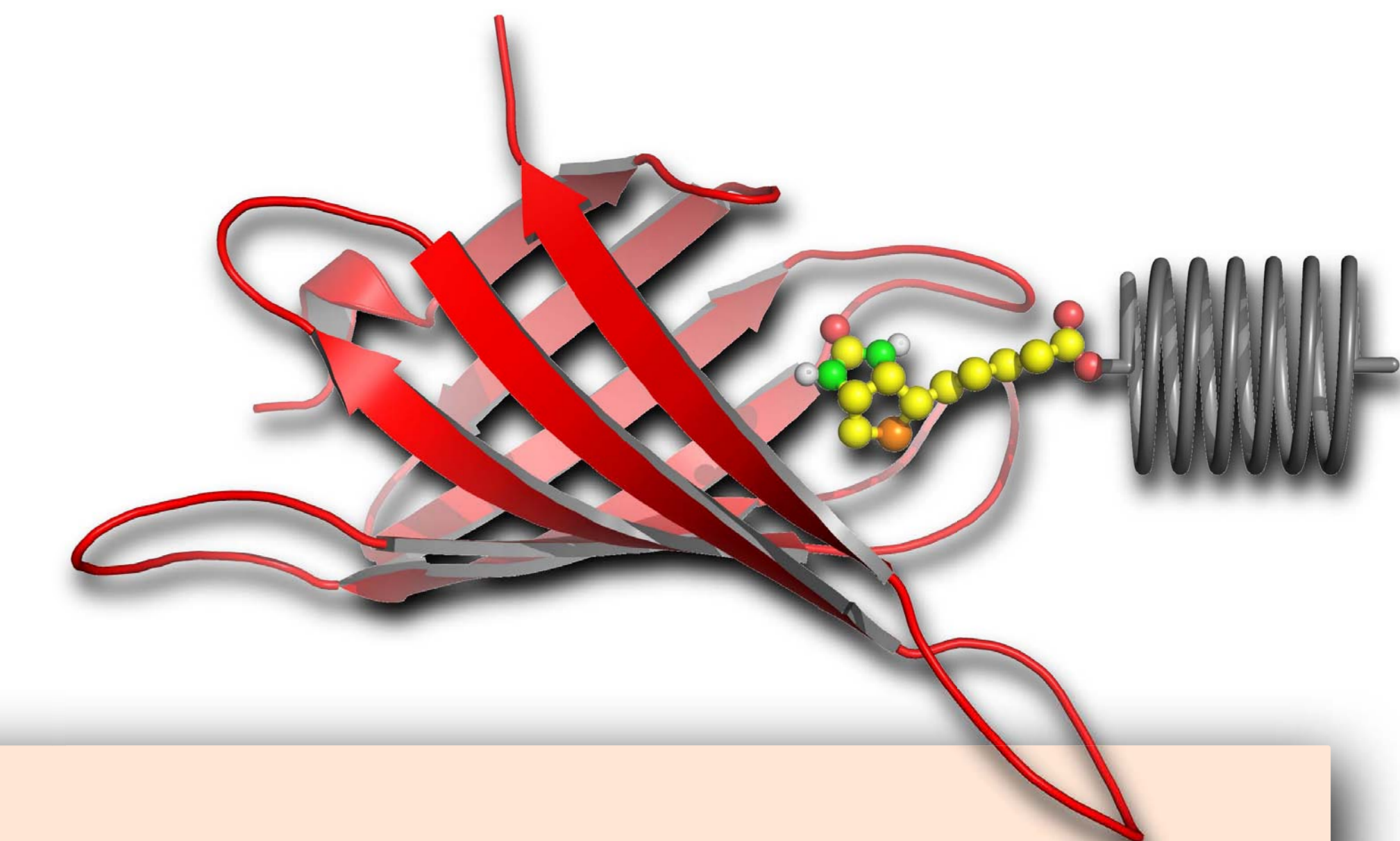




MAX-PLANCK-GESELLSCHAFT

# Distance Reconstruction in smFRET Experiments through Combination with Dye Dynamics from MD Simulations.

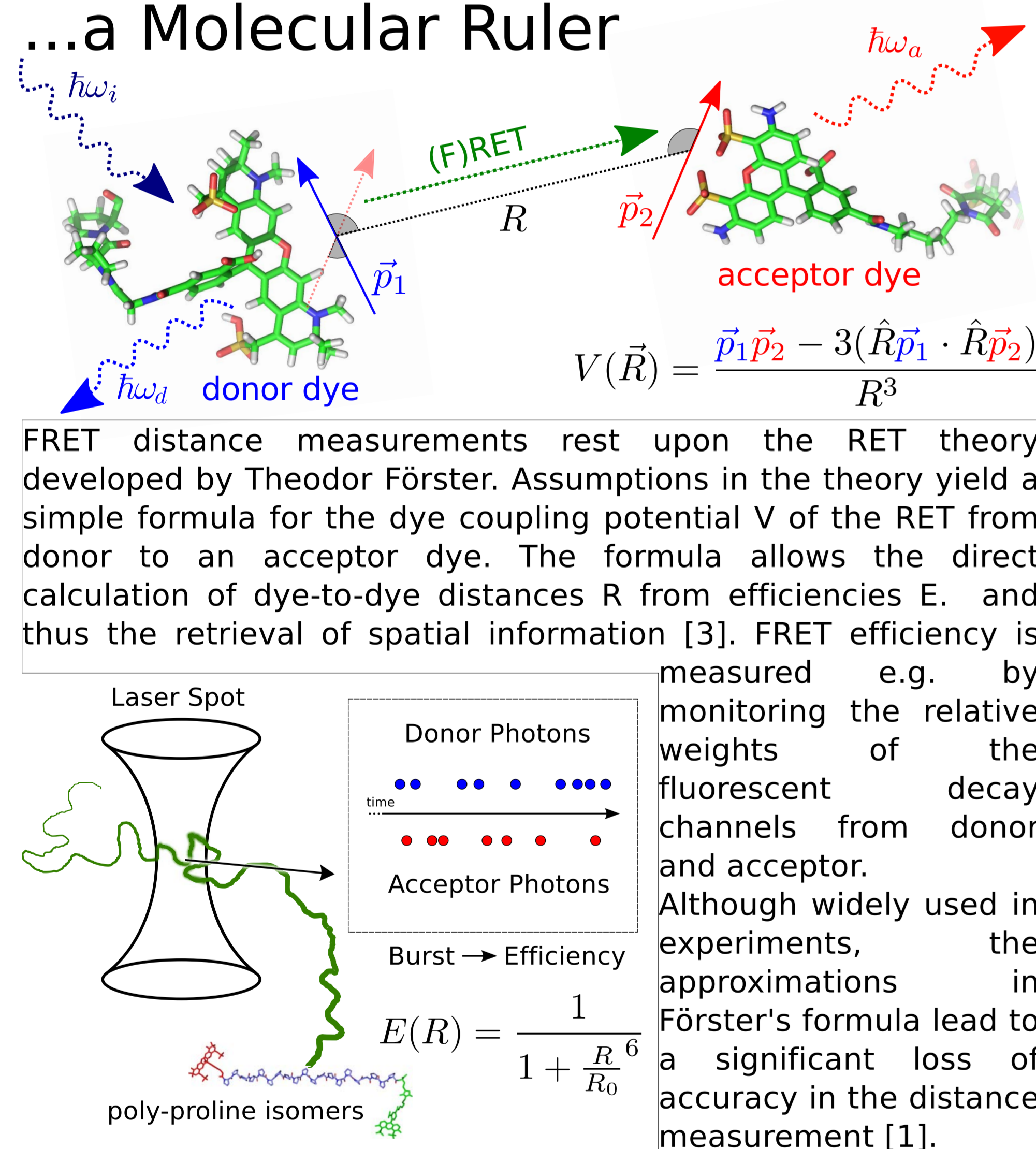
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## Abstract

