Transition State and Encounter Complex for fast Association of Cytochrome c2 with Bacterial Reaction Center

[Osamu Miyashita et al.; PNAS; November 16, 2004; 16174-16179]

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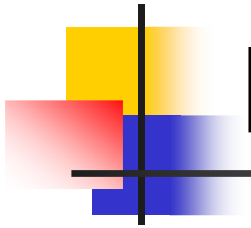


Motivation

- Photosynthesis is properly one of the most important processes in biological systems
- A better understanding could lead to:
  - New methods in hydrogen production

    [http://www.zukunft-in-brand.de]
  - Development of better photo-voltaic cells

    [http://www.elfenbeinturm.net/archiv/2003/11.html]
- Rudimental reactions in photosynthesis/respiration are the electron-transfer reactions

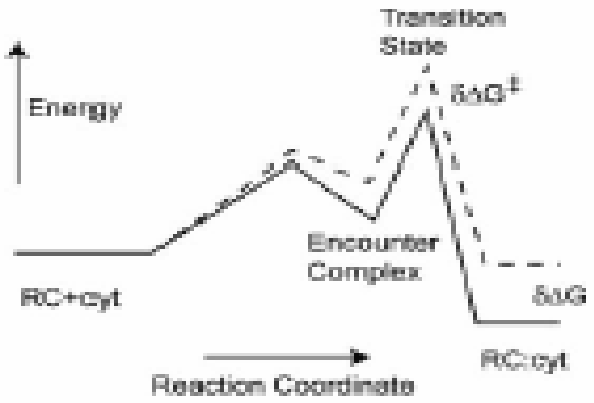
Electron-Transfer Reactions and the Two-Step Model

- Two electron-transfer proteins associate to form an electron donor-acceptor complex
- Electrostatic interactions can enhance the association
- A two-step association process is proposed

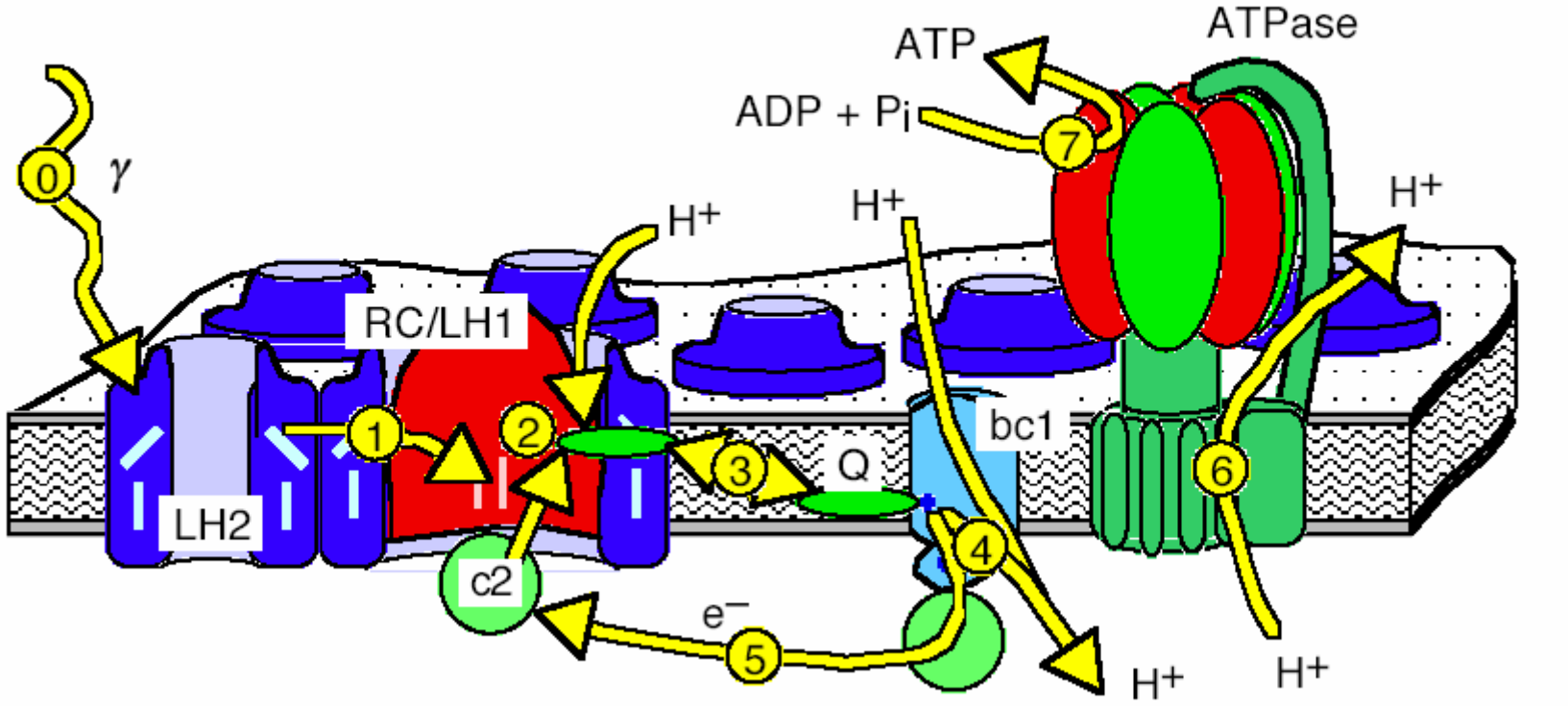
  - Formation of a loosly bound encounter complex (EC)

  - Formation of the transition state (TS)

