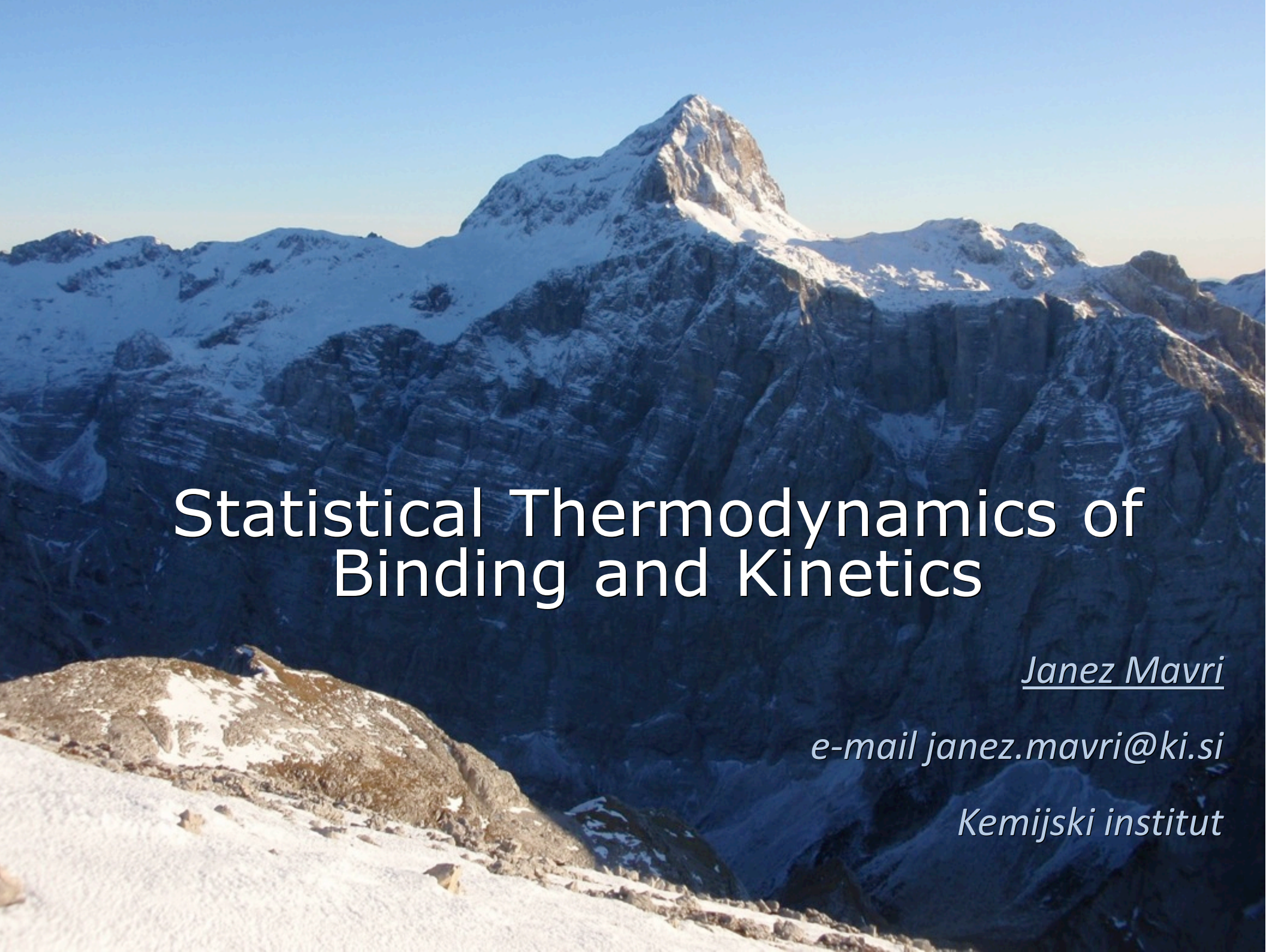

Statistical Thermodynamics of Binding and Kinetics

Janez Mavri

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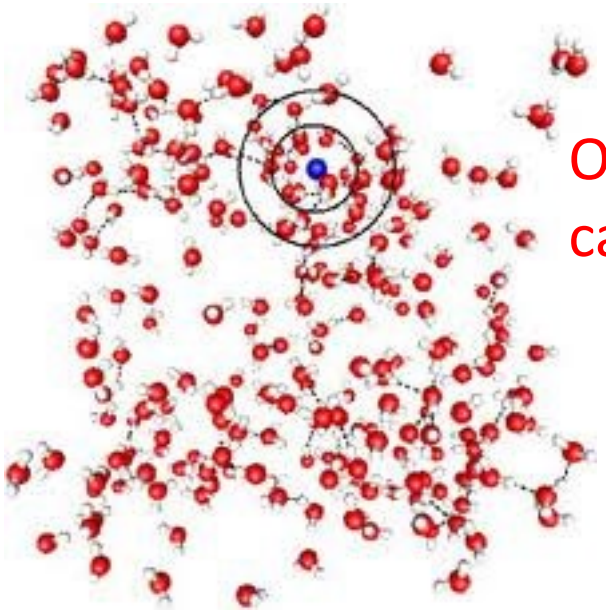
Kemijski institut

Textbooks say that all hydrated proteins represent soft condensed matter. What does it mean:



One single geometry of several water molecules can not describe aqueous solution !

Several milions of configuration are within k_BT =0.59 kcal/mol per degree of freedom at room temperature. The idea of thermal averaging is to search the phase space numerically and to calculate ensemble averages and dynamic quantities.

Biological macromolecules are the molecules of life

A single residue mutation of an enzyme can lead to pathology e.g. monoamine oxidase mutants can cause autism or extremely aggressive behavior.

By binding of ligands function of macromolecules are changed and physiological answer follows.

