Mapping Conformational Changes in Proteins through Crystallographic Refinement and Computational Approaches

Shekhar C Mande, CDFD, Hyderabad

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Laboratory Activities

Crystallography (Biochemical and Biophysical analysis) of *M. tuberculosis* proteins:

        Hsp60, Hsp65, Hsp10
        AhpC, Trx's, TrxR, Glutaredoxin
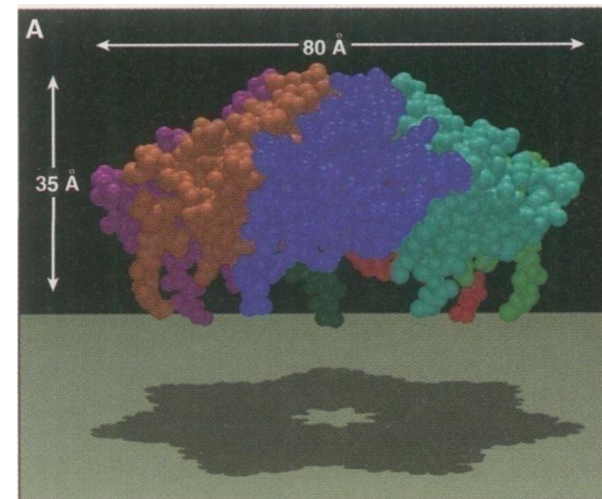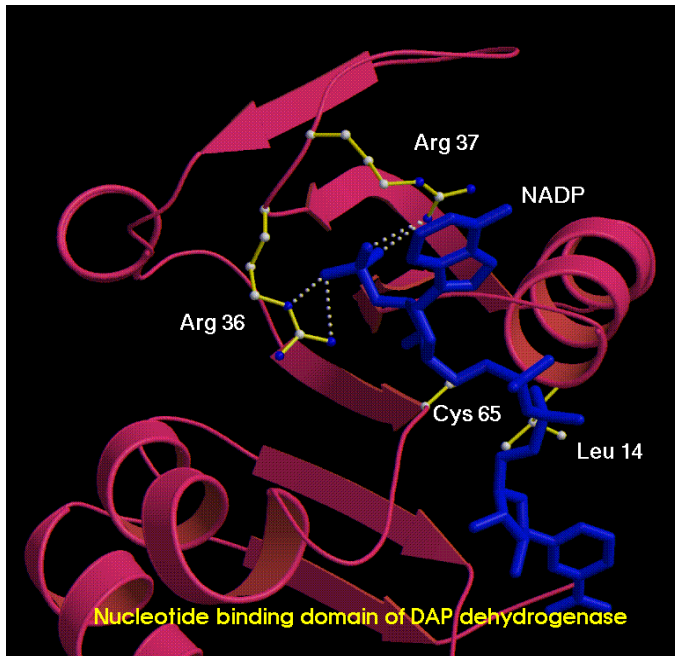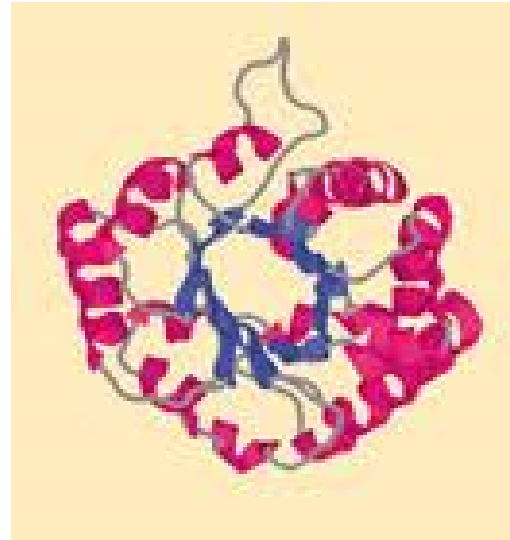   Chorismate mutase, toxin-antitoxin, CRP

Networks

        Genome-wide protein interaction networks
        Dynamics of networks
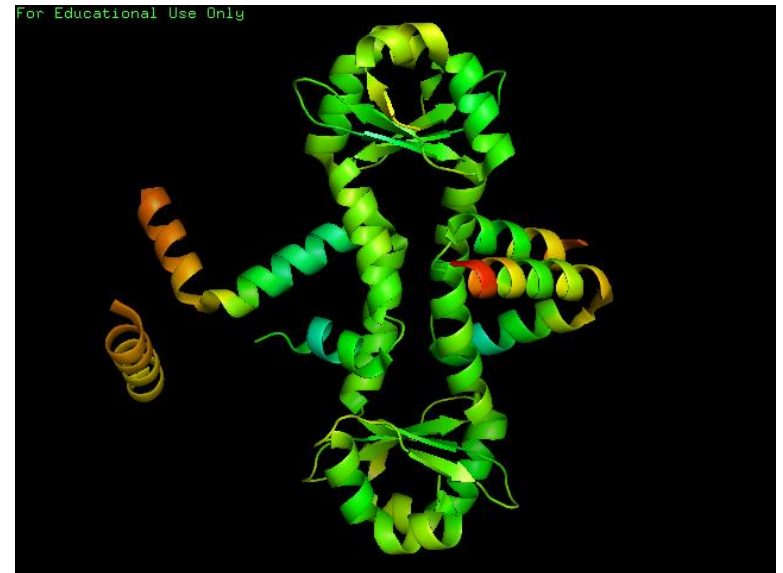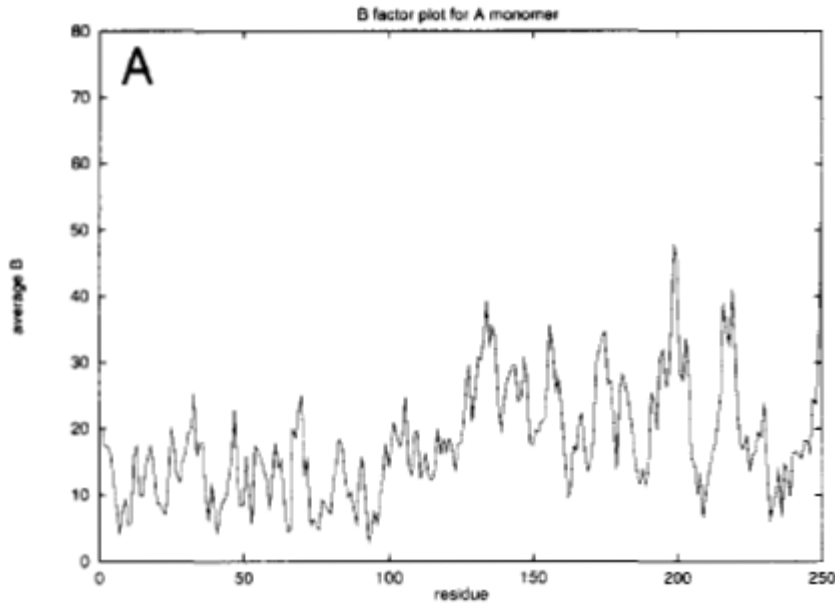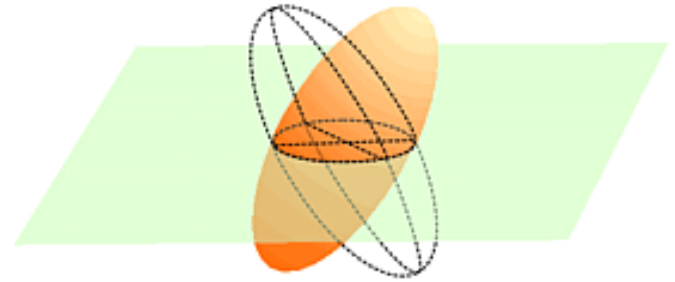   Prediction of genome-wide lethalities, synthetic lethalities

Protein Structures:
Beyond pretty pictures

Arg 37

NADP

Arg 36

Cys 65

Leu 14

Nucleotide binding domain of DAP dehydrogenase

A

80 Å

35 Å

Thermal fluctuations in proteins:
B-factor

$$B = 8 \pi^2 \langle u^2 \rangle$$

B factor plot for A monomer

A typical B-factor represents isotropic movement of an atom around its mean position

