ÚFE Institute of Photonics and Electronics
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Doctoral thesis review from the reviewer Michal Cifra

Title of the doctoral thesis:
REORGANIZATION OF IN VITRO RECONSTITUTED ACTIN-BASED NETWORKS

Author of the doctoral thesis:
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This PhD thesis investigates the cytoskeleton's role in cellular motion and structural reorganization, focusing on two main areas: neuronal growth cone navigation during brain development and the creation of chiral actin flows during cellular division. The thesis first explores the crosstalk between actin filaments and microtubules in neuronal growth cones, particularly the role of the microtubule polymerase CKAP5. Using TIRF microscopy and in vitro assays, it demonstrates that CKAP5 can bundle actin filaments, crosslink them with microtubules, and enable dynamic interactions between these cytoskeletal components.
Additionally, the research examines the actomyosin cortex's dynamic remodeling to create chiral actin flows, crucial for establishing left-right body asymmetry in dividing embryos. The study finds that while myosins and formins are involved in symmetry breaking, the friction between growing actin filaments and surfaces induces chirality with a counterclockwise bias. Lastly, the thesis introduces a methodological advancement that simplifies the preparation phase for in vitro reconstitution assays using fluorescence microscopy.
A large number of reconstitution assays were designed, optimized, performed, and both quantitatively and qualitatively analyzed and interpreted. The thesis is well-written, with the results clearly described with high level of graphics quality. Notably, I did not find any typographical errors, which is quite rare.

Questions:

1. Given the nature of TIRF microscopy, all data are from the cytoskeletal fibers behavior on a surface, which is basically 2D, maybe 2.5D system. How can be these findings transferred to the 3D reality of cells?
    a. Especially, what about the case of the chirality emergence? There the dimensionality could play an important role.
2. p.36 “and Matlab (R2020b)” what part of the analysis exactly was done in Matlab?
3. Results, part 4.1 CKAP5 – how does the employed CKAP5 concentration (1-10 nM) compares to CKAP5 biological concentrations?
4. Discussion, part 4.1, p.63 - What is the nature of the intermolecular forces underlying MT-CKAP5 and actin-CKAP5 (TOG domains) interactions, respectively? How could this be uncovered and tested?
5. Fig. 4.3: there is a remarkable difference of density of CKAP5 coverage and then the actin on MTs for 1 nM vs 4 nM CKAP5 concentration – why so? What might be a factor determining the critical concentration required for the actin-MT crosslinking?

6. p. 62 – “CKAP5 is bundling and binding only to thick (~ 32 filament) bundles” – how can that work from a molecular structural perspective?
7. p. 71- how did you define “chirality initiation time” (Fig. 4.17F)?
8. p. 74 - “which indicated that chiral reorganization of the bead-centered network was induced at 17.8 μm diameter for both CW and CCW-oriented actin networks” What might be the underlying characteristics which defines this spatial scale (17.8 μm diameter of oriented actin networks)? How this could be tested?
9. SciRep paper – which type of PMT was used?

Comments

10. It would be practical to provide also molar concentrations within the same context, e.g. “Experiments were initiated by the introduction of a mixture composed of 10 nM CKAP5-mNG and 40 μg/ml stabilized actin filaments”

Overall, the thesis is of a very high quality, and I recommend its defense.

Prague, 25th May, 2024
Ing. Michal Cifra, PhD.
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