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Prediction of First Hyperpolarizability of Fluorescent Proteins

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Abstract. In an attempt to investigate the predictability of nonlinear optical properties of fluorescent proteins, we have experimentally and theoretically determined the first hyperpolarizability of eGFP, eYFP, ZsYellow, DsRed, mStrawberry and mCherry. Our first comparisons between experiment and calculations meet our expectations and point out some key features, e.g. the strong dependence on x-ray data and sensitivity to environmental factors like pH and ionic strength.

Keywords: First Hyperpolarizability, Nonlinear Optics, Fluorescent Protein, Time-Dependent Hartree-Fock, eGFP, eYFP, ZsYellow, DsRed, mStrawberry, mCherry
PACS: 42.65.Kg; 42.65.Re; 78.47.jh; 87.15.-v; 87.64.km

INTRODUCTION

Physicists have been trying to explain naturally occurring phenomena by the so-called "Laws of Physics" for ages. Nowadays they have gotten to the point that they can predict a vast number of situations or events. For the last decennia biophysicists have been trying to make up a complementary set of laws of biophysics in order to be able to predict biomolecular interactions, optical, physical and other properties of biomolecules and much more. Here, we need to take into account that biomolecular systems are much more complex than small molecules. Because of the size of biomolecules, the calculation of their properties requires larger computational resources than that of small organic molecules. So, scientists have already developed Molecular Mechanics (MM) and Molecular Dynamics (MD) algorithms for modeling biomolecular dynamics and interactions[1,2], while the computation of the linear and nonlinear optical properties of biomolecules - to our knowledge - still requires quantum chemical treatments. Nevertheless, these calculations can only retain those molecular fragments responsible for the major part of the optical responses while the effects of the surrounding can be incorporated using electrostatic schemes[3].

Our research contributes to the prediction of the first hyperpolarizability (β), a second order nonlinear optical property, of a set of fluorescent proteins (FPs), specifically concentrated on the included chromophores. Usually we can assume that small organic chromophores move freely in solution and they can only interact with each other and with the solvent. The chromophores of FPs on the other hand are located inside a so-called beta-barrel, protected from the environment by the 3D structure of the rest of the protein, and are kept in position by interactions with neighboring atoms inside the protein (Fig. 1). This produces a lot more possible interactions for the chromophore than in the case of small organic molecules in solution, and hence many more factors to take into account in the computations.

METHODS

For both experimental and computational methods, we refer to previous publications (Refs. 4 and 5). Nevertheless, the structure of mStrawberry – obtained from geometry optimization under constraints – has been determined here at the DFT level using the B3LYP hybrid exchange-correlation functional whereas the geometry reported in ref. 4 was determined using the semiempirical PM3 Hamiltonian.

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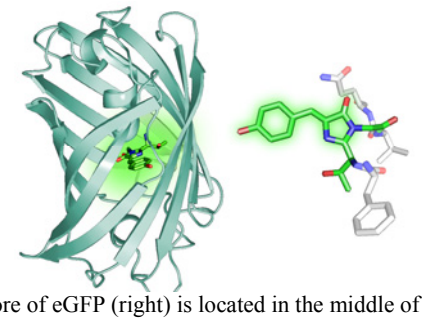

FIGURE 1. The chromophore of eGFP (right) is located in the middle of a so-called beta-barrel (left).

RESULTS

The nonlinear optical properties determined by Hyper Rayleigh Scattering (HRS) are listed in Table 1, followed by the computed values for all measured fluorescent proteins. In this table $\beta_{HRS,800}$ is the dynamic hyperpolarizability using 800 nm laser light after correction for fluorescence contribution, and $\beta_{HRS,\infty}$ is the static hyperpolarizability, being $\beta_{HRS,800}$ after an additional correction for resonance enhancement using the two-state approximation (TSA), since we are measuring under resonance conditions. Even though we did not expect two-photon fluorescence contribution at 400 nm for all chromophores, all measurements show a certain amount of emission at 400 nm, but at the same time enabled us to correct for this contribution performing demodulation of the apparent β.[6,7]

TABLE 1. Experimental (lines 1+2) and calculated β_{HRS} values for 6 fluorescent proteins

