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# Master Biomedizin 2023

- 1) UniProt
- 2) Homology
- 3) MSA
- 4) Phylogeny

# UniProt database

1

- a. What is the AC of the UniProt entry for the human insulin? **P01308**
- b. How many isoforms for this protein are described in that entry? **2 isoforms**
- c. How many times has this entry been modified? **271 times; currently in version 272**  
... and the protein sequence? **None; currently in version 1**
- d. With how many proteins does the human insulin interact? **524 interactors (BioGRID), 20 interactors (IntAct); databases do not always agree**

## Protein-protein interaction databases

Nov 2023

|               |   |        |   |
|---------------|---|--------|---|
| BioGRID       | 109842 <a href="#">↗</a> 524 interactors  | IntAct | P01308 <a href="#">↗</a> 20 interactors |
| ComplexPortal | CPX-943 <a href="#">↗</a> Insulin complex | MINT   | P01308 <a href="#">↗</a>                |
| DIP           | DIP-6024N <a href="#">↗</a>               | STRING | 9606.ENSP00000380432 <a href="#">↗</a>  |

Feb 2022

## Protein-protein interaction databases

|                      |                         |
|----------------------|-------------------------|
| BioGRID <sup>i</sup> | 109842, 487 interactors |
| DIP <sup>i</sup>     | DIP-6024N               |
| IntAct <sup>i</sup>  | P01308, 18 interactors  |
| MINT <sup>i</sup>    | P01308                  |
| STRING <sup>i</sup>  | 9606.ENSP00000380432    |

## Protein-protein Interaction databases

|                      |                        |
|----------------------|------------------------|
| BioGRID <sup>i</sup> | 109842, 24 interactors |
| DIP <sup>i</sup>     | DIP-6024N              |
| IntAct <sup>i</sup>  | P01308, 18 interactors |
| MINT <sup>i</sup>    | P01308                 |
| STRING <sup>i</sup>  | 9606.ENSP00000380432   |

Feb 2021

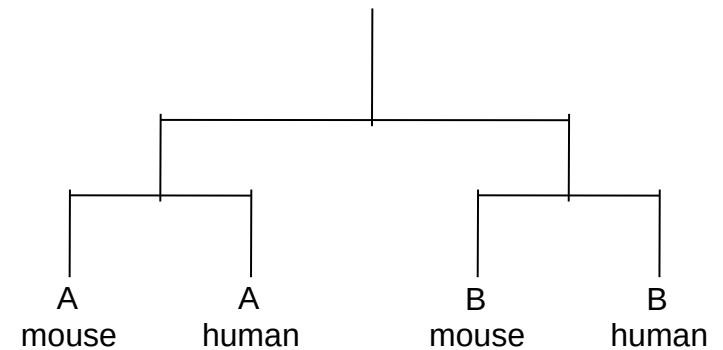
# Homology

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## 2

Classify the following protein pairs based on their evolutionary relationship.  
Note: proteins A and B have a common ancestor.

- a. Protein A mouse / Protein A human → Orthologs
- b. Protein A mouse / Protein B mouse → Paralogs
- c. Protein A mouse / Protein B human → Homologs
- d. Protein A human / Protein B mouse → Homologs
- e. Protein A human / Protein B human → Paralogs
- f. Protein B mouse / Protein B human → Orthologs



## 3

a. Using the human protein “P21741”, find its orthologous proteins in frog (*Xenopus laevis*) and get their UniProt AC. P48530, P48531

b. Check the identity between the orthologs (human – frog proteins).

P21741-P48530 = 61.1%, P21741-P48531 = 60.4%

c. Check the identity between the paralogs (frog – frog proteins).

P48530-P48531 = 97.9%. Note: may also be done with “alignments”.



Human  
(*Homo sapiens*)



Frog  
(*Xenopus laevis*)

## 4

a. Based on the sequence of the “ATP synthase subunit a” protein from the extinct mammoth (*Mammuthus primigenius*) [Q38PR7], was the mammoth closer to the asian elephant (*Elephas maximus*) or to the african elephant (*Loxodonta africana*)? Use only SwissProt proteins.

*M. primigenius* (Q38PR7) – *E. maximus* (Q2I3G9) = 95.5%

*M. primigenius* (Q38PR7) – *L. africana* (Q9TA24) = 93.2%

b. Is there evidence enough to conclude if they are / are not closer? No.

c. Could you check with the “cytochrome b” protein too? [P92658] Use only SwissProt proteins.

*M. primigenius* (P92658) – *E. maximus* (O47885) = 96.3%

*M. primigenius* (P92658) – *L. africana* (P24958) = 97.9%



Woolly mammoth  
(*Mammuthus primigenius*)



Asian elephant  
(*Elephas maximus*)



African elephant  
(*Loxodonta africana*)

## 5

a. Based solely on the sequence of the “Cytochrome b” protein (Q8SG72) from the extinct dodo (*Raphus cucullatus*), was the dodo closer to the Nicobar pigeon “*Caloenas nicobarica*” or to the chicken (*Gallus gallus*)? Use NCBI Blast.

