Dynamical Analysis of Networks: How to Identify Important Nodes with Applications to Protein Engineering

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DIMACS/CCICADA Workshop
on Stochastic Networks: Reliability, Resiliency, and Optimization

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- Introduction to proteins and protein modeling
- Mathematical framework for network modeling
- Luciferase bioluminescence

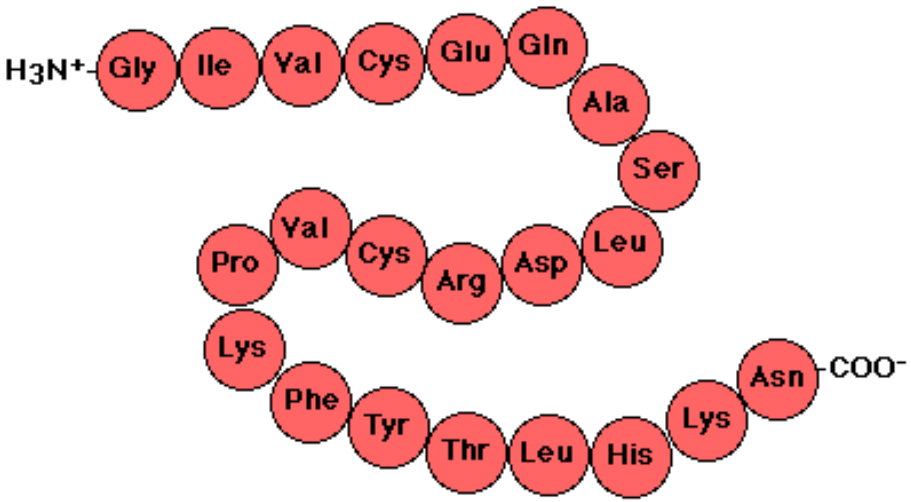
Mathematical challenge in biology: a lesson in complexity

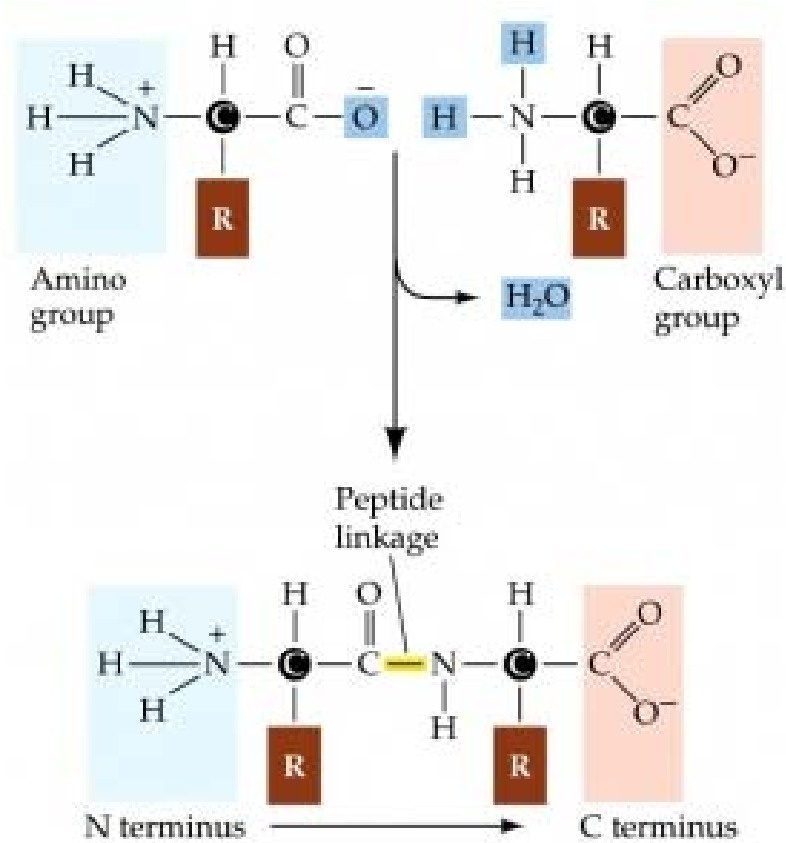
- High dimensionality
- Nonlinearity
- Stochasticity

Introduction to proteins

Protein sequence

- Sequence: the order of amino acids (20 amino acids)



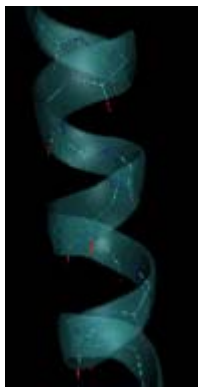


- Mutation: change in sequence

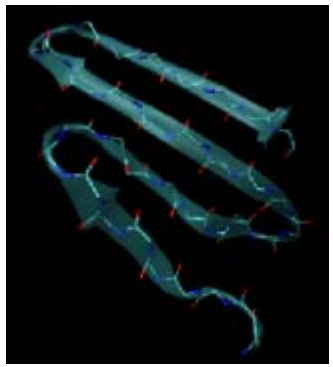
Protein structure

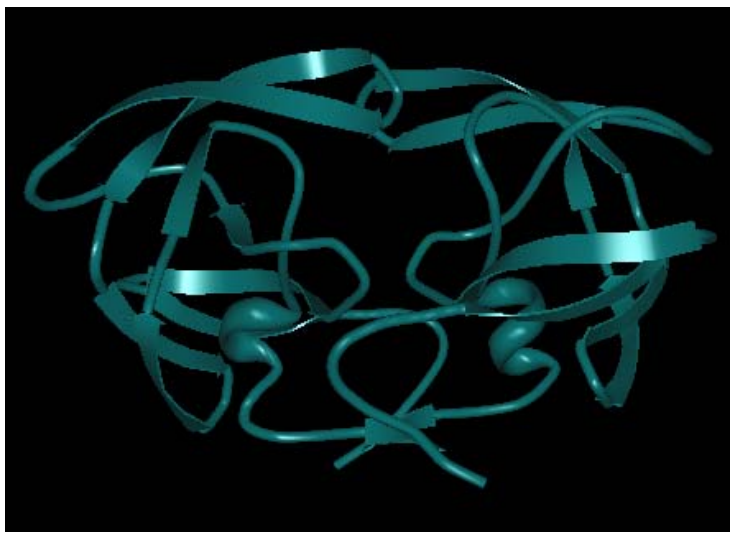
- Secondary structure

α helix

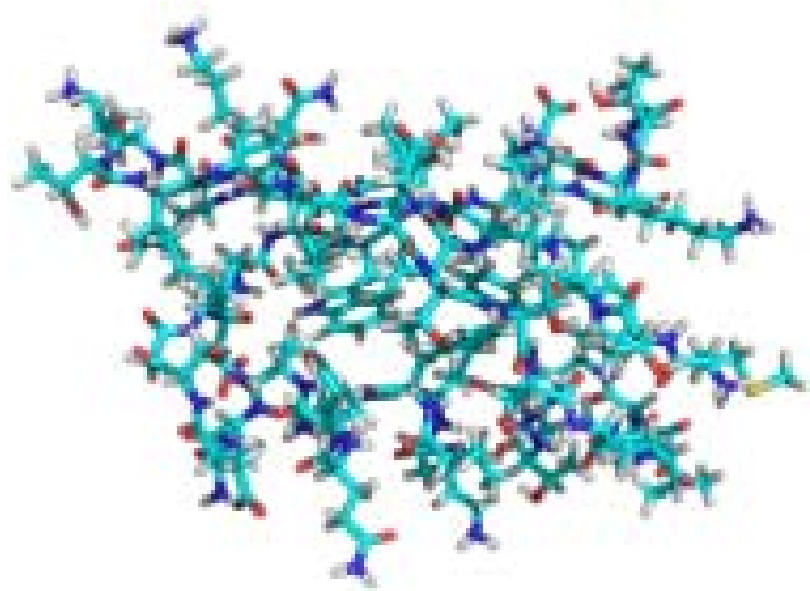


β sheet



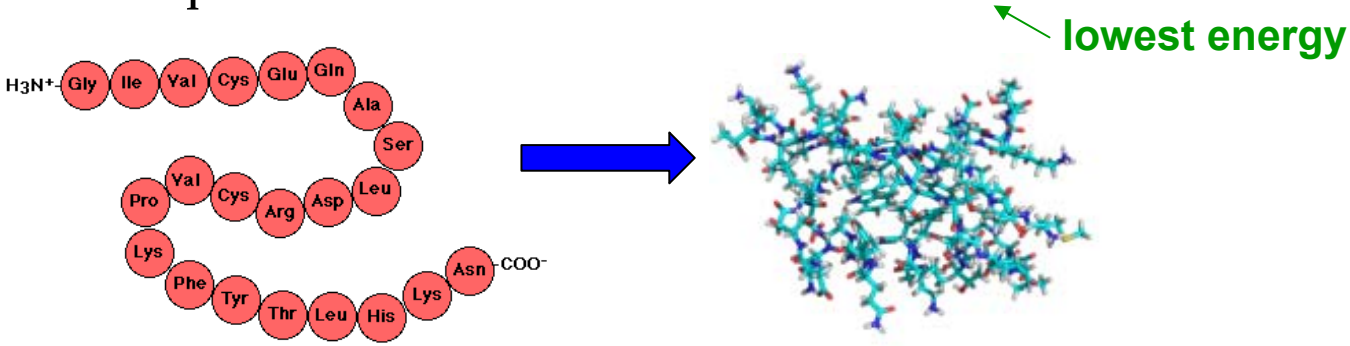


- Tertiary structure



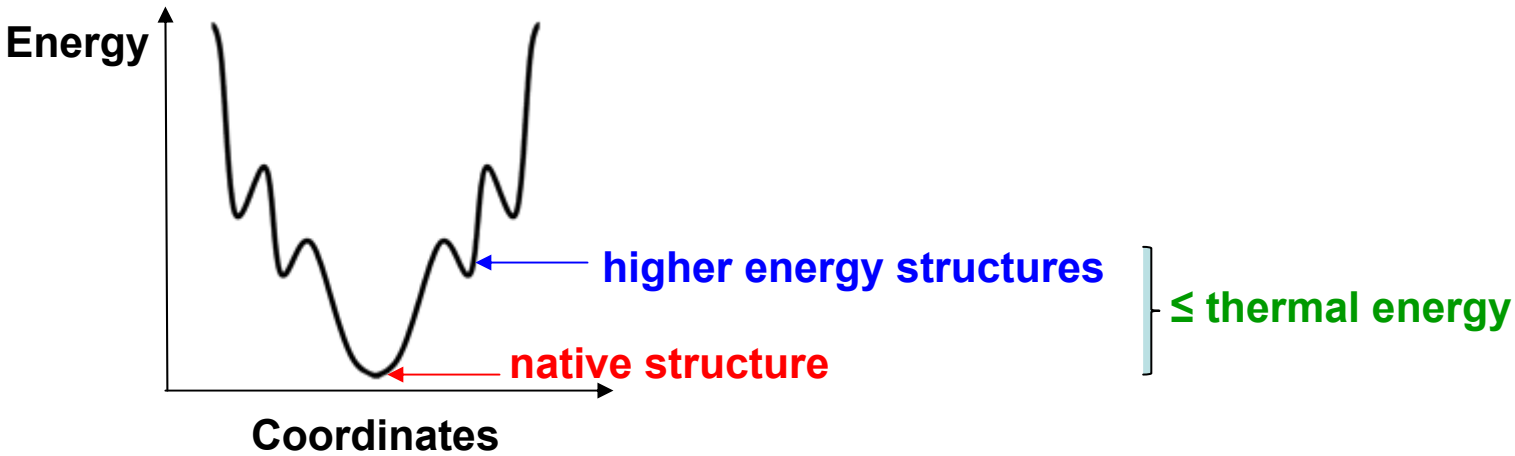
Protein folding: from sequence to structure