  → Bound and active state

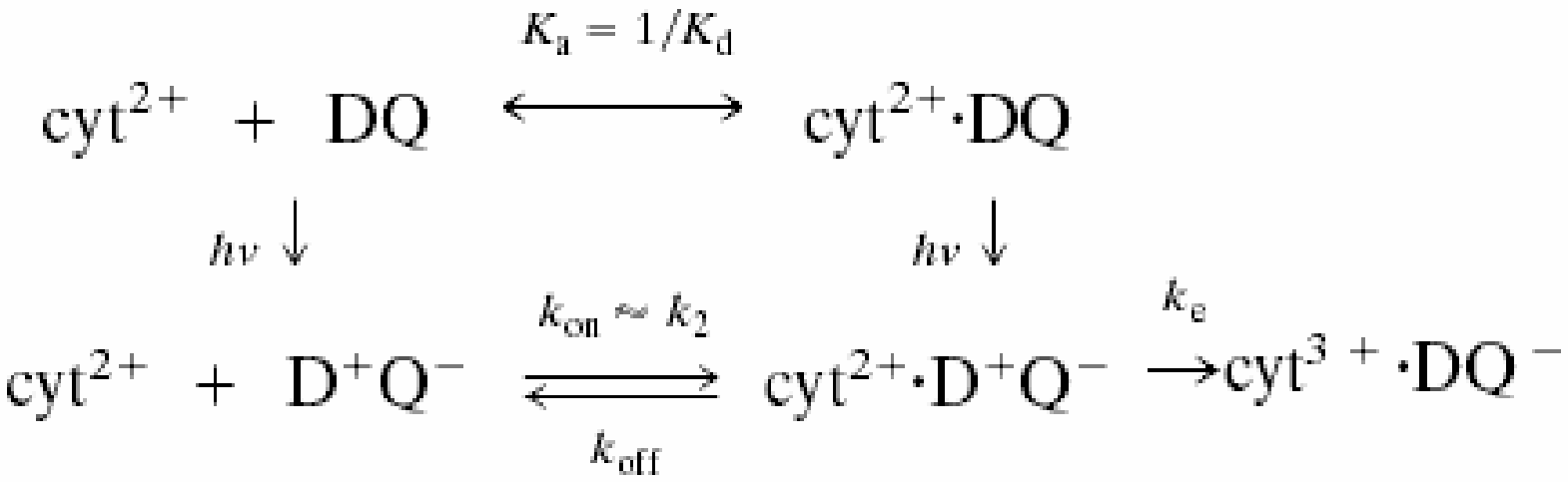


Investigated System (1)



Investigated System (2)

- Second-order rate constant k_2 for association including electron transfer has been measured using laser-flash techniques to be $10^9 s^{-1} M^{-1}$

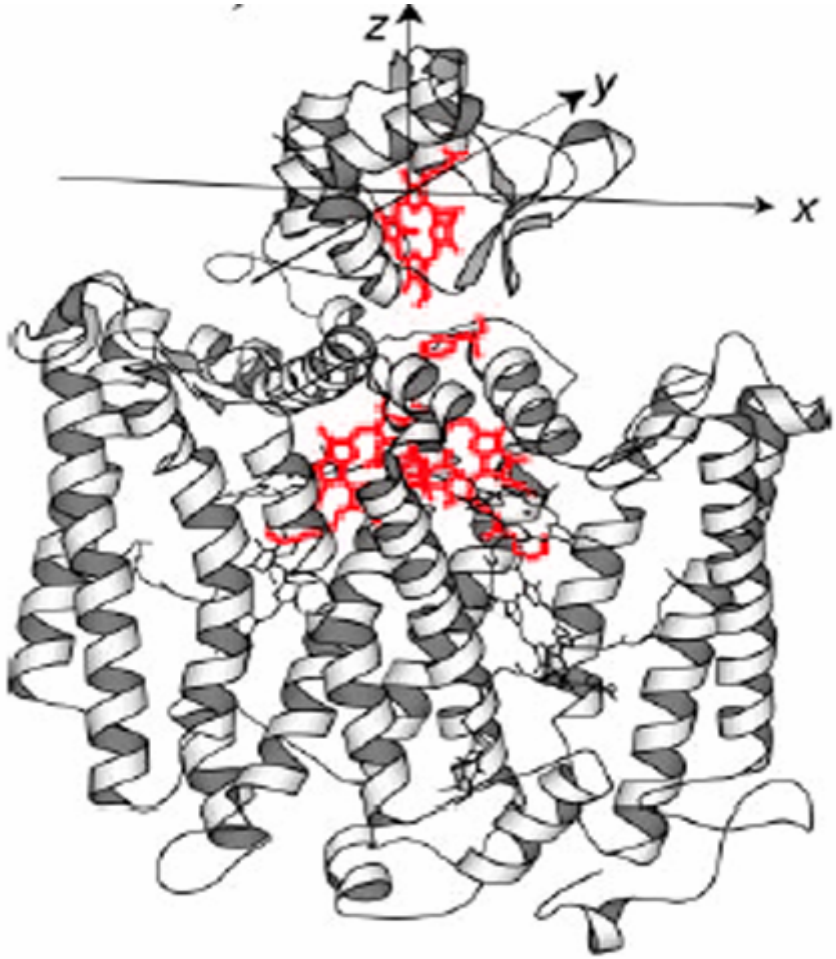


- k_2 is approximately k_{on} and thus a measure for the formation of the final state at which electron transfer occurs

Investigated System (3)

- Structure of the c2/RC complex determined by x-ray crystallopraphy
- Crystallized c2/RC complex has the same electron-transfer rate as in solution

