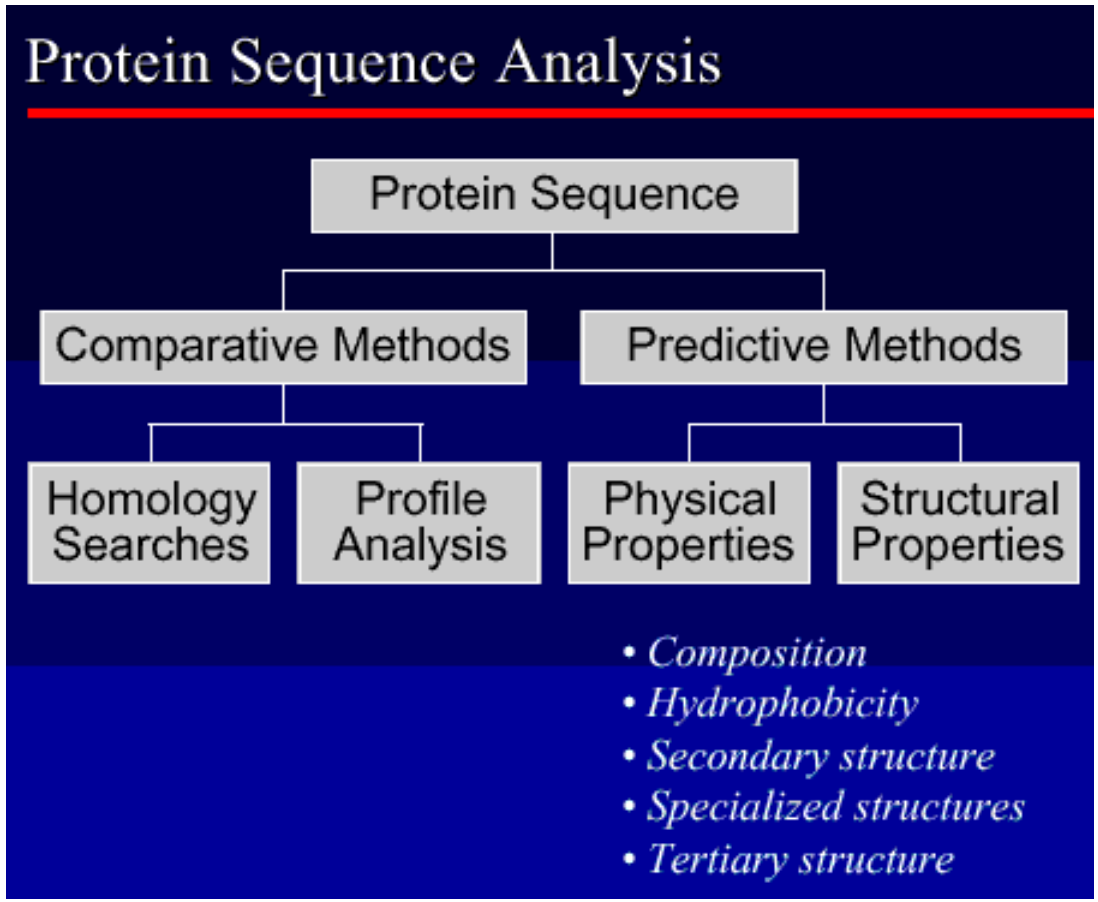
Working with a single protein sequence



Doing biochemistry on a computer

ExPASy: www.expasy.ch
Swiss EMBnet: www.ch.embnet.org

Physico-chemical properties of a protein

Primary structure analysis: www.expasy.ch/tools/#primary
Click the ProtParam link

Primary structure analysis

- ProtParam - Physico-chemical parameters of a protein sequence (amino-acid and atomic compositions, isoelectric point, extinction coefficient, etc.)
- Compute pI/Mw - Compute the theoretical isoelectric point (*pI*) and molecular weight (*Mw*) from a UniProt Knowledgebase entry or for a user sequence
- ScanSite pI/Mw - Compute the theoretical *pI* and *Mw*, and multiple phosphorylation states
- MW, pI, Titration curve - Computes *pI*, composition and allows to see a titration curve

- Radar - De novo repeat detection in protein sequences
- REP - Searches a protein sequence for repeats
- REPRO - De novo repeat detection in protein sequences
- TRUST - De novo repeat detection in protein sequences

- SAPS - Statistical analysis of protein sequences at EMBnet-CH [Also available at EBI]

- Coils - Prediction of coiled coil regions in proteins (Lupas's method) at EMBnet-CH [Also available at PBIL]
- Paircoil - Prediction of coiled coil regions in proteins (Berger's method)
- Multicoil - Prediction of two- and three-stranded coiled coils
- 2ZIP - Prediction of Leucine Zippers

- PESTfind - Identification of PEST regions at EMBnet Austria

- HLA_Bind - Prediction of MHC type I (HLA) peptide binding
- PEPVAC - Prediction of supertypic MHC binders
- RANKPEP - Prediction of peptide MHC binding
- SYFPEITHI - Prediction of MHC type I and II peptide binding