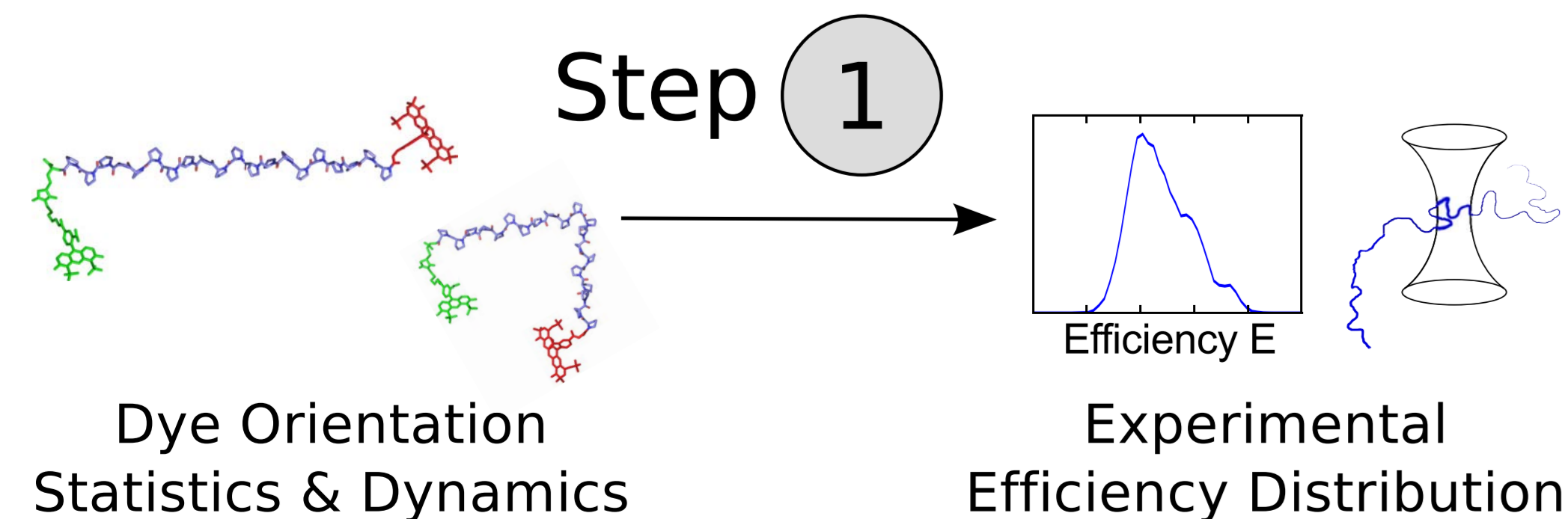
Fluorescence resonance energy transfer (FRET) experiments probe molecular distances via the efficiency of energy transfer from an excited donor dye to its acceptor counterpart. To study flexibilities and structural changes in bio-molecules, single molecule FRET settings allow probing distance distributions and fluctuations. However, the measured energy transfer efficiency depends not only on the distance between the two dyes, but also on their **mutual orientation**, which is typically inaccessible to experiments. Thus, assumptions on the orientation distributions have to be made such as an isotropic orientation distribution. These **approximations severely limit the accuracy** of the distance distributions extracted from FRET experiments alone. Here we show that estimates of the distance distributions can be improved considerably by combining measured efficiency distributions with dye orientation statistics obtained from molecular dynamics (MD) simulations. Quantitative agreement of calculated efficiency distributions from simulations with the experimental efficiency distributions was obtained for poly-proline chains with Alexa488 and Alexa594 dyes attached. The experimentally observed heterogeneity was related to the presence of cis isomers and previously undetected distinct dye conformers. This agreement of our in silico FRET approach with experiments substantiates using the obtained dye orientation dynamics from MD simulations in the distance reconstruction. We propose a transfer function based approach and show that the poly-proline all-trans peak location in the reconstructed distances deviates by only 0.35 Å from the true peak location in contrast to a 6.2 Å deviation when employing the isotropic approximation of the dye orientation.

