Simulacija reakcij v raztopini in v encimih s kvantno kemijskimi orodji in tehniko EVB

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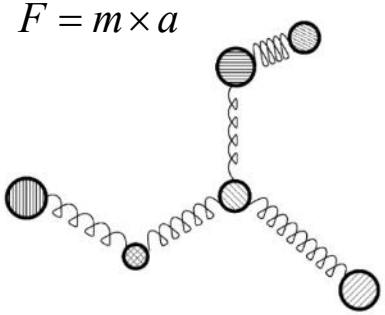
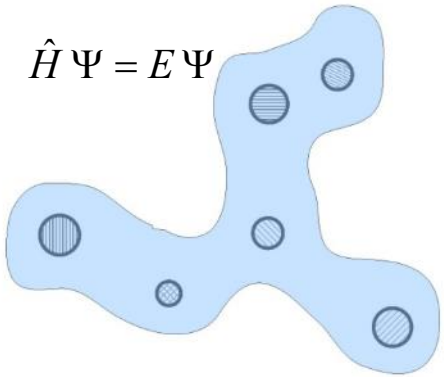
Physical background

$F = m \times a$



$\hat{H}\Psi = E\Psi$



Classical (MM)

- ❑ Empirical force field.
- ❑ Newtonian mechanics.
- ❑ No info on electron structure.
- ❑ "Permanent" chemical bonds.
- ❑ Approximate but allows for modeling of large systems.
- ❑ 10.000 atoms: fraction of a second.

Quantum-chemical (QM)

- ❑ Schrödinger equation for a many-electron system.
- ❑ Complete electron structure available.
- ❑ Bonds can break and form.
- ❑ Accurate but size limited.
- ❑ 10 atoms: a second to many days.

St. Martin's Church, Dobrepolje, Slovenia

Erwin Schrödinger (1887-1961)

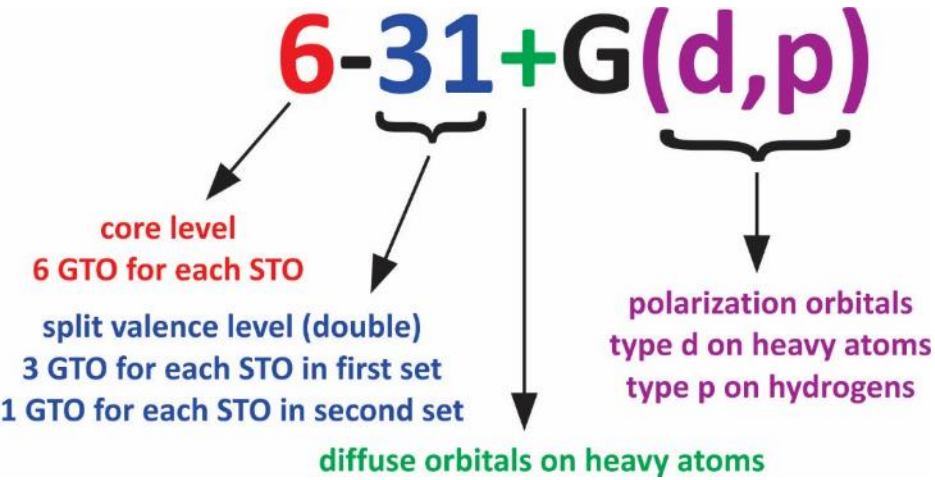
The Schrödinger equation for the electronic structure

$$\hat{H}\,\Psi = E\,\Psi$$

$$\hat{H} \equiv -\sum_i \frac{\partial^2}{\partial x_i^2} + \frac{\partial^2}{\partial y_i^2} + \frac{\partial^2}{\partial z_i^2} + \sum_{\alpha,\beta} \frac{1}{r_{\alpha\beta}} - \sum_{i,\alpha} \frac{1}{r_{i\alpha}} + \sum_{i,j} \frac{1}{r_{ij}}$$

- ❑ The SE is an analog of 2nd Newton's law.
- ❑ The Hamiltonian operator *H* includes kinetic and potential energy.
- ❑ The wavefunction Ψ is related to the probability density (electron density in the case of molecular systems).
- ❑ SE is a partial differential equation of the second order.
- ❑ Due to pairwise electron-electron repulsion terms ($1/r_{ij}$), the variables are not separable.
- ❑ No analytical solution for a many-electron system, numerical solving only.
- ❑ Extremely demanding numerical procedure.

