Conformation, allostery

Petr Louša

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Outline

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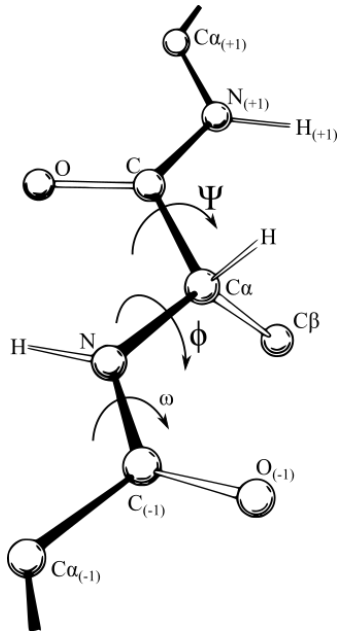
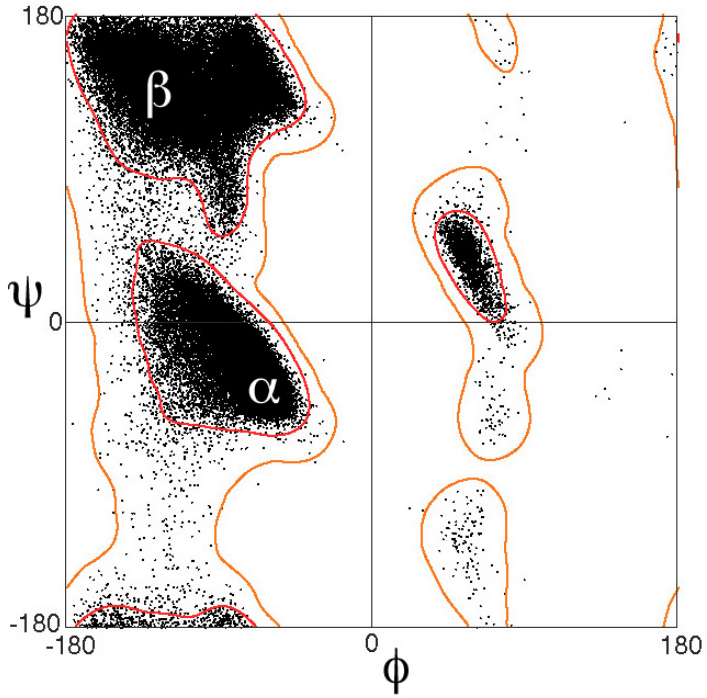
Conformation of proteins

constitution topology of molecule – *isopropanol, n-propanol*

configuration bond arrangement – *cis/trans, R/S*

conformation 3D structure – rotation around single bonds





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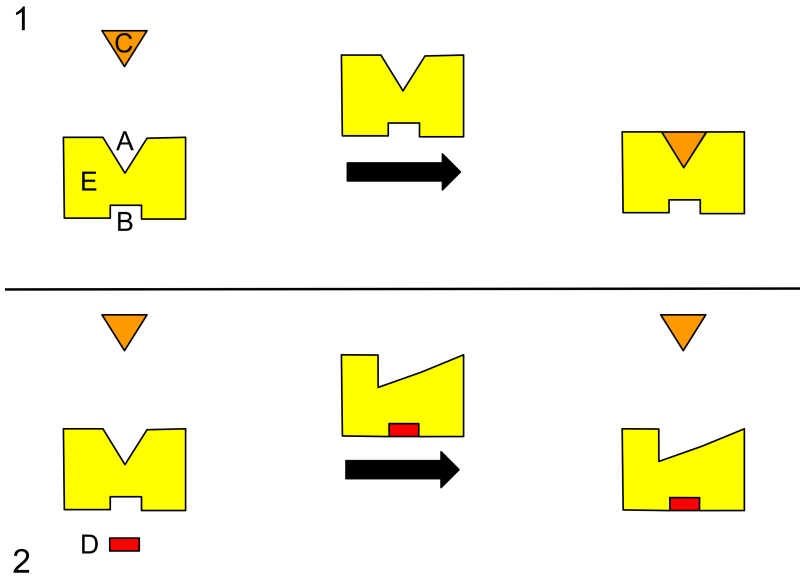
Allostery

- Change of conformation after ligand binding.
- $\Rightarrow$ Change of K_{d} for other ligands.
- Typical example hemoglobin.
- Often multimeric proteins with more equivalent active sites.



