Using spin chemistry and photochemistry in the chiral model systems to study the role of D amino acids in the Alzheimer's disease

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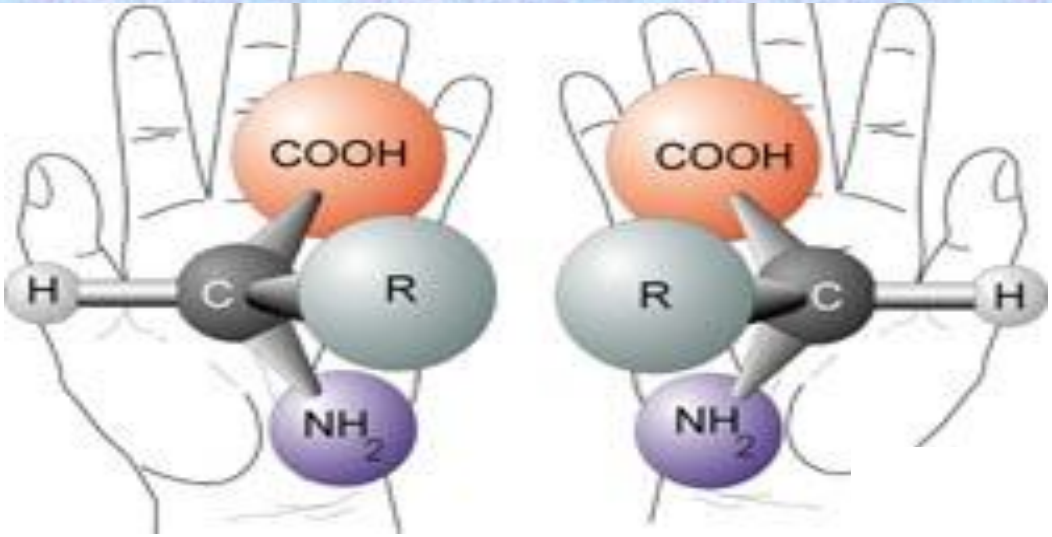
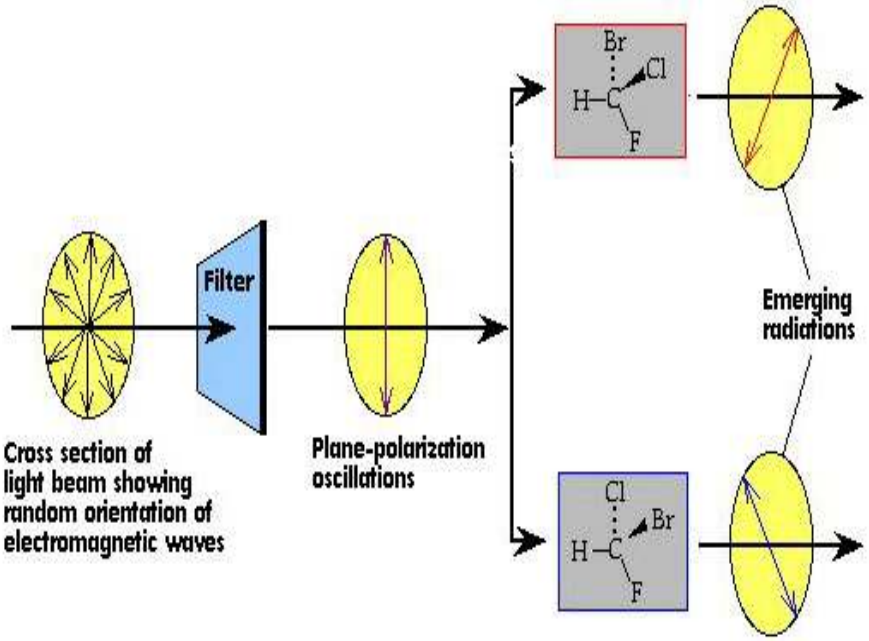
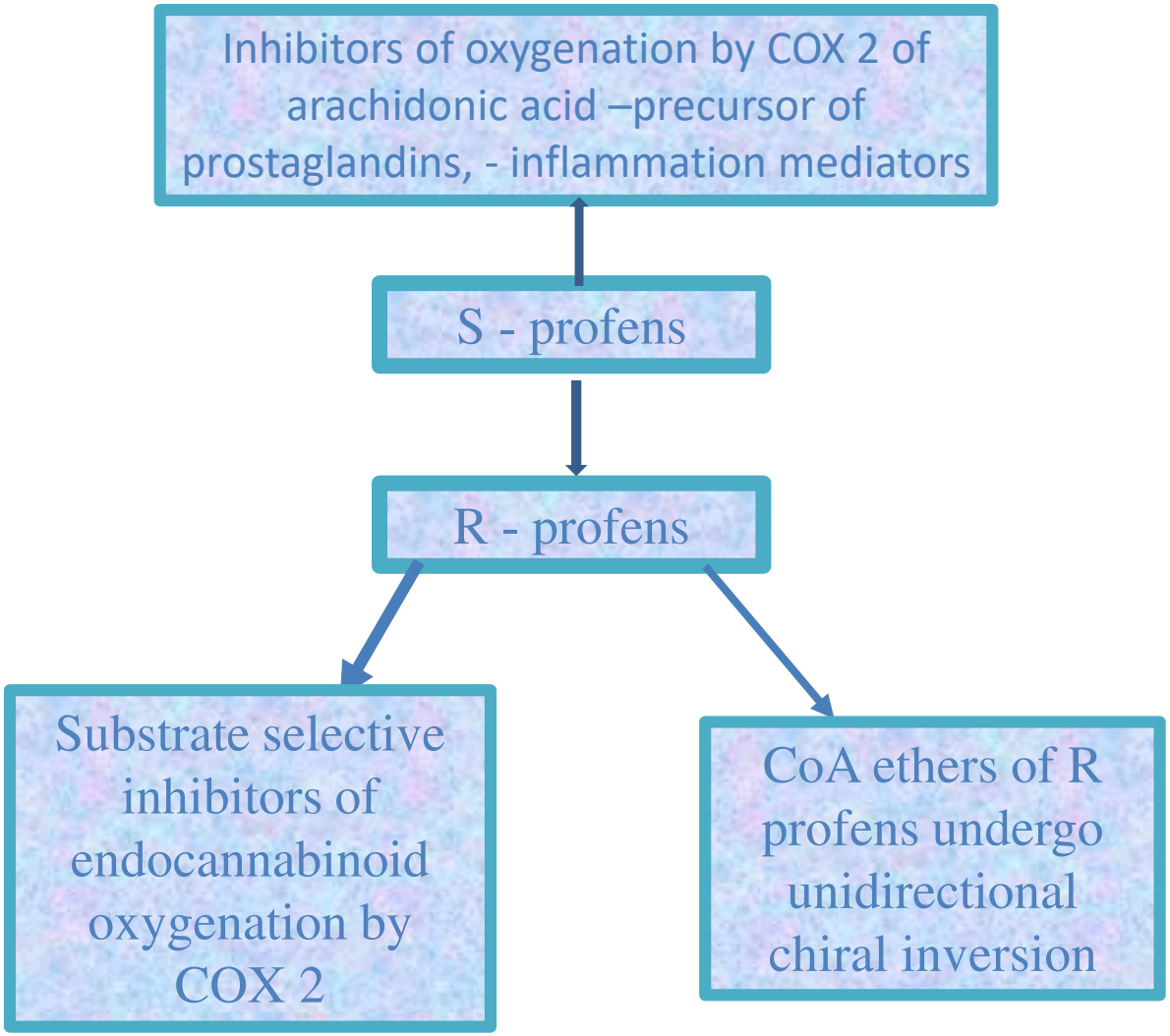
Chiral enantiomers



All physico chemical properties of enantiomers are identical except for the direction of plane polarized light rotation



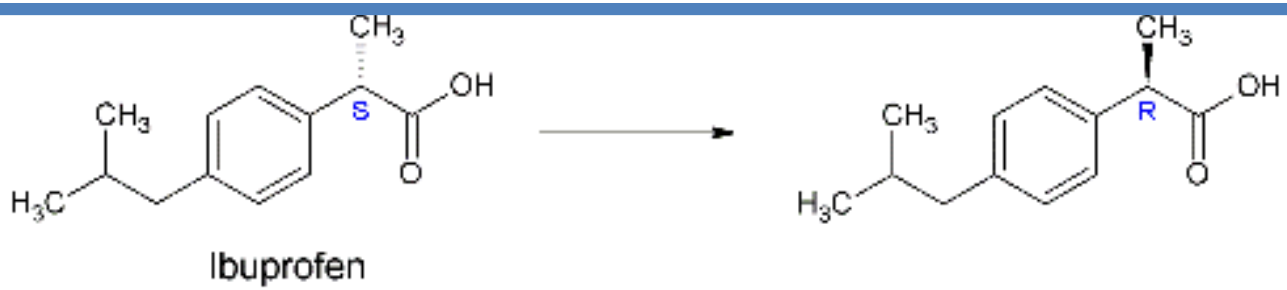
Different therapeutic activities of NSAIDs on the example of naproxen



