



Welcome to Biophysics (BT 301)

Navin Gupta
Assistant Professor,
Dept of BSBE, IIT Guwahati
Email: cngupta@iitg.ac.in

Ack: Few images are from Google

REFRESHER ON PROTEINS



PROTEIN FOLDING (PF) PROBLEM

Anfinsen's experiment (1950s)

HP Model of Protein Folding (PF)

Complexity of folding

Energetics of Folding

Levinthal Paradox

Current Active Researchers in PF

- Integrated Lecture from Textbook/Journal Papers of Researchers
 - working in the field of Protein Folding



Structure of Proteins; How are they formed??

REFRESHER ON PROTEINS



- 20 types of amino acids form various proteins
- Each amino acid consists of NH_2 , COOH and side group (R)
- Every **amino acid can be hydrophobic or hydrophilic.**
- Proteins formed due to condensation process of amino acids resulting in a CO-NH (Peptide bond)

Conformation

- Active Form of Protein
- Folded 3D structure of Protein

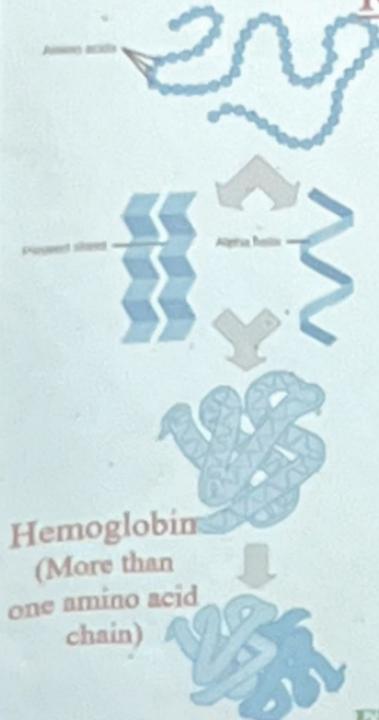
Denatured

- Unfolded structure
- Inactive Form



Structure of Proteins??

REFRESHER ON PROTEIN STRUCTURE



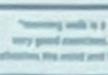
Alphabets \leftrightarrow Primary structure



Words \leftrightarrow Secondary structure



Sentences \leftrightarrow Tertiary structure



Paragraph \leftrightarrow Quaternary structure

Figure from Rob Phillips Physical Cell Biology

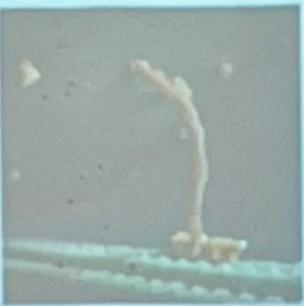


What Other facts do we know about Proteins

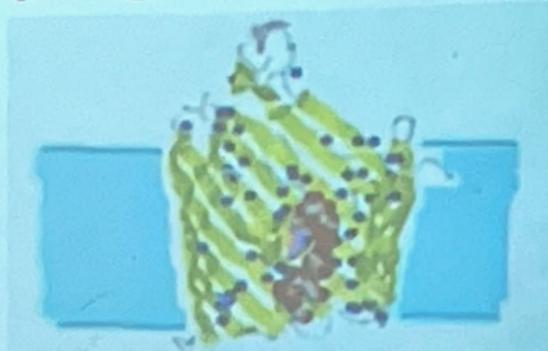


REFRESHER ON PROTEINS

Proteins Supervise everything in body



Muscles contract because
proteins crawl on top of
each other*



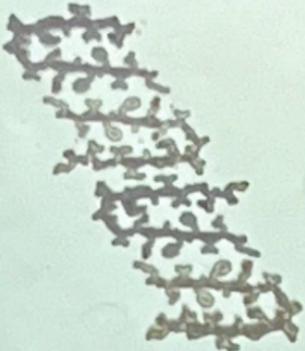
Proteins build up Structure



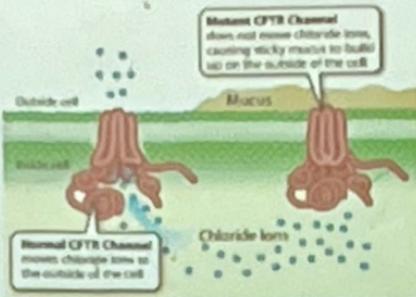
REFRESHER ON PROTEINS

Proteins are machines of Life

Everything that happens in our body happens because a protein supervises it, even when things go wrong



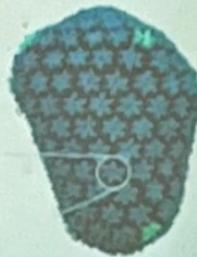
Protein aggregates cause
Alzheimer's disease



A single “unlucky” mutation causes cystic fibrosis



p24



HIV capsid

Bacteria and virus need proteins to function



Why is Protein Shape Important



Protein Shape Importance determines function

Proteins are long molecules, their shape is determined by the interactions between their components



