

BIOPYTON PIPELINE PROJECT

HOMOLOGY BASED FUNCTIONAL PREDICTION OF AN
HYPOTHETICAL PROTEIN SEQUENCES USING
BIOINFORMATICS TOOLS

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STEP - 1

BIOLOGICAL SEQUENCE SELECTION



Hypothetical Protein
from UniProt:

- go to Google open uniport
- search for an hypothetical protein
- download it
- an hypothetical protein was successful selected from uniport

SEQUENCE QUALITY & PROTEIN ANALYSIS

- 1) The selected hypothetical protein was checked for :
- length of the protein sequence
- determine the composition of amino acids
- The selected protein was analysed through biopython library

```
from Bio import SeqIO
from Bio.SeqUtils.ProtParam import ProteinAnalysis

protein_seq = SeqIO.read("uniprot.s.fasta","fasta")
print(protein_seq.seq)

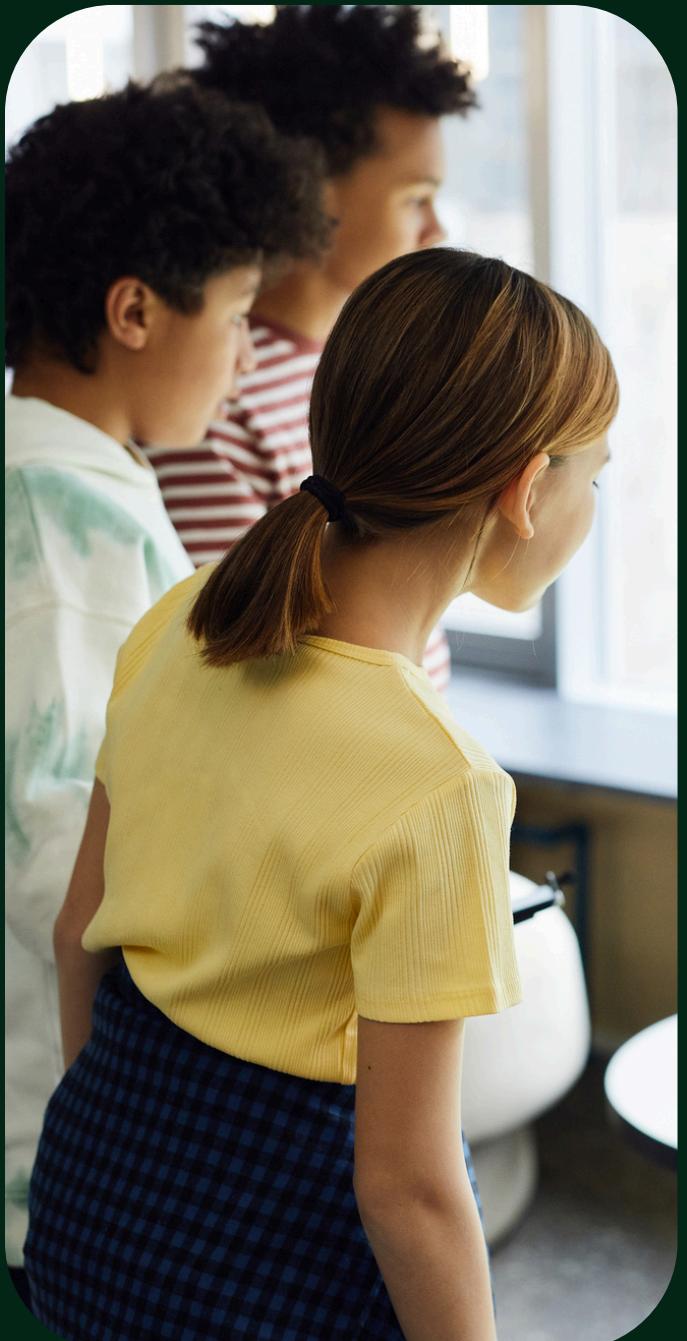
print(len(protein_seq))
result=ProteinAnalysis(protein_seq)
print(result)

percent_AA=(result.get_amino_acids_percent())
print(percent_AA)
```

```
14
PROBLEMS OUTPUT DEBUG CONSOLE TERMINAL PORTS
powershell + v ④ ... |
```

```
58394160583941604, 'R': 0.06569343065693431, 'S': 0.08029197080291971, 'T': 0.0364963503649635, 'V': 0.072992700729927005, 'W': 0.0072992700729927005, 'Y': 0.0364963503649635}
PS C:\Users\swaro\Desktop\BIOPYTHON & C:\Users\swaro\AppData\Local\Programs\Python\Python311\python.exe c:/Users/swaro/Desktop/BIOPYTHON/project1.py
GLRAGTLGRAGAGRGAPEGPGPSGQAQGGSIHSGRIAIVHNPLSVLIRPLPSVLDPAKVQSLVDTIREPPDSVPPIDVLWIKGAQGDDYFYSFGGCHRYAAQQLQRETIPAKLVQSTLSDLRVYLGASTPDLQ
137
<Bio.SeqUtils.ProtParam.ProteinAnalysis object at 0x0000029A6157E390>
C:\Users\swaro\AppData\Local\Programs\Python\Python311\lib\site-packages\Bio\SeqUtils\ProtParam.py:106: BiopythonDeprecationWarning: The get_amino_acids_percent method has been deprecated and will
ly be removed from Biopython in the near future. Please use the amino_acids_percent attribute instead.
warnings.warn(
[A': 0.09489051094890512, 'C': 0.0072992700729927005, 'D': 0.058394160583941604, 'E': 0.021897810218978103, 'F': 0.014598540145985401, 'G': 0.145985401459854, 'H': 0.021897810218978103, 'I': 0.051094891, 'K': 0.021897810218978103, 'L': 0.10218978102189782, 'M': 0.0072992700729927005, 'N': 0.0072992700729927005, 'P': 0.08759124087591241, 'Q': 0.058394160583941604, 'R': 0.065693430656934
'S': 0.008029197080291971, 'T': 0.0364963503649635, 'V': 0.072992700729927005, 'W': 0.0072992700729927005, 'Y': 0.0364963503649635}
PS C:\Users\swaro\Desktop\BIOPYTHON |
```

