
0  0.000219      0.000219
```

```
TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          3              111                   43            32

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                20  12.396396                    46.51  0.690079  0.001197

     p_fdr  p_bonferroni
0  0.00479       0.00479
```

```
Interpro Domains

                                          annotation  total_reference  \
0           ABC transporter-like, ATP-binding domain              111
2                                  AAA+ ATPase domain              111
3  P-loop containing nucleoside triphosphate hydr…             111
1               ABC transporter-like, conserved site              111

   annotated_reference  total_subset  annotated_subset  expected  \
0                   17            32                17  4.900901
2                   18            32                17  5.189189
3                   19            32                17  5.477477
1                   16            32                16  4.612613

   percentage_of_annotated       lfc             p         p_fdr  p_bonferroni
0                   100.00  1.794416  1.241102e-11  9.928813e-10  9.928813e-10
2                    94.44  1.711954  1.897301e-10  5.059469e-09  1.517841e-08
3                    89.47  1.633951  1.528203e-09  3.056406e-08  1.222563e-07
1                   100.00  1.794416  7.369041e-11  2.947616e-09  5.895232e-09
```

Cluster 1.1

Enrichment in oxidoreductase-driven transport, as well as associated complexes. Electron transport and respiratory system are enriched as well. Majority of proteins has alpha helix, thre remainder could be unknown secondary structure or active part of a complex. The annotations related to catalytic complexes and respiratory system only apply to 6/29 proteins. We should look at the annotations of the remaining proteins.

Cluster 1.2

This cluster contains 19/23 outer membrane proteins and 11/14 periplasmic space proteins, both cellular components are enriched. Some beta barrel channels can be part of both membranes, so there could be some overlap. We get transporters for peptides, amides and siderophores (i.e. iron).

The TCDB class 1.B is enriched (18/21 proteins), which contains the beta barrel channels. Usher proteins related to cellular assembly are also in this cluster. Do they facilitate the transport between two bacteria? If that is the case then we should exclude them from the dataset

Cluster 1.3

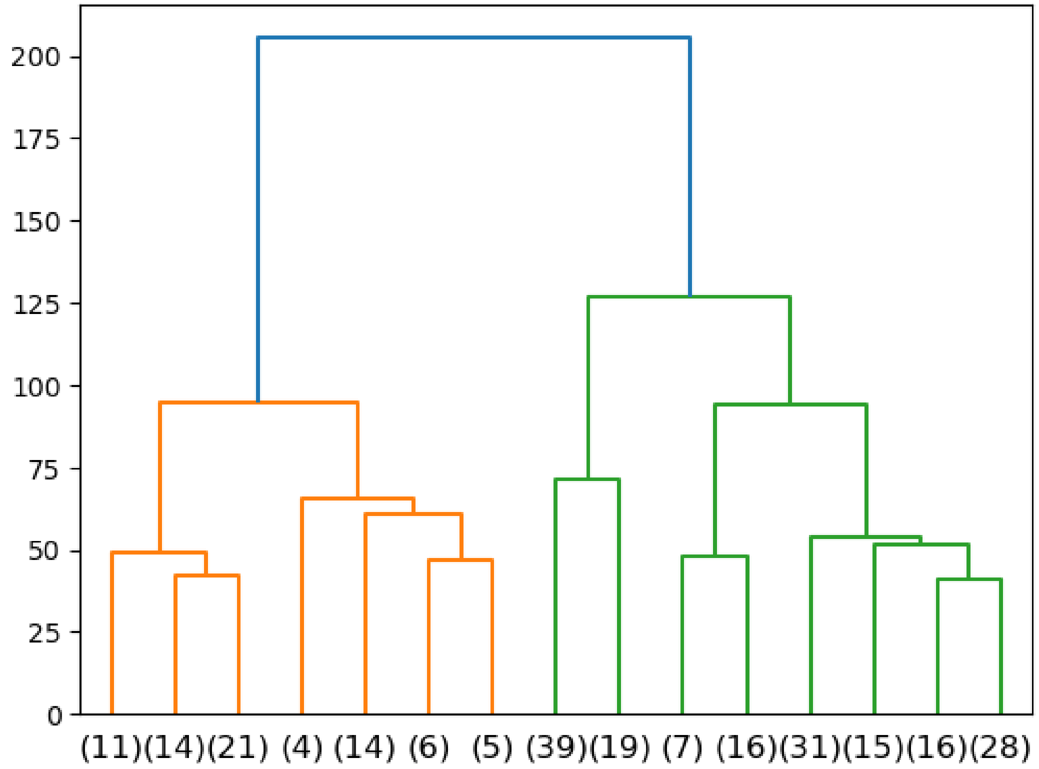
Ths cluster contains all of the remaining known transmembrane helix proteins, i.e. the ones that were not in cluster 1. The Major Facilitator Superfamily (2.A) is enriched in this cluster, it contains 10/19 MFS proteins. Domains related to a amino acid permease in the form of an ABC transporter is also enriched.

Cluster 1.4

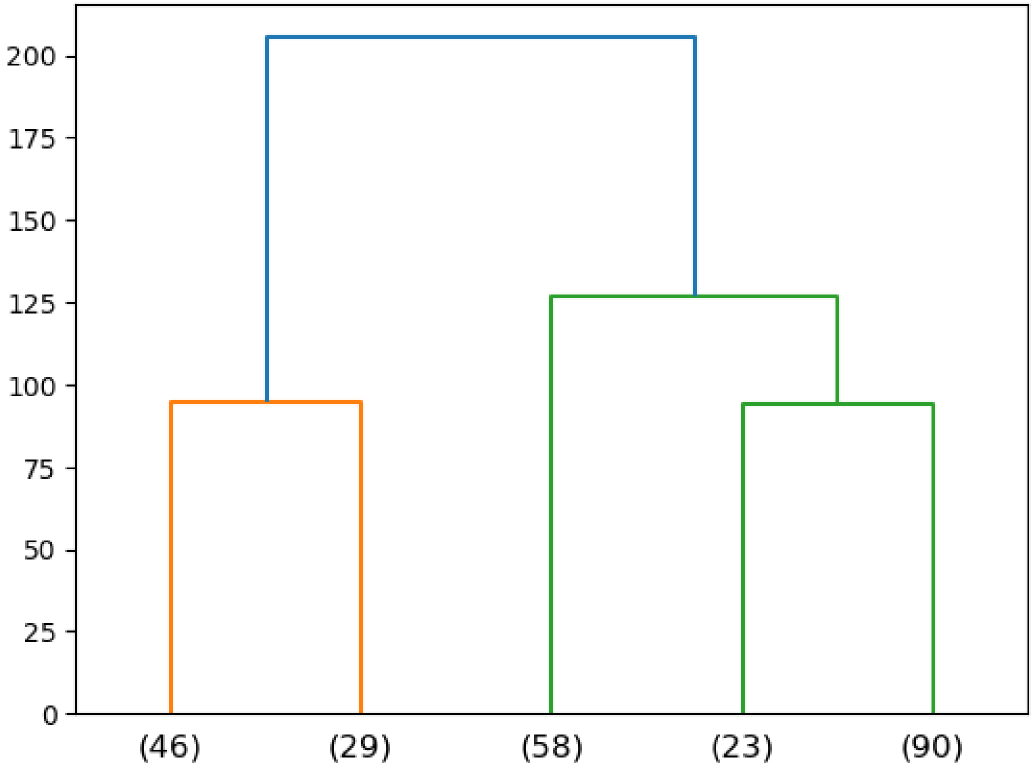
Cluster 4 contains molecule-binding proteins from protein complexes in the plasma membrane that transport a variety of small molecules, such as ions, carbohydrates, ribonucleotides, etc. Domains and families related to ABC transport and active transport in general are enriched.

Right cluster:

[ ]:
```
reference_set_rightcluster = labels_twoclusters[labels_twoclusters == 2].index.
  ↪tolist()
feature_pssm_rightcluster = feature_pssm.loc[reference_set_rightcluster]
linkage_rightcluster = get_linkage(feature_data=feature_pssm_rightcluster)
dendrogram_rightcluster = dendrogram_plot(linkage_rightcluster)
```



```
[ ]: dendrogram_rightcluster = dendrogram_plot(linkage_rightcluster,max_leaves=5)
     labels_rightcluster = get_cluster_labels(linkage_rightcluster, n_clusters=5,␣
      ↪index = feature_pssm_rightcluster.index)
```



```
[ ]: cluster_enrichment_analysis(cluster_labels=labels_rightcluster,␣
      ↪reference_set=reference_set_rightcluster, annotations_dict=records_all_dict,␣
      ↪p_cutoff=0.05)
```

```
============================================================
CLUSTER 1
============================================================
Keywords

            annotation  total_reference  annotated_reference  total_subset  \
0  Transmembrane helix              246                  215            46

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                46  40.203252                     21.4  0.194322  0.001011

      p_fdr  p_bonferroni
0  0.041445      0.041445

GO Cellular Component
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

GO Molecular Function

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

GO Biological Process

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

TCDB Class

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

TCDB Mechanism

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

Interpro Domains

                        annotation  total_reference  annotated_reference  \
0  Phosphotransferase system, EIIC              246                    7

   total_subset  annotated_subset  expected  percentage_of_annotated  \
0            46                 7  1.308943                    100.0

        lfc         p     p_fdr  p_bonferroni
0  2.418953  0.000005  0.000496      0.000496

============================================================
CLUSTER 2
============================================================
Keywords
```

```
                  annotation  total_reference  annotated_reference  \
0                ATP-binding              246                   21   
2         Nucleotide-binding              246                   23   
6  Direct protein sequencing              246                   18   
1                     Signal              246                    8   
8                Translocase              246                   17   
7                  Cytoplasm              246                   10   
3        Cell outer membrane              246                    4   
4                      Porin              246                    4   
5  Transmembrane beta strand              246                    4   

   total_subset  annotated_subset  expected  percentage_of_annotated  \
0            29                10  2.475610                    47.62   
2            29                10  2.711382                    43.48   
6            29                 8  2.121951                    44.44   
1            29                 6  0.943089                    75.00   
8            29                 6  2.004065                    35.29   
7            29                 5  1.178862                    50.00   
3            29                 4  0.471545                   100.00   
4            29                 4  0.471545                   100.00   
5            29                 4  0.471545                   100.00   

        lfc         p     p_fdr  p_bonferroni  
0  2.014144  0.000016  0.000731      0.000731  
2  1.882900  0.000045  0.000675      0.002025  
6  1.914609  0.000277  0.001781      0.012467  
1  2.669496  0.000039  0.000871      0.001742  
8  1.582033  0.007849  0.039243      0.353190  
7  2.084534  0.002691  0.015135      0.121081  
3  3.084534  0.000160  0.001436      0.007178  
4  3.084534  0.000160  0.001436      0.007178  
5  3.084534  0.000160  0.001436      0.007178  
```

GO Cellular Component

```
                          annotation  total_reference  annotated_reference  \
0            membrane protein complex              246                   87   
1          protein-containing complex              246                   92   
6  transmembrane transporter complex              246                   77   
7                 transporter complex              246                   79   
2                 cell outer membrane              246                    4   
3                      outer membrane              246                    4   
5                             cytosol              246                    6   
4                        pore complex              246                    3   

   total_subset  annotated_subset   expected  percentage_of_annotated  \
0            29                22  10.256098                    25.29   
1            29                22  10.845528                    23.91   
6            29                16   9.077236                    20.78   
```

```
7            29                16   9.313008                    20.25
2            29                 4   0.471545                   100.00
3            29                 4   0.471545                   100.00
5            29                 4   0.707317                    66.67
4            29                 3   0.353659                   100.00

        lfc         p     p_fdr  p_bonferroni
0  1.101022  0.000003  0.000082      0.000082
1  1.020403  0.000009  0.000134      0.000268
6  0.817747  0.003960  0.017536      0.122752
7  0.780753  0.005411  0.020970      0.167756
2  3.084534  0.000160  0.001413      0.004945
3  3.084534  0.000160  0.001413      0.004945
5  2.499571  0.002014  0.010404      0.062422
4  3.084534  0.001491  0.009243      0.046216
```

GO Molecular Function

```
                                           annotation  total_reference  \
0                                             binding              246
22                                    protein binding              246
5   ATPase-coupled transmembrane transporter activity              246
6                              ATP-dependent activity              246
18  primary active transmembrane transporter activity              246
3                              small molecule binding              246
4                     carbohydrate derivative binding              246
14                       nucleoside phosphate binding              246
21                                      anion binding              246
20         passive transmembrane transporter activity              246
19                                   channel activity              246
17                    organic cyclic compound binding              246
16                      heterocyclic compound binding              246
15                          purine nucleotide binding              246
12         purine ribonucleoside triphosphate binding              246
13                                 nucleotide binding              246
11                             ribonucleotide binding              246
10                      purine ribonucleotide binding              246
9                           adenyl nucleotide binding              246
8                       adenyl ribonucleotide binding              246
7                                         ATP binding              246
23                                        ion binding              246
1                          wide pore channel activity              246
2                                      porin activity              246

    annotated_reference  total_subset  annotated_subset  expected  \
0                    60            29                16  7.073171
22                   39            29                10  4.597561
5                    24            29                 9  2.829268
6                    24            29                 9  2.829268
```

```
18                   28            29                 9  3.300813
3                    17            29                 8  2.004065
4                    17            29                 8  2.004065
14                   16            29                 7  1.886179
21                   18            29                 7  2.121951
20                   18            29                 7  2.121951
19                   18            29                 7  2.121951
17                   17            29                 7  2.004065
16                   17            29                 7  2.004065
15                   16            29                 7  1.886179
12                   16            29                 7  1.886179
13                   16            29                 7  1.886179
11                   16            29                 7  1.886179
10                   16            29                 7  1.886179
9                    15            29                 7  1.768293
8                    15            29                 7  1.768293
7                    15            29                 7  1.768293
23                   23            29                 7  2.711382
1                     4            29                 4  0.471545
2                     4            29                 4  0.471545

    percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
0                     26.67  1.177643  0.000139  0.016559      0.016559
22                    25.64  1.121059  0.006811  0.035237      0.810451
5                     37.50  1.669496  0.000498  0.009112      0.059230
6                     37.50  1.669496  0.000498  0.009112      0.059230
18                    32.14  1.447104  0.001886  0.011814      0.224474
3                     47.06  1.997071  0.000167  0.004426      0.019915
4                     47.06  1.997071  0.000167  0.004426      0.019915
14                    43.75  1.891888  0.000839  0.007396      0.099842
21                    38.89  1.721963  0.001966  0.011141      0.233951
20                    38.89  1.721963  0.001966  0.011141      0.233951
19                    38.89  1.721963  0.001966  0.011141      0.233951
17                    41.18  1.804426  0.001309  0.008902      0.155791
16                    41.18  1.804426  0.001309  0.008902      0.155791
15                    43.75  1.891888  0.000839  0.007396      0.099842
12                    43.75  1.891888  0.000839  0.007396      0.099842
13                    43.75  1.891888  0.000839  0.007396      0.099842
11                    43.75  1.891888  0.000839  0.007396      0.099842
10                    43.75  1.891888  0.000839  0.007396      0.099842
9                     46.67  1.984998  0.000514  0.006797      0.061176
8                     46.67  1.984998  0.000514  0.006797      0.061176
7                     46.67  1.984998  0.000514  0.006797      0.061176
23                    30.43  1.368326  0.009833  0.048753      1.000000
1                    100.00  3.084534  0.000160  0.007593      0.018982
2                    100.00  3.084534  0.000160  0.007593      0.018982
```

GO Biological Process

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
1        3.A              246                   56            29
0        1.B              246                    4            29

   annotated_subset  expected  percentage_of_annotated       lfc        p  \
1                13  6.601626                    23.21  0.977618  0.00421
0                 4  0.471545                   100.00  3.084534  0.00016

      p_fdr  p_bonferroni
1  0.010524      0.021049
0  0.000798      0.000798

TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
1          3              246                   60            29
0          1              246                   12            29

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
1                13  7.073171                    21.67  0.878083  0.008412
0                 5  1.414634                    41.67  1.821499  0.007106

      p_fdr  p_bonferroni
1  0.016823      0.033646
0  0.028423      0.028423

Interpro Domains

                                          annotation  total_reference  \
0               ABC transporter-like, conserved site              246
1                                 AAA+ ATPase domain              246
2           ABC transporter-like, ATP-binding domain              246
3  P-loop containing nucleoside triphosphate hydr…              246

   annotated_reference  total_subset  annotated_subset  expected  \
0                   16            29                 9  1.886179
1                   18            29                 9  2.121951
2                   18            29                 9  2.121951
3                   19            29                 9  2.239837

   percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
0                    56.25  2.254459  0.000008  0.000544      0.000544
```

```
1                    50.00  2.084534  0.000030  0.000786      0.001965
2                    50.00  2.084534  0.000030  0.000786      0.001965
3                    47.37  2.006531  0.000053  0.000861      0.003443

============================================================
CLUSTER 3
============================================================
Keywords

            annotation  total_reference  annotated_reference  total_subset  \
0  Transmembrane helix              246                  215            58
1        Transmembrane              246                  219            58

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                58  50.691057                    26.98  0.194322  0.000126
1                58  51.634146                    26.48  0.167727  0.000435

      p_fdr  p_bonferroni
0  0.005911      0.005911
1  0.010219      0.020438

GO Cellular Component

        annotation  total_reference  annotated_reference  total_subset  \
0  plasma membrane              246                  222            58

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                58  52.341463                    26.13  0.148099  0.001084

      p_fdr  p_bonferroni
0  0.020593      0.020593

GO Molecular Function

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

GO Biological Process

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        2.A              246                  133            58
```

```
   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                46  31.357724                    34.59  0.552813  0.000006

      p_fdr  p_bonferroni
0  0.000024      0.000024

TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          2              246                  133            58

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                46  31.357724                    34.59  0.552813  0.000006

      p_fdr  p_bonferroni
0  0.000024      0.000024

Interpro Domains

                      annotation  total_reference  annotated_reference  \
0  Major facilitator superfamily              246                   26
1                    EamA domain              246                    5

   total_subset  annotated_subset  expected  percentage_of_annotated  \
0            58                15  6.130081                    57.69
1            58                 5  1.178862                   100.00

        lfc         p     p_fdr  p_bonferroni
0  1.290984  0.000074  0.005719      0.005719
1  2.084534  0.000636  0.024479      0.048958

============================================================
CLUSTER 4
============================================================
Keywords

           annotation  total_reference  annotated_reference  total_subset  \
0         ATP-binding              246                   21            23
1  Nucleotide-binding              246                   23            23
2          Translocase              246                   17            23
3       Phosphoprotein              246                   16            23
4            Cytoplasm              246                   10            23
5                 Zinc              246                    7            23
6        Metal-binding              246                    5            23

   annotated_subset  expected  percentage_of_annotated       lfc  \
0                11  1.963415                    52.38  2.486067
1                11  2.150407                    47.83  2.354822
2                 8  1.589431                    47.06  2.331490
3                 7  1.495935                    43.75  2.226307
```

```
4                 5  0.934959                    50.00  2.418953
5                 4  0.654472                    57.14  2.611598
6                 3  0.467480                    60.00  2.681987

              p     p_fdr  p_bonferroni
0  7.347041e-08  0.000003      0.000003
1  2.549162e-07  0.000005      0.000011
2  2.377248e-05  0.000333      0.000998
3  1.631512e-04  0.001713      0.006852
4  8.523928e-04  0.007160      0.035800
5  1.713178e-03  0.011992      0.071953
6  6.361637e-03  0.038170      0.267189
```

GO Cellular Component

```
                          annotation  total_reference  annotated_reference  \
1           membrane protein complex              246                   87
3         protein-containing complex              246                   92
0  transmembrane transporter complex              246                   77
2                transporter complex              246                   79
4    extrinsic component of membrane              246                    2

   total_subset  annotated_subset  expected  percentage_of_annotated  \
1            23                16  8.134146                    18.39
3            23                16  8.601626                    17.39
0            23                15  7.199187                    19.48
2            23                15  7.386179                    18.99
4            23                 2  0.186992                   100.00

        lfc         p     p_fdr  p_bonferroni
1  0.976009  0.000492  0.006648      0.013295
3  0.895391  0.001068  0.007206      0.028824
0  1.059057  0.000459  0.012399      0.012399
2  1.022062  0.000643  0.005787      0.017361
4  3.418953  0.008396  0.045336      0.226680
```

GO Molecular Function

```
                                           annotation  total_reference  \
13                                            binding              246
2   ATPase-coupled transmembrane transporter activity              246
3                              ATP-dependent activity              246
16  primary active transmembrane transporter activity              246
0                       heterocyclic compound binding              246
19                                 catalytic activity              246
17                                        ion binding              246
1                     organic cyclic compound binding              246
12                          purine nucleotide binding              246
18                                      anion binding              246
14                             small molecule binding              246
```

```
15                    carbohydrate derivative binding              246
11                       nucleoside phosphate binding              246
10                                 nucleotide binding              246
9          purine ribonucleoside triphosphate binding              246
8                              ribonucleotide binding              246
7                       purine ribonucleotide binding              246
6                           adenyl nucleotide binding              246
5                       adenyl ribonucleotide binding              246
4                                         ATP binding              246
29  protein-phosphocysteine-sugar phosphotransfera…              246
26                         lipid transporter activity              246
27      xenobiotic transmembrane transporter activity              246
20      hydrolase activity, acting on acid anhydrides              246
22                            ATP hydrolysis activity              246
23   ribonucleoside triphosphate phosphatase activity              246
24                           pyrophosphatase activity              246
25                                 hydrolase activity              246
21  hydrolase activity, acting on acid anhydrides,…              246
28     zinc efflux transmembrane transporter activity              246

    annotated_reference  total_subset  annotated_subset  expected  \
13                   60            23                15  5.609756
2                    24            23                10  2.243902
3                    24            23                10  2.243902
16                   28            23                10  2.617886
0                    17            23                 9  1.589431
19                   31            23                 9  2.898374
17                   23            23                 9  2.150407
1                    17            23                 9  1.589431
12                   16            23                 8  1.495935
18                   18            23                 8  1.682927
14                   17            23                 8  1.589431
15                   17            23                 8  1.589431
11                   16            23                 8  1.495935
10                   16            23                 8  1.495935
9                    16            23                 8  1.495935
8                    16            23                 8  1.495935
7                    16            23                 8  1.495935
6                    15            23                 8  1.402439
5                    15            23                 8  1.402439
4                    15            23                 8  1.402439
29                   16            23                 5  1.495935
26                    6            23                 4  0.560976
27                    9            23                 4  0.841463
20                    3            23                 3  0.280488
22                    3            23                 3  0.280488
23                    3            23                 3  0.280488
24                    3            23                 3  0.280488
```

```
25                    3            23                 3  0.280488
21                    3            23                 3  0.280488
28                    2            23                 2  0.186992

    percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
13                    25.00  1.418953  0.000014  0.000127      0.001775
2                     41.67  2.155918  0.000006  0.000208      0.000729
3                     41.67  2.155918  0.000006  0.000208      0.000729
16                    35.71  1.933526  0.000031  0.000231      0.003924
0                     52.94  2.501415  0.000002  0.000137      0.000205
19                    29.03  1.634681  0.000605  0.003811      0.076217
17                    39.13  2.065316  0.000039  0.000273      0.004911
1                     52.94  2.501415  0.000002  0.000137      0.000205
12                    50.00  2.418953  0.000013  0.000160      0.001682
18                    44.44  2.249028  0.000040  0.000267      0.005081
14                    47.06  2.331490  0.000024  0.000193      0.002995
15                    47.06  2.331490  0.000024  0.000193      0.002995
11                    50.00  2.418953  0.000013  0.000160      0.001682
10                    50.00  2.418953  0.000013  0.000160      0.001682
9                     50.00  2.418953  0.000013  0.000160      0.001682
8                     50.00  2.418953  0.000013  0.000160      0.001682
7                     50.00  2.418953  0.000013  0.000160      0.001682
6                     53.33  2.512062  0.000007  0.000149      0.000892
5                     53.33  2.512062  0.000007  0.000149      0.000892
4                     53.33  2.512062  0.000007  0.000149      0.000892
29                    31.25  1.740881  0.010003  0.042013      1.000000
26                    66.67  2.833990  0.000783  0.003656      0.098719
27                    44.44  2.249028  0.005416  0.024371      0.682378
20                   100.00  3.418953  0.000723  0.003874      0.091044
22                   100.00  3.418953  0.000723  0.003874      0.091044
23                   100.00  3.418953  0.000723  0.003874      0.091044
24                   100.00  3.418953  0.000723  0.003874      0.091044
25                   100.00  3.418953  0.000723  0.003874      0.091044
21                   100.00  3.418953  0.000723  0.003874      0.091044
28                   100.00  3.418953  0.008396  0.036477      1.000000
```

```
GO Biological Process
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
TCDB Class
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
```

```
Index: []
```

TCDB Mechanism

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

Interpro Domains

```
                                           annotation  total_reference  \
0                                  AAA+ ATPase domain              246   
1            ABC transporter-like, ATP-binding domain              246   
2   P-loop containing nucleoside triphosphate hydr…             246   
3                ABC transporter-like, conserved site              246   
4        ABC transporter type 1, transmembrane domain              246   
5   ABC transporter type 1, transmembrane domain s…             246   
9                              PTS EIIA type-2 domain              246   
8                Phosphotransferase/anion transporter              246   
7                       Acriflavin resistance protein              246   
6   Multidrug efflux transporter AcrB TolC docking…             246   
10                P-type ATPase, cytoplasmic domain N              246   
11                P-type ATPase, phosphorylation site              246   
12                P-type ATPase, A domain superfamily              246   
13                               HAD-like superfamily              246   
14                                    HAD superfamily              246   
15                                      P-type ATPase              246   
16        P-type ATPase, haloacid dehalogenase domain              246   

    annotated_reference  total_subset  annotated_subset  expected  \
0                    18            23                 9  1.682927   
1                    18            23                 9  1.682927   
2                    19            23                 9  1.776423   
3                    16            23                 7  1.495935   
4                     5            23                 4  0.467480   
5                     5            23                 4  0.467480   
9                     4            23                 3  0.373984   
8                     4            23                 3  0.373984   
7                     3            23                 3  0.280488   
6                     3            23                 3  0.280488   
10                    2            23                 2  0.186992   
11                    2            23                 2  0.186992   
12                    2            23                 2  0.186992   
13                    2            23                 2  0.186992   
14                    2            23                 2  0.186992   
15                    2            23                 2  0.186992   
16                    2            23                 2  0.186992   
```

```
    percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
0                     50.00  2.418953  0.000003  0.000123      0.000185
1                     50.00  2.418953  0.000003  0.000123      0.000185
2                     47.37  2.340950  0.000006  0.000111      0.000332
3                     43.75  2.226307  0.000163  0.002447      0.009789
4                     80.00  3.097024  0.000279  0.003040      0.016721
5                     80.00  3.097024  0.000279  0.003040      0.016721
9                     75.00  3.003915  0.002712  0.017128      0.162712
8                     75.00  3.003915  0.002712  0.017128      0.162712
7                    100.00  3.418953  0.000723  0.005781      0.043354
6                    100.00  3.418953  0.000723  0.005781      0.043354
10                   100.00  3.418953  0.008396  0.035981      0.503733
11                   100.00  3.418953  0.008396  0.035981      0.503733
12                   100.00  3.418953  0.008396  0.035981      0.503733
13                   100.00  3.418953  0.008396  0.035981      0.503733
14                   100.00  3.418953  0.008396  0.035981      0.503733
15                   100.00  3.418953  0.008396  0.035981      0.503733
16                   100.00  3.418953  0.008396  0.035981      0.503733

============================================================
CLUSTER 5
============================================================
Keywords

            annotation  total_reference  annotated_reference  total_subset  \
0  Transmembrane helix              246                  215            90   
1        Transmembrane              246                  219            90   
2        Cell membrane              246                  228            90   
3             Membrane              246                  232            90   

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                90  78.658537                    41.86  0.194322   
1                90  80.121951                    41.10  0.167727   
2                90  83.414634                    39.47  0.109624   
3                90  84.878049                    38.79  0.084534   

              p     p_fdr  p_bonferroni  
0  2.171405e-07  0.000013      0.000013  
1  1.838997e-06  0.000055      0.000110  
2  1.876446e-04  0.003753      0.011259  
3  1.359406e-03  0.020391      0.081564  

GO Cellular Component

        annotation  total_reference  annotated_reference  total_subset  \
0  plasma membrane              246                  222            90   
1         membrane              246                  228            90   

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                 90  81.219512                    40.54  0.148099  0.000009
1                 90  83.414634                    39.47  0.109624  0.000188

      p_fdr  p_bonferroni
0  0.000327      0.000327
1  0.003471      0.006943
```

```
GO Molecular Function
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
GO Biological Process
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
TCDB Class
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
TCDB Mechanism
```

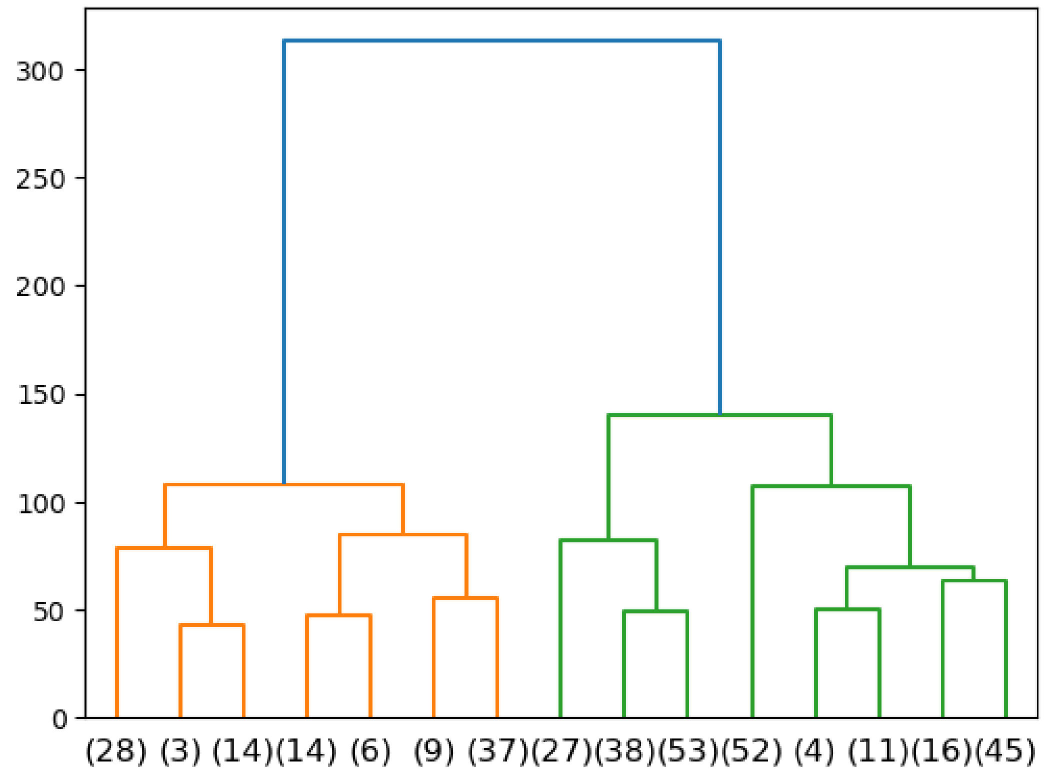
```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
Interpro Domains
```