	EGFP	EYFP	ZsYellow	DsRed	mStrawberry	mCherry
$\beta_{HRS,800}$	107 ± 17	37 ± 4	83 ± 4	81 ± 8	104 ± 5	134 ± 16.5
$\beta_{HRS,\infty}$ (TSA)	33 ± 5	14 ± 2	35 ± 2	39 ± 4	54 ± 3	71 ± 9
$\beta_{HRS,\infty}$ HF/IEFPCM (solvent = H_2O)	31	31	39	39	84	65
$\beta_{HRS,1900}$ HF/IEFPCM (solvent = H_2O)	14	15	20	21	44	39
$\beta_{HRS,1064}$ HF/IEFPCM (solvent = H_2O)	25	26	48	44	175	124

In order to better map the real structure and properties, in addition to evaluating β_{HRS} for the chromophore, additional calculations were carried out by considering seemingly important amino acid residues surrounding the chromophore. For eYFP it is widely recognized that the π-stacked Tyrosine203 residue in the structure contributes to the bathochromic shift in its linear optical properties. Therefore, we performed the calculation of the eYFP chromophore plus its TYR203 residue. As to compare with another yellow FP, we also checked the calculated value for the chromophore of ZsYellow including its adjacent Histidine201 residue, showing no stacking. These results are shown in Table 2.

TABLE 2. Additional calculated β_{HRS} values for chromophore models including neighboring residues.

	EYFP + Tyr203	ZsYellow + His201
$\beta_{HRS,\infty}$ HF/IEFPCM (solvent = H_2O)	25	34
$\beta_{HRS,1900}$ HF/IEFPCM (solvent = H_2O)	12	19
$\beta_{HRS,1064}$ HF/IEFPCM (solvent = H_2O)	21	49

DISCUSSION

Our previous reports already showed that computation of β_{HRS} of the chromophores of FPs, although not straightforward, is possible. Most calculated values are in acceptable agreement with the experimental data. We would like to stress though that there are a number of factors that seem to be very important to take into account in the calculations.

Dependence on Experimental Data

As long as protein structures cannot be completely predicted, our calculations will depend on x-ray structures. Hence, our computational contribution is limited by the number of proteins of which the tertiary structure (3D,

intramolecular interactions) or even quarternary structure (intermolecular interactions) has been resolved, and also by the resolution of these structures. It is important to note here that a crystallographic (x-ray) structure does not necessarily represent the correct structure of the protein in solution, since the proteins were crystallized in different and sometimes extreme conditions.

Bond Length Alternation

The structure of the conjugated system in the chromophore of DsRed, mStrawberry and mCherry at first sight look identical (Fig 2A), while we both measured and calculated different values for all three chromophores. A closer look at the chromophores reveals indeed small but important differences. First of all we observe considerable differences in the bond length alternations of the chromophores π-conjugated path. These are probably caused by interactions with the surrounding residues affecting the behavior of the electron cloud of the conjugated system, Van der Waals forces pushing atoms aside or strong H-bonds twisting or stretching bonds inside the chromophore. These differences in bond length alternation have a significant effect on the calculated first hyperpolarizabilities.

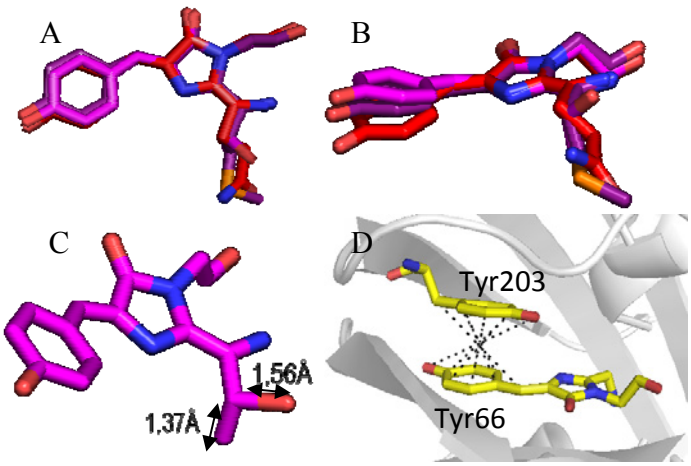

FIGURE 2. [A] The conjugated system of the chromophores of DsRed, mStrawberry and mCherry look almost identical while [B] shows the difference in planarity of the chromophores. [C] We found at least 2 dubious bond lengths in the chromophore of mStrawberry (2H5P). [D] Tyr203 is located centrosymmetrically compared to the Tyr66 residue of the chromophore.