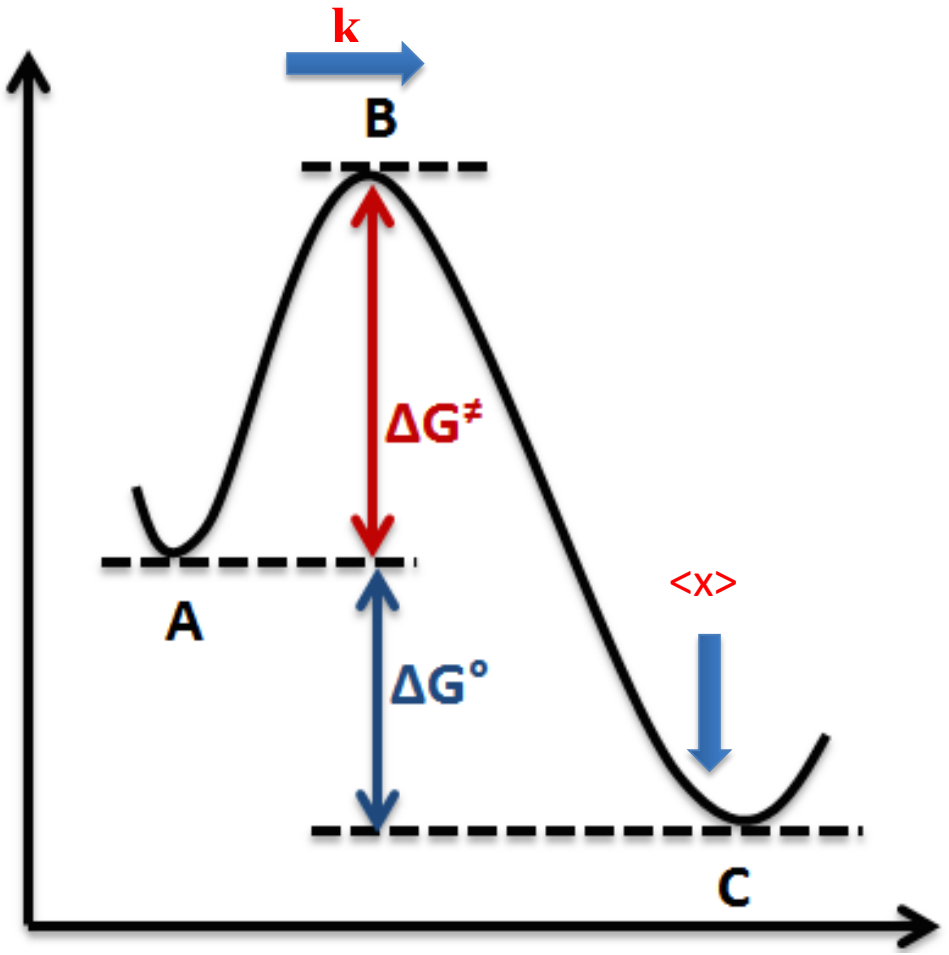
Corpora non agunt nisi fixata.

Paul Ehrlich 1854-1915

Free energy differences provide information about:

Structure
Stability

Dynamics



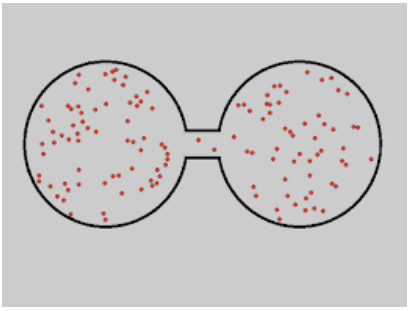
Structure = mean structure at the (global) minimum <x>
Stability = free energy of binding $\Delta G_{AC} = -k_B T * \ln K_{AC}$
Dynamics = the rate of barrier crossing reflected as chemical kinetics with the rate constant k
$k = k_B T/h * \exp(\Delta G^{\#} / k_B T)$

for liquid and solid state enthalpy basically equals energy

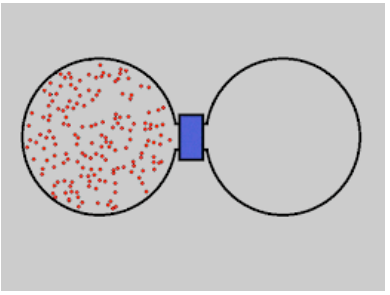
$G = H - TS$

high energy

low energy



high entropy



low entropy

Vršič 1611m

Transition State

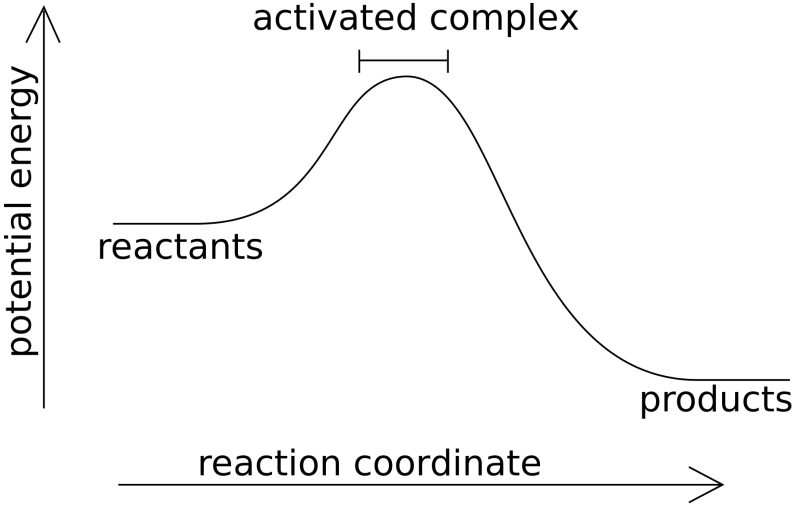
Kranjska Gora 806m

Reactants

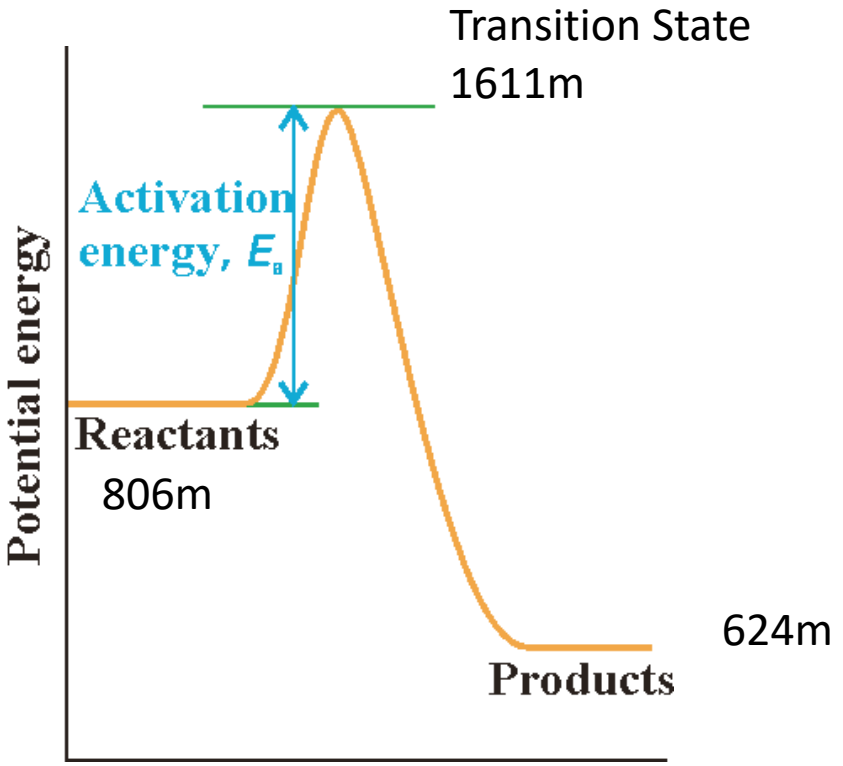
Minimum energy path looks like:

Trenta 624m

Products



Minimum energy path looks like:



A group of 100 cyclists arrives and after some time we observe their populations:

R 8
TS 2
P 90

This is experiment and entropy effects are included

k_BT at room temperature is 0.59 kcal/mol

$K_{RP} = 90/8 = 11.25$

$\Delta G_{RP} = -k_BT*\ln K_{RP} = -0.59\text{kcal/mol}*\ln 11.25 = -1.43$ kcal/mol

A group of 100 cyclists arrives and after some time we observe their populations:

R	8
TS	2
P	90

These are experimental populations and entropy effects are included

The equilibrium constant between R and the TS is
$K_{R\text{-}TS} = 2/8 = 0.25$

And the corresponding free energy of activation is
$\Delta G^{\#} = -k_B T * \ln K_{R\text{-}TS} = -0.59 \text{kcal/mol} * \ln 0.25 = 0.82 \text{ kcal/mol}$

The transition state assumes that the reactants and the transition state are at thermal equilibrium and the rate constant *k* can be calculated analytically from $\Delta G^{\#}$.
Please note that $k_B T/h$ at room temperature is 6 *10^12/s
and that $k_B T$ at room temperature is 0.59 kcal/mol

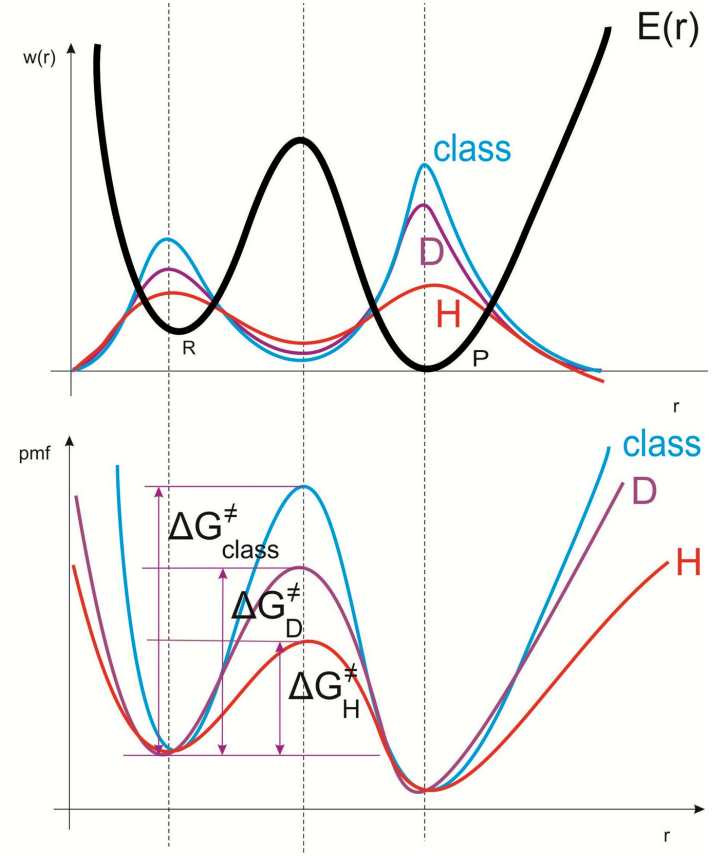
$k = k_B T/h * \exp(\Delta G^{\#}/k_B T) = 6.10^{12}/s * \exp(-0.82/0.59) = 1.5.10^{12}\ s^{-1}$

This is a little fast for real cyclists …, in reality they need two hours to cross Vršič …
Moreover, the saddle point is always crowded, therefore the transition state theory is not strictly valid for cyclists …

Tunneling is change of the probability density at the region of the barrier relative to the classical treatment of the particles due to quantum nature of their motion.

For chemical reactions this means effective increase of the probability density at the barrier region giving rise to lowering of the barrier in terms of free energy and increased rate of crossing.

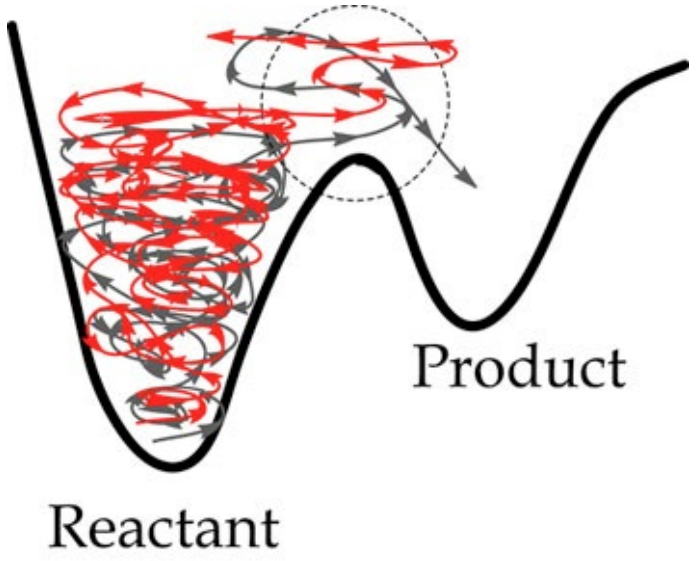
Tunneling is not a dynamical phenomenon and is perfectly compatible with the transition state theory. Technically speaking it can be calculated also by Monte Carlo methodology.



Dynamical effects are deviations from the transitions state theory due to barrier recrossing.

