Oxidant-responsive Ferrocene-based Cyclodextrin Complex Coacervate Core Micelles

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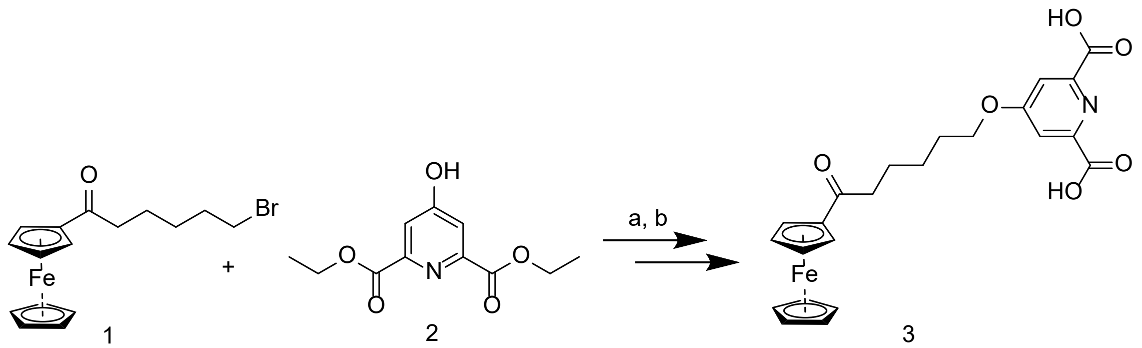
Synthesis of ferrocene-modified dipicolinic acid (3)



Scheme 1. Synthesis of ferrocene-modified dipicolinc acid (Fc-DPA); a) K_2CO_3, butanone, refluxed overnight and b) 2% NaOH, MeOH/H_2O, N_2.

399 mg (1.1 mmol) (6-bromo-1-oxohexyl) ferrocene (1) and 250 mg (1.0 mmol) diethyl 4-hydroxy 2,6-pyridinedicarboxylate (2) were dissolved in 10 mL butanone with 1.2 equivalents of K_2CO_3. The mixture was refluxed overnight. The next day the solid was filtered off from the solution and the butanone was evaporated. The crude was purified by column chromatography (1:30 w/w crude/silica) in 2:1 cyclohexane:ethyl acetate.

The product was then deprotected by stirring it in a 2% NaOH solution of degassed MeOH/water 98:2. After 2 hours, the orange precipitate was collected by filtration, washed with a small amount of cold MeOH and dried under vacuum. The overall yield of product 3 was 60%.

^{1}H-NMR spectrum (600 MHz, D_2O): ppm 7.53 (s, 2H, C**H**pyr), 4.87 (d, 2H, $\mathbf{H_g}$-(Fc))-4.74 (d, 2H, $\mathbf{H_h}$-(Fc)),4.30 (m, 5H, $\mathbf{H_i}$-(Fc)), 4.26 (t, 2H, J=12.04 Hz, C$\mathbf{H_2}$-O-pyr), 3.37 (MeOH), 2.84 (t, 2H, J=15.23 Hz, C$\mathbf{H_2}$-C(=O)-(Fc)), 1.89 (m, 2H, C$\mathbf{H_2}$-CH_2-O-Pyr), 1.77 (m, 2H, C$\mathbf{H_2}$-CH_2-C(=O)-Fc), 1.58 (m, 2H, C$\mathbf{H_2}$-CH_2-CH_2-C(=O)-(Fc))

^{13}C-NMR spectrum (600 MHz, D_2O): ppm 212 (**C**(=O)-(Fc)), 172 (Pyr-**C**O_2), 166 (O-**C**-(Pyr)), 154 (**C**(Pyr)-CO_2), 111 (**C**H-(Pyr)), 77.7 (**C**-(C=O)-(Fc)), 74 ($\mathbf{C_h}$-(Fc)), 70.3 ($\mathbf{C_i}$-(Fc)), 69.8 (**C**$_g$-(Fc)), 68.4 (**C**H_2-O-pyr), 39.2 (**C**H_2-C(=O)-Fc), 27.7 (**C**H_2-CH_2-O-(pyr)), 24.8 (C**H_2**-CH_2-CH_2-C(=O)-(Fc)), 24.5 (C**H_2**-CH_2-C(=O)-Fc)

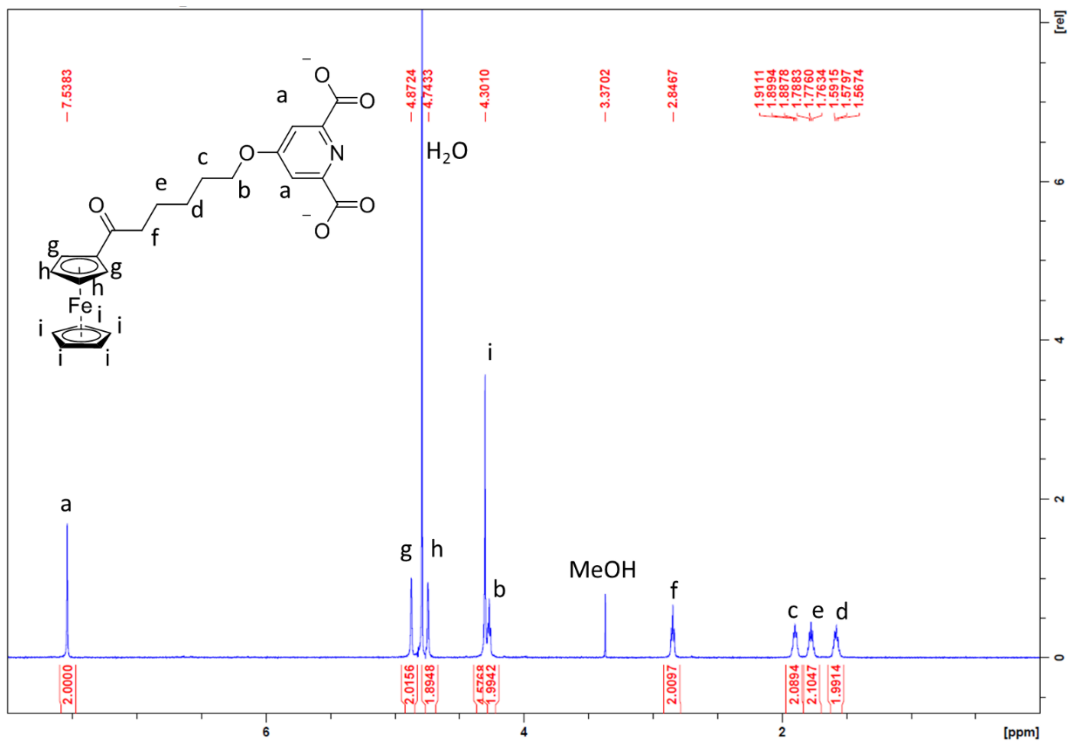


Figure S1. **^{1}H NMR peak assignment of ferrocene-modified dipicolinic acid (Fc-DPA) in D_2O.**

