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π-Stacking interactions in YFP, quantum mechanics and force field evaluations in the S_0 and S_1 states

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ABSTRACT

We study the π-stacking interaction between the chromophore and Tyr203 in the Yellow Fluorescent Protein (YFP) in order to (i) evaluate the contribution of the internal interaction energy of the isolated Chromophore-Tyrosine complex (E_{int}) to the 26 nm red shift observed from GFP to YFP, (ii) compare the effects of E_{int} and of the proteic environment. To that end, we perform quantum mechanical and force field (*ff*) calculations of the isolated complex in S_0 and S_1 states on a large sample of geometries, together with molecular dynamics simulations and potential of mean force analysis. The calculated absorption wavelengths are found red shifted with respect to the isolated chromophore by 12–19 nm, that represents a large part of the GFP-YFP shift. We find that the effect of the protein is determinant on the dynamics of the complex while the error that results from using a classical*ff* is of limited effect.

1. Introduction

π-Stacking interactions are found frequently in biological systems like proteins. The resulting structure of the stacked complex is a compromise between the attraction of the two partners and the constraints coming from the protein (covalent bonds, hydrogen bonds, electrostatic effects, steric effects). We try here to evaluate the respective contributions of the two factors by comparing the results of quantum mechanics calculations of the interaction energy of the isolated complex with the results of molecular dynamics simulations of the whole protein, using the Yellow Fluorescent Protein (YFP) [1] as a test case.

In fact, among the Green Fluorescent Protein (GFP) family, the yellow variant YFP has a key role as one of the most widely used acceptors in Förster resonance energy transfer (FRET) applications in molecular biology [1,2], due to the red shift of the absorption and emission wavelengths with respect to GFP. In GFP-S65T the absorption wavelengths of the anionic chromophore is 488 nm [3] while it is 514 nm in YFP [4]. This 26 nm shift is usually ascribed to a π -stacking interaction between the chromophore (that part of the protein responsible for absorption and emission of visible light) and the phenol ring of a neighbour tyrosine (Tyr203, see Fig. 1) in YFP [4,5].

Recently, it has been clearly demonstrated that the shift in YFP comes from the combination of two effects, the π-stacking and the electrostatic interaction with the protein [6].

In addition, a large number of both experimental and theoretical studies on small dimers of aromatic rings in gas phase have characterised the intrinsic properties of π -stacking effects as subtle interactions [7–12] that require high level of calculations to be quantitatively described, especially when one partner is electronically excited [13–15].

Of course, if the stacked dimer is buried inside the protein the π -stacking conformations are dependent on geometrical constraints and on the global dynamics of the protein. These effects may be of the same order of magnitude or even greater than the π -stacking stabilisation. Thus, π-stacking analysis inside proteins requires an accurate calculation of the interaction energy of the stacked partners on a large sample of conformations and long molecular dynamics simulations based on a force field description of this interaction.

In this work we address also the question of the relevance of such an approach and particularly of the relevance of a force field description of stacked complexes. The reliability of classical force fields has been yet evaluated for complexes of aromatic aminoacid residues [7,9,10,16] but never for complexes involving aromatic molecules like fluorescent protein chromophores.

We performed accurate *ab initio* quantum mechanics calculations and classical force field calculations of the intermolecular interaction energy of the complex chromophore-tyrosine in the

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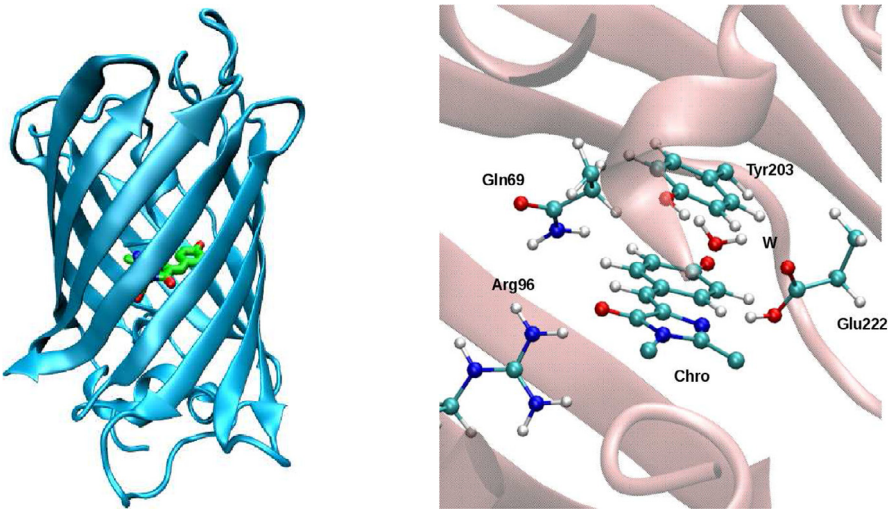


