Posudek práce

předložené na Přírodovědecké fakultě JU

❑ posudek vedoucího	☑ posudek oponenta
☑ bakalářské práce	❑ diplomové práce

Autor:	Karel Divoky
Název práce:	Fluorescentní protein citlivý na elektrické napětí, využívající motorický protein prestin
Studijní program a obor:	Biofyzika, Biofyzika
Rok odevzdání:	2011

Jméno a tituly oponenta:	doc. RNDr. Rudiger Ettrich, PhD.
Pracoviště:	Ústav Systémové Biologie a Ekologie AVČR
Kontaktní e-mail:	ettrich@nh.usbe.cas.cz

Odborná úroveň práce:
☑ vynikající ❑ velmi dobrá ❑ průměrná ❑ podprůměrná ❑ nevyhovující

Věcné chyby:
❑ téměř žádné ☑ vzhledem k rozsahu přiměřený počet ❑ méně podstatné četné ❑ závažné

Výsledky:
❑ originální ☑ původní i převzaté ❑ netriviální kompilace ❑ citované z literatury ❑ opsané

Rozsah práce:
❑ veliký ☑ standardní ❑ dostatečný ❑ nedostatečný

Grafická, jazyková a formální úroveň:
❑ vynikající ☑ velmi dobrá ❑ průměrná ❑ podprůměrná ❑ nevyhovující

Tiskové chyby:
☑ téměř žádné ❑ vzhledem k rozsahu a tématu přiměřený počet ❑ četné

Celková úroveň práce:
☑ vynikající ❑ velmi dobrá ❑ průměrná ❑ podprůměrná ❑ nevyhovující

Slovní vyjádření, komentáře a připomínky oponenta:

In his bachelor thesis Karel Divoky describes the preparation, expression and characterization of a series of fluorescence labeled constructs based on the motor protein prestin, with the aim to get a genetically encoded optical probe of cell membrane voltage. For this puropse Karel Divoky used

four different plasmids encoding fusion proteins of prestin with yellow fluorescence protein YFP, expressed these in mammalian cells, and measured their linear dichroism by two photon polarization microscopy. Karel Divoky used the full spectrum of molecular biology methods in protein expression and purification, which means transformation, DNA isolation, restriction, electrophoresis, PCR, cell passaging, and transfection. In the next step the transfected HEK cells after 24-48h in the incubator were examined using the two photon polarization microscope. This technique was recently developed and patented in the Lazar lab and the instrument used by Karel Divoky is the only one existing to my knowledge to date. The instrument is modular throughout and still far from being commerical, which in fact is this is an excellent opportunity for a student of biophysics to play with the set up and to gain a deep understanding of the underlying physics and its realization within the set-up. I therefore consider the topic, with its combination of both, molecular and cell biology as well as physics to be an optimal choice for a bachelor thesis.
The bachelor thesis of Karel Divoky follows the standard scheme for this type of publication, with the first half being a literature introduction into the topic and methodology, followed by the experimental or research part divided into aim, materials and methods, results, discussion and conclusions. The thesis closes with the conclusion that the very ambitious aim of having a genetically encoded optical probe was not fullfilled to the full extend, mainly due to problems on the molecular biology side, but that the preliminary results give a good starting point for the continuation of the work and hope to reach the aim on a later stage. I think this does not matter at all, or even I would say the value of participation in a non-standard hot topic project is a much higher value than could be the fullfillment of a easy-to-reach standard aim one often finds in bachelor works. Facing non-standard problems that one can solve only by scientific thinking and hard work is an experience that must be highly appreciated.
I certainly can recommend Karel Divoky for being awarded the Bsc. degree.

Případné otázky při obhajobě a náměty do diskuze:

The construct A744YFP-P.1-pEPAx2.4 was successfully transfected to HEK293 cells and the two photon fuorescence was observed. However, the construct is an intermediate construct and was not in an expression vector. In the discussion I miss a detailed scientific discussion of this preliminary result, which means figure 12 in comparison with figure 13, and how the result can be interpreted. It is always important for a student to be aware of the fact that most of the readers of publications are never doing excatly the same, and therefore do not have the same deep knowledge, especially not in the case when one works with a unique set up. But in fact these are the readers one needs to reach AND convince of the results. In the introduction there is mentioned that the linear dichroism can be identified in the figures as the presence of green and red color and the absence of yellow. In figure 12 I can hardly identify any color by eye, but of course there are ways to determine the colors and actually in the figure legend a number is given for the LD. Could you please discuss, explain and compare both figures (12,13) in a way one would like to read in a scientific paper, maybe even repeating explanations from the introduction? Pretend I am the grant agency financing your work and you want to convince me with your results that I should continue paying your research.

Práci
☑ doporučuji
☐ nedoporučuji
uznat jako bakalářskou.

Navrhuji hodnocení stupněm:
☑ výborně ☐ velmi dobře ☐ dobře ☐ neprospěl/a

Místo, datum a podpis oponenta: Nové Hrady, 19.1.2011, Rudiger Ettrich