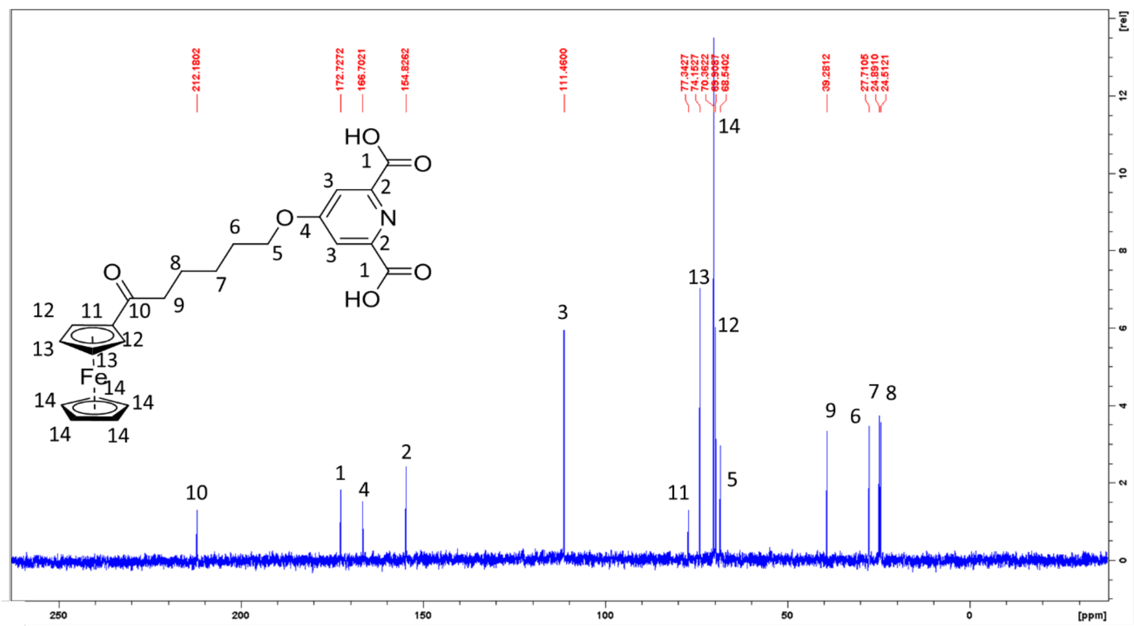


Figure S2. **^{13}C NMR peak assignment of ferrocene-modified dipicolinic acid (Fc-DPA) in D_2O.**

Micelle characterization

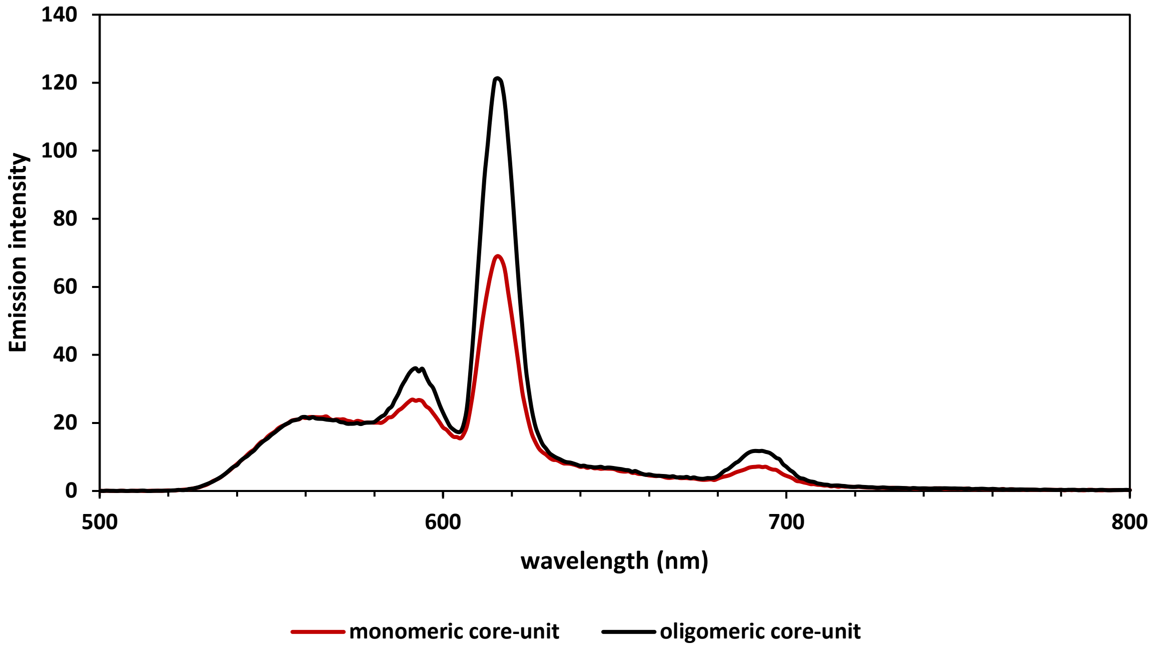


Figure S3. Emission spectrum of europium ions in the Fc-core-units, exciting the DPA at 280 nm (antenna phenomenon). The europium emission is not normalized for the europium concentration. As mentioned in the main text, the oligomeric core-units contains double the concentration of europium ions, compared to the monomeric core-units.

