Formation of Biological Structures

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Semmelweis University

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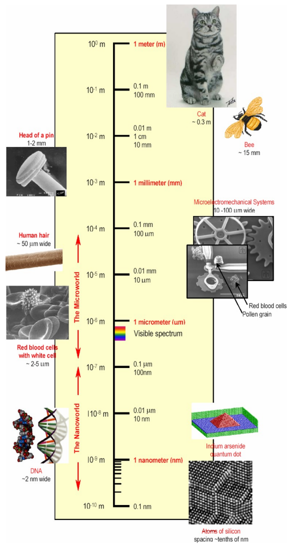
How Big Are They?



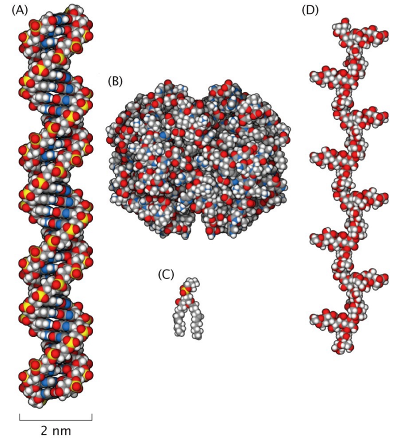


Figure 1.1 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

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"Plenty of Room at the Bottom"

" The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom. It is not an attempt to violate any laws; it is something, in principle, that can be done; but in practice, it has not been done because we are too big."

Richard Feynman, 1959

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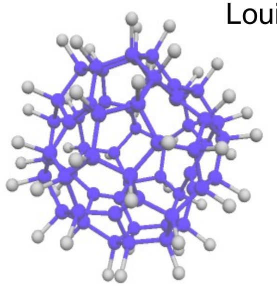
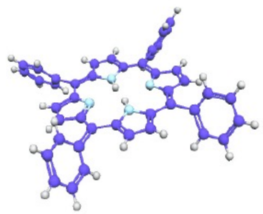
Wave – Particle Duality

Louis De Broglie: $\lambda = h/p$



fluorofullerene
$C_{60}F_{48}$
1632 Da



tetraphenylporphyrin $C_{44}H_{30}N_4$

L Hackermuller, S Uttenthaler, K Hornberger, E Reiger, B Brezger, A Zeilinger, M Arndt; Phys. Rev. Lett. 91 (2003) 90408

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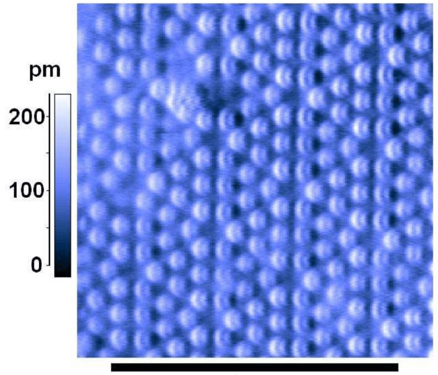
Wave – Particle Duality



Scanning Tunneling Microscope (STM) image

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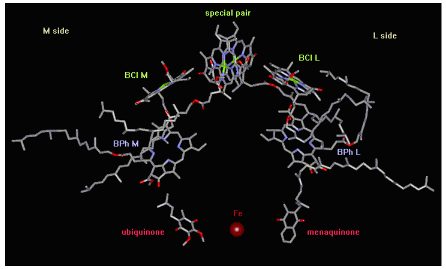
Structure – Function Relationship

From the molecular level to ecosystems, there is a strong relationship between structure and function of biological systems.

Hartmut Michel, Johann Deisenhofer, Robert Huber
1982 – 3D structure of the bacterial photosynthetic reaction center
1988 – Nobel prize



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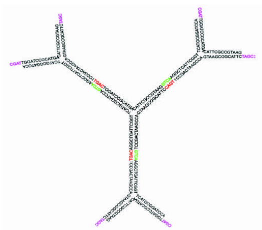
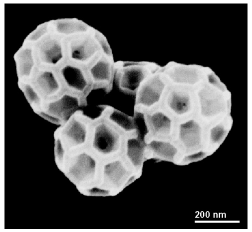
Self Assembly

Molecular recognition
(e.g. self assembly of DNA molecules into „balls").