Types of allostery

- By type of K_d change:
  - positive – K_d decreases – next substrate binds more easily – **activation**
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- Non-regulatory allostery
  - protein needs other component for its function, but is not regulated by it – e.g. ions, vitamins

Hill equation I

- Quantification of cooperativity

$$\begin{aligned} \mathrm{R} + \mathrm{L} &\rightleftharpoons \mathrm{RL} & K_\mathrm{d}^{(1)} \\ \mathrm{RL} + \mathrm{L} &\rightleftharpoons \mathrm{RL}_2 & K_\mathrm{d}^{(2)} \\ &\vdots & \\ \mathrm{RL}_{n-1} + \mathrm{L} &\rightleftharpoons \mathrm{RL}_n & K_\mathrm{d}^{(n)} \end{aligned}$$

- Constants $K_\mathrm{d}^{(i)}$ differ – system is cooperative.

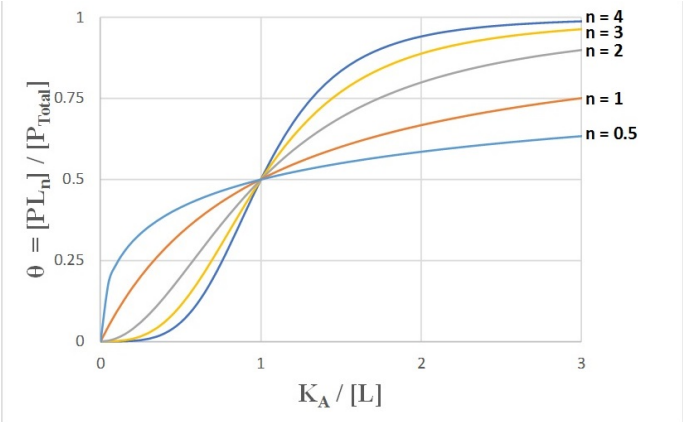
Hill equation II

- Assumption – $[\mathrm{RL}]_i = 0$
- Only one equation remains $\Rightarrow$ Hill analysis.

$$\mathrm{R} + n\mathrm{L} \rightleftharpoons \mathrm{RL}_n \qquad \hat{K}_\mathrm{d} \tag{1}$$

$$\hat{K}_\mathrm{d} = \frac{[\mathrm{R}][\mathrm{L}]^n}{[\mathrm{RL}]_n} \tag{2}$$

$$\hat{K}_\mathrm{d} = (K_\mathrm{d})^n \tag{3}$$

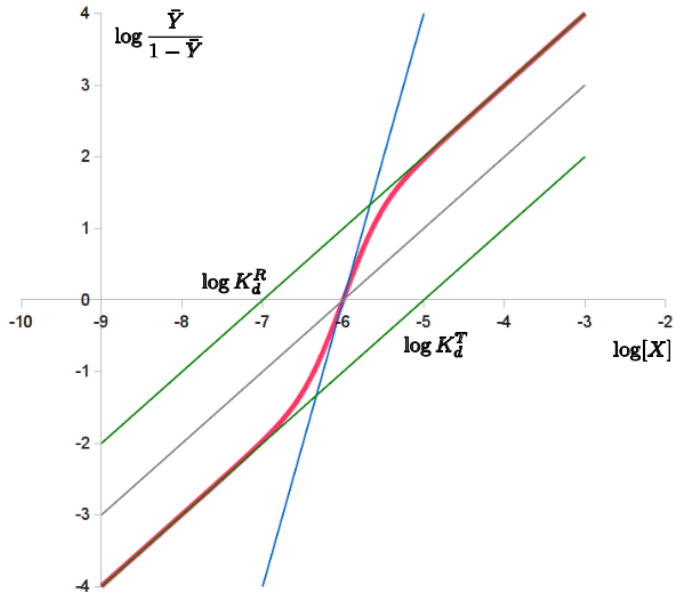


Analysis of experiment

- Measure fraction of bound receptors and linearize:

$$y = \frac{[\mathrm{RL}]_n}{[\mathrm{R_{tot}}]} \tag{4}$$

$$\log\left(\frac{y}{1-y}\right) = n\log[\mathrm{L}] - \log\hat{K}_\mathrm{d} \tag{5}$$



Microskopic models I – MWC model I

- Monod, Wyman, Changeux
- Two-state receptor system – can “switch” only in free form.
- Form A is dominant in free form.

$$[\mathrm{A}] \gg [\mathrm{B}]$$

- Affinity to B is significantly larger.
- Assumes change of protein structure after binding – “locking” in B state.