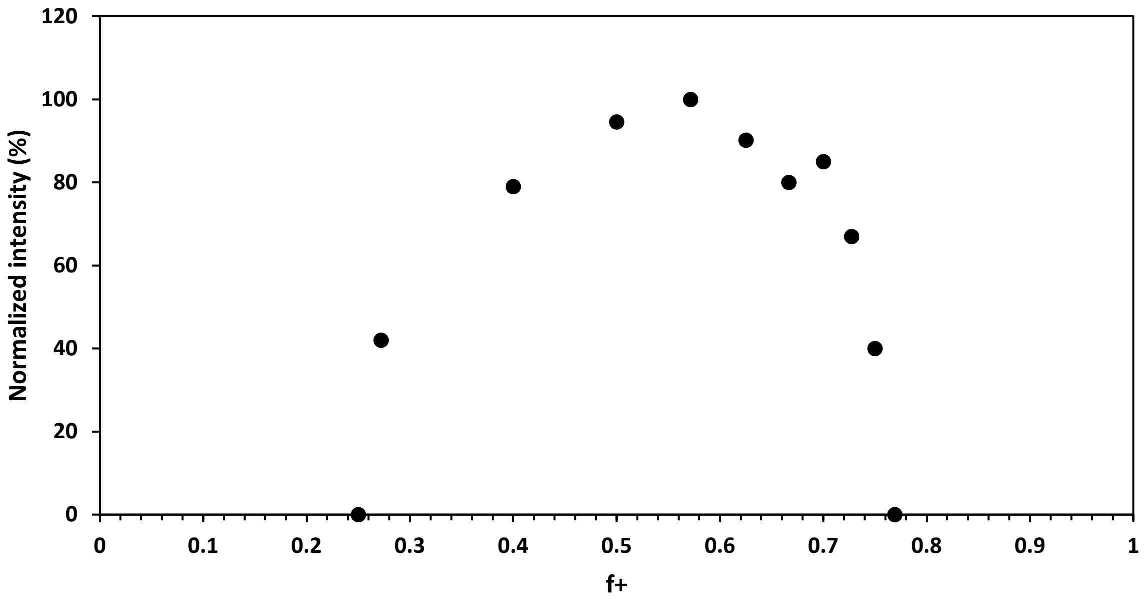
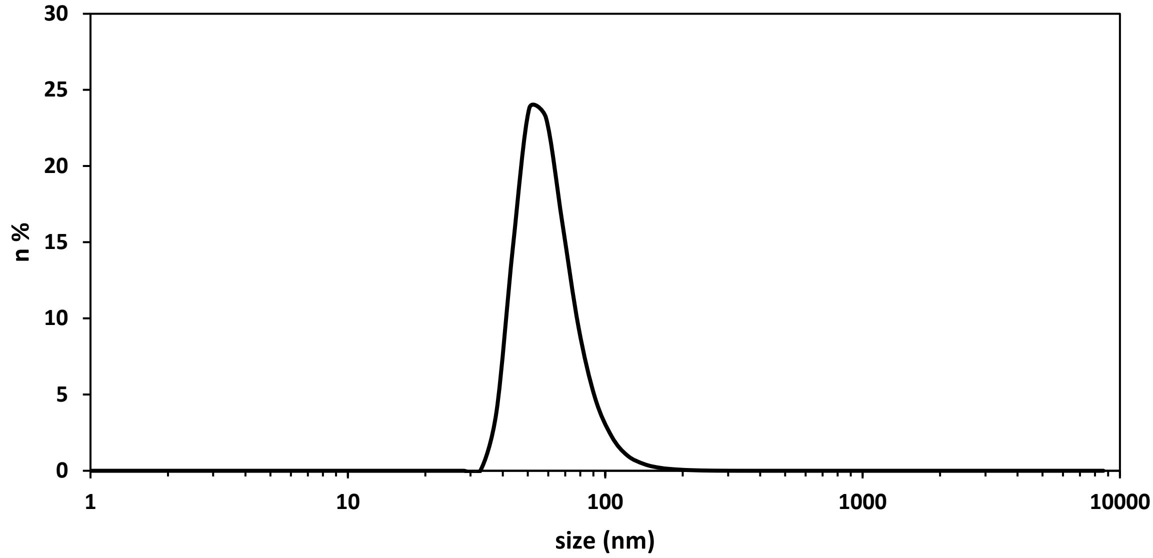


Figure S4. Determination of positive fraction (f+), by titrating the block copolymer to the core-units. The f+ determines the optimum concentration of block copolymer to reach the neutralization of the core-unit charges. In this case, 0.57 mM was determined as the optimum block copolymer concentration.



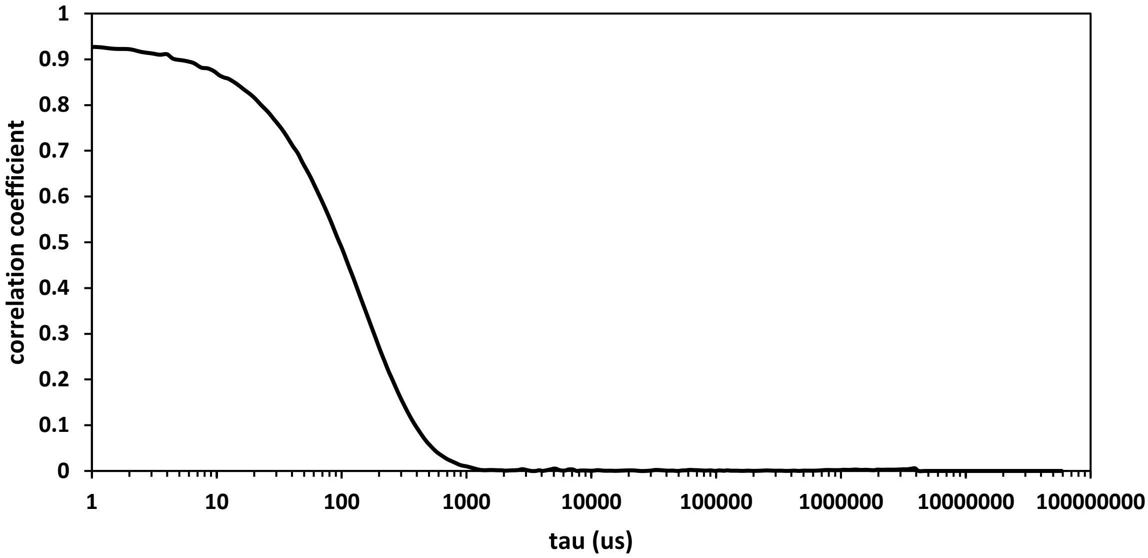
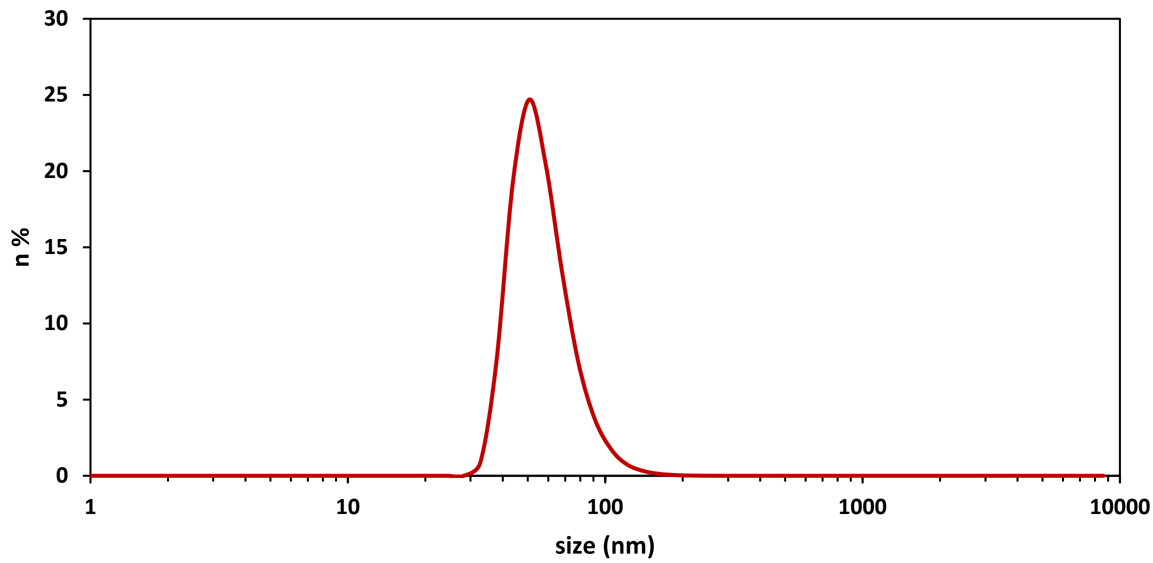


Figure S5. Size distribution (above) and correlogram (below) of oligomeric core-unit based Fc-C4Ms, measured at the DLS.