Quantum chemistry job notation (Pople basis sets)



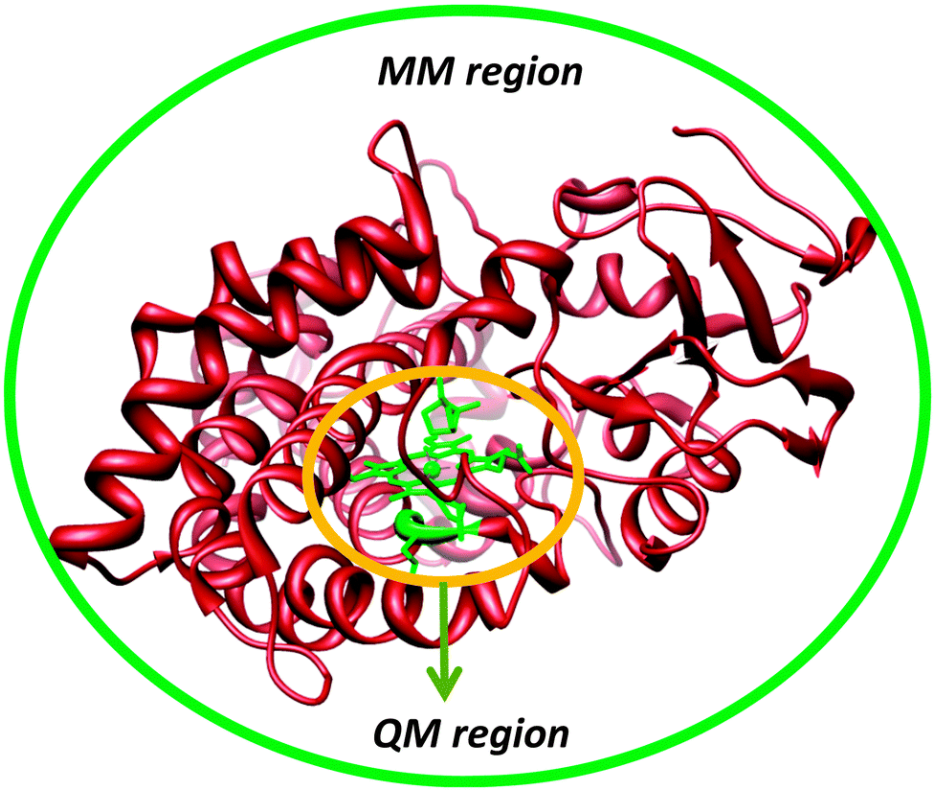
Other examples: 3-21G,6-31G, 6-311G, 6-311++G(d,p), 6-311++G(3df,3pd),…

Popular model Hamiltonians:

- ❑ HF (Hartree-Fock)
- ❑ Post-HF methods: MP2, CISD, CCSD(T),…
- ❑ DFT methods: B3LYP, PBE, M06,…

Between the atolls, Maldives

Hybrid/Multiscale methods (QM/MM)



- ❑ Central part of interest (e.g. enzyme active site) treated by QM whereas the environment (the e.g. rest of the enzyme, solvent) is modeled by MM.
- ❑ In principle multiscale treatment combine advantages of both QM and MM approaches.
- ❑ Multiscale methods allow for the modeling of reactions embedded in the realistic environment. ☺
- ❑ However, performance of a QM/MM calculation is rate-limited by the slowest component. 😳 ☹ 😠
- ❑ In most cases this is the QM treatment, given that it is based on quantization of electronic structure.
- ❑ Empirical Valence Bond (EVB) **IS** a multiscale technique, but it is **NOT** based on quantization of electronic structure, therefore it does not suffer from performance limitations typical of QM/MM. ☺

Usain Bolt, 100 & 200 m WR holder, Athletissima Lausanne, Switzerland

Free energy and kinetics

$$k = \frac{k_B T}{h} \exp\left(-\frac{\Delta G^{\ddagger}}{k_B T}\right)$$

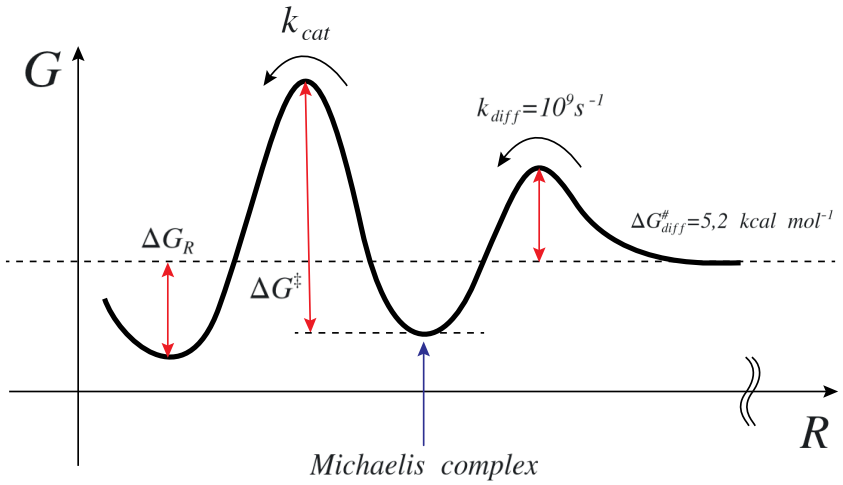
Transition state theory: the measurable reaction rate (k) is exponentially related to the free energy barrier ($\Delta G^{\ddagger}$).