Planarity

Another important but related difference appearing in the x-ray structures is the degree of planarity of the chromophores. The interaction of the chromophore with its surrounding is so that the chromophores of mStrawberry and mCherry considerably deviate from perfect planarity (Fig 2B). The exact effect of this deviation is unclear, since the x-ray data of mStrawberry revealed an additional defect, which will be discussed in the next paragraph.

Accuracy of X-ray Structure

The previous paragraphs already suggest that an accurate x-ray structure is essential. During our investigation we encountered some irregularities in the crystal structure of mStrawberry (2H5P and ref. 8), being a couple of dubious bond lengths in the first residue of the chromophore (Thr66). (Fig. 2D) Even though these bonds are no part of the chromophore π-conjugated system, they are included in the model used for the computation of β. This way they could influence the calculation and give us erratic results. However, this kind of chromophores is not flexible in its environment, so even though we found dubious bond lengths, we have to respect the given structure instead of having the system relax to its lowest energy state. Full geometry relaxation is therefore not an option knowing that the bond length alternation and planarity are essential factors for our calculations. Refinement of the existing data of the 2H5P crystal structure was not successful, so we started to purify mStrawberry in order to make a crystal structure concentrating on the chromophore and surroundings.

Dependence on Surrounding Conditions

Interactions with Neighbouring Residues

As previously mentioned, it is widely known that the π-stacked Tyrosine203 residue in eYFP causes a bathochromic shift in the linear absorption and fluorescence spectra compared to eGFP. Our results in Table 2 show that it is essential to include this residue in our calculations too. Table 2 gives better estimates for $\beta_{HRS,\infty}$ of both eYFP and ZsYellow compared with the experimental results in Table 1. Note that in eYFP this residue introduces a centrosymmetric element in the structure, causing a significant drop of β.

The interactions with the surrounding amino acid residues are also very important because they can cause the chromophore structure to deviate from lowest energy conformation. Changes in the bond length alternation and planarity of the chromophore produce large deviations in the calculated β values. This way, changes in pH causing a H-bond to appear or break might have a different effect on β than the difference in calculated value of the protonated versus the deprotonated chromophore.

Solvent: pH and Ionic Strength

It is commonly known that the linear optical properties of several FPs are very sensitive to changes in pH of the environment[8]. Preliminary results show that the nonlinear optical properties also change upon changes in acidity of the buffer.

Next to the pH of the solution, the ionic strength of the buffer composition has a surprisingly large influence on β, knowing that in general the ionic strength does not alter the linear optical properties. Ionic strength and pH of the buffer used in experiments determine the exact conformation and the behavior of the electron cloud of the conjugated system in a chromophore, and hence the optical properties of the molecules.

CONCLUSIONS

Existing models to calculate the first hyperpolarizability of small organic molecules are a good start to predict β of the chromophores inside fluorescent proteins. Nevertheless, this situation is much more complex: the nonlinear optical properties are very sensitive to changes in buffering conditions (pH, ionic strength…) and the presence of centrosymmetrical elements. Correct and detailed x-ray structures are essential to the calculations, and even then we will need to find algorithms to find out how many of the surrounding atoms and details from the environment are crucial to include into the model of the chromophore.

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REFERENCES

1. J. Aqvist, V.B. Luzhkov and B.O. Brandsdal, *Acc. Chem. Res.* 35 (6) 358-365 (2002)
2. N. Huang, C. Kalyanaraman, K. Bernacki and M.P. Jacobson, *Phys. Chem. Chem. Phys.* **8** (44) 5166-5177 (2006)
3. M.B. Kanoun and B. Champagne, Int. J. Quantum Chem., *in press*
4. E. De Meulenaere, I. Asselberghs, M. de Wergifosse, E. Botek, S. Spaepen, B. Champagne, J. Vanderleyden and K. Clays, *J. Mater. Chem.* **19**, 7514-7519 (2009).
5. E. De Meulenaere, M. de Wergifosse, E. Botek, S. Spaepen, B. Champagne, J. Vanderleyden and K. Clays, *J. Nonl. Opt. Phys. Mat.* **19**, 1-13 (2010).
6. G. Olbrechts, R. Strobbe, K. Clays and A. Persoons, *Rev. Sci. Instrum*. **69** (6), 2233-2241 (1998)
7. K. Clays, K. Wostyn, K. Binnemans and A. Persoons, *Rev. Sci. Instrum*. **72** (8), 3215-3220 (2001).
8. X. Shu, N. C. Shaner, C. A. Yarbrough, R. Y. Tsien and S. J. Remington, *Biochemistry* **45**, 9639-9647 (2006)