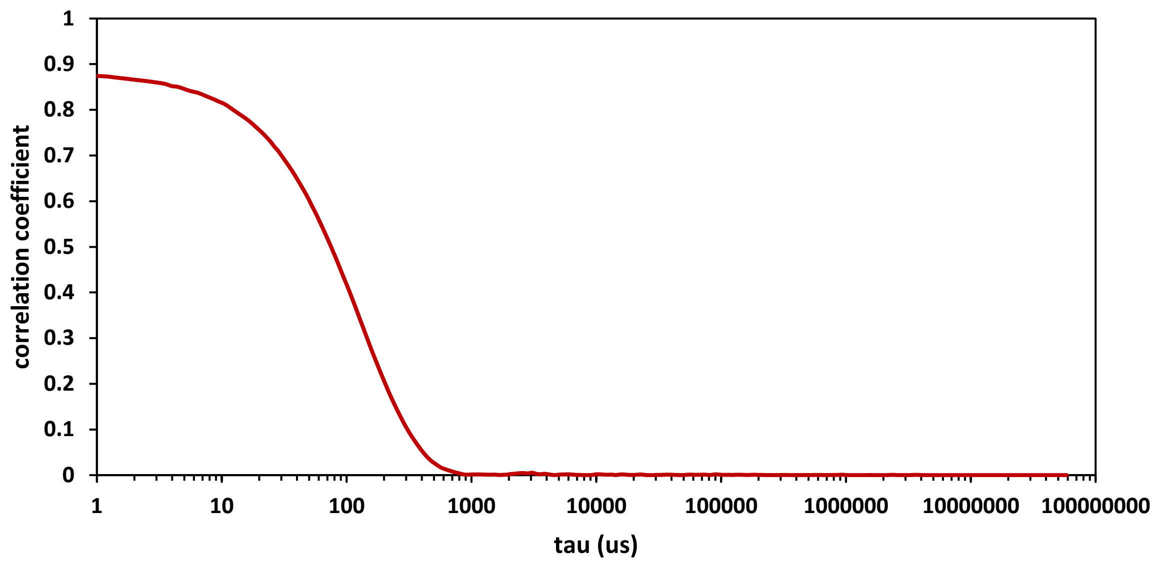


Figure S6. Size distribution (above) and correlogram (below) of monomeric core-unit based Fc-C4Ms, measured at the DLS.

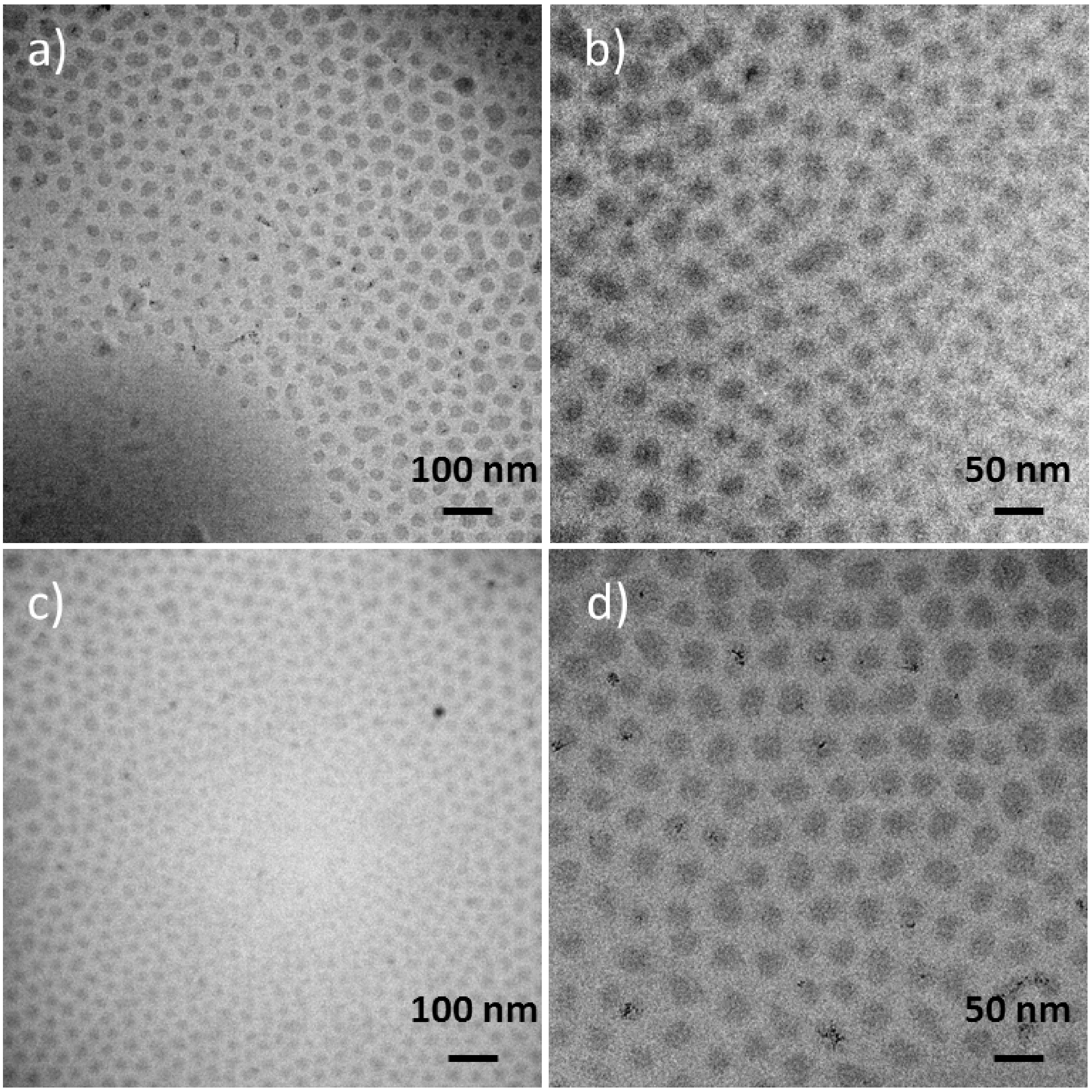


Figure S7. Cryo-TEM pictures of oligomeric core-unit based Fc-C4Ms a) and b) and monomeric core-unit based Fc-C4Ms c) and d).