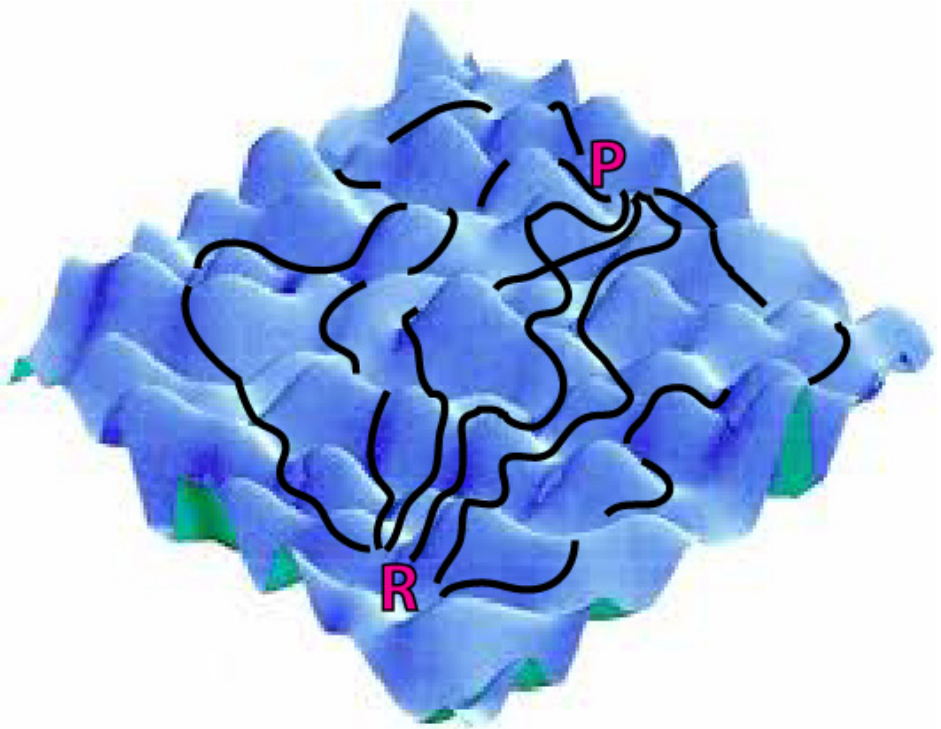
$$G = E + PV - TS$$

- ❑ In the condensed phase the essential part originates from both potential energy *E* and the entropic contribution *TS*.
- ❑ Highly accurate quantum calculations yield only the potential energy *E* but no fluctuations.
- ❑ In order to compute *G*, thermal averaging is needed → phase space sampling → MD of sufficient length.

The timescale issue

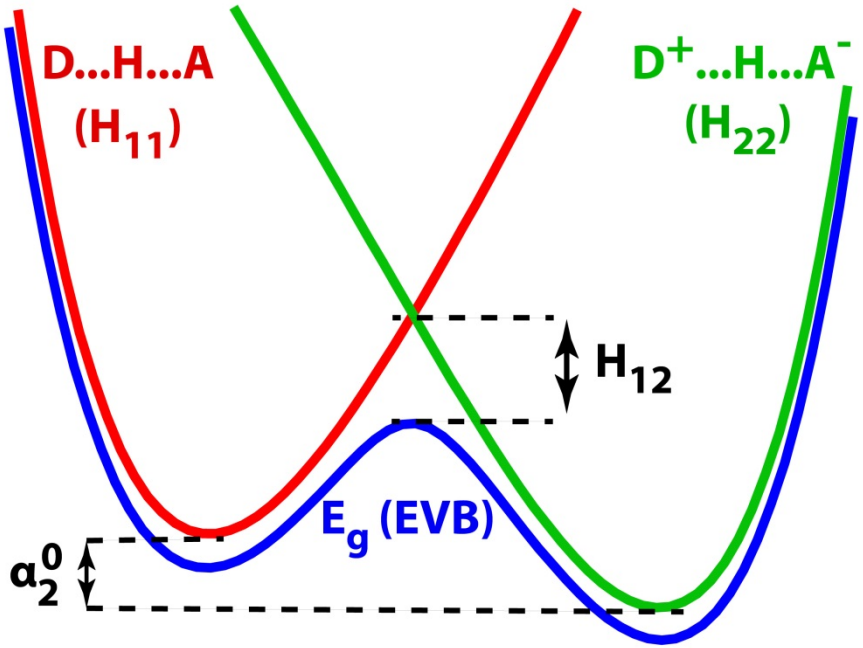
$$k_{cat} = \frac{k_B T}{h} e^{-\frac{\Delta G^{\ddagger}}{k_B T}}$$





- ❑ It takes a while (nanoseconds or more) for the reaction profile to converge.
- ❑ Reaction path sampling: "*throwing ropes over rough mountain passes in the dark*".
- ❑ Classical methods: poor for reactivity.
- ❑ QM/MM methods: accurate, but limited timescale (~100 ps). Why? **Because the cost of QM/MM is determined by the cost of the QM part,** given that QM is based on quantization of the electronic structure (not the case with EVB).