## Single Molecule FRET... ...a Molecular Ruler



## Idea: Combination of Simulation and Experiment

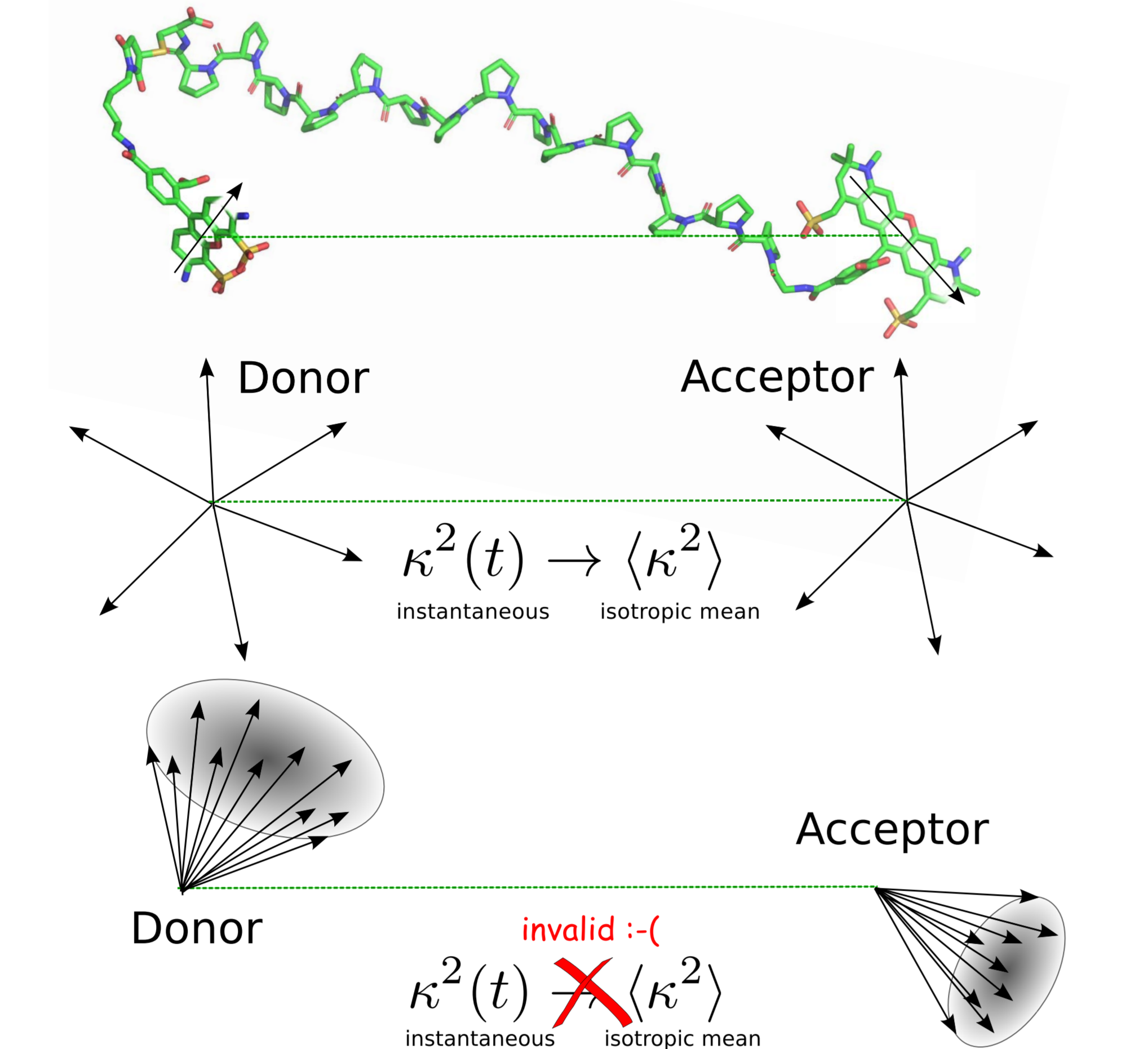
To overcome the  $\kappa^2$ -problem, we have combined experiments with MD simulations. Dye orientation statistics and dynamics from MD serves to calculate the orientation factor  $\kappa^2$  in the Förster formula for  $R_0$ . Moreover, distributions of  $\kappa^2$  from simulations are employed replacing a mean orientation factor in  $R_0$ .



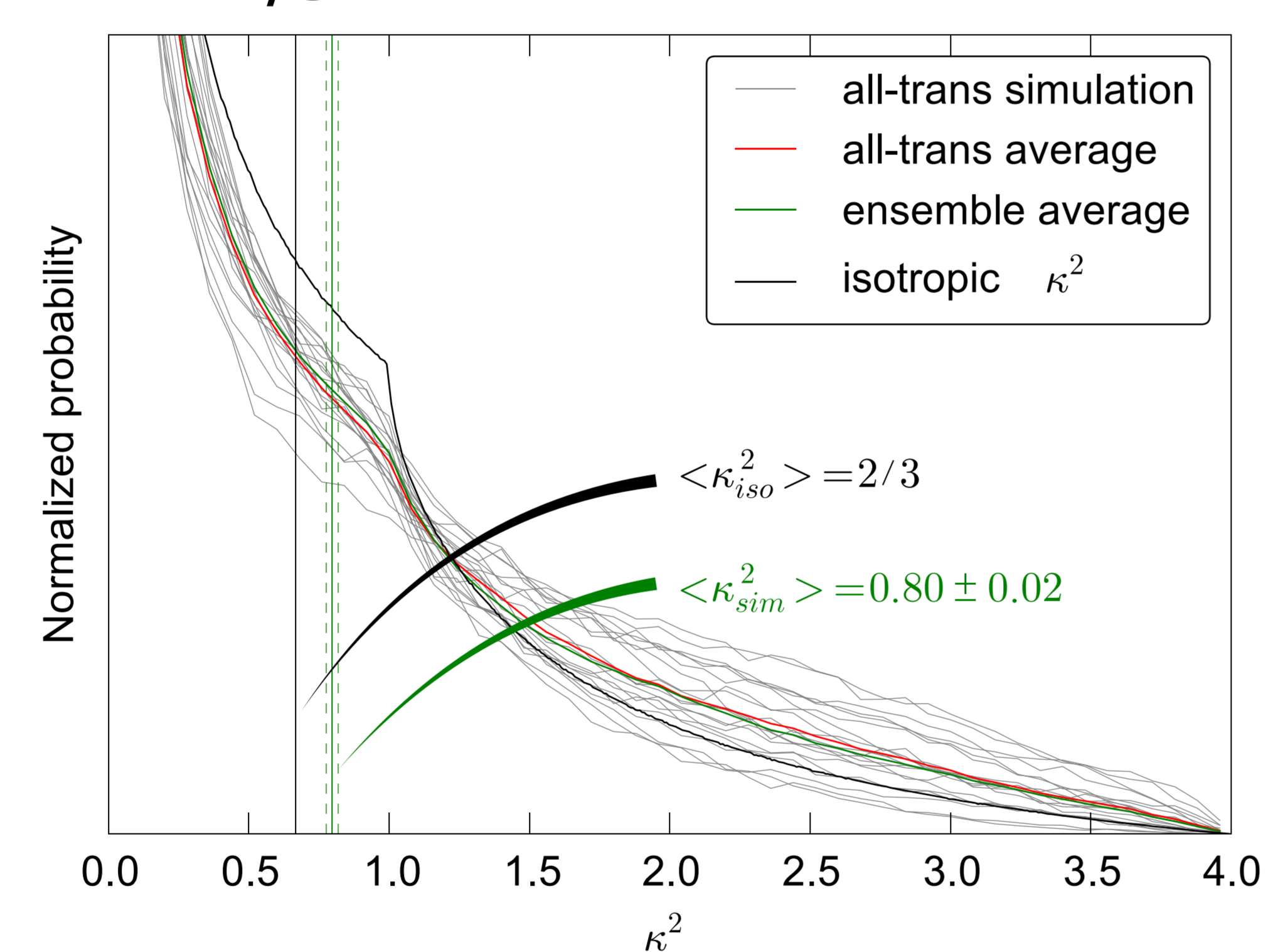
Do simulation ensembles accurately describe the experiment? To answer this question, we developed a method to simulate smFRET allowing the comparison of efficiency distributions with experiments.