Similar likes similar

Being part of a chain

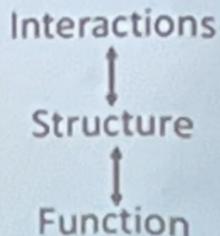


Figure from Rob Phillips
Physical Cell Biology



HP Model for Protein Folding

Figure from Rob Phillips Physical Cell Biology



HP Model for Amino Acids

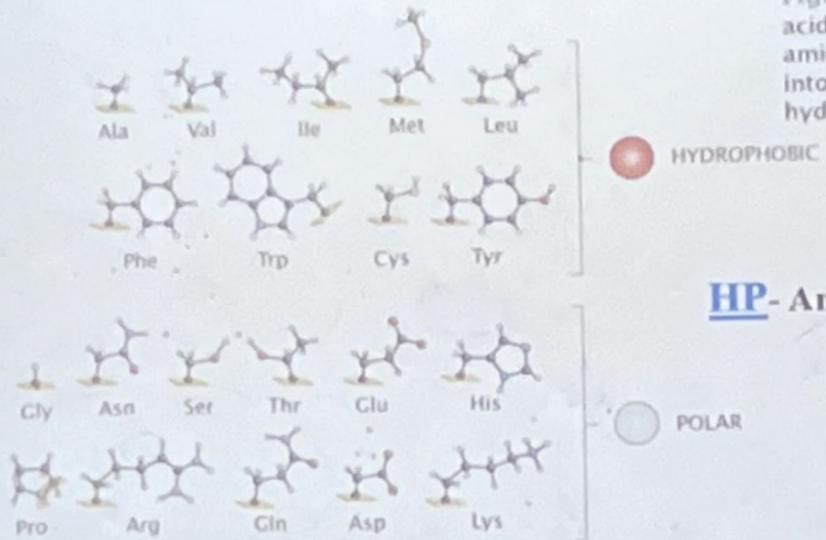
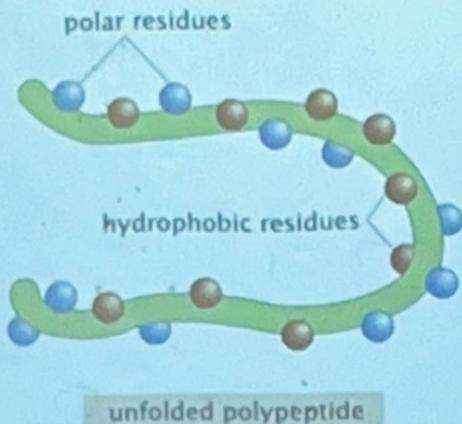


Figure 8.28: Mapping of the amino acids onto an HP alphabet. The 20 amino acids are coarsely separated into two categories, namely, hydrophobic (H) or polar (P).

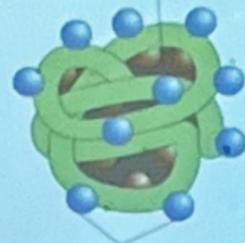
HP- Amino acids classified based on similar properties

Figure from Rob Phillips Physical Cell Biology

Schematic of Protein Folding (HP-Model)



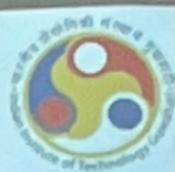
free energy lowered by sequestering hydrophobic residues



polar residues participate in hydrogen bond network

folded conformation in aqueous environment

Figure from Rob Phillips Physical Cell Biology



ANFINSENS EXPERIMENT(1950)



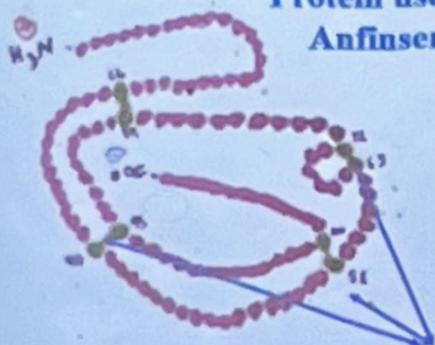
ANFINSENS EXPERIMENT

Overview:

- Christian studied a particular type of enzyme called ribonuclease. This protein consisted of 124 amino acids and had a tertiary structure with four disulfide bonds.

Protein used by

Anfinsen's



Disulphide bonds

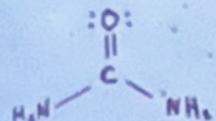
-The plan was to destroy the tertiary structure of ribonuclease by using appropriate agents and then investigate the conditions under which the proper tertiary structure reformed.

ANFINSENS EXPERIMENT



Anfinsen Experiment

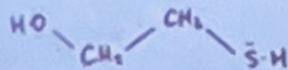
In 1950's, Christian Anfinsen conducted a series of experiments that ultimately showed that the information needed to form the three-dimensional active protein lies in its sequence of amino acids. Later experiments generalized this idea that the primary structure determines the conformation of the protein.



Urea

- Urea readily disrupts non-covalent bonds such as ionic interactions and hydrogen bonds.

Denaturing agents which break protein molecule



β -mercaptoethanol

- β -mercaptoethanol breaks down disulfide bonds via an oxidation-reduction reaction.



ANFINSSENS EXPERIMENT

RNAse A



+ 8 M urea
 β -mercaptoethanol

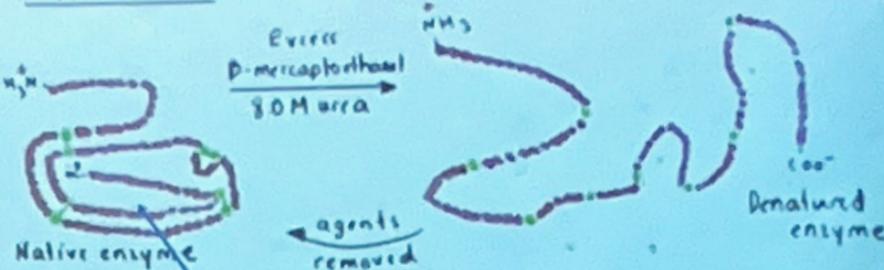


Denatured
disulfide bonds reduced

RNAse A

-90% active

Experiment # 1:

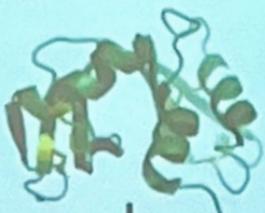


When the native enzyme was treated with excess β -mercaptoethanol and 8.0 M urea, all disulfide bonds and hydrogen bonds were broken and the protein denatured. When the denaturing agents were removed, the enzyme eventually reformed its original tertiary structure.

