Unraveling functional hole hopping pathways in the [4Fe4S]-containing DNA primase

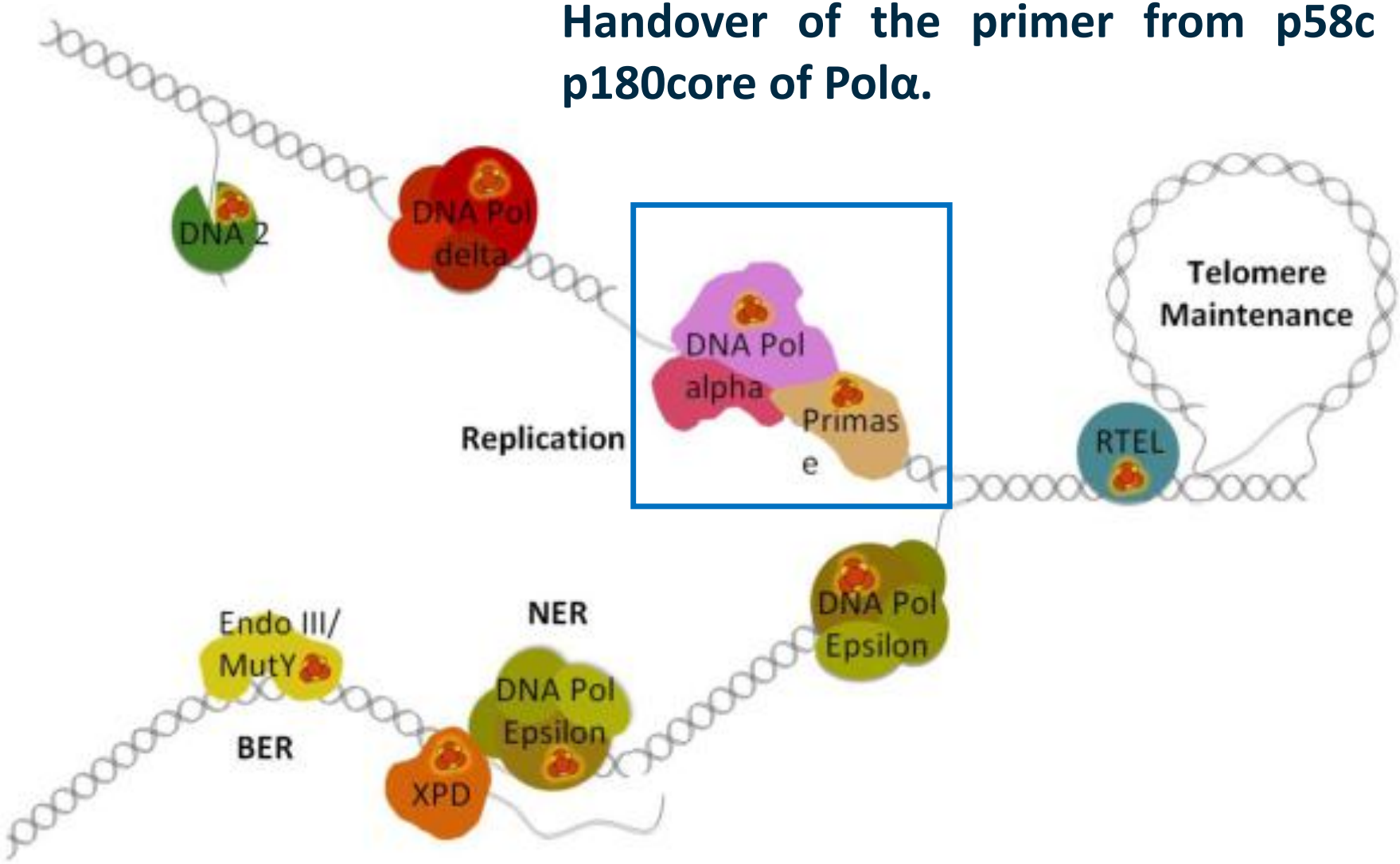
"Blue Waters has enabled me to develop force field parameters for $[Fe_4S_4]^{2+/3+}$ cluster and EHPath.py."

Darius Teo, Ph.D. candidate

Beratan group, Duke University

Emerging roles of Fe-S cluster enzymes in DNA replication and repair

Handover of the primer from p58c of primase to p180core of Polα.



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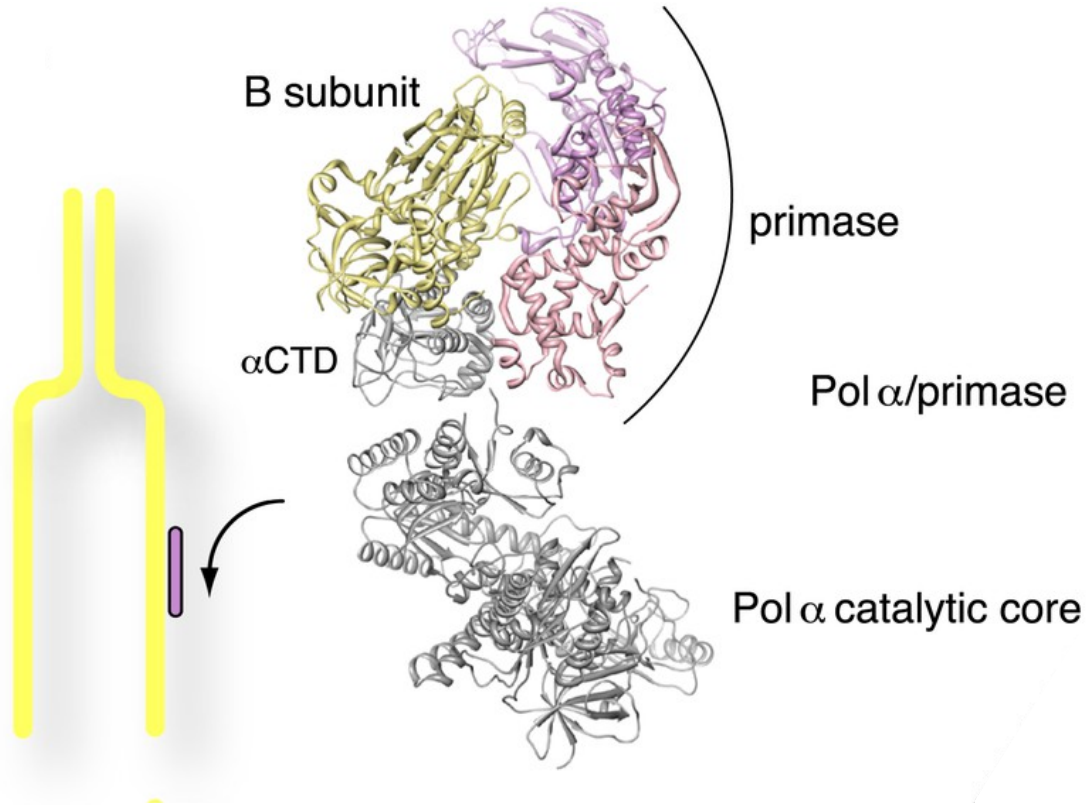
Fuss, J. O. et al., 2015. *Biochim. Biophys. Acta -Molecular Cell Research*, *1853*(6), pp.1253-1271.