The transition state theory is perfectly valid for the reactions in enzymes and solutions since the reactive species are in thermal equilibrium with the rest of enzyme and solvent.
This is so called strong coupling regime.

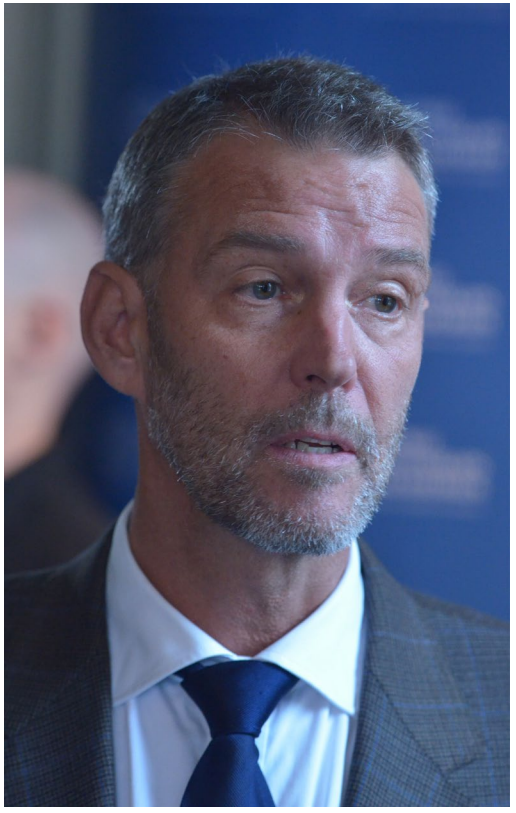
Deviation from the transition state theory can be observed in the weak coupling regime= Lindemann regime, where reaction dynamics is controlled by reaction energy dissipation. Several barriers recrossings occur.



Beware of improper models

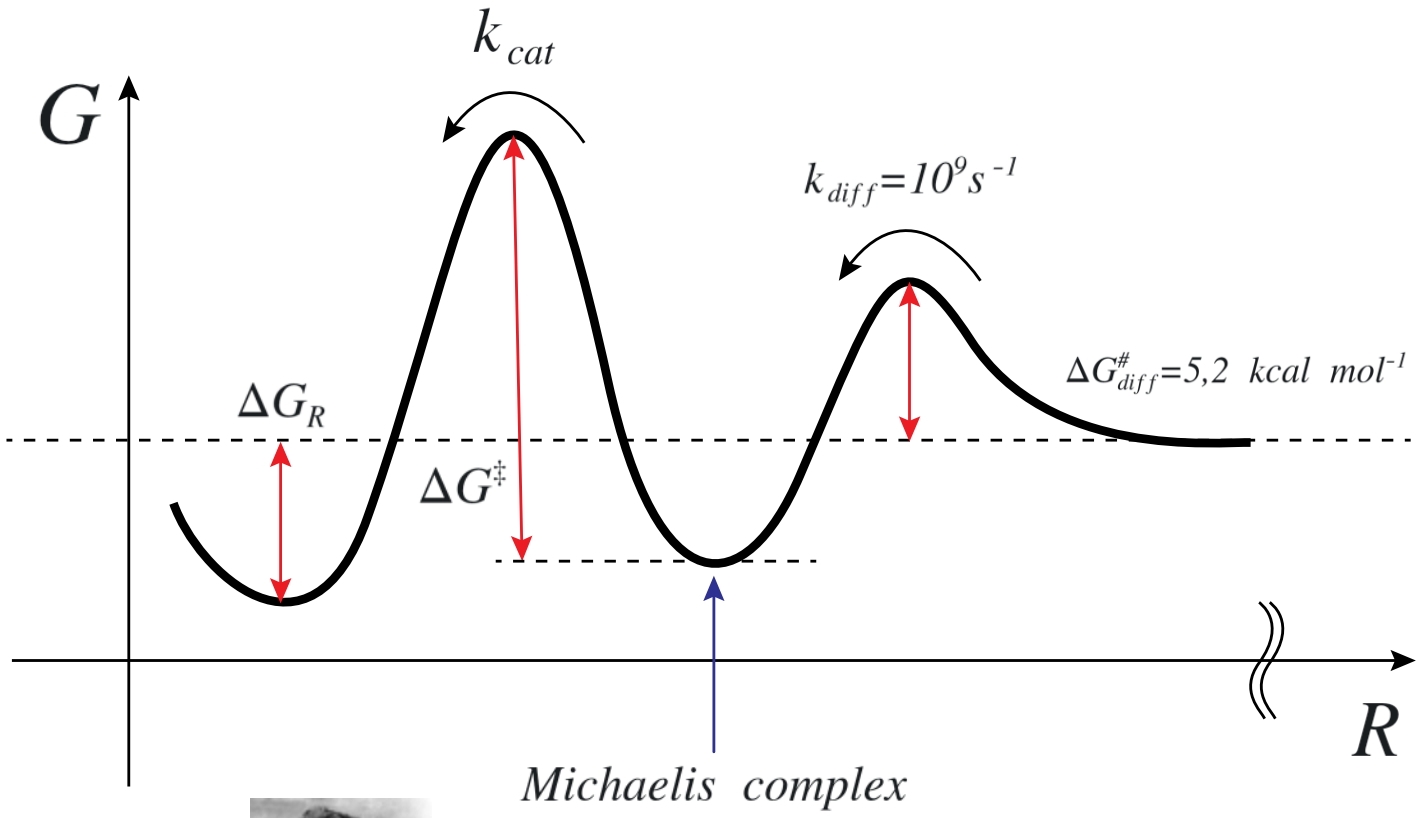
Dynamical and other esoteric effects including promoting modes, coherence, nonequilibrium solvation, sterical strain … do not contribute to catalysis and binding.

By this cartoon Johan Åqvist, University of Uppsala, Sweden, represents the essential aim of biomolecular simulation.