- Protein sequence determines native structure



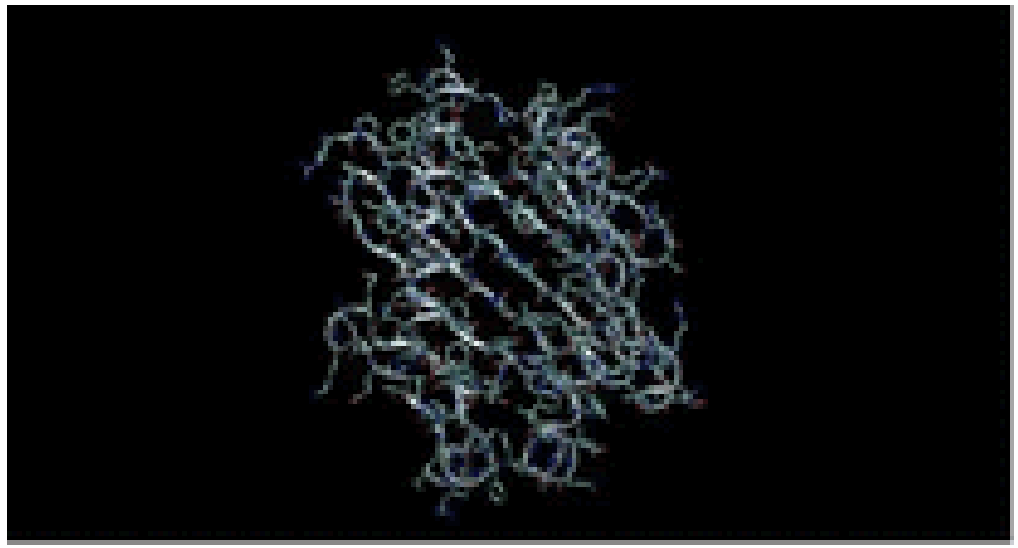
- Computational approach:

  modeling the atomic interactions and sampling different structures



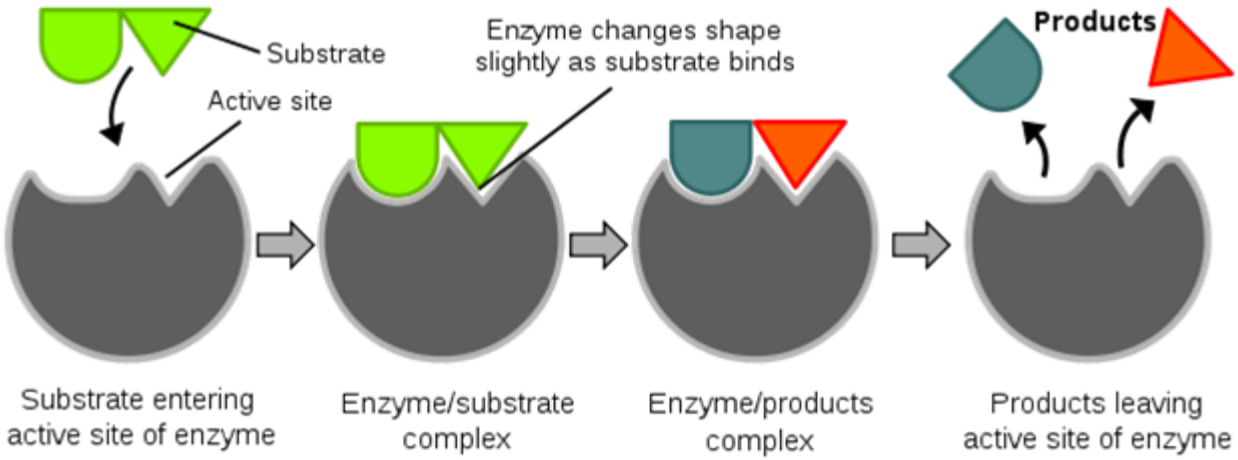
Protein dynamics

- Proteins occupy an ensemble of conformations at room temperature



Protein function

- Proteins are the most functionally diverse molecules in living organisms
- Proteins function by binding to other molecules (ligands, proteins).
- Enzyme proteins catalyzes the chemical reactions by binding to reactant (substrate).



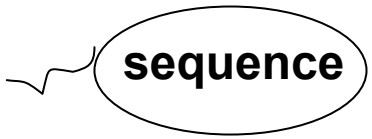
Active site crucial to enzymatic activity

- The major targets of prescription drugs are proteins.

Biological question: protein sequence ⇔? function

- change in sequence → what's the change in function?
- how to change sequence ? ← desirable function

♦ **Experimental approach: mutagenesis experiments**

Mutation 

Replace one residue type by another

limits: lack of details and adequate sampling

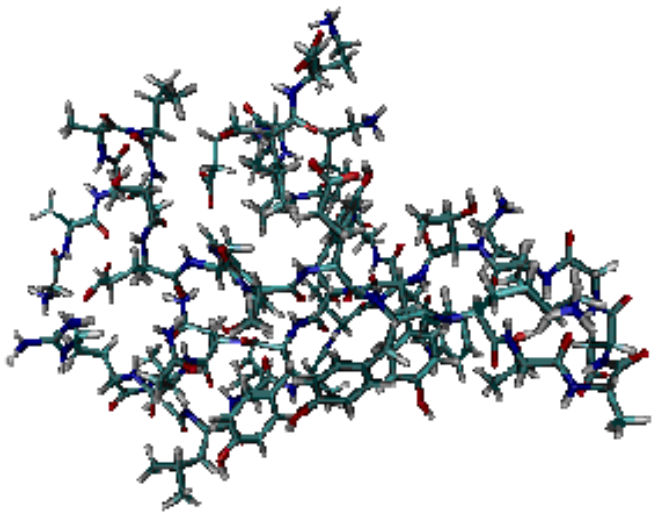
Structure modeling of protein

energy function

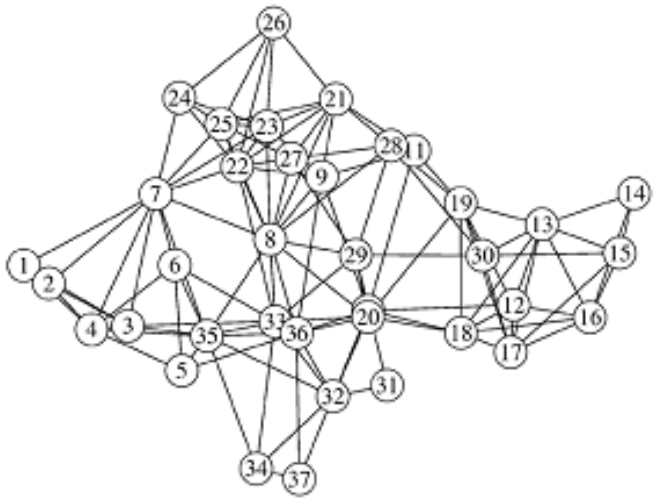
Structure → Topology → ergy or dynam

↓

Function



Atomistic model



Elastic network model

Structure based modeling of protein: bottom-top approach

- **Protein as a system of interacting components**

  atoms or residues?

- **Protein modeled by classical mechanics**

$$m_i a_i = \frac{-\partial V}{\partial r_i}$$

- **Modeling starts with structure, generates information about dynamics and energetics.**

  ↓ structural details of a biological process

  ↓ quantitative measure of molecular interactions

Use protein modeling to address:

protein sequence ↔ function

- change in sequence → what's the change in function?

Answer: protein function is computed as a chemical or physical property (based on energetics)

- how to change sequence ? ← desirable function

Use protein modeling to address:

protein sequence $\xrightarrow{?}$ function

- change in sequence $\rightarrow$ what's the change in function?

Answer: protein function is computed as a chemical or physical property (based on energetics)

- ~~how to change sequence $\leftarrow$ desirable function?~~

how to identify important residues?

Answer: Important residues interact strongly with protein's functional sites.

