Report on the doctoral thesis by Petr Pospíšil (Fluorescence Spectroscopy: Advanced Methods and Their Defined Applications in Protein Science)

The dissertation thesis deals with application of various methods of fluorescence spectroscopy to study properties of various chromophores, mostly in proteins, except the chapter 3.4 that somehow does not match the title of the thesis as it describes spectroscopic properties of metallocorolles in solution. The thesis is divided into three main chapters – Introduction, Methods and Results. It is written in a good English in a readable style allowing to access the information easily even for the readers who are not experts in the field.

The introductory part explains basics of interaction of light and matter, summarizing all important aspects of basic photoinduced processes in molecules. It may serve as a useful reference material for anybody who will follow the project. All important aspects of the topic are included and explained in an understandable way. Yet, there are some formulations that deserve explanation. As an example, concept of "molecular contact" when describing the quenching is unclear to me and the author may explain what he actually means by this term during the thesis defense. Also, I would not include quenching in Jablonski diagram, but I understand this is a matter of taste. Along the same lines, putting the rates associated with quenching into the figure is problematic, because there are a number of examples when the quenching rate is much faster than 10^{-11} s, the upper limit mentioned in the figure (e.g. electron injection in dye-sensitized solar cells or charge separation in photosynthetic reaction centers).

The second chapter provides description of all experimental methods used in the thesis. Again, a good reference text for potential successors, except for a few issues that somewhat lower the quality of the text. For example, the very first subchapter 2.1 would deserve at least one equation describing the Beer-Lambert law, if the author think that a scheme of absorption measurement is too elementary to show it in the thesis. I understand that the author deals mainly with fluorescence measurements, but to describe the Beer-Lambert law in words (moreover without mentioning the existence of extinction coefficient) is a bit off for a text like this.

Yet, these minor mistakes do not impair the quality of the work done by the author. The core of the doctoral thesis lies in Chapter 3 that summarizes the results presented in the thesis. It is clear that the author mastered a number of sophisticated fluorescence techniques and applied them successfully to study various systems. I especially value the range of different fluorescence techniques used in the thesis. I am somehow missing the statement clarifying the role of the author in each project (paper), because all project are clearly collaborative projects. This should be clarified during the thesis defense along with answering some questions outlined below:

1. On p. 10 the author writes that "…the forbidden transitions can be allowed…by intersystem crossing from an excited state…". How the ISC can make the transition allowed? I would say that ISC is the consequence of the fact that the transition is partially allowed, not the reason for it.

2. On p. 26, when describing the time-dependent fluorescence shift, the author claims that the process starts from the initial non-equilibrium FC state when the excited molecule is in the lowest vibrational state. Can the author explain in more detail what he means? After vertical

transition from the relaxed ground state the molecule reach FC region of the excited state that is certainly (except maybe some very special cases) not the lowest vibrational state.

3. In Chapter 3.2, the origin of the multiexponential decay of Badan-labelled CYPs is assigned to conformations for which Badan can be quenched by Forster transfer to heme. How far is the heme from Badan? Is the observed rate feasible for the expected distance?

4. When exciting Badan at 373 nm (Chapter 3.2.), the heme in CYP will be also partially excited. Can this fact affect the interpretation of the results?

5. What are the major limitations of the used quantum chemical methods for calculations of electronic properties of studied systems? For example, in calculations of electronic properties of molecules, it is known that TDDFT is notoriously problematic in calculations of charge-transfer states that could be present for example in metallocorroles that are the subject of Chapter 3.4.

Overall, I have no reservations that this thesis fulfills the requirements of the doctoral thesis. The candidate did a great piece of scientific work and proved that he is able to contribute significantly to the development in the field. The thesis in my opinion fulfills the criteria for being accepted as a ground for awarding Petr Pospíšil with the title Ph.D. and should be accepted for the defense.

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