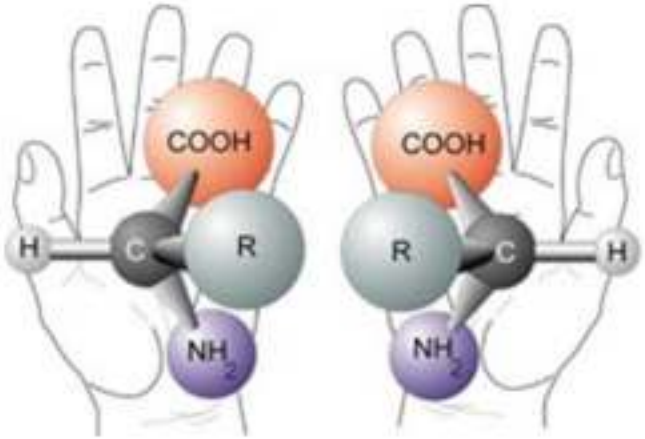
V.V. Voevodsky Institute of Chemical Kinetics and Combustion,

Novosibirsk, Russia

Stereoselectivity of photoinduced interaction of chiral drug S-ketoprofen with enantiomers of tryptophan in phospholipid membranes

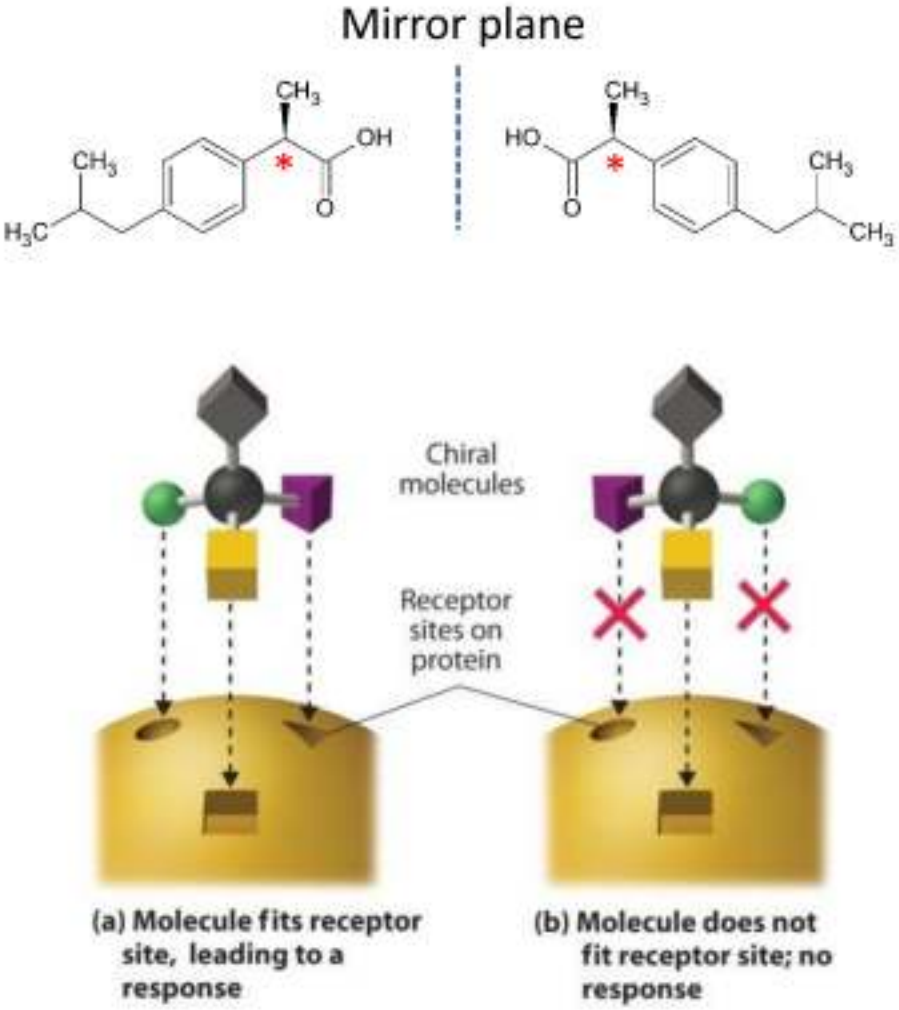
Nikolay E. Polyakov,

Anna V. Mastova, Olga Yu. Selyutina

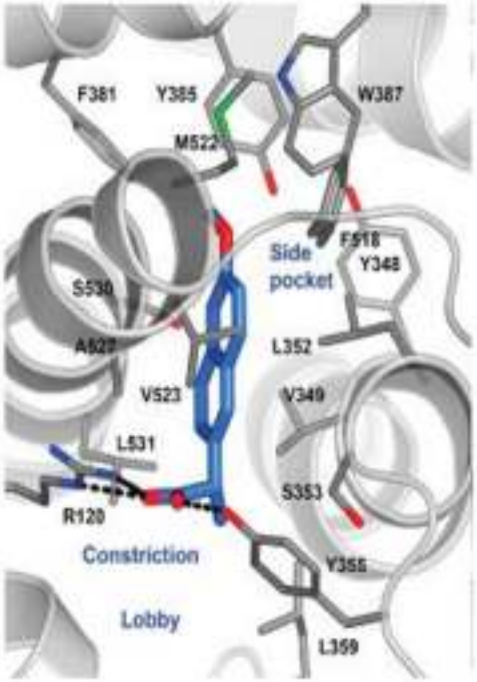


Chirality

- All chemical properties of enantiomers in homogeneous solutions are identical.
- However in living systems, enantiomers of drugs have different, and often opposite, therapeutic properties.
- The physicochemical reasons for this difference is still under debates.
- We assume that the stereoselectivity appears in the interaction of chiral drugs with another chiral molecules, for example, amino acid residues or lipid molecules.
- Another question is the role of chiral isomers of amino acids involved in various peptides in the development of many diseases including Alzheimer, Parkinson, and a number of other pathological conditions.

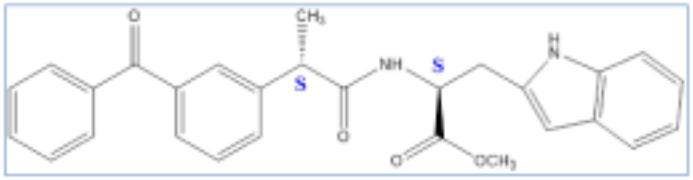


NSAIDs, derivatives of propionic acid, are among the most striking examples of differences in the medicinal properties of enantiomers.

