Electronic Supplementary Information

Fatty Acid based Transient Nanostructures for Temporal Regulation of Artificial Peroxidase Activity

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1. Materials

Stearic acid (**C18A)**, Propanoic acid (**C3A**), *N,N*-dimethylaminomethyl ferrocene (**Fc-NMe_2**), Ferric perchlorate along with other chemicals were purchased from Sigma Aldrich Merck. HPLC grade acetonitrile, dimethyl sulfoxide and water were procured from Merck.

2. EXPERIMENTAL METHODS

Gelation

In a typical experiment, **C18A** (70 mg) was dissolved in DMSO (100 μL) by heating at 70 °C in a glass vial with inner diameter of 10 mm and 1 equiv. **Fc-NMe_2** (60 mg, 48.5 μL) was added to the vial and vortexed for 10 s. To this mixture, water (900 μL) was added and vortexed for 10 s which resulted in formation of a self-supporting gel, checked by vial inversion method. The final solvent composition was 10% (v/v) DMSO-water and final concentration of both **C18A** and **Fc-NMe_2** were 245 mM. The pH was found to be in the range of 8.4±0.15. The gel was stable for more than a month. Transient gels were formed by adding aqueous solution of oxidant $Fe(ClO_4)_3$ of varying concentration (final concentrations in the range 2.5 mM to 12.5 mM) instead of water. The pH of the system was measured to be in the range of 8.3 to 8.1. After autonomous gel to sol conversion (245 mM of **C18A** and **Fc-NMe_2** and 10 mM oxidizing agent), refueling was done with 4mM **Fc-NMe_2** which resulted in reformation of gel. The pH showed a minute increment of 0.1 and remained in the range of pH 8.3 ± 0.1. The transition times for autonomous gel to sol formation and upon refueling sol to gel formation were monitored by vial inversion method.

UV Vis spectroscopy study

UV-Vis spectra were recorded in Lamda 35 Perkin Elmer UV Vis spectrophotometer. Demountable quartz cells of path length of 0.1 mm or cuvettes of path length 10 mm were used for recording the absorbance of different samples. For measuring the hemin spectra in **C18A-Fc-NMe_2** at t= 0h, 3h, respective background spectra were corrected with the transient assemblies of **C18A-Fc-NMe_2** without hemin of ages t= 0h, 3h.

Fluorescence spectroscopy study

Fluorescence spectra were recorded in JASCO FP-8600 fluorimeter. In a typical experiment, **C18A** or **C3A** was dissolved in DMSO along with pyrene (final concentration 5μM) and **Fc-NMe_2** as mentioned above. To this mixture, water was added to keep the final concentration

of **C18A**, **C3A**, **Fc-NMe$_2$** at 245 mM each (solvent composition of 10%v/v, DMSO/water). **C18A-Fc-NMe$_2$** system formed gel within few seconds where **C3A**-**Fc-NMe$_2$** system remained sol. Afterwards, 50 μL of this gel or sol was taken out from the vial and placed in a cuvette for the measurement. The spectra were recorded by exciting pyrene at 337 nm.

Transmission Electron Microscopy (TEM)

TEM experiments were performed by casting the samples on carbon coated copper grid (200 mesh Cu grid from Agar Scientific, UK). In a typical experiment, 10 μL of sample was taken out at different time intervals and casted on grid. The samples were allowed to adsorb for 45 s followed by wicking off the excess samples with filter paper. Grid were dried for few hours before imaging. Images were recorded in JEOL JEM-2100F electron microscopes.

Scanning Electron Microscopy (SEM)

SEM samples were prepared following similar protocol as followed for TEM. Briefly, samples were casted without dilution on silicon wafer and dried before proceeding for imaging. SEM images were recorded on a Carl Ziess SUPRA 55VP instrument.

Atomic Force Microscopy (AFM)

The samples were placed on a silicon chip (3.5'' diameter diced fresh silicon wafer) and air dried. Tapping mode analysis was carried out on a Bruker Multimode 8 scanning probe microscope with silicon cantilever (Bruker).

Rheology measurement

The viscoelastic behaviors of the gels were characterized by AR-G2 rheometer from TA instrument equipped with 60 mm parallel plate at 25 °C. To determine the linear viscoelastic region of the gel, strain sweep was performed with varying the strain from 0.1 % to 100 % at a fixed oscillatory frequency of 1 Hz. For mechanical strength of the gel, frequency sweep experiment was carried at a fixed strain of 0.1%. For variable time experiment, series of samples in vials were aged for different time intervals. Then storage modulus (G') of each aged sample was measured at fixed strain of 0.1 % and fixed oscillatory frequency of 1 Hz. The storage moduli were plotted against time to get time resolved gel to sol transition.