Need to account for dynamic behaviour of proteins

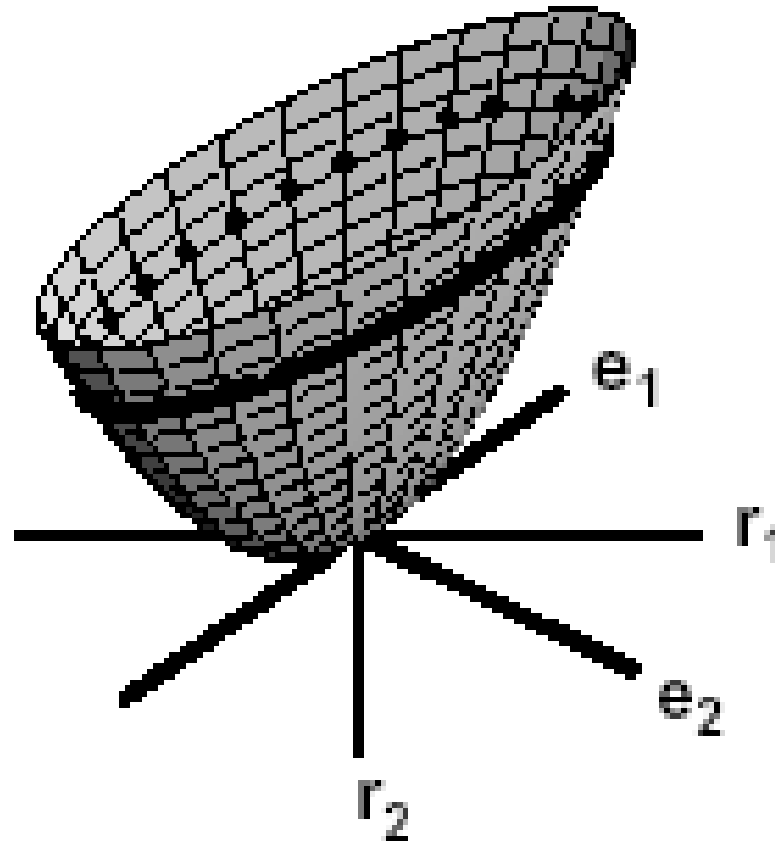$$U(\chi) = \sum_i (\chi_i - \chi_i^0) \frac{\partial U}{\partial \chi_i}\bigg|_{\chi = \chi_o} +$$

$$\frac{1}{2!} \sum_{ij} \frac{\partial^2 U}{\partial \chi_i \partial \chi_j}\bigg|_{\chi = \chi_o} (\chi_i - \chi_i^0)(\chi_j - \chi_j^0) +$$

$$\frac{1}{3!} \sum_{ijk} \frac{\partial^3 U}{\partial \chi_i \partial \chi_j \partial \chi_k}\bigg|_{\chi = \chi_o} (\chi_i - \chi_i^0)(\chi_j - \chi_j^0)(\chi_k - \chi_k^0) + \ldots\ldots$$

Harmonic approximation

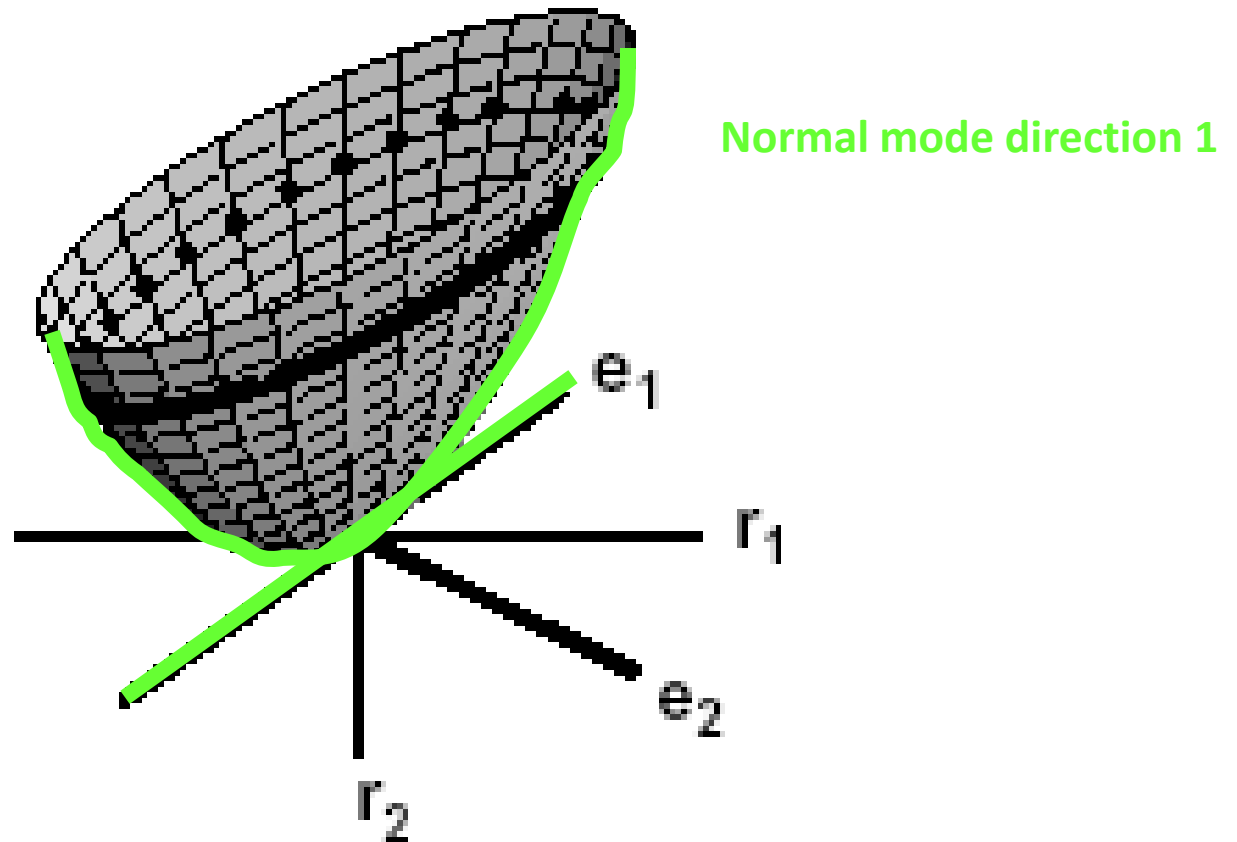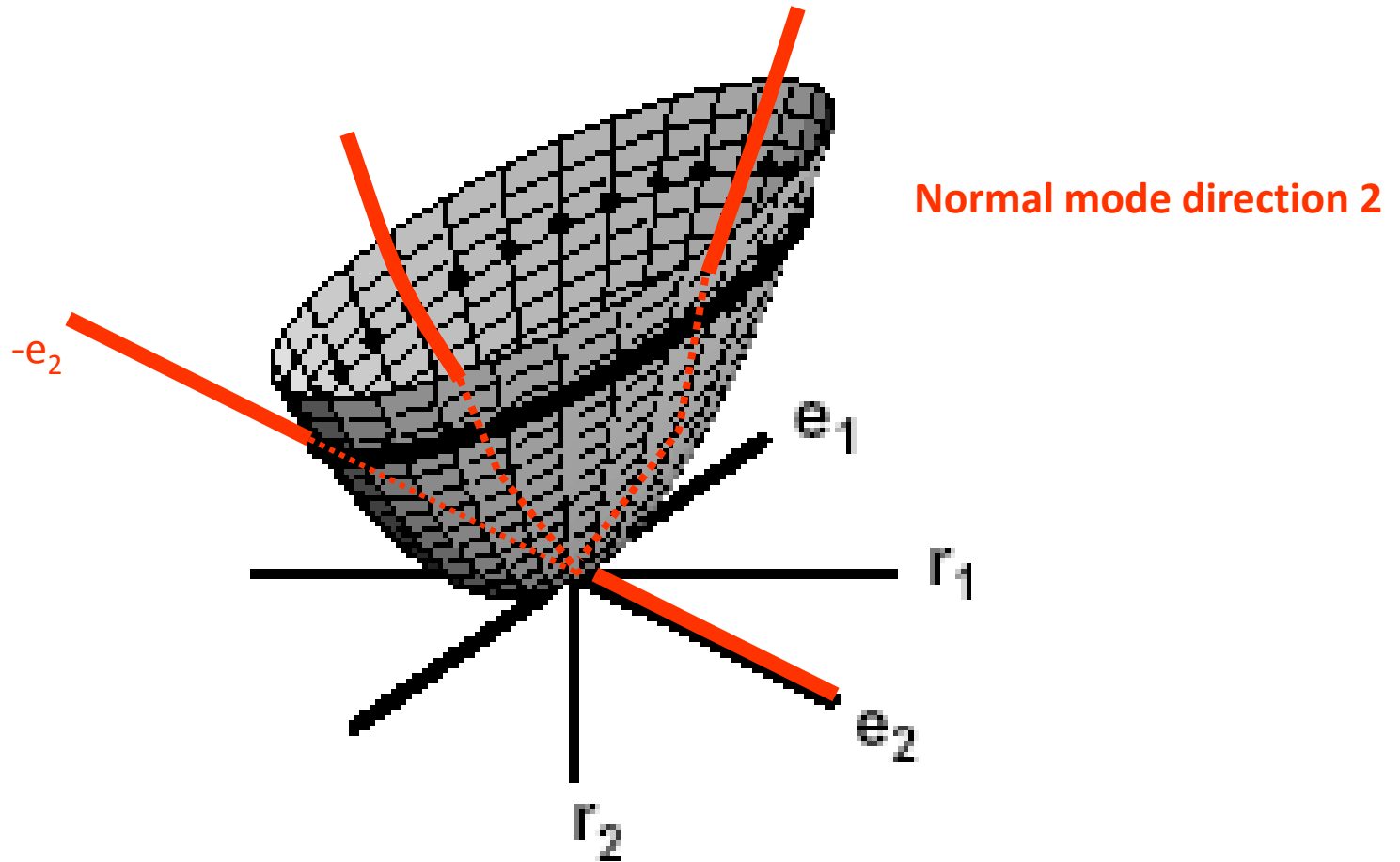$$U(\chi) \cong \frac{1}{2} \sum_{ij} \frac{\partial^2 U}{\partial \chi_i \partial \chi_j}\bigg|_{r = r_o} (\chi_i - \chi_i^0)(\chi_j - \chi_j^0)$$

