FÖRSTER RESONANCE ENERGY TRANSFER APPLIED TO DRUG DESIGN

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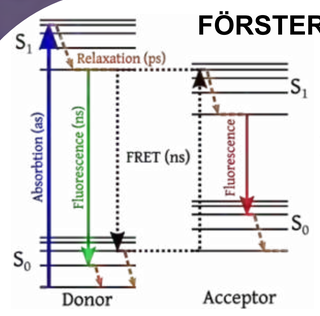
ABSTRACT

The Förster resonance energy transfer (FRET) technique is an important tool in structural biology, due to its ability to monitor and measure distances in biological systems.[1] Albeit FRET is widely used to measure distances in fluorophore-tagged proteins, intrinsic FRET processes in protein-ligand complexes prevent straightforward application of Förster theory due to the lack of rotational freedom of the Trp and ligands involved and their relatively short separations.

In this contribution, we aim at developing a novel technique to discover binding sites and characterize ligand binding modes in proteins by combining fluorescence spectroscopy with a novel multiscale computational methodology. Our methodology combines classical molecular dynamics (MD) simulations of predicted binding modes with polarizable quantum/molecular mechanical (QM/MM) calculations of FRET properties beyond Förster dipole and dielectric screening approximations.[1][2] We apply this approach to study the binding of several ligands to Human Serum Albumin (HSA), which presents several advantages to assess the novel methodology, like the presence of multiple binding sites, a single Trp residue, and binding FRET data reported for multiple ligands.[2][3]
The ultimate objective of the project is to assess the ability of FRET simulations to characterize allosteric and cryptic binding sites and ligand binding modes for drug discovery targets.

FÖRSTER RESONANCE ENERGY TRANSFER



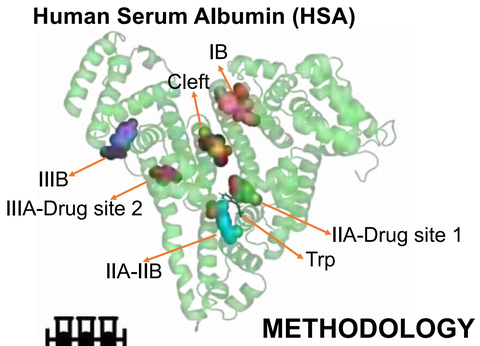
The electronic excitation energy transfer from a donor to an acceptor molecule through non-radiative dipole-dipole coupling.

Application of Förster theory limitated in case of restricted rotational freedom of D/A (Assumption of isotropic orientational factor.

GOAL OF THE PROJECT : Developing a new technique to to characterize allosteric and cryptic binding sites and ligand binding modes for drug discovery targets by combining FRET assays & simulations.

TARGET PROTEIN & LIGANDS

Human Serum Albumin (HSA)

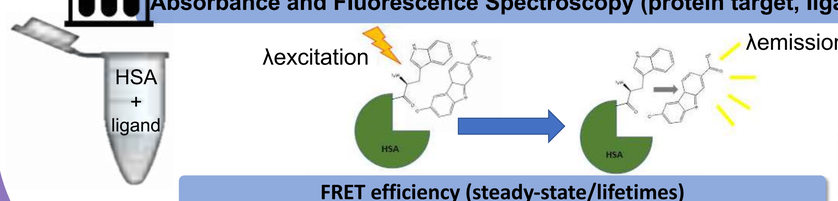


Initial fluorescent ligand library
8 ligands

To test our method, we first used HSA for our experiments because:
- HSA has 6 known binding sites
- There are lots of experimental & theoretical resuls available for HSA with different ligands
- It has 1 Trp

METHODOLOGY – Experimental

Absorbance and Fluorescence Spectroscopy (protein target, ligands, complexes)



FRET efficiency (steady-state/lifetimes)

$$E = \frac{1}{1+\left(\frac{R}{R_0}\right)^6}$$

From donor lifetime $E = 1 - \frac{\tau_{DA}}{\tau_D}$

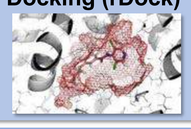
From donor fluorescence $E = 1 - \frac{I_D}{I_{DA}}$

From acceptor fluorescence $E = \frac{I_{AD}A_{AA} - I_{AA}A_{AD}}{I_{AA}A_{DD}}$

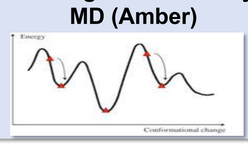
METHODOLOGY – Computational

Binding site identification (MDmix)

Binding mode identification Docking (rDock)



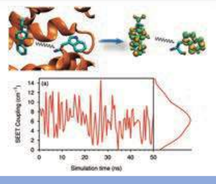
Binding mode stability MD (Amber)