RNA-DNA primer synthesis during DNA replication of the lagging strand



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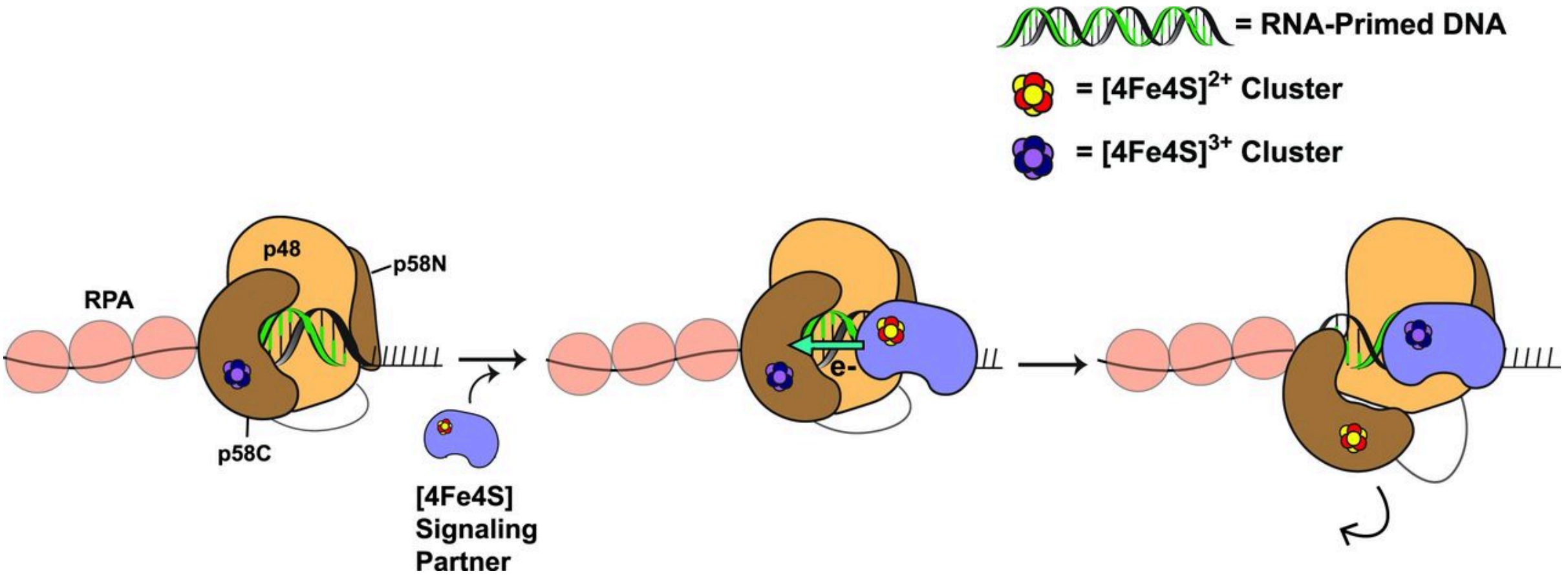
Perera, R.L., Torella, R., Klinge, S., Kilkenny, M.L., Maman, J.D. and Pellegrini, L., 2013. *Elife*, 2.

