

# Nonequilibrium Molecular Dynamics of Trp Zwitterion in Water: Picosecond Fluorescence Measurements vs. Computer Simulations

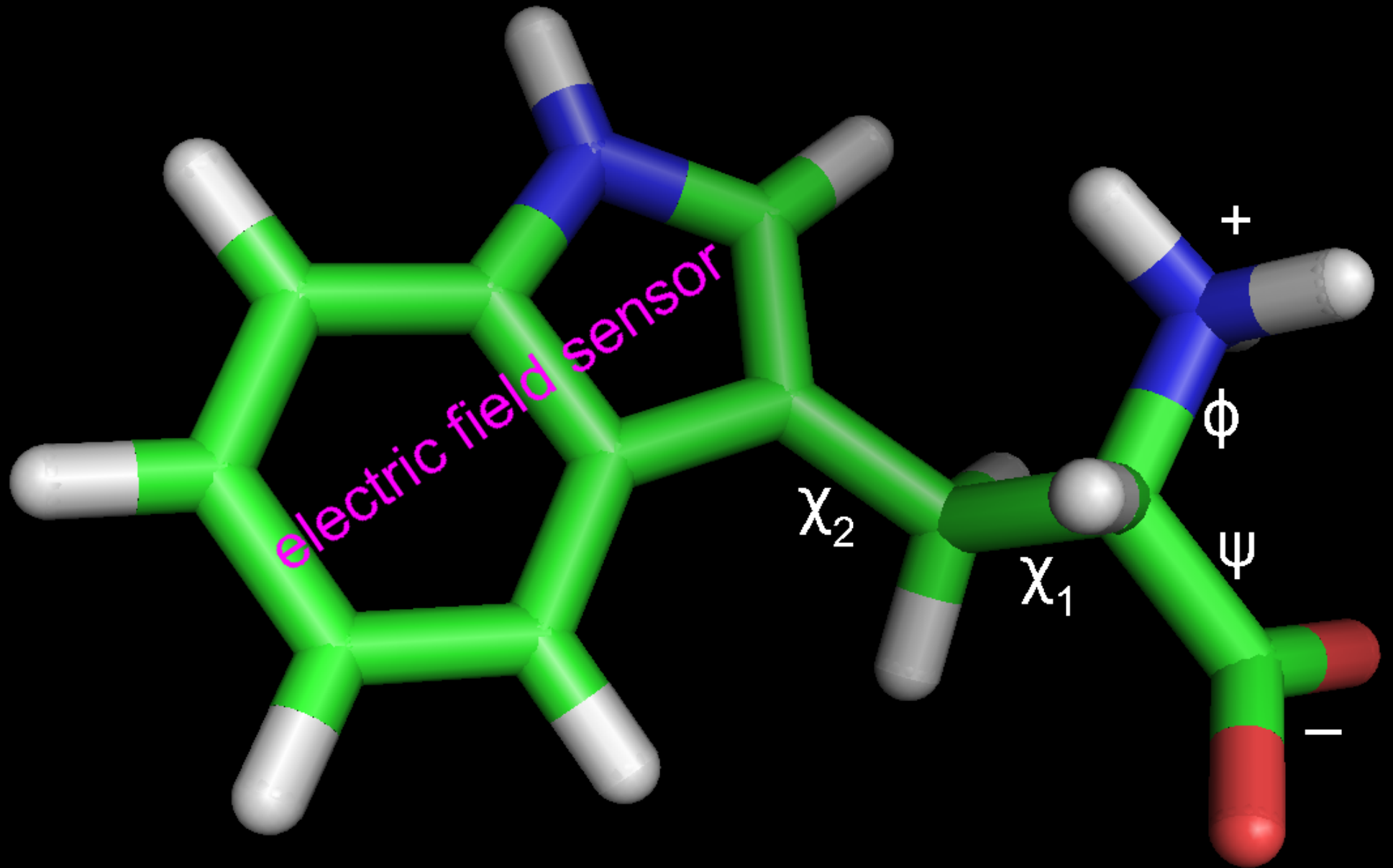
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- Molecular Dynamics (MD) simulations are widely used to understand protein function.
- Functionally significant protein motions, such as folding and unfolding, binding of enzymes to coenzymes and substrates, consist of a large number of elementary steps in protein conformational space.
- Each elementary step involves a change in the conformation of just one sidechain or in the backbone conformation.
- Although one elementary conformational step may have little functional significance, the rates of all functionally significant protein motions are completely determined by the rates of the underlying elementary steps.

- Elementary conformational changes: time scale from 1 ps to 1 ns, corresponding to the frequency range from 300 MHz to 300 GHz.
- The current generation of empirical forcefields was trained on infrared and Raman spectroscopy data, which cover the frequency range from 300 GHz to 400 THz. This range does not cover elementary conformational changes.
- The bandwidth of the signals recorded in NMR is less than 300MHz. This bandwidth is too low to track elementary conformational changes.
- The time-resolved fluorescence equipment in our lab has the bandwidth of about 10 GHz, which covers the lower part of the elementary conformation frequency range.

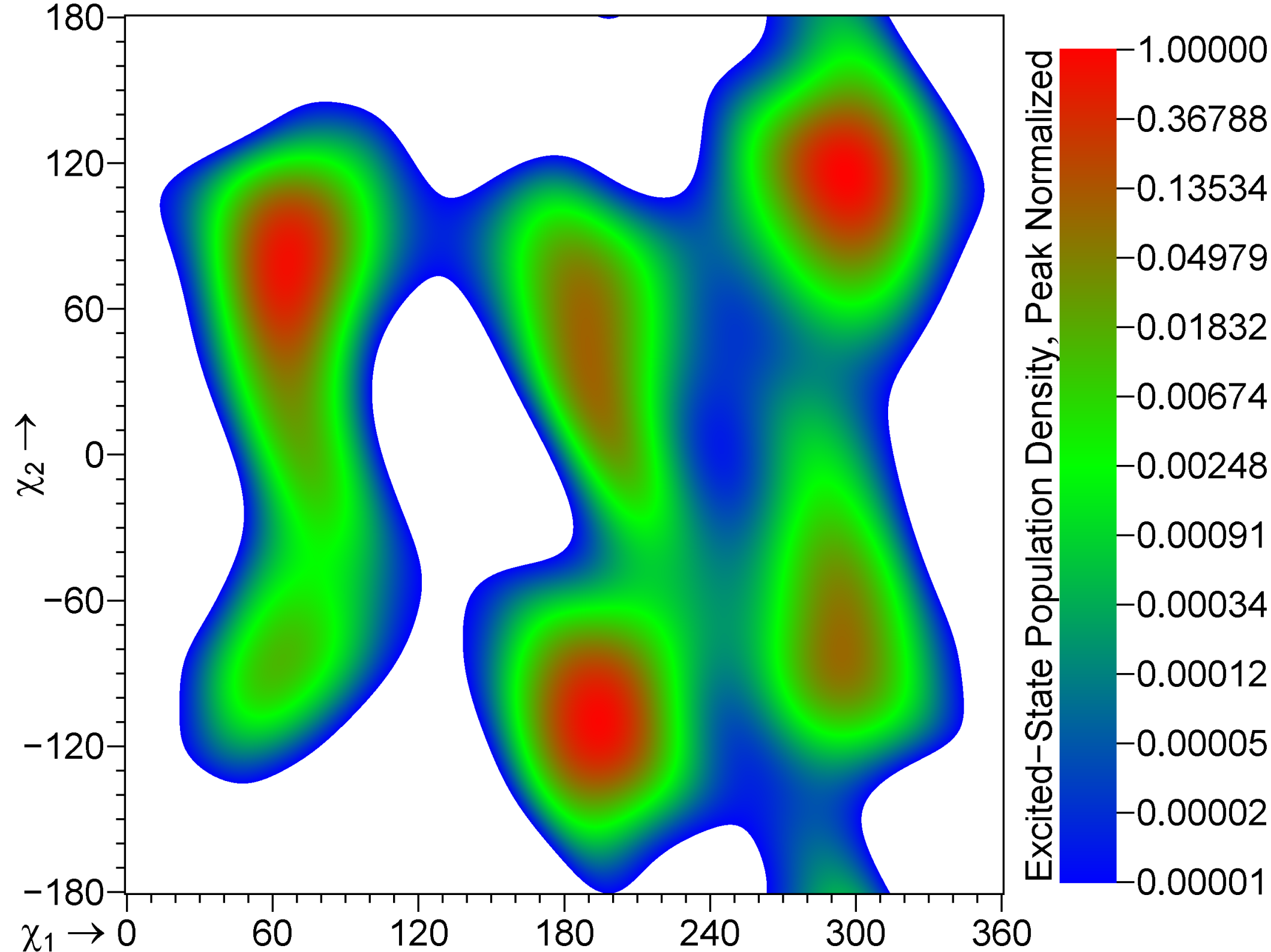
# The smallest model system: Trp zwitterion

