Testing_notebook

March 7, 2024

1 Import Packages and Define Functions

[1]:
```python
# import python embedding from TimsRust
import timsrust_pyo3

from pyteomics import mzml
from psims.transform.mzml import MzMLTransformer, cvstr
from pyteomics.auxiliary import unitfloat
from pyteomics import mgf
from pyteomics import mztab

import os
from typing import Dict, List, Tuple
import fileinput
import json

import numpy as np
import pandas as pd
import matplotlib.pyplot as plt


def flatten(nested_list):
    """
    Flatten a nested list into a single flat list.

    Parameters:
        nested_list (list): The nested list to be flattened.

    Returns:
        list: The flattened list.
    """
    flattened_list = [item for sublist in nested_list for item in sublist]
    return flattened_list

#dataclass
class DenseFrame:
    rt: float
    intensities: list[int]
    mzs: list[float]
    imss: list[float]

    #classmethod
    def from_frame(
        cls, frame: timsrust_pyo3.PyFrame, reader: timsrust_pyo3.TimsReader
    ):
        mzs = reader.resolve_mzs(frame.tof_indices)
        out_imss = [None] * len(mzs)
        last_so = 0
        for ims, so in zip(
            reader.resolve_scans(list(range(1, len(frame.scan_offsets) + 1))),
            frame.scan_offsets,
            strict=True,
        ):
            out_imss[last_so:so] = [ims] * (so - last_so)
            last_so = so

        return cls(
            rt=frame.rt,
            intensities=frame.intensities,
            mzs=mzs,
            imss=out_imss,
        )

class Precursor:
    mz: float
    rt: float
    im: float
    charge: int
    intensity: float
    index: int
    frame_index: int
    collision_energy: float

    #classmethod
    def from_precursor(cls, precursor, reader):
        mz = reader.resolve_mzs(frame.tof_indices)
        rt = frame.rt
        im = 0.0  # Placeholder value, replace with actual logic
        charge = 0  # Placeholder value, replace with actual logic
        intensity = 0.0  # Placeholder value, replace with actual logic
        index = 0  # Placeholder value, replace with actual logic
        frame_index = 0  # Placeholder value, replace with actual logic
        collision_energy = 0.0  # Placeholder value, replace with actual logic
        return cls(
            mz=mz,
            rt=rt,
            im=im,
            charge=charge,
            intensity=intensity,
            index=index,
            frame_index=frame_index,
            collision_energy=collision_energy,
        )

def in_minutes(x):
    '''Convert a time quantity to minutes

    Parameters
    ----------
    x: unitfloat
        A float representing a quantity of time annotated with a time unit

    Returns
    -------
    unitfloat:
        The time after conversion to minutes
    '''
    try:
        unit = x.unit_info
    except AttributeError:
        return x
    if unit == 'minute':
        return x
    elif unit == 'second':
        y = unitfloat(x / 60., 'minute')
        return y
    elif unit == 'hour':
        y = unitfloat(x * 60, 'minute')
        return y
    else:
        warnings.warn("Time unit %r not recognized" % unit)
    return x

def in_seconds(x):
    '''Convert a time quantity to seconds

    Parameters
    ----------
    x: unitfloat
        A float representing a quantity of time annotated with a time unit
    Returns
    -------
    unitfloat:
        The time after conversion to seconds
    '''
    try:
        unit = x.unit_info
    except AttributeError:
        return x
    if unit == 'second':
        return x
    elif unit == 'minute':
        y = unitfloat(x * 60., 'second')
        return y
    elif unit == 'hour':
        y = unitfloat(x * 3600, 'second')
        return y
    else:
        warnings.warn("Time unit %r not recognized" % unit)
    return x
```

2 Retrieve spectra data

[2]:
```
path = "/Users/daviddornig/miniconda3/envs/casanovo_env/lib/python3.10/
  ↪site-packages/Casaval/Testing/example_data/5909_HeLa200ng_1hour_9-14-2023.d"
reader = timsrust_pyo3.TimsReader(path)
```

[3]:
```
TR_all_spectra = reader.read_all_spectra()
```

[4]:
```
TR_spectra_1 = TR_all_spectra[0]
```

[5]:
```
TR_precursor_1 = TR_all_spectra[0].precursor
```

[6]:
```
TR_all_spectra[0]
```

[6]:
```
PySpectrum(index=0, len(mz_values)=67, len(intensities)=67, precursor=(index=1,
frame_index=8, mz=663.2879991882091, im=0.8166844415024028, charge=2,
intensity=1777))
```

[7]:
```
TR_precursor_1.mz
```

[7]:
```
663.2879991882091
```

[8]:
```
reader.read_spectrum(index=0)
```

[8]:
```
PySpectrum(index=0, len(mz_values)=67, len(intensities)=67, precursor=(index=1,
frame_index=8, mz=663.2879991882091, im=0.8166844415024028, charge=2,
intensity=1777))
```

[9]:
```
TR_spectra_1
```

[9]:
```
PySpectrum(index=0, len(mz_values)=67, len(intensities)=67, precursor=(index=1,
frame_index=8, mz=663.2879991882091, im=0.8166844415024028, charge=2,
intensity=1777))
```

[10]:
```
mz_list = TR_all_spectra[0].mz_values
print(mz_list)
```

```
[273.03466354161986, 360.92334212510104, 400.35373277781986, 401.8133103861163,
407.28968561994867, 411.9606209715564, 419.3447774187975, 428.82073145081455,
431.31027364990433, 435.3131368165745, 435.7279407249644, 436.30438496747695,
441.2709076625036, 443.3078997274028, 456.35934079821664, 459.2823697135421,
461.80106136935785, 462.31308821225906, 462.7812813889207, 463.31761960345636,
469.82235562601835, 471.3759916106486, 476.8339136909532, 479.89080257934086,
482.6247081534055, 484.8695263286511, 486.3399744023621, 486.8027739148428,
486.8306184970462, 492.8602086197886, 497.2334970551532, 507.8885452440419,
512.2778749444939, 516.2773941641501, 516.3275761830735, 519.9219113950902,
522.349096619367, 522.4500515243793, 526.9053457203823, 528.7936004552736,
535.3469621998132, 538.8566375378165, 540.8322005116535, 545.0335273609973,
551.8717282692417, 562.7009303653501, 566.3628281289388, 566.8434653915355,
567.3430935046222, 571.7478025801689, 573.9829994640005, 628.5522275047583,
646.3430334387486, 685.7229213285268, 720.734206102824, 733.6875279803832,
757.7501161473994, 780.6883960819159, 820.6577716123243, 832.8864017060561,
898.7264950798839, 912.5743191027257, 916.5959978264356, 917.6278866173682,
920.478146093064, 965.4919164660057, 987.6505337591028]
```

[11]:
```
TR_all_frames = reader.read_all_frames()
```

[12]:
```
TR_all_frames_indexcorrected = {}

for frame in TR_all_frames:
    TR_all_frames_indexcorrected[frame.index] = frame
```

[13]:
```
TR_all_frames_indexcorrected[1]
```

[13]:
```
PyFrame(index=1, rt=0.639134, frame_type=0, len(scan_offsets)=928,
len(tof_indices)=48951, len(intensities)=48951)
```

3 Inspect provided mzml files with pyteomics

[14]:
```
mzml_file = '/Users/daviddornig/miniconda3/envs/casanovo_env/lib/python3.10/
↪site-packages/Casaval/Testing/example_data/5909_HeLa200ng_1hour_9-14-2023.
↪mzml'
```

[15]:
```
with mzml.read(mzml_file) as mzml_data:
    for spectrum in mzml_data:
        # Access spectrum attributes
        mz_values = spectrum['m/z array']
        intensity_values = spectrum['intensity array']
        retention_time = spectrum['scanList']['scan'][0]['scan start time']
```

[16]:
```
with mzml.read(mzml_file) as mzml_data:
    # Initialize an empty dictionary to store spectra data
    MZML_spectra_data = {}

    # Iterate over spectra in the mzML file
    for spectrum in mzml_data:
        # Get spectrum ID
        spectrum_id = spectrum['index']

        # Store spectrum data in the dictionary
        MZML_spectra_data[spectrum_id] = {
            'mz_values': spectrum['m/z array'],
            'intensity_values': spectrum['intensity array'],
            'retention_time': spectrum['scanList']['scan'][0]['scan start␣
↪time'],
            'ion_mobility': spectrum['total ion current']
        }
```

[17]:
```
with mzml.read(mzml_file) as mzml_data:
    # Initialize an empty dictionary to store spectra data
    MZML_spectra_data_detailed = {}

    # Iterate over spectra in the mzML file
    for index, spectrum in enumerate(mzml_data):
        # Store spectrum data in the dictionary
        MZML_spectra_data_detailed[index] = spectrum
```

[18]:
```
limit = 10
count = 0
for count in MZML_spectra_data_detailed:
    if count <= limit:
        print(MZML_spectra_data_detailed[count])
    else:
        break
```

```
{'index': 0, 'defaultArrayLength': 17373, 'id': 'index=1', 'scanList': {'count':
1, 'scan': [{'scanWindowList': {'count': 1, 'scanWindow': [{'scan window lower
limit': 100.0, 'scan window upper limit': 1700.0}]}, 'scan start time':
0.010652233333333334}], 'no combination': ''}, 'ms level': 1, 'total ion
current': 4758905.0, 'base peak intensity': 115579.0, 'base peak m/z':
1221.9882233393096, 'positive scan': '', 'centroid spectrum': '', 'MS1
spectrum': '', 'count': 2, 'm/z array': array([ 100.57569506,  101.09210999,
101.36510722, …, 1698.5229779 ,
       1699.56335017, 1699.85601226]), 'intensity array': array([102.,  66.,
67., …, 101., 122.,  87.], dtype=float32)}
{'index': 1, 'defaultArrayLength': 17444, 'id': 'index=2', 'scanList': {'count':
1, 'scan': [{'scanWindowList': {'count': 1, 'scanWindow': [{'scan window lower
limit': 100.0, 'scan window upper limit': 1700.0}]}, 'scan start time':
0.012646316666666666}], 'no combination': ''}, 'ms level': 1, 'total ion
current': 4777778.0, 'base peak intensity': 114200.0, 'base peak m/z':
1221.9856117368256, 'positive scan': '', 'centroid spectrum': '', 'MS1
spectrum': '', 'count': 2, 'm/z array': array([ 100.15214803,  100.42861666,
100.65481395, …, 1698.89062307,
       1699.17967582, 1699.96007596]), 'intensity array': array([ 80.,  84.,
95., …, 121., 114.,  84.], dtype=float32)}
{'index': 2, 'defaultArrayLength': 17217, 'id': 'index=3', 'scanList': {'count':
1, 'scan': [{'scanWindowList': {'count': 1, 'scanWindow': [{'scan window lower
limit': 100.0, 'scan window upper limit': 1700.0}]}, 'scan start time':
0.0148106}], 'no combination': ''}, 'ms level': 1, 'total ion current':
4671823.0, 'base peak intensity': 114345.0, 'base peak m/z': 1221.9859431775146,
'positive scan': '', 'centroid spectrum': '', 'MS1 spectrum': '', 'count': 2,
'm/z array': array([ 100.24057654,  100.43019758,  101.60984731, …,
1698.98460378,
       1699.38126203, 1699.87552396]), 'intensity array': array([ 92.,  74.,
57., …, 136.,  62.,  25.], dtype=float32)}
{'index': 3, 'defaultArrayLength': 17101, 'id': 'index=4', 'scanList': {'count':
1, 'scan': [{'scanWindowList': {'count': 1, 'scanWindow': [{'scan window lower
limit': 100.0, 'scan window upper limit': 1700.0}]}, 'scan start time':
0.016976516666666667}], 'no combination': ''}, 'ms level': 1, 'total ion
current': 4684455.0, 'base peak intensity': 112523.0, 'base peak m/z':
1221.985758344349, 'positive scan': '', 'centroid spectrum': '', 'MS1 spectrum':
'', 'count': 2, 'm/z array': array([ 100.5408926 ,  101.96254952,  102.05399167,
…, 1698.22392982,
       1698.75703389, 1699.09514323]), 'intensity array': array([ 82., 132.,
84., …, 111.,  39.,  68.], dtype=float32)}
{'index': 4, 'defaultArrayLength': 17311, 'id': 'index=5', 'scanList': {'count':
1, 'scan': [{'scanWindowList': {'count': 1, 'scanWindow': [{'scan window lower
limit': 100.0, 'scan window upper limit': 1700.0}]}, 'scan start time':
0.01913025}], 'no combination': ''}, 'ms level': 1, 'total ion current':
4751362.0, 'base peak intensity': 115445.0, 'base peak m/z': 1221.9860861378704,
'positive scan': '', 'centroid spectrum': '', 'MS1 spectrum': '', 'count': 2,
'm/z array': array([ 101.10479937,  101.19364735,  101.69573515, …,
1698.93908858,
       1699.20568627, 1699.34224441]), 'intensity array': array([185.,  89.,
54., …,  62.,  46.,  61.], dtype=float32)}
{'index': 5, 'defaultArrayLength': 17438, 'id': 'index=6', 'scanList': {'count':
1, 'scan': [{'scanWindowList': {'count': 1, 'scanWindow': [{'scan window lower
limit': 100.0, 'scan window upper limit': 1700.0}]}, 'scan start time':
0.021203816666666667}], 'no combination': ''}, 'ms level': 1, 'total ion
current': 4806505.0, 'base peak intensity': 114527.0, 'base peak m/z':
1221.9870186046128, 'positive scan': '', 'centroid spectrum': '', 'MS1
spectrum': '', 'count': 2, 'm/z array': array([ 101.20634311,  101.65755829,
101.74664876, …, 1698.48397013,
       1698.56460608, 1699.03011959]), 'intensity array': array([128., 106.,
58., …,  75., 121., 103.], dtype=float32)}
{'index': 6, 'defaultArrayLength': 17429, 'id': 'index=7', 'scanList': {'count':
1, 'scan': [{'scanWindowList': {'count': 1, 'scanWindow': [{'scan window lower
limit': 100.0, 'scan window upper limit': 1700.0}]}, 'scan start time':
0.023337516666666665}], 'no combination': ''}, 'ms level': 1, 'total ion
current': 4756743.0, 'base peak intensity': 113811.0, 'base peak m/z':
1221.9865880885236, 'positive scan': '', 'centroid spectrum': '', 'MS1
spectrum': '', 'count': 2, 'm/z array': array([ 100.7228813 ,  101.57804644,
102.21820397, …, 1699.01711889,
       1699.43145084, 1699.5505457 ]), 'intensity array': array([ 72.,  51.,
69., …,  74., 155.,  97.], dtype=float32)}
{'index': 7, 'defaultArrayLength': 17275, 'id': 'index=8', 'scanList': {'count':
1, 'scan': [{'scanWindowList': {'count': 1, 'scanWindow': [{'scan window lower
limit': 100.0, 'scan window upper limit': 1700.0}]}, 'scan start time':
0.025463799999999998}], 'no combination': ''}, 'ms level': 1, 'total ion
current': 4697231.0, 'base peak intensity': 111403.0, 'base peak m/z':
1221.9873663032945, 'positive scan': '', 'centroid spectrum': '', 'MS1
spectrum': '', 'count': 2, 'm/z array': array([ 100.42703599,  100.50451488,
100.51084101, …, 1698.97160326,
       1699.42678703, 1699.77797045]), 'intensity array': array([ 52.,  55.,
10., …,  52., 181.,  63.], dtype=float32)}
{'index': 8, 'defaultArrayLength': 67, 'id': 'index=9', 'scanList': {'count': 1,
'scan': [{'scanWindowList': {'count': 1, 'scanWindow': [{'scan window lower
limit': 100.0, 'scan window upper limit': 1700.0}]}, 'scan start time':
0.025463799999999998}], 'no combination': ''}, 'precursorList': {'count': 1,
'precursor': [{'spectrumRef': 'index=8', 'isolationWindow': {'isolation window
target m/z': 663.2879991882091, 'isolation window lower offset':
0.5917277272992578, 'isolation window upper offset': 1.4082722727007422},
'selectedIonList': {'count': 1, 'selectedIon': [{'selected ion m/z':
663.2879991882091, 'charge state': 2, 'inverse reduced ion mobility':
0.8293334816068573}]}, 'activation': {'collision energy': 33.38565022421525,
'CID': ''}}]}, 'ms level': 2, 'total ion current': 5412.0, 'positive scan': '',
'centroid spectrum': '', 'MSn spectrum': '', 'count': 2, 'm/z array':
array([273.03166788, 360.92126028, 400.35207354, 401.81166621,
       407.2880975 , 411.95908009, 419.3433101 , 428.8193565 ,
       431.30892258, 435.31182382, 435.72663165, 436.30308133,
       441.26965055, 443.30666152, 456.35822121, 459.28127612,
       461.79999001, 462.31202136, 462.78021864, 463.31656156,
       469.82135411, 471.37500346, 476.83297206, 479.88988674,
       482.62381522, 484.8686521 , 486.33911237, 486.80191571,
       486.82976052, 492.8594002 , 497.2327242 , 507.88785782,
       512.27722228, 516.27677298, 516.32695539, 519.92131876,
       522.34852293, 522.44947862, 526.90480747, 528.79307685,
       535.34648927, 538.85619168, 540.83176987, 545.03312907,
       551.87138264, 562.70066836, 566.36259454, 566.84323553,
       567.34286753, 571.74761095, 573.98282533, 628.55250547,
       646.34347461, 685.72376389, 720.7354609 , 733.68894991,
       757.7518703 , 780.69049324, 820.66052472, 832.88936895,
       898.73069474, 912.57878847, 916.60054575, 917.63245471,
       920.48276989, 965.4974121 , 987.65644293]), 'intensity array': array([
56., 138.,  63., 115., 115.,  76., 112., 111., 120.,  24.,  45.,
       134.,  74.,  87.,  90., 110., 115.,  87., 107., 101.,  97.,  47.,
       145.,  99.,  32.,  45.,  95., 259.,  10.,  95.,  91., 120.,  93.,
        80.,  86., 138., 109.,  74.,  86.,  84., 124.,  44.,  32.,  84.,
       111.,  71., 137.,  88.,  44.,  64., 111.,  10.,  23.,  49.,  64.,
        10.,  59.,  67.,  68.,  79.,  83.,  32.,  57.,  34.,  67.,  11.,
        24.], dtype=float32)}
{'index': 9, 'defaultArrayLength': 17411, 'id': 'index=10', 'scanList':
{'count': 1, 'scan': [{'scanWindowList': {'count': 1, 'scanWindow': [{'scan
window lower limit': 100.0, 'scan window upper limit': 1700.0}]}, 'scan start
time': 0.0311456}], 'no combination': ''}, 'ms level': 1, 'total ion current':
4758368.0, 'base peak intensity': 110802.0, 'base peak m/z': 1221.9860752210172,
'positive scan': '', 'centroid spectrum': '', 'MS1 spectrum': '', 'count': 2,
'm/z array': array([ 100.17740957,  100.4649811 ,  100.84957903, …,
1699.3487512 ,
       1699.58936744, 1699.96658393]), 'intensity array': array([72., 43., 80.,
…, 83., 48., 86.], dtype=float32)}
{'index': 10, 'defaultArrayLength': 17418, 'id': 'index=11', 'scanList':
{'count': 1, 'scan': [{'scanWindowList': {'count': 1, 'scanWindow': [{'scan
window lower limit': 100.0, 'scan window upper limit': 1700.0}]}, 'scan start
time': 0.039993799999999996}], 'no combination': ''}, 'ms level': 1, 'total ion
current': 4777093.0, 'base peak intensity': 115464.0, 'base peak m/z':
1221.9860157008086, 'positive scan': '', 'centroid spectrum': '', 'MS1
spectrum': '', 'count': 2, 'm/z array': array([ 100.07007102,  100.38277575,
101.25237212, …, 1698.32144648,
       1699.07563989, 1699.67765596]), 'intensity array': array([117.,  56.,
111., …,  83.,  40., 211.], dtype=float32)}
```

4 Write a .mzML file corresponding to provided data !incomplete! (complete data conversion better with ProteoWizard msconvert?)

4.1 Compare the file keys

[19]:
```
TR_all_spectra[0]
```

[19]:
```
PySpectrum(index=0, len(mz_values)=67, len(intensities)=67, precursor=(index=1,
frame_index=8, mz=663.2879991882091, im=0.8166844415024028, charge=2,
intensity=1777))
```

[20]:
```
MZML_spectra_data_detailed[0].keys()
```

[20]:
```
dict_keys(['index', 'defaultArrayLength', 'id', 'scanList', 'ms level', 'total
ion current', 'base peak intensity', 'base peak m/z', 'positive scan', 'centroid
spectrum', 'MS1 spectrum', 'count', 'm/z array', 'intensity array'])
```

[21]:
```
MZML_spectra_data_detailed[8]
```

[21]:
```
{'index': 8,
 'defaultArrayLength': 67,
 'id': 'index=9',
 'scanList': {'count': 1,
  'scan': [{'scanWindowList': {'count': 1,
     'scanWindow': [{'scan window lower limit': 100.0 m/z,
       'scan window upper limit': 1700.0 m/z}]},
    'scan start time': 0.025463799999999998 minute}],
  'no combination': ''},
 'precursorList': {'count': 1,
  'precursor': [{'spectrumRef': 'index=8',
    'isolationWindow': {'isolation window target m/z': 663.2879991882091 m/z,
     'isolation window lower offset': 0.5917277272992578 m/z,
     'isolation window upper offset': 1.4082722727007422 m/z},
    'selectedIonList': {'count': 1,
     'selectedIon': [{'selected ion m/z': 663.2879991882091 m/z,
       'charge state': 2,
       'inverse reduced ion mobility': 0.8293334816068573 volt-second per square
centimeter}]},
    'activation': {'collision energy': 33.38565022421525 electronvolt,
     'CID': ''}}]},
 'ms level': 2,
 'total ion current': 5412.0,
 'positive scan': '',
 'centroid spectrum': '',
 'MSn spectrum': '',
 'count': 2,
 'm/z array': array([273.03166788, 360.92126028, 400.35207354, 401.81166621,
        407.2880975 , 411.95908009, 419.3433101 , 428.8193565 ,
        431.30892258, 435.31182382, 435.72663165, 436.30308133,
        441.26965055, 443.30666152, 456.35822121, 459.28127612,
        461.79999001, 462.31202136, 462.78021864, 463.31656156,
        469.82135411, 471.37500346, 476.83297206, 479.88988674,
        482.62381522, 484.8686521 , 486.33911237, 486.80191571,
        486.82976052, 492.8594002 , 497.2327242 , 507.88785782,
        512.27722228, 516.27677298, 516.32695539, 519.92131876,
        522.34852293, 522.44947862, 526.90480747, 528.79307685,
        535.34648927, 538.85619168, 540.83176987, 545.03312907,
        551.87138264, 562.70066836, 566.36259454, 566.84323553,
        567.34286753, 571.74761095, 573.98282533, 628.55250547,
        646.34347461, 685.72376389, 720.7354609 , 733.68894991,
        757.7518703 , 780.69049324, 820.66052472, 832.88936895,
        898.73069474, 912.57878847, 916.60054575, 917.63245471,
        920.48276989, 965.4974121 , 987.65644293]),
 'intensity array': array([ 56., 138.,  63., 115., 115.,  76., 112., 111., 120.,
24.,  45.,
        134.,  74.,  87.,  90., 110., 115.,  87., 107., 101.,  97.,  47.,
        145.,  99.,  32.,  45.,  95., 259.,  10.,  95.,  91., 120.,  93.,
         80.,  86., 138., 109.,  74.,  86.,  84., 124.,  44.,  32.,  84.,
        111.,  71., 137.,  88.,  44.,  64., 111.,  10.,  23.,  49.,  64.,
         10.,  59.,  67.,  68.,  79.,  83.,  32.,  57.,  34.,  67.,  11.,
         24.], dtype=float32)}
```

Provided mzML file has a lot of more parameters for reproducible and comparable ms data: 1. spectrum index (index (counted from 0), id (counted from 1)) 2. defaultArrayLength - Length of mz Arrays 3. scanList provides scanData (scan window lower limit, upper limit, scan start time, no combination) 4. ms level 5. total ion current 6. base peak intensity 7. base peak m/z 8. positive scan 9. centroid spectrum 10. MS1 spectrum 11. count 12. m/z array 13. intensity array

If a precursor exists, there is additionally:

15. precursor list with count, and precursor - data: [spectrum index, isolationWindow (with m/z), offsets from window]
16. selection ion list with count and activation data: [energy, CID], and ion - data: [ion m/z, ion charge, inversed ion mobility]

5 Casanovo prefers .mgf, .h5, .hdf5 files for training

For example their own provided .mgf file:

[22]:
```python
path = "/Users/daviddornig/miniconda3/envs/casanovo_env/lib/python3.10/
↪site-packages/Casaval/Testing/example_data/sample_preprocessed_spectra.mgf"
```

[23]:
```python
from pyteomics import mgf
```

```
# Dictionary to store spectra
example_spectra_dict = {}

# Open the MGF file and iterate through spectra
with mgf.read(path) as spectra:
    for index, spectrum in enumerate(spectra):
        example_spectra_dict[index] = spectrum
```

5.1 Parameter dictionary structure comparison (TimsRust and example mgf)

[24]:
```
example_spectra_dict[0]
```

[24]:
```
{'params': {'title': '0',
  'pepmass': (451.25348, None),
  'charge': [2],
  'scans': 'F1:2478',
  'rtinseconds': 824.574 second,
  'seq': 'IAHYNKR'},
 'm/z array': array([ 63.9948349 ,  70.06543732,  84.08129883,  85.08439636,
         86.09666443, 110.0710907 , 129.102005  , 138.065979  ,
        157.13291931, 175.11853027, 185.12837219, 209.10263062,
        273.13378906, 301.1282959 , 303.21221924, 304.17529297,
        322.18591309, 350.67874146, 417.25521851, 580.31854248,
        630.36572266, 717.37670898, 753.37487793, 788.42077637,
        866.45446777]),
 'intensity array': array([0.06119308, 0.06860413, 0.22455615, 0.06763621,
0.22344913,
        0.30348614, 0.09322319, 0.07667152, 0.14716865, 0.19198035,
        0.09717457, 0.13139844, 0.09324287, 0.08515828, 0.07235292,
        0.07120858, 0.1583406 , 0.07397215, 0.1498218 , 0.31572264,
        0.06255879, 0.59908968, 0.09976937, 0.35858697, 0.12016355]),
 'charge array': masked_array(data=[--, --, --, --, --, --, --, --, --, --, --,
--, --, --,
                    --, --, --, --, --, --, --, --, --, --, --],
              mask=[ True,  True,  True,  True,  True,  True,  True,  True,
                     True,  True,  True,  True,  True,  True,  True,  True,
                     True,  True,  True,  True,  True,  True,  True,  True,
                     True],
        fill_value=0,
             dtype=int64)}
```

[25]:
```
type(example_spectra_dict[0]['params']['rtinseconds'])
```

[25]:
```
pyteomics.auxiliary.structures.unitfloat
```

[26]:
```

```

```
TR_all_spectra[0]
#invoke parameters with .mz_values, .intensities, .precursor, .im, .charge, .
↪intensity per precursor
```

[26]:
```
PySpectrum(index=0, len(mz_values)=67, len(intensities)=67, precursor=(index=1,
frame_index=8, mz=663.2879991882091, im=0.8166844415024028, charge=2,
intensity=1777))
```

[27]:
```
TR_all_frames[7]
```

[27]:
```
PyFrame(index=8, rt=1.527828, frame_type=0, len(scan_offsets)=928,
len(tof_indices)=48758, len(intensities)=48758)
```

6 Convert TimsRust dictionary to .mgf

6.1 Define placeholder dict:

[28]:
```
from pyteomics.auxiliary import unitfloat
from typing import Dict, List, Tuple

# Define the placeholder dictionary with specified data types
TR_to_mgf_spectrum_dict: Dict[str, Dict[str, object]] = {
    'params': {
        'title': str, # File name: Precursor Index
        'pepmass': Tuple[float, float],
        'charge': List[int],
        'ion_mobility': float,
        'scans': str,
        'rtinseconds': unitfloat, # pyteomics own datatype for values with units
        'casanovo_seq': str, # For later evaluation, the added casanovo␣
↪prediction seq
        'seq': str # Database sequence as 'seq' to keep casanovo's definition␣
↪of this parameter
    },
    'm/z array': List[float],
    'intensity array': List[float]
}
```

[29]:
```
# Example:
TR_to_mgf_spectrum_dict['params']['title'] = 'HeLa:0' # Precursor Index
TR_to_mgf_spectrum_dict['params']['pepmass'] = (500.0, 1.0)
TR_to_mgf_spectrum_dict['params']['charge'] = [1]
TR_to_mgf_spectrum_dict['params']['ion_mobility'] = 0.0
TR_to_mgf_spectrum_dict['params']['scans'] = 'F1' # Frame/Scan Index
TR_to_mgf_spectrum_dict['params']['rtinseconds'] = unitfloat(10.0, 'second')
TR_to_mgf_spectrum_dict['params']['casanovo_seq'] = 'ACDEFGH'
TR_to_mgf_spectrum_dict['params']['seq'] = 'ABCDEFGHIJ'
```

```
TR_to_mgf_spectrum_dict['m/z array'] = [100.0, 200.0, 300.0]
TR_to_mgf_spectrum_dict['intensity array'] = [10.0, 20.0, 30.0]

print(TR_to_mgf_spectrum_dict)
```

```
{'params': {'title': 'HeLa:0', 'pepmass': (500.0, 1.0), 'charge': [1],
'ion_mobility': 0.0, 'scans': 'F1', 'rtinseconds': 10.0, 'casanovo_seq':
'ACDEFGH', 'seq': 'ABCDEFGHIJ'}, 'm/z array': [100.0, 200.0, 300.0], 'intensity
array': [10.0, 20.0, 30.0]}
```

6.2 Define class type for a spectrum in mgf:

[30]:
```
class TR_to_MGF_Spectrum:
    def __init__(self, title: str, pepmass: Tuple[float, float], charge:␣
 ↪List[int], ion_mobility: float, scans: str, rtinseconds: unitfloat,␣
 ↪casanovo_seq: str, casanovo_aa_scores: List[float], seq: str, mz_array:␣
 ↪List[float], intensity_array: List[float]):
        self.params = {
            'title': title,
            'pepmass': pepmass,
            'charge': charge,
            'ion_mobility': ion_mobility,
            'scans': scans,
            'rtinseconds': rtinseconds,
            'casanovo_seq': casanovo_seq,
            'casanovo_aa_scores': [],
            'seq': seq
        }
        self.mz_array = mz_array
        self.intensity_array = intensity_array

    def to_dict(self) -> dict:
        return {
            'params': self.params,
            'm/z array': self.mz_array,
            'intensity array': self.intensity_array
        }
```

[31]:
```
spectrum = TR_to_MGF_Spectrum(
    title='Precursor Index',
    pepmass=(500.0, 1.0),
    charge=[1],
    ion_mobility=1.0,
    scans='Scan1',
    rtinseconds=unitfloat(10.0, 'second'),
    casanovo_seq='',
    casanovo_aa_scores = [],
    seq='',
    mz_array=[100.0, 200.0, 300.0],
    intensity_array=[10.0, 20.0, 30.0]
)
```

[32]:
```
spectrum.to_dict()
```

[32]:
```
{'params': {'title': 'Precursor Index',
  'pepmass': (500.0, 1.0),
  'charge': [1],
  'ion_mobility': 1.0,
  'scans': 'Scan1',
  'rtinseconds': 10.0 second,
  'casanovo_seq': '',
  'casanovo_aa_scores': [],
  'seq': ''},
 'm/z array': [100.0, 200.0, 300.0],
 'intensity array': [10.0, 20.0, 30.0]}
```

7 Convert TimsRust retrieved data to a list of mgf ready spectra dictionaries

7.1 Call example data of the first precursor of HeLa for inspection of available metadata

[33]:
```
TR_all_spectra[0]
```

[33]:
```
PySpectrum(index=0, len(mz_values)=67, len(intensities)=67, precursor=(index=1,
frame_index=8, mz=663.2879991882091, im=0.8166844415024028, charge=2,
intensity=1777))
```

[34]:
```
frame_index = TR_all_spectra[0].precursor.frame_index

print(TR_all_frames[frame_index])
print(TR_all_frames_indexcorrected[frame_index])
```

```
PyFrame(index=9, rt=1.655497, frame_type=1, len(scan_offsets)=928,
len(tof_indices)=9, len(intensities)=9)
PyFrame(index=8, rt=1.527828, frame_type=0, len(scan_offsets)=928,
len(tof_indices)=48758, len(intensities)=48758)
```

[35]:
```
MZML_spectra_data_detailed[8]
```

[35]:
```
{'index': 8,
 'defaultArrayLength': 67,
 'id': 'index=9',
 'scanList': {'count': 1,
  'scan': [{'scanWindowList': {'count': 1,
     'scanWindow': [{'scan window lower limit': 100.0 m/z,
       'scan window upper limit': 1700.0 m/z}]},
    'scan start time': 0.025463799999999998 minute}],
  'no combination': ''},
 'precursorList': {'count': 1,
  'precursor': [{'spectrumRef': 'index=8',
    'isolationWindow': {'isolation window target m/z': 663.2879991882091 m/z,
     'isolation window lower offset': 0.5917277272992578 m/z,
     'isolation window upper offset': 1.4082722727007422 m/z},
    'selectedIonList': {'count': 1,
     'selectedIon': [{'selected ion m/z': 663.2879991882091 m/z,
       'charge state': 2,
       'inverse reduced ion mobility': 0.8293334816068573 volt-second per square
centimeter}]},
    'activation': {'collision energy': 33.38565022421525 electronvolt,
     'CID': ''}}]},
 'ms level': 2,
 'total ion current': 5412.0,
 'positive scan': '',
 'centroid spectrum': '',
 'MSn spectrum': '',
 'count': 2,
 'm/z array': array([273.03166788, 360.92126028, 400.35207354, 401.81166621,
        407.2880975 , 411.95908009, 419.3433101 , 428.8193565 ,
        431.30892258, 435.31182382, 435.72663165, 436.30308133,
        441.26965055, 443.30666152, 456.35822121, 459.28127612,
        461.79999001, 462.31202136, 462.78021864, 463.31656156,
        469.82135411, 471.37500346, 476.83297206, 479.88988674,
        482.62381522, 484.8686521 , 486.33911237, 486.80191571,
        486.82976052, 492.8594002 , 497.2327242 , 507.88785782,
        512.27722228, 516.27677298, 516.32695539, 519.92131876,
        522.34852293, 522.44947862, 526.90480747, 528.79307685,
        535.34648927, 538.85619168, 540.83176987, 545.03312907,
        551.87138264, 562.70066836, 566.36259454, 566.84323553,
        567.34286753, 571.74761095, 573.98282533, 628.55250547,
        646.34347461, 685.72376389, 720.7354609 , 733.68894991,
        757.7518703 , 780.69049324, 820.66052472, 832.88936895,
        898.73069474, 912.57878847, 916.60054575, 917.63245471,
        920.48276989, 965.4974121 , 987.65644293]),
 'intensity array': array([ 56., 138.,  63., 115., 115.,  76., 112., 111., 120.,
24.,  45.,
        134.,  74.,  87.,  90., 110., 115.,  87., 107., 101.,  97.,  47.,
        145.,  99.,  32.,  45.,  95., 259.,  10.,  95.,  91., 120.,  93.,
         80.,  86., 138., 109.,  74.,  86.,  84., 124.,  44.,  32.,  84.,
        111.,  71., 137.,  88.,  44.,  64., 111.,  10.,  23.,  49.,  64.,
         10.,  59.,  67.,  68.,  79.,  83.,  32.,  57.,  34.,  67.,  11.,
         24.], dtype=float32)}
```

7.2 Prepare for saving TR data to mgf format:

7.2.1 Get file specifications:

```
[36]: import os

file_path = "/Users/daviddornig/Documents/SoftwareProject/
 ↪5909_HeLa200ng_1hour_9-14-2023.d"
file_name = os.path.basename(file_path)
file_name_without_extension = os.path.splitext(os.path.basename(file_path))[0]
file_extension = os.path.splitext(os.path.basename(file_path))[1]

print(file_name_without_extension)  # Output: 5909_HeLa200ng_1hour_9-14-2023.d
```

```
5909_HeLa200ng_1hour_9-14-2023
```

```
[37]: example_title = f'{TR_all_spectra[0].precursor.index}'
example_split = example_title.split(':')
example_split
```

```
[37]: ['1']
```

7.2.2 Structure TR spectra as mgf spectra:

```
[38]: TR_to_mgf_spectra_list = []
for spectrum in TR_all_spectra:
    spectrum_instance = TR_to_MGF_Spectrum(
        title=spectrum.precursor.index,
        #f'{file_name_without_extension}:Precursor_{spectrum.precursor.index}'
        pepmass=(spectrum.precursor.mz, None),
        charge=[spectrum.precursor.charge],
        ion_mobility=spectrum.precursor.im,
        scans=f'F{spectrum.precursor.frame_index}:{spectrum.precursor.
 ↪intensity}',
        rtinseconds=unitfloat(TR_all_frames_indexcorrected[spectrum.precursor.
 ↪frame_index].rt, 'second'),
        casanovo_seq='',
        casanovo_aa_scores=[],
        seq='',
        mz_array=spectrum.mz_values,
        intensity_array=spectrum.intensities
    )
    TR_to_mgf_spectra_list.append(spectrum_instance.to_dict())
```

```
[39]: example_rt = in_minutes(unitfloat(TR_all_frames_indexcorrected[spectrum.
 ↪precursor.frame_index].rt, 'second'))
```

```
example_rt
```

```
[39]: 59.92563591666667 minute
```

```
[40]: len(TR_to_mgf_spectra_list)
```

```
[40]: 151503
```

```
[41]: TR_to_mgf_spectra_list[700]
```

```
[41]: {'params': {'title': 701,
        'pepmass': (1147.9568944899602, None),
        'charge': [2],
        'ion_mobility': 1.11599490046092,
        'scans': 'F3531:4750.0',
        'rtinseconds': 423.862084 second,
        'casanovo_seq': '',
        'casanovo_aa_scores': [],
        'seq': ''},
       'm/z array': [528.1771000505856, 987.4026703653274, 1322.586725143252],
       'intensity array': [78.0, 60.0, 31.0]}
```

7.3 Save as .mgf file:

```
[42]: from pyteomics import mgf

      mgf.write(TR_to_mgf_spectra_list, 'output.mgf')
```

```
[42]: <pyteomics.auxiliary.file_helpers._file_obj at 0x2188afbe0>
```

8 Inspection: Functions to open and write .mgf files for predictions:

8.1 Read MGF file:

```
[43]: # Read the MGF file and create Spectrum objects
      mgf_path = 'output.mgf'
      opened_MGF_spectra = mgf.read(mgf_path, convert_arrays=True)
```

```
[44]: opened_MGF_spectra['151502']
```

```
[44]: {'params': {'title': '151502',
        'pepmass': (1221.9879333107788, None),
        'rtinseconds': 3589.934304 second,
        'charge': [1],
        'ion_mobility': '1.3690286963761604',
        'scans': 'F33121:50923.0',
  'casanovo_seq': '',
  'casanovo_aa_scores': '[]',
  'seq': ''},
 'm/z array': array([ 248.91790045,  250.92294504,  277.92815861,  318.17916291,
        346.00916032,  391.9424282 ,  510.17348558,  533.94265766,
        553.93781316,  553.94895153,  715.94391359,  715.95657642,
        715.96501836,  716.92772652,  716.96996537,  744.43020118,
        857.943003  ,  858.95521006,  877.9499306 ,  877.96862731,
        878.9458073 ,  878.95983776,  898.94877819,  899.96595563,
        969.95756987, 1039.95854862, 1039.99924635, 1040.02468284,
       1040.96096197, 1040.98641022, 1042.9010096 , 1220.5052228 ,
       1220.64300403, 1221.56358216, 1221.75656168, 1221.93301343,
       1221.97712836, 1221.99918613, 1222.01021508, 1222.02124409,
       1222.04330225, 1222.1646257 , 1222.88717638, 1222.98095815,
       1223.03060878, 1223.06370976, 1223.09129425, 1223.78652612,
       1223.97968114, 1224.03487108, 1224.09006228, 1224.9070374 ,
       1224.96776941, 1225.27697387, 1225.35428108, 1225.48129108,
       1225.54755978, 1226.23244125, 1226.26006145, 1228.34352056,
       1230.27934941, 1235.05351891, 1330.22210235]),
 'intensity array': array([3.200e+01, 6.700e+01, 1.050e+02, 8.100e+01,
1.000e+01, 1.710e+02,
       1.000e+01, 3.500e+01, 8.700e+01, 1.350e+02, 1.200e+02, 9.400e+01,
       6.600e+01, 8.200e+01, 7.900e+01, 2.500e+01, 1.030e+02, 4.100e+01,
       4.780e+02, 4.480e+02, 2.100e+01, 1.870e+02, 4.800e+01, 2.600e+01,
       1.000e+02, 6.060e+02, 2.300e+01, 3.900e+01, 1.690e+02, 3.600e+01,
       4.800e+01, 6.200e+01, 9.300e+01, 3.300e+01, 1.100e+01, 1.500e+02,
       2.010e+04, 2.610e+02, 1.590e+02, 7.700e+01, 5.400e+01, 4.200e+01,
       1.100e+01, 4.381e+03, 7.900e+01, 3.600e+01, 2.400e+01, 1.000e+01,
       9.530e+02, 3.400e+01, 1.260e+02, 2.700e+01, 1.100e+01, 1.000e+01,
       9.400e+01, 3.500e+01, 2.700e+01, 2.400e+01, 1.400e+01, 4.400e+01,
       2.600e+01, 4.000e+01, 5.800e+01]),
 'charge array': array([0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
0, 0, 0,
       0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
       0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0])}
```

8.2 Load prediction data

[45]:
```python
import pandas as pd
from pyteomics import mztab
```

8.2.1 load database predictions:

[46]:
```python
database_path = '../Casaval/Testing/example_data/
 ↪5909_HeLa200ng_1hour_9-14-2023-database_predictions.csv'

database_df_origin = pd.read_csv(database_path)
```

```
[47]: database_df_origin = database_df_origin.sort_values(by='Precursor Id',␣
       ↪ascending=True)
      database_df_origin
```

```
[47]:                                          Peptide  -10lgP       Mass  Length  \
      22345                                   HAVSEGTK   53.98   827.4137       8
      36749                                    KTESHHK   31.98   865.4406       7
      26963                                  RGNVAGDSK   47.92   902.4570       9
      14713                         VSDSGSHSGSDSHSGASR   64.05  1715.7095      18
      38847                                    HGEAQVK   27.22   767.3926       7
      …                                            …     …         …     …
      17755                A(+42.01)ALDSLSLFTSLGLSEQK   59.94  1921.0044      18
      24526                         M(+42.01)ELITILEK   51.19  1130.6257       9
      19022  FQSSAVMALQEASEAYLVGLFEDTNLC(+57.02)AIHAK   58.27  3512.6956      32
      6627                           ELDRDTVFALVNYIFFK   77.35  2089.0884      17
      31607                        A(+42.01)GILFEDIFDVK   41.12  1407.7285      12

             ppm        m/z     RT      1/k0 Range  Area HeLa200ng  Fraction  \
      22345 -2.3   414.7132   3.83  0.7669-0.7954             0.0         1
      36749 -3.2   433.7262   5.86  0.7430-0.7715             0.0         1
      26963 -5.4   452.2333   6.97  0.7692-0.7977             0.0         1
      14713 -6.0   572.9070   6.99  0.8249-0.8533             0.0         1
      38847 -5.8   384.7014   6.99  0.7556-0.7840             0.0         1
      …      …          …      …             …              …       …
      17755 -7.1   961.5026  56.35  1.2753-1.3031             0.0         1
      24526 -5.3   566.3171  56.47  0.8918-0.9201          1682.9         1
      19022 -4.9  1171.9000  56.46  1.1326-1.1606             0.0         1
      6627   6.5   697.3746  56.48  0.9495-0.9777             0.0         1
      31607 -4.3   704.8685  56.56  0.9980-1.0261             0.0         1

             Precursor Id                      Source File  #Feature  \
      22345           251  5909_HeLa200ng_1hour_9-14-2023.d         0
      36749           339  5909_HeLa200ng_1hour_9-14-2023.d         0
      26963           387  5909_HeLa200ng_1hour_9-14-2023.d         0
      14713           410  5909_HeLa200ng_1hour_9-14-2023.d         0
      38847           423  5909_HeLa200ng_1hour_9-14-2023.d         0
      …               …                               …       …
      17755        151080  5909_HeLa200ng_1hour_9-14-2023.d         0
      24526        151090  5909_HeLa200ng_1hour_9-14-2023.d         1
      19022        151105  5909_HeLa200ng_1hour_9-14-2023.d         0
      6627         151120  5909_HeLa200ng_1hour_9-14-2023.d         0
      31607        151276  5909_HeLa200ng_1hour_9-14-2023.d         0

             #Feature HeLa200ng                                    Accession  \
      22345                  0  Q99880|H2B1L_HUMAN:O60814|H2B1K_HUMAN:P58876|H…
      36749                  0  Q6FI13|H2A2A_HUMAN:Q99878|H2A1J_HUMAN:Q96KK5|H…
      26963                  0                                P68104|EF1A1_HUMAN
```

```
14713                 0          Q96N21-2|AP4AT_HUMAN:Q96N21|AP4AT_HUMAN
38847                 0                               P18669|PGAM1_HUMAN
…                 …                                                    …
17755                 0                                 P47897|SYQ_HUMAN
24526                 1                                Q14974|IMB1_HUMAN
19022                 0                                 Q71DI3|H32_HUMAN
6627                  0                              P01009-2|A1AT_HUMAN
31607                 0                               P52434|RPAB3_HUMAN

                      PTM                              AScore  Found By
22345                 NaN                                 NaN  PEAKS DB
36749                 NaN                                 NaN  PEAKS DB
26963                 NaN                                 NaN  PEAKS DB
14713                 NaN                                 NaN  PEAKS DB
38847                 NaN                                 NaN  PEAKS DB
…                       …                                   …       …
17755  Acetylation (N-term)   A1:Acetylation (N-term):1000.00  PEAKS DB
24526  Acetylation (N-term)   M1:Acetylation (N-term):1000.00  PEAKS DB
19022  Carbamidomethylation  C27:Carbamidomethylation:1000.00  PEAKS DB
6627                  NaN                                 NaN  PEAKS DB
31607  Acetylation (N-term)   A1:Acetylation (N-term):1000.00  PEAKS DB

[38894 rows x 18 columns]
```

8.2.2 load casanovo predictions:

[48]:
```
casanovo_path = '../Casaval/Testing/example_data/
 ↪5909_HeLa200ng_1hour_9-14-2023-casanovo_predictions/casanovo_20240106003148.
 ↪mztab'

file = mztab.MzTab(casanovo_path)
casanovo_df_origin = pd.DataFrame(file.spectrum_match_table)
```

[49]:
```
casanovo_df_origin['Precursor Id'] = casanovo_df_origin['spectra_ref'].str.
 ↪extract(r'scan=(\d+)', expand=False).astype(int)
# add column for Precursor Id
```