Fig. 1. Left: the overall structure of YFP. Right: the chromophore (Chro, aromatic part only), Tyr203 and the side chains of neighbouring amino-acids.

ground state S_0 and in the excited state S_1, in a large sample of geometries that covers the ones explored in a standard force field molecular dynamics. All along this work the chromophore is taken in its anionic form (deprotonated at the phenolic oxygen). The π-stacking contribution to the absorption red shift observed beween GFP and YFP is quantified. We compare the π-stacking energy landscapes in the S_0 and S_1 states, and examine the validity of a standard force field description. We analyse the effects of the internal forces of the complex and those of the proteic environment.

2. Methods

2.1. Molecular dynamics

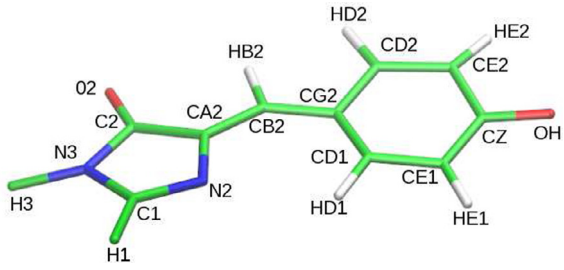
Molecular dynamics (MD) calculations of the YPF protein with the chromophore in the electronic ground state were carried out using the AMBER 10 suite [17]. The starting coordinates were taken from the X-ray structure of Wachter et al. [4] in the Protein Data Bank. The protein was solvated in a water box. All titrable amino-acids were taken in their standard protonation state at neutral pH except Glu222 which was taken neutral in order to allow hydrogen bonding with the imidazolinone moiety of the chromophore.

The AMBER 1999 force field was used for all standard amino acids. The chromophore force field as wellas the initial equilibration protocol of the system (protein + solvent) are the same as those used in a preceding work in the case of GFP [18]. Point charges for the aromatic part of the chromophore are shown in Table 1.

A special attention was paid here to the orientation of the hydroxyl group OH of Tyr203. In the X-ray structure, the phenol oxygen of Tyr203 is H-bonded to a water molecule W, which is also H-bonded to Glu222 (see Fig. 1, right). Two short simulations were run with the OH group pointing in either direction. Finally the geometry with OH pointing towards W was retained because it leads to a strong Tyr203-W-Glu222 cluster (% of existence of the H-bond equal to 93% for Tyr203-W and 92% for W-Glu222), in agreement with the X-ray structure, while these percentages are much smaller with the opposite direction of the OH group.

Then a 12 ns-long simulation with constant temperature (300 K) and pressure (1 atm) was run.

Table 1
Charges (ua) of the chromophore (HBI) in the ground and excited states.



	S_0	S_1
Imidazolinone		
C1	+0.1318	+0.1226
N2	−0.4101	−0.4976
CA2	−0.0307	+0.1595
C2	+0.3785	+0.4051
O2	−0.5789	−0.5711
N3	+0.0683	+0.0392
H1	−0.0408	−0.0514
H3	−0.0289	−0.0197
Sum	−0.5108	−0.4139
Difference q(S1)-q(S0		+0.0974
Bridge		
CB2	−0.1238	−0.3601
HB2	+0.1293	+0.1346
Sum	+0.0055	−0.2255
Sifference		−0.2200
Phenolate		
CG2	+0.0034	+0.2211
CD1,CD2	−0.1225	−0.2391
HD1,HD2	+0.1457	+0.1665
CE1,CE2	−0.4186	−0.3364
HE1,HE2	+0.1416	+0.1372
CZ	+0.7125	+0.6509
OH	−0.7032	−0.6894
sum	−0.4949	−0.3610
difference		+0.1339

2.2. Force field intermolecular energy.

Evaluation of the interaction energy between the chromophore and Tyr203 was performed in the force field (ff) approximation.