

***Physical Chemistry
of Redox-Active Proteins / Enzymes :***

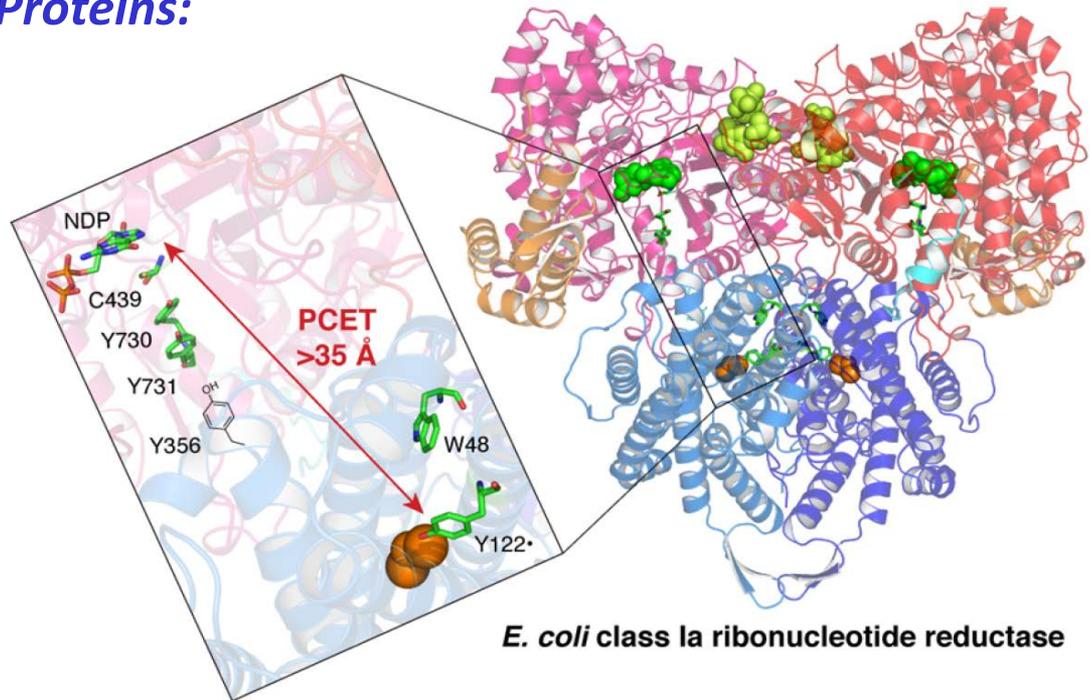
*Electron Transfer Kinetics, Marcus Theory,
Adiabatic and Non-Adiabatic Reaction Dynamics,
Landau-Zener model*

- lecture 13 -

Examples of Electron Transfer in Proteins:

Ribonucleotide reductase:

(proton-coupled electron transfer)