Agents removed: Disulphide bonds reformed by interaction of denatured protein with oxygen



RNase A

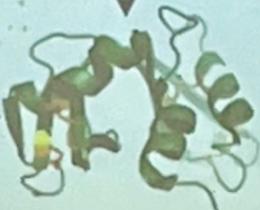


+ 8 M urea



Denatured

Dialyze



RNase A

ANFINSENS EXPERIMENT



RNase A
100% active

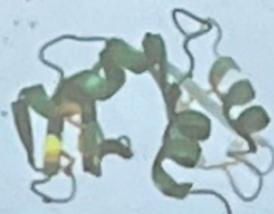


+ 8 M urea

β -mercaptoethanol

Denatured, disulfide
bonds reduced
0% active

Dialyze



~90% active

ANFINSENS EXPERIMENT

Re-oxidize
in urea

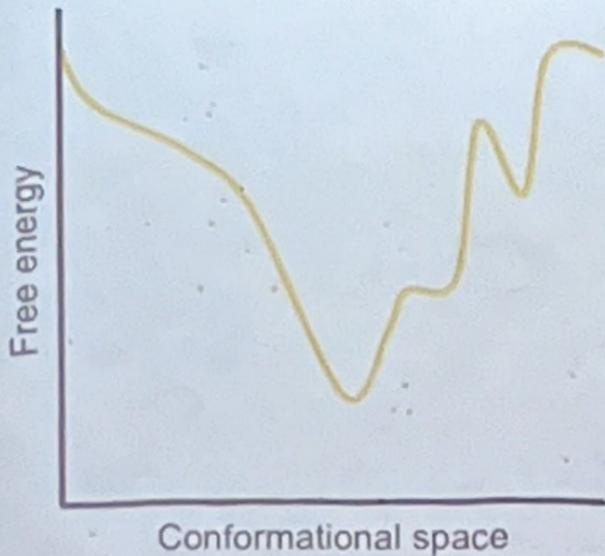
Dialyze



~1% active



Protein Folding studied using Protein folding funnel.



Conformational Space illustrates all the many confirmations the proteins can take

The nature of protein folding pathways



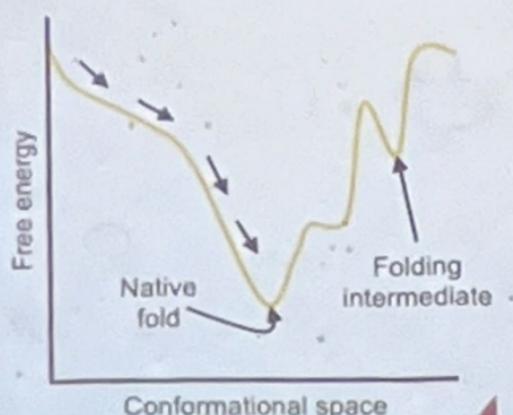
S. Walter Englander¹ and Leland Mayne

¹Johnson Research Foundation, Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

Edited by Alan R. Fersht, Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom, and approved September 23, 2014 (received for review June 24, 2014)

Viewing Protein Folding Through a Funnel

(B) the new view of multiple routes through a funneled landscape

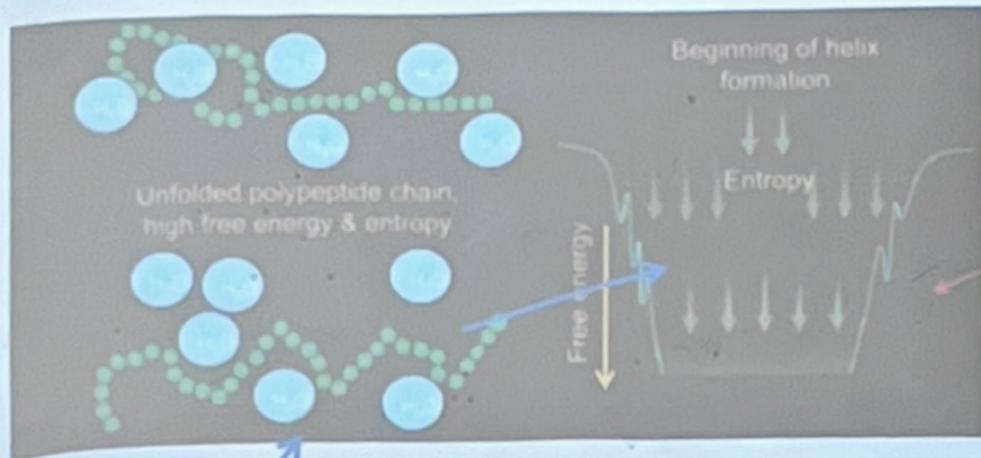


How Does a Protein Reach its Ordered Folded State from its Unfolded Ensemble?

What is the nature of the ordering in a folded protein, and how does that ordering arise from its highly disordered denatured state? The basic ideas are expressed through statistical mechanics. The relative stabilities of states depend on their free energies. At equilibrium, the probability of occupying a state depends on its Boltzmann's weight, $\exp(-\Delta G/k_B T)$, where ΔG is the difference in free energies of the states, native

and unfolded in this case. k_B is Boltzmann's constant and T is temperature.^{40,42} Small proteins typically fold cooperatively, i.e. through relatively sharp transitions between the disordered and ordered states.^{50,51}