*R. cucullatus* – *C. nicobarica* = 98.50%

*R. cucullatus* – *G. gallus* = 92.51%

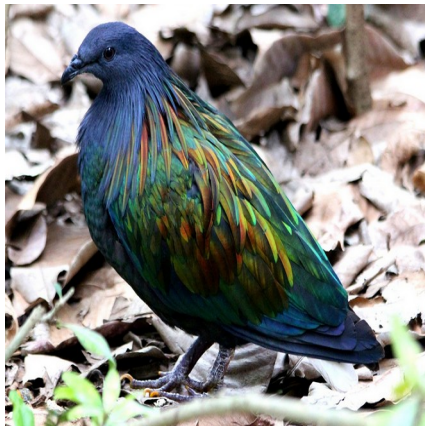
b. There are more than 300 species of pigeons. Do the results differ if you consider the street pigeon (*Columba livia*)?

*R. cucullatus* – *C. livia* = 96.25%

*R. cucullatus* – *G. gallus* = 92.51%



Dodo  
(*Raphus cucullatus*)



Nicobar pigeon  
(*Caloenas nicobarica*)



Chicken (rooster)  
(*Gallus gallus*)



Pigeon  
(*Columba livia*)

## 6

**a.** The UniProt entry “P04585” contains the Gag-Pol polyprotein from the virus HV1H2. Do you think it would resemble any protein in the human proteome (*Homo sapiens*)?

Many retroviral proteins integrated in the human genome.

**b.** The Gag-Pol polyprotein is composed of more than one protein. Can you identify them? Use only SwissProt proteins.

N-terminal → Gag      .... and maybe more!  
C-terminal → Pol

# MSA

## 7

Given the following alignments,

classify them in:

- Pairwise / multiple
- Local / global

calculate their:

- % similarity
- % identity

```
>Protein_A
KKKYYWKKKT
>Protein_B
AKKYYW
>Protein_C
RKRWWWRT
```

a) Protein\_A YYWW  
Protein\_B YYWW  
\*\*\*\*

Pairwise  
Local  
100% similarity  
100% identity

b) Protein\_A KKKYYWKKKT  
Protein\_B AKKYYW---  
\*\*\*\*\*

Pairwise  
Global  
60% similarity  
60% identity

c) Protein\_A KKKYYWKKKT  
Protein\_B AKKYYW---  
Protein\_C AKRWWWRT  
\*:::\*\*

Multiple  
Global  
60% similarity  
30% identity



## 8

**a.** The proteins “P11582” and “P02226” are paralogs, but they differ in length (152 vs 161 amino acids). Is there an extra region in P02226, or the extra amino acids are dispersed along the protein? Use UniProt.

Extra region in P02226: “IGNESN”.

**b.** How could that have happened in evolution?

Deletion in P11582, or insertion in P02226.

**c.** Protein “P02226” is a globin. If you blast P02226 and use only SwissProt proteins as database, how many results do you get? Is this protein present in any bacterial proteome?

159 results; 15 in Bacteria and 144 in Eukaryota.

// FYI: Protein entry P02226 is one of the oldest in SwissProt; check the date! November 1, 1988.

## 9

a. Both “P17861” (XBP1\_HUMAN) and “Q3SZZ2” (XBP1\_BOVIN) are “X-box binding protein 1” proteins. Can you detect which region/s of these proteins is/are important for their function? Why? Use Clustal Omega.

What should you do to detect them? **No. They are too similar. We would need a protein from a more distant organism.**

b. Add the proteins “G5EE07” (G5EE07\_CAEEL) and “Q8UVQ5” (Q8UVQ5\_DANRE) to the study. Are you able to identify that region/s now? Why? Use Clustal Omega.

**Yes. They are not as similar.**

c. Check the positional annotations in the entry of the human protein. Was the region you identified annotated as a domain?

**bZIP (basic-leucine zipper) domain in positions:**

70-133 (human)

70-133 (cattle)

61-117 (worm)

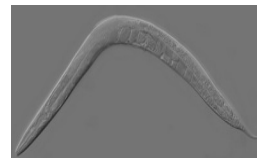
69-132 (zebrafish)



Human  
(*Homo sapiens*)



Cattle  
(*Bos taurus*)



Worm  
(*Caenorhabditis elegans*)



Zebra fish  
(*Danio rerio*)

## 10

a. Using the following set of orthologous proteins, predict which regions are important for their function. Use T-Coffee.

Q90WY9  
P97801  
Q98SU9  
Q9W6S8  
Q09808

Boxed regions.

b. Check your predictions in their UniProt entries.