Microskopic models I – MWC model II

- Assumes constant microscopic K_d.

$$\mathrm{A} \rightleftharpoons \mathrm{B}$$

$$\mathrm{A} + \mathrm{L} \rightleftharpoons \mathrm{AL} \qquad \mathrm{B} + \mathrm{L} \rightleftharpoons \mathrm{BL}$$

$$\mathrm{AL} + \mathrm{L} \rightleftharpoons \mathrm{AL_2} \qquad \mathrm{BL} + \mathrm{L} \rightleftharpoons \mathrm{BL_2}$$

- Ligand binds preferentially to B and shifts the equilibrium of free forms.

- Problem?

Microskopic models II – KNF model

- Koshland, Nemethy, Filmer
- Generalization of MWC model.
- Assumes different B constants for sequential equilibria.
- K_d constants are free parameters for fitting.
- Each ligand binding changes the binding site for other ligands.

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- Disadvantage: too many free parameters (K_d constants) – better for experiment fitting than for predictions.

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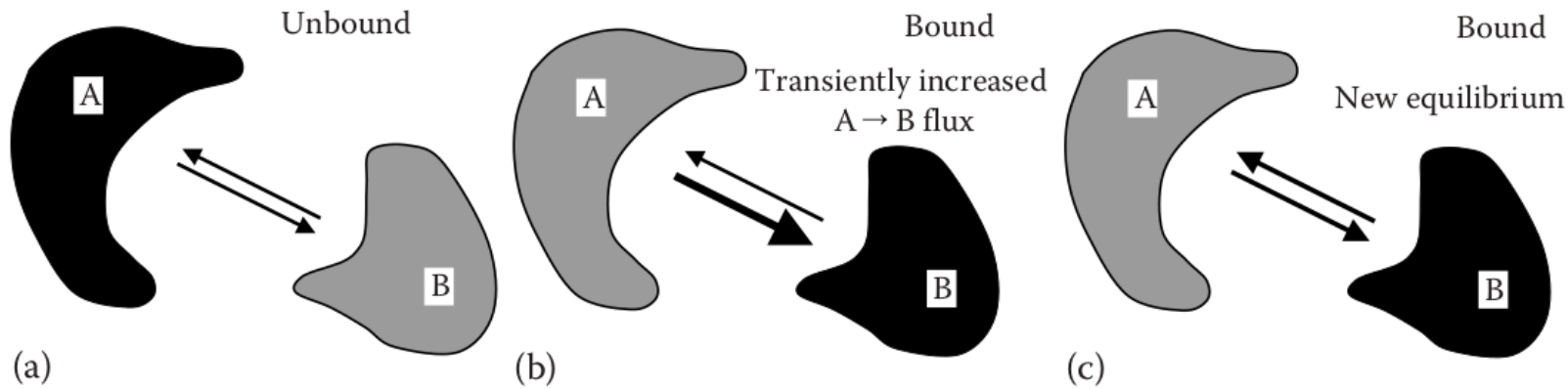
Kinetic view to allostery

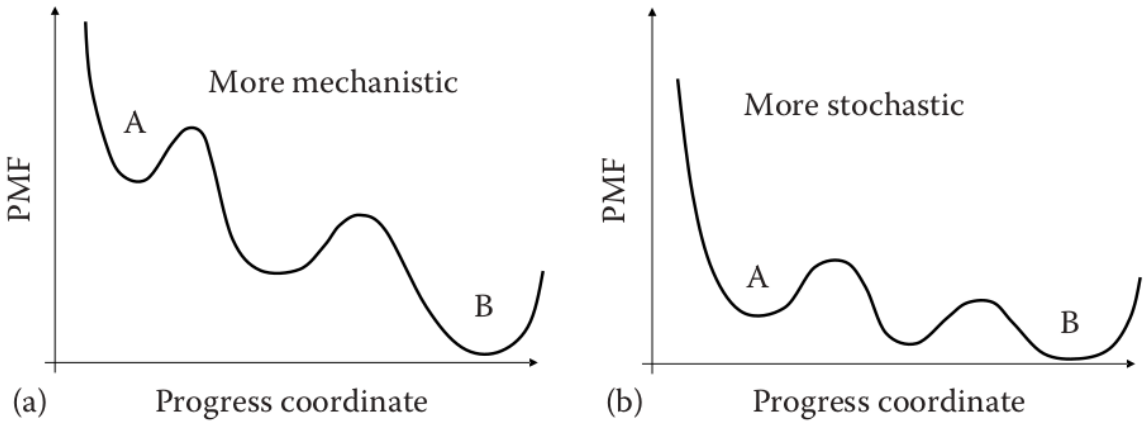
- Ligand binding changes equilibrium between two “states” (MWC model).
- After binding, structural ensemble changes – B forms dominate.
- Caused by changes of kinetic parameters of the transition.



