Ultrabright Turn-On Fluorescent Probe based on Squaraine Dendrimer

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Fluorescent probes, which lights up only in response to a specific target or event, have attracted considerable attention in the recent years due to its advantage of wash-free fluorescence imaging *in vivo*.[1],[2] As they are practically non-fluorescent in their free form at polar media, they can drastically decrease the background and thus improve the signal-to-background ratio in fluorescence imaging. We recently showed such background-free imaging by using a new concept of fluorogenic dye based on a squaraine dimer that unfolds on changing environment from aqueous to organic and thus turns on its fluorescence on binding to a target protein.[3] In continuation with our effort, we decided to increase the fluorogenic response together with the brightness (expressed as ε × quantum yield) of the probe. To this end, we coupled eight squaraine dyes to a lysine-based dendrimer. This design should increase the brightness by the factor of eight provided the quantum yield of the dendrimer remains the same. However, the developed octamer exhibits low quantum yield even after unfolding in the organic solvents, which hints the possibility of self-quenching between the overcrowded squaraine dyes. To circumvent this problem, we placed a PEG_8 linker between lysine core and the squaraine dye that enables environment sensitive folding and unfolding of the individual dyes that are sufficiently far from each other. Incorporation of PEG groups should also minimize the nonspecific interactions at the cell surface, which will be beneficiary for target-selective cellular imaging. Photophysical characterization shows that the PEGylated octamer exhibits poor fluorescence in water due to the folded form with π-stacked self-quenched dyes, whereas in organic solvents, the octamer unfolds and exhibits bright fluorescence. Remarkably, PEGylated octamer shows 335-fold enhancement of the fluorescence quantum yield in dioxane when compared to water. In order to convert this PEGylated octamer into fluorogenic probe for biomolecules, it was modified with different ligands and further tested in cells expressing corresponding target biomolecules. The obtained environment-sensitive dendrimer constitutes a promising building block for construction of ultrabright probes with turn-on response to biomolecular targets.

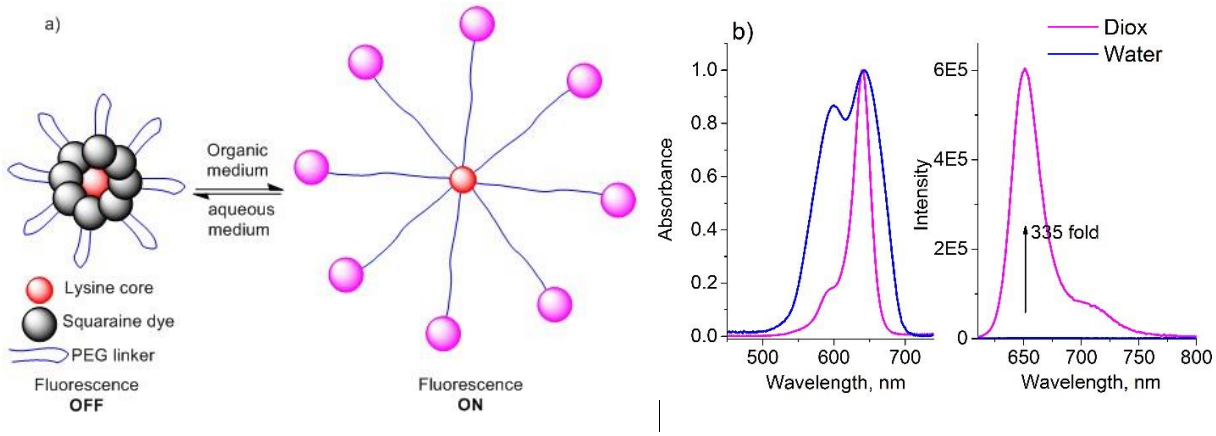

Figure 1. a) Schematic representation of environment-dependent folding and unfolding of PEGylated octamer; b) Absorption and fluorescence spectra of PEGylated octamer in water and dioxane.

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References:

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