Conformational diseases

When little mistake might cause large troubles
Objectives: Understanding protein misfolding diseases

- Fate of the newly synthesized polypeptide chain: to fold, non-fold or misfold;
- Abundance of conformational diseases;
- What can cause protein to misfold?
- Molecular mechanisms of protein misfolding;
- Major consequences of misfolding: α-Synuclein and Parkinson’s disease as a case study;
- Nanotools for megaproblems: Nanomedicine and protein misfolding diseases
- Bioinformatics and protein misfolding diseases
The route from the DNA code to the protein

The DNA is responsible for coding for all proteins. Each amino acid is designated by one or more set of triplet nucleotides. The code is produced from one strand of the DNA by a process called "transcription". This produces mRNA which then is sent out of the nucleus where the message is translated into proteins. The cartoon to the left shows the basic sequence of transcription and translational events.
Many proteins have complex 3D shapes which determine their functions.
Mechanisms of protein folding

Folding through the funnels

Frame-work model


O.B. Ptitsyn DAN SSSR 210 (1973) 1213-1215
Protein non-folding problem

Protein stays substantially unfolded

Protein partially folds to a pre-molten globule-like state

Protein partially folds to a molten globule-like state
Protein misfolding problem

Native MG → Ordered

Native coil → Native PMG

Amyloid fibril
Abundance of conformational diseases

- More than 450 different disorders, which are known to affect all organs;
- They can be hereditary or sporadic;
- More than 25 different proteins in amyloidoses only, which prior to fibrillation have different structures (β-sheets, α-helices, β-helices, or contain both α-helices and β-sheets);
- They may be globular proteins with rigid 3D-structure or be intrinsically disordered (even natively unfolded)
<table>
<thead>
<tr>
<th>Disease</th>
<th>Protein</th>
<th>Site of folding</th>
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<tbody>
<tr>
<td>Hypercholesterolaemia</td>
<td>Low-density lipoprotein receptor</td>
<td>ER</td>
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<tr>
<td>Cystic fibrosis</td>
<td>Cystic fibrosis trans-membrane regulator</td>
<td>ER</td>
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<td>Phenylketonuria</td>
<td>Phenylalanine hydroxylase</td>
<td>Cytosol</td>
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<td>Huntington’s disease</td>
<td>Huntingtin</td>
<td>Cytosol</td>
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<td>Marfan syndrome</td>
<td>Fibrillin</td>
<td>ER</td>
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<td>Osteogenesis imperfecta</td>
<td>Procollagen</td>
<td>ER</td>
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<td>Sickle cell anaemia</td>
<td>Haemoglobin</td>
<td>Cytosol</td>
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<tr>
<td>αl-Antitrypsin deficiency</td>
<td>αl-Antitrypsin</td>
<td>ER</td>
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<td>Tay-Sachs disease</td>
<td>β-Hexosaminidase</td>
<td>ER</td>
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<td>Scurvy</td>
<td>Collagen</td>
<td>ER</td>
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<tr>
<td>Alzheimer’s disease</td>
<td>β-Amyloid/ presenilin, tau protein</td>
<td>ER</td>
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<tr>
<td>Parkinson’s disease</td>
<td>α-Synuclein</td>
<td>Cytosol</td>
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<td>Scrapie/ Creutzfeldt-J akob disease, kuru</td>
<td>Prion protein</td>
<td>ER</td>
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<tr>
<td>Familial amyloidoses</td>
<td>Transthyretin/ lysozyme</td>
<td>ER</td>
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<tr>
<td>Retinitis pigmentosa</td>
<td>Rhodopsin</td>
<td>ER</td>
</tr>
<tr>
<td>Cataracts</td>
<td>Crystallins</td>
<td>Cytosol</td>
</tr>
<tr>
<td>Cancer</td>
<td>p53</td>
<td>Cytosol</td>
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</table>
What can cause protein to misfold?
When good proteins go bad