Proposed mechanism of primer handoff driven by DNA charge transfer



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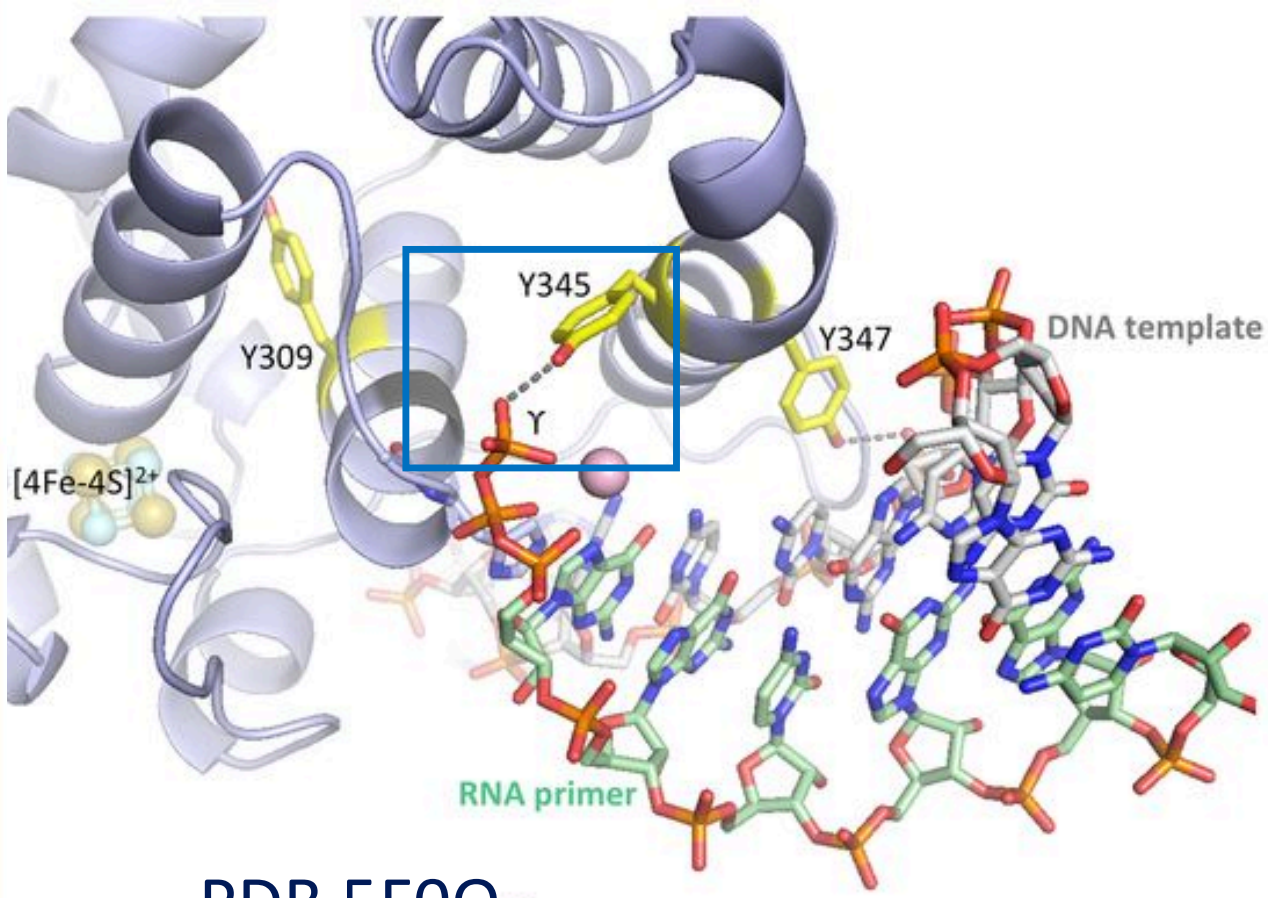
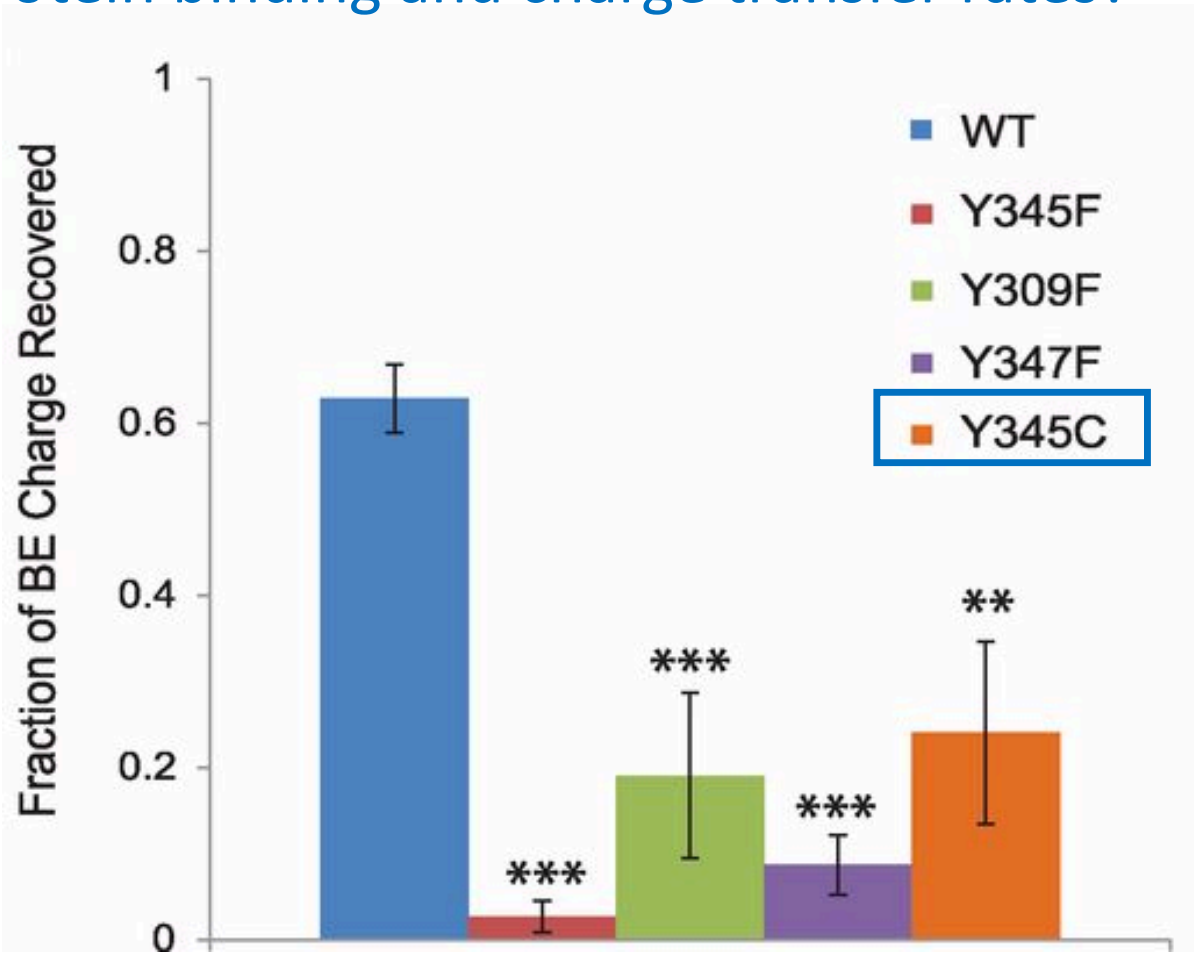
O'Brien, E et al., 2017. *Science*, *355*(6327), p.eaag1789.