STEP -3



SEQUENCE FILTERING & VALIDATION

- it doesn't have any low quality sequences
Or short sequence
- The length of this protein sequences is 137, it's not too long and too short ,suitable for validation.
- it shows valid amino acid composition
- this sequence is suitable for analysis



STEP 4

HOMOLOGY SEARCH

- Homology evidence comes from BLAST and database annotations linking this sequence to known sulfiredoxin proteins with high similarity and conserved domains involved in redox regulation

```
from Bio.Blast import NCBIWWW
from Bio import SeqIO
record= SeqIO.read("uniprot.s.fasta","fasta")
result= NCBIWWW.qblast(
    program="blastp",
    database="nr",
    sequence= record.seq
)
with open ("re_blast.xml","w") as b:
    b.write(result.read())
print("code is correct")
```

PROBLEMS OUTPUT DEBUG CONSOLE TERMINAL PO

```
PS C:\Users\swaro\Desktop\BIOPYTHON> & C:\Users\swaro\Desktop\BIOPYTHON>
code is correct
PS C:\Users\swaro\Desktop\BIOPYTHON>
```

• The existence of homologous sequences in organisms like mouse (*Mus musculus*) indicates evolutionary conservation of the protein's role in responding to oxidative stress

```
blast.xml
<?xml version="1.0" encoding="US-ASCII"?>
<!DOCTYPE BlastOutput PUBLIC "-//NCBI//NCBI BlastOutput/EN" "http://www.ncbi.nlm.nih.gov/dtd/NCBI_BlastOutput.dtd">
<BlastOutput>
    <BlastOutput_program>blastp</BlastOutput_program>
    <BlastOutput_version>BLASTP 2.17.0+</BlastOutput_version>
    <BlastOutput_reference>Stephen F. Altschul, Thomas L. Madden, Alejandro A. Sch&uuml;ffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David
    <BlastOutput_db>nr</BlastOutput_db>
    <BlastOutput_query-ID>Query_3066923</BlastOutput_query-ID>
    <BlastOutput_query-def>unnamed protein product</BlastOutput_query-def>
    <BlastOutput_query-len>137</BlastOutput_query-len>
    <BlastOutput_param>
        <Parameters>
            <Parameters_matrix>BLOSUM62</Parameters_matrix>
            <Parameters_expect>10</Parameters_expect>
            <Parameters_gap-open>11</Parameters_gap-open>
            <Parameters_gap-extend>1</Parameters_gap-extend>
            <Parameters_filter>F</Parameters_filter>
        </Parameters>
    </BlastOutput_param>
    <BlastOutput_iterations>
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            <Iteration_query-def>unnamed protein product</Iteration_query-def>
            <Iteration_query-len>137</Iteration_query-len>
        <Iteration_hits>
            <Hit>
                <Hit_num>1</Hit_num>
                <Hit_id>ref|NP_542763.1|</Hit_id>
                <Hit_def>sulfiredoxin-1 [Homo sapiens] &gt;sp|Q9BYN0.2| RecName: Full=Sulfiredoxin-1 [Homo sapiens] &gt;emb|CAL38018.1| hypothetical protein, par
                <Hit_accession>NP_542763</Hit_accession>
                <Hit_len>137</Hit_len>
                <Hit_hsps>
                    <Hsp>
                        <Hsp_num>1</Hsp_num>
                        <Hsp_bit-score>273.478</Hsp_bit-score>
                        <Hsp_score>698</Hsp_score>