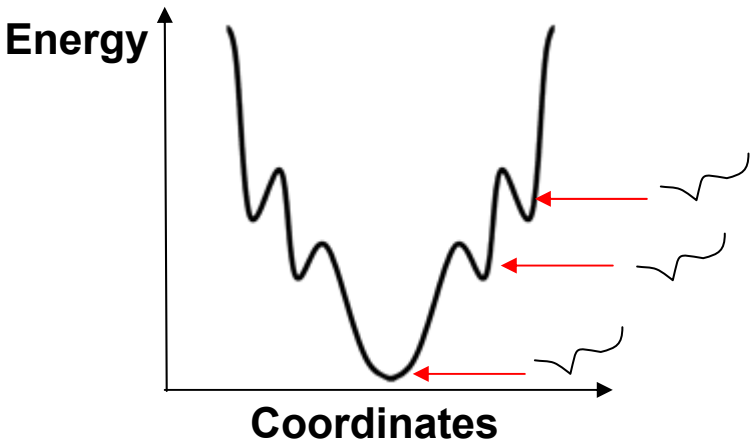
$\downarrow$

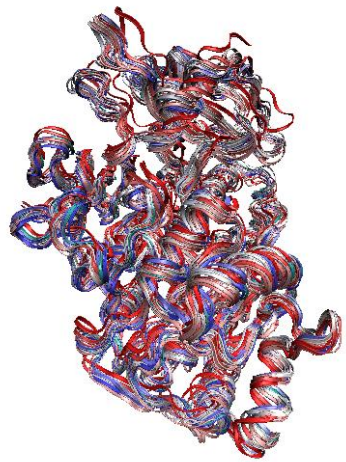
dynamic correlations

Structure vs dynamics

- For a given sequence
  - Protein native structure ↔ energy minimum
  - Protein dynamics ↔ shape of energy function

- Mutation effects may affect the shape of energy function



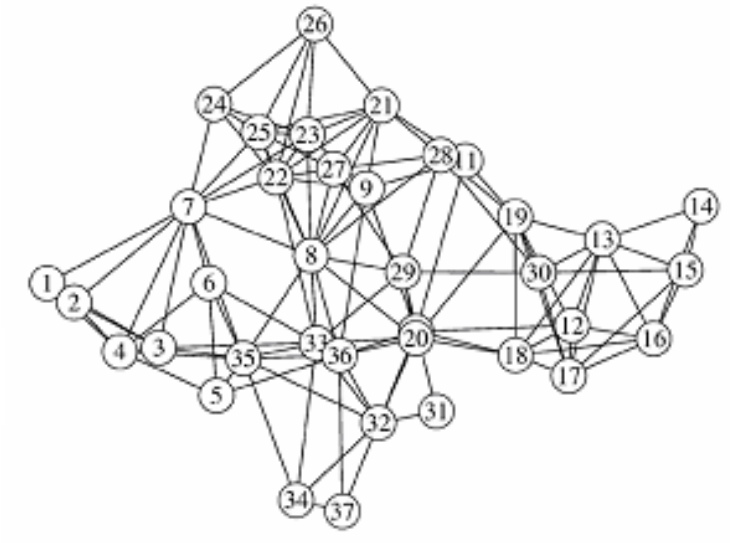


Protein dynamics

- Computation of protein dynamics
  - How to sample the protein energy space efficiently?

Elastic network modeling of proteins

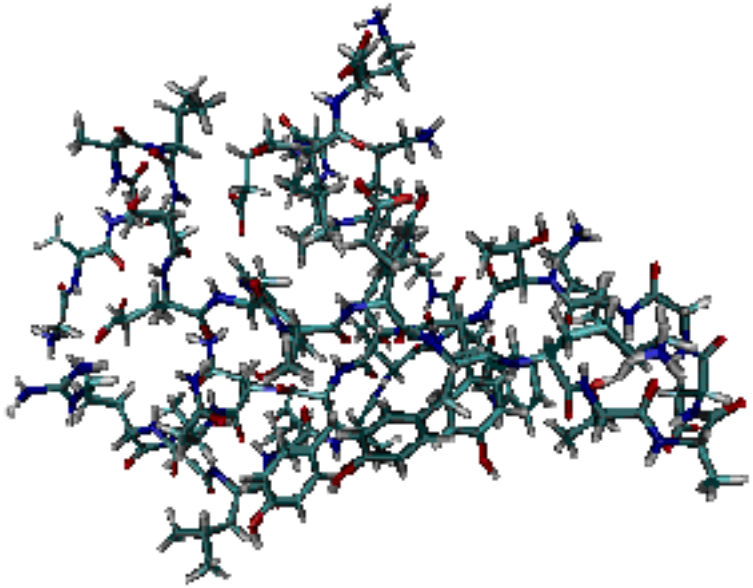
Elastic network model



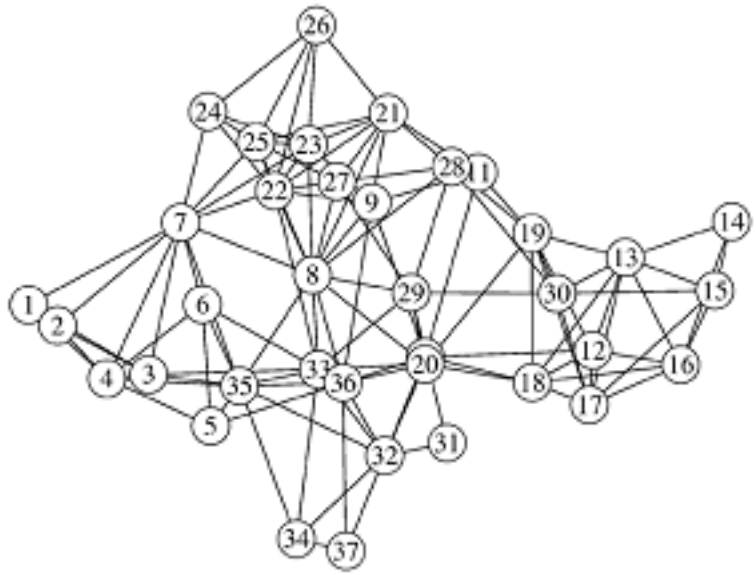
- **Starts with protein crystal structure**
- **Each amino acid is replaced by node (Cα atom)**
- **Any pair within a cutoff distance is governed by**

$$V = \frac{1}{2} C (d_{ij} - d_{ij}^{0})^2$$

Atomistic model vs elastic network model



Atomistic model



Elastic network model

- **Elastic network model is**
  - **computationally simple**
  - **energetic inaccurate**

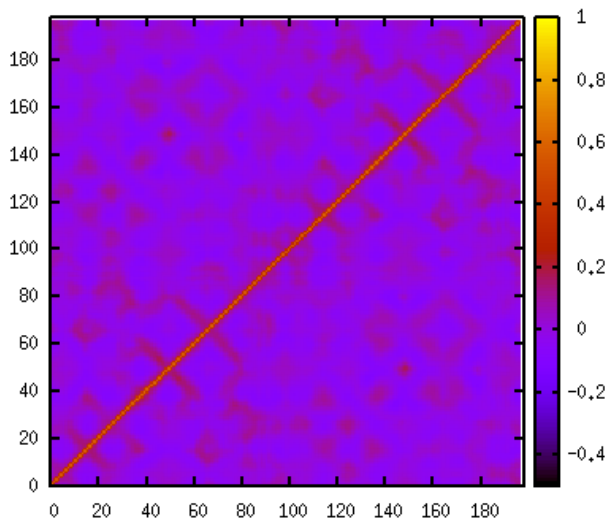
Dynamic measures

- Fluctuation and covariance $R_{ij} = < (R_i - < R_i >)(R_j - < R_j >) >$

- Correlation $C_{ij} = \dfrac{< R_{ij} >}{[< R_{ii} >< R_{jj} >]^{1/2}}$

- Correlation matrix

$$\begin{bmatrix} C_{11} & C_{12} & C_{13} & 6 \\ C_{21} & C_{22} & C_{23} & 6 \\ C_{31} & C_{32} & C_{33} & 6 \\ 7 & 7 & 7 & 9 \end{bmatrix}$$



ENM: protein dynamics in a closed form

$$R_{ij} = < (R_i - < R_i >)(R_j - < R_j >) > = (\frac{1}{Z}) \int (R_{ij})\, e^{-E/kBT}\, d\Delta R$$
$$= \frac{k_B T}{C} [\mathrm{H}^{-1}]_{ij}$$

- **Hessian matrix H**

$$\begin{bmatrix} \mathrm{H}_{11} & 6 & \mathrm{H}_{1N} \\ 7 & 9 & 7 \\ \mathrm{H}_{N1} & 6 & \mathrm{H}_{NN} \end{bmatrix}$$

$$\mathrm{H}_{ij} = \begin{bmatrix} \frac{\partial^2 E}{\partial x_i \partial x_j} & \frac{\partial^2 E}{\partial x_i \partial y_j} & \frac{\partial^2 E}{\partial x_i \partial z_j} \\ \frac{\partial^2 E}{\partial y_i \partial x_j} & \frac{\partial^2 E}{\partial y_i \partial y_j} & \frac{\partial^2 E}{\partial y_i \partial z_j} \\ \frac{\partial^2 E}{\partial z_i \partial x_j} & \frac{\partial^2 E}{\partial z_i \partial y_j} & \frac{\partial^2 E}{\partial z_i \partial z_j} \end{bmatrix}$$

From elastic network model $\frac{\partial^2 E}{\partial x_i \partial y_j} = \frac{-c(x_i - x_j)(y_i - y_j)}{d_{ij}^2}$

- **Normal mode analysis** $[\mathrm{H}^{-1}]_{ij} = \sum_k [\frac{v_k v_k^{\mathrm{T}}}{\lambda_k}]_{ij}$

