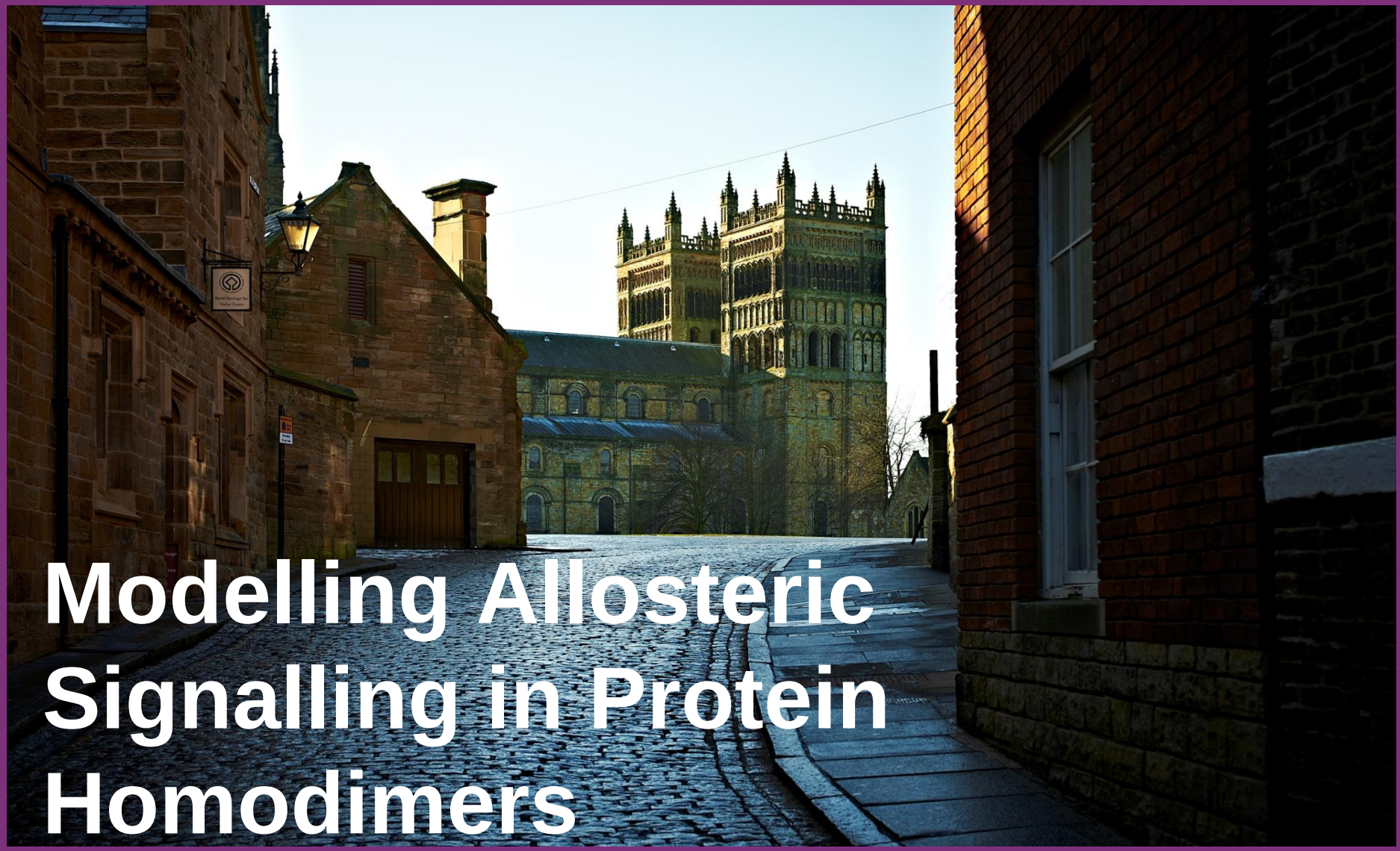

Modelling Allosteric Signalling in Protein Homodimers

Durham University

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Department of Chemistry
Durham University, UK

Biophysical Sciences Institute

Acknowledgements

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Modelling Allosteric Signalling in Protein Homodimers

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- David Burnell
- Prof. Tom McLeish (Physics)
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Biophysical Sciences Institute

- Dr. Martin Cann
- Dr. Philip Townsend
- Dr. Ehmke Pohl

EPSRC

Engineering and Physical Sciences Research Council

Protein Allostery

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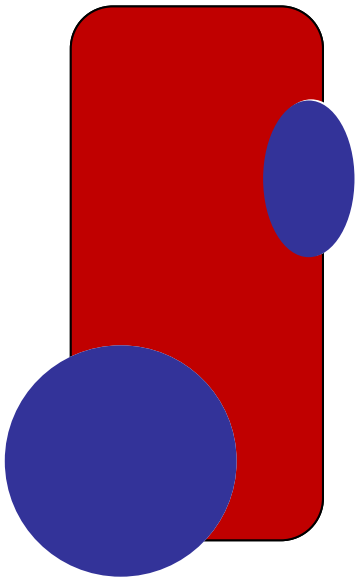
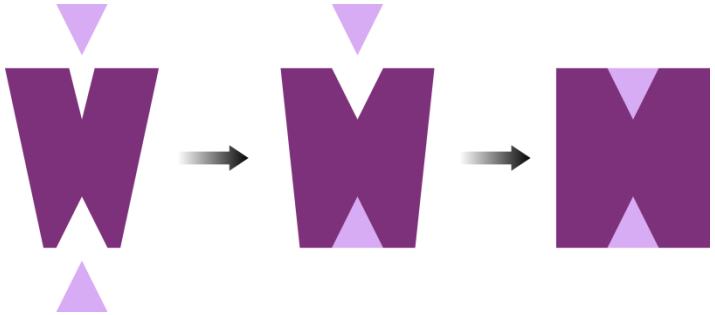
- Effect of binding one molecule on a second binding



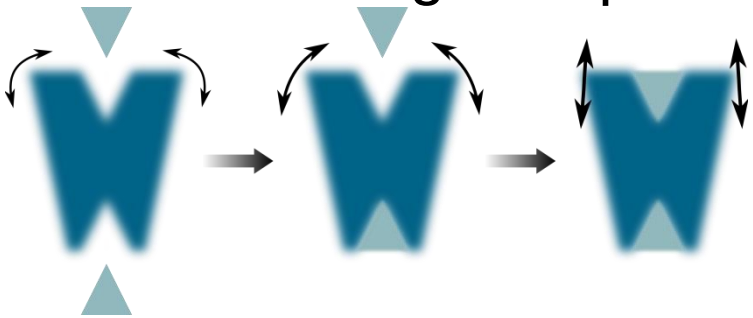
- Central role in biochemical pathways
  - Binding an activator causes binding to DNA
  - Binding a ligand enhances or inhibits binding elsewhere to regulate a process

Allosteric Binding

- Classically explained by conformational change

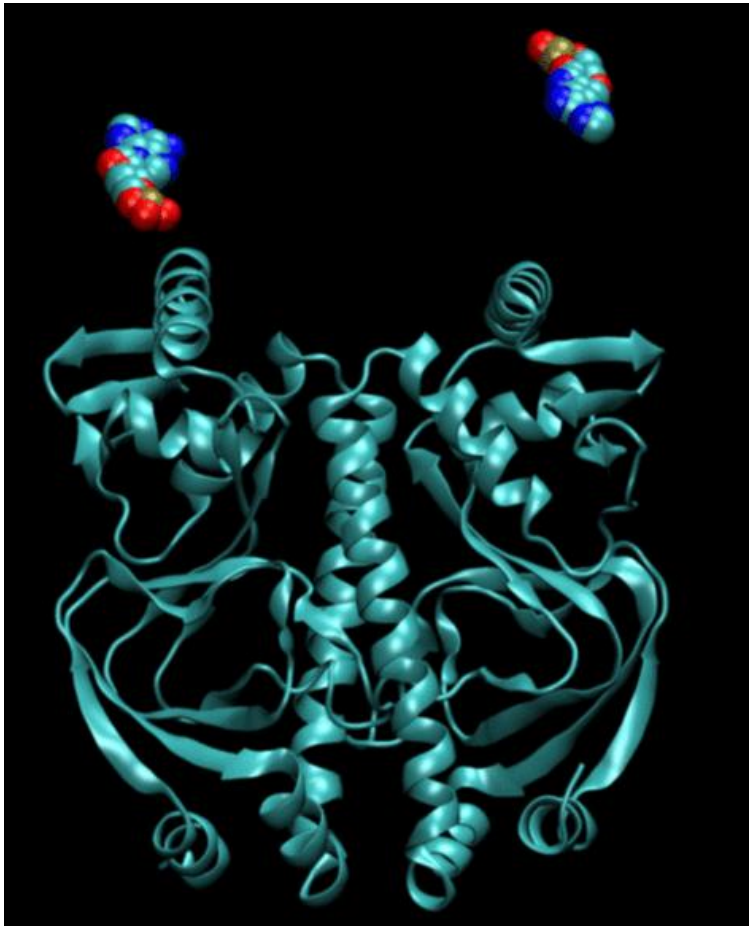


- Free energy contributions from changes in protein vibrations



$$\Delta G = \Delta H - T\Delta S$$

Allosteric Binding – example of CAP



- Catabolite Gene Activator Protein (CAP)

$$\Delta\Delta G = \Delta G_2 - \Delta G_1$$

- Negative cooperativity

$\Delta\Delta G > 0$

(affinity for binding 2nd ligand is reduced)

- Positive cooperativity

$\Delta\Delta G < 0$

Catabolite Activated Protein (CAP) homodimer shows negative co-operativity between two identical binding sites for cyclic AMP (cAMP) without a change in structure

Aims of this talk

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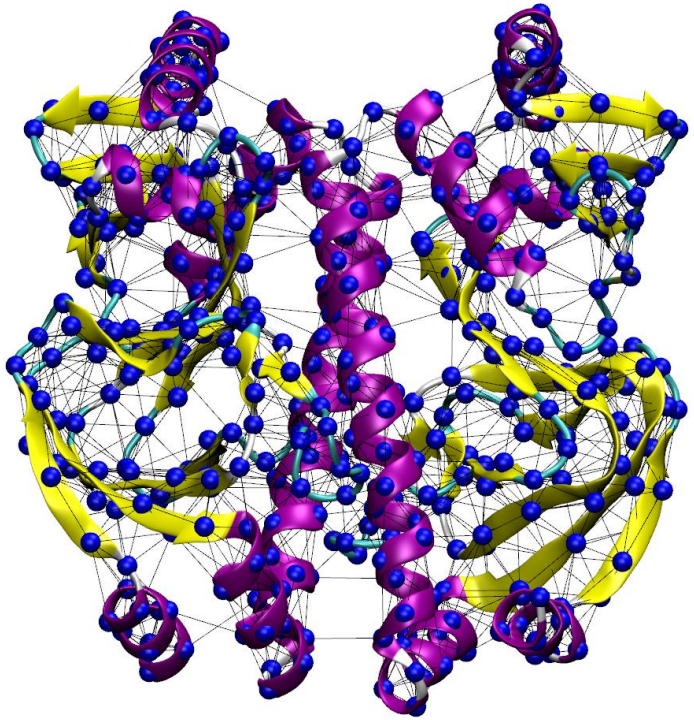
1. Use multi/many-scale models to investigate dynamic allostery for (the protein dimer) CAP
   - Elastic Network Model insights
   - Super-coarse-grained models
   - Atomistic Models
2. Use the models to show how we can control dynamic allostery by selected mutation
   - hence control dynamic landscape of a protein
   - provide a new route for drug design?
3. Point to some other ways in which proteins have evolved to harness dynamic pathways

Elastic Network Model (ENM)

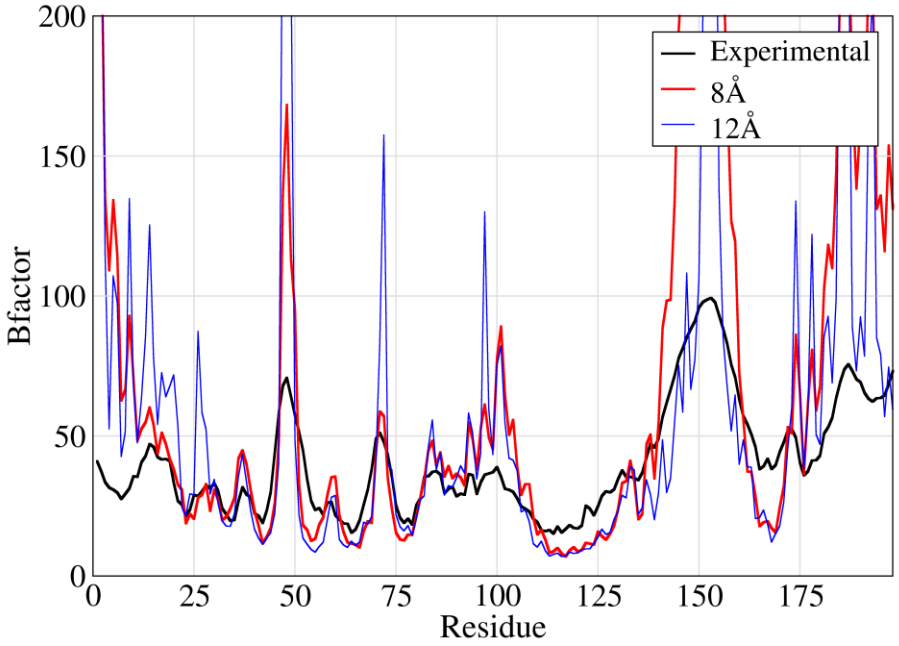
- Join C_α atoms with Hookean springs
- Diagonalize mass-weighted Hessian matrix
- Eigenvectors – normal modes
- Eigenvalues – frequencies
- Low frequency modes most important for motion