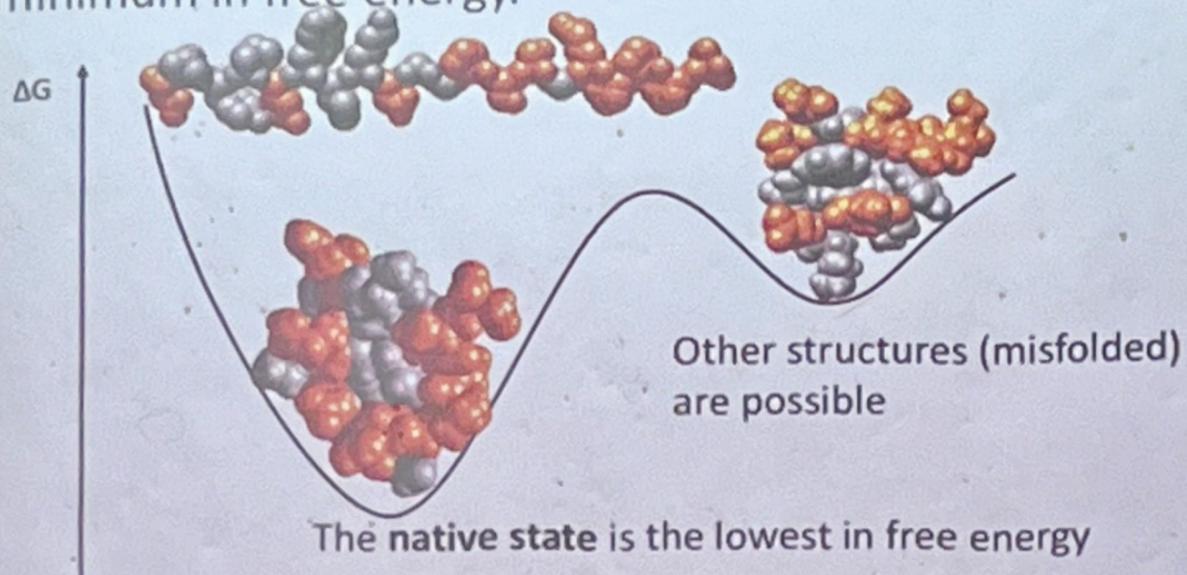
Middle of Funnel (During Protein Folding)



Entropy of the amino acid chain decreases in Middle of funnel

Entropy of surrounding water molecules increases down the funnel

Physics teaches us that the most stable structure is the minimum in free energy.



Slide for Dr. Emiliano Brini Talk on MELD method for protein folding prediction



Protein Folding inside a CELL

insight review articles

Cited more than 5000 times

Protein folding and misfolding

Christopher M. Dobson

University of Cambridge, Department of Chemistry, Lensfield Road, Cambridge CB2 1EW, UK (e-mail: cmd44@cam.ac.uk)

The manner in which a newly synthesized chain of amino acids transforms itself into a perfectly folded protein depends both on the intrinsic properties of the amino-acid sequence and on multiple contributing influences from the crowded cellular milieu.



Anfinsens and other research done seem to suggest that if our understanding of energetic forces that drive protein folding are sufficiently precise we can predict structure of small proteins knowing the primary structure.



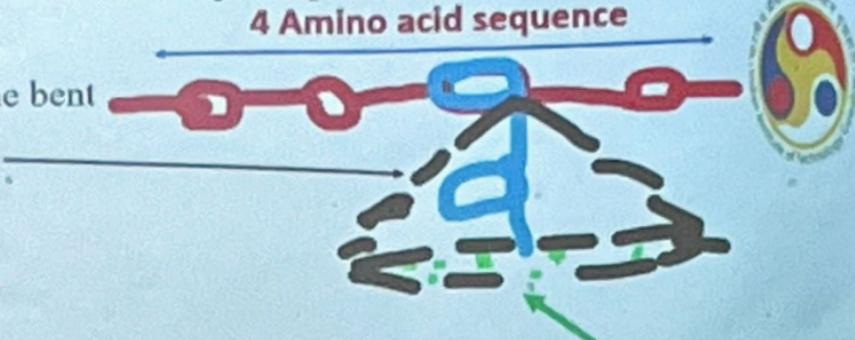
Protein Folding Computationally

Lets Discuss the Complexity of Folding Problem?

Why is determining Protein Folding a difficult problem??

- Folded structure of sequence determined by sequence of successive solid bend angles.

Cone is the range around which the bent sequence can wriggle



- We can limit the chain orientations in cone (say) **discretize to seven solid angles in cone[In green]**. So we have search space **of 7^4**
- Hence determining **the shape** is the greatest computational problem.

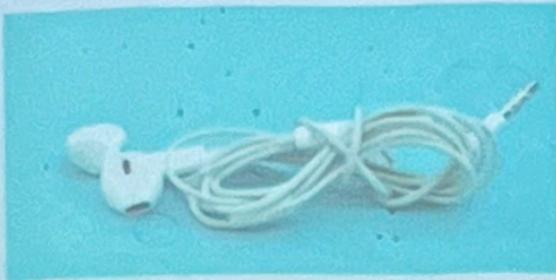
Content from Prof. Steven Skiena lectures and Rob Philips (Physical Cell Biology)



Laymans view of Protein Folding Problem

Physics works, but the phase space of a protein is huge!

You need to see many of these



before seeing this

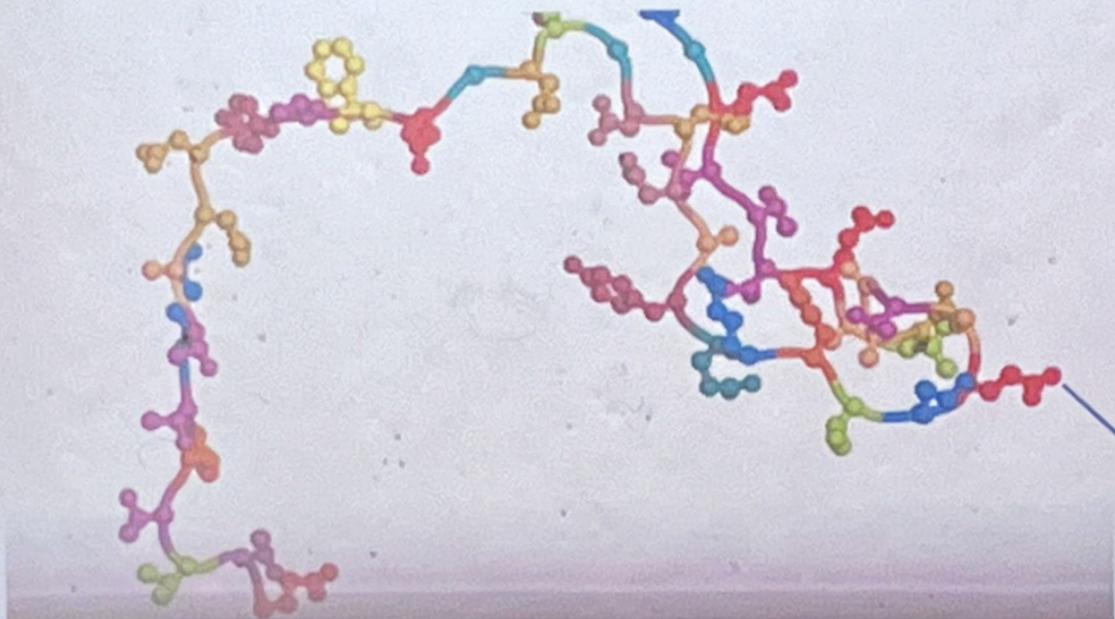
We need to reduce the search space!