High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) was performed on Waters HPLC system equipped with UV-Vis detector using Atlantis® T3 C18 5 μm, 4.6 X 250 mm analytical HPLC

column. The flow rate was maintained at 1 mL/min with a gradient elution method in acetonitrile (with 0.1 % TFA) and water (with 0.1 % TFA). Samples were diluted 25 times just before injecting. The consumption of **Fc-NMe$_2$** in the transient systems were determined by measuring the peak area obtained from chromatograms and utilizing a calibration plot of area versus concentration prepared by injecting known concentrations of **Fc-NMe$_2$**.

Activity measurement of hemin towards substrate pyrogallol

Activity of hemin was monitored spectrophotometrically using varying concentrations of pyrogallol in presence of H_2O_2.[1,2] For the temporal monitoring of activity, separate vials containing hemin with **C18A**-**Fc-NMe$_2$** or **C3A**-**Fc-NMe$_2$** in presence of 10 mM $Fe(ClO_4)_3$ as oxidizing agent were prepared. Typically, each multiple vial contained total volume 200 μL, 3.33 μL hemin (final conc. was 0.1 mM), 2 μL molecular histidine (final conc. was 1 mM) and 2 μL pyrogallol (varying stock concentration). After ageing for different time intervals, 2 μL of H_2O_2 (final conc. 30 mM) was added to initiate the oxidation of pyrogallol to purpurogallin. The absorbance change was monitored at λ_{max} = 420 nm for the initial 3 min (ε420 nm is 2640$M^{-1}cm^{-1}$ in water). For checking the effect of refueling, vials of **C18A**-**Fc-NMe$_2$** were mixed with batches of 4 mM **Fc-NMe$_2$** after each cycle of gel melting. For control **C3A**-**Fc-NMe$_2$** system, after similar time intervals as done in case of **C18A**-**Fc-NMe$_2$**, 4 mM **Fc-NMe$_2$** was added to investigate its effect on hemin activity (Figure 4c in main manuscript). For controls, kinetics of these systems was monitored without the addition of H_2O_2 and were found to be negligible. This suggests that in these systems, H_2O_2 acts as the sole oxidant.

Figure S1. Vial image of gel of **C18A-Fc-NMe$_2$**.

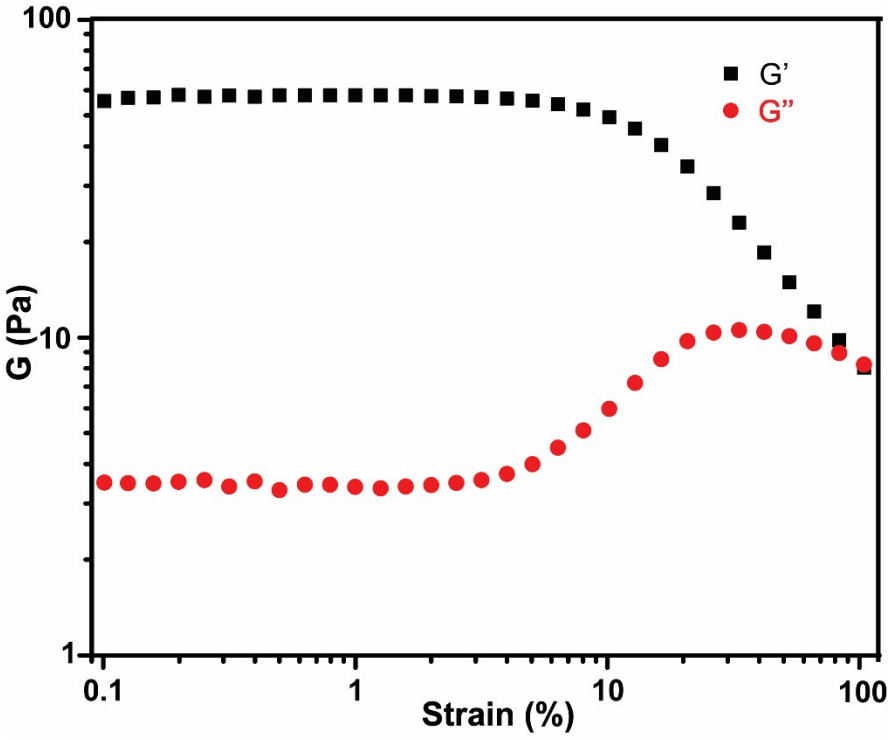

Figure S2. Strain sweep of gel of **C18A-Fc-NMe$_2$** at a fixed frequency of 1 Hz.