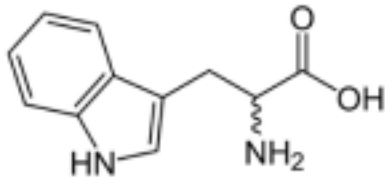


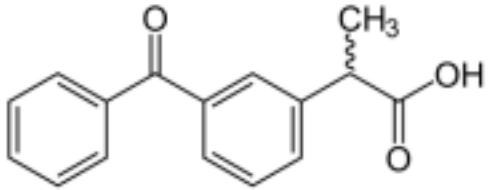
Naproxen at the active site of cyclooxygenase 2. [K. C. Duggan, M. J. Walters, J. Musee at al., J. Biol. Chem. 285, 45, 34950-34959, 2010]

Approach



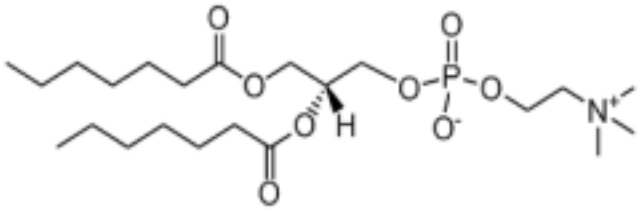
• An original approach has been developed to model biological activity of chiral drugs. The approach is based on the study of elementary process of photoinduced electron transfer between the drug molecule and the chiral amino acids located in the active centers of cell receptors or enzymes.



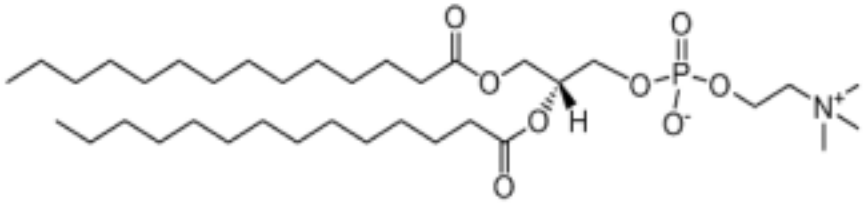


Another reason of our interest to photoinduced reactions of Ketoprofen (KP) is its high photosensitivity. KP is considered to be the strongest photosensitizer among non-steroidal anti-inflammatory drugs (NSAIDs). The photosensitizing reactions are caused by substituted benzophenone chromophore. It produces various toxic effects through the formation of active paramagnetic intermediates and photoproducts able to attack biological targets. However, the detailed mechanisms of photoinduced interaction of KP with biological molecules are still under debates.

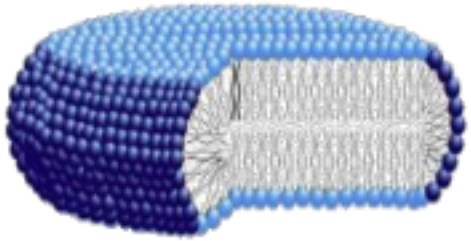
Model lipid membrane



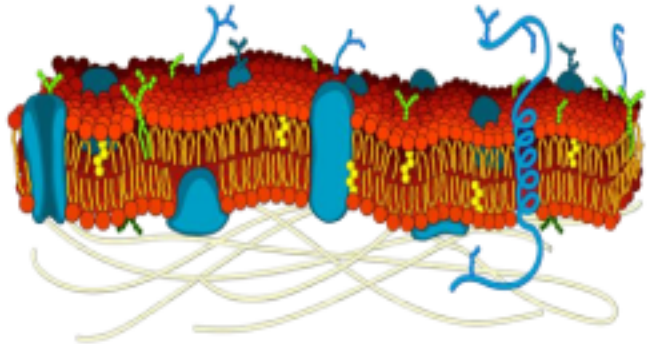
DHPC



DMPC



Bicelle

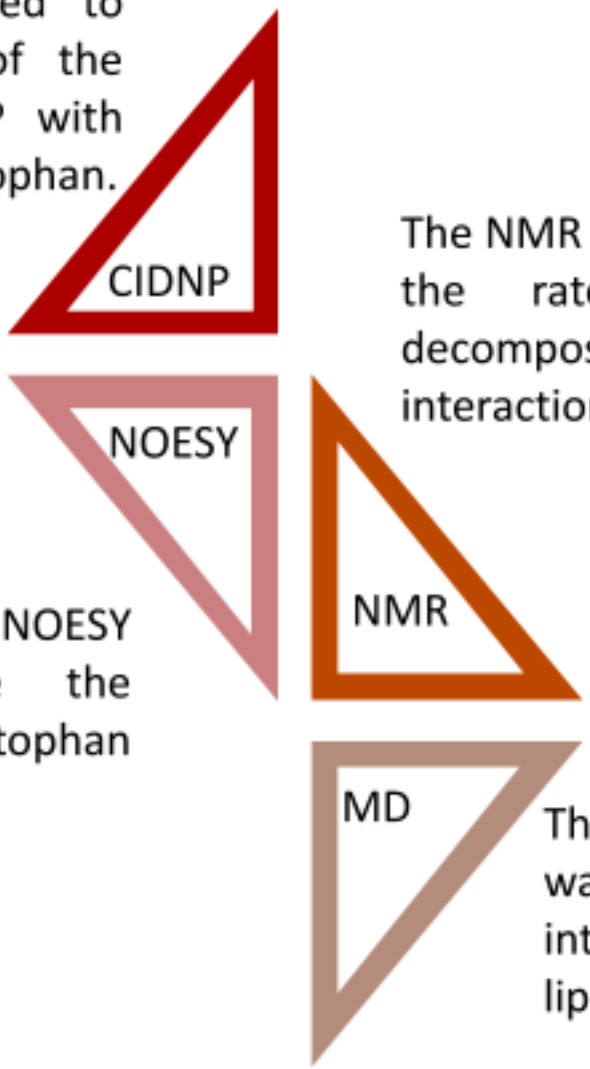


Cell membranes ensure the stable functioning of the cell and the transport of small molecules and ions between the cell and the environment; They fix the position of membrane-bound proteins and influence their structure and activity.

Damage to cell membranes can cause various phototoxic and photoallergic reactions.

Methods

The CIDNP method was used to determine the mechanism of the photoinduced reaction of KP with lipids and the amino acid tryptophan.

