A spectroscopic and molecular dynamics study on the aggregation process of a long-acting

lipidated therapeutic peptide: the case of Semaglutide.

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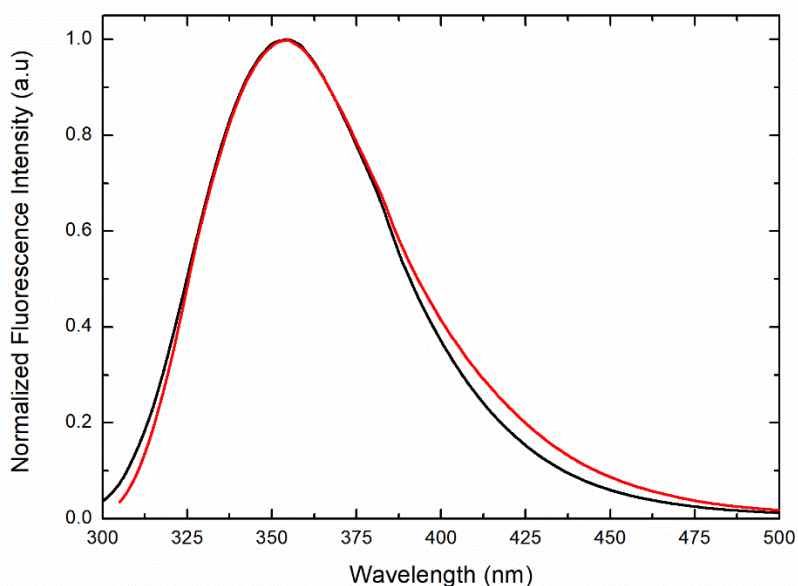
Electronic Supplementary Information

Fig. S1 Fluorescence spectra of SMG. Black line: λ_{ex}=280 nm; red: λ_{ex}=295 nm. The spectra were normalized to unit at the maximum to emphasize shape similarity.
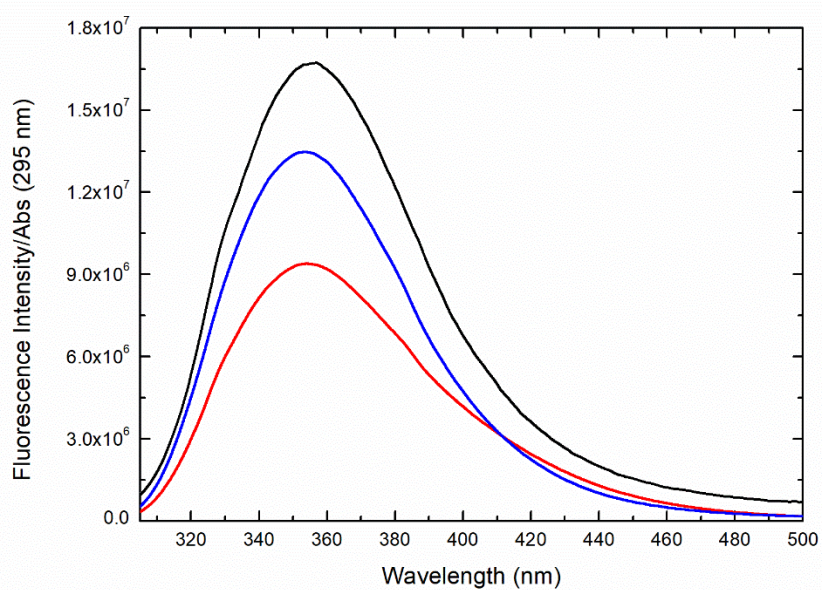
Fig. S2 Fluorescence emission spectra of SMG solutions (phosphate buffer, pH 8) normalized by the absorption at λ_{ex}=295 nm. Black line: 4 µM; blue: 30 µM; red: 20 µM.
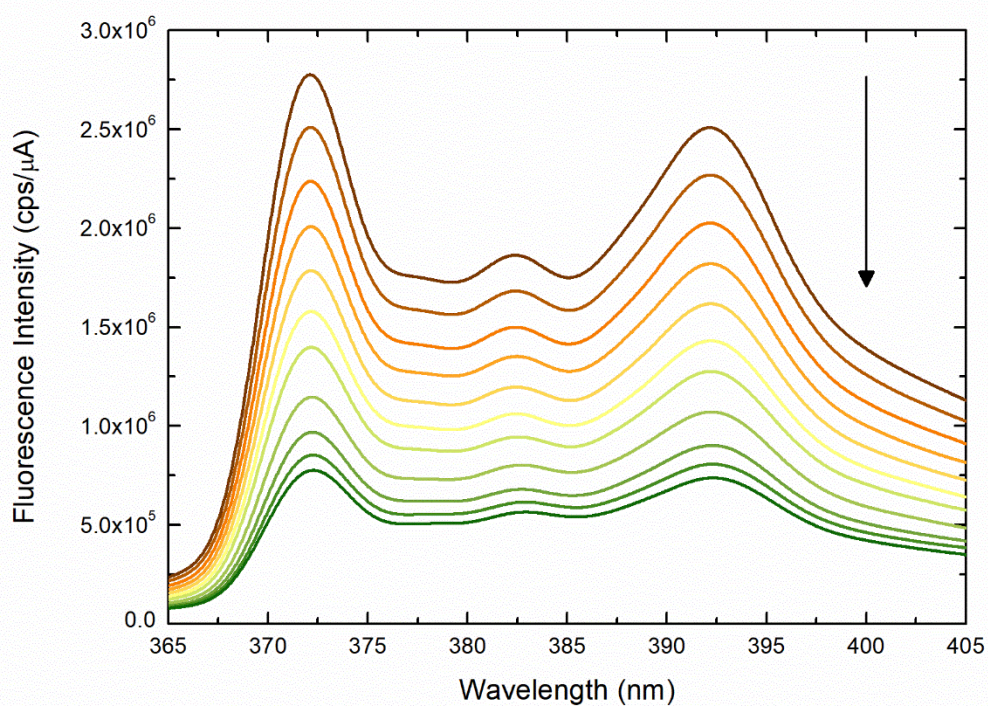