```

```

project2.py > ...
from Bio.Blast import NCBIXML
with open ("re_blast.xml") as b:
    blast_record=NCBIXML.read(b)

print(len(blast_record.alignments))
alignment_1=blast_record.alignments[0]
#print(alignment_1.title)
#print(alignment_1.length)
for s in blast_record.alignments:
    print(s.title)
print(len(alignment_1.hsps))
first_hsp = alignment_1.hsps[0]
print(first_hsp.score)
print(first_hsp.expect)
print("Query sequence")
print(first_hsp.query)
print("Mached sequence")
print(first_hsp.sbjct)
print("alignment seq")
print(first_hsp.match)
print("query range:",first_hsp.query_start,"_",first_hsp.query_end)
print("subject range:",first_hsp.query_start,"_",first_hsp.sbjct_end)

ref|XP_037667572.1| sulfiredoxin-1 [cholepus didactylus]
emb|VCW69597.1| unnamed protein product [Gulo gulo]
ref|XP_027414720.1| sulfiredoxin-1 [Bos indicus x Bos taurus]
ref|XP_045663500.1| sulfiredoxin-1 [Ursus americanus]
ref|XP_017751243.1| PREDICTED: sulfiredoxin-1 isoform X1 [Rhinopithecus bieti]
ref|NP_001192917.1| sulfiredoxin-1 [Bos taurus] >tpg|DAA23029.1| TPA: sulfiredoxin 1 homolog [Bos taurus]
1
698.0
6.51413e-92
Query sequence
MGLRAGGTGRAGAGRGAPEGPSPGGAQGGSIHSGRIAAVHNPLSVLIRPLPSVLDAKQSLVDTIREDPDSVPPIDVLWIKGAQGGDYFYSFGGCHRYAAYQQLQRETIPAKLVQSTLSDLRVYLGASTPDLQ
Mached sequence
MGLRAGGTGRAGAGRGAPEGPSPGGAQGGSIHSGRIAAVHNPLSVLIRPLPSVLDAKQSLVDTIREDPDSVPPIDVLWIKGAQGGDYFYSFGGCHRYAAYQQLQRETIPAKLVQSTLSDLRVYLGASTPDLQ
alignment seq
MGLRAGGTGRAGAGRGAPEGPSPGGAQGGSIHSGRIAAVHNPLSVLIRPLPSVLDAKQSLVDTIREDPDSVPPIDVLWIKGAQGGDYFYSFGGCHRYAAYQQLQRETIPAKLVQSTLSDLRVYLGASTPDLQ
query range: 1 _ 137
subject range: 1 _ 137
PS C:\Users\swaro\Desktop\BIOPYTHON>

```

FUNCTIONAL ANNOTATION

Protein Name: Sulfiredoxin-1 (SRXN1)

Function: Involved in cellular defense against oxidative stress by repairing over-oxidized peroxiredoxins and restoring their antioxidant activity.