NMA

$$U(r) = 0.5 \, (r - R_{min})' \cdot K(R_{min}) \cdot (r - R_{min})$$

NMA

Normal mode direction 1

$$U(r) = 0.5 \, (r - R_{min})' \cdot K(R_{min}) \cdot (r - R_{min})$$
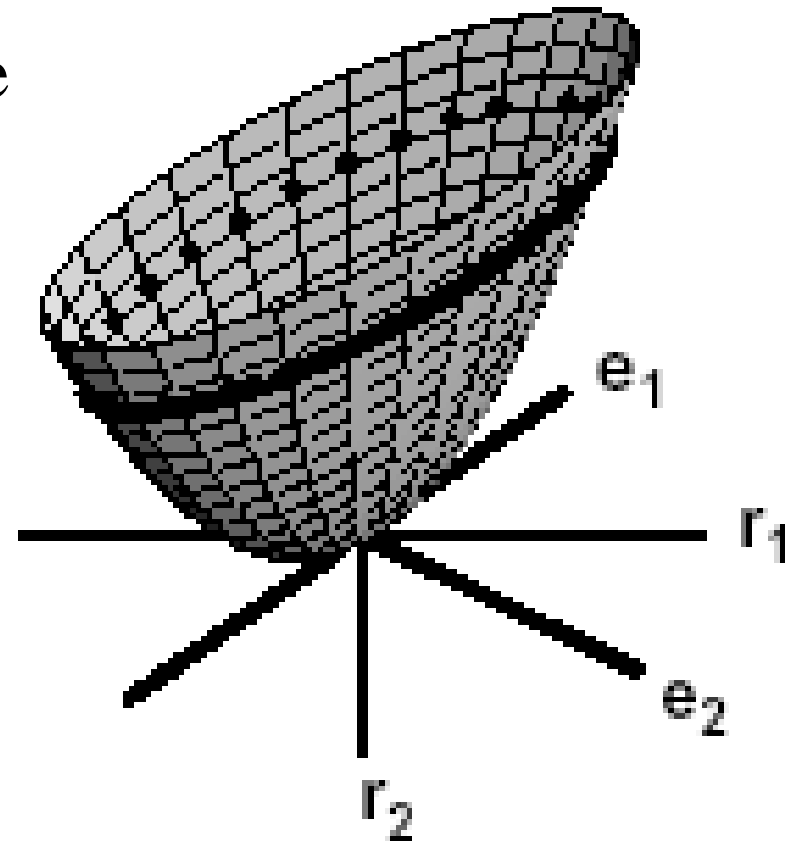
NMA

Normal mode direction 2

$$U(r) = 0.5 \, (r - R_{min})' \cdot K(R_{min}) \cdot (r - R_{min})$$

Properties of NMA

- The eigenvalues describe the energetic cost of displacing the system by one length unit along the eigenvectors.

- For a given amount of energy, the molecule can move more along the low frequency normal modes

- The first six eigenvalues are 0, corresponding to rigid body movements of the protein
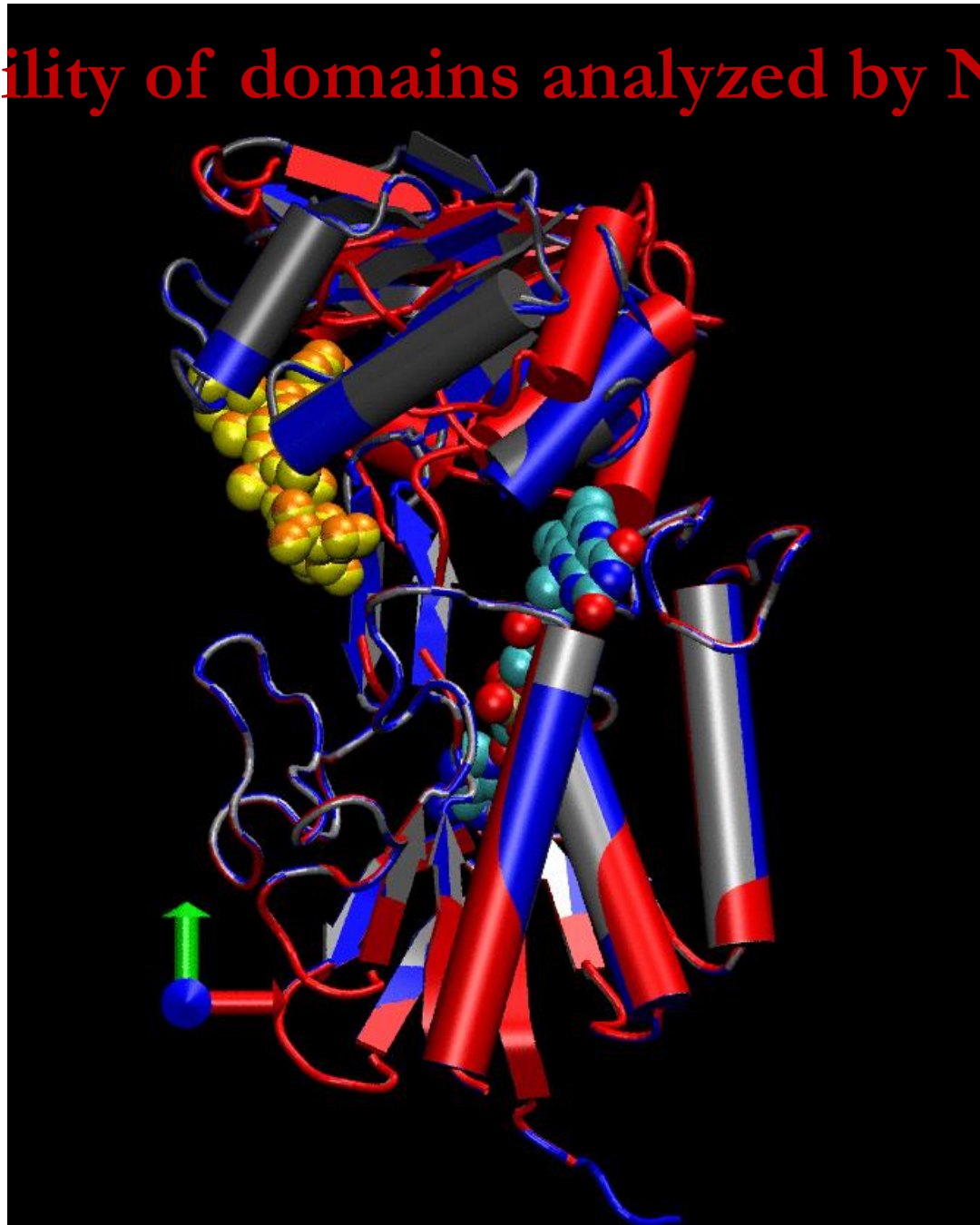
M. tuberculosis thioredoxin reductase