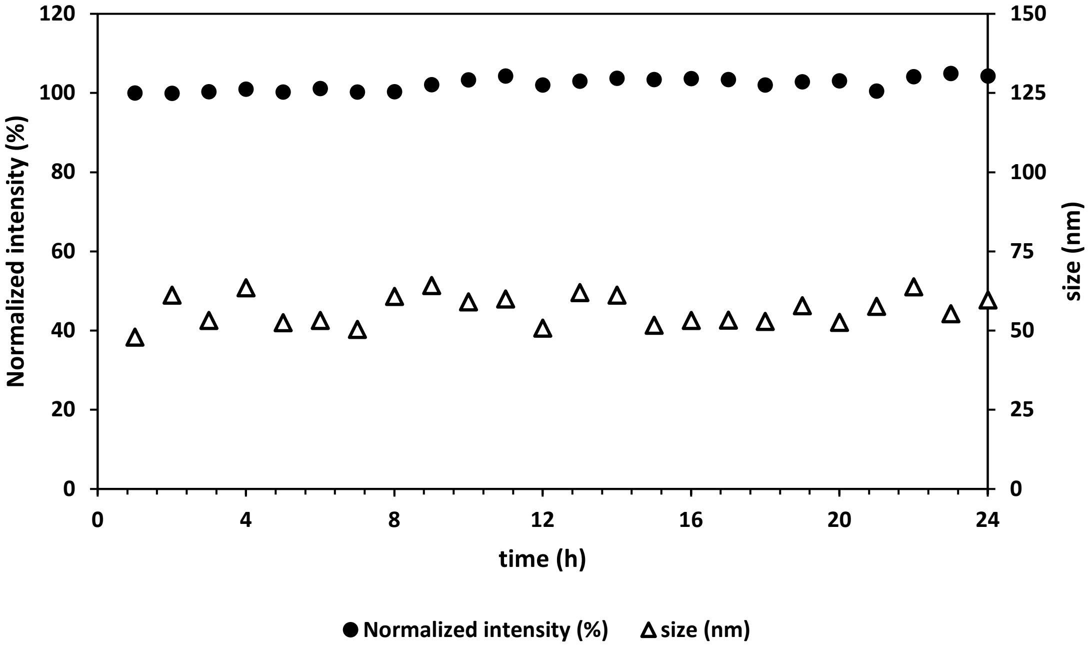


Figure S8. Stability in time of oligomeric core-unit based Fc-C4Ms, measured at the DLS.

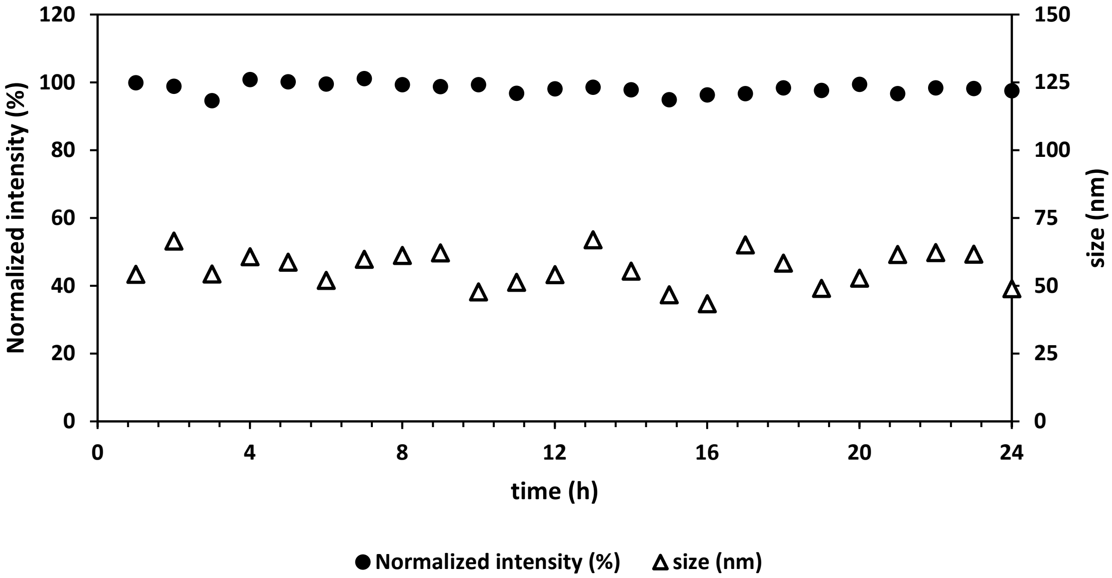


Figure S9. Stability in time of monomeric core-unit based Fc-C4Ms, measured at the DLS.

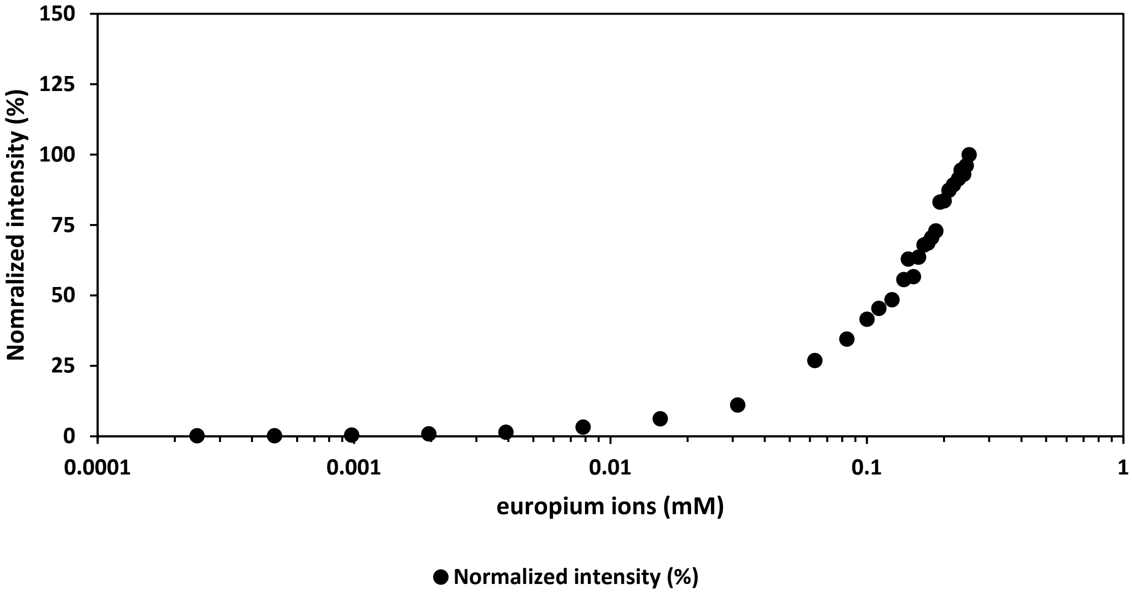


Figure S10. Critical Micelle Concentration (CMC) determination for the oligomeric core-unit based Fc-C4Ms, measured at the DLS.