## The $\kappa^2$ Problem

The pivotal assumption in Förster's RET theory is that dye orientations of donor and acceptor are isotropic and mutually uncorrelated leading to an orientation factor  $\kappa^2$  is 2/3. However, **anisotropies or mutual correlation** of the dye orientation are often **non-negligible** due to the interaction of dyes and their linkers with bio-molecular surfaces and their spatially restricted orientation space. In those cases, the above approximation of  $\kappa^2$  reduces the accuracy of calculated distances from FRET efficiency measurements.

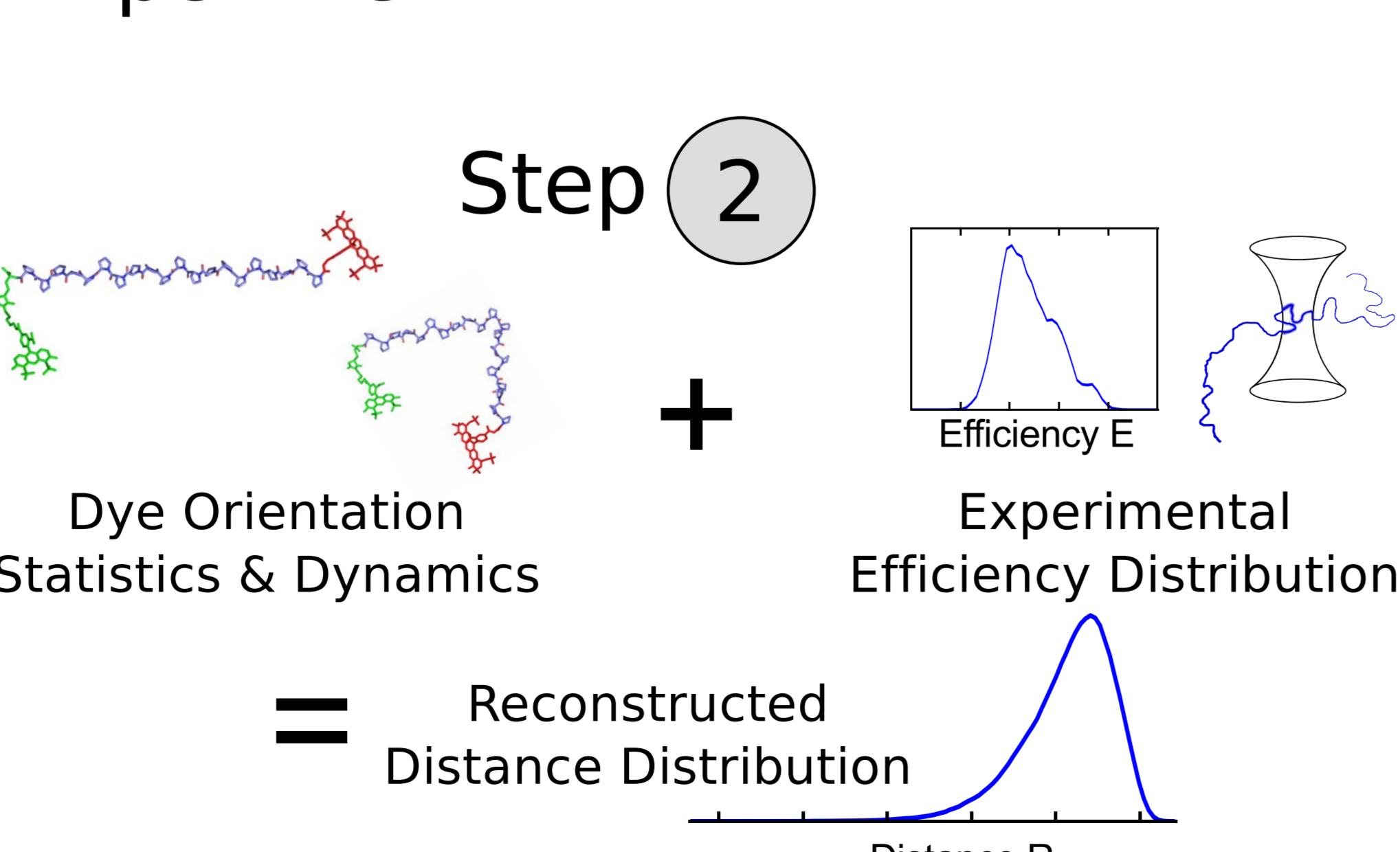


## $\kappa^2 \neq 2/3$



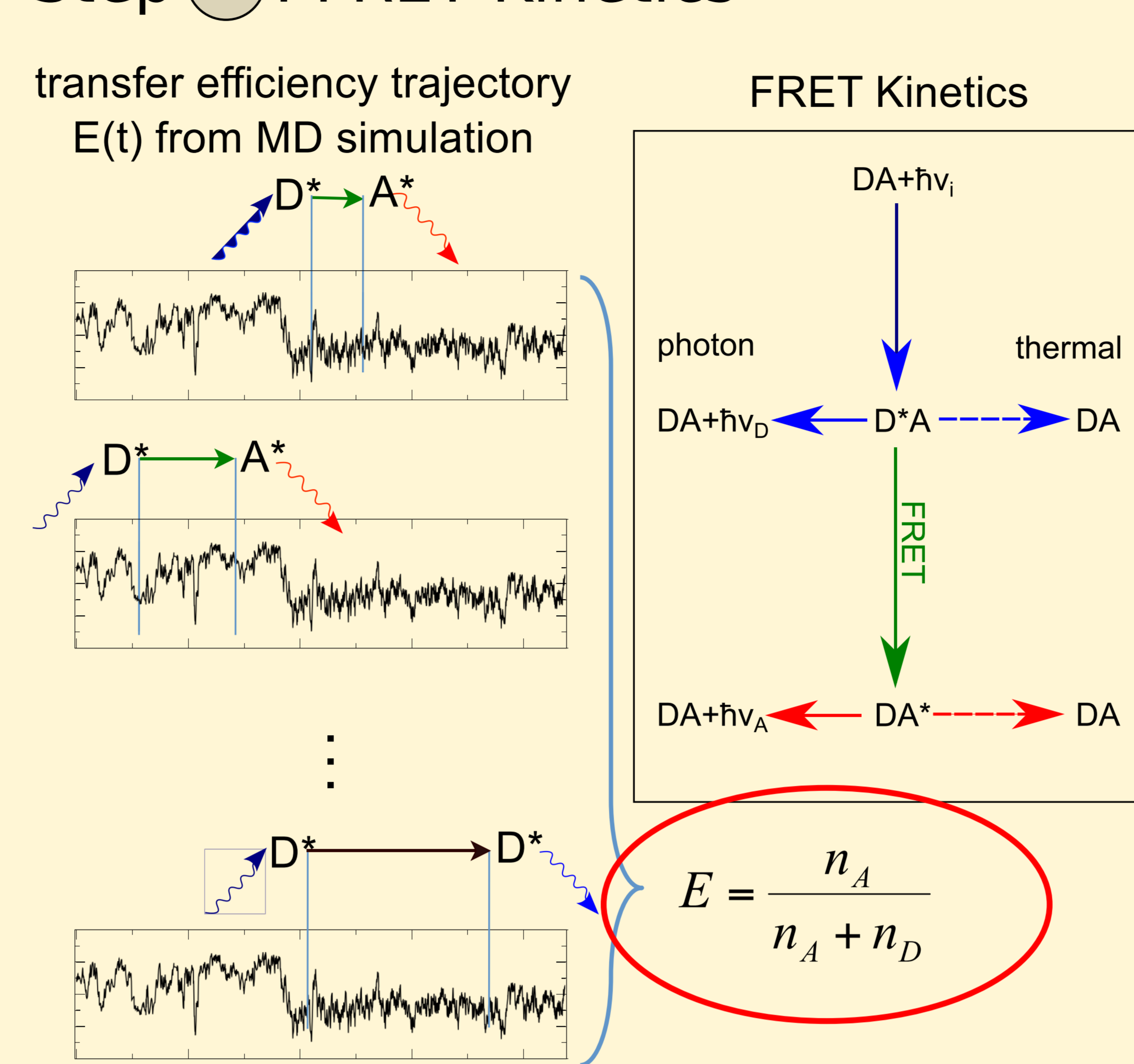
From MD simulations, the **distribution** of  $\kappa^2$  was determined. The **ensemble average of  $\kappa^2$  is larger than 2/3**. Thus, an **isotropic approximation will lead to a systematic error** in distance measurements. Distributions of  $\kappa^2$  for each simulation also vary between simulations. Therefore, multiple simulations improve the convergence of  $\kappa^2$ .

## Step 2



Combined simulation and experiments dropping  $\kappa^2$  assumptions in  $R_0$  and replaced them with dye dynamics and statistics from the MD simulations.

## Step 1a: FRET Kinetics



To calculate efficiency distributions from MD simulations, we employed a Monte Carlo (MC) approach to probe the kinetic pathways of FRET experiments. All probabilities in the kinetic scheme are considered as constant except the FRET probability  $p_f(t)$  which is calculated from the coupling between the dyes in the MD simulation and changes with every time-step.

Individual photons are generated by starting from an arbitrary point in time on a random trajectory in the ensemble with the donor D in the excited state. Random numbers are employed to discriminate between the pathways until de-excitation occurs. This process is repeated until the desired photons for a photon burst are obtained.

## Step 1b: Dye Orientation Heterogeneity and Comparison to Experiment

Efficiencies are calculated from photon bursts. Each photon burst in experiments is from a single molecule diffusing through the laser spot (ms timescale). Therefore, multiple simulations (each 100 ns) might be required to achieve the appropriate sampling of the dye conformations in one single burst.