v_k **and** λ_k **are the eigenvector and eigenvalue of mode** ***k***

Comparison with experiments

- **Debye-Waller factor:** $\beta = \frac{8\pi^2}{3} < R_{ii} >$

Figure 1

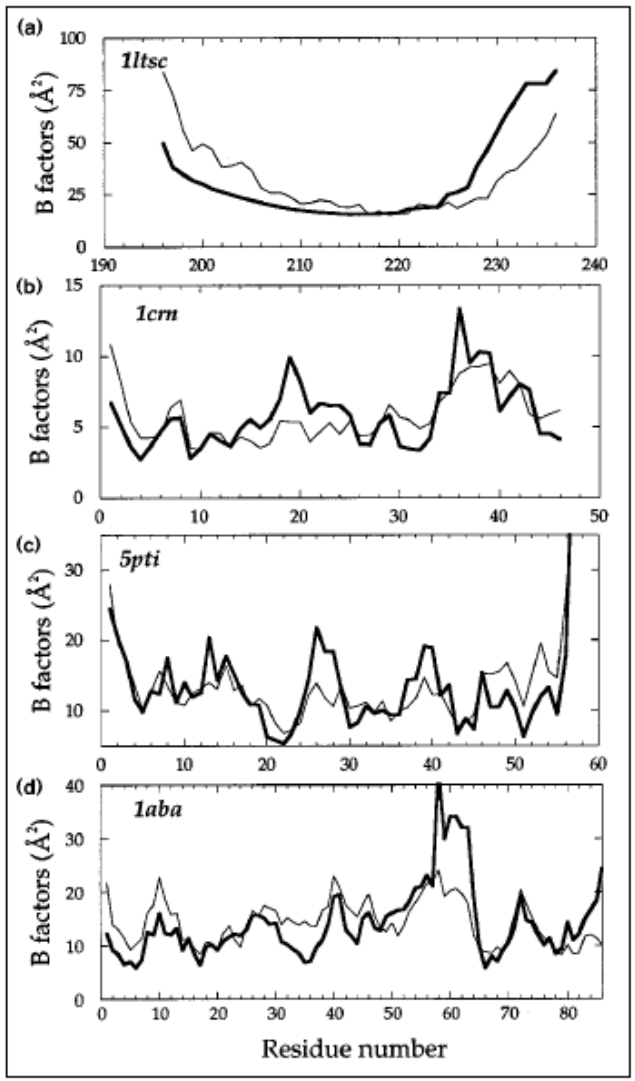
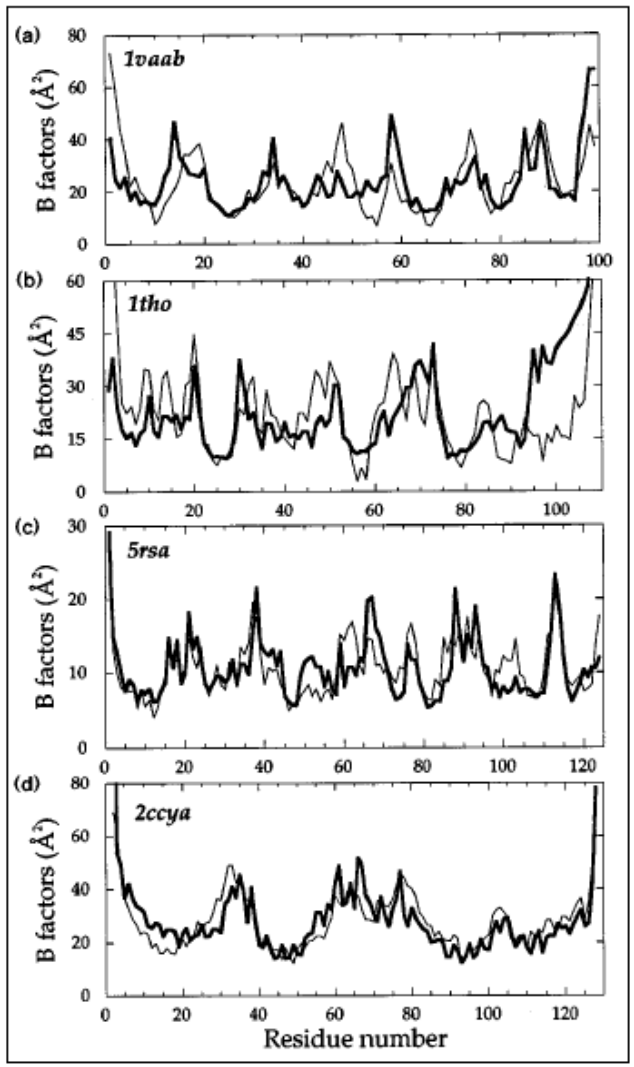


Figure 2



Exp.

Theory

I. Bahar, A. R. Atilgan, and B. Erman (1997) *Folding & Design* **2**, 173-181

Use protein dynamics to identify important residues

- **Fact:** Proteins carry out function by binding to other molecules.

- **Hypotheses**
  - Functionally important residues interact strongly with the functional sites.
  - Residues involved in the conformational changes of binding are functionally important.

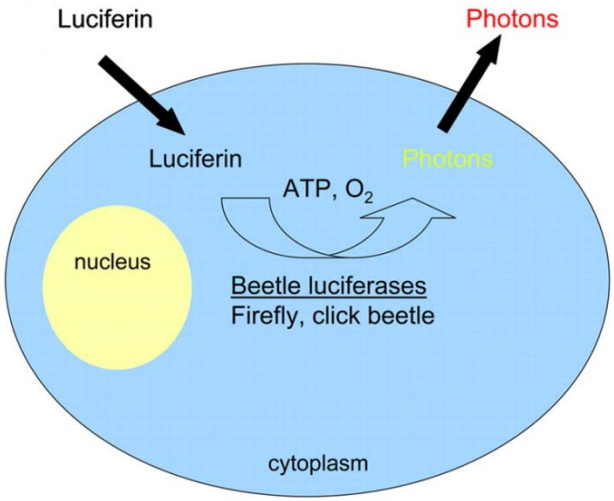
- **Advantage:** Mechanistic understanding of catalytic reaction is not necessary.

How sequence distribution of luciferase affects the color emission of bioluminescence

Y. Mao, "Dynamics Studies of Luciferase Using Elastic Network Model: How the Sequence Distribution of Luciferase Determines its Color"
Protein Engineering Design & Selection 2011, 24: 341-349.

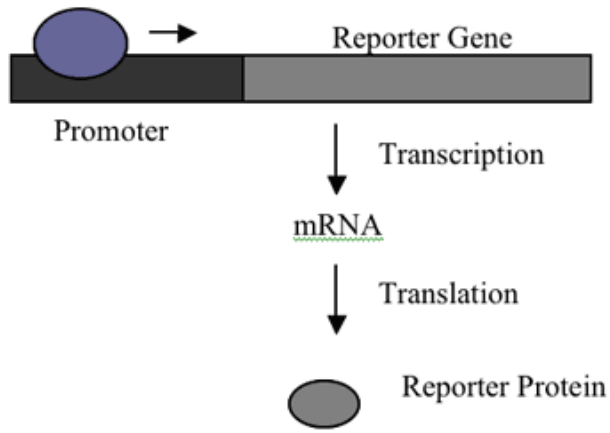
Bioluminescence

- **Conversion of chemical energy into light**

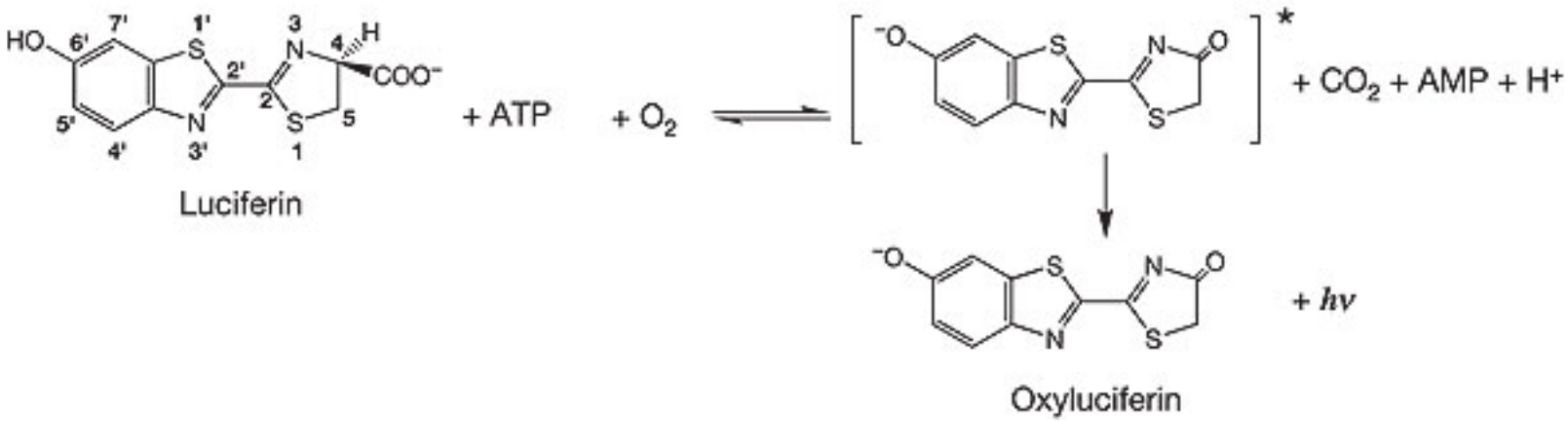


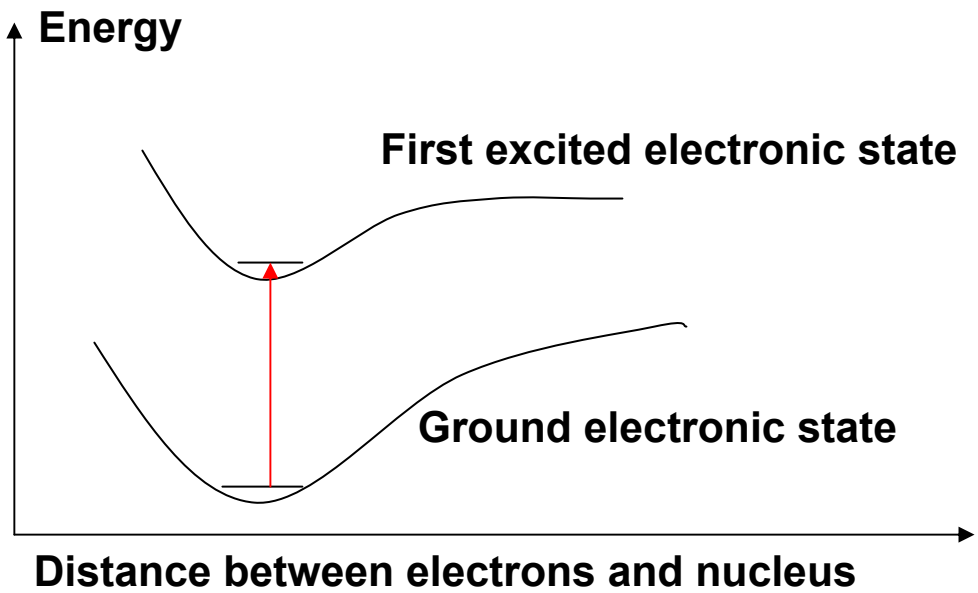
- **Protein engineering challenge: create a red-emitting system?**