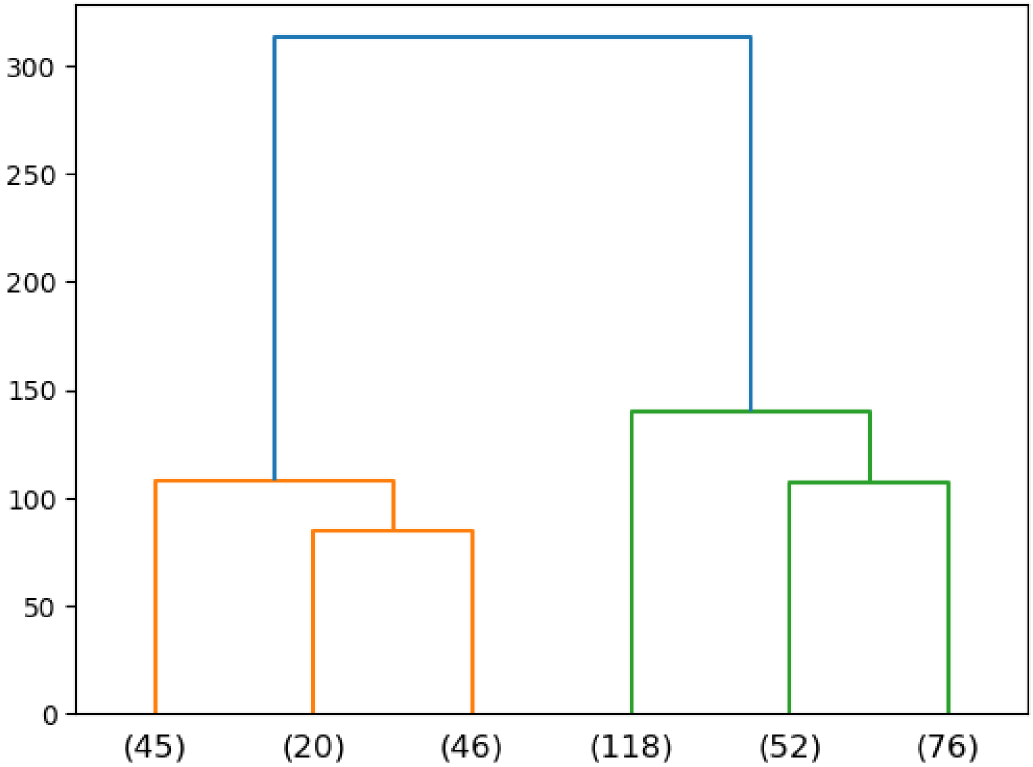
```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

Five clusters If we look at the dendrogram again, it seems like we can divide the left cluster into two sub-clusters, and the right cluster into three:

```
[ ]: dendrogram = dendrogram_plot(linkage, max_leaves=15)
```



```
[ ]: dendrogram = dendrogram_plot(linkage, max_leaves=6)
```



Clusters 1 is now divided into the new clusters 1 and 2, while the old cluster 2 is now clusters 3, 4 and 5.

[ ]:
```
labels_clusters = get_cluster_labels(linkage, n_clusters=5, index=feature_pssm.
  ↪index.tolist())
labels_clusters.value_counts()
```

[ ]:
```
3    118
5     76
2     66
4     52
1     45
dtype: int64
```

[ ]:
```
# TODO analysis!
# TODO left and right clusters: analysis separately? Would make more sense with␣
  ↪reference set
# Problem: The clusters still contain small outliers.
# Next: better filtering with annotations, then try again. Maybe: Only keep␣
  ↪proteins with specific substrate annotation?
```

```
cluster_enrichment_analysis(cluster_labels=labels_clusters,␣
 ↪reference_set=reference_set_transmembrane_transporters,␣
 ↪annotations_dict=records_all_dict, p_cutoff=0.05)
```

```
============================================================
CLUSTER 1
============================================================
Keywords

            annotation  total_reference  annotated_reference  total_subset  \
0  Cell inner membrane              357                  302            45
1        Cell membrane              357                  304            45
3  Transmembrane helix              357                  268            45
2         3D-structure              357                  116            45

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                45  38.067227                    14.90  0.241376  0.000308
1                45  38.319328                    14.80  0.231853  0.000425
3                42  33.781513                    15.67  0.314155  0.000916
2                25  14.621849                    21.55  0.773802  0.000541

      p_fdr  p_bonferroni
0  0.018796      0.018796
1  0.012955      0.025910
3  0.013964      0.055856
2  0.011003      0.033010

GO Cellular Component

               annotation  total_reference  annotated_reference  total_subset  \
0         plasma membrane              357                  285            45
1  oxidoreductase complex              357                   11            45
2       catalytic complex              357                   12            45

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                44  35.924370                    15.44  0.292541  0.000264
1                 6   1.386555                    54.55  2.113458  0.000828
2                 6   1.512605                    50.00  1.987927  0.001497

      p_fdr  p_bonferroni
0  0.009238      0.009238
1  0.014482      0.028963
2  0.017469      0.052406

GO Molecular Function

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
```

```
Index: []

GO Biological Process

                                          annotation  total_reference  \
0     generation of precursor metabolites and energy              357
1                           electron transport chain              357
2               respiratory electron transport chain              357
3                                aerobic respiration              357
4  energy derivation by oxidation of organic comp…              357
5                               cellular respiration              357
6        electron transport coupled proton transport              357
7  energy coupled proton transmembrane transport,…              357

   annotated_reference  total_subset  annotated_subset  expected  \
0                   10            45                 6  1.260504
1                   10            45                 6  1.260504
2                   10            45                 6  1.260504
3                    5            45                 4  0.630252
4                    5            45                 4  0.630252
5                    5            45                 4  0.630252
6                    5            45                 4  0.630252
7                    5            45                 4  0.630252

   percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
0                     60.0  2.250962  0.000416  0.046982      0.093964
1                     60.0  2.250962  0.000416  0.046982      0.093964
2                     60.0  2.250962  0.000416  0.046982      0.093964
3                     80.0  2.665999  0.001015  0.038248      0.229487
4                     80.0  2.665999  0.001015  0.038248      0.229487
5                     80.0  2.665999  0.001015  0.038248      0.229487
6                     80.0  2.665999  0.001015  0.038248      0.229487
7                     80.0  2.665999  0.001015  0.038248      0.229487

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        3.D              357                   10            45

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 6  1.260504                     60.0  2.250962  0.000416

      p_fdr  p_bonferroni
0  0.003326      0.003326

TCDB Mechanism

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
```

```
Index: []

Interpro Domains

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

============================================================
CLUSTER 2
============================================================
Keywords

                   annotation  total_reference  annotated_reference  \
0                      Signal              357                   46
13               3D-structure              357                  116
3   Direct protein sequencing              357                   55
1         Cell outer membrane              357                   27
2   Transmembrane beta strand              357                   27
6                 ATP-binding              357                   42
7          Nucleotide-binding              357                   44
12                 Translocase              357                   37
4                   Periplasm              357                   10
5               Disulfide bond              357                   10
11                      Porin              357                   12
8                    TonB box              357                    6
9         Fimbrium biogenesis              357                    6
10                   Receptor              357                    7

    total_subset  annotated_subset   expected  percentage_of_annotated  \
0             66                36   8.504202                    78.26
13            66                33  21.445378                    28.45
3             66                27  10.168067                    49.09
1             66                23   4.991597                    85.19
2             66                23   4.991597                    85.19
6             66                20   7.764706                    47.62
7             66                20   8.134454                    45.45
12            66                15   6.840336                    40.54
4             66                 9   1.848739                    90.00
5             66                 9   1.848739                    90.00
11            66                 8   2.218487                    66.67
8             66                 6   1.109244                   100.00
9             66                 6   1.109244                   100.00
10            66                 6   1.294118                    85.71

         lfc             p         p_fdr  p_bonferroni
0   2.081749  2.509921e-22  1.882441e-20  1.882441e-20
```

```
13  0.621799  8.171944e-04  4.377827e-03  6.128958e-02
3   1.408914  9.833544e-09  1.843790e-07  7.375158e-07
1   2.204061  3.735733e-15  1.120720e-13  2.801800e-13
2   2.204061  3.735733e-15  1.120720e-13  2.801800e-13
6   1.364997  3.310541e-06  3.547008e-05  2.482906e-04
7   1.297883  8.443522e-06  7.915802e-05  6.332642e-04
12  1.132823  7.660688e-04  4.419628e-03  5.745516e-02
4   2.283383  1.345864e-06  1.835269e-05  1.009398e-04
5   2.283383  1.345864e-06  1.835269e-05  1.009398e-04
11  1.850424  2.479974e-04  1.549984e-03  1.859980e-02
8   2.435386  3.296341e-05  2.602375e-04  2.472256e-03
9   2.435386  3.296341e-05  2.602375e-04  2.472256e-03
10  2.212994  1.969352e-04  1.342740e-03  1.477014e-02

GO Cellular Component

                                           annotation  total_reference  \
5                          protein-containing complex              357
4                            membrane protein complex              357
6                                 transporter complex              357
7                   transmembrane transporter complex              357
11                    plasma membrane protein complex              357
9    ATPase dependent transmembrane transport complex              357
10     ATP-binding cassette (ABC) transporter complex              357
0                                 cell outer membrane              357
1                                      outer membrane              357
8   ATP-binding cassette (ABC) transporter complex…              357
2            outer membrane-bounded periplasmic space              357
3                                   periplasmic space              357
12                                 MacAB-TolC complex              357
13        macrolide transmembrane transporter complex              357

    annotated_reference  total_subset  annotated_subset   expected  \
5                   155            66                46  28.655462
4                   147            66                45  27.176471
6                   130            66                37  24.033613
7                   127            66                36  23.478992
11                   91            66                27  16.823529
9                    81            66                25  14.974790
10                   77            66                24  14.235294
0                    27            66                22   4.991597
1                    27            66                22   4.991597
8                    63            66                22  11.647059
2                    16            66                14   2.957983
3                    16            66                14   2.957983
12                    3            66                 3   0.554622
13                    3            66                 3   0.554622

    percentage_of_annotated       lfc             p         p_fdr  \
5                     29.68  0.682824  1.766584e-06  1.589926e-05
4                     30.61  0.727567  8.904808e-07  9.617193e-06
6                     28.46  0.622472  2.533416e-04  1.954349e-03
7                     28.35  0.616626  3.813533e-04  2.574135e-03
11                    29.67  0.682479  1.688688e-03  7.599097e-03
9                     30.86  0.739392  1.423236e-03  7.685473e-03
10                    31.17  0.753562  1.590306e-03  7.806955e-03
0                     81.48  2.139930  1.130564e-13  4.070030e-12
1                     81.48  2.139930  1.130564e-13  4.070030e-12
8                     34.92  0.917538  4.302753e-04  2.581652e-03
2                     87.50  2.242741  1.414229e-09  2.181953e-08
3                     87.50  2.242741  1.414229e-09  2.181953e-08
12                   100.00  2.435386  6.085422e-03  2.434169e-02
13                   100.00  2.435386  6.085422e-03  2.434169e-02

    p_bonferroni
5   9.539555e-05
4   4.808597e-05
6   1.368044e-02
7   2.059308e-02
11  9.118916e-02
9   7.685473e-02
10  8.587651e-02
0   6.105044e-12
1   6.105044e-12
8   2.323487e-02
2   7.636836e-08
3   7.636836e-08
12  3.286128e-01
13  3.286128e-01
```

```
GO Molecular Function
                                           annotation  total_reference  \
0                                             binding              357
22                                    protein binding              357
3                                         ion binding              357
9                                    channel activity              357
10         passive transmembrane transporter activity              357
4                     carbohydrate derivative binding              357
5                       heterocyclic compound binding              357
6                     organic cyclic compound binding              357
7                              small molecule binding              357
8                                       anion binding              357
11                       nucleoside phosphate binding              357
12                                 nucleotide binding              357
16                      purine ribonucleotide binding              357
19                           purine nucleotide binding              357
18         purine ribonucleoside triphosphate binding              357
```

```
17                     ribonucleotide binding            357
13                                ATP binding            357
15                  adenyl nucleotide binding            357
14              adenyl ribonucleotide binding            357
1                 wide pore channel activity             357
2                             porin activity             357
24        amide transmembrane transporter activity       357
20  siderophore uptake transmembrane transporter a…    357
21              fimbrial usher porin activity            357
23  siderophore-iron transmembrane transporter act…    357
25                   glycosaminoglycan binding           357

    annotated_reference  total_subset  annotated_subset   expected  \
0                   119            66                45  22.000000
22                   77            66                25  14.235294
3                    47            66                22   8.689076
9                    44            66                20   8.134454
10                   44            66                20   8.134454
4                    37            66                19   6.840336
5                    38            66                19   7.025210
6                    38            66                19   7.025210
7                    36            66                18   6.655462
8                    37            66                18   6.840336
11                   34            66                17   6.285714
12                   34            66                17   6.285714
16                   33            66                16   6.100840
19                   33            66                16   6.100840
18                   33            66                16   6.100840
17                   33            66                16   6.100840
13                   32            66                16   5.915966
15                   32            66                16   5.915966
14                   32            66                16   5.915966
1                    19            66                15   3.512605
2                    19            66                15   3.512605
24                   35            66                13   6.470588
20                    6            66                 6   1.109244
21                    6            66                 6   1.109244
23                   10            66                 6   1.848739
25                    3            66                 3   0.554622

    percentage_of_annotated       lfc             p         p_fdr  \
0                     37.82  1.032421  1.177922e-10  2.379402e-08
22                    32.47  0.812456  5.677372e-04  4.986214e-03
3                     46.81  1.340229  1.269099e-06  6.408948e-05
9                     45.45  1.297883  8.443522e-06  1.624373e-04
10                    45.45  1.297883  8.443522e-06  1.624373e-04
4                     51.35  1.473860  1.410170e-06  5.697088e-05
5                     50.00  1.435386  2.431397e-06  7.556035e-05
```

```
6                      50.00  1.435386  2.431397e-06  7.556035e-05
7                      50.00  1.435386  4.906263e-06  1.238831e-04
8                      48.65  1.395858  8.218351e-06  1.844563e-04
11                     50.00  1.435386  9.797265e-06  1.583238e-04
12                     50.00  1.435386  9.797265e-06  1.583238e-04
16                     48.48  1.390992  3.223413e-05  3.519618e-04
19                     48.48  1.390992  3.223413e-05  3.519618e-04
18                     48.48  1.390992  3.223413e-05  3.519618e-04
17                     48.48  1.390992  3.223413e-05  3.519618e-04
13                     50.00  1.435386  1.937052e-05  2.608564e-04
15                     50.00  1.435386  1.937052e-05  2.608564e-04
14                     50.00  1.435386  1.937052e-05  2.608564e-04
1                      78.95  2.094349  5.135662e-09  4.149615e-07
2                      78.95  2.094349  5.135662e-09  4.149615e-07
24                     37.14  1.006543  4.809425e-03  3.886015e-02
20                    100.00  2.435386  3.296341e-05  3.097027e-04
21                    100.00  2.435386  3.296341e-05  3.097027e-04
23                     60.00  1.698421  3.677758e-03  3.095447e-02
25                    100.00  2.435386  6.085422e-03  4.727905e-02

    p_bonferroni
0   2.379402e-08
22  1.146829e-01
3   2.563579e-04
9   1.705592e-03
10  1.705592e-03
4   2.848544e-04
5   4.911423e-04
6   4.911423e-04
7   9.910651e-04
8   1.660107e-03
11  1.979047e-03
12  1.979047e-03
16  6.511294e-03
19  6.511294e-03
18  6.511294e-03
17  6.511294e-03
13  3.912846e-03
15  3.912846e-03
14  3.912846e-03
1   1.037404e-06
2   1.037404e-06
24  9.715038e-01
20  6.658609e-03
21  6.658609e-03
23  7.429072e-01
25  1.000000e+00
```

```
GO Biological Process
                                      annotation  total_reference  \
0                 cellular component assembly              357   
5              cellular component organization              357   
6  cellular component organization or biogenesis              357   
7             iron ion transmembrane transport              357   
1                     cell projection assembly              357   
2                               pilus assembly              357   
3                           pilus organization              357   
4                 cell projection organization              357   

   annotated_reference  total_subset  annotated_subset  expected  \
0                   10            66                 9  1.848739   
5                   13            66                 9  2.403361   
6                   13            66                 9  2.403361   
7                   10            66                 7  1.848739   
1                    6            66                 6  1.109244   
2                    6            66                 6  1.109244   
3                    6            66                 6  1.109244   
4                    6            66                 6  1.109244   

   percentage_of_annotated       lfc         p     p_fdr  p_bonferroni  
0                    90.00  2.283383  0.000001  0.000357      0.000357  
5                    69.23  1.904871  0.000060  0.002429      0.015791  
6                    69.23  1.904871  0.000060  0.002429      0.015791  
7                    70.00  1.920813  0.000419  0.013879      0.111033  
1                   100.00  2.435386  0.000033  0.002496      0.008735  
2                   100.00  2.435386  0.000033  0.002496      0.008735  
3                   100.00  2.435386  0.000033  0.002496      0.008735  
4                   100.00  2.435386  0.000033  0.002496      0.008735  
TCDB Class
  annotation  total_reference  annotated_reference  total_subset  \
1        3.A              357                   93            66   
0        1.B              357                   25            66   

   annotated_subset   expected  percentage_of_annotated       lfc  \
1                29  17.193277                    31.18  0.754208   
0                21   4.621849                    84.00  2.183847   

              p         p_fdr  p_bonferroni  
1  3.512964e-04  8.782409e-04  1.756482e-03  
0  1.499811e-13  7.499054e-13  7.499054e-13  
TCDB Mechanism
  annotation  total_reference  annotated_reference  total_subset  \
1          3              357                  103            66   
```

```
0          1              357                   42            66

   annotated_subset   expected  percentage_of_annotated       lfc  \
1                29  19.042017                    28.16  0.606867
0                24   7.764706                    57.14  1.628031

              p         p_fdr  p_bonferroni
1  2.748784e-03  5.497568e-03  1.099514e-02
0  1.521792e-09  6.087168e-09  6.087168e-09
```

Interpro Domains

```
                                           annotation  total_reference  \
4   P-loop containing nucleoside triphosphate hydr…              357
1                                  AAA+ ATPase domain                357
5            ABC transporter-like, ATP-binding domain                357
6                ABC transporter-like, conserved site                357
0           Peptide/nickel binding protein, MppA-type                357
2                     Solute-binding protein family 5                357
3              Solute-binding protein family 5 domain                357
13            Fimbrial membrane usher, conserved site                357
18                PapC, N-terminal domain superfamily                357
17                            PapC, N-terminal domain                357
16           PapC-like, C-terminal domain superfamily                357
15                       PapC-like, C-terminal domain                357
14     Outer membrane usher protein FimD, plug domain                357
12                       Outer membrane usher protein                357
11    Solute-binding protein family 5, conserved site                357
10  TonB-dependent receptor-like, beta-barrel doma…              357
9           TonB-dependent receptor-like, beta-barrel                357
8    TonB-dependent receptor, plug domain superfamily                357
7                TonB-dependent receptor, plug domain                357
19                           TonB box, conserved site                357
20                TonB-dependent siderophore receptor                357
21            TonB-dependent receptor, conserved site                357
22  RND efflux pump, membrane fusion protein, barr…              357
23           RND efflux pump, membrane fusion protein                357
24  Oligopeptide/dipeptide ABC transporter, C-term…              357
25                  Vitamin B12 transporter BtuB-like                357
26       Cation efflux system protein CusB,  domain 1                357
27            Molybdate/tungstate binding, C-terminal                357
28                       TonB-dependent receptor-like                357

    annotated_reference  total_subset  annotated_subset  expected  \
4                    38            66                18  7.025210
1                    36            66                18  6.655462
5                    35            66                17  6.470588
6                    32            66                16  5.915966
0                     8            66                 8  1.478992
```

```
2                      9            66                 8  1.663866
3                      9            66                 8  1.663866
13                     6            66                 6  1.109244
18                     6            66                 6  1.109244
17                     6            66                 6  1.109244
16                     6            66                 6  1.109244
15                     6            66                 6  1.109244
14                     6            66                 6  1.109244
12                     6            66                 6  1.109244
11                     6            66                 6  1.109244
10                     6            66                 6  1.109244
9                      6            66                 6  1.109244
8                      6            66                 6  1.109244
7                      6            66                 6  1.109244
19                     5            66                 5  0.924370
20                     5            66                 5  0.924370
21                     4            66                 4  0.739496
22                     4            66                 4  0.739496
23                     4            66                 4  0.739496
24                     5            66                 4  0.924370
25                     3            66                 3  0.554622
26                     3            66                 3  0.554622
27                     3            66                 3  0.554622
28                     3            66                 3  0.554622

    percentage_of_annotated       lfc             p    p_fdr  p_bonferroni
4                     47.37  1.357384  1.342661e-05  0.000360      0.001799
1                     50.00  1.435386  4.906263e-06  0.000329      0.000657
5                     48.57  1.393566  1.635775e-05  0.000365      0.002192
6                     50.00  1.435386  1.937052e-05  0.000371      0.002596
0                    100.00  2.435386  9.498614e-07  0.000127      0.000127
2                     88.89  2.265461  7.285900e-06  0.000279      0.000976
3                     88.89  2.265461  7.285900e-06  0.000279      0.000976
13                   100.00  2.435386  3.296341e-05  0.000327      0.004417
18                   100.00  2.435386  3.296341e-05  0.000327      0.004417
17                   100.00  2.435386  3.296341e-05  0.000327      0.004417
16                   100.00  2.435386  3.296341e-05  0.000327      0.004417
15                   100.00  2.435386  3.296341e-05  0.000327      0.004417
14                   100.00  2.435386  3.296341e-05  0.000327      0.004417
12                   100.00  2.435386  3.296341e-05  0.000327      0.004417
11                   100.00  2.435386  3.296341e-05  0.000327      0.004417
10                   100.00  2.435386  3.296341e-05  0.000327      0.004417
9                    100.00  2.435386  3.296341e-05  0.000327      0.004417
8                    100.00  2.435386  3.296341e-05  0.000327      0.004417
7                    100.00  2.435386  3.296341e-05  0.000327      0.004417
19                   100.00  2.435386  1.902151e-04  0.001243      0.025489
20                   100.00  2.435386  1.902151e-04  0.001243      0.025489
21                   100.00  2.435386  1.082999e-03  0.006310      0.145122
```

```
22                   100.00  2.435386  1.082999e-03  0.006310      0.145122
23                   100.00  2.435386  1.082999e-03  0.006310      0.145122
24                    80.00  2.113458  4.654134e-03  0.024946      0.623654
25                   100.00  2.435386  6.085422e-03  0.029653      0.815447
26                   100.00  2.435386  6.085422e-03  0.029653      0.815447
27                   100.00  2.435386  6.085422e-03  0.029653      0.815447
28                   100.00  2.435386  6.085422e-03  0.029653      0.815447

============================================================
CLUSTER 3
============================================================
Keywords

            annotation  total_reference  annotated_reference  total_subset  \
0  Transmembrane helix              357                  268           118
1        Transmembrane              357                  295           118
2        Cell membrane              357                  304           118
4             Membrane              357                  331           118
3  Cell inner membrane              357                  302           118
5              Symport              357                   51           118

   annotated_subset    expected  percentage_of_annotated       lfc  \
0               118   88.582633                    44.03  0.413691
1               118   97.507003                    40.00  0.275209
2               118  100.481793                    38.82  0.231853
4               118  109.406162                    35.65  0.109093
3               116   99.820728                    38.41  0.216713
5                26   16.857143                    50.98  0.625152

              p         p_fdr  p_bonferroni
0  3.160906e-19  2.275852e-17  2.275852e-17
1  7.320306e-13  2.635310e-11  5.270620e-11
2  6.580012e-11  1.579203e-09  4.737608e-09
4  1.825299e-05  2.628431e-04  1.314216e-03
3  1.553242e-08  2.795835e-07  1.118334e-06
5  3.272024e-03  3.926428e-02  2.355857e-01

GO Cellular Component

                   annotation  total_reference  annotated_reference  \
0             plasma membrane              357                  285
1                    membrane              357                  316
2  cellular anatomical entity              357                  334

   total_subset  annotated_subset    expected  percentage_of_annotated  \
0           118               118   94.201681                    41.40
1           118               118  104.448179                    37.34
2           118               118  110.397759                    35.33

        lfc             p         p_fdr  p_bonferroni
```

```
0  0.324962  3.986291e-15  1.395202e-13  1.395202e-13
1  0.176000  2.043211e-08  3.575619e-07  7.151238e-07
2  0.096076  6.781986e-05  7.912317e-04  2.373695e-03
```

```
GO Molecular Function
```

```
                                          annotation  total_reference  \
0  secondary active transmembrane transporter act…              357
3      active ion transmembrane transporter activity              357
1                                  symporter activity              357
2                    solute:cation symporter activity              357
4                    solute:proton symporter activity              357

   annotated_reference  total_subset  annotated_subset   expected  \
0                   88           118                51  29.086835
3                   83           118                41  27.434174
1                   51           118                31  16.857143
2                   43           118                27  14.212885
4                   32           118                20  10.577031

   percentage_of_annotated       lfc             p     p_fdr  p_bonferroni
0                    57.95  0.810131  2.200118e-08  0.000005      0.000005
3                    49.40  0.579650  3.132826e-04  0.019502      0.078007
1                    60.78  0.878908  1.028833e-05  0.001281      0.002562
2                    62.79  0.925760  1.930407e-05  0.001602      0.004807
4                    62.50  0.919065  3.328999e-04  0.016578      0.082892
```

```
GO Biological Process
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
↪p_bonferroni]
Index: []
```

```
TCDB Class
```

```
  annotation  total_reference  annotated_reference  total_subset  \
0        2.A              357                  152           118

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                89  50.240896                    58.55  0.824943


              p         p_fdr  p_bonferroni
0  6.477707e-19  3.238854e-18  3.238854e-18
```

```
TCDB Mechanism
```

```
  annotation  total_reference  annotated_reference  total_subset  \
0          2              357                  152           118
```

```
   annotated_subset   expected  percentage_of_annotated       lfc  \
0                89  50.240896                    58.55  0.824943

              p         p_fdr  p_bonferroni
0  6.477707e-19  2.591083e-18  2.591083e-18

Interpro Domains

                                     annotation  total_reference  \
0                   MFS transporter superfamily              357
2          Major facilitator superfamily domain              357
1                 Major facilitator superfamily              357
3                    ABC transporter, BtuC-like              357
4  ABC transporter, permease protein, BtuC-like              357

   annotated_reference  total_subset  annotated_subset   expected  \
0                   46           118                29  15.204482
2                   42           118                26  13.882353
1                   31           118                21  10.246499
3                    7           118                 7   2.313725
4                    6           118                 6   1.983193

   percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
0                    63.04  0.931556  0.000008  0.001077      0.001077
2                    61.90  0.905260  0.000042  0.002005      0.006016
1                    67.74  1.035258  0.000038  0.002684      0.005367
3                   100.00  1.597137  0.000381  0.013637      0.054549
4                   100.00  1.597137  0.001195  0.034191      0.170953

============================================================
CLUSTER 4
============================================================
Keywords

            annotation  total_reference  annotated_reference  total_subset  \
0  Transmembrane helix              357                  268            52
1        Transmembrane              357                  295            52
3  Cell inner membrane              357                  302            52
4        Cell membrane              357                  304            52
2              Symport              357                   51            52

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                52  39.036415                    19.40  0.413691
1                52  42.969188                    17.63  0.275209
3                52  43.988796                    17.22  0.241376
4                52  44.280112                    17.11  0.231853
2                18   7.428571                    35.29  1.276840

              p     p_fdr  p_bonferroni
0  8.311004e-08  0.000003      0.000003
```

```
1  2.051081e-05  0.000400      0.000800
3  7.813293e-05  0.000762      0.003047
4  1.137826e-04  0.000888      0.004438
2  4.268621e-05  0.000555      0.001665
```

```
GO Cellular Component
```

```
        annotation  total_reference  annotated_reference  total_subset  \
0  plasma membrane              357                  285            52
1         membrane              357                  316            52

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                52  41.512605                    18.25  0.324962  0.000003
1                52  46.028011                    16.46  0.176000  0.001027

      p_fdr  p_bonferroni
0  0.000077      0.000077
1  0.013860      0.027720
```

```
GO Molecular Function
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
GO Biological Process
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
TCDB Class
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
TCDB Mechanism
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
Interpro Domains
```

```
                                   annotation  total_reference  \
0  Major facilitator,  sugar transporter-like              357
1     MFS transporter, metabolite:H symporter              357

   annotated_reference  total_subset  annotated_subset  expected  \
0                    7            52                 6  1.019608
1                    4            52                 4  0.582633

   percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
0                    85.71  2.556948  0.000046  0.004085      0.004085
1                   100.00  2.779341  0.000407  0.018103      0.036206

============================================================
CLUSTER 5
============================================================
Keywords
                  annotation  total_reference  annotated_reference  \
0         Nucleotide-binding              357                   44
1                ATP-binding              357                   42
7                Translocase              357                   37
2             Phosphoprotein              357                   22
4                Transferase              357                   23
6  Phosphotransferase system              357                   26
3                  Cytoplasm              357                   13
5                     Kinase              357                   18

   total_subset  annotated_subset  expected  percentage_of_annotated  \
0            76                23  9.366947                    52.27
1            76                21  8.941176                    50.00
7            76                15  7.876751                    40.54
2            76                14  4.683473                    63.64
4            76                13  4.896359                    56.52
6            76                12  5.535014                    46.15
3            76                10  2.767507                    76.92
5            76                10  3.831933                    55.56

        lfc         p     p_fdr  p_bonferroni
0  1.295983  0.000001  0.000085      0.000085
1  1.231853  0.000009  0.000388      0.000777
7  0.929290  0.003948  0.041457      0.331654
2  1.579776  0.000010  0.000287      0.000861
4  1.408731  0.000136  0.002278      0.011390
6  1.116376  0.002844  0.034122      0.238856
3  1.853341  0.000019  0.000397      0.001589
5  1.383856  0.001116  0.015628      0.093766

