Supplementary Data

Comparative effect of cationic gemini surfactant and its monomeric counterpart on the conformational stability and activity of lysozyme

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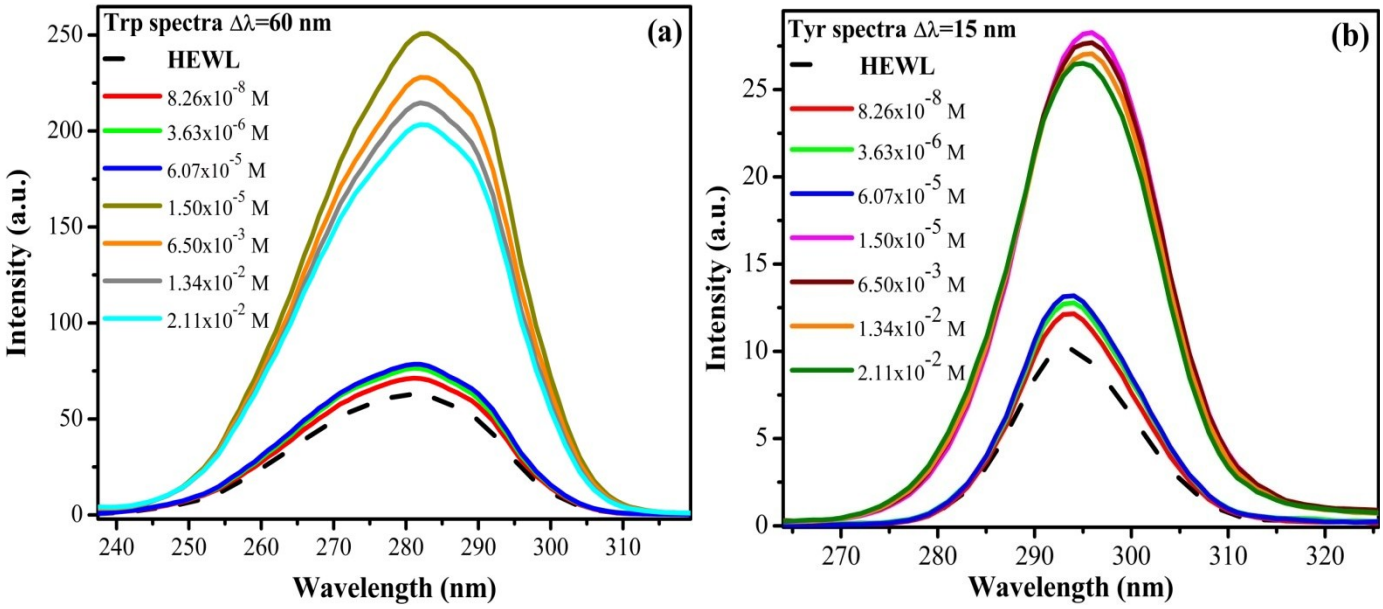

Fig 1S: Synchronous fluorescence spectra of HEWL at varying concentration of M_{16} (a) Δλ=60 nm and (b) Δλ=15 nm at 298K.