During this summer school we will learn how to calculate the free energy differences by various tools

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

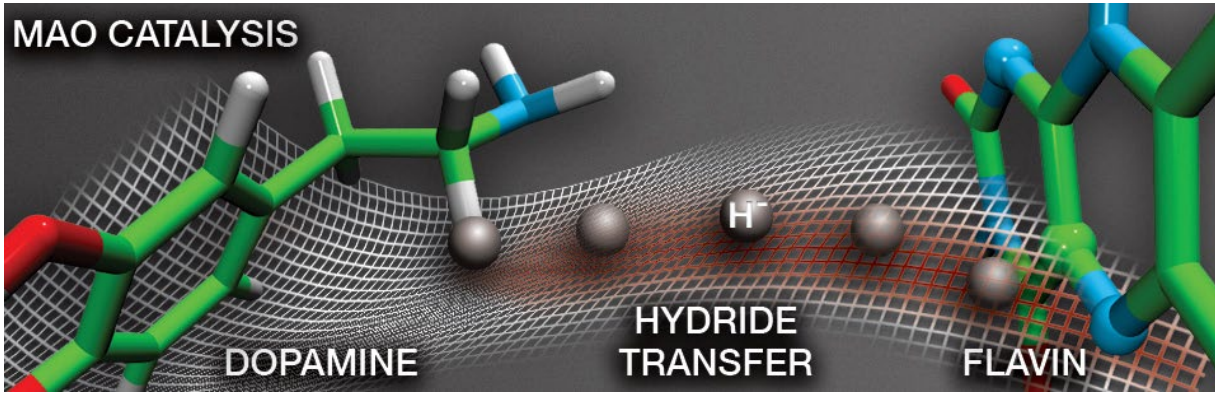


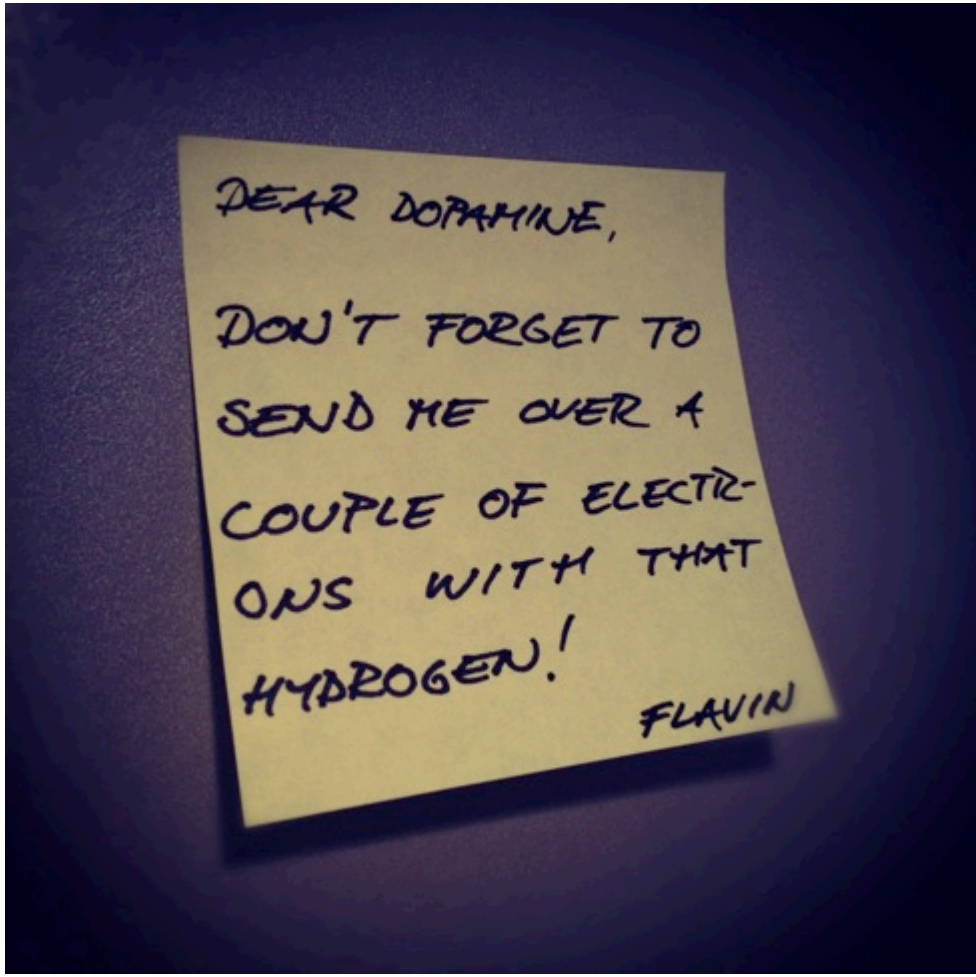
Leonor Michaelis

Maud Menten

L. Michaelis and Miss Maud L. Menten (1913), "Die Kinetik der Invertinwirkung", *Biochem Z* **49**: 333–369

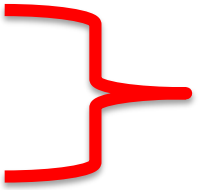
Monoamine oxidases metabolize neurotransmitters dopamine and serotonin.
Rate limiting step is abstraction of hydride from methylene group.





Dopamin Decomposition

Environment	Activation free energy (kcal/mol)
Gas phase	32.8± 0.9
Water	26.5± 1.3
Enzyme	16.1± 0.8



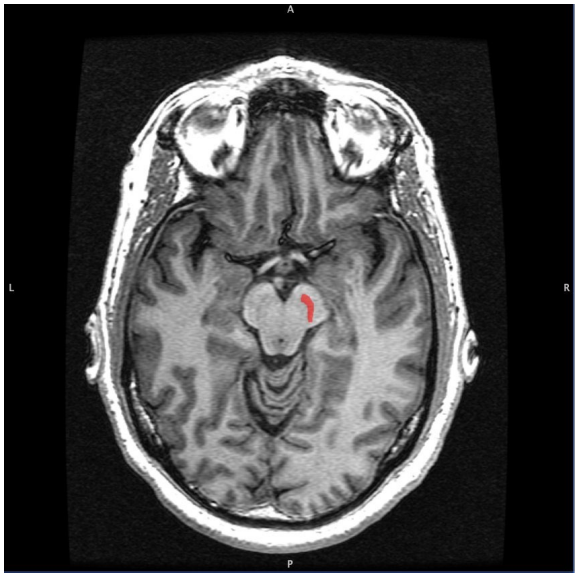
Enzyme(Experiment) 16.5

MAO B provides 10.4 kcal/mol to catalysis

Dopamine is beside MAO B-catalyzed decomposition also rapidly autoxidized in aqueous solution

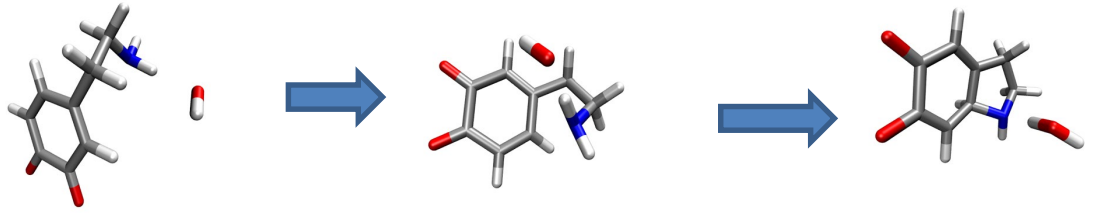
Dopamine is in aqueous solution at pH=7.4 autoxidized to dopaminochrome with the rate constant of 0.147 s^{-1}. As as side product hydrogen peroxide is formed.

