Protein Structures: Experiments and Modeling

Patrice Koehl
Structural Bioinformatics: Proteins

Proteins: Sources of Structure Information

Proteins: Homology Modeling

Proteins: Ab initio prediction

Proteins: Quality of Structures/Models
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Proteins: Quality of Structures/Models
Proteins: Finding the Primary Structure

Methods for finding the sequence of a protein:

- Translating gene sequence
  - For proteins from prokaryotes, direct translation
  - For proteins from eukaryotes, we need the sequence of mRNA or cDNA

- Edman degradation
  limited to “small” proteins, up to 50 amino acids
  for automated sequencer

- Mass spectrometry
Proteins: Finding the Primary Structure

EDMAN DEGRADATION

Ala --- Gly --- Phe --- Asp --- Asn

(labeling amino-terminal residue)

Ala --- Gly --- Phe --- Asp --- Asn

.removing the 1st residue

Ala --- Gly --- Phe --- Asp --- Asn

(labeling the 2nd residue)

Gly --- Phe --- Asp --- Asn

.removing the 2nd residue

Gly

Phe --- Asp --- Asn

(Phenyl isothiocyanate or PITC)

(Trifluoroacetic acid, TFA)

(http://en.wikibooks.org/wiki/Structural_Biochemistry/Proteins/Protein_sequence_determination_techniques)
Proteins: Finding the Tertiary Structure

Methods for finding the 3D structure of a protein:

- Circular Dichroism
  (low resolution; provides information on secondary structure)

- X-ray crystallography
  (high resolution; finds structure of a protein in a crystal)

- NMR spectroscopy
  (high resolution; finds structure of a protein in solution)
Proteins: Circular Dichroism

Circular dichroism (CD) spectroscopy measures differences in the absorption of left-handed polarized light versus right-handed polarized light which arise due to structural asymmetry.

Different secondary structures in proteins have different CD spectra as they have different asymmetry.

CD therefore can detect secondary structures in protein
Proteins: X-ray Crystallography

Bragg’s Law:

$$2d \sin(\theta) = n\lambda$$

From the “pattern of diffraction”, i.e. the maximum of intensities observed, we can find the angles of diffraction and for each angle we get the corresponding $d$ using Bragg’s law.
General principle of X-ray crystallography applied to proteins:

1) We need a crystal

2) From the diffraction pattern, we get the crystal organization

3) From the diffraction intensities, we get the electron densities

4) Once the electron density map we fit a structure that matches with this density

5) From the atomic model, we can compute a theoretical diffraction map; if it matches with the experimental one, we are done; otherwise refine
Getting the Diffraction Pattern

Rosalyn Franklin:
Diffraction pattern for DNA
One hidden problem: diffraction patterns provide intensities; for Fourier transform, need intensity and phase. A significant step in X-ray crystallography is the solve the “phase problem”.
Fitting the structure: Influence of the resolution

2.6 Å resolution

1.2 Å resolution
## Resolution of X-ray structures

<table>
<thead>
<tr>
<th>Resolution (Å)</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;4.0</td>
<td>Individual coordinates meaningless</td>
</tr>
<tr>
<td>3.0 - 4.0</td>
<td>Fold possibly correct, but errors are very likely.</td>
</tr>
<tr>
<td>2.5 - 3.0</td>
<td>Fold likely correct except that some surface loops might be mismodelled.</td>
</tr>
<tr>
<td>2.0 - 2.5</td>
<td>Many small errors can normally be detected. Fold normally correct and number of errors in surface loops is small.</td>
</tr>
<tr>
<td></td>
<td>Water molecules and small ligands become visible.</td>
</tr>
<tr>
<td>1.5 - 2.0</td>
<td>Many small errors can normally be detected. Folds are extremely rarely incorrect, even in surface loops.</td>
</tr>
<tr>
<td>0.5 - 1.5</td>
<td>In general, structures have almost no errors at this resolution. geometry studies are made from these structures.</td>
</tr>
</tbody>
</table>

Large molecular assemblies: X-ray crystallography and Cryo-EM

*X-ray structure*

(180 copies of the same protein)

(Norwalk virus: http://www.bcm.edu/molvir/norovirus)
Large molecular assemblies: X-ray crystallography and Cryo-EM

Cryo-EM:

- Microscopy technique; as such, do not need crystal (closer to physiological conditions)

- Not high-resolution enough to provide atomic details; used in combination with modeling
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Why we need Homology Modeling?

- Aim to solve the structure of all proteins: this is too much work experimentally!

- Solve enough structures so that the remaining structures can be inferred from those experimental structures

- The number of experimental structures needed depend on our abilities to generate a model.
Why we need Homology Modeling?

Proteins with known structures

Unknown proteins
Why does Homology Modeling Work?