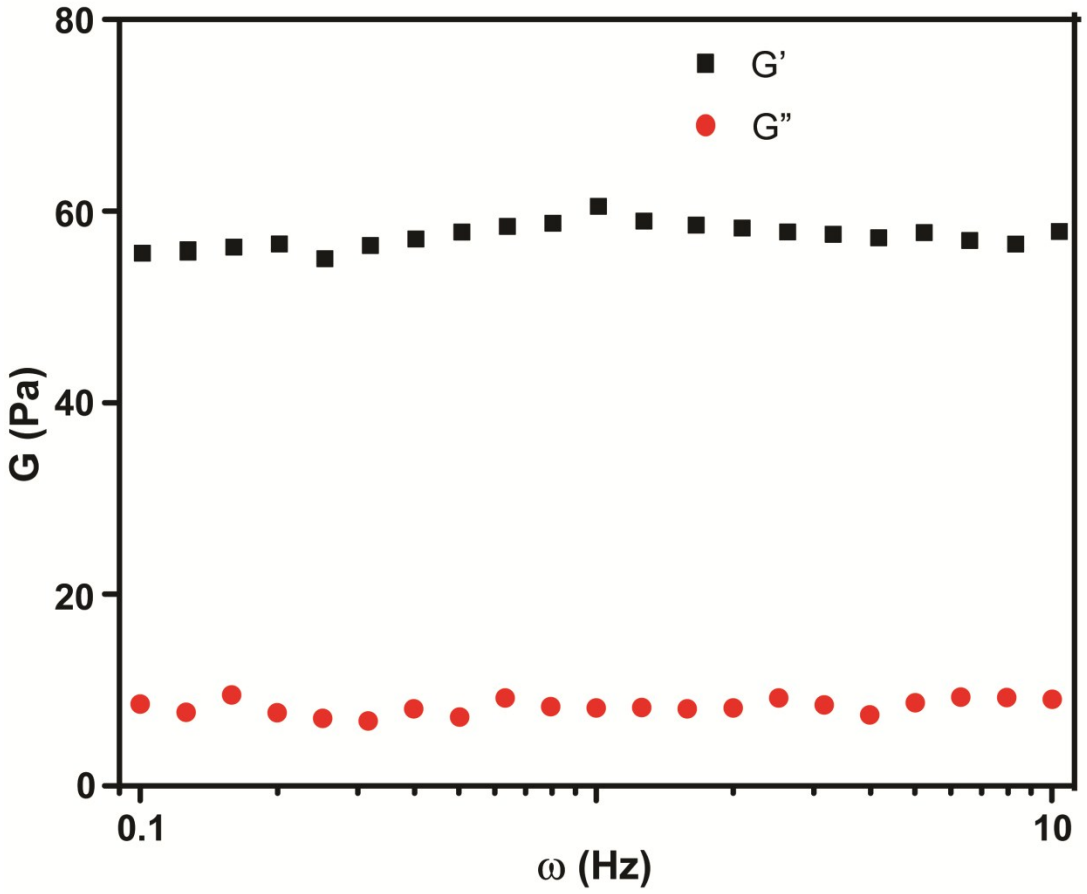

Figure S3. Frequency sweep of gel of **C18A-Fc-NMe$_2$** at a fixed strain of 0.1%.

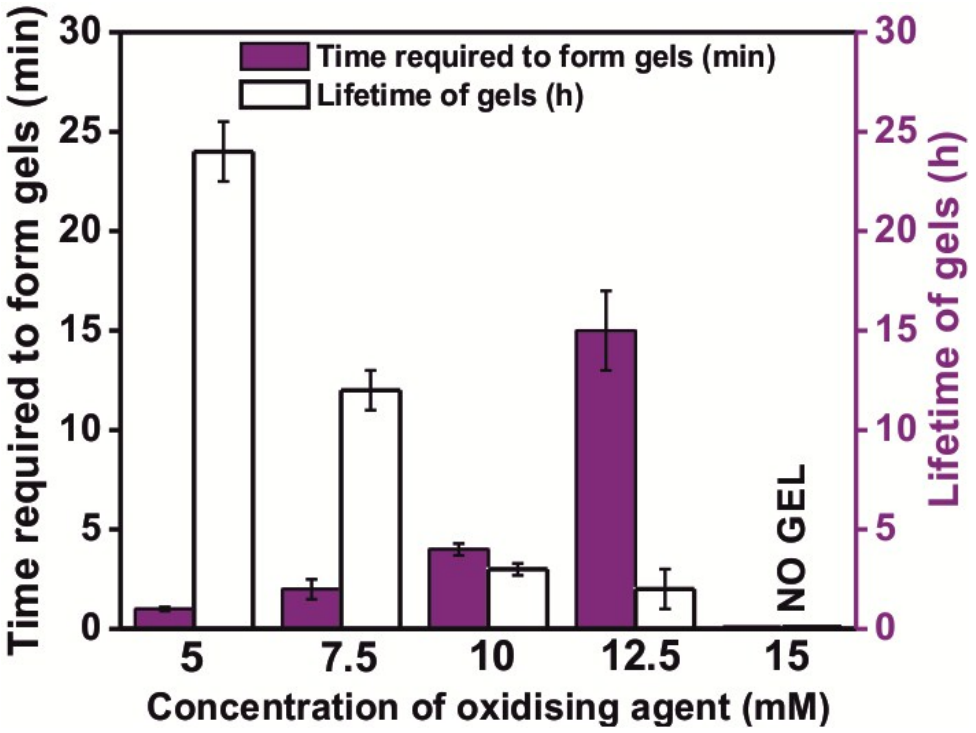

Figure S4. Gel formation time and life times of gels in presence of different concentrations of $Fe(ClO_4)_3$.