DNA-binding, charge transfer–deficient p58C (primase) mutants

How does the mutation affect RNA/DNA-protein binding and charge transfer rates?



PDB 5F0Q



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O'Brien, E et al., 2017. *Science*, *355*(6327), p.eaag1789.

Objectives

1) Develop AMBER force field parameters for the [4Fe4S] cluster in 2+/3+ state.
   - Broken-symmetry DFT for geometry optimization
   - Generate force constants and RESP charges
   - Validate parameters using MD simulations
2) Charge transfer pathway analysis using a hopping program
   - EHPath.py
3) Examine binding between primase and RNA/DNA duplex
   - MMPBSA.py

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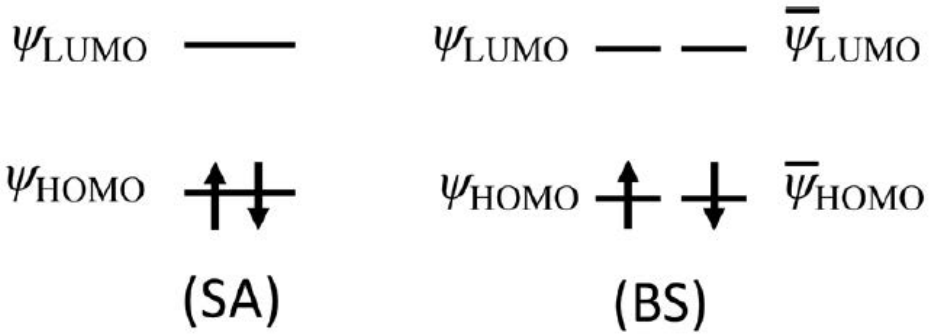
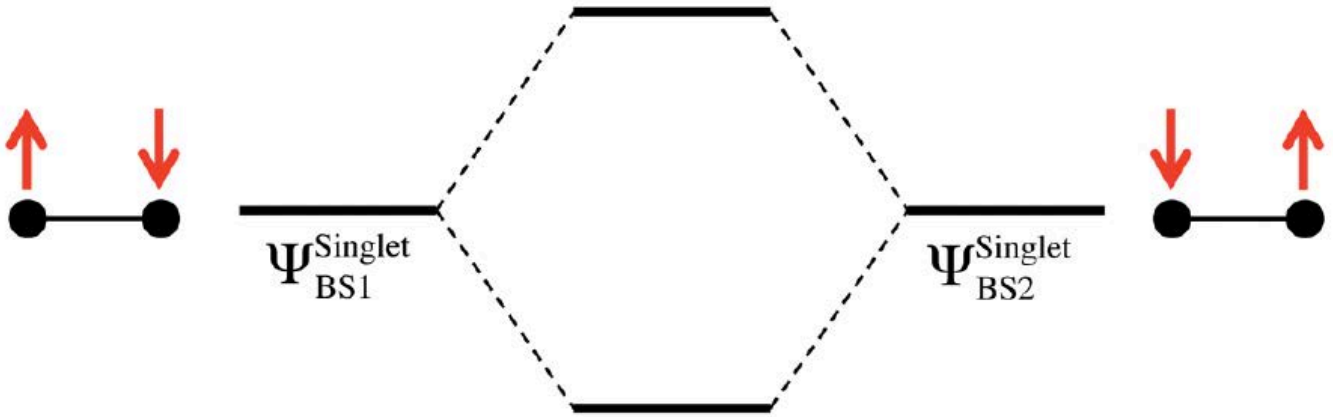
Broken-symmetry method



$$\psi_{HOMO}^{BS} = \cos\theta\psi_{HOMO} + \sin\theta\psi_{LUMO'}$$

$$\overline{\psi}_{HOMO}^{BS} = \cos\theta\psi_{HOMO} - \sin\theta\psi_{LUMO'}$$

$$\Psi^{Triplet} = \frac{1}{\sqrt{2}}\left(\Psi_{BS1}^{Singlet} + \Psi_{BS2}^{Singlet}\right)$$



$$\Psi^{Singlet} = \frac{1}{\sqrt{2}}\left(\Psi_{BS1}^{Singlet} - \Psi_{BS2}^{Singlet}\right)$$

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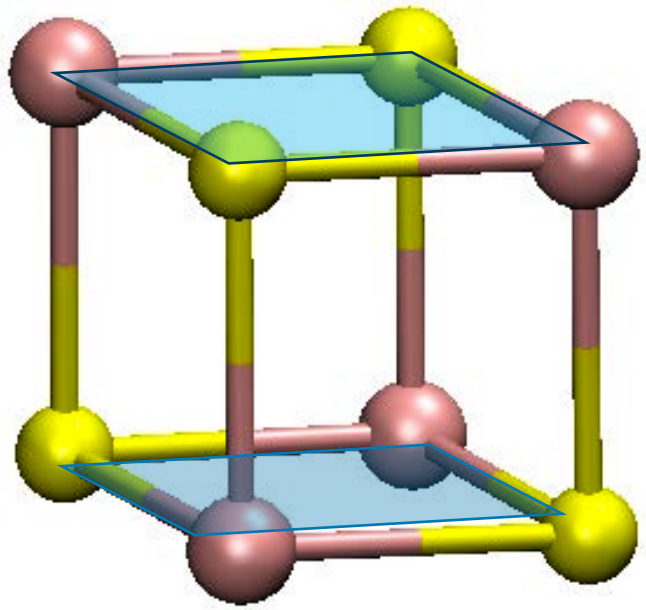
Kitagawa, Y. et al., 2018. In *Symmetry (Group Theory) and Mathematical Treatment in Chemistry*.