GO Cellular Component
                                         annotation  total_reference  \
1                        protein-containing complex              357
0                          membrane protein complex              357
2                 transmembrane transporter complex              357
3                               transporter complex              357
4                   plasma membrane protein complex              357
6  ATPase dependent transmembrane transport complex              357
5    ATP-binding cassette (ABC) transporter complex              357
7                                           cytosol              357

   annotated_reference  total_subset  annotated_subset   expected  \
1                  155            76                54  32.997199
0                  147            76                53  31.294118
2                  127            76                45  27.036415
3                  130            76                45  27.675070
4                   91            76                33  19.372549
6                   81            76                30  17.243697
5                   77            76                29  16.392157
7                    8            76                 6   1.703081

   percentage_of_annotated       lfc             p         p_fdr  p_bonferroni
1                    34.84  0.710616  4.122939e-08  1.071964e-06  2.143928e-06
0                    36.05  0.760101  1.384027e-08  7.196939e-07  7.196939e-07
2                    35.43  0.735021  1.791565e-06  3.105379e-05  9.316136e-05
3                    34.62  0.701338  4.274637e-06  5.557029e-05  2.222811e-04
4                    36.26  0.768452  8.504471e-05  8.844650e-04  4.422325e-03
6                    37.04  0.798893  1.390684e-04  1.033080e-03  7.231559e-03
5                    37.66  0.823047  1.326587e-04  1.149709e-03  6.898253e-03
7                    75.00  1.816815  1.527043e-03  9.925780e-03  7.940624e-02
```

GO Molecular Function

```
                                           annotation  total_reference  \
14                                            binding              357
11  primary active transmembrane transporter activity              357
0   ATPase-coupled transmembrane transporter activity              357
18                                 catalytic activity              357
1                              ATP-dependent activity              357
19                                        ion binding              357
8                                       anion binding              357
13                    organic cyclic compound binding              357
12                      heterocyclic compound binding              357
7                     carbohydrate derivative binding              357
2                              small molecule binding              357
9                                  nucleotide binding              357
10                       nucleoside phosphate binding              357
6                           purine nucleotide binding              357
5          purine ribonucleoside triphosphate binding              357
4                              ribonucleotide binding              357
3                       purine ribonucleotide binding              357
```

```
15                                       ATP binding            357
16                      adenyl ribonucleotide binding            357
17                          adenyl nucleotide binding            357
20  transferase activity, transferring phosphorus-…            357
21  phosphotransferase activity, alcohol group as …            357
22                               transferase activity            357
23  protein-phosphocysteine-sugar phosphotransfera…            357

    annotated_reference  total_subset  annotated_subset   expected  \
14                  119            76                38  25.333333
11                   51            76                21  10.857143
0                    39            76                20   8.302521
18                   50            76                20  10.644258
1                    39            76                20   8.302521
19                   47            76                19  10.005602
8                    37            76                17   7.876751
13                   38            76                17   8.089636
12                   38            76                17   8.089636
7                    37            76                17   7.876751
2                    36            76                17   7.663866
9                    34            76                16   7.238095
10                   34            76                16   7.238095
6                    33            76                16   7.025210
5                    33            76                16   7.025210
4                    33            76                16   7.025210
3                    33            76                16   7.025210
15                   32            76                15   6.812325
16                   32            76                15   6.812325
17                   32            76                15   6.812325
20                   27            76                13   5.747899
21                   27            76                13   5.747899
22                   27            76                13   5.747899
23                   23            76                11   4.896359

    percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
14                    31.93  0.584963  0.000525  0.007729      0.115930
11                    41.18  0.951745  0.000385  0.007085      0.085016
0                     51.28  1.268379  0.000010  0.001437      0.002155
18                    40.00  0.909925  0.000891  0.010362      0.196886
1                     51.28  1.268379  0.000010  0.001437      0.002155
19                    40.43  0.925191  0.001065  0.011767      0.235338
8                     45.95  1.109862  0.000330  0.008589      0.073007
13                    44.74  1.071388  0.000496  0.008117      0.109574
12                    44.74  1.071388  0.000496  0.008117      0.109574
7                     45.95  1.109862  0.000330  0.008589      0.073007
2                     47.22  1.149391  0.000215  0.015854      0.047562
9                     47.06  1.144390  0.000365  0.007686      0.080700
10                    47.06  1.144390  0.000365  0.007686      0.080700
```

```
6                     48.48  1.187459  0.000233  0.009371      0.051538
5                     48.48  1.187459  0.000233  0.009371      0.051538
4                     48.48  1.187459  0.000233  0.009371      0.051538
3                     48.48  1.187459  0.000233  0.009371      0.051538
15                    46.88  1.138743  0.000616  0.008002      0.136026
16                    46.88  1.138743  0.000616  0.008002      0.136026
17                    46.88  1.138743  0.000616  0.008002      0.136026
20                    48.15  1.177405  0.001113  0.011177      0.245902
21                    48.15  1.177405  0.001113  0.011177      0.245902
22                    48.15  1.177405  0.001113  0.011177      0.245902
23                    47.83  1.167722  0.003073  0.028294      0.679059
```

```
GO Biological Process
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
TCDB Class
```

```
  annotation  total_reference  annotated_reference  total_subset  \
0        3.A              357                   93            76
1        4.A              357                   25            76

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                31  19.798319                    33.33  0.646890  0.001091
1                12   5.322129                    48.00  1.172959  0.001853

      p_fdr  p_bonferroni
0  0.007635      0.007635
1  0.006486      0.012972
```

```
TCDB Mechanism
```

```
  annotation  total_reference  annotated_reference  total_subset  \
1          3              357                  103            76
0          4              357                   25            76

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
1                32  21.927171                    31.07  0.545352  0.003730
0                12   5.322129                    48.00  1.172959  0.001853

      p_fdr  p_bonferroni
1  0.009324      0.018648
0  0.009266      0.009266
```

```
Interpro Domains
```

```
                                          annotation  total_reference  \
0  P-loop containing nucleoside triphosphate hydr…             357
1           ABC transporter-like, ATP-binding domain             357
2                                 AAA+ ATPase domain             357
5                ABC transporter-like, conserved site             357
3          PTS system IIB component-like superfamily             357
4  Phosphotransferase system, EIIB component, typ…             357
7  Phosphotransferase system, EIIB component, type 2             357
6        ABC transporter type 1, transmembrane domain             357
9  ABC transporter type 1, transmembrane domain s…             357
8  Phosphotransferase system, fructose-specific I…             357

   annotated_reference  total_subset  annotated_subset  expected  \
0                   38            76                19  8.089636
1                   35            76                18  7.450980
2                   36            76                18  7.663866
5                   32            76                16  6.812325
3                    8            76                 7  1.703081
4                    8            76                 7  1.703081
7                    7            76                 6  1.490196
6                    5            76                 5  1.064426
9                    6            76                 5  1.277311
8                    4            76                 4  0.851541

   percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
0                    50.00  1.231853  0.000028  0.004405      0.004405
1                    51.43  1.272495  0.000030  0.002294      0.004588
2                    50.00  1.231853  0.000049  0.002545      0.007634
5                    50.00  1.231853  0.000145  0.003742      0.022455
3                    87.50  2.039208  0.000105  0.003607      0.016231
4                    87.50  2.039208  0.000105  0.003607      0.016231
7                    85.71  2.009460  0.000460  0.008918      0.071346
6                   100.00  2.231853  0.000393  0.008707      0.060949
9                    83.33  1.968818  0.001963  0.030423      0.304227
8                   100.00  2.231853  0.001928  0.033202      0.298821
```

1.6 Hierarchical clustering using only keywords

Filtering for proteins with direct substrate annotation

[ ]:

```
keywords_transport = {
    "Ion transport",
    "Anion exchange",
    "Protein transport",
    "Sodium/potassium transport",
    "Polysaccharide transport",
    "Bacteriocin transport",
    "Peptide transport",
    "Translocation",
    "Bacterial flagellum protein export",
    "Amino-acid transport",
    "Electron transport",
    "Lipid transport",
    "mRNA transport",
    "Neurotransmitter transport",
    "Oxygen transport",
    "Phosphate transport",
    "Ammonia transport",
    "Phosphonate transport",
    "Viral movement protein",
    "Sulfate transport",
    "Sugar transport",
    "Calcium transport",
    "Cobalt transport",
    "Copper transport",
    "Hydrogen ion transport",
    "Iron transport",
    "Zinc transport",
    "Nickel transport",
    "Potassium transport",
    "Sodium transport",
    "Chloride",
}
```

[ ]:

```
sequences_all

# TODO uniref keywords are predicted, not experimental!
keywords_ecoli_substrate = keywords_ecoli[keywords_ecoli.keyword.
 ↪isin(keywords_transport)].reset_index(drop=True)
keywords_ecoli_substrate
```

[ ]:

```
    Uniprot                keyword
0    P02916        Sugar transport
1    P02925        Sugar transport
2    P02929   Bacteriocin transport
3    P02929      Protein transport
4    P03959          Ion transport
..      …                      …
530  P52636     Electron transport
531  P68646     Electron transport
532  Q46833      Protein transport
533  P45757      Protein transport
534  P23481     Electron transport

[535 rows x 2 columns]
```

```
[ ]: sequences_substrate_keywords = sequences_all.loc[keywords_ecoli_substrate.
      ↪Uniprot.unique()]
     sequences_substrate_keywords =␣
      ↪sequences_substrate_keywords[sequences_substrate_keywords.index.
      ↪isin(go_annotations_tmtp.Uniprot.unique())]
```

```
[ ]: feature_pssm_keywords = calculate_pssm_feature(
         sequences=sequences_substrate_keywords.sequence,
         tmp_folder="../data/intermediate/blast/pssm_uniref50_3it/",
         blast_db="../data/raw/uniref/uniref50/uniref50.fasta",
         iterations=3,
         verbose=False,
         psiblast_threads=80
     )
     feature_pssm_keywords
```

```
[ ]:                AA        AR        AN        AD        AC        AQ        AE  \
     P02916  0.819672  0.370902  0.387295  0.325820  0.336066  0.397541  0.350410
     P03959  0.936430  0.342298  0.420538  0.322738  0.586797  0.386308  0.344743
     P05825  0.724662  0.339527  0.407095  0.371622  0.234797  0.385135  0.337838
     P0AAD6  0.787425  0.281437  0.419162  0.215569  0.541916  0.299401  0.251497
     P0AAD8  0.791531  0.390879  0.469055  0.397394  0.677524  0.462541  0.377850
     …            …         …         …         …         …         …         …
     P0A843  0.717949  0.384615  0.474359  0.500000  0.205128  0.474359  0.576923
     P45539  0.911765  0.289593  0.371041  0.255656  0.470588  0.343891  0.280543
     P75826  0.714286  0.411429  0.414286  0.365714  0.551429  0.431429  0.397143
     P76128  0.928736  0.250575  0.310345  0.287356  0.225287  0.381609  0.335632
     P31448  0.847059  0.285714  0.344538  0.270588  0.473950  0.339496  0.295798

                   AG        AH        AI  …        VL        VK        VM  \
     P02916  0.530738  0.319672  0.497951  …  0.665984  0.415984  0.633197
     P03959  0.616137  0.371638  0.501222  …  0.660147  0.337408  0.745721
     P05825  0.530405  0.315878  0.378378  …  0.592905  0.290541  0.500000
     P0AAD6  0.565868  0.323353  0.661677  …  0.703593  0.338323  0.706587
     P0AAD8  0.625407  0.423453  0.674267  …  0.713355  0.410423  0.732899
     …            …         …         … …       …         …         …
     P0A843  0.641026  0.320513  0.346154  …  0.641026  0.410256  0.589744
     P45539  0.640271  0.278281  0.597285  …  0.690045  0.414027  0.678733
     P75826  0.597143  0.457143  0.565714  …  0.642857  0.434286  0.631429
     P76128  0.452874  0.280460  0.379310  …  0.648276  0.340230  0.588506
     P31448  0.606723  0.278992  0.610084  …  0.719328  0.287395  0.687395

                   VF        VP        VS        VT        VW        VY        VV
     P02916  0.594262  0.411885  0.510246  0.606557  0.446721  0.508197  0.725410
     P03959  0.647922  0.418093  0.559902  0.603912  0.518337  0.518337  0.792176
     P05825  0.530405  0.300676  0.398649  0.496622  0.427365  0.423986  0.717905
     P0AAD6  0.682635  0.356287  0.535928  0.568862  0.544910  0.592814  0.781437
```

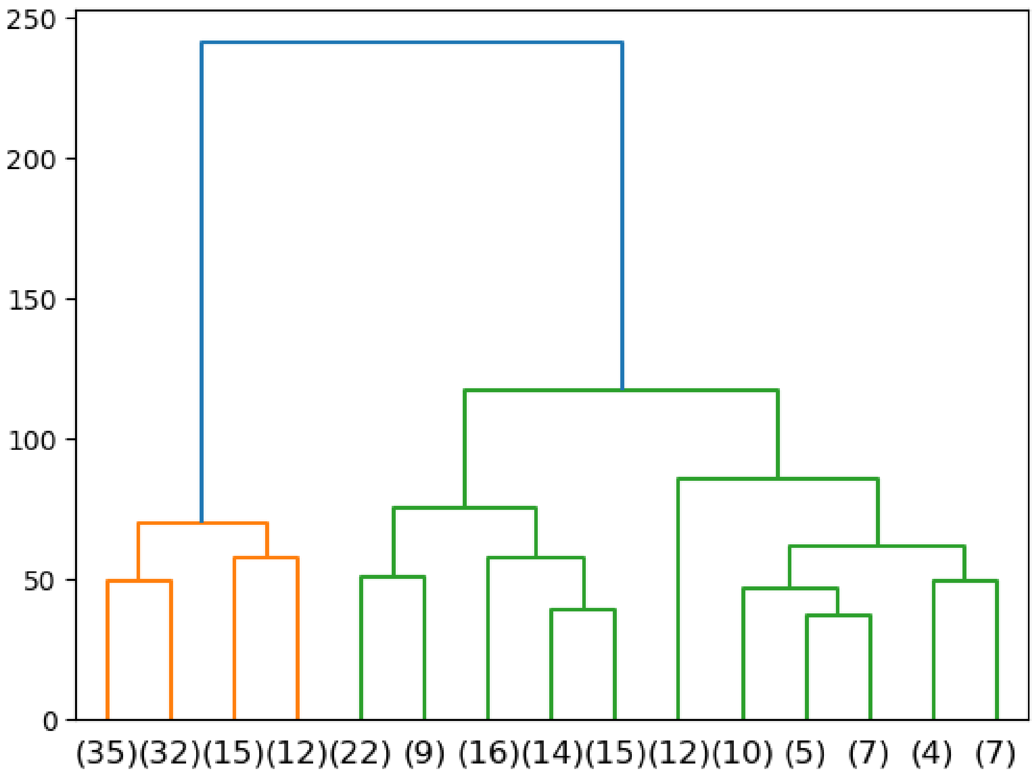
```
P0AAD8  0.742671  0.420195  0.570033  0.625407  0.563518  0.605863  0.785016
…            …         …         …         …         …         …         …
P0A843  0.525641  0.384615  0.423077  0.500000  0.320513  0.435897  0.743590
P45539  0.606335  0.484163  0.601810  0.662896  0.472851  0.486425  0.830317
P75826  0.634286  0.468571  0.531429  0.582857  0.488571  0.520000  0.728571
P76128  0.581609  0.308046  0.404598  0.535632  0.425287  0.508046  0.859770
P31448  0.655462  0.364706  0.492437  0.571429  0.549580  0.504202  0.852101

[215 rows x 400 columns]
```

[ ]:

```

```

[ ]:

```
import matplotlib.pyplot as plt
linkage = get_linkage(feature_pssm_keywords)
dendrogram = dendrogram_plot(linkage)
plt.show()
labels = get_cluster_labels(linkage=linkage, n_clusters=2,␣
  ↪index=feature_pssm_keywords.index.tolist())
display(labels.value_counts())
records_not_go_dict = {k:v for k,v in records_all_dict.items() if "GO" not in k}
records_not_go_dict["Keywords substrates"] = keywords_ecoli_substrate.
  ↪to_records(index=False).tolist()
reference_set = set(sequences_substrate_keywords.index.tolist())
cluster_enrichment_analysis(labels, reference_set,␣
  ↪annotations_dict=records_not_go_dict,p_cutoff=0.05)
```



```
2    121
1     94
dtype: int64

============================================================
CLUSTER 1
============================================================
Keywords
            annotation  total_reference  annotated_reference  total_subset  \
2  Cell inner membrane              215                  181            94
3        Cell membrane              215                  181            94
0  Transmembrane helix              215                  157            94
1        Transmembrane              215                  175            94
4              Symport              215                   31            94

   annotated_subset   expected  percentage_of_annotated       lfc  \
2                90  79.134884                    49.72  0.185611
3                90  79.134884                    49.72  0.185611
0                89  68.641860                    56.69  0.374717
1                89  76.511628                    50.86  0.218126
4                21  13.553488                    67.74  0.631725
```

```
              p         p_fdr  p_bonferroni
2  1.818258e-05  3.688467e-04  1.290964e-03
3  1.818258e-05  3.688467e-04  1.290964e-03
0  2.674631e-11  1.898988e-09  1.898988e-09
1  4.026345e-06  1.429352e-04  2.858705e-04
4  3.303760e-03  4.691339e-02  2.345669e-01

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        2.A              215                   83            94

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                59  36.288372                    71.08  0.701208

              p         p_fdr  p_bonferroni
0  1.139016e-10  4.556065e-10  4.556065e-10

TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          2              215                   83            94

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                59  36.288372                    71.08  0.701208

              p         p_fdr  p_bonferroni
0  1.139016e-10  4.556065e-10  4.556065e-10

Interpro Domains

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

Keywords substrates

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

============================================================
CLUSTER 2
============================================================
Keywords

                  annotation  total_reference  annotated_reference  \
6               3D-structure              215                   78
```

```
0  Direct protein sequencing              215                   44
1                      Signal              215                   29
5          Nucleotide-binding              215                   30
4                 ATP-binding              215                   28
8                 Translocase              215                   23
2         Cell outer membrane              215                   18
3  Transmembrane beta strand              215                   18
7                       Porin              215                   12

   total_subset  annotated_subset   expected  percentage_of_annotated  \
6           121                56  43.897674                    71.79
0           121                40  24.762791                    90.91
1           121                28  16.320930                    96.55
5           121                26  16.883721                    86.67
4           121                25  15.758140                    89.29
8           121                20  12.944186                    86.96
2           121                18  10.130233                   100.00
3           121                18  10.130233                   100.00
7           121                12   6.753488                   100.00

        lfc             p     p_fdr  p_bonferroni
6  0.351282  3.981792e-04  0.005119      0.035836
0  0.691826  4.054122e-08  0.000004      0.000004
1  0.778704  3.260382e-07  0.000015      0.000029
5  0.622879  1.637954e-04  0.002457      0.014742
4  0.665831  7.510187e-05  0.001352      0.006759
8  0.627696  1.097769e-03  0.010978      0.098799
2  0.829330  1.763293e-05  0.000453      0.001587
3  0.829330  1.763293e-05  0.000453      0.001587
7  0.829330  7.855134e-04  0.008837      0.070696
```

TCDB Class

```
  annotation  total_reference  annotated_reference  total_subset  \
1        3.A              215                   64           121
0        1.B              215                   18           121

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
1                45  36.018605                    70.31  0.321183  0.005004
0                18  10.130233                   100.00  0.829330  0.000018

      p_fdr  p_bonferroni
1  0.017514      0.035028
0  0.000123      0.000123
```

TCDB Mechanism

```
  annotation  total_reference  annotated_reference  total_subset  \
1          3              215                   68           121
0          1              215                   26           121
```

```
   annotated_subset   expected  percentage_of_annotated       lfc         p  \
1                49  38.269767                    72.06  0.356577  0.001101
0                23  14.632558                    88.46  0.652452  0.000226

      p_fdr  p_bonferroni
1  0.002753      0.005507
0  0.001129      0.001129
```

```
Interpro Domains
```

```
                                          annotation  total_reference  \
0  P-loop containing nucleoside triphosphate hydr…              215
1                                 AAA+ ATPase domain              215
2           ABC transporter-like, ATP-binding domain              215
3               ABC transporter-like, conserved site              215

   annotated_reference  total_subset  annotated_subset   expected  \
0                   25           121                23  14.069767
1                   23           121                21  12.944186
2                   22           121                20  12.381395
3                   20           121                18  11.255814

   percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
0                    92.00  0.709035  0.000051  0.014001      0.014001
1                    91.30  0.698085  0.000162  0.022085      0.044171
2                    90.91  0.691826  0.000284  0.025833      0.077499
3                    90.00  0.677327  0.000852  0.046505      0.232527
```

```
Keywords substrates
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
[ ]: reference_set = set(sequences_substrate_keywords.index.tolist())
     cluster_enrichment_analysis(labels, reference_set,␣
      ↪annotations_dict=records_not_go_dict,p_cutoff=0.05)
```

```
============================================================
CLUSTER 1
============================================================
Keywords
```

```
            annotation  total_reference  annotated_reference  total_subset  \
2  Cell inner membrane              215                  181            94
3        Cell membrane              215                  181            94
0  Transmembrane helix              215                  157            94
```

```
1        Transmembrane              215                  175            94
4              Symport              215                   31            94

   annotated_subset   expected  percentage_of_annotated       lfc  \
2                90  79.134884                    49.72  0.185611
3                90  79.134884                    49.72  0.185611
0                89  68.641860                    56.69  0.374717
1                89  76.511628                    50.86  0.218126
4                21  13.553488                    67.74  0.631725

              p         p_fdr  p_bonferroni
2  1.818258e-05  3.688467e-04  1.290964e-03
3  1.818258e-05  3.688467e-04  1.290964e-03
0  2.674631e-11  1.898988e-09  1.898988e-09
1  4.026345e-06  1.429352e-04  2.858705e-04
4  3.303760e-03  4.691339e-02  2.345669e-01

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        2.A              215                   83            94

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                59  36.288372                    71.08  0.701208

              p         p_fdr  p_bonferroni
0  1.139016e-10  4.556065e-10  4.556065e-10

TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          2              215                   83            94

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                59  36.288372                    71.08  0.701208

              p         p_fdr  p_bonferroni
0  1.139016e-10  4.556065e-10  4.556065e-10

Interpro Domains

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

Keywords substrates

Empty DataFrame
```

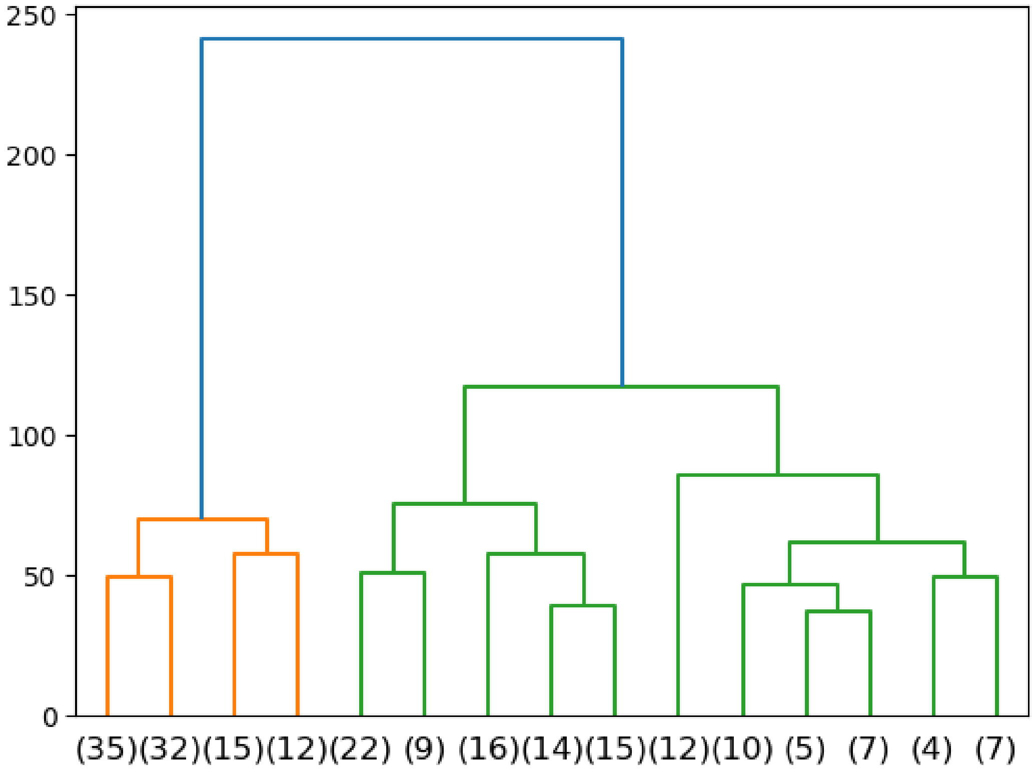
```
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

============================================================
CLUSTER 2
============================================================
Keywords

                  annotation  total_reference  annotated_reference  \
6               3D-structure              215                   78
0   Direct protein sequencing              215                   44
1                     Signal              215                   29
5          Nucleotide-binding              215                   30
4                ATP-binding              215                   28
8                 Translocase              215                   23
2         Cell outer membrane              215                   18
3   Transmembrane beta strand              215                   18
7                      Porin              215                   12

   total_subset  annotated_subset   expected  percentage_of_annotated  \
6           121                56  43.897674                    71.79
0           121                40  24.762791                    90.91
1           121                28  16.320930                    96.55
5           121                26  16.883721                    86.67
4           121                25  15.758140                    89.29
8           121                20  12.944186                    86.96
2           121                18  10.130233                   100.00
3           121                18  10.130233                   100.00
7           121                12   6.753488                   100.00

        lfc             p     p_fdr  p_bonferroni
6  0.351282  3.981792e-04  0.005119      0.035836
0  0.691826  4.054122e-08  0.000004      0.000004
1  0.778704  3.260382e-07  0.000015      0.000029
5  0.622879  1.637954e-04  0.002457      0.014742
4  0.665831  7.510187e-05  0.001352      0.006759
8  0.627696  1.097769e-03  0.010978      0.098799
2  0.829330  1.763293e-05  0.000453      0.001587
3  0.829330  1.763293e-05  0.000453      0.001587
7  0.829330  7.855134e-04  0.008837      0.070696

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
1        3.A              215                   64           121
0        1.B              215                   18           121
```

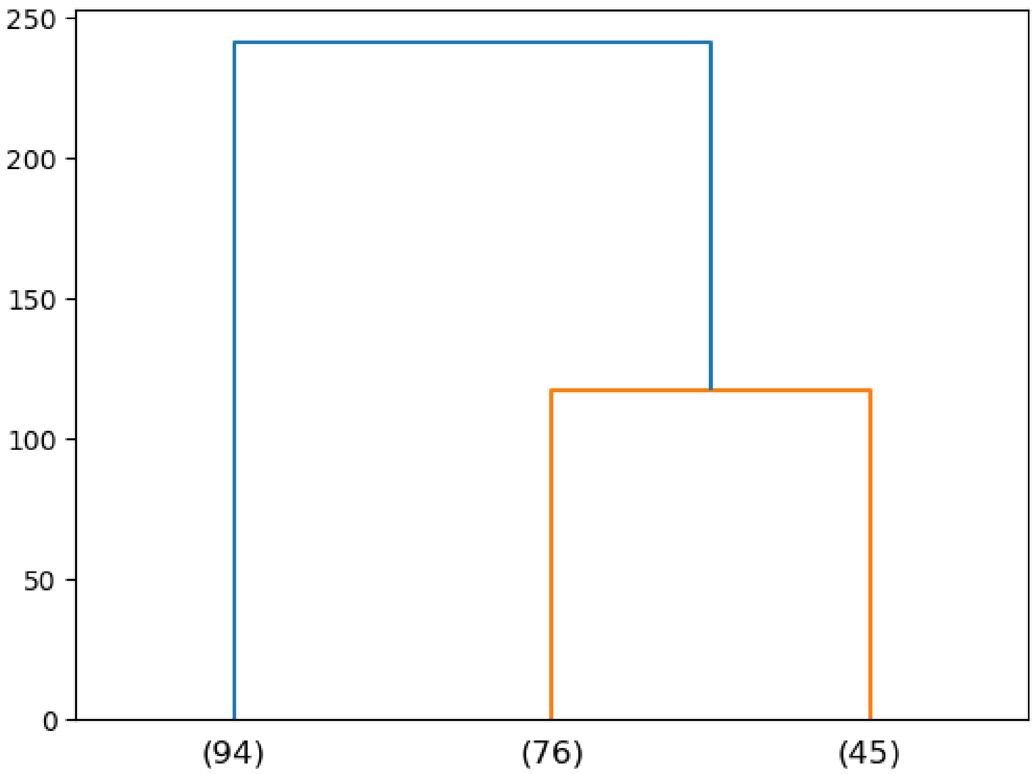
```
   annotated_subset   expected  percentage_of_annotated       lfc         p  \
1                45  36.018605                    70.31  0.321183  0.005004
0                18  10.130233                   100.00  0.829330  0.000018

      p_fdr  p_bonferroni
1  0.017514      0.035028
0  0.000123      0.000123

TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
1          3              215                   68           121
0          1              215                   26           121

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
1                49  38.269767                    72.06  0.356577  0.001101
0                23  14.632558                    88.46  0.652452  0.000226

      p_fdr  p_bonferroni
1  0.002753      0.005507
0  0.001129      0.001129

Interpro Domains

                                           annotation  total_reference  \
0  P-loop containing nucleoside triphosphate hydr…               215
1                                  AAA+ ATPase domain              215
2             ABC transporter-like, ATP-binding domain              215
3                 ABC transporter-like, conserved site              215

   annotated_reference  total_subset  annotated_subset   expected  \
0                   25           121                23  14.069767
1                   23           121                21  12.944186
2                   22           121                20  12.381395
3                   20           121                18  11.255814

   percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
0                    92.00  0.709035  0.000051  0.014001      0.014001
1                    91.30  0.698085  0.000162  0.022085      0.044171
2                    90.91  0.691826  0.000284  0.025833      0.077499
3                    90.00  0.677327  0.000852  0.046505      0.232527

Keywords substrates

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset, annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr, p_bonferroni]
Index: []
```

1.6.1 With 3 clusters

Now, we get different enriched TCDB classes for each cluster: 2A 3A an 1B.

[ ]:
```
import matplotlib.pyplot as plt
n_clusters = 3
linkage = get_linkage(feature_pssm_keywords)
dendrogram = dendrogram_plot(linkage)
plt.show()
dendrogram = dendrogram_plot(linkage,max_leaves=n_clusters)
plt.show()
labels = get_cluster_labels(linkage=linkage, n_clusters=3,␣
 ↪index=feature_pssm_keywords.index.tolist())
display(labels.value_counts())
records_not_go_dict = {k:v for k,v in records_all_dict.items() if "GO" not in k}
records_not_go_dict["Keywords substrates"] = keywords_ecoli_substrate.
 ↪to_records(index=False).tolist()
reference_set = set(sequences_substrate_keywords.index.tolist())
cluster_enrichment_analysis(labels, reference_set,␣
 ↪annotations_dict=records_not_go_dict,p_cutoff=0.05)
```





```
1    94
2    76
3    45
dtype: int64

============================================================
CLUSTER 1
============================================================
Keywords

            annotation  total_reference  annotated_reference  total_subset  \
2  Cell inner membrane              215                  181            94
3        Cell membrane              215                  181            94
0  Transmembrane helix              215                  157            94
1        Transmembrane              215                  175            94
4              Symport              215                   31            94

   annotated_subset   expected  percentage_of_annotated       lfc  \
2                90  79.134884                    49.72  0.185611
3                90  79.134884                    49.72  0.185611
0                89  68.641860                    56.69  0.374717
1                89  76.511628                    50.86  0.218126
4                21  13.553488                    67.74  0.631725
```