Chiral inversion

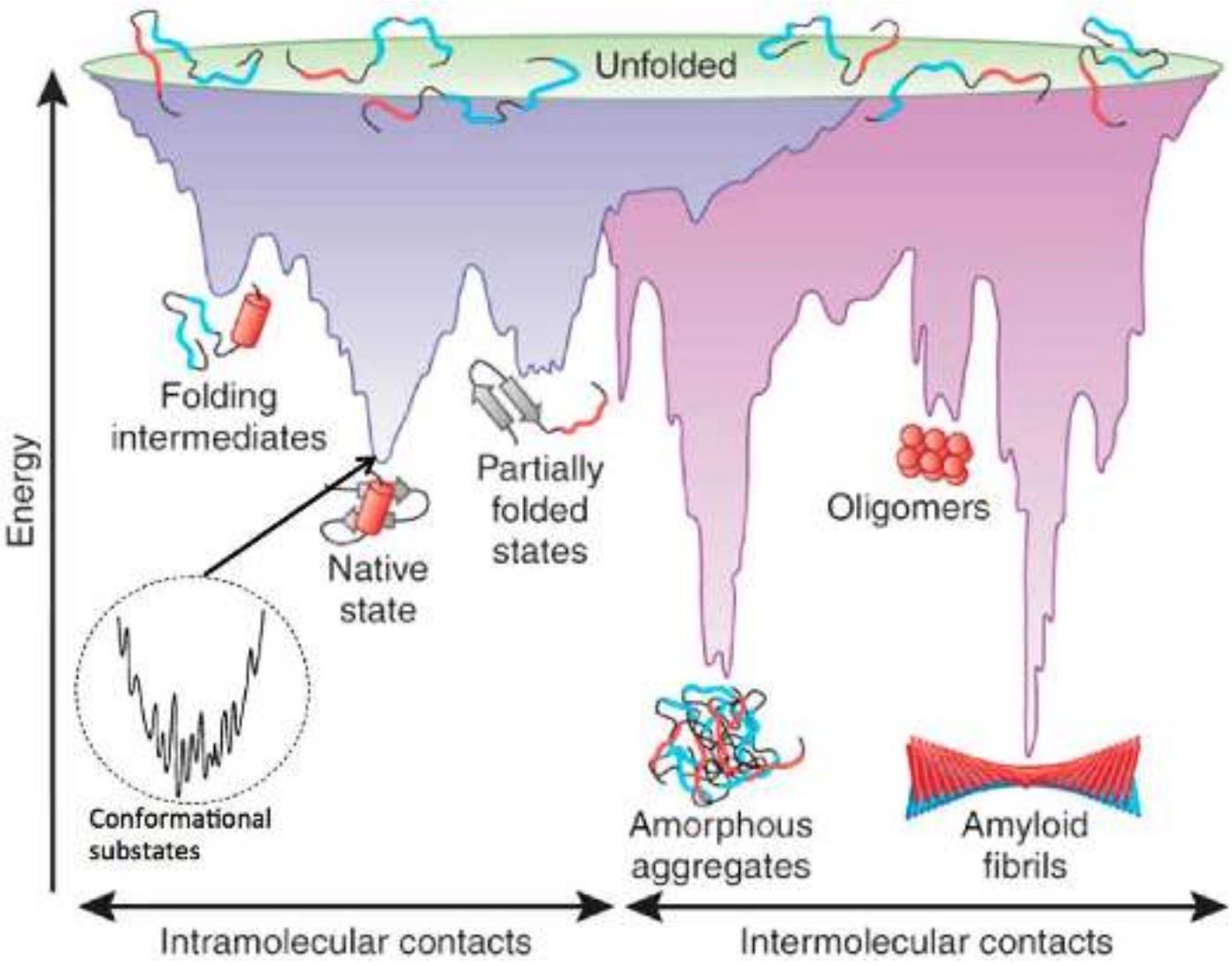
L – Tryptophan ⟶ **D Tryptophan**

It is well known that optical isomers may undergo so-called chiral inversion – conversion of one optical configuration to another one. This can happen both in vivo and in vitro under various conditions, e.g. change of temperature, solvent, pressure, action of enzymes and the influence of other chiral substances.



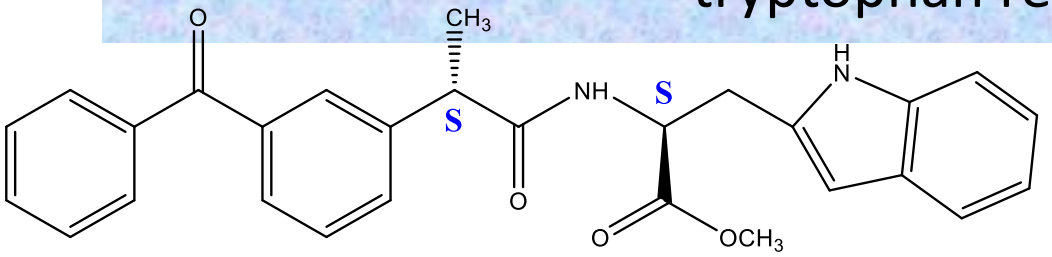
Ibuprofen

It was shown that the presence of D-isomers of amino acids causes disruptions in the folding processes, leading to the aggregation of internally disordered amyloid proteins with the formation of oligomers or fibrils of large sizes that have a toxic effect and literally destroy the brain. So, one of the causes of Alzheimer's, Parkinson's, and type 2 diabete may be the age-related change in the optical configuration of amino acids from L to D in the islet amyloid polypeptide.

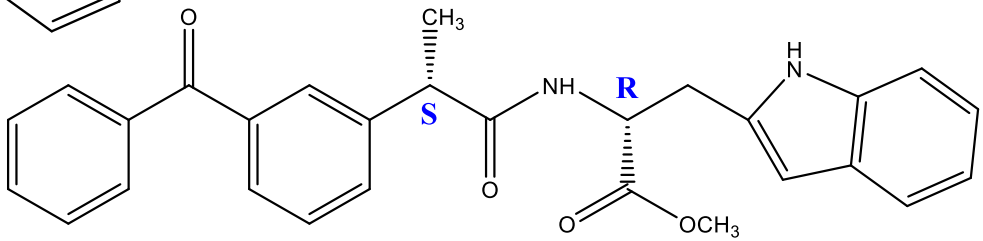


Schematic energy landscape for protein folding and aggregation. The surface shows the multitude of conformations "funneling" towards the native state via intramolecular contact formation, or towards the formation of amyloid fibrils via intermolecular contacts. The landscape is represented by the free energy of the protein as a function of some reaction coordinate planar slices through the 3D surface). Entropy is schematized as width within any particular sub-funnel.

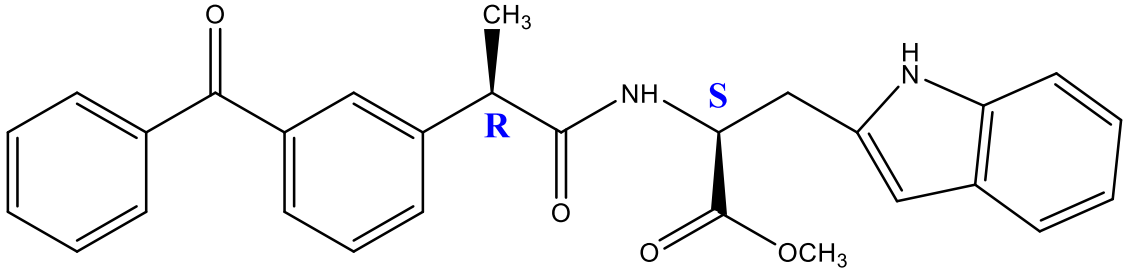
Model systems to study the nature of the difference in L and D tryptophan reactivity