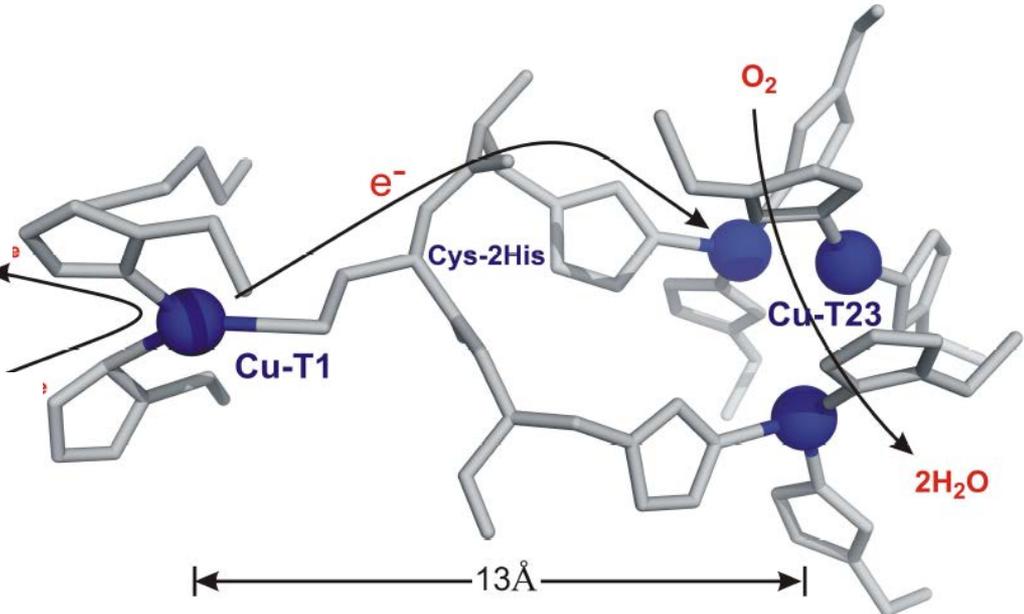


E. coli class Ia ribonucleotide reductase

Multicopper reductase

Oxidized substrate

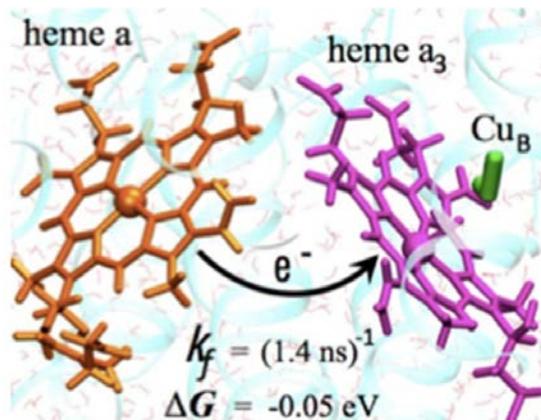
Reduced substrate



Electron transfers

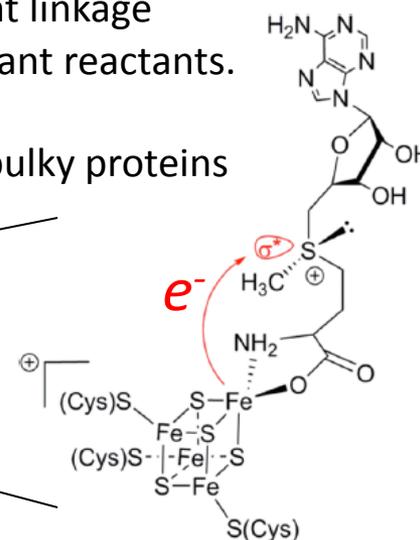
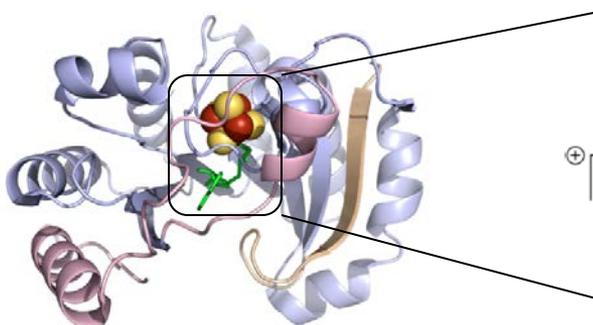
• **Outer-sphere ET** event that occurs between chemical species that remain separate and intact before, during, and after the ET event. Long-range ETs in biology are all of the outer-sphere type.

cytochrome C oxidase:



Inner-sphere ET a redox chemical reaction that proceeds via a covalent linkage — a strong electronic interaction— between the oxidant and the reductant reactants. A ligand bridges the two metal redox centers during the ET event. It is rare in biological systems, where redox sites are often shielded by bulky proteins

A radical S-adenosylmethionine enzyme:



Classical Marcus theory (for outer-sphere ET)

1) Eyring's TST (lecture 8)

$$k = \tau_{el} \frac{k_B T}{h} \exp\left[-\frac{\Delta G^\ddagger}{k_B T}\right]$$

Electron Transmission coefficient

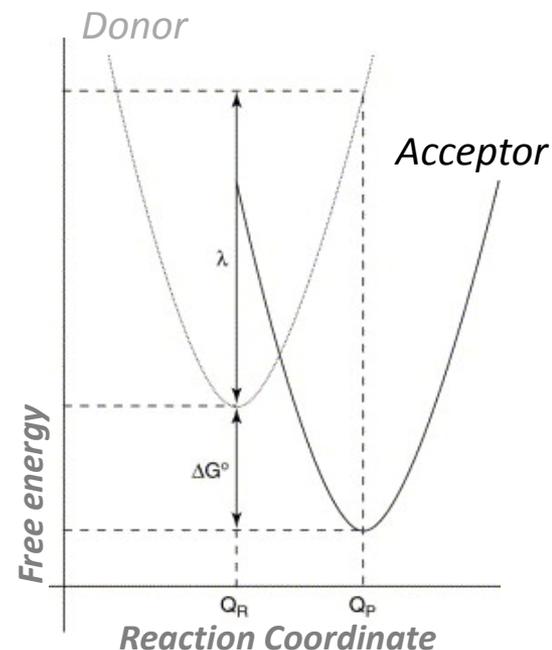
2) Reactant and product free-energy surfaces considered as two equivalent parabolas, then:

$$\Delta G^\ddagger = \frac{(\lambda + \Delta G^\circ)^2}{4\lambda}$$

$$k_{ET} = A \exp\left[-\frac{(\lambda + \Delta G^\circ)^2}{4\lambda k_B T}\right]$$



This term depends on the nature of ET (unimolecular, bimolecular...)



λ – reorganization energy (always positive)

(the free energy change due to the (R→P) nuclear rearrangement with no ET)

