## **Supporting Information**

## Utilizing an Aggregate Forming Microenvironment Sensitive Coumarin-

Cholesterol Conjugate as a Sensor of Pluronic Organization and Micro-

## polarity

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Table S1: Few important parameters of P123 and F127.

Trade name	P123	F127
Formula	PEO <sub>20</sub> -PPO <sub>70</sub> -PEO <sub>20</sub>	PEO <sub>100</sub> -PPO <sub>64</sub> -PEO <sub>100</sub>
Core size (Å)	42.1	37.5
Corona thickness (Å)	24.4	70.2
HLB	8	22



Figure S1: Normalized fluorescence spectra of Cum-Chl in presence of 10% (a) P123 and (b) F127 with increasing temperature; at  $\lambda_{ex}$  440 nm.



Figure S2: Variation of steady state fluorescence anisotropy ( $r_{SS}$ ) of Cum-Chl monomer in presence of 10% P123 with increasing temperature; at  $\lambda_{ex}$  440 nm.

Table S2: Fluorescence lifetime data of Cum-Chl monomer and aggregate form in presence of 10% P123 with increasing temperature, ( $\lambda_{ex} = 444 \text{ nm}$ ,  $\lambda_{em} = 470 \text{ nm}$  for monomer and  $\lambda_{ex} = 444 \text{ nm}$ ,  $\lambda_{em} = 566 \text{ nm}$  for aggregate).

Temperature	$\tau_1$ (ns) ( $\beta_1$ )	$\tau_2$ (ns) ( $\beta_2$ )	$\tau_{aveg}$	$\chi^2$	
( <sup>°</sup> C)			(ns)		
5	0.66 (0.89)	2.03 (0.11)	0.81	1.38	
11	0.43 (0.78)	2.05 (0.22)	0.79	1.19	
15	0.31 (0.57)	2.59 (0.43)	1.29	1.25	Monomer
20	0.98 (0.44)	2.66 (0.56)	1.92	1.04	
24	0.81 (0.48)	2.61 (0.52)	1.75	1.06	
5	7.83 (0.53)	26.46 (0.47)	16.59	1.22	
11	4.87 (0.52)	25.83 (0.48)	14.93	1.34	
15	2.40 (0.92)	19.10 (0.08)	3.74	1.17	Aggregate
20	2.11 (0.93)	18.45 (0.07)	3.25	1.30	
24	2.05 (0.93)	17.87 (0.07)	3.16	1.33	





Figure S3: Residue distribution plots of Cum-Chl monomer and aggregate form in presence of 10% P123 with increasing temperature (corresponds to Table S2).



Figure S4: Normalized fluorescence spectra of Cum in presence of 10% (a) P123 and (b) F127 with increasing temperature; at  $\lambda_{ex}$  440 nm.

Table S3: Fluorescence lifetime data of Cum in presence of 10% P123 with increasing temperature ( $\lambda_{ex} = 444 \text{ nm}$ ,  $\lambda_{em} = 480 \text{ nm}$ ).

Temperature (°C)	$\tau_1$ (ns) ( $\beta_1$ )	$\tau_2$ (ns) ( $\beta_2$ )	$\tau_{aveg}$ (ns)	$\chi^2$
5	0.18 (0.99)	0.92 (0.01)	0.19	1.21
11	0.20 (0.99)	1.87 (0.01)	0.22	1.01
15	0.21 (0.96)	2.42 (0.04)	0.30	1.22
20	0.86 (0.63)	2.56 (0.37)	1.50	1.01
24	0.83 (0.63)	2.51 (0.37)	1.45	1.08



Figure S5: Residue distribution plots of Cum in presence of 10% P123 with increasing temperature (corresponds to Table S3).

Table S4: Fluorescence lifetime data of Cum in presence of 10% F127 with increasing temperature ( $\lambda_{ex} = 444 \text{ nm}$ ,  $\lambda_{em} = 480 \text{ nm}$ ).

Temperature (°C)	$\tau_1$ (ns) ( $\beta_1$ )	$\tau_2$ (ns) ( $\beta_2$ )	$\tau_{aveg}$ (ns)	$\chi^2$
13	0.12 (0.99)	1.28 (0.01)	0.13	1.24
17	0.14 (0.99)	1.73 (0.01)	0.16	1.02
21	0.49 (0.76)	2.12 (0.24)	0.88	1.21
27	0.57 (0.74)	2.14 (0.26)	0.98	1.08
34	0.48 (0.76)	2.02 (0.24)	0.85	1.10



Figure S6: Residue distribution plots of Cum in presence of 10% F127 with increasing temperature (corresponds to Table S4).



Figure S7: Intrinsic fluorescence of 10% P123 and F127, (a) emission spectra at  $\lambda_{ex}$  440 nm, (b) excitation spectra at  $\lambda_{em}$  470 nm and (c) excitation spectra at  $\lambda_{em}$  566 nm.





Figure S8: Normalized fluorescence spectra of Cum-Chl, with increasing % of P123 at (a)  $10^{\circ}C$  and (b)  $35^{\circ}C$  and with increasing % of F127 at (c)  $10^{\circ}C$  and (d)  $35^{\circ}C$ ; at  $\lambda_{ex}$  440 nm.



Figure S9: Fluorescence excitation spectra of Cum-Chl ( $\lambda_{em} = 566$  nm) in presence of (a) P123 and (b) F127 at 10°C, inset shows normalized spectra, fluorescence excitation spectra of Cum-Chl ( $\lambda_{em} = 470$  nm) in presence of F127 at (c) 10°C and (d) 35°C.



Figure 10: Normalized fluorescence spectra of Cum, with increasing % of P123 at (a)  $10^{\circ C}$  and (b)  $35^{\circ C}$  and with increasing % of F127 at (c)  $10^{\circ C}$  and (d)  $35^{\circ C}$ ; at  $\lambda_{ex}$  440 nm.