S-KP-S-Trp



S-KP-R-Trp

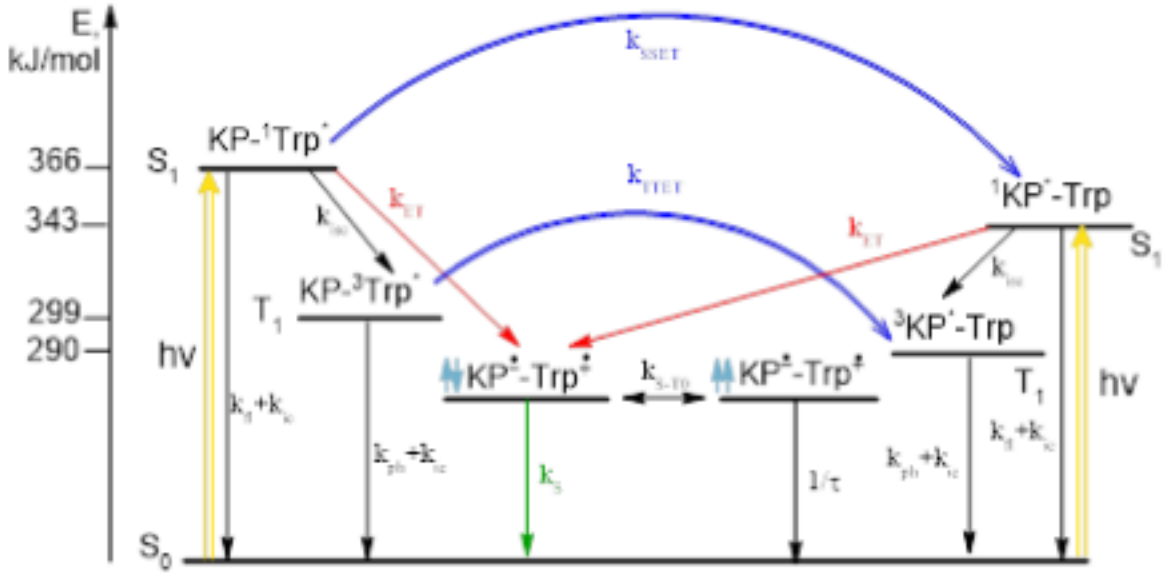


R-KP-S-Trp

The current trend to study distinguish between L and D amino acids in highly disordered proteins and peptides is the use of molecular modeling methods by the example of short peptides. The use of methods in silica to solve this problem is proposed in the literature because highly disordered proteins cannot be studied either by high-resolution magnetic resonance or by means of EX-rays.

Our approach is to study photoinduced ET and RET in these model dyads, including the L or D residues of Trp, in order to trace the difference between the reactivity of Trp isomers using the analysis of spin effects and fluorescence quenching data.

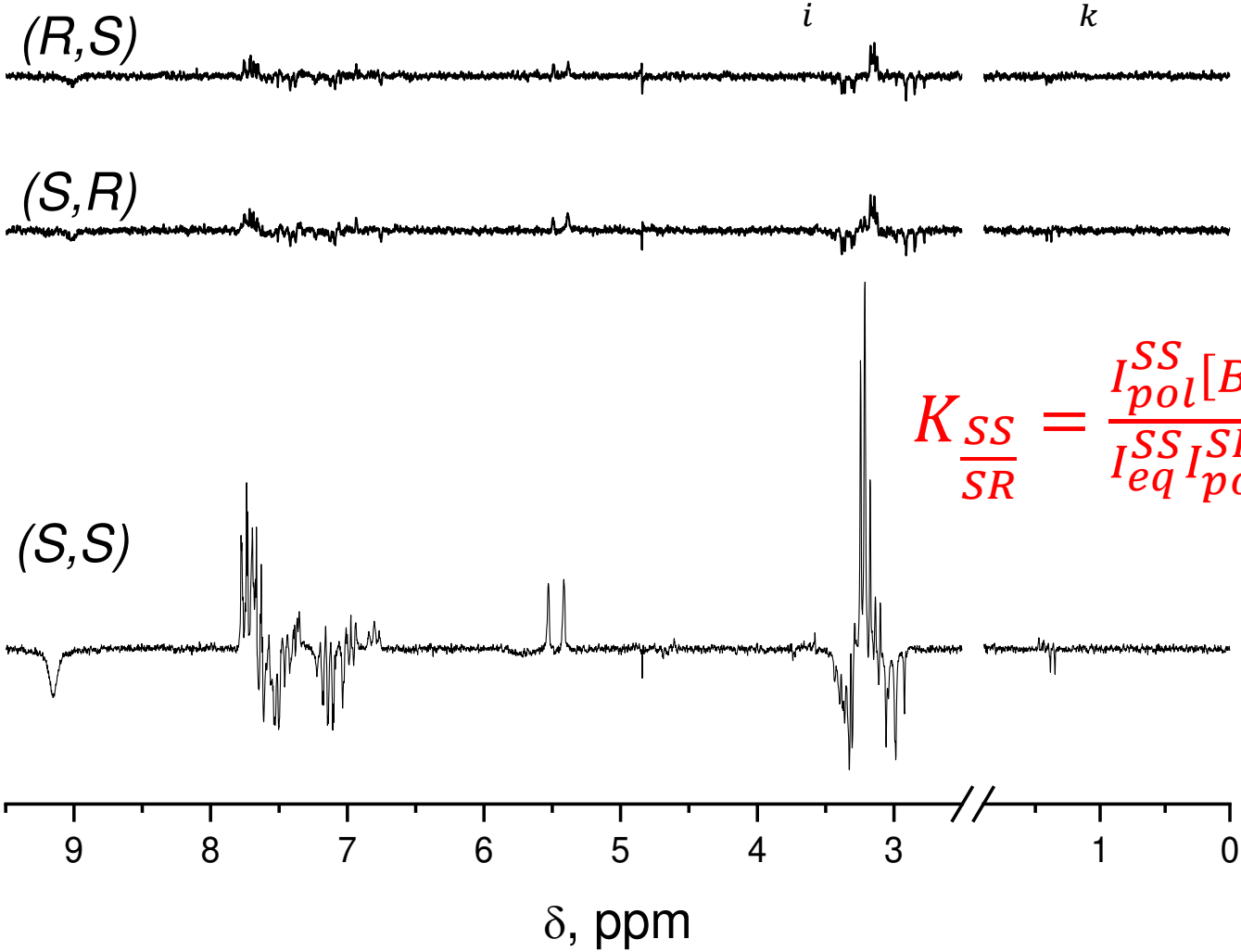
Scheme of the possible photoinduced transformations in (R/S)KP-(R/S)Trp



Ways of excitation degradation in donor-acceptor linked system. In this scheme S^1 is the singlet excited state of Trp, k_{ET} is the rate constant of electron transfer, k_{RET} is the rate constant of singlet-singlet energy transfer, S and T_0 are the collective spin states of the biradical-zwitterion in high magnetic field, k_{S-T0} is the intersystem crossing constant, k_{BET} is the rate constant of back ET, k_P – rate constant of by-products formation.

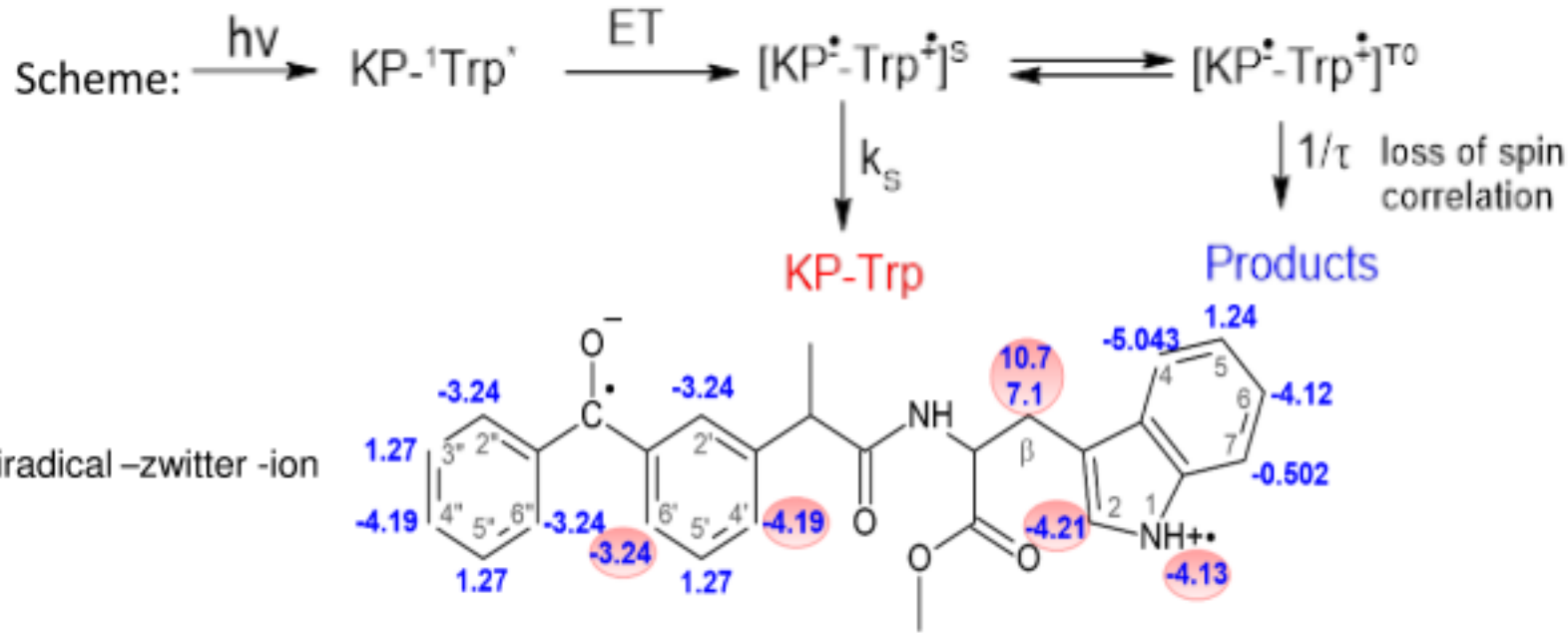
CIDNP pattern of SS, SR and RS configurations of KP –Trp dyad; K SS/SR – ratio of CIDNP enhancement coefficients –spin selectivity (SpS). SpS means the difference in hyperpolarization of different optical configuration

$$\hat{H} = g_1\beta B\hat{S}_{1z} + g_2\beta B\hat{S}_{2z} + \sum_i a_{1i}\hat{S}_{1z}\hat{I}_{iz} + \sum_k a_{2k}\hat{S}_{2z}\hat{I}_{kz}$$



$$K_{\frac{SS}{SR}} = \frac{I_{pol}^{SS}[BZ]^{SS} I_{eq}^{SR}}{I_{eq}^{SS} I_{pol}^{SR}[BZ]^{SR}} = 10$$

