



Fluorescence Spectroscopy: Advanced methods and their defined applications in protein science

Submitted by Mgr. Petr Pospíšil

PhD advisor's opinion on the PhD work

Mgr. Petr Pospíšil joined my laboratory in July 2012 with the unfortunate starting situation of having been already two years enrolled in the PhD program of the Faculty of Mathematics and Physics, Charles University in Prague without publishable results. On the other hand, Mgr. Petr Pospíšil finalized already all experiments for his PhD thesis in December 2016, which means that it took him about four and half year to acquire enough scientific results in order to compile and submit a PhD thesis, based on three already published publications and two publications which are indeed in preparation. In my opinion this testifies that Mgr. Petr Pospíšil has been an efficient PhD student, despite his quite unfortunate start in the PhD program of the Faculty of Mathematics and Physics.

The basic line of his work in my laboratory was to use state of the art (fluorescence) spectroscopy techniques to answer actual question in protein and DNA sciences. Regarding these fluorescence techniques, he mastered in my laboratory the time-dependent fluorescence shift (TDFS) technique as well as fluorescence cross-correlation spectroscopy (FCCS). Moreover, during his stays at EPFL, Lausanne, and Rutherford Appleton Laboratories, Harwell Oxford he got acquainted with fluorescence up-conversion and time-resolved infrared (TRIR) experiments, respectively. Viewing the variety of these techniques addressing processes from milliseconds to femtoseconds as well as from single molecules to ensemble of molecules, one can conclude that Mgr. Petr Pospíšil became a spectroscopist with rather broad knowledge. This is certainly also one reason that after his contract in my Institute ended, he immediately found a post-doc position in another, spectroscopy oriented lab.

Specifically, Mgr. Petr Pospíšil used the TDFS technique to map DNA interactions with proteins and lipids by a newly synthesized fluorene dye, leading to a publication in Chemical Sciences. Again it was the TDFS technique which allowed to investigate the effect of the membrane lateral pressure on the hydration and dynamics at the active site of a ATPase. The results of this study were summarized in a paper which appeared in *Angewandte Chemie*. A careful TDFS study in cytochromes P450 led to a detailed understanding of the interaction of BADAN, a typical TDFS probe, and tryptohan residues in proteins. This study which is essential for the general application of the TDFS technique appeared in *Journal of Physical Chemistry B*. The fourth topic involved investigations of heavy atom effects on the excited state relaxation processes by the fluorescence up-conversion approach in iodinated metalloporphyrins, which are promising dyes for biological imaging. The obtained findings are presently summarised for publication. In the last project FCCS was applied to investigate the cooperative binding of two antimicrobial peptides, PGLa and Mag2, to lipid bilayers on a single molecule level.

There is another positive aspect in the PhD work of Mgr. Petr Pospíšil: Due to the interdisciplinary character of those research topic he has collaborated with a series of different scientist. Within the Institute there should be at least mentioned Dr. Mariana Amaro and Prof. Tony Vlček, outside the Institute Profs Karim Fahmy (Dresden), Michal Hocek (Prague), Burkhard Bechinger (Strasbourg) and Majed Chergui (Lausanne).

Just by summarizing his activities, it is evident that Mgr. Petr Pospíšil has been a very good PhD student. There is absolutely no doubt that his PhD thesis should be accepted by the PhD examination board. For the case Mgr. Petr Pospíšil will successfully defend his thesis, he should be awarded with the title PhD.

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Prof. Dr. Martin Hof, DSc.