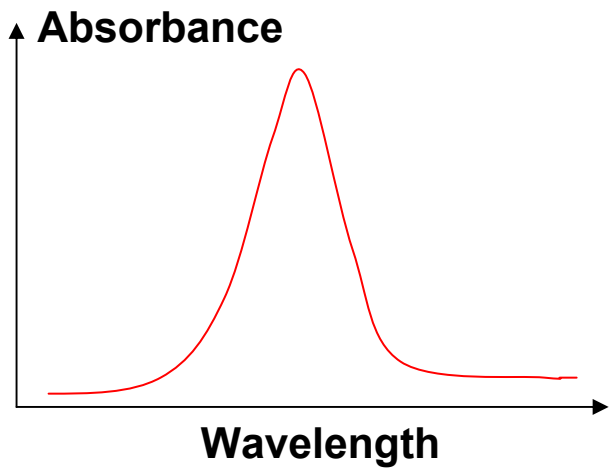
Bioluminescence reporter gene imaging



Excitation of luciferin

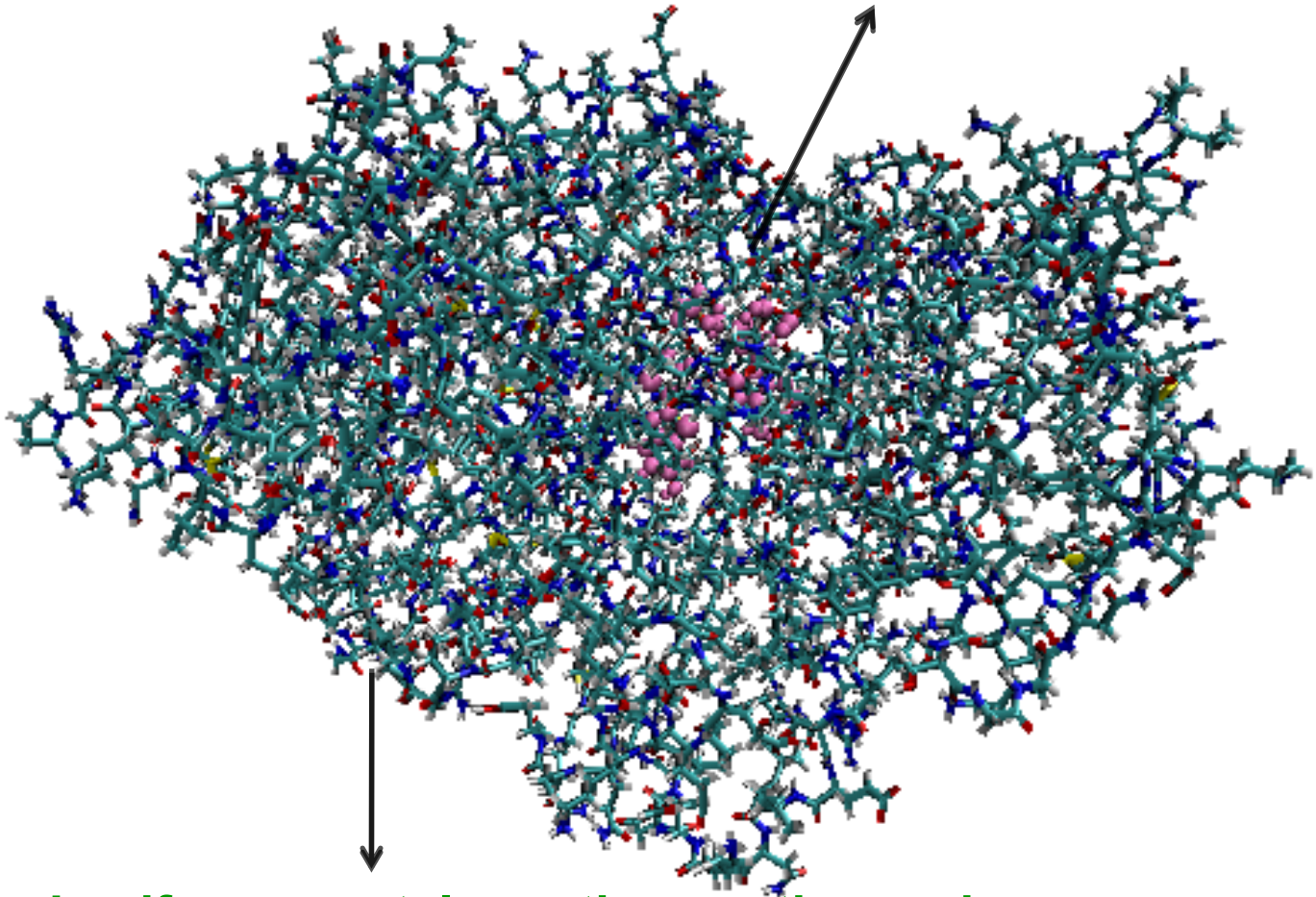






Atomic structure of luciferase/luciferin complex

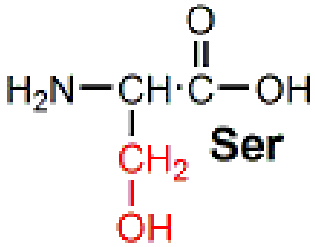
Luciferin: reactant for bioluminescence reaction

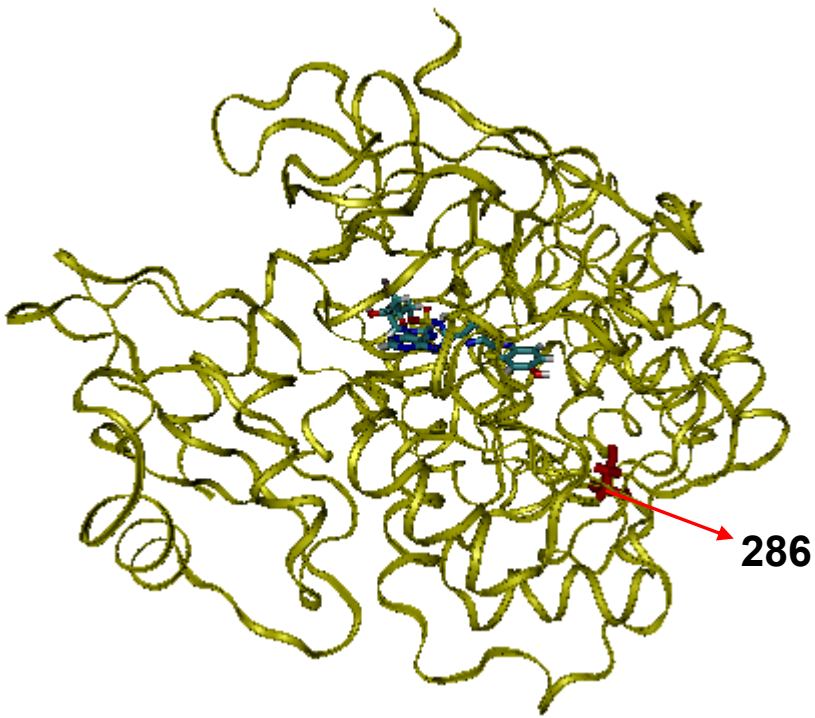


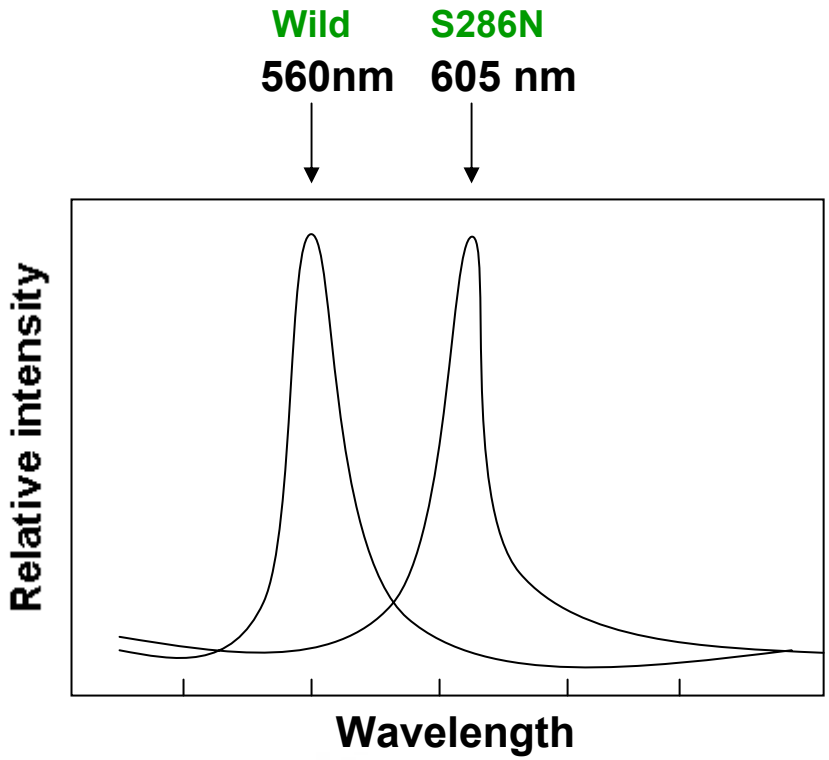
Luciferase: catalyzes the reaction and influences the outcome of reaction (frequency)

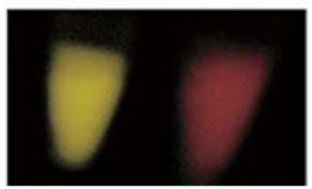
Spectral shift: luciferase-luciferin interactions

Mutation at site 286: Ser ⟶ Asn









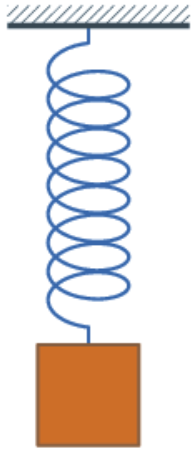
Nakatsu et al., ***Nature*** 440, p 372-376, 2006

Application of elastic network model to luciferase

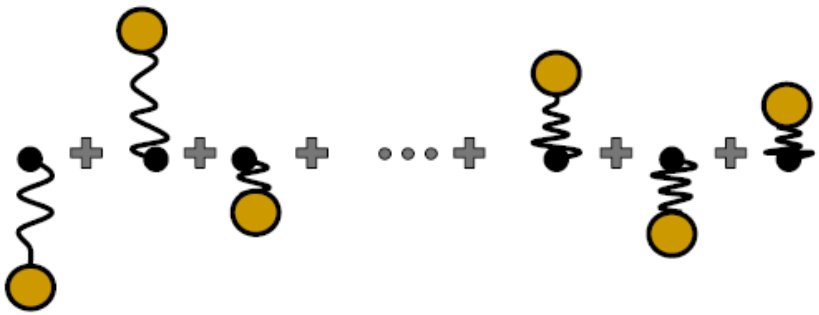
- **Goal: to identify residues whose mutations may have the potential to change the bioluminescence frequency**

- **Approach:**
  - **validates the linkage between protein global dynamics and function**
  - **identifies the important residues**
  - **probes the nature of couplings between the important residues and the active site**

Meaning of Normal modes



- **Normal mode: all parts move with the same frequency and phase**
- **Any motion of the system can be thought as a combination of its normal modes.**