[50]:
```
casanovo_df_origin
```

[50]:
```
                         sequence  PSM_ID accession unique database  \
PSM_ID
1                     TLHTLLLDNRK       1      None   None     None
2                      NDLLLEKEEK       2      None   None     None
3        KPREC+57.021ESC+57.021R       3      None   None     None
4                        PMGLLRLK       4      None   None     None
5                           LNNNN       5      None   None     None
…                             …       …         …      …        …
```

```
149272             HDC+57.021DK  149272      None   None     None
149273             EC+57.021FER  149273      None   None     None
149274              QYGPKPPTVLK  149274      None   None     None
149275        LC+57.021EDRLDNGK  149275      None   None     None
149276                  KEEDNER  149276      None   None     None

       database_version      search_engine  search_engine_score[1]  \
PSM_ID
1                  None  (Casanovo, 4.0.1)               -0.596066
2                  None  (Casanovo, 4.0.1)               -0.652129
3                  None  (Casanovo, 4.0.1)               -0.650732
4                  None  (Casanovo, 4.0.1)               -0.479740
5                  None  (Casanovo, 4.0.1)               -0.803877
…                     …                  …                       …
149272             None  (Casanovo, 4.0.1)               -0.635977
149273             None  (Casanovo, 4.0.1)               -0.697781
149274             None  (Casanovo, 4.0.1)               -0.398575
149275             None  (Casanovo, 4.0.1)               -0.358718
149276             None  (Casanovo, 4.0.1)               -0.608214

       modifications retention_time  charge  exp_mass_to_charge  \
PSM_ID
1               None           None     2.0           663.28800
2               None           None     1.0          1221.99000
3               None           None     1.0          1223.99230
4               None           None     1.0           922.00525
5               None           None     1.0           466.81647
…                  …              …       …                   …
149272          None           None     1.0           623.02484
149273          None           None     1.0           622.02673
149274          None           None     1.0          1223.98800
149275          None           None     1.0          1221.98790
149276          None           None     1.0           922.00850

        calc_mass_to_charge            spectra_ref   pre  post start   end  \
PSM_ID
1                662.390785       ms_run[1]:scan=1  None  None  None  None
2               1230.657608       ms_run[1]:scan=2  None  None  None  None
3               1221.546302       ms_run[1]:scan=3  None  None  None  None
4                927.580820       ms_run[1]:scan=4  None  None  None  None
5                588.273613      ms_run[1]:scan=23  None  None  None  None
…                         …                      …     …     …     …     …
149272           674.256251  ms_run[1]:scan=151499  None  None  None  None
149273           740.303201  ms_run[1]:scan=151500  None  None  None  None
149274          1227.709578  ms_run[1]:scan=151501  None  None  None  None
149275          1219.573562  ms_run[1]:scan=151502  None  None  None  None
149276           919.411564  ms_run[1]:scan=151503  None  None  None  None
```

```
                                 opt_ms_run[1]_aa_scores  Precursor Id
PSM_ID
1       0.62897,0.46597,0.40492,0.38024,0.31451,0.3153…            1
2       0.48481,0.22510,0.24477,0.27449,0.28019,0.3035…            2
3       0.44714,0.24382,0.22875,0.23995,0.36384,0.2729…            3
4       0.70436,0.43039,0.47188,0.53315,0.39718,0.4157…            4
5                 0.21670,0.21841,0.22674,0.20244,0.21438           23
…                                                    …             …
149272            0.32813,0.38435,0.62690,0.32973,0.33301       151499
149273            0.35393,0.30745,0.23200,0.51399,0.25483       151500
149274  0.72467,0.43650,0.43237,0.49246,0.78193,0.7950…       151501
149275  0.81150,0.66726,0.75720,0.48835,0.42572,0.5005…       151502
149276  0.45166,0.29354,0.26007,0.27790,0.27601,0.4011…       151503

[149276 rows x 20 columns]
```

[51]:
```
casanovo_df_origin[casanovo_df_origin['Precursor Id'] == 251]
```

[51]:
```
        sequence  PSM_ID accession unique database database_version  \
PSM_ID
145     HAVSGTEK     145      None   None     None             None

            search_engine  search_engine_score[1] modifications  \
PSM_ID
145     (Casanovo, 4.0.1)                0.934061          None

       retention_time  charge  exp_mass_to_charge  calc_mass_to_charge  \
PSM_ID
145              None     2.0           414.71317           414.714138

               spectra_ref   pre  post start   end  \
PSM_ID
145     ms_run[1]:scan=251  None  None  None  None

                                 opt_ms_run[1]_aa_scores  Precursor Id
PSM_ID
145     0.95846,0.95933,0.96202,0.71932,0.96250,0.9620…          251
```

[52]:
```
database_df_origin[database_df_origin['Precursor Id'] == 339]
```

[52]:
```
       Peptide  -10lgP      Mass  Length  ppm       m/z    RT     1/k0 Range  \
36749  KTESHHK   31.98  865.4406       7 -3.2  433.7262  5.86  0.7430-0.7715

       Area HeLa200ng  Fraction  Precursor Id  \
36749             0.0         1           339
```

```
                            Source File  #Feature  #Feature HeLa200ng  \
36749  5909_HeLa200ng_1hour_9-14-2023.d         0                   0

                                              Accession  PTM AScore  Found By
36749  Q6FI13|H2A2A_HUMAN:Q99878|H2A1J_HUMAN:Q96KK5|H…  NaN     NaN  PEAKS DB
```

8.3 Write prediction sequences to opened MGF spectra:

8.3.1 add database predictions:

[53]:
```
precursor_id_list = database_df_origin['Precursor Id'].tolist()
#precursor_id_list
```

8.3.2 Using pyteomics write

[142]:
```
# Write database predictions to mgf:
mgf_path = 'output.mgf'
opened_MGF_spectra = mgf.read(mgf_path, convert_arrays=True)

new_MGF_spectra_list = []

for spectrum in opened_MGF_spectra:
    precursor_id_list = database_df_origin['Precursor Id'].tolist()
    precursor_id_MGF = int(spectrum['params']['title'])
    if precursor_id_MGF in precursor_id_list:
        peptide = database_df_origin.loc[database_df_origin['Precursor Id'] ==␣
 ↪precursor_id_MGF, 'Peptide'].iloc[0]
        spectrum['params']['seq'] = peptide
        #print(f"Peptide added: {peptide}, of Precursor Id. {precursor_id_MGF}")
    new_MGF_spectra_list.append(spectrum)
```

[143]:
```
new_MGF_spectra_list[250]
```

[143]:
```
{'params': {'title': '251',
  'pepmass': (414.7131510926927, None),
  'rtinseconds': 229.928144 second,
  'charge': [2],
  'ion_mobility': '0.771090028717373',
  'scans': 'F1925:4698.0',
  'casanovo_seq': 'HAVSGTEK',
  'casanovo_aa_scores':
'[0.95846,0.95933,0.96202,0.71932,0.96250,0.96205,0.96254,0.95851]',
  'seq': 'HAVSEGTK'},
 'm/z array': array([209.10764765, 225.10350547, 245.8042115 , 249.13945602,
        258.1078381 , 258.14585492, 262.16597184, 270.10491026,
        274.09919805, 289.78649918, 290.15988832, 290.16526258,
        306.12279194, 308.17970747, 320.17450054, 329.15342409,
        357.1392097 , 377.20234282, 380.19234998, 383.16328161,
        395.20026649, 395.20653852, 405.70261393, 412.23281995,
        415.88574241, 416.21072582, 417.21865879, 418.22135861,
        425.18287088, 438.19449456, 445.203117  , 452.23379741,
        455.22437941, 466.20831376, 468.21325428, 482.19161102,
        482.19853904, 503.24911411, 506.22611961, 510.24831323,
        521.25364269, 522.26257161, 524.25807202, 525.25182605,
        545.25820207, 553.27342598, 554.94814791, 563.26611814,
        580.83640671, 581.25848864, 581.27750495, 583.26832869,
        583.27975815, 602.30637461, 620.32538804, 620.33717498,
        622.33864714, 664.30892957, 673.34943831, 674.3527041 ,
        691.35656936, 691.37316071, 692.35655332, 693.35310614]),
 'intensity array': array([ 81.,  16., 170.,  83.,  56.,  79., 151., 208., 131.,
88., 112.,
         86.,  87.,  74., 153., 128.,  76., 107.,  88.,  66.,  66.,  86.,
        111.,  56.,  37.,  62.,  64.,  93.,  81.,  62.,  92.,  99.,  53.,
        117.,  67.,  37., 101.,  87., 169.,  21., 262.,  67.,  42., 123.,
         46.,  74.,  53., 193.,  52.,  54.,  57.,  82.,  87., 134.,  63.,
         29.,  45.,  26.,  61.,  80., 365.,  91., 174.,  32.]),
 'charge array': array([0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
0, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0])}
```

[ ]:
```
mgf.write(new_MGF_spectra_list, 'HeLa_database_seq.mgf')
```

Pyteomics write mgf takes a lot of time to finish!! … (30 min for 1.7GB data)

8.3.3 speeding up with fileinput?

[55]:
```
import fileinput
```

[56]:
```
database_df_origin['Precursor Id'].values
```

[56]:
```
array([   251,    339,    387, …, 151105, 151120, 151276])
```

[57]:
```
mgf_path = 'output.mgf'

def add_database_data(line, database_df, precursor_id):
    if line.startswith('TITLE='):
        precursor_id[0] = int(line.split('=')[1].strip())  # Extract and save␣
↪the precursor ID
    elif line.startswith('SEQ='):
        if precursor_id[0] in database_df_origin['Precursor Id'].values:
            peptide = database_df_origin.loc[database_df_origin['Precursor Id']␣
↪== precursor_id[0], 'Peptide'].iloc[0]
            # Replace the SEQ line with the peptide sequence
            return f'SEQ={peptide}\n'
    return line

with fileinput.FileInput(mgf_path, inplace=True, backup='.bak') as file:
    precursor_id = [None]  # Placeholder for precursor ID
    for line in file:
        print(add_database_data(line, database_df_origin, precursor_id), end='')
```

Editing of mgf with fileinput is much faster!!! (10 min for 1.7 GB data)

8.3.4 add casanovo predictions

[58]:
```
mgf_path = 'output.mgf'

def add_casanovo_data(line, casanovo_df, precursor_id):
    if line.startswith('TITLE='):
        precursor_id[0] = int(line.split('=')[1].strip())  # Extract and save␣
 ↪the precursor ID
    elif line.startswith('CASANOVO_SEQ='):
        if precursor_id[0] in casanovo_df_origin['Precursor Id'].values:
            peptide = casanovo_df_origin.loc[casanovo_df_origin['Precursor Id']␣
 ↪== precursor_id[0], 'sequence'].iloc[0]
            # Replace the SEQ line with the peptide sequence
            return f'CASANOVO_SEQ={peptide}\n'
    elif line.startswith('CASANOVO_AA_SCORES='):
        if precursor_id[0] in casanovo_df_origin['Precursor Id'].values:
            aa_scores = casanovo_df_origin.loc[casanovo_df_origin['Precursor␣
 ↪Id'] == precursor_id[0], 'opt_ms_run[1]_aa_scores'].iloc[0]
            # Replace the CASANOVO_AA_SCORES line with the list of aa_scores
            return f'CASANOVO_AA_SCORES=[{aa_scores}]\n'
    return line

with fileinput.FileInput(mgf_path, inplace=True, backup='.bak') as file:
    precursor_id = [None]  # Placeholder for precursor ID
    for line in file:
        print(add_casanovo_data(line, casanovo_df_origin, precursor_id), end='')
```

9 Evaluation: Metadata, metrics and functions

Open prediction added HeLA sample mgf again:

[59]:
```
mgf_path = 'output.mgf'
opened_MGF_spectra_pred = mgf.read(mgf_path, convert_arrays=True)
```

[60]:
```
len(opened_MGF_spectra_pred)
```

[60]:
```
151503
```

```
[61]: def get_line_count(file_path: str) -> int:
          with open(file_path, 'r') as file:
              line_count = sum(1 for line in file)
          return line_count

      line_count = get_line_count("output.mgf")
      print("Line count:", line_count)
```

```
Line count: 73497466
```

```
[62]: !wc -l output.mgf
```

```
 73497466 output.mgf
```

```
[63]: #Make dataframe:

      # Initialize empty lists to store data (takes a lot of time...)
      params_list = []
      mz_array_list = []
      intensity_array_list = []

      # Iterate through spectra and extract data
      for spectrum in opened_MGF_spectra_pred:
          params = spectrum['params']
          mz_array = spectrum['m/z array']
          intensity_array = spectrum['intensity array']

          # Append data to lists
          params_list.append(params)
          mz_array_list.append(mz_array)
          intensity_array_list.append(intensity_array)
```

```
[185]: import json

       # Create DataFrame for 'params' and expand the dictionary into columns
       params_df = pd.DataFrame(params_list)

       # Create DataFrame for 'm/z array' and 'intensity array'
       mz_intensity_df = pd.DataFrame({
           'm/z array': mz_array_list,
           'intensity array': intensity_array_list
       })

       # Concatenate all DataFrames
       df = pd.concat([params_df, mz_intensity_df], axis=1)
```

```
# Define a function to convert string representation of list to actual list of␣
↪floats
def parse_float_list(string_list):
    if string_list == '[]':  # Check for empty string
        return []  # Return empty list
    else:
        return json.loads(string_list)  # Convert string to list of floats␣
↪using json module

# Apply the function to the entire column
df['casanovo_aa_scores'] = df['casanovo_aa_scores'].apply(lambda x:␣
↪parse_float_list(x))

# Save the DataFrame as CSV (takes too long...)
#df.to_csv('output.csv', index=False)
```

[186]:
```
df
```

[186]:
```
         title                      pepmass  rtinseconds charge  \
0            1   (663.2879991882091, None)     1.527828   [2+]
1            2  (1221.9899373704318, None)     2.399628   [1+]
2            3  (1223.9922918996538, None)     2.973578   [1+]
3            4   (922.0052771097749, None)     2.973578   [1+]
4            5   (535.7742023095526, None)     2.973578   [1+]
…          …                          …          …      …
151498  151499   (623.0248420939173, None)  3584.723493   [1+]
151499  151500   (622.0267592077544, None)  3585.272101   [1+]
151500  151501  (1223.9880700318918, None)  3586.245334   [1+]
151501  151502  (1221.9879333107788, None)  3589.934304   [1+]
151502  151503   (922.0084868635723, None)  3595.538155   [1+]

             ion_mobility         scans            casanovo_seq  \
0       0.8166844415024028       F8:1777.0             TLHTLLLDNRK
1       1.2557798217225913      F16:3885.0              NDLLLEKEEK
2       1.3628076304583132      F21:3454.0  KPREC+57.021ESC+57.021R
3        1.113046705532348      F21:2708.0                PMGLLRLK
4       0.8977651224932436      F21:4667.0
…                       …           …                       …
151498  0.9613576822314688   F33077:2258.0             HDC+57.021DK
151499  0.9782158702844477  F33082:25826.0            EC+57.021FER
151500  1.3611104452168818   F33090:1846.0             QYGPKPPTVLK
151501  1.3690286963761604  F33121:50923.0        LC+57.021EDRLDNGK
151502   1.177092421069372  F33167:23236.0                 KEEDNER

                                    casanovo_aa_scores seq  \
0       [0.62897, 0.46597, 0.40492, 0.38024, 0.31451, …
1       [0.48481, 0.2251, 0.24477, 0.27449, 0.28019, 0…
```

```
2        [0.44714, 0.24382, 0.22875, 0.23995, 0.36384, …
3        [0.70436, 0.43039, 0.47188, 0.53315, 0.39718, …
4                                                       []
…                                                     …  ..
151498       [0.32813, 0.38435, 0.6269, 0.32973, 0.33301]
151499        [0.35393, 0.30745, 0.232, 0.51399, 0.25483]
151500   [0.72467, 0.4365, 0.43237, 0.49246, 0.78193, 0…
151501   [0.8115, 0.66726, 0.7572, 0.48835, 0.42572, 0…
151502   [0.45166, 0.29354, 0.26007, 0.2779, 0.27601, 0…

                                                m/z array  \
0        [273.03466354161986, 360.92334212510104, 400.3…
1        [553.9489515333205, 558.5288494547539, 877.954…
2        [230.92269514520777, 245.9377834925359, 319.93…
3        [614.6492959691126, 616.6141580080161, 624.611…
4                 [412.85761609768576, 418.72477380794015]
…                                                     …
151498   [200.99180651257458, 290.92891283118473, 393.9…
151499   [188.0090480275357, 209.95023734750205, 225.93…
151500   [227.93117309197157, 250.83549331644747, 250.8…
151501   [248.9179004506173, 250.9229450401413, 277.928…
151502   [207.91631035695485, 229.92175940366593, 229.9…

                                          intensity array
0        [56.0, 138.0, 63.0, 115.0, 115.0, 76.0, 112.0,…
1        [85.0, 11.0, 61.0, 49.0, 31.0, 62.0, 45.0, 45…
2        [98.0, 77.0, 81.0, 71.0, 125.0, 96.0, 141.0, 6…
3        [75.0, 67.0, 24.0, 45.0, 39.0, 53.0, 85.0, 76…
4                                            [38.0, 57.0]
…                                                     …
151498   [10.0, 65.0, 62.0, 71.0, 71.0, 102.0, 70.0, 49…
151499   [59.0, 110.0, 157.0, 104.0, 88.0, 10.0, 186.0,…
151500   [189.0, 31.0, 28.0, 133.0, 64.0, 136.0, 106.0,…
151501   [32.0, 67.0, 105.0, 81.0, 10.0, 171.0, 10.0, 3…
151502   [77.0, 96.0, 67.0, 119.0, 112.0, 142.0, 84.0, …

[151503 rows x 11 columns]
```

[66]:
```
casanovo_df = df[df['casanovo_seq'] != '']
database_df = df[df['seq'] != '']
matched_df = df[(df['seq'] != '') & (df['casanovo_seq'] != '')]
```

9.1 Duplicates:

[67]:
```
casanovo_duplicates = casanovo_df[casanovo_df.duplicated(subset='title',␣
↪keep=False)]
casanovo_duplicates['title'].unique()
```

[67]:
```
array([], dtype=object)
```

[68]:
```
database_duplicates = database_df_origin[database_df_origin.
↪duplicated(subset='Precursor Id', keep=False)]
database_duplicates['Precursor Id'].unique()
```

[68]:
```
array([ 10071,  21011,  22802,  30255,  35089,  35150,  35216,  36656,
        39033,  39887,  39950,  40856,  43822,  47243,  47287,  47416,
        47713,  47850,  49695,  50653,  51310,  52182,  52432,  52574,
        55300,  56513,  56857,  57379,  58070,  60423,  62073,  63971,
        65216,  65383,  65667,  68077,  68822,  72499,  72982,  74036,
        74166,  74315,  74730,  74872,  75585,  75637,  75730,  76413,
        76873,  76965,  77111,  78365,  79670,  79752,  80309,  83443,
        83870,  84008,  84175,  85365,  86625,  90838,  91189,  91997,
        92048,  92683,  93697,  94012,  94190,  94673,  98292, 101597,
       102843, 103765, 104099, 104190, 104657, 104772, 106448, 106496,
       106667, 106858, 108295, 108328, 109530, 110136, 114303, 115641,
       116622, 117155, 117841, 118483, 120232, 122420, 123716, 124011,
       125134, 125274, 126041, 126995, 128061, 128670, 129793, 130433,
       131062, 131262, 133703, 135388, 139583, 140522, 144643, 145687,
       146466, 148852])
```

[69]:
```
database_df_origin.loc[database_df_origin['Precursor Id'] == 10071, 'Peptide']
```

[69]:
```
27384    IINHSVDK
27383    ILNHSVDK
Name: Peptide, dtype: object
```

[70]:
```
#opened_MGF_spectra_pred['10071']
```

The sequence with the highest index was added. The casanovo evaluate script accounts for aminoacids with equivalent aminoacid mass.

[71]:
```
opened_MGF_spectra_pred[38770]
```

[71]:
```
{'params': {'title': '38771',
  'pepmass': (714.8580883863376, None),
  'rtinseconds': 1384.866382 second,
  'charge': [2],
  'ion_mobility': '1.024561009229294',
  'scans': 'F12562:4428.0',
  'casanovo_seq': 'MVGDEALQHPTTK',
  'casanovo_aa_scores': '[0.68934,0.55402,0.52167,0.85233,0.85514,0.78124,0.7340
5,0.78229,0.57947,0.47785,0.81282,0.74030,0.81484]',
  'seq': ''},
 'm/z array': array([ 186.23956774,  195.09130293,  203.12878357,  221.12609597,
         231.12170424,  231.46717639,  269.16203096,  271.13514993,
         302.15026222,  308.17139963,  316.15920733,  323.16502197,
         330.17021238,  334.05984142,  335.14770544,  355.1654286 ,
         363.15649593,  368.19719288,  389.24253137,  389.24875595,
         392.43290061,  400.18330615,  410.17914177,  415.20079744,
         416.22038074,  417.20254805,  419.20265275,  426.19508992,
         428.24598943,  435.27364198,  435.29668042,  436.22201246,
         438.21430789,  440.18466473,  440.19790353,  446.25554065,
         446.26553797,  461.21139549,  466.215126  ,  469.16949986,
         470.27380915,  489.22823159,  490.64935959,  493.16493934,
         498.28229848,  503.26680841,  510.24831323,  525.20121221,
         525.24459536,  526.19947791,  529.23625178,  530.23471062,
         535.29951404,  546.76951212,  553.26229439,  553.2808471 ,
         564.29616245,  565.31214483,  569.23089103,  570.24753704,
         582.25536207,  583.31785714,  583.33690711,  584.31268097,
         584.35081369,  585.32361832,  590.79394446,  591.30018036,
         594.08066313,  594.31138252,  598.27905005,  603.24363645,
         610.29242989,  611.29828522,  615.30651028,  616.28515787,
         620.29395675,  623.74041377,  632.19206129,  637.2951286 ,
         638.27516061,  638.29110232,  638.31102972,  639.27987658,
         639.29184225,  641.2996686 ,  641.31964291,  652.29624304,
         654.31226661,  654.32437213,  657.36640152,  662.31408932,
         664.29673203,  666.29455359,  667.28032684,  669.35608754,
         676.34916855,  682.3068199 ,  682.31506108,  682.32742293,
         693.37387528,  694.35037565,  695.46068014,  707.34653321,
         711.37995018,  713.37567848,  717.350171  ,  723.37503874,
         725.3535217 ,  725.36201887,  725.52772356,  726.35227938,
         726.36078239,  727.35597862,  736.34340309,  739.33002793,
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         754.35382198,  759.40982523,  764.36059408,  765.34220417,
         767.32916276,  767.33790233,  781.3540927 ,  783.38820644,
         798.38732384,  798.39623852,  798.40961064,  799.40391852,
         803.08779064,  810.35968937,  810.36867065,  811.36590178,
         812.36824232,  824.41288518,  825.41418573,  828.38995113,
         852.42107352,  853.42080206,  854.64707504,  880.40089462,
         895.4285895 ,  895.47107406,  896.45322025,  898.42385589,
         910.42637551,  913.42753043,  913.44183346,  913.4561366 ,
         914.42901283,  914.46717539,  915.43104394,  923.43828398,
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         991.44175537,  992.43045165,  992.47020853, 1015.43715392,
        1025.50055097, 1026.48081127, 1027.50704893, 1028.50850376,
        1035.47143071, 1110.55753359, 1113.90350621, 1127.5654901 ,
        1128.56686585, 1128.57746479, 1129.56868605, 1151.53916471,
        1152.55648295, 1180.58950601, 1180.60034649, 1182.6121161 ,
        1183.57251258, 1197.98701654, 1198.58223237, 1198.59861655,
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        1297.67489567]),
 'intensity array': array([132., 222.,  85., 139.,  95.,  97., 122.,  48., 145.,
86.,  85.,
        136., 100.,  45.,  58.,  41.,  44., 121.,  66.,  99.,  58.,  86.,
         78.,  63.,  87.,  95.,  78.,  82., 101.,  86.,  38.,  58.,  91.,
        210., 114., 169., 342.,  86.,  53.,  43.,  26.,  11.,  55., 115.,
        118.,  99., 105., 110., 119., 100., 102.,  27., 104., 110.,  49.,
         62.,  89., 108.,  52.,  51.,  62., 335., 125., 223.,  66.,  67.,
        103.,  60.,  10.,  57.,  21.,  89.,  37., 130.,  32.,  78.,  61.,
         90.,  86.,  85., 146., 682.,  95., 148.,  67., 105.,  10., 124.,
         35., 114.,  43.,  68.,  85.,  83.,  56.,  84.,  50.,  33., 173.,
         91.,  29., 121.,  10.,  10.,  55.,  10.,  91.,  23.,  45.,  53.,
        110.,  12.,  41.,  50.,  95.,  66.,  71.,  82.,  32.,  58.,  11.,
         54.,  56.,  95.,  94.,  56.,  54.,  26.,  52.,  33.,  35.,  79.,
         86., 154.,  80., 121.,  52., 101., 111.,  61., 102.,  48.,  57.,
         48.,  23.,  30.,  10.,  93.,  49.,  90.,  28.,  61.,  38., 287.,
         28.,  31., 282.,  78.,  74.,  51.,  44.,  77.,  16.,  59.,  77.,
         45.,  69.,  12.,  10.,  68.,  10.,  25.,  10.,  58.,  10.,  32.,
         26.,  59.,  53.,  13.,  45.,  64.,  29.,  42.,  39.,  29., 214.,
        163.,  70., 103.,  21.,  59.,  58.]),
 'charge array': array([0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
0, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0])}
```

9.2 Modifications:

TOKENS

Amino acid modifications:

"M+15.995": 147.035400 # Met oxidation: 131.040485 + 15.994915

"N+0.984": 115.026943 # Asn deamidation: 114.042927 + 0.984016

"Q+0.984": 129.042594 # Gln deamidation: 128.058578 + 0.984016

N-terminal modifications:

"+42.011": 42.010565 # Acetylation

"+43.006": 43.005814 # Carbamylation

"-17.027": -17.026549 # NH3 loss

"+43.006-17.027" : 25.980265 # Carbamylation and NH3 loss

canonical:

"C+57.021"

G,A,S,P,V,T,L,I,N,D,Q,K,E,M,H,F,R,W (U, O)

9.2.1 Modification df subsets

[72]:
```
database_modifications_df = df[df['seq'].str.contains(r'\d')]
casanovo_modifications_df = df[df['casanovo_seq'].str.contains(r'\d')]
database_modifications_df.head()
```

[72]:
```
    title                        pepmass  rtinseconds charge         ion_mobility  \
480   481   (782.3185350800387, None)   420.350962   [2+]  0.9845111201974767
503   504   (652.7659935780628, None)   421.521961   [2+]  0.9299330927684736
583   584   (703.2715306865842, None)   422.692786   [2+]  0.9261423663707522
593   594   (573.7785047994056, None)   422.692786   [2+]  0.8692402766260495
596   597  (382.85352207661816, None)   422.692786   [3+]  0.7065246012239691

              scans                  casanovo_seq  \
480   F3498:3666.0  GQSSQVC+57.021HGM+15.995PTSR
503  F3509:54700.0          AEGDDC+57.021GNPAGSR
583  F3520:31885.0           HGDEEASTDEC+57.021R
593  F3520:62796.0                     QRVQDESQR
596  F3520:37342.0             C+57.021NTDQAGRPK

                                      casanovo_aa_scores  \
480  [0.66546, 0.61213, 0.69182, 0.82841, 0.72443, …
503  [0.67412, 0.44788, 0.44003, 0.68788, 0.79082, …
583  [0.77353, 0.68559, 0.87545, 0.73799, 0.86894, …
593  [0.94731, 0.94808, 0.73508, 0.95385, 0.9503, 0…
596  [0.97438, 0.96625, 0.97582, 0.97792, 0.97762, …

                          seq  \
480  AESSQTC(+57.02)HSEQGDK
503   AAGDGDC(+57.02)GTTHSR
583   HGGSDNASTDC(+57.02)ER
593         RQ(+.98)VQDESQR
596      C(+57.02)NTDQAGRPK

                                               m/z array  \
480  [268.0399833129692, 303.12997620694784, 374.24…
503  [244.09079359843835, 269.08439465734784, 286.1…
583  [179.3128996919326, 226.08916534781523, 250.02…
```

```
593  [198.09494897278134, 199.0752954657264, 200.07…
596  [185.04024163307307, 197.1081632909489, 198.11…

                                      intensity array
480  [71.0, 88.0, 86.0, 94.0, 53.0, 83.0, 47.0, 83…
503  [89.0, 11.0, 84.0, 89.0, 91.0, 69.0, 107.0, 64…
583  [10.0, 120.0, 51.0, 94.0, 75.0, 74.0, 79.0, 13…
593  [239.0, 212.0, 63.0, 104.0, 11.0, 132.0, 134.0…
596  [96.0, 493.0, 55.0, 184.0, 106.0, 87.0, 53.0, …
```

9.2.2 Get all Modifications in database and change its modification tokens:

[73]:
```python
import re
from collections import defaultdict

# Assuming database_modifications_df is your DataFrame
modifications_dict = defaultdict(list)

pattern_to_find = r'\((.*?)\)'

for index, row in database_modifications_df.iterrows():
    seq = row['seq']
    titles = row['title']
    patterns = re.findall(pattern_to_find, seq)
    for pattern in patterns:
        full_pattern = f'({pattern})'  # Add parentheses to the pattern
        modifications_dict[full_pattern].append(titles)

# Convert defaultdict to a regular dictionary
modifications_dict = dict(modifications_dict)
```

[74]:
```python
modifications_dict.keys()
```

[74]:
```
dict_keys(['(+57.02)', '(+.98)', '(+42.01)', '(+15.99)'])
```

[75]:
```python
lengths = {key: len(value) for key, value in modifications_dict.items()}
lengths
```

[75]:
```
{'(+57.02)': 9564, '(+.98)': 3152, '(+42.01)': 1465, '(+15.99)': 503}
```

[76]:
```python
modifications_dict['(+57.02)'][0:10]
```

[76]:
```
['481', '504', '584', '597', '646', '646', '665', '727', '727', '753']
```

[77]:
```python
database_modifications_df[database_modifications_df['title'] == '2868']
```

```
[77]:      title                      pepmass  rtinseconds charge        ion_mobility  \
     2867  2868  (619.2743426911019, None)   505.809337   [2+]  0.8773655991885236

                scans      casanovo_seq  \
     2867  F4301:2715.0  QEM+15.995QEVQSSR

                                        casanovo_aa_scores                 seq  \
     2867  [0.96698, 0.98005, 0.98017, 0.97804, 0.98051, …  QEM(+15.99)QEVQSSR

                                                 m/z array  \
     2867  [213.09337303026976, 226.48545662847033, 229.1…

                                           intensity array
     2867  [76.0, 10.0, 125.0, 87.0, 87.0, 66.0, 107.0, 9…
```

```
[78]: def rearrange_modifications(peptide_sequence):
          # Use regular expression to find all occurrences of the modification pattern
          matches = re.finditer(r'([A-Z])\(([-+]?\d+\.\d+)\)', peptide_sequence)

          # Iterate through matches and replace the original format with the␣
       ↪rearranged format
          for match in matches:
              amino_acid = match.group(1)
              numeric_part = match.group(2)
              original_format = match.group(0)
              rearranged_format = f'{numeric_part}{amino_acid}'

              # If the match is at the beginning of the sequence, replace directly
              if peptide_sequence.startswith(original_format):
                  peptide_sequence = peptide_sequence.replace(original_format,␣
       ↪rearranged_format, 1)
              else:
                  # Replace the original format with the rearranged format in the␣
       ↪peptide_sequence
                  peptide_sequence = peptide_sequence.replace(original_format,␣
       ↪rearranged_format, 1)

          return peptide_sequence
```

```
[79]: rearrange_modifications('A(+42.01)GILFEDIFDVK')
```

```
[79]: '+42.01AGILFEDIFDVK'
```

```
[80]: def modify_peptide_sequences(column):

          # change all Amino acid modifications:
          aa_changes = [
              (r'\(\+57\.02\)', '+57.021'),
              (r'\(\+\.98\)', '+0.984'),
              (r'\(\+15\.99\)', '+15.995'),
          ]

          # change all N-term modifications:
          N_changes = [
              (r'\+42\.01', '+42.011'),
              (r'\+43\.01', '+43.006'),
              (r'\-17\.03', '-17.027'),
              (r'\+43\.01\(\-17\.03\)', '+43.006-17.027')
          ]

          modified_column = column.copy()

          for pattern, replacement in aa_changes:
              modified_column = modified_column.apply(lambda x: re.sub(pattern,␣
      ↪replacement, x))

          # rearrange N-term modifications A(+42.01) -> +42.01A
          modified_column = modified_column.apply(lambda x:␣
      ↪rearrange_modifications(x))

          for pattern, replacement in N_changes:
              modified_column = modified_column.apply(lambda x: re.sub(pattern,␣
      ↪replacement, x))


          return modified_column
```

[140]:
```
database_modifications_df.loc[:, 'Modified seq'] =␣
↪modify_peptide_sequences(database_modifications_df.loc[:,'seq']).values
modify_peptide_sequences(database_modifications_df.loc[:,'seq'])
```

[140]:
```
480                          AESSQTC+57.021HSEQGDK
503                           AAGDGDC+57.021GTTHSR
583                           HGGSDNASTDC+57.021ER
593                                RQ+0.984VQDESQR
596                               C+57.021NTDQAGRPK
                            …
151072     +42.0111DDDIAALVVDN+0.984GSGMC+57.021K
151079              +42.0111AALDSLSLFTSLGLSEQK
151089                          +42.0111MELITILEK
151104    FQSSAVMALQEASEAYLVGLFEDTNLC+57.021AIHAK
151275                       +42.0111AGILFEDIFDVK
Name: seq, Length: 10990, dtype: object
```

```
[82]: database_modifications_df.head()
```

```
[82]:     title                          pepmass  rtinseconds charge          ion_mobility  \
480    481    (782.3185350800387, None)   420.350962    [2+]   0.9845111201974767
503    504    (652.7659935780628, None)   421.521961    [2+]   0.9299330927684736
583    584    (703.2715306865842, None)   422.692786    [2+]   0.9261423663707522
593    594    (573.7785047994056, None)   422.692786    [2+]   0.8692402766260495
596    597   (382.85352207661816, None)   422.692786    [3+]   0.7065246012239691

              scans                  casanovo_seq  \
480   F3498:3666.0  GQSSQVC+57.021HGM+15.995PTSR
503  F3509:54700.0          AEGDDC+57.021GNPAGSR
583  F3520:31885.0           HGDEEASTDEC+57.021R
593  F3520:62796.0                     QRVQDESQR
596  F3520:37342.0             C+57.021NTDQAGRPK

                                    casanovo_aa_scores  \
480  [0.66546, 0.61213, 0.69182, 0.82841, 0.72443, …
503  [0.67412, 0.44788, 0.44003, 0.68788, 0.79082, …
583  [0.77353, 0.68559, 0.87545, 0.73799, 0.86894, …
593  [0.94731, 0.94808, 0.73508, 0.95385, 0.9503, 0…
596  [0.97438, 0.96625, 0.97582, 0.97792, 0.97762, …

                          seq  \
480  AESSQTC(+57.02)HSEQGDK
503   AAGDGDC(+57.02)GTTHSR
583   HGGSDNASTDC(+57.02)ER
593         RQ(+.98)VQDESQR
596      C(+57.02)NTDQAGRPK

                                             m/z array  \
480  [268.0399833129692, 303.12997620694784, 374.24…
503  [244.09079359843835, 269.08439465734784, 286.1…
583  [179.3128996919326, 226.08916534781523, 250.02…
593  [198.09494897278134, 199.0752954657264, 200.07…
596  [185.04024163307307, 197.1081632909489, 198.11…

                                       intensity array          Modified seq
480  [71.0, 88.0, 86.0, 94.0, 53.0, 83.0, 47.0, 83…  AESSQTC+57.021HSEQGDK
503  [89.0, 11.0, 84.0, 89.0, 91.0, 69.0, 107.0, 64…    AAGDGDC+57.021GTTHSR
583  [10.0, 120.0, 51.0, 94.0, 75.0, 74.0, 79.0, 13…    HGGSDNASTDC+57.021ER
593  [239.0, 212.0, 63.0, 104.0, 11.0, 132.0, 134.0…          RQ+0.984VQDESQR
596  [96.0, 493.0, 55.0, 184.0, 106.0, 87.0, 53.0, …       C+57.021NTDQAGRPK
```

```
[187]: df['seq'] = modify_peptide_sequences(df['seq']).values
```

```
[84]: database_modifications_df = df[df['seq'].str.contains(r'\d')]
      database_modifications_df
```

```
[84]:         title                      pepmass  rtinseconds charge  \
480         481   (782.3185350800387, None)   420.350962   [2+]
503         504   (652.7659935780628, None)   421.521961   [2+]
583         584   (703.2715306865842, None)   422.692786   [2+]
593         594   (573.7785047994056, None)   422.692786   [2+]
596         597  (382.85352207661816, None)   422.692786   [3+]
…           …                          …          …    …
151072  151073   (911.9029864084164, None)  3377.129390   [2+]
151079  151080   (961.5026393091026, None)  3381.112196   [2+]
151089  151090   (566.3165152650313, None)  3385.366051   [2+]
151104  151105   (1171.900026588638, None)  3387.814265   [3+]
151275  151276   (704.8685231178537, None)  3393.665178   [2+]

             ion_mobility          scans  \
480     0.9845111201974767   F3498:3666.0
503     0.9299330927684736  F3509:54700.0
583     0.9261423663707522  F3520:31885.0
593     0.8692402766260495  F3520:62796.0
596     0.7065246012239691  F3520:37342.0
…                      …              …
151072  1.1627110544648063  F31273:2432.0
151079    1.25887097544921  F31308:1908.0
151089  0.8900965335502041  F31346:2194.0
151104   1.120991341576058  F31369:9550.0
151275  0.9906925497189761  F31424:2499.0

                                   casanovo_seq  \
480                GQSSQVC+57.021HGM+15.995PTSR
503                        AEGDDC+57.021GNPAGSR
583                         HGDEEASTDEC+57.021R
593                                   QRVQDESQR
596                           C+57.021NTDQAGRPK
…                                             …
151072    +43.006GC+57.021DLAALVVDNGSGMC+57.021K
151079                +42.011AALDSLSLTFSLGLSQEK
151089                         +42.011MELLTLLEK
151104  QQDAAVMALQELYASSTLSTQGLSTLTC+57.021ALHAK
151275                                GLSEFDELFDVK

                                   casanovo_aa_scores  \
480     [0.66546, 0.61213, 0.69182, 0.82841, 0.72443, …
503     [0.67412, 0.44788, 0.44003, 0.68788, 0.79082, …
583     [0.77353, 0.68559, 0.87545, 0.73799, 0.86894, …
593     [0.94731, 0.94808, 0.73508, 0.95385, 0.9503, 0…
```

```
596     [0.97438, 0.96625, 0.97582, 0.97792, 0.97762, …
…                                                  …
151072  [0.86087, 0.91799, 0.90924, 0.91923, 0.91784, …
151079  [0.90429, 0.81302, 0.90067, 0.92657, 0.92622, …
151089  [0.97946, 0.97909, 0.9821, 0.98179, 0.98214, 0…
151104  [0.73876, 0.69725, 0.76276, 0.73163, 0.75209, …
151275  [0.62313, 0.76164, 0.8619, 0.87266, 0.73913, 0…

                                           seq  \
480                     AESSQTC+57.021HSEQGDK
503                      AAGDGDC+57.021GTTHSR
583                      HGGSDNASTDC+57.021ER
593                             RQ+0.984VQDESQR
596                           C+57.021NTDQAGRPK
…                                           …
151072    +42.011DDDIAALVVDN+0.984GSGMC+57.021K
151079              +42.011AALDSLSLFTSLGLSEQK
151089                     +42.011MELITILEK
151104  FQSSAVMALQEASEAYLVGLFEDTNLC+57.021AIHAK
151275                  +42.011AGILFEDIFDVK

                                                m/z array  \
480     [268.0399833129692, 303.12997620694784, 374.24…
503     [244.09079359843835, 269.08439465734784, 286.1…
583     [179.3128996919326, 226.08916534781523, 250.02…
593     [198.09494897278134, 199.0752954657264, 200.07…
596     [185.04024163307307, 197.1081632909489, 198.11…
…                                                  …
151072  [231.06175272141326, 232.0627610127923, 255.17…
151079  [201.11930377954843, 239.06454038495326, 242.1…
151089  [197.12588150885645, 214.07087132311415, 215.1…
151104  [253.19952964469232, 260.12149907488987, 311.2…
151275  [143.12058171064643, 217.08650947938978, 217.0…

                                          intensity array
480     [71.0, 88.0, 86.0, 94.0, 53.0, 83.0, 47.0, 83…
503     [89.0, 11.0, 84.0, 89.0, 91.0, 69.0, 107.0, 64…
583     [10.0, 120.0, 51.0, 94.0, 75.0, 74.0, 79.0, 13…
593     [239.0, 212.0, 63.0, 104.0, 11.0, 132.0, 134.0…
596     [96.0, 493.0, 55.0, 184.0, 106.0, 87.0, 53.0, …
…                                                  …
151072  [83.0, 75.0, 79.0, 136.0, 43.0, 65.0, 100.0, 5…
151079  [138.0, 72.0, 66.0, 67.0, 103.0, 22.0, 60.0, 1…
151089  [103.0, 111.0, 155.0, 101.0, 72.0, 49.0, 93.0,…
151104  [62.0, 198.0, 64.0, 76.0, 86.0, 110.0, 180.0, …
151275  [131.0, 147.0, 42.0, 90.0, 128.0, 148.0, 40.0,…
```

```
[10990 rows x 11 columns]
```

9.2.3 Add booleans to the df for modifications:

```
[188]: df['db_modified'] = df['seq'].str.contains(r'\d')
       df['cs_modified'] = df['casanovo_seq'].str.contains(r'\d')
```

```
[86]: df[(df['db_modified'] == True) & (df['cs_modified'] == False)]
```

```
[86]:          title                     pepmass  rtinseconds charge  \
      593        594  (573.7785047994056, None)   422.692786   [2+]
      737        738  (543.7670949222372, None)   425.032819   [2+]
      970        971  (574.2384531045504, None)   428.543221   [2+]
      1096      1097  (559.2561248409539, None)   430.884694   [2+]
      1103      1104  (626.2380117103042, None)   430.884694   [2+]
      …          …                          …          …     …
      150978  150979  (822.3866636811822, None)  3335.786313   [2+]
      150989  150990  (889.4897518429245, None)  3338.021350   [2+]
      150993  150994  (686.8878603341283, None)  3338.021350   [2+]
      151022  151023  (894.9612997974076, None)  3349.586481   [2+]
      151275  151276  (704.8685231178537, None)  3393.665178   [2+]

                   ion_mobility          scans       casanovo_seq  \
      593     0.8692402766260495  F3520:62796.0          QRVQDESQR
      737     0.8508074986103232  F3542:41287.0          LQNQQDPML
      970     0.9053471778036298  F3575:70153.0        GFGNDVSGSHGT
      1096    0.8700056693139301  F3597:23636.0          EQQPSSASER
      1103    0.9021636231062317  F3597:18695.0        QSSSSTTSQNVK
      …                      …              …                  …
      150978  1.0249836013922387  F30895:2313.0     DPVQEAWAEDVNAAK
      150989  1.0966087822261015  F30916:3515.0  TANNGPLNLPLLADTVR
      150993  1.0011790533507448  F30916:2298.0        SWVQPLGLGFLR
      151022   1.159747965087771  F31023:2024.0  SLNNWFATAAGHGPAHK
      151275  0.9906925497189761  F31424:2499.0        GLSEFDELFDVK

                                          casanovo_aa_scores  \
      593     [0.94731, 0.94808, 0.73508, 0.95385, 0.9503, 0…
      737     [0.87686, 0.9108, 0.94183, 0.80763, 0.91846, 0…
      970     [0.40655, 0.41975, 0.55974, 0.43787, 0.77412, …
      1096    [0.97881, 0.97929, 0.98644, 0.98609, 0.98653, …
      1103    [0.93575, 0.84697, 0.87281, 0.96328, 0.96305, …
      …                                                   …
      150978  [0.80596, 0.68117, 0.93392, 0.94799, 0.95424, …
      150989  [0.63923, 0.57067, 0.8893, 0.88621, 0.88524, 0…
      150993  [0.84655, 0.6409, 0.84236, 0.83859, 0.76745, 0…
      151022  [0.70805, 0.42782, 0.49562, 0.78185, 0.81249, …
      151275  [0.62313, 0.76164, 0.8619, 0.87266, 0.73913, 0…
```

```
                              seq  \
593                RQ+0.984VQDESQR   
737                LQNQQN+0.984GQR   
970              GSEEVDSHC+57.021K   
1096             HVSPSC+57.021STSR   
1103            GGDEYDNHC+57.021GK   
…                               …   
150978         DPVQ+0.984EAWAEDVDLR   
150989  +42.011EGGLGPLN+0.984IPLLADVTR   
150993       +42.011VMVQ+0.984PINLIFR   
151022 +42.011AGLEVLFASAAPAITC+57.021R   
151275           +42.011AGILFEDIFDVK   

                                                m/z array  \
593     [198.09494897278134, 199.0752954657264, 200.07…   
737     [216.09750441508407, 225.12954087641108, 230.1…   
970     [219.07596337088495, 233.14782169808066, 256.0…   
1096    [161.12354617046634, 195.07808295216427, 209.0…   
1103    [199.0752954657264, 200.053599326878, 204.1350…   
…                                                   …   
150978  [213.09107025378887, 215.5575249708841, 242.14…   
150989  [222.15709403106132, 269.0895700621734, 270.06…   
150993  [228.13603718887236, 231.11690783315618, 246.1…   
151022  [201.1304896789157, 245.12948626579828, 256.17…   
151275  [143.12058171064643, 217.08650947938978, 217.0…   

                                          intensity array  db_modified  \
593     [239.0, 212.0, 63.0, 104.0, 11.0, 132.0, 134.0…         True   
737     [112.0, 150.0, 84.0, 83.0, 142.0, 79.0, 160.0,…         True   
970     [138.0, 110.0, 149.0, 35.0, 95.0, 101.0, 101.0…         True   
1096    [143.0, 181.0, 148.0, 58.0, 181.0, 80.0, 74.0,…         True   
1103    [87.0, 59.0, 95.0, 103.0, 101.0, 124.0, 111.0,…         True   
…                                                   …          …   
150978  [112.0, 39.0, 104.0, 78.0, 122.0, 207.0, 91.0,…         True   
150989  [99.0, 51.0, 99.0, 71.0, 88.0, 164.0, 118.0, 1…         True   
150993  [150.0, 22.0, 99.0, 222.0, 92.0, 67.0, 52.0, 5…         True   
151022  [161.0, 105.0, 117.0, 118.0, 98.0, 126.0, 64.0…         True   
151275  [131.0, 147.0, 42.0, 90.0, 128.0, 148.0, 40.0,…         True   

        cs_modified  
593           False  
737           False  
970           False  
1096          False  
1103          False  
…             …  
```

```
150978        False
150989        False
150993        False
151022        False
151275        False

[2143 rows x 13 columns]
```

9.3 Evaluation

9.3.1 Get functions and amino acid dictionary:

[87]:
```
import os

# Import the required modules
from Casaval.casaeval.evaluation.evaluate  import aa_match_batch,␣
 ↪aa_match_metrics, aa_precision_recall  # from casanovo/denovo/evaluate.py,␣
 ↪modified functions for evaluation
from Casaval.casaeval.evaluation.masses import PeptideMass # get PeptideMass␣
 ↪for a dictionary of all tokens
import numpy as np
```

[88]:
```
# call dictionary, which maps AminoAcids to their masses for tokenization
aadict = PeptideMass(residues='massivekb').masses
aadict
```

[88]:
```
{'G': 57.021463735,
 'A': 71.037113805,
 'S': 87.032028435,
 'P': 97.052763875,
 'V': 99.068413945,
 'T': 101.047678505,
 'C+57.021': 160.030644505,
 'L': 113.084064015,
 'I': 113.084064015,
 'N': 114.04292747,
 'D': 115.026943065,
 'Q': 128.05857754,
 'K': 128.09496305,
 'E': 129.042593135,
 'M': 131.040484645,
 'H': 137.058911875,
 'F': 147.068413945,
 'R': 156.10111105,
 'Y': 163.063328575,
 'W': 186.07931298,
 '+42.011': 42.010565,
 '+43.006': 43.005814,
 '-17.027': -17.026549,
 '+43.006-17.027': 25.980265,
 'M+15.995': 147.03539964499998,
 'N+0.984': 115.02694346999999,
 'Q+0.984': 129.04259353999998}
```

[89]:
```
def calculate_metrics(df, aadict, cum_mass_threshold=np.inf):
    casanovo_pred = df['casanovo_seq'].tolist()
    database_pred = df['seq'].tolist()
    aa_score_list = df['casanovo_aa_scores'].tolist()

    # Use casanovo's evaluation functions to get boolean lists and metrics:
    aa_bool = aa_match_batch(
                database_pred, casanovo_pred, aadict, aa_score_list,␣
 ↪cum_mass_threshold
            )

    aa_precision, aa_recall, pep_precision = aa_match_metrics(*aa_bool)

    casanovo_pred = df['casanovo_seq']

    # Initialize counters
    n_total_aa = aa_bool[2]
    n_total_correct_aa = 0
    n_total_wrong_aa = 0

    n_total_peptide = len(aa_bool[0])
    n_total_correct_peptide = 0
    n_total_wrong_peptide = 0

    # Iterate through aa_bool[0]
    for array in aa_bool[0]:

        # Update total number of correct amino acids
        n_total_correct_aa += sum(array[0])

        if all(array[0]):
            # Update total number of correct peptides
            n_total_correct_peptide += 1
        else:
            # Update total number of wrong peptides
            n_total_wrong_peptide += 1

    # Update total number of wrong amino acids
    n_total_wrong_aa += n_total_aa - n_total_correct_aa
    return {
        'aa_precision of total pred. AA in %': round(aa_precision*100,2),
        'aa_recall of total true AA in %': round(aa_recall*100,2),
        'pep_precision in %': round(pep_precision*100,2),
        'n_total_aa': n_total_aa,
        'n_total_correct_aa': n_total_correct_aa,
        'correct_aa in %': round(n_total_correct_aa*100/n_total_aa,2),
        'n_total_wrong_aa': n_total_wrong_aa,
        'wrong_aa in %': round(n_total_wrong_aa*100/n_total_aa,2),
        'n_total_peptide': n_total_peptide,
        'n_total_correct_peptide': n_total_correct_peptide,
        'correct_peptide in %': round(n_total_correct_peptide*100/
↪n_total_peptide,2),
        'n_total_wrong_peptide': n_total_wrong_peptide,
        'wrong_peptide in %': round(n_total_wrong_peptide*100/
↪n_total_peptide,2),
    }
```