*High sequence identity*

*High structure similarity*

Homology Modeling: How it works
Homology Modeling: How it works

- Find template
- Align target sequence with template
- Generate model:
  - add loops
  - add sidechains
- Refine model
Homology Modeling: Input

The query, also called target sequence

The template structure and sequence
(need high quality structure)

The sequence alignment between query and template sequence
(Probably the most important input!)
Wallner B, Elofsson A.
All are not equal: a benchmark of different homology modeling programs.
Homology Modeling: Which program to use?

1) **Web service: SwissModel**
   http://swissmodel.expasy.org/

   3 modes:
   - fully automatic
   - “Alignment mode”: you provide your own target-template alignment
   - “Project mode”: provides an environment to edit alignment

2) **Software: Modeller**
   http://www.salilab.org/modeller/

   Probably the best maintained software the homology modeling
Do not start a homology modeling project before checking...

- **Swiss-Model** repository
  (http://swissmodel.expasy.org/repository/)
  - Companion to the Swiss-Model tools – over 3.0 million models of protein domains computed from over 2.2 million sequences.

- **ModBase**
  (http://modbase.compbio.ucsf.edu/)
  - Companion to **Modeller**

**ModBase Statistics**

- Number of Models: 10408644
- Number of Unique Sequences modeled: 2430327
- Unique sequences attempted: 3367150
- Number of PDB chains: 38355

Statistics updated at Fri Oct 1 22:06:13 PDT 2010
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Secondary Structure Prediction

- Given a protein sequence $a_1a_2\ldots a_N$, secondary structure prediction aims at defining the state of each amino acid $a_i$ as being either H (helix), E (extended=strand), or O (other) (Some methods have 4 states: H, E, T for turns, and O for other).

- The quality of secondary structure prediction is measured with a “3-state accuracy” score, or $Q_3$. $Q_3$ is the percent of residues that match “reality” (X-ray structure).
Determine Secondary Structure positions in known protein structures using DSSP or STRIDE:

Early methods for Secondary Structure Prediction

- **Chou and Fasman**
  

- **GOR**
  
Start by computing amino acids propensities to belong to a given type of secondary structure:

\[
\begin{align*}
    P(i / \text{Helix}) & \quad P(i / \text{Beta}) & \quad P(i / \text{Turn}) \\
    P(i) & \quad P(i) & \quad P(i)
\end{align*}
\]

Propensities > 1 mean that the residue type I is likely to be found in the corresponding secondary structure type.
### Chou and Fasman

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>α-Helix</th>
<th>β-Sheet</th>
<th>Turn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>1.29</td>
<td>0.90</td>
<td>0.78</td>
</tr>
<tr>
<td>Cys</td>
<td>1.11</td>
<td>0.74</td>
<td>0.80</td>
</tr>
<tr>
<td>Leu</td>
<td>1.30</td>
<td>1.02</td>
<td>0.59</td>
</tr>
<tr>
<td>Met</td>
<td>1.47</td>
<td>0.97</td>
<td>0.39</td>
</tr>
<tr>
<td>Glu</td>
<td>1.44</td>
<td>0.75</td>
<td>1.00</td>
</tr>
<tr>
<td>Gin</td>
<td>1.27</td>
<td>0.80</td>
<td>0.97</td>
</tr>
<tr>
<td>His</td>
<td>1.22</td>
<td>1.08</td>
<td>0.69</td>
</tr>
<tr>
<td>Lys</td>
<td>1.23</td>
<td>0.77</td>
<td>0.96</td>
</tr>
<tr>
<td>Val</td>
<td>0.91</td>
<td>1.49</td>
<td>0.47</td>
</tr>
<tr>
<td>Ile</td>
<td>0.97</td>
<td>1.45</td>
<td>0.51</td>
</tr>
<tr>
<td>Phe</td>
<td>1.07</td>
<td>1.32</td>
<td>0.58</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.72</td>
<td>1.25</td>
<td>1.05</td>
</tr>
<tr>
<td>Trp</td>
<td>0.99</td>
<td>1.14</td>
<td>0.75</td>
</tr>
<tr>
<td>Thr</td>
<td>0.82</td>
<td>1.21</td>
<td>1.03</td>
</tr>
<tr>
<td>Gly</td>
<td>0.56</td>
<td>0.92</td>
<td>1.64</td>
</tr>
<tr>
<td>Ser</td>
<td>0.82</td>
<td>0.95</td>
<td>1.33</td>
</tr>
<tr>
<td>Asp</td>
<td>1.04</td>
<td>0.72</td>
<td>1.41</td>
</tr>
<tr>
<td>Asn</td>
<td>0.90</td>
<td>0.76</td>
<td>1.23</td>
</tr>
<tr>
<td>Pro</td>
<td>0.52</td>
<td>0.64</td>
<td>1.91</td>
</tr>
<tr>
<td>Arg</td>
<td>0.96</td>
<td>0.99</td>
<td>0.88</td>
</tr>
</tbody>
</table>