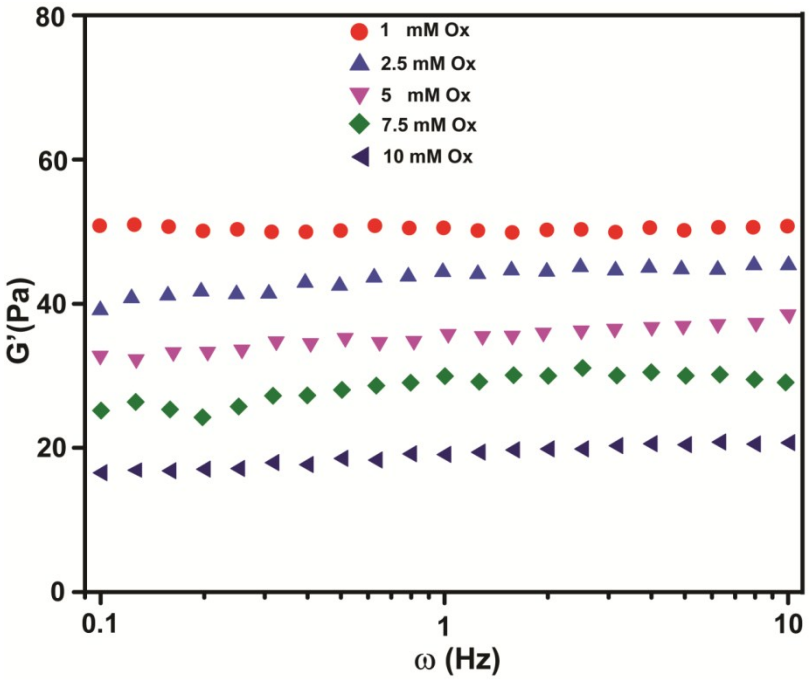

Figure S5. G' (storage modulus) versus frequency sweep of gel in presence of different concentration of oxidizing agent $Fe(ClO_4)_3$ at a fixed strain of 0.1%.

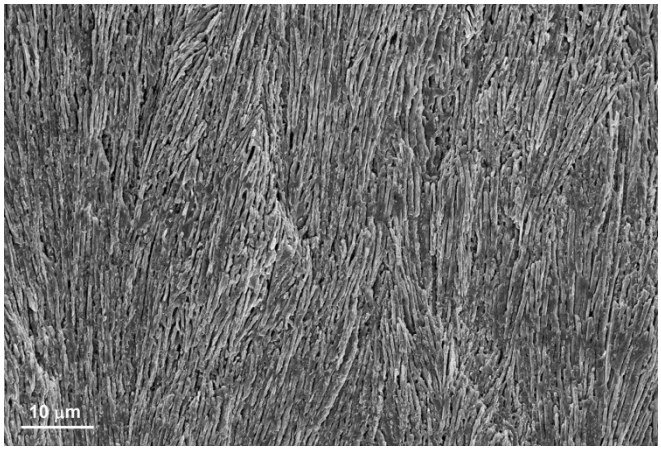

Figure S6. Representative SEM image of **C18A-Fc-NMe$_2$** hydrogel showing fibrous like morphology with stacks of hexagons (Figure 2e,g in main text).