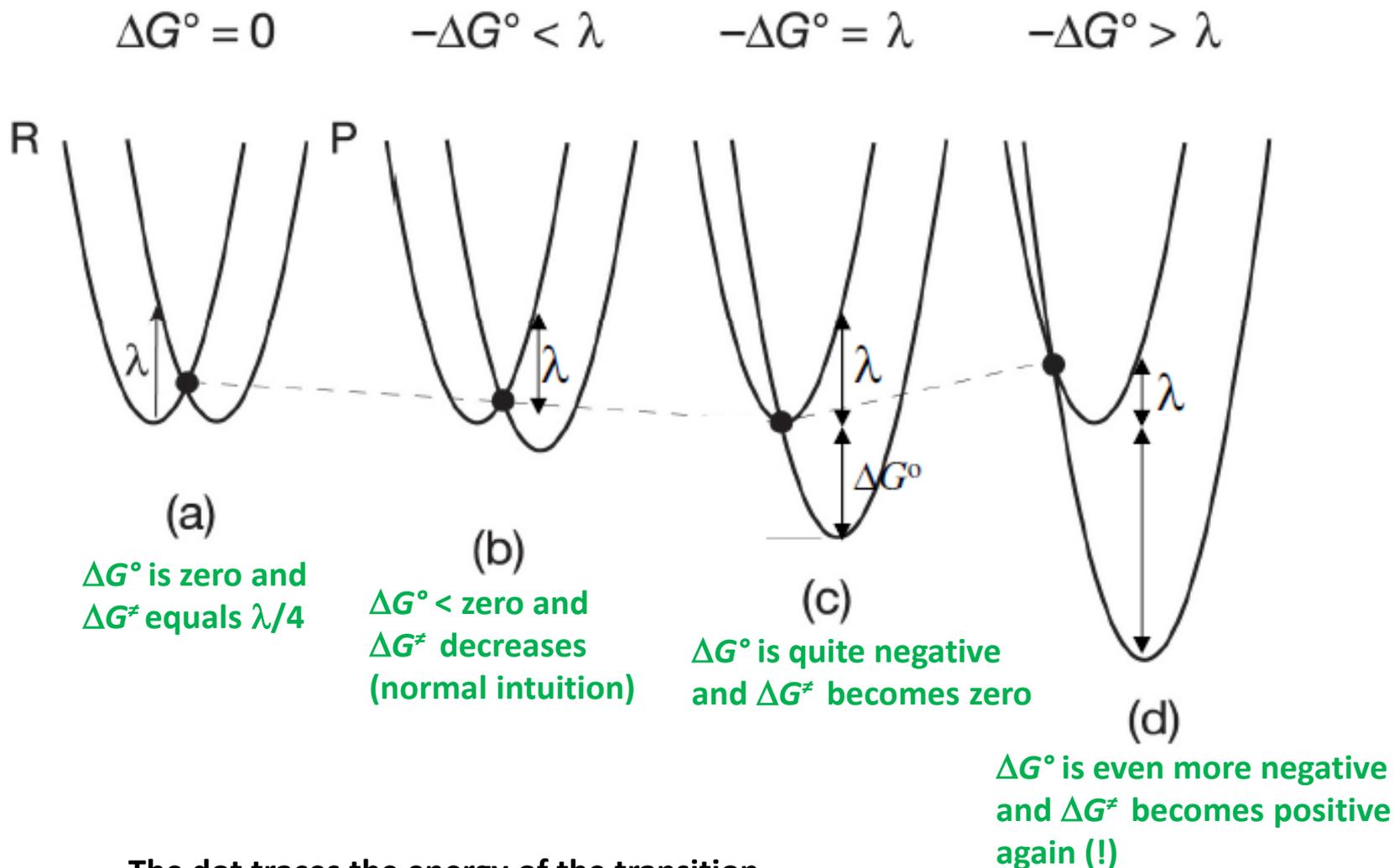
$$\lambda = \lambda_i + \lambda_o \rightarrow \text{Reorganization energy of a solvent}$$

Reorganization energy of a solute

ΔG° reaction free energy (thermodynamic driving force)

$$\Delta G^\circ = -F\Delta E^0 = -F(E_A^0 - E_D^0)$$

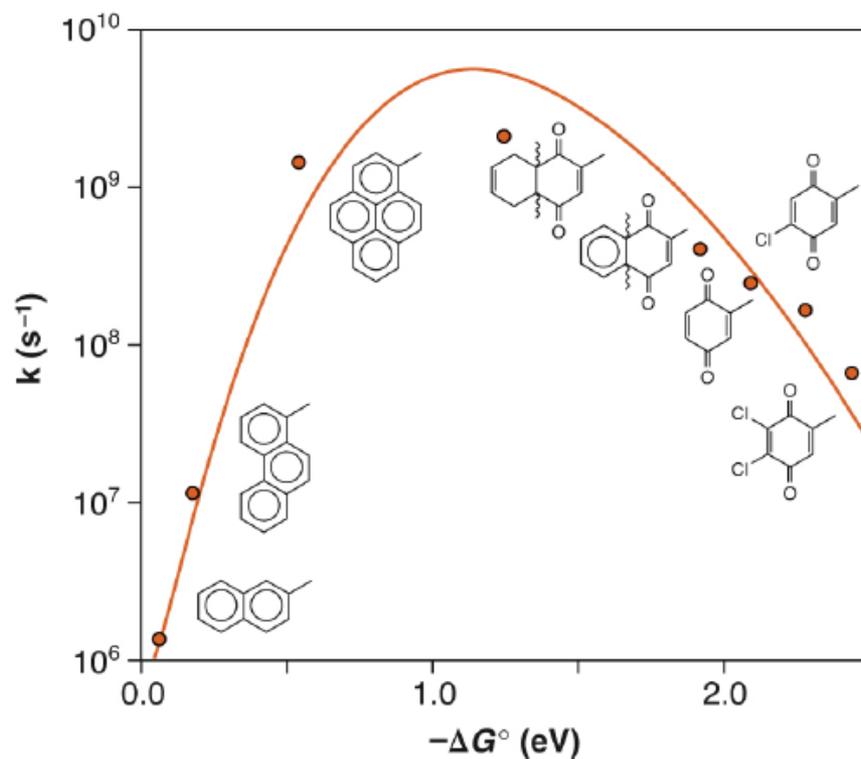
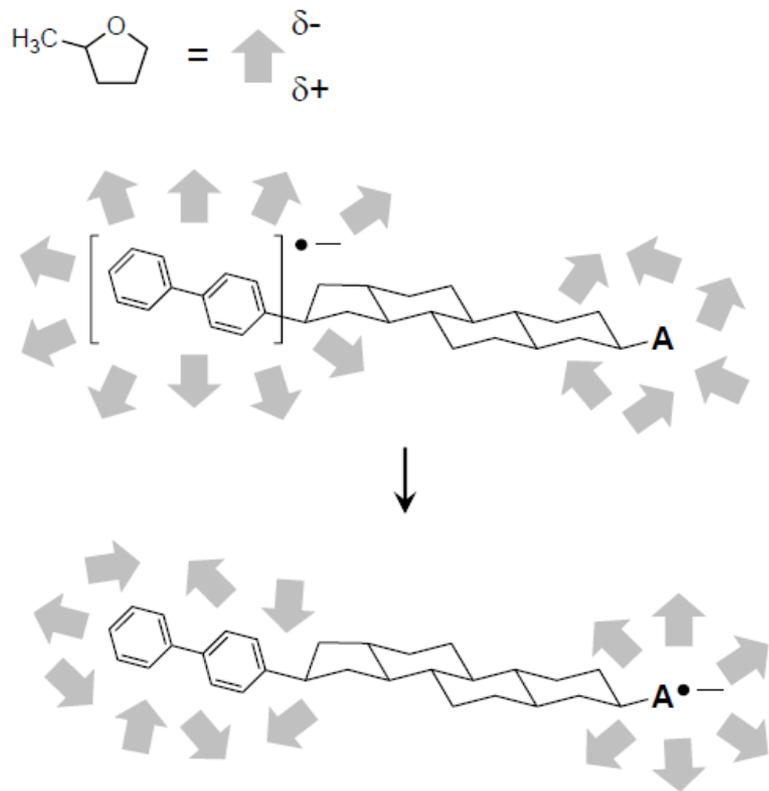
The Marcus Predictions → Inverted Region