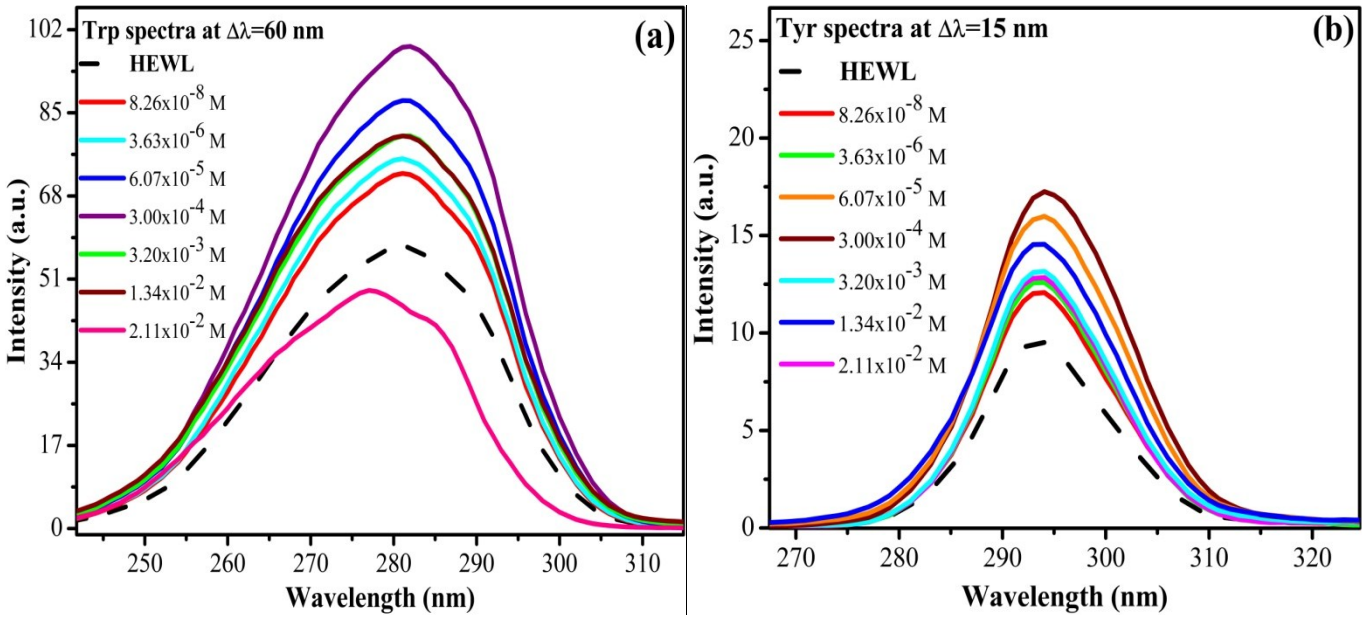

Fig 2S: Synchronous fluorescence spectra of HEWL at varying concentration of G_{16} (a) Δλ=60 nm (b) Δλ=15 nm at 298K.

Table S1. Calculation of α–helical content of HEWL in different M_{16} and G_{16} concentrations[a]

Concentration of surfactant (M)	α- helix (%)
M_{16}	
0	41.54[b]
8.26×10^{-8} M	42.84
6.39×10^{-7} M	43.33
2.10×10^{-4} M	43.69
1.50×10^{-3} M	44.56
3.20×10^{-3} M	39.33
>3.20×10^{-3} M	Cannot be determined[c]
G_{16}	
0	42.10[b]
8.26×10^{-8} M	44.68
2.10×10^{-4} M	43.69
3.00×10^{-4} M	41.90
1.50×10^{-3} M	41.12
>1.50×10^{-3} M	Cannot be determined[c]

[a]Uncertainty in results is ±1%, [b]Native HEWL[1], [c]Due to noise in CD spectra at higher concentration of M_{16} and G_{16}.

Table S2: Fluorescence lifetime parameters of HEWL at various M_{16} concentrations

M_{16} (M)	α_1 (%)	τ_1 (ns)	α_2 (%)	τ_2 (ns)	α_3 (%)	τ_3 (ns)	τ_{avg}	χ^2
0	11.08	0.36	60.81	1.37	28.11	2.81	2.03	0.86
8.26×10^{-8}	12.09	0.39	60.66	1.38	27.25	2.82	2.02	0.86
6.39×10^{-7}	15.02	0.45	63.68	1.50	21.30	2.99	2.03	0.82
3.63×10^{-6}	10.87	0.33	60.80	1.36	28.33	2.83	2.04	0.87
4.68×10^{-6}	16.31	0.51	60.84	1.51	22.85	3.01	2.07	0.84
6.07×10^{-5}	10.22	0.30	62.54	1.37	27.24	2.85	2.04	0.83
2.10×10^{-4}	15.00	0.51	62.95	1.65	22.05	3.31	2.26	0.84
3.00×10^{-4}	14.72	0.53	55.63	1.77	29.65	4.82	3.48	0.93
1.50×10^{-3}	06.87	0.64	58.49	2.36	34.63	5.28	3.98	0.87
3.20×10^{-3}	17.91	0.66	52.37	2.48	29.73	5.28	3.88	0.82
6.50×10^{-3}	13.93	0.72	55.22	2.28	30.85	5.02	3.68	0.97
1.34×10^{-2}	14.06	0.71	55.85	2.04	30.09	4.73	3.43	0.93
2.11×10^{-2}	15.62	0.62	52.77	1.87	31.61	4.51	3.32	0.92

$(\tau) = (\alpha_1 \tau_1 + \alpha_2 \tau_2) / (\alpha_1 + \alpha_2)$ [b] $\pm$ 0.05. The magnitude of χ^2 denotes the goodness of the fit.