```
              p         p_fdr  p_bonferroni
2  1.818258e-05  3.688467e-04  1.290964e-03
3  1.818258e-05  3.688467e-04  1.290964e-03
0  2.674631e-11  1.898988e-09  1.898988e-09
1  4.026345e-06  1.429352e-04  2.858705e-04
4  3.303760e-03  4.691339e-02  2.345669e-01

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        2.A              215                   83            94

   annotated_subset   expected  percentage_of_annotated       lfc  \
0               59  36.288372                    71.08  0.701208

              p         p_fdr  p_bonferroni
0  1.139016e-10  4.556065e-10  4.556065e-10

TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          2              215                   83            94

   annotated_subset   expected  percentage_of_annotated       lfc  \
0               59  36.288372                    71.08  0.701208

              p         p_fdr  p_bonferroni
0  1.139016e-10  4.556065e-10  4.556065e-10

Interpro Domains

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

Keywords substrates

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

============================================================
CLUSTER 2
============================================================
Keywords

           annotation  total_reference  annotated_reference  total_subset  \
0  Nucleotide-binding              215                   30            76
1         ATP-binding              215                   28            76

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                 19  10.604651                    63.33  0.841302  0.000734
1                 18   9.897674                    64.29  0.862835  0.000811

      p_fdr  p_bonferroni
0  0.048425      0.048425
1  0.026760      0.053519

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        3.A              215                   64            76

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                 33  22.623256                    51.56  0.544659  0.001142

     p_fdr  p_bonferroni
0  0.00685       0.00685

TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          3              215                   68            76

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                 34  24.037209                     50.0  0.500265  0.001998

      p_fdr  p_bonferroni
0  0.009989      0.009989

Interpro Domains

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

Keywords substrates

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

============================================================
CLUSTER 3
```

```
============================================================
Keywords

                  annotation  total_reference  annotated_reference  \
8               3D-structure              215                   78
0                     Signal              215                   29
4  Direct protein sequencing              215                   44
1        Cell outer membrane              215                   18
2  Transmembrane beta strand              215                   18
3                      Porin              215                   12
5                   Receptor              215                    6
6                   TonB box              215                    5
7             Disulfide bond              215                    5
9                  Periplasm              215                    6

   total_subset  annotated_subset   expected  percentage_of_annotated  \
8            45                26  16.325581                    33.33
0            45                25   6.069767                    86.21
4            45                24   9.209302                    54.55
1            45                18   3.767442                   100.00
2            45                18   3.767442                   100.00
3            45                12   2.511628                   100.00
5            45                 6   1.255814                   100.00
6            45                 5   1.046512                   100.00
7            45                 5   1.046512                   100.00
9            45                 5   1.255814                    83.33

        lfc             p         p_fdr  p_bonferroni
8  0.671377  8.122045e-04  5.775676e-03  5.198109e-02
0  2.042215  1.584897e-16  1.014334e-14  1.014334e-14
4  1.381871  1.573314e-08  2.013842e-07  1.006921e-06
1  2.256340  2.369936e-14  6.067037e-13  1.516759e-12
2  2.256340  2.369936e-14  6.067037e-13  1.516759e-12
3  2.256340  1.930303e-09  3.088485e-08  1.235394e-07
5  2.256340  6.370263e-05  6.794947e-04  4.076968e-03
6  2.256340  3.344388e-04  2.853878e-03  2.140408e-02
7  2.256340  3.344388e-04  2.853878e-03  2.140408e-02
9  1.993305  1.688120e-03  1.080397e-02  1.080397e-01
TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        1.B              215                   18            45

   annotated_subset  expected  percentage_of_annotated      lfc             p  \
0                18  3.767442                    100.0  2.25634  2.369936e-14

          p_fdr  p_bonferroni
0  1.421962e-13  1.421962e-13
```

TCDB Mechanism

```
  annotation  total_reference  annotated_reference  total_subset  \
0          1              215                   26            45   

   annotated_subset  expected  percentage_of_annotated       lfc  \
0                19   5.44186                    73.08  1.803828   

              p         p_fdr  p_bonferroni  
0  8.208449e-10  3.283379e-09  3.283379e-09  
```

Interpro Domains

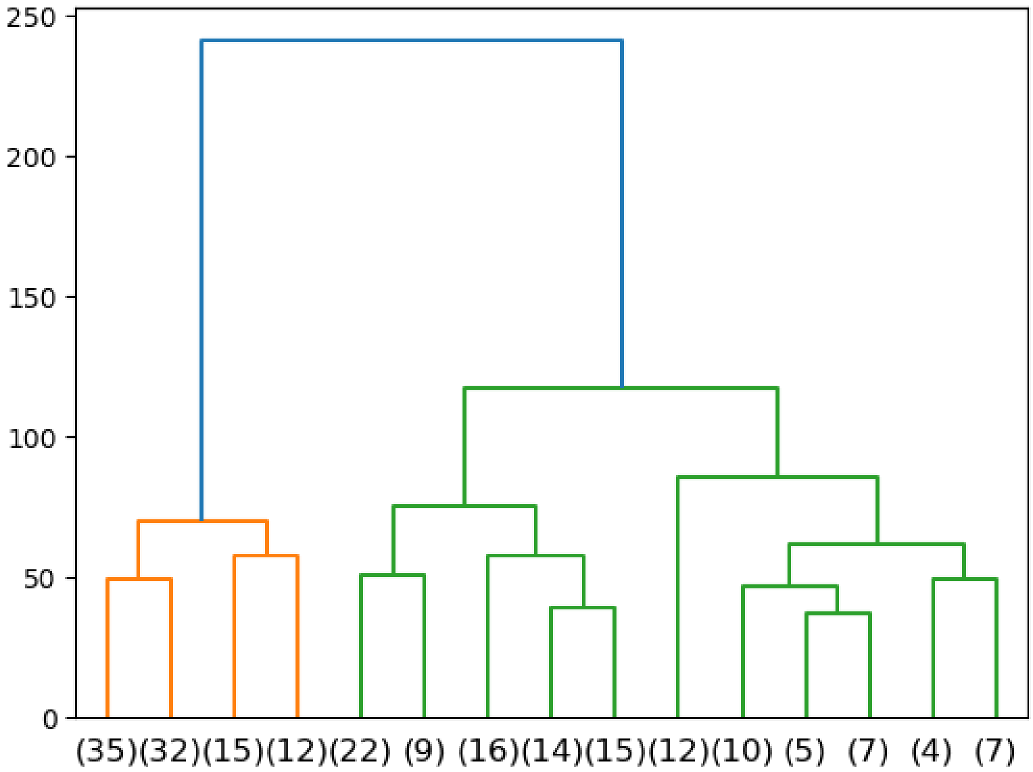
```
                                           annotation  total_reference  \
0                TonB-dependent receptor, plug domain              215   
1    TonB-dependent receptor, plug domain superfamily              215   
2           TonB-dependent receptor-like, beta-barrel              215   
3                            TonB box, conserved site              215   
4   TonB-dependent receptor-like, beta-barrel doma…              215   
5             TonB-dependent receptor, conserved site              215   
6                 TonB-dependent siderophore receptor              215   
7                            Porin domain superfamily              215   
8           Peptide/nickel binding protein, MppA-type              215   
9                     Solute-binding protein family 5              215   
10    Solute-binding protein family 5, conserved site              215   
11             Solute-binding protein family 5 domain              215   

    annotated_reference  total_subset  annotated_subset  expected  \
0                     5            45                 5  1.046512   
1                     5            45                 5  1.046512   
2                     5            45                 5  1.046512   
3                     5            45                 5  1.046512   
4                     5            45                 5  1.046512   
5                     4            45                 4  0.837209   
6                     4            45                 4  0.837209   
7                     4            45                 4  0.837209   
8                     4            45                 4  0.837209   
9                     4            45                 4  0.837209   
10                    4            45                 4  0.837209   
11                    4            45                 4  0.837209   

    percentage_of_annotated      lfc         p     p_fdr  p_bonferroni  
0                     100.0  2.25634  0.000334  0.013378      0.040133  
1                     100.0  2.25634  0.000334  0.013378      0.040133  
2                     100.0  2.25634  0.000334  0.013378      0.040133  
3                     100.0  2.25634  0.000334  0.013378      0.040133  
4                     100.0  2.25634  0.000334  0.013378      0.040133  
5                     100.0  2.25634  0.001721  0.022948      0.206536  
6                     100.0  2.25634  0.001721  0.022948      0.206536  
```

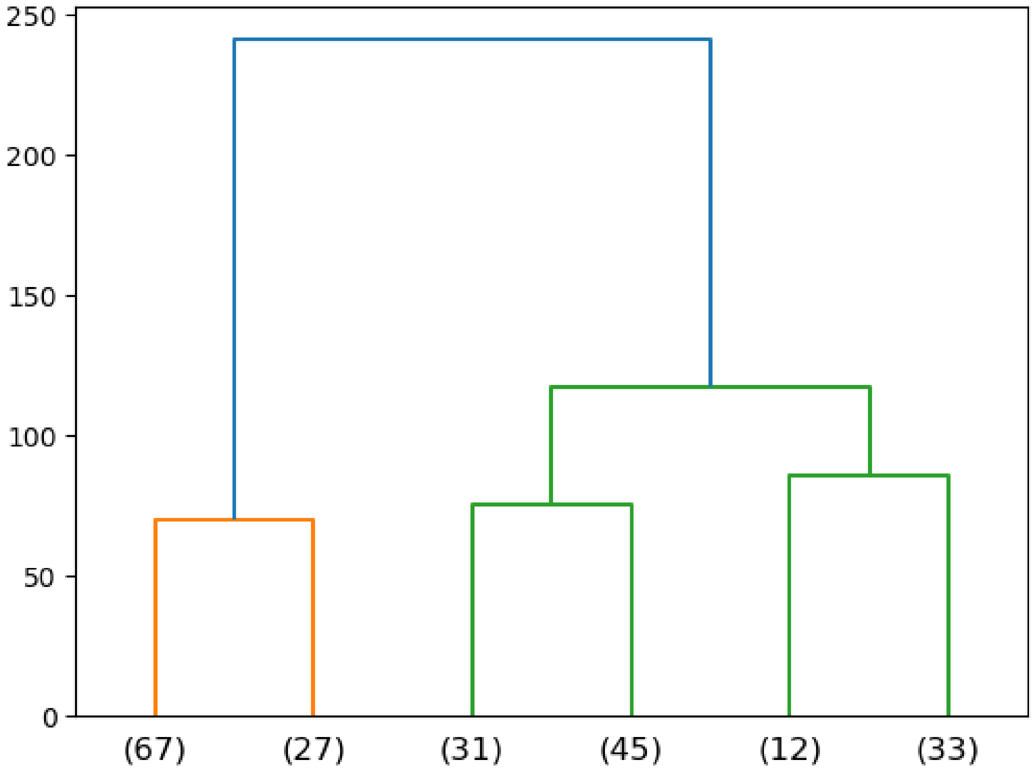
```
7                     100.0  2.25634  0.001721  0.022948      0.206536
8                     100.0  2.25634  0.001721  0.022948      0.206536
9                     100.0  2.25634  0.001721  0.022948      0.206536
10                    100.0  2.25634  0.001721  0.022948      0.206536
11                    100.0  2.25634  0.001721  0.022948      0.206536

Keywords substrates

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

1.6.2 With 6 clusters

The dendrogram looks like we can split the clusters again:

[ ]:
```python
import matplotlib.pyplot as plt
n_clusters = 6
linkage = get_linkage(feature_pssm_keywords)
dendrogram = dendrogram_plot(linkage)
plt.show()
dendrogram = dendrogram_plot(linkage,max_leaves=n_clusters)
plt.show()
labels = get_cluster_labels(linkage=linkage, n_clusters=n_clusters,␣
 ↪index=feature_pssm_keywords.index.tolist())
display(labels.value_counts())
records_not_go_dict = {k:v for k,v in records_all_dict.items() if "GO" not in k}
records_not_go_dict["Keywords substrates"] = keywords_ecoli_substrate.
 ↪to_records(index=False).tolist()
reference_set = set(sequences_substrate_keywords.index.tolist())
cluster_enrichment_analysis(labels, reference_set,␣
 ↪annotations_dict=records_not_go_dict,p_cutoff=0.05)
```


250
200
150
100
50
0
(35)(32)(15)(12)(22) (9) (16)(14)(15)(12)(10) (5) (7) (4) (7)


250
200
150
100
50
0
(67) (27) (31) (45) (12) (33)

```
1    67
4    45
6    33
3    31
2    27
5    12
dtype: int64
============================================================
CLUSTER 1
============================================================
Keywords
            annotation  total_reference  annotated_reference  total_subset  \
0  Transmembrane helix              215                  157            67
1        Transmembrane              215                  175            67
2  Cell inner membrane              215                  181            67
3        Cell membrane              215                  181            67
4             Membrane              215                  199            67
5              Symport              215                   31            67

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                67  48.925581                    42.68  0.453572
1                67  54.534884                    38.29  0.296982
2                67  56.404651                    37.02  0.248347
3                67  56.404651                    37.02  0.248347
4                67  62.013953                    33.67  0.111568
5                17   9.660465                    54.84  0.815370

              p         p_fdr  p_bonferroni
0  4.350402e-12  2.479729e-10  2.479729e-10
1  4.677616e-08  1.333121e-06  2.666241e-06
2  7.877550e-07  1.282915e-05  4.490204e-05
3  7.877550e-07  1.282915e-05  4.490204e-05
4  1.943137e-03  2.215176e-02  1.107588e-01
5  2.698185e-03  2.563275e-02  1.537965e-01
TCDB Class
  annotation  total_reference  annotated_reference  total_subset  \
0        2.A              215                   83            67

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                47  25.865116                    56.63  0.861653

              p         p_fdr  p_bonferroni
0  2.207186e-10  8.828743e-10  8.828743e-10
```

```
TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          2              215                   83            67

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                47  25.865116                    56.63  0.861653

              p         p_fdr  p_bonferroni
0  2.207186e-10  8.828743e-10  8.828743e-10

Interpro Domains

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

Keywords substrates

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
============================================================
CLUSTER 2
============================================================
Keywords

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

TCDB Class

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

TCDB Mechanism

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
Interpro Domains

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

Keywords substrates

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

============================================================
CLUSTER 3
============================================================
Keywords

           annotation  total_reference  annotated_reference  total_subset  \
0         ATP-binding              215                   28            31
1  Nucleotide-binding              215                   30            31
2         Translocase              215                   23            31

   annotated_subset  expected  percentage_of_annotated       lfc  \
0                17  4.037209                    60.71  2.074104
1                17  4.325581                    56.67  1.974569
2                13  3.316279                    56.52  1.970874

              p         p_fdr  p_bonferroni
0  3.971159e-10  2.104714e-08  2.104714e-08
1  1.913646e-09  5.071162e-08  1.014232e-07
2  4.203403e-07  7.426013e-06  2.227804e-05

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        3.A              215                   64            31

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                19  9.227907                    29.69  1.041924  0.000079

      p_fdr  p_bonferroni
0  0.000317      0.000317

TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          3              215                   68            31
```

```
   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                19  9.804651                    27.94  0.954461  0.000224

      p_fdr  p_bonferroni
0  0.000896      0.000896

Interpro Domains

                                        annotation  total_reference  \
0         ABC transporter-like, ATP-binding domain              215
1                               AAA+ ATPase domain              215
2  P-loop containing nucleoside triphosphate hydr…              215
3             ABC transporter-like, conserved site              215
4  Oligopeptide/dipeptide ABC transporter, C-term…              215

   annotated_reference  total_subset  annotated_subset  expected  \
0                   22            31                15  3.172093
1                   23            31                15  3.316279
2                   25            31                15  3.604651
3                   20            31                13  2.883721
4                    3            31                 3  0.432558

   percentage_of_annotated       lfc             p         p_fdr  p_bonferroni
0                    68.18  2.241456  6.552396e-10  5.372965e-08  5.372965e-08
1                    65.22  2.177325  1.738092e-09  7.126177e-08  1.425235e-07
2                    60.00  2.057031  9.852818e-09  2.693103e-07  8.079310e-07
3                    65.00  2.172508  3.724617e-08  7.635465e-07  3.054186e-06
4                   100.00  2.793997  2.752004e-03  4.513287e-02  2.256644e-01

Keywords substrates

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

============================================================
CLUSTER 4
============================================================
Keywords

            annotation  total_reference  annotated_reference  total_subset  \
0  Transmembrane helix              215                  157            45
1        Transmembrane              215                  175            45
2  Cell inner membrane              215                  181            45
3        Cell membrane              215                  181            45

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                45  32.860465                    28.66  0.453572
```

```
1                45  36.627907                    25.71  0.296982
2                45  37.883721                    24.86  0.248347
3                45  37.883721                    24.86  0.248347

              p     p_fdr  p_bonferroni
0  9.293357e-08  0.000005      0.000005
1  2.720170e-05  0.000666      0.001333
2  1.554647e-04  0.002177      0.007618
3  1.554647e-04  0.002177      0.007618
```

```
TCDB Class
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
TCDB Mechanism
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
Interpro Domains
```

```
                        annotation  total_reference  annotated_reference  \
0  Phosphotransferase system, EIIC              215                   12
1        ABC transporter, permease              215                    5

   total_subset  annotated_subset  expected  percentage_of_annotated  \
0            45                 9  2.511628                     75.0
1            45                 5  1.046512                    100.0

        lfc         p     p_fdr  p_bonferroni
0  1.841302  0.000051  0.005308      0.005308
1  2.256340  0.000334  0.017391      0.034782
```

```
Keywords substrates
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
============================================================
CLUSTER 5
============================================================
Keywords
```

```
           annotation  total_reference  annotated_reference  total_subset  \
0  Electron transport              215                    5            12
1   Respiratory chain              215                    2            12

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 3  0.279070                     60.0  3.426265  0.001262
1                 2  0.111628                    100.0  4.163230  0.002869

      p_fdr  p_bonferroni
0  0.042923      0.042923
1  0.048772      0.097544

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        3.D              215                    4            12

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 3  0.223256                     75.0  3.748193  0.000522

      p_fdr  p_bonferroni
0  0.002086      0.002086

TCDB Mechanism

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

Interpro Domains

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

Keywords substrates

           annotation  total_reference  annotated_reference  total_subset  \
0  Electron transport              215                    5            12

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 3   0.27907                     60.0  3.426265  0.001262

      p_fdr  p_bonferroni
0  0.008837      0.008837

============================================================
CLUSTER 6
```

```
============================================================
Keywords
                 annotation  total_reference  annotated_reference  \
0                     Signal              215                   29
4  Direct protein sequencing              215                   44
9               3D-structure              215                   78
1        Cell outer membrane              215                   18
2  Transmembrane beta strand              215                   18
3                      Porin              215                   12
5                   Receptor              215                    6
6                   TonB box              215                    5
7             Disulfide bond              215                    5
8                  Periplasm              215                    6

   total_subset  annotated_subset   expected  percentage_of_annotated  \
0            33                23   4.451163                    79.31
4            33                21   6.753488                    47.73
9            33                20  11.972093                    25.64
1            33                18   2.762791                   100.00
2            33                18   2.762791                   100.00
3            33                12   1.841860                   100.00
5            33                 6   0.920930                   100.00
6            33                 5   0.767442                   100.00
7            33                 5   0.767442                   100.00
8            33                 5   0.920930                    83.33

        lfc             p         p_fdr  p_bonferroni
0  2.369380  6.372331e-18  3.058719e-16  3.058719e-16
4  1.636685  2.375738e-09  2.280708e-08  1.140354e-07
9  0.740325  1.804311e-03  8.660694e-03  8.660694e-02
1  2.703799  1.432497e-17  2.750394e-16  6.875985e-16
2  2.703799  1.432497e-17  2.750394e-16  6.875985e-16
3  2.703799  2.381448e-11  2.857738e-10  1.143095e-09
5  2.703799  8.662305e-06  6.929844e-05  4.157906e-04
6  2.703799  6.496728e-05  4.157906e-04  3.118430e-03
7  2.703799  6.496728e-05  4.157906e-04  3.118430e-03
8  2.440764  3.464922e-04  1.847958e-03  1.663162e-02
TCDB Class
  annotation  total_reference  annotated_reference  total_subset  \
0        1.B              215                   18            33

   annotated_subset  expected  percentage_of_annotated       lfc  \
0                18  2.762791                    100.0  2.703799

              p         p_fdr  p_bonferroni
0  1.432497e-17  4.297491e-17  4.297491e-17
```

```
TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          1              215                   26            33

   annotated_subset  expected  percentage_of_annotated       lfc  \
0                18  3.990698                    69.23  2.173284

              p         p_fdr  p_bonferroni
0  1.217010e-11  3.651029e-11  3.651029e-11

Interpro Domains

                                           annotation  total_reference  \
0                TonB-dependent receptor, plug domain              215
1    TonB-dependent receptor, plug domain superfamily              215
2           TonB-dependent receptor-like, beta-barrel              215
3                            TonB box, conserved site              215
4   TonB-dependent receptor-like, beta-barrel doma…              215
5             TonB-dependent receptor, conserved site              215
6                 TonB-dependent siderophore receptor              215
7                            Porin domain superfamily              215
8           Peptide/nickel binding protein, MppA-type              215
9                     Solute-binding protein family 5              215
10    Solute-binding protein family 5, conserved site              215
11             Solute-binding protein family 5 domain              215
12                        Porin, gammaproteobacterial              215
13                          Porin, Gram-negative type              215
14                       TonB-dependent receptor-like              215

    annotated_reference  total_subset  annotated_subset  expected  \
0                     5            33                 5  0.767442
1                     5            33                 5  0.767442
2                     5            33                 5  0.767442
3                     5            33                 5  0.767442
4                     5            33                 5  0.767442
5                     4            33                 4  0.613953
6                     4            33                 4  0.613953
7                     4            33                 4  0.613953
8                     4            33                 4  0.613953
9                     4            33                 4  0.613953
10                    4            33                 4  0.613953
11                    4            33                 4  0.613953
12                    3            33                 3  0.460465
13                    3            33                 3  0.460465
14                    3            33                 3  0.460465

    percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
0                     100.0  2.703799  0.000065  0.001603      0.004808
```

```
1                     100.0  2.703799  0.000065  0.001603      0.004808
2                     100.0  2.703799  0.000065  0.001603      0.004808
3                     100.0  2.703799  0.000065  0.001603      0.004808
4                     100.0  2.703799  0.000065  0.001603      0.004808
5                     100.0  2.703799  0.000473  0.003887      0.034979
6                     100.0  2.703799  0.000473  0.003887      0.034979
7                     100.0  2.703799  0.000473  0.003887      0.034979
8                     100.0  2.703799  0.000473  0.003887      0.034979
9                     100.0  2.703799  0.000473  0.003887      0.034979
10                    100.0  2.703799  0.000473  0.003887      0.034979
11                    100.0  2.703799  0.000473  0.003887      0.034979
12                    100.0  2.703799  0.003340  0.017656      0.247187
13                    100.0  2.703799  0.003340  0.017656      0.247187
14                    100.0  2.703799  0.003340  0.017656      0.247187
```

```
Keywords substrates
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

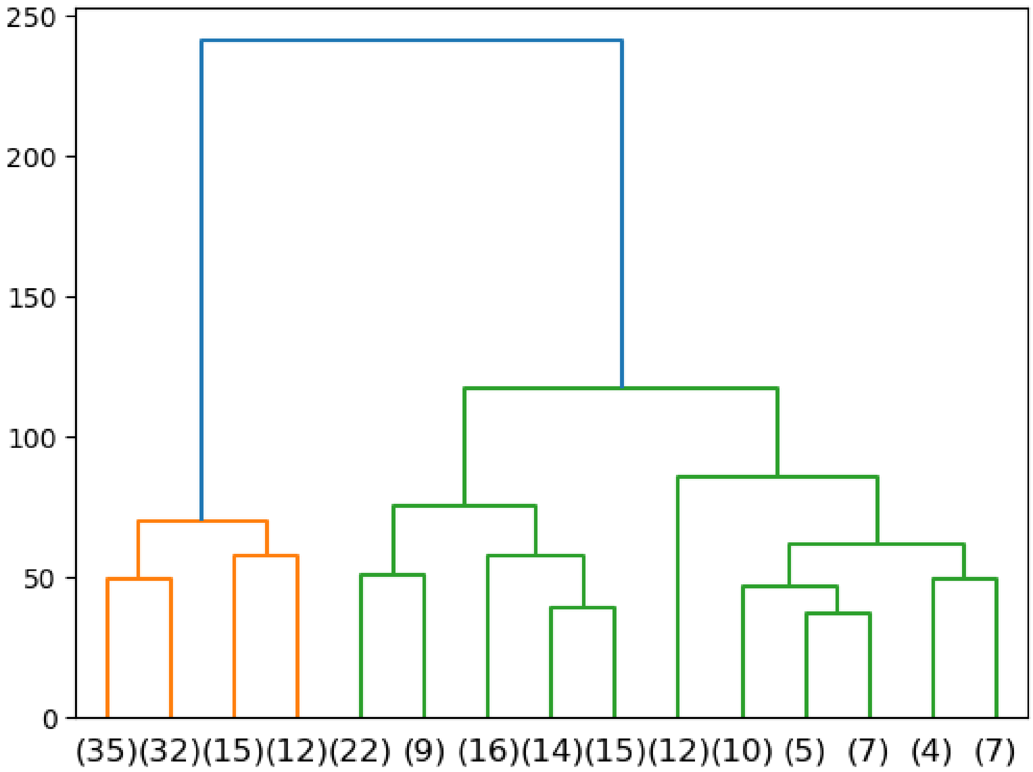
1.6.3 Substrate distribution

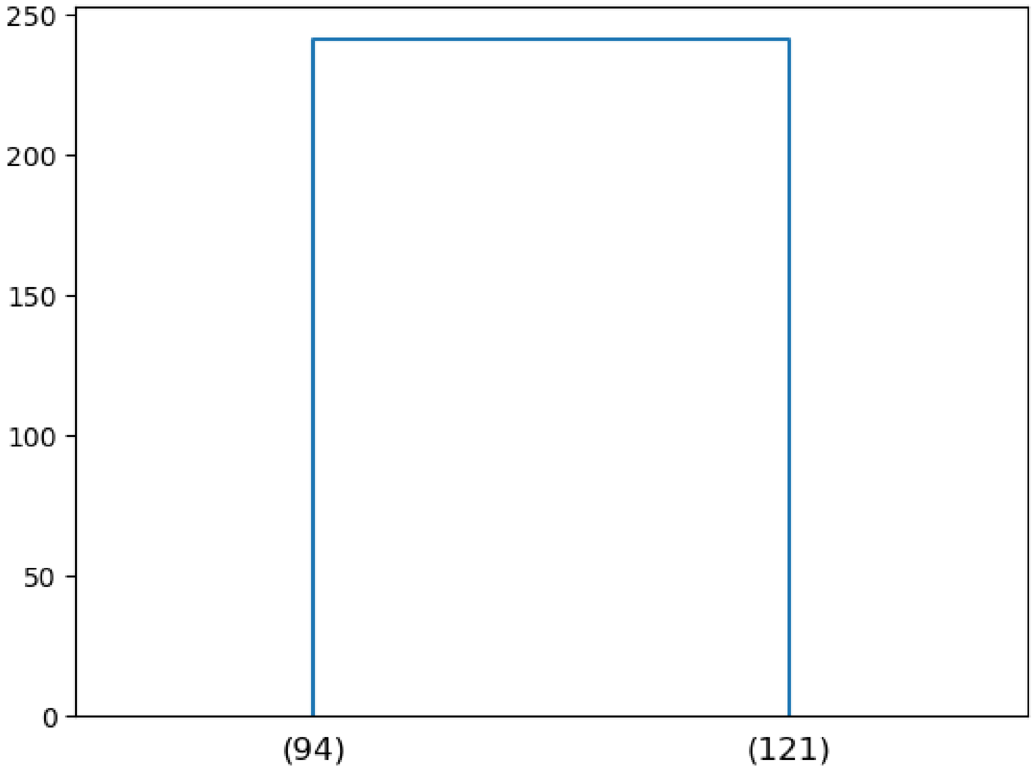
What is the distribution of substrates in two clusters, and in 6 clusters?

Two clusters

[ ]:

```
import matplotlib.pyplot as plt
n_clusters = 2
linkage = get_linkage(feature_pssm_keywords)
dendrogram = dendrogram_plot(linkage)
plt.show()
dendrogram = dendrogram_plot(linkage,max_leaves=n_clusters)
plt.show()
labels = get_cluster_labels(linkage=linkage, n_clusters=n_clusters,␣
 ↪index=feature_pssm_keywords.index.tolist())
display(labels.value_counts())
records_not_go_dict = dict()
records_not_go_dict["Keywords substrates"] = keywords_ecoli_substrate.
 ↪to_records(index=False).tolist()
reference_set = set(sequences_substrate_keywords.index.tolist())
cluster_enrichment_analysis(labels, reference_set,␣
 ↪annotations_dict=records_not_go_dict,p_cutoff=1)
```


250
200
150
100
50
0
(35)
(32)
(15)
(12)
(22)
(9)
(16)
(14)
(15)
(12)
(10)
(5)
(7)
(4)
(7)


250
200
150
100
50
0
(94)
(121)

```
2    121
1     94
dtype: int64

============================================================
CLUSTER 1
============================================================
Keywords substrates

                annotation  total_reference  annotated_reference  \
5            Ion transport              215                   81
1     Amino-acid transport              215                   48
15         Sugar transport              215                   56
12       Protein transport              215                   27
4        Peptide transport              215                   19
6           Iron transport              215                   16
0         Sodium transport              215                    8
2           Zinc transport              215                    7
9      Potassium transport              215                   10
3      Phosphate transport              215                    4
14  Hydrogen ion transport              215                   10
7         Nickel transport              215                    3
13        Copper transport              215                    3
8        Sulfate transport              215                    1
11                Chloride              215                    2
10        Cobalt transport              215                    2
16           Translocation              215                    8

    total_subset  annotated_subset   expected  percentage_of_annotated  \
5             94                37  35.413953                    45.68
1             94                27  20.986047                    56.25
15            94                19  24.483721                    33.93
12            94                11  11.804651                    40.74
4             94                10   8.306977                    52.63
6             94                 8   6.995349                    50.00
0             94                 7   3.497674                    87.50
2             94                 5   3.060465                    71.43
9             94                 5   4.372093                    50.00
3             94                 3   1.748837                    75.00
14            94                 3   4.372093                    30.00
7             94                 2   1.311628                    66.67
13            94                 1   1.311628                    33.33
8             94                 1   0.437209                   100.00
11            94                 1   0.874419                    50.00
10            94                 1   0.874419                    50.00
16            94                 1   3.497674                    12.50
```