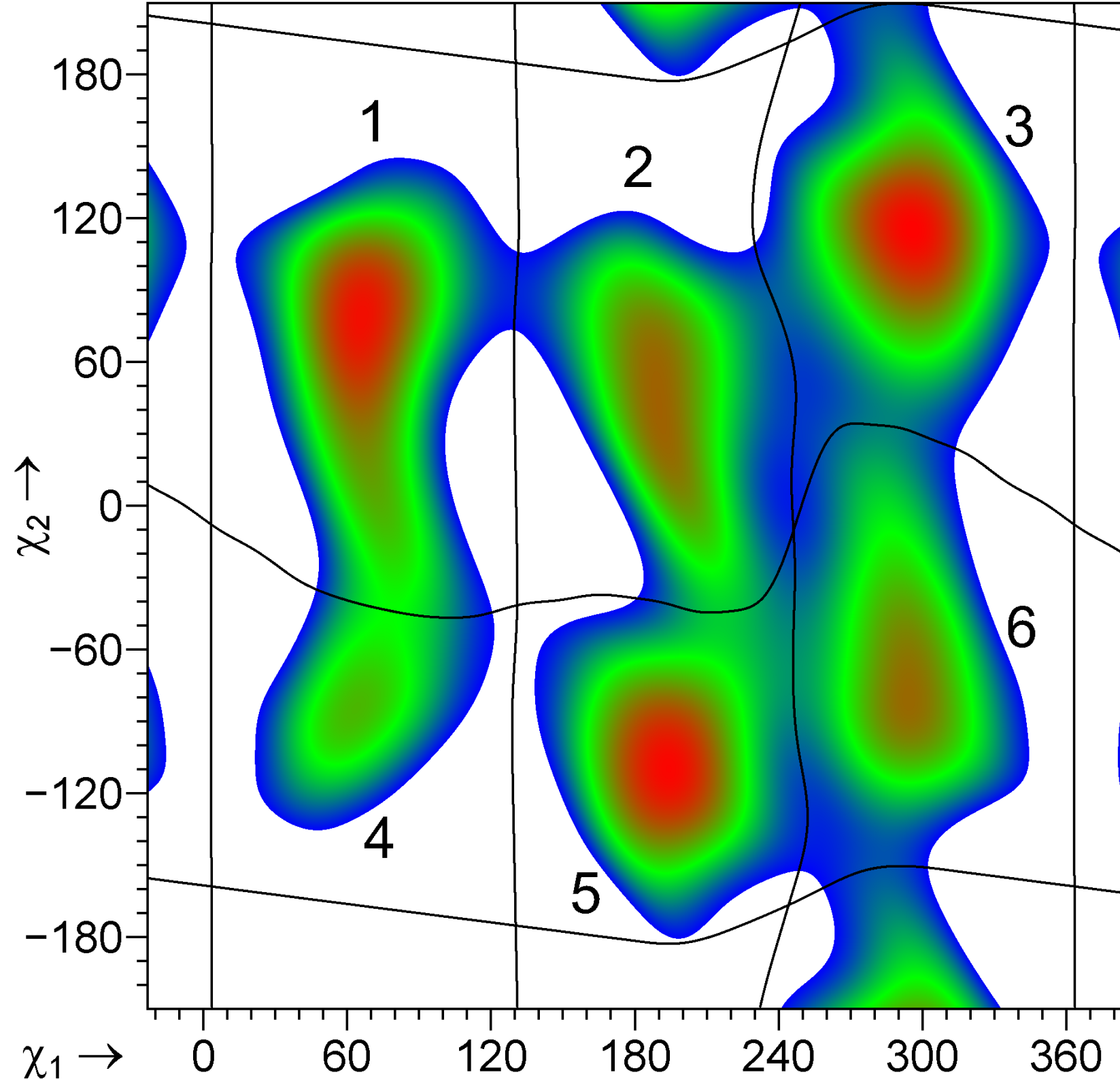


Rotation about the angle  $\psi$  results in the exchange of two identical oxygens with each other. Rotation about the angle  $\phi$  results in a cyclic permutation of three identical hydrogens. Rotations about the angles  $\chi_1$  and  $\chi_2$  may result in new stable conformations of the Trp zwitterion.

## MD SIMULATION - DETAILS

- CHARMM22 forcefield
- Trp atomic charges from *J. Phys. Chem. B* **2010**, *114*, 11323
- 496 explicit TIP3P water molecules
- 24.8Å×24.8Å×24.8Å box, periodic boundary conditions, T=278°K
- 150 trajectories starting from different values of  $\chi_1$  and  $\chi_2$
- each trajectory: 0.5ns Trp ground state + 10.0ns Trp excited state





- Population density distribution within the boundaries of one rotamer reaches equilibrium in less than 20 ps.
- Equilibration of population density between different rotamers requires more than 10 ns.
- At times  $> 20$  ps the conformational dynamics of Trp zwitterion can be described by the system of six linear differential equations:

$$\frac{dp_n}{dt} = \sum_{m \neq n} (C_{mn} p_m - C_{nm} p_n) - D_n p_n$$

$p_n$  - the population of the rotamer  $n$  ( $n=1,2,\dots,6$ )

$C_{mn}$  - the conversion rate from rotamer  $m$  to rotamer  $n$

$D_n$  - the sum of the radiative and nonradiative decay rate for the rotamer  $n$