- **Favors α-Helix**
- **Favors β-strand**
- **Favors turn**
Predicting helices:
- find nucleation site: 4 out of 6 contiguous residues with $P(\alpha)>1$
- extension: extend helix in both directions until a set of 4 contiguous residues has an average $P(\alpha) < 1$ (breaker)
- if average $P(\alpha)$ over whole region is $>1$, it is predicted to be helical

Predicting strands:
- find nucleation site: 3 out of 5 contiguous residues with $P(\beta)>1$
- extension: extend strand in both directions until a set of 4 contiguous residues has an average $P(\beta) < 1$ (breaker)
- if average $P(\beta)$ over whole region is $>1$, it is predicted to be a strand
### Position-specific parameters for turn:

Each position has distinct amino acid preferences.

**Examples:**

- At position 2, Pro is highly preferred; Trp is disfavored
- At position 3, Asp, Asn and Gly are preferred
- At position 4, Trp, Gly and Cys preferred

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>$f(i)$</th>
<th>$f(i+1)$</th>
<th>$f(i+2)$</th>
<th>$f(i+3)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>0.060</td>
<td>0.076</td>
<td>0.035</td>
<td>0.058</td>
</tr>
<tr>
<td>Arg</td>
<td>0.070</td>
<td>0.106</td>
<td>0.099</td>
<td>0.085</td>
</tr>
<tr>
<td>Asp</td>
<td>0.147</td>
<td>0.110</td>
<td>0.179</td>
<td>0.081</td>
</tr>
<tr>
<td>Asn</td>
<td>0.161</td>
<td>0.083</td>
<td>0.191</td>
<td>0.091</td>
</tr>
<tr>
<td>Cys</td>
<td>0.149</td>
<td>0.050</td>
<td>0.117</td>
<td>0.128</td>
</tr>
<tr>
<td>Glu</td>
<td>0.056</td>
<td>0.060</td>
<td>0.077</td>
<td>0.064</td>
</tr>
<tr>
<td>Gln</td>
<td>0.074</td>
<td>0.098</td>
<td>0.037</td>
<td>0.098</td>
</tr>
<tr>
<td>Gly</td>
<td>0.102</td>
<td>0.085</td>
<td>0.190</td>
<td>0.152</td>
</tr>
<tr>
<td>His</td>
<td>0.140</td>
<td>0.047</td>
<td>0.093</td>
<td>0.054</td>
</tr>
<tr>
<td>Ile</td>
<td>0.043</td>
<td>0.034</td>
<td>0.013</td>
<td>0.056</td>
</tr>
<tr>
<td>Leu</td>
<td>0.061</td>
<td>0.025</td>
<td>0.036</td>
<td>0.070</td>
</tr>
<tr>
<td>Lys</td>
<td>0.055</td>
<td>0.115</td>
<td>0.072</td>
<td>0.095</td>
</tr>
<tr>
<td>Met</td>
<td>0.068</td>
<td>0.082</td>
<td>0.014</td>
<td>0.055</td>
</tr>
<tr>
<td>Phe</td>
<td>0.059</td>
<td>0.041</td>
<td>0.065</td>
<td>0.065</td>
</tr>
<tr>
<td>Pro</td>
<td>0.102</td>
<td>0.301</td>
<td>0.034</td>
<td>0.068</td>
</tr>
<tr>
<td>Ser</td>
<td>0.120</td>
<td>0.139</td>
<td>0.125</td>
<td>0.106</td>
</tr>
<tr>
<td>Thr</td>
<td>0.086</td>
<td>0.108</td>
<td>0.065</td>
<td>0.079</td>
</tr>
<tr>
<td>Trp</td>
<td>0.077</td>
<td>0.013</td>
<td>0.064</td>
<td>0.167</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.082</td>
<td>0.065</td>
<td>0.114</td>
<td>0.125</td>
</tr>
<tr>
<td>Val</td>
<td>0.062</td>
<td>0.048</td>
<td>0.028</td>
<td>0.053</td>
</tr>
</tbody>
</table>
Chou and Fasman

Predicting turns:
- for each tetrapeptide starting at residue i, compute:
  - $P_{\text{Turn}}$ (average propensity over all 4 residues)
  - $F = f(i) \cdot f(i+1) \cdot f(i+2) \cdot f(i+3)$

- if $P_{\text{Turn}} > P_{\alpha}$ and $P_{\text{Turn}} > P_{\beta}$ and $P_{\text{Turn}} > 1$ and $F > 0.000075$
  tetrapeptide is considered a turn.

Chou and Fasman prediction:

http://fasta.bioch.virginia.edu/fasta_www/chofas.htm
The GOR method

Position-dependent propensities for helix, sheet or turn is calculated for each amino acid. For each position $j$ in the sequence, eight residues on either side are considered.