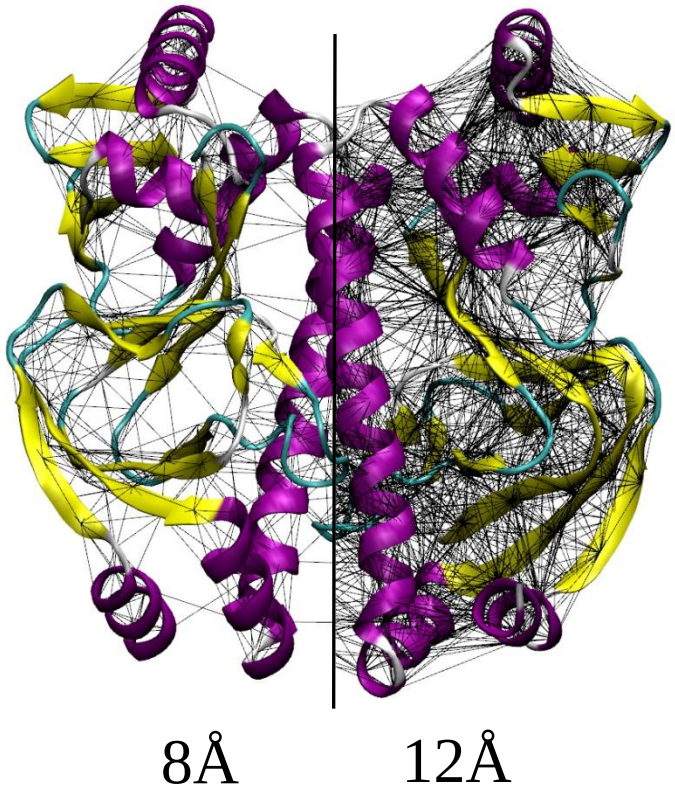
Catabolite Gene Activator Protein (CAP)



$$V_{ij} = \begin{cases} \frac{k_{ij}}{2}\left(r_{ij} - R_{ij}\right)^2 & R_{ij}^2 \leq R_c^2 \\ 0 & R_{ij}^2 > R_c^2 \end{cases}$$

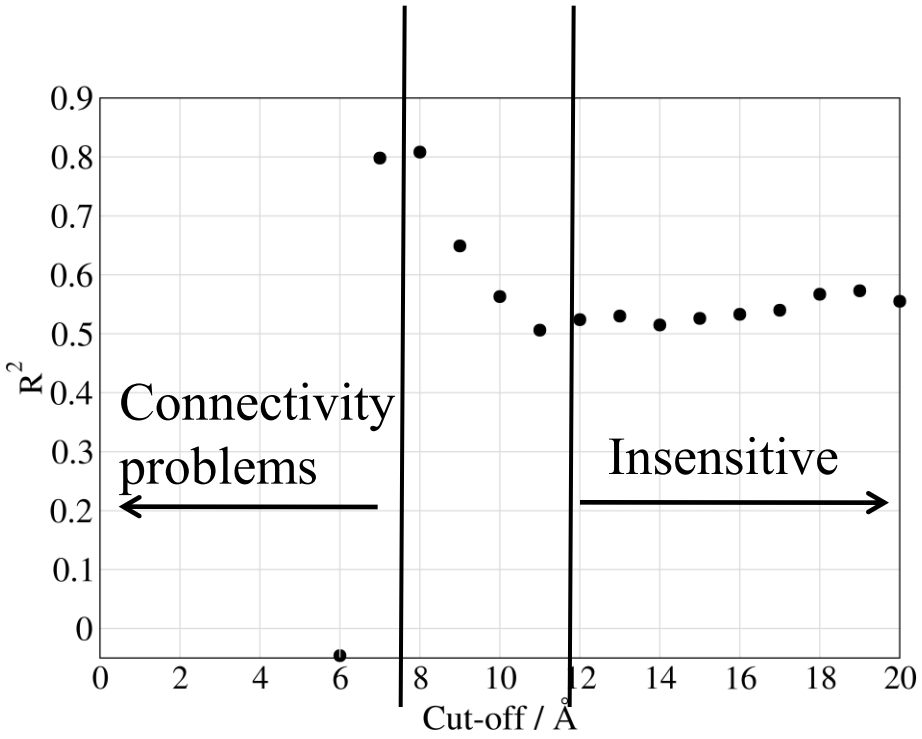
ENM cutoff

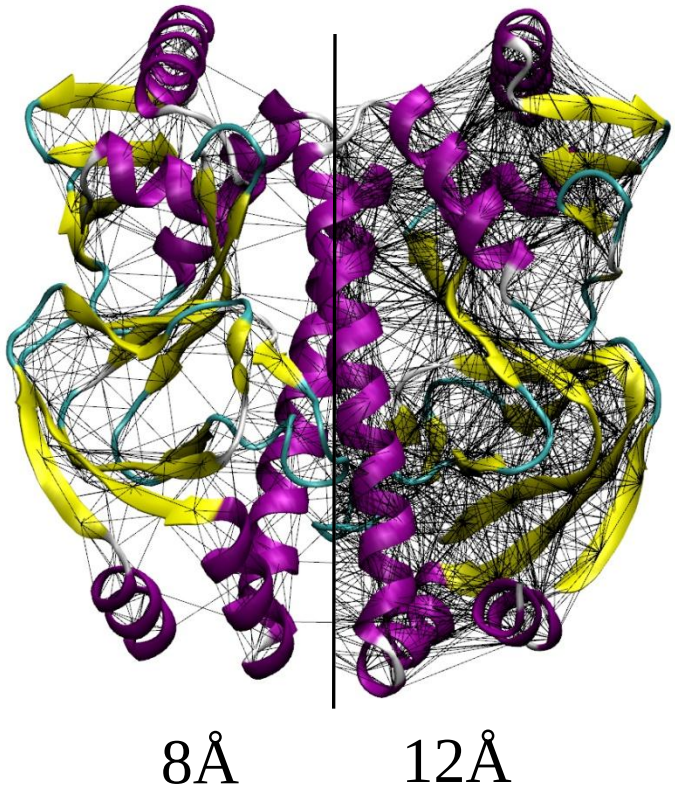




- Smaller cut-off is better

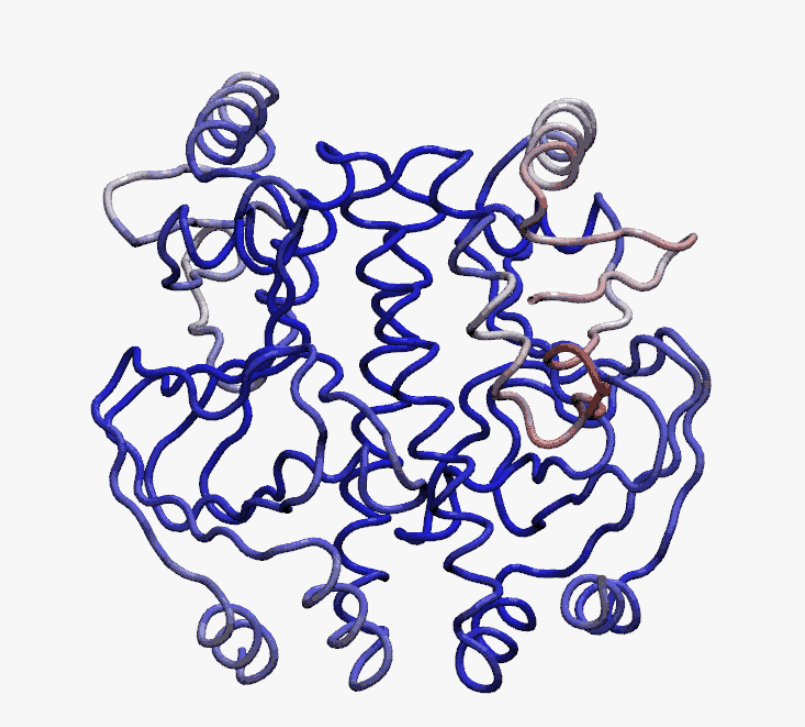
ENM cutoff

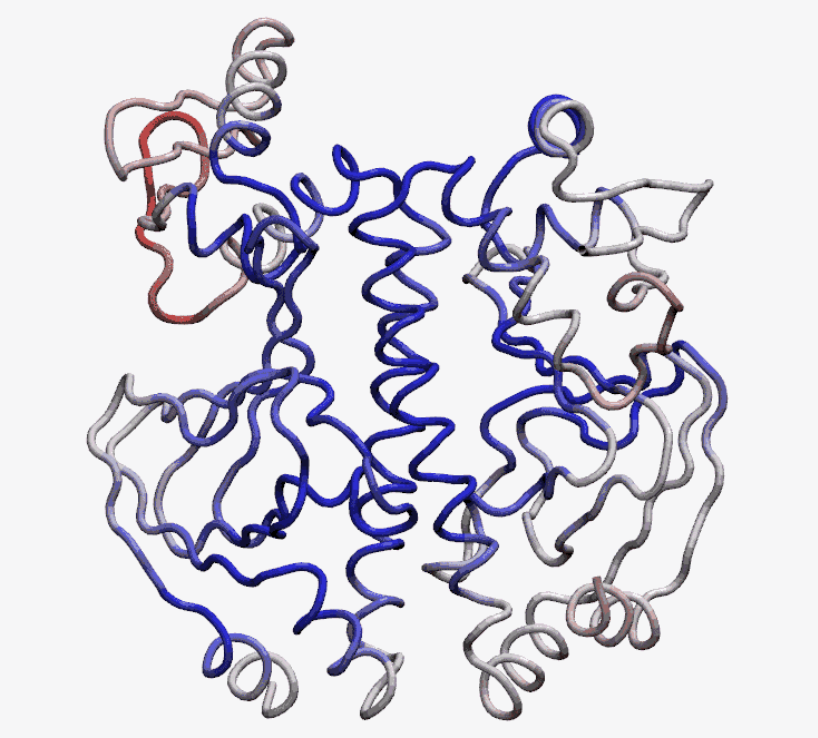




- Smaller cut-off is better

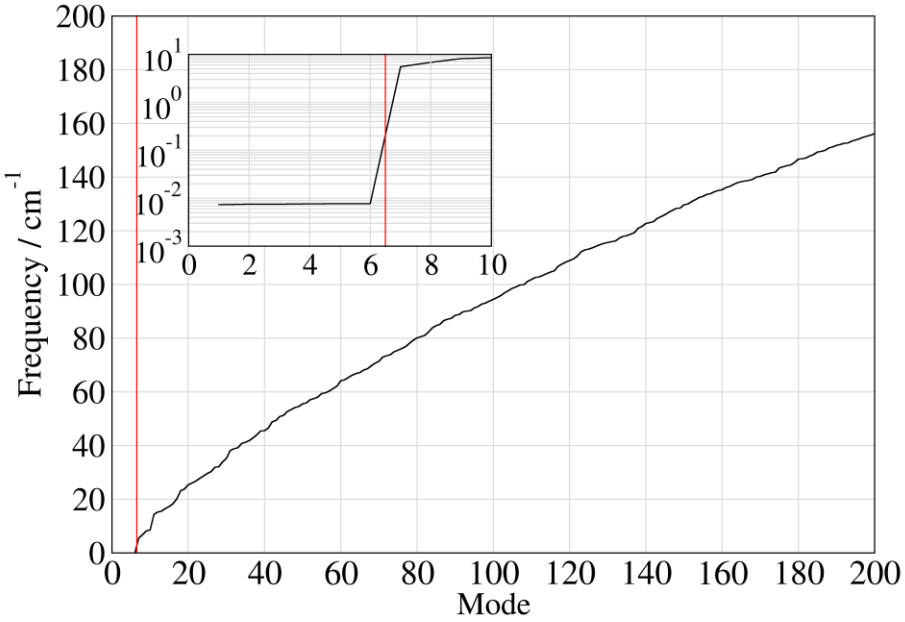
Modes



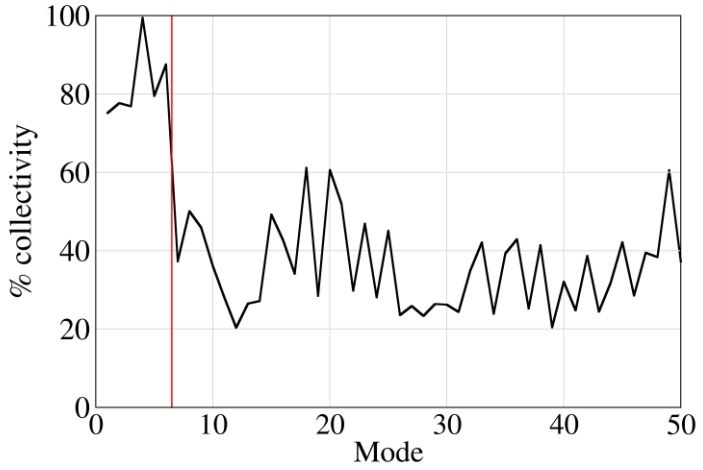


Note: - movements of the whole protein
- importance of the strength of hydrophobic interactions between helices