Functions of the Thioredoxin System
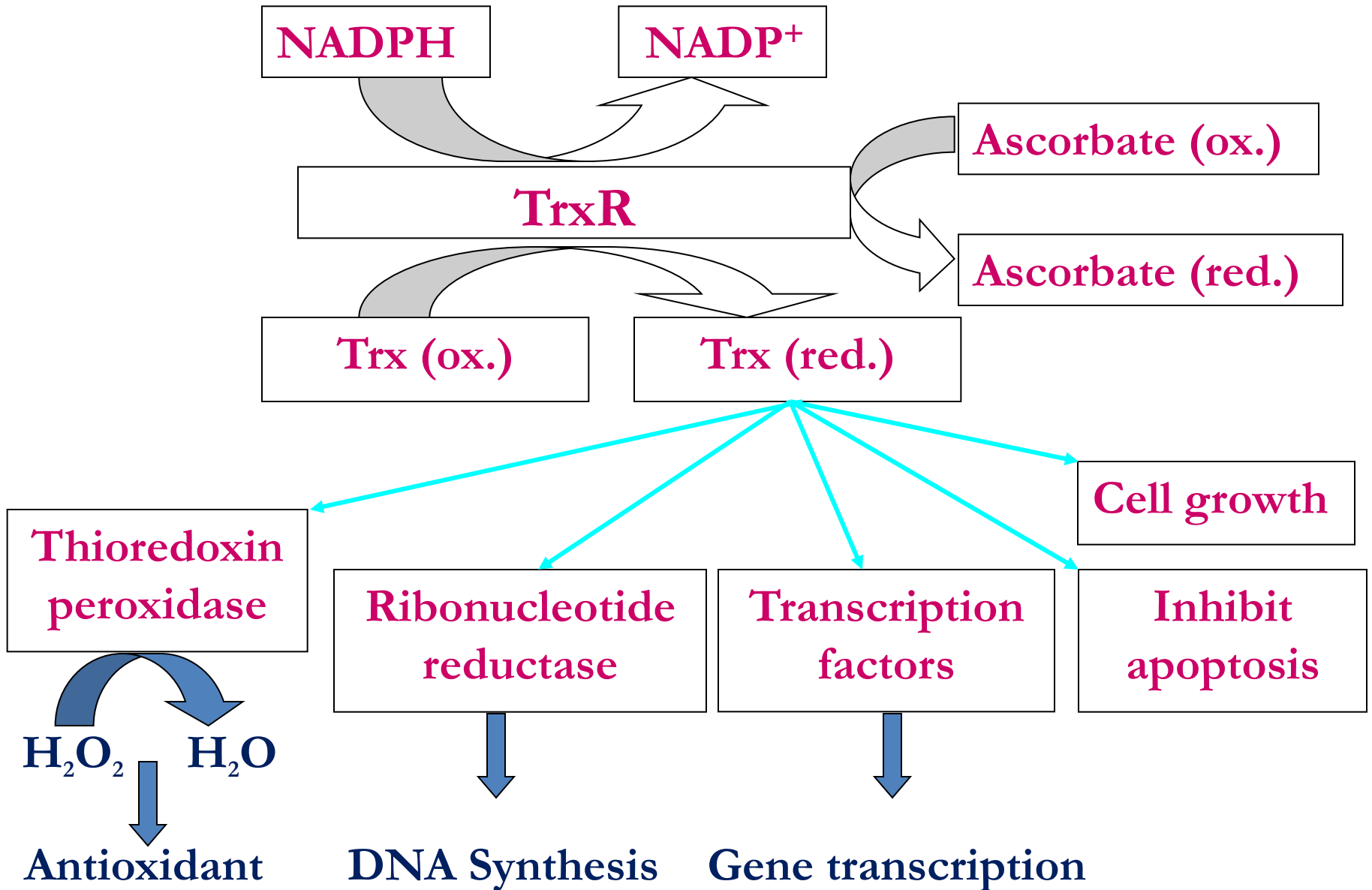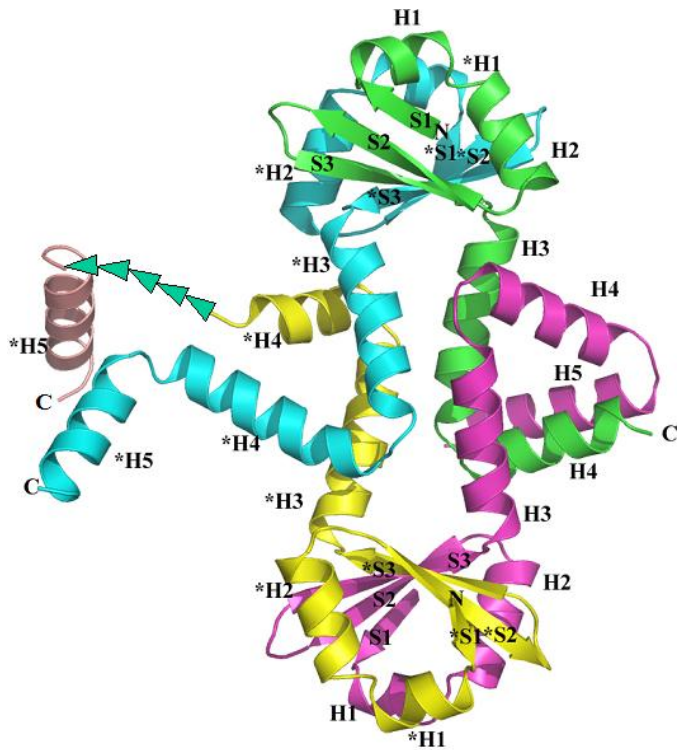
Domain Flexibility of *Mtb* TrxR
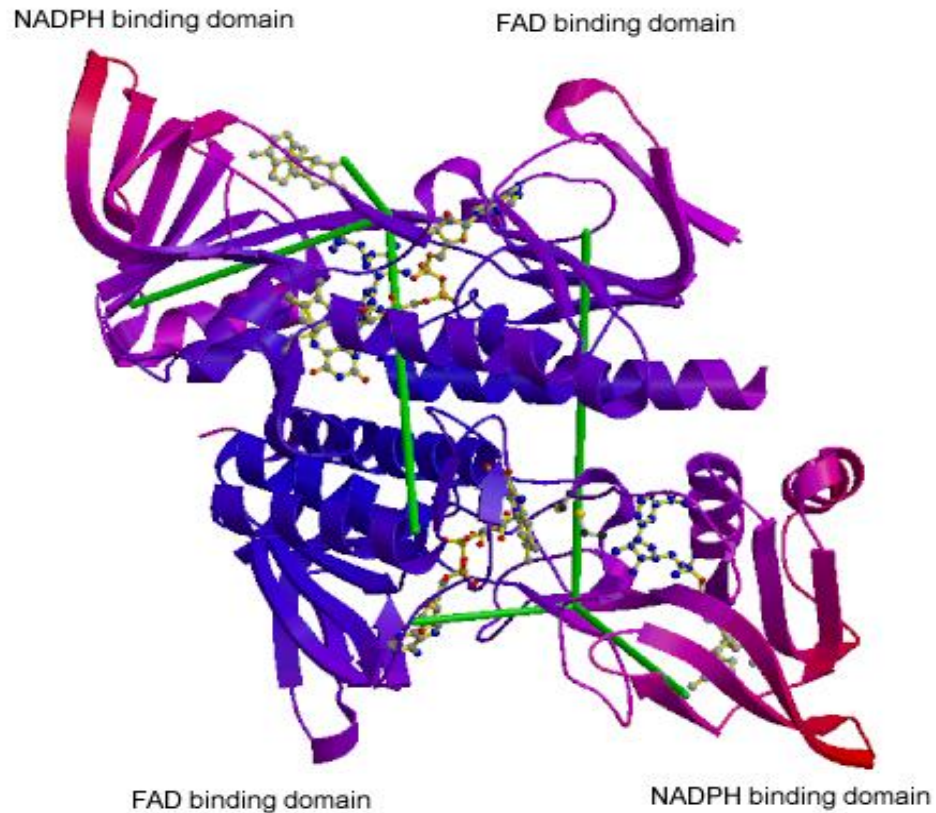