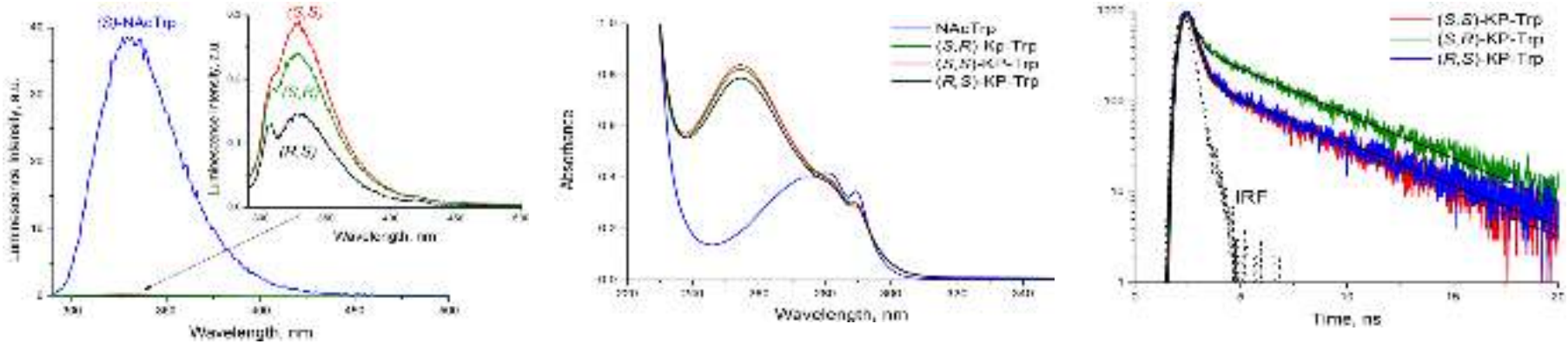
Conclusion:The lack of proportionality between CIDNP and HFI may be a feature of the coupled system, and differences between diastereomers may mean that the spin density distribution is different for the (S,S) and (R,S) configurations.



Relationships between CIDNP coefficients and HFI constants in the biradica l- zwitter- ion

Position	4'/6'	β-CH_2	2	1
$HFI_i/HFI_{(1)}$*	0.90	2.6-1.7	1.02	1
$K_i/K_{(1)}$	0.6-0.5	1.4-1.3	0.3-0.1	1
K_{SS}	1.4 ± 0.1	3.0 ± 0.3	0.6 ± 0.1	2.2 ± 0.2
K_{SR}	0.11 ± 0.01	0.3 ± 0.1	0.03 ± 0.01	0.21 ± 0.02
$K_{SS/SR}$	13 ± 1	10 ± 1	20 ± 2	10 ± 1

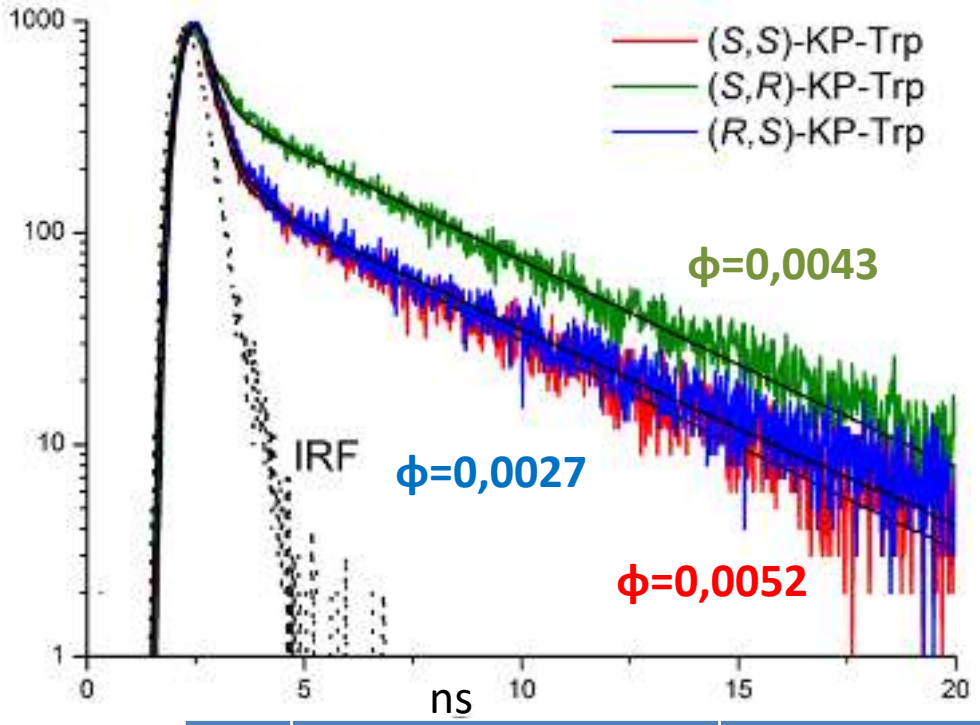
Spectral characteristics of the KR-Trp dyad's diastereomers



(**left**) Fluorescence spectra of is isoabsorptive solutions of *(S)*-NAcTrp and *(S,S)*-, *(S,R)*-, *(R,S)*-optical isomers of KP-Trp dyad in acetonitrile (λ_{ex} = 280 nm) in 1 cm cuvette. Concentrations were about $6x10^{-5}$ M; (**middle**) absorbance spectra of (*S*)-NAcTrp and (*S,S*)-, (*S,R*)-, (*R,S*)-optical isomers of KP-Trp dyad in acetonitrile in 1 cm cuvette. (**right**) Fluorescence decay traces of (*S,S*)-, (*S,R*)-, (*R,S*)-optical isomers of KP-Trp dyad in acetonitrile at 330 nm (λ_{ex} = 270 nm), IRF—instrument response function;

The photophysical properties of Trp are highly sensitive to its (local) environment. Current consensus is that Trp emission originates from only the 1La state, except when the local environment is completely nonpolar. Therefore, the fluorescence of Trp is strongly affected by the polarity of its surrounding, promoting emission from both the 1La and 1Lb state. (Amar Ghisaidoobe, S. Chung, Int. J. Mol. Sci. 2014, 15)

Fluorescence kinetics of solutions (S, S) -; (S R) -; (R, S) - optical isomers of the KP-Trp dyad in acetonitrile (λex = 280 nm). C ~ 6 10-5.



$$\phi_0 / \phi = 1 + kq\tau 0$$

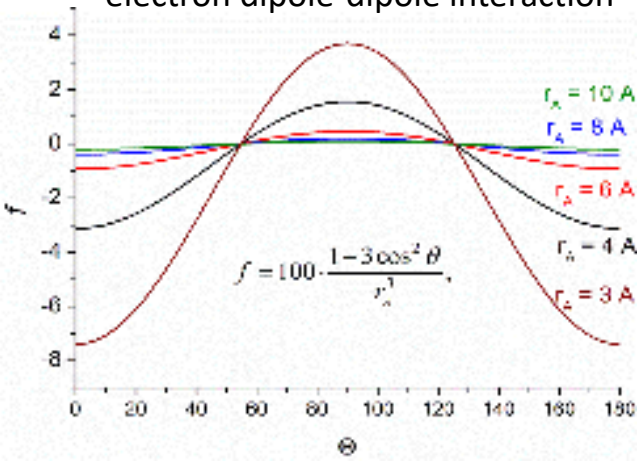
where ϕ0 and τ0 are the quantum yield of fluorescence and the lifetime of free tryptophan (0.16 and 3.6 ns); ϕ is the quantum yield of fluorescence of tryptophan in the dyad kq - quenching constant (kq = kET + kRET).