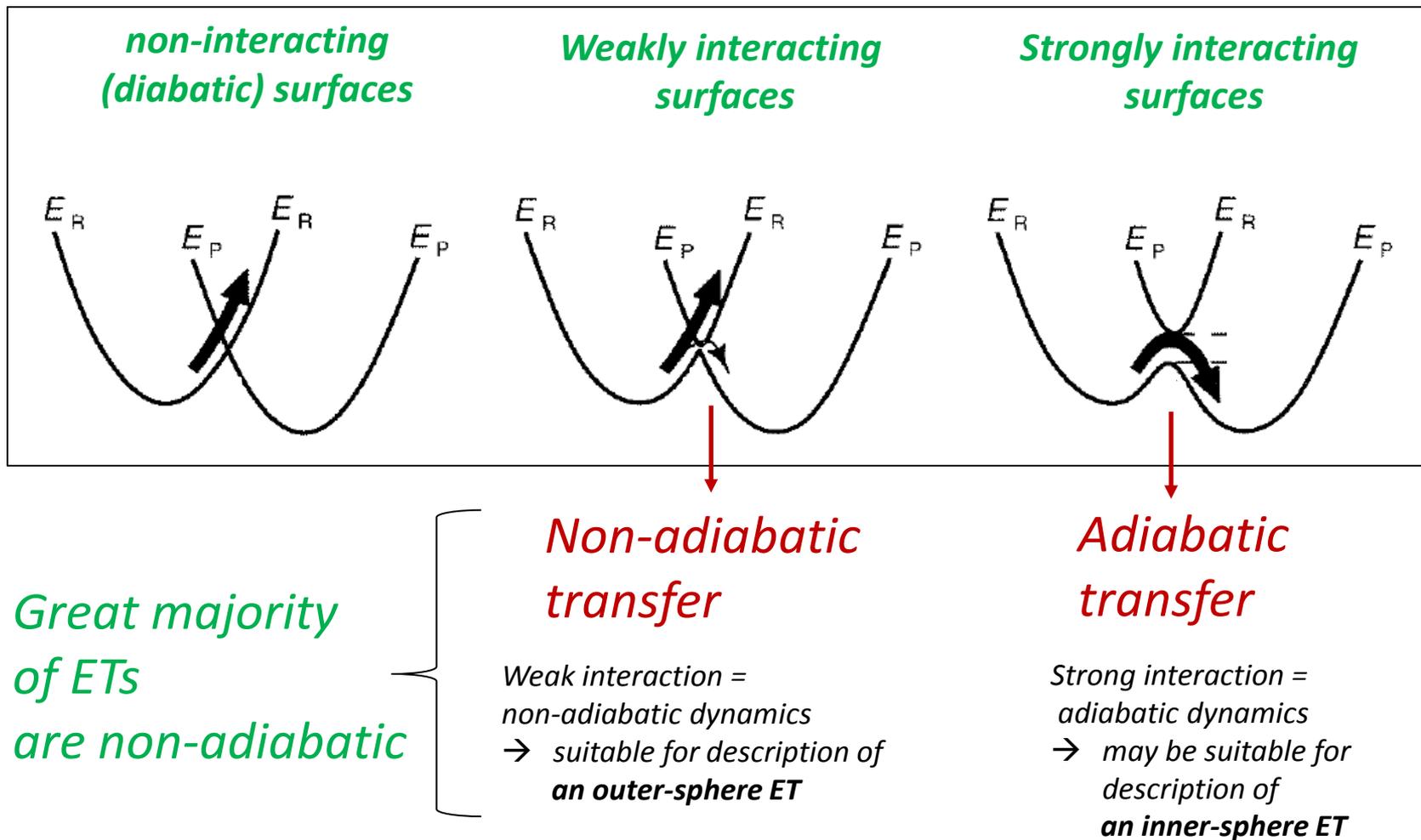
- The dot traces the energy of the transition State as ΔG° becomes more negative

