

Development of a synthetic 'cilium localization sequence' for selective delivery of cargo to the primary cilium

Ioannis, Tsakoumagkos

ioannis.tsakoumagkos@unige.ch

The primary cilium is a protruding microtubule-based organelle, present in one copy in almost all mammalian cells. Although the ciliary membrane is continuous with the plasma membrane and the primary cilium is not physically separated from the cytosol, it maintains a unique protein and lipid composition.^{1,2} Intriguingly, the cilium lacks the machinery required for protein synthesis and the entry, retention and exit of proteins is mediated by complex ciliary transport mechanisms, the details of which are not yet fully determined.^{3,4,5} While ciliary proteins often bear specific signal sequences called Ciliary Targeting Sequences (CTS) to ensure their targeting to the cilium, synthetic modalities for ciliary targeting have not been reported.⁶ In this work, we focused on the development of a synthetic ciliary targeting sequence, synCTS, based on the structure of INPP5E, a protein transported into the primary cilium by the carrier protein PDE6d. Fluorescence polarisation assay of the synCTS and PDE6d showed a strong binding affinity in the low nM range, and verified the importance of the farnesyl group for this synCTS-PDE6d interaction. To evaluate the properties of synCTS, we developed a ciliary chloroalkane penetration assay (cilium-CAPA) in NIH/3T3 cells stably expressing an ARL13b-HaloTag-eGFP fusion protein. Using this assay, we validated the dose-dependent ciliary localization of the synCTS-candidates. Moreover, we demonstrated that the non-farnesylated peptide showed decreased ciliary localization in comparison to the farnesylated one.

References:

- [1] Nachury, M. V.; Mick, D. U., *Nat. Rev. Mol. Cell Biol.* **2019**, 20, 389–405..
 - [2] Fry, A. M.; Leaper, M. J.; Bayliss, R., *Organogenesis* **2014**, 10, 62–68.
 - [3] Breslow, D. K.; Koslover, E. F.; Spakowitz, A. J.; Nachury, M. V., *J. Cell Biol.* **2013**, 203, 129–147.
 - [4] Shi, X.; Garcia, G.; Van De Weghe, J. C.; McGorty, R.; Pazour, G. J.; Doherty, D.; Huang, B.; Reiter, J. F., *Nat. Cell Biol.* **2017**, 19, 1178–1188.
 - [5] Malicki, J.; Avidor-Reiss, T., *Organogenesis* **2014**, 10, 138–157.
 - [6] Cilleros-Rodriguez, D.; Martin-Morales, R., *eLife* **2022**, 11, e78383.
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