- DNA mutation causes the production of a protein that cannot fold when initially synthesized by a ribosome;
- Mutations cause the production of a protein that is destabilized and thus unfolds easier once folded;
- Stresses during the lifetime of the protein modify it causing it to be destabilized and partially unfolded;
- Environmental factors (metals, pesticides, toxins) can modify proteins making them more prone to misfold;
- Posttranslational modifications can change protein structure;
- Proteolytic cleavages can produce amyloidogenic peptides;
Folding-misfolding alternative

Protein Aggregation

- When proteins are partially or completely unfolded they are more susceptible to aggregation;
- Exposure of hydrophobic segments to water is generally unfavorable;
- During aggregation, several destabilized proteins bind together;
- Aggregates are highly stable;
- Once the polymerized species becomes large enough, it becomes insoluble and falls out of solution;
3D structure and cross-$\beta$ model for insulin fibrils

Protein Aggregates

- Insoluble;
- Usually very large;
- Very difficult for cells to breakdown;
- Not easy to transport;
- Continues to grow over time and may even recruit properly folded protein;
- Often (but not always) toxic to cells;
Multi-Facial Aggregates
Molecular Mechanisms of Protein Misfolding Diseases

- Altered protein structure and enhanced aggregation
  - AD, PD, prion disease, AL and AA amyloidoses, type II diabetes, dialysis-related amyloidosis

- Point mutations and enhanced protein deposition
  - Familial amyloid polyneuropathy, systemic non-neuropathic amyloidosis, familial cases of AD and PD

- Altered protein structure and impaired protein function
  - SOD1, p53

- Changes in supra-molecular structure
  - Muscular dystrophies, cataracts, sickle cell anemia

- Altered protein structure and changes in cellular/nuclear function
  - Polyglutamine diseases

- Posttranslational modifications
  - Glycation (cataracts, muscular dystrophies); phosphorylation (AD)
Protein Misfolding Diseases:

Alzheimer’s Disease: Aβ protein

- Over time more and more destabilized protein forms and eventually with age there is enough to aggregate.
- Aggregated protein forms long fibers that wrap themselves around the base of the nerve cells.

www.ahaf.org/alzdis/about/AmyloidPlaques.htm

PET Scan of Normal Brain  PET Scan of AD Brain

www.Namenda.com
Protein Misfolding Diseases:
Mad Cow Disease and Prion Protein

- Similar to Alzheimer’s diseases in that long neurofibrillary tangles are formed by aggregated prion protein;

- The protein is the infectious particle playing a game of “aggregation tag” in your cells.
Protein Misfolding Diseases: Huntington’s disease and Huntingtin

- Polyglutamine repeat in the Huntingtin protein of some people causes self association of the protein in neurons;
- Cytoplasmic inclusions are formed that kill nerve cells
Protein Misfolding Diseases:
Amyotrophic Lateral Sclerosis and SOD1

Normal SOD1 performs the crucial role of scavenging potentially damaging superoxide ions.

There are 114 SOD1 mutants implicated in familial ALS cases.

Mutations in SOD1 causes protein destabilization and enhanced propensity to aggregate. Aggregated SOD1 leads to the death of the motor neurons, which control movement. When this happens, the brain can no longer direct the muscles of the body. This eventually leads to the muscle atrophy.
Protein p53 is the tumor suppressor, which is a transcription factor that is at the center of a network of interactions that affect the cell cycle and apoptosis;

- It is inactivated in virtually all cancers;
- In about 50% of cancers p53 is directly inactivated by mutation;
- There are >250 cancer-associated mutations in p53 gene, vast majority is located in DNA-binding domain.
Protein Misfolding Diseases:
Sickle Cell Anemia and Hemoglobin

- Aggregate is not toxic to cells, but causes cells to be elongated;
- Limits cells ability to bind oxygen

http://rad.usuhs.mil/sickle/index.html
Protein Misfolding Diseases:

Cataract and crystallin proteins

- Cataract: crystallin proteins
  - The leading cause of blindness world-wide;
  - 50% of people over the age of 80 have cataracts;
  - Likelihood of developing cataracts increases exponentially with age
Protein Misfolding Diseases: AD and tau protein

- In norm, tau interacts with tubulin and promote its assembly into microtubules;
- Regulated by alternative splicing and phosphorylation: Six splicing isoforms, multiple phosphorylation sites;
- Hyperphosphorylated tau self-assemble into tangles of paired helical filaments and straight filaments in AD and other taupathies.
Protein Misfolding Diseases: Parkinson’s disease and α-synuclein

• Second most common neurodegenerative disease;
• An “environmental” disease (pesticides and metals);
• Due to death of dopaminergic neurons in the substantia nigra;
• Pathological hallmark is intracellular proteinaceous inclusions called Lewy bodies and Lewy neurites;
• Major protein component in LBs and LNs is α-synuclein;
• Substantial evidence implicates the aggregation of α-synuclein as a key factor in the etiology of Parkinson’s disease
The basal ganglia controls movement and balance in the body.
Nerve cells in _basal ganglia_ send messages that signal the body to move.

In PD, many nerve cells are damaged and do not produce enough dopamine to carry signals properly.
Where is *substantia nigra*?

Cross-section of the brain
What is *substantia nigra* doing?

In the *substantia nigra*, the neurotransmitter dopamine is produced and stored. The *substantia nigra* is connected to other parts of the brain by nerve cells that transmit dopamine.
Brain regions involved in Parkinson’s disease
Pathology of Parkinson’s disease: Neurodegeneration
Pathology of Parkinson’s disease: Lewy bodies in *substantia nigra*
Structure of Lewy body

Typical LBs appear as intracytoplasmic inclusions, 5-25 μm in diameter with a dense core of filamentous and granular material that is surrounded by radially oriented filaments.
The relation between $\alpha$-synuclein aggregation and Parkinson’s disease

- Parkinson’s disease is a movement disorder first described by James Parkinson in 1817.

- Symptoms are: paucity of spontaneous movement (bradykinesia, hypokinesia), muscle rigidity, characteristic tremors, shuffling gait, and impaired balance

- Normally affects people > 60 years of age (~ 1 M in US)

- Pathologically, PD is characterized by the presence of Lewy Bodies (cell body) and Lewy neurites (axon) in the substantia nigra

- In 1997 the gene responsible for familial early onset PD was shown to be $\alpha$-synuclein. Only 3 kindreds shown to have familial early-onset PD

- $\alpha$-Synuclein shown to be major component of Lewy Bodies and Lewy neurites
α-Synuclein and Parkinson’s disease

- α-Synuclein is the major fibrillar component of LBs;
- Point mutations in α-synuclein cause early-onset of PD;
- Triplication of α-synuclein cause early-onset of PD;
- Transgenic mice and flies producing α-synuclein develop the motor deficits and neuronal inclusions reminiscent to PD;
Structural features and conformational behavior of α-synuclein
Fibrillation of α-synuclein in vitro

Effect of decrease in pH (A) or temperature increase (B) on fibrillation kinetics of human α-synuclein

Formation of the partially folded intermediate (low pH or high temperatures) accelerates fibrillation process

Uversky et al. (2001) J. Biol. Chem. 276, 10737
Factors modulating structure and aggregation of α-synuclein

- Macro-environment: pH, temperature;
- Amino acid substitutions;
- PD environmental risk factors (metals, pesticides);
- Oxidative modification (nitration and methionine oxidation);
- Crowded environment;
- Membrane field;
- Structure of water;
- Interaction with binding partners;
α-Synuclein is a protein-chameleon
Misfolding or misrecognition?