Protein Folding Live



Example:1 Protein Molecule Visualized (Long and stringy)



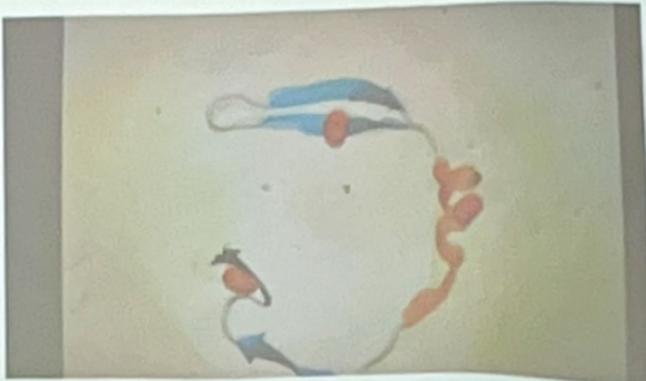
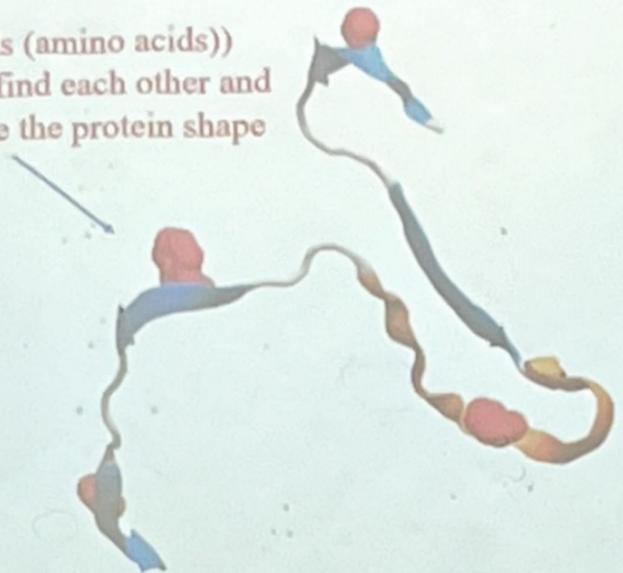
False colors indicate the various amino acids

Lines are bonds

Protein Structure can be viewed on a)NMR b)X-crystallography



Red beads (amino acids)
needs to find each other and
determine the protein shape

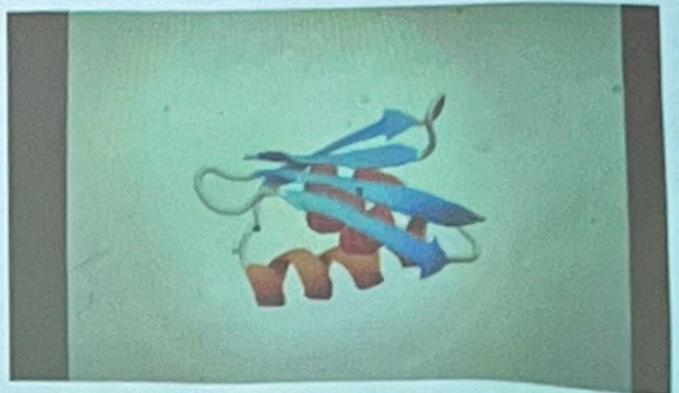
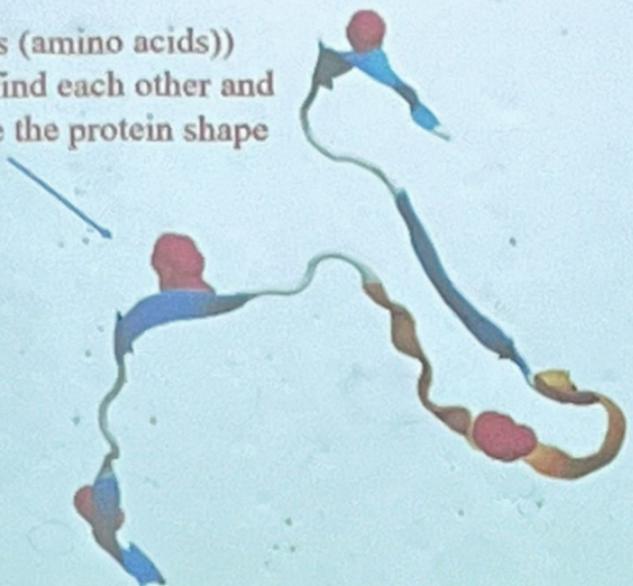


Protein shape determines protein function

Content from Prof. Ken Dill TED talk, State University of New York at Stony Brook