Processivity vs. Stochasticity

Processivity

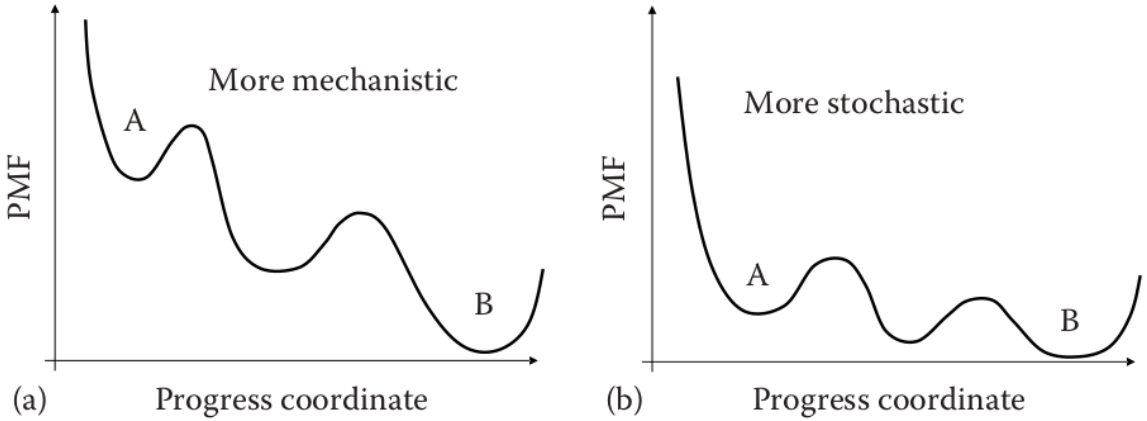
- Ability to run irreversibly in one direction.
- E.g. ATP synthase, motor proteins, polymerases
- Free energy decreases in larger jumps – irreversibility.
- Source of energy needed – ATP, GTP, proton gradient.
- ATP – ca $20k_{\mathrm{B}}T$ of energy.



Processivita vs. Stochasticity

Stochasticity

- Many reversible steps.
- E.g. glycolysis.
- Reversibility – $\Delta G \approx k_{\mathrm{B}}T$
- Even many reversible steps can lead to irreversible event – ΔG adds up.



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1. What structure does given sequence of amino acids take?

2. How does the protein fold?

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   - Homology modeling
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     - MD of stretched chain
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     - Evolution covariation – requires hundreds of homologous *sequences*
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2. How does the protein fold?
   - Folding process
   - Kinetics
   - Transit states
   - Intermediates

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  - Often capable of multiple de- and renaturation.
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- Only with “helpers” – chaperons.
  - Hydrophobic boxes.
  - Proteins can search through the configuration space more easily.

Folding as conformation change

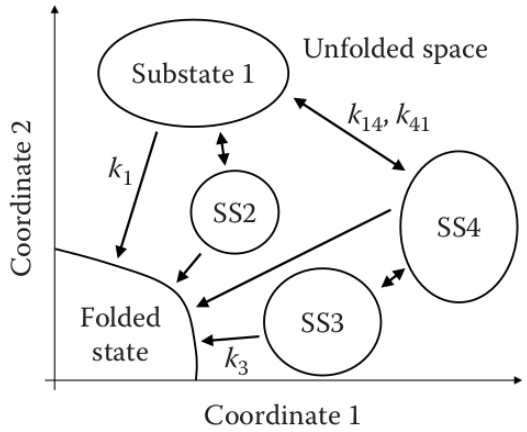
- Folding follows the same physics as other structural changes.

Differences:

- Large ranges of equilibrium and nonequilibrium conditions.
- De-/renaturation can go very slowly and reversibly or “immediately”.
- “Unfolded state” is **not** a state.
- Many different substates – difficult to characterize.
- Differ also in the denaturation process – temperature, pH, chemical agents,...