- ProtScale - Amino acid scale representation (Hydrophobicity, other conformational parameters, etc.)
- Drawhca - Draw an HCA (Hydrophobic Cluster Analysis) plot of a protein sequence
- Protein Colourer - Tool for coloring your amino acid sequence
- Three To One - Tool to convert a three-letter coded amino acid sequence to single letter code
- Colorseq - Tool to highlight (in red) a selected set of residues in a protein sequence
- HelixWheel / HelixDraw - Representations of a protein fragment as a helical wheel

- RandSeq - Random protein sequence generator

P00533: 1-1210
Number of amino acids: 1210
Molecular weight: 134277.4
Theoretical pI: 6.26
Amino acid composition: Ala (A) 72 6.0% Arg (R) 60 5.0% Asn (N) 66 5.5% Asp (D) 61 5.0% Cys (C) 60 5.0% Gln (Q) 49 4.0% Glu (E) 77 6.4% Gly (G) 85 7.0% His (H) 31 2.6% Ile (I) 69 5.7% Leu (L) 111 9.2% Lys (K) 66 5.5% Met (M) 25 2.1% Phe (F) 36 3.0% Pro (P) 75 6.2% Ser (S) 84 6.9% Thr (T) 64 5.3% Trp (W) 13 1.1% Tyr (Y) 36 3.0% Val (V) 70 5.8% Asx (B) 0 0.0% Glx (Z) 0 0.0% Xaa (X) 0 0.0%
Total number of negatively charged residues (Asp + Glu): 138
Total number of positively charged residues (Arg + Lys): 126
Atomic composition: Carbon C 5875 Hydrogen H 9284 Nitrogen N 1646 Oxygen O 1786 Sulfur S 85
Formula: $C_{5875}H_{9284}N_{1646}O_{1786}S_{85}$
Total number of atoms: 18676
Extinction coefficients: Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm. Ext. coefficient 128890 Abs 0.1% (=1 g/l) 0.960, assuming ALL Cys residues appear as half cystines Ext. coefficient 125140 Abs 0.1% (=1 g/l) 0.932, assuming NO Cys residues appear as half cystines
Estimated half-life: The N-terminal of the sequence considered is M (Met). The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).
Instability index: The instability index (II) is computed to be 44.59 This classifies the protein as unstable.
Aliphatic index: 80.74
Grand average of hydropathicity (GRAVY): -0.316

***References and documentation are available

Digesting a protein in a computer

http://www.expasy.org/tools/peptidecutter/

Separate the domains in your protein
Identify potential post-translational modification by mass spectrometry
Remove a tag protein when you express a fusion protein
Make sure that the protein you are cloning isn’t sensitive to some endogenous proteases

Primary structure analysis

1. Hydrophobic regions: membrane spanning

   http://www.expasy.ch/tools/protscale.html

   Hphob. / Kyte & Doolittle: the recommended threshold value is 1.6

   Compare with another scale

   TMHMM: http://www.cbs.dtu.dk/services/TMHMM/

2. Coiled-coil regions: potential protein-protein interaction

   http://www.ch.embnet.org/software/COILS_form.html

3. Hydrophilic stretches: looping out at the surface

   FESTfind:

(http://www.bioinformatrix.com/net/modules.php?name=Web_Links)

Predicting post-translational modifications

PROSITE patterns

Small well-conserved segments

PKA phosphorylation

[RK] – x – [ST]: ex. RGT, KCS, KET

prokaryotic C4 Zn-finger

C-[DES] – x – C – x(3) – I – x(3) – R – x(4) – P – x(4) – C – x(2) – C

Scan prosite

http://www.expasy.ch/tools/scanprosite/

read PDOC

be careful with species information

how to remove false positives

how to find genuine negatives

everything is not in PROCITE

Finding domains

Domains: Independent globular folding units

A portion of protein that can be active on its own

Results from an alignment between the profile (domain) and your sequence

The main domain collections

PROCITE-Profile	www.expasy.ch/procite
PfamA	www.sanger.ac.uk/Software/Pfam
PfamB	www.sanger.ac.uk/Software/Pfam
PRINTSs	www.bioinf.man.ac.uk/dbbrosers/PRINTS
PRODOM	prodes.roulouse.inra.fr/prodom/doc
SMART	smart.embl~heidelberg.de
TIGRFAM	www.tigr.org/TIGRFAMs
BLOCKs	www.blocks.fhcrc.org

Finding domains with InterProScan

http://www.ebi.ac.uk/InterProScan/

integration of many databases

Finding domains with the CD server

CD: conserved domains server at NCBI

come along with a score

www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi

deselect Low Complexity check box

- sequences that contain repeated residues are low-complexity sequences
- an amino acid is over represented in many interesting domains
- ex. Leucine zippers, glycine-rich domains

ragged ends indicate partial matches: mostly insignificant

different colors from different domains

E-values need to be below 0.01 to mean something

Assignments

Perform the following analysis and describe any significant findings

1. Physico-chemical properties
2. Protease digestion
3. Primary structure analysis
4. Prosite pattern
5. Domain pattern