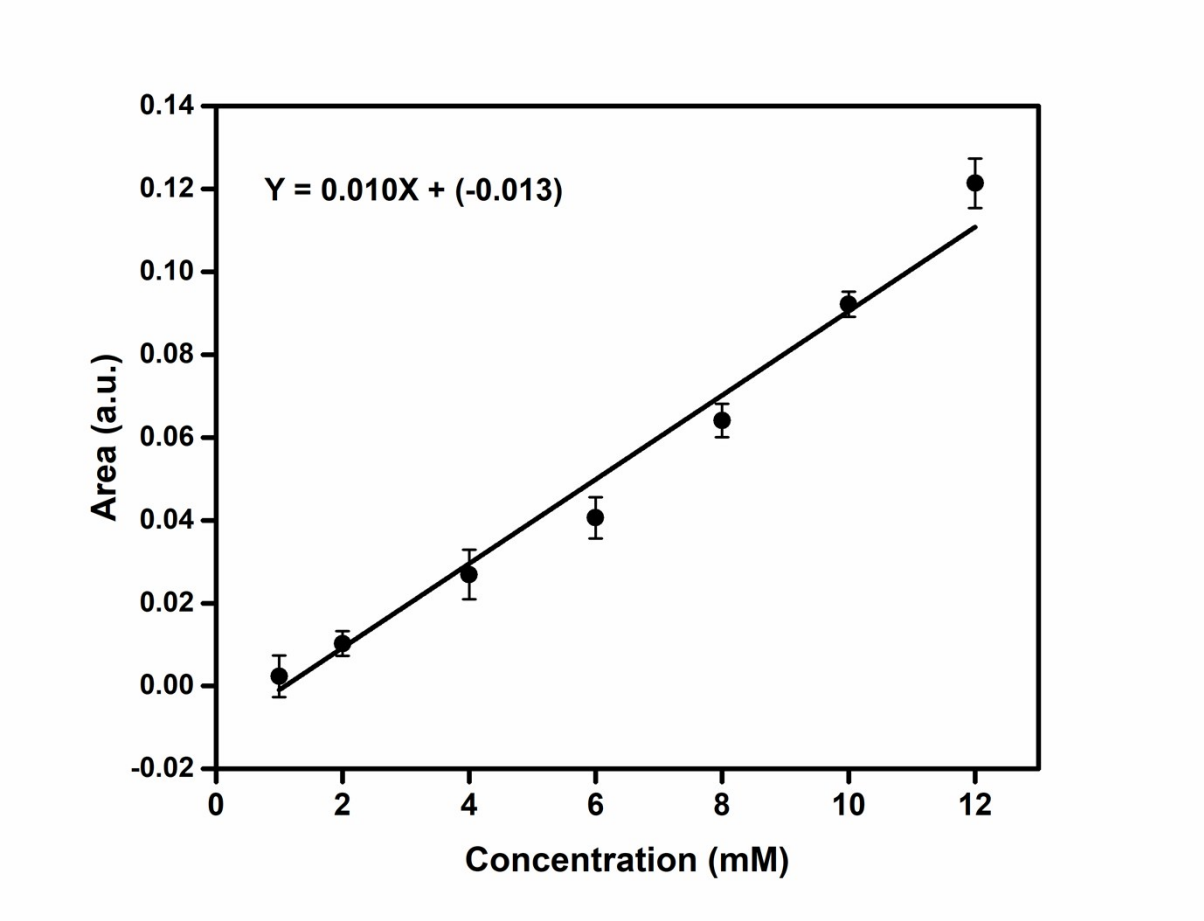

Figure S7. HPLC calibration curve of peak area vs concentration of **Fc-NMe$_2$.**

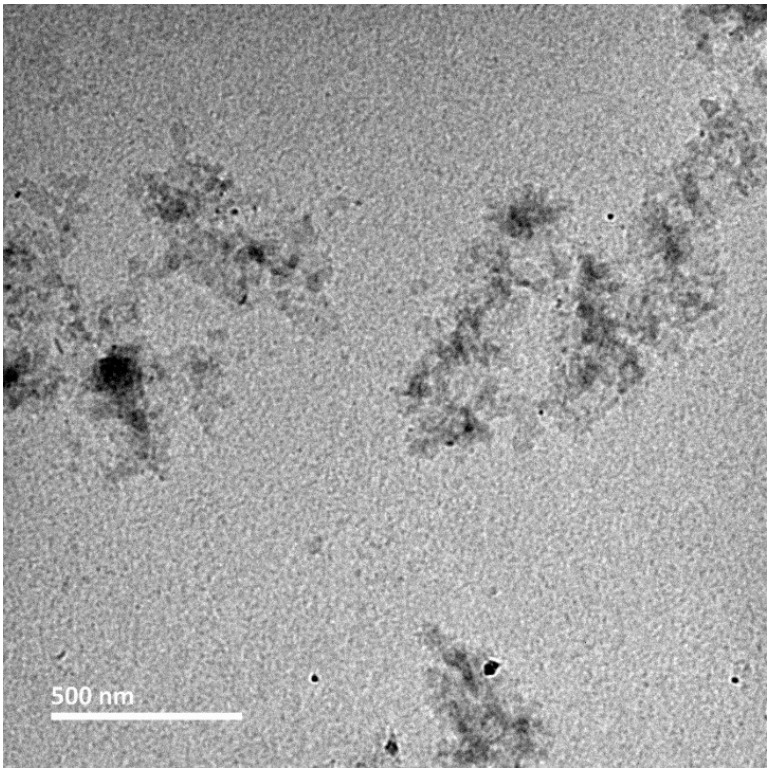

Figure S8. Representative TEM image of sample of **C3A-Fc-NMe$_2$** system.