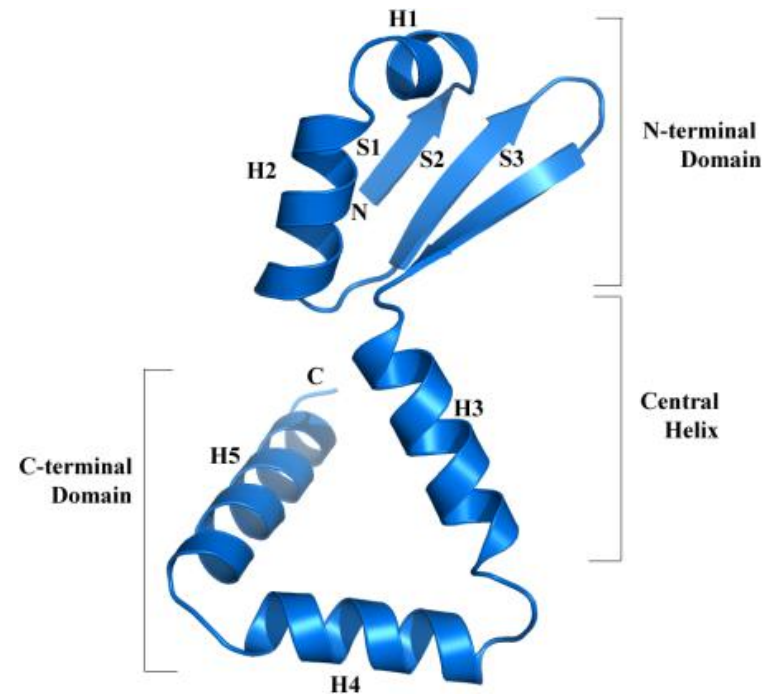# Flexibility of domains analyzed by NMA
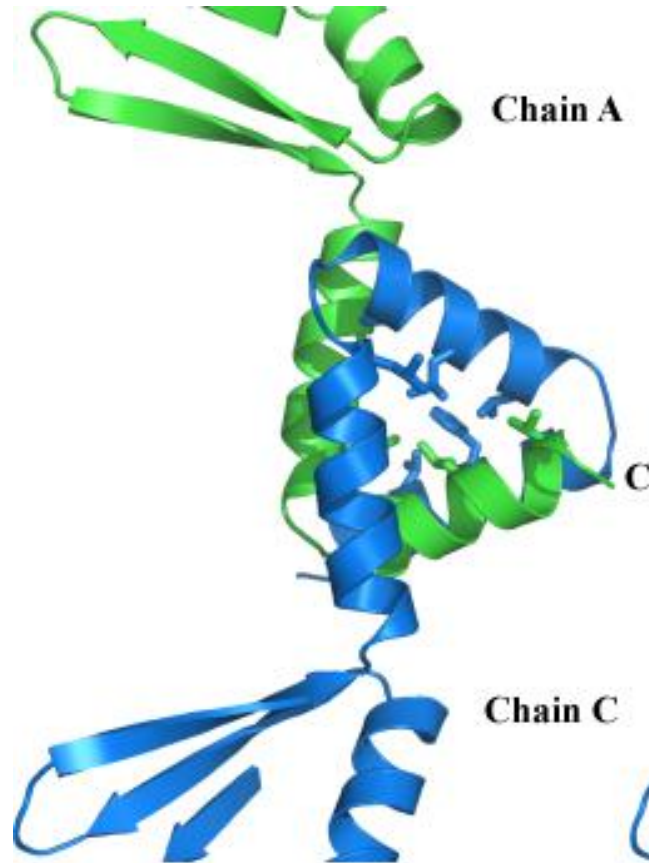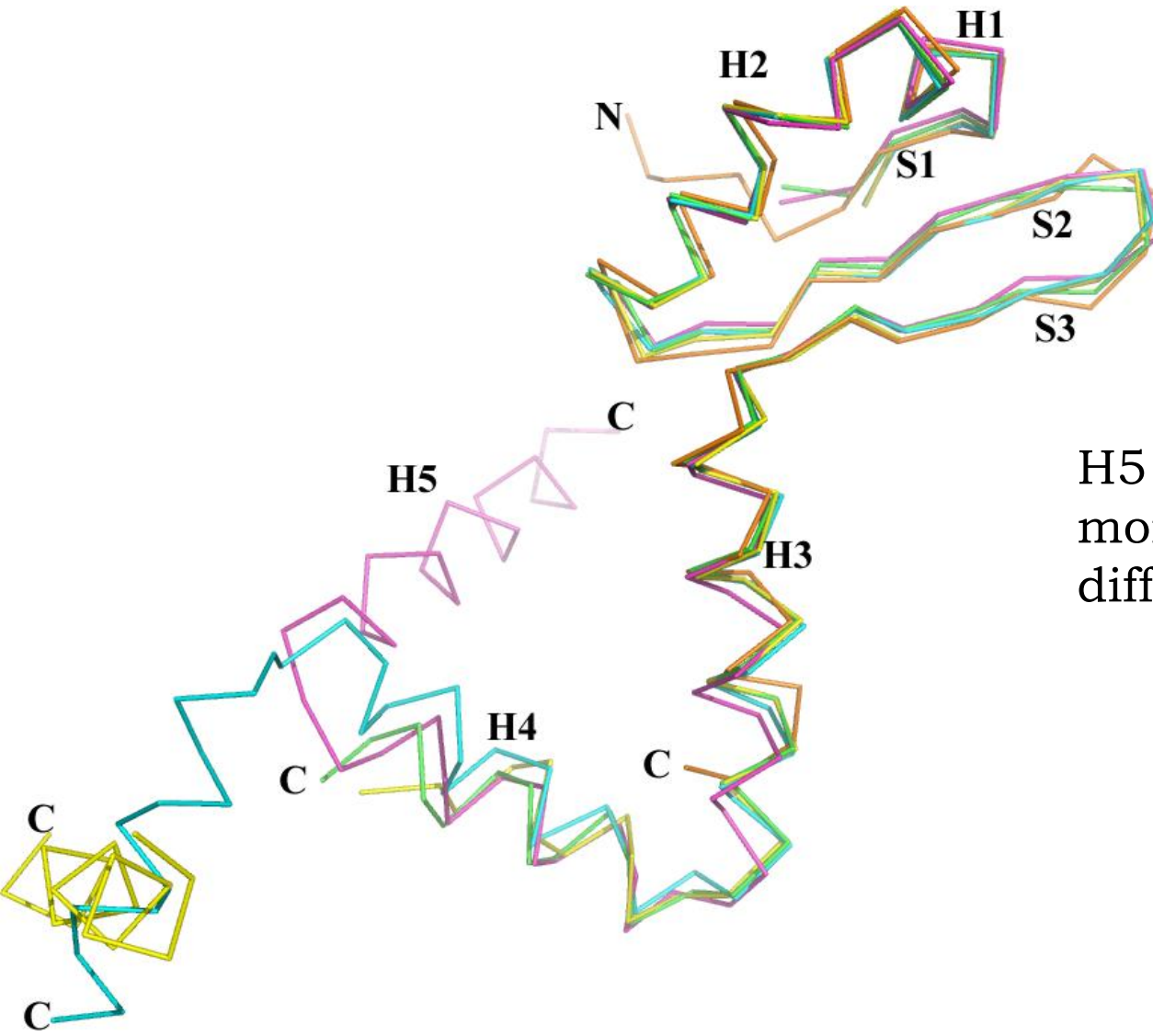
M. tuberculosis YefM anti-toxin