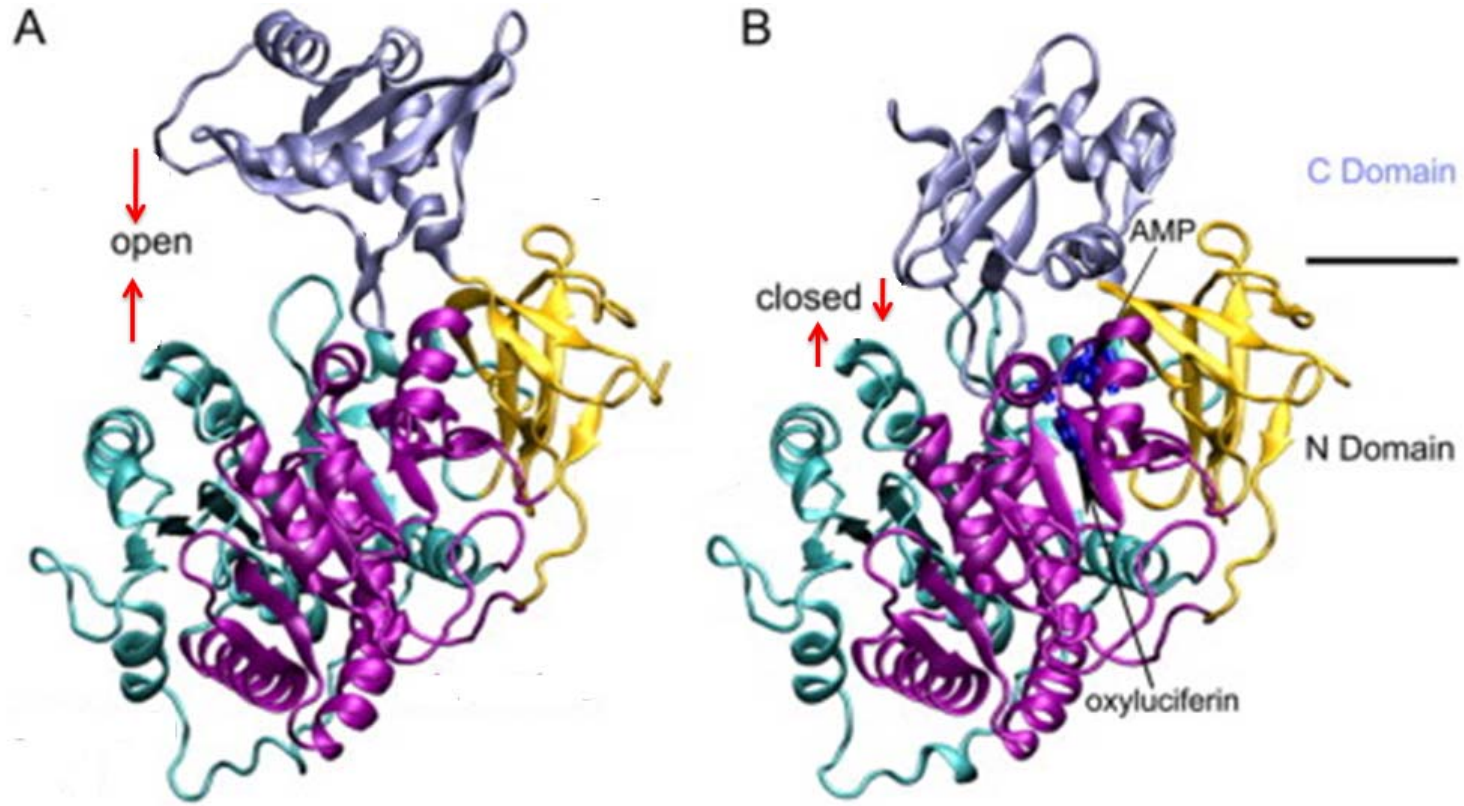
Global motions ⟷ Local motions
Low frequencies High frequencies

Question: what is the biological meaning of these modes?

Functionally most important motion: binding-induced change

Unbound of Luciferase (without substrate)

Bound form of Luciferase (with substrate)

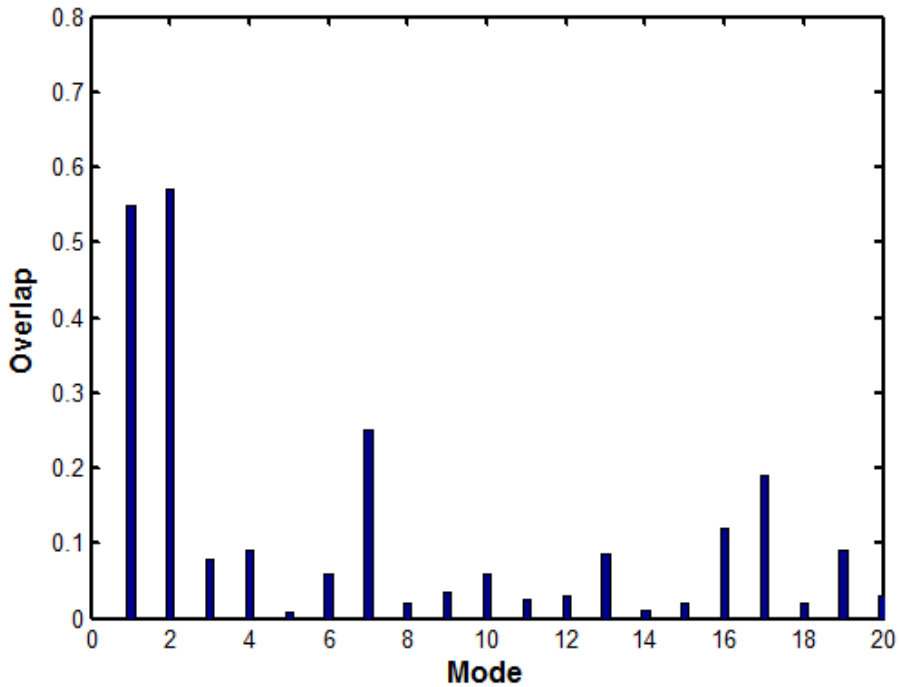


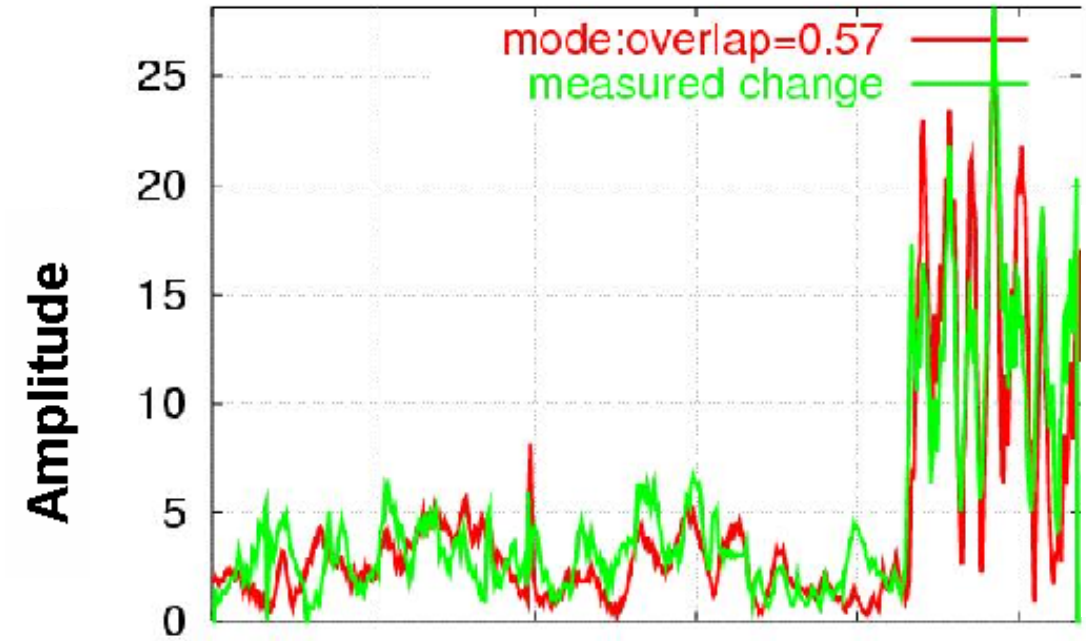
Normal mode analysis: biological meanings of normal modes

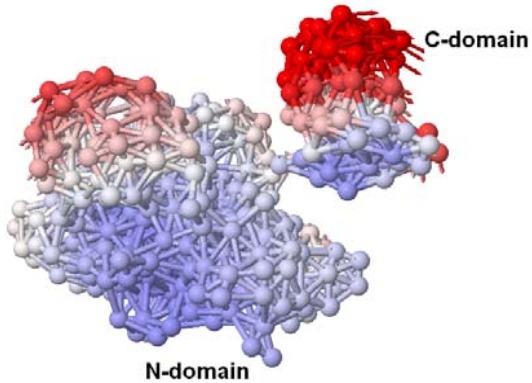
- **Description of conformational change by normal modes**

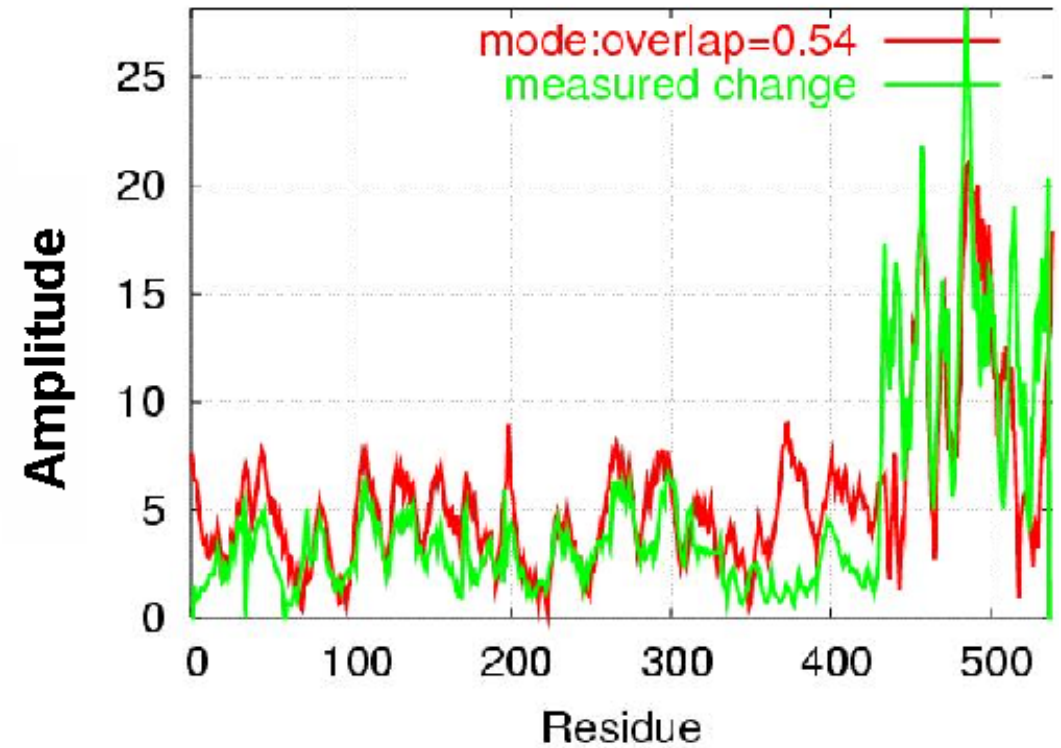
Luciferase (unbound form) ⟶ Luciferase + substrate (bound form)

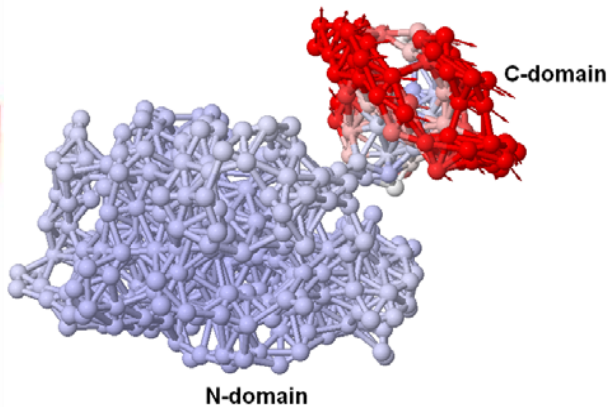
Overlap between the modes and conformational change
= cosine between two vectors




mode:overlap=0.57
measured change
Amplitude
25
20
15
10
5
0


C-domain
N-domain


mode:overlap=0.54
measured change
Amplitude
25
20
15
10
5
0
0
100
200
300
400
500
Residue


C-domain
N-domain

Summary from normal mode analysis

- The two lowest-frequency modes adequately account for the observed conformational changes induced by binding.

