Assessing drug-protein binding by simulation of stereoselective energy transfer dynamics: electronic interactions between tryptophan and flurbiprofen

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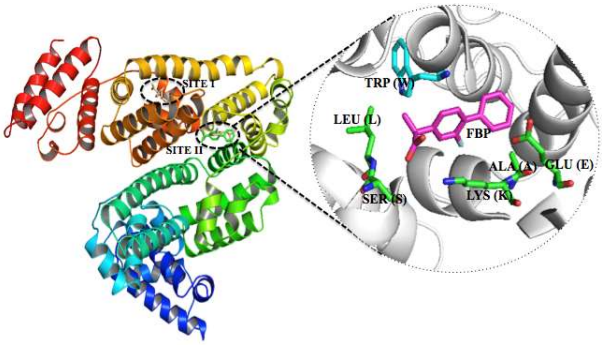
Protein fluorescence decays are difficult to interpret and often involving several energy transfer processes among Trp residues or Trp-ligands. In this study, we simulate EET rates by computing MD-averaged electronic couplings V. Fluorescence decays have been observed for the HSA protein bound to the S and R enantiomers of FBP. So far, our results in the HSA-FBP system agree with the experimental hypothesis (stereoselective energy transfer) and strongly support the binding modes proposed for the R and S enantiomers in HSA.

I. INTRODUCTION

The fluorescence of proteins is a complex process often involving several electronic energy transfer (EET) reactions between aromatic amino acids (typically arising from tryptophan), before light emission. In protein-ligand complexes, the ligand can also modify the fluorescence properties by participating in those EET processes, as well as by contributing to electron transfer reactions or the formation of exciplexes. The complex interpretation of optical experiments, however, precludes in a full explotation of the structural information encapsulated in such experiments and related to the drug-binding events observed. The detailed understanding of drug-protein binding is determinant for drug action and drug transport and disposition, which are regulated by various transport proteins such as HSA-*Human Serum Albumin* (*Fig.*1)[1].



Fi gure 1. Drug binding to site 2 in HSA. The detailed binding conformation is show for flurbiprofen.

In this context, we intended to explore the potential of simulation techniques MD/QM-MM in describing EET processes and fluorescence protein-ligand systems in order to determine the binding modes of protein ligands by comparison with fluorescence experiments.[2]

II. COMPUTATIONAL DETAILS

We have simulated how energy transfers involving different flurbiprofen enantiomers modulate the fluorescence properties of model tryptophan-flurbiprofen (TRP-FBP) and flurbiprofen-HSA (*human serum albumin*) complexes *(Fig.2)*, where stereoselective dynamic quenchings have been recently observed.[2] To this aim, we combine classical MD techniques with a polarizable QM/MM methodology that we have recently developed[3] and applied to study the light-harvesting properties of photosynthetic systems.[4,5]

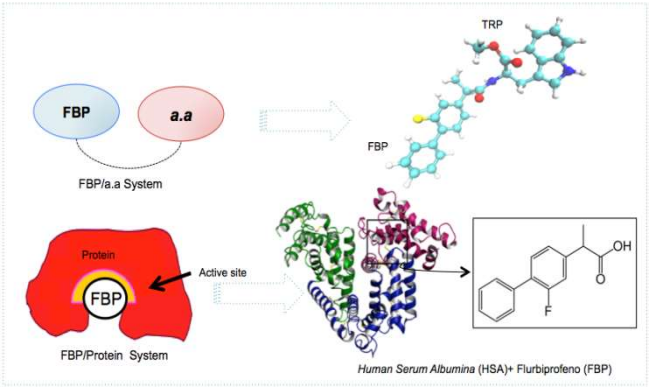


Figure 2. Model system (FBP/TRP) and biological system (HSA/TRP)

The QM/MMpol linear response approach

The QM/MMpol model[3] combines a quantum-chemical description of the pigment's excited states (TD-DFT, CIS or ZINDO) with a polarizable MM description of the surrounding environment, where MM atoms are assigned with a partial charge and an isotropic polarizability:

$$\hat{H}_{eff}\left|\Psi\right\rangle = \left(\hat{H}_{QM} + \hat{H}_{QM/MM} + \hat{H}_{MM}\right)\left|\Psi\right\rangle = E\left|\Psi\right\rangle$$