	k_{ET}	k_{RET}
(R,S)/(S,R)	1	1.6
(S,S)/(R,S)	?	0.5

	(R,S)-KP-Trp				(S,R)-KP-Trp				(S,S)-KP-Trp			
ε	τ_1, ns	A_1, %	τ_2, ns	A_2, %	τ_1, ns	A_1, %	τ_2, ns	A_2, %	τ_1, ns	A_1, %	τ_2, ns	A_2, %
36.8	0.2±0.1	49	4.5±0.5	51	0.1±0.1	27	4.4±0.4	73	0.1±0.1	50	4.3±0.4	50
30.25	0.2±0.1	55	4.6±0.5	45	0.2±0.1	27	4.3±0.4	73	0.1±0.1	44	4.1±0.4	56
23.85	0.1±0.1	57	4.4±0.5	43	0.2±0.1	27	4.2±0.4	73	0.1±0.1	49	4.2±0.4	51
17.7	0.2±0.1	57	4.4±0.4	43	0.2±0.1	26	4.1±0.4	74	0.1±0.1	51	4.1±0.4	49
11.9	0.2±0.1	56	4.4±0.4	44	0.2±0.1	24	4.0±0.4	76	0.2±0.1	45	4.0±0.4	55
6.19	0.2±0.1	51	4.1±0.4	49	0.3±0.1	26	3.7±0.4	74	0.2±0.1	50	3.9±0.4	50

Spin selectivity relationship with optical configuration of the dyad and MD results.

Conditions for the manifestation of electron-electron dipole-dipole interaction



Dependence of the electron dipole-dipole interaction on the angle θ between radius vector connecting the paramagnetic centers r_a and the direction of the external magnetic field. With r_a=4 A and parameter f=1, everything is averaged. When the particle size R is about 16 A, no averaging occurs.

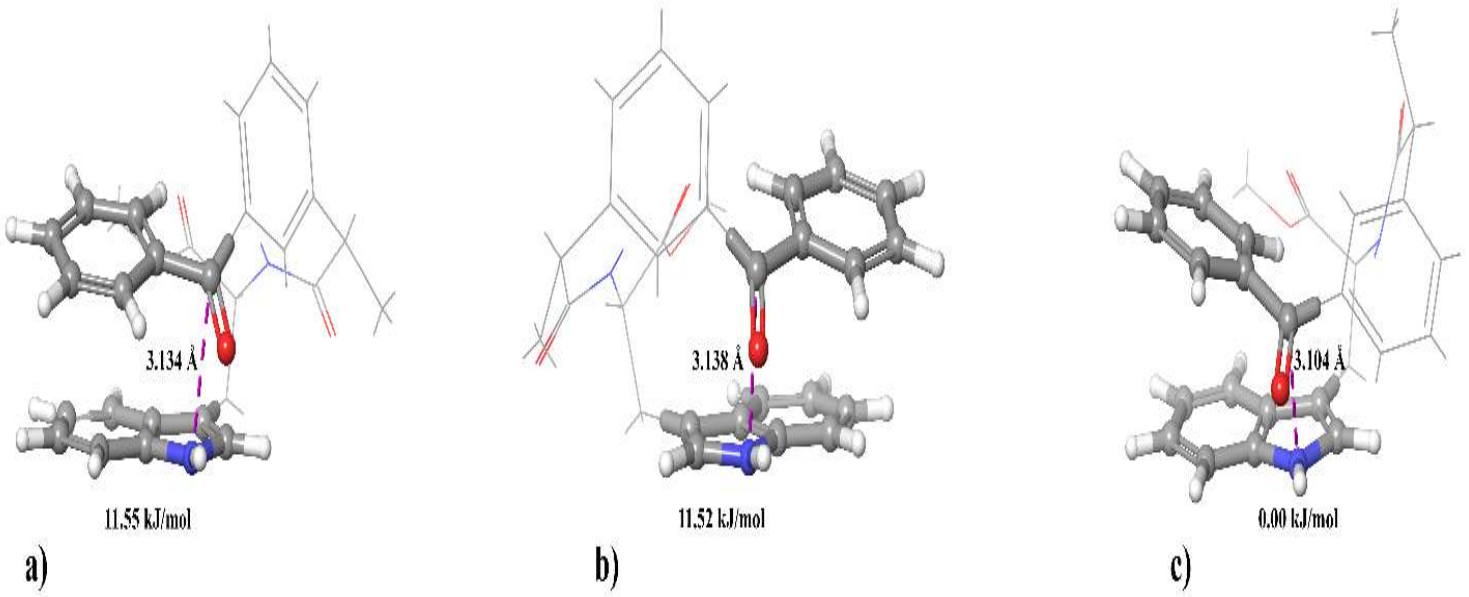
	C_{SS}, mM	K_{SS}	C_{RS}, mM	K_{RS}
Addition of (S,R)- into the (S,S)+(R,S) mixture	1.2	4.00	3.6	0.64
	1.2	3.20	5.8	0.43
	1.3	2.70	9.2	0.31
Addition of (S,S)- into the (S,S)+(R,S) mixture	0.9	2.16	5.0	0.23
	3.5	2.24	5.2	0.28
	5.7	1.95	5.4	0.25

Dependence of the ratio of the CIDNP enhancement coefficient (K) on the concentration ratio during UV irradiation of diastereomers of KP –Trp dyad mixture.

The comparison of the possibility of dimer formation for various optical configurations of dyads Kp –Trp (IV) and NPX –Trp (III). The molecular dynamics (MD) simulations were performed using GROMACS 2018.4 package.

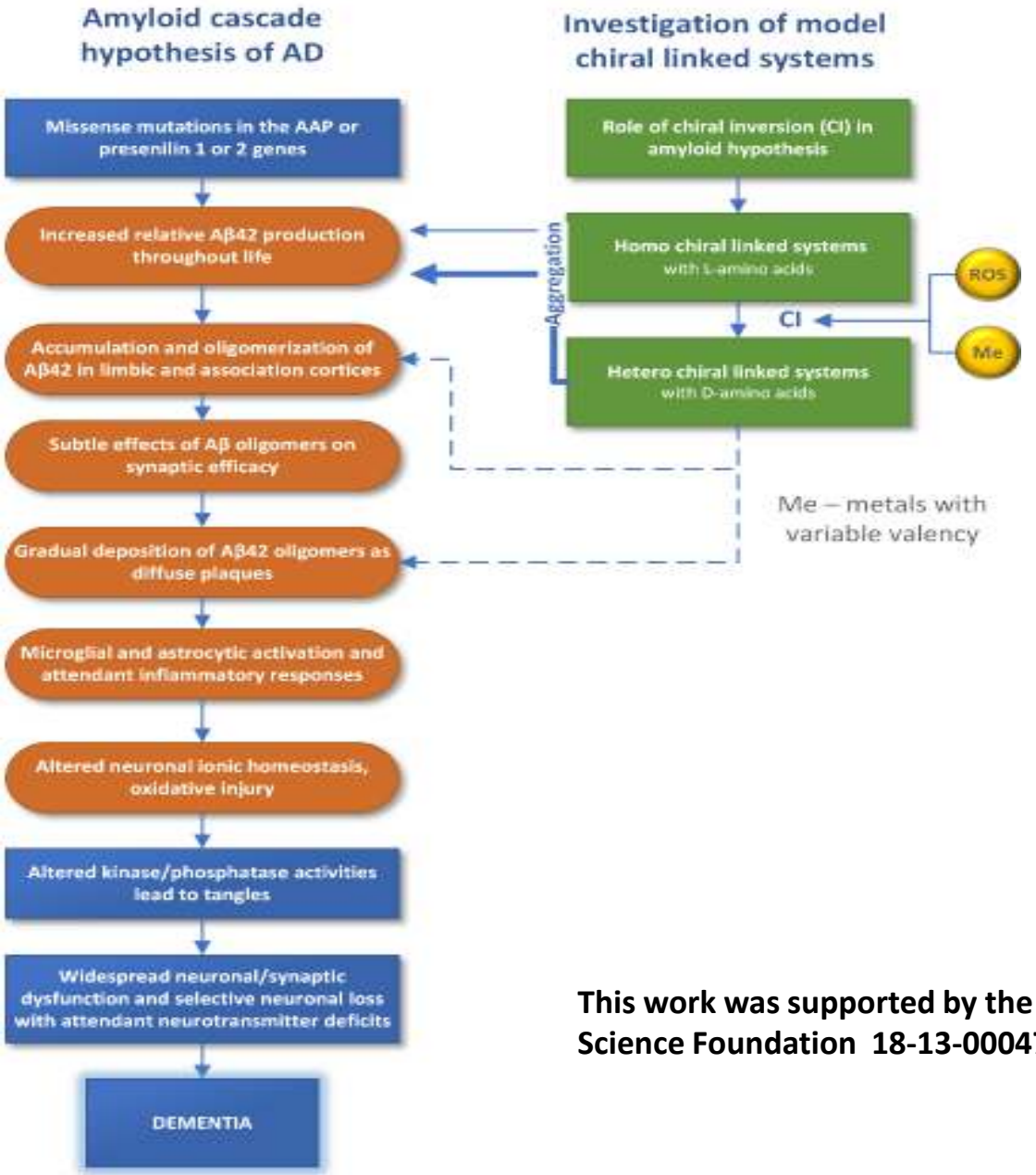
	The fraction of time spent in dimer form, %
(R,S)-III	0.81±0.08
(S,S)-III	0.46±0.05
(R,S)-IV	0.6±0.03
(S,S)-IV	0.38±0.04

Molecular modeling: the results of quantum chemical calculations for dyad Kp-Trp different optical configuration using the Jaguar software, the M06-2X-D3 / cc-pVTZ (-f) method made by Sofia Borisevich.