Define the elements of a 6×6 matrix **A**

$$A_{nn} = D_n + \sum_{m \neq n} C_{nm}$$

$$A_{mn} = -C_{nm} \quad (m \neq n)$$

Numerically solve eigenvalue-eigenvector problem

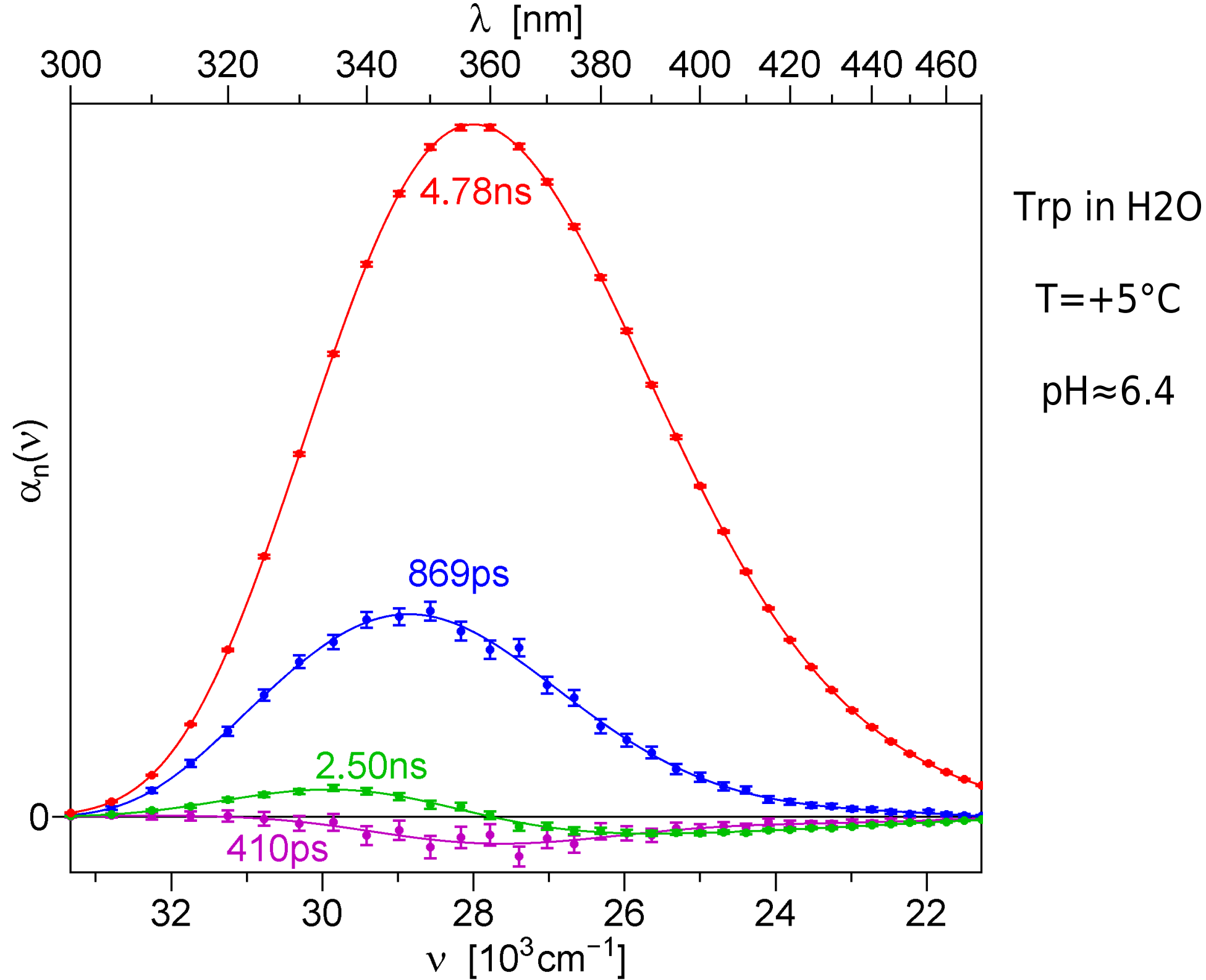
$$\mathbf{A} \mathbf{V}_k = \lambda_k \mathbf{V}_k$$

$$\mathbf{U}_k \mathbf{A} = \lambda_k \mathbf{U}_k$$

Populations are expressed in terms of the eigenvalues and eigenvectors:

$$p_n(t) = \sum_{l=1}^6 V_{nl} \alpha_l \exp(-\lambda_l t)$$

$$\alpha_k = \sum_{n=1}^6 U_{nk} p_n(0)$$



$$F(\nu, t) = \sum_{n=1}^6 s_n(\nu) p_n(t)$$

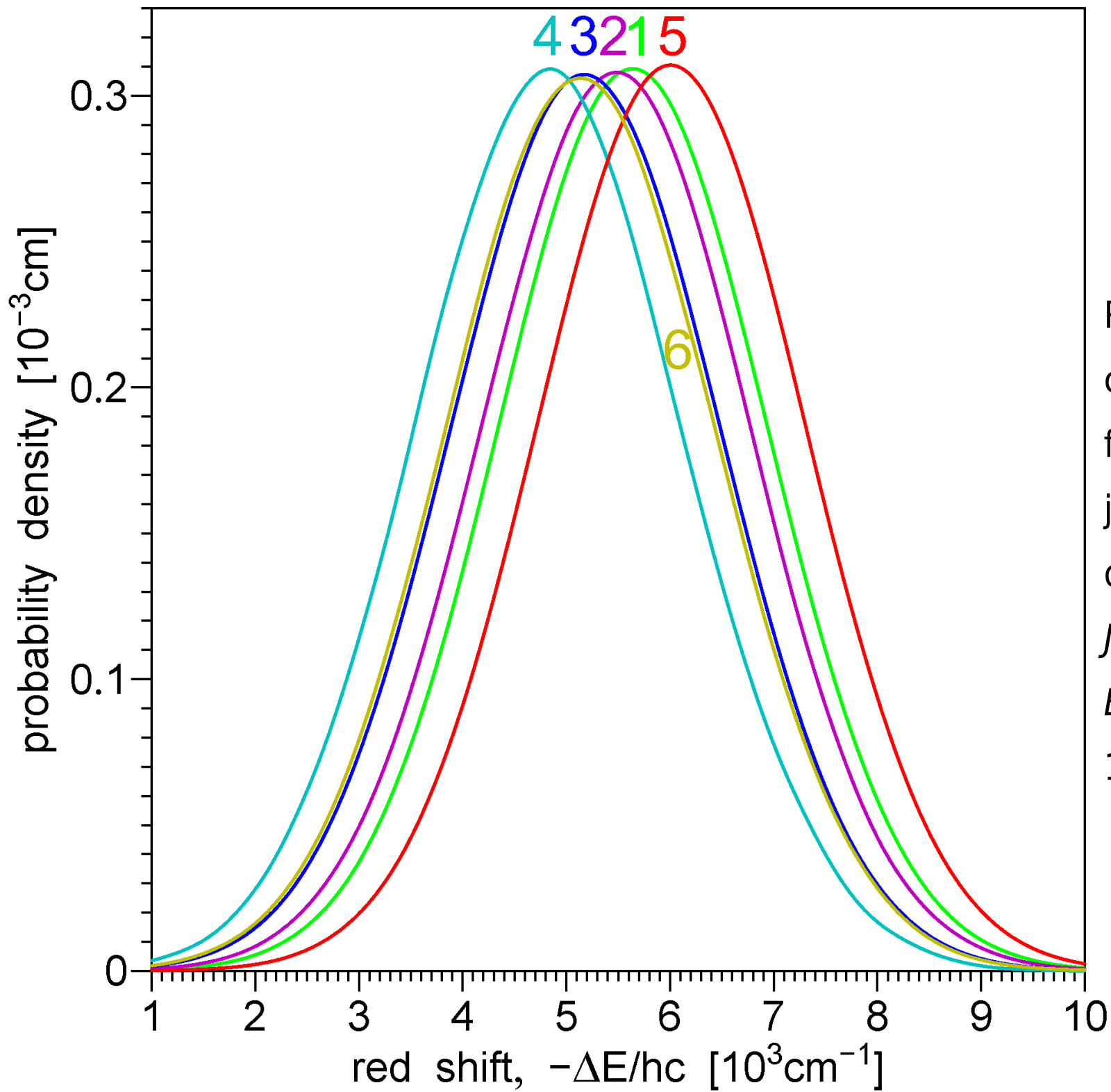
$$F(\nu, t) = \sum_{k=1}^6 \alpha_k f_k(\nu) \exp(-t/\tau_k)$$

Where

$$f_k(\nu) = \sum_{n=1}^6 V_{nk} s_n(\nu) \quad - \text{ amplitude spectra}$$

$$\tau_k = 1/\lambda_k \quad - \text{ "lifetimes" or (better) taus}$$

Goal: match computed and experimental taus and amplitude spectra



Red shift was calculated from MD trajectories as described in *J. Phys. Chem. B* **2010**, *114*, 11323

$$\frac{dp_n}{dt} = \sum_{m \neq n} (C_{mn} p_m - C_{nm} p_n) - D_n p_n$$

$C_{mn}$  - the conversion rate from rotamer  $m$  to rotamer  $n$

$$C_{mn} = \frac{\text{number of } m \rightarrow n \text{ border crossings by trajectories}}{\text{total time spent as rotamer } m}$$