Biological Process: Redox homeostasis and response to oxidative stress. Cellular Location: Cytoplasm.

```

PS C:\Users\swaro\Desktop\BIOPYTHON> & C:\Users\swaro\AppData\Local\Programs\Python\Python311\python.exe c:/Users/swaro/Desktop/BIOPYTHON/project2.py
50
ref|NP_542763.1| sulfiredoxin-1 [Homo sapiens] >sp|Q9BYN0.2| RecName: Full=Sulfiredoxin-1 [Homo sapiens] >emb|CAL38018.1| hypothetical protein, partial [synthetase] >gb|AAH32604.1| sulfiredoxin 1 homolog (S. cerevisiae) [Homo sapiens] >gb|AAH47707.1| sulfiredoxin 1 homolog (S. cerevisiae) [Homo sapiens] >gb|KAI2593776.1| sulfiredoxin-1 [Homo sapiens] >gb|KAI4004484.1| sulfiredoxin 1 [Homo sapiens]
ref|XP_004061692.3| sulfiredoxin-1 [Gorilla gorilla gorilla]
ref|XP_525238.3| sulfiredoxin-1 [Pan troglodytes]
ref|XP_055121824.1| sulfiredoxin-1 [Symphalangus syndactylus]
gb|PNI62280.1| SRXN1 isoform 1 [Pan troglodytes]
ref|XP_003273506.2| sulfiredoxin-1 [Nomascus leucogenys]
ref|XP_002830202.4| sulfiredoxin-1 [Pongo abelii] >ref|XP_054322668.1| sulfiredoxin-1 [Pongo pygmaeus]
ref|XP_031998363.2| sulfiredoxin-1 [Hylobates moloch]
ref|XP_020756532.1| sulfiredoxin-1 [Odocoileus virginianus]
ref|XP_028380132.1| sulfiredoxin-1 [Phyllostomus discolor]
emb|CAI9154461.1| unnamed protein product [Rangifer tarandus platyrhynchus] >emb|CAM9498264.1| unnamed protein product [Rangifer tarandus platyrhynchus] >emb|CAN8467750.1| unnamed protein product [Rangifer tarandus]
ref|XP_010350134.2| sulfiredoxin-1 [Saimiri boliviensis] >ref|XP_017370780.1| sulfiredoxin-1 [Cebus imitator] >ref|XP_032151253.1| sulfiredoxin-1 [Sapajus apella] >gb|KAF6089488.1| sulfiredoxin 1 [Phyllostomus discolor]
ref|XP_008139209.1| sulfiredoxin-1 isoform XI [Eptesicus fuscus]
ref|XP_036100730.1| sulfiredoxin-1 [Molossus molossus] >gb|KAM7126439.1| sulfiredoxin-1 [Molossus nigricans]
ref|XP_045688825.1| sulfiredoxin-1 [Phyllostomus hastatus]
gb|KAM5220210.1| sulfiredoxin-1 [Hipposideros larvatus]
ref|XP_004014508.1| sulfiredoxin-1 [Ovis aries] >ref|XP_013824239.1| PREDICTED: sulfiredoxin-1 [Capra hircus] >ref|XP_040103571.1| sulfiredoxin-1 [Oryx dammah] >ref|XP_043740010.1| sulfiredoxin-1 [Cervus canadensis] >ref|XP_055290766.1| sulfiredoxin-1 [Moschus berezovskii] >ref|XP_055290767.1| sulfiredoxin-1 [Muntiacus reevesi] >ref|XP_069402979.1| sulfiredoxin-1 [Ovis canadensis] >gb|KAI4538381.1| hypothetical protein MG293_011784 [Ovis ammon polii] >gb|KAI4538382.1| sulfiredoxin 1 [Capra ibex] >gb|KAG5201743.1| hypothetical protein JEQ12_004506 [Ovis aries] >gb|XDA82514.1| hypothetical protein R6Z07_012614 [Oreamnos americanus] >gb|KAF6089488.1| sulfiredoxin 1 [Phyllostomus discolor]
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ref|XP_045688825.1| sulfiredoxin-1 [Phyllostomus hastatus]
gb|KAM5220210.1| sulfiredoxin-1 [Hipposideros larvatus]
ref|XP_004014508.1| sulfiredoxin-1 [Ovis aries] >ref|XP_013824239.1| PREDICTED: sulfiredoxin-1 [Capra hircus] >ref|XP_040103571.1| sulfiredoxin-1 [Oryx dammah] >ref|XP_043740010.1| sulfiredoxin-1 [Cervus canadensis] >ref|XP_055290766.1| sulfiredoxin-1 [Moschus berezovskii] >ref|XP_055290767.1| sulfiredoxin-1 [Muntiacus reevesi] >ref|XP_069402979.1| sulfiredoxin-1 [Ovis canadensis] >gb|KAI4
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BIOLOGICAL INTERPRETATION

- 1) What does this sequence likely do?
It functions as Sulfiredoxin-1 (SRXN1), an enzyme that protects cells from oxidative stress by repairing over-oxidized peroxiredoxins.
- 2) Why do they think so?
Because the sequence shows strong similarity to known sulfiredoxin proteins and contains conserved residues required for this repair activity.
- 3) What evidence supports their claim?
BLAST homology results, UniProt functional annotations, and experimental studies showing higher oxidative damage in cells lacking SRXN1.

"I analyzed an hypothetical sequence, assessed its quality, identified homologous sequences through database searches, and predicted its biological function using computational and bioinformatics methods."

THANK YOU

Sowmyaballa