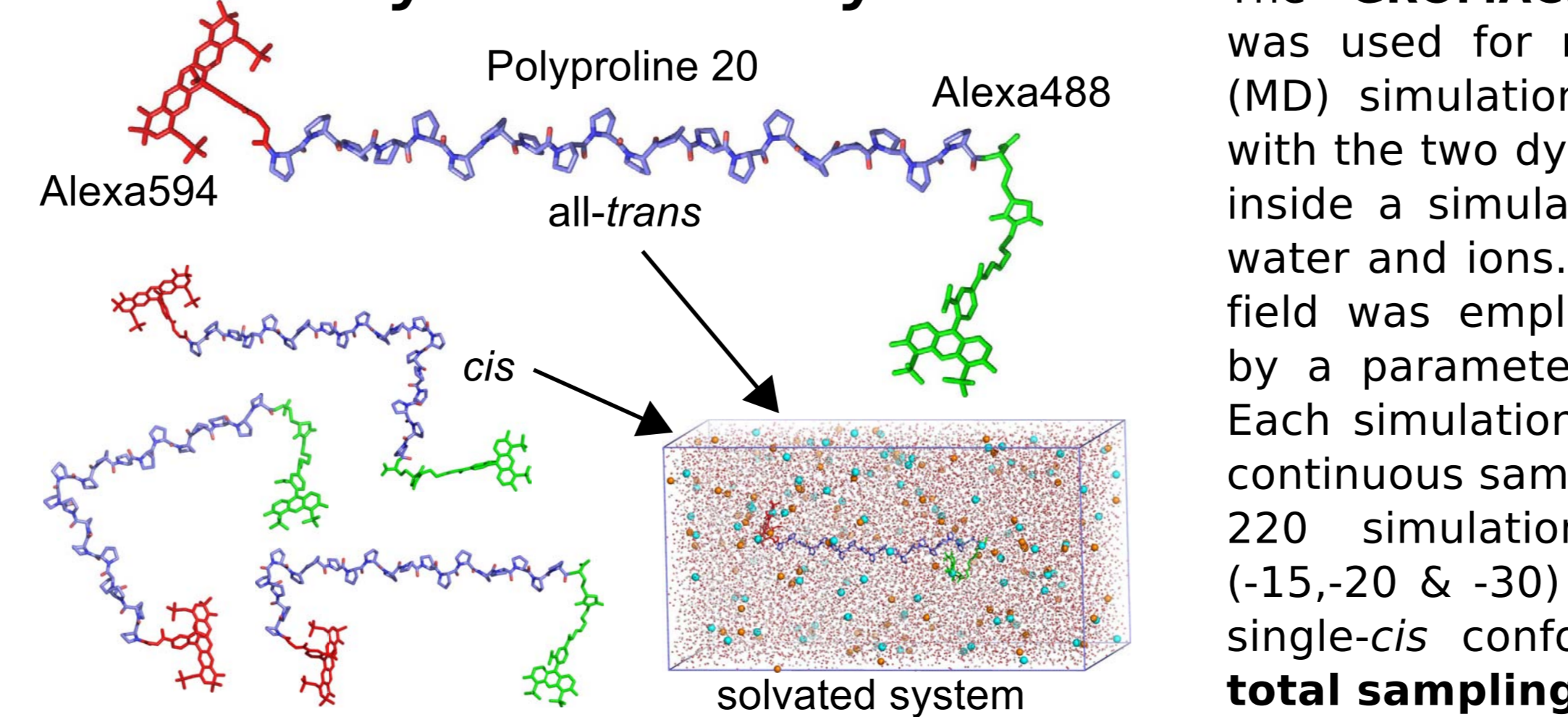
Our simulations revealed a difference in dynamic behavior of the open and closed conformation. While the reorientation of the open conformation is too slow compared to the sampling of a single simulation, transitions between multiple closed states are even slow compared to the burst timescale.

We therefore employed two methods to form bursts from multiple simulations. Each method differs in the averaging of the dye motion.

First, bursts are formed from photons of all trajectories of the same isomer. Each efficiency is then an average of the entire dye motion in our simulation ensemble. Second, each burst is formed from photons of a single trajectory only. Here, each efficiency is an average of the fast dye motion within a single simulation only.

Comparison of the resulting efficiency distributions to the experiment leads to agreement in different regions (see solid vs stroke lines) for each method. The open conformation leads to large dye-to-dye distances, therefore agreement on the low efficiency side is found. Vice versa, the closed conformation results in small dye-to-dye distances resulting in agreement on the high efficiency side. In summary, agreement with experiment was established and more importantly without any fit parameter present in the model [1].