$C_{mn}$  in  $10^9 \text{ s}^{-1}$  units

	n=1	n=2	n=3	n=4	n=5	n=6
m=1	-----	0.0142	0.00000	0.5727	0.00095	0.00000
m=2	0.0638	-----	0.1276	0.00000	1.0420	0.2977
m=3	0.00000	0.0472	-----	0.00000	0.0171	0.0750
m=4	30.3464	0.00000	0.00000	-----	0.7462	0.00000
m=5	0.0045	0.2130	0.0067	0.00000	-----	0.0919
m=6	0.00000	0.4929	0.4452	0.00000	1.1131	-----

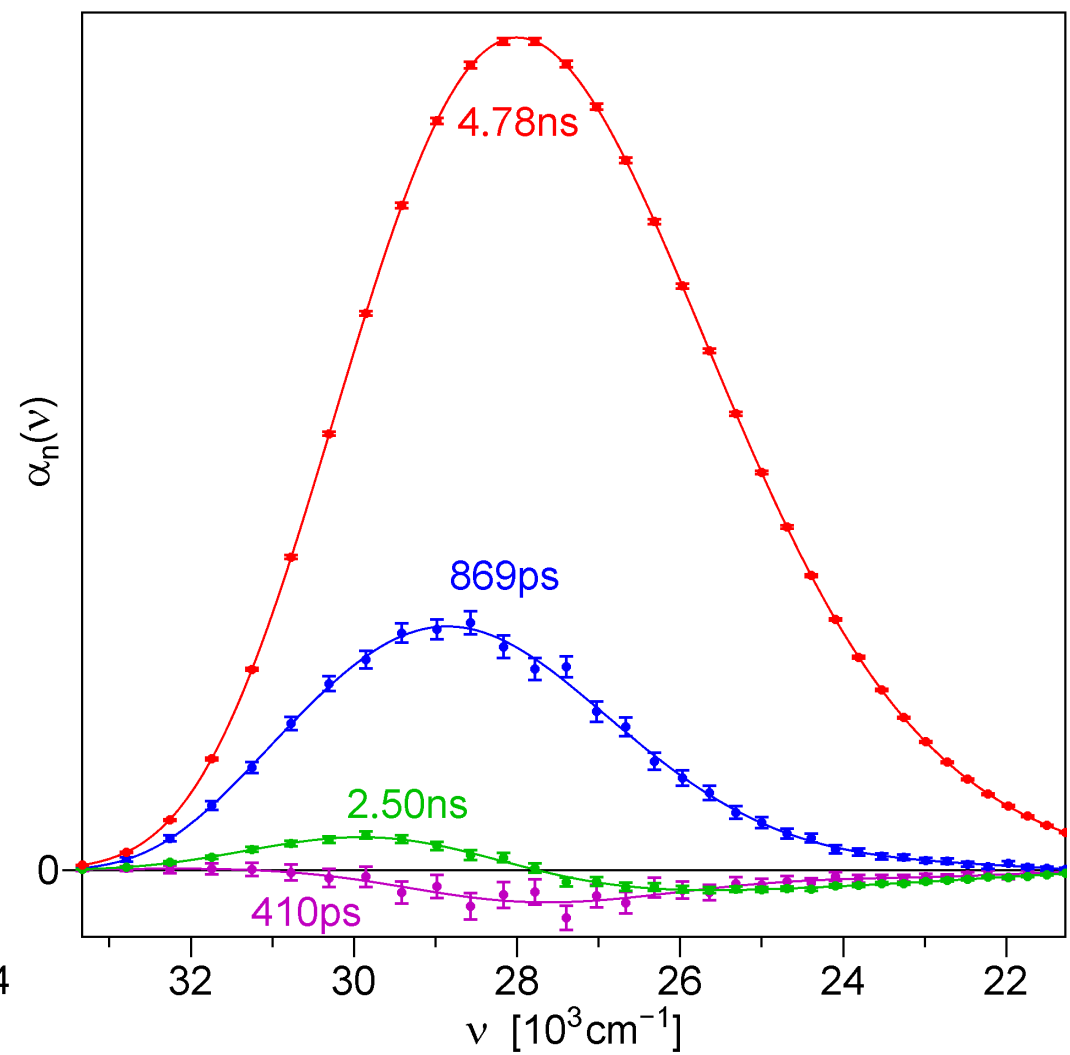
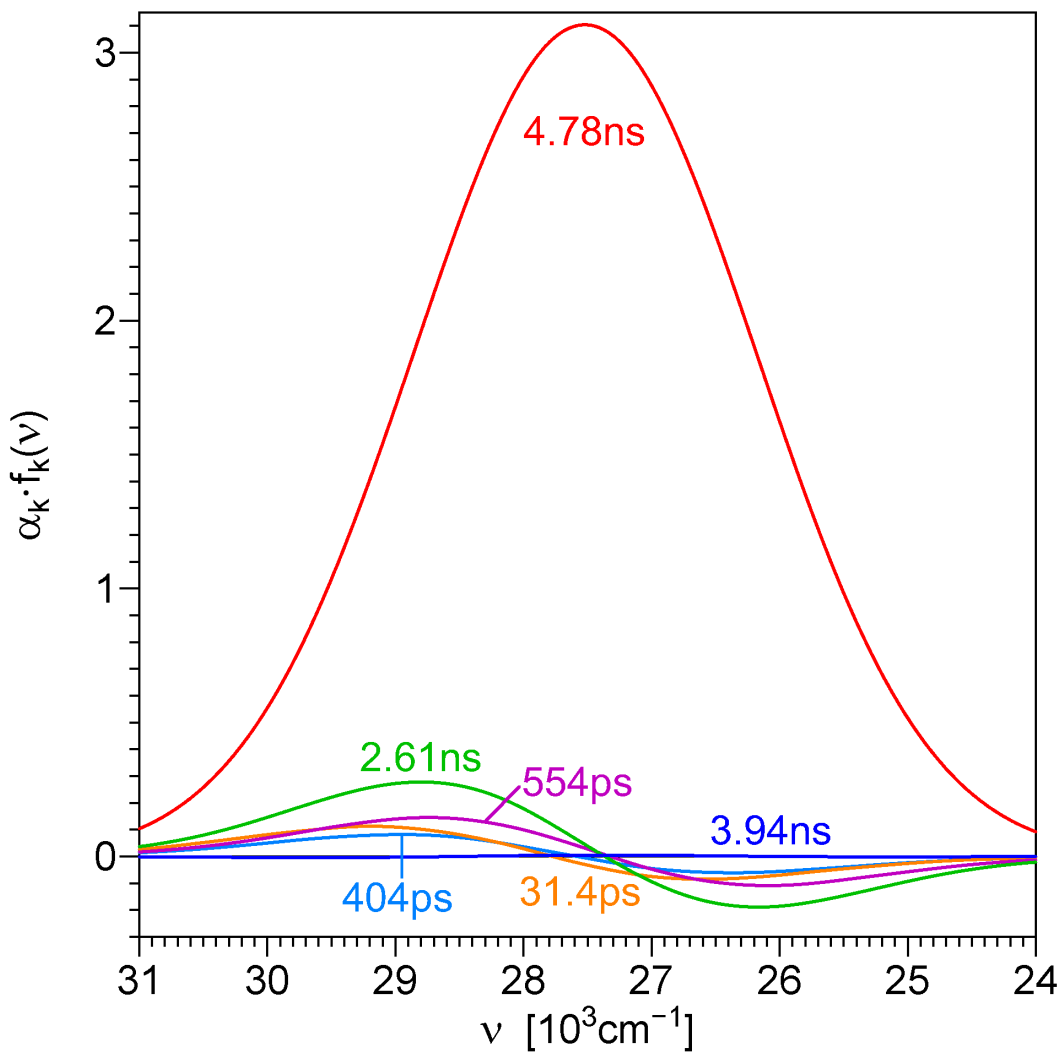
$$\frac{dp_n}{dt} = \sum_{m \neq n} (C_{mn} p_m - C_{nm} p_n) - D_n p_n$$