Modeling and computational setup

B3LYP/6-31G**, COSMO

PDB 5F0Q



Charge = -2
S = 9/2

Charge = -1
S = 9/2

Charge = -1
S = 4

Charge = -1
S = 9/2

Fe-coordinated Cys are included in the treatment but not shown here

$Fe_4S_4^{3+}$

$Fe_4S_4^{2+}$

6 assignments

3 assignments

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Structural comparison of $Fe_4S_4^{3+}$ DFT structures with crystal structure

Structures	RMSD (Å)
$\mathbf{1}_{3+}$	0.283
$\mathbf{2}_{3+}$	0.278
$\mathbf{3}_{3+}$	0.258
$\mathbf{4}_{3+}$	0.311
$\mathbf{5}_{3+}$	0.279
$\mathbf{6}_{3+}$	0.307

$[Fe_4S_4]$ cluster of primase was likely crystallized in the oxidized state of 3+, as the (aerobic) sitting-drop vapor diffusion protocol was utilized and generated needle-like prisms over 2-4 days.

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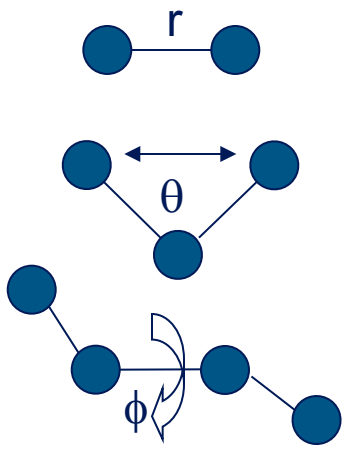
Overview of force field parameters

Bonds $k_r(r - r_0)^2$

Angles $k_\theta(\theta - \theta_0)^2$

Torsions $\sum_n k_n(\cos n\varphi)$



Nonbonded:

Lennard-Jones $\varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right]$

Electrostatic $\frac{q_i q_j}{r_{ij}}$

Sources of parameters:

- Gas-phase QM
- Macroscopic properties *via* liquid state simulation, e.g., density, heat capacity, compressibility (esp. OPLS)
- Spectroscopic and crystallographic data (small molecules)

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All-Atom Force Fields: e.g., CHARMM, AMBER, OPLS, GROMOS

Bond and angle force constants

$$[\mathbf{k}_{AB}] = -\begin{bmatrix} \frac{\partial^2 E}{\partial x_A \partial x_B} & \frac{\partial^2 E}{\partial x_A \partial y_B} & \frac{\partial^2 E}{\partial x_A \partial z_B} \\ \frac{\partial^2 E}{\partial y_A \partial x_B} & \frac{\partial^2 E}{\partial y_A \partial y_B} & \frac{\partial^2 E}{\partial y_A \partial z_B} \\ \frac{\partial^2 E}{\partial z_A \partial x_B} & \frac{\partial^2 E}{\partial z_A \partial y_B} & \frac{\partial^2 E}{\partial z_A \partial z_B} \end{bmatrix}$$

Visual Force Field Derivation Toolkit (VFFDT)

$$k_r = \sum_{i=1}^{3} \lambda_i^{AB} |\hat{u}^{AB} \cdot \hat{v}_i^{AB}|$$

$$\frac{1}{k_\theta} = \frac{1}{R_{AB}^2 k_{PA}} + \frac{1}{R_{CB}^2 k_{PC}}$$

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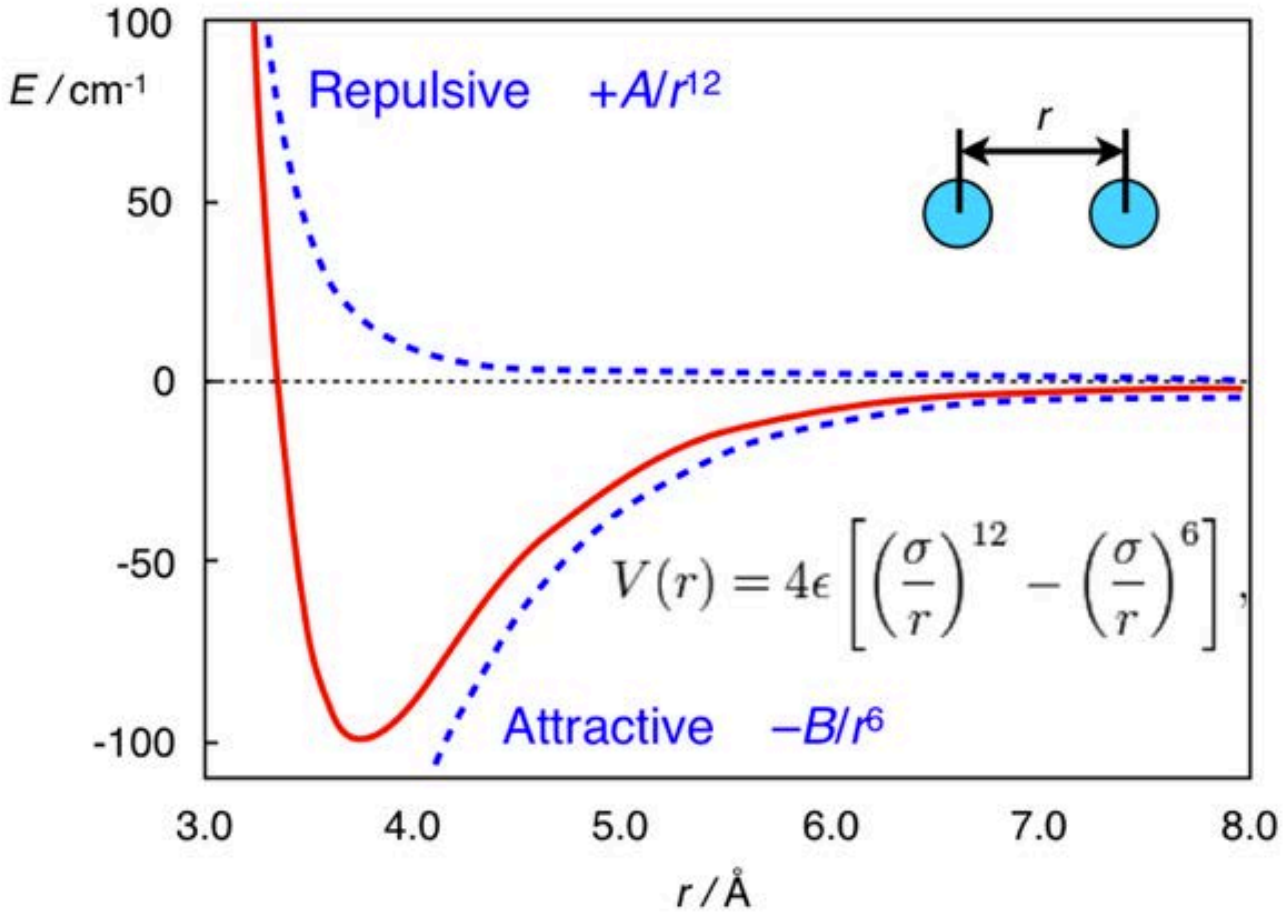
Seminario, J.M., 1996. *Int. J. Quantum Chem.*, *60*(7), pp.1271-1277.