Excited states (TD-DFT) , FRET from MD trajectories, TrESP & QM/MMPol

Donor - Trp Acceptor - Ligand

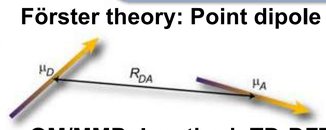
Binding models validation



Simulating FRET rates, efficiencies from R_0, τ_D, donor and acceptor transition dipole moments (μ^T) and coupling between donor and acceptor (V_{DA})

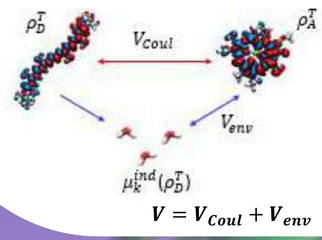
$$k_{theo} = V_{DA}^2 \frac{3}{2}\frac{R_0^6}{\tau_D}\frac{n^4}{(\mu_D^T)^2(\mu_A^T)^2} \qquad E_{theo} = \langle \frac{1}{1+\frac{1}{\tau_D \langle k_{theo}\rangle_{fast}}} \rangle_{slow}$$

Förster theory: Point dipole approximation + continuous dielectric environment



$$V \approx \frac{1}{n^2} V_{dip-dip}$$

QM/MMPol method: TD-DFT Transition densities + polarizable MM environment



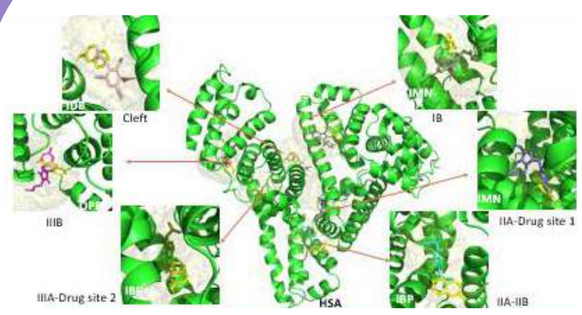
$$V = V_{Coul} + V_{env}$$

Förster theory assumes D/A rotational freedom and doesn't take into account deviations from screening factor due to heterogeneities in the environment and deviations from the ideal dipole approximation while calculating the dipole dipole coupling.

QM/MMPol uses TD-DFT transition densities instead of point dipoles and takes environmental effect into consideration in atomic detail, so it can go beyond Förster approximations.

RESULTS – Docking & MD Simulations

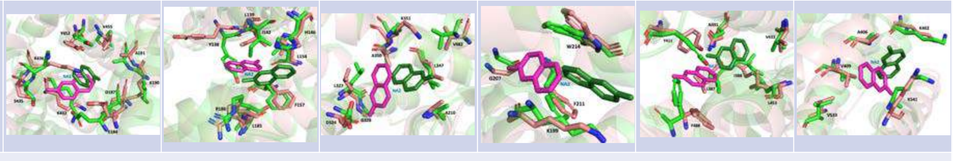
Docking for 2-naphthol



Each ligand in the initial library is docked at all the binding sites of HSA. The results are shown for 2-naphthol in comparison with the reference ligand for each binding site.

After docking, the stability of the 3 best docking poses is explored by MD:
- 3×100 ns replicas for each pose
- Amber ff19SB and gaff2 force fields (proteins and ligands)

Docking & MD Results for 2-naphthol

Binding site	Cleft	IB	IIA-IIB	IIA	IIIA	IIIB
Docking Score (kJ/mol)	-17.8	-15.1	-17.0	-20.2	-21.5	-15.1
Binding pose						
	Initial position before MD		Final position after MD		NA2 --> 2-naphthol	
Distance Trp - L (Å)	18.4	23.7	9.9	4.8	17.8	36.9

RESULTS – Experimental & Theoretical FRET Efficiency

For 2-naphthol

E_{exp}	R_0 (Angstrom)	R_{exp} (Angstrom)	Binding site	E_{theo}	R_{theo} (Angstrom)
0.11	32.8	46.5	**Cleft**	0.98	18.4
			IB	0.94	23.7
			IIA-IIB	1.00	9.9
			IIA	1.00	4.8
			IIIA	0.96	17.8
			IIIB	0.48	36.9

Excited state calculations are performed using time dependent DFT with B3LYP and CAM-B3LYP functionals. Transition densities are derived from TD-DFT calculations. Then the FRET efficiency is calculated from the electronic couplings computed along MD trajectories by using transition densities instead of dipoles.

Conclusion & Future Perspective:

We observed that the efficiency values were higher for the ligands in the binding sites closer to Trp which was what we were expecting.
However, these efficiencies assume that the proteins are fully complexed with ligand. So, a correction based on the Kd values will be applied to experimental FRET efficiencies.
Also, theoretical efficiencies will be further improved taking into account dynamic and static disorder effects.

Regarding experimental results some inaccuracies have been found so far in order to obtain robust estimates of efficiencies, and we plan to perform the fluorescence experiments in an AB2 spectrometer measuring fluorescence and absorption in more complete titration curves obtained at different ligand concentrations.

ACKNOWLEDGEMENTS

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