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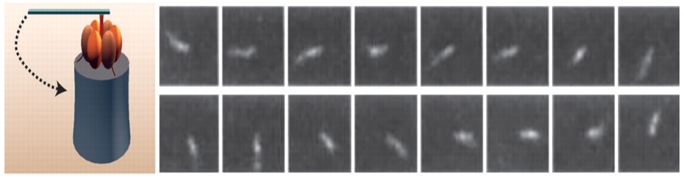
F_1-ATPase Driven Nickel Propeller



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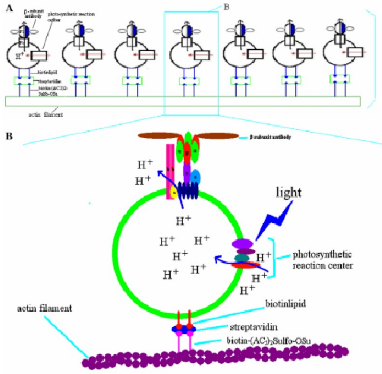
Light - Driven Swimming Structure



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Biopolymers

reaction	$t_{1/2}$ @ 25 °C	$t_{1/2}$ @ 100 °C	typical number of monomeric units in a polymer molecule	number of different monomers
DNA hydrolysis	140 000 years	22 years	$3 \cdot 10^9$ (human DNA)	4
RNA hydrolysis	4 years	9 days	few dozen (tRNA)	4
protein hydrolysis	400 years	5.5 weeks	few hundred	20

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Biopolymers

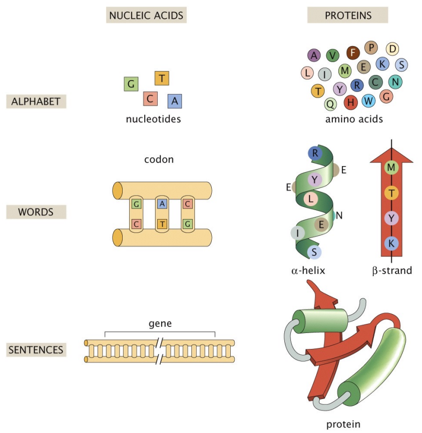

Figure 1.2 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

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Role of RNA in Living Systems

- messenger (mRNS)
- ribosomal (rRNS)
- transfer (tRNS)
- regulator
- enzyme (ribozyme)
- switch (riboswitch)
- virus gene RNS

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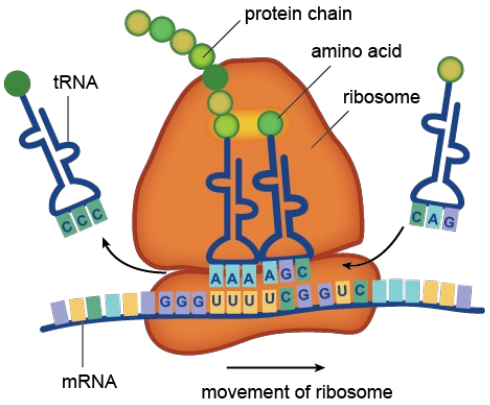
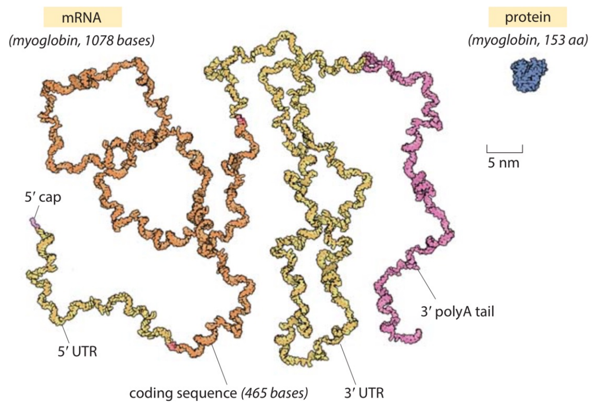
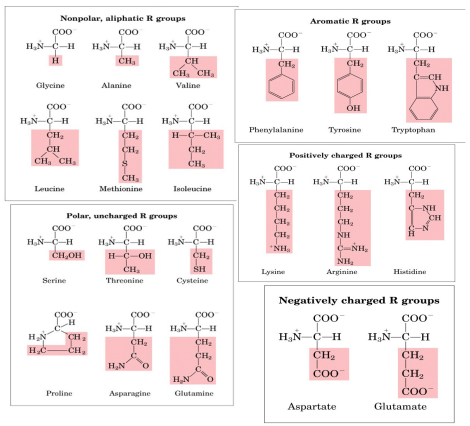
Ribosome Function