Zheng, S. et al., 2016. *J. Chem. Inf. Model.*, *56*(4), pp.811-818.

12-6 Lennard-Jones parameters

- Dispersion and short-range repulsion are then combined in the Lennard-Jones formula: $A/r^{12} - B/r^{6}$
- LJ parameters are scaled according to formal charges of Fe in the cluster
- i.e., $Fe^{2.5+}$ parameters are derived as the average of the Fe^{2+} and Fe^{3+} parameters



RESP Charges: B3LYP/6-31G* in order for compatibility with ff99SB

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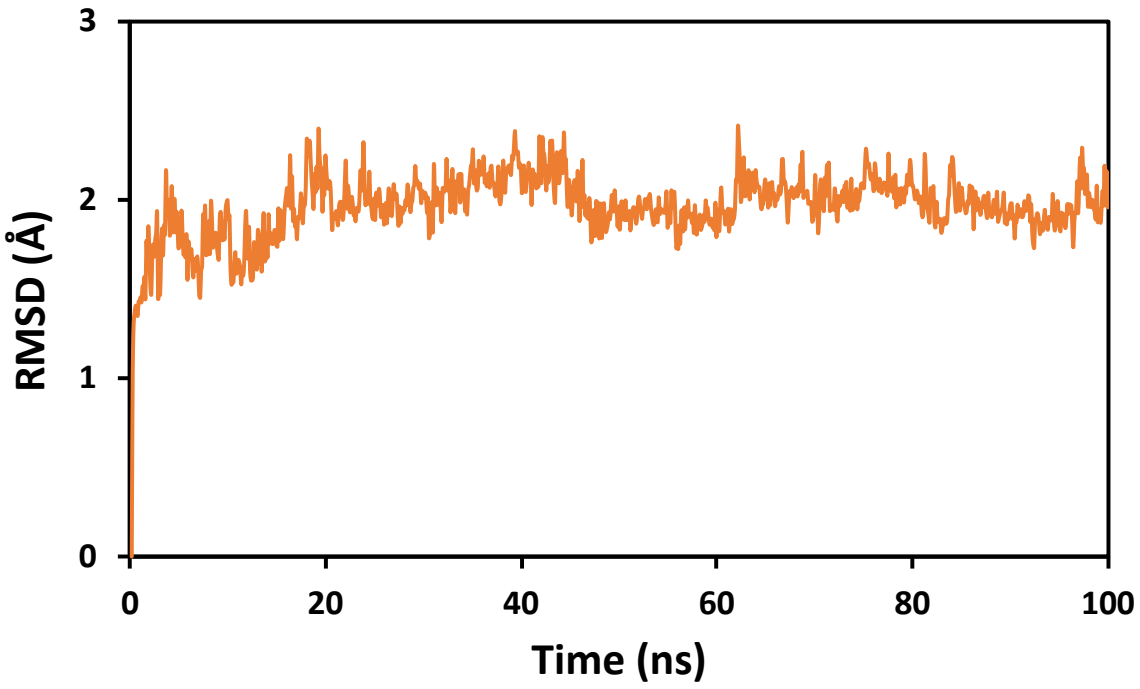
Li, P. et al., 2013. *JCTC*, *9*(6), pp.2733-2748.

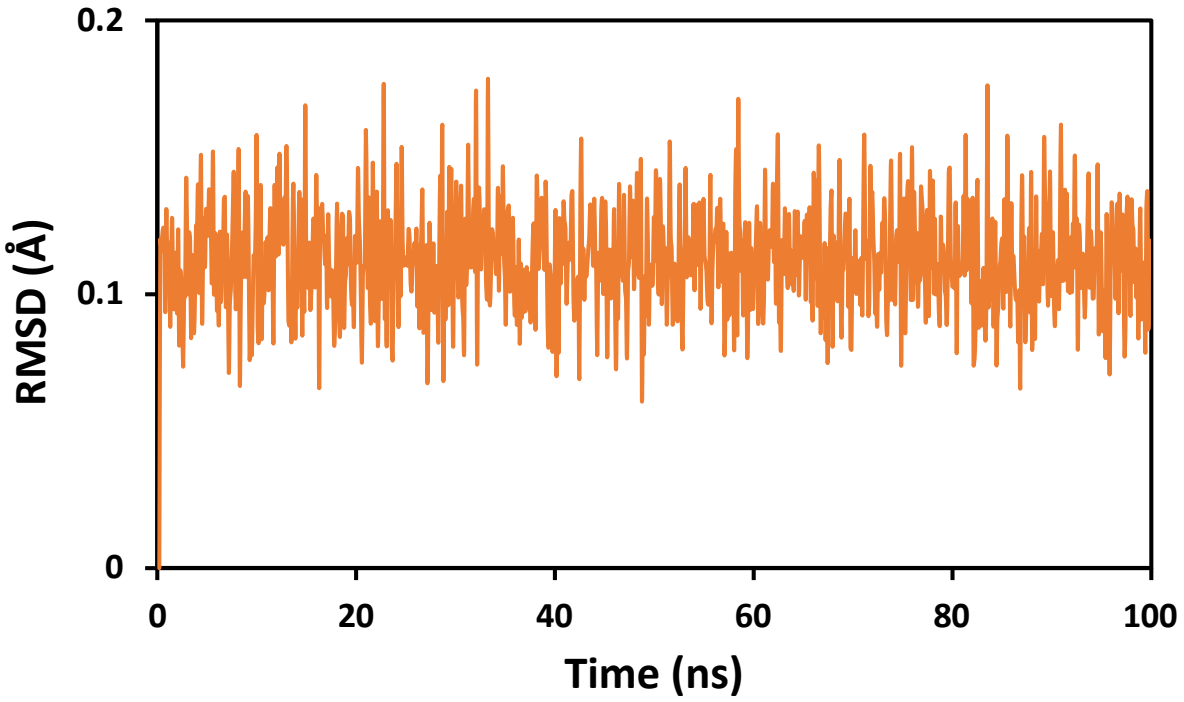
Li, P. et al., 2014. *J. Phys. Chem. B*, *119*(3), pp.883-895.