Variation of Mode Frequency



- Large number of contributing modes
- Low frequency modes involve whole protein



Calculating Allostery with the ENM

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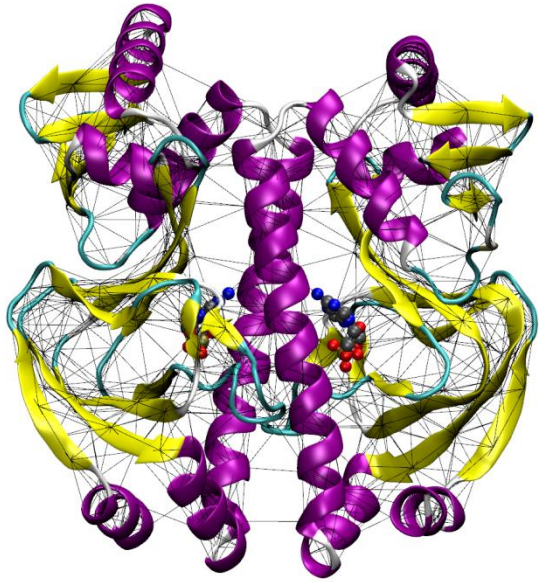
$$\mathrm{CAP_{apo}} + 2\mathrm{cAMP} \underset{K_{1,\mathrm{unbind}}}{\overset{K_{1,\mathrm{bind}}}{\rightleftharpoons}} \mathrm{CAP_{holo1}} + \mathrm{cAMP} \underset{K_{2,\mathrm{unbind}}}{\overset{K_{2,\mathrm{bind}}}{\rightleftharpoons}} \mathrm{CAP_{holo2}}$$

- Experimental Allostery coefficient,

$$\frac{K_2}{K_1} = \frac{K_{2,\mathrm{unbind}}}{K_{1,\mathrm{unbind}}} = \frac{K_{1,\mathrm{bind}}}{K_{2,\mathrm{bind}}}$$



- Calculate vibrational free energy

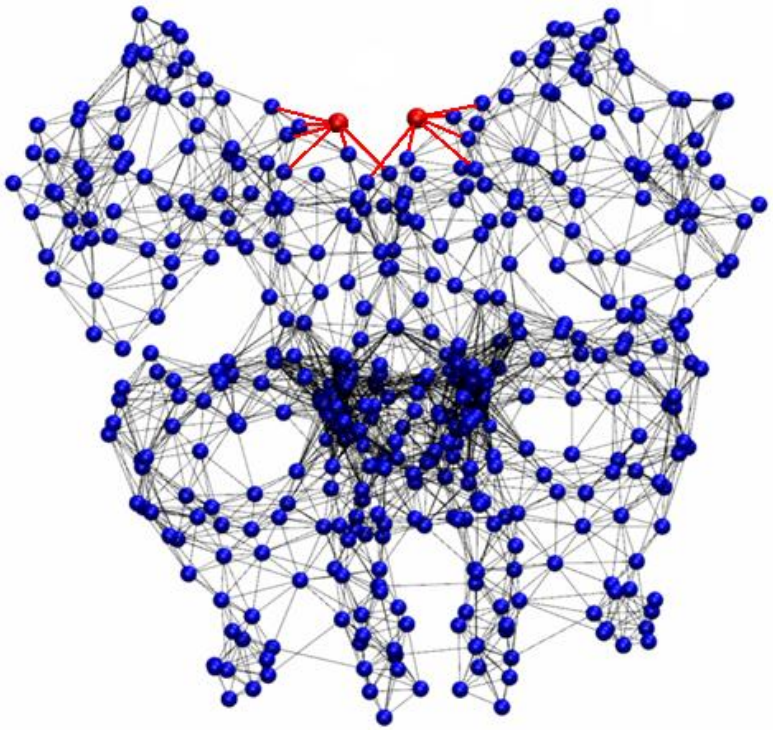
$$\Delta\Delta G = \left(\Delta G_{\mathrm{holo2}} - \Delta G_{\mathrm{holo1}}\right) - \left(\Delta G_{\mathrm{holo1}} - \Delta G_{\mathrm{apo}}\right) = \frac{kT}{2} \ln\left(\frac{\left|\mathrm{H}_{\mathrm{holo\,2}}\right|\left|\mathrm{H}_{\mathrm{apo}}\right|}{\left|\mathrm{H}_{\mathrm{holo1}}\right|^2}\right)$$

- Determine K_2/K_1

ENM predicts $K_2/K_1 > 1$ negative cooperativity
(reduced affinity – as seen experimentally)

Protein Mutations in the ENM

- Mutations represented by varying residue spring constant
- Can investigate sensitivity of vibrational contribution to $\Delta\Delta G$



CAP mutations

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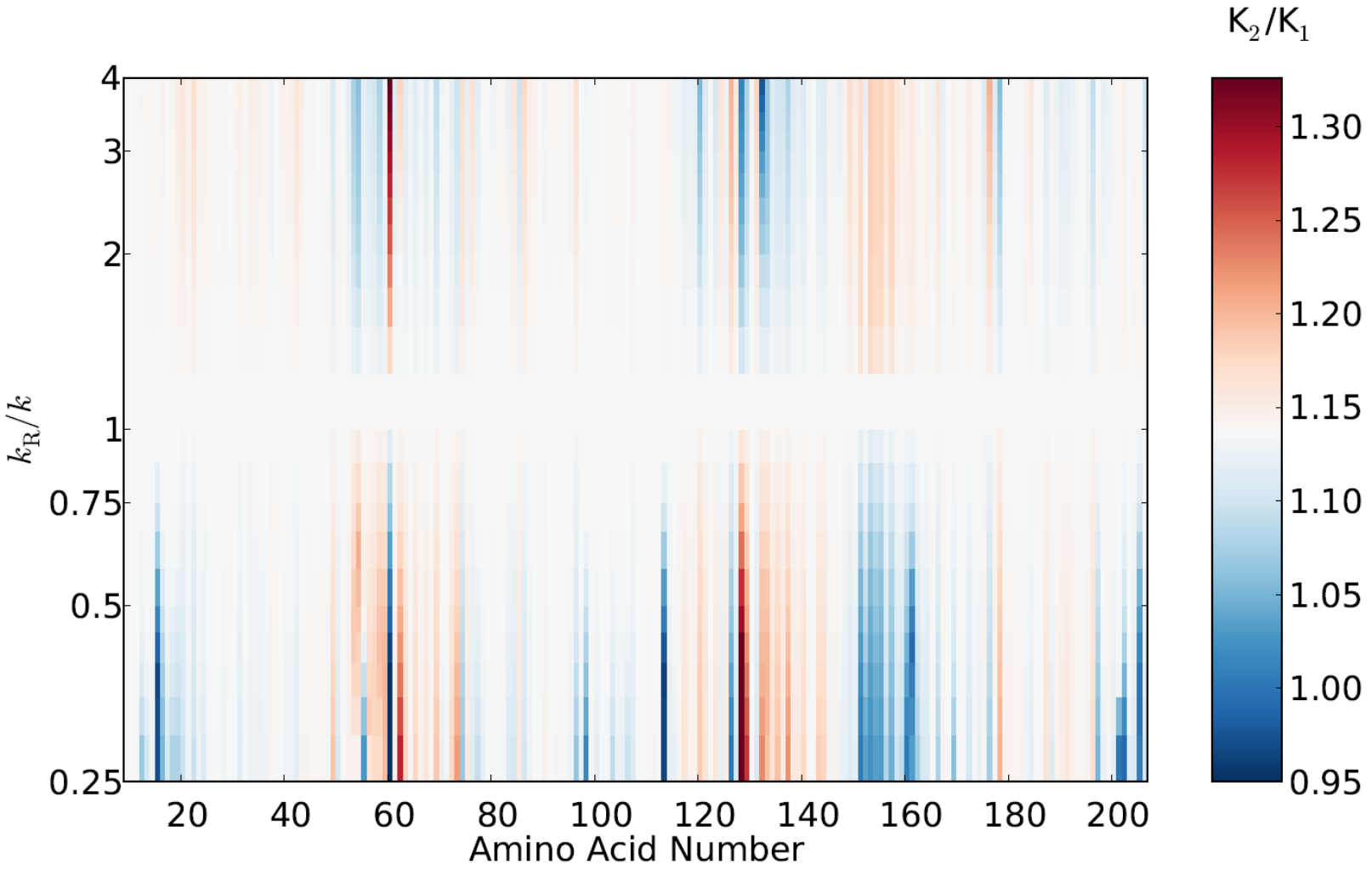
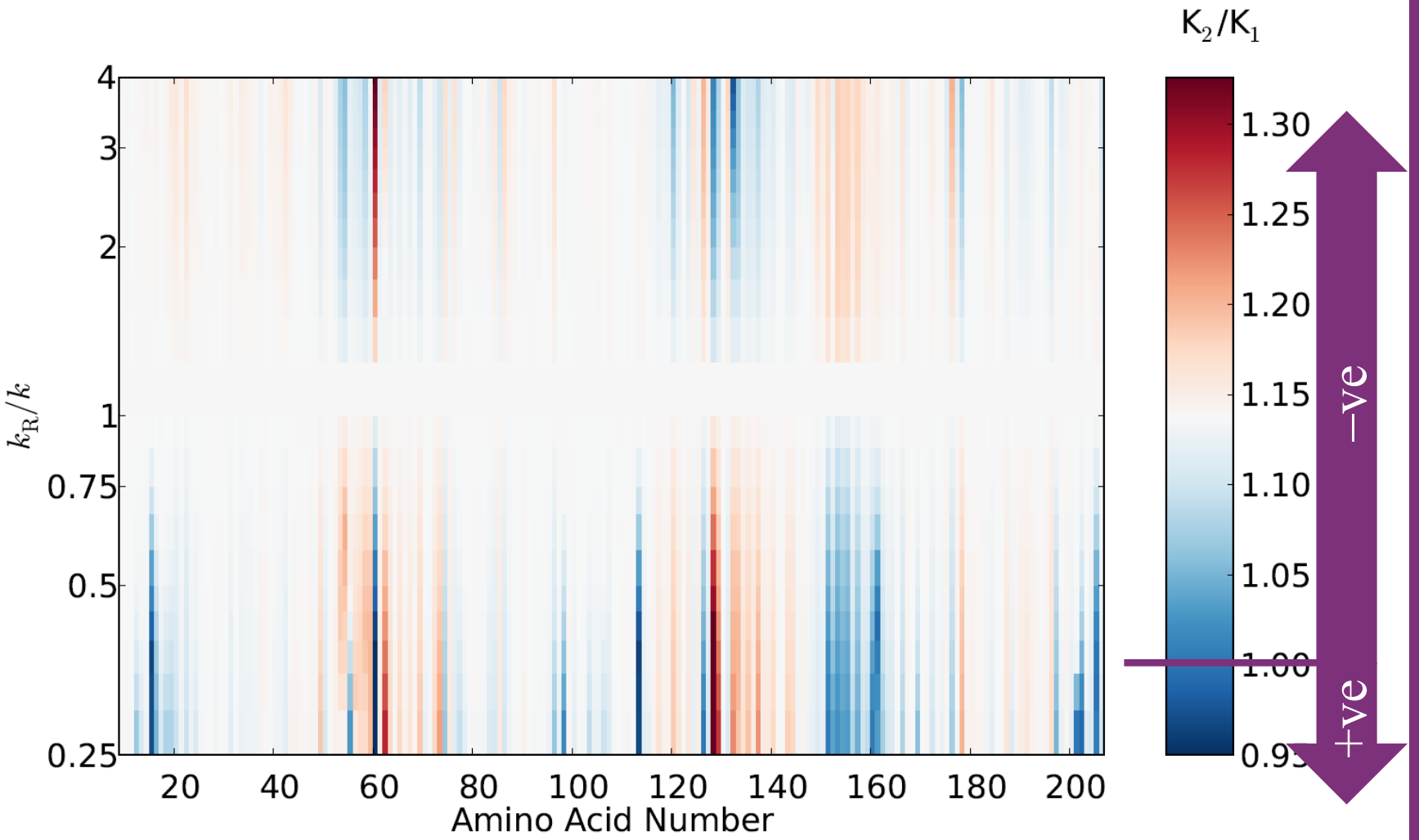