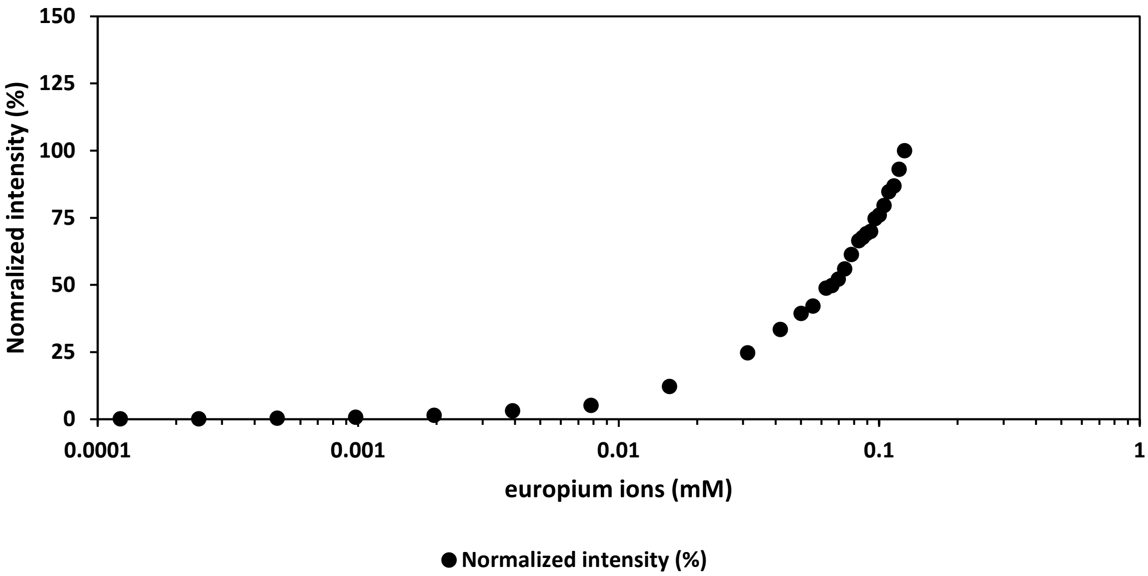


Figure S11. Critical Micelle Concentration (CMC) determination for the monomeric core-unit based Fc-C4Ms, measured at the DLS.

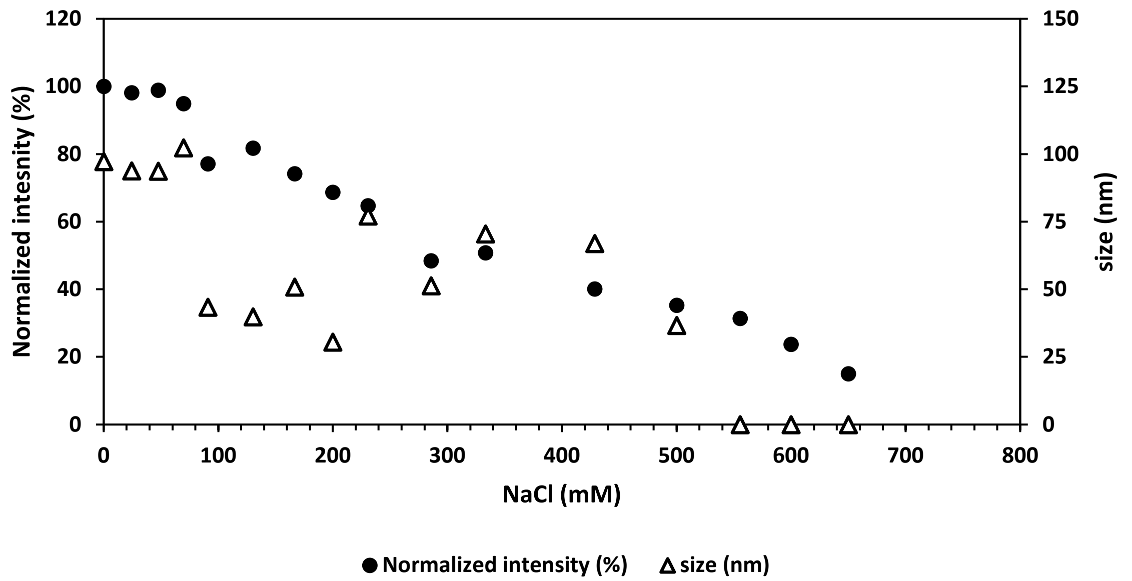


Figure S12. Critical Salt Concentration (CSC) determination for the oligomeric core-unit based Fc-C4Ms, measured at the DLS.

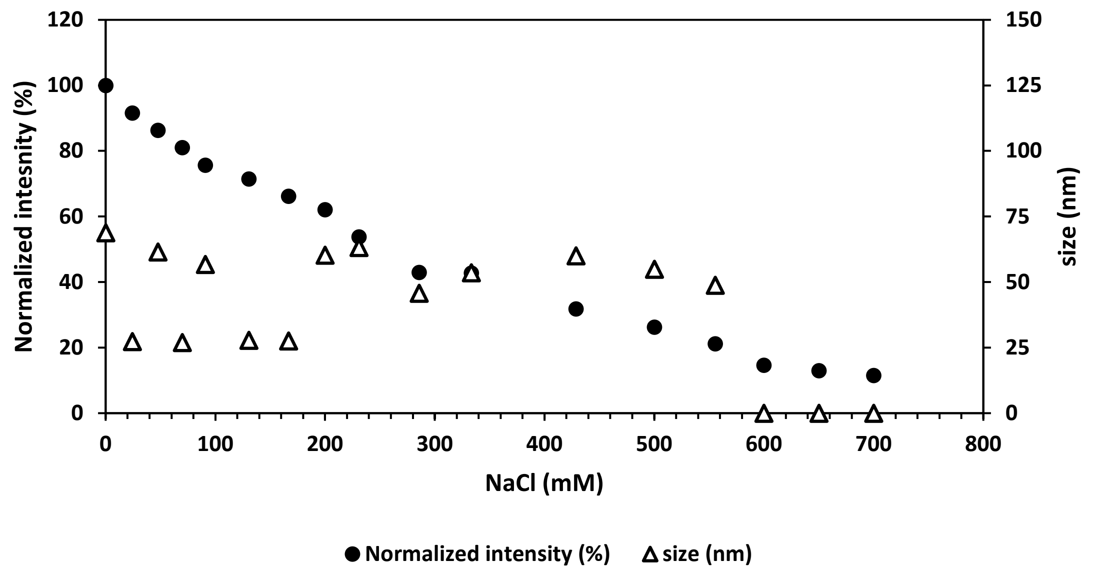


Figure S13. Critical Salt Concentration (CSC) determination for the monomeric core-unit based Fc-C4Ms, measured at the DLS.