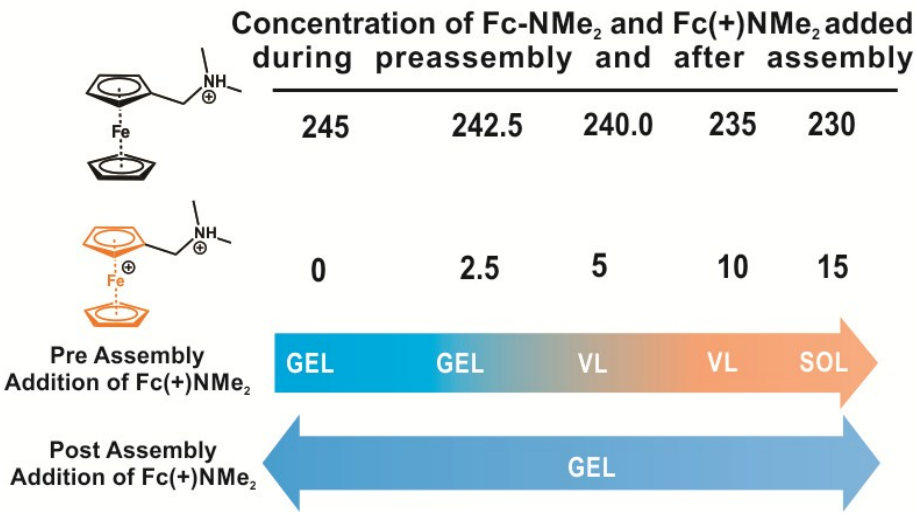

Figure S9. Effect of addition of **Fc(+)-NMe_2** in varying ratios to the pre-assembled and post-assembled mixture of **C18A** and **Fc-NMe_2**, (total concentration of **Fc-NMe_2** and **Fc(+)-NMe_2** constant at 245 mM. VL=viscous liquid).

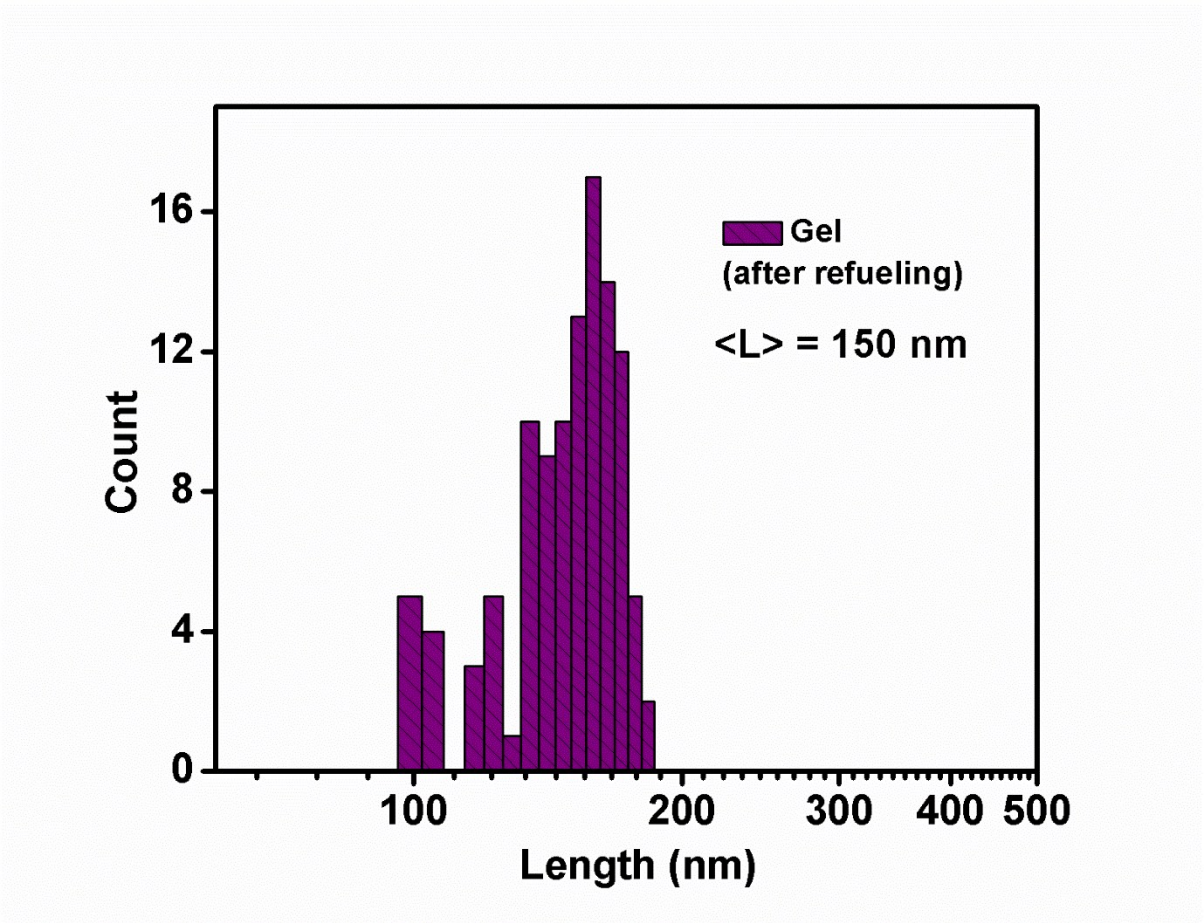

Figure S10. Histogram of refueled gel showing size distributions of hexagons (distance between the apexes)