The Marcus Inverted Region → Experimental Confirmation

“**inverted region**”: redox reactions get faster as they get more spontaneous only up to a certain point; beyond this point, in the inverted region, they get slower for more negative ΔG° .



Adiabatic vs. non-adiabatic ETs



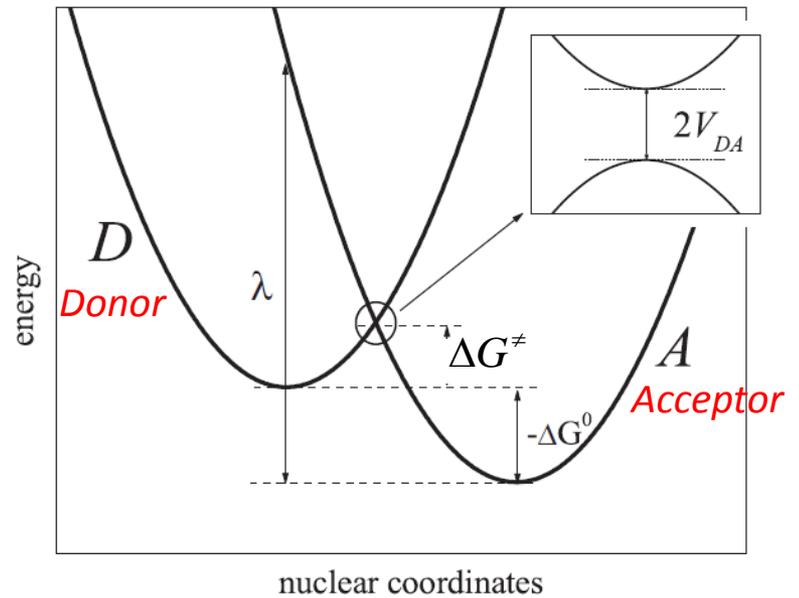
The arrows indicate the relative probability of crossing to the product surface (E_R to E_P)

Semi-classical Marcus theory for non-adiabatic ETs:

the ET reaction in the non-adiabatic limit can be considered analogously to an optical transition between two electronic states within the Franck-Condon approximation.

1) Reactant and product free-energy surfaces considered as two equivalent parabolas

2) Fermi's Golden Rule (a simple formula for the constant transition rate from one energy eigenstate of a quantum system into other energy eigenstates, **effected by a perturbation**)



$$k = \frac{2\pi}{\hbar} |V_{DA}|^2 \times \text{FCWD}$$

Electronic coupling
Franck-Condon weighted density of states

where $|V_{AD}|^2 = |\langle \Psi_A | \hat{V} | \Psi_D \rangle|^2 = V_0^2 e^{-\beta R_{AD}}$ and $\text{FCWD} = \frac{1}{\sqrt{4\pi\lambda k_B T}} \exp\left[-\frac{(\lambda + \Delta G^\circ)^2}{4\lambda k_B T}\right]$

FCWD which is a sum of products of overlap integral of the vibrational and solvational wavefunctions of the reactants with those of the products weighted by Boltzmann factors. In the high temperature (classical) limit, the FCWD reduces to the above shown FCWD formula.

3) Applying 1 & 2:

Marcus equation in the non-adiabatic limit

$$k_{ET} = \frac{2\pi}{\hbar} V_0^2 e^{-\beta R_{AD}} \frac{1}{\sqrt{4\pi\lambda k_B T}} \exp\left[-\frac{(\lambda + \Delta G^\circ)^2}{4\lambda k_B T}\right]$$

In proteins
typically $1-10^{-3} \text{ cm}^{-1}$

Coupling decay for electron tunneling

Rate constant decreases exponentially with distance consistent with the exponential radial dependence of the WF, if ET between D and A not enhanced by electronic mixing with the intervening space i.e., unless transported by localization and hopping or by resonance in general, **no more than 20 Å**.

Assumptions:

- The nonadiabatic ET model embodied in the equation rests on the assumption that the electronic transition from the reactant potential energy surface (D + A) to the product surface (D⁺ + A⁻) is much lower than the frequency of nuclear motion on these surfaces.