H_2O_2-response studies

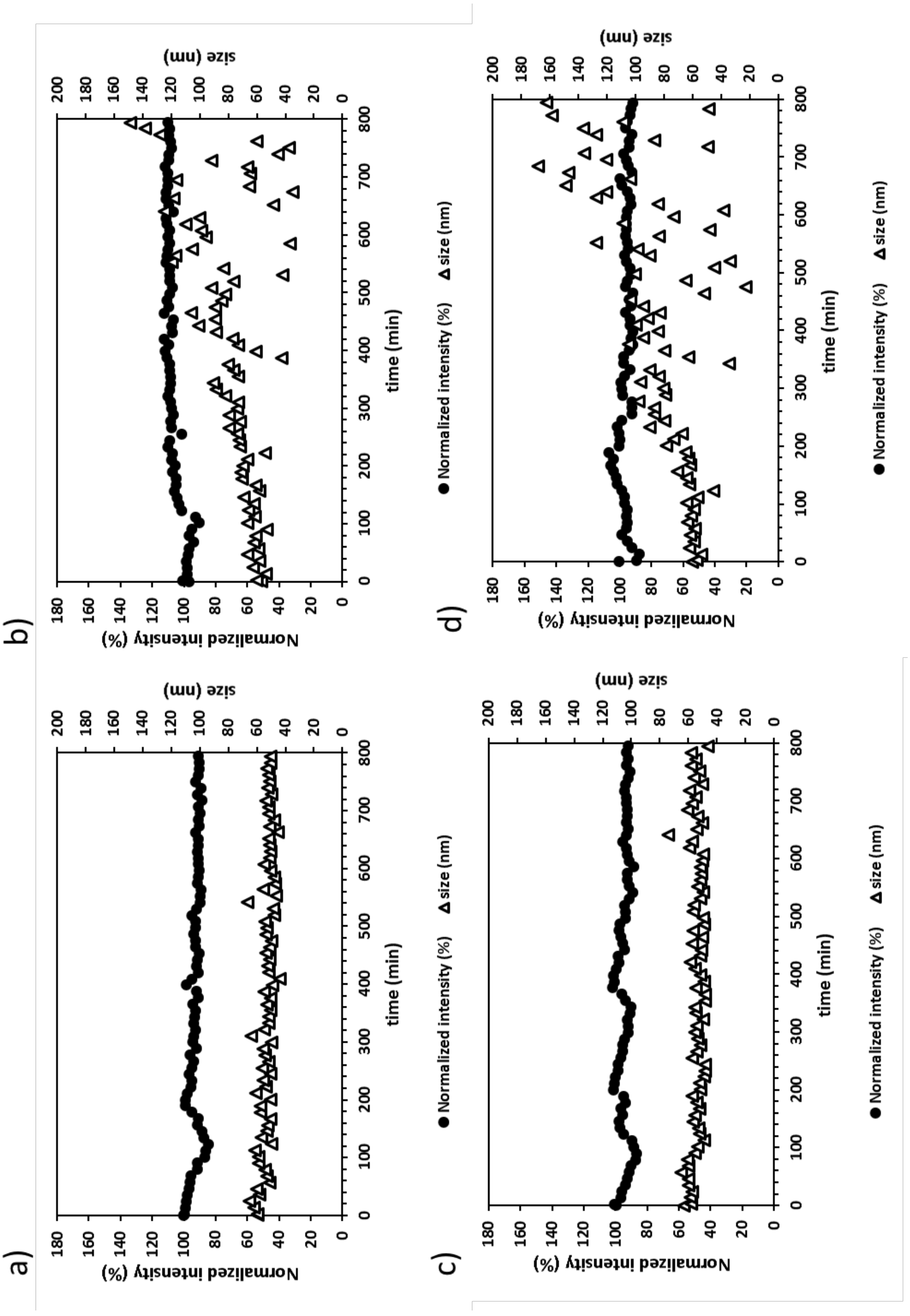


Figure S14. Normalized intensity and size over time of Fc-C4Ms after the addition of 325 equivalents a) and b) and 650 equivalents c) and d) of H_2O_2 , for oligomeric core-unit a) and c) and monomeric core-unit based micelles b) and d), measured at the DLS.

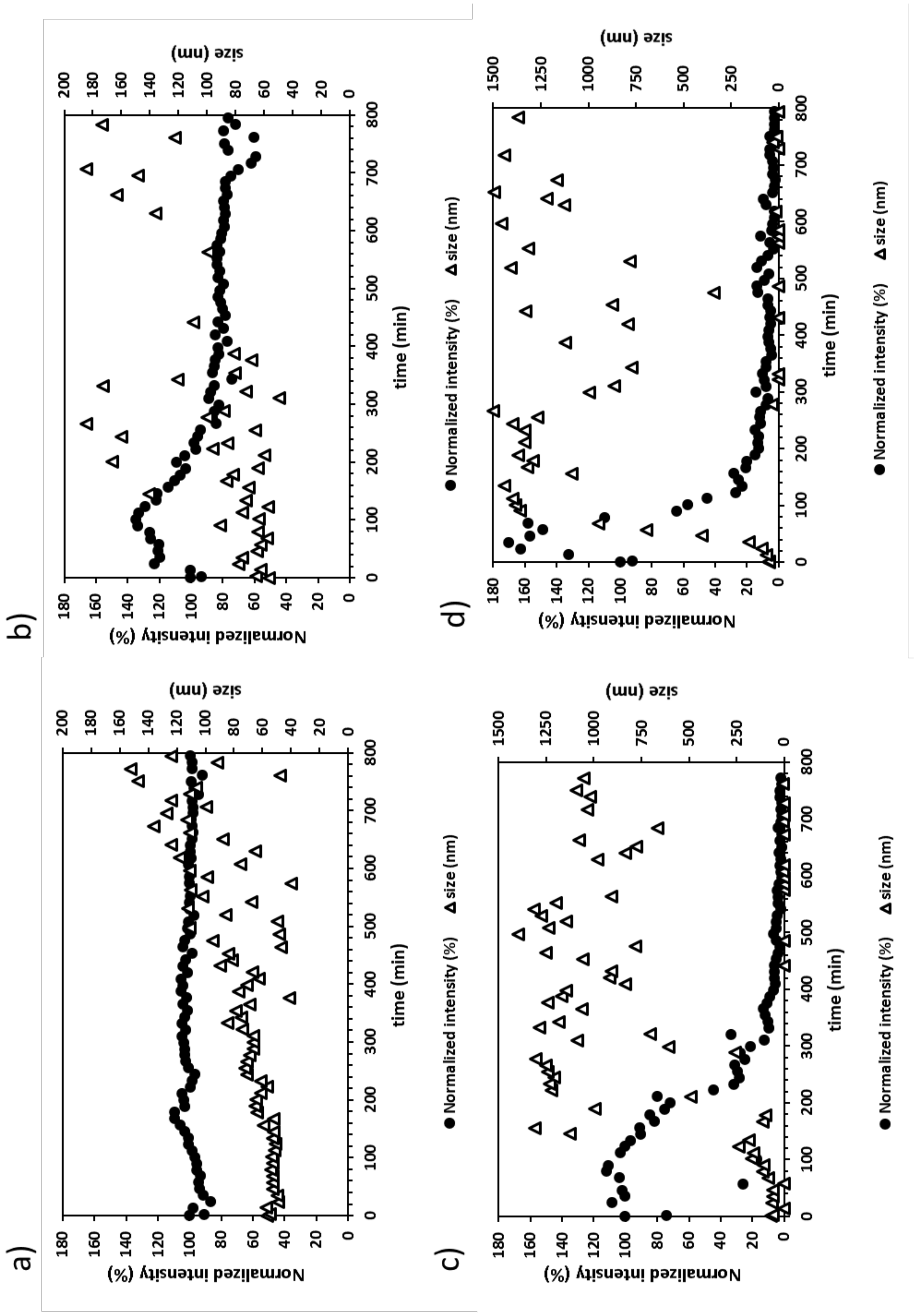


Figure S15. Normalized intensity and size over time of Fc-C4Ms after the addition of 970 equivalents a) and b) and 1500 equivalents c) and d) of H_2O_2 , for oligomeric core-unit a) and c) and monomeric core-unit based micelles b) and d), measured at the DLS.