A helix propensity table contains information about propensity for residues at 17 positions when the conformation of residue $j$ is helical. The helix propensity tables have $20 \times 17$ entries. Build similar tables for strands and turns.

**GOR simplification:**
The predicted state of AA$_j$ is calculated as the sum of the position-dependent propensities of all residues around AA$_j$.

GOR can be used at: [http://abs.cit.nih.gov/gor/](http://abs.cit.nih.gov/gor/) (current version is GOR IV)
Both Chou and Fasman and GOR have been assessed and their accuracy is estimated to be Q3=60-65%.
Secondary Structure Prediction

- **Available servers:**

  - **JPRED**: [http://www.compbio.dundee.ac.uk/~www-jpred/](http://www.compbio.dundee.ac.uk/~www-jpred/)
  - **PSIPRED**: [http://bioinf.cs.ucl.ac.uk/psipred/](http://bioinf.cs.ucl.ac.uk/psipred/)
  - **NNPREDICT**: [http://www.cmpharm.ucsf.edu/~nomi/nnpredict.html](http://www.cmpharm.ucsf.edu/~nomi/nnpredict.html)
  - **Chou and Fassman**: [http://fasta.bioch.virginia.edu/fasta_www/chofas.htm](http://fasta.bioch.virginia.edu/fasta_www/chofas.htm)

- **Interesting paper:**

Protein Structure Prediction

One popular model for protein folding assumes a sequence of events:

- Hydrophobic collapse
- Local interactions stabilize secondary structures
- Secondary structures interact to form motifs
- Motifs aggregate to form tertiary structure
Protein Structure Prediction

A physics-based approach:

- find conformation of protein corresponding to a thermodynamics minimum (free energy minimum)

- cannot minimize internal energy alone! Needs to include solvent

- simulate folding…a very long process!

Folding time are in the ms to second time range
Folding simulations at best run 1 ns in one day…
I AM One IN A MILLION

By downloading the Folding@home software you are supplying us with the computer power we need to find new cures. And by spreading the word you’re giving even more people the chance to contribute. In our book, that certainly makes you one in a million.
This section describes how Folding@home simulations work and why our methods benefit from distributed computing. The descriptions found here may be slightly technical, but aims for people who would like to learn more about how this project works and how their computer is involved.

**TABLE OF CONTENTS**

- Why do proteins fold?
- How does Folding@home simulate protein folding?
- What are Markov State Models?
- What do MSMs look like?
- What is adaptive sampling, and how is it related to MSMs?
- How do you build an MSM using adaptive sampling?
- Why is this approach particularly useful?
- What are some of the applications for these techniques?
- Who answered these questions?
Folding @ Home: Results

Predicted folding time (nanoseconds)

Experimental measurement (nanoseconds)

Experiments:

**villin:**
Raleigh, et al, SUNY, Stony Brook

**BBAW:**
Gruebele, et al, UIUC

**beta hairpin:**
Eaton, et al, NIH

**alpha helix:**
Eaton, et al, NIH

**PPA:**
Gruebele, et al, UIUC

http://pande.stanford.edu/
Protein Structure Prediction

DECOYS:
Generate a large number of possible shapes

DISCRIMINATION:
Select the correct, native-like fold

Need good decoy structures
Need a good energy function
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Proteins: Quality of Structures/Models
What is a “good” protein structure?

-The structure must have good stereochemistry
  -Bond lengths and bond angles close to ideal values
  -Backbone dihedral angles in “allowed” regions of the Ramachandran plot
  -Side-chains close to “rotamer” states

-The structure should be energetically favorable
  - No “clashes” (such as two atoms too close to each other)
  - Hydrophobic residues should be mostly buried
  - Polar residues should be on the surface. No charges buried.

I advise running Procheck to check the quality of a protein structure:

-As a standalone program:
  http://www.ebi.ac.uk/thornton-srv/software/PROCHECK/

-Or through the PDBsum web portal:
  http://www.ebi.ac.uk/pdbsum/
What have we learnt?

- X-ray crystallography is the method of choice for the elucidation of the high-resolution structure of a protein.
- Alternative methods include NMR and Cryo-EM.
- The mapping between protein sequence and protein structure is not one-to-one: for one sequence, there is (usually) one structure, but for one structure, there can be many sequences.
What have we learnt?

- Homology modeling is a computational technique that predicts the structure of a target protein based on a template structure and an alignment between the target and template sequences.
- The quality of the alignment defines the quality of the homology models.
- Two good programs for homology modeling: Swiss-Model (web-based) and Modeller (standalone software)
- The swiss model repository and ModBase are useful databases of protein structure models generated by homology modeling.
- It is usually good practice to check the quality of a protein structure or model. ProCheck is a useful tool for this: it checks both the stereochemistry and energetics of the protein.