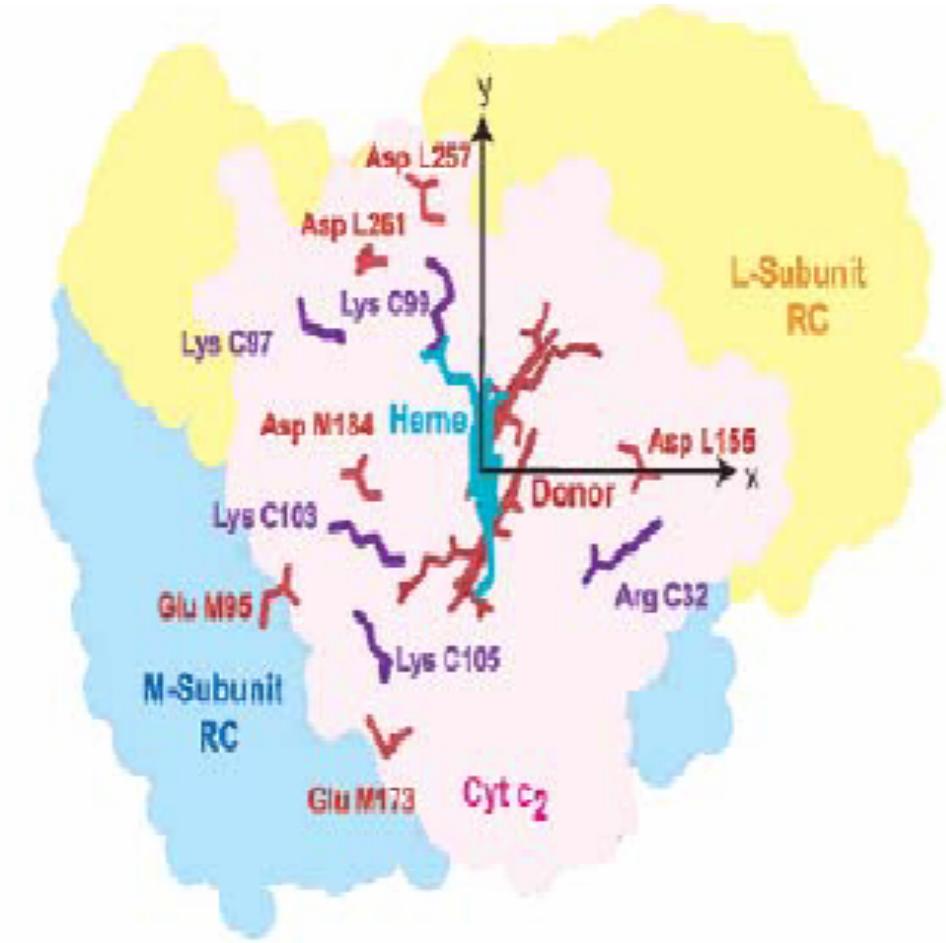
→ Represents the active state



Model to Examine the Structures of EC and TS

- TS was studied by analyzing the set of measured values for the 2nd order rate constants
[Tetreault et al; Biochemistry 41; 5807-5815]

- EC was modeled as an ensemble of configurations stabilized by electrostatic interactions and analyzed by continuum electrostatic calculation for different configurations of c2 and RC



Methods: Protein Association

- Second-order rate constant for association k_{on} is related to the free energy barrier of the TS (Transition State Theory)

→ k_2 also related

- Thus the change in interaction free energy of the TS

$$\delta\Delta G^{\ddagger} = \Delta G^{\ddagger}_{\text{mut}} - \Delta G^{\ddagger}_{\text{native}}$$

is related to the ratio of the second-order rate constants for electron transfer

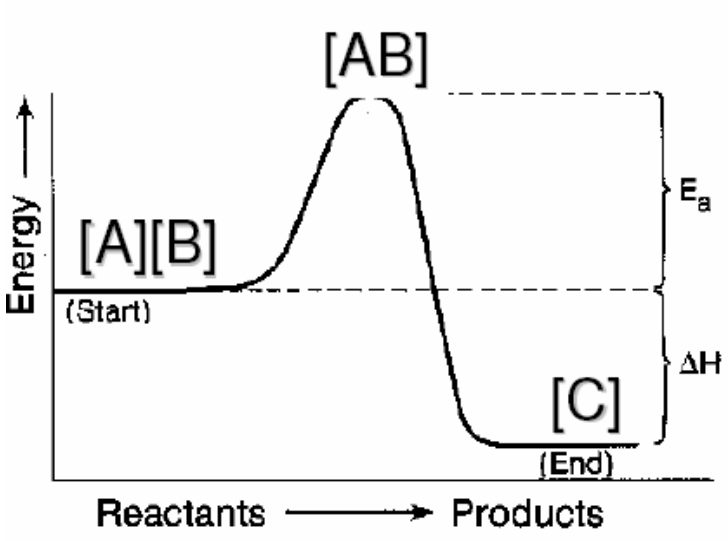
$$\delta\Delta G^{\ddagger}_{\text{exp}} = -RT \ln \frac{k_2^{\text{mut}}}{k_2^{\text{native}}}$$

- Changes in interaction free energy of the TS, $\delta\Delta G^{\ddagger}_{\text{exp}}$,were compared to the changes in the calculated electrostatic energy for the Cyt/RC complex in different docking positions[8]

Transition State Theory (1)

[Computational Chemistry II; Lecture 10; Iris Antes]

Reaction: $A + B \xrightarrow{K} [AB] \xrightarrow{k} C$



1st TST assumption:

$$K = \frac{[AB]}{[A][B]}$$

The rate of the overall reaction becomes:

$$\frac{d[C]}{dt} = k[AB] = kK[A][B]$$

Transition State Theory (2)

[Computational Chemistry II; Lecture 10; Iris Antes]