9.3.2 Get metrics for matched df:

```
[90]: matched_df = df[(df['seq'] != '') & (df['casanovo_seq'] != '')]

      casanovo_pred = matched_df['casanovo_seq'].tolist()
      database_pred = matched_df['seq'].tolist()
```

```
[91]: matched_df
```

```
[91]:          title                         pepmass  rtinseconds charge  \
      250        251    (414.7131510926927, None)   229.928144   [2+]
      338        339    (433.7261934825522, None)   351.727794   [2+]
      386        387    (452.2333325909003, None)   418.405295   [2+]
      409        410    (572.9069691658391, None)   419.180763   [3+]
      422        423   (384.70134439684443, None)   419.180763   [2+]
      …          …                            …          …     …
      151079  151080    (961.5026393091026, None)  3381.112196   [2+]
      151089  151090    (566.3165152650313, None)  3385.366051   [2+]
      151104  151105   (1171.900026588638, None)  3387.814265   [3+]
      151119  151120    (697.3745460832697, None)  3388.984397   [3+]
      151275  151276    (704.8685231178537, None)  3393.665178   [2+]

                   ion_mobility          scans  \
      250      0.771090028717373   F1925:4698.0
      338     0.7480718215926132   F2934:1829.0
      386     0.7729811003309721  F3480:18282.0
      409     0.8259100150097249  F3487:15217.0
      422     0.7600746026175902  F3487:12144.0
```

```
…                     …           …
151079    1.25887097544921  F31308:1908.0
151089  0.8900965335502041  F31346:2194.0
151104   1.120991341576058  F31369:9550.0
151119  0.9447715275631229  F31380:4070.0
151275  0.9906925497189761  F31424:2499.0

                                   casanovo_seq  \
250                                    HAVSGTEK
338                                     QPGYHHK
386                                   RGNVAGDSK
409            -17.027QAAC+57.021DHSGSDSHSSQR
422                                      SRDEDR
…                                           …
151079                +42.011AALDSLSLTFSLGLSQEK
151089                        +42.011MELLTLLEK
151104  QQDAAVMALQELYASSTLSTQGLSTLTC+57.021ALHAK
151119                        LEDNSLYNLMVNGLHSFK
151275                              GLSEFDELFDVK

                                   casanovo_aa_scores  \
250     [0.95846, 0.95933, 0.96202, 0.71932, 0.9625, 0…
338     [0.8761, 0.8782, 0.82104, 0.59657, 0.82445, 0…
386     [0.96216, 0.97383, 0.97015, 0.97438, 0.97623, …
409     [0.83947, 0.60469, 0.86706, 0.80854, 0.85195, …
422     [0.48144, 0.40026, 0.33183, 0.29243, 0.35022, …
…                                                  …
151079  [0.90429, 0.81302, 0.90067, 0.92657, 0.92622, …
151089  [0.97946, 0.97909, 0.9821, 0.98179, 0.98214, 0…
151104  [0.73876, 0.69725, 0.76276, 0.73163, 0.75209, …
151119  [0.54334, 0.39658, 0.58759, 0.64371, 0.55222, …
151275  [0.62313, 0.76164, 0.8619, 0.87266, 0.73913, 0…

                                           seq  \
250                                   HAVSEGTK
338                                    KTESHHK
386                                  RGNVAGDSK
409                          VSDSGSHSGSDSHSGASR
422                                    HGEAQVK
…                                          …
151079               +42.011AALDSLSLFTSLGLSEQK
151089                       +42.011MELITILEK
151104  FQSSAVMALQEASEAYLVGLFEDTNLC+57.021AIHAK
151119                       ELDRDTVFALVNYIFFK
151275                    +42.011AGILFEDIFDVK

                                               m/z array  \
250     [209.10764765158007, 225.10350547020948, 245.8…
338     [266.0704599178995, 284.1693012729633, 284.174…
386     [172.14680911293425, 197.10373386089654, 197.1…
409     [210.25663775256817, 294.1423328124341, 369.15…
422     [306.12279194097084, 350.1855285513594, 382.13…
…                                                     …
151079  [201.11930377954843, 239.06454038495326, 242.1…
151089  [197.12588150885645, 214.07087132311415, 215.1…
151104  [253.19952964469232, 260.12149907488987, 311.2…
151119  [243.13793120208354, 247.14156409470553, 251.1…
151275  [143.12058171064643, 217.08650947938978, 217.0…

                                          intensity array  db_modified  \
250     [81.0, 16.0, 170.0, 83.0, 56.0, 79.0, 151.0, 2…        False
338     [52.0, 83.0, 40.0, 93.0, 76.0, 52.0, 292.0, 19…        False
386     [129.0, 142.0, 168.0, 86.0, 25.0, 95.0, 204.0,…        False
409     [31.0, 77.0, 48.0, 93.0, 128.0, 11.0, 168.0, 1…        False
422     [28.0, 83.0, 98.0, 135.0, 75.0, 81.0, 87.0, 38…        False
…                                                     …          …
151079  [138.0, 72.0, 66.0, 67.0, 103.0, 22.0, 60.0, 1…         True
151089  [103.0, 111.0, 155.0, 101.0, 72.0, 49.0, 93.0,…         True
151104  [62.0, 198.0, 64.0, 76.0, 86.0, 110.0, 180.0, …         True
151119  [59.0, 110.0, 68.0, 72.0, 108.0, 191.0, 97.0, …        False
151275  [131.0, 147.0, 42.0, 90.0, 128.0, 148.0, 40.0,…         True

        cs_modified
250           False
338           False
386           False
409            True
422           False
…             …
151079         True
151089         True
151104         True
151119        False
151275        False

[38777 rows x 13 columns]
```

[92]:
```python
matched_df.reset_index(drop=True, inplace=True)
matched_df
```

[92]:
```
        title                    pepmass  rtinseconds charge  \
0         251  (414.7131510926927, None)   229.928144   [2+]
1         339  (433.7261934825522, None)   351.727794   [2+]
2         387  (452.2333325909003, None)   418.405295   [2+]
```

```
3         410   (572.9069691658391, None)   419.180763   [3+]
4         423  (384.70134439684443, None)   419.180763   [2+]
…        …                          …          …     …
38772  151080   (961.5026393091026, None)  3381.112196   [2+]
38773  151090   (566.3165152650313, None)  3385.366051   [2+]
38774  151105   (1171.900026588638, None)  3387.814265   [3+]
38775  151120   (697.3745460832697, None)  3388.984397   [3+]
38776  151276   (704.8685231178537, None)  3393.665178   [2+]

             ion_mobility          scans  \
0       0.771090028717373   F1925:4698.0
1      0.7480718215926132   F2934:1829.0
2      0.7729811003309721  F3480:18282.0
3      0.8259100150097249  F3487:15217.0
4      0.7600746026175902  F3487:12144.0
…                     …             …
38772    1.25887097544921  F31308:1908.0
38773  0.8900965335502041  F31346:2194.0
38774   1.120991341576058  F31369:9550.0
38775  0.9447715275631229  F31380:4070.0
38776  0.9906925497189761  F31424:2499.0

                                  casanovo_seq  \
0                                     HAVSGTEK
1                                      QPGYHHK
2                                    RGNVAGDSK
3              -17.027QAAC+57.021DHSGSDSHSSQR
4                                       SRDEDR
…                                            …
38772                 +42.011AALDSLSLTFSLGLSQEK
38773                         +42.011MELLTLLEK
38774  QQDAAVMALQELYASSTLSTQGLSTLTC+57.021ALHAK
38775                        LEDNSLYNLMVNGLHSFK
38776                              GLSEFDELFDVK

                                     casanovo_aa_scores  \
0      [0.95846, 0.95933, 0.96202, 0.71932, 0.9625, 0…
1      [0.8761, 0.8782, 0.82104, 0.59657, 0.82445, 0…
2      [0.96216, 0.97383, 0.97015, 0.97438, 0.97623, …
3      [0.83947, 0.60469, 0.86706, 0.80854, 0.85195, …
4      [0.48144, 0.40026, 0.33183, 0.29243, 0.35022, …
…                                                   …
38772  [0.90429, 0.81302, 0.90067, 0.92657, 0.92622, …
38773  [0.97946, 0.97909, 0.9821, 0.98179, 0.98214, 0…
38774  [0.73876, 0.69725, 0.76276, 0.73163, 0.75209, …
38775  [0.54334, 0.39658, 0.58759, 0.64371, 0.55222, …
38776  [0.62313, 0.76164, 0.8619, 0.87266, 0.73913, 0…
```

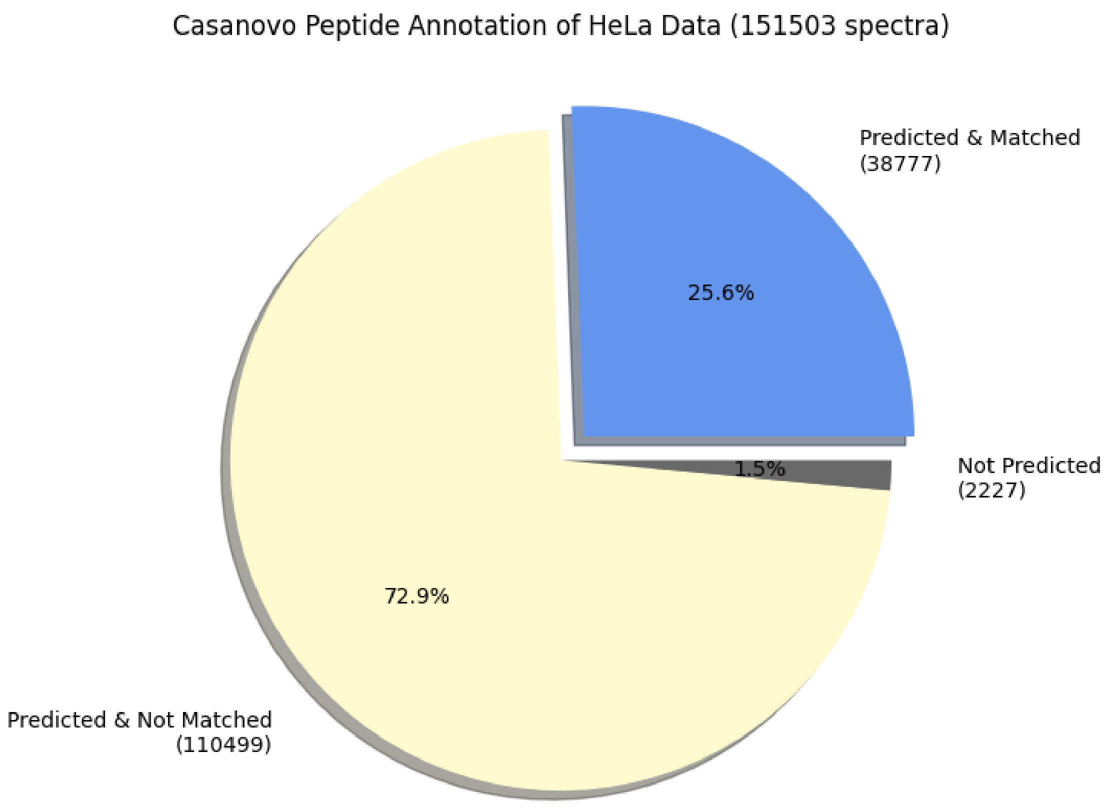
```
                                        seq  \
0                                  HAVSEGTK
1                                   KTESHHK
2                                 RGNVAGDSK
3                        VSDSGSHSGSDSHSGASR
4                                   HGEAQVK
…                                       …
38772              +42.011AALDSLSLFTSLGLSEQK
38773                      +42.011MELITILEK
38774  FQSSAVMALQEASEAYLVGLFEDTNLC+57.021AIHAK
38775                      ELDRDTVFALVNYIFFK
38776                   +42.011AGILFEDIFDVK

                                                m/z array  \
0      [209.10764765158007, 225.10350547020948, 245.8…
1      [266.0704599178995, 284.1693012729633, 284.174…
2      [172.14680911293425, 197.10373386089654, 197.1…
3      [210.25663775256817, 294.1423328124341, 369.15…
4      [306.12279194097084, 350.1855285513594, 382.13…
…                                                   …
38772  [201.11930377954843, 239.06454038495326, 242.1…
38773  [197.12588150885645, 214.07087132311415, 215.1…
38774  [253.19952964469232, 260.12149907488987, 311.2…
38775  [243.13793120208354, 247.14156409470553, 251.1…
38776  [143.12058171064643, 217.08650947938978, 217.0…

                                         intensity array  db_modified  \
0      [81.0, 16.0, 170.0, 83.0, 56.0, 79.0, 151.0, 2…        False
1      [52.0, 83.0, 40.0, 93.0, 76.0, 52.0, 292.0, 19…        False
2      [129.0, 142.0, 168.0, 86.0, 25.0, 95.0, 204.0,…        False
3      [31.0, 77.0, 48.0, 93.0, 128.0, 11.0, 168.0, 1…        False
4      [28.0, 83.0, 98.0, 135.0, 75.0, 81.0, 87.0, 38…        False
…                                                   …          …
38772  [138.0, 72.0, 66.0, 67.0, 103.0, 22.0, 60.0, 1…         True
38773  [103.0, 111.0, 155.0, 101.0, 72.0, 49.0, 93.0,…         True
38774  [62.0, 198.0, 64.0, 76.0, 86.0, 110.0, 180.0, …         True
38775  [59.0, 110.0, 68.0, 72.0, 108.0, 191.0, 97.0, …        False
38776  [131.0, 147.0, 42.0, 90.0, 128.0, 148.0, 40.0,…         True

       cs_modified
0            False
1            False
2            False
3             True
4            False
…            …
```

```
38772         True
38773         True
38774         True
38775        False
38776        False

[38777 rows x 13 columns]
```

[151]:
```
matched_metrics = calculate_metrics(matched_df, aadict)
matched_metrics
```

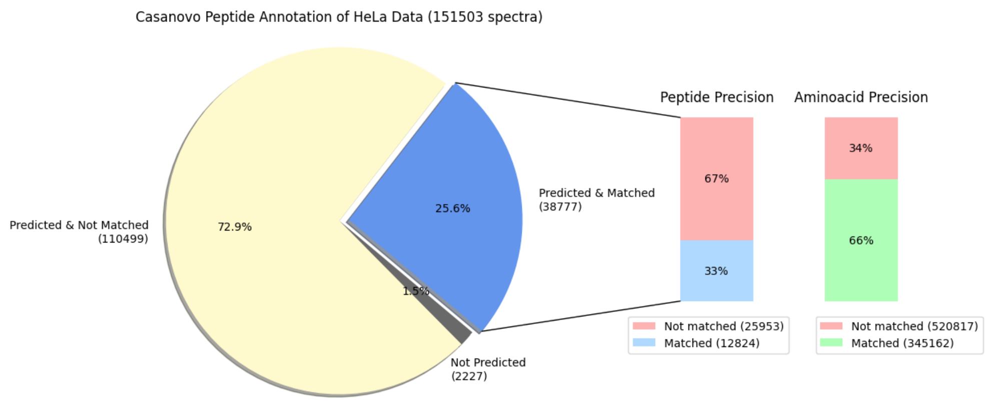
[151]:
```
{'aa_precision of total pred. AA in %': 66.27,
 'aa_recall of total true AA in %': 66.95,
 'pep_precision in %': 33.07,
 'n_total_aa': 520817,
 'n_total_correct_aa': 345162,
 'correct_aa in %': 66.27,
 'n_total_wrong_aa': 175655,
 'wrong_aa in %': 33.73,
 'n_total_peptide': 38777,
 'n_total_correct_peptide': 12824,
 'correct_peptide in %': 33.07,
 'n_total_wrong_peptide': 25953,
 'wrong_peptide in %': 66.93}
```

[94]:
```
predicted_matched = matched_df.shape[0]
predicted_not_matched = df[(df['seq'] == '') & (df['casanovo_seq'] != '')].
 ↪shape[0]
not_predicted = df[(df['seq'] == '') & (df['casanovo_seq'] == '')].shape[0]

# Plotting pie chart for peptides
labels = [f'Predicted & Matched\n({predicted_matched})',
          f'Predicted & Not Matched\n({predicted_not_matched})',
          f'Not Predicted\n({not_predicted})']
sizes = [predicted_matched, predicted_not_matched, not_predicted]
colors = ['cornflowerblue', 'lemonchiffon', 'dimgray']
explode = (0.1, 0, 0)  # explode the Predicted & Matched section
plt.figure(figsize=(10, 7))
plt.pie(sizes, explode=explode, labels=labels, colors=colors, autopct='%1.
 ↪1f%%', shadow=True, startangle=0, labeldistance=1.2)
plt.title('Casanovo Peptide Annotation of HeLa Data (151503 spectra)')
plt.show()
```



[95]:
```
import matplotlib.pyplot as plt
import numpy as np
from matplotlib.patches import ConnectionPatch

def plot_matched_metrics(df, matched_df, matched_metrics):
    # Define your data
    predicted_matched = matched_df.shape[0]
    predicted_not_matched = df[(df['seq'] == '') & (df['casanovo_seq'] != '')].
  ↪shape[0]
    not_predicted = df[(df['seq'] == '') & (df['casanovo_seq'] == '')].shape[0]

    #fig, (ax1, ax2, ax3) = plt.subplots(1, 3, figsize=(10, 4))
    #fig.subplots_adjust(wspace=0)

    fig = plt.figure(figsize=(10,5))
    left, bottom, width, height = 0.1, 0.1, 0.6, 0.6

    x_position = 0.1  # Fraction of figure width
    y_position = 0.3  # Fraction of figure height
    width = 0.8         # Fraction of figure width
    height = 0.6        # Fraction of figure height

    ax1 = fig.add_axes([0, 0, width, height])

    size_scale = 0.8

    ax2 = fig.add_axes([0.55, 0.1, size_scale*width, size_scale*height])

    ax3 = fig.add_axes([0.73, 0.1, size_scale*width, size_scale*height])

    # Additional bar chart 1
    # Define data for bar chart 1
    overall_ratios = [predicted_matched, predicted_not_matched, not_predicted]
    labels = [f'Predicted & Matched\n({predicted_matched})',
              f'Predicted & Not Matched\n({predicted_not_matched})',
              f'Not Predicted\n({not_predicted})']
    explode = (0.1, 0, 0)
    angle = -40
    colors = ['cornflowerblue', 'lemonchiffon', 'dimgray']
    wedges, texts, _ = ax1.pie(overall_ratios, autopct='%1.1f%%',␣
↪startangle=angle, colors=colors, labels=labels, explode=explode, radius=1.8,␣
↪shadow = True)
    ax1.set_title('Casanovo Peptide Annotation of HeLa Data (151503 spectra)',␣
↪y=1.3)


    x,y = texts[2].get_position()
    texts[2].set_position((x - 0.3, y- 0.2))

    # Bar chart 1 for Peptide Precision
    pep_ratios = [matched_metrics['pep_precision in %']/100,␣
↪1-matched_metrics['pep_precision in %']/100]
    pep_labels = [f'Matched ({matched_metrics["n_total_correct_peptide"]})',␣
↪f'Not matched ({matched_metrics["n_total_wrong_peptide"]})']
    bottom = 1
    width = .2
    pep_bar_colors = ['#60B5FE', '#FE6969']
    for j, (height, label, color) in enumerate(reversed([*zip(pep_ratios,␣
↪pep_labels, pep_bar_colors)])):
        bottom -= height
        bc = ax2.bar(0, height, width, bottom=bottom, color=color, label=label,␣
↪alpha=0.5)
        ax2.bar_label(bc, labels=[f"{height:.0%}"], label_type='center')

    ax2.set_title('Peptide Precision')
    ax2.legend(loc='best', bbox_to_anchor=(0.15, -0.3, 0.5, 0.5))
    ax2.axis('off')
    ax2.set_xlim(- 3.5 * width, 3.5 * width)

    # Bar chart 2 for AA Precision
    aa_ratios = [matched_metrics['aa_precision of total pred. AA in %']/100,␣
↪1-matched_metrics['aa_precision of total pred. AA in %']/100]
    aa_labels = [f"Matched ({matched_metrics['n_total_correct_aa']})", f"Not␣
↪matched ({matched_metrics['n_total_aa']})"]
    bottom = 1
    width = .2
    aa_bar_colors = ['#60FE70', '#FE6969']
    for j, (height, label, color) in enumerate(reversed([*zip(aa_ratios,␣
↪aa_labels, aa_bar_colors)])):
        bottom -= height
        bc = ax3.bar(0, height, width, bottom=bottom, color=color, label=label,␣
↪alpha=0.5)
        ax3.bar_label(bc, labels=[f"{height:.0%}"], label_type='center')

    ax3.set_title('Aminoacid Precision')
    ax3.legend(loc='best', bbox_to_anchor=(0.4, -0.3, 0.5, 0.5))
    ax3.axis('off')
    ax3.set_xlim(- 3.5 * width, 3.5 * width)

    # Set up connections between the plots
    theta1, theta2 = wedges[0].theta1, wedges[0].theta2
    center, r = wedges[0].center, wedges[0].r
    bar_height = sum(pep_ratios)
    x = r * np.cos(np.pi / 180 * theta2) + center[0]
    y = r * np.sin(np.pi / 180 * theta2) + center[1]
    con = ConnectionPatch(xyA=(-width / 2, bar_height), coordsA=ax2.transData,␣
↪xyB=(x, y), coordsB=ax1.transData)
    con.set_color([0, 0, 0])
    con.set_linewidth(1)
    ax2.add_artist(con)

    # Draw bottom connecting line
    x = r * np.cos(np.pi / 180 * theta1) + center[0]
    y = r * np.sin(np.pi / 180 * theta1) + center[1]
    con = ConnectionPatch(xyA=(-width / 2, 0), coordsA=ax2.transData, xyB=(x,␣
↪y), coordsB=ax1.transData)
    con.set_color([0, 0, 0])
    ax2.add_artist(con)
    con.set_linewidth(1)

    plt.show()
```

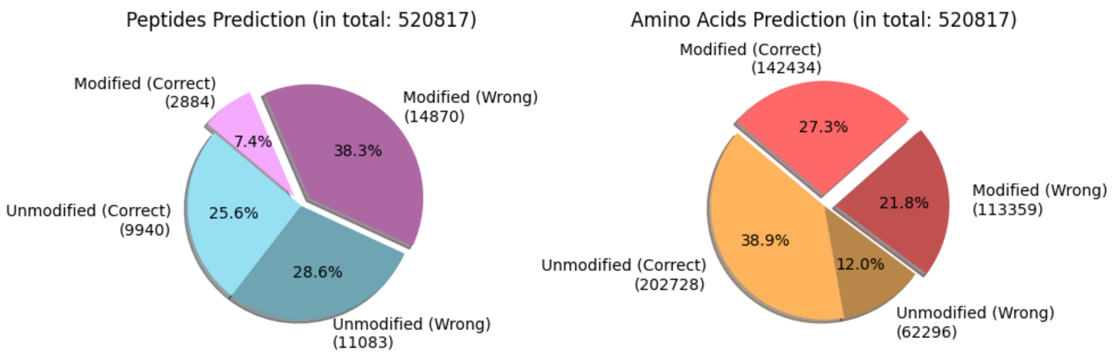
```
plot_matched_metrics(df, matched_df, matched_metrics)
```



