Rational FRET biosensor design based on molecular dynamics simulations

Shourjya Sanyal * †, Donal MacKernan † and David F. Coker ** †‡

*shourjya.sanyal@ucdconnect.ie **david.coker@ucd.ie

† Department of Physics, and Complex Adaptive Systems Laboratory, University College Dublin, Dublin 4, Ireland

‡ Department of Chemistry, Boston University, Massachusetts 02215, United States

Abstract

Fluorescence resonance energy transfer (FRET) is used to identify signaling dynamics of biochemical pathways, to be used as an important tool in optical microscopy. [1,2]

The standard experimental setup uses high concentration of intra-molecular fluorophores to achieve target signal strength which,problematically, often scales non-linearly to target biological observables.[3] Our work aims to design and identify linkers which addresses this issue at a molecular level.

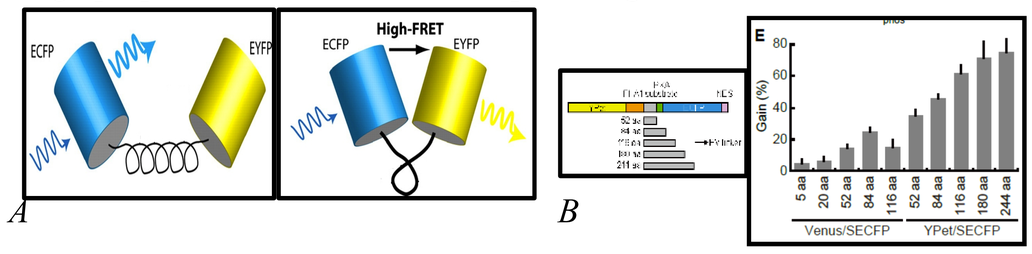
Our approach combines rare event methods and ab-initio computations to better understand the molecular and electronic processes relevant to optical microscopy.

The work involves parameterising the chromophores using different force fields, and understanding the effect of aqueous environment and protein scaffold on the chromophores. This leads to efforts in theoretical analysis and prediction of features in the emission spectrum for ECFP chromophores.

Introduction

• Experimentally developed linkers, tend to be specific to the fluorophore pair used and non-directional with limited optimisation.

• Our MD approach involves biased sampling of FRET rate at representative relative orientations of donor and acceptor to determine design properties of linker, towards optimising it.



A B

Fig.1. A.FRET dependence on linker. B. Experimental Efforts.[4]

Simulation of FRET Fluorophores

• A difficulty that must be faced before simulation and even forcefield parametrization is what precisely is the chromophore ? This is due to the fact that chromophores are not standard amino-acids but in fact chemical by-products thereof. This ideally involves combining homology modeling, and ab-intio optical calculations with known steric constraints and experimental absorption and emission spectroscopy.

• Here, hydrogen and terminal groups were added to the structure obtained from diffraction data [6], using library charges from Automated Topology Builder (http://compbio.biosci.uq.edu.au/atb/). This contains a library of optimized geometry at the B3LYP/6-31G* level of theory as a function of the molecular symmetry.

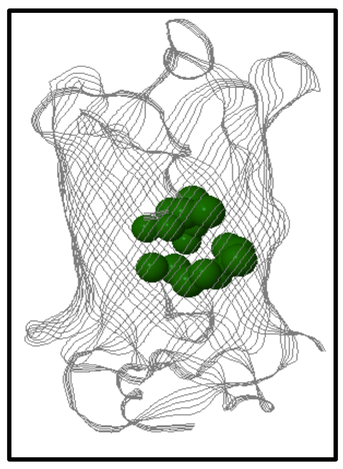


Fig.2. Scaffold showing chromophore inside. Molecular dynamics was performed on the equilibrated system for 2ns using Lincs/SHAK, and Particle Mesh Ewald.

Proposed Work Plan

The aim of the project is to enhance signal-to-noise ratio and signal strength in Intra-molecular FRET by developing models for optimised linkers in a rational progression described below.

• Modeling Biomolecular CFP-Linker-YFP system for FRET probes.
• Modeling protein linked CFP-Linker-YFP system as rational biosensors.
• Developing uni-directional FRET probes.