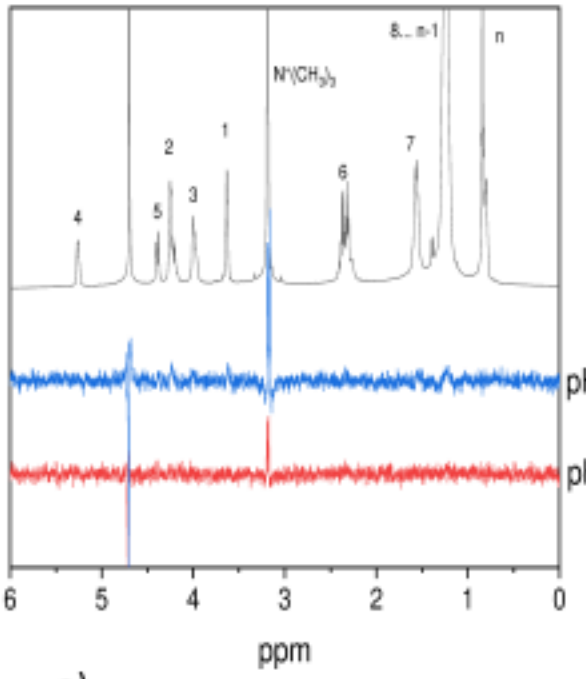


The NMR method was used to determine the rates and degrees of KP decomposition, as well as to study the interaction of KP with Trp enantiomers.

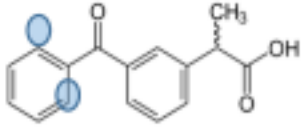
One-dimensional selective NOESY was used to determine the interaction of KP and tryptophan with the lipid bilayer.

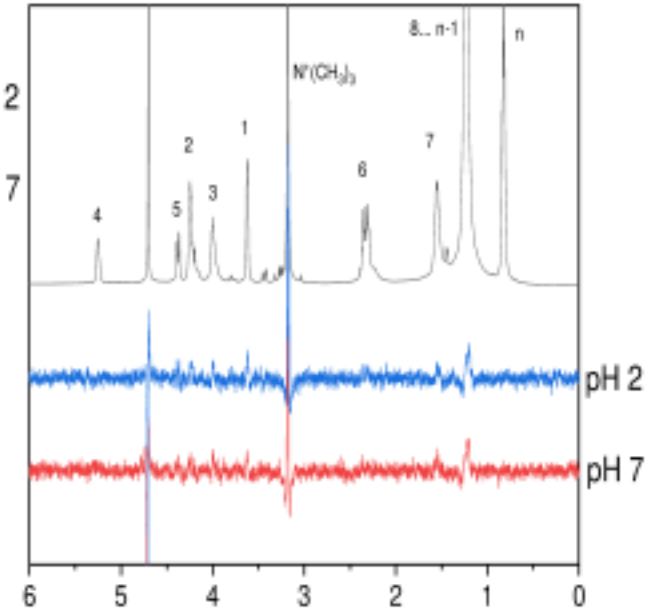
The Molecular Dynamics method was used to visualize the interaction of KP and Trp with the lipid bilayer.

Selective NOESY

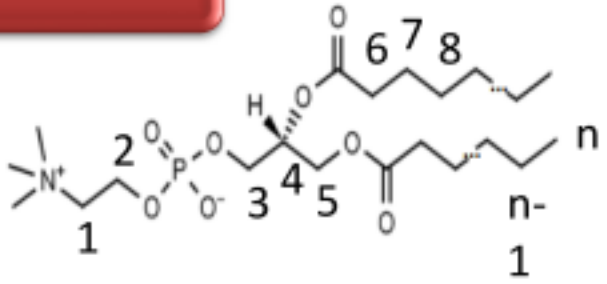


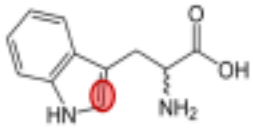
a)

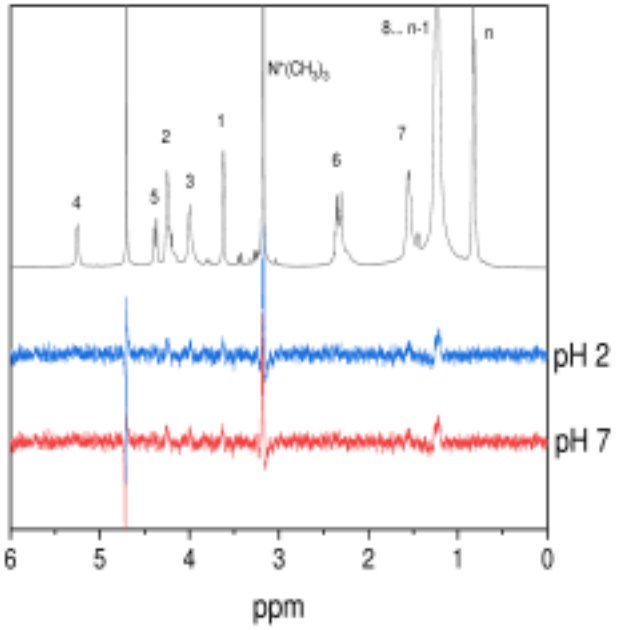




b)