Fig S3. Fluorescence spectra of Pyrene (1µM) adding increasing aliquots (25 µl) of 66 µM SMG solution (phosphate buffer, pH 8, 25°C).
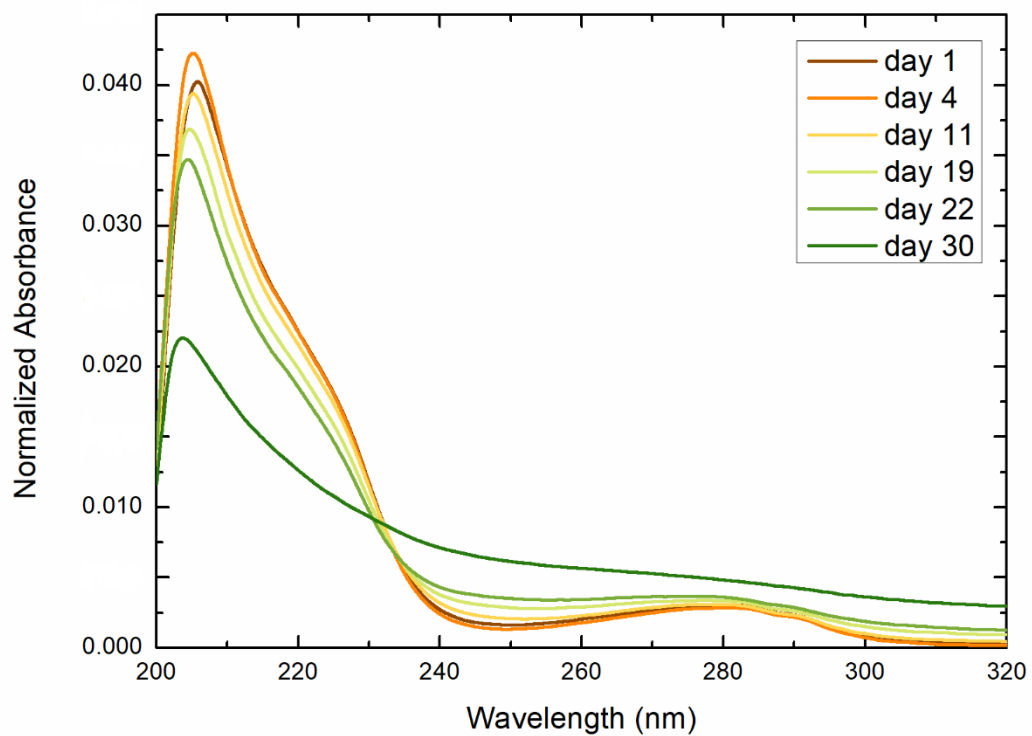
Fig. S4 Absorption spectra of SMG (phosphate buffer, pH 8, 25°C) at different times (days). The absorption bands were normalized to unit area for better comparison.
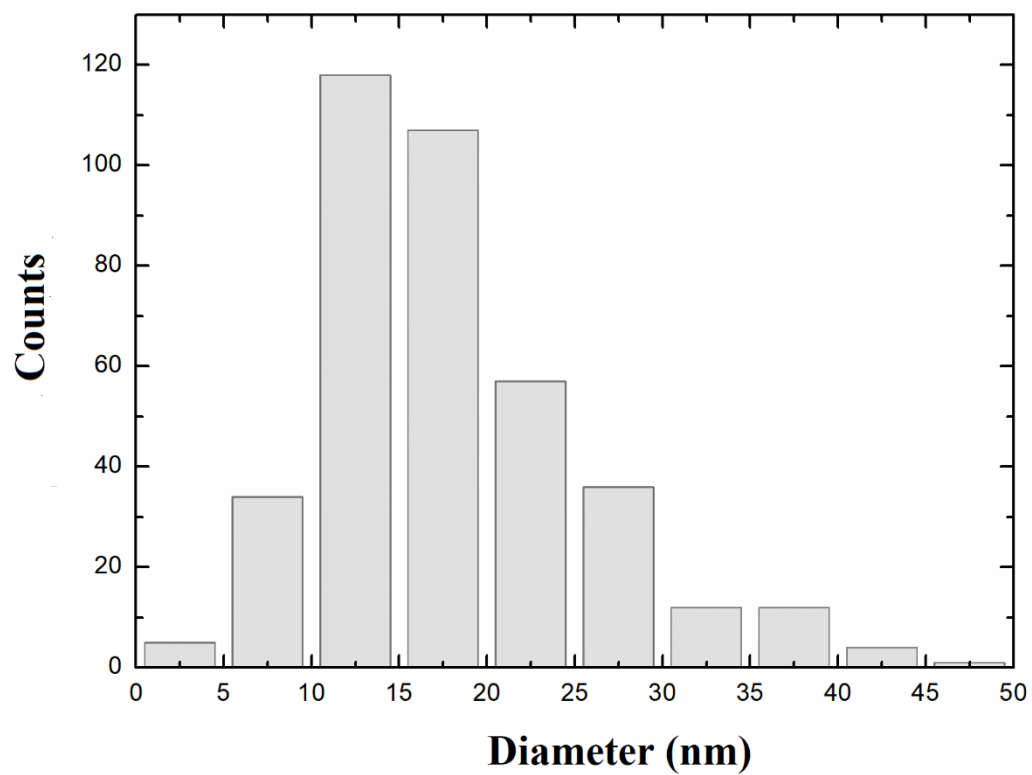
Fig. S5 Size distribution of the globular structures imaged by AFM upon deposition on mica of a freshly-prepared 30 µM SMG aqueous solution (phosphate buffer, pH 8, T=25°C).