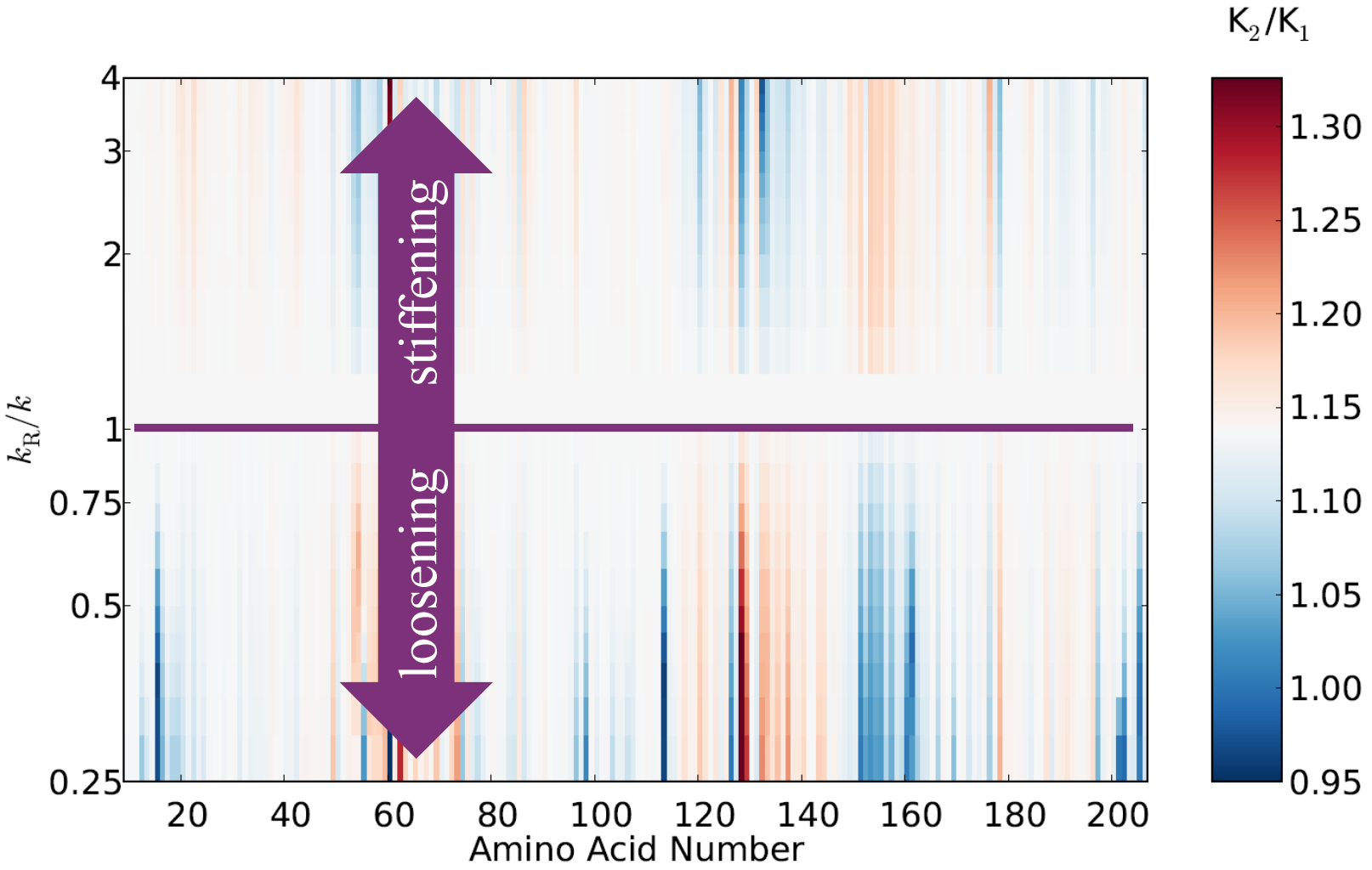
CAP mutations

Durham University



CAP mutations

Durham University



Protein Engineering

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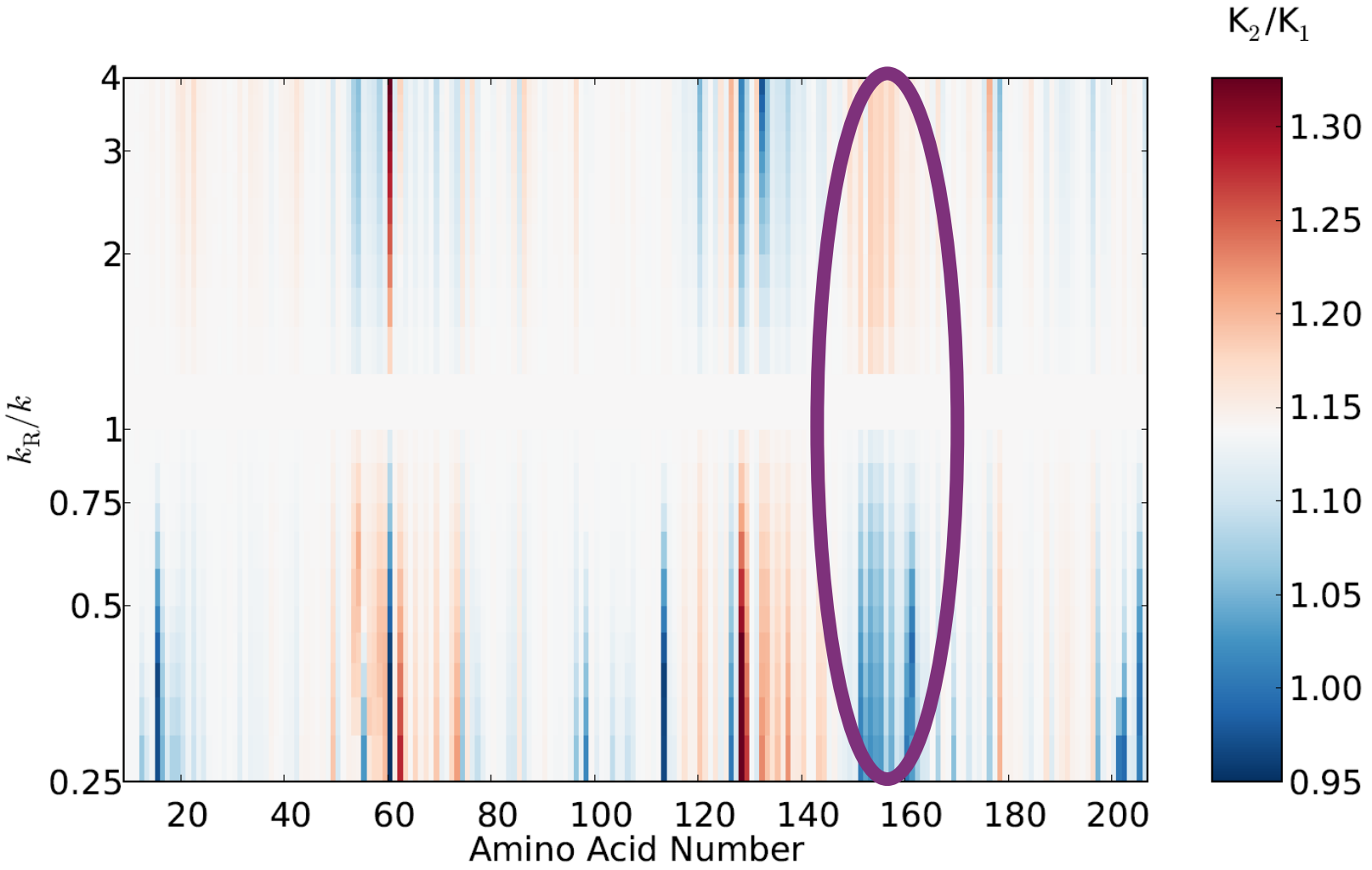
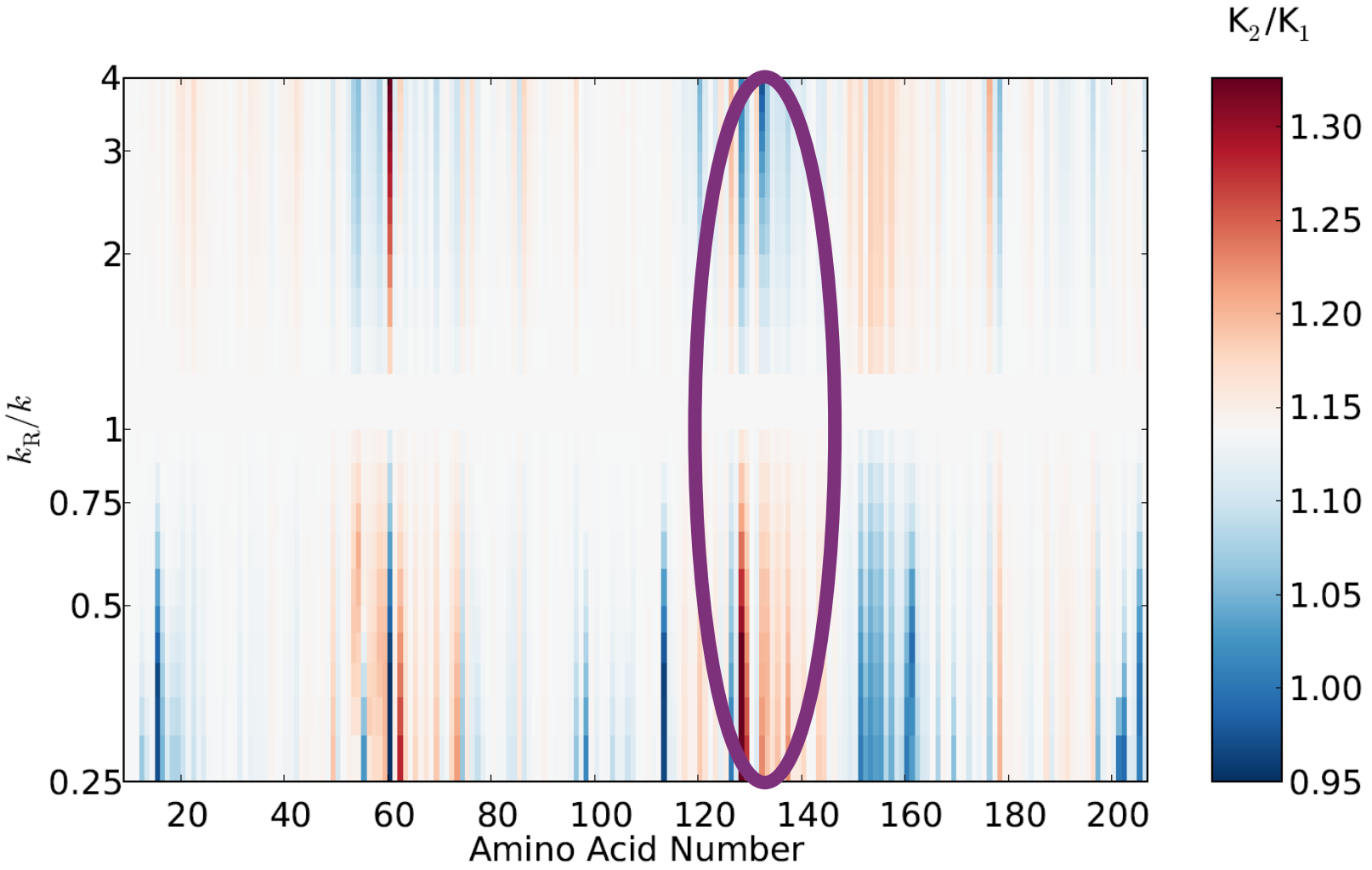
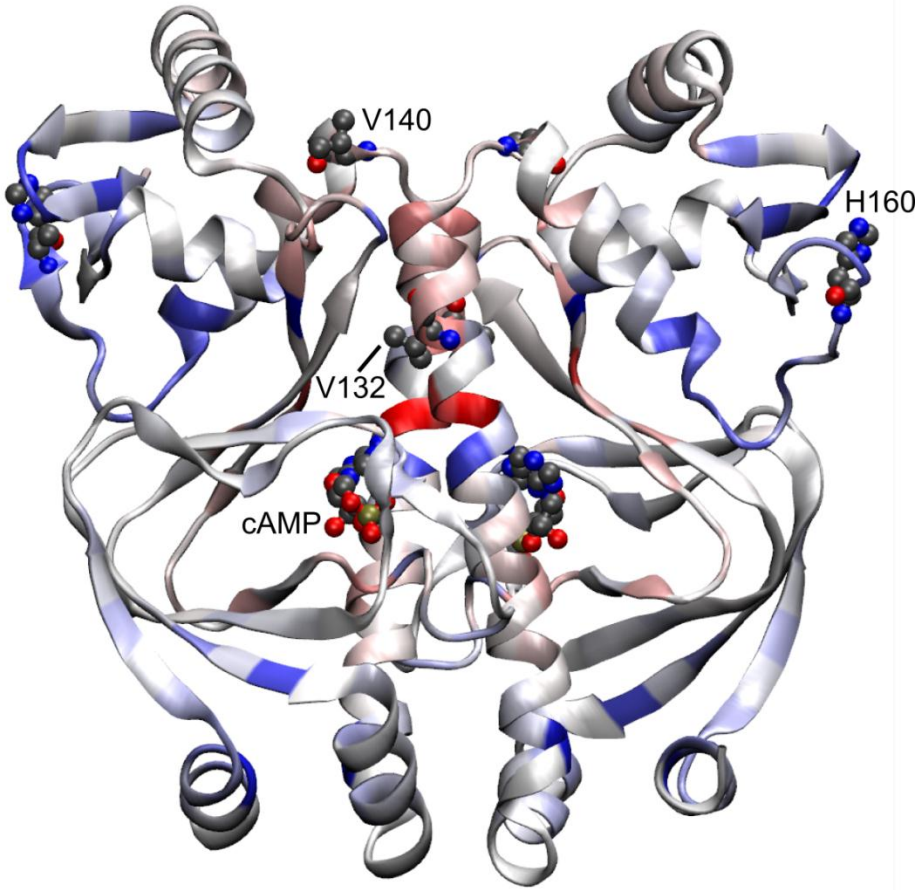