Overall structure
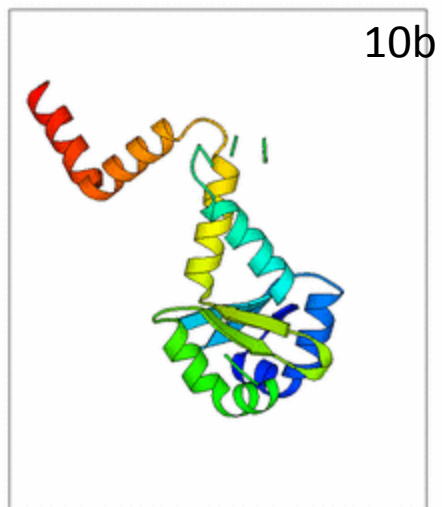

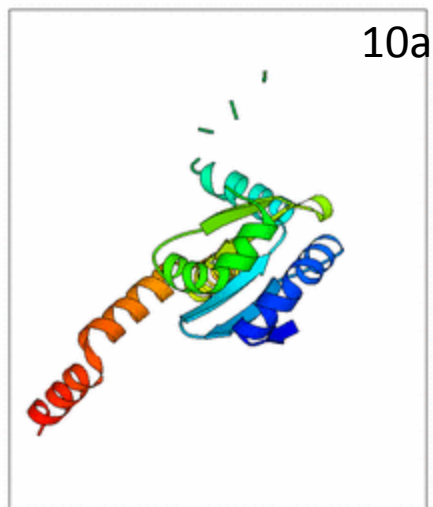Tetramer

Monomer

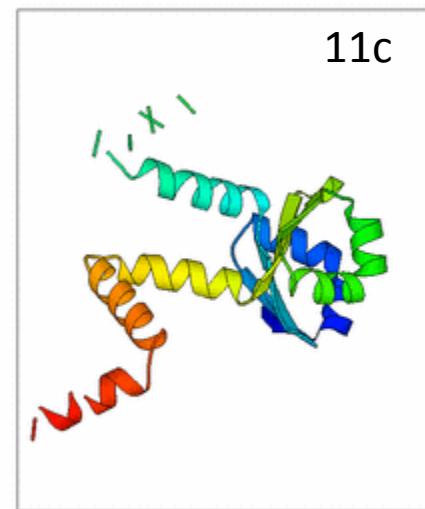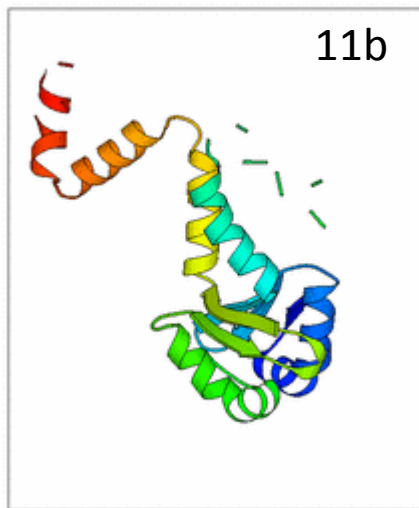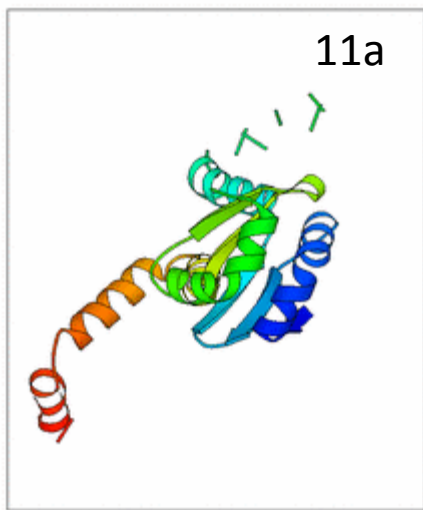# Interactions within dimers