Validation of force field parameters for the $[4Fe4S]^{3+}$ cluster

Using ‘average’ parameters,



Cluster + Protein + DNA



Cluster

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EHPath.py

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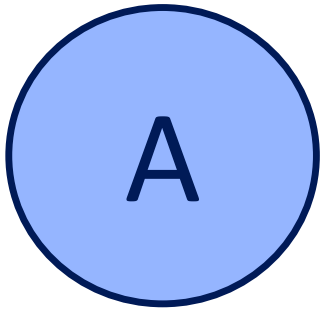
Charge transfer between donor and acceptor



$k_{DA} = ?$

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Marcus theory of charge transfer

$$k_{DA} = \frac{2\pi}{\hbar} \langle V_{DA}{}^2 \rangle \frac{1}{\sqrt{4\pi\lambda_{DA} T}} \mathrm{e}^{-\frac{(\Delta G^\circ + \lambda_{DA})^2}{4\lambda_{DA} k_B T}}$$

V_{DA} - electronic coupling, decays with donor/acceptor distance.

ΔG° - free energy change of the CT reaction.

λ_{DA} - reorganization energy, depends on changes of solvation and donor/acceptor geometries upon CT.

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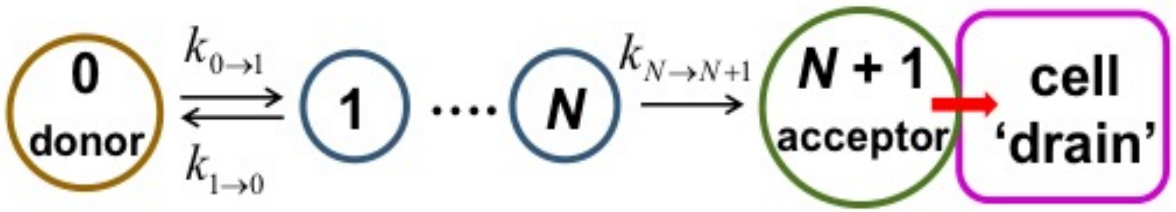
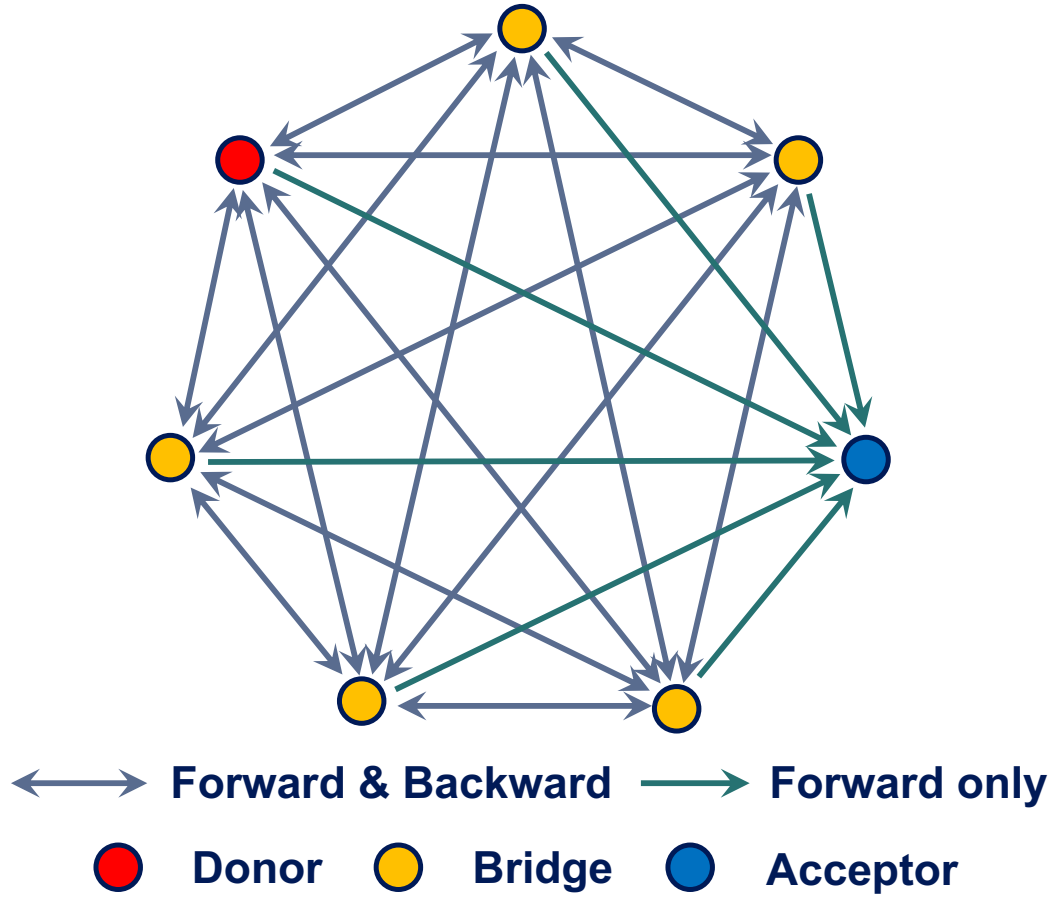
Kinetic model and mean residence time