Protein Engineering

Durham University



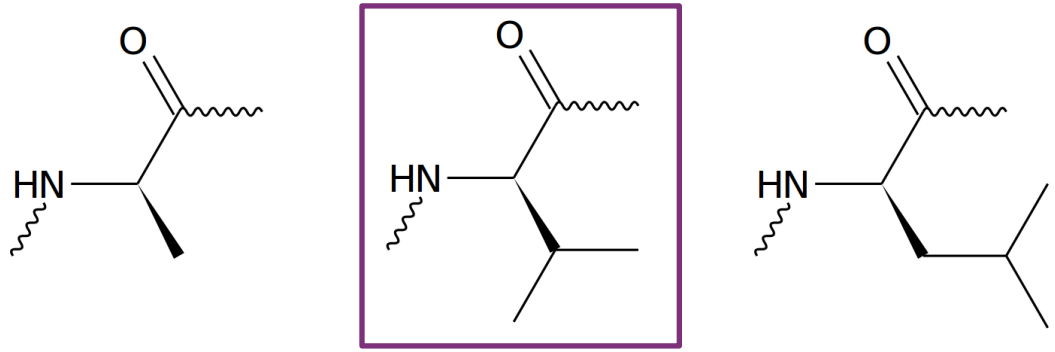
Protein Engineering with the ENM



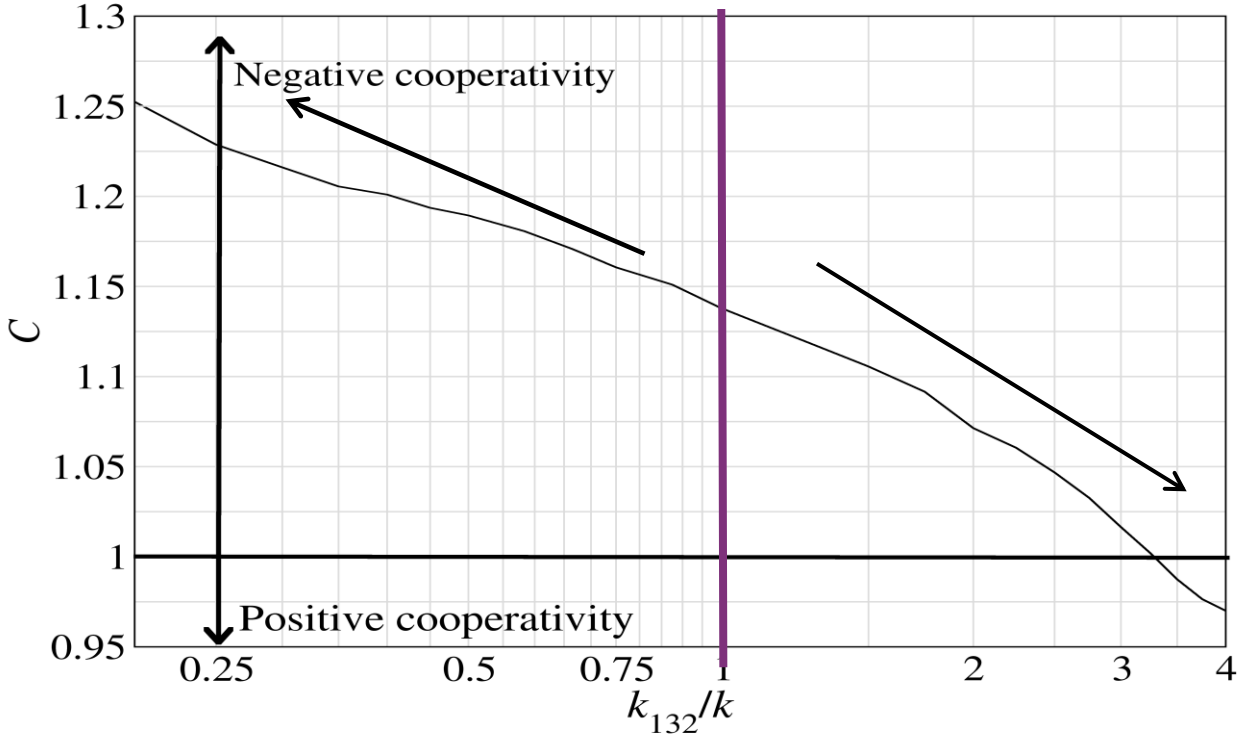
Decreasing stiffness in red regions leads to increased –ve cooperativity

Decreasing stiffness in blue regions leads to +ve cooperatvity

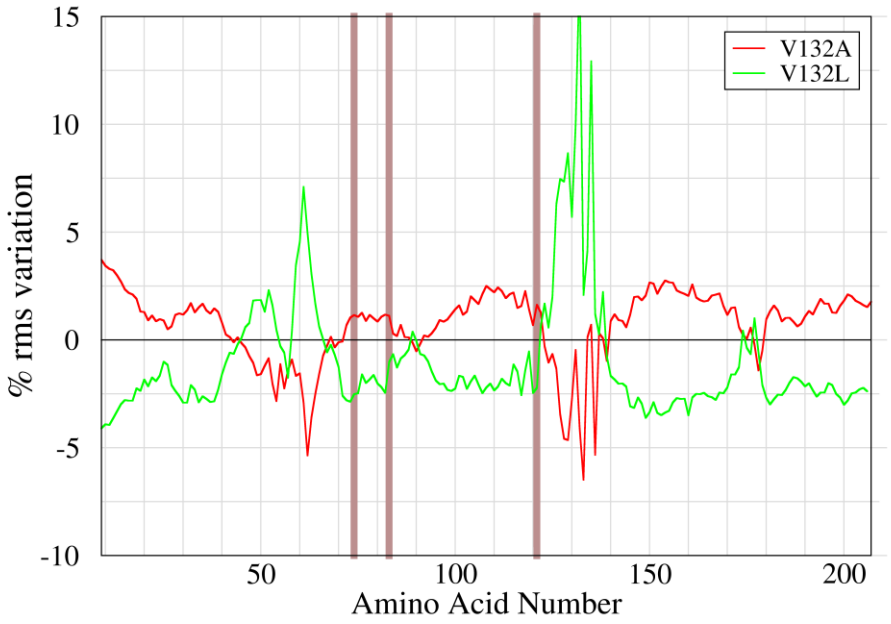
Protein engineering in practice - Variation in V132



Alanine Valine Leucine



Motion Variation



- At the dimer interface changes in hydrophobic interaction have big effect on motion across protein
- Mutation to alanine or leucine have opposite effects

cAMP affinities for proteins

- The ratio of the second to first dissociation constants for cAMP (K_2/K_1) for wild type and mutant CAP proteins

	CAP protein	K_2/K_1 (ENM)	K_2/K_1 (ITC)		
☺	Wild Type	1.13	1.6	✓	
☺	V132A/*k*=0.25	↑	↑	✓	
☺	V132L/*k*=0.25	↓ +ve	↓ +ve	✓	
☺	H160L/*k*=0.25	↑	↑	✓	H-bond removal
☯	V140A V140/*k*=0.25 V179/*k*=4	↓ +ve	↓ +ve	✓	X-ray shows conformational change
☺	V140L/*k*=4	↑	↑	✓	

☺High resolution X-ray – confirm no change in protein structure

Super-Coarse Graining

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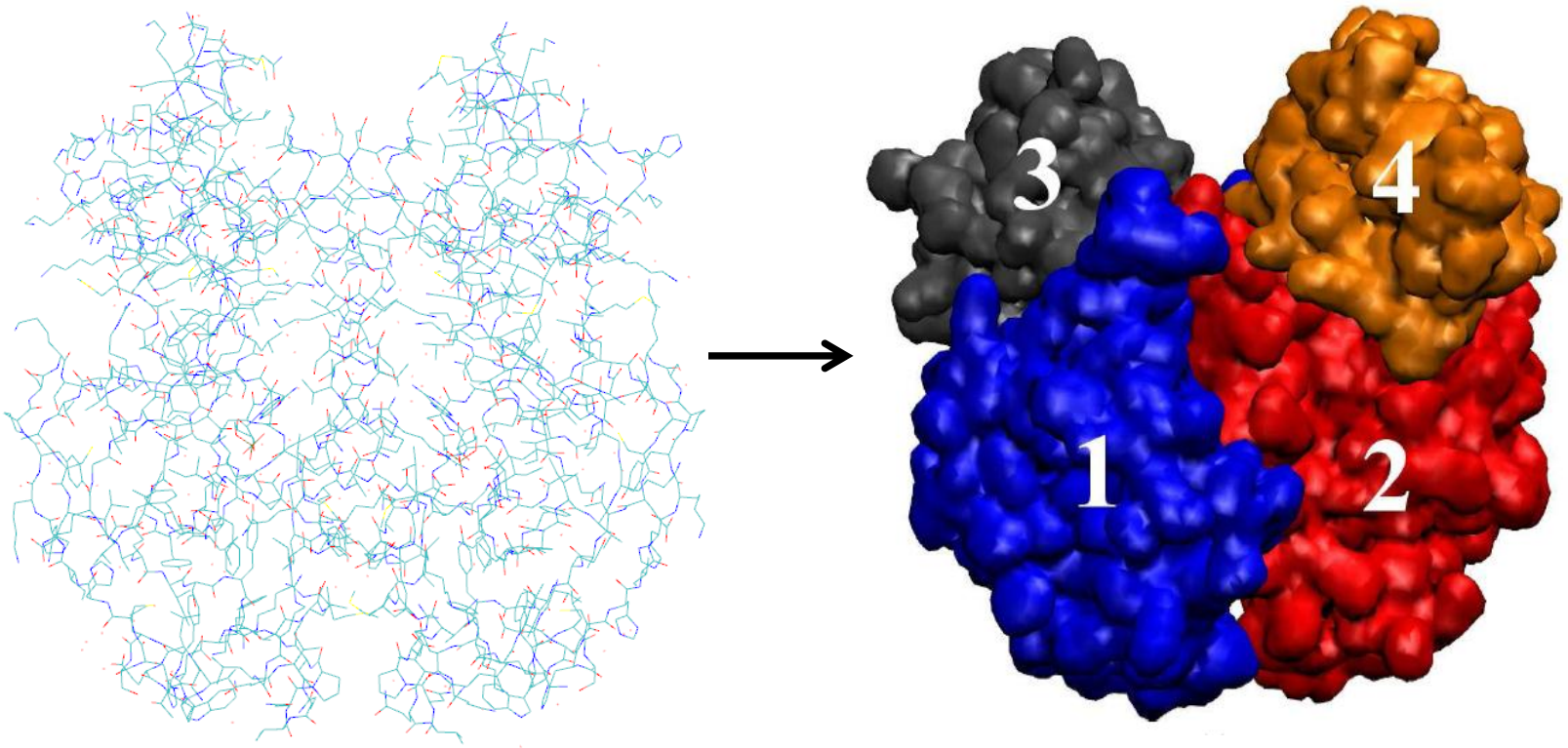
- ENM provides valuable insights
- ENMs can predict motion and allostery

But….

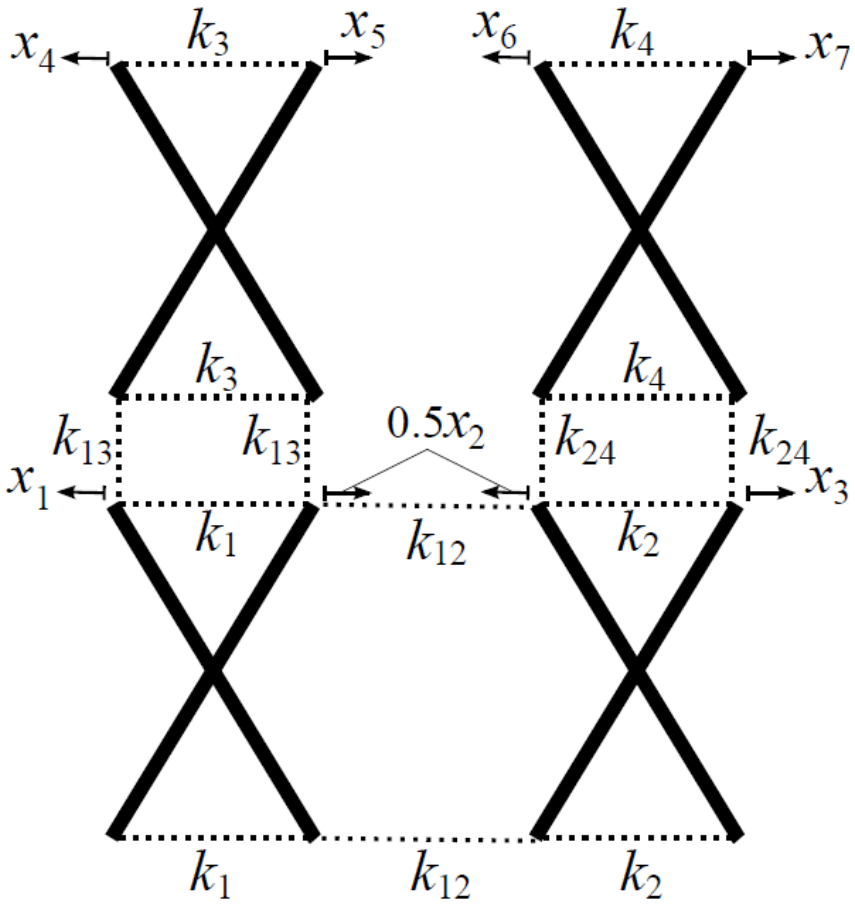
- A 3N x 3N interaction matrix to work with
- & it looks like there are some generic features that could be captured by a simpler model

Rotational Translational Block method



- Now (4 x 6) x (4 x 6) matrix – interactions easier to identify

Super-Coarse Grain Models



- CAP monomer approximated as 4 'scissor' domains
- Internal spring constants $k_1 = k_2$, $k_3 = k_4$
- 3 independent coupling spring constants k_{12}, k_{13}, and k_{24}

Super-Coarse Grained Models

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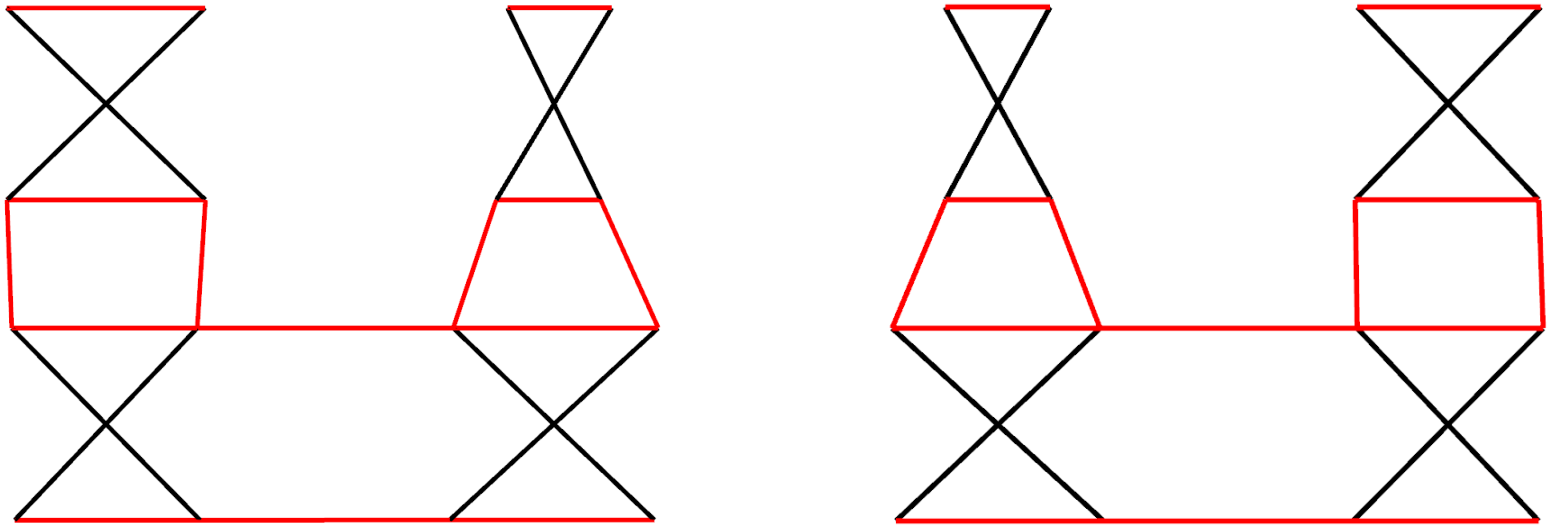