The optimized structures of (a) (R,S)-; (b) (S,R)-; (c) (S,S)- of dyad Kp -Trp. Values of Gibbs energy are compared with those for (S,S)-configuration.

$$k_{RET} = \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6$$



This work was supported by the grants of Russian Science Foundation 18-13-00047, extended

Conclusion

- The structure and reactivity of diastereomers of the (R,S)KP-(R,S)Trp dyad were compared using a number of experimental and computational physical methods.
- Differences in the efficiency of fluorescence quenching through the electron and energy transfer mechanisms in L and D tryptophan as part of dyad with KP have been demonstrated.
- The high sensitivity of the CIDNP to the optical configuration of diastereomers was demonstrated: a change in the configuration of one chiral center leads to changes in the CIDNP coefficients of all polarized nuclei of the molecule.
- Analysis of CIDNP effects detected in the KP - Trp dyad and its conformation MD simulating show the differences in the dyads with L- and D-tryptophan: greater stability of the (*S,S*)-configuration in comparison with other analogs, different probability of dimer formation, and different distribution of spin density for (*S,S*)- and (*R,S*)-configuration.
- These results: greater stability of (S, S)-homo configurations and a greater propensity of heteroanalogues: (R,S), (S,R) to association are consistent with the results of the behavior of D amino acids in Aβ-amyloids (heterostructure), namely in the part of oligomers and fibrils formation.

This work was supported by the grants of Russian Science Foundation 18-13-00047, extended.

Publications

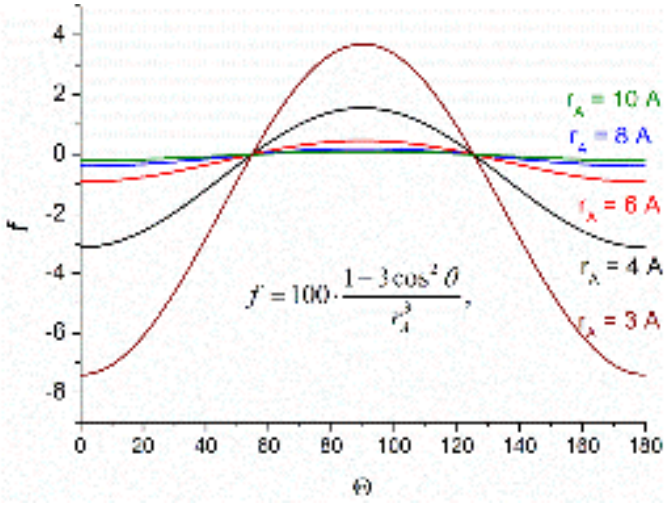
1.Role of chiral configuration in the photoinduced interaction of D- and L-tryptophan with optical isomers of ketoprofen in linked systems, Aleksandra A. Ageeva , Ilya M. Magin, Alexander B. Doktorov , Victor F. Plyusnin , Polina S. Kuznetsova , Alexander A. Stepanov , Alexander A. Alekseev , Nikolay E. Polyakov, Tatyana V. Leshina

2.Optical configuration effect on the structure and reactivity of diastereomers revealed by spin effects and molecular dynamics calculations, Aleksandra A. Ageeva, Alexander B. Doktorov, Olga Yu. Selyutina, Ilya M. Magin, Margarita G. Ilyina, Sophia S. Borisevich, Ruslan Yu. Rubtsov, Sergey L. Khursan, Alexander A. Stepanov, Sergey F. Vasilevsky, Nikolay E. Polyakov Tatyana V. Leshina

3.Role of Association in Chiral Catalysis: From Asymmetric Synthesis to Spin Selectivity, Aleksandra A. Ageeva, Ekaterina A. Khramtsova, Ilya M. Magin, Peter A. Purtov, Miguel A. Miranda,[c] and Tatyana V. Leshina, Chem. Eur. J. 2018, 24, 18587 – 18600

4.Chiral linked systems as a model for understanding D-amino acids influence on the structure and properties of amyloid peptides, Aleksandra A. Ageeva , Aleksander B. Doktorov , Nikolay E. Polyakov and Tatyana V. Leshina, J. Mol. Sci. **2022**, 23, 3060.

Conditions for the manifestation of electron-electron dipole-dipole interaction



Dependence of the electron dipole-dipole interaction on the angle θ between radius vector connecting the paramagnetic centers r_a and the direction of the external magnetic field. With r_a=4 A and parameter f=1, everything is averaged. When the particle size R is about 16 A, no averaging occurs.

Within the framework of the approach used, the characteristic quantity that determines whether the magnetic dipole-dipole interaction of electrons is averaged is the product of the amplitude of the magnetic dipole-dipole interaction (A) by the characteristic time of rotational correlation (τ_c). When the value of this product is much greater than 1, the dipole-dipole interaction is averaged. An estimate of the corresponding characteristic correlation time at an amplitude of the dipole-dipole interaction of 6.068×10^9 in frequency units (at r_a = 3 A) gives a value of 0.16 ns. Further estimation of the characteristic particle size at which the dipole-dipole interaction is averaged was carried out using the following formula:

$$\tau_c = \frac{1}{2D} = \frac{3\eta V}{k_B T} = \frac{4\pi\eta R^3}{k_B T},$$

where τ_c is the characteristic time of rotational diffusion, D is the rotational diffusion coefficient, η is the viscosity of acetonitrile (0.35×10^{-3} Pa·s), k_B is the Boltzmann constant, V and R are the volume and radius of the particle.

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.

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.

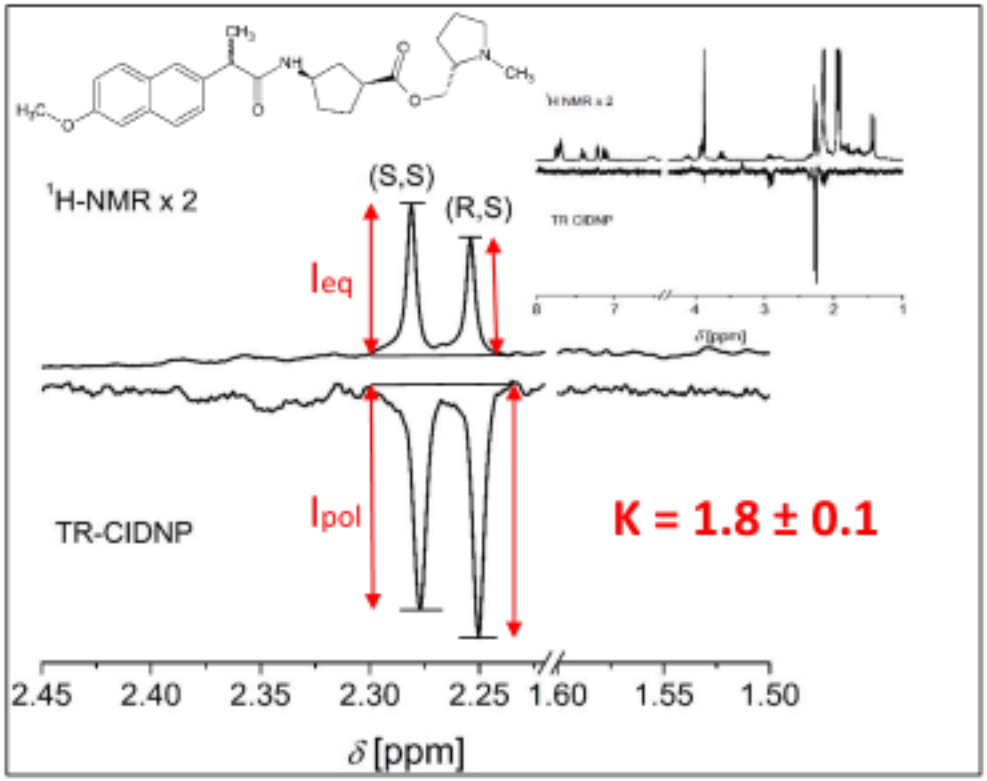
Determination of CIDNP enhancement coefficients