Addition of a non-responsive bislinker

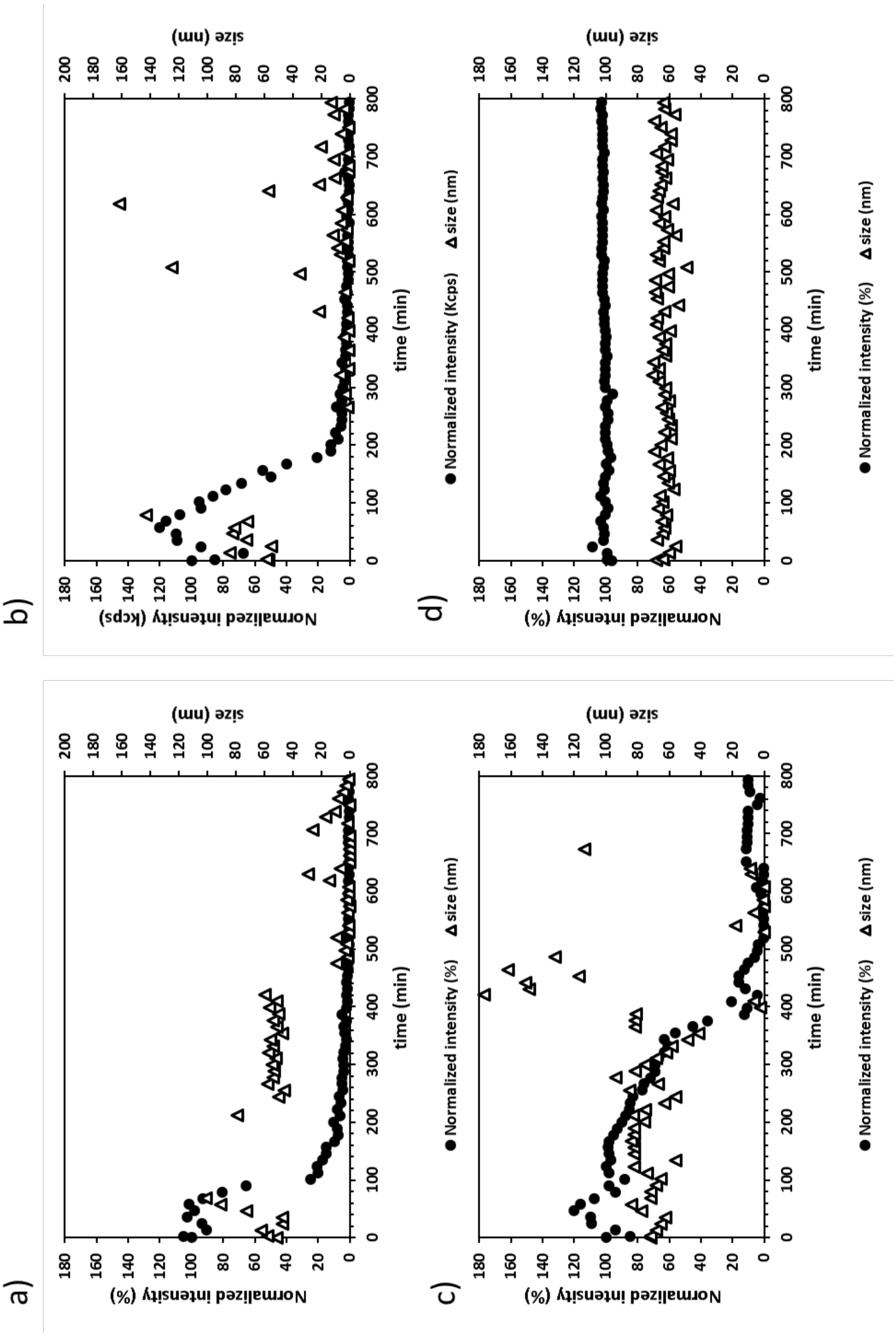


Figure S16. Normalized intensity and size over time after the addition of 1300 eq of H_2O_2 to a Fc-C4Ms sample, by substituting increasing amount of Fc-DPA with Ad-Glu-Ad bislinker, a) 10%, b)50 %, c) 90% and d) 100%, measured at the DLS.

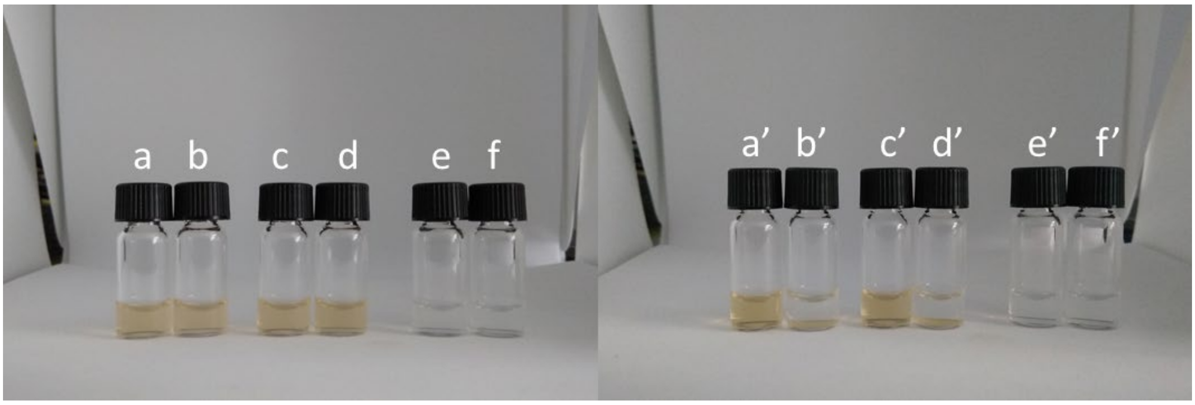


Figure S17. Pictures of the destabilization study of oligomeric a) and b) and monomeric c) and d) core-unit Fc-C4Ms. The vials e and f represent the control C4Ms, prepared with Ad-Glu-Ad bislinker. The vials b,d and f were treated with 1300 eq of H_2O_2.

Table S1. Intensity and size values of the Fc-based C4Ms controls, measured at the DLS.

number	*name*	*I (Mcps)*	*size (nm)*
1	*Eu*	*0.1*	*na*
2	*βCD-DPA*	*na*	*na*
3	*Fc-DPA*	*na*	*na*
4	*BP*	*na*	*na*
5	*Eu βCD-DPA*	*na*	*na*
6	*Eu Fc-DPA*	*na*	*na*
7	*Eu BP*	*na*	*na*
8	*Eu Fc-DPA BP*	*0.3*	*10*
9	*Eu βCD-DPA BP*	*0.4*	*na*
10	*Fc-DPA BP*	*0.2*	*10*
11	*Fc-DPA βCDDPA*	*na*	*na*
12	*βCD-DPA BP*	*na*	*na*
13	*monomeric core-unit based Fc-C4Ms*	*22*	*60*
14	*oligomeric core-unit based Fc-C4Ms*	*45*	*60*