EVB methodology



- Simple but efficient multiscale (QM/MM) methodology applicable to enzymatic reactions.
- 2-state representation of a reactive system (bond breaking and making) with included quantum coupling.
- **Tunable parameters H_{12} and α_2^0 are calibrated to computed or experimental parameters of a reference reaction, usually (but not necessarily) in the solution.**
- **All parameters (H_{12}, α_2^0, H_{11} and H_{22}) transferrable between various types of environment.**
- EVB simulations support timescales typical of classical MD (inexpensive comparing to AIMD).

$$\begin{vmatrix} H_{11} - E_g & H_{12} \\ H_{12} & H_{22} - E_g \end{vmatrix} = 0$$

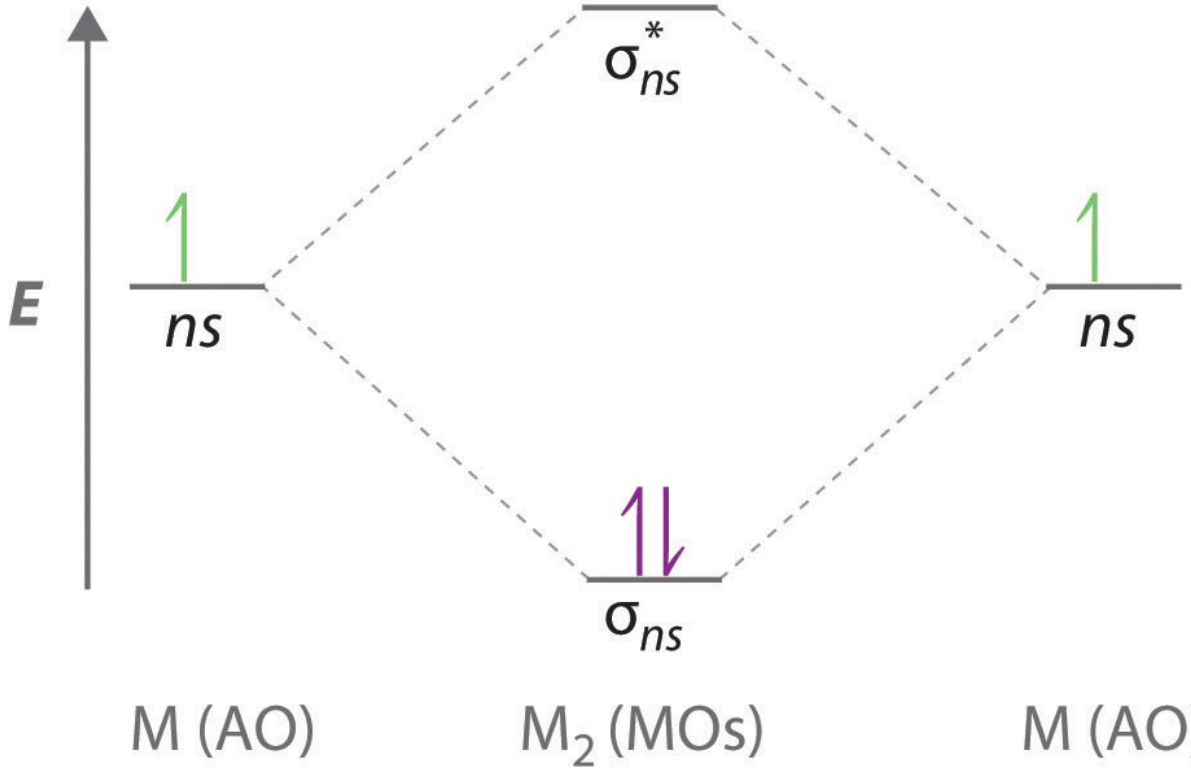
$$E_g = \frac{1}{2}\left[(H_{11} + H_{22}) - \sqrt{(H_{11} - H_{22})^2 + 4H_{12}^2}\right]$$

Janez Mavri & Arieh Warshel
QAMTS Darmstadt, 2010

“2 states in, 2 states out”

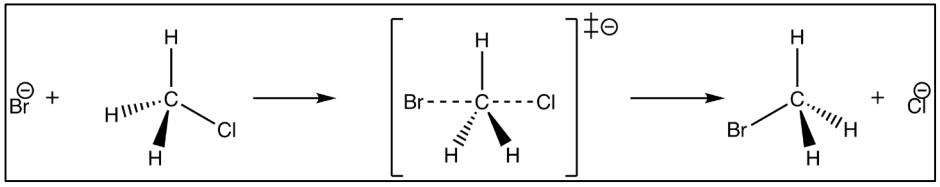
❑ When treating certain states (electronic, as shown below) quantum-mechanically, the fundamental principle postulates that the number of “output” states obtained by the treatment is the same as the number of “input” states subject to the treatment.

❑ Quantum treatment of the electronic structure typically involves dozens (hundreds) of states and is very demanding; however, useful cases of much lower complexity exist, and EVB is one of those cases: **quantum coupling of empirical valence states.**



About reactive FF & FEP

Mind that the involved species have different equilibrium geometries, force constants and atomic charges in the R and P state!