$D_n$  - the sum of the radiative and nonradiative decay rate for the rotamer  $n$

- Radiative decay rate was calculated individually for each rotamer from the shape of its emission spectrum, the magnitude of the transition dipole moment, the solvent refractive index, etc., as described in *J. Fluorescence* **2003**, 13, 201. Blue-shifted rotamers have slightly higher radiative decay rates than the red-shifted ones.
- Nonradiative decay rate was first assumed to be equal for all 6 rotamers. The value of the nonradiative decay rate was selected so that the tau of the dominant component obtained by solving the rate equations would equal the tau of the dominant component in the analysis of experimental data.

$$Q_1 = Q_2 = Q_3 = Q_4 = Q_5 = Q_6 = 0.149 \cdot 10^9 \text{ s}^{-1}$$

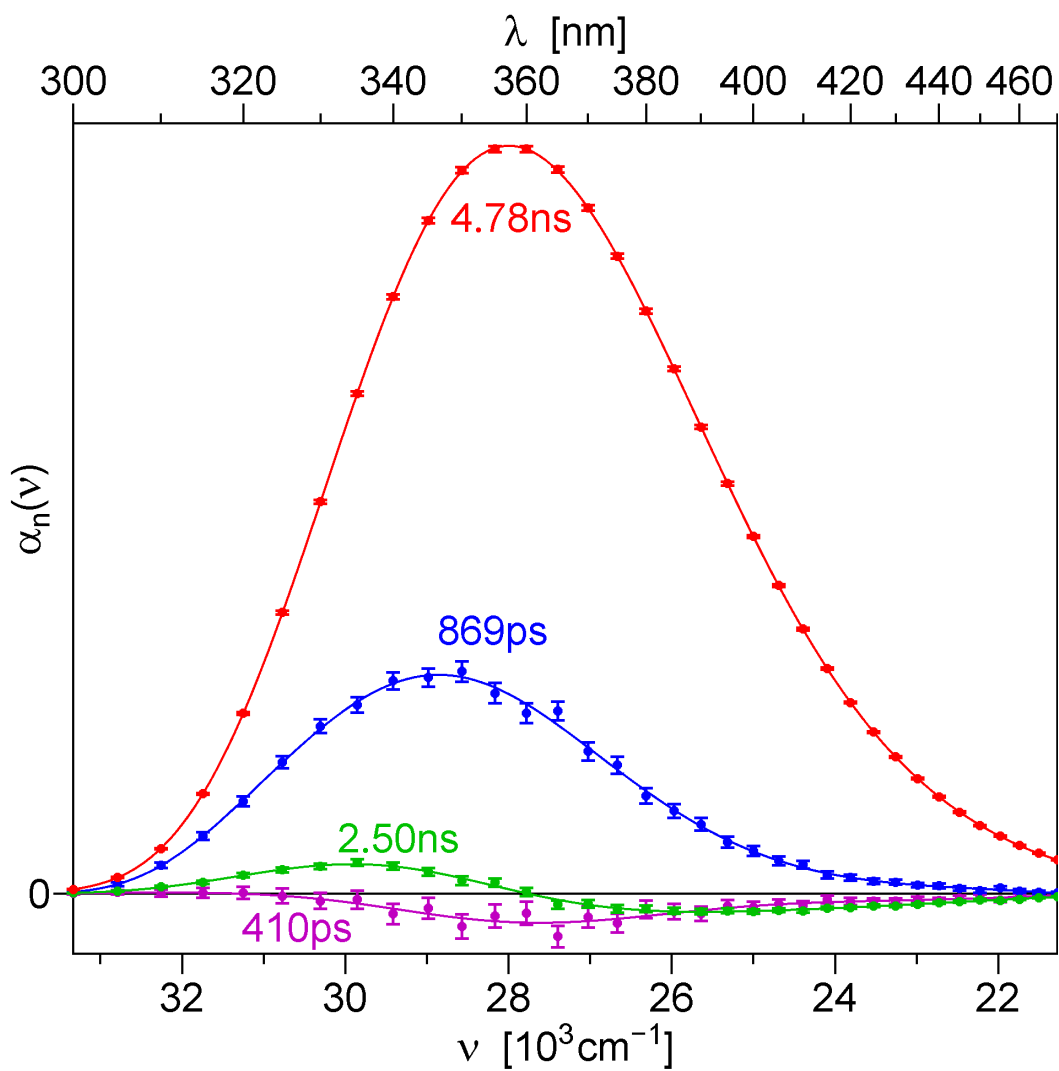
Experiment



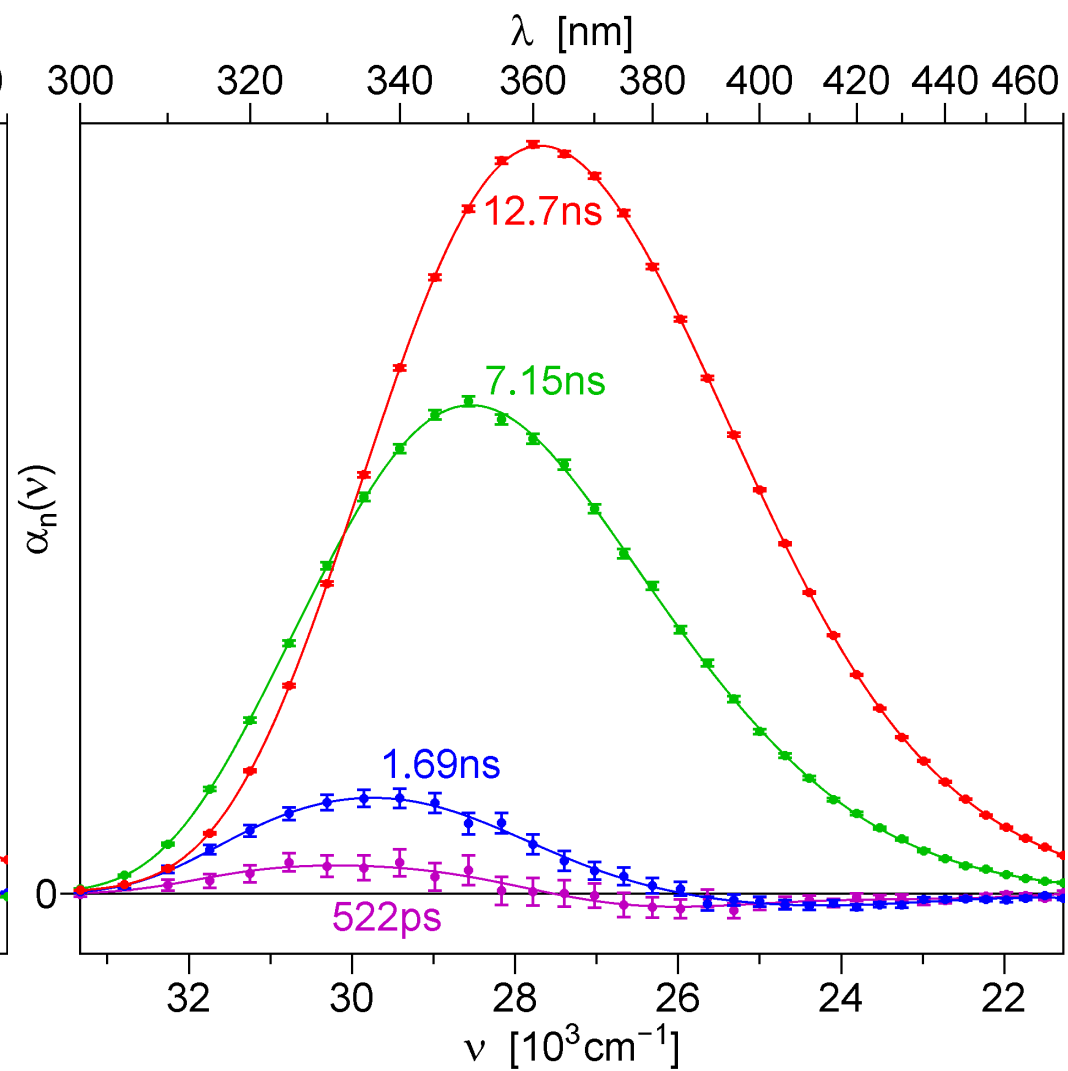
- Under the assumption that the nonradiative decay rate is equal for all 6 rotamers there is no agreement between the theoretical and experimental taus and amplitude spectra.
- The difference in the nonradiative decay rate between the rotamers is likely due to the proton transfer from the amino group to carbon four of the indole ring (atom CE3 in CHARMM nomenclature). This mechanism has been described in *J. Am. Chem. Soc.* **2002**, 124, 6398.
- More than two-fold difference between the taus experimentally measured in H<sub>2</sub>O and in D<sub>2</sub>O serves as reliable evidence of proton transfer (next slide) →