Buried area approximately 480 Å^2
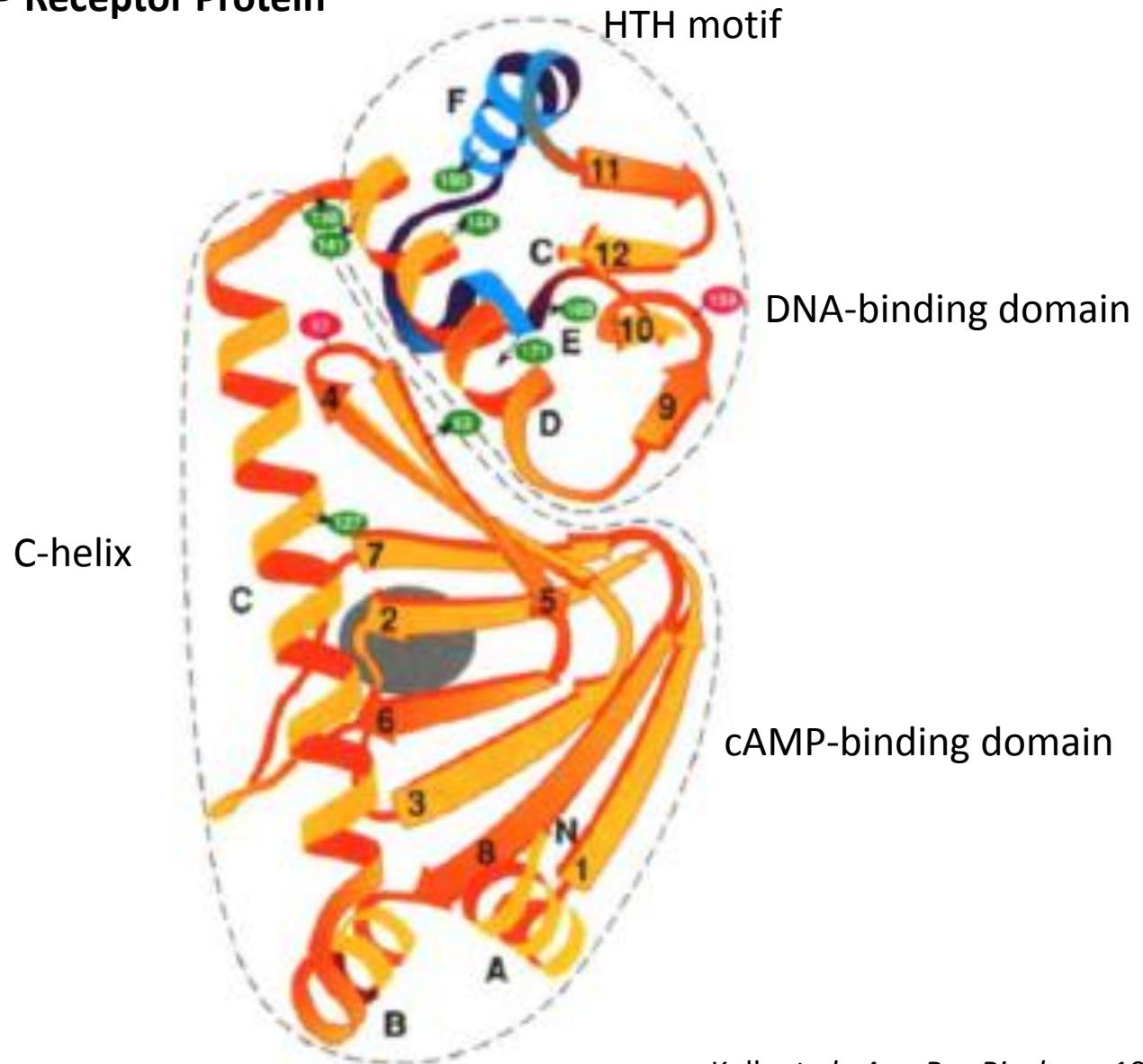
Conformational variability at the C-terminal

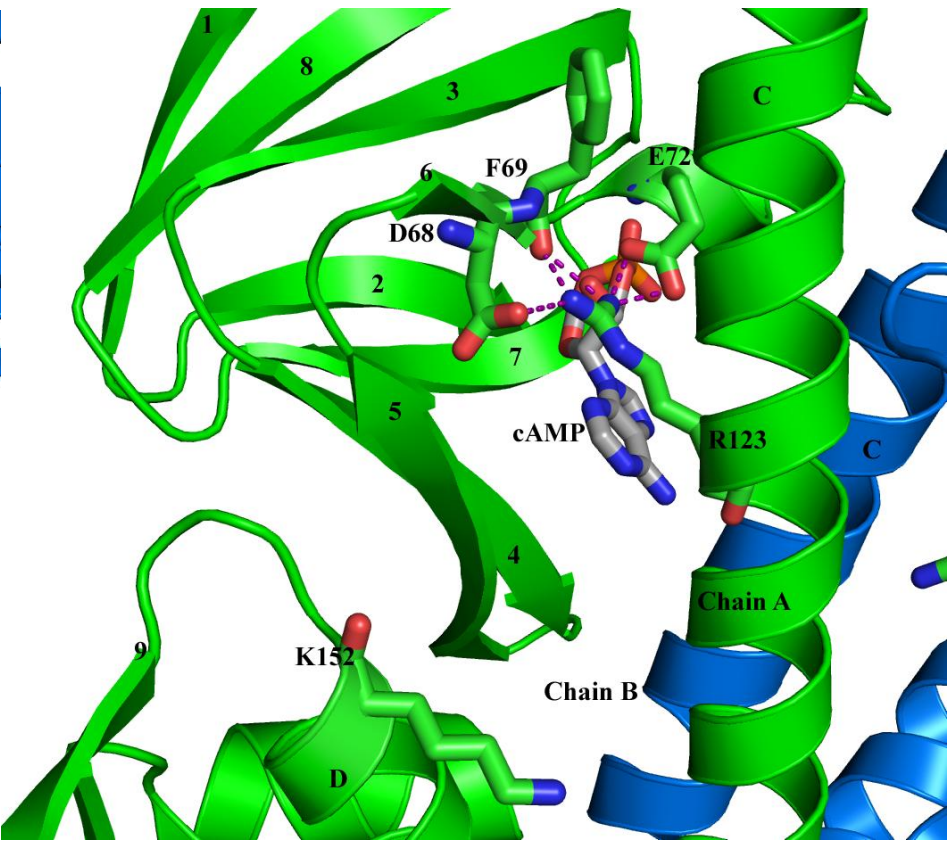H5 helices in all the monomers adopt different conformations
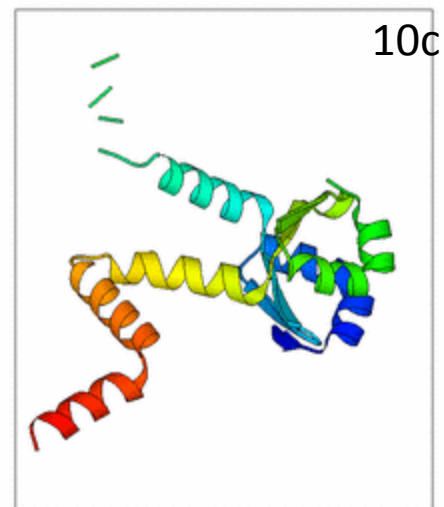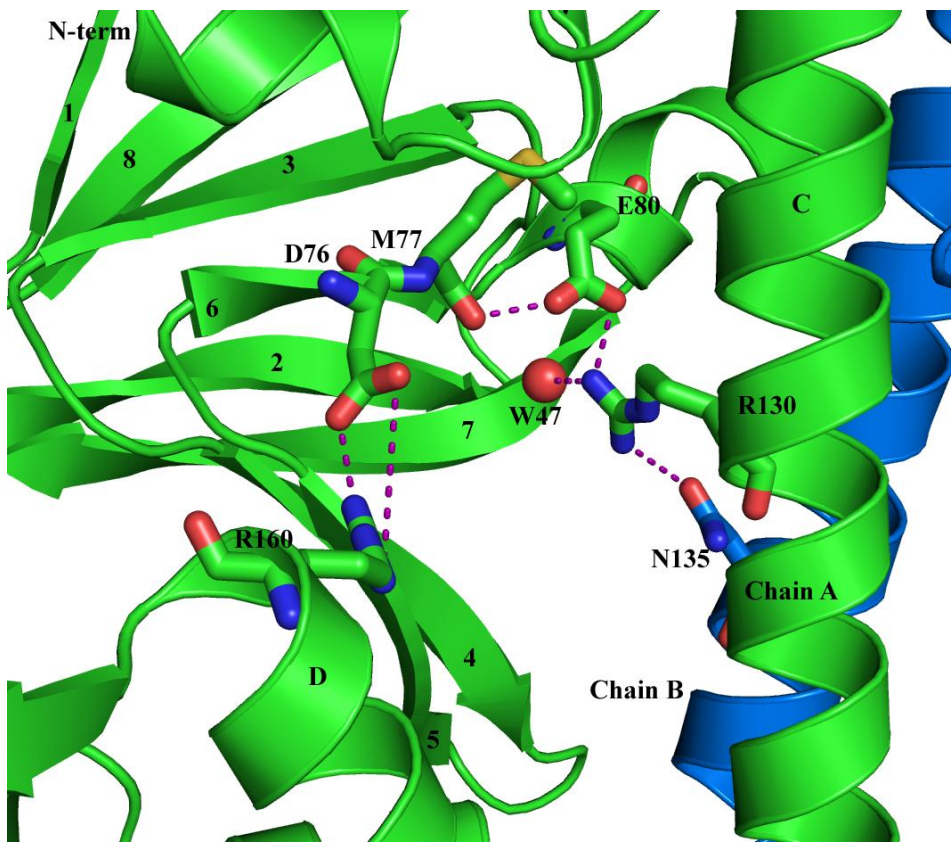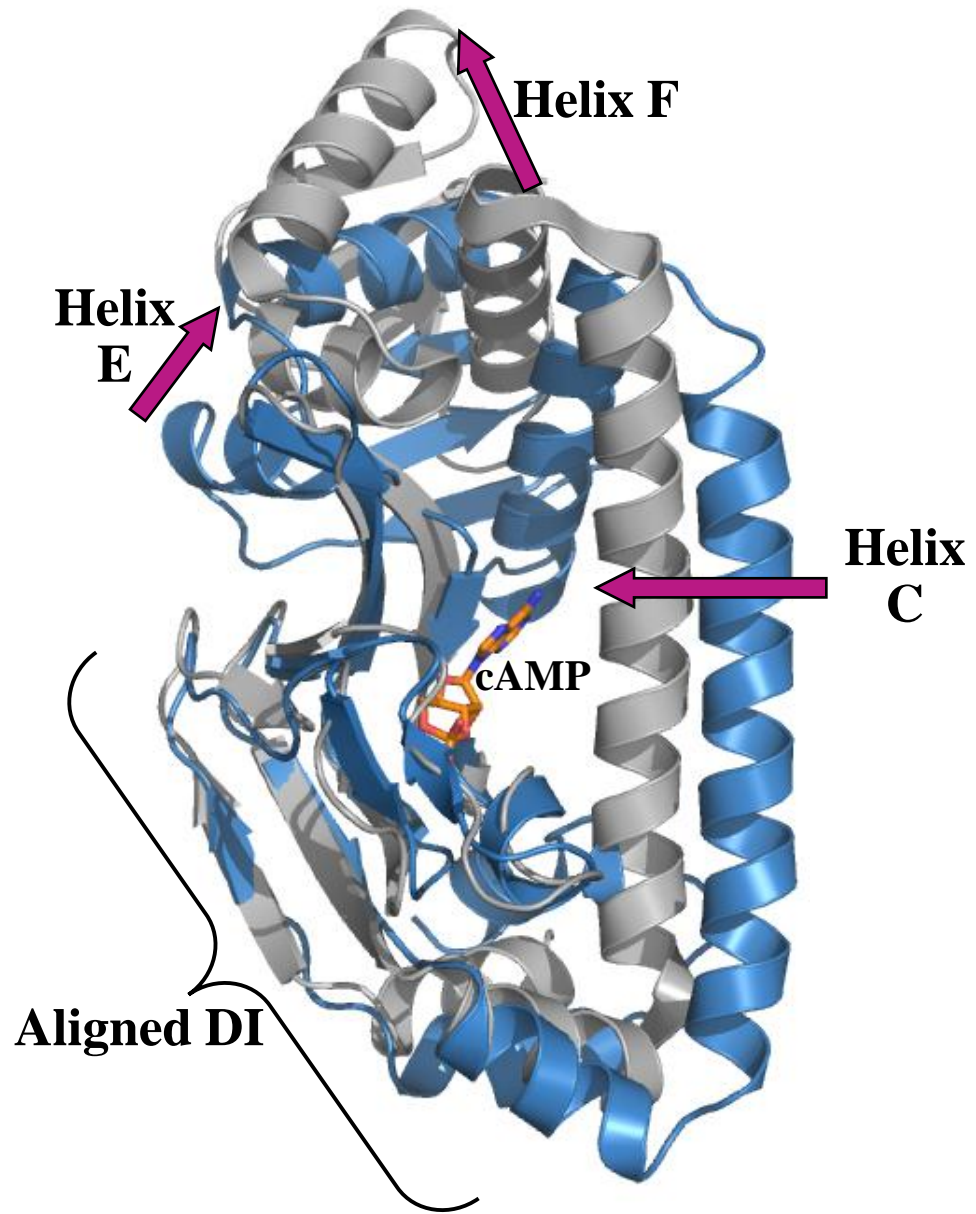
Conformational Transitions in the Cyclic AMP Receptor Protein