9.3.3 Get metrics for modified dfs:

[97]:
```
def calculate_modified_metrics(matched_df, aadict):
    mod_df = matched_df[(matched_df['db_modified'] == True) |␣
 ↪(matched_df['cs_modified'] == True)]
    non_mod_df = matched_df[(matched_df['db_modified'] != True) &␣
 ↪(matched_df['cs_modified'] != True)]
    mod_metrics = calculate_metrics(mod_df, aadict)
    non_mod_metrics = calculate_metrics(non_mod_df, aadict)

    return mod_metrics, non_mod_metrics

mod_metrics, non_mod_metrics = calculate_modified_metrics(matched_df, aadict)
```

[98]:
```
from typing import Dict, Any, Tuple, List

def plot_modified_metrics(matched_metrics: Dict[str, Any], mod_metrics:␣
 ↪Dict[str, Any], non_mod_metrics: Dict[str, Any]) -> None:
    peptides_total: int = matched_metrics['n_total_aa']
    peptides_unmodified: int = peptides_total - mod_metrics['n_total_peptide']
    peptides_modified: int = mod_metrics['n_total_peptide']
    peptides_modified_correct: int = mod_metrics['n_total_correct_peptide']
    peptides_unmodified_correct: int =␣
 ↪non_mod_metrics['n_total_correct_peptide']
    peptides_modified_wrong: int = mod_metrics['n_total_wrong_peptide']
    peptides_unmodified_wrong: int = non_mod_metrics['n_total_wrong_peptide']

    amino_acids_total: int = matched_metrics['n_total_aa']
    amino_acids_of_modified_peptides: int = mod_metrics['n_total_aa']
    amino_acids_of_unmodified_peptides: int = non_mod_metrics['n_total_aa']
    amino_acids_of_modified_correct: int = mod_metrics['n_total_correct_aa']
    amino_acids_of_unmodified_correct: int =␣
↪non_mod_metrics['n_total_correct_aa']
    amino_acids_of_modified_wrong: int = mod_metrics['n_total_wrong_aa']
    amino_acids_of_unmodified_wrong: int = non_mod_metrics['n_total_wrong_aa']

    # Plotting pie chart for peptides
    labels_peptides: List[str] = [f'Unmodified␣
↪(Correct)\n({peptides_unmodified_correct})',
                                  f'Unmodified␣
↪(Wrong)\n({peptides_unmodified_wrong})',
                                  f'Modified␣
↪(Wrong)\n({peptides_modified_wrong})',
                                  f'Modified␣
↪(Correct)\n({peptides_modified_correct})']
    sizes_peptides: List[int] = [peptides_unmodified_correct,␣
↪peptides_unmodified_wrong, peptides_modified_wrong,␣
↪peptides_modified_correct]
    colors_peptides: List[str] = ['#97E0F4', '#6FA4B3', '#AD68A4', '#F6AAFF']
    explode_peptides: Tuple[float, ...] = (0, 0, 0.1, 0.1)  # explode the␣
↪modified (correct) section
    plt.figure(figsize=(10, 5))
    plt.subplot(1, 2, 1)
    plt.pie(sizes_peptides, explode=explode_peptides, labels=labels_peptides,␣
↪colors=colors_peptides, autopct='%1.1f%%', shadow=True, startangle=140,␣
↪labeldistance=1.15)
    plt.title(f'Peptides Prediction (in total: {peptides_total})', y=1.10)

    # Plotting pie chart for amino acids
    labels_aa: List[str] = [f'Unmodified␣
↪(Correct)\n({amino_acids_of_unmodified_correct})',
                            f'Unmodified␣
↪(Wrong)\n({amino_acids_of_unmodified_wrong})',
                            f'Modified␣
↪(Wrong)\n({amino_acids_of_modified_wrong})',
                            f'Modified␣
↪(Correct)\n({amino_acids_of_modified_correct})']
    sizes_aa: List[int] = [amino_acids_of_unmodified_correct,␣
↪amino_acids_of_unmodified_wrong, amino_acids_of_modified_wrong,␣
↪amino_acids_of_modified_correct]
    colors_aa: List[str] = ['#FEB45D', '#B9864A', '#C15151', '#FE6868']
    explode_aa: Tuple[float, ...] = (0, 0, 0.1, 0.1)  # explode the incorrect␣
↪slice
    plt.subplot(1, 2, 2)
    plt.pie(sizes_aa, explode=explode_aa, labels=labels_aa, colors=colors_aa,␣
 ↪autopct='%1.1f%%', shadow=True, startangle=140, labeldistance=1.2)
    plt.title(f'Amino Acids Prediction (in total: {amino_acids_total})', y=1.10)
    plt.subplots_adjust(wspace=0.5)
    plt.tight_layout()
    plt.show()

plot_modified_metrics(matched_metrics, mod_metrics, non_mod_metrics)
```



9.3.4 Get aminoacid prediction score metrics:

Inspect Boolean of aa_match_batch() of casanovo:

[99]:
```
matched_df_filtered = matched_df[matched_df['seq'].str.len() >␣
 ↪matched_df['casanovo_seq'].str.len()]
matched_df_filtered.index.tolist()[0:20]
```

[99]:
```
[4,
 19,
 20,
 25,
 28,
 31,
 42,
 45,
 47,
 48,
 53,
 90,
 104,
 115,
 119,
 122,
 125,
 127,
 130,
 134]
```

[100]:
```
def tokenize(peptide):
    if isinstance(peptide, str):
        tokenized_peptide = re.split(r"(?<=.)(?=[A-Z])", peptide)

    return tokenized_peptide
```

[127]:
```
aa_bool = aa_match_batch(
                database_pred, casanovo_pred, aadict, aa_score_list,␣
 ↪cum_mass_threshold=np.inf
            )
```

[128]:
```
from itertools import chain


boolean_aa_score_list = []

for array in aa_bool[0]:
    boolean_aa_score_list.append(array[0])
flat_boolean_aa_score_list = list(chain.from_iterable(boolean_aa_score_list))
len(flat_boolean_aa_score_list)
```

[128]:
```
526557
```

[129]:
```
def extract_correct_aa_scores(df, row_index, aa_scores_column):
    # Get the amino acid scores as a list of floats
    aa_score_list = df.iloc[row_index][aa_scores_column]

    # Tokenize the sequences
    seq = tokenize(df.iloc[row_index]['seq'])
    casanovo_seq = tokenize(df.iloc[row_index]['casanovo_seq'])
    row_index_matched_df = df.index.tolist()[row_index]
    # Initialize list to store correct amino acid scores

    forward_aa_score_list = []
    forward_matches = []
    # Forward comparison
    index = 0
    for step in range(0, min(len(seq), len(casanovo_seq))):
        boolean = boolean_aa_score_list[row_index_matched_df][index]
        cas_aa = casanovo_seq[index]
        db_aa = seq[index]
        if cas_aa == db_aa:
            forward_aa_score_list.append(aa_score_list[index])
            forward_matches.append(cas_aa)
        else:
            forward_aa_score_list.append(0.0)
            forward_matches.append('')
        index += 1

    reversed_aa_score_list = []
    reversed_matches = []
    # Reversed comparison
    index_db = len(seq) - 1
    index_cas = len(casanovo_seq) - 1

    for step in range(0, min(len(seq), len(casanovo_seq))):
        boolean = boolean_aa_score_list[row_index_matched_df][max(index_db,␣
↪index_cas)]
        cas_aa = casanovo_seq[index_cas]
        db_aa = seq[index_db]
        if cas_aa == db_aa:
            reversed_aa_score_list.append(aa_score_list [index_cas])
            reversed_matches.append(cas_aa)
        else:
            reversed_aa_score_list.append(0.0)
            reversed_matches.append('')
        index_db -= 1
        index_cas -= 1

    return forward_aa_score_list, forward_matches, reversed_aa_score_list,␣
↪reversed_matches

# Example usage:
correct_aa_scores = extract_correct_aa_scores(matched_df_filtered, 1,␣
↪'casanovo_aa_scores')
print(correct_aa_scores)
```

```
([0.0, 0.0, 0.60493, 0.0, 0.61474, 0.60406, 0.60601, 0.75741, 0.0, 0.0, 0.0,
0.0, 0.0], ['', '', 'P', '', 'N', 'S', 'T', 'Q', '', '', '', '', ''], [0.57806,
0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0], ['K', '', '', '',
'', '', '', '', '', '', '', '', ''])
```

[130]:
```
aa_score_list = matched_df['casanovo_aa_scores'].tolist()
```

Plot aminoacid precision and recall at score thresholds between 0 and 1:

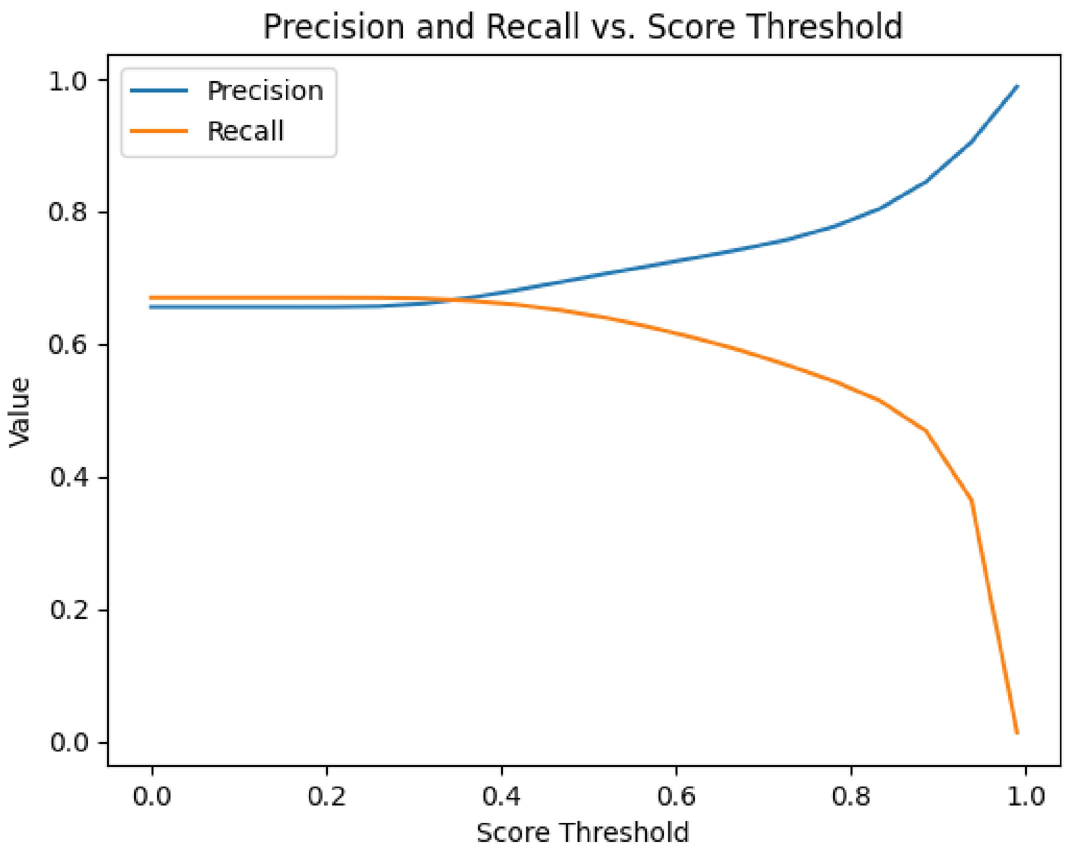
[132]:
```
matched_df
casanovo_pred = matched_df['casanovo_seq']
database_pred = matched_df['seq']
```

[133]:
```
def aa_metrics_at_score_threshold(df, aadict, cum_mass_threshold,␣
↪score_threshold):
    casanovo_pred = df['casanovo_seq']
    database_pred = df['seq']
    aa_score_list = df['casanovo_aa_scores'].tolist()

    aa_bool = aa_match_batch(database_pred, casanovo_pred, aadict,␣
↪aa_score_list, cum_mass_threshold=np.inf)
    n_total_aa = aa_bool[1]

    aa_scores_correct = []
    aa_scores_all = []

    for array in aa_bool[0]:
        aa_scores_all.append(array[2])
        indices = np.argwhere(array[0]).flatten()
        for index in indices:
            aa_scores_correct.append(array[2][index])

    aa_scores_all = list(chain.from_iterable(aa_scores_all))

    aa_precision, aa_recall = aa_precision_recall(aa_scores_correct,␣
↪aa_scores_all, n_total_aa, score_threshold)

    return aa_precision, aa_recall
```

```
[134]: aa_precision, aa_recall = aa_metrics_at_score_threshold(matched_df, aadict, np.
↪Inf, 0.95)
```

```
[135]: def plot_precision_recall(df, aadict, cum_mass_threshold, score_threshold_step␣
↪= 10):
    precisions = []
    recalls = []

    score_thresholds = np.linspace(0, 0.99, score_threshold_step)

    for step, threshold in enumerate(score_thresholds):
        print(step)
        aa_precision, aa_recall = aa_metrics_at_score_threshold(df, aadict, np.
↪Inf, threshold)
        precisions.append(aa_precision)
        recalls.append(aa_recall)

    plt.plot(score_thresholds, precisions, label='Precision')
    plt.plot(score_thresholds, recalls, label='Recall')
    plt.xlabel('Score Threshold')
    plt.ylabel('Value')
    plt.title('Precision and Recall vs. Score Threshold')
    plt.legend()
    plt.show()

    return score_thresholds, precisions, recalls

# Plot precision and recall
precision_recall_data = plot_precision_recall(matched_df, aadict, np.Inf,␣
 ↪score_threshold_step = 20)
```

```
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
```



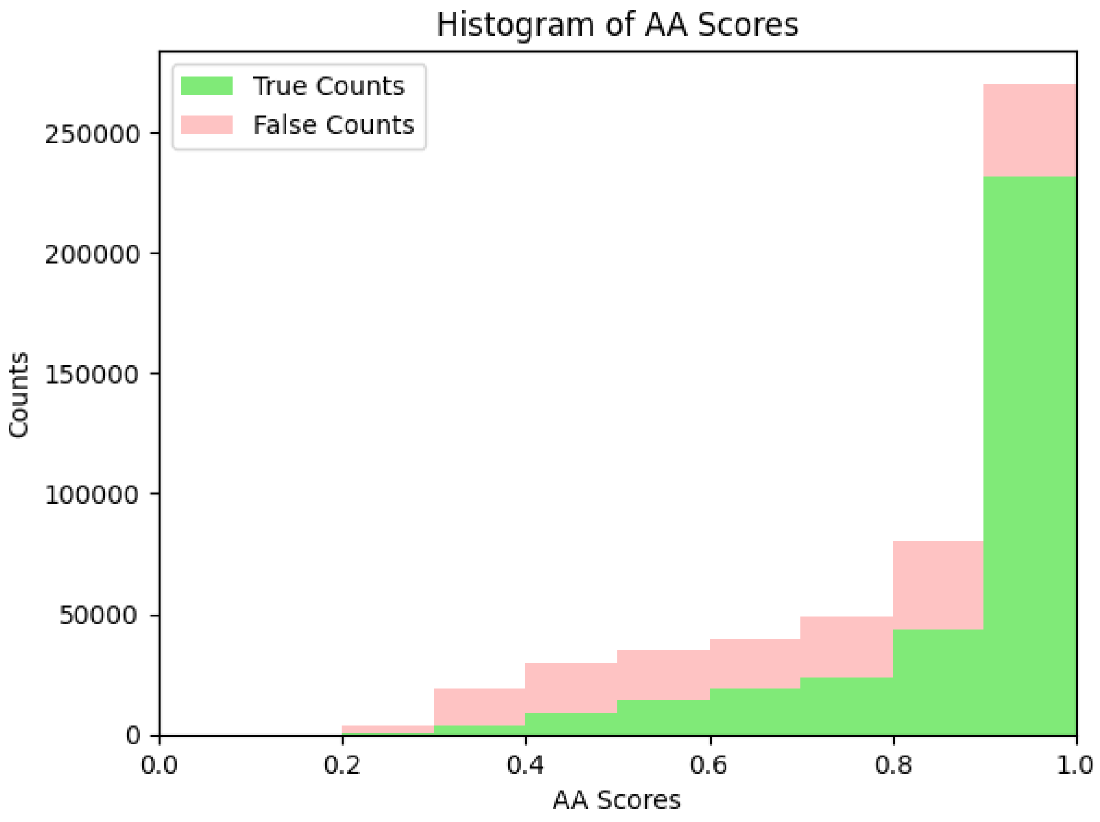
[172]:
```
score_pr_df = pd.DataFrame({'score thresholds': precision_recall_data[0],␣
 ↪'precisions': precision_recall_data[1], 'recalls': precision_recall_data[2]})
score_pr_df
```

[172]:
```
    score thresholds  precisions   recalls
0           0.000000    0.655507  0.669523
1           0.052105    0.655507  0.669523
2           0.104211    0.655507  0.669523
3           0.156316    0.655507  0.669523
4           0.208421    0.655530  0.669515
5           0.260526    0.656394  0.669325
6           0.312632    0.660846  0.668152
7           0.364737    0.669262  0.664883
8           0.416842    0.680633  0.659179
9           0.468947    0.693166  0.650374
10          0.521053    0.705963  0.638945
11          0.573158    0.718142  0.624232
12          0.625263    0.730314  0.607543
13          0.677368    0.743192  0.588694
14          0.729474    0.757572  0.566960
```

```
15          0.781579    0.776980  0.542959
16          0.833684    0.804024  0.513782
17          0.885789    0.844147  0.468609
18          0.937895    0.904259  0.364249
19          0.990000    0.988032  0.013772
```

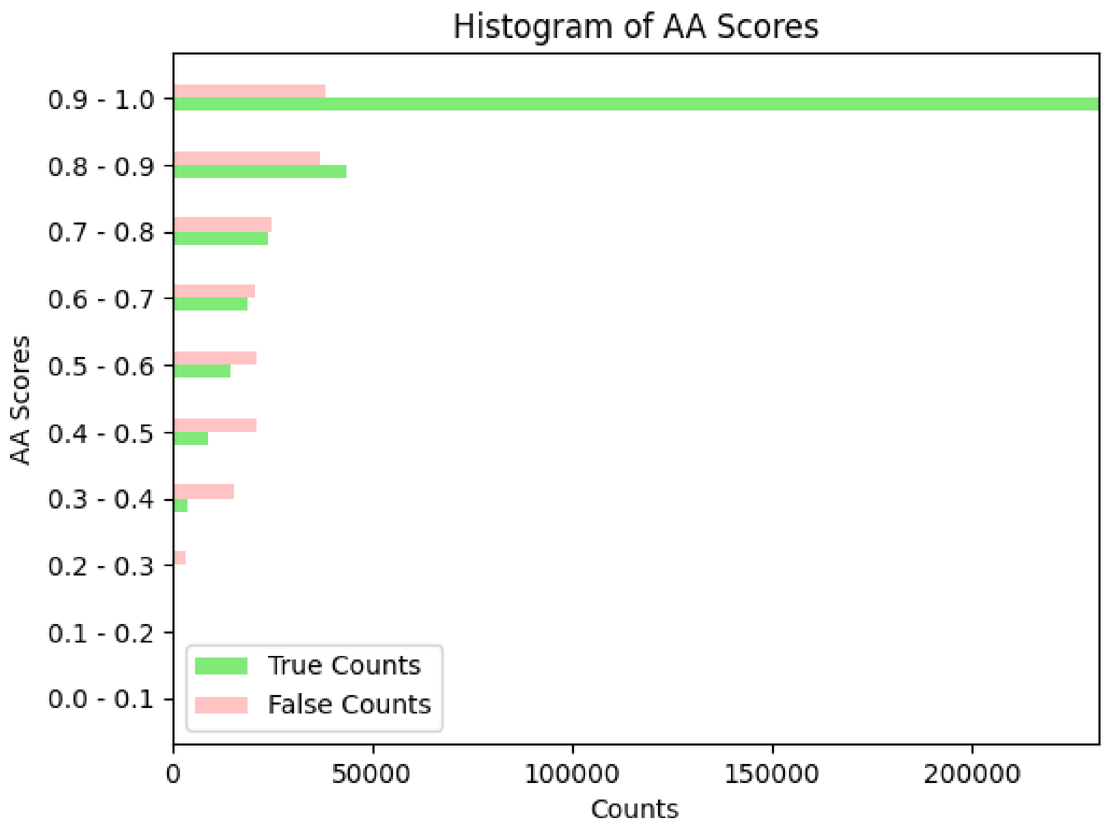
Plot correctly and falsely matched aminoacid counts by their prediction score:

[136]:
```
def plot_aa_scores_histogram(df, aadict, num_bins=9, cum_mass_threshold=np.inf):

    casanovo_pred = df['casanovo_seq']
    database_pred = df['seq']
    df['casanovo_aa_scores'].tolist()
    aa_score_list = df['casanovo_aa_scores'].tolist()

    aa_bool = aa_match_batch(database_pred, casanovo_pred, aadict,␣
 ↪aa_score_list, cum_mass_threshold)
    aa_scores_all = []
    match_boolean_all = []
    for array in aa_bool[0]:
        match_boolean_all.append(array[0])
        aa_scores_all.append(array[2])

    aa_scores_all = list(chain.from_iterable(aa_scores_all))
    match_boolean_all = list(chain.from_iterable(match_boolean_all))

    # Convert lists to arrays
    aa_scores = np.array(aa_scores_all)
    match_boolean = np.array(match_boolean_all)

    # Define bins
    bins = [0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1]
    x_positions = [bin_value + 0.05 for bin_value in bins[:-1]]
    # Calculate histograms for True and False counts
    true_counts, _ = np.histogram(aa_scores[match_boolean], bins=bins)
    false_counts, _ = np.histogram(aa_scores[~match_boolean], bins=bins)

    # Plot histograms
    plt.bar(x_positions, true_counts, width=np.diff(bins), color='#2BDD1F',␣
 ↪alpha=0.6, label='True Counts')
    plt.bar(x_positions, false_counts, width=np.diff(bins), color='#FE6969',␣
 ↪alpha=0.4, bottom=true_counts, label='False Counts')
    plt.xlabel('AA Scores')
    plt.ylabel('Counts')
    plt.title('Histogram of AA Scores')
    plt.legend()
    # Set x-axis ticks to bin edges
    #plt.xticks(bins[:-1], bins[:-1])
    plt.xlim(0,1)
    plt.show()
    #plt.savefig("histogram_of_aa_scores.png")
    return true_counts, false_counts

true_counts, false_counts = plot_aa_scores_histogram(matched_df, aadict,␣
 ↪num_bins=9, cum_mass_threshold=np.inf)
```



[137]:
```
import matplotlib.pyplot as plt
import numpy as np
from itertools import chain

def plot_aa_scores_horizontal(df, aadict, cum_mass_threshold=np.inf):
    # Extract relevant data from the DataFrame
    casanovo_pred = df['casanovo_seq']
    database_pred = df['seq']
    aa_score_list = df['casanovo_aa_scores'].tolist()

    # Perform AA matching batch
    aa_bool = aa_match_batch(database_pred, casanovo_pred, aadict,␣
↪aa_score_list, cum_mass_threshold)

    # Extract match boolean and AA scores
    match_boolean_all = [array[0] for array in aa_bool[0]]
    aa_scores_all = [array[2] for array in aa_bool[0]]

    # Flatten lists
    aa_scores_all = list(chain.from_iterable(aa_scores_all))
    match_boolean_all = list(chain.from_iterable(match_boolean_all))

    # Convert lists to arrays
    aa_scores = np.array(aa_scores_all)
    match_boolean = np.array(match_boolean_all)

    # Define bins
    bins = [0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1]

    # Calculate histograms for True and False counts
    true_counts, _ = np.histogram(aa_scores[match_boolean], bins=bins)
    false_counts, _ = np.histogram(aa_scores[~match_boolean], bins=bins)

    # Calculate bar heights
    bar_height = 0.2

    # Calculate bar positions
    y_positions = np.arange(len(bins[:-1]))

    # Plot histograms using Matplotlib
    plt.barh(y_positions - bar_height/2, true_counts, height=bar_height,␣
↪color='#2BDD1F', alpha=0.6, label='True Counts')
    plt.barh(y_positions + bar_height/2, false_counts, height=bar_height,␣
↪color='#FE6969', alpha=0.4, label='False Counts')

    # Add group labels
    tick_labels = [f'{bins[i]:.1f} - {bins[i+1]:.1f}' for i in␣
↪range(len(bins)-1)]
    plt.yticks(y_positions, tick_labels)

    plt.xlabel('Counts')
    plt.ylabel('AA Scores')
    plt.title('Histogram of AA Scores')
    plt.xlim(0, max(max(true_counts), max(false_counts))+10)
    plt.legend()
    plt.show()

    return true_counts, false_counts
```

```
true_counts, false_counts = plot_aa_scores_horizontal(matched_df, aadict,␣
    ↪cum_mass_threshold=np.inf)
```



```
[176]: bin_labels = ['0 - 0.1', '0.1 - 0.2', '0.2 - 0.3', '0.3 - 0.4', '0.4 - 0.5', '0.
    ↪5 - 0.6', '0.6 - 0.7', '0.7 - 0.8', '0.8 - 0.9', '0.9 - 1']

       # Create a DataFrame
       aa_scores_hist_df = pd.DataFrame({'bin range': bin_labels, 'true_counts':␣
    ↪true_counts, 'false_counts': false_counts})

       aa_scores_hist_df
```

```
[176]:    bin range  true_counts  false_counts
       0    0 - 0.1            0             0
       1  0.1 - 0.2            0             4
       2  0.2 - 0.3          502          3282
       3  0.3 - 0.4         3688         15388
       4  0.4 - 0.5         9011         21058
       5  0.5 - 0.6        14378         20882
```

```
6  0.6 - 0.7        18782         20739
7  0.7 - 0.8        23680         24895
8  0.8 - 0.9        43378         36858
9    0.9 - 1       231743         38289
```