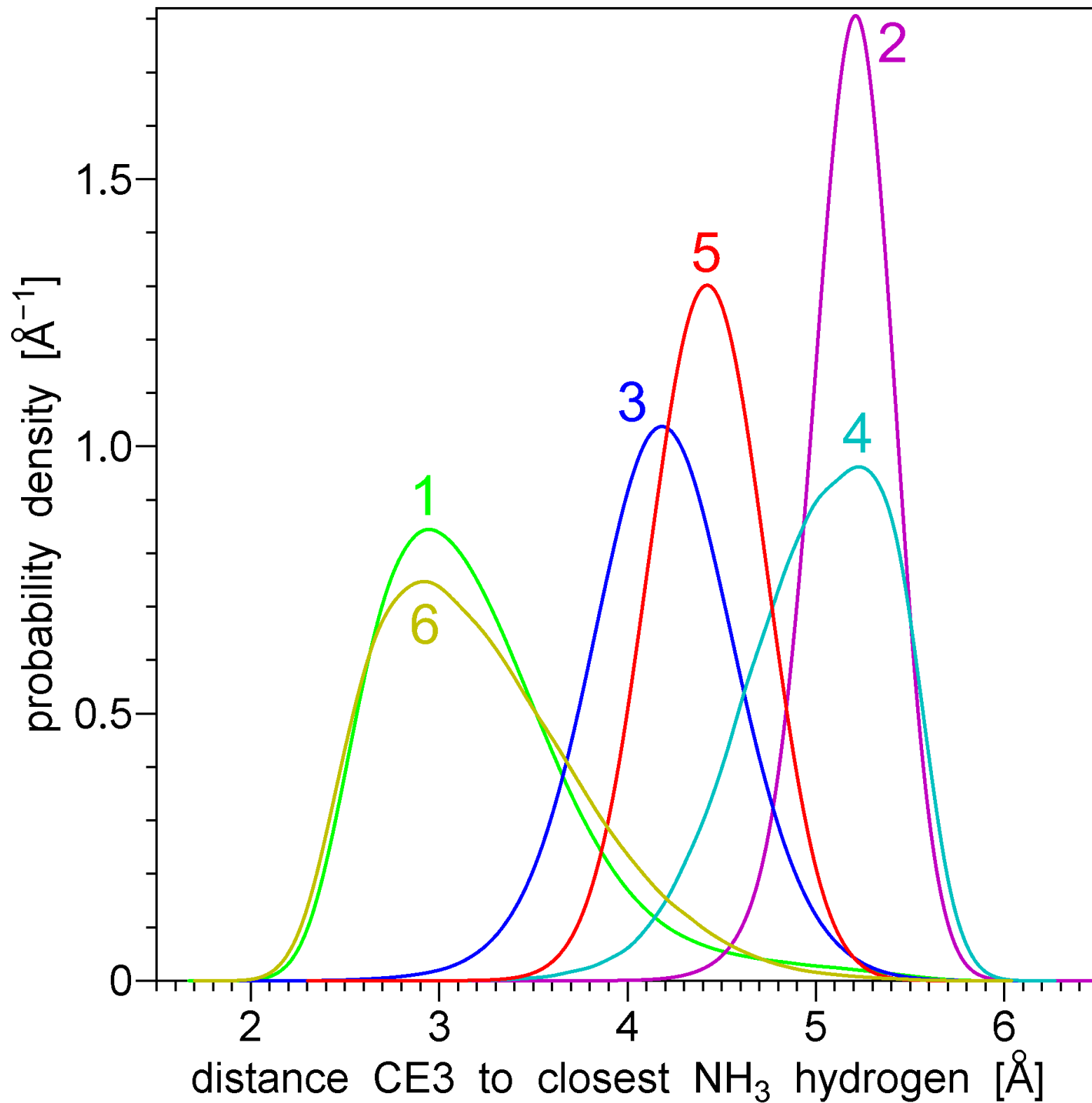


Trp in H2O T=+5°C pH≈6.4



Trp in D2O T=+5°C pD≈6.4

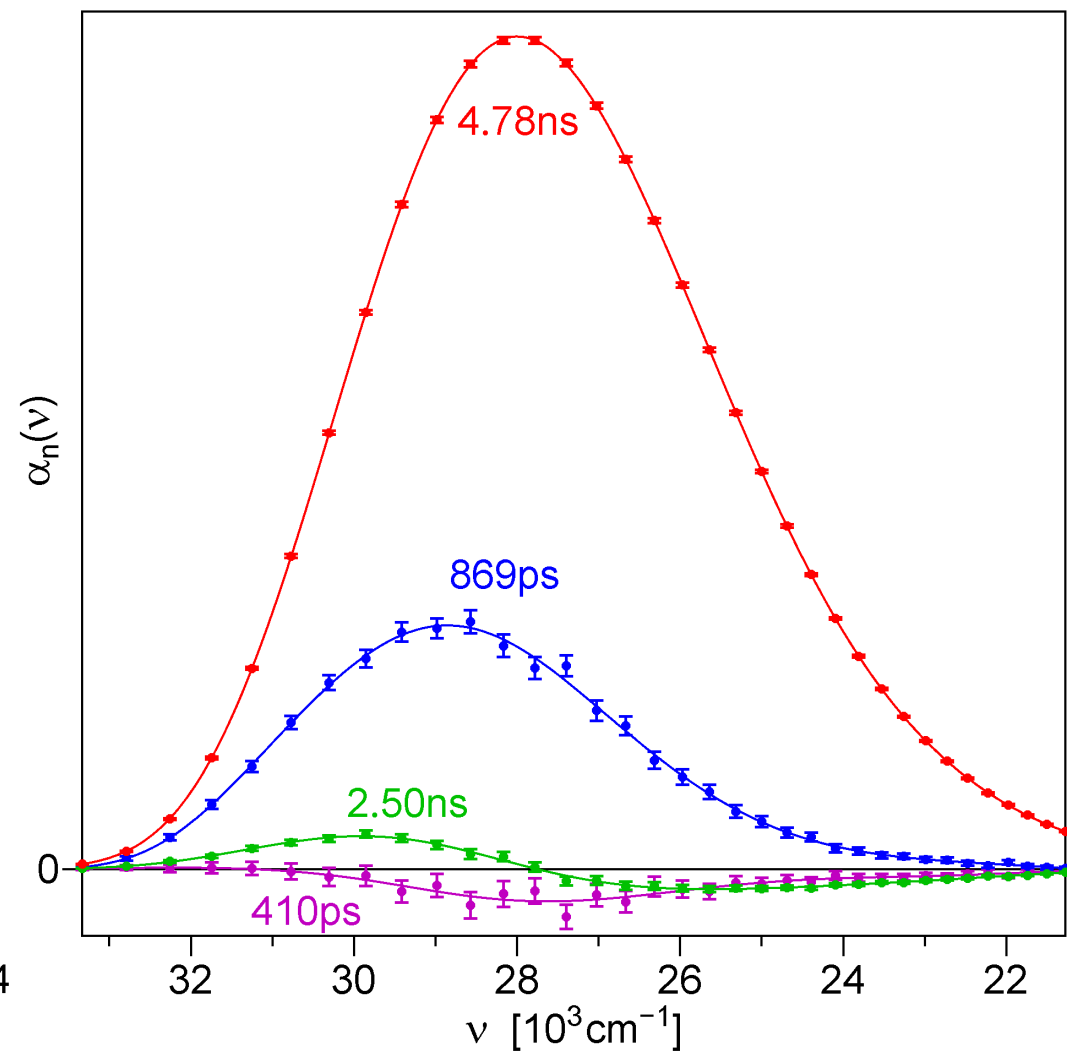
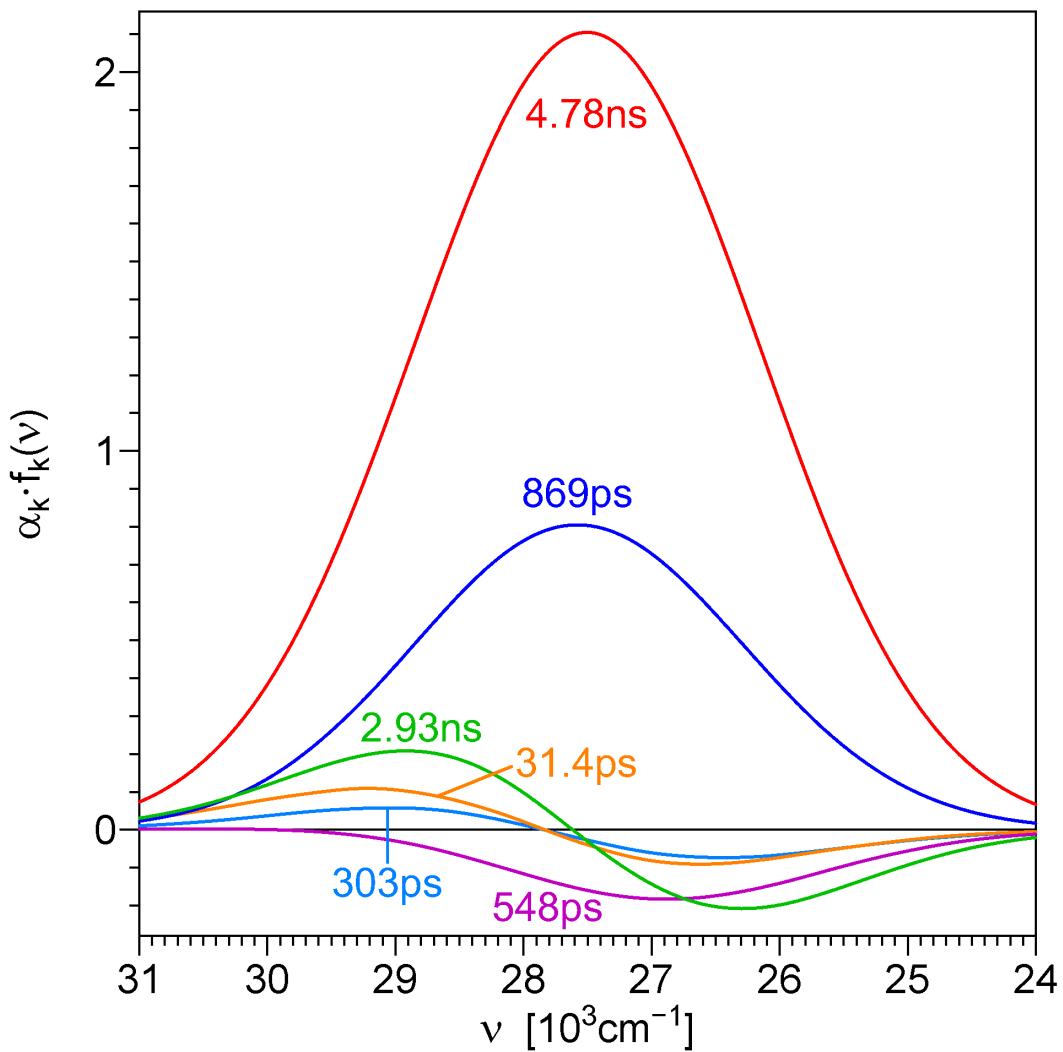




- Proton transfer rate falls exponentially with the distance.
- The rate is significant at 2Å distance and falls to zero at 3Å distance.
- Only rotamers 1 and 6 allow distances short enough for proton transfer.
- We assume that there is one nonradiative decay rate for the rotamers 1 & 6 and the second (smaller) nonradiative decay rate for the other rotamers.
- The values of these two rates were selected so that the taus of the two dominant components obtained by solving the rate equations would equal the taus of the dominant components in the analysis of experimental data.

$$Q_1 = Q_6 = 1.071 \cdot 10^9 \text{ s}^{-1}$$
$$Q_2 = Q_3 = Q_4 = Q_5 = 0.106 \cdot 10^9 \text{ s}^{-1}$$