Fig. S6 Size distribution (length) of the rod structures imaged by AFM upon deposition on mica of an aged 30 μM SMG aqueous solution (phosphate buffer, pH 8, T=25°C).
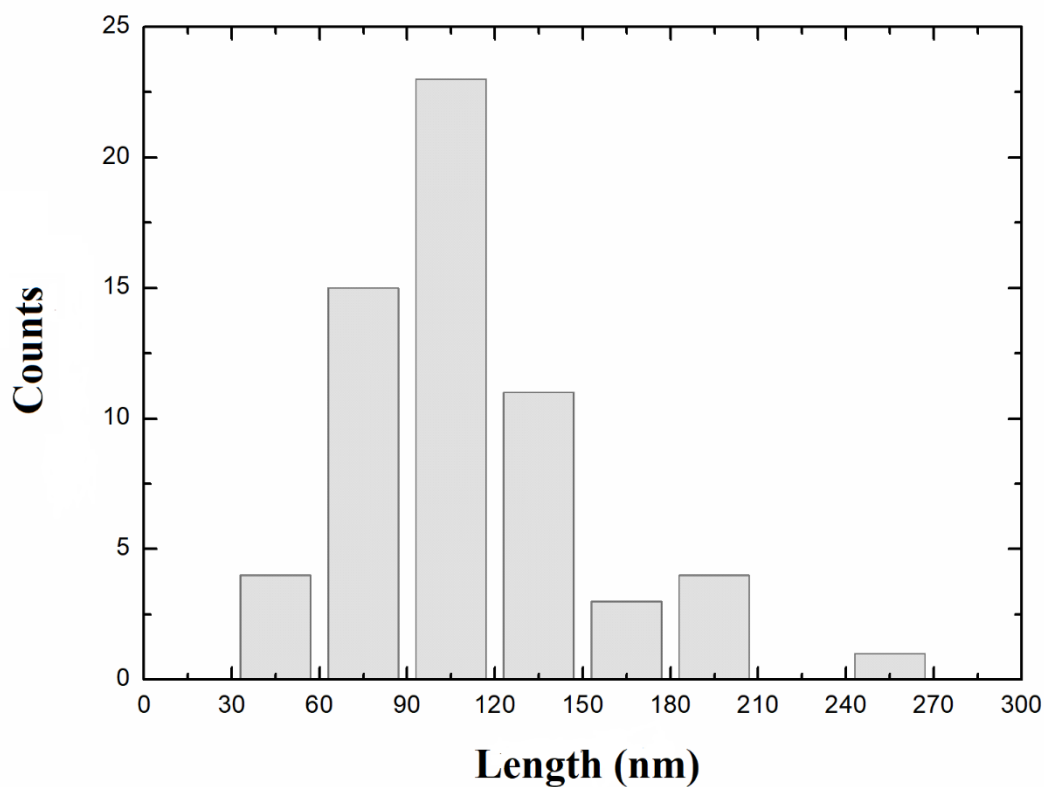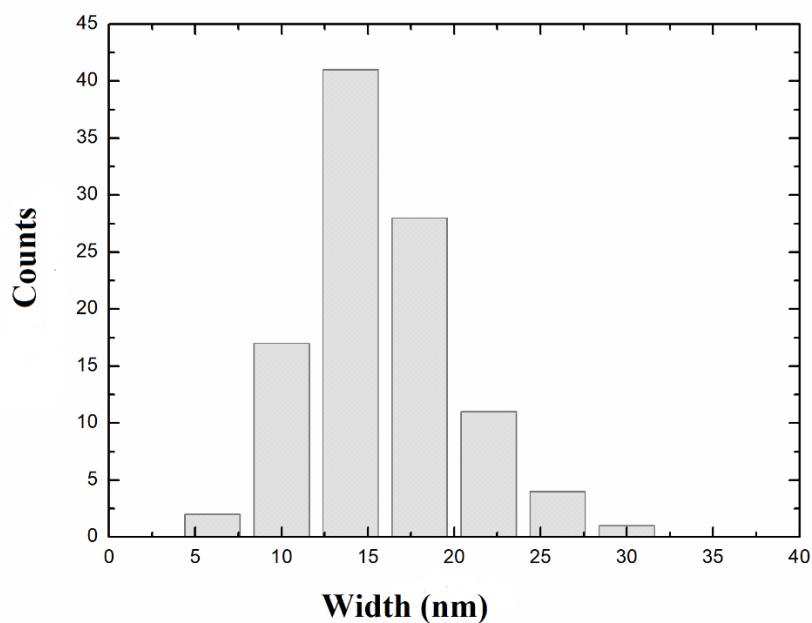