```
          lfc         p     p_fdr  p_bonferroni
5    0.063207  0.378534  1.000000      1.000000
1    0.363529  0.034671  0.294706      0.589412
15  -0.365823  0.970412  1.000000      1.000000
12  -0.101852  0.703867  0.920442      1.000000
4    0.267605  0.280260  0.952885      1.000000
6    0.193604  0.392779  0.953892      1.000000
0    1.000959  0.013600  0.231195      0.231195
2    0.708177  0.132877  0.752969      1.000000
9    0.193604  0.461912  0.785250      1.000000
3    0.778566  0.222586  0.945993      1.000000
14  -0.543362  0.891102  1.000000      1.000000
7    0.608641  0.405873  0.862481      1.000000
13  -0.391359  0.823688  1.000000      1.000000
8    1.193604  0.437209  0.825840      1.000000
11   0.193604  0.684416  1.000000      1.000000
10   0.193604  0.684416  1.000000      1.000000
16  -1.806396  0.990935  0.990935      1.000000

============================================================
CLUSTER 2
============================================================
Keywords substrates

                  annotation  total_reference  annotated_reference  \
9              Ion transport              215                   81
1            Sugar transport              215                   56
18      Amino-acid transport              215                   48
5          Protein transport              215                   27
14         Peptide transport              215                   19
11            Iron transport              215                   16
2              Translocation              215                    8
3     Hydrogen ion transport              215                   10
0         Electron transport              215                    5
10       Potassium transport              215                   10
8           Copper transport              215                    3
17            Zinc transport              215                    7
4            Lipid transport              215                    2
7   Polysaccharide transport              215                    1
12                  Chloride              215                    2
13          Cobalt transport              215                    2
6          Ammonia transport              215                    1
15          Nickel transport              215                    3
16       Phosphate transport              215                    4
19          Sodium transport              215                    8

    total_subset  annotated_subset   expected  percentage_of_annotated  \
9            121                44  45.586047                    54.32
```

```
1            121                37  31.516279                    66.07
18           121                21  27.013953                    43.75
5            121                16  15.195349                    59.26
14           121                 9  10.693023                    47.37
11           121                 8   9.004651                    50.00
2            121                 7   4.502326                    87.50
3            121                 7   5.627907                    70.00
0            121                 5   2.813953                   100.00
10           121                 5   5.627907                    50.00
8            121                 2   1.688372                    66.67
17           121                 2   3.939535                    28.57
4            121                 2   1.125581                   100.00
7            121                 1   0.562791                   100.00
12           121                 1   1.125581                    50.00
13           121                 1   1.125581                    50.00
6            121                 1   0.562791                   100.00
15           121                 1   1.688372                    33.33
16           121                 1   2.251163                    25.00
19           121                 1   4.502326                    12.50
```

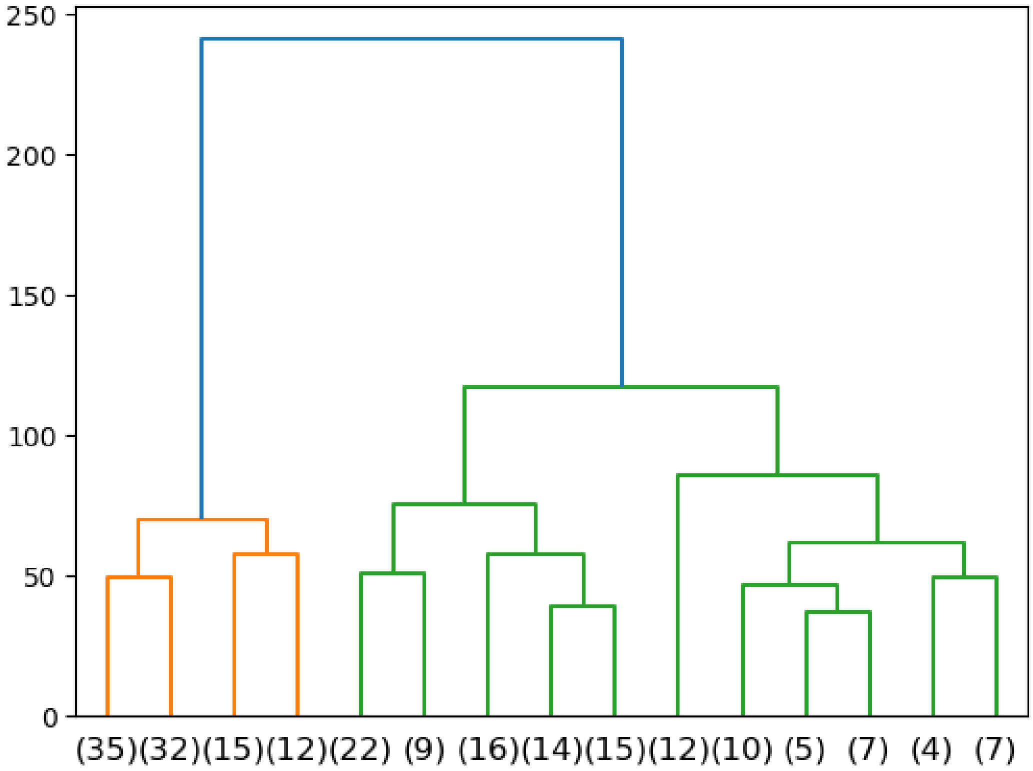
```
         lfc         p     p_fdr  p_bonferroni
9  -0.051089  0.723286  1.000000           1.0
1   0.231428  0.058461  0.584615           1.0
18 -0.363315  0.984031  1.000000           1.0
5   0.074442  0.452417  1.000000           1.0
14 -0.248673  0.855797  1.000000           1.0
11 -0.170670  0.785344  1.000000           1.0
2   0.636685  0.068861  0.459076           1.0
3   0.314756  0.288868  1.000000           1.0
0   0.829330  0.054417  1.000000           1.0
10 -0.170670  0.770120  1.000000           1.0
8   0.244367  0.594127  1.000000           1.0
17 -0.978025  0.971512  1.000000           1.0
4   0.829330  0.315584  1.000000           1.0
7   0.829330  0.562791  1.000000           1.0
12 -0.170670  0.809998  1.000000           1.0
13 -0.170670  0.809998  1.000000           1.0
6   0.829330  0.562791  1.000000           1.0
15 -0.755633  0.917933  1.000000           1.0
16 -1.170670  0.964773  1.000000           1.0
19 -2.170670  0.998879  0.998879           1.0
```

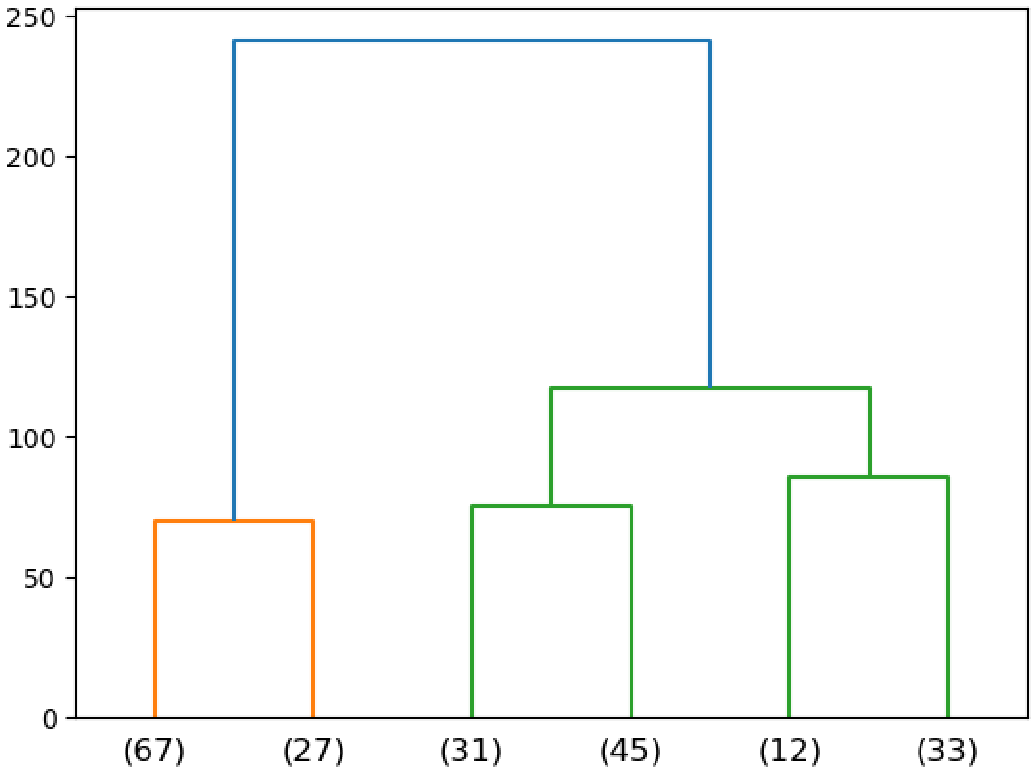
No separation along substrate classes at all.

Six clusters:

```python
import matplotlib.pyplot as plt
n_clusters = 6
linkage = get_linkage(feature_pssm_keywords)
```

```
dendrogram = dendrogram_plot(linkage)
plt.show()
dendrogram = dendrogram_plot(linkage,max_leaves=n_clusters)
plt.show()
labels = get_cluster_labels(linkage=linkage, n_clusters=n_clusters,␣
 ↪index=feature_pssm_keywords.index.tolist())
display(labels.value_counts())
records_not_go_dict = dict()
records_not_go_dict["Keywords substrates"] = keywords_ecoli_substrate.
 ↪to_records(index=False).tolist()
reference_set = set(sequences_substrate_keywords.index.tolist())
cluster_enrichment_analysis(labels, reference_set,␣
 ↪annotations_dict=records_not_go_dict,p_cutoff=1)
```





```
1    67
4    45
6    33
3    31
2    27
5    12
dtype: int64
```

```
============================================================
CLUSTER 1
============================================================
Keywords substrates

                annotation  total_reference  annotated_reference  \
12           Ion transport              215                   81
4     Amino-acid transport              215                   48
15         Sugar transport              215                   56
3        Protein transport              215                   27
0        Peptide transport              215                   19
8           Iron transport              215                   16
2      Potassium transport              215                   10
5         Sodium transport              215                    8
1      Phosphate transport              215                    4
```

```
6         Nickel transport              215                    3
11          Zinc transport              215                    7
13  Hydrogen ion transport              215                   10
7        Sulfate transport              215                    1
9         Cobalt transport              215                    2
10        Copper transport              215                    3
14           Translocation              215                    8

    total_subset  annotated_subset   expected  percentage_of_annotated  \
12            67                24  25.241860                    29.63
4             67                18  14.958140                    37.50
15            67                13  17.451163                    23.21
3             67                11   8.413953                    40.74
0             67                10   5.920930                    52.63
8             67                 6   4.986047                    37.50
2             67                 5   3.116279                    50.00
5             67                 4   2.493023                    50.00
1             67                 3   1.246512                    75.00
6             67                 2   0.934884                    66.67
11            67                 2   2.181395                    28.57
13            67                 2   3.116279                    20.00
7             67                 1   0.311628                   100.00
9             67                 1   0.623256                    50.00
10            67                 1   0.934884                    33.33
14            67                 1   2.493023                    12.50

         lfc         p     p_fdr  p_bonferroni
12 -0.072784  0.700406  0.862038      1.000000
4   0.267066  0.183756  0.588018      1.000000
15 -0.424812  0.953875  0.953875      1.000000
3   0.386648  0.176152  0.704606      1.000000
0   0.756104  0.034926  0.558808      0.558808
8   0.267066  0.376297  0.668972      1.000000
2   0.682104  0.165663  0.883535      1.000000
5   0.682104  0.212004  0.565344      1.000000
1   1.267066  0.090755  0.726036      1.000000
6   1.097141  0.229670  0.524960      1.000000
11 -0.125251  0.698822  0.931762      1.000000
13 -0.639824  0.873972  0.998826      1.000000
7   1.682104  0.311628  0.623256      1.000000
9   0.682104  0.527146  0.843434      1.000000
10  0.097141  0.675884  0.983104      1.000000
14 -1.317896  0.952551  1.000000      1.000000

============================================================
CLUSTER 2
============================================================
Keywords substrates
```

```
               annotation  total_reference  annotated_reference  total_subset  \
3           Ion transport              215                   81            27
2    Amino-acid transport              215                   48            27
7         Sugar transport              215                   56            27
0          Zinc transport              215                    7            27
1        Sodium transport              215                    8            27
5          Iron transport              215                   16            27
4                Chloride              215                    2            27
6  Hydrogen ion transport              215                   10            27

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
3                13  10.172093                    16.05  0.353895  0.161286
2                 9   6.027907                    18.75  0.578268  0.113168
7                 6   7.032558                    10.71 -0.229087  0.759061
0                 3   0.879070                    42.86  1.770913  0.044017
1                 3   1.004651                    37.50  1.578268  0.064515
5                 2   2.009302                    12.50 -0.006695  0.624592
4                 1   0.251163                    50.00  1.993305  0.235905
6                 1   1.255814                    10.00 -0.328623  0.746655

      p_fdr  p_bonferroni
3  0.322572      1.000000
2  0.301781      0.905343
7  0.759061      1.000000
0  0.352135      0.352135
1  0.258061      0.516122
5  0.832790      1.000000
4  0.377448      1.000000
6  0.853320      1.000000

============================================================
CLUSTER 3
============================================================
Keywords substrates

                annotation  total_reference  annotated_reference  \
5            Ion transport              215                   81
8          Sugar transport              215                   56
12    Amino-acid transport              215                   48
7        Protein transport              215                   27
1   Hydrogen ion transport              215                   10
0         Copper transport              215                    3
3            Translocation              215                    8
4      Potassium transport              215                   10
10          Iron transport              215                   16
11       Peptide transport              215                   19
2          Lipid transport              215                    2
6       Electron transport              215                    5
9           Zinc transport              215                    7
```

```
    total_subset  annotated_subset   expected  percentage_of_annotated  \
5             31                12  11.679070                    14.81   
8             31                 8   8.074419                    14.29   
12            31                 5   6.920930                    10.42   
7             31                 4   3.893023                    14.81   
1             31                 3   1.441860                    30.00   
0             31                 2   0.432558                    66.67   
3             31                 2   1.153488                    25.00   
4             31                 2   1.441860                    20.00   
10            31                 2   2.306977                    12.50   
11            31                 2   2.739535                    10.53   
2             31                 1   0.288372                    50.00   
6             31                 1   0.720930                    20.00   
9             31                 1   1.009302                    14.29   

         lfc         p     p_fdr  p_bonferroni  
5   0.039109  0.523812  1.000000      1.000000  
8  -0.013358  0.590665  0.853183      1.000000  
12 -0.469038  0.872833  0.872833      1.000000  
7   0.039109  0.570372  0.926855      1.000000  
1   1.057031  0.160652  1.000000      1.000000  
0   2.209034  0.055135  0.716755      0.716755  
3   0.793997  0.324424  1.000000      1.000000  
4   0.472068  0.436783  1.000000      1.000000  
10 -0.206003  0.704944  0.833115      1.000000  
11 -0.453931  0.795375  0.861657      1.000000  
2   1.793997  0.268159  1.000000      1.000000  
6   0.472068  0.544549  1.000000      1.000000  
9  -0.013358  0.669365  0.870175      1.000000  

============================================================
CLUSTER 4
============================================================
Keywords substrates

               annotation  total_reference  annotated_reference  \
0          Sugar transport              215                   56   
1     Amino-acid transport              215                   48   
12           Ion transport              215                   81   
7        Protein transport              215                   27   
2      Potassium transport              215                   10   
8        Peptide transport              215                   19   
4            Translocation              215                    8   
5   Hydrogen ion transport              215                   10   
3                 Chloride              215                    2   
6       Electron transport              215                    5   
9           Zinc transport              215                    7   
10        Sodium transport              215                    8   
```

```
11          Iron transport              215                   16

    total_subset  annotated_subset   expected  percentage_of_annotated  \
0             45                17  11.720930                    30.36
1             45                12  10.046512                    25.00
12            45                11  16.953488                    13.58
7             45                 5   5.651163                    18.52
2             45                 3   2.093023                    30.00
8             45                 3   3.976744                    15.79
4             45                 2   1.674419                    25.00
5             45                 2   2.093023                    20.00
3             45                 1   0.418605                    50.00
6             45                 1   1.046512                    20.00
9             45                 1   1.465116                    14.29
10            45                 1   1.674419                    12.50
11            45                 1   3.348837                     6.25

         lfc         p     p_fdr  p_bonferroni
0   0.536448  0.036453  0.473885      0.473885
1   0.256340  0.274624  1.000000      1.000000
12 -0.624079  0.988735  0.988735      1.000000
7  -0.176620  0.710650  1.000000      1.000000
2   0.519374  0.349548  1.000000      1.000000
8  -0.406625  0.804855  1.000000      1.000000
4   0.256340  0.526827  1.000000      1.000000
5  -0.065588  0.657686  1.000000      1.000000
3   1.256340  0.375571  1.000000      1.000000
6  -0.065588  0.694776  1.000000      1.000000
9  -0.551015  0.811817  1.000000      1.000000
10 -0.743660  0.852529  1.000000      1.000000
11 -1.743660  0.980034  1.000000      1.000000

============================================================
CLUSTER 5
============================================================
Keywords substrates

               annotation  total_reference  annotated_reference  total_subset  \
2         Sugar transport              215                   56            12
0      Electron transport              215                    5            12
6           Ion transport              215                   81            12
1       Ammonia transport              215                    1            12
3        Cobalt transport              215                    2            12
4        Nickel transport              215                    3            12
5  Hydrogen ion transport              215                   10            12

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
2                 6  3.125581                    10.71  0.940838  0.059633
0                 3  0.279070                    60.00  3.426265  0.001262
```

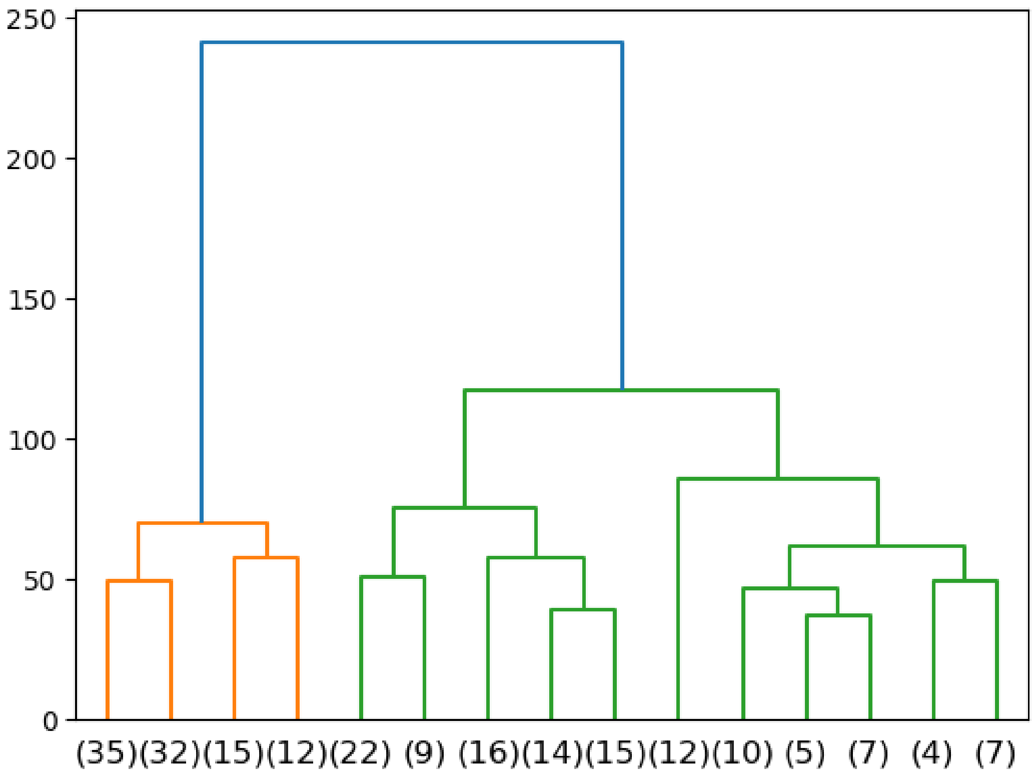
```
6                 3  4.520930                     3.70 -0.591657  0.895530
1                 1  0.055814                   100.00  4.163230  0.055814
3                 1  0.111628                    50.00  3.163230  0.108759
4                 1  0.167442                    33.33  2.578268  0.158970
5                 1  0.558140                    10.00  0.841302  0.444053

      p_fdr  p_bonferroni
2  0.139145      0.417434
0  0.008837      0.008837
6  0.895530      1.000000
1  0.195349      0.390698
3  0.190328      0.761313
4  0.222558      1.000000
5  0.518061      1.000000

============================================================
CLUSTER 6
============================================================
Keywords substrates
                  annotation  total_reference  annotated_reference  \
0              Ion transport              215                   81
2          Protein transport              215                   27
9            Sugar transport              215                   56
1             Iron transport              215                   16
6          Peptide transport              215                   19
10      Amino-acid transport              215                   48
3              Translocation              215                    8
4   Polysaccharide transport              215                    1
5            Lipid transport              215                    2
7        Phosphate transport              215                    4
8     Hydrogen ion transport              215                   10

    total_subset  annotated_subset   expected  percentage_of_annotated  \
0             33                18  12.432558                    22.22
2             33                 7   4.144186                    25.93
9             33                 6   8.595349                    10.71
1             33                 5   2.455814                    31.25
6             33                 4   2.916279                    21.05
10            33                 4   7.367442                     8.33
3             33                 3   1.227907                    37.50
4             33                 1   0.153488                   100.00
5             33                 1   0.306977                    50.00
7             33                 1   0.613953                    25.00
8             33                 1   1.534884                    10.00

         lfc         p     p_fdr  p_bonferroni
0   0.533874  0.025130  0.276433      0.276433
2   0.756266  0.093839  0.344078      1.000000
```

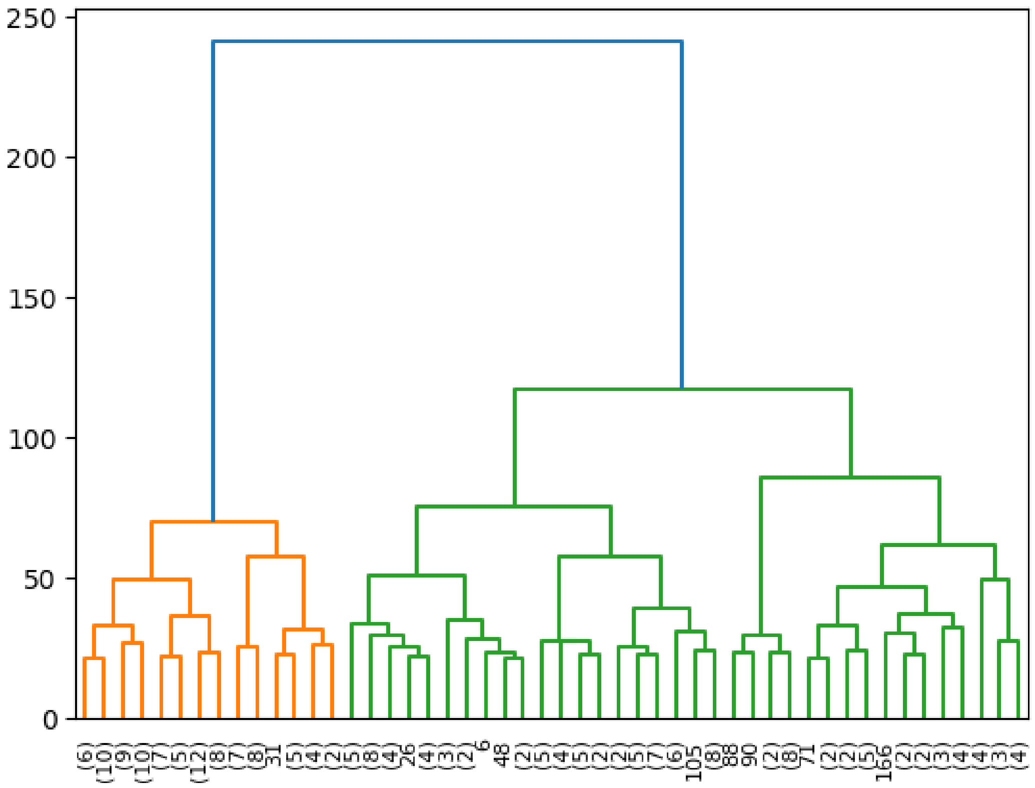
```
9   -0.518594  0.912855  1.000000      1.000000
1    1.025727  0.077690  0.427295      0.854589
6    0.455871  0.329017  0.517026      1.000000
10  -0.881164  0.967419  0.967419      1.000000
3    1.288761  0.107330  0.295157      1.000000
4    2.703799  0.153488  0.337674      1.000000
5    1.703799  0.284025  0.520713      1.000000
7    0.703799  0.489133  0.672558      1.000000
8   -0.618129  0.818324  1.000000      1.000000
```

50 Clusters

[ ]:

```
import matplotlib.pyplot as plt
n_clusters = 50
linkage = get_linkage(feature_pssm_keywords)
dendrogram = dendrogram_plot(linkage)
plt.show()
dendrogram = dendrogram_plot(linkage,max_leaves=n_clusters)
plt.show()
labels = get_cluster_labels(linkage=linkage, n_clusters=n_clusters,␣
  ↪index=feature_pssm_keywords.index.tolist())
display(labels.value_counts())
records_not_go_dict = dict()
records_not_go_dict["Keywords substrates"] = keywords_ecoli_substrate.
  ↪to_records(index=False).tolist()
reference_set = set(sequences_substrate_keywords.index.tolist())
cluster_enrichment_analysis(labels, reference_set,␣
  ↪annotations_dict=records_not_go_dict,p_cutoff=1)
```


250
200
150
100
50
0
(35)
(32)
(15)
(12)
(22)
(9)
(16)
(14)
(15)
(12)
(10)
(5)
(7)
(4)
(7)


250
200
150
100
50
0
(6)
(10)
(9)
(10)
(7)
(5)
(12)
(8)
(7)
(8)
31
(5)
(4)
(2)
(5)
(8)
(4)
26
(4)
(3)
(2)
6
48
(2)
(5)
(4)
(5)
(2)
(2)
(5)
(7)
(6)
105
(8)
88
90
(2)
(8)
71
(2)
(2)
(5)
166
(2)
(2)
(3)
(4)
(4)
(3)
(4)

```
7      12
2      10
4      10
3       9
33      8
8       8
10      8
16      8
38      8
5       7
9       7
31      7
32      6
1       6
42      5
6       5
11      5
15      5
27      5
25      5
30      5
13      4
26      4
47      4
48      4
50      4
18      4
17      4
46      3
49      3
20      3
21      2
22      2
43      2
29      2
14      2
41      2
37      2
39      2
28      2
44      2
40      1
35      1
36      1
23      1
```

```
34     1
24     1
12     1
19     1
45     1
dtype: int64
============================================================
CLUSTER 1
============================================================
Keywords substrates
         annotation  total_reference  annotated_reference  total_subset  \
1     Ion transport              215                   81             6
0    Iron transport              215                   16             6
2  Nickel transport              215                    3             6

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
1                 6  2.260465                     7.41  1.408343  0.002538
0                 5  0.446512                    31.25  3.485158  0.000007
2                 1  0.083721                    33.33  3.578268  0.081777

      p_fdr  p_bonferroni
1  0.003807      0.007615
0  0.000021      0.000021
2  0.081777      0.245331
============================================================
CLUSTER 2
============================================================
Keywords substrates
               annotation  total_reference  annotated_reference  total_subset  \
3           Ion transport              215                   81            10
0       Peptide transport              215                   19            10
2       Protein transport              215                   27            10
1     Potassium transport              215                   10            10
4        Nickel transport              215                    3            10
5        Sodium transport              215                    8            10
6  Hydrogen ion transport              215                   10            10
7    Amino-acid transport              215                   48            10

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
3                 6  3.767442                     7.41  0.671377  0.124483
0                 3  0.883721                    15.79  1.763300  0.047373
2                 3  1.255814                    11.11  1.256340  0.116124
1                 2  0.465116                    20.00  2.104337  0.071852
4                 1  0.139535                    33.33  2.841302  0.133740
5                 1  0.372093                    12.50  1.426265  0.321271
6                 1  0.465116                    10.00  1.104337  0.385416
```

```
7                 1  2.232558                     2.08 -1.158698  0.924883

      p_fdr  p_bonferroni
3  0.248966      0.995865
0  0.378986      0.378986
2  0.309664      0.928992
1  0.287409      0.574819
4  0.213984      1.000000
5  0.428361      1.000000
6  0.440475      1.000000
7  0.924883      1.000000

============================================================
CLUSTER 3
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
1       Sugar transport              215                   56             9
5         Ion transport              215                   81             9
0        Zinc transport              215                    7             9
2      Cobalt transport              215                    2             9
3   Phosphate transport              215                    4             9
4        Iron transport              215                   16             9
6  Amino-acid transport              215                   48             9

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
1                 5  2.344186                     8.93  1.092841  0.053624
5                 3  3.390698                     3.70 -0.176620  0.727008
0                 2  0.293023                    28.57  2.770913  0.029412
2                 1  0.083721                    50.00  3.578268  0.082156
3                 1  0.167442                    25.00  2.578268  0.158257
4                 1  0.669767                     6.25  0.578268  0.508278
6                 1  2.009302                     2.08 -1.006695  0.902063

      p_fdr  p_bonferroni
1  0.187685      0.375370
5  0.848176      1.000000
0  0.205887      0.205887
2  0.191697      0.575092
3  0.276949      1.000000
4  0.711590      1.000000
6  0.902063      1.000000

============================================================
CLUSTER 4
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
5  Amino-acid transport              215                   48            10
8        Ion transport              215                   81            10
4    Protein transport              215                   27            10
7      Sugar transport              215                   56            10
0     Copper transport              215                    3            10
1   Phosphate transport              215                    4            10
2         Translocation              215                    8            10
3     Sodium transport              215                    8            10
6    Peptide transport              215                   19            10

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
5                 3  2.232558                     6.25  0.426265  0.393125
8                 3  3.767442                     3.70 -0.328623  0.798447
4                 2  1.255814                     7.41  0.671377  0.364515
7                 2  2.604651                     3.57 -0.381090  0.785422
0                 1  0.139535                    33.33  2.841302  0.133740
1                 1  0.186047                    25.00  2.426265  0.174601
2                 1  0.372093                    12.50  1.426265  0.321271
3                 1  0.372093                    12.50  1.426265  0.321271
6                 1  0.883721                     5.26  0.178337  0.611776

      p_fdr  p_bonferroni
5  0.589688           1.0
8  0.798447           1.0
4  0.656127           1.0
7  0.883600           1.0
0  1.000000           1.0
1  0.785706           1.0
2  0.826124           1.0
3  0.826124           1.0
6  0.786569           1.0

============================================================
CLUSTER 5
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
2       Sugar transport              215                   56             7
0      Sodium transport              215                    8             7
5         Ion transport              215                   81             7
1   Phosphate transport              215                    4             7
3     Peptide transport              215                   19             7
4     Protein transport              215                   27             7
6  Amino-acid transport              215                   48             7