Experiment



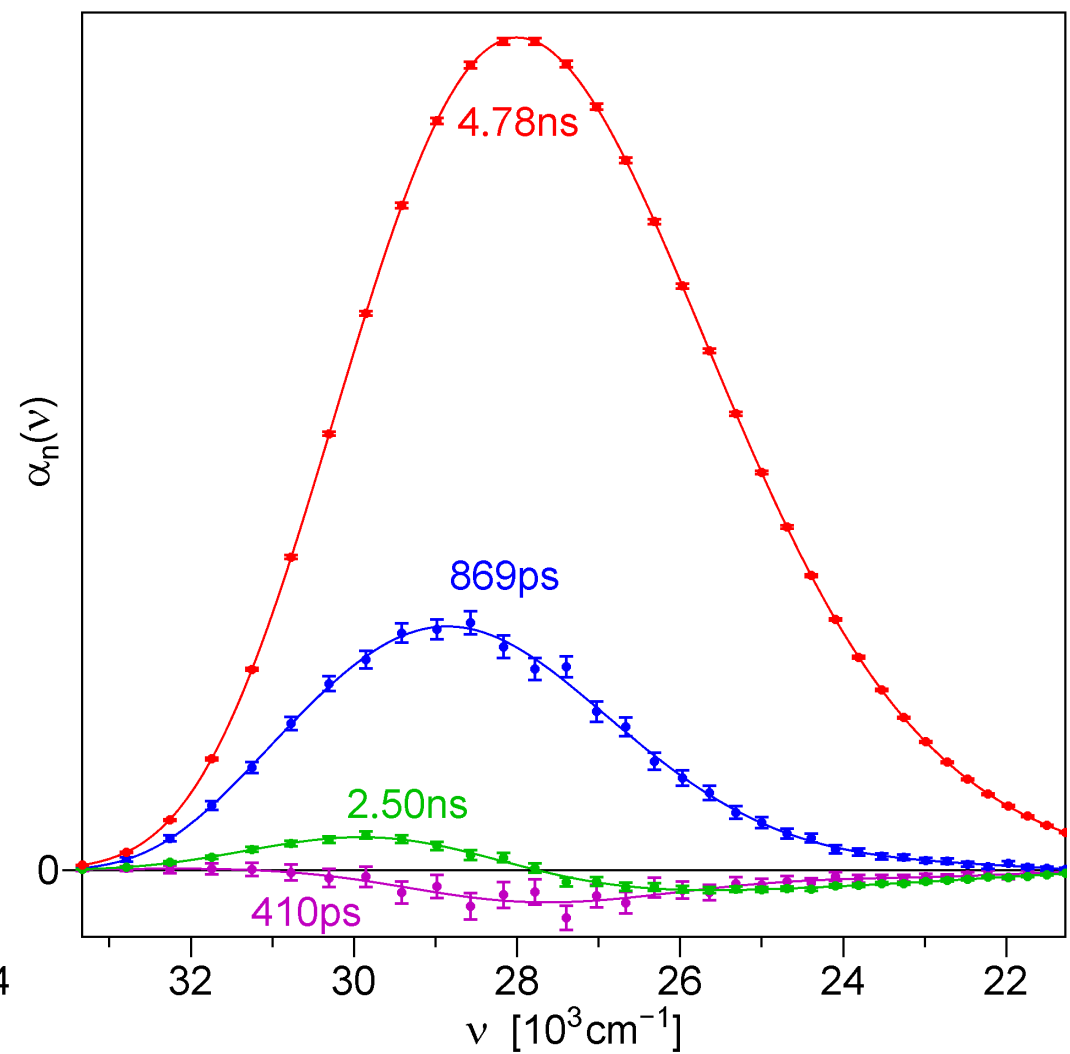
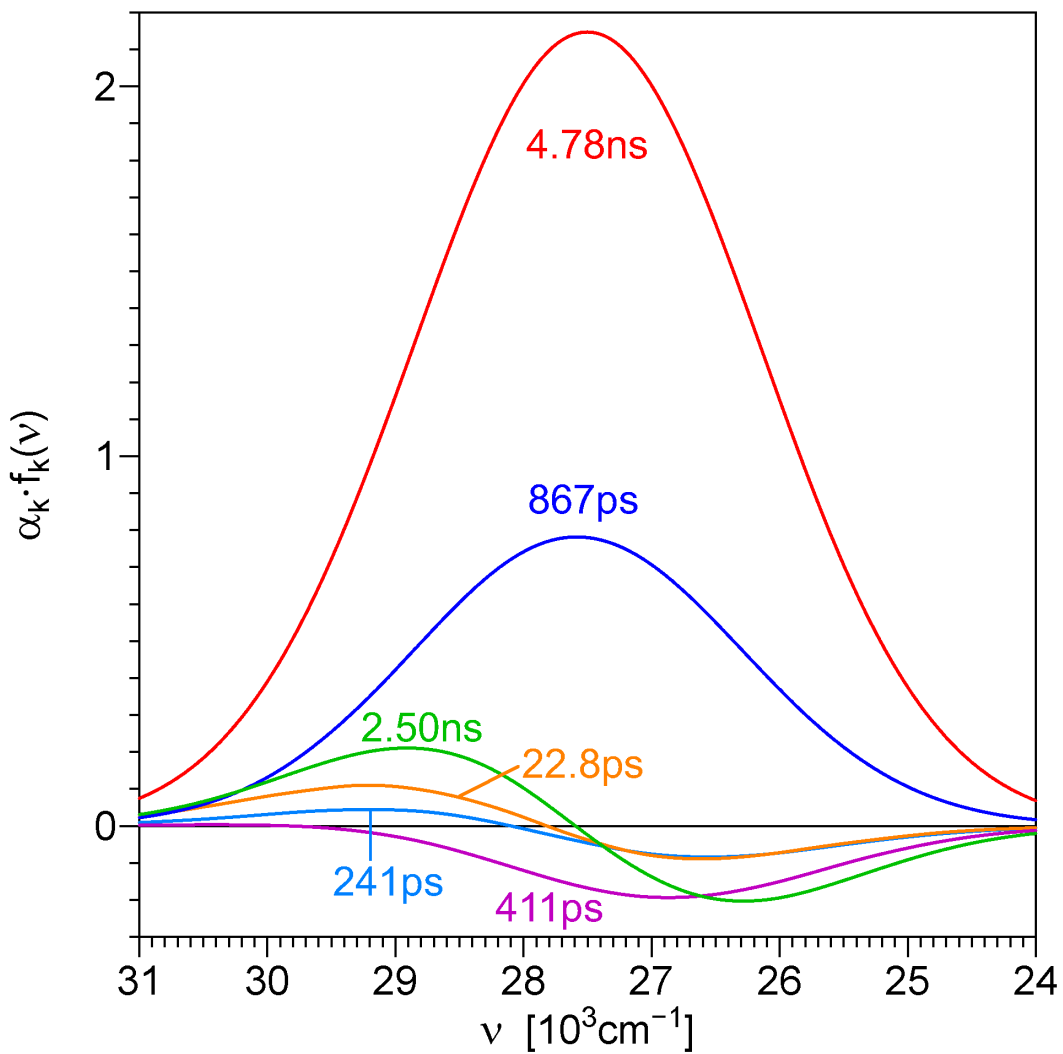
- Now we have good agreement between the theoretical and experimental shapes of all four amplitude spectra.
- The tau values of 4.78ns ( $\tau_1$ ) and 869ps ( $\tau_3$ ) are the same in theory and in the experiment.
- The  $\tau_2$  value of 2.93ns (theory) is greater than 2.50ns (experiment) and the  $\tau_4$  value of 548ps (theory) is greater than 410ps (experiment).
- The theoretical and experimental values of  $\tau_2$  and  $\tau_4$  cannot be made equal by changing the decay rates only.
- The next step is to scale all conversion rates  $C_{mn}$  by one scale factor and adjust the value of this factor.

$$Q_1 = Q_6 = 1.060 \cdot 10^9 \text{s}^{-1}$$

$$Q_2 = Q_3 = Q_4 = Q_5 = 0.100 \cdot 10^9 \text{s}^{-1}$$

$$C_{mn} \rightarrow 1.38 \cdot C_{mn}$$

Experiment



- Now we have a good agreement between the theoretical and experimental shapes of all four amplitude spectra and between the theoretical and experimental values of  $\tau_1$ ,  $\tau_2$ ,  $\tau_3$  and  $\tau_4$ .
- $\tau_6=22.8\text{ps}$  is too fast for our experimental time resolution ( $\sim 65\text{ps}$ ).
- $\tau_5=241\text{ps}$  is difficult to resolve because its amplitude spectrum is too weak as compared to the other four amplitude spectra.
- Elementary conformational changes of Trp sidechain are 1.38-fold faster in the experiment as compared to MD simulations.
- In accordance with the Arrhenius equation, to reach an agreement between MD and experiment it is necessary to lower all potential barriers between Trp sidechain rotamers by  $\ln(1.38) \cdot k_B T$ , which equals  $0.178\text{kcal/mol}$ .

# CONCLUSIONS

- Time-resolved fluorescence emission of Trp zwitterion in water is accurately described by the model of six discrete rotamers with conversion rates of the same order of magnitude as their decay rates.
- Population decay of each rotamer is a linear combination of six exponential terms  $\exp(-t/\tau_n)$ . The six time constants  $\tau_n$  are not lifetimes and do not correspond to individual rotamers, as it was earlier postulated.
- To reach an agreement between MD simulations using CHARMM22 forcefield and experimental data it is necessary to lower the torsional parameters for  $\chi_1$  and  $\chi_2$  angles of Trp by 0.178kcal/mol.
- Picosecond-resolution fluorescence spectroscopy can be used as a method to fine-tune forcefield parameters.



## ACKNOWLEDGEMENTS

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