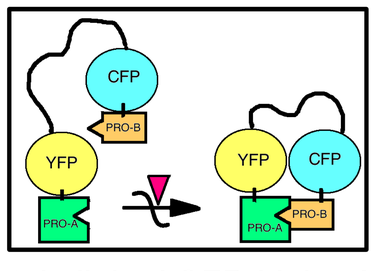
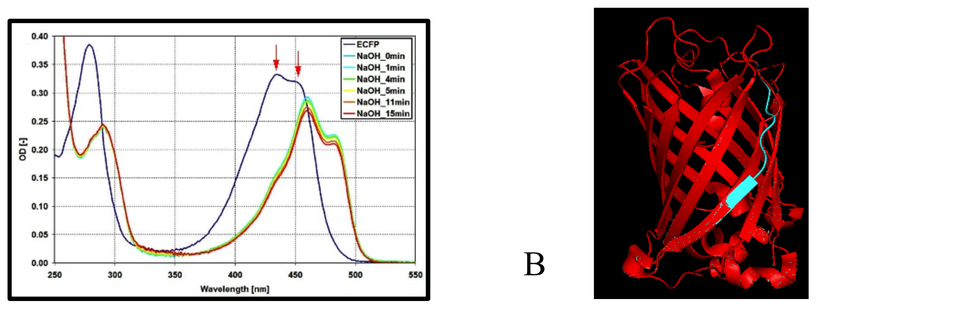


Fig 5. Protein linked CFP-Linker-YFP system

Features in ECFP Emission Spectrum

• Lelimousin et al. [5] have shown that the chromophore,freed from its specific interactions with the protein, retains two peaks in its absorption spectrum.

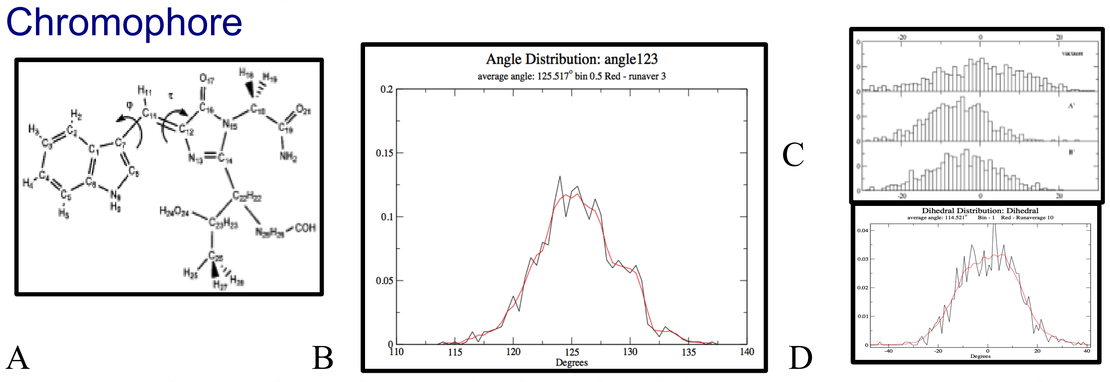
• Demachy et.al. [6] have shown that there is no difference between the positions of the absorption spectra of the two conformations A′ and B′ with either density functional. (TD-DFT with B3LYP and BP86)



A B

Fig 3. A. Emission spectrum of ECFP chromophore showing two humps.[5] B. ECFP Conformation B' (Blue) superimposed on Conformation A' (Red)

Bridging Bonds and Dihedrals as Order parameters for systematic study of the different conformations of the ECFP Chromophore



A B C D

Fig 4.(A) Chromophore showing bridging bond C7-C11-C12. (B) Two peaks corresponding to states, in angle distribution of C7-C11-C12 from 2ns NVE simulation with chromophore in scaffold in water box.
Fluctuations of Dihedral angle φ (C8-C7-C11-C12)from mean value (C) from 1ns simulation reported in [6] (D) from 2ns simulation reported here.

Reference

1.Janetopoulos et.al., J Cell Sci.122, 2597-2603 (2009)
2. Karynov et.al. Science.Vol. 290 no. 5490, 333-337 (2000)
3. Marc R. Birtwistle et.al., Volume 6.Issue 11 (2011)
4.Naoki Komatsu et.al., Molecular Biology of the Cell. 22(23):4647-4656. (2011)
5. Lelimousin et al., Biochemistry. Vol. 48, No. 42.(2009)
6. Demachy et.al.,J. Phys. Chem. B. Vol.109, No. 50.(2005)

Acknowledgement :

I am grateful to Dr.Jens Rauch and Dr.Drieke Vandamme for fruitful discussions. I am also indebted to SBI, Ireland and Prof.Dr.Walter Kolch for his support.