9.3.5 Analyze Dark Data and get peptides with high mean score (0.9 - 1.0):

[113]:
```
casanovo_unmatched_df = df[(df['seq'] == '') & (df['casanovo_seq'] != '')]
```

[114]:
```
casanovo_unmatched_df
```

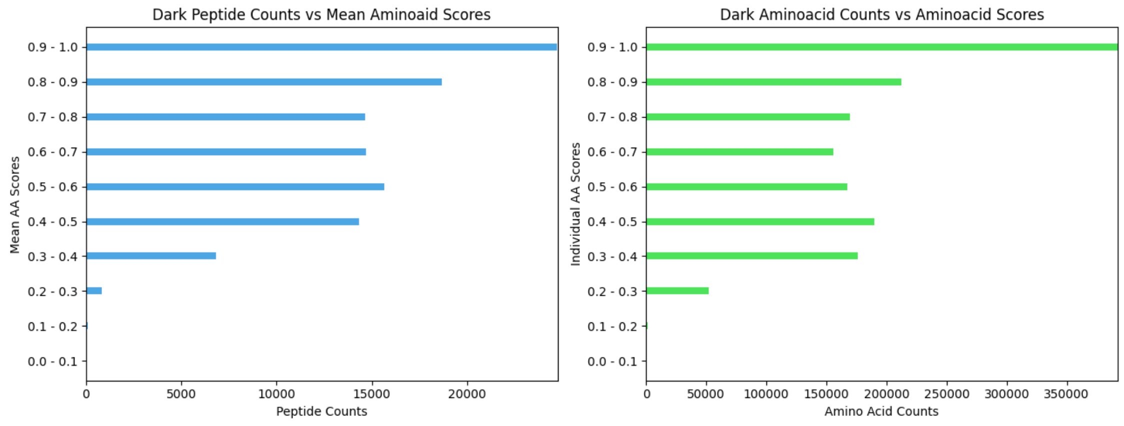
[114]:
```
         title                    pepmass  rtinseconds charge  \
0            1   (663.2879991882091, None)     1.527828   [2+]
1            2  (1221.9899373704318, None)     2.399628   [1+]
2            3  (1223.9922918996538, None)     2.973578   [1+]
3            4   (922.0052771097749, None)     2.973578   [1+]
22          23   (466.8164532109858, None)     2.973578   [1+]
…          …                          …          …     …
151498  151499   (623.0248420939173, None)  3584.723493   [1+]
151499  151500   (622.0267592077544, None)  3585.272101   [1+]
151500  151501  (1223.9880700318918, None)  3586.245334   [1+]
151501  151502  (1221.9879333107788, None)  3589.934304   [1+]
151502  151503   (922.0084868635723, None)  3595.538155   [1+]

              ion_mobility           scans             casanovo_seq  \
0       0.8166844415024028       F8:1777.0              TLHTLLLDNRK
1       1.2557798217225913      F16:3885.0               NDLLLEKEEK
2       1.3628076304583132      F21:3454.0  KPREC+57.021ESC+57.021R
3        1.113046705532348      F21:2708.0                 PMGLLRLK
22      0.8072465531378396      F21:5141.0                    LNNNN
…                      …              …                        …
151498  0.9613576822314688    F33077:2258.0             HDC+57.021DK
151499  0.9782158702844477   F33082:25826.0             EC+57.021FER
151500  1.3611104452168818    F33090:1846.0              QYGPKPPTVLK
151501  1.3690286963761604   F33121:50923.0       LC+57.021EDRLDNGK
151502   1.177092421069372   F33167:23236.0                  KEEDNER

                                     casanovo_aa_scores seq  \
0       [0.62897, 0.46597, 0.40492, 0.38024, 0.31451, …
1       [0.48481, 0.2251, 0.24477, 0.27449, 0.28019, 0…
2       [0.44714, 0.24382, 0.22875, 0.23995, 0.36384, …
3       [0.70436, 0.43039, 0.47188, 0.53315, 0.39718, …
22           [0.2167, 0.21841, 0.22674, 0.20244, 0.21438]
…                                                    …  ..
151498       [0.32813, 0.38435, 0.6269, 0.32973, 0.33301]
151499        [0.35393, 0.30745, 0.232, 0.51399, 0.25483]
151500  [0.72467, 0.4365, 0.43237, 0.49246, 0.78193, 0…
```

```
151501  [0.8115, 0.66726, 0.7572, 0.48835, 0.42572, 0…
151502  [0.45166, 0.29354, 0.26007, 0.2779, 0.27601, 0…

                                              m/z array  \
0       [273.03466354161986, 360.92334212510104, 400.3…
1       [553.9489515333205, 558.5288494547539, 877.954…
2       [230.92269514520777, 245.9377834925359, 319.93…
3       [614.6492959691126, 616.6141580080161, 624.611…
22                                 [561.2985454672379]
…                                                   …
151498  [200.99180651257458, 290.92891283118473, 393.9…
151499  [188.0090480275357, 209.95023734750205, 225.93…
151500  [227.93117309197157, 250.83549331644747, 250.8…
151501  [248.9179004506173, 250.9229450401413, 277.928…
151502  [207.91631035695485, 229.92175940366593, 229.9…

                                        intensity array  db_modified  \
0       [56.0, 138.0, 63.0, 115.0, 115.0, 76.0, 112.0,…        False
1       [85.0, 11.0, 61.0, 49.0, 31.0, 62.0, 45.0, 45…       False
2       [98.0, 77.0, 81.0, 71.0, 125.0, 96.0, 141.0, 6…        False
3       [75.0, 67.0, 24.0, 45.0, 39.0, 53.0, 85.0, 76…       False
22                                               [72.0]        False
…                                                   …          …
151498  [10.0, 65.0, 62.0, 71.0, 71.0, 102.0, 70.0, 49…        False
151499  [59.0, 110.0, 157.0, 104.0, 88.0, 10.0, 186.0,…        False
151500  [189.0, 31.0, 28.0, 133.0, 64.0, 136.0, 106.0,…        False
151501  [32.0, 67.0, 105.0, 81.0, 10.0, 171.0, 10.0, 3…        False
151502  [77.0, 96.0, 67.0, 119.0, 112.0, 142.0, 84.0, …        False

        cs_modified
0             False
1             False
2              True
3             False
22            False
…                 …
151498         True
151499         True
151500        False
151501         True
151502        False

[110499 rows x 13 columns]
```

[152]:
```
casanovo_unmatched_df.loc[:,'mean_aa_score'] = [np.mean(scores) for scores in␣
↪casanovo_unmatched_df['casanovo_aa_scores'].tolist()]
```

```
[116]: def plot_dark_scores_horizontal(df, aadict, cum_mass_threshold=np.inf):
           # Extract relevant data from the DataFrame
           casanovo_pred = df['casanovo_seq']
           aa_score_list = df['casanovo_aa_scores'].tolist()

           aa_scores_all = list(chain.from_iterable(aa_score_list))
           # Calculate mean scores of peptides
           mean_scores_of_peptides = [np.mean(scores) for scores in aa_score_list]

           # Define bins
           bins = [0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1]

           # Calculate histograms for mean scores of peptides
           peptide_counts, _ = np.histogram(mean_scores_of_peptides, bins=bins)
           aa_counts, _ = np.histogram(aa_scores_all, bins=bins)

           # Calculate bar heights
           bar_height = 0.2

           # Calculate bar positions
           y_positions = np.arange(len(bins[:-1]))

           # Create subplots
           fig, axs = plt.subplots(1, 2, figsize=(13, 5))

           # Plot histograms using Matplotlib
           axs[0].barh(y_positions, peptide_counts, height=bar_height,␣
        ↪color='#1F90DD', alpha=0.8, label='Peptide Counts')
           axs[1].barh(y_positions, aa_counts, height=bar_height, color='#1FDD31',␣
        ↪alpha=0.8, label='Amino Acid Counts')

           # Add group labels
           tick_labels = [f'{bins[i]:.1f} - {bins[i+1]:.1f}' for i in␣
        ↪range(len(bins)-1)]
           axs[0].set_yticks(y_positions)
           axs[0].set_yticklabels(tick_labels)
           axs[1].set_yticks(y_positions)
           axs[1].set_yticklabels(tick_labels)

           axs[0].set_xlabel('Peptide Counts')
           axs[1].set_xlabel('Amino Acid Counts')
           axs[0].set_ylabel('Mean AA Scores')
           axs[1].set_ylabel('Individual AA Scores')
           axs[0].set_title('Dark Peptide Counts vs Mean Aminoaid Scores')
           axs[1].set_title('Dark Aminoacid Counts vs Aminoacid Scores')
           axs[0].set_xlim(0, max(peptide_counts) + 10)
           axs[1].set_xlim(0, max(aa_counts) + 10)
```

```
    plt.tight_layout()
    plt.show()

    return mean_scores_of_peptides, peptide_counts, aa_counts

mean_scores_of_peptides, peptide_counts, aa_counts =␣
 ↪plot_dark_scores_horizontal(casanovo_unmatched_df, aadict,␣
 ↪cum_mass_threshold=np.inf)
```



```
[117]: peptide_counts
```

```
[117]: array([    0,    74,   848,  6812, 14336, 15664, 14682, 14674, 18669,
              24740])
```

```
[118]: aa_counts
```

```
[118]: array([     4,     871,  52235, 175749, 190098, 167571, 155712, 169365,
              212372, 392379])
```

```
[119]: high_mean_aa_score_peptides =␣
        ↪casanovo_unmatched_df[casanovo_unmatched_df['mean_aa_score'] >= 0.9]
```

```
[120]: high_mean_aa_score_peptides
```

```
[120]:         title                       pepmass  rtinseconds charge  \
       406      407    (842.9035361246644, None)   419.180763   [2+]
       450      451    (516.7634567388639, None)   420.350962   [2+]
       452      453   (344.84357427933645, None)   420.350962   [3+]
       456      457    (516.7632365678688, None)   420.350962   [2+]
       468      469    (340.2016005669674, None)   420.350962   [2+]
       …        …                            …          …     …
```

```
151008  151009   (704.8338525680721, None)  3343.022244   [2+]
151083  151084   (829.9228812513311, None)  3383.174162   [2+]
151093  151094   (711.8400118275151, None)  3386.643982   [2+]
151113  151114   (645.8417350087976, None)  3388.984397   [2+]
151181  151182   (704.8331039130268, None)  3391.325472   [2+]

              ion_mobility           scans        casanovo_seq  \
406     1.0147815679606023   F3487:42381.0       EQQQQQQQQQQQK
450     0.8106028537237576   F3498:43764.0          APGTPHSHTK
452     0.6609748466561632   F3498:22807.0          APGTPHSHTK
456     0.8445387827269866   F3498:17965.0          APGTPHSHTK
468     0.6917447890982745   F3498:12259.0             GPGPPPR
…                        …               …                   …
151008  0.9636146248747218  F30963:51657.0        PMELFSELAEDK
151083  1.0649016345019877   F31326:1911.0     DLADELALVDVLEDK
151093  0.9730302243010961  F31358:11884.0        PMELFTELADEK
151113  0.9627503300680705   F31380:8936.0  C+57.021LESLLAVFQK
151181  0.9695210842293459  F31402:14021.0        PMELFSELAEDK

                                       casanovo_aa_scores seq  \
406     [0.97043, 0.97145, 0.97275, 0.97308, 0.97314, …
450     [0.94065, 0.92083, 0.97895, 0.98049, 0.98108, …
452     [0.93299, 0.96249, 0.95395, 0.96814, 0.96855, …
456     [0.96732, 0.96846, 0.97218, 0.97168, 0.97201, …
468     [0.94588, 0.96228, 0.96045, 0.96072, 0.96042, …
…                                                    …  ..
151008  [0.98327, 0.98011, 0.98263, 0.98454, 0.98444, …
151083  [0.95472, 0.95315, 0.95598, 0.95783, 0.95878, …
151093  [0.89934, 0.76858, 0.91037, 0.94778, 0.94086, …
151113  [0.98118, 0.97888, 0.98084, 0.96764, 0.98145, …
151181  [0.9695, 0.95785, 0.97148, 0.97296, 0.97325, 0…

                                                m/z array  \
406     [167.09287736323137, 240.099827596804, 242.110…
450     [196.14593405359054, 208.12562969795786, 209.1…
452     [225.0964051661733, 231.13369547797018, 235.12…
456     [210.13542282493052, 235.1223054025792, 238.11…
468     [237.1366934967142, 237.14398122140636, 249.13…
…                                                    …
151008  [201.01417153017272, 201.10588111090868, 201.1…
151083  [201.12154093453705, 202.46383268131925, 228.0…
151093  [201.10364404301725, 201.10811819124248, 243.1…
151113  [228.68915279954516, 229.1020412787415, 229.10…
151181  [200.8911793309175, 201.1103552840188, 216.143…

                                          intensity array  db_modified  \
406     [85.0, 472.0, 136.0, 80.0, 74.0, 103.0, 114.0,…        False
```

```
450     [33.0, 106.0, 99.0, 126.0, 119.0, 101.0, 114.0…        False
452     [90.0, 34.0, 150.0, 145.0, 62.0, 93.0, 201.0, …        False
456     [179.0, 99.0, 63.0, 79.0, 15.0, 257.0, 66.0, 1…        False
468     [94.0, 59.0, 114.0, 100.0, 173.0, 59.0, 75.0, …        False
…                                                       …          …
151008  [72.0, 562.0, 65.0, 147.0, 87.0, 142.0, 95.0, …        False
151083  [52.0, 24.0, 73.0, 133.0, 87.0, 153.0, 99.0, 9…        False
151093  [122.0, 298.0, 86.0, 124.0, 96.0, 61.0, 52.0, …        False
151113  [104.0, 269.0, 75.0, 80.0, 32.0, 106.0, 31.0, …        False
151181  [38.0, 952.0, 83.0, 120.0, 74.0, 961.0, 92.0, …        False

        cs_modified  mean_aa_score
406           False       0.954533
450           False       0.967511
452           False       0.941116
456           False       0.948805
468           False       0.928171
…               …            …
151008        False       0.975562
151083        False       0.924545
151093        False       0.901087
151113         True       0.971375
151181        False       0.951958

[24740 rows x 14 columns]
```

[158]:
```
duplicate_sequences =␣
↪high_mean_aa_score_peptides[high_mean_aa_score_peptides['casanovo_seq'].
↪duplicated(keep=False)]
duplicate_counts = duplicate_sequences['casanovo_seq'].value_counts()
duplicate_counts[0:20]
```

[158]:
```
casanovo_seq
C+57.021DSSPDSAEDVRK           35
YPLEHGLLTNWDDMEK               32
+42.011DDDLAALVVDNGSGMC+57.021K    27
EDQTEYLEER                     22
QEYDESGPSLVHR                  22
EDTEEHHLR                      21
VAPEEHPVLLTEAPLNPK             20
QVHPDTGLSSK                    19
HLQLALR                        18
LENHEGVR                       17
YRPGTVALR                      16
LLSNASC+57.021TTNC+57.021LAPLAK    15
DSYVGDEAQSK                    15
-17.027QEYDESGPSLVHR          15
```

```
PEEHPVLLTEAPLNPK                 14
LSGLLYEETR                       13
+42.011SETAPAAPAAPAPAEK          13
DATNVGDEGGFAPNLLENK              13
PDTGLSSK                         13
TLTLEVEPSDTLENVK                 12
Name: count, dtype: int64
```

[159]:
```
modified_sequences =␣
↪high_mean_aa_score_peptides[high_mean_aa_score_peptides['cs_modified'] ==␣
↪True]
modified_counts = modified_sequences['casanovo_seq'].value_counts()
modified_counts[0:20]
```

[159]:
```
casanovo_seq
C+57.021DSSPDSAEDVRK                     35
+42.011DDDLAALVVDNGSGMC+57.021K          27
LLSNASC+57.021TTNC+57.021LAPLAK          15
-17.027QEYDESGPSLVHR                     15
+42.011SETAPAAPAAPAPAEK                  13
C+57.021DVDLR                            12
+42.011SETAPAAPAAAPPAEK                  11
+43.006APLDPVAGYK                        11
C+57.021EMEQQNQEYK                       11
+42.011ADKPDMGELASFDK                    11
C+57.021NLLAEK                           10
LQC+57.021YNC+57.021PNPTADC+57.021K       9
EPAC+57.021DDPDTEQAALAAVDYLNK             9
-17.027QTVQEAWAEDVDLR                     9
+42.011SDAAVDTSSELTTK                     9
SYC+57.021AELAHNVSSK                      9
+43.006LPLEHGLLTNWDDMEK                   9
VDC+57.021TAHSDVC+57.021SAQGVR            8
+43.006C+57.021DSSPDSAEDVRK               8
LKPDPNTLC+57.021DEFK                      8
Name: count, dtype: int64
```

[160]:
```
non_modified_sequences =␣
↪high_mean_aa_score_peptides[high_mean_aa_score_peptides['cs_modified'] ==␣
↪False]
non_modified_counts = non_modified_sequences['casanovo_seq'].value_counts()
non_modified_counts[0:20]
```

[160]:
```
casanovo_seq
YPLEHGLLTNWDDMEK       32
QEYDESGPSLVHR          22
EDQTEYLEER             22
```

```
EDTEEHHLR              21
VAPEEHPVLLTEAPLNPK     20
QVHPDTGLSSK            19
HLQLALR                18
LENHEGVR               17
YRPGTVALR              16
DSYVGDEAQSK            15
PEEHPVLLTEAPLNPK       14
DATNVGDEGGFAPNLLENK    13
LSGLLYEETR             13
PDTGLSSK               13
SYELPDGQVLTLGNER       12
VDNDENEHQLSLR          12
TLTLEVEPSDTLENVK       12
RVTLMPK                11
FFESFGDLSSADALLGNPK    11
NDEELNK                11
Name: count, dtype: int64
```

[166]:
```python
1-len(high_mean_aa_score_peptides['casanovo_seq'].unique())/
 ↪len(high_mean_aa_score_peptides['casanovo_seq'])
```

[166]:
```
0.25719482619240097
```

[123]:
```python
df[(df['casanovo_seq'] != '') & (df['seq'] == '')]
```

[123]:
```
         title                      pepmass  rtinseconds charge  \
0            1   (663.2879991882091, None)     1.527828   [2+]
1            2  (1221.9899373704318, None)     2.399628   [1+]
2            3  (1223.9922918996538, None)     2.973578   [1+]
3            4   (922.0052771097749, None)     2.973578   [1+]
22          23   (466.8164532109858, None)     2.973578   [1+]
…          …                          …          …    …
151498  151499   (623.0248420939173, None)  3584.723493   [1+]
151499  151500   (622.0267592077544, None)  3585.272101   [1+]
151500  151501  (1223.9880700318918, None)  3586.245334   [1+]
151501  151502  (1221.9879333107788, None)  3589.934304   [1+]
151502  151503   (922.0084868635723, None)  3595.538155   [1+]

             ion_mobility          scans             casanovo_seq  \
0       0.8166844415024028      F8:1777.0              TLHTLLLDNRK
1       1.2557798217225913     F16:3885.0               NDLLLEKEEK
2       1.3628076304583132     F21:3454.0  KPREC+57.021ESC+57.021R
3        1.113046705532348     F21:2708.0                 PMGLLRLK
22      0.8072465531378396     F21:5141.0                    LNNNN
…                        …              …                        …
151498  0.9613576822314688   F33077:2258.0               HDC+57.021DK
```

```
151499  0.9782158702844477  F33082:25826.0            EC+57.021FER
151500  1.3611104452168818   F33090:1846.0             QYGPKPPTVLK
151501  1.3690286963761604  F33121:50923.0        LC+57.021EDRLDNGK
151502   1.177092421069372  F33167:23236.0                  KEEDNER

                                   casanovo_aa_scores seq  \
0       [0.62897, 0.46597, 0.40492, 0.38024, 0.31451, …
1       [0.48481, 0.2251, 0.24477, 0.27449, 0.28019, 0…
2       [0.44714, 0.24382, 0.22875, 0.23995, 0.36384, …
3       [0.70436, 0.43039, 0.47188, 0.53315, 0.39718, …
22           [0.2167, 0.21841, 0.22674, 0.20244, 0.21438]
…                                                     …  ..
151498       [0.32813, 0.38435, 0.6269, 0.32973, 0.33301]
151499        [0.35393, 0.30745, 0.232, 0.51399, 0.25483]
151500  [0.72467, 0.4365, 0.43237, 0.49246, 0.78193, 0…
151501  [0.8115, 0.66726, 0.7572, 0.48835, 0.42572, 0…
151502  [0.45166, 0.29354, 0.26007, 0.2779, 0.27601, 0…

                                            m/z array  \
0       [273.03466354161986, 360.92334212510104, 400.3…
1       [553.9489515333205, 558.5288494547539, 877.954…
2       [230.92269514520777, 245.9377834925359, 319.93…
3       [614.6492959691126, 616.6141580080161, 624.611…
22                                   [561.2985454672379]
…                                                     …
151498  [200.99180651257458, 290.92891283118473, 393.9…
151499  [188.0090480275357, 209.95023734750205, 225.93…
151500  [227.93117309197157, 250.83549331644747, 250.8…
151501  [248.9179004506173, 250.9229450401413, 277.928…
151502  [207.91631035695485, 229.92175940366593, 229.9…

                                      intensity array  db_modified  \
0       [56.0, 138.0, 63.0, 115.0, 115.0, 76.0, 112.0,…        False
1       [85.0, 11.0, 61.0, 49.0, 31.0, 62.0, 45.0, 45…        False
2       [98.0, 77.0, 81.0, 71.0, 125.0, 96.0, 141.0, 6…        False
3       [75.0, 67.0, 24.0, 45.0, 39.0, 53.0, 85.0, 76…        False
22                                             [72.0]        False
…                                                     …            …
151498  [10.0, 65.0, 62.0, 71.0, 71.0, 102.0, 70.0, 49…        False
151499  [59.0, 110.0, 157.0, 104.0, 88.0, 10.0, 186.0,…        False
151500  [189.0, 31.0, 28.0, 133.0, 64.0, 136.0, 106.0,…        False
151501  [32.0, 67.0, 105.0, 81.0, 10.0, 171.0, 10.0, 3…        False
151502  [77.0, 96.0, 67.0, 119.0, 112.0, 142.0, 84.0, …        False

        cs_modified
0             False
1             False
```

```
2              True
3             False
22            False
…             …
151498         True
151499         True
151500        False
151501         True
151502        False

[110499 rows x 13 columns]
```

```
[180]: casanovo_unmatched_df.shape[0]
```

```
[180]: 110499
```

```
[182]: high_mean_aa_score_peptides.shape[0]
```

```
[182]: 24740
```