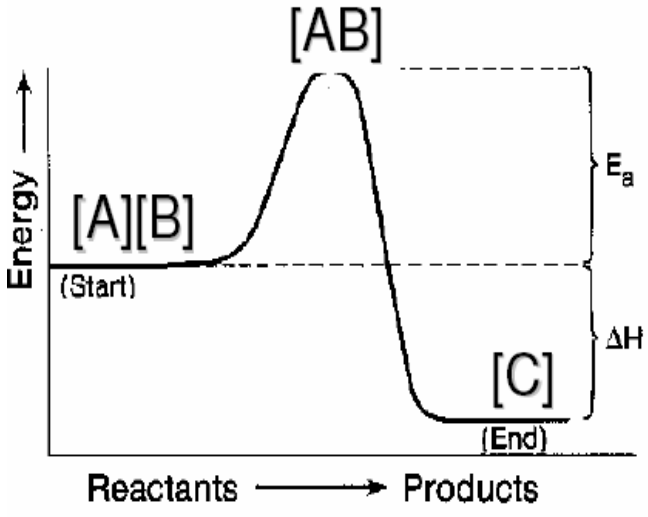
2nd TST assumption: $\nu \infty [AB] = K[A][B]$

$$\nu = kK[A][B] \qquad \nu = \frac{k_B T}{h} K[A][B] \qquad kK = \frac{k_B T}{h} K$$

Remember the definition of Gibbs free energy with respect to the equilibrium constant

$$\Delta G = -RT \ln K \qquad K = \exp\left(\frac{\Delta G}{RT}\right)$$

$$kK = \left[\frac{k_b T}{h}\right] \exp\left(\frac{\Delta G}{RT}\right)$$



Methods: Calculation of Electrostatic Energies

- Two different methods were used to calculate the electrostatic binding energy

  - Full-continuum electrostatics calculation

    → Used to calculate the electrostatic energy of the EC for native Cyt and RC, but too time consuming to perform all the calculations

  - Coulomb‘s law calculation

    → Used to calculate the electrostatic

    energy of the Cys/RC complex in the TS

Full continuum electrostatics calculation

[Computational Chemistry I; Lecture 9; V. Helms & M. Hutter]

- Proteins are treated as point charges within a region having dielectric constant $\varepsilon_{in} = 10$
- Proteins are surrounded by a solvent having dielectric constant $\varepsilon_{ext} = 80$
- Electrostatic energy can be obtained by solving the Poisson-Boltzmann equation. One numerical method to do so is the so called finite difference method (Warwick & Watson 1982) which is implemented in the program DELPHI

$$\nabla \varepsilon(r) \nabla \phi(r) - \kappa' \phi(r) = -4\pi\rho(r)$$

$$\kappa^2 = \frac{\kappa'^2}{\varepsilon} = \frac{8\pi N_A e^2 I}{1000 \varepsilon k_b T}$$

$$I = \tfrac{1}{2} \sum_i c_i z_i^2$$

Determining the TS Ensemble by using the Correlation Coefficient

- For each Cyt/RC configuration, the effects of mutating charged residues on the Coulombic energy were calculated by using $\varepsilon = 10$

$$\delta\Delta G_{coul} = \Delta G_{coul}^{mut} - \Delta G_{coul}^{nat}$$

- These free energy changes were correlated to the experimental changes in free energy

$$\delta\Delta G_{\mathrm{exp}}^{\ddagger} = -RT \ln \frac{k_2^{\mathrm{mut}}}{k_2^{\mathrm{native}}}$$

- The correlation coefficient was found to be R=0.89

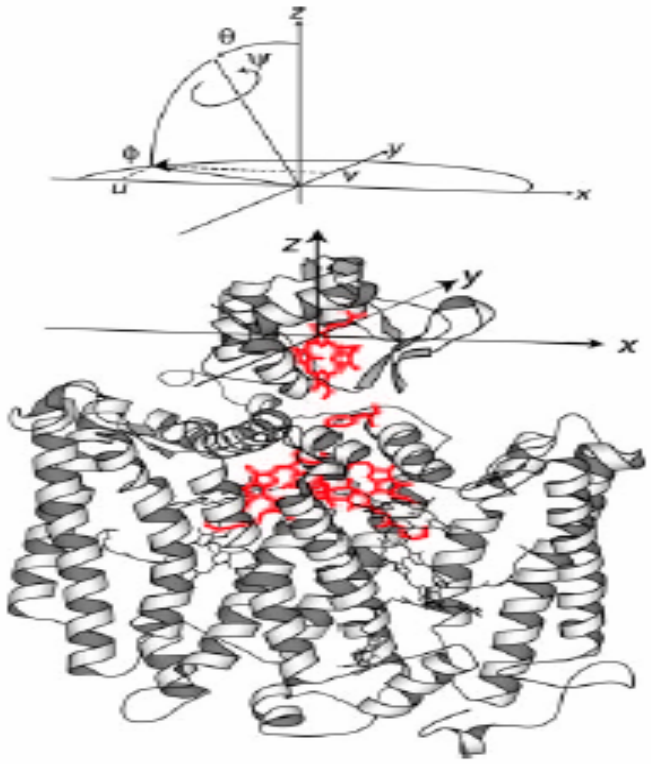
Monte Carlo Sampling

[Computational Chemistry I; Lecture 3; V. Helms & M. Hutter]

- Construct starting configuration(s)
- Perform random changes in the degree(s) of freedom
- Calculate the energy change ΔE depending on these changes
- If $\Delta E < 0$ accept the new configuration
- Else if $\Delta E > 0$ calculate the likelihood w and create a random number r within the interval [0,1] $w = \exp\left(-\frac{\Delta E}{k_B T}\right)$
- Accept the new configuration if $w \geq r$, else reject it

Monte Carlo Sampling of the TS Configuration (1)

- Configuration space sampled by Metropolis Monte Carlo Algorithm with acceptance ratio taken as $\exp(\frac{-\Delta R}{T_R})$
- Four starting structures z = 2, 4, 6, 8
- Translation of Cyt along x, y ,z by a grid of 2Å
- Additional rotation around the center by angles ϕ, θ and ψ by a grid of 5°