Overall folding rate

- Unfolded protein can have multiple substates.
- Only some allow transition to folded state.
- Depends on particular rate constants.
- Depends on substate populations.
- Population can differ based on the denaturation process.



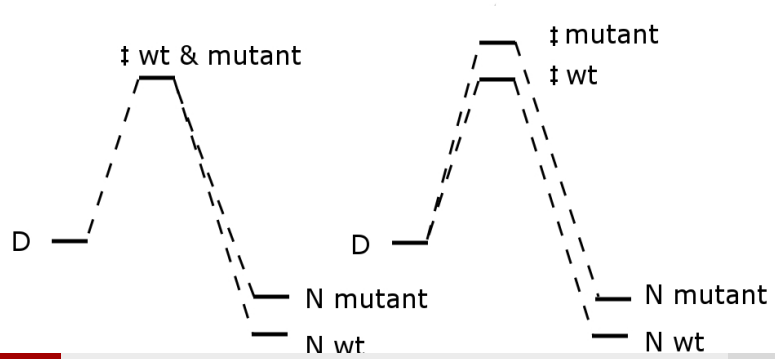
Φ values analysis

- Alan Fersht
- Influence of individual residues on folding and transient states.
- Compare ΔG profiles for wild type and mutant.

$$\Delta\Delta G_{ij} = \Delta G_{ij}(\text{mut}) - \Delta G_{ij}(\text{wt}) \tag{6}$$

$$\Phi_{\mathrm{F}} = \frac{\Delta\Delta G_{\mathrm{D}\ddagger}}{\Delta\Delta G_{\mathrm{DN}}} \tag{7}$$

- $\Phi_{\mathrm{F}} = 0$ residuum is unfolded in transient state.
- $\Phi_{\mathrm{F}} = 1$ residuum is folded in transient state.



Conformation, allostery – exercise

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Hill equation

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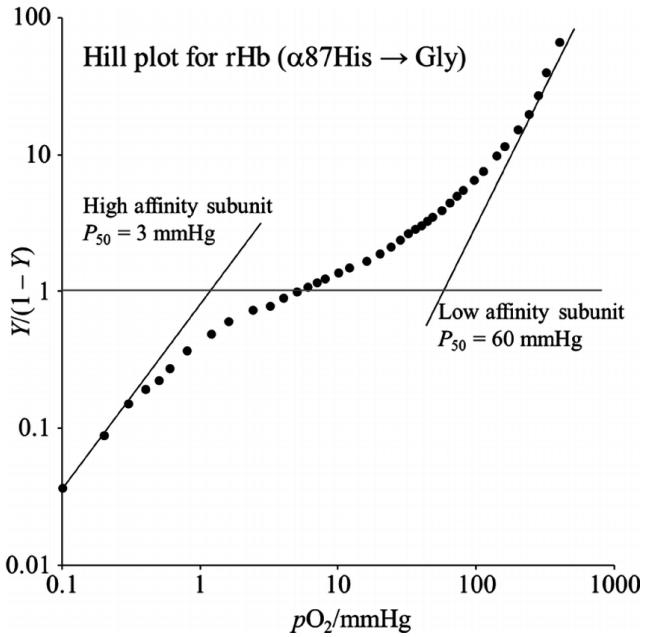

Free energy differences

Assume free energy differences between states A and B of:

1. $1\ k_{\mathrm{B}}T$
2. $5\ k_{\mathrm{B}}T$
3. $20\ k_{\mathrm{B}}T$

Calculate ratio of forward and backward reaction. Think, whether it is processive or stochastic process.

Free energy differences – solution

$$\Delta G = -RT \ln K \tag{8}$$

$$\ln K = \frac{\Delta G}{RT} \tag{9}$$

$$K = \frac{k_{\text{on}}}{k_{\text{off}}} = \exp \frac{\Delta G}{RT} \tag{10}$$

1. $1\ k_{\text{B}}T \Rightarrow K = 2.7$
2. $5\ k_{\text{B}}T \Rightarrow K = 150$
3. $20\ k_{\text{B}}T \Rightarrow K = 4.85 \cdot 10^{8}$

References

- Zuckerman, Daniel M. *Statistical Physics of Biomolecules. An Introduction*
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- Wikipedia