- It validates the applicability of the elastic network model to luciferase.

Perturbation analysis: identifying important residues

Change in sequence ? → **Change in function**

- **Experimentally random mutagenesis**

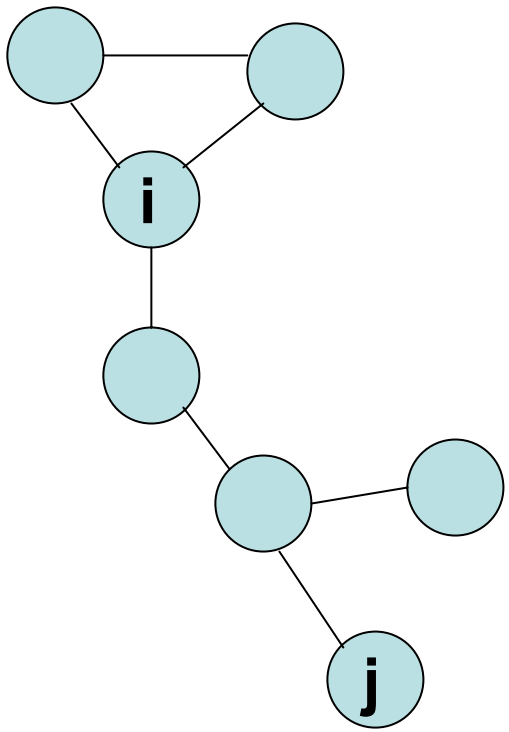
  Replace one residue type by another at position i

- **Computationally perturbation analysis**

  Change in the force constant of residue i

  Change in the fluctuations of the active site

Perturbation analysis



- **Introduce an energy perturbation at node i**

$$E_i' = \frac{1}{2}\sum_k C'(d_{ik} - d_{ik}^0)^2$$

- **Measure the difference in the fluctuation at node j**

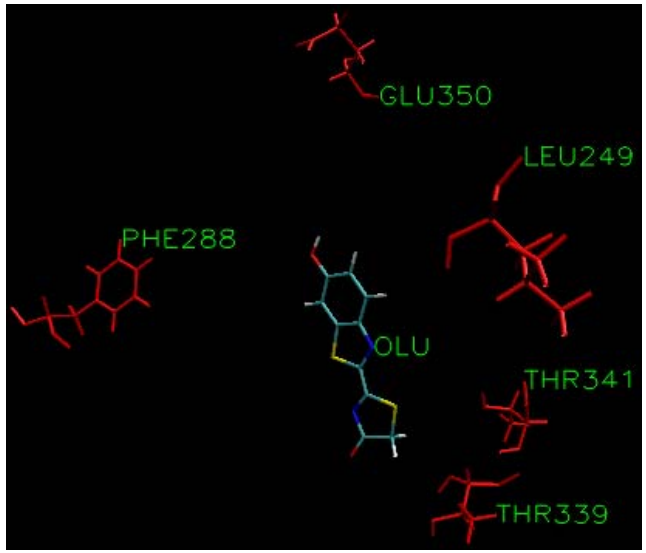
the perturbation based correlation

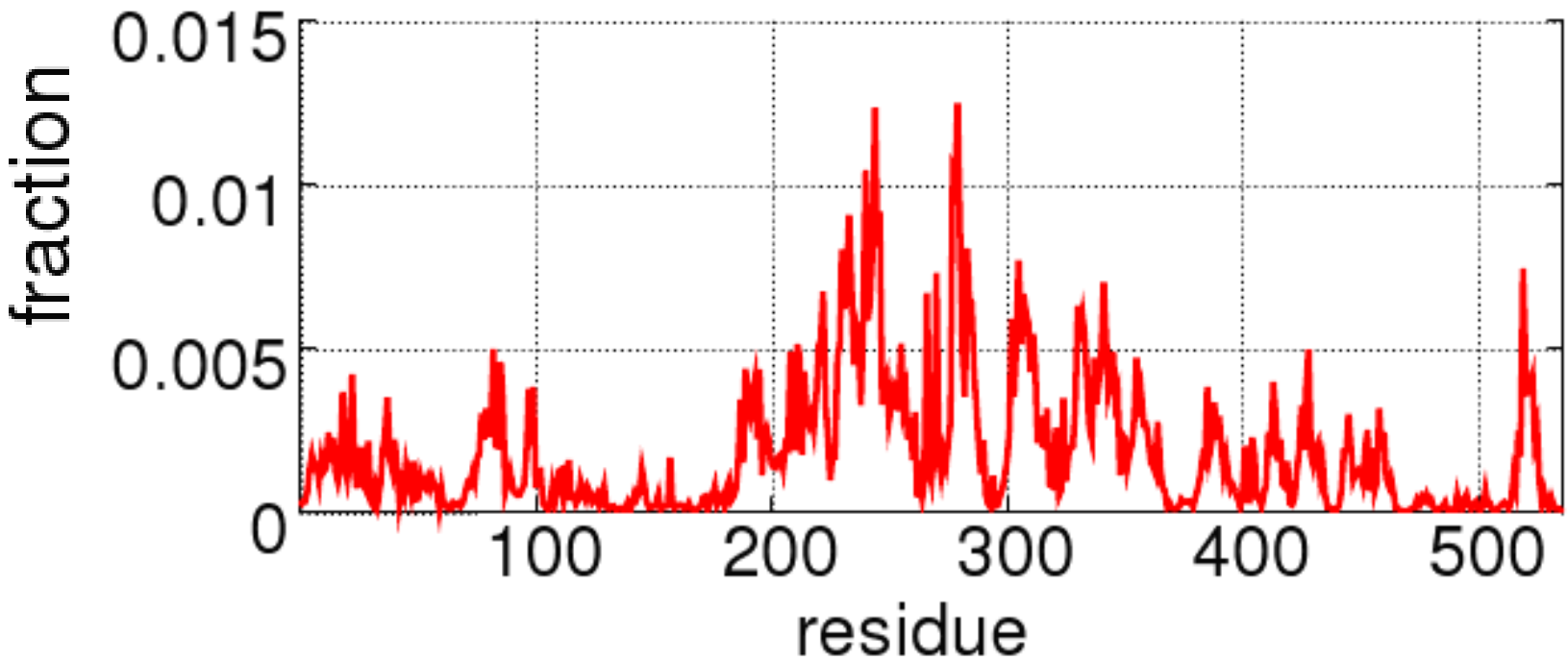
$$\frac{R_{jj}' - R_{jj}}{R_{jj}}$$

Application of perturbation analysis to luciferase

- **node *j* = residues at the binding site (249, 288, 339, 341 and 350)**
  node *i* = all the other residues
- **the fraction of change in the fluctuation of *j***

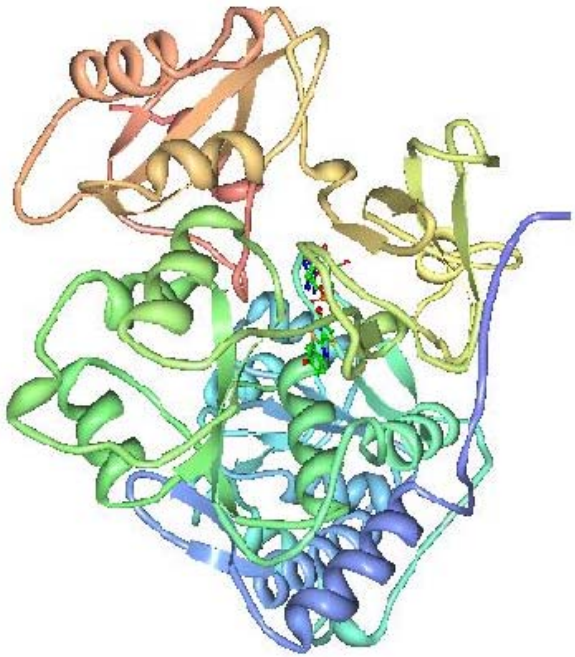
$$\frac{R_{jj}'-R_{jj}}{R_{jj}}$$



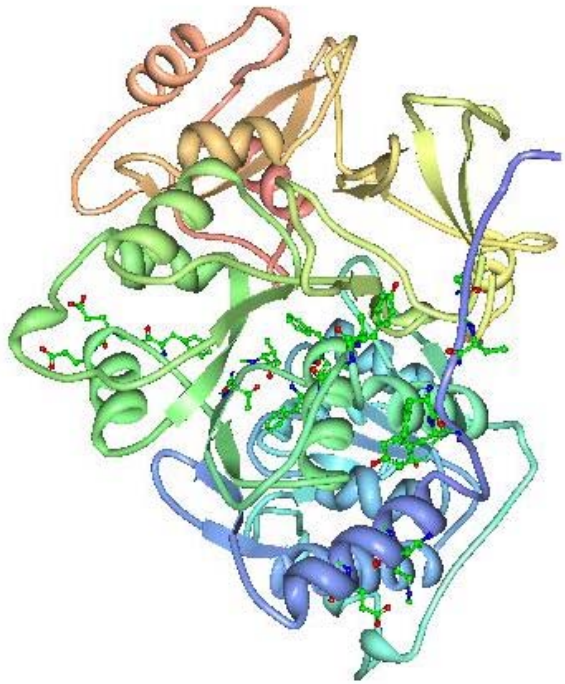


The important residues

91,217, 220, 229, 230, 231, 237, 238,
240, 241, 242, 243, 245, 246, 248, 250,
251, 252, 253, 254, 255, 264, 275, 279
286, 287, 289, 290, 292, 293, 294,311,
313, 314, 315, 316, 317, 318, 320, 339,
340, 341, 342, 350, 351, 354, 364, 437, 528