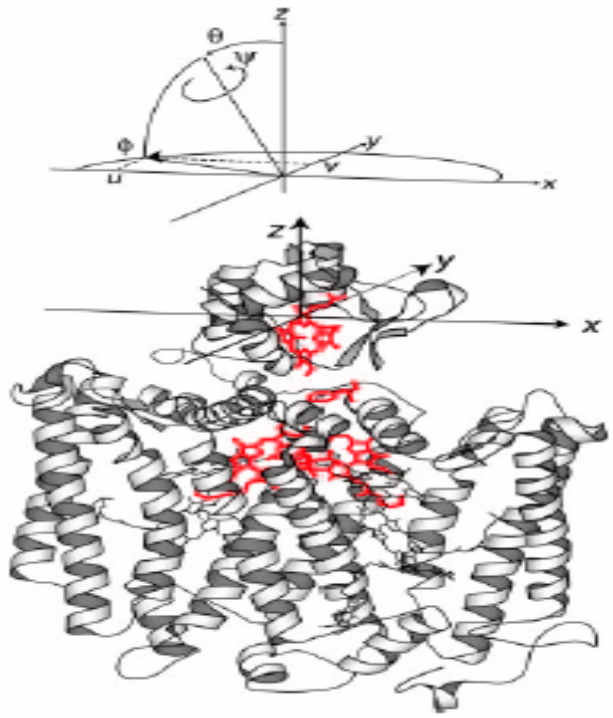


Monte Carlo Sampling of the TS Configuration (2)

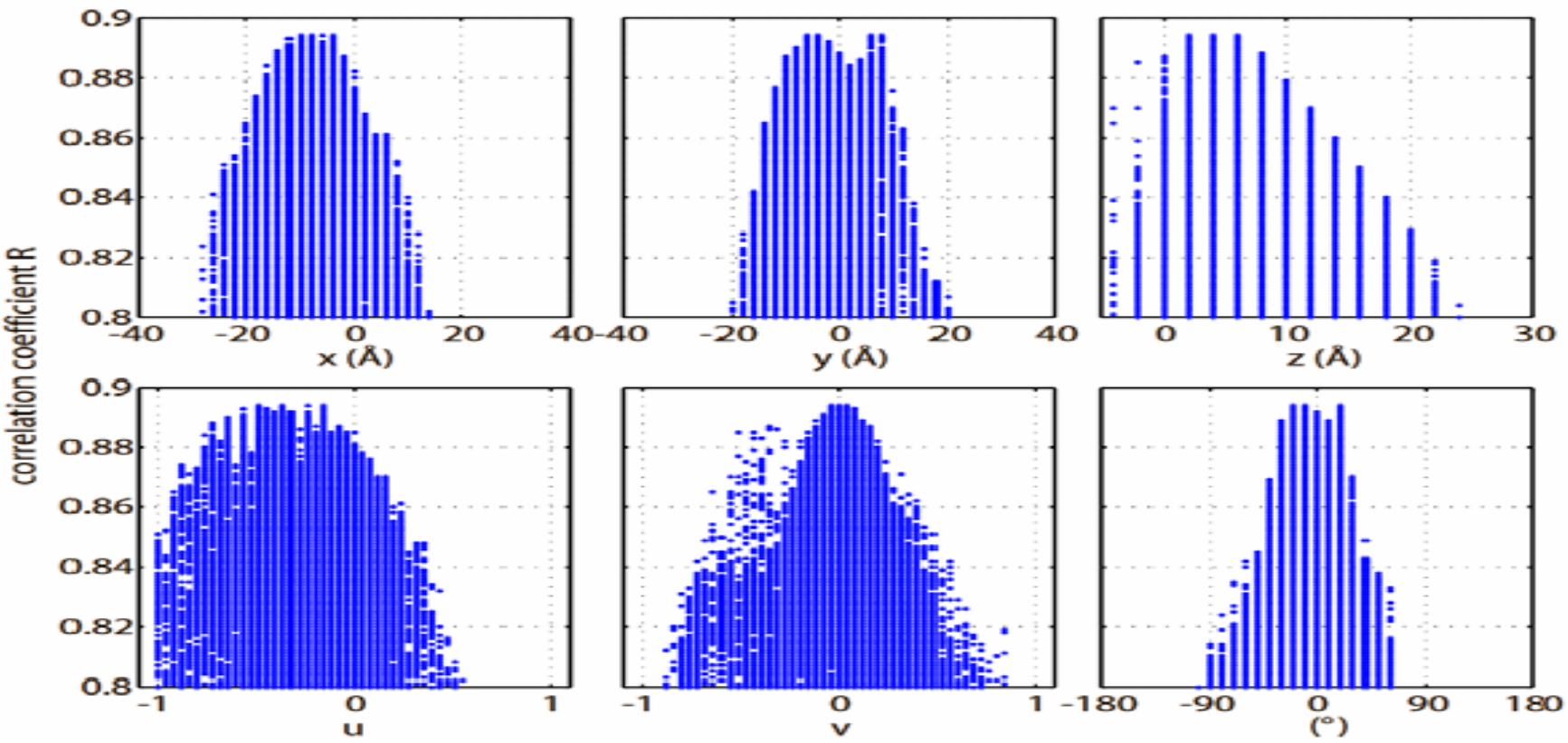
- 4000 MC steps from each structure with $T_R = 0.1$
- Selection of 40 structures with 100 steps interval from each of the four trajectories → 4*40 structures in total
- 200 MC steps with $T_R = 0.01$
- Selection of 80 structures with 100 steps interval from the four trajectories → 4*80 structures in total
- 200 MC steps with $T_R = 0.001$

Monte Carlo Sampling of the EC Configuration Space

- Configuration space sampled by Metropolis Monte Carlo Algorithm with acceptance ratio taken to be $\exp(\frac{-\Delta G_{elec}}{k_B T})$
- 40 independent MC samplings of 1000 steps starting from the TS structure
- Grid size was set to
  - 4Å for x and y
  - 2Å for z
  - 20° for ϕ and ψ
  - 5° for θ