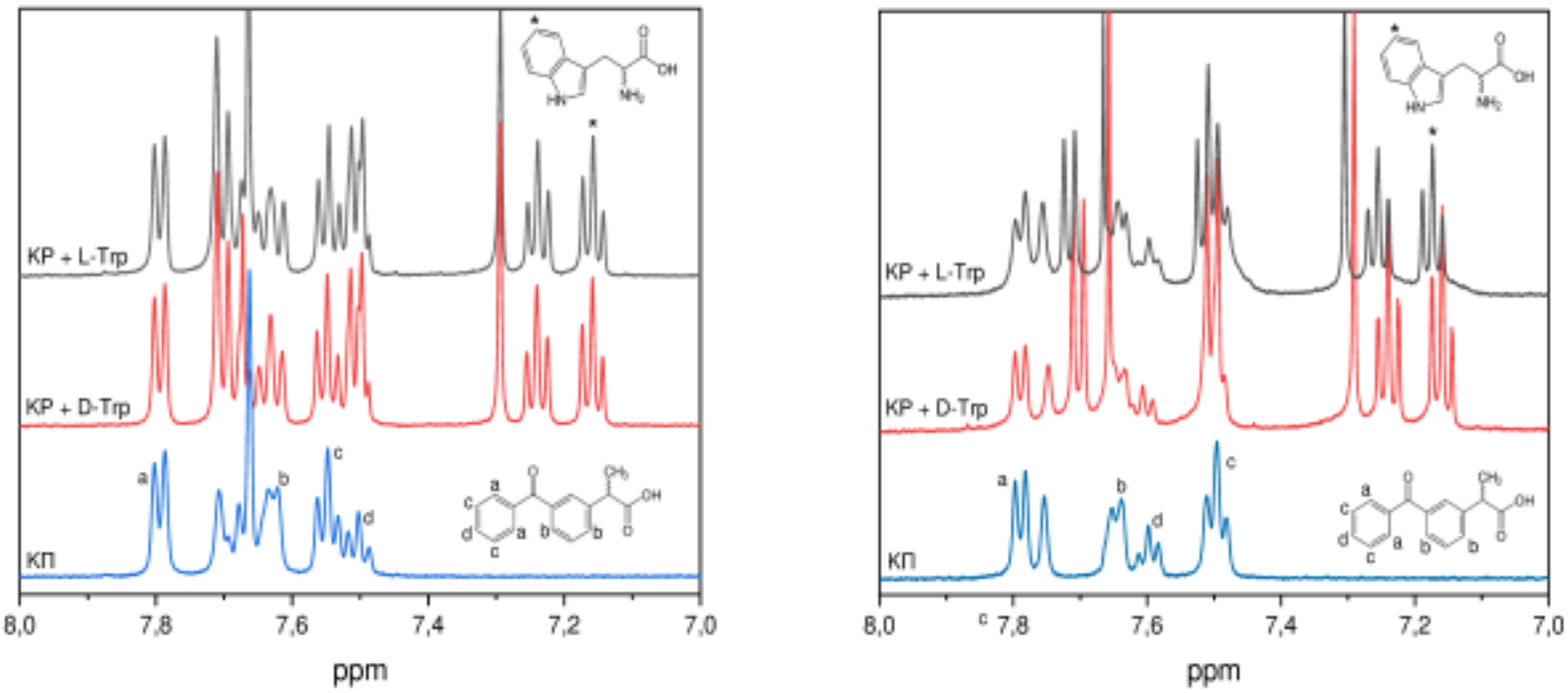




c)

1D NOESY and ^{1}H NMR spectra a) 2 mM KP; b) 2 mM KP + 4 mM Trp (KP saturation) ; c) 2 mM KP + 4 mM Trp (Trp saturation) in bicelles at different pH values.

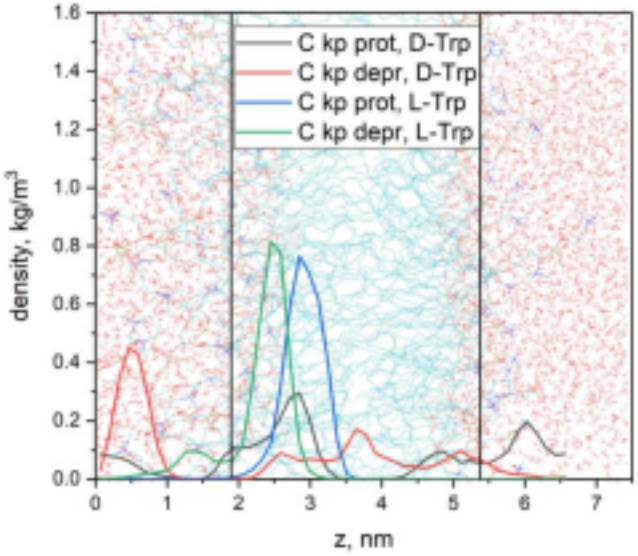
^{1}H NMR: stereoselectivity of KP-Trp interaction

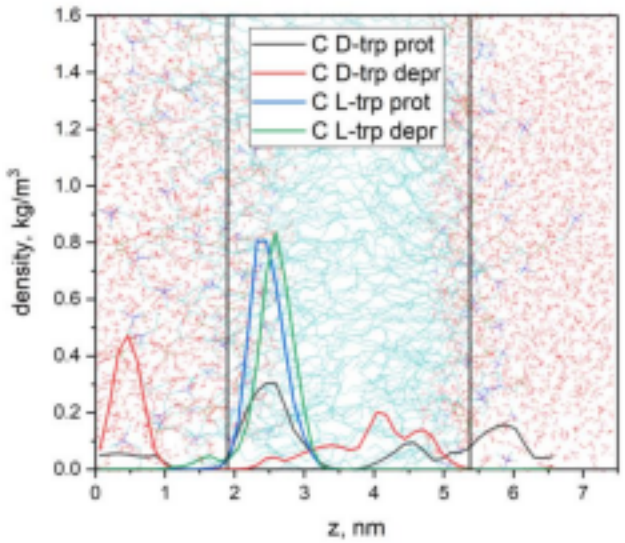


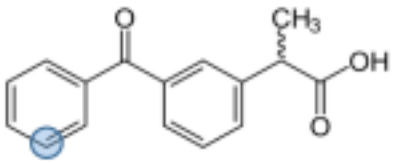
1H NMR spectra of S-KP (2mM) in the absence (blue) and presence (black and red) of L/D-Trp (4mM) in bicelles: a) in buffer at pH=7.4; b) at pH=4.

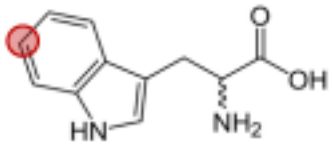
Molecular Dynamics

MD simulation shows significant stereo selectivity and pH sensitivity of Trp interaction with KP and lipid membrane.