If not the case, then adiabatic approach

Solvent-controlled
adiabatic processes:

$$k_{ET,adiab} = \frac{k_{ET,nonadiab}}{1 - \kappa}$$

Adiabaticity factor

$$\kappa = \frac{2\pi V_{DA}^2 \langle \tau \rangle}{\hbar \lambda}$$

Solvent relaxation time (typically 10^{-11} s)

For $\kappa \rightarrow 1$ $V_{DA} = 60 \text{ cm}^{-1}$

Input Quantities for non-adiabatic Marcus equation

- *Electronic coupling, V_{DA}*

→ QM calculations of electronic matrix element or surface splitting

- *Reorganization energies (λ)*

→ outer-sphere (solvent) : dielectric continuum model or MD

→ inner-sphere (solute modes) : QM calculations of solute

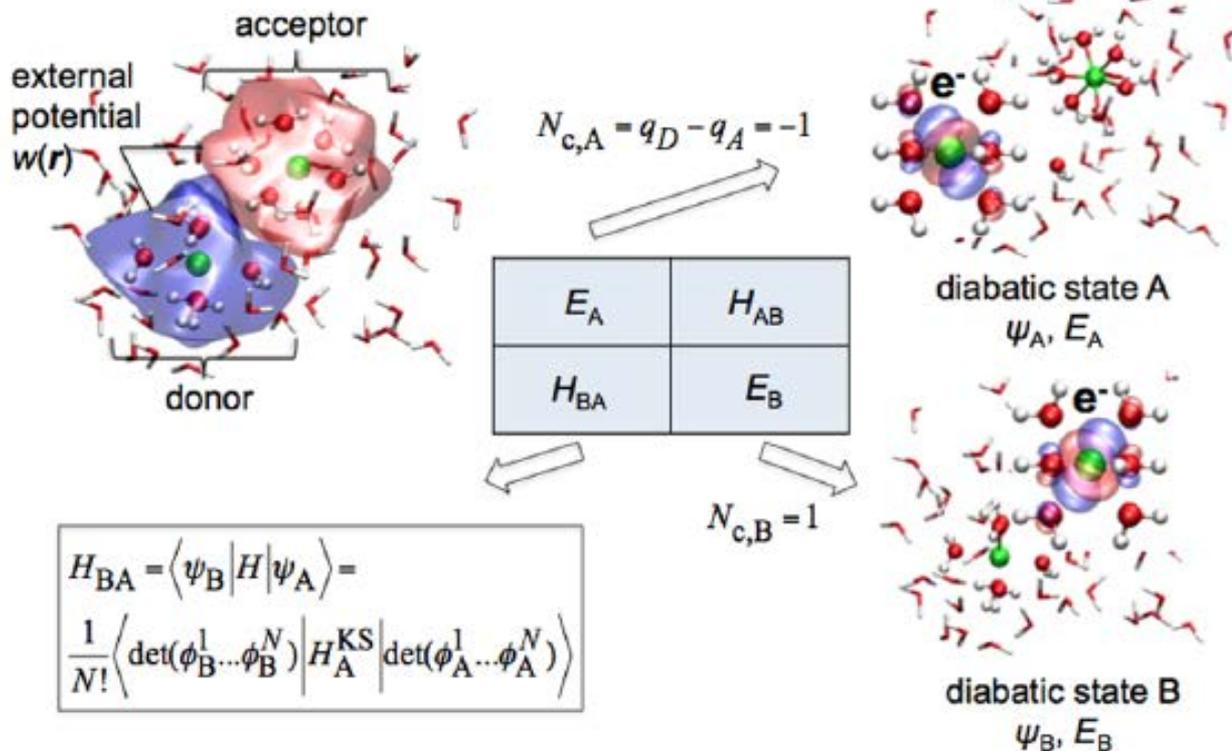
- *Free energy of reaction for ground state (ΔG^0)*

→ Calculated or experimental reduction potentials (**how to calculate reduction potential – see e.g. lecture 5**)

Electronic coupling, V_{DA} : Computational Evaluation

Constrained DFT

e.g.:



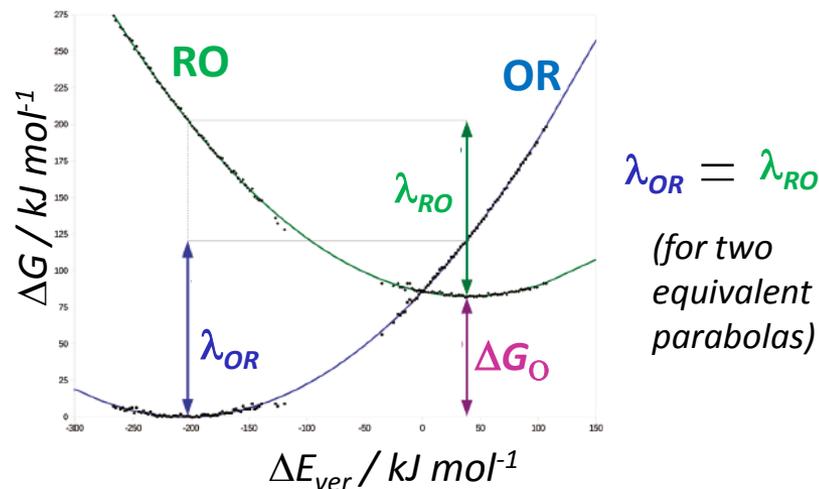
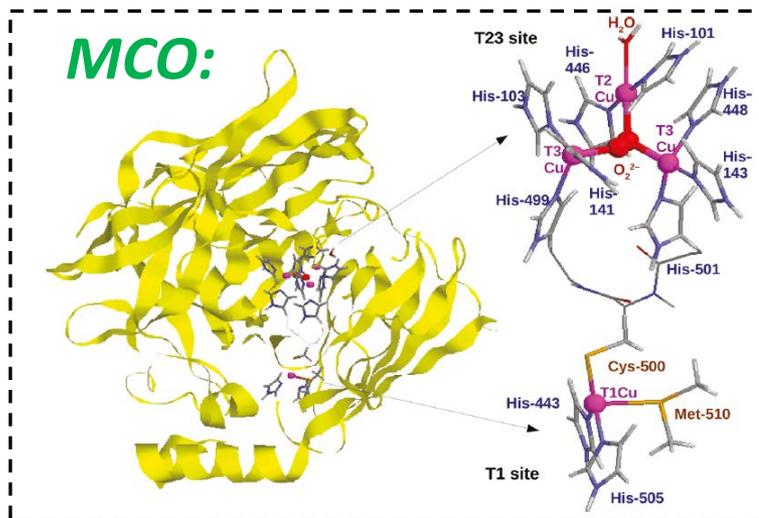
and many other empirical or semiempirical approaches...