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
2                 3  1.823256                     5.36  0.718446  0.263382
0                 2  0.260465                    25.00  2.940838  0.023243
```

```
5                  2  2.637209                     2.47 -0.399012  0.813317
1                  1  0.130233                    25.00  2.940838  0.124841
3                  1  0.618605                     5.26  0.692910  0.481770
4                  1  0.879070                     3.70  0.185950  0.614691
6                  1  1.562791                     2.08 -0.644125  0.834246

      p_fdr  p_bonferroni
2  0.614557      1.000000
0  0.162703      0.162703
5  0.948870      1.000000
1  0.436943      0.873886
3  0.843098      1.000000
4  0.860568      1.000000
6  0.834246      1.000000

============================================================
CLUSTER 6
============================================================
Keywords substrates
             annotation  total_reference  annotated_reference  total_subset  \
0  Amino-acid transport              215                   48             5
1       Sugar transport              215                   56             5

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                  4  1.116279                     8.33  1.841302  0.009364
1                  1  1.302326                     1.79 -0.381090  0.782450

      p_fdr  p_bonferroni
0  0.018727      0.018727
1  0.782450      1.000000

============================================================
CLUSTER 7
============================================================
Keywords substrates
               annotation  total_reference  annotated_reference  total_subset  \
0    Amino-acid transport              215                   48            12
2       Peptide transport              215                   19            12
4       Protein transport              215                   27            12
5         Sugar transport              215                   56            12
1       Sulfate transport              215                    1            12
3  Hydrogen ion transport              215                   10            12
6           Ion transport              215                   81            12

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                  6  2.679070                    12.50  1.163230  0.028579
2                  2  1.060465                    10.53  0.915303  0.286882
4                  2  1.506977                     7.41  0.408343  0.459761
```

```
5                 2  3.125581                         3.57 -0.644125  0.867556
1                 1  0.055814                       100.00  4.163230  0.055814
3                 1  0.558140                        10.00  0.841302  0.444053
6                 1  4.520930                         1.23 -2.176620  0.997172

      p_fdr  p_bonferroni
0  0.200054      0.200054
2  0.669391      1.000000
4  0.643666      1.000000
5  1.000000      1.000000
1  0.195349      0.390698
3  0.777092      1.000000
6  0.997172      1.000000

============================================================
CLUSTER 8
============================================================
Keywords substrates

            annotation  total_reference  annotated_reference  total_subset  \
0   Potassium transport              215                   10             8
1     Peptide transport              215                   19             8
2     Protein transport              215                   27             8
4         Ion transport              215                   81             8
3  Amino-acid transport              215                   48             8

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 3  0.372093                    30.00  3.011227  0.003628
1                 3  0.706977                    15.79  2.085228  0.024831
2                 3  1.004651                    11.11  1.578268  0.064515
4                 3  3.013953                     3.70 -0.006695  0.637586
3                 2  1.786047                     4.17  0.163230  0.566488

      p_fdr  p_bonferroni
0  0.018138      0.018138
1  0.062079      0.124157
2  0.107525      0.322576
4  0.637586      1.000000
3  0.708111      1.000000

============================================================
CLUSTER 9
============================================================
Keywords substrates

            annotation  total_reference  annotated_reference  total_subset  \
0  Amino-acid transport              215                   48             7
2         Ion transport              215                   81             7
1      Sodium transport              215                    8             7
3       Sugar transport              215                   56             7
```

```
   annotated_subset  expected  percentage_of_annotated        lfc         p  \
0                 4  1.562791                     8.33   1.355875  0.045630
2                 2  2.637209                     2.47  -0.399012  0.813317
1                 1  0.260465                    12.50   1.940838  0.236077
3                 1  1.823256                     1.79  -0.866517  0.883210

      p_fdr  p_bonferroni
0  0.182521      0.182521
2  1.000000      1.000000
1  0.472155      0.944310
3  0.883210      1.000000

============================================================
CLUSTER 10
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
2         Ion transport              215                   81             8
4  Amino-acid transport              215                   48             8
0      Sodium transport              215                    8             8
1              Chloride              215                    2             8
3        Zinc transport              215                    7             8
5       Sugar transport              215                   56             8

   annotated_subset  expected  percentage_of_annotated        lfc         p  \
2                 5  3.013953                     6.17   0.730271  0.135366
4                 3  1.786047                     6.25   0.748193  0.253828
0                 2  0.297674                    25.00   2.748193  0.030406
1                 1  0.074419                    50.00   3.748193  0.073201
3                 1  0.260465                    14.29   1.940838  0.236077
5                 1  2.083721                     1.79  -1.059162  0.914653

      p_fdr  p_bonferroni
2  0.270731      0.812194
4  0.304594      1.000000
0  0.182436      0.182436
1  0.219604      0.439209
3  0.354116      1.000000
5  0.914653      1.000000

============================================================
CLUSTER 11
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
1         Ion transport              215                   81             5
2  Amino-acid transport              215                   48             5
```

```
0        Zinc transport              215                    7             5
3        Iron transport              215                   16             5

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
1                 3  1.883721                     3.70  0.671377  0.276158
2                 2  1.116279                     4.17  0.841302  0.310384
0                 1  0.162791                    14.29  2.618910  0.153875
3                 1  0.372093                     6.25  1.426265  0.323255

      p_fdr  p_bonferroni
1  0.552316      1.000000
2  0.413846      1.000000
0  0.615498      0.615498
3  0.323255      1.000000

============================================================
CLUSTER 12
============================================================
Keywords substrates

               annotation  total_reference  annotated_reference  total_subset  \
0  Hydrogen ion transport              215                   10             1
1           Ion transport              215                   81             1

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.046512                    10.00  4.426265  0.046512
1                 1  0.376744                     1.23  1.408343  0.376744

      p_fdr  p_bonferroni
0  0.093023      0.093023
1  0.376744      0.753488

============================================================
CLUSTER 13
============================================================
Keywords substrates

        annotation  total_reference  annotated_reference  total_subset  \
0  Sugar transport              215                   56             4
1   Zinc transport              215                    7             4
2    Ion transport              215                   81             4

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 3  1.041860                     5.36  1.525800  0.055156
1                 1  0.130233                    14.29  2.940838  0.124841
2                 1  1.506977                     1.23 -0.591657  0.851668

      p_fdr  p_bonferroni
0  0.165469      0.165469
1  0.187261      0.374522
```

```
2  0.851668      1.000000

============================================================
CLUSTER 14
============================================================
Keywords substrates

        annotation  total_reference  annotated_reference  total_subset  \
0   Iron transport              215                   16             2
1  Sugar transport              215                   56             2
2    Ion transport              215                   81             2

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.148837                     6.25  2.748193  0.143621
1                 1  0.520930                     1.79  0.940838  0.453988
2                 1  0.753488                     1.23  0.408343  0.612649

      p_fdr  p_bonferroni
0  0.430863      0.430863
1  0.680982      1.000000
2  0.612649      1.000000

============================================================
CLUSTER 15
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
2         Ion transport              215                   81             5
0        Iron transport              215                   16             5
3  Amino-acid transport              215                   48             5
1        Zinc transport              215                    7             5

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
2                 3  1.883721                     3.70  0.671377  0.276158
0                 2  0.372093                    12.50  2.426265  0.045616
3                 2  1.116279                     4.17  0.841302  0.310384
1                 1  0.162791                    14.29  2.618910  0.153875

      p_fdr  p_bonferroni
2  0.368210      1.000000
0  0.182464      0.182464
3  0.310384      1.000000
1  0.307749      0.615498

============================================================
CLUSTER 16
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
1  Amino-acid transport              215                   48             8
0     Peptide transport              215                   19             8
2     Protein transport              215                   27             8
4       Sugar transport              215                   56             8
3   Potassium transport              215                   10             8
5         Ion transport              215                   81             8

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
1                 3  1.786047                     6.25  0.748193  0.253828
0                 2  0.706977                    10.53  1.500265  0.150402
2                 2  1.004651                     7.41  0.993305  0.264579
4                 2  2.083721                     3.57 -0.059162  0.663105
3                 1  0.372093                    10.00  1.426265  0.321271
5                 1  3.013953                     1.23 -1.591657  0.979007

      p_fdr  p_bonferroni
1  0.761484      1.000000
0  0.902415      0.902415
2  0.529159      1.000000
4  0.795726      1.000000
3  0.481906      1.000000
5  0.979007      1.000000

============================================================
CLUSTER 17
============================================================
Keywords substrates

        annotation  total_reference  annotated_reference  total_subset  \
1    Ion transport              215                   81             4
0  Lipid transport              215                    2             4
2  Sugar transport              215                   56             4

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
1                 2  1.506977                     2.47  0.408343  0.484802
0                 1  0.037209                    50.00  4.748193  0.036948
2                 1  1.041860                     1.79 -0.059162  0.703851

      p_fdr  p_bonferroni
1  0.727203      1.000000
0  0.110845      0.110845
2  0.703851      1.000000

============================================================
CLUSTER 18
============================================================
Keywords substrates

               annotation  total_reference  annotated_reference  total_subset  \
2           Ion transport              215                   81             4
```

```
0        Copper transport              215                    3             4
1      Electron transport              215                    5             4
3  Hydrogen ion transport              215                   10             4

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
2                 3  1.506977                     3.70  0.993305  0.151287   
0                 2  0.055814                    66.67  5.163230  0.000778   
1                 1  0.093023                    20.00  3.426265  0.090440   
3                 1  0.186047                    10.00  2.426265  0.174601   

      p_fdr  p_bonferroni  
2  0.201717      0.605150  
0  0.003110      0.003110  
1  0.180879      0.361758  
3  0.174601      0.698406  
============================================================
CLUSTER 19
============================================================
Keywords substrates

            annotation  total_reference  annotated_reference  total_subset  \
0  Potassium transport              215                   10             1   
1        Ion transport              215                   81             1   

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.046512                    10.00  4.426265  0.046512   
1                 1  0.376744                     1.23  1.408343  0.376744   

      p_fdr  p_bonferroni  
0  0.093023      0.093023  
1  0.376744      0.753488  
============================================================
CLUSTER 20
============================================================
Keywords substrates

               annotation  total_reference  annotated_reference  total_subset  \
1         Sugar transport              215                   56             3   
0  Hydrogen ion transport              215                   10             3   
2           Ion transport              215                   81             3   

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
1                 2  0.781395                     3.57  1.355875  0.166884   
0                 1  0.139535                    10.00  2.841302  0.133740   
2                 1  1.130233                     1.23 -0.176620  0.759952   

      p_fdr  p_bonferroni  
1  0.250325      0.500651  
```

```
0  0.401220      0.401220
2  0.759952      1.000000

============================================================
CLUSTER 21
============================================================
Keywords substrates

          annotation  total_reference  annotated_reference  total_subset  \
0      Translocation              215                    8             2   
1  Protein transport              215                   27             2   

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 2  0.074419                    25.00  4.748193  0.001217   
1                 2  0.251163                     7.41  2.993305  0.015258   

      p_fdr  p_bonferroni  
0  0.002434      0.002434  
1  0.015258      0.030515  

============================================================
CLUSTER 22
============================================================
Keywords substrates

        annotation  total_reference  annotated_reference  total_subset  \
0  Sugar transport              215                   56             2   

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 2   0.52093                     3.57  1.940838  0.066942   

      p_fdr  p_bonferroni  
0  0.066942      0.066942  

============================================================
CLUSTER 23
============================================================
Keywords substrates

        annotation  total_reference  annotated_reference  total_subset  \
0  Sugar transport              215                   56             1   

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.260465                     1.79  1.940838  0.260465   

      p_fdr  p_bonferroni  
0  0.260465      0.260465  

============================================================
CLUSTER 24
============================================================
Keywords substrates

               annotation  total_reference  annotated_reference  total_subset  \
0  Hydrogen ion transport              215                   10             1
1           Ion transport              215                   81             1

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.046512                    10.00  4.426265  0.046512
1                 1  0.376744                     1.23  1.408343  0.376744

      p_fdr  p_bonferroni
0  0.093023      0.093023
1  0.376744      0.753488

============================================================
CLUSTER 25
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
0  Amino-acid transport              215                   48             5
1       Sugar transport              215                   56             5

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 4  1.116279                     8.33  1.841302  0.009364
1                 1  1.302326                     1.79 -0.381090  0.782450

      p_fdr  p_bonferroni
0  0.018727      0.018727
1  0.782450      1.000000

============================================================
CLUSTER 26
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
0       Sugar transport              215                   56             4
1  Amino-acid transport              215                   48             4

   annotated_subset  expected  percentage_of_annotated      lfc         p  \
0                 3  1.041860                     5.36  1.52580  0.055156
1                 1  0.893023                     2.08  0.16323  0.638936

      p_fdr  p_bonferroni
0  0.110313      0.110313
1  0.638936      1.000000

============================================================
CLUSTER 27
============================================================
Keywords substrates

            annotation  total_reference  annotated_reference  total_subset  \
0      Sugar transport              215                   56             5   
2        Ion transport              215                   81             5   
1  Potassium transport              215                   10             5   

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 3  1.302326                     5.36  1.203872  0.112344   
2                 2  1.883721                     2.47  0.086415  0.623898   
1                 1  0.232558                    10.00  2.104337  0.213720   

      p_fdr  p_bonferroni  
0  0.337031      0.337031  
2  0.623898      1.000000  
1  0.320580      0.641159  
============================================================
CLUSTER 28
============================================================
Keywords substrates

          annotation  total_reference  annotated_reference  total_subset  \
0  Peptide transport              215                   19             2   
1  Protein transport              215                   27             2   
2      Ion transport              215                   81             2   

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.176744                     5.26  2.500265  0.169311   
1                 1  0.251163                     3.70  1.993305  0.235905   
2                 1  0.753488                     1.23  0.408343  0.612649   

      p_fdr  p_bonferroni  
0  0.507933      0.507933  
1  0.353858      0.707716  
2  0.612649      1.000000  
============================================================
CLUSTER 29
============================================================
Keywords substrates

           annotation  total_reference  annotated_reference  total_subset  \
0  Electron transport              215                    5             2   
1      Zinc transport              215                    7             2   
2       Ion transport              215                   81             2   

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.046512                    20.00  4.426265  0.046077   
1                 1  0.065116                    14.29  3.940838  0.064203   
```

```
2                 1  0.753488                     1.23  0.408343  0.612649

      p_fdr  p_bonferroni
0  0.138231      0.138231
1  0.096305      0.192610
2  0.612649      1.000000

============================================================
CLUSTER 30
============================================================
Keywords substrates

          annotation  total_reference  annotated_reference  total_subset  \
1    Sugar transport              215                   56             5
0      Translocation              215                    8             5
2  Protein transport              215                   27             5

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
1                 3  1.302326                     5.36  1.203872  0.112344
0                 2  0.186047                    25.00  3.426265  0.011498
2                 2  0.627907                     7.41  1.671377  0.119712

      p_fdr  p_bonferroni
1  0.168516      0.337031
0  0.034493      0.034493
2  0.119712      0.359137

============================================================
CLUSTER 31
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
1       Sugar transport              215                   56             7
3         Ion transport              215                   81             7
0   Potassium transport              215                   10             7
2        Iron transport              215                   16             7
4  Amino-acid transport              215                   48             7

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
1                 3  1.823256                     5.36  0.718446  0.263382
3                 3  2.637209                     3.70  0.185950  0.529967
0                 2  0.325581                    20.00  2.618910  0.036184
2                 1  0.520930                     6.25  0.940838  0.422678
4                 1  1.562791                     2.08 -0.644125  0.834246

      p_fdr  p_bonferroni
1  0.658454      1.000000
3  0.662459      1.000000
0  0.180921      0.180921
```

```
2  0.704463      1.000000
4  0.834246      1.000000

============================================================
CLUSTER 32
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
0  Amino-acid transport              215                   48             6
1      Sodium transport              215                    8             6
2     Peptide transport              215                   19             6
3     Protein transport              215                   27             6
4       Sugar transport              215                   56             6
5         Ion transport              215                   81             6

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 4  1.339535                     8.33  1.578268  0.023426
1                 1  0.223256                    12.50  2.163230  0.205673
2                 1  0.530233                     5.26  0.915303  0.429947
3                 1  0.753488                     3.70  0.408343  0.557530
4                 1  1.562791                     1.79 -0.644125  0.840463
5                 1  2.260465                     1.23 -1.176620  0.943861

      p_fdr  p_bonferroni
0  0.140558      0.140558
1  0.617018      1.000000
2  0.859895      1.000000
3  0.836295      1.000000
4  1.000000      1.000000
5  0.943861      1.000000

============================================================
CLUSTER 33
============================================================
Keywords substrates

               annotation  total_reference  annotated_reference  total_subset  \
2         Sugar transport              215                   56             8
4    Amino-acid transport              215                   48             8
6           Ion transport              215                   81             8
0                Chloride              215                    2             8
1  Hydrogen ion transport              215                   10             8
3       Peptide transport              215                   19             8
5       Protein transport              215                   27             8

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
2                 3  2.083721                     5.36  0.525800  0.346615
4                 2  1.786047                     4.17  0.163230  0.566488
6                 2  3.013953                     2.47 -0.591657  0.871895
```

```
0                 1  0.074419                    50.00  3.748193  0.073201
1                 1  0.372093                    10.00  1.426265  0.321271
3                 1  0.706977                     5.26  0.500265  0.529109
5                 1  1.004651                     3.70 -0.006695  0.664707

      p_fdr  p_bonferroni
2  0.808767       1.00000
4  0.793084       1.00000
6  0.871895       1.00000
0  0.512410       0.51241
1  1.000000       1.00000
3  0.925940       1.00000
5  0.775492       1.00000

============================================================
CLUSTER 34
============================================================
Keywords substrates

               annotation  total_reference  annotated_reference  total_subset  \
0  Hydrogen ion transport              215                   10             1
1           Ion transport              215                   81             1

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.046512                    10.00  4.426265  0.046512
1                 1  0.376744                     1.23  1.408343  0.376744

      p_fdr  p_bonferroni
0  0.093023      0.093023
1  0.376744      0.753488

============================================================
CLUSTER 35
============================================================
Keywords substrates

           annotation  total_reference  annotated_reference  total_subset  \
0  Electron transport              215                    5             1

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.023256                     20.0  5.426265  0.023256

      p_fdr  p_bonferroni
0  0.023256      0.023256

============================================================
CLUSTER 36
============================================================
Keywords substrates

           annotation  total_reference  annotated_reference  total_subset  \
0  Electron transport              215                    5             1

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.023256                     20.0  5.426265  0.023256  

      p_fdr  p_bonferroni  
0  0.023256      0.023256  

============================================================
CLUSTER 37
============================================================
Keywords substrates

         annotation  total_reference  annotated_reference  total_subset  \
0  Cobalt transport              215                    2             2   
1  Nickel transport              215                    3             2   
2   Sugar transport              215                   56             2   
3     Ion transport              215                   81             2   

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.018605                    50.00  5.748193  0.018561   
1                 1  0.027907                    33.33  5.163230  0.027777   
2                 1  0.520930                     1.79  0.940838  0.453988   
3                 1  0.753488                     1.23  0.408343  0.612649   

      p_fdr  p_bonferroni  
0  0.074245      0.074245  
1  0.055553      0.111106  
2  0.605318      1.000000  
3  0.612649      1.000000  

============================================================
CLUSTER 38
============================================================
Keywords substrates

               annotation  total_reference  annotated_reference  total_subset  \
0         Sugar transport              215                   56             8   
4           Ion transport              215                   81             8   
1       Ammonia transport              215                    1             8   
2      Electron transport              215                    5             8   
3  Hydrogen ion transport              215                   10             8   

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 5  2.083721                     8.93  1.262766  0.029793   
4                 2  3.013953                     2.47 -0.591657  0.871895   
1                 1  0.037209                   100.00  4.748193  0.037209   
2                 1  0.186047                    20.00  2.426265  0.174214   
3                 1  0.372093                    10.00  1.426265  0.321271   
```

```
      p_fdr  p_bonferroni
0  0.148964      0.148964
4  0.871895      1.000000
1  0.093023      0.186047
2  0.290357      0.871070
3  0.401588      1.000000

============================================================
CLUSTER 39
============================================================
Keywords substrates

          annotation  total_reference  annotated_reference  total_subset  \
0      Translocation              215                    8             2   
1  Protein transport              215                   27             2   
2      Ion transport              215                   81             2   

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.074419                    12.50  3.748193  0.073201   
1                 1  0.251163                     3.70  1.993305  0.235905   
2                 1  0.753488                     1.23  0.408343  0.612649   

      p_fdr  p_bonferroni  
0  0.219604      0.219604  
1  0.353858      0.707716  
2  0.612649      1.000000  

============================================================
CLUSTER 40
============================================================
Keywords substrates

          annotation  total_reference  annotated_reference  total_subset  \
0      Translocation              215                    8             1   
1  Protein transport              215                   27             1   

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.037209                     12.5  4.748193  0.037209   
1                 1  0.125581                      3.7  2.993305  0.125581   

      p_fdr  p_bonferroni  
0  0.074419      0.074419  
1  0.125581      0.251163  

============================================================
CLUSTER 41
============================================================
Keywords substrates

               annotation  total_reference  annotated_reference  total_subset  \
0           Translocation              215                    8             2   
```

```
1  Hydrogen ion transport              215                  10             2
2       Protein transport              215                  27             2
3           Ion transport              215                  81             2

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.074419                    12.50  3.748193  0.073201
1                 1  0.093023                    10.00  3.426265  0.091067
2                 1  0.251163                     3.70  1.993305  0.235905
3                 1  0.753488                     1.23  0.408343  0.612649

      p_fdr  p_bonferroni
0  0.292806      0.292806
1  0.182134      0.364269
2  0.314540      0.943621
3  0.612649      1.000000

============================================================
CLUSTER 42
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
1  Amino-acid transport              215                   48             5
2       Sugar transport              215                   56             5
0   Phosphate transport              215                    4             5

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
1                 2  1.116279                     4.17  0.841302  0.310384
2                 2  1.302326                     3.57  0.618910  0.389455
0                 1  0.093023                    25.00  3.426265  0.090440

      p_fdr  p_bonferroni
1  0.465576      0.931152
2  0.389455      1.000000
0  0.271319      0.271319

============================================================
CLUSTER 43
============================================================
Keywords substrates

       annotation  total_reference  annotated_reference  total_subset  \
0  Iron transport              215                   16             2
1   Ion transport              215                   81             2

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 2  0.148837                    12.50  3.748193  0.005216
1                 2  0.753488                     2.47  1.408343  0.140839

      p_fdr  p_bonferroni
```

```
0  0.010433      0.010433
1  0.140839      0.281678

============================================================
CLUSTER 44
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
0     Peptide transport              215                   19             2
1     Protein transport              215                   27             2
2  Amino-acid transport              215                   48             2

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 1  0.176744                     5.26  2.500265  0.169311
1                 1  0.251163                     3.70  1.993305  0.235905
2                 1  0.446512                     2.08  1.163230  0.397479

      p_fdr  p_bonferroni
0  0.507933      0.507933
1  0.353858      0.707716
2  0.397479      1.000000

============================================================
CLUSTER 45
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
0  Amino-acid transport              215                   48             1

   annotated_subset  expected  percentage_of_annotated      lfc         p  \
0                 1  0.223256                     2.08  2.16323  0.223256

      p_fdr  p_bonferroni
0  0.223256      0.223256

============================================================
CLUSTER 46
============================================================
Keywords substrates

          annotation  total_reference  annotated_reference  total_subset  \
0  Peptide transport              215                   19             3
1  Protein transport              215                   27             3

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 3  0.265116                    15.79  3.500265  0.000593
1                 3  0.376744                    11.11  2.993305  0.001791

      p_fdr  p_bonferroni
```

```
0  0.001187      0.001187
1  0.001791      0.003582

============================================================
CLUSTER 47
============================================================
Keywords substrates

        annotation  total_reference  annotated_reference  total_subset  \
0   Iron transport              215                   16             4
2    Ion transport              215                   81             4
1  Lipid transport              215                    2             4

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 3  0.297674                    18.75  3.333155  0.001308
2                 3  1.506977                     3.70  0.993305  0.151287
1                 1  0.037209                    50.00  4.748193  0.036948

      p_fdr  p_bonferroni
0  0.003925      0.003925
2  0.151287      0.453862
1  0.055423      0.110845

============================================================
CLUSTER 48
============================================================
Keywords substrates

        annotation  total_reference  annotated_reference  total_subset  \
0    Ion transport              215                   81             4
1  Sugar transport              215                   56             4

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 4  1.506977                     4.94  1.408343  0.019219
1                 2  1.041860                     3.57  0.940838  0.278611

      p_fdr  p_bonferroni
0  0.038438      0.038438
1  0.278611      0.557221

============================================================
CLUSTER 49
============================================================
Keywords substrates

      annotation  total_reference  annotated_reference  total_subset  \
0  Ion transport              215                   81             3

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 3  1.130233                      3.7  1.408343  0.052236
```

```
      p_fdr  p_bonferroni
0  0.052236      0.052236

============================================================
CLUSTER 50
============================================================
Keywords substrates
                 annotation  total_reference  annotated_reference  \
1             Ion transport              215                   81
2           Sugar transport              215                   56
0  Polysaccharide transport              215                    1

   total_subset  annotated_subset  expected  percentage_of_annotated  \
1             4                 4  1.506977                     4.94
2             4                 2  1.041860                     3.57
0             4                 1  0.018605                   100.00

        lfc         p     p_fdr  p_bonferroni
1  1.408343  0.019219  0.028828      0.057657
2  0.940838  0.278611  0.278611      0.835832
0  5.748193  0.018605  0.055814      0.055814
```

1.6.4 Trying ProtNLM instead

[ ]:

```
feature_protnlm_keywords = feature_protnlm_all.loc[sequences_substrate_keywords.
 ↪index]
feature_protnlm_keywords
```

[ ]:

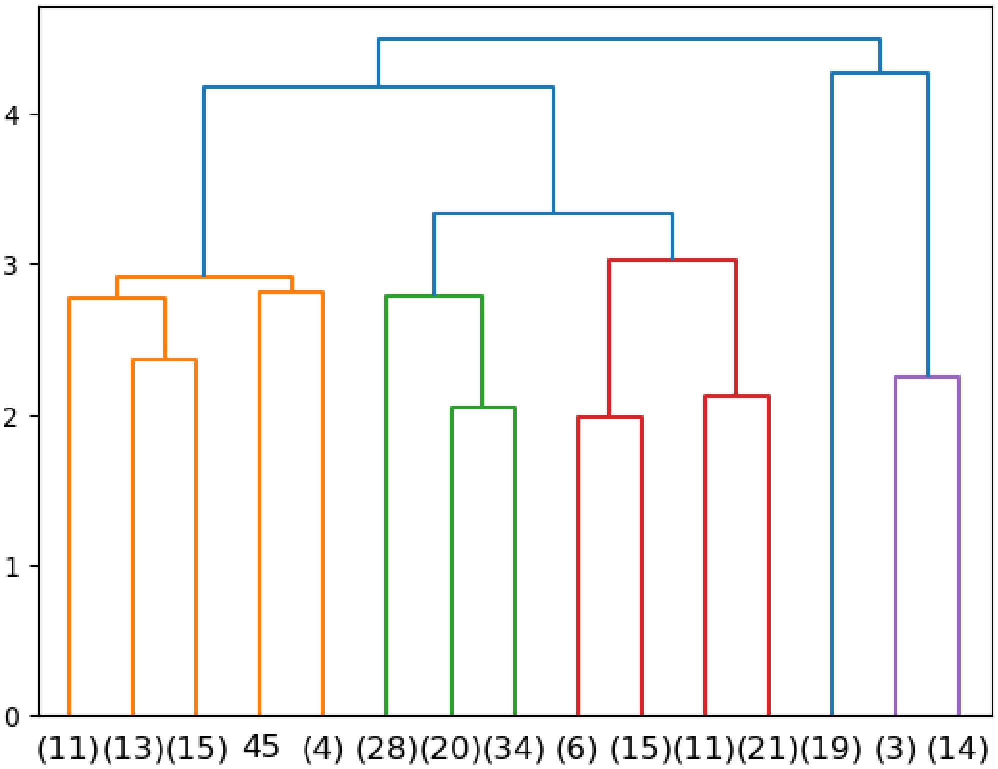
```
                0         1         2         3         4         5         6     \
Uniprot
P02916   0.010300  0.080383  0.056885  0.034698  0.031525  0.022995 -0.030945
P03959   0.025909  0.126099  0.006119  0.035278 -0.004730  0.042938 -0.029327
P05825   0.053955  0.033478  0.056885  0.033112  0.042786 -0.027786 -0.003395
P0AAD6   0.018661  0.125000  0.028625  0.026184  0.010612  0.024185 -0.019943
P0AAD8   0.008408  0.095642  0.017944  0.023651  0.000017  0.017029 -0.007919
…             …         …         …         …         …         …         …
P0A843  -0.004459  0.017624  0.008377  0.001963 -0.023682  0.003561  0.051147
P45539  -0.025604  0.040894 -0.008926  0.015884 -0.047333  0.015182 -0.015015
P75826   0.033661  0.057709  0.028580  0.044678  0.015495  0.015656  0.011452
P76128   0.004066  0.061127  0.011909  0.042267 -0.004524 -0.005066 -0.048584
P31448   0.013634  0.117554  0.018234  0.038971 -0.007210  0.011536 -0.030243