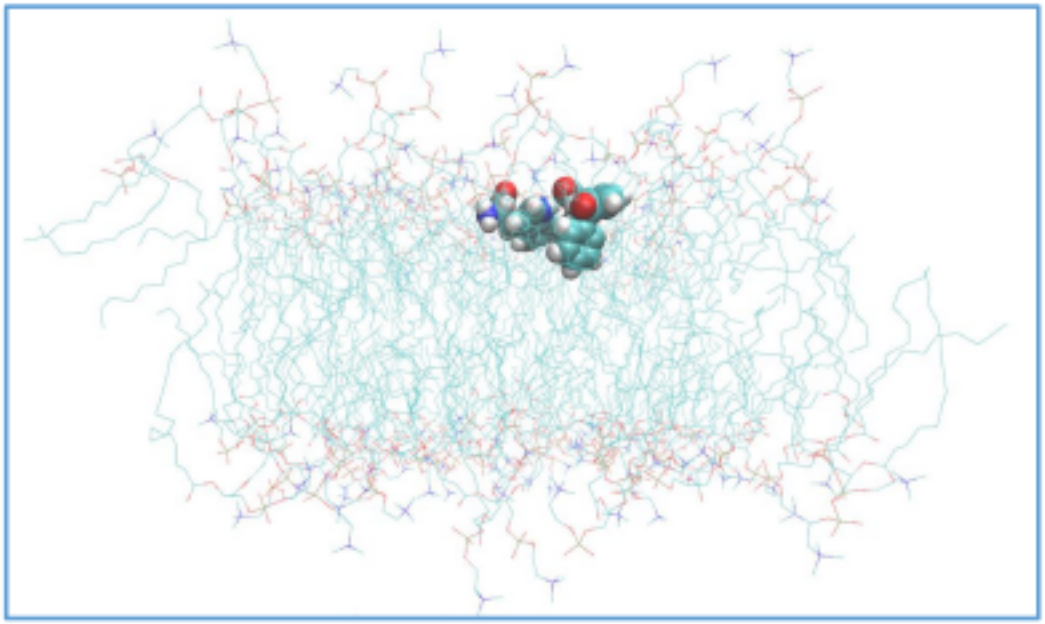




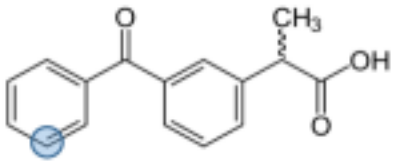
It is noticeable, that the localization significantly differs for L- and D-Trp. L-Trp is located predominantly inside the bilayer, while D-Trp could leave the bilayer more freely. Therefore, D-tryptophan demonstrated less affinity to the lipid membrane.

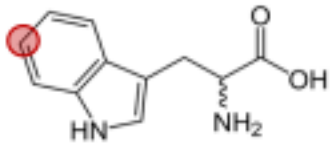
Molecular Dynamics

The associate of deprotonated KP with D-Trp was observed.



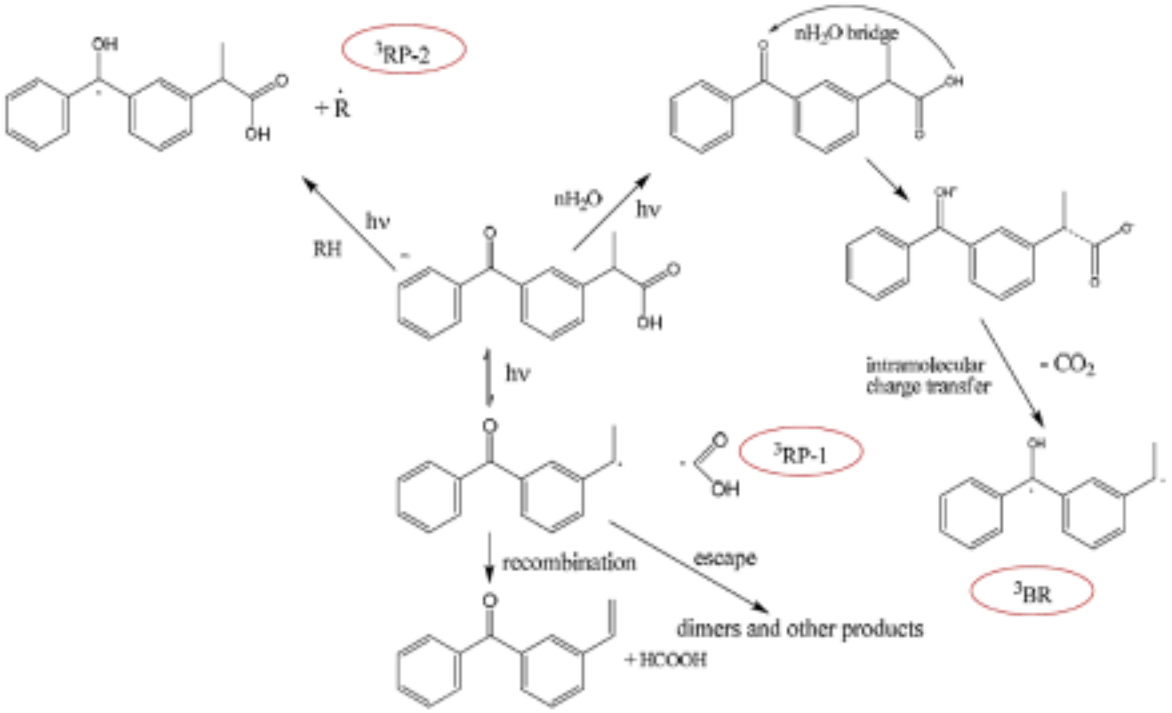
Snapshot of MD trajectory with deprotonated KP associate with D-Trp. Water molecules are not shown.

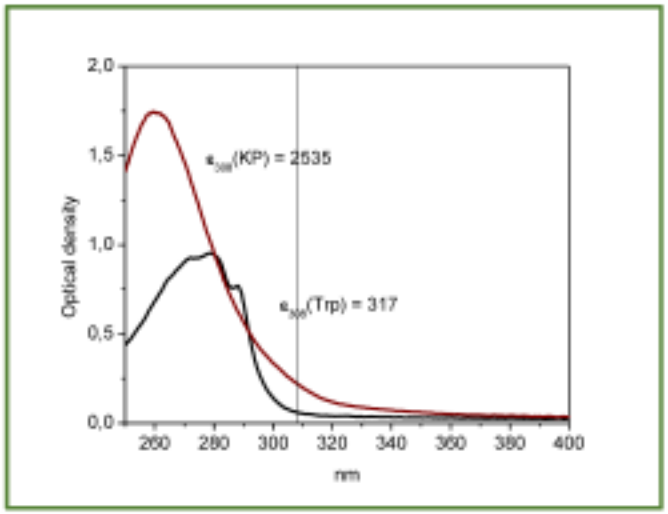




We have also calculated mean distances between selected C atoms of KP and Trp from MD trajectories. For protonated form of KP mean distance is 2.5±0.4 nm, for deprotonated form mean distance is 1.2±0.3 nm. Therefore, in the case of deprotonated KP molecule the distance between aromatic protons of KP and Trp is 2 times lower than in the case of protonated KP molecule.