(more in Chem Rev 2015, Jochen Blumberger)

Ab initio QM (CASPT2, CI...) – suitable only for very small systems

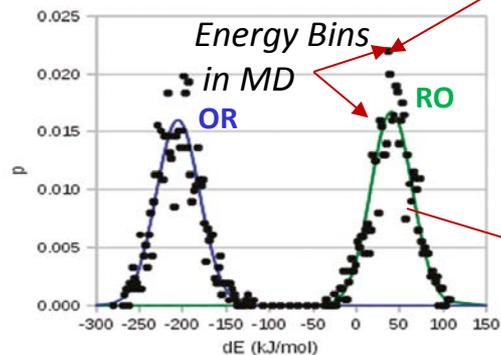
Reorganization energy (and reduction potential) : Computational Evaluation

Example (taken from J. Phys. Chem B Ryde et al 2015) :



- Energies (E): QM/MM-2QM ; sampling MD (together: QTCP-2QM)
- $\Delta E_{ver} = E_{OR} - E_{RO}$ is **vertical energy gap** (defined as the reaction coordinate)

Then, $\Delta G = RT \ln p(\Delta E_{ver})$ Eq. 1



Probability of observing a certain value of ΔE_{ver}

Gaussian fit (Marcus Theory)
=> the ΔG surfaces are parabolic (OR and RO) according Eq. 1

Alternative approach:

$$\lambda = \frac{\langle \Delta E_{ver} \rangle_{OR} - \langle \Delta E_{ver} \rangle_{RO}}{2} \quad \text{Eq. 2}$$

$$\Delta G_0 = \frac{\langle \Delta E_{ver} \rangle_{OR} + \langle \Delta E_{ver} \rangle_{RO}}{2} \quad \text{Eq. 3}$$

$\langle \rangle$ Averages over MD simulations

Electron transfers in proteins through tunneling and hopping

- The tunneling time tables compiled by Moser, Dutton, Winkler and Gray :
a single step tunneling gives D---A distances up to ~20 Å.
- **Many protein structures support ET over longer distances**
(hydrogenases, complexes of the respiratory chain, CO-dehydrogenases, multi-heme cytochromes...)
- The solution for **very long ranged ET in biology** is to arrange redox active cofactors in chains at close cofactor **spacings of typically 10-15 Å, enabling consecutive electron tunneling steps over shorter distances (=hopping).**

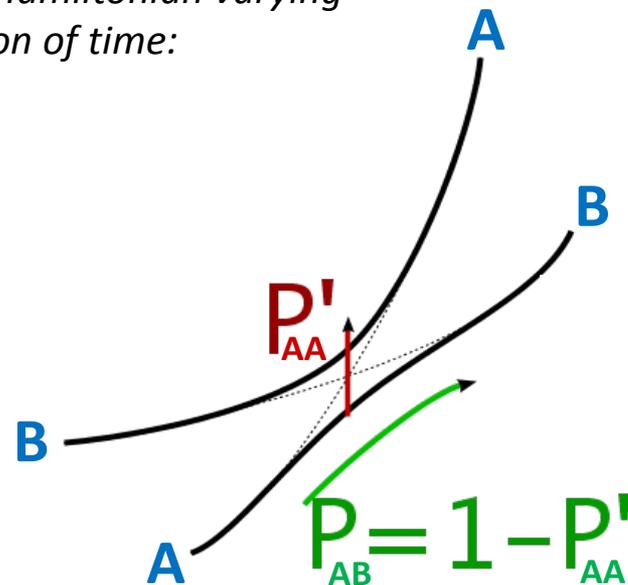
(in addition to metal containing cofactors, ionizable protein residues such as tryptophan, tyrosine or cysteines are good mediators for hopping mechanism)

Landau-Zener transition dynamics

Analytic solution to the equations of motion governing the transition dynamics of a two-level quantum mechanical system, with a time-dependent Hamiltonian varying such that the energy separation of the two states is a linear function of time:

The probability that the system will cross from state A to B:

$$P_{AB} = 1 - \exp \left[- \frac{2\pi}{\hbar} \frac{|H_{AB}|^2}{\frac{d(V_A - V_B)}{dQ} \frac{dQ}{dt}} \right]$$



$|H_{AB}|^2 = |\langle \Psi_A | \hat{V} | \Psi_B \rangle|^2$ — Electronic coupling between A and B

$V_A(Q); V_B(Q)$ — Born-Oppenheimer potential energy surfaces

$\frac{dQ}{dt}$ — Velocity of the system moving along the reaction coordinate Q

At non-zero velocities, transitions occur with probability as described by the Landau-Zener formula...