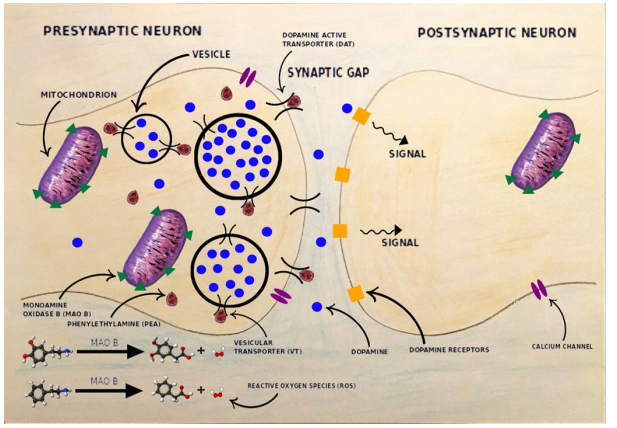
Clinical manifestation: Parkinson disease that is initially localized to nigrostriatal pathway.



We suggest the mechanism of dopamine autoxidation

Dopamine is in aqueous solution at pH=7.4 autoxidated to dopaminochrome. Calculations of the reaction profile on the M06-2X/6-31+G(d,p) level with solvent reaction field.





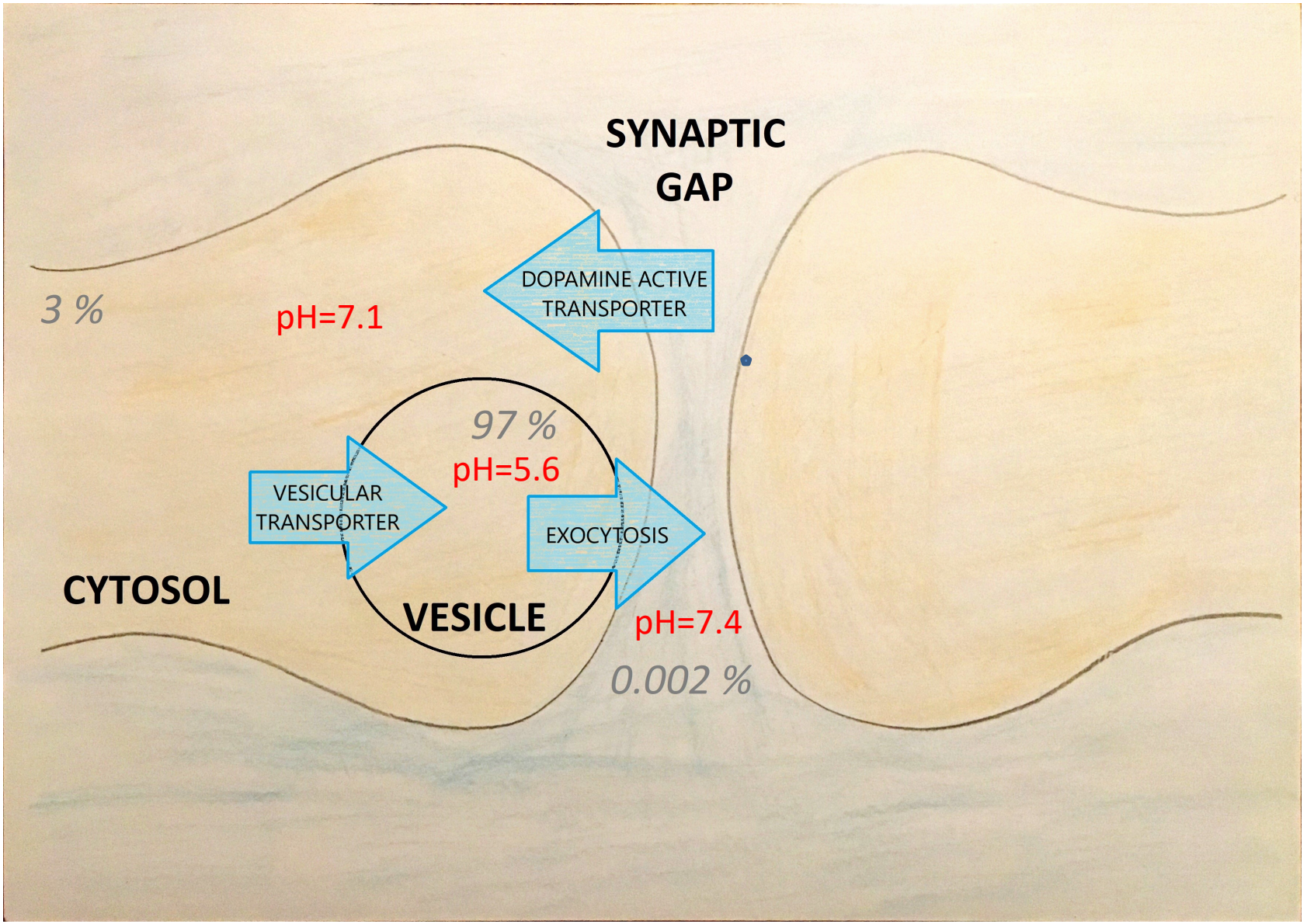
The rate constant is controlled by pH

$$\Delta G^{\neq} = 6.78\ kcal\ \ mol^{-1} + k_B T \ln(10)\,(15.7 - pH) + k_B T \ln(10)\,(9.58 - pH)$$

pH	k_{rate} (s^{-1})	$t_{1/2}$
5.6	0.00000059 1	13.5 days
7.1	0.000586	19.7 min
7.4	0.00233	4.95 min
8.0	0.0368	18.8 s
9.5 8	52.8	0.0131 s