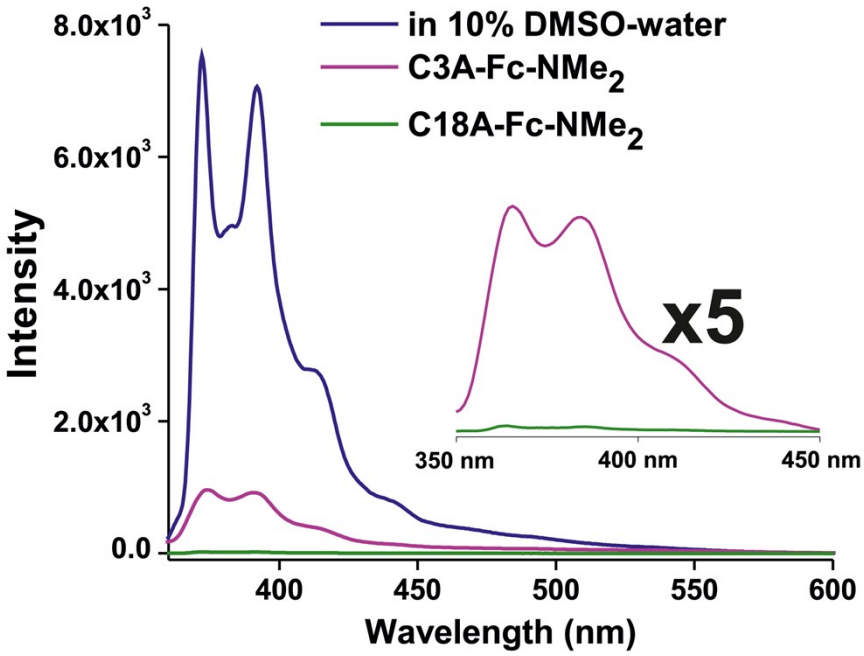

Figure S11. Fluorescence spectra of pyrene (5 μM) in **C18A-Fc-NMe$_2$** and **C3A-Fc-NMe$_2$** in 10% (v/v) DMSO-water