Super Coarse-Grained CAP

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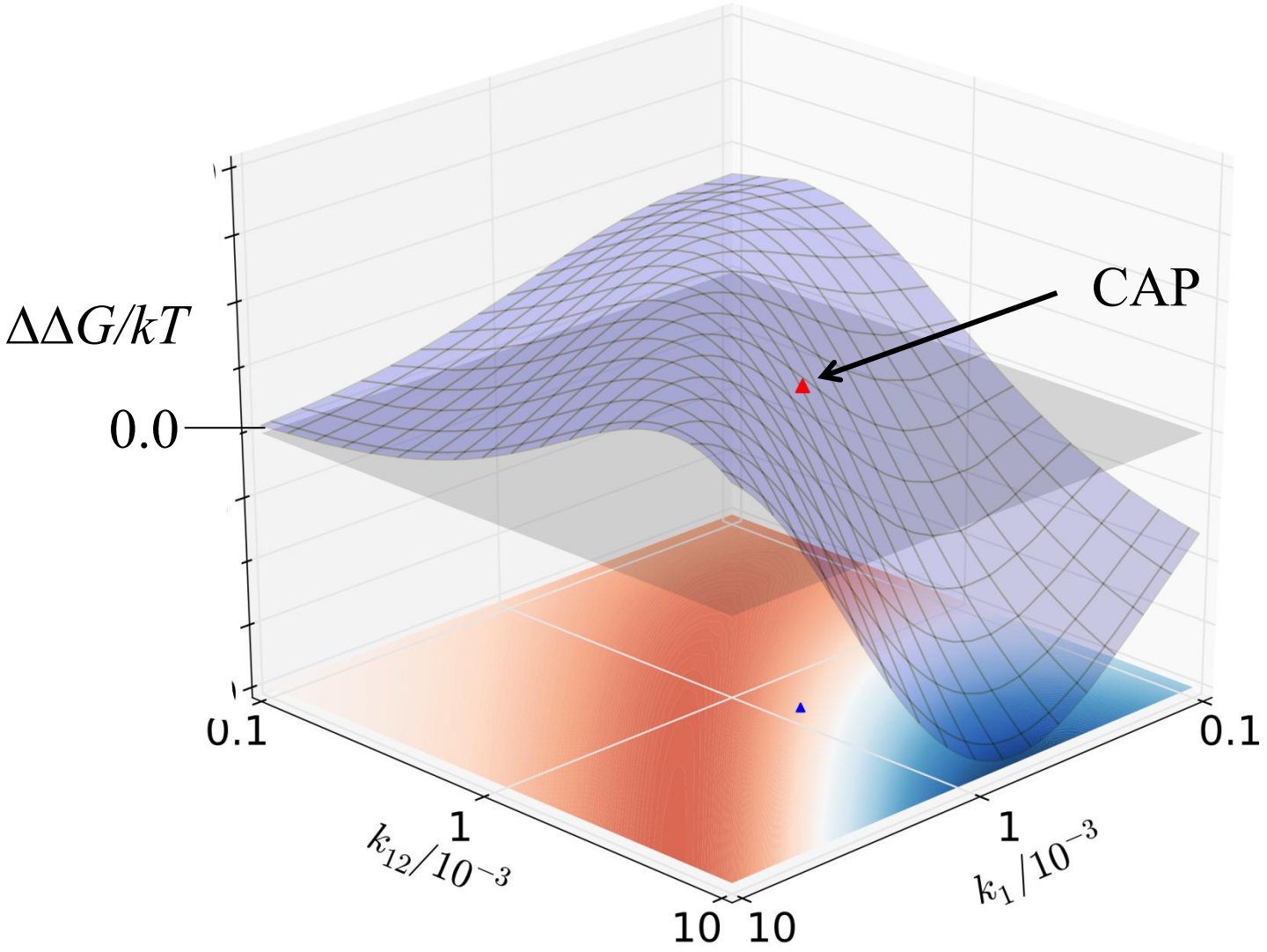
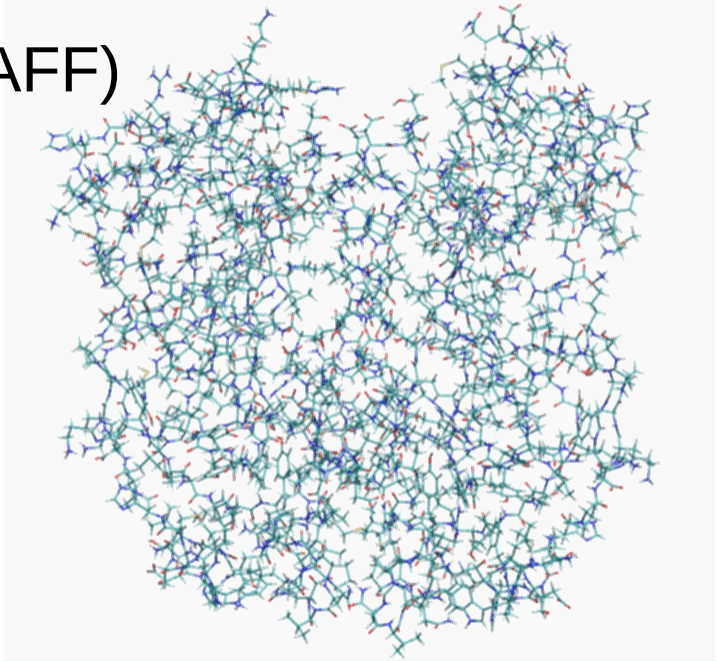


Atomic Simulations

- Full atomistic simulations in water (AMBER, 2 fs time step, ff99SB/GAFF)
- 200+ ns molecular dynamics
- 6457 protein atoms
  - 401 amino acids
  - 10297 water molecules



$$V = \sum_{\text{bonds}} \frac{1}{2} k_b (l - l_0) + \sum_{\text{angles}} \frac{1}{2} k_a (\theta - \theta_0) + \sum_{\text{torsions}} \frac{1}{2} V_n \left(1 + \cos(n\varphi - \gamma)\right)$$

$$+ \sum_{i,j} \frac{1}{4\pi\varepsilon\varepsilon_0} \frac{q_i q_j}{r_{ij}} + \sum_{i,j} 4\varepsilon_{ij} \left(\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right)$$

Atomistic Simulations

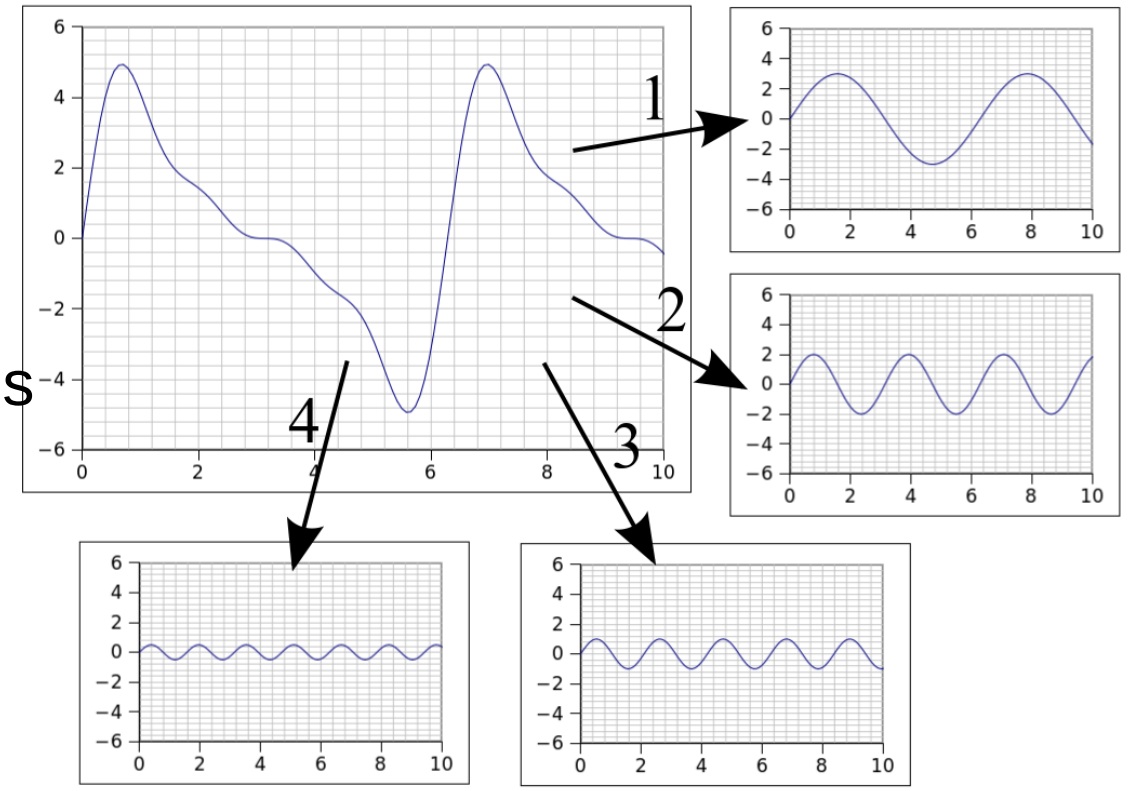
- Analysis of 200 ns trajectory by principal component analysis (PCA)

$$\mathbf{F} = \left\langle \mathbf{M}^{1/2} \left(\mathbf{x} - \langle \mathbf{x} \rangle \right) \left(\mathbf{M}^{1/2} \left(\mathbf{x} - \langle \mathbf{x} \rangle \right) \right)^{T} \right\rangle$$

- calculate and diagonalize the (mass-weighted) covariance matrix.
- Pulls out key