Results (1): TS Configuration

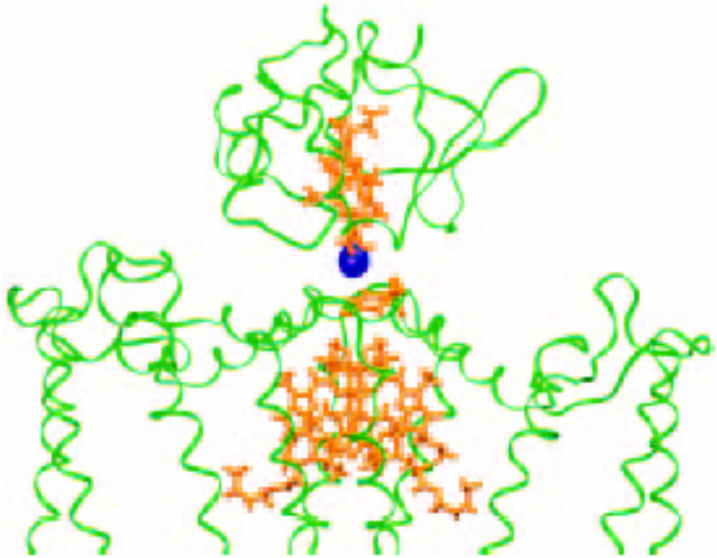


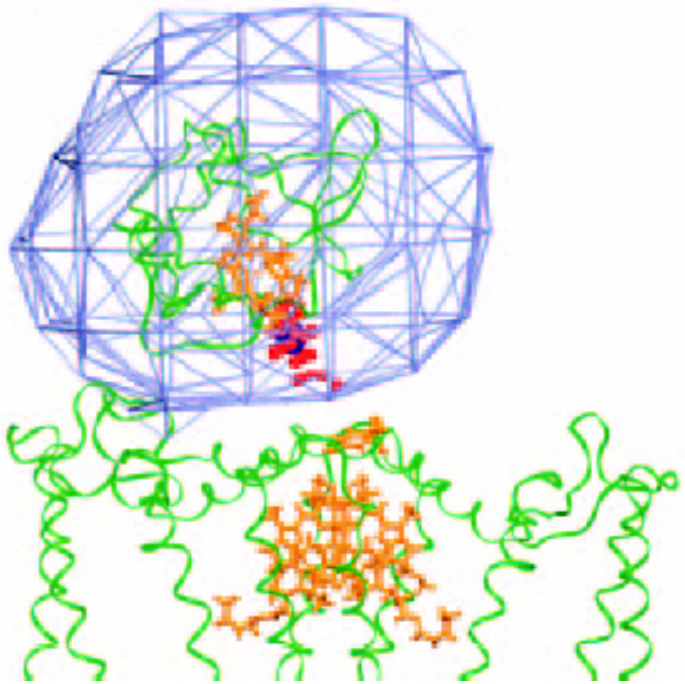
u = sinθ cosϕ v=sinθ sinϕ

Results (2): TS Configuration

Ensemble	Cutoff	No. of states	x	y	z	u	v	ψ
TS	−1%	750	−9.0 ± 2.3	−4.1 ± 2.8	5.2 ± 1.3	−0.40 ± 0.13	−0.00 ± 0.06	350 ± 9
TS	−5%	14,332	−8.3 ± 5.1	−4.0 ± 4.2	6.0 ± 2.5	−0.32 ± 0.26	−0.01 ± 0.13	350 ± 14
EC	$+1\,k_BT$	51	−6.4 ± 5.3	4.2 ± 3.2	5.0 ± 1.3	−0.05 ± 0.25	0.07 ± 0.15	27 ± 68
EC	$+2\,k_BT$	769	−5.0 ± 5.0	2.7 ± 4.1	5.2 ± 1.4	−0.06 ± 0.22	0.04 ± 0.17	24 ± 64

Average ± SD, $u = \sin\theta\cos\phi$, $v = \sin\theta\cos\phi$, distances are in angstroms, angles are in degrees.