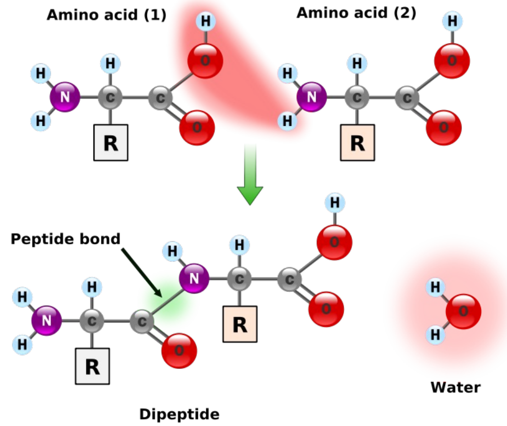
Relationship Between RNA Code and Protein Sequence



The Twenty Standard Amino Acids



The Peptide Bond



Role of Proteins in Living Systems

- chemical catalysis
- transport
- energy conversion and storage
- coordinated movement
- mechanical skeleton
- immune response
- molecular recognition
- passing information
- gene regulation
- growth and differentiation

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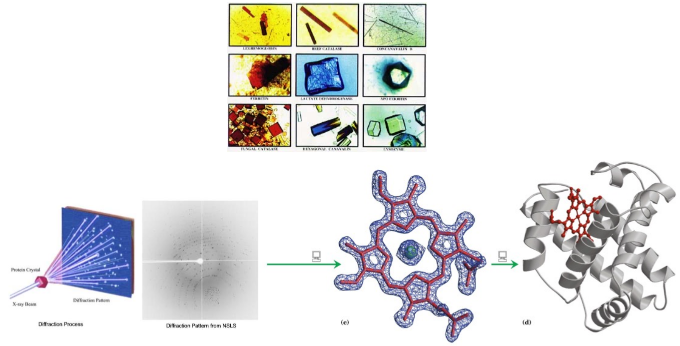
X-ray Crystallography



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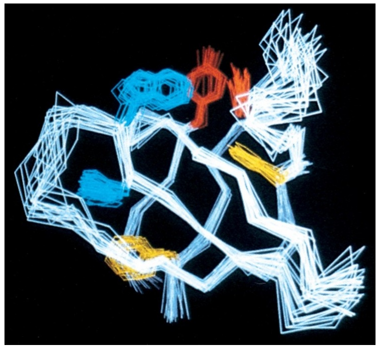
NMR Structure Determination



NMR structure of the 64 amino acid
SH3 domain of the Src protein

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Interactions Stabilizing the Native State

short range repulsion

Van der Waals interaction

electrostatic interaction

hydrogen bonding

hydrophobic interaction

disulfide bridge

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Short Range Repulsion

Due to the exchange (Pauli) interaction, at short distances there is a strong repulsion between electrons.

The potential energy of the repulsion increases quickly with decreasing distance (~ $1/r^{12}$).

Atoms can be considered hard spheres with a given radius (Van der Waals radius).

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Van der Waals Interaction

Occurs between any two atoms due to the interaction of induced dipole moments.

Dependence on the distance of the interaction energy: ~ $1/r^6$

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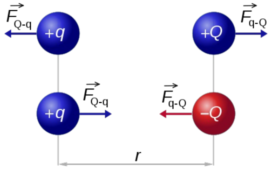
Electrostatic Interaction

Distance dependence of the interaction energy of the Coulomb force:

$$E = \frac{q \cdot Q}{4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon_r \cdot r}$$



The relative dielectric constant inside the protein is approx. 4, and 80 in water.

Salt bridges between ion pairs (Lys, Arg and Glu, Asp).

There is a large hydrate shell around charges in water.

Mobile ions can strongly shield charges.

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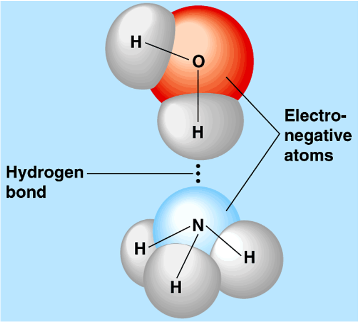
Hydrogen Bonding

Attraction force between a H atom of a more electronegative atom or group (hydrogen bond donor) and another atom bearing a lone pair of electrons (hydrogen bond acceptor).