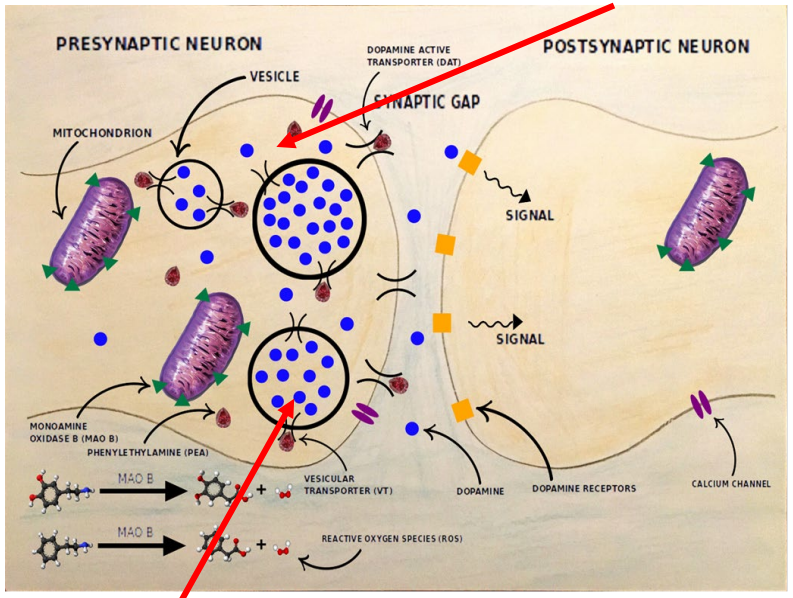
Nejc Umek, Blaž Geršak, Neli Vintar, Maja Šoštarič, Janez Mavri, Dopamine Autoxidation is Controlled by Acidic pH, Front.Mol. Neurosci, Mol. NeuroSci. 11 (2018) article 467

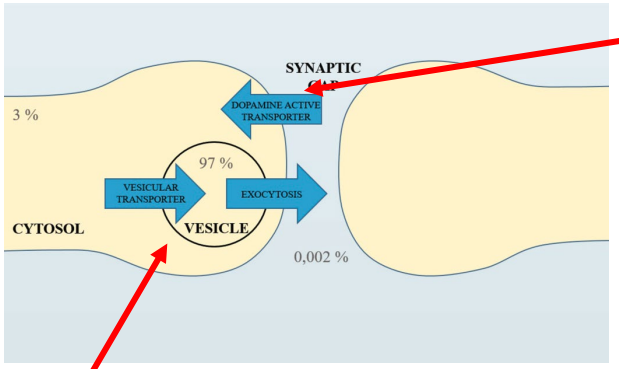
Large majority of dopamine is stored in the vesicles with low pH



Can Amphetamine or Cocaine Induce M. Parkinson/Parkinsonism?

Cytosol has pH value of 7.1, dopamine is autoxidized here





DAT permeability decreases for 55% upon cocaine application: experimental data for *Drosophila Melanogaster*

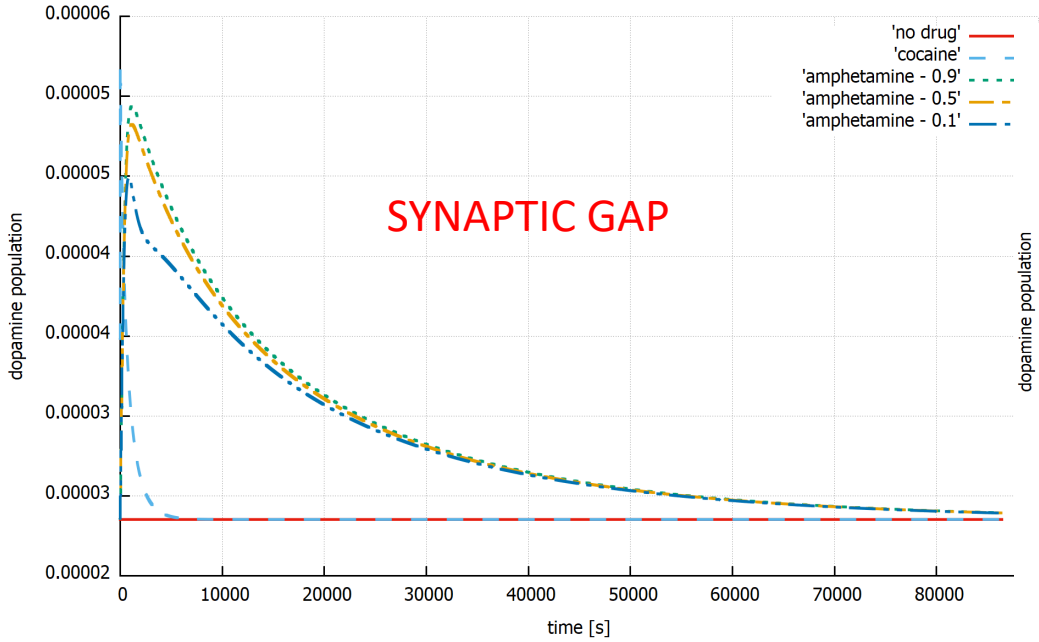
Upon amphetamine application beside DAT also VT permeability is decreased, we tried with 10%, 50% and 90% since no experimental data are available

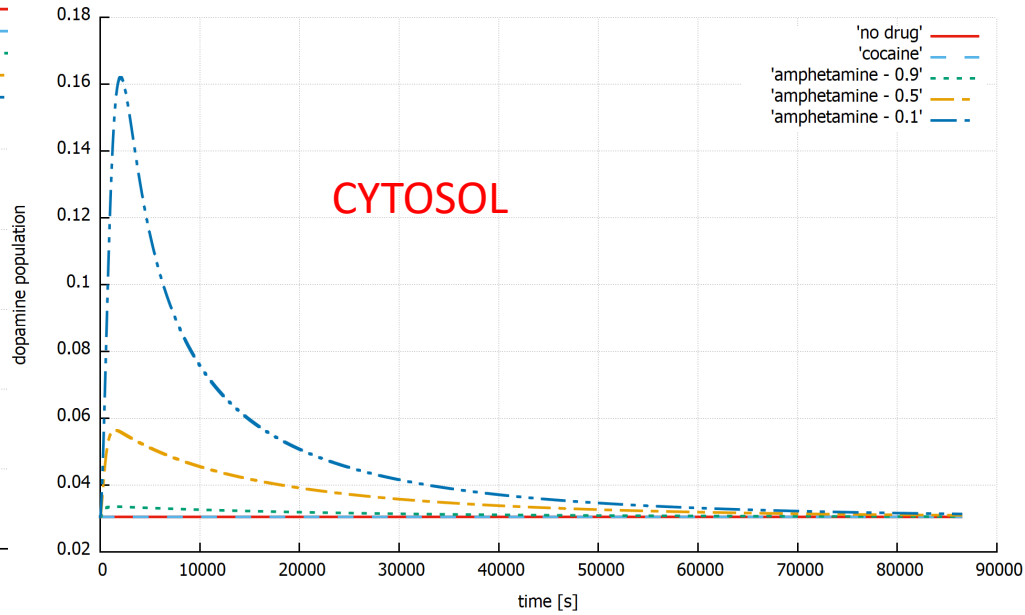
Interior of the vesicle is acidic with pH=5.6, dopamine is not autoxidized here