The electronic coupling (V) between relevant excited states, is obtained perturbatively from the transition

densities computed for the non-interacting donor (D) and acceptor (A):

$$V = V_0 + V_{\text{env}}$$

$$V_0 = \int d\vec{r}' \int d\vec{r} \rho_A^{T*}(\vec{r}') \left[\frac{1}{|\vec{r} - \vec{r}'|} + g_{xc} \right] \rho_D^T(\vec{r}) - \omega_0 \int d\vec{r}' \int d\vec{r} \rho_A^{T*}(\vec{r}') \rho_D^T(\vec{r})$$

Direct interaction between D/A transition densities. Includes Coulomb, exchange-correlation and overlap terms.

$$V_{\text{env}} = -\sum_k \vec{\mu}_k^{ind} \left(\rho_D^T \right) \int d\vec{r} \frac{\rho_A^T(\vec{r}) \left(\vec{r}_k - \vec{r} \right)}{\left| \vec{r}_k - \vec{r} \right|^3}$$

Environment-mediated D/A interaction described in terms of the MM polarization response.

III. Results and Discussion

In order to interpret the fluorescence experiments on the Flurbiprofen complex with HSA protein, we studied the ability of different methods in order to describe the electronic states involved. The table shows the results of the transition energies obtained for the π→π* state of flurbiprofen (FBP) and the states La and Lb of tryptophan (TRP). The transitions energies for the Flurbiprofen lowest p → p* state and the tryptophan La state are in excellent agreement with the experimental values, which indicates the goodness of the semiempirical ZINDO method in order to describe the properties of the system.

	Flurbiprofen (donor)			Tryptophan La state			Tryptophan Lb state	
	exp / eV	ΔE / eV	f	exp / eV	ΔE / eV	f	ΔE / eV	f
Protein *S*	4.29	4.12	0.56	4.16	4.21	0.18	4.01	0.05
Protein *R*		4.11	0.56		4.18	0.19	4.01	0.06
Model *S*		4.26	0.46		4.14	0.17	4.02	0.04
Model *R*		4.25	0.45		4.13	0.17	3.99	0.04

Then we proceeded to estimate the rate of energy transfer (EET) for the two enantiomers of FBP, to investigate whether changes in fluorescence (experimentally observed) are due to processes EET, and to validate the binding mode of each enantiomer predicted theoretically. The results ZINDO level found for HSA-FBP biological system suggest a process EET approximately 30% faster than FBP for TRP in the case of the *S* enantiomer, according to the experimental observation, giving validity to the proposed model.

The results of coupling squared (V^2) calculated ZINDO level are shown in Figure 3. For the FBP-TRP model in solution, found comparable results between *R* and *S* enantiomers. These results are in contrast with the experimental observation where it postulates a EET 2-3 times faster for the *R* enantiomer .

However, this system is very flexible from the conformational point of view. Currently, there are increasing in the number of structures studied to converge predictions, besides, it is necessary to study the validity of the conformational preferences predicted by MD simulations that could be affecting our results significantly.

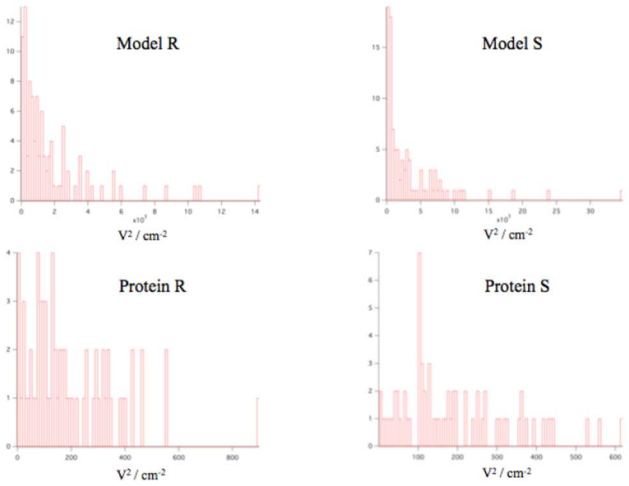


Figure 3: Coupling distribution (V2) for the model (FBP / TRP) and to the biological system (HSA / TRP) calculated over the simulation.

In general, preliminary analysis of MD-QM/MMpol simulations at the ZINDO semiempirical level (100 snapshots) predict a distribution of squared couplings along the simulation comparable for the *S* and *R* enantiomers, opposite to the experiments. In contrast, the results for the protein systems suggest a slightly faster EET from FBP to Trp in the *S* case, in agreement with the experimental observation. Nowadays, we are extending the simulations to more structures in order to properly converge the estimated EET rates. We are also performing *ab inito* CIS and TD-DFT calculations in order to verify the semiempirical results. Overall, the results obtained strongly support the hypothesis that changes in the fluorescence of the HSA-FBP system arise from EET processes.

Acknowledgment

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