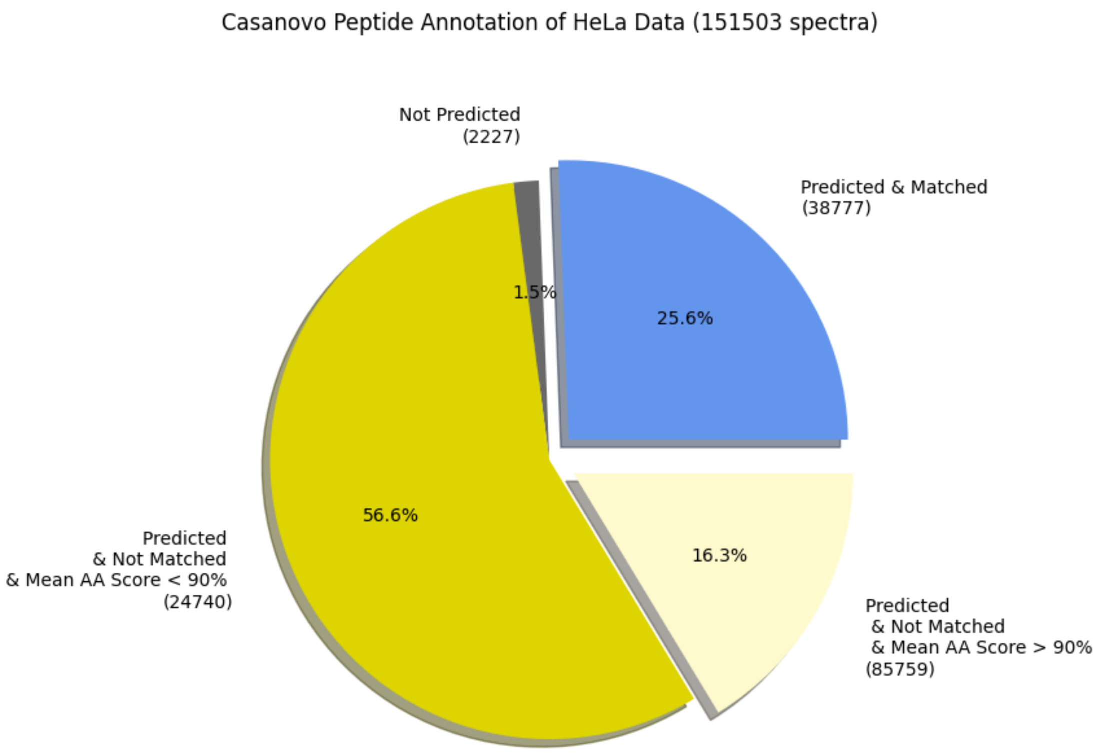
```
[124]: 110499 - 24740
```

```
[124]: 85759
```

```
[125]: 24740/38777
```

```
[125]: 0.6380070660443046
```

```
[190]: predicted_matched = matched_df.shape[0]
predicted_not_matched = df[(df['seq'] == '') & (df['casanovo_seq'] != '')].
 ↪shape[0]
not_predicted = df[(df['seq'] == '') & (df['casanovo_seq'] == '')].shape[0]

total_dark_annotation = casanovo_unmatched_df.shape[0]
high_score_dark_annotation = high_mean_aa_score_peptides.shape[0]
low_score_dark_annotation = total_dark_annotation - high_mean_aa_score_peptides.
 ↪shape[0]



# Plotting pie chart for peptides
labels = [f'Predicted & Matched\n({predicted_matched})',
          f'Not Predicted\n({not_predicted})',
          f'Predicted \n & Not Matched \n & Mean AA Score < 90%␣
 ↪\n({high_score_dark_annotation})',
            f'Predicted \n & Not Matched \n & Mean AA Score > 90%␣
↪\n({low_score_dark_annotation})',
        ]
sizes = [predicted_matched, not_predicted, low_score_dark_annotation, ␣
↪high_score_dark_annotation]
colors = ['cornflowerblue', 'dimgray',  '#DDD401', 'lemonchiffon']
explode = (0.1, 0, 0, 0.1)  # explode the Predicted & Matched section
plt.figure(figsize=(10, 7))
plt.pie(sizes, explode=explode, labels=labels, colors=colors, autopct='%1.
↪1f%%', shadow=True, startangle=0, labeldistance=1.2)
plt.title('Casanovo Peptide Annotation of HeLa Data (151503 spectra)', y = 1.1)
plt.show()
```



[214]:
```
duplicate_count= duplicate_sequences.shape[0]
duplicate_count
```

[214]:
```
9746
```

[220]:
```
duplicate_sequences =␣
↪high_mean_aa_score_peptides[high_mean_aa_score_peptides['casanovo_seq'].
↪duplicated(keep=False)]
```

```
duplicate_counts = duplicate_sequences['casanovo_seq'].value_counts()
duplicate_ratio = 1 - (len(high_mean_aa_score_peptides['casanovo_seq'].
 ↪unique()) / len(high_mean_aa_score_peptides['casanovo_seq']))
duplicate_ratio
```

```
[220]: 0.25719482619240097
```

```
[218]: 9746*100/110499
```

```
[218]: 8.819989321170327
```

```
[304]: high_mean_aa_score_peptides
```

```
[304]:          title                         pepmass  rtinseconds charge  \
406        407   (842.9035361246644, None)   419.180763   [2+]
450        451   (516.7634567388639, None)   420.350962   [2+]
452        453  (344.84357427933645, None)   420.350962   [3+]
456        457   (516.7632365678688, None)   420.350962   [2+]
468        469   (340.2016005669674, None)   420.350962   [2+]
…          …                           …        …     …
151008  151009   (704.8338525680721, None)  3343.022244   [2+]
151083  151084   (829.9228812513311, None)  3383.174162   [2+]
151093  151094   (711.8400118275151, None)  3386.643982   [2+]
151113  151114   (645.8417350087976, None)  3388.984397   [2+]
151181  151182   (704.8331039130268, None)  3391.325472   [2+]

             ion_mobility           scans        casanovo_seq  \
406     1.0147815679606023   F3487:42381.0       EQQQQQQQQQQQK
450     0.8106028537237576   F3498:43764.0          APGTPHSHTK
452     0.6609748466561632   F3498:22807.0          APGTPHSHTK
456     0.8445387827269866   F3498:17965.0          APGTPHSHTK
468     0.6917447890982745   F3498:12259.0             GPGPPPR
…                      …             …                   …
151008  0.9636146248747218  F30963:51657.0         PMELFSELAEDK
151083  1.0649016345019877   F31326:1911.0      DLADELALVDVLEDK
151093  0.9730302243010961  F31358:11884.0         PMELFTELADEK
151113  0.9627503300680705   F31380:8936.0  C+57.021LESLLAVFQK
151181  0.9695210842293459  F31402:14021.0         PMELFSELAEDK

                                          casanovo_aa_scores seq  \
406     [0.97043, 0.97145, 0.97275, 0.97308, 0.97314, …
450     [0.94065, 0.92083, 0.97895, 0.98049, 0.98108, …
452     [0.93299, 0.96249, 0.95395, 0.96814, 0.96855, …
456     [0.96732, 0.96846, 0.97218, 0.97168, 0.97201, …
468     [0.94588, 0.96228, 0.96045, 0.96072, 0.96042, …
…                                                  …  ..
151008  [0.98327, 0.98011, 0.98263, 0.98454, 0.98444, …
```

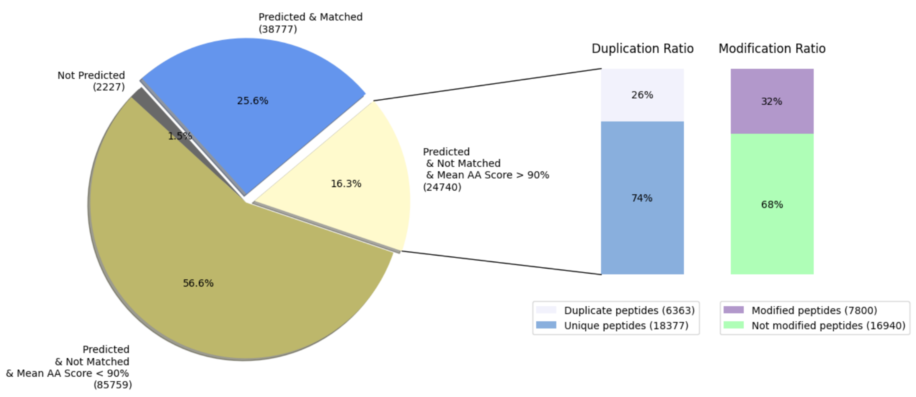
```
151083  [0.95472, 0.95315, 0.95598, 0.95783, 0.95878, …
151093  [0.89934, 0.76858, 0.91037, 0.94778, 0.94086, …
151113  [0.98118, 0.97888, 0.98084, 0.96764, 0.98145, …
151181  [0.9695, 0.95785, 0.97148, 0.97296, 0.97325, 0…

                                              m/z array  \
406     [167.09287736323137, 240.099827596804, 242.110…
450     [196.14593405359054, 208.12562969795786, 209.1…
452     [225.0964051661733, 231.13369547797018, 235.12…
456     [210.13542282493052, 235.1223054025792, 238.11…
468     [237.1366934967142, 237.14398122140636, 249.13…
…                                                   …
151008  [201.01417153017272, 201.10588111090868, 201.1…
151083  [201.12154093453705, 202.46383268131925, 228.0…
151093  [201.10364404301725, 201.10811819124248, 243.1…
151113  [228.68915279954516, 229.1020412787415, 229.10…
151181  [200.8911793309175, 201.1103552840188, 216.143…

                                        intensity array  db_modified  \
406     [85.0, 472.0, 136.0, 80.0, 74.0, 103.0, 114.0,…        False
450     [33.0, 106.0, 99.0, 126.0, 119.0, 101.0, 114.0…        False
452     [90.0, 34.0, 150.0, 145.0, 62.0, 93.0, 201.0, …        False
456     [179.0, 99.0, 63.0, 79.0, 15.0, 257.0, 66.0, 1…        False
468     [94.0, 59.0, 114.0, 100.0, 173.0, 59.0, 75.0, …        False
…                                                   …          …
151008  [72.0, 562.0, 65.0, 147.0, 87.0, 142.0, 95.0, …        False
151083  [52.0, 24.0, 73.0, 133.0, 87.0, 153.0, 99.0, 9…        False
151093  [122.0, 298.0, 86.0, 124.0, 96.0, 61.0, 52.0, …        False
151113  [104.0, 269.0, 75.0, 80.0, 32.0, 106.0, 31.0, …        False
151181  [38.0, 952.0, 83.0, 120.0, 74.0, 961.0, 92.0, …        False

        cs_modified  mean_aa_score
406           False       0.954533
450           False       0.967511
452           False       0.941116
456           False       0.948805
468           False       0.928171
…             …            …
151008        False       0.975562
151083        False       0.924545
151093        False       0.901087
151113         True       0.971375
151181        False       0.951958

[24740 rows x 14 columns]
```

```
[326]: import matplotlib.pyplot as plt
       import numpy as np
       from matplotlib.patches import ConnectionPatch

       def plot_dark_metrics(df, matched_df, casanovo_unmatched_df,␣
        ↪high_mean_aa_score_peptides, modified_bool):
           # Define your data
           predicted_matched = matched_df.shape[0]
           predicted_not_matched = df[(df['seq'] == '') & (df['casanovo_seq'] != '')].
        ↪shape[0]
           not_predicted = df[(df['seq'] == '') & (df['casanovo_seq'] == '')].shape[0]
           total_dark_annotation = casanovo_unmatched_df.shape[0]
           high_score_dark_annotation = high_mean_aa_score_peptides.shape[0]
           low_score_dark_annotation = total_dark_annotation -␣
        ↪high_mean_aa_score_peptides.shape[0]

           duplicate_sequences =␣
        ↪high_mean_aa_score_peptides[high_mean_aa_score_peptides['casanovo_seq'].
        ↪duplicated(keep=False)]
           duplicate_data = duplicate_sequences['casanovo_seq'].value_counts()
           unique_counts = len(high_mean_aa_score_peptides['casanovo_seq'].unique())
           duplicate_counts = high_score_dark_annotation - unique_counts
           duplicate_ratio = 1 - (unique_counts / high_score_dark_annotation)

           dark_metrics = {'total_predicted_not_matched':int(total_dark_annotation),
                           'high_score_annotations':int(high_score_dark_annotation),␣
        ↪'duplicates': int(duplicate_counts),
                                   'duplicate_ratio': round(duplicate_ratio,2),␣
        ↪'unique_ratio': round(1-duplicate_ratio,2)}

           dark_df = pd.DataFrame.from_dict({'metrics_of_unmatched_data':
        ↪dark_metrics}).reindex(index=['total_predicted_not_matched',
                                                                                          ␣
        ↪             'high_score_annotations',
                                                                                          ␣
        ↪                'duplicates',
                                                                                          ␣
        ↪             'duplicate_ratio',
                                                                                          ␣
        ↪             'unique_ratio'])

           #fig, (ax1, ax2, ax3) = plt.subplots(1, 3, figsize=(10, 4))
           #fig.subplots_adjust(wspace=0)

           fig = plt.figure(figsize=(10,5))
           left, bottom, width, height = 0.1, 0.1, 0.6, 0.6
```

```
    x_position = 0.1  # Fraction of figure width
    y_position = 0.3  # Fraction of figure height
    width = 0.8       # Fraction of figure width
    height = 0.6      # Fraction of figure height

    ax1 = fig.add_axes([0, 0, width, height])



    # Define data for pie chart
    overall_ratios = [predicted_matched, not_predicted,␣
↪low_score_dark_annotation,  high_score_dark_annotation]
    labels = [f'Predicted & Matched\n({predicted_matched})',
              f'Not Predicted\n({not_predicted})',
                f'Predicted \n & Not Matched \n & Mean AA Score < 90%␣
↪\n({low_score_dark_annotation})',
                f'Predicted \n & Not Matched \n & Mean AA Score > 90%␣
↪\n({high_score_dark_annotation})',
          ]
    explode = (0.1, 0, 0, 0.1)
    angle = 40
    colors = ['cornflowerblue', 'dimgray',  'darkkhaki', 'lemonchiffon']
    wedges, texts, _ = ax1.pie(overall_ratios, autopct='%1.1f%%',␣
↪startangle=angle, colors=colors, labels=labels, explode=explode, radius=1.8,␣
↪shadow = True)
    ax1.set_title('Casanovo Peptide Annotation of HeLa Data (151503 spectra)␣
↪After Casanovo Prediction Evaluation', y=1.5)

    x,y = texts[2].get_position()
    texts[2].set_position((x - 0.3, y- 0.2))

    if modified_bool:

        modified_sequences =␣
↪high_mean_aa_score_peptides[high_mean_aa_score_peptides['cs_modified'] ==␣
↪True]
        modified_count = modified_sequences.shape[0]
        modified_ratio = modified_count/high_score_dark_annotation
        non_modified_sequences =␣
↪high_mean_aa_score_peptides[high_mean_aa_score_peptides['cs_modified'] ==␣
↪False]
        non_modified_count = non_modified_sequences.shape[0]
        non_modified_ratio = non_modified_count/high_score_dark_annotation
        dark_metrics = {'total_predicted_not_matched':
↪int(total_dark_annotation),
                    'high_score_annotations':int(high_score_dark_annotation),␣
↪'duplicates': int(duplicate_counts),
                            'duplicate_ratio': round(duplicate_ratio,2),␣
↪'unique_ratio': round(1-duplicate_ratio,2),
                       'modified': int(modified_count), 'modified_ratio':
↪round(modified_ratio,2), 'non_modified_ratio': round(non_modified_ratio,2)}

        dark_df = pd.DataFrame.from_dict({'metrics_of_unmatched_data':
↪dark_metrics}).reindex(index=['total_predicted_not_matched',
                                                                              ␣
↪                 'high_score_annotations',
                                                                              ␣
↪                    'duplicates',
                                                                              ␣
↪                 'duplicate_ratio',
                                                                              ␣
↪                 'unique_ratio', 'modified', 'modified_ratio',
                                                                              ␣
↪                   'non_modified_ratio'])

        size_scale = 1

        ax2 = fig.add_axes([0.55, 0.1, size_scale*width, size_scale*height])
        ax3 = fig.add_axes([0.73, 0.1, size_scale*width, size_scale*height])

        bbox_to_anchor_1 = (0.3, -0.3, 0.5, 0.5)
        bbox_to_anchor_2 =(0.4, -0.3, 0.5, 0.5)

      # Bar chart 1 for Duplication analysis
        pep_ratios = [1-duplicate_ratio, duplicate_ratio]
        pep_labels = [f'Unique peptides ({unique_counts})', f'Duplicate␣
↪peptides ({duplicate_counts})']
        bottom = 1
        width = .2
        pep_bar_colors = ['#1560bd', 'lavender']
        for j, (height, label, color) in enumerate(reversed([*zip(pep_ratios,␣
↪pep_labels, pep_bar_colors)])):
            bottom -= height
            bc = ax2.bar(0, height, width, bottom=bottom, color=color,␣
↪label=label, alpha=0.5)
            ax2.bar_label(bc, labels=[f"{height:.0%}"], label_type='center')

        ax2.set_title('Duplication Ratio')
        ax2.legend(loc='best', bbox_to_anchor=bbox_to_anchor_1)
        ax2.axis('off')
        ax2.set_xlim(- 3.5 * width, 3.5 * width)

            # Bar chart 2 for Modification analysis
        aa_ratios = [non_modified_ratio, modified_ratio,]
        aa_labels = [f"Not modified peptides ({non_modified_count})",␣
↪f"Modified peptides ({modified_count})"]
        bottom = 1
        width = .2
        aa_bar_colors = ['#60FE70', 'rebeccapurple']
        for j, (height, label, color) in enumerate(reversed([*zip(aa_ratios,␣
↪aa_labels, aa_bar_colors)])):
            bottom -= height
            bc = ax3.bar(0, height, width, bottom=bottom, color=color,␣
↪label=label, alpha=0.5)
            ax3.bar_label(bc, labels=[f"{height:.0%}"], label_type='center')

        ax3.set_title('Modification Ratio')
        ax3.legend(loc='best', bbox_to_anchor=bbox_to_anchor_2)
        ax3.axis('off')
        ax3.set_xlim(- 3.5 * width, 3.5 * width)


    else:
        size_scale = 1

        ax2 = fig.add_axes([0.65, 0.15, 0.6, size_scale*height])

        bbox_to_anchor_1 =(0.15, -0.6, 0.5, 0.5)

        # Bar chart 1 for Duplication analysis
        pep_ratios = [1-duplicate_ratio, duplicate_ratio]
        pep_labels = [f'Unique ({unique_counts})', f'Duplicates␣
↪({duplicate_counts})']
        bottom = 1
        width = .2
        pep_bar_colors = ['#1560bd', 'lavender']
        for j, (height, label, color) in enumerate(reversed([*zip(pep_ratios,␣
↪pep_labels, pep_bar_colors)])):
            bottom -= height
            bc = ax2.bar(0, height, width, bottom=bottom, color=color,␣
↪label=label, alpha=0.5)
            ax2.bar_label(bc, labels=[f"{height:.0%}"], label_type='center')

        ax2.set_title('Duplication Ratio')
        ax2.legend(bbox_to_anchor = bbox_to_anchor_1, loc='best')
        ax2.axis('off')
        ax2.set_xlim(- 3.5 * width, 3.5 * width)

    print(dark_df)
    connected_section = 3
    # Set up connections between the plots
    theta1, theta2 = wedges[connected_section].theta1,␣
 ↪wedges[connected_section].theta2
    center, r = wedges[connected_section].center, wedges[0].r
    bar_height = sum(pep_ratios)
    x = r * np.cos(np.pi / 180 * theta2) + center[0]
    y = r * np.sin(np.pi / 180 * theta2) + center[1]
    con = ConnectionPatch(xyA=(-width / 2, bar_height), coordsA=ax2.transData,␣
 ↪xyB=(x, y), coordsB=ax1.transData)
    con.set_color([0, 0, 0])
    con.set_linewidth(1)
    ax2.add_artist(con)

    # Draw bottom connecting line
    x = r * np.cos(np.pi / 180 * theta1) + center[0]
    y = r * np.sin(np.pi / 180 * theta1) + center[1]
    con = ConnectionPatch(xyA=(-width / 2, 0), coordsA=ax2.transData, xyB=(x,␣
 ↪y), coordsB=ax1.transData)
    con.set_color([0, 0, 0])
    ax2.add_artist(con)
    con.set_linewidth(1)

    plt.show()

    return dark_df

output = plot_dark_metrics(df, matched_df, casanovo_unmatched_df,␣
 ↪high_mean_aa_score_peptides, modified_bool=True)
```

```
                           metrics_of_unmatched_data
total_predicted_not_matched                110499.00
high_score_annotations                      24740.00
duplicates                                   6363.00
duplicate_ratio                                 0.26
unique_ratio                                    0.74
modified                                     7800.00
modified_ratio                                  0.32
non_modified_ratio                              0.68
```

Casanovo Peptide Annotation of HeLa Data (151503 spectra) After Casanovo Prediction Evaluation



```
[179]: eval_df = pd.DataFrame.from_dict(
           {'all matched predictions': matched_metrics},
            orient='index'
       )
       mod_eval_df = pd.DataFrame.from_dict({'subset with modifications': mod_metrics,
            'subset without modifications': non_mod_metrics},
            orient='index'
       )
       concatenated_df = pd.concat([eval_df, mod_eval_df])
       concatenated_df
```

```
[179]:                               aa_precision of total pred. AA in %  \
       all matched predictions                                      66.27
       subset with modifications                                    55.68
       subset without modifications                                 76.49

                                     aa_recall of total true AA in %  \
       all matched predictions                                  66.95
       subset with modifications                                56.62
       subset without modifications                             76.80

                                     pep_precision in %  n_total_aa  \
       all matched predictions                    33.07      520817
       subset with modifications                  16.24      255793
       subset without modifications               47.28      265024

                                     n_total_correct_aa  correct_aa in %  \
       all matched predictions                   345162            66.27
       subset with modifications                 142434            55.68
       subset without modifications              202728            76.49

                                     n_total_wrong_aa  wrong_aa in %  \
       all matched predictions                    175655        33.73
       subset with modifications                  113359        44.32
       subset without modifications                62296        23.51

                                     n_total_peptide  n_total_correct_peptide  \
       all matched predictions                 38777                    12824
       subset with modifications               17754                     2884
       subset without modifications            21023                     9940

                                     correct_peptide in %  n_total_wrong_peptide  \
       all matched predictions                      33.07                  25953
       subset with modifications                    16.24                  14870
       subset without modifications                 47.28                  11083

                                     wrong_peptide in %
       all matched predictions                    66.93
       subset with modifications                  83.76
       subset without modifications               52.72
```

[338]:
```
high_mean_aa_score_peptides_sorted = high_mean_aa_score_peptides.
 ↪sort_values(by='mean_aa_score', ascending=False)
# Get the integer-based index of the columns
precursor_index = high_mean_aa_score_peptides_sorted.columns.get_loc('title')
casanovo_seq_index = high_mean_aa_score_peptides_sorted.columns.
 ↪get_loc('casanovo_seq')
mean_aa_score_index = high_mean_aa_score_peptides_sorted.columns.
 ↪get_loc('mean_aa_score')

# Select the first 20 rows and specific columns using integer-based indexers
subset = high_mean_aa_score_peptides_sorted.iloc[0:20, [precursor_index,␣
 ↪casanovo_seq_index, mean_aa_score_index]]
subset
```

[338]:
```
         title                           casanovo_seq  mean_aa_score
8726      8727                SAC+57.021GVC+57.021PGR       0.991478
31683    31684                               APMTHLVR       0.991478
77431    77432  LLSNASC+57.021TTN+0.984C+57.021LAPLAK       0.991280
19189    19190                               HLTGEFEK       0.991241
90553    90554                             EYWMDPEGEMK       0.991213
42190    42191                         VC+57.021NPLLTK       0.991189
117524  117525                              MFLSFPTTK       0.991181
66219    66220                              FGTLNLVHPK       0.991177
79805    79806                           TPALVNAAVTYSK       0.991176
42197    42198                         VC+57.021NPLLTK       0.991144
99372    99373                           REDLVVAPAGLTLK       0.991114
80428    80429                ATC+57.021LGNNSAAAVSMLK       0.991086
59300    59301                     GC+57.021GTVLLSGPR       0.991083
```

```
59365    59366                    GC+57.021GTVLLSGPR       0.991076
5193      5194            -17.027QHSNAAQTQTGEANR       0.991066
143644  143645                  +43.006YAVLYQPLFDK       0.991052
8683      8684              EYVTC+57.021HTC+57.021R       0.991018
10679    10680                     LFC+57.021SEHRPK       0.991011
59351    59352                    GC+57.021GTVLLSGPR       0.991009
59419    59420                    GC+57.021GTVLLSGPR       0.991000
```

```
[ ]:
```

```
[ ]:
```