active site



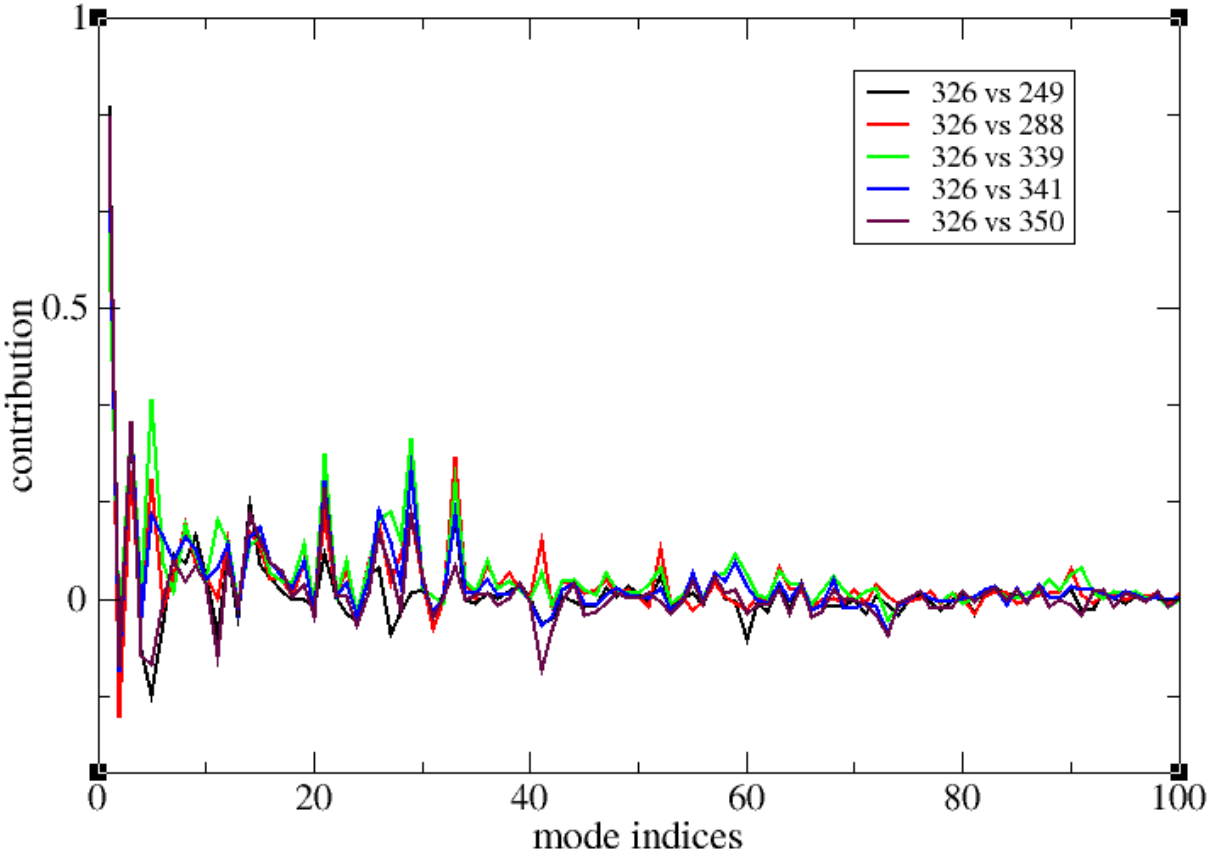
important residues

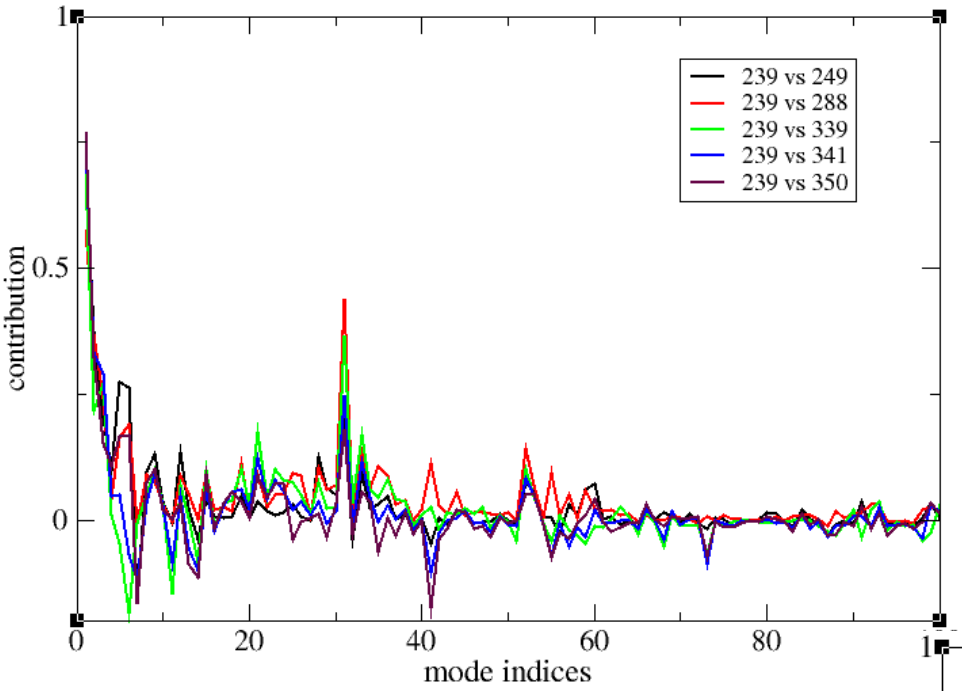
The mode decomposition analysis

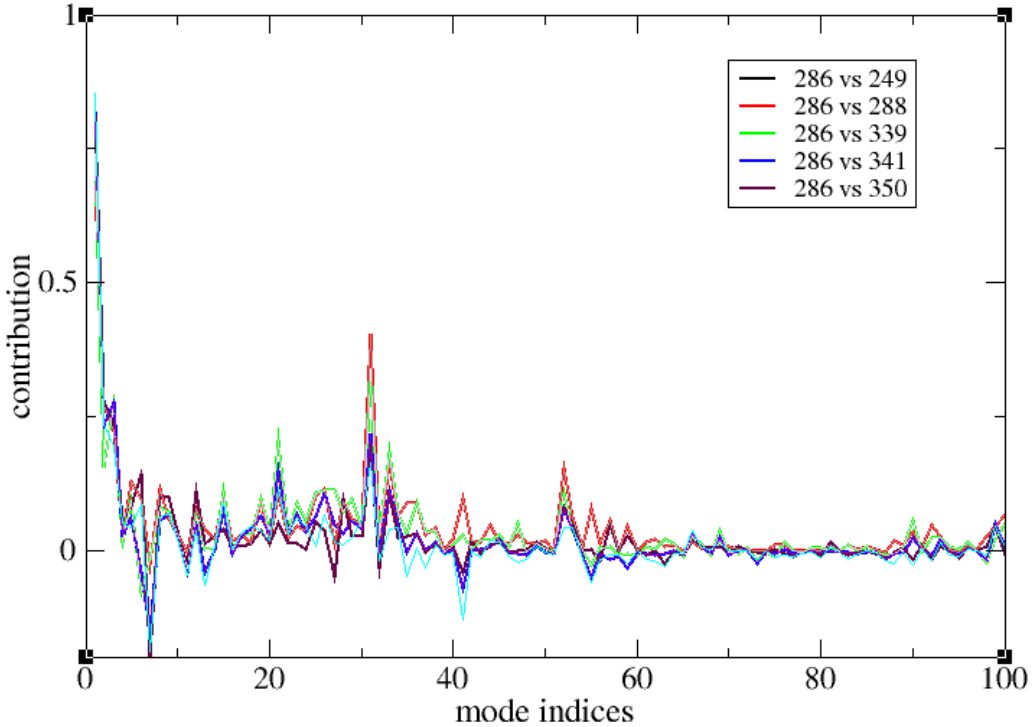
$$R_{ij} = \frac{k_B T}{C} \sum_k [\frac{v_{ik} v_{jk}^{\mathrm{T}}}{\lambda_k}]_{ij}$$

- **Contribution of the *k*th mode to the correlation of residues *i* and *j***

$$(\frac{v_{ik} v_{jk}}{\lambda_k}) / \sqrt{\sum_{m<M} (\frac{v_{im} v_{jm}}{\lambda_m})^2}$$




239 vs 249
239 vs 288
239 vs 339
239 vs 341
239 vs 350
contribution
mode indices
0
0.5
1
0
20
40
60
80
100


286 vs 249
286 vs 288
286 vs 339
286 vs 341
286 vs 350
contribution
mode indices
0
0.5
0
20
40
60
80
100

Application of elastic network model to luciferase

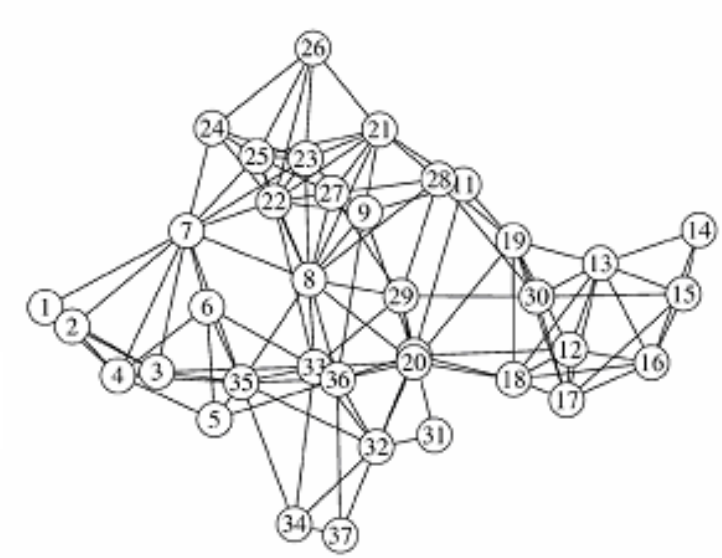
- Network modeling captures the motions essential to luciferase function.
- Perturbation approach identifies the important residues.
- Lowest frequency modes are mainly responsible for the couplings between the remote important residues and the active site.

Future plan

- **Why do such simplified network models work?**

information encoded in global topology

local stochasticity



- **When do such simplified network models work?**

Acknowledgements

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