Uversky et al. (2005) J. Mol. Recognit. 18(5):343
Multifactorial Model for Parkinson’s Disease

Genes
- e.g. A30P
- e.g. A53T

Genetic susceptibility

Environment
- e.g. pesticides
- and metals

α-Synuclein aggregation

Loss of dopaminergic neurons

Parkinson’s disease
There is a unifying mechanism of protein aggregation and fibrillation which involves formation of aggregation-prone partially folded conformation(s).
Nanotools for Megaproblems:
Nanomedicine and protein misfolding diseases

- Why are nanomedicine and nanotools?
- What are nanotools for?
- Nano-glasses, nano-toolboxes and nano-containers
Nanomedicine

According to Wikipedia, nanomedicine is the medical application of nanotechnology and related research. It covers areas such as nanoparticle drug delivery and possible future applications of molecular nanotechnology.

Consider this point wider: To work with misfolded proteins (subcellular level) one need nano-glasses and nanotools
Nano-glasses:
New means to see deeper

www.tigerpath.com/
Active nano-glasses or nanotools?
Atomic Force Microscopy

Concept of AFM and the optical lever: (left) a cantilever touching a sample; (right) the optical lever.
Measuring the strength of protein-protein interactions by AFM
Fatal attraction: AFM analysis of protein aggregation

McAllister et al. (2005) JMB, 354(5):1028-42
Nanotweezers: Probing the inter-filament interaction

AFM pulling experiments: The AFM tip was guided to selected points on the α-synuclein fibril (indicated by arrows) and the interaction between filaments within the fibril was measured by pulling the tip away from the surface (see insert (i)). The characteristic saw-tooth pattern indicates the unzipping of the fibril.

Nano-containers: Systems for the Targeted Drug Delivery

Nanocontainers for protein delivery: the core represents the polyion complex of proteins and synthetic polyelectrolytes of opposite charge. The PEO-PPO-PEO chains grafted to the polyelectrolyte within the core form a micelle-like structure around the core, with the hydrophobic PPO [poly(propylene oxide)] blocks near the core and the hydrophilic PEO [poly(ethylene oxide)] blocks forming the exterior shell. Additional non-modified PEO-PPO-PEO chains fill in to optimize the stability of the micelle-like structure.

Using this approach, a model impermeable protein, horse radish peroxidase (HRP), was successfully delivered into the living bovine brain microvessel endothelial cells.
Where is the place for bioinformatics in this picture?

- Database development and maintenance;
- Development of the informatics tools for the large scale analyses;
- Classification and systemization;
- Development of the predictive tools;
- Systemic analysis;
- Model development;
- Drug development;
- etc., etc., etc.
Understanding the role of charge and hydrophobicity in protein aggregation

Many, but not all, globular proteins have been shown to have compact intermediate state(s) under equilibrium conditions in vitro.

Those who form intermediates are more prone to aggregate, whereas non-formers are less “sticky”.

Charge to hydrophobicity ratio of a polypeptide chain may represent a key determinant in this respect.

Role of charge in aggregation

Aggregation is favoured by macromolecules with an opposite charge

Amount of precipitation of proteins induced at neutral pH by amyloid fibrils of Aβ_{25-35} (expressed as ratio of total precipitate and amount of initial Aβ_{23-35} fibrils) plotted against the isoelectric points (pI) of the proteins.

AD-related proteins were shown to form a highly connected and statistically significant protein interaction sub-network.

Application of the integrated approach to the analysis of human interaction data and Alzheimer disease proteins allowed validation of the existing disease protein targets and predict novel ones not present in the initial list of disease-associated proteins.

Chen et al. (2006) Pacific Symposium on Biocomputing 11:367
Bioinformatics tools in treasure hunting for new drugs

**Panel 1:**
Why are you digging a hole?

**Panel 2:**
I'm looking for buried treasure!

**Panel 3:**
What have you found?
A few dirty rocks, a weird root, and some disgusting drugs.

**Panel 4:**
On your first try??

**Panel 5:**
There's treasure everywhere!
Bioinformatics, intrinsic disorder and drug discovery

Chen et al. (2006) TIB, in press
Thanks for your patience !!!