Levels of dopamine within three compartments (gap, presynaptic neuron cytosol, vesicle) was described by a system of three ordinary differential equations that was solved numerically by Runge-Kutta 4-th order method

Elevated dopamine levels in the synaptic gap are responsible for psychotropic effect

Elevated dopamine levels in cytosol are a measure for neurodegeneration.



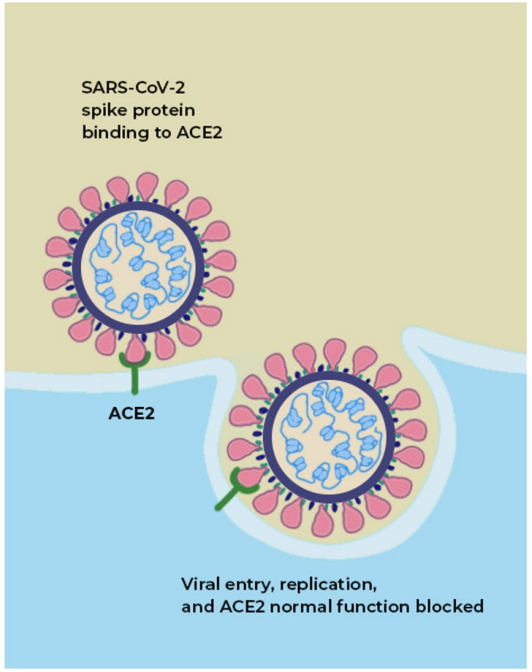


The results give evidence that recreational use of cocaine is in terms of neurodegeneration safe, while amphetamines are problematic.

D. Pregeljc, D. Teodorescu-Perijoc, R. Vianello, N. Umek and J. Mavri, How Important is the Use of Cocaine and Amphetamines in the Development of Parkinson Disease? A Computational Study, sub. Neurotoxicity Research 9/2019

34% of Covid-19 patients with severe course of pathology develop neurological symptoms ranging from anosmia to parkinsonism and dementia.

"Growing evidence suggests that the coronavirus causes 'brain fog' and other neurological symptoms through multiple mechanisms."
Nature **595**, 484-485 (2021)



SARS-CoV-2 enters neurons by spike protein binding to receptor ACE2 that internalizes together with the virus.

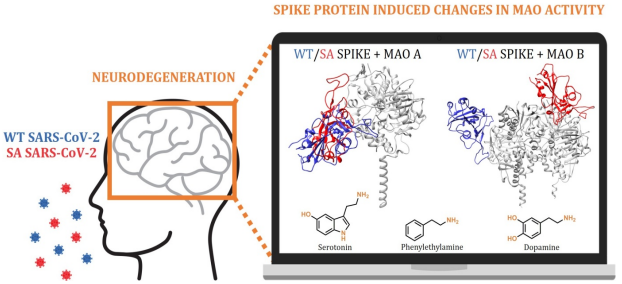
Upon SARS-CoV-2 infection expression of ACE2 is downregulated.

ACE2 expression is correlated with the expression of aromatic L-amino acid decarboxylase that produces monoamines from the aromatic amino acids and this effects decreases dopamine levels.

SARS-CoV-2 spike protein inhibits monoamine oxidase B and this effect increases dopamine levels.

The levels of phenylethylamine (endogenous amphetamine) are substantially increased giving rise to psychotropic effect and dopamine autoxidation.

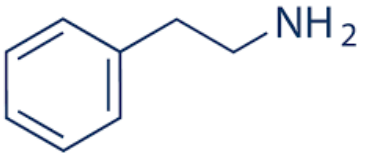
M. Cuperlovic-Culf et al. , Metabolomics and computational analysis of the role of monoamine oxidase activity in delirium and SARS-COV-2 infection. *Sci Rep*, 2021, 11, 10629.



By molecular simulation we clearly demonstrated that SARS-CoV-2 spike protein and monoamine oxidase B form very stable complexes. The effects of spike protein mutations (WT and South African strain) were considered.

Hok L, et al. Relationship between COVID-19 infection and neurodegeneration: Computational insight into interactions between the SARS-CoV-2 spike protein and the monoamine oxidase enzymes. bioRxiv, 2021.
doi: https://doi.org/10.1101/2021.08.30.458208, https://www.biorxiv.org/content/10.1101/2021.08.30.458208v1

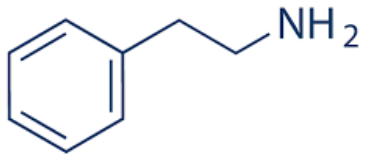
Dopaminergic neurons of SARS-CoV-2 patients with severe course of disease are exposed to steady and high doses of phenylethylamine (PEA)



PEA is oily liquid with a fishy smell

PEA is a substance with psychotropic effect that is eliminated after about 30 minutes

No differential equations involving experimental PEA pharmacokinetics will be necessary



We simulated MAO catalyzed PEA decomposition

Gabriel Oanca, Miha Purg Janez Mavri, Jean C. Shih, Jernej Stare, Insights into enzyme point mutation effect by molecular simulation: phenylethylamine oxidation catalyzed by monoamine oxidase A
Phys.Chem.Chem.Phys., 2016, 18, 13346

Gabriel Oanca, Jernej Stare, Robert Vianello, Janez Mavri,
Multiscale simulation of monoamine oxidase catalyzed decomposition of phenylethylamine analogs, European Journal of Pharmacology 817 (2017) 46–50