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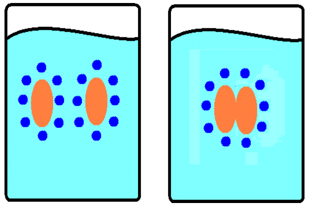
Hydrophobic Interaction

observed tendency of nonpolar surfaces to adhere in an aqueous solution and exclude water molecule

entropic effect originating from the disruption of hydrogen bonds of liquid water by the nonpolar solute



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Disulfide Bridge

stabilizes the native structure
decreases the conformational entropy of the unfolded protein:

$$\Delta S = -2.1\ J/K - 1.5 \cdot R \cdot \ln n$$

n is the number of AAs between the two bonded AAs.

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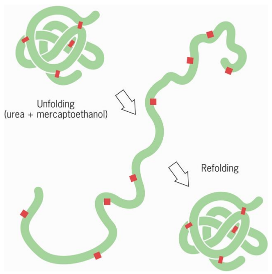
Anfinsen's Dogma



Refolding of Ribonuclease A

Christian B. Anfinsen

The information of the 3D protein structure is encoded in the 1 D AA sequence.

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Importance of the Protein Folding Problem

One of the most important questions of molecular biophysics.

We sequence genomes, we build databases, but we can't predict protein structure and function based on the genetic information.

There are roughly two dozen conformational diseases:

Misfolded proteins and deposition of amyloid plaques was observed in various diseases (pl. Creutzfeld-Jakob disease, Alzheimer disease, Parkinson disease).

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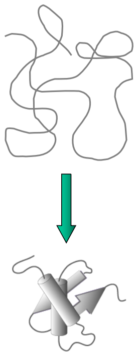
In vitro and *in vivo* Folding

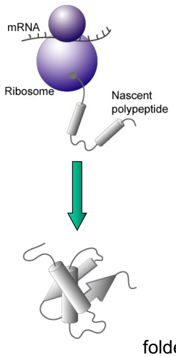
in vitro



unfolded protein

folded protein

in vivo



folded protein

differences:

- entire sequence / co-translational folding
- crowded intracellular environment

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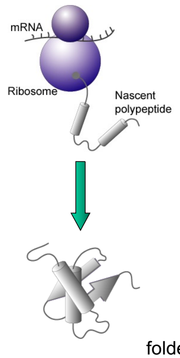
Co-Translational Folding

The N terminal of the nascent polypeptide chain starts to fold before completion of the translation.

20-30 AAs of the C terminal are protected within the ribosome.

in vivo



folded protein

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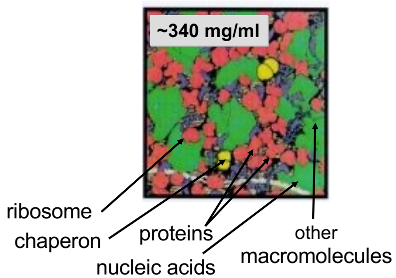
Molecular Crowding

in vitro

***E. coli* citosol**



In vitro experiments
- lack of binding partner molecules
- lack of posttranslational modifications
- very different physico-chemical environment than in a cell

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Effect of Molecular Crowding

Molecular crowding:
a large fraction of the volume of the cytoplasm is filled by other molecules than water.

- dissociation constants decrease
- speed of protein-protein association increases
- association of fully or partially denatured proteins speeds up

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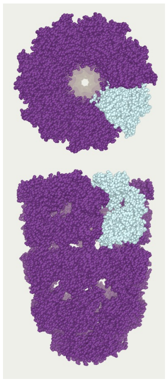
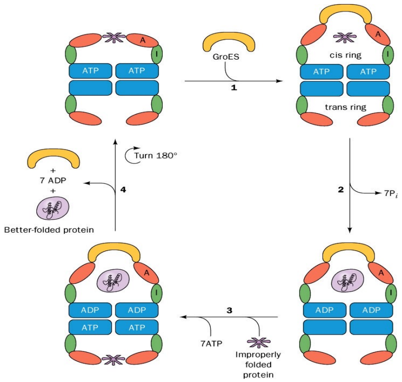
GroEL/ES Chaperon Cycle