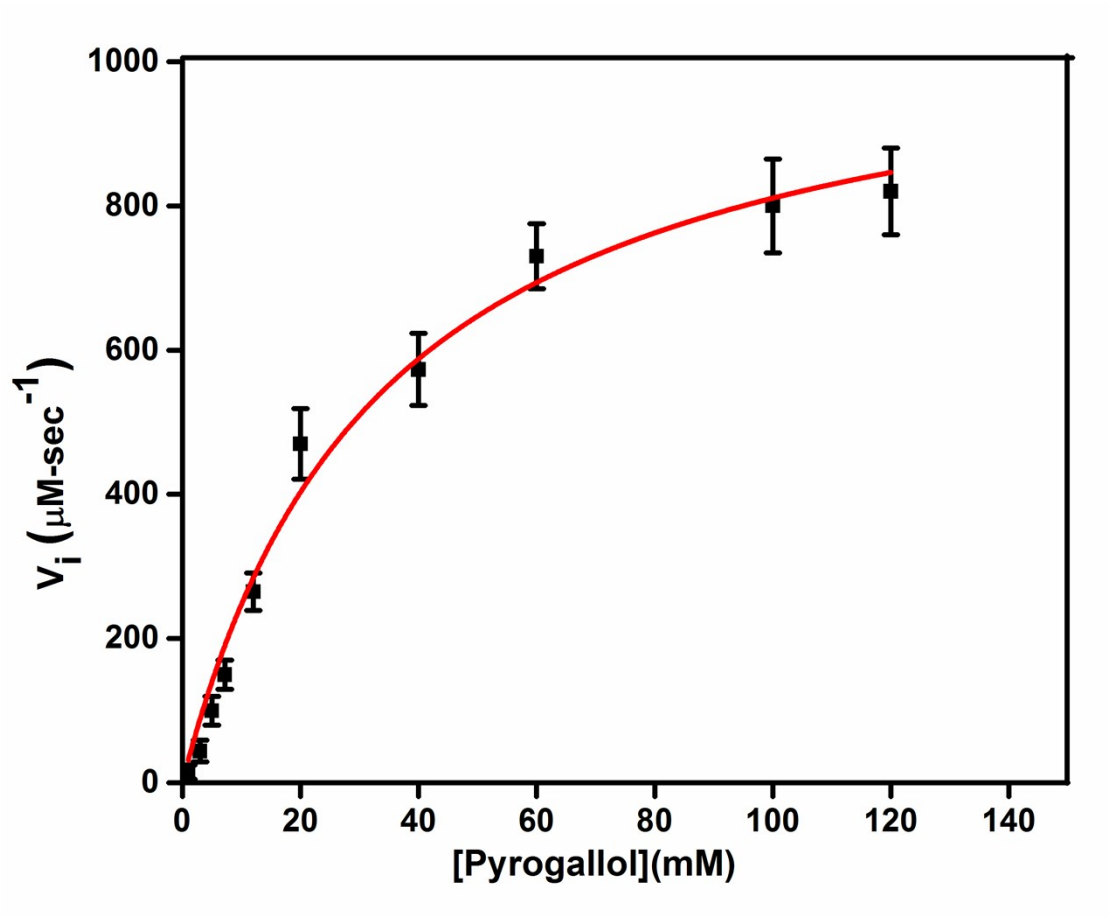

Figure S12. Representative Michaelis Menten plot of gel-hemin-histidine system. [Hemin] = 0.1 mM, [histidine] = 1.0 mM, [H_2O_2] = 30 mM.

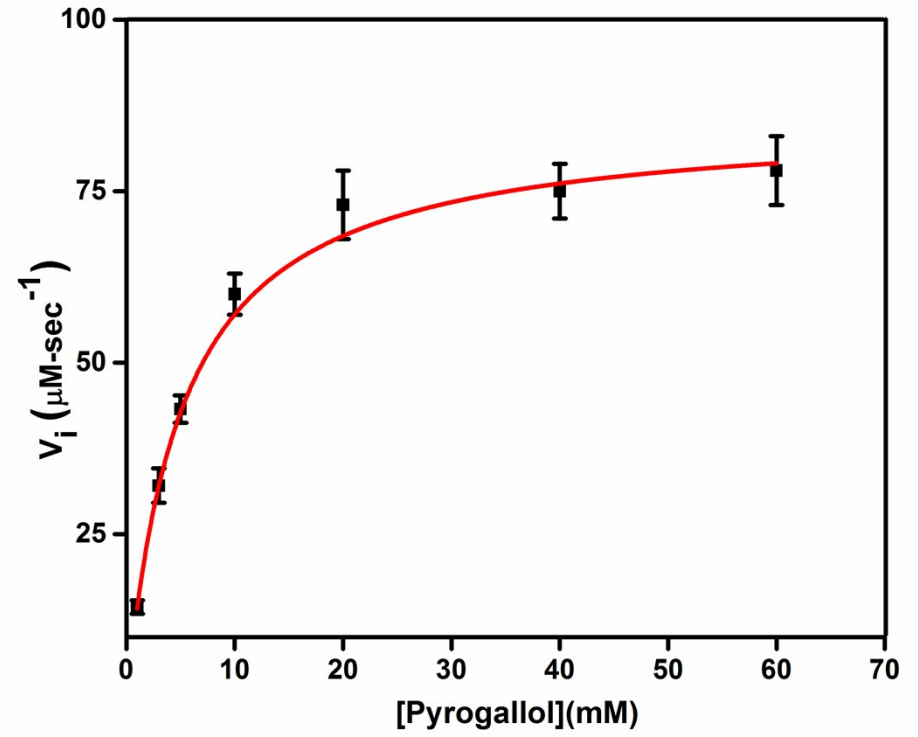

Figure S13. Representative Michaelis Menten plot of sol-hemin-histidine system. [Hemin] = 0.1 mM, [histidine] = 1.0 mM, [H_2O_2] = 30 mM.

Table S1: Kinetic parameters of the systems used. [Hemin] = 0.1 mM, [histidine] = 1.0 mM, [H_2O_2] = 30 mM.

Systems	k_{cat} (sec^{-1})	k_m (mM)	k_{cat}/k_m (mM^{-1} sec^{-1})
Gel (**C18 system**)	10.6 ± 1	30.1 ± 3	0.35 ± 0.04
Sol (**C18 system**)	0.84 ± 0.2	4.5 ± 0.5	0.19 ± 0.05
Refuelled Gel (**C18 system**)	8.3 ± 0.8	26 ± 3.5	0.32 ± 0.05
Sol (**C3 system**)	0.38 ± 0.09	1.9 ± 0.3	0.20 ± 0.06

References

1. Q. Wang, Z. Yang, X. Zhang, X. Xiao, C. K. Chang and B. Xu, *Angew. Chem. Int. Ed*., 2007, **46**, 4285–4289.
2. N. Kapil, A. Singh and D. Das*, Angew. Chem. Int. Ed*., 2015, **54**, 6492-6495.