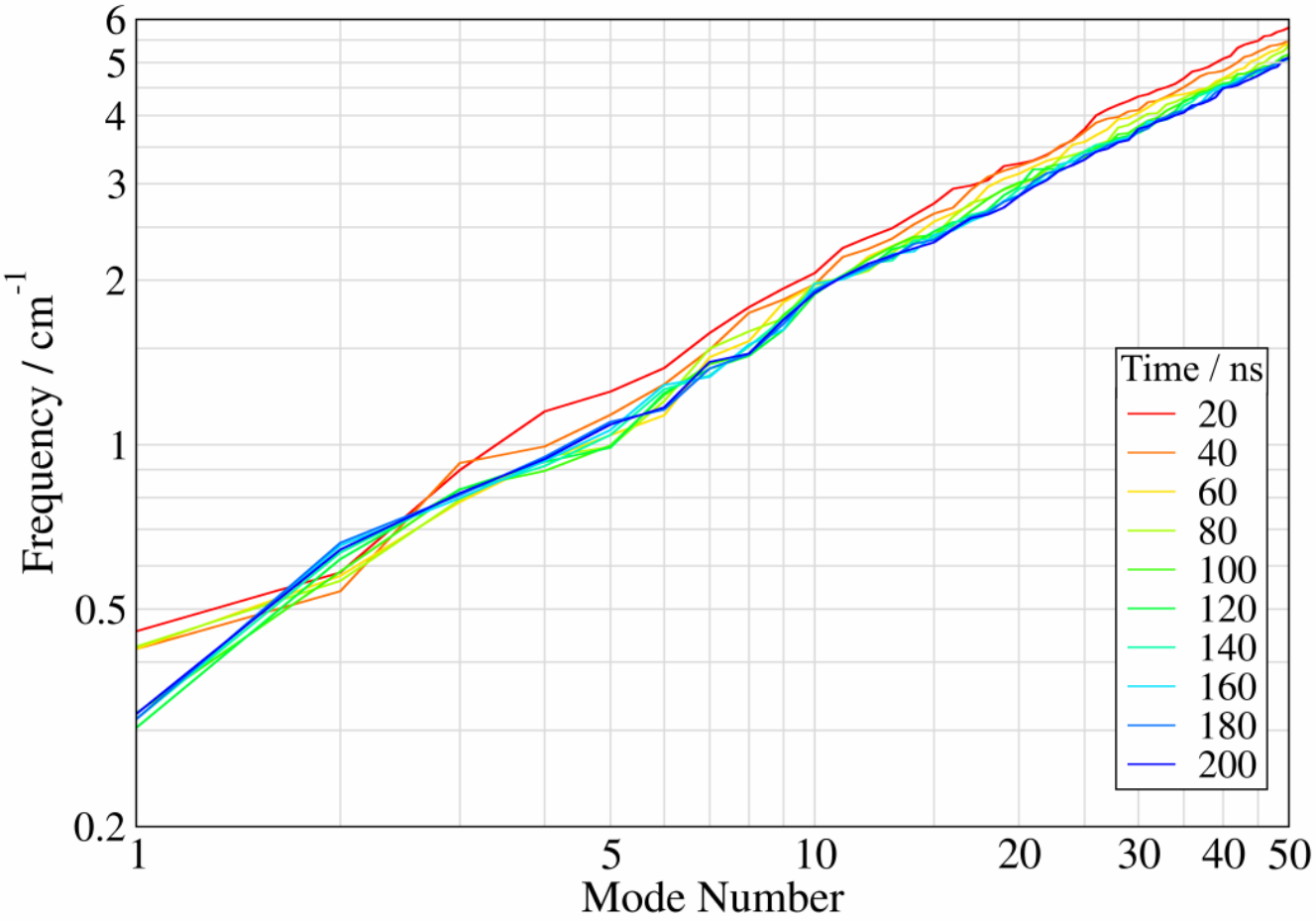
dynamical modes

of motion & frequencies



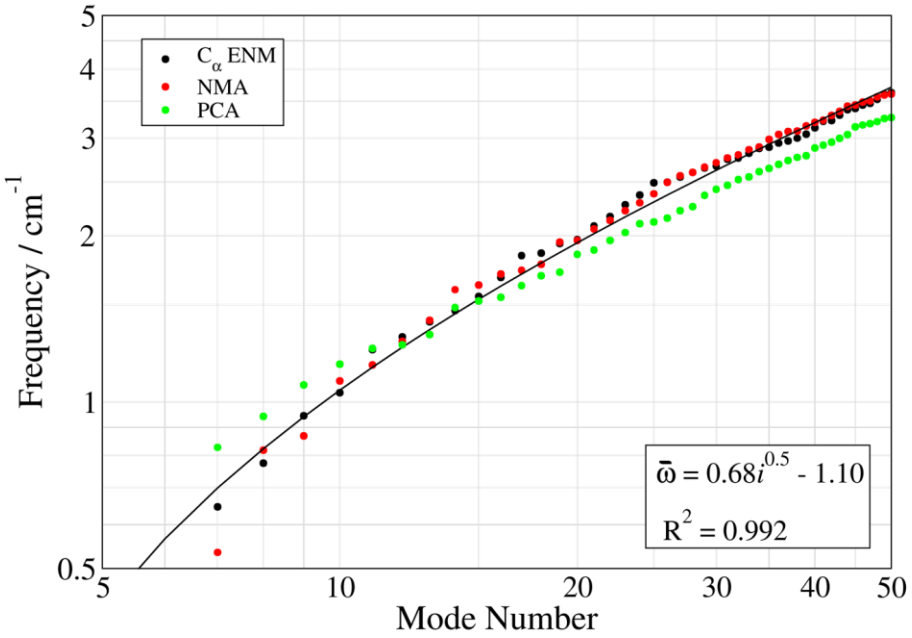
PCA comparison

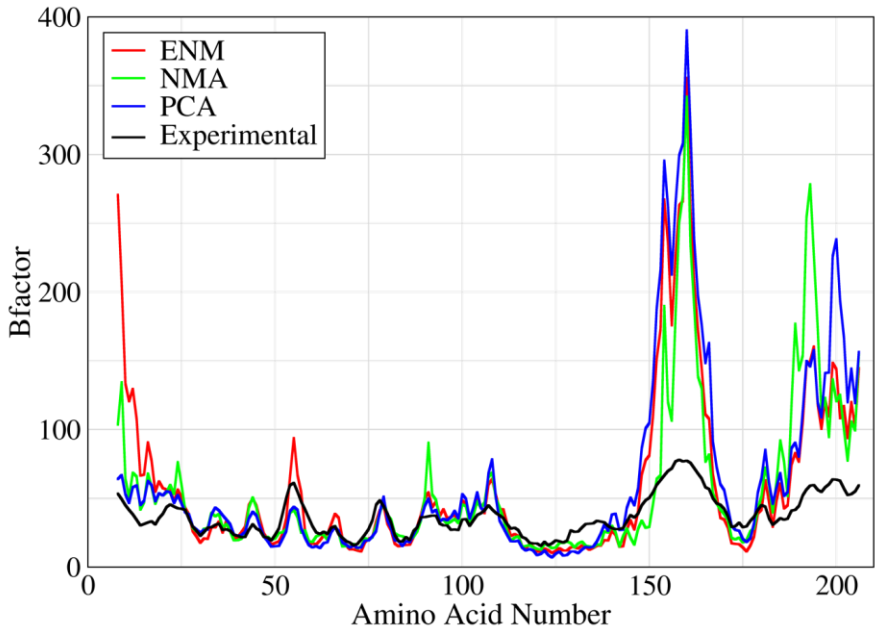
- Test repeatability of analysis



Comparisons

- ENM, PCA, and atomic NMA produce similar frequencies and atomic motions





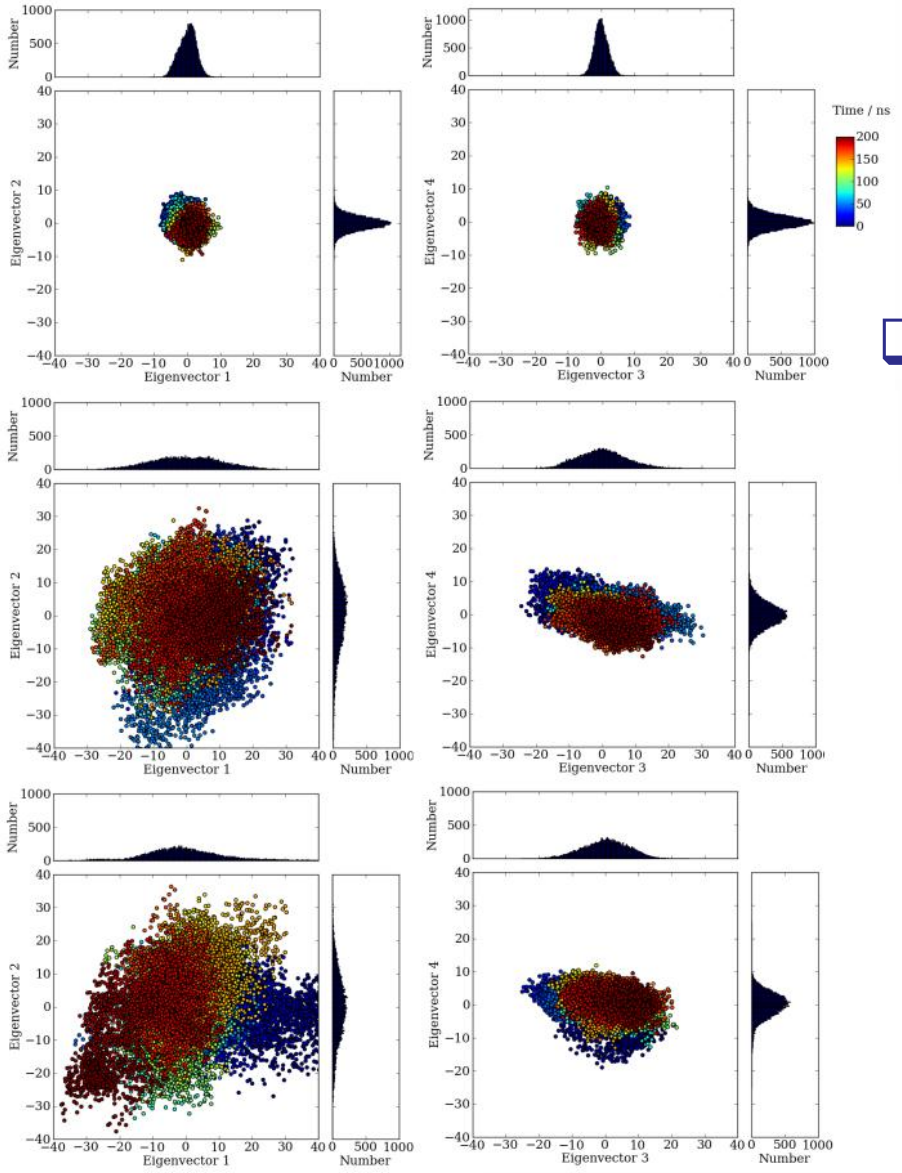
C_α and NMA data scalled to fit PCA data (x0.447)

Motion of protein

apo-CAP

$holo_1$-CAP

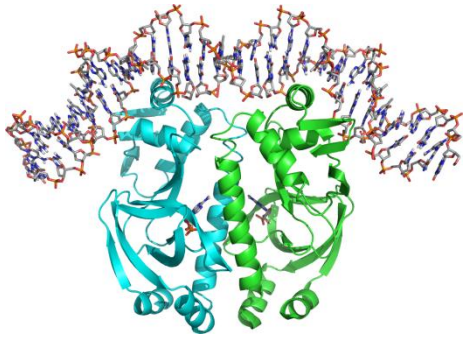
$holo_2$-CAP



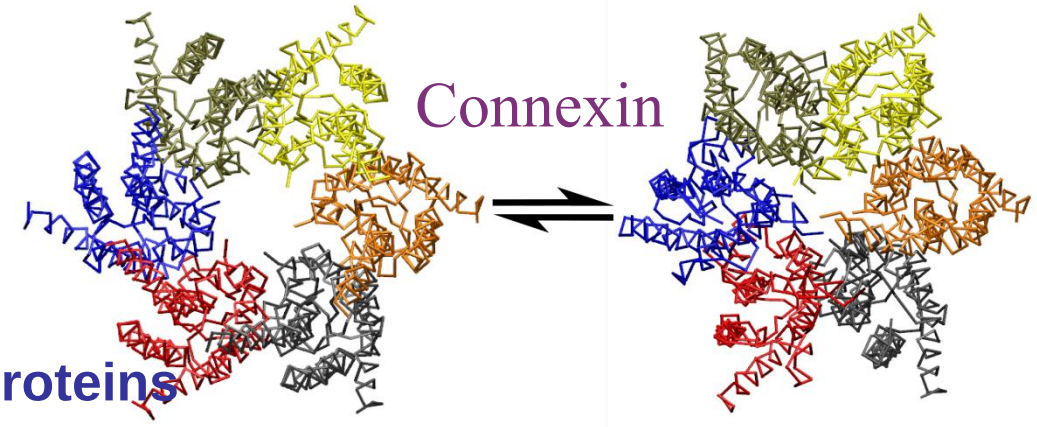
- Protein dynamics changes on ligand binding

Final ideas

- Allosteric binding modelled at different levels suggests proteins may have evolved to harness dynamic pathways
- Also shown for LacR, GlxR (larger effects)
- Possibility of studying dynamic contribution to DNA binding