Results (3): TS Configuration and Comparison with the EC

Ensemble	Cutoff	No. of states	x	y	z	u	v	ψ
TS	−1%	750	−9.0 ± 2.3	−4.1 ± 2.8	5.2 ± 1.3	−0.40 ± 0.13	−0.00 ± 0.06	350 ± 9
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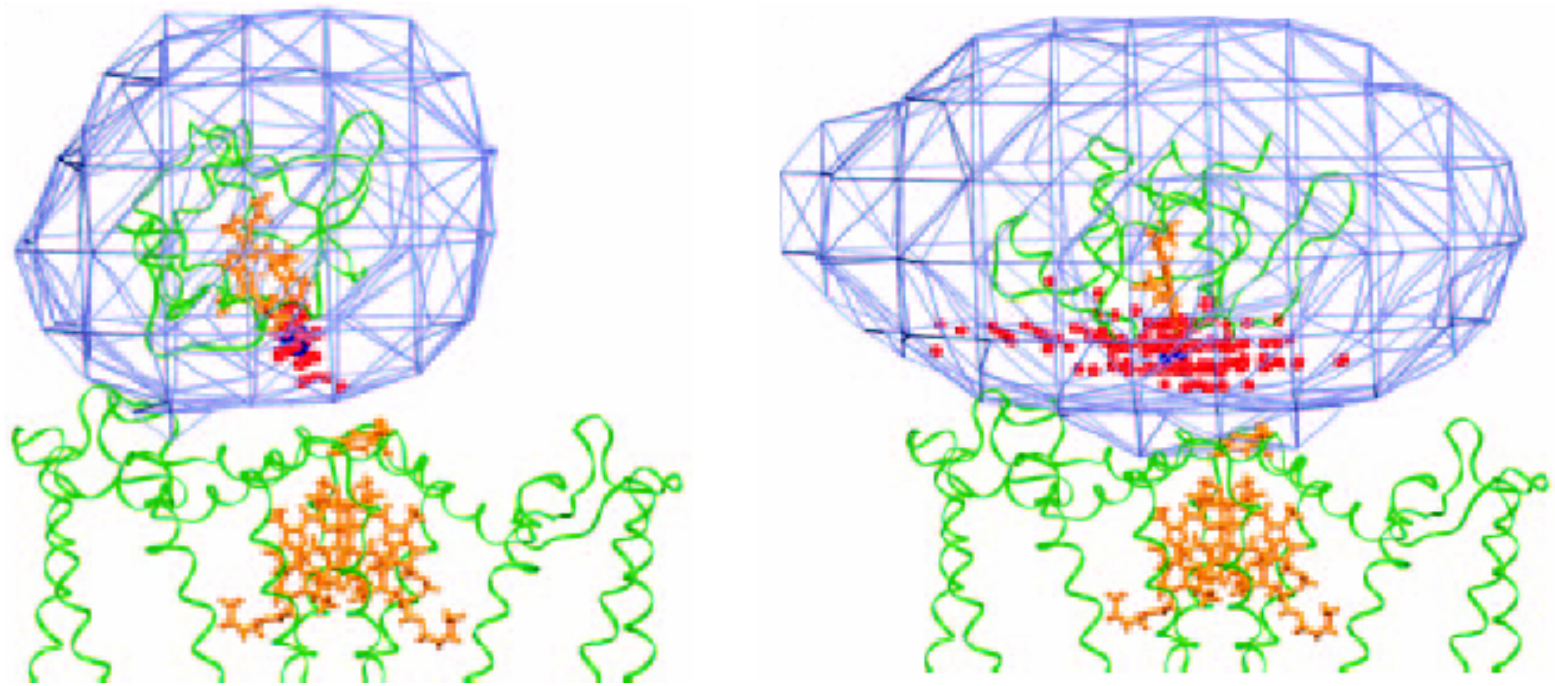
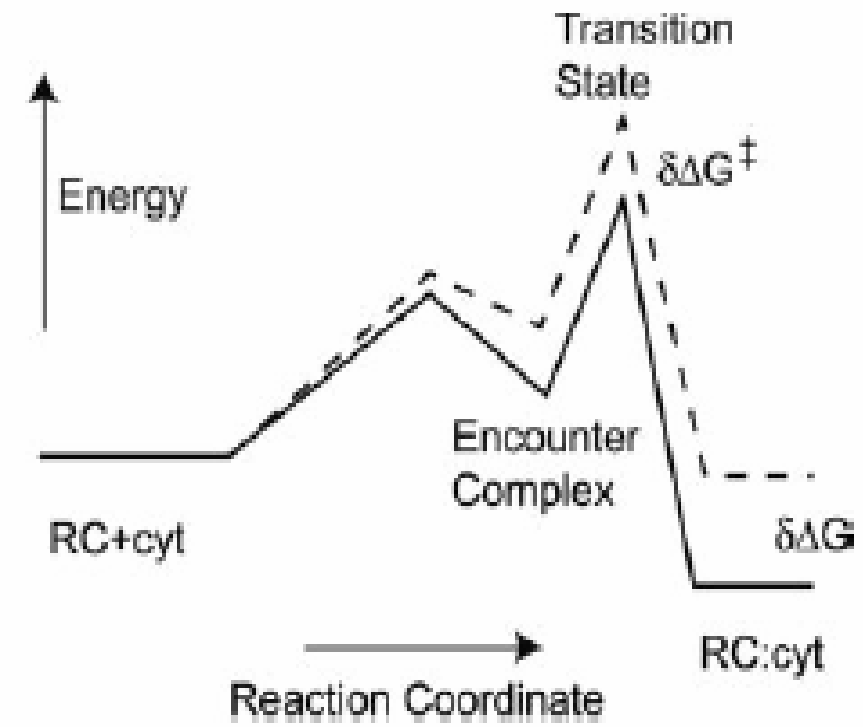
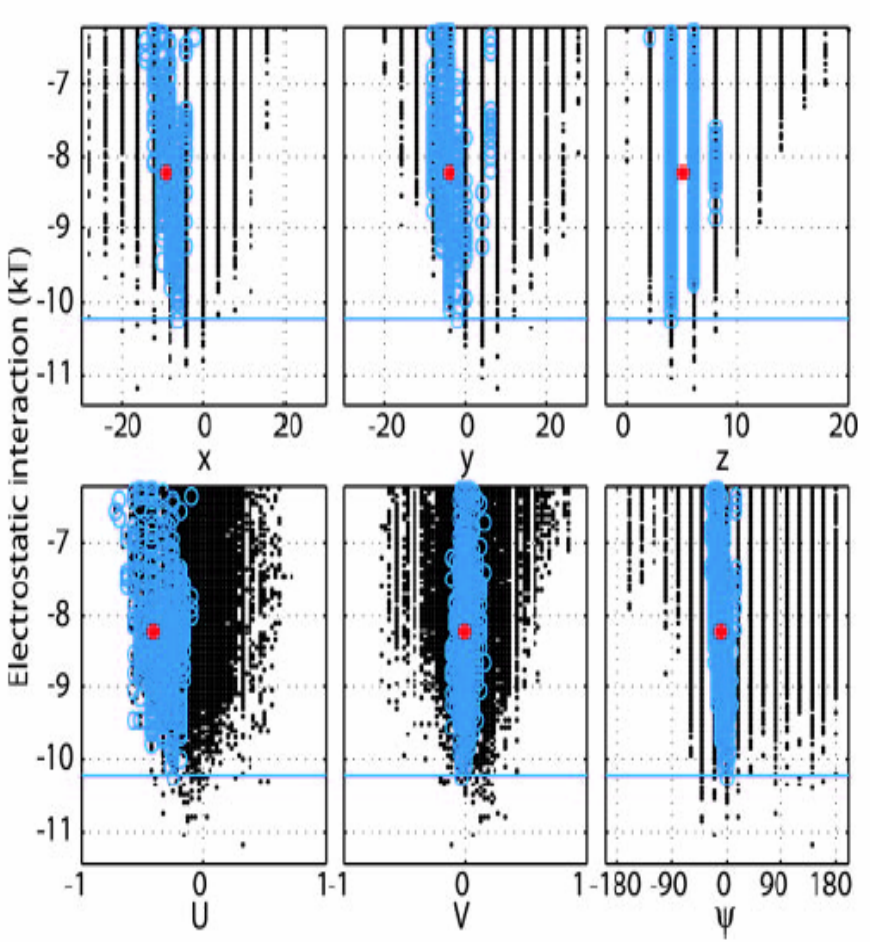
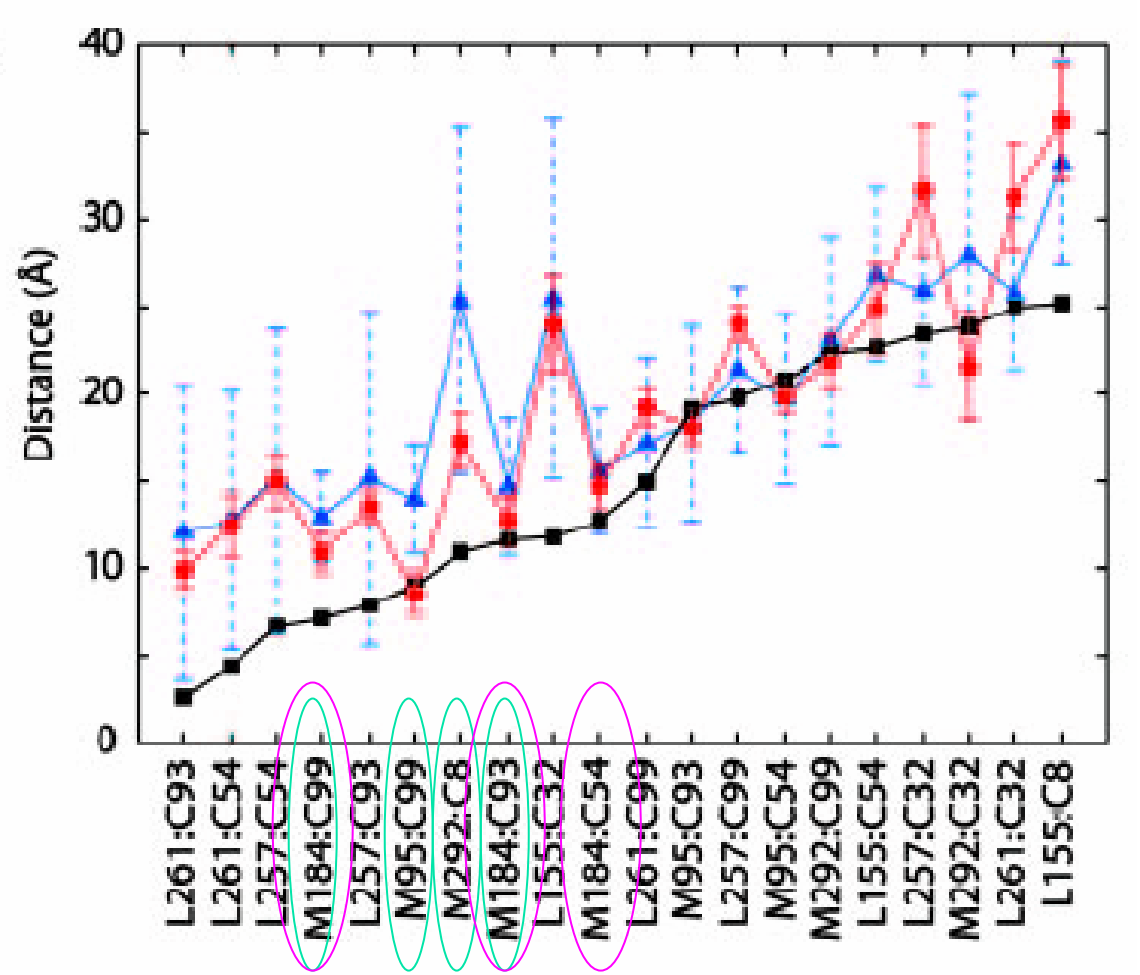