                7         8         9     …      1014      1015      1016  \
Uniprot                                   …
P02916  -0.037567  0.042816 -0.010796  … -0.024963 -0.022446 -0.000171
P03959  -0.038696  0.002594 -0.082703  … -0.004604 -0.014725 -0.009979
P05825  -0.013596  0.064453  0.022583  … -0.014297 -0.019241 -0.038879
```

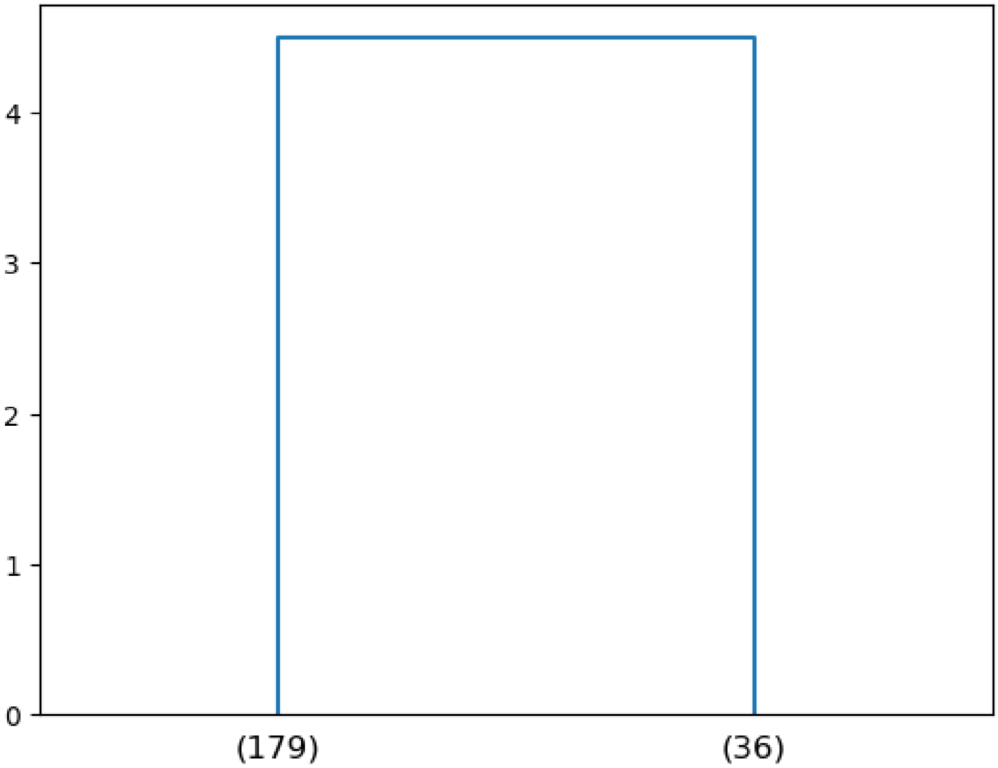
```
P0AAD6  -0.036438  0.029083 -0.038940  … -0.034515  0.003023  0.027237
P0AAD8  -0.038269  0.038330 -0.037720  … -0.039276 -0.008675  0.033600
…             …         …        …   …        …         …         …
P0A843  -0.023010  0.000399  0.009888  … -0.075500 -0.008049 -0.060822
P45539  -0.017899 -0.021454 -0.056213  … -0.012024 -0.051117  0.008102
P75826  -0.051300  0.054230  0.007256  … -0.033234  0.044037  0.013901
P76128  -0.051849  0.059906  0.004223  … -0.019302 -0.012032  0.002796
P31448  -0.014648  0.033691 -0.064209  … -0.044098 -0.032776 -0.011055

             1017      1018      1019      1020      1021      1022      1023
Uniprot
P02916  -0.046082  0.042053 -0.053955 -0.039642 -0.031525 -0.041718  0.017944
P03959  -0.030670  0.023117 -0.051910 -0.019653 -0.017334 -0.018646  0.016357
P05825  -0.015221  0.074341 -0.015099 -0.027710 -0.004265 -0.045197 -0.001211
P0AAD6  -0.035645  0.059235 -0.050781 -0.029648 -0.003340 -0.012543  0.006695
P0AAD8  -0.036194  0.047882 -0.047424 -0.025833 -0.008163  0.005672  0.011299
…             …         …        …         …         …         …         …
P0A843  -0.071838 -0.059418 -0.048035 -0.044891 -0.002670  0.017578  0.062439
P45539  -0.100830  0.038452  0.015594 -0.020660 -0.055634  0.046509 -0.002180
P75826  -0.058044 -0.000068 -0.022720 -0.043152  0.003742 -0.020599  0.015640
P76128  -0.012848  0.086853 -0.019775 -0.067627 -0.031647 -0.037079  0.016907
P31448  -0.034088  0.032196 -0.015244 -0.041290  0.003870 -0.010788  0.009956

[215 rows x 1024 columns]
```

ProtNLM 2 clusters

[ ]:

```python
import matplotlib.pyplot as plt
n_clusters = 2
linkage = get_linkage(feature_protnlm_keywords,standardize=False)
dendrogram = dendrogram_plot(linkage)
plt.show()
dendrogram = dendrogram_plot(linkage,max_leaves=n_clusters)
plt.show()
labels = get_cluster_labels(linkage=linkage, n_clusters=n_clusters,␣
  ↪index=feature_protnlm_keywords.index.tolist())
display(labels.value_counts())
records_not_go_dict = {k:v for k,v in records_all_dict.items() if "GO" not in k}
records_not_go_dict["Keywords substrates"] = keywords_ecoli_substrate.
  ↪to_records(index=False).tolist()
reference_set = set(sequences_substrate_keywords.index.tolist())
cluster_enrichment_analysis(labels, reference_set,␣
  ↪annotations_dict=records_not_go_dict,p_cutoff=0.05)
```


0
1
2
3
4
(11)
(13)
(15)
45
(4)
(28)
(20)
(34)
(6)
(15)
(11)
(21)
(19)
(3)
(14)


0
1
2
3
4
(179)
(36)

```
1    179
2     36
dtype: int64

============================================================
CLUSTER 1
============================================================
Keywords

            annotation  total_reference  annotated_reference  total_subset  \
1  Cell inner membrane              215                  181           179
2        Cell membrane              215                  181           179
3        Transmembrane              215                  175           179
0  Transmembrane helix              215                  157           179
4              Symport              215                   31           179

   annotated_subset    expected  percentage_of_annotated       lfc  \
1               164  150.693023                    90.61  0.122083
2               164  150.693023                    90.61  0.122083
3               158  145.697674                    90.29  0.116947
0               157  130.711628                   100.00  0.264377
4                31   25.809302                   100.00  0.264377

              p         p_fdr  p_bonferroni
1  5.591880e-09  2.102547e-07  5.256368e-07
2  5.591880e-09  2.102547e-07  5.256368e-07
3  2.252471e-07  5.293308e-06  2.117323e-05
0  5.031195e-26  4.729323e-24  4.729323e-24
4  2.097203e-03  3.942743e-02  1.971371e-01

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        2.A              215                   83           179
1        4.A              215                   23           179

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                83  69.102326                    100.0  0.264377
1                23  19.148837                    100.0  0.264377

              p         p_fdr  p_bonferroni
0  2.712901e-09  1.899030e-08  1.899030e-08
1  1.143163e-02  4.001071e-02  8.002142e-02

TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          2              215                   83           179
```

```
1          4              215                   23            179

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                83  69.102326                    100.0  0.264377
1                23  19.148837                    100.0  0.264377

              p         p_fdr  p_bonferroni
0  2.712901e-09  1.356450e-08  1.356450e-08
1  1.143163e-02  2.857908e-02  5.715815e-02
```

```
Interpro Domains
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
Keywords substrates
```

```
Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []
```

```
============================================================
CLUSTER 2
============================================================
Keywords
```

```
                   annotation  total_reference  annotated_reference  \
11              Ion transport              215                   81
2                 ATP-binding              215                   28
3          Nucleotide-binding              215                   30
0         Cell outer membrane              215                   18
1   Transmembrane beta strand              215                   18
5                      Signal              215                   29
6                 Translocase              215                   23
9   Direct protein sequencing              215                   44
4                       Porin              215                   12
10             Iron transport              215                   16
13                       Iron              215                   18
7                    Receptor              215                    6
8                    TonB box              215                    5
12                     Repeat              215                   10

    total_subset  annotated_subset   expected  percentage_of_annotated  \
11            36                20  13.562791                    24.69
2             36                19   4.688372                    67.86
```

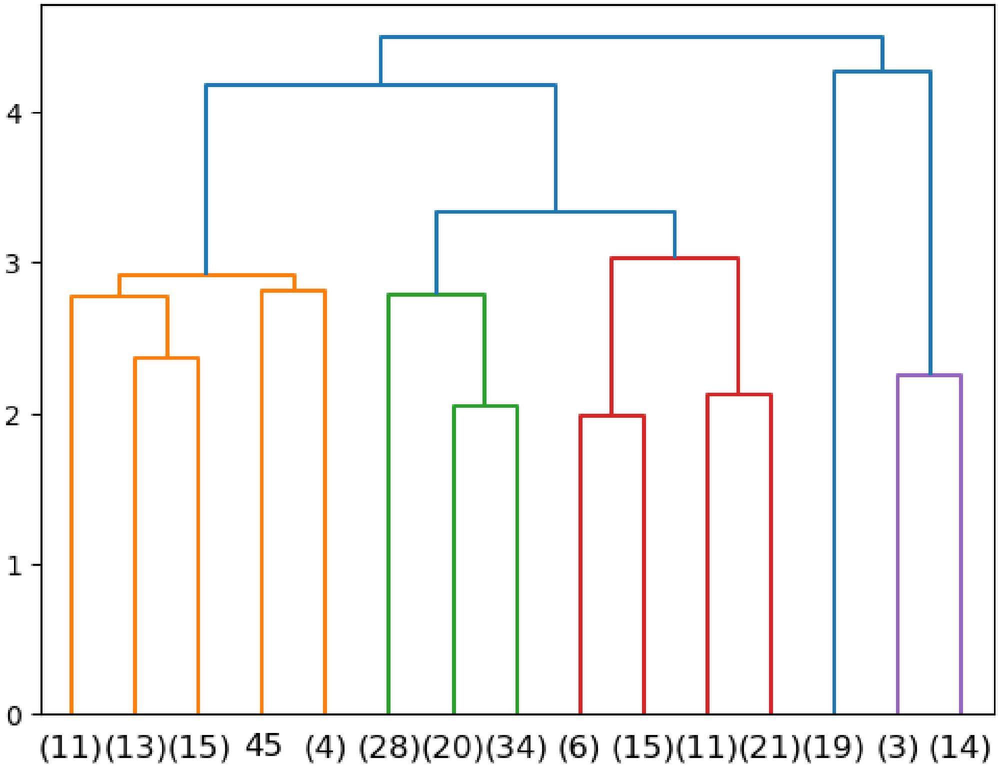
```
3             36                19   5.023256                    63.33
0             36                17   3.013953                    94.44
1             36                17   3.013953                    94.44
5             36                17   4.855814                    58.62
6             36                14   3.851163                    60.87
9             36                14   7.367442                    31.82
4             36                11   2.009302                    91.67
10            36                 7   2.679070                    43.75
13            36                 7   3.013953                    38.89
7             36                 6   1.004651                   100.00
8             36                 5   0.837209                   100.00
12            36                 5   1.674419                    50.00

         lfc             p         p_fdr  p_bonferroni
11  0.560346  1.346474e-02  4.712658e-02  5.655189e-01
2   2.018840  3.592812e-11  5.029937e-10  1.508981e-09
3   1.919305  2.373020e-10  2.491671e-09  9.966684e-09
0   2.495806  2.138096e-14  5.986668e-13  8.980003e-13
1   2.495806  2.138096e-14  5.986668e-13  8.980003e-13
5   1.807750  2.103379e-08  1.472365e-07  8.834191e-07
6   1.862061  3.444156e-07  2.066494e-06  1.446546e-05
9   0.926191  4.135863e-03  1.737062e-02  1.737062e-01
4   2.452737  7.302113e-09  6.133775e-08  3.066887e-07
10  1.385623  7.686359e-03  2.934791e-02  3.228271e-01
13  1.215698  1.641162e-02  4.923487e-02  6.892881e-01
7   2.578268  1.523371e-05  7.997697e-05  6.398157e-04
8   2.578268  1.031961e-04  4.815817e-04  4.334236e-03
12  1.578268  1.349402e-02  4.359606e-02  5.667487e-01
TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        1.B              215                   18            36
1        3.A              215                   64            36

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                17   3.013953                    94.44  2.495806
1                17  10.716279                    26.56  0.665731

              p         p_fdr  p_bonferroni
0  2.138096e-14  4.276192e-14  4.276192e-14
1  1.205218e-02  1.205218e-02  2.410437e-02
TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          1              215                   26            36
1          3              215                   68            36

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                17   4.353488                    65.38  1.965291
1                17  11.386047                    25.00  0.578268

              p         p_fdr  p_bonferroni
0  1.707540e-09  3.415081e-09  3.415081e-09
1  2.422165e-02  2.422165e-02  4.844331e-02

Interpro Domains

                                          annotation  total_reference  \
0            ABC transporter-like, ATP-binding domain              215
3   P-loop containing nucleoside triphosphate hydr…              215
1                                  AAA+ ATPase domain              215
2                ABC transporter-like, conserved site              215
6           TonB-dependent receptor-like, beta-barrel              215
7                            TonB box, conserved site              215
8   TonB-dependent receptor-like, beta-barrel doma…              215
5    TonB-dependent receptor, plug domain superfamily              215
4                TonB-dependent receptor, plug domain              215
9             TonB-dependent receptor, conserved site              215
10               TonB-dependent siderophore receptor              215
11                          Porin domain superfamily              215
12                       Porin, gammaproteobacterial              215
13                         Porin, Gram-negative type              215
14  Oligopeptide/dipeptide ABC transporter, C-term…              215
15  ABC-type amino acid transport system, ATPase c…              215
16                      TonB-dependent receptor-like              215

    annotated_reference  total_subset  annotated_subset  expected  \
0                    22            36                19  3.683721
3                    25            36                19  4.186047
1                    23            36                19  3.851163
2                    20            36                17  3.348837
6                     5            36                 5  0.837209
7                     5            36                 5  0.837209
8                     5            36                 5  0.837209
5                     5            36                 5  0.837209
4                     5            36                 5  0.837209
9                     4            36                 4  0.669767
10                    4            36                 4  0.669767
11                    4            36                 4  0.669767
12                    3            36                 3  0.502326
13                    3            36                 3  0.502326
14                    3            36                 3  0.502326
15                    3            36                 3  0.502326
16                    3            36                 3  0.502326

    percentage_of_annotated       lfc             p         p_fdr  \
0                     86.36  2.366764  1.360968e-14  7.893612e-13
```

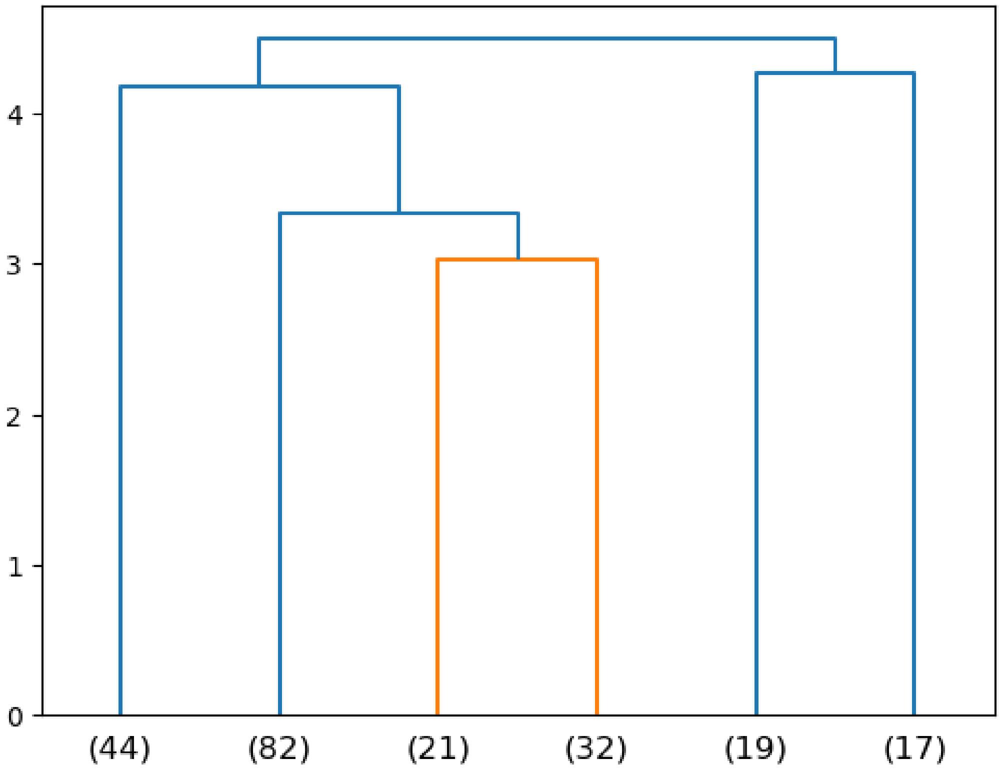
```
3                     76.00  2.182339  1.203118e-12  1.744521e-11
1                     82.61  2.302633  7.171710e-14  2.079796e-12
2                     85.00  2.343803  1.118304e-12  2.162054e-11
6                    100.00  2.578268  1.031961e-04  8.550533e-04
7                    100.00  2.578268  1.031961e-04  8.550533e-04
8                    100.00  2.578268  1.031961e-04  8.550533e-04
5                    100.00  2.578268  1.031961e-04  8.550533e-04
4                    100.00  2.578268  1.031961e-04  8.550533e-04
9                    100.00  2.578268  6.804492e-04  3.587823e-03
10                   100.00  2.578268  6.804492e-04  3.587823e-03
11                   100.00  2.578268  6.804492e-04  3.587823e-03
12                   100.00  2.578268  4.371371e-03  1.690263e-02
13                   100.00  2.578268  4.371371e-03  1.690263e-02
14                   100.00  2.578268  4.371371e-03  1.690263e-02
15                   100.00  2.578268  4.371371e-03  1.690263e-02
16                   100.00  2.578268  4.371371e-03  1.690263e-02

    p_bonferroni
0   7.893612e-13
3   6.978084e-11
1   4.159592e-12
2   6.486162e-11
6   5.985373e-03
7   5.985373e-03
8   5.985373e-03
5   5.985373e-03
4   5.985373e-03
9   3.946605e-02
10  3.946605e-02
11  3.946605e-02
12  2.535395e-01
13  2.535395e-01
14  2.535395e-01
15  2.535395e-01
16  2.535395e-01

Keywords substrates

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,
 annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,
 p_bonferroni]
Index: []
```

ProtNLM 4 clusters

```
[ ]: import matplotlib.pyplot as plt
     n_clusters = 6
     linkage = get_linkage(feature_protnlm_keywords,standardize=False)
```

```
dendrogram = dendrogram_plot(linkage)
plt.show()
dendrogram = dendrogram_plot(linkage,max_leaves=n_clusters)
plt.show()
labels = get_cluster_labels(linkage=linkage, n_clusters=n_clusters,␣
 ↪index=feature_protnlm_keywords.index.tolist())
display(labels.value_counts())
records_not_go_dict = {k:v for k,v in records_all_dict.items() if "GO" not in k}
records_not_go_dict["Keywords substrates"] = keywords_ecoli_substrate.
 ↪to_records(index=False).tolist()
reference_set = set(sequences_substrate_keywords.index.tolist())
cluster_enrichment_analysis(labels, reference_set,␣
 ↪annotations_dict=records_not_go_dict,p_cutoff=0.05)
```





```
2    82
1    44
4    32
3    21
5    19
6    17
dtype: int64
============================================================
CLUSTER 1
============================================================
Keywords
               annotation  total_reference  annotated_reference  total_subset  \
0               Cytoplasm              215                   10            44
5          Phosphoprotein              215                   19            44
6             Transferase              215                   19            44
2  Hydrogen ion transport              215                   10            44
1           ATP synthesis              215                    7            44
3           Translocation              215                    8            44
4                   CF(1)              215                    4            44

   annotated_subset  expected  percentage_of_annotated       lfc        p  \
0                 9  2.046512                    90.00  2.136758  0.000003
5                 9  3.888372                    47.37  1.210759  0.005354
6                 9  3.888372                    47.37  1.210759  0.005354
2                 8  2.046512                    80.00  1.966833  0.000057
1                 7  1.432558                   100.00  2.288761  0.000010
3                 7  1.637209                    87.50  2.096116  0.000068
4                 4  0.818605                   100.00  2.288761  0.001568

      p_fdr  p_bonferroni
0  0.000132      0.000132
5  0.041187      0.267714
6  0.041187      0.267714
2  0.000957      0.002870
1  0.000251      0.000502
3  0.000848      0.003390
4  0.015681      0.078407

TCDB Class

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

TCDB Mechanism

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

Interpro Domains

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

Keywords substrates

               annotation  total_reference  annotated_reference  total_subset  \
0  Hydrogen ion transport              215                   10            44
1           Translocation              215                    8            44

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 8  2.046512                     80.0  1.966833  0.000057
1                 7  1.637209                     87.5  2.096116  0.000068
```

```
      p_fdr  p_bonferroni
0  0.000689      0.000689
1  0.000407      0.000814

============================================================
CLUSTER 2
============================================================
Keywords

                   annotation  total_reference  annotated_reference  \
1         Transmembrane helix              215                  157
2               Transmembrane              215                  175
3         Cell inner membrane              215                  181
4               Cell membrane              215                  181
6                    Membrane              215                  199
0                     Symport              215                   31
9             Sugar transport              215                   56
10  Phosphotransferase system              215                   24
8                    Antiport              215                   12
5                      Sodium              215                    9
7            Sodium transport              215                    8

    total_subset  annotated_subset   expected  percentage_of_annotated  \
1             82                82  59.879070                    52.23
2             82                82  66.744186                    46.86
3             82                82  69.032558                    45.30
4             82                82  69.032558                    45.30
6             82                82  75.897674                    41.21
0             82                31  11.823256                   100.00
9             82                31  21.358140                    55.36
10            82                15   9.153488                    62.50
8             82                10   4.576744                    83.33
5             82                 9   3.432558                   100.00
7             82                 8   3.051163                   100.00

         lfc             p         p_fdr  p_bonferroni
1   0.453572  1.515750e-15  4.244100e-14  8.488200e-14
2   0.296982  3.116372e-10  5.817227e-09  1.745168e-08
3   0.248347  1.249118e-08  1.554458e-07  6.995063e-07
4   0.248347  1.249118e-08  1.554458e-07  6.995063e-07
6   0.111568  3.184434e-04  2.547547e-03  1.783283e-02
0   1.390641  1.472106e-15  8.243794e-14  8.243794e-14
9   0.537482  1.870410e-03  1.047430e-02  1.047430e-01
10  0.712569  9.344745e-03  4.757325e-02  5.233057e-01
8   1.127606  1.390931e-03  8.654680e-03  7.789212e-02
5   1.390641  1.283685e-04  1.198106e-03  7.188636e-03
7   1.390641  3.590848e-04  2.513594e-03  2.010875e-02

TCDB Class
```

```
  annotation  total_reference  annotated_reference  total_subset  \
0        2.A              215                   83            82   
1        4.A              215                   23            82   

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                61  31.655814                    73.49  0.946339   
1                14   8.772093                    60.87  0.674434   

              p         p_fdr  p_bonferroni  
0  1.400965e-17  7.004827e-17  7.004827e-17  
1  1.698887e-02  4.247218e-02  8.494437e-02  
TCDB Mechanism
  annotation  total_reference  annotated_reference  total_subset  \
0          2              215                   83            82   
1          4              215                   23            82   

   annotated_subset   expected  percentage_of_annotated       lfc  \
0                61  31.655814                    73.49  0.946339   
1                14   8.772093                    60.87  0.674434   

              p         p_fdr  p_bonferroni  
0  1.400965e-17  5.603862e-17  5.603862e-17  
1  1.698887e-02  3.397775e-02  6.795550e-02  
Interpro Domains
                                          annotation  total_reference  \
0                        MFS transporter superfamily              215   
1               Major facilitator superfamily domain              215   
2                    Phosphotransferase system, EIIC              215   
3  Phosphotransferase system, EIIC component, type 1              215   
4                      Major facilitator superfamily              215   
5                        Glucose permease domain IIB              215   
6  Phosphotransferase system EIIB, cysteine phosp…              215   
7   Phosphotransferase system, IIB component, type 1              215   
8                 Amino acid permease/ SLC12A domain              215   

   annotated_reference  total_subset  annotated_subset  expected  \
0                   15            82                15  5.720930   
1                   13            82                13  4.958140   
2                   12            82                11  4.576744   
3                    7            82                 7  2.669767   
4                    6            82                 6  2.288372   
5                    6            82                 6  2.288372   
6                    6            82                 6  2.288372   
7                    6            82                 6  2.288372   
8                    6            82                 6  2.288372   
```

```
   percentage_of_annotated       lfc             p     p_fdr  p_bonferroni
0                   100.00  1.390641  2.215815e-07  0.000028      0.000028
1                   100.00  1.390641  1.917445e-06  0.000120      0.000240
2                    91.67  1.265110  1.305564e-04  0.005440      0.016320
3                   100.00  1.390641  9.958619e-04  0.031121      0.124483
4                   100.00  1.390641  2.738620e-03  0.048904      0.342328
5                   100.00  1.390641  2.738620e-03  0.048904      0.342328
6                   100.00  1.390641  2.738620e-03  0.048904      0.342328
7                   100.00  1.390641  2.738620e-03  0.048904      0.342328
8                   100.00  1.390641  2.738620e-03  0.048904      0.342328

Keywords substrates

         annotation  total_reference  annotated_reference  total_subset  \
1   Sugar transport              215                   56            82
0  Sodium transport              215                    8            82

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
1                31  21.358140                    55.36  0.537482  0.001870
0                 8   3.051163                   100.00  1.390641  0.000359

      p_fdr  p_bonferroni
1  0.012158      0.024315
0  0.004668      0.004668

============================================================
CLUSTER 3
============================================================
Keywords

            annotation  total_reference  annotated_reference  total_subset  \
0  Transmembrane helix              215                  157            21

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
0                21  15.334884                    13.38  0.453572  0.000918

      p_fdr  p_bonferroni
0  0.018369      0.018369

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        3.A              215                   64            21

   annotated_subset  expected  percentage_of_annotated       lfc  \
0                19  6.251163                    29.69  1.603803

              p         p_fdr  p_bonferroni
0  1.489553e-09  1.489553e-09  1.489553e-09

TCDB Mechanism
```

```
  annotation  total_reference  annotated_reference  total_subset  \
0          3              215                   68            21

   annotated_subset  expected  percentage_of_annotated      lfc             p  \
0                19   6.64186                    27.94  1.51634  5.444385e-09

          p_fdr  p_bonferroni
0  5.444385e-09  5.444385e-09

Interpro Domains

                                          annotation  total_reference  \
0  ABC transporter type 1, transmembrane domain M…              215
1                              MetI-like superfamily              215
2                         ABC transporter, BtuC-like              215
3                          ABC transporter, permease              215
4        ABC transporter, permease protein, BtuC-like              215
5  ABC transporter type 1, GsiC-like, N-terminal …              215
6  Amino acid ABC transporter, permease protein, …              215
7  Oligopeptide transport permease C-like, N-term…              215

   annotated_reference  total_subset  annotated_subset  expected  \
0                   13            21                10  1.269767
1                   13            21                10  1.269767
2                    6            21                 6  0.586047
3                    5            21                 5  0.488372
4                    5            21                 5  0.488372
5                    3            21                 3  0.293023
6                    2            21                 2  0.195349
7                    2            21                 2  0.195349

   percentage_of_annotated       lfc             p         p_fdr  p_bonferroni
0                    76.92  2.977364  1.845054e-09  1.845054e-08  2.767581e-08
1                    76.92  2.977364  1.845054e-09  1.845054e-08  2.767581e-08
2                   100.00  3.355875  4.243995e-07  2.121997e-06  6.365992e-06
3                   100.00  3.355875  5.570243e-06  1.856748e-05  8.355365e-05
4                   100.00  3.355875  5.570243e-06  1.856748e-05  8.355365e-05
5                   100.00  3.355875  8.142749e-04  2.035687e-03  1.221412e-02
6                   100.00  3.355875  9.128450e-03  1.825690e-02  1.369268e-01
7                   100.00  3.355875  9.128450e-03  1.825690e-02  1.369268e-01

Keywords substrates

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

============================================================
CLUSTER 4
============================================================
Keywords

         annotation  total_reference  annotated_reference  total_subset  \
2      3D-structure              215                   78            32   
1            Signal              215                   29            32   
0         Periplasm              215                    6            32   
3  Copper transport              215                    3            32   

   annotated_subset   expected  percentage_of_annotated       lfc         p  \
2                20  11.609302                    25.64  0.784719  0.001010   
1                11   4.316279                    37.93  1.349643  0.000805   
0                 6   0.893023                   100.00  2.748193  0.000007   
3                 3   0.446512                   100.00  2.748193  0.003037   

      p_fdr  p_bonferroni  
2  0.020531      0.061593  
1  0.024545      0.049091  
0  0.000432      0.000432  
3  0.046310      0.185238  

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        9.A              215                    2            32   

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 2  0.297674                    100.0  2.748193  0.021561   

      p_fdr  p_bonferroni  
0  0.043121      0.129363  

TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          9              215                    2            32   

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 2  0.297674                    100.0  2.748193  0.021561   

      p_fdr  p_bonferroni  
0  0.043121      0.086242  

Interpro Domains

                                           annotation  total_reference  \
0   ABC transporter type 1, transmembrane domain s…              215   
1     P-type ATPase, transmembrane domain superfamily              215   
2           Peptide/nickel binding protein, MppA-type              215   
3                     Solute-binding protein family 5              215   
```

```
4     Solute-binding protein family 5, conserved site              215
5              Solute-binding protein family 5 domain              215
6         ABC transporter type 1, transmembrane domain              215
7                  P-type ATPase, cytoplasmic domain N              215
8                 P-type ATPase, phosphorylation site              215
9                P-type ATPase, A domain superfamily              215
10                               HAD-like superfamily              215
11                                    HAD superfamily              215
12                                      P-type ATPase              215
13         P-type ATPase, haloacid dehalogenase domain              215

    annotated_reference  total_subset  annotated_subset  expected  \
0                     4            32                 4  0.595349
1                     4            32                 4  0.595349
2                     4            32                 4  0.595349
3                     4            32                 4  0.595349
4                     4            32                 4  0.595349
5                     4            32                 4  0.595349
6                     3            32                 3  0.446512
7                     3            32                 3  0.446512
8                     3            32                 3  0.446512
9                     3            32                 3  0.446512
10                    3            32                 3  0.446512
11                    3            32                 3  0.446512
12                    3            32                 3  0.446512
13                    3            32                 3  0.446512

    percentage_of_annotated       lfc         p     p_fdr  p_bonferroni
0                     100.0  2.748193  0.000415  0.010800      0.037801
1                     100.0  2.748193  0.000415  0.010800      0.037801
2                     100.0  2.748193  0.000415  0.010800      0.037801
3                     100.0  2.748193  0.000415  0.010800      0.037801
4                     100.0  2.748193  0.000415  0.010800      0.037801
5                     100.0  2.748193  0.000415  0.010800      0.037801
6                     100.0  2.748193  0.003037  0.026318      0.276339
7                     100.0  2.748193  0.003037  0.026318      0.276339
8                     100.0  2.748193  0.003037  0.026318      0.276339
9                     100.0  2.748193  0.003037  0.026318      0.276339
10                    100.0  2.748193  0.003037  0.026318      0.276339
11                    100.0  2.748193  0.003037  0.026318      0.276339
12                    100.0  2.748193  0.003037  0.026318      0.276339
13                    100.0  2.748193  0.003037  0.026318      0.276339
Keywords substrates

        annotation  total_reference  annotated_reference  total_subset  \
0  Copper transport              215                    3            32

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 3  0.446512                   100.0  2.748193  0.003037

      p_fdr  p_bonferroni
0  0.039477      0.039477

============================================================
CLUSTER 5
============================================================
Keywords

           annotation  total_reference  annotated_reference  total_subset  \
0         ATP-binding              215                   28            19
1  Nucleotide-binding              215                   30            19
2         Translocase              215                   23            19
3              Repeat              215                   10            19

   annotated_subset  expected  percentage_of_annotated       lfc  \
0                19  2.474419                    67.86  2.940838
1                19  2.651163                    63.33  2.841302
2                14  2.032558                    60.87  2.784058
3                 4  0.883721                    40.00  2.178337

              p         p_fdr  p_bonferroni
0  9.200664e-21  2.300166e-19  2.300166e-19
1  7.276889e-20  9.096111e-19  1.819222e-18
2  2.282096e-12  1.901746e-11  5.705239e-11
3  6.609229e-03  4.130768e-02  1.652307e-01