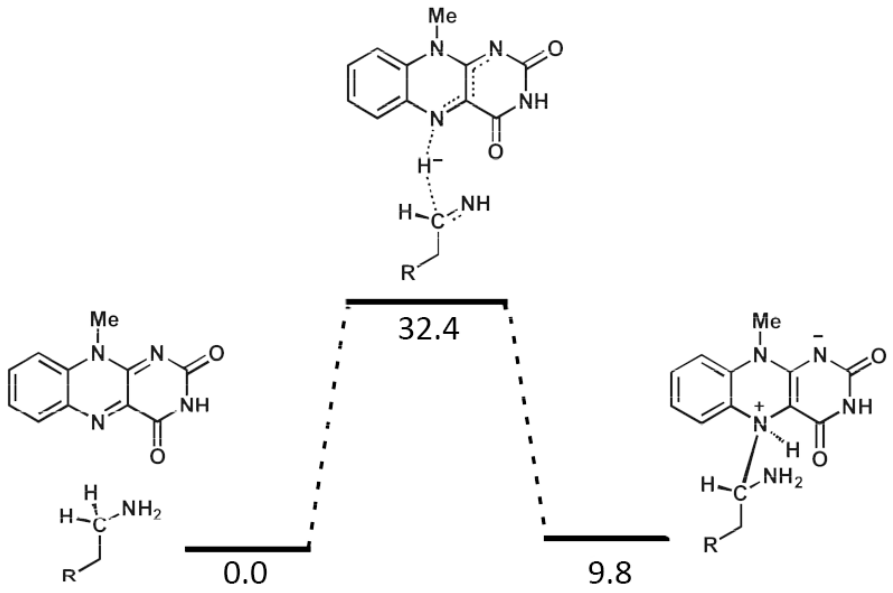


- Morse potentials (rather than harmonic) are used on breaking and forming bonds.
- In order to make the system go uphill and cross the barrier during MD, a coupling parameter λ is being used to blend the Hamiltonians corresponding to reactants (H_{11}) and products (H_{22}).
- Starting in the reactant well (λ = 1), a sequence of MDs is run with incrementally increasing λ by a small amount (0.02) until the state of products (λ = 0) is reached.
- Free energy is calculated by using the perturbation formula.

$$\boldsymbol{H(\lambda) = \lambda H_{11} + (1-\lambda)H_{22}}$$

$$\boldsymbol{\Delta G = \sum \Delta g = -k_B T \sum log \left\langle exp(-\frac{\Delta H}{k_B T}) \right\rangle_A}$$

EVB calibration



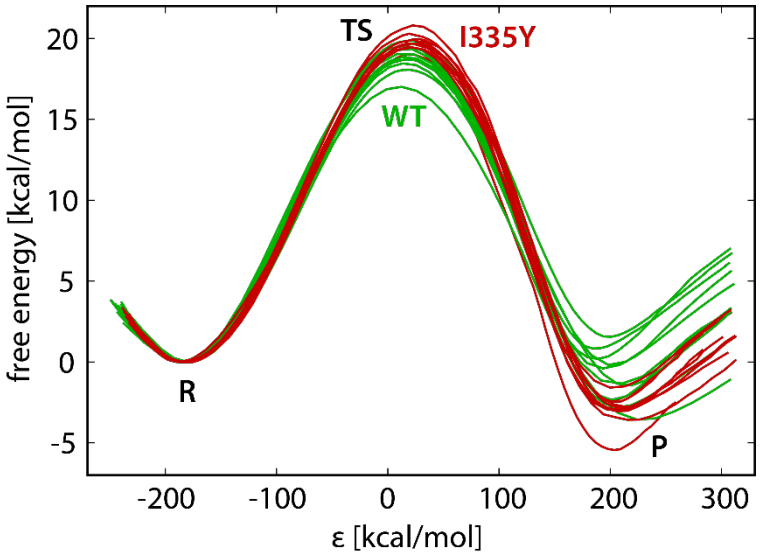
Reaction mechanism characterized by M06-2X/6-31+G(d,p) gas phase calculations.

The reaction mechanism has to be pre-determined, EVB is not capable of determining the mechanisms!

- ❑ Characterize R, P and TS of the reactive step by ***gas-phase*** quantum calculations.
- ❑ Then, perform classical FEP simulation of the same reaction ***in the gas phase***.
- ❑ Fit parameters H_{12} and α_2^0 such that the resulting EVB reaction energy and barrier match the computed values.
- ❑ Alternatively, use ***experimental data for the enzymatic reaction*** and fit the parameters for the FEP simulation ***in the enzyme***.
- ❑ Use the fitted values of H_{12} and α_2^0 in all subsequent EVB calculations.
- ❑ Once calibrated, the EVB model can be used in any type of environment (solution, enzyme), among the rest for the studies of:
  - Enzyme mutations
  - Protonation states of ionizable residues
  - Ionic strength
  - Nuclear quantum effects

PEA decomposition by MAO A: I335Y point mutation effect

- EVB parameters (H_{12} and α_2^0) calibrated for the simulation in WT MAO.
- The calibrated parameters are then used to compute the free energy profile in I335Y MAO.