Fig. S7 Size distribution (width) of the rod structures imaged by AFM upon deposition on mica of an aged 30 μM SMG aqueous solution (phosphate buffer, pH 8, T=25°C).

Table T1 Time decay parameters of SMG in water/glycerol 1:2 (v/v) solutions for different temperatures.

T(°C)	τ_1 (ns)	α_1	τ_2 (ns)	α_2	τ_3 (ns)	α_3	$<\tau>$ (ns)
3.1	0.88	0.23	4.38	0.43	7.59	0.34	4.7
5.8	0.82	0.23	4.62	0.52	7.88	0.25	4.6
10.0	0.91	0.22	4.54	0.54	7.74	0.24	4.5
14.8	0.74	0.20	4.24	0.54	7.59	0.26	4.4
20.7	0.83	0.20	4.15	0.58	7.51	0.22	4.3
25.2	0.97	0.18	4.17	0.62	7.62	0.20	4.3
29.8	0.99	0.20	4.17	0.64	7.73	0.16	4.1
34.4	1.11	0.20	4.04	0.63	7.54	0.17	4.1

Table T2. Fluorescence time decays of SMG aged solutions (phosphate buffer, pH 8, T=25°C

λ_{ex}=298 nm; λ_{em} = 350 nm

Concentration (μM)	α_1	τ_1 (ns)	α_2	τ_2 (ns)	α_3	τ_3 (ns)	$\langle \tau \rangle$ (ns)	χ^2
4	0.89	1.2	0.05	3.7	0.06	9.9	1.9	1.04
20	0.74	0.7	0.24	3.3	0.02	13.6	1.7	1.09
30	0.80	1.4	0.14	5.6	0.06	13.8	2.7	1.12

λ_{ex}=298 nm; λ_{em} = 420 nm

Concentration (μM)	α_1	τ_1 (ns)	α_2	τ_2 (ns)	$\langle \tau \rangle$ (ns)	χ^2
4	0.25	0.94	0.75	3.72	3.02	2.71
20	0.35	0.89	0.65	3.85	2.82	2.77
30	0.18	0.34	0.82	3.33	2.80	2.59

λ_{ex}=344 nm; λ_{em} = 420 nm

Concentration (μM)	α_1	τ_1 (ns)	α_2	τ_2 (ns)	α_3	τ_3 (ns)	$\langle \tau \rangle$ (ns)	χ^2
4	0.35	1	0.2	3.1	0.45	6.2	3.8	1.06
20	0.54	0.2	0.17	1.7	0.29	5.5	2.0	1.12
30	0.44	1.2	0.47	4.7	0.09	10.5	3.8	1.12

Fractal autocatalytic aggregation model (Pasternack *et al.* 1998):

$$\lambda(t) = \lambda_0 + \frac{(\lambda_0 - \lambda_i)}{\left\{1 + (m-1)\left[k_0 t + \frac{(k_c t)^{n+1}}{n+1}\right]\right\}^{\frac{1}{m-1}}}$$

Parameter	Value	Standard Error
λ_i (nm)	409.1	0.2
λ_0 (nm)	353.8	0.2
m	2.1	0.6
n	14	3
k_0 (d^{-1})	1.5×10^{-3}	4×10^{-4}
k_c (d^{-1})	4.72×10^{-2}	4×10^{-4}
$\chi^2 = 0.0673$		$R^2 = 0.9999$