Table S3: Fluorescence lifetime parameters of HEWL at various G_{16} concentrations

G_{16} (M)	α_1 (%)	τ_1 (ns)	α_2 (%)	τ_2 (ns)	α_3 (%)	τ_3 (ns)	τ_{avg}	χ^2
0	11.08	0.36	60.81	1.37	28.11	2.81	2.03	0.86
8.26×10^{-8}	14.48	0.49	61.90	1.51	23.63	3.01	2.09	0.76
6.39×10^{-7}	14.82	0.48	60.98	1.50	24.21	2.98	2.08	0.79
3.63×10^{-6}	14.65	0.50	56.98	1.44	28.37	2.90	2.10	0.87
4.68×10^{-6}	11.40	0.39	59.23	1.41	29.37	2.91	2.12	0.83
6.07×10^{-5}	18.68	0.61	62.17	1.79	19.15	5.03	3.15	0.78
2.10×10^{-4}	18.34	0.61	59.62	1.84	22.04	5.16	3.39	0.85
3.00×10^{-4}	18.88	0.63	59.98	1.85	21.14	5.21	3.37	0.85
1.50×10^{-3}	19.27	0.67	60.77	1.74	19.69	5.56	3.51	0.87
3.20×10^{-3}	19.27	0.67	60.77	1.87	19.69	5.61	3.55	0.85
6.50×10^{-3}	18.25	0.72	65.32	1.87	14.33	6.35	3.61	0.97
1.34×10^{-2}	29.06	0.70	64.41	2.11	06.53	8.86	3.80	0.93
2.11×10^{-2}	32.20	0.77	59.84	2.21	07.96	9.49	4.42	0.92

$(\tau) = (\alpha_1 \tau_1 + \alpha_2 \tau_2) / (\alpha_1 + \alpha_2)$ [b] $\pm$ 0.05. The magnitude of χ^2 denotes the goodness of the fit.

Table S4. Critical micelle concentration (*cmc*) values of surfactants in different media[a]

Surfactant	*cmc*	
	Pure water[2-3]	HEWL (0.3 mg)
M_{16}	2.40×10^{-4} M	2.60×10^{-4} M
G_{16}	3.63×10^{-6} M	3.91×10^{-6} M

[a]Uncertainty of *cmc* is ±0.1

Table S5. Relative activity of HEWL in different M_{16} and G_{16} concentrations[c]

Concentration of surfactant (M)	RA[a] (%)	Relative error[b] (5%)
	M_{16}	
0	100	5.00
8.30×10^{-8}	72.89	3.64
6.39×10^{-7}	80.75	4.03
3.63×10^{-6}	82.11	4.10
1.20×10^{-4}	86.17	4.30
2.10×10^{-4}	86.99	4.34
3.00×10^{-4}	75.88	3.79
3.20×10^{-3}	36.04	1.80
$>3.20\times10^{-3}$	No activity	-
	G_{16}	
0	100	5.00
8.30×10^{-8}	61.24	3.06
6.30×10^{-7}	66.12	3.30
3.63×10^{-6}	76.15	3.80
3.40×10^{-5}	82.92	4.14
1.20×10^{-4}	92.14	4.60
2.10×10^{-4}	94.30	4.71
3.00×10^{-4}	117.61	5.88
$>3.00\times10^{-4}$	No activity	-

[a]%RA calculated using eq 6, where the initial slope is fitted by taking adj. R-square value ~0.99
[b]Relative error is calculated through OriginPro 8.5. [c]Uncertainty in %RA is ±5%.

References:

1 A. Sethuraman and G. Belfort, *Biophys. J.*, 2005, **88**, 1322-1333.

2 D. Tikariha, N. Singh, M. L. Satnami, K. K. Ghosh, N. Barbero and P. Quagliotto, *Colloids Surf., A*, 2012, **411**, 1-11.

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