Structural investigation of the transmembrane C domain of the mannitol permease from Escherichia coli using 5-FTrp fluorescence spectroscopy
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Impact of the C\(_{10}\)E\(_5\) detergent micelle on the solvent accessibility and rotational mobility of tryptophan octyl ester

Tortech et al. [4] have addressed the impact of the detergent belt on the solvent accessibility and rotational mobility of a hydrophobic Trp derivative, tryptophan octyl ester (TOE), embedded in the micelle. In the study of Tortech et al. several detergents were investigated including the neutral PEG-based detergent C\(_{12}\)E\(_8\) [4]. The blue \(\lambda_{\text{max}}\) of \(~335\) nm of TOE when dissolved in detergent solution demonstrated that the indole moiety of TOE is embedded in the micelle. They found that the C\(_{12}\)E\(_8\) micelle is highly permeable to iodide and minimally affects the rotational mobility of the Trp derivative [4]. As this information was not available for the C\(_{10}\)E\(_5\) detergent, we performed similar Stern-Volmer experiments as reported by Tortech et al. TOE dissolved in C\(_{10}\)E\(_5\) buffer showed a \(\lambda_{\text{max}}\) of \(333\) nm and the probe was found to be efficiently quenched by KI, yielding a \(K_\text{sv}\) value of \(3.5\) M\(^{-1}\) (Fig. S1). The \(\tau_{\text{av}}\) \((\tau_{\text{av}} = \sum \alpha \tau_i)\) of TOE is \(1.8\) ns (Fig. S2A), yielding a \(k_q\) value of \(1.9 \times 10^9\) M\(^{-1}\)s\(^{-1}\). This \(k_q\) value is \(46\%\) of the value of \(k_q\) for NATA (N-acetyl-tryptophanamide) in this buffer \((4.2 \times 10^9\) M\(^{-1}\)s\(^{-1}\) [4]). When corrected for the slow translational diffusional rate constant of TOE in C\(_{10}\)E\(_5\) micelles, compared to free NATA, as detailed in [4], a \(k_q\) value of \(60\%\) of the value of NATA is obtained. For TOE in C\(_{12}\)E\(_8\) micelles a corrected \(k_q\) value of \(83\%\) of the value of NATA was reported [4]. These measurements show that the C\(_{10}\)E\(_5\) micelles are permeable to iodide,
although less than C_{12}E_8 micelles. We showed before that introduction of a fluor atom at the 5 position of Trp does not affect the KI quenching [5].

The rotational mobility of TOE in C_{10}E_5 is high as a rotational correlation time (\( \phi \)) of 0.3 ns was measured (\( \beta = 0.123 \)) together with a small fraction (\( \beta = 0.004 \)) showing a \( \phi = 2.1 \) ns (Fig. S2B). Taken together, the presence of C_{10}E_5 has only a small impact on the iodide quenching data and the rotational mobility data of the 5-FTrp side chain, presented in this manuscript.

**Fig. S1.** Stern-Volmer plot of Trp octyl ester (TOE) in C_{10}E_5 micelles with KI as quencher*. 

![Stern-Volmer plot](image)

*Conditions: (■) 5 \( \mu \)M TOE in 10 mM sodium phosphate buffer, pH= 7.5 at 20 °C, supplemented with 4 mM C_{10}E_5, (●) 5 \( \mu \)M TOE in 20 mM Tris-HCl, pH= 8.4 at 23 °C. Aliquots of KI and KCl were used so that [KI] + [KCl] = 0.2 M in each measurement. Excitation wavelength was 280 nm, Ex and Em bandpasses were set at 1.25 nm and 3 nm, respectively. Other experimental details are presented in the Material and Methods section.
Fig. S2. Fluorescence decay kinetics (A) and anisotropy decay kinetics (B) of Trp octyl ester (TOE) embedded in C₁₀E₅ micelles. Upper panels: experimental decays, lower panels – reduced residuals.

\( \chi^2 = 1.18 \)

\( \chi^2 = 1.11 \)

\( \text{Intensity (counts)} \)

\( \text{Anisotropy} \)

Conditions: 5 µM TOE in 10 mM sodium phosphate buffer, pH= 7.5 at 20 °C. The excitation wavelength was 295 nm. Other experimental conditions are as presented in the Materials and Methods section. Three lifetimes were resolved: \( \tau_1 = 0.04 \) ns (\( \alpha = 0.23 \)), \( \tau_2 = 0.99 \) ns (\( \alpha = 0.25 \)), and \( \tau_3 = 2.99 \) ns (\( \alpha = 0.52 \)). Fitting of the anisotropy data gave two rotational correlation times: \( \phi_1 = 0.33 \) ns (\( \beta = 0.123 \)) and \( \phi_2 = 2.10 \) ns (\( \beta = 0.04 \)).
Fig. S3. Fluorescence decays of single 5-FTrp containing mutants of EII\textsuperscript{mtl}.
Upper panels: the experimental decays, middle panels the reduced residuals, lower panels the autocorrelation function of the residuals.
Fig. S4 Anisotropy decays of single 5-FTrp containing mutants of EII<sup>mtl</sup> in absence (black) and in presence (violet) of 50 µM mannitol. The upper panels are anisotropy decays, the middle panels are weighed residuals for the anisotropy decay of mutants in absence of mannitol, and the lower panels are the weighed residuals for the anisotropy decays of mutants in presence of 50 µM mannitol.
References