Photolysis of KP

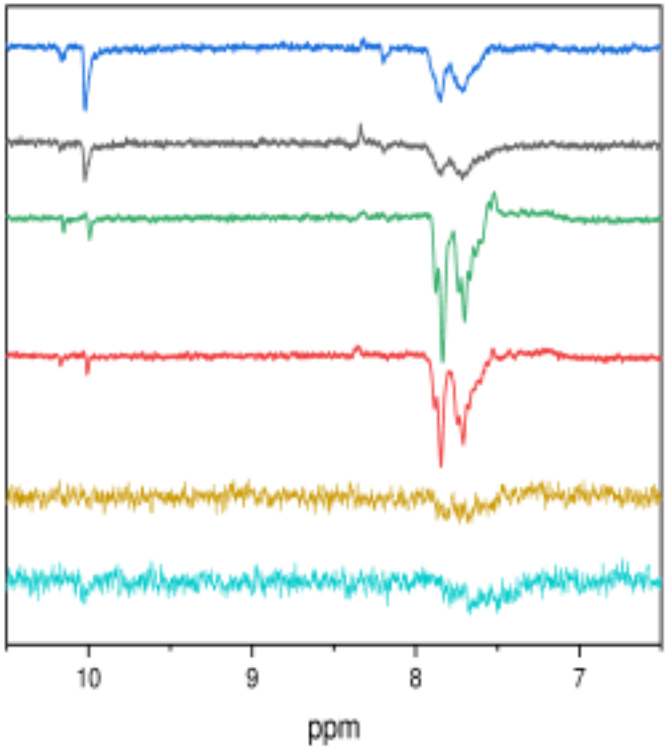




Absorption spectra of KP (0.1 mM) and Trp (0.2 mM) in bicelles at pH 7.4. Extinction coefficients are shown for wavelength of laser light, 308 nm.

CIDNP

Bicelle at pH=3.8



KP+L-Trp long irradiation

KP+D-Trp long irradiation

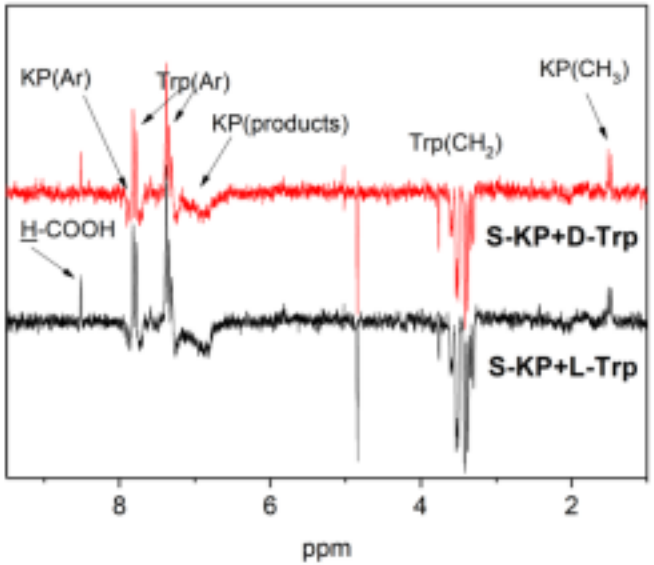
KP+L-Trp 64 laser pulses

KP+D-Trp 64 laser pulses

KP at pH=3.8, long irradiation

KP at pH=7.4, long irradiation

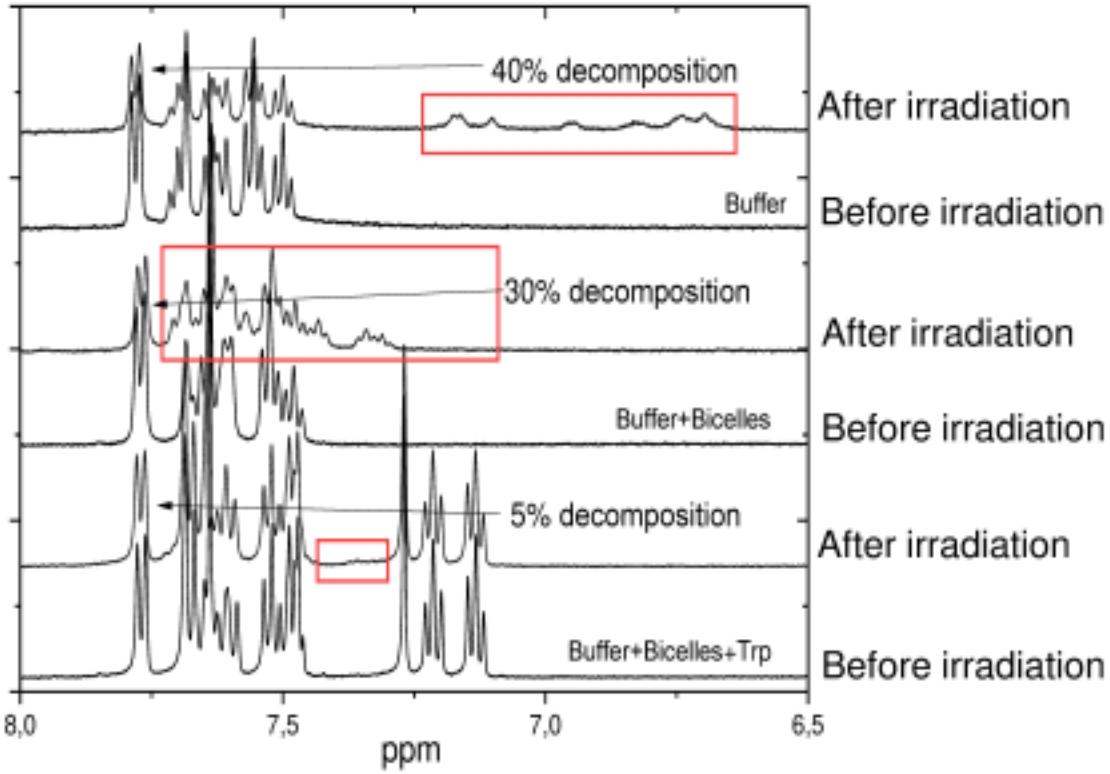
PBS at pH=7.4

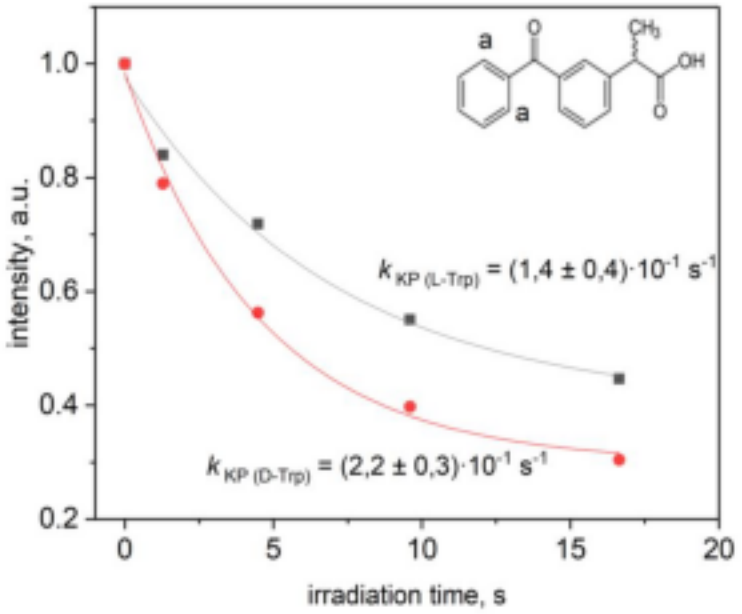


- The presence of polarization on the KP protons indicates the reversibility of the photoinduced electron transfer.
- Signals at 10 ppm belong to lipid oxidation products and are not observed in the absence of tryptophan.