Discussion (1)





Discussion (2)



EC

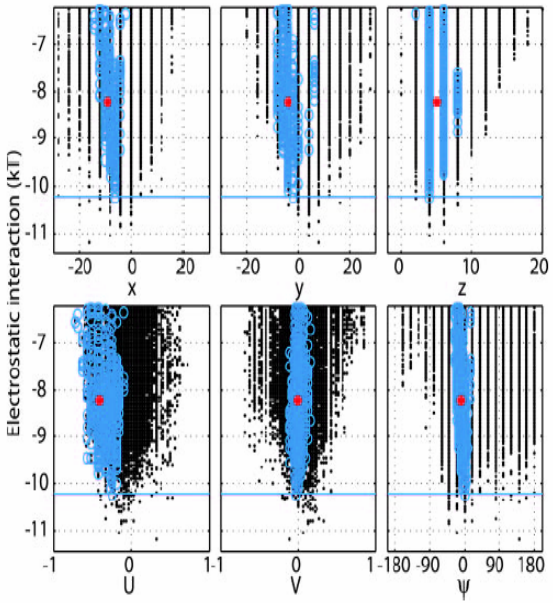
TS

Bound Complex

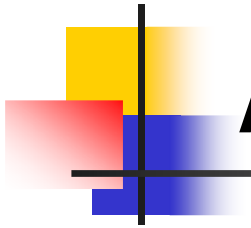
Disussion (3)

- Close resemblance between minimum energy configuration of EC and TS helps to increase the rate process of protein asssociation

- The energies of the TS ensemble are within $2k_BT$ of the minimum EC coordinates, allowing an easy access to the TS configuration



- Association process resembles a binding funnel in which a smaller number of lower energy configurations are brought close to the target configuration of the final stat



Acknowledgement

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