TCDB Class

  annotation  total_reference  annotated_reference  total_subset  \
0        3.A              215                   64            19

   annotated_subset  expected  percentage_of_annotated       lfc  \
0                17  5.655814                    26.56  1.587728

              p         p_fdr  p_bonferroni
0  2.154527e-08  2.154527e-08  2.154527e-08

TCDB Mechanism

  annotation  total_reference  annotated_reference  total_subset  \
0          3              215                   68            19

   annotated_subset  expected  percentage_of_annotated       lfc  \
0                17  6.009302                     25.0  1.500265

              p         p_fdr  p_bonferroni
0  6.679566e-08  6.679566e-08  6.679566e-08

Interpro Domains
```

```
                                          annotation   total_reference  \
0            ABC transporter-like, ATP-binding domain              215
1                                   AAA+ ATPase domain              215
2   P-loop containing nucleoside triphosphate hydr…              215
3                ABC transporter-like, conserved site              215
4   Oligopeptide/dipeptide ABC transporter, C-term…              215
5   ABC-type amino acid transport system, ATPase c…              215
6   ABC transporter, maltose/maltodextrin import, …              215
7             Molybdate/tungstate binding, C-terminal              215
8                       Nucleic acid-binding, OB-fold              215
9                                MalK, OB fold domain              215
10                             CBS domain superfamily              215

    annotated_reference  total_subset  annotated_subset  expected  \
0                    22            19                19  1.944186
1                    23            19                19  2.032558
2                    25            19                19  2.209302
3                    20            19                17  1.767442
4                     3            19                 3  0.265116
5                     3            19                 3  0.265116
6                     2            19                 2  0.176744
7                     2            19                 2  0.176744
8                     2            19                 2  0.176744
9                     2            19                 2  0.176744
10                    2            19                 2  0.176744

    percentage_of_annotated       lfc             p         p_fdr  \
0                     86.36  3.288761  2.051430e-24  5.128575e-23
1                     82.61  3.224631  1.179572e-23  1.474465e-22
2                     76.00  3.104337  2.359145e-22  1.965954e-21
3                     85.00  3.265800  2.877353e-20  1.798346e-19
4                    100.00  3.500265  5.932574e-04  2.696625e-03
5                    100.00  3.500265  5.932574e-04  2.696625e-03
6                    100.00  3.500265  7.433167e-03  2.064769e-02
7                    100.00  3.500265  7.433167e-03  2.064769e-02
8                    100.00  3.500265  7.433167e-03  2.064769e-02
9                    100.00  3.500265  7.433167e-03  2.064769e-02
10                   100.00  3.500265  7.433167e-03  2.064769e-02

    p_bonferroni
0   5.128575e-23
1   2.948931e-22
2   5.897862e-21
3   7.193383e-19
4   1.483144e-02
5   1.483144e-02
6   1.858292e-01
7   1.858292e-01
```

```
8   1.858292e-01
9   1.858292e-01
10  1.858292e-01

Keywords substrates

Empty DataFrame
Columns: [annotation, total_reference, annotated_reference, total_subset,␣
 ↪annotated_subset, expected, percentage_of_annotated, lfc, p, p_fdr,␣
 ↪p_bonferroni]
Index: []

============================================================
CLUSTER 6
============================================================
Keywords

                   annotation  total_reference  annotated_reference  \
0         Cell outer membrane              215                   18
1   Transmembrane beta strand              215                   18
2                      Signal              215                   29
13              Transmembrane              215                  175
5               Ion transport              215                   81
8                3D-structure              215                   78
3                       Porin              215                   12
7   Direct protein sequencing              215                   44
4                    Receptor              215                    6
6                    TonB box              215                    5
10             Iron transport              215                   16
12                       Iron              215                   18
9              Disulfide bond              215                    5
11      Host-virus interaction              215                    2

    total_subset  annotated_subset   expected  percentage_of_annotated  \
0             17                17   1.423256                    94.44
1             17                17   1.423256                    94.44
2             17                17   2.293023                    58.62
13            17                17  13.837209                     9.71
5             17                16   6.404651                    19.75
8             17                13   6.167442                    16.67
3             17                11   0.948837                    91.67
7             17                11   3.479070                    25.00
4             17                 6   0.474419                   100.00
6             17                 5   0.395349                   100.00
10            17                 5   1.265116                    31.25
12            17                 5   1.423256                    27.78
9             17                 3   0.395349                    60.00
11            17                 2   0.158140                   100.00
```

```
         lfc             p         p_fdr  p_bonferroni
0   3.578268  2.734726e-24  4.922507e-23  7.383760e-23
1   3.578268  2.734726e-24  4.922507e-23  7.383760e-23
2   2.890212  7.884509e-18  7.096058e-17  2.128817e-16
13  0.296982  2.591634e-02  4.998151e-02  6.997411e-01
5   1.320880  7.034408e-07  3.165484e-06  1.899290e-05
8   1.075768  5.422188e-04  1.626657e-03  1.463991e-02
3   3.535199  1.648835e-13  1.112963e-12  4.451854e-12
7   1.660730  4.099476e-05  1.383573e-04  1.106858e-03
4   3.660730  9.679287e-08  5.226815e-07  2.613407e-06
6   3.660730  1.693875e-06  6.533518e-06  4.573463e-05
10  1.982658  4.295251e-03  1.054289e-02  1.159718e-01
12  1.812733  7.619602e-03  1.582533e-02  2.057293e-01
9   2.923764  3.760980e-03  1.015465e-02  1.015465e-01
11  3.660730  5.911758e-03  1.330146e-02  1.596175e-01
```

TCDB Class

```
  annotation  total_reference  annotated_reference  total_subset  \
0        1.B              215                   18            17   

   annotated_subset  expected  percentage_of_annotated       lfc  \
0                17  1.423256                    94.44  3.578268   

              p         p_fdr  p_bonferroni  
0  2.734726e-24  2.734726e-24  2.734726e-24  
```

TCDB Mechanism

```
  annotation  total_reference  annotated_reference  total_subset  \
0          1              215                   26            17   

   annotated_subset  expected  percentage_of_annotated       lfc  \
0                17  2.055814                    65.38  3.047753   

              p         p_fdr  p_bonferroni  
0  4.747105e-19  4.747105e-19  4.747105e-19  
```

Interpro Domains

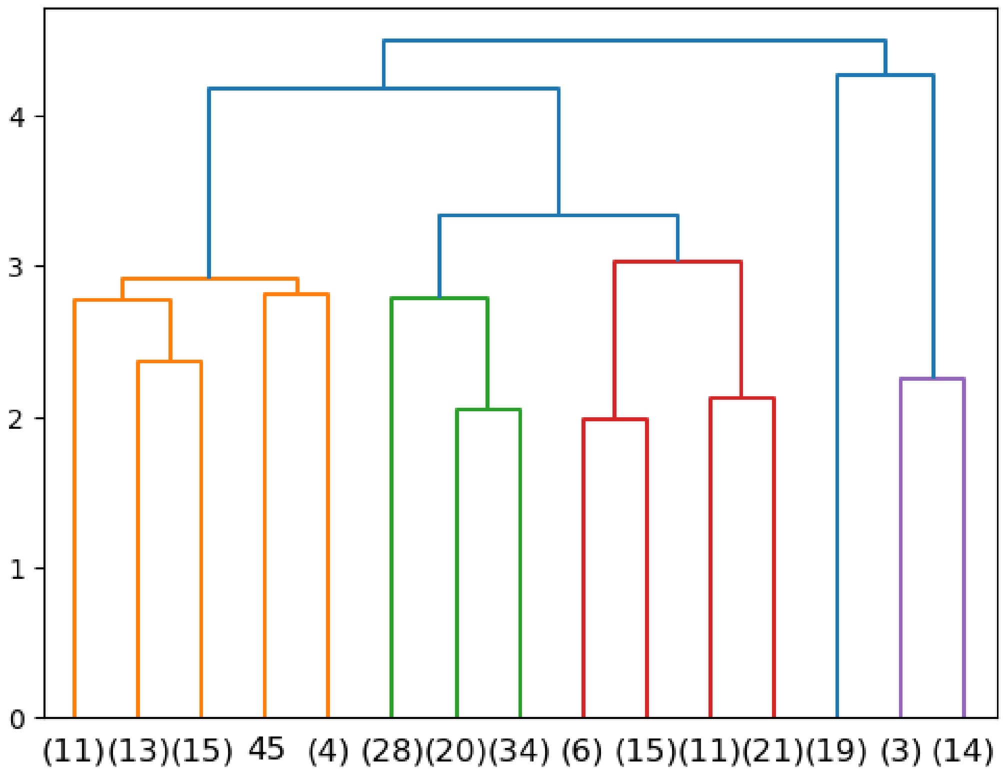
```
                                           annotation  total_reference  \
0                TonB-dependent receptor, plug domain              215   
2           TonB-dependent receptor-like, beta-barrel              215   
3                            TonB box, conserved site              215   
4   TonB-dependent receptor-like, beta-barrel doma…              215   
1    TonB-dependent receptor, plug domain superfamily              215   
5             TonB-dependent receptor, conserved site              215   
6                 TonB-dependent siderophore receptor              215   
7                            Porin domain superfamily              215   
10                       TonB-dependent receptor-like              215   
8                         Porin, gammaproteobacterial              215   
```

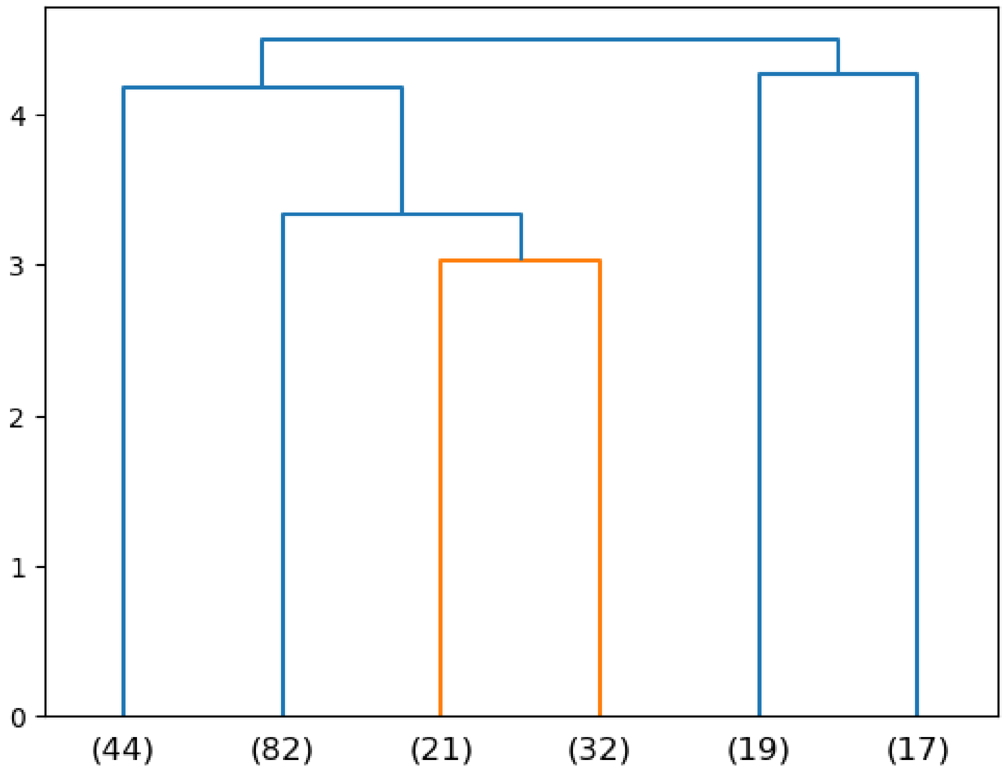
```
9                           Porin, Gram-negative type              215
11                  Vitamin B12 transporter BtuB-like              215
12                   Porin domain, Gram-negative type              215
13          Porin, Gram-negative type, conserved site              215
14                                   Porin, LamB-type              215
15                       Porin, LamB-type superfamily              215
16                  Oligogalacturonate-specific porin              215

    annotated_reference  total_subset  annotated_subset  expected  \
0                     5            17                 5  0.395349
2                     5            17                 5  0.395349
3                     5            17                 5  0.395349
4                     5            17                 5  0.395349
1                     5            17                 5  0.395349
5                     4            17                 4  0.316279
6                     4            17                 4  0.316279
7                     4            17                 4  0.316279
10                    3            17                 3  0.237209
8                     3            17                 3  0.237209
9                     3            17                 3  0.237209
11                    2            17                 2  0.158140
12                    2            17                 2  0.158140
13                    2            17                 2  0.158140
14                    2            17                 2  0.158140
15                    2            17                 2  0.158140
16                    2            17                 2  0.158140

    percentage_of_annotated      lfc         p     p_fdr  p_bonferroni
0                     100.0  3.66073  0.000002  0.000019      0.000056
2                     100.0  3.66073  0.000002  0.000019      0.000056
3                     100.0  3.66073  0.000002  0.000019      0.000056
4                     100.0  3.66073  0.000002  0.000019      0.000056
1                     100.0  3.66073  0.000002  0.000019      0.000056
5                     100.0  3.66073  0.000027  0.000130      0.000907
6                     100.0  3.66073  0.000027  0.000130      0.000907
7                     100.0  3.66073  0.000027  0.000130      0.000907
10                    100.0  3.66073  0.000416  0.001374      0.013739
8                     100.0  3.66073  0.000416  0.001374      0.013739
9                     100.0  3.66073  0.000416  0.001374      0.013739
11                    100.0  3.66073  0.005912  0.013454      0.195088
12                    100.0  3.66073  0.005912  0.013454      0.195088
13                    100.0  3.66073  0.005912  0.013454      0.195088
14                    100.0  3.66073  0.005912  0.013454      0.195088
15                    100.0  3.66073  0.005912  0.013454      0.195088
16                    100.0  3.66073  0.005912  0.013454      0.195088
```

```
Keywords substrates

       annotation  total_reference  annotated_reference  total_subset  \
0   Ion transport              215                   81            17
1  Iron transport              215                   16            17

   annotated_subset  expected  percentage_of_annotated       lfc  \
0                16  6.404651                    19.75  1.320880
1                 5  1.265116                    31.25  1.982658

              p     p_fdr  p_bonferroni
0  7.034408e-07  0.000004      0.000004
1  4.295251e-03  0.010738      0.021476
```

What is the substrate dist.?

```
[ ]: import matplotlib.pyplot as plt
     n_clusters = 6
     linkage = get_linkage(feature_protnlm_keywords,standardize=False)
     dendrogram = dendrogram_plot(linkage)
     plt.show()
     dendrogram = dendrogram_plot(linkage,max_leaves=n_clusters)
     plt.show()
     labels = get_cluster_labels(linkage=linkage, n_clusters=n_clusters,␣
       ↪index=feature_protnlm_keywords.index.tolist())
     display(labels.value_counts())
     records_not_go_dict = dict()
     records_not_go_dict["Keywords substrates"] = keywords_ecoli_substrate.
       ↪to_records(index=False).tolist()
     reference_set = set(sequences_substrate_keywords.index.tolist())
     cluster_enrichment_analysis(labels, reference_set,␣
       ↪annotations_dict=records_not_go_dict,p_cutoff=1.1)
```


4
3
2
1
0
(11)
(13)
(15)
45
(4)
(28)
(20)
(34)
(6)
(15)
(11)
(21)
(19)
(3)
(14)


4
3
2
1
0
(44)
(82)
(21)
(32)
(19)
(17)

```
2    82
1    44
4    32
3    21
5    19
6    17
dtype: int64

============================================================
CLUSTER 1
============================================================
Keywords substrates
                annotation  total_reference  annotated_reference  \
6            Ion transport              215                   81   
10         Sugar transport              215                   56   
0   Hydrogen ion transport              215                   10   
9     Amino-acid transport              215                   48   
1            Translocation              215                    8   
5        Protein transport              215                   27   
3           Zinc transport              215                    7   
2         Cobalt transport              215                    2   
4       Electron transport              215                    5   
8      Potassium transport              215                   10   
11          Iron transport              215                   16   
7         Nickel transport              215                    3   

    total_subset  annotated_subset   expected  percentage_of_annotated  \
6             44                18  16.576744                    22.22   
10            44                 9  11.460465                    16.07   
0             44                 8   2.046512                    80.00   
9             44                 8   9.823256                    16.67   
1             44                 7   1.637209                    87.50   
5             44                 7   5.525581                    25.93   
3             44                 3   1.432558                    42.86   
2             44                 2   0.409302                   100.00   
4             44                 2   1.023256                    40.00   
8             44                 2   2.046512                    20.00   
11            44                 2   3.274419                    12.50   
7             44                 1   0.613953                    33.33   

         lfc         p     p_fdr  p_bonferroni  
6   0.118836  0.370942  0.635901      1.000000  
10 -0.348669  0.874303  0.953785      1.000000  
0   1.966833  0.000057  0.000689      0.000689  
9  -0.296201  0.826530  0.991836      1.000000  
```

```
1   2.096116  0.000068  0.000407      0.000814
5   0.341229  0.299871  0.599742      1.000000
3   1.066369  0.152884  0.458652      1.000000
2   2.288761  0.041121  0.164486      0.493458
4   0.966833  0.271379  0.651309      1.000000
8  -0.033167  0.643991  0.858655      1.000000
11 -0.711239  0.878572  0.878572      1.000000
7   0.703799  0.498697  0.748046      1.000000

============================================================
CLUSTER 2
============================================================
Keywords substrates

                annotation  total_reference  annotated_reference  \
1          Sugar transport              215                   56
2     Amino-acid transport              215                   48
12           Ion transport              215                   81
0         Sodium transport              215                    8
5        Peptide transport              215                   19
8        Protein transport              215                   27
7      Potassium transport              215                   10
3                 Chloride              215                    2
6      Phosphate transport              215                    4
10  Hydrogen ion transport              215                   10
4        Ammonia transport              215                    1
9       Electron transport              215                    5
11          Zinc transport              215                    7

    total_subset  annotated_subset   expected  percentage_of_annotated  \
1             82                31  21.358140                    55.36
2             82                25  18.306977                    52.08
12            82                20  30.893023                    24.69
0             82                 8   3.051163                   100.00
5             82                 8   7.246512                    42.11
8             82                 8  10.297674                    29.63
7             82                 3   3.813953                    30.00
3             82                 2   0.762791                   100.00
6             82                 2   1.525581                    50.00
10            82                 2   3.813953                    20.00
4             82                 1   0.381395                   100.00
9             82                 1   1.906977                    20.00
11            82                 1   2.669767                    14.29

         lfc         p     p_fdr  p_bonferroni
1   0.537482  0.001870  0.012158      0.024315
2   0.449535  0.019150  0.082983      0.248950
12 -0.627281  0.999598  0.999598      1.000000
0   1.390641  0.000359  0.004668      0.004668
```

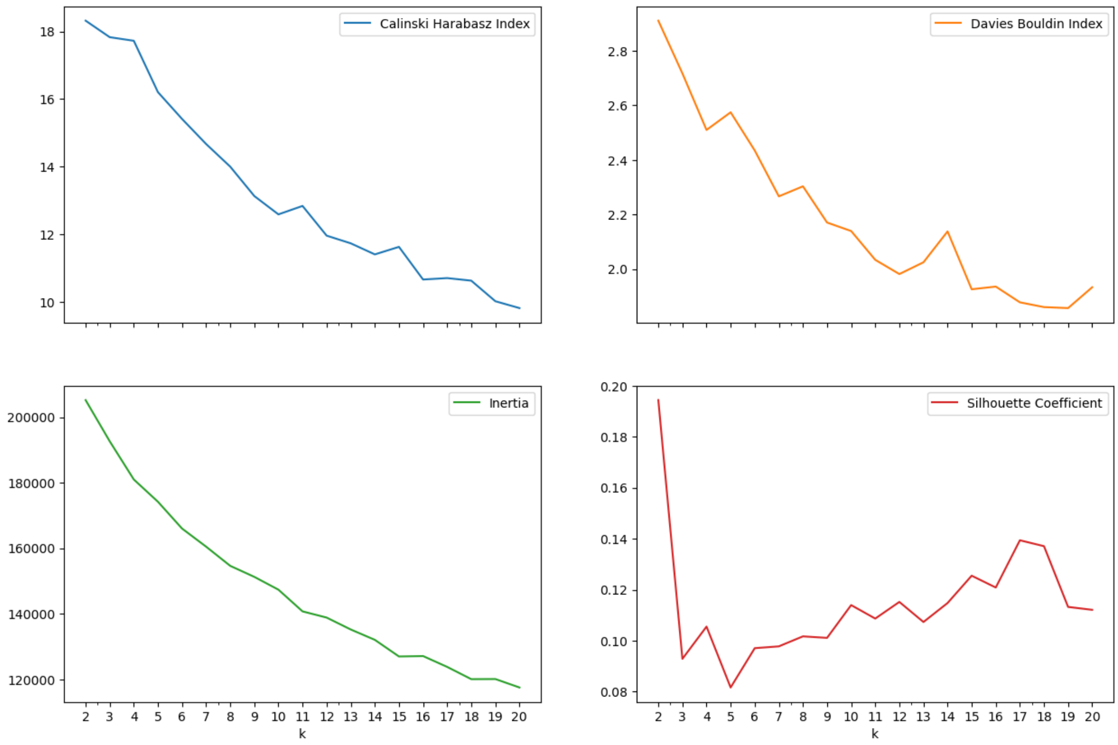
```
5   0.142713  0.443887  0.961756      1.000000
8  -0.364247  0.883362  1.000000      1.000000
7  -0.346325  0.807017  1.000000      1.000000
3   1.390641  0.144360  0.469170      1.000000
6   0.390641  0.493016  0.915600      1.000000
10 -0.931287  0.945376  1.000000      1.000000
4   1.390641  0.381395  0.991628      1.000000
9  -0.931287  0.912021  1.000000      1.000000
11 -1.416714  0.967414  1.000000      1.000000

============================================================
CLUSTER 3
============================================================
Keywords substrates
             annotation  total_reference  annotated_reference  total_subset  \
6         Ion transport              215                   81            21
0        Iron transport              215                   16            21
2     Peptide transport              215                   19            21
3     Protein transport              215                   27            21
7  Amino-acid transport              215                   48            21
8       Sugar transport              215                   56            21
1      Nickel transport              215                    3            21
4   Phosphate transport              215                    4            21
5        Zinc transport              215                    7            21

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
6                 8  7.911628                     9.88  0.016025  0.571014
0                 5  1.562791                    31.25  1.677804  0.011737
2                 4  1.855814                    21.05  1.107948  0.098155
3                 4  2.637209                    14.81  0.600988  0.260140
7                 4  4.688372                     8.33 -0.229087  0.735239
8                 4  5.469767                     7.14 -0.451479  0.849499
1                 2  0.293023                    66.67  2.770913  0.025757
4                 1  0.390698                    25.00  1.355875  0.339115
5                 1  0.683721                    14.29  0.548521  0.518217

      p_fdr  p_bonferroni
6  0.734160      1.000000
0  0.105634      0.105634
2  0.294464      0.883391
3  0.585315      1.000000
7  0.827144      1.000000
8  0.849499      1.000000
1  0.115906      0.231811
4  0.610407      1.000000
5  0.777326      1.000000

============================================================
CLUSTER 4
============================================================
Keywords substrates
              annotation  total_reference  annotated_reference  total_subset  \
5          Ion transport              215                   81            32
6      Protein transport              215                   27            32
2      Peptide transport              215                   19            32
1    Potassium transport              215                   10            32
11  Amino-acid transport              215                   48            32
0       Copper transport              215                    3            32
12       Sugar transport              215                   56            32
4     Electron transport              215                    5            32
9         Iron transport              215                   16            32
3      Sulfate transport              215                    1            32
7        Lipid transport              215                    2            32
8         Zinc transport              215                    7            32
10         Translocation              215                    8            32

    annotated_subset   expected  percentage_of_annotated       lfc         p  \
5                 15  12.055814                    18.52  0.315233  0.166697
6                  6   4.018605                    22.22  0.578268  0.191685
2                  5   2.827907                    26.32  0.822193  0.131155
1                  4   1.488372                    40.00  1.426265  0.044596
11                 4   7.144186                     8.33 -0.836770  0.960237
0                  3   0.446512                   100.00  2.748193  0.003037
12                 3   8.334884                     5.36 -1.474200  0.997255
4                  2   0.744186                    40.00  1.426265  0.160882
9                  2   2.381395                    12.50 -0.251807  0.722884
3                  1   0.148837                   100.00  2.748193  0.148837
7                  1   0.297674                    50.00  1.748193  0.276114
8                  1   1.041860                    14.29 -0.059162  0.681944
10                 1   1.190698                    12.50 -0.251807  0.730875

       p_fdr  p_bonferroni
5   0.361176      1.000000
6   0.355986      1.000000
2   0.568338      1.000000
1   0.289875      0.579750
11  1.000000      1.000000
0   0.039477      0.039477
12  0.997255      1.000000
4   0.418293      1.000000
9   0.939750      1.000000
3   0.483721      1.000000
7   0.448685      1.000000
8   0.985030      1.000000
10  0.863762      1.000000
```

```
============================================================
CLUSTER 5
============================================================
Keywords substrates

             annotation  total_reference  annotated_reference  total_subset  \
0  Amino-acid transport              215                   48            19
5       Sugar transport              215                   56            19
8         Ion transport              215                   81            19
2        Iron transport              215                   16            19
4     Peptide transport              215                   19            19
7     Protein transport              215                   27            19
1   Phosphate transport              215                    4            19
3        Zinc transport              215                    7            19
6   Potassium transport              215                   10            19

   annotated_subset  expected  percentage_of_annotated       lfc         p  \
0                 7  4.241860                    14.58  0.722658  0.099919
5                 5  4.948837                     8.93  0.014839  0.582897
8                 4  7.158140                     4.94 -0.839585  0.969734
2                 2  1.413953                    12.50  0.500265  0.424041
4                 2  1.679070                    10.53  0.252338  0.519096
7                 2  2.386047                     7.41 -0.254622  0.722186
1                 1  0.353488                    25.00  1.500265  0.311218
3                 1  0.618605                    14.29  0.692910  0.481770
6                 1  0.883721                    10.00  0.178337  0.611776

      p_fdr  p_bonferroni
0  0.899274      0.899274
5  0.874346      1.000000
8  0.969734      1.000000
2  1.000000      1.000000
4  0.934373      1.000000
7  0.812459      1.000000
1  1.000000      1.000000
3  1.000000      1.000000
6  0.786569      1.000000

============================================================
CLUSTER 6
============================================================
Keywords substrates

                 annotation  total_reference  annotated_reference  \
0             Ion transport              215                   81
1            Iron transport              215                   16
4           Sugar transport              215                   56
2  Polysaccharide transport              215                    1
3           Lipid transport              215                    2
```

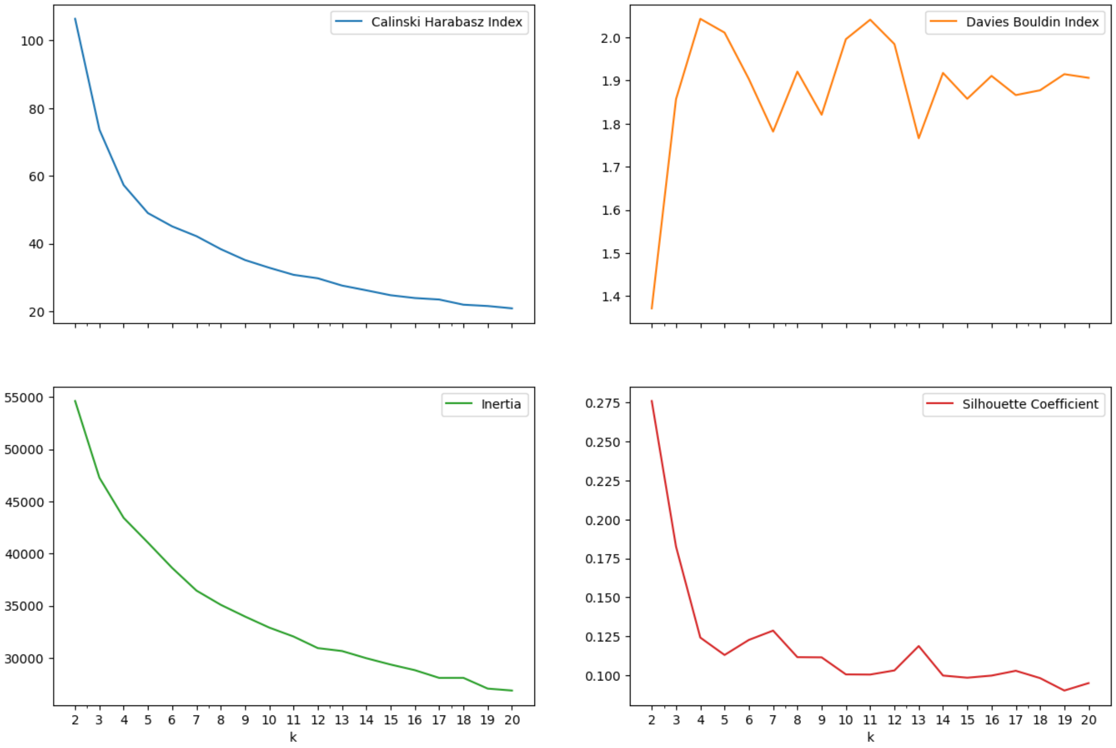
```
   total_subset  annotated_subset  expected  percentage_of_annotated  \
0            17                16  6.404651                    19.75
1            17                 5  1.265116                    31.25
4            17                 4  4.427907                     7.14
2            17                 1  0.079070                   100.00
3            17                 1  0.158140                    50.00

        lfc             p     p_fdr  p_bonferroni
0  1.320880  7.034408e-07  0.000004      0.000004
1  1.982658  4.295251e-03  0.010738      0.021476
4 -0.146625  6.923713e-01  0.692371      1.000000
2  3.660730  7.906977e-02  0.131783      0.395349
3  2.660730  1.522278e-01  0.190285      0.761139
```

```
[ ]: clustering_quality_plots(feature_protnlm_keywords)
```

```
[ ]: array([[<AxesSubplot:xlabel='k'>, <AxesSubplot:xlabel='k'>],
            [<AxesSubplot:xlabel='k'>, <AxesSubplot:xlabel='k'>]], dtype=object)
```



```
[ ]: clustering_quality_plots(feature_pssm_keywords)
[ ]: array([[<AxesSubplot:xlabel='k'>, <AxesSubplot:xlabel='k'>],
            [<AxesSubplot:xlabel='k'>, <AxesSubplot:xlabel='k'>]], dtype=object)
```


Calinski Harabasz Index
Davies Bouldin Index
Inertia
Silhouette Coefficient
k
k