$$\tau = \sum_{n=0}^{N} \tau_n = \sum_{n=0}^{N-1} \frac{1}{k_{n\to n+1}} \left(\sum_{j=0}^{N-n-1} \prod_{i=n+1}^{N-j} \frac{k_{i\to i-1}}{k_{i\to i+1}} + 1 \right) + \frac{1}{k_{N\to N+1}}$$

$$\tau_{approx} \cong \sum_{n=0}^{N} \frac{1}{k_{n\to n+1}}$$



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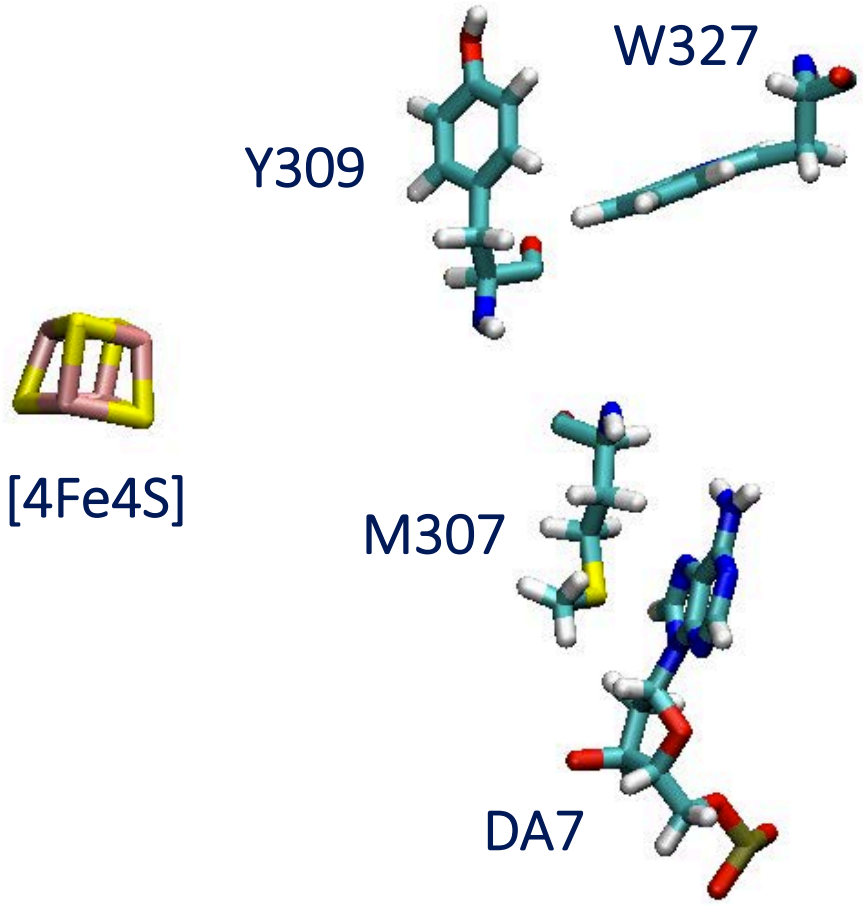
Teo, R. D. et. al, 2019. *Chem*, *5*(1), pp.122-137.

Pathway analysis in wild-type p58c-DNA/RNA using EHPath.py

Using MD snapshots from 20 - 100ns,

Top hopping pathways	% of pathways
[4Fe4S]-DA7	1.2
[4Fe4S]-Y309-DA7	54.3
[4Fe4S]-Y309-M307-DA7	33.3
[4Fe4S]-Y309-W327-DA7	11.1

At 20 ns,



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MMPBSA.py

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Miller III, B.R. et. al. *JCTC*, *8*(9), pp.3314-3321.

Free energy calculations using MMPBSA.py

$$\Delta G_{\text{binding,solvated}} = \Delta G_{\text{complex, solvated}} - [\Delta G_{\text{receptor, solvated}} + \Delta G_{\text{ligand,solvated}}]$$

$$\Delta G_{\text{solvated}} \cong \langle E_{\text{gas}} \rangle + \langle \Delta G_{\text{solvation}} \rangle - T\langle S_{\text{solute}} \rangle$$

$$= \frac{1}{N}\sum_{i=1}^{N} E_{i,\text{gas}} + \frac{1}{N}\sum_{i=1}^{N} \Delta G_{i,\text{solvation}} - \frac{T}{N}\sum_{i=1}^{N} S_{i,\text{solute}}$$

- E_{gas} – molecular mechanical energies (bonded, electrostatic, VDW)
- $\Delta G_{solvation}$ – polar (implicit solvent models) and non-polar
- S_{solute} – vibrational contribution calculated by normal mode analysis or quasi-harmonic approximation
- Single trajectory protocol (STP)

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Miller III, B.R. et. al. *JCTC*, *8*(9), pp.3314-3321.

$[4Fe4S]^{3+}$-DNA/RNA binding free energy (MM/PBSA)

Differences (Complex – Receptor – Ligand):

Energy Component	Average	Std. Dev.	Std. Err. of Mean
VDWAALS	−120.9776	8.1879	1.1465
EEL	−3093.7918	82.3014	11.5245
EPB	3078.1227	80.0224	11.2054
ENPOLAR	−12.2814	0.5799	0.0812
EDISPER	0.0000	0.0000	0.0000
DELTA G gas	−3214.7693	82.0505	11.4894
DELTA G solv	3065.8414	79.9099	11.1896
DELTA TOTAL	−148.9280	9.9197	1.3890

Using Quasi-harmonic Entropy Approximation: DELTA G binding = −7.8911

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Acknowledgements

- Professor David Beratan
- Professor Agostino Migliore
- Dr. Victor Anisimov
- Beratan group
- Dr. Tomasz Janowski
- Tom Milledge
- Blue Waters and NCSA staff


Thank you for your attention!