88-148 Tudor  
10-41 P1  
92-204 Required for interaction with RPP20/POP7  
235-262 P2  
274-288 Required for interaction with SYNCRIP

c. Do you think the ortholog from *S. pombe* (“Q09808”) interacts with RPP20/POP7?

No, it does not have the region required for the interaction with RPP20/POP7.

## 11

a. Using the following set of orthologous proteins, do you think the evolution is pressuring them? Use UniProt.

Q02078  
Q5REW7  
Q2MJT0  
Q60929  
A2VDZ3  
A2ICN5  
Q9W6U8

Yes, they conserve most of the sequence but have some differences.

b. Have they evolved? Did they gain or lose any domain/motif/region?

Differences in:

Beta domain (LCR Glu, E)

Glutamines. Maximum Q stretch:

Chicken = 3 Q

Pig = 4 Q

Cattle = 5 Q

Mouse = 6 Q

Rat = 7 Q

Orangutan = 9 Q

Human = 11 Q

# SeaView

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Go to “<http://doua.prabi.fr/software/seaview>” (or search in Google “SeaView alignment”), download and start SeaView, with which we will generate the phylogenetic trees. Steps:

1. Go to -> <http://doua.prabi.fr/software/seaview>
2. Click on “MS Windows” to download the software
3. Click on downloaded file "seaview5.exe"
4. Ausführen
5. Extract
6. Go to folder "seaview"
7. Click on "seaview.exe"

If you have problems, do not worry → Use UniProt

# Phylogeny

## 12

A patient comes to the hospital. He was just bitten by a snake. We have the sequence of the mitochondrial gene ND4 of 24 species of snake ("*snakes.fasta*"; <https://cbdm.uni-mainz.de/mb23/>). We have three antidotes available. Given the following information, which antidote would you administer the patient?

- 1) The snake that bit the patient is in terrarium #1.
- 2) The most distant snake species is in terrarium #12.
- 3) Antidote1 is indicated against bites from the species in terrarium #3.
- 4) Antidote2 is indicated against bites from the species in terrarium #11.
- 5) Antidote3 is indicated against bites from the species in terrarium #17.
- 6) Snakes in terrariums #15 and #20 are non-venomous.

No antidote! The snake seems not venomous



## 13

**a.** All of the sequences in “*file1.fasta*” (<https://cbdm.uni-mainz.de/mb23/>) are homologs. How many groups of orthologs would you say there are in this file? Use SeaView.

Two groups of orthologs: Protein A & protein B.

**b.** What could you say about the history of this protein family?

*E. coli* has only one protein, and then it duplicated to form A and B. It is possible that *X.laevis\_B* duplicated later to form B and C.

**c.** Would you say there is any wrongly annotated sequence?

*X.tropicalis\_B* is wrongly annotated. It should be *X.tropicalis\_A*, because they are in the same branch. The actual *X.tropicalis\_B* is either not in the dataset or was lost during evolution.

## 14

**a.** Using “*file2.fasta*” (<https://cbdm.uni-mainz.de/mb23/>), can you approximate to which taxonomic division belongs “proteinX”? *Primates*.

**b.** From which organism could it be? After guessing, check it.

*Homo sapiens* (human) or *Pan troglodytes* (chimpanzee); they are 100% identical.



## 15

Human hemoglobin consists of four protein subunits: two from the alpha globin gene cluster (located on chromosome 16) and two more from the beta globin gene cluster (located on chromosome 11). But there are at least nine different globin genes in these clusters, which are: zeta, mu, alpha, theta1, epsilon, gamma1, gamma2, delta and beta. Use the proteins in “*file3.fasta*” (<https://cbdm.uni-mainz.de/mb23/>), which includes an hemoglobin protein from *Gallus gallus* to be used as outgroup.

**a.** Sort them either in cluster alpha or cluster beta.

Alpha: zeta, mu, alpha, theta1.

Beta: epsilon, gamma1, gamma2, delta and beta.

**b.** Why do you think they are clustered in either cluster alpha or cluster beta?

Paralogous expansion from one ancestral alpha and one ancestral beta.

# Phylogeny

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## 16

The sequences in “*file4.fasta*” (<https://cbdm.uni-mainz.de/mb23/>) are orthologs from dolphin, human, cattle, zebra fish, chicken, platypus and frog.

**a.** Build a phylogenetic tree using these sequences.

**b.** Check in UniProt > Taxonomy (<https://www.uniprot.org/taxonomy/>) if the phylogenetic tree built only from that one protein family is coherent with evolution.

Dolphin = *Delphinus delphis*

Human = *Homo sapiens*

Cattle = *Bos taurus*

Zebra fish = *Danio rerio*

Chicken = *Gallus gallus*

Platypus = *Ornithorhynchus anatinus*

Frog = *Xenopus laevis*

No, the tree fails to relate all amniotes in one branch.