The CIDNP enhancement coefficient for one RP is equal to the intensity of the polarized proton signal (I_{pol}) divided by the equilibrium intensity of the signal in the NMR spectrum (I_{eq}) and the concentration of [*BZ*]. For convenience, we will use the ratio of CIDNP enhancement coefficients derived from

$$K = \frac{I_{pol}^{RS} \times I_{eq}^{SS} \times [BZ]_{SS}}{I_{eq}^{RS} \times I_{pol}^{SS} \times [BZ]_{RS}}$$

where [*BZ*] is determined by

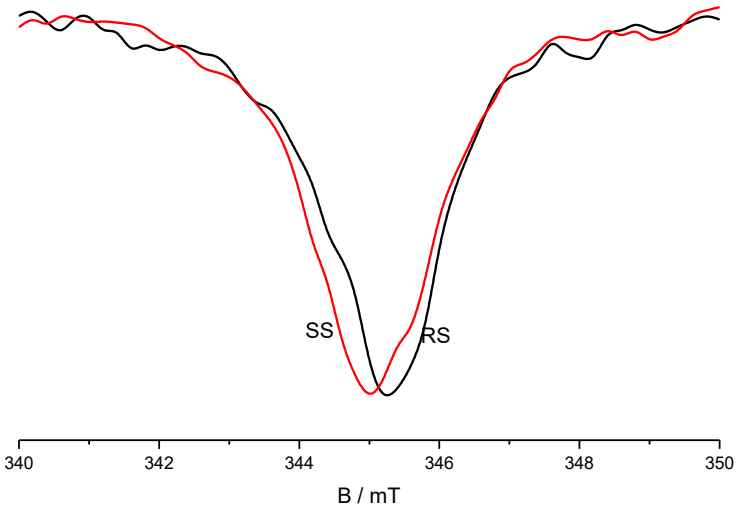
$$[BZ] = (1 - 10^{-A}) \frac{\lambda E (1 - \varphi_{fl})}{hcN_A V}$$



CH_3 proton signals of N-methylpyrrolidine fragment in mixture of diastereomers (R,S)/(S,S)=0,8 in CD_3CN

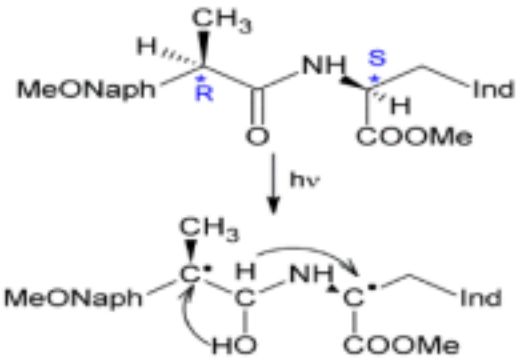
(*A* is the absorption of a 5 mM solution of dyads at 308 nm; λ is the wavelength of the laser light; *E* is the energy of the incident laser light; φ_{fl} are the fluorescence quantum yields of the studied dyads; *V* is the irradiated volume fraction of the sample; N_A is Avogadro's constant; *h* is Plank's constant; and *c* is the speed of light).

CIDEP spectra of biradical –zwitter- ions diastereomers of KP –Trp dyad

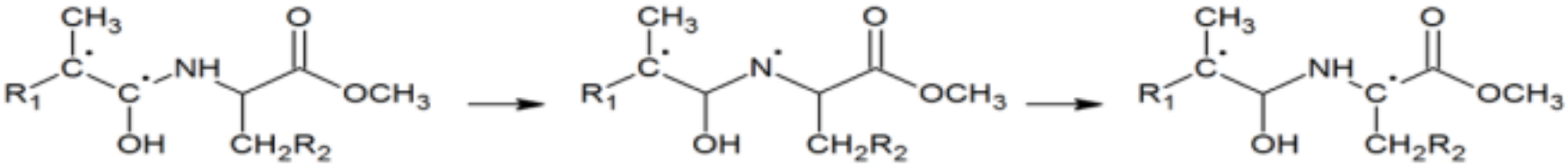


CIDEP spectra of (R) KP- (S) Trp and (S) KP- (S) Trp dyads in toluene at 280K. Excitation wavelength 355 nm.

Photoinduced chiral inversion of (R) NPX –(S) Trp dyad by radical mechanism



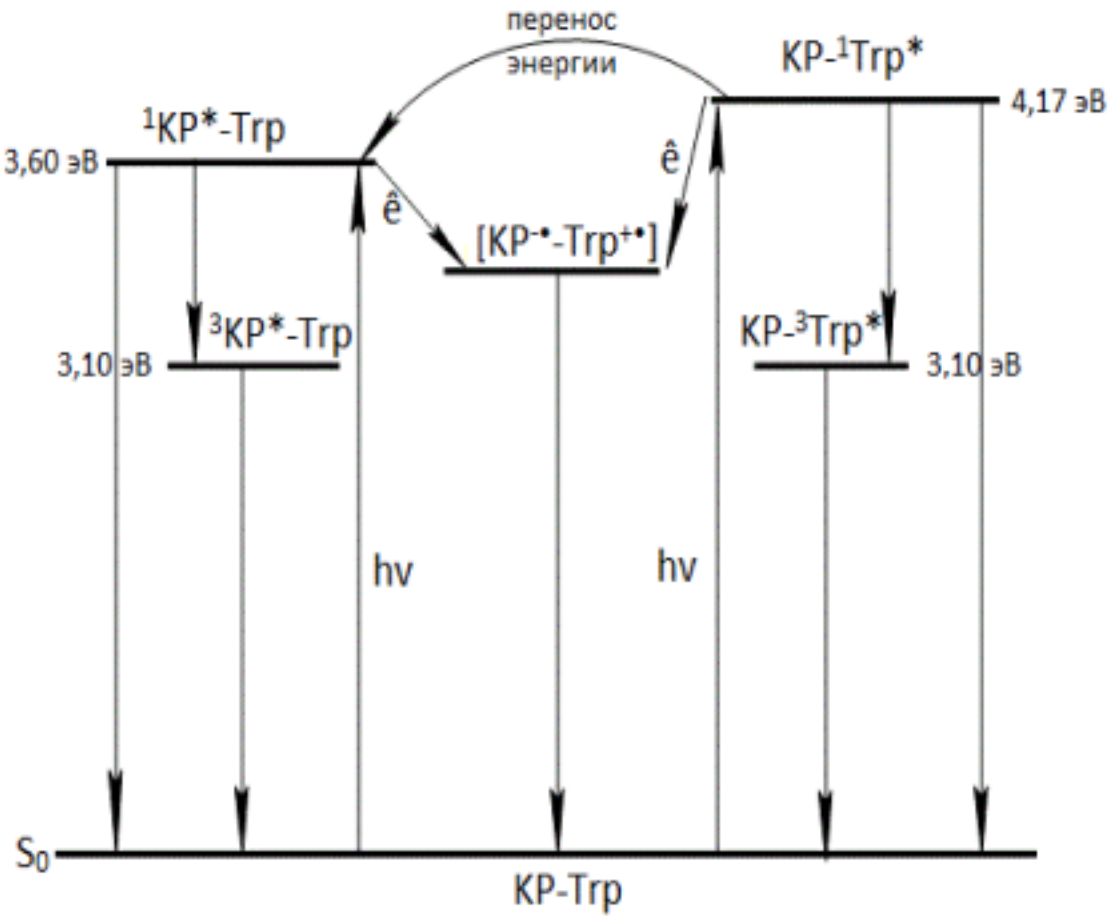
(MeONaph - 6-methoxynaphtalene, Ind - indole)



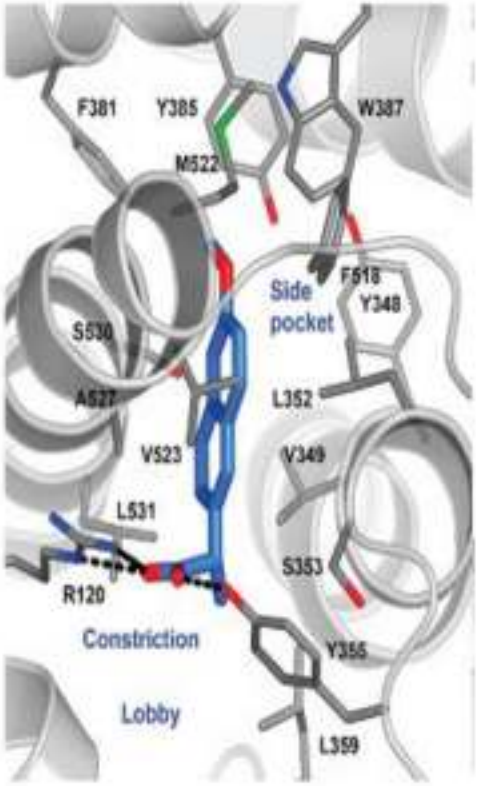
Перенос электрона

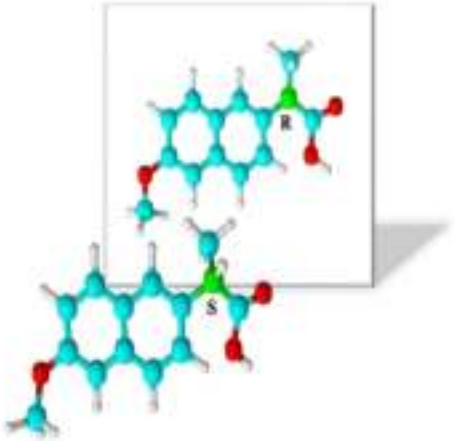
$$\Delta G_{et} = E_{ox} - E_{red} - E_{0-0} + \frac{2.6\ eV}{\varepsilon} - 0.13\ eV$$

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.