Red beads (amino acids)
needs to find each other and
determine the protein shape

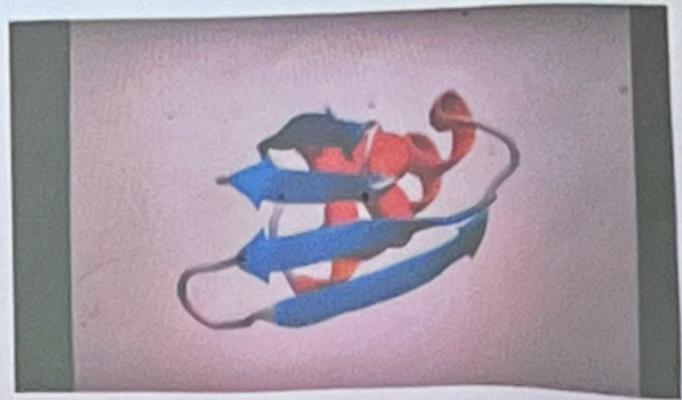
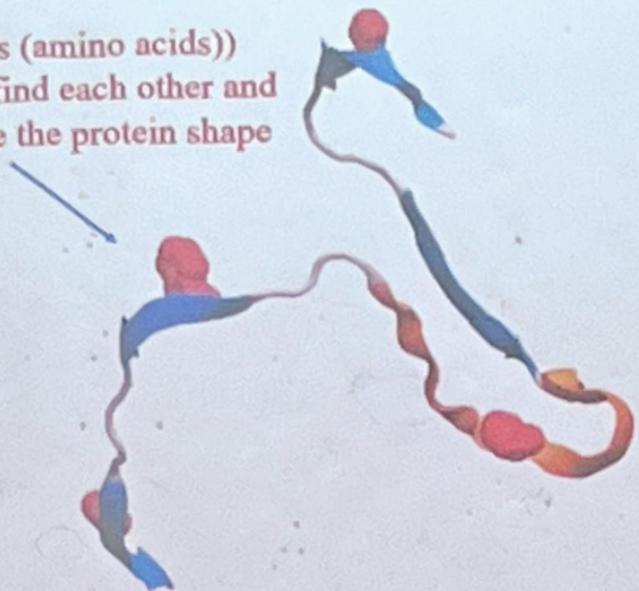


Protein shape determines protein function

Content from Prof. Ken Dill TED talk, State University of New York at Stony Brook



Red beads (amino acids)
needs to find each other and
determine the protein shape

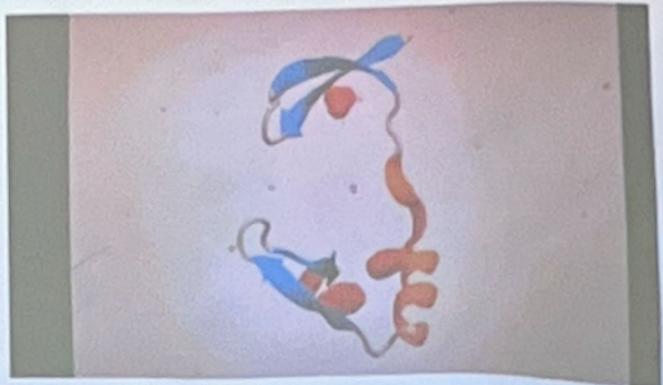
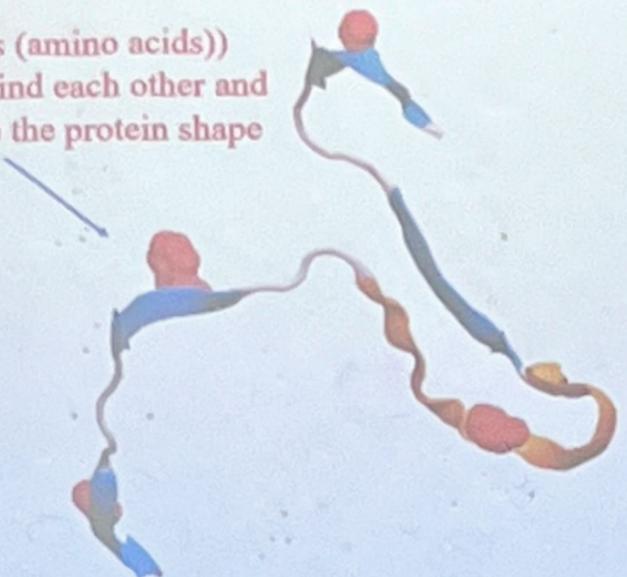


Protein shape determines protein function

Content from Prof. Ken Dill TED talk, State University of New York at Stony Brook



Red beads (amino acids)
needs to find each other and
determine the protein shape



Protein shape determines protein function

Content from Prof. Ken Dill TED talk, State University of New York at Stony Brook



Levinthal Paradox



Levinthal's paradox

ROBERT ZWANZIG, ATTILA SZABO, AND BIMAN BAGCHI*

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, Building 2, National Institutes of Health, Bethesda, MD 20892

Contributed by Robert Zwanzig, October 7, 1991

ABSTRACT Levinthal's paradox is that finding the native folded state of a protein by a random search among all possible configurations can take an enormously long time. Yet proteins can fold in seconds or less. Mathematical analysis of a simple model shows that a small and physically reasonable energy bias against locally unfavorable configurations, of the order of a few kT , can reduce Levinthal's time to a biologically significant size.