Landau-Zener transition model

- *The LZ theory can be used to derive the Marcus equation for the non-adiabatic ET (page 9)*
- *The LZ theory can be used for spin-forbidden reactions* (calculation of rate constant is relatively complex – not discussed here)

$$|V_{DA}|^2 = \left| \langle \Psi_D | H_{SOC} | \Psi_A \rangle \right|^2$$

An electronic coupling between two spin states is due to their (relativistic) spin-orbit interaction = spin-orbit coupling

If SOC small
(non-adiabatic limit)

If SOC large
(adiabatic limit)
e.g. transition metals
Of second & third row

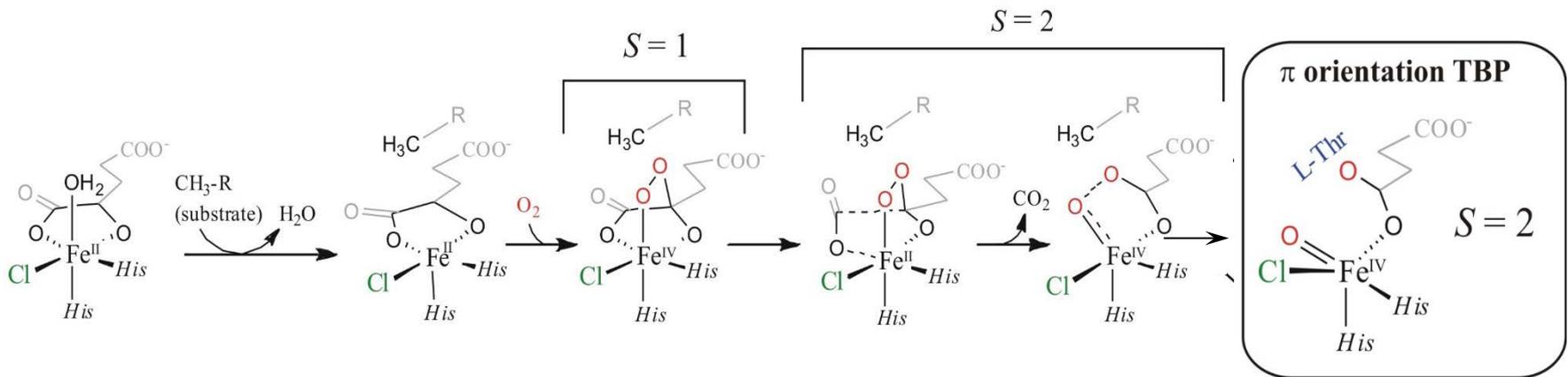
According to the **spin selection rule arising from the Wigner-Eckart theorem**, two arbitrary spin states do interact if their total spin angular momenta R and S observe this condition: **$|R-S| \leq 1 \leq |R+S|$**

e.g. S=5/2 do not directly spin-orbit couple to S=1/2 but S=3/2 does

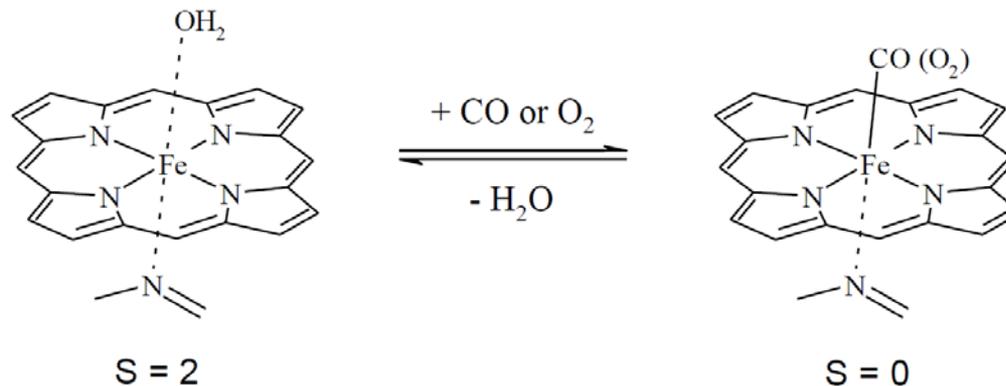
Example of multi-spin state reactivity in enzymes / proteins

- O_2 activation in α -ketoglutarate dependent mononuclear non-heme iron enzymes:

e.g., active site of syringomycin halogenase – first part of the catalytic cycle:



- O_2 /CO binding to heme-Fe complex in myoglobin



Proton-coupled Electron Transfers and H-atom Transfers

- **Concepts and theory from Marcus theory for electron transfer and analogous theories for proton transfer** (theory is complex and provided in some Hammes-Shiffer's review (e.g. JACS 2015)).
- Proton transfer can be electronically non-adiabatic, adiabatic, or in between depending on relative timescales of electronic transition (τ_e) and proton tunneling (τ_p)
 - Electronically nonadiabatic PT: electrons do not respond instantaneously to proton motion, $\tau_e \gg \tau_p$ (PCET)
 - Electronically adiabatic PT: electrons respond instantaneously to proton motion, $\tau_e \ll \tau_p$ (HAT)

e.g. *Orbital Analysis of HAT vs PCET at Transition State*

