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POSTER ABSTRACT PRESENTATIONS

SESSION TITLE: STRUCTURE OF MEMBRANE PROTEINS

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Abstract P-17: Photophysical Properties of Freely Diffusing and Immobilized Fluorescent Conjugate Based on Calcium-Binding Protein Recoverin and Alexa647 Dye

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Background: Recoverin is a calcium sensor membrane-associated protein that inhibits rhodopsin kinase thereby participating in the regulation of visual transduction. Here we examined calcium-induced conformational changes in recoverin conjugated with fluorescent dye Alexa647.
Methods: Photophysical properties of immobilized and freely diffusing recoverin were investigated using fluorescence lifetime imaging microscopy and fluorescence emission spectroscopy. In solution, the formation and dissociation of the Ca^{2+}-recoverin complex manifested as changes in Alexa647 spectra and the lifetime. In contrast, immobilization of recoverin on the microscopy glass via biotin–NeutrAvidin–biotinylated polyethylene glycol (PEG) tether inhibited changes in fluorescent signal. That can be provided by PEG as it prevented the calcium-induced changes in spectrum and lifetime of recoverin-bound Alexa647 in solution. The use of another immobilization facilitator, bovine serum albumin (BSA), did not affect calcium-induced changes in fluorescence of the conjugate in solution but produced the matrix, which was ineffective in recoverin immobilization.
Results: Microscale thermophoresis demonstrated that biotinylated recoverin interacted with NeutrAvidin in solution indicating that immobilization affinity depended mainly on the geometry of the glass coating surface.

Conclusion: Our results highlight the challenge of specific protein immobilization that does not affect protein functionality. By the example of recoverin, we showed that the employment of two common immobilization facilitators, PEG and BSA, yielded surfaces with different space geometry, which differently affect NeutrAvidin-based immobilization affinity as well as Ca^{2+}-dependent conformational changes of the biotinylated protein.

Key Words: Recoverin • microscale thermophoresis • protein immobilization

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