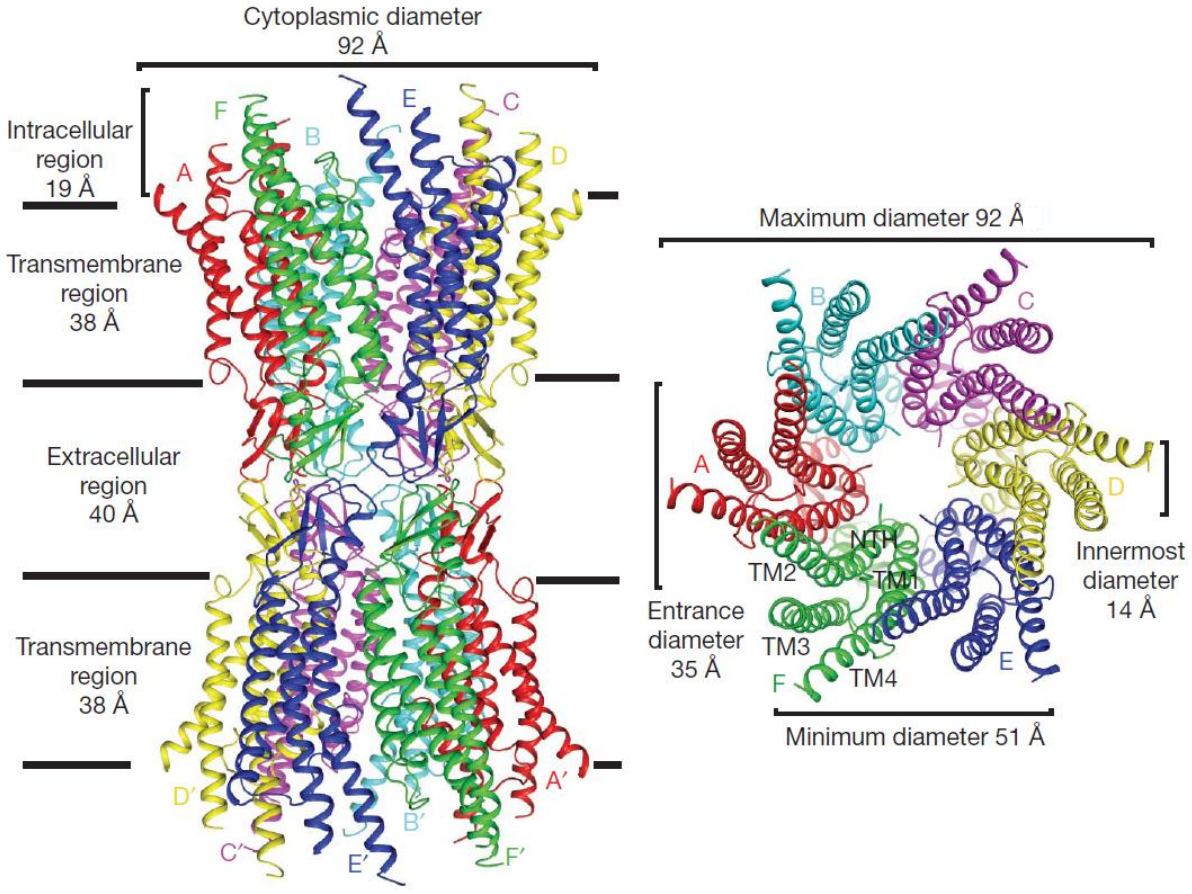
CAP/DNA



- Also see how dynamics is of key importance for other proteins

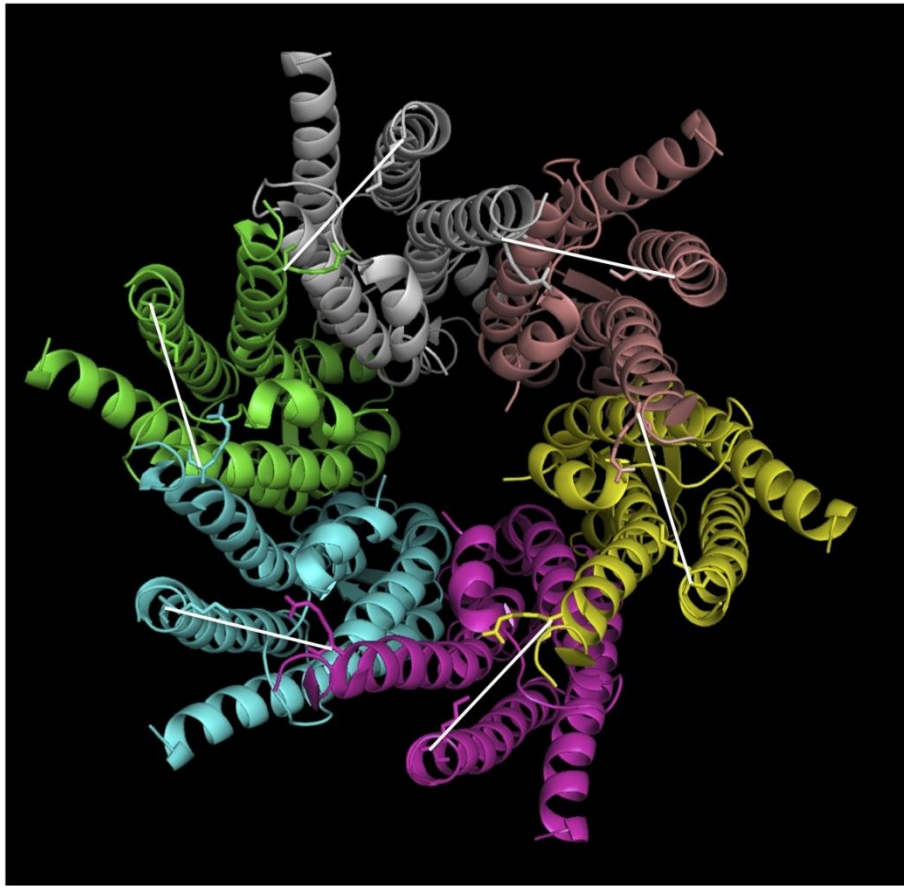
Connexin 26 (a gap junction protein)

- 6 monomers form a funnel pore
- 2 pore units form the inter-cellular pathway



CO_2 binding

- CO_2 binds between monomers at the end of the channel



Connexin ENM

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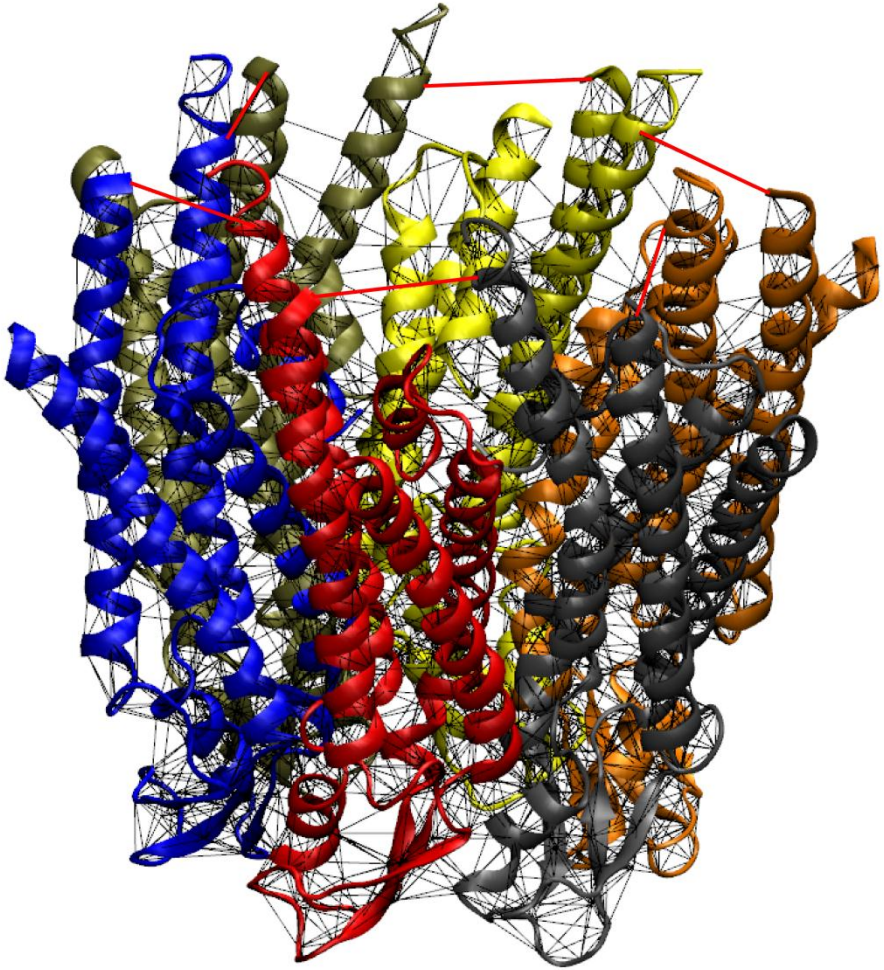
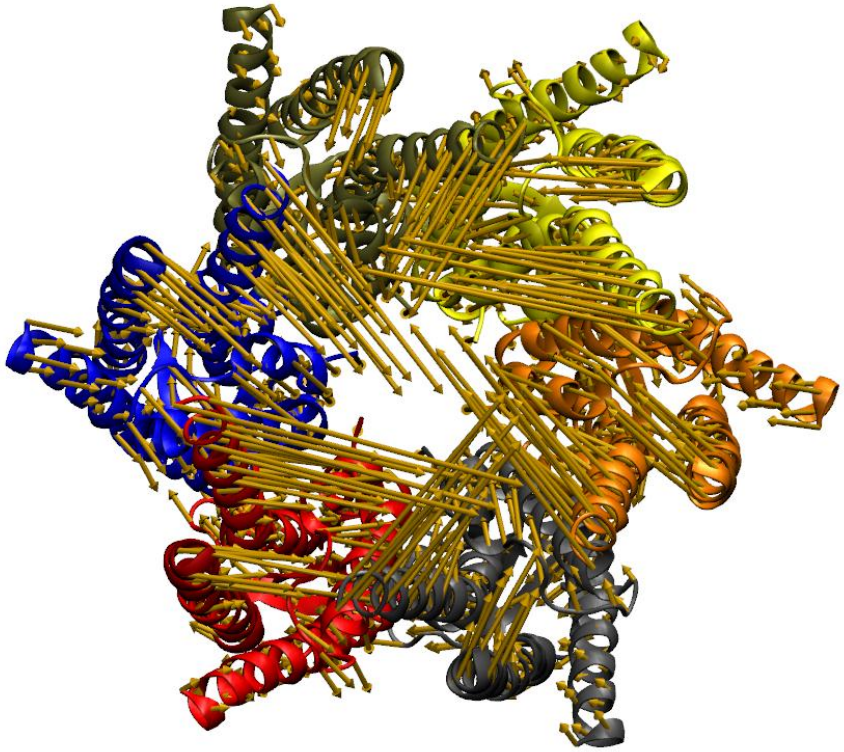


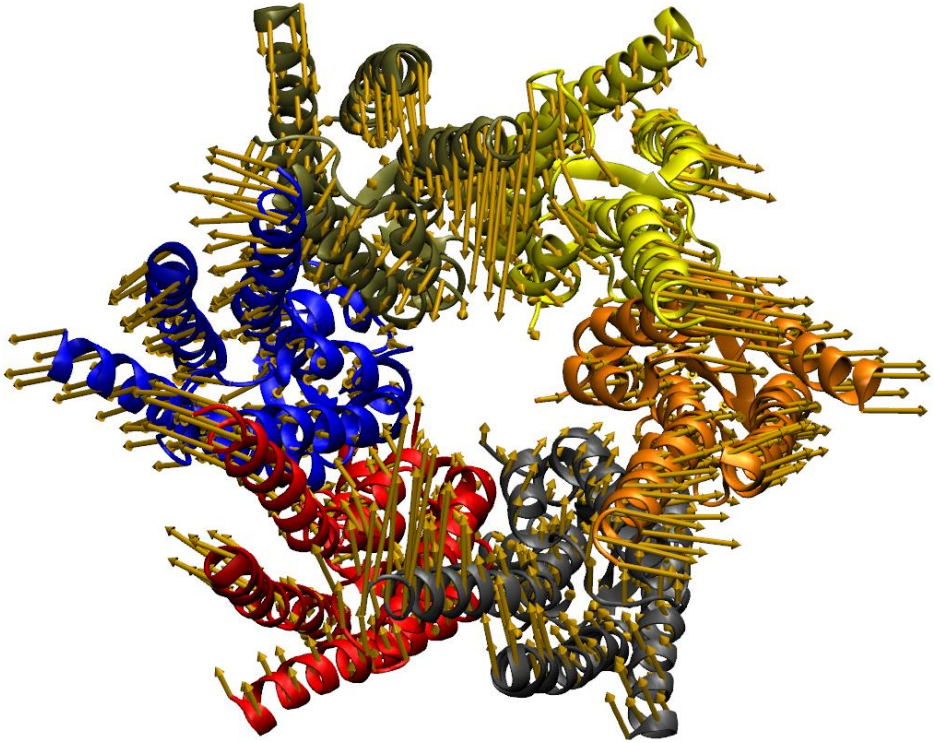
$$V_{ij} = \begin{cases} \frac{k_{ij}}{2}\left(r_{ij} - R_{ij}\right)^2 & R_{ij}^2 \leq R_c^2 \\ 0 & R_{ij}^2 > R_c^2 \end{cases}$$

Variation in Main mode

Durham University



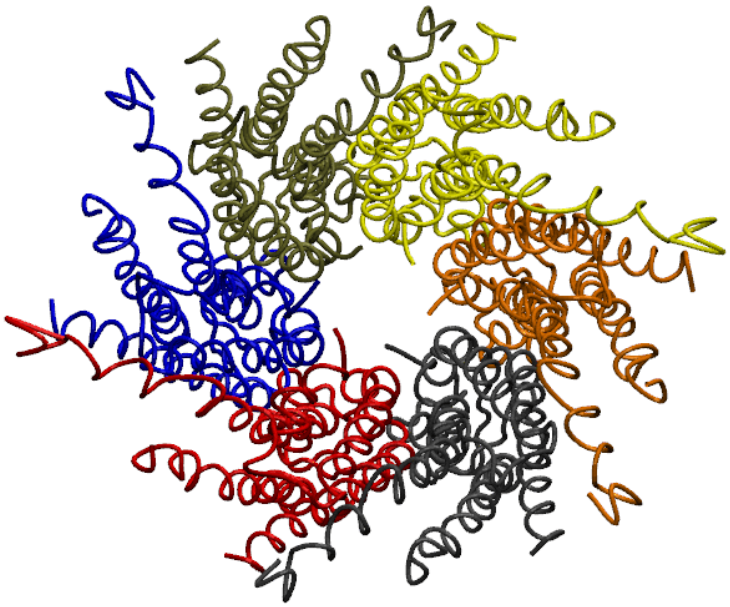
No CO_2



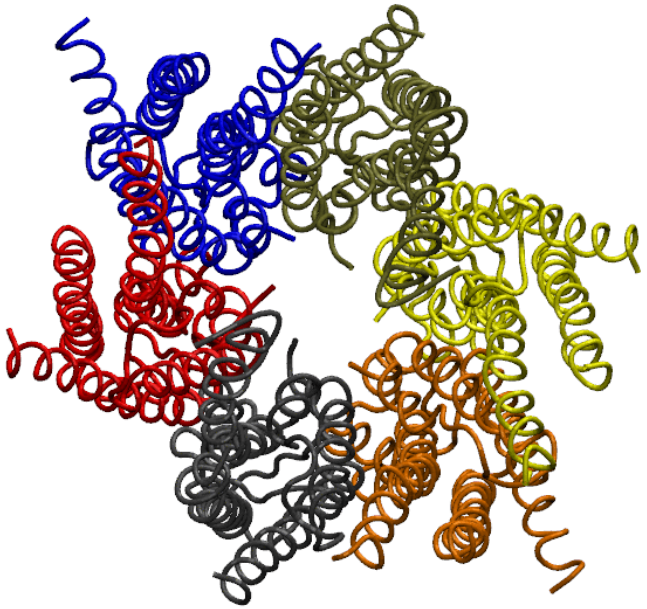
CO_2

Variation in Main mode

Durham University



No CO_2



CO_2

When CO_2 is bound the channel never closes

Summary

- Calculations point to interesting phenomenon "dynamic allostery"
- Thermodynamics basis for this is the vibrational contribution to $\Delta\Delta G$
- Simulations of ENM, super-CG and atomistic models provide valuable insights
- Third site mutation provides a mechanism to control this effect
- Potential for many interesting insights from the role of dynamics in protein function