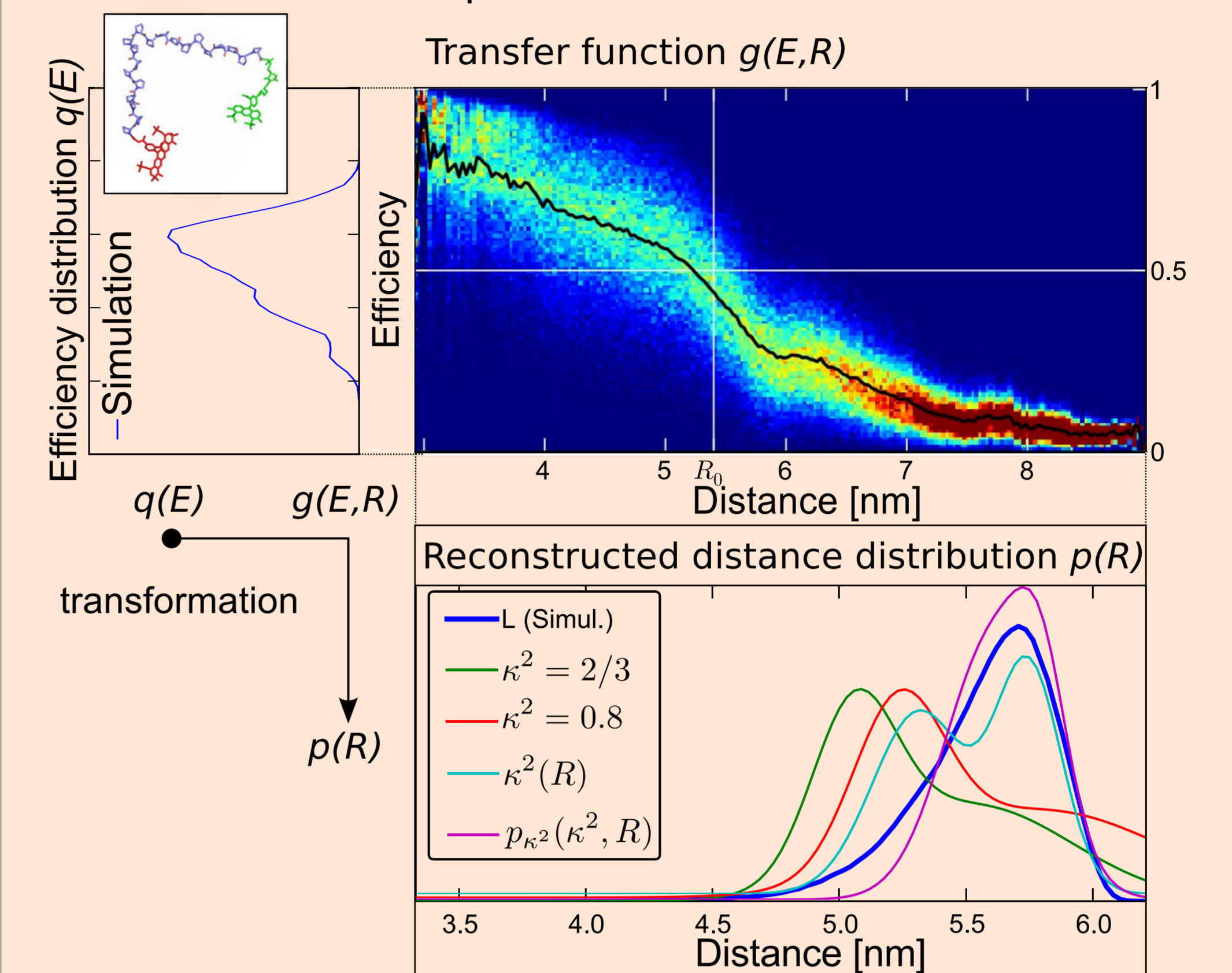
## Model System: Poly-Proline



The GROMACS 4.0.7 package was used for molecular dynamics (MD) simulations. The poly-proline with the two dyes attached was put inside a simulation box, filled with water and ions. The OPLS/AA force-field was employed and extended by a parameter set for the dyes. Each simulation consists of 100 ns continuous sampling. We performed 220 simulations of poly-proline (-15, -20 & -30) in the all-trans and single-cis conformation yielding a **total sampling of 22  $\mu$ s**.

## Step 2: Distance Reconstruction

Simulated efficiencies - known distance distribution. Reconstruction input: efficiencies from simulation

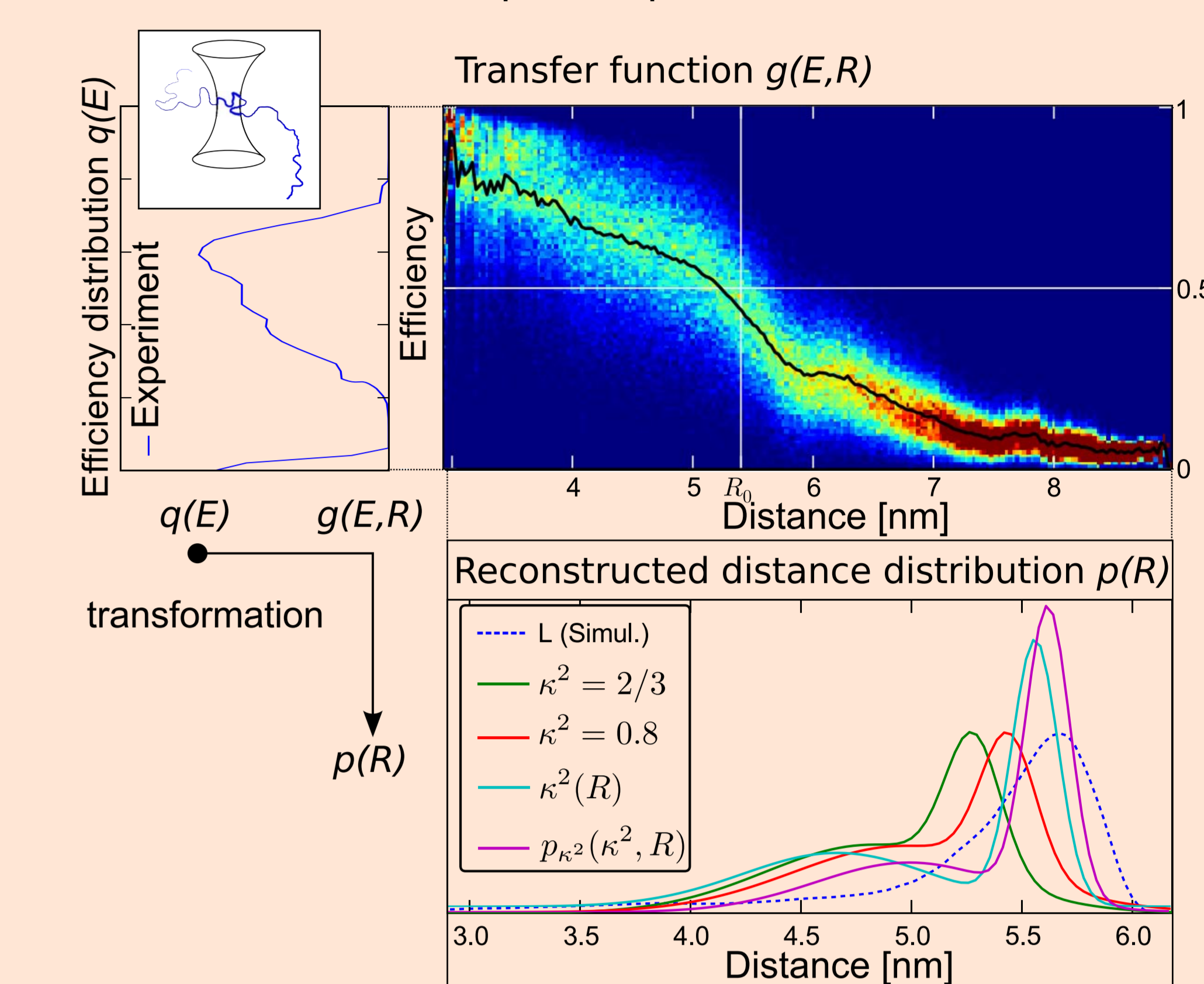


For the distance reconstruction, we developed a transfer function formalism in which the conversion of distances to efficiencies is given by

$$q(E) = \int_0^{R_{max}} g(E,R)p(R)dR$$

with  $q(E)$  and  $p(R)$  as probability distributions of efficiencies and distances, respectively. The transfer function  $g(E,R)$  is refined for each approximation level. Inversion of the formalism allows calculation of distance distributions  $p(R)$  from experimental efficiency distributions  $q(E)$ .

Measured efficiencies - unknown distance distribution. Reconstruction input: experimental efficiencies



To obtain distances we systematically reduced the levels of approximation for the dye orientation in the RET theory and thereby refine the transfer function  $g(E,R)$  [1].