Structure of the cAMP Receptor Protein

HTH motif

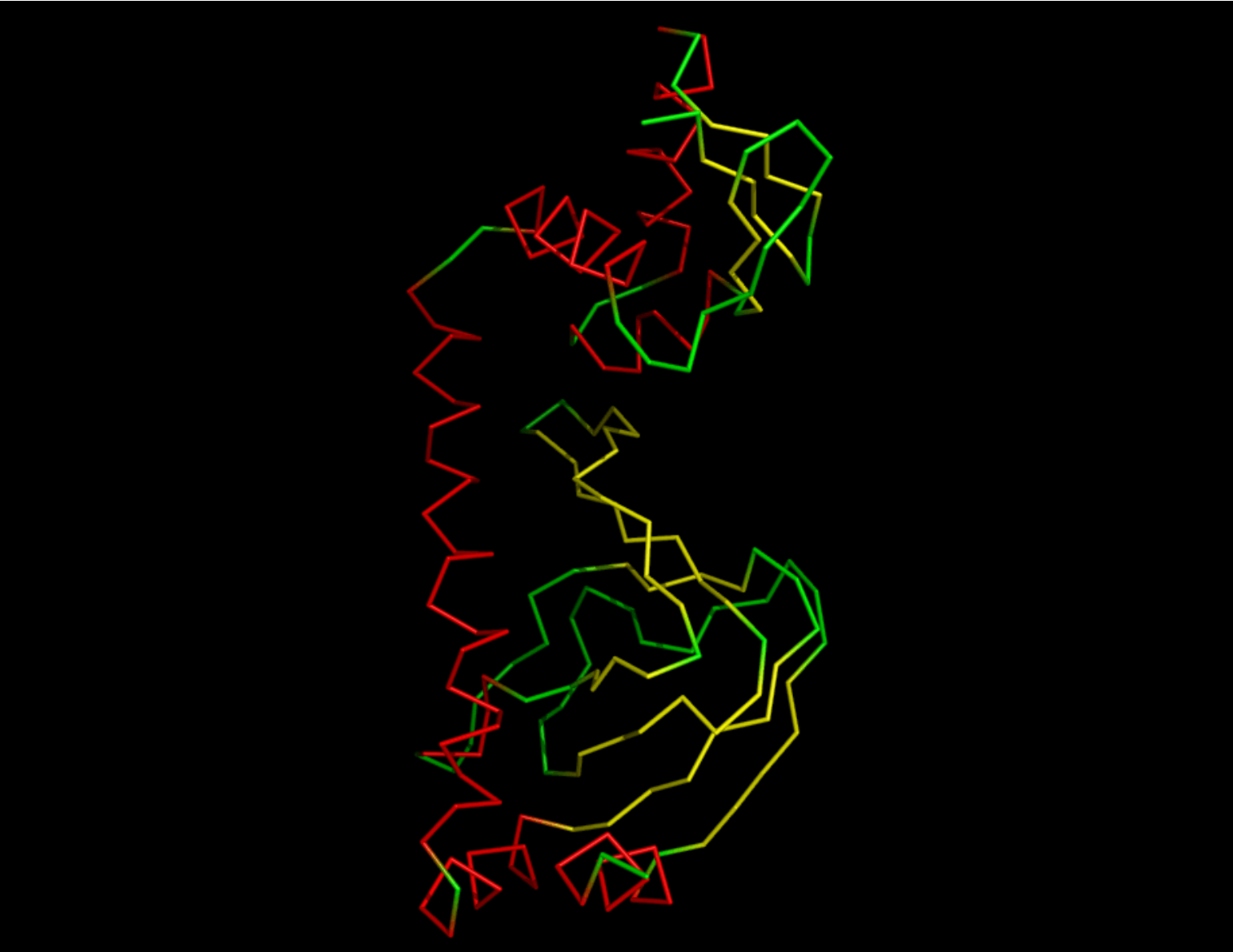DNA-binding domain

C-helix

cAMP-binding domain

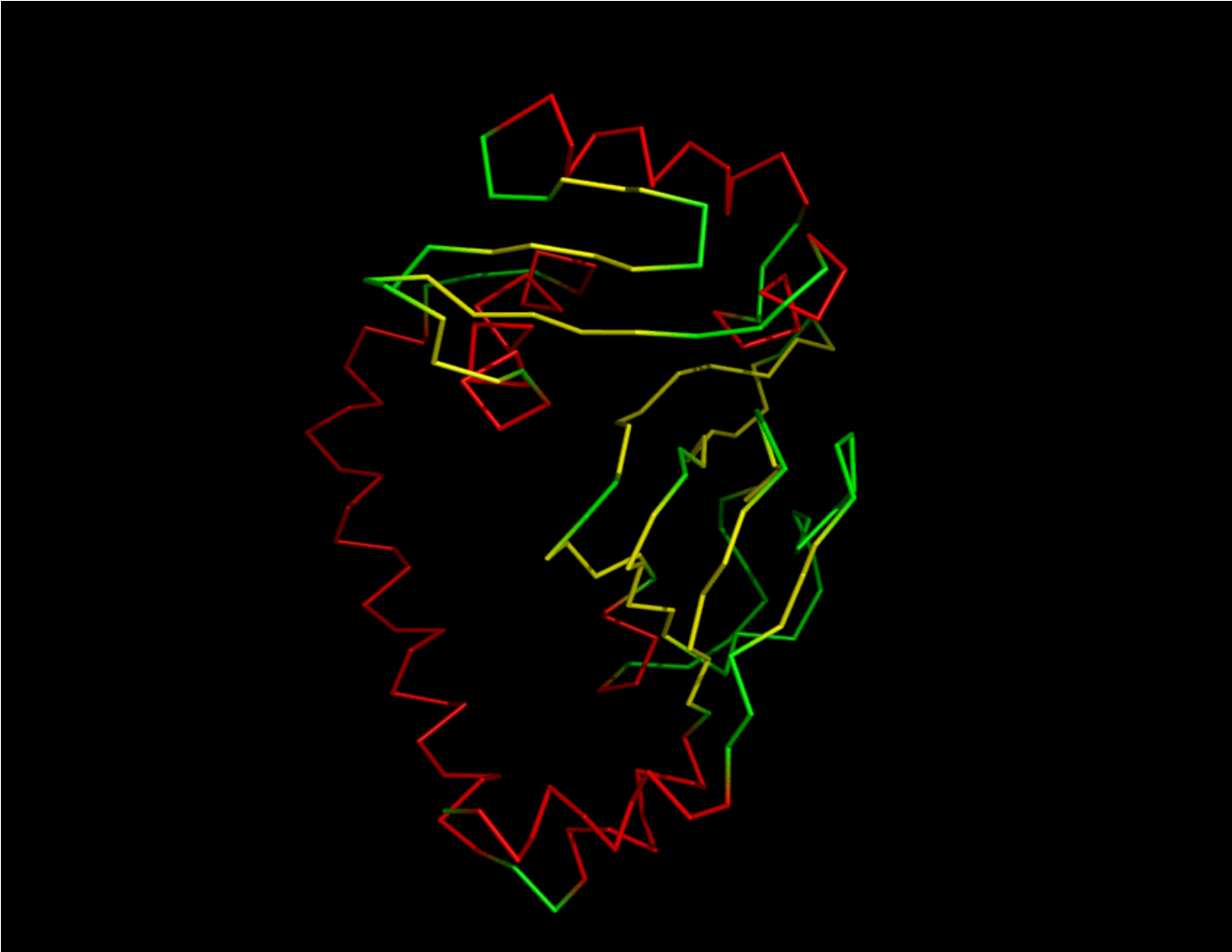Kolb *et al.*, *Ann Rev Biochem*, 1993

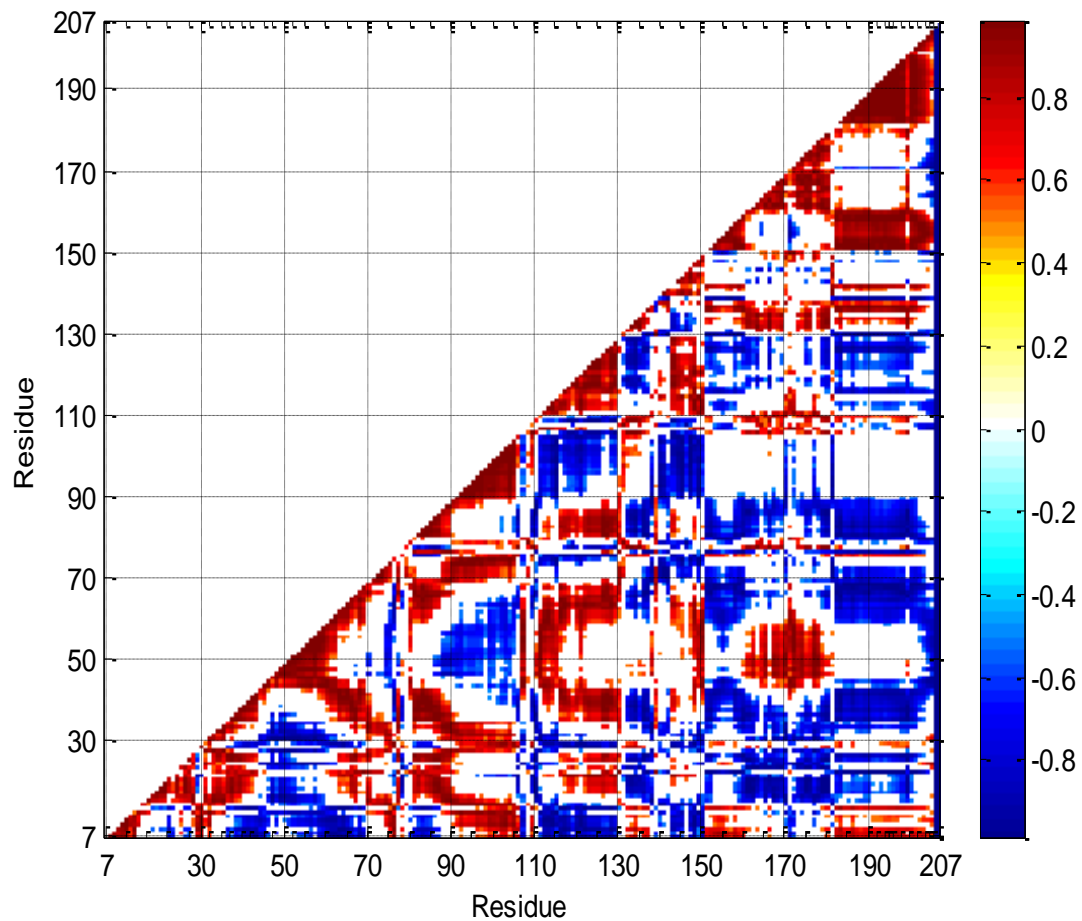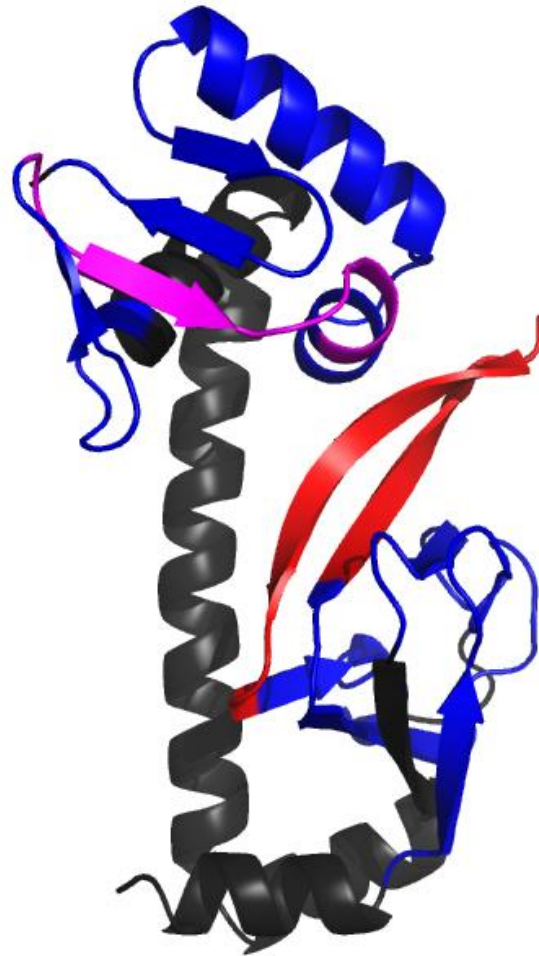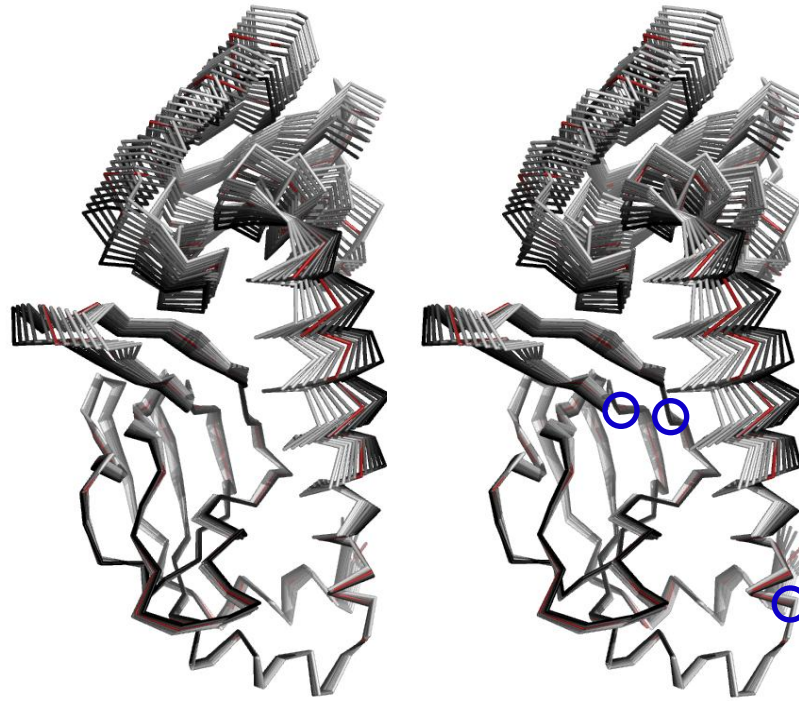# cAMP binding site comparison

1G6N chain B

3H3U chain B

Dynamic Cross Correlation Map for 1G6N and model of *E. coli* cAMP-free CRP.

Normal Mode Analysis of 1G6N and *E.coli* model based on 3H3U structure as a reference. Mode 13 shows 57.9 % collectivity and 43.9 % overlap.

Summary of Overall Conformational Changes Effected by cAMP-binding

1. In absence of cAMP, the cAMP-binding and DNA-binding domains interact closely with each other, reducing mobility of the DNA-binding domain

2. The reduced mobility prevents sequence specific recognition of DNA

3. Binding of cAMP triggers reorientation of side chains (especially Arg 123) in the binding pocket of CRP

4. The cAMP-binding domain is drawn towards the C-helix closing over the bound cAMP

5. Conformational change in the cAMP-binding domain forces out the DNA-binding domain away from the C-helix

6. The DNA-binding domain remains sufficiently flexible, poised for sequence-specific DNA recognition

Acknowledgements