- Computed increase in free energy barrier: **1.25** kcal/mol. Experimental: **1.09** kcal/mol.
- EVB simulation correctly reproduces the effect of point mutation on enzyme kinetics.

G. Oanca et al., *PCCP* **2016**, *18*, 13346.

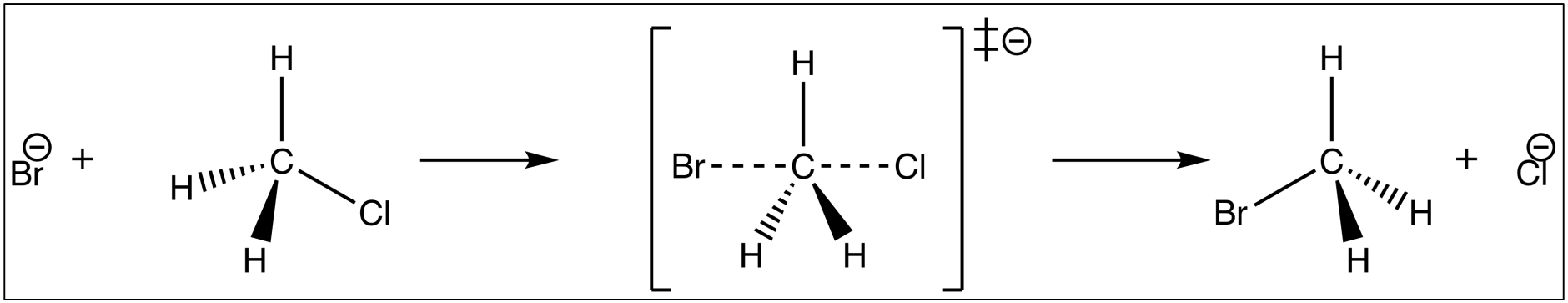
FAD

C-H...N5 reacting moiety

PEA

Ile335 / Tyr335

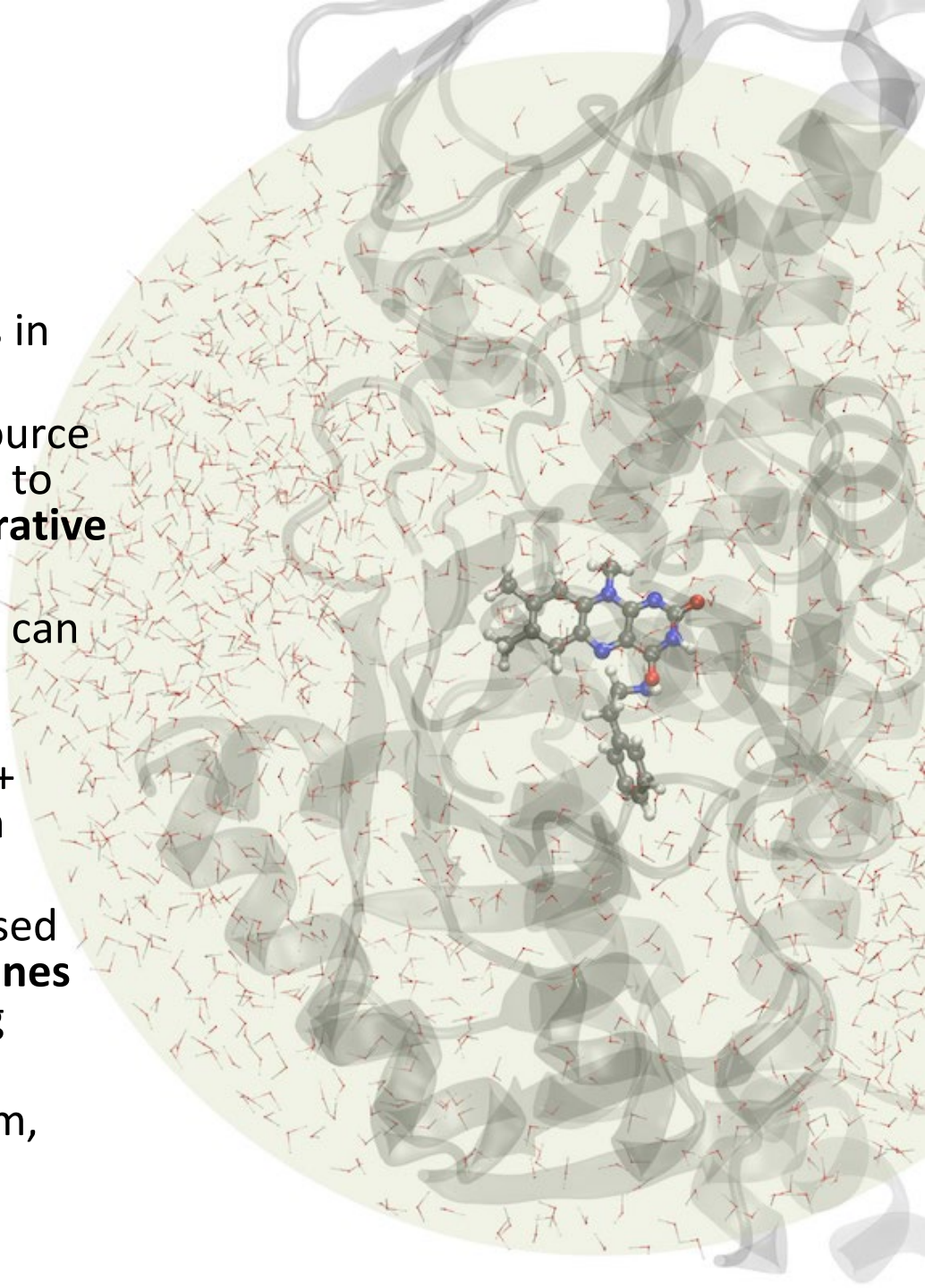
EVB simulation of an SN2 reaction



- Build models of R, P and TS and optimize them in the gas phase at the M06-2X/6-31+G(d,p) level of theory using the *Gaussian16* program.
- Calculate $\Delta E^{\ddagger}$ and ΔE_R for the gas-phase reaction.
- Prepare and run (program package *Q5*) classical simulation of the gas-phase reaction using a two-state force field and free energy perturbation methodology.
- Calibrate EVB parameters (H_{12} and α_0) for the classical simulation using the $\Delta E^{\ddagger}$ and ΔE_R as target values.