Levinthal's paradox

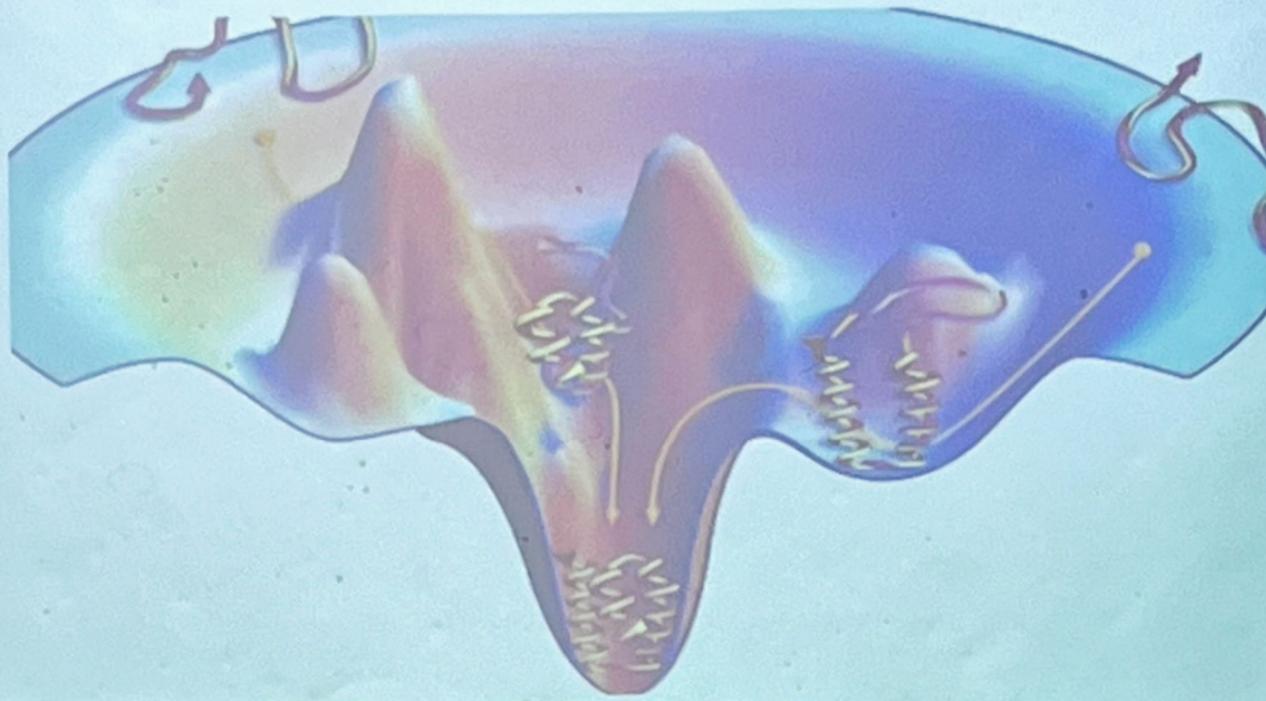
ROBERT ZWANZIG, ATTILA SEABO, AND BIRMAN BAGCHI*

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, Building 3, National Institutes of Health,
Bethesda, MD 20892

Contributed by Robert Zwanzig, October 7, 1991

How long does it take for a protein to fold up into its native structure? In a standard illustration of the Levinthal paradox, each bond connecting amino acids can have several (e.g., three) possible states, so that a protein of, say, 101 amino acids could exist in $3^{100} = 5 \times 10^{47}$ configurations. Even if the protein is able to sample new configurations at the rate of 10^{13} per second, or 3×10^{20} per year, it will take 10^{27} years to try them all. Levinthal concluded that random searches are not an effective way of finding the correct state of a folded protein. Nevertheless, proteins do fold, and in a time scale of seconds or less. This is the paradox.

Nice Summary video from Prof.Ken Dill (2012)



Active Researchers in Protein Folding

(Pioneers/Active Researchers) in Protein Folding



Ken A. Dill

Director of Laufer Center, Stony Brook University

Verified email at laufercenter.org

Statistical physics of protein

FOLLOW

Cited by

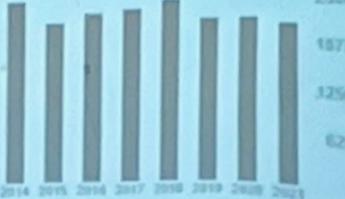
[VIEW ALL](#)

All
Since 2016

53744
13726

h-index
58

110-Index
206



TITLE

CryoFold: Determining protein structures and data-guided ensembles from cryo-EM density maps
M Shukla, G Inyang, C Gupta, D Sarkar, G Debussche, NJ Silcox,
Matter

CITED BY

YEAR

2021

MELD-accelerated molecular dynamics help determine amyloid fibril structures
U Bhattacharya, KA Dill
Communications Biology 4 (1), 1-11

2021

Nanoscale Catalyst Chemosis Can Drive the Assembly of Functional Pathways
C Kothiyal, L Aquazzano, K Dill
The Journal of Physical Chemistry B 125 (31), 6781-6795

2021

The protein folding problem: The role of theory
J Nussinov, GS Djinov, RM Ratan, KA Dill
Journal of Molecular Biology, 167126

3 2021

Public access

[VIEW ALL](#)

4 articles

[107 articles](#)

(Pioneers/Active Researchers) in Protein Folding



Christopher Dobson

University of Cambridge

Verified email at cam.ac.uk · Home page
protein science

FOLLOW

Cited by

VIEW ALL

All Since 2017

Citations	130815	45351
H-index	170	97
H0-index	821	542

TITLE

CITED BY

YEAR

N-Terminal Acetylation of α -Synuclein Slows down Its Aggregation Process and Alters the Morphology of the Resulting Aggregates

2022

R Bell, RJ Drush, M Castellana-Cruz, M Ossler, R Stasik, A Nimm, ...