Justification of the selected models: the possible mechanism of chirality influence in Drug-Enzyme Interaction



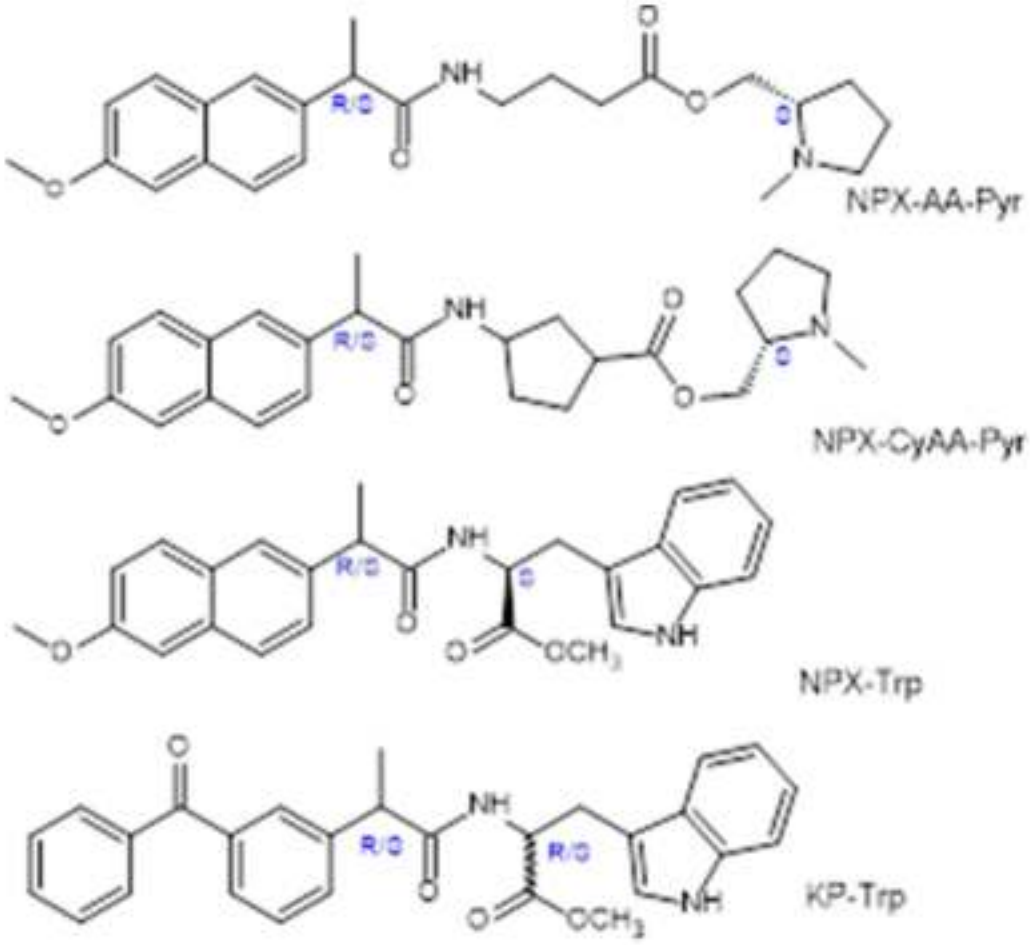


Naproxen at the active site of cyclooxygenase 2

In the enzyme's active sites chiral drugs interact with other chiral species – amino acid residues

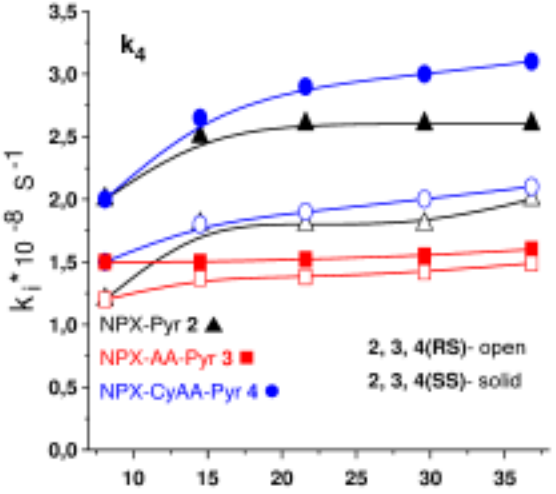
*K. C. Duggan, M. J. Walters, J. Musee at al., **J. Biol. Chem**. 285, 45, 34950-34959, **2010***

Models for studying of the chiral configuration influence on the reactivity of enantiomers in the composition of diastereomers



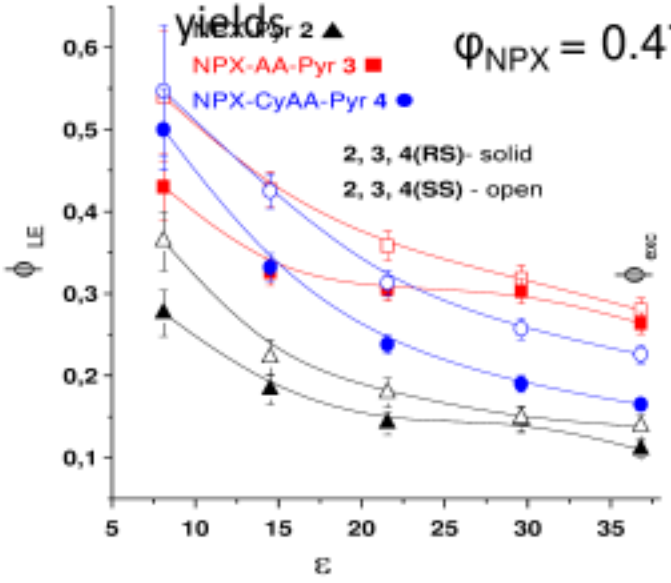
Photochemistry Study

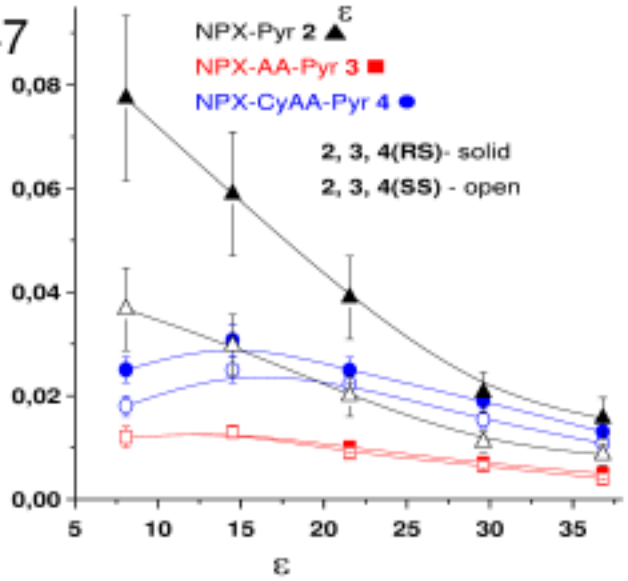
Rate constants of exciplex formation



Fluorescence quantum yields

$\varphi_{NPX} = 0.47$





Problem statement: the establishment of the nature of the difference in biological activity of enantiomers of both chiral drugs (xenobiotics) and amino acids (structural components of biomolecules) are the challenging problems.

Content: Using model processess and physical methods to study the role of optical configuration in biological activity of chiral systems.

- The concept of chiral systems and their role in modern pharmacology and medicine.
- Model systems: donor-acceptor dyad's diastereomers containing L or D tryptophan (donor) and NSAID (acceptor) – (R/S) ketoprofen (KP);
- Model processes – photoinduced intramolecular transfer of an electron and energy, as well as a hydrogen atom.
- Research methods: NMR, spin effects (CIDNP and CIDEP), fluorescence spectroscopy and molecular modeling.
- Illustration of the developed approach application on the example of Alzheimer's disease.