NMR after photolysis

In contrast to a homogeneous phosphate buffer solution, where the amino acid tryptophan accelerates the photodecomposition of KP due to intramolecular hydrogen transfer, tryptophan in a lipid membrane significantly reduces the rate of photodegradation due to a reversible electron (or hydrogen) transfer reaction.

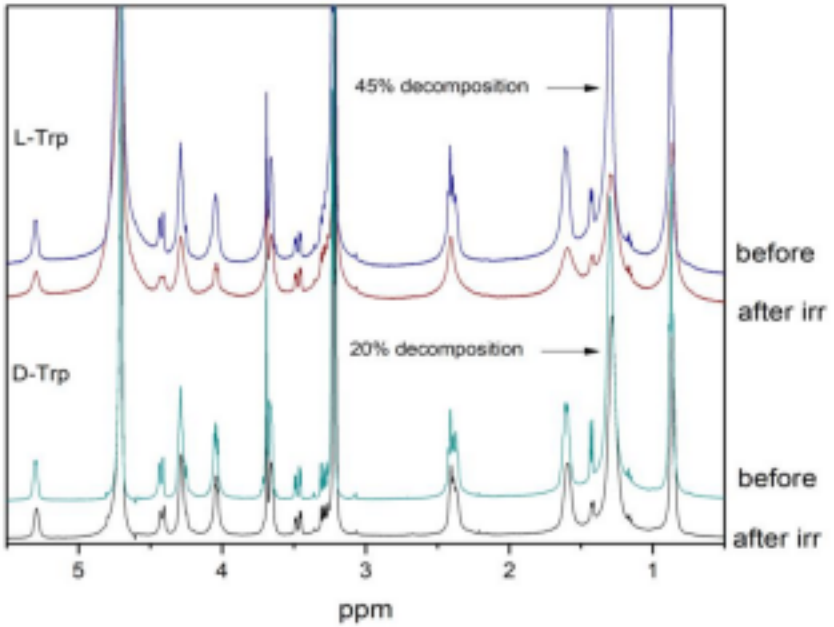




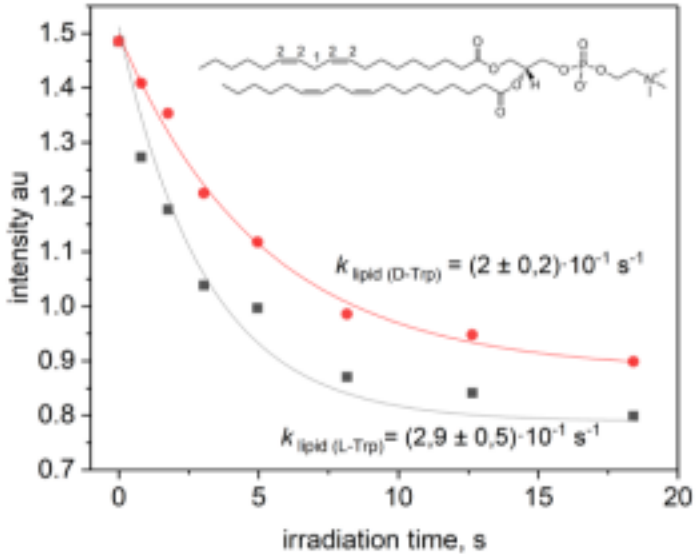
Decay kinetics of KP under irradiation with L/D-Trp in bicelles.

NMR after photolysis

NMR analysis shows significant stereo selectivity of lipid photo-oxidation induced by KP in the presence of Trp enantiomers.



NMR spectra of bicelles before and after photolysis in the presence of KP and L/D-Trp at pH=7.4



Decay kinetics of 1-H lipid protons at 2.7 ppm on irradiation time in DHPC/DLPC bicelles at pH=3.3.

These results correlate with data from molecular modeling of lipids in the presence of KP and L/D-Trp. The free exchange of KP and Trp molecules across the bilayer may explain the slower rate of lipid degradation reaction in the case of D-Trp.

Conclusion

- Ketoprofen molecules are able to penetrate lipid membranes and interact stereoselectively with enantiomers of amino acid tryptophan.
- The main channels of ketoprofen photolysis in lipid membranes are radical reactions with lipid molecules and with amino acid tryptophan, in contrast to monomolecular decomposition in homogeneous solutions.
- The stereoselectivity of the oxidation of lipid molecules during the photolysis of ketoprofen with tryptophan enantiomers in the lipid bilayer was found.

Publications:

Mastova A. V., Selyutina O.Y., Polyakov N.E. Photoinduced Oxidation of Lipid Membranes in the Presence of the Nonsteroidal Anti-Inflammatory Drug Ketoprofen // Membranes 2022, 12, 251.
https://doi.org/10.3390/membranes12030251

Mastova A. V., Selyutina O.Y., Polyakov N.E. Stereoselectivity of Interaction of Nonsteroidal Anti-Inflammatory Drug S-Ketoprofen with L/D-Tryptophan in Phospholipid Membranes // Membranes 2022, 12, 460.
https://doi.org/10.3390/membranes12050460

Ageeva, A.A.; Doktorov, A.B.; Polyakov, N.E.; Leshina, T.V. Chiral Linked Systems as a Model for Understanding D-Amino Acids Influence on the Structure and Properties of Amyloid Peptides. // Int. J. Mol. Sci. 2022, 23, 3060.
https://doi.org/10.3390/ijms23063060

This study was supported by the Russian Science Foundation, grant 18-13-00047.

Thank you for your kind attention!