Biochemistry 61 (17), 1743-1750

Amyloid Formation, Protein Homeostasis and Human Disease

2022

F Chiti, CM Dobson

Method of tracking a plurality of objects

2022

J-J Boulard, C-M Boulard, D Choula, T Koenigsmann, P Koenigsmann, M Koenigsmann



AlphaFold from Google Deepmind

[nature](#) > [articles](#) > article



Article | Open Access | Published: 15 July 2021

Highly accurate protein structure prediction with AlphaFold

John Jumper , Richard Evans, Alexander Pritzel, Tim Green, Michael Figurnov, Olaf Ronneberger,



- announcement and Copyright/Service Update: Update the reading file for the AlphaFold PR. 20 hours ago 81 commits
- update 20 hours ago
- update 20 hours ago
- update 20 days ago

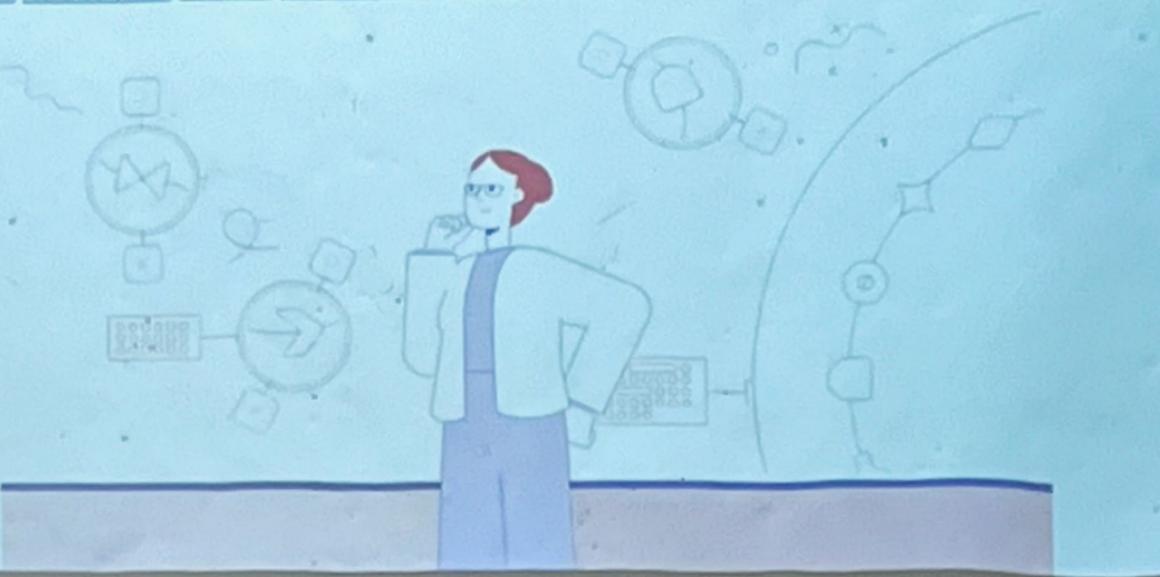
AlphaFold from Google Deepmind



Article | Open Access | Published: 15 July 2021

Highly accurate protein structure prediction with AlphaFold

John Jumper , Richard Evans, Alexander Pritzel, Tim Green, Michael Figurnov, Olaf Ronneberger,

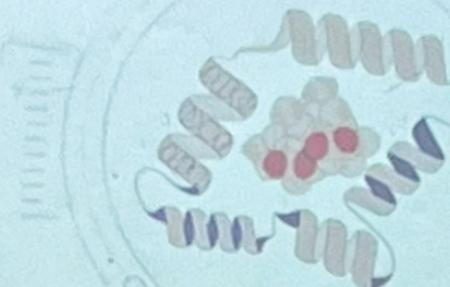


AlphaFold from Google Deepmind

Article | Open Access | Published: 15 July 2021

Highly accurate protein structure prediction with AlphaFold

[John Jumper](#) , [Richard Evans](#), [Alexander Pritzel](#), [Tim Green](#), [Michael Figurnov](#), [Dilat Ronneberger](#),



AlphaFold from Google Deepmind

Article | Open Access | Published: 15 July 2021



Highly accurate protein structure prediction with AlphaFold

John Jumper Richard Evans, Alexander Pritzel, Tim Green, Michael Equumov, Olaf Ronneberger,



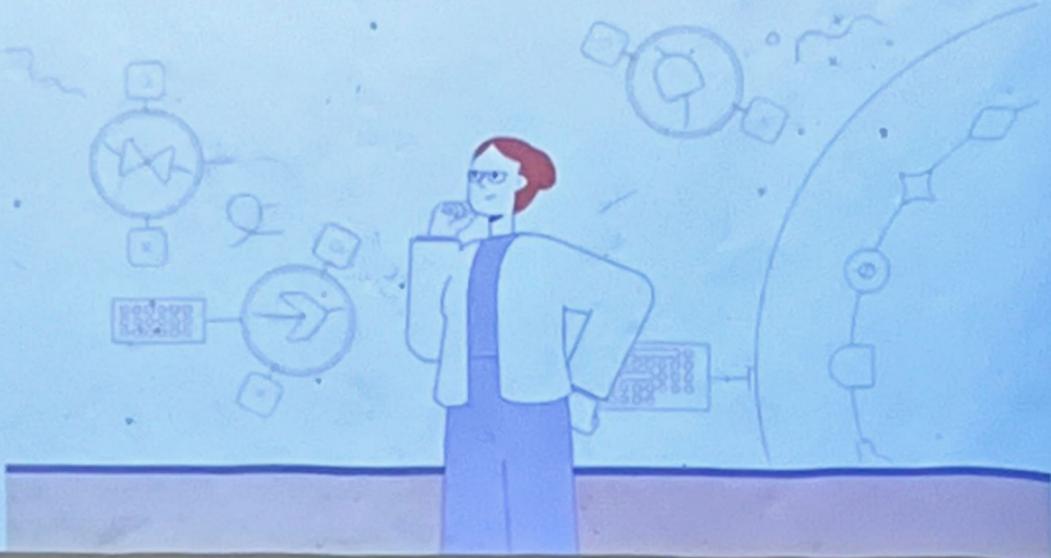
AlphaFold from Google Deepmind

Article | Open Access | Published: 15 July 2021



Highly accurate protein structure prediction with AlphaFold

[John Jumper](#) [Richard Evans](#), [Alexander Pritzel](#), [Tim Green](#), [Michael Figurnov](#), [Olaf Ronneberger](#),





THANKS