**UNIVERSITY OF SAARLAND**

**Structural Bioinformatics Assignment 5**

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**1.**

There are methods like X-ray crystallography, NMR and cryo-EM.

X-ray crystallography sends X-ray beams to crystallized molecules. By doing so it makes it possible to observe the beams’ situation towards crystallized molecules like their angle and intensities. Measuring these will give the whereabouts of the density of the electrons within the crystallized molecule, creating and essential fundamental for the prediction of the whole molecule. Position of the atoms, chemical bonds, etc. can be predicted with the knowledge of electron densities (Eckert, 2012).

Knowing that all of the nuclei are electrically charged and most of them have spins, NMR applies an external magnetic field to them, making it possible for an energy exchange from base to higher energy levels. NMR basically measures this exchange transfer in terms of signals such as wavelength and frequencies. So that, three-dimensional structure can be measured in solution(Fürtig et al., 2003).

Cryo-EM uses electron cannon and the lenses, which are electric coils through which the current flows, create a magnetic field, thereby focusing an electron beam that will eventually form the image. It allows direct observation (without staining or fixation) of native conformational states at atomic resolution (Ohi et al., 2004).

If we want to have high resolution and are dealing with water soluble proteins such as membrane proteins or macromolecules, we can use X-ray crystallization since the resolution is not limited by the molecular weight. But, it might be a bit challenging to crystallize water soluble proteins.

If we want to study molecular dynamics after measuring the structure and if we have large amounts of pure samples and our sample doesn’t have large molecular wright, then we can use NMR to measure three-dimensional structure.

If we only have large molecules in terms of molecular weight and looking for a method that is rather easy than the previous ones (crystallization, sample preparation) then we can use cryo-EM. It is also good if we are looking for moving, heterogonous molecules. But, it comes with rather low resolution.

**2.**

In model optimization short runs of molecular dynamics are performed. It has advantage on energy minimization processes with its self-parameterizing force field. However, in terms of model evaluation, molecular dynamics doesn’t work well because of its force field being not that accurate and having an energy landscape with a lot of local minima.

**3.**

It is better to have more sequence identity and sequence length when it comes to choosing a template. If we have more than 75% sequence identity it would be a good start. Having less than this percentage might require alignment correction.

**4.**

BLAST is using a word to word searches in queries and looks for words that are similar. It creates a word sequence and extends from there. It is called a word alignment and by setting a cut-off value, we can decide when for the alignment process to stop.

PSI-BLAST uses BLAST but it also uses position-specific scoring matrix to set an alignment profile.

HMMer makes a similarity search with given database of sequences in the database of set of HMMs. It is considered slow since it has to be tested for each HMM.

HHblits uses queries as HMM also. Usually those HMM queries are the result of PSI-BLAST. It also uses the same comparison as HMMer does. And, it is also slow.

Hhsenser uses relatively the same method as HMMer and HHblits but its cutoffs are stricter than the other two, making it more sensitive in the cost of being slower than the two.

HHpred is used to search HMM-HMM in Protein Data Bank database. It can be used with single sequences or alignments. It also has collection of predictions for 3D structures in the PDB database that has been calculated before. Firstly, it looks for homologs by using PSI-BLAST, then starts an HMM construction and comparison. Then, it uses MODELLER.

**REFERENCES**

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