Techniques

- ^{1}H pseudo steady state (PSS) CIDNP experiments were performed on DPX-200 NMR spectrometer (Bruker, 200 MHz ^{1}H operating frequency, τ(90) = 3.0 μs). EMG 101 MSC excimer laser (Lambda Physik, 308 nm, 100 mJ at output window, 20 mJ per pulse in sample volume, with pulse duration 15 ns).
- Spectra and kinetic curves of luminescence were recorded with an Edinburgh Instruments FLSP-920 spectrofluorimeter with either a Xenon lamp or a laser diode EPLED-320 (λex = 320 nm, pulse duration 0.6 ns) as excitation sources. The kinetic traces were fitted by biexponential decay functions using a reconvolution procedure. For the correct selection of the weak band of exciplex luminescence the spectrum was recorded twice: without a filter and with the step 395 nm filter. The absorbance at an excitation wavelength was kept ca. 0.1. UV/Visible absorption spectra were recorded using an Agilent 8453 spectrophotometer (Agilent Technologies).
- All experiments were performed at room temperature 296K.

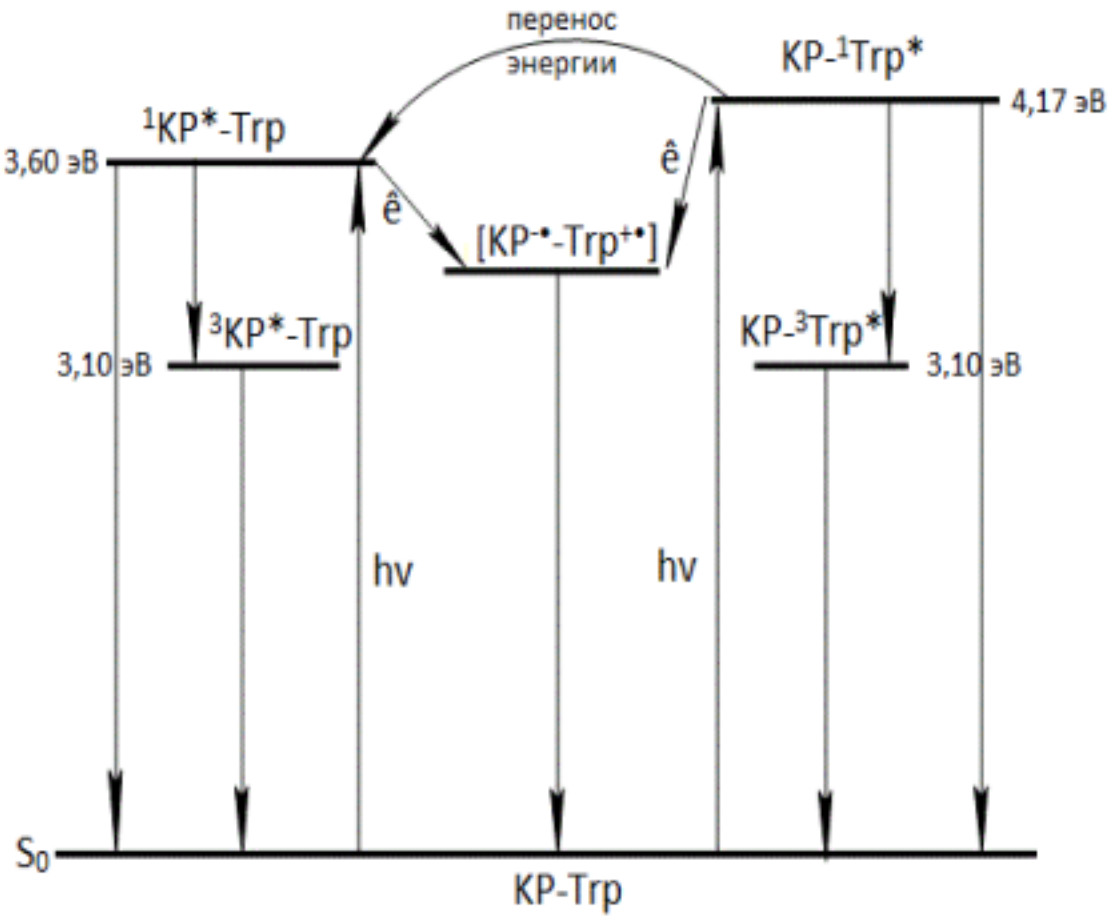
Molecular Modeling description

- For each system, 10 MD trajectories with a duration of 100 ns were obtained. Using the standard GROMACS utilities, the time dependence of the distance between the marked atoms was obtained . Further, the average time spent by these groups at a distance of less than 0.6 nm and less than 0.45 nm from each other was calculated. It should be noted that the figures were obtained for launches with a length of 100 ns, i.e., it is more correct to correlate the results with the fraction of time that the atoms are at a distance of less than 0.6 nm. That is, 0.2% and 0.05% of the time spent by the (R, S) and (S, S) -diads at these distances.
- Also, the average angles between the marked planes were determined during the calculated time for distances of less than 0.6 nm and less than 0.45 nm. The results of the analysis of MD trajectories are shown in Tables.

Перенос электрона

$$\Delta G_{et} = E_{ox} - E_{red} - E_{0-0} + \frac{2.6\ eV}{\varepsilon} - 0.13\ eV$$

Eox (Trp) = 1.015эВ
Ered (КР) = -1.44 эВ
E0-0 = 4.17 эВ – энергия синглетного возбужденного состояния триптофана.
Член T ΔS в ацетонитриле не превышает 0.1 эВ, и отражает вклад от растворителя.



Proposed Scheme of intramolecular electron transfer

$$\textbf{Rehm} - \textbf{Weller equation}$$

$$\Delta G = \left[E_{ox}\left(\frac{D}{D^{+}}\right) - E_{red}\left(\frac{A^{-}}{A}\right)\right]_{\varepsilon_0} - -\frac{e^2}{\varepsilon \cdot r} + \Delta G_{solv} - E(\,^1NPX^*),$$

$$\Delta G_{solv} = \frac{e^2}{2}\left(\frac{1}{R^{D^+}} + \frac{1}{R^{A^-}}\right)\left(\frac{1}{\varepsilon} - \frac{1}{\varepsilon_0}\right);$$

E_{ox}(Pyr)=1.0eV,

E_{red}(2-methoxynaphtalene) = -2.6 eV, and $E_{0\text{-}0}$(NPX) = 3.69 eV.

$w_{intra} = 1.8\times10^{8}\ s^{-1}$ (*S*,*S*); $2.8\times10^{8}\ s^{-1}$ (*R*,*S*);

$w_{inter} = 2\times10^{9}\times10^{-3}\ s^{-1}$.