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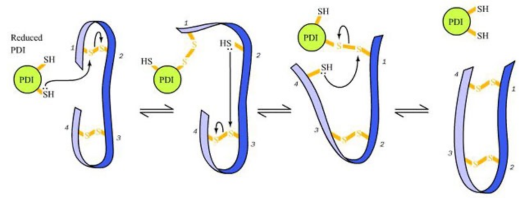
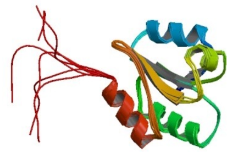
Protein Disulfide Isomerase Function



structure of the human protein disulfide isomerase



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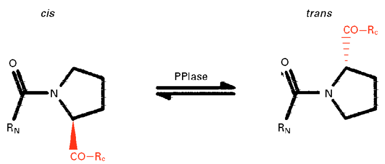
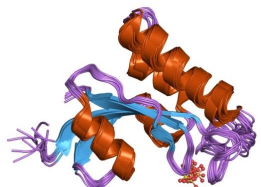
Proline Cis/Trans Isomerase

Due to the activation barrier between the cis and trans prolines, the presence of cis prolines in the native structure:
- speeds up early folding steps
- slows down the final formation of the native structure.

PPIase (peptidyl-prolyl isomerase)





PPIase structure

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Fate of the Protein in Eukaryotic Cells

cytosol .... protein synthesis and folding,
extracellular volume .... export of folded protein
mitochondrion .... limited protein synthesis
chloroplast .... limited protein synthesis
endoplasmic reticulum .... import of unfolded protein
peroxisome .... import of folded protein
nucleus .... import of folded protein
lysosome .... import of unfolded protein

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Levinthal's Paradox - Calculation

Cyrus Levinthal

Consider a protein of 151 AAs. Assume all the 150 bonds connecting them have only two possible conformations. Assume that a reorientation of the bonds happens in 10^{-13}s.

A random search through the phase space would last:

$2^{150} * 10^{-13}$s= $4.6 \cdot 10^{24}$years.

Age of Earth: $4.6 \cdot 10^9$ years
Age of the Universe: $13.7 \cdot 10^9$ years
Proteins typically fold on the ms to s timescale.

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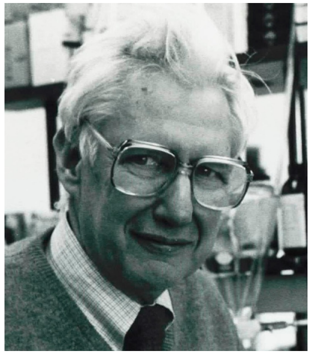
Levinthal's Paradox - Conclusion

The phase space of a protein is way too big to find the native structure by random search.

Cyrus Levinthal
1922 - 1990



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Kinetic Pathways and Intermediate States

All proteins have a most stable conformation.

The protein can find this conformation by following a kinetic pathway and adopting specific intermediate states.

In vivo, trapping of the protein in intermediate states is prevented by protein disulfide isomerases, peptidyl prolyl isomerases and chaperones.

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Energy Landscape Models

At constant pressure and temperature every thermodynamic system tends to minimize Free enthalpy (Gibbs free energy).

A free enthalpy (Gibbs free energy) value is associated to every conformation of the protein.

The protein does not search through the entire phasespace, but starts to "flow" towards lower free enthalpies.

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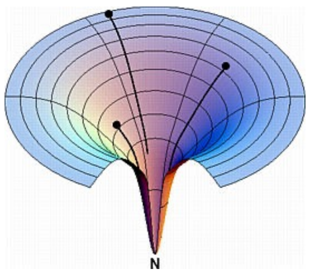
Smooth Funnel



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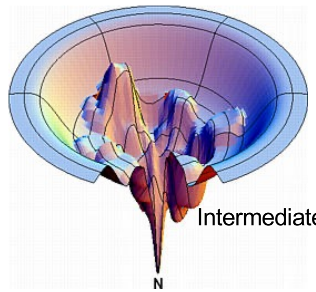
Rugged Funnel



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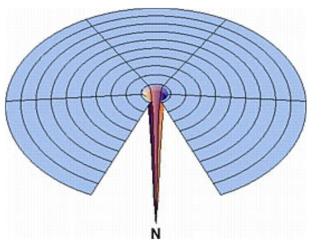
Energy Landscape View of Levinthal's Paradox



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Comparison of the Two Folding Models

Pathways	Landscape
Given pathways	Energy landscape
Well distinguished intermediates	Multitude of intermediates
Consecutive steps	Parallel folding routes
Classical chemical kinetics applied to protein folding	Statistical physics developed to understand spin glasses

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