- Compute the EVB free energy profiles in aqueous solution using the calibrated parameters.

MAO enzymes

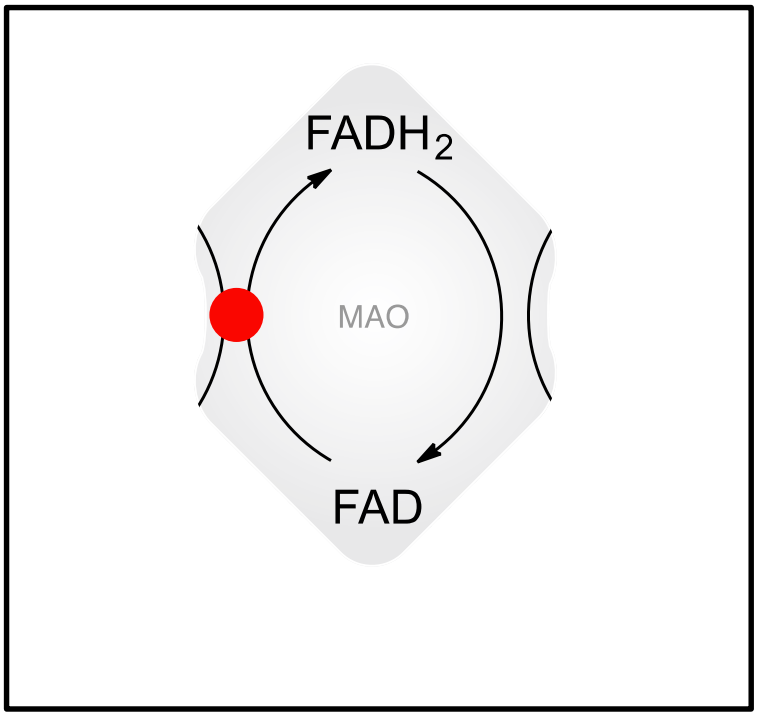
- **Monoamine oxidases** (MAO) are enzymes that decompose monoaminergic neurotransmitters in the central nervous system.
- The action of MAO represents a source of oxidative stress and contributes to the development of **neurodegenerative diseases** (Alzheimer, Parkinson).
- Deficient or elevated MAO activity can lead to **neurological disorders** (depression, autism).
- About 50 % of population aged 85+ suffer from dementia – “a trillion dollar disease“.
- The work of our laboratory is focused on gaining an insight into **how amines are decomposed by MAO** by using methods of **molecular simulation**.
- Elucidating the reaction mechanism, mutation effects, **kinetics**.

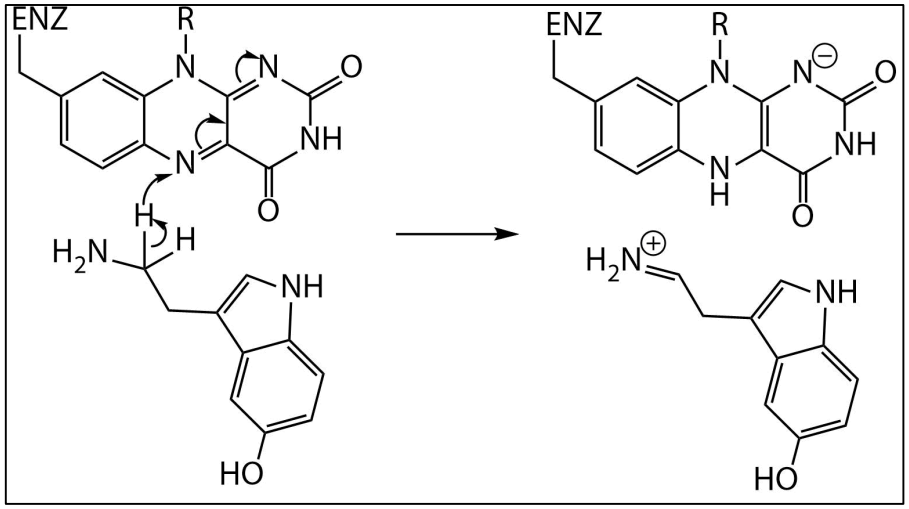


Reaction mechanism

Reaction between the substrate (monoamine) and MAO cofactor flavin adenine dinucleotide (FAD).