The first step is to drop the assumption that the dye orientations are isotropic (green). Instead the mean  $\kappa^2$  of 0.8 from the simulations is used (red). Next, we assume that  $\kappa^2$  depends on the distance (cyan). Finally, we use the full dye orientation information from the simulations to obtain distributions of  $\kappa^2$  (purple).

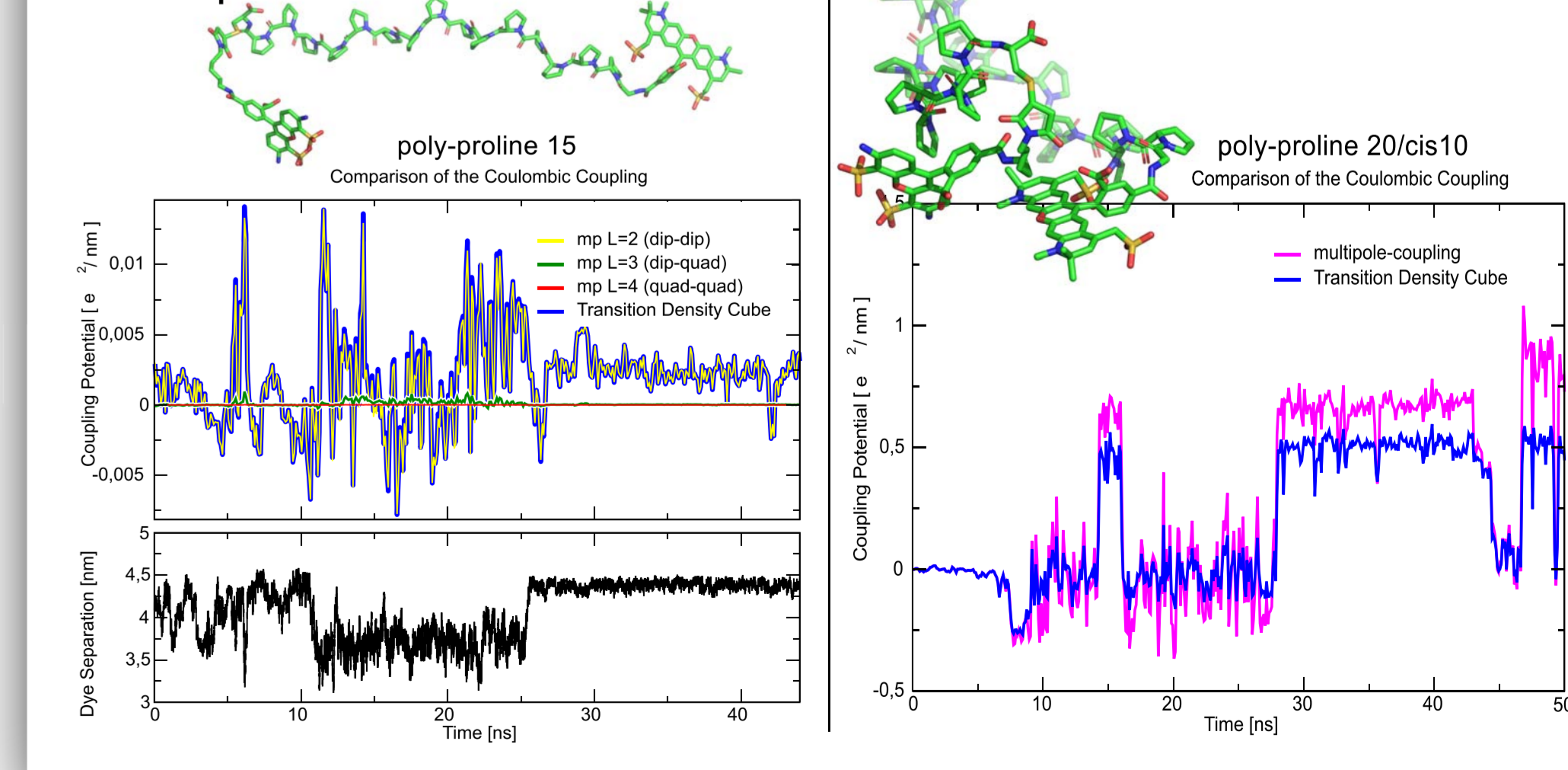
To assess the improvement of each step, we reconstruct (known) distances using the calculated FRET efficiency distribution  $q(E)$ . First (left panel), the efficiency distribution  $q(E)$  from simulations is used and the reconstructed distances are compared to the known result.

Second, we apply this to the experimental distribution  $q(E)$  (right panel). For the synthetic case an improvement from 6.2 Å to only 0.35 Å error of the peak location is achieved. This suggests that a similar improvement is obtained when using experimental efficiency distributions.

## Validity of the Dipole-Dipole Approximation

Is the dipole-dipole approximation a valid assumption? To test this, we employed multipole coupling and direct electron transition density coupling [2] instead of Förster's approximation. Thereby, accurate coupling is achieved for...  
... dyes with significant moments beyond dipoles.  
... for dye coupling at short distances.

Below, two examples are depicted:  
Left side: Multipole coupling potential for an extended poly-proline 15. For the Alexa488 - Alexa594 dye pair and a separation of ~4nm, dominant contribution is of order  $L=2$ . Higher moments than the dipole ones are negligible.  
Right side: Poly-proline 20 with central cis-bond, comparison between multipole coupling up to  $L=8$  and a direct method (Transition Density Cube [2]). The difference between both methods suggests, that for small distances ~1nm, direct methods should be used to achieve an accurate coupling description.



## References

- [1] Structural Heterogeneity and Quantitative FRET Efficiency Distributions of Polyprolines through a Hybrid Atomistic Simulation and Monte Carlo Approach, Hoefling, M.; Lima, N.; Hänni, D.; Schuler, B.; Seidel, C. A. M. & Grubmüller, H.; PLoS ONE, Vol. 6, pp. e19791, 2011
- [2] Calculation of Couplings and Energy-Transfer Pathways Between the Pigments of LH2 by the Ab Initio Transition Density Cube Method Krueger, B. P.; Scholes, G. D. & Fleming, G. R.; J. Phys. Chem. B, Vol. 102, pp. 5378-5386, 1998
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