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PII: S0005-2736(17)30029-9
DOI: doi:10.1016/j.bbamem.2017.01.021
Reference: BBAMEM 82403

To appear in: *BBA - Biomembranes*

Received date: 4 October 2016
Revised date: 31 December 2016
Accepted date: 19 January 2017

Please cite this article as: Volodymyr V. Shvadchak, Oleksandr Kucherak, Kseniia Afitska, Dmytro Dziuba, Dmytro A. Yushchenko, Environmentally sensitive probes for monitoring protein-membrane interactions at nanomolar concentrations, *BBA - Biomembranes* (2017), doi:10.1016/j.bbamem.2017.01.021

Environmentally sensitive probes for monitoring protein-membrane interactions at nanomolar concentrations

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Keywords: solvatochromic probes, fluorescence, protein-membrane interaction, affinity, binding stoichiometry, α-synuclein

Highlights

- A series of thiol-reactive highly solvatochromic probes was synthesized
- The sensitivities of the probes for protein-membrane interaction were compared
- Effects of scattering on probe sensitivity were analyzed
- Developed protocol for determining Kd and stoichiometry of protein-membrane binding
- Affinity and stoichiometry of α-synuclein to lipid membranes was determined

Abstract

Solvatochromic probes are suitable tools for quantitative characterization of protein-membrane interactions. Based on diverse fluorophores these probes have different fluorescent properties and therefore demonstrate different responses when applied for sensing the interactions of biomolecules. Surprisingly, to the best of our knowledge, no systematic comparison of the sensitivities of solvatochromic dyes for monitoring protein-membrane interactions was described. Hence, a rational choice of an optimal environmentally sensitive probe for such experiments is usually not a straightforward task.

In this work we developed a series of thiol-reactive fluorescent probes based on the fluorophores with high sensitivity to their environment and compared them with two widely used DNS and DMN probes. We investigated the responses of these probes to the interaction of probe-labeled presynaptic protein α-synuclein with lipid membranes. We observed that newly synthesized probes based on fluorene and chromone dyes, which combine the strongest brightness and significant changes of fluorescence intensity, demonstrated the highest sensitivity to interaction of α-synuclein with lipid membranes. They are especially beneficial for sensing in scattering media such as solutions of lipid vesicles.

We show that the described probes permit quantitative measurements of α-synuclein binding to lipid membranes at low nanomolar concentrations. We developed a detailed protocol for measuring K_d and binding stoichiometry for interaction of soluble peripheral proteins with membranes based on the response of the environmentally sensitive fluorescent probes. We applied this protocol for quantification of the affinity of α-synuclein to anionic membranes and found that it is substantially higher than it was earlier reported.