Products are aldehyde and ammonia (primary amines); hydrogen peroxide is produced due to FAD regeneration.

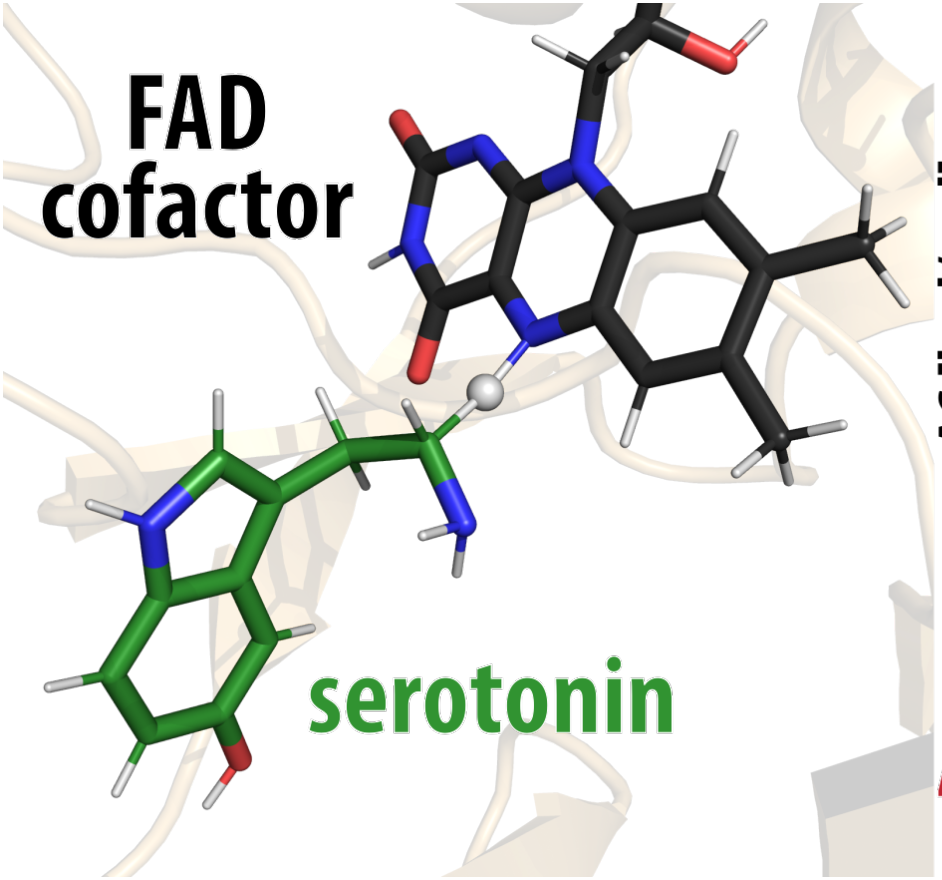


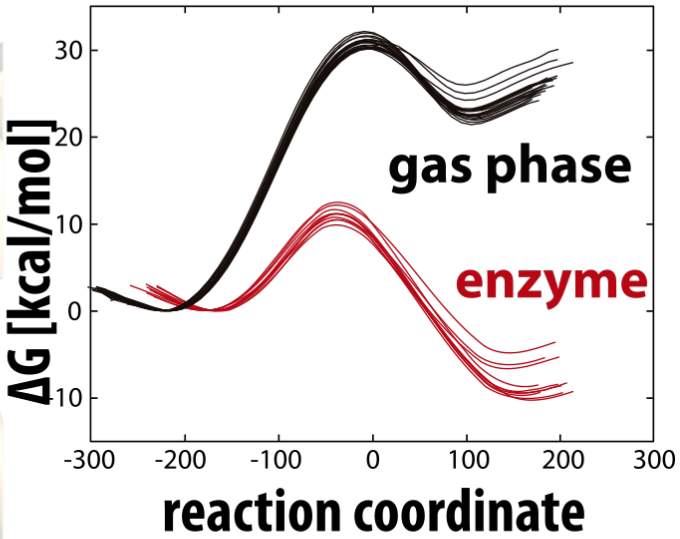


Rate-limiting step is hydrogen transfer from the substrate to the flavin cofactor (ie. C-H bond cleavage and N-H bond forming).

Three suggested mechanisms: radical, polar nucleophilic and hydride.

MAO A + serotonin





$\Delta G^{\ddagger}_{exp} = 16.0$ kcal/mol

$\Delta G^{\ddagger}_{calc} = 14.8$ kcal/mol