

Supporting Information for

Entropically and Enthalpically Driven Self-Assembly of Naphthalimide Based Luminescent Organic π -Amphiphile in Water.

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Materials and Methods: All the reagents were purchased from commercial sources and used as such without further purification. ^1H NMR spectra were recorded on a Bruker DPX-300 MHz and all the spectra were calibrated against TMS. UV-Vis spectra were recorded in a HITACHI UH4150 UV/Vis spectrophotometer. The mass spectra were recorded with Water's Q-tof Micro YA263 mass spectrometer. AFM images were collected using Bruker Innova-S2 instrument in the tapping mode. Fluorescence spectra were recorded on the HORIBA scientific Fluoromax-4 fluorescence spectrophotometer. For the DLS measurement, Malvern Nanozetasizer (NANO-ZS) was utilized. The ITC experiment was performed using a Microcal-200 ITC ultrasensitive isothermal titration micro-calorimeter.

UV-visible spectroscopic studies: A stock solution of NMI-W (1.0 mM) was made separately in THF and water solvent. After that, they were left to equilibrate for two hours at room temperature. An aliquot (100 μl) of each stock solution was transferred to a glass vial, then, a necessary amount of THF or water solvent was added to it to accomplish the final concentration of 0.1mM. Prior to performing spectral measurements, the Solutions were given a one-hour equilibration period at room temperature.

X-Ray diffraction: On a Rigaku SmartLab diffractometer, XRD data were acquired using Cu K α radiation ($\lambda=0.15406$), 40 kV of voltage, and 40 mA of current. Data were collected at a sample interval of 0.02°/step, ranging from 2 to 30°.

Transmission Electron Microscopic (TEM) Measurement: In order to capture the TEM image, the water solution of NMI-W was drop casted on a 300-mesh carbon-coated copper grid and air dried for 24 hours before the measurements were carried out.

Determination of Critical Aggregation constant (CAC) by DiI encapsulation: At first stock solution of DiI having a concentration of 1mM was made in Acetone solvent. Then 20 μ l aliquot of this stock solution was added into 1ml 0.1mM NMI-W water solution and kept for 3 hours to evaporate the acetone solvent. This was used as a mother solution of DiI-encapsulated NMI-W. Now, several screw-capped vials were filled with varying concentrations of DiI-encapsulated NMI-W solution (0.1mM, 0.09mM, 0.08mM, 0.07mM, 0.06mM, 0.04mM, 0.03mM, 0.02mM, 0.01mM, 0.005mM and 0.002mM per 1ml HPLC water). Next, DiI-encapsulated NMI-W solutions were used to measure UV-Vis spectra and finally, CAC was calculated using UV spectrum data.

Isothermal Titration Calorimetric (ITC) Experiments: In a typical ITC measurement, both the sample cell and reference cell were filled with deionized water. The 40 μ L NMI-W sample solution having a concentration of 1mM was taken in the syringe. Then the sample (each injection volume 1 μ L, total number of injection-39) was injected with a time interval of 120 sec between each drop with continuous stirring at 400 rpm with maintaining temperature at 25°C. The free energy (ΔG) was calculated by the following equation-1, where R, T, and CAC are the universal gas constant, temperature, and critical aggregation constant respectively. The entropy of aggregation can be calculated from Gibb's-Helmholtz equation-2.

$$\Delta G = RT \ln CAC \dots \dots \dots \text{eq.1}$$

$$\Delta G = \Delta H - T\Delta S \dots \dots \dots \text{eq.2}$$

Dynamic Light Scattering (DLS) Study: The 0.1mM NMI-W sample solution was prepared for DLS measurements. The data were recorded at 25⁰C, 30⁰C, 40⁰C, 50⁰C, 60⁰C and 70⁰C temperature.

Fluorescence Microscopy Studies: For the Fluorescence microscopy experiment, R6G or DiI-encapsulated NMI-W was drop casted on cleaned glass slides. Then the glass slides were kept for 24 hours to evaporate the solvent completely, after that another cover glass was placed on it. Finally, the images were recorded on a fluorescence microscope (OLIMPUS CKX-53) in 10 x magnification.

Atomic Force Microscopic (AFM) Experiment: In the AFM experiment, a microscopic cover glass was coated with 20 μ L NMI-W sample solution (concentration = 0.1mM) and left to air dry for the entire night before taking pictures. Tapping mode was used to take the images.

Synthesis of the Napthalimide Derivative (NMI-2): The synthetic procedure of NMI-W was outlined as below:

Compound NMI-W-NO₂: In a round flask with 40 mL of dried toluene, Compound A (0.217g, 1.33 mmol) (synthesized based on the previous report)¹ and 4-Nitro 1,8 Napthalic Anhydride (0.304g, 1.25 mmol) were added. The mixture was subsequently stirred at 100⁰C for 24 hours under an argon atmosphere. The reaction was stopped and let it cool to room temperature. Then the solution was evaporated to get the crude product. Using silica gel as the stationary phase and 20% ethyl acetate in hexane as the eluent, it was purified by column chromatography, yielding 72% of the compound NMI-W-NO₂ as a light yellow solid. ¹H-NMR (300MHz, CDCl₃ TMS): δ (ppm) =

8.84 (d, 1H), 8.72 (d, 1H), 8.68 (d, 1H), 8.42 (d, 1H), 8.00 (t, 1H), 4.46 (t, 2H), 3.85 (t, 2H), 3.70 (t, 2H), 3.63 (t, 2H), 3.58 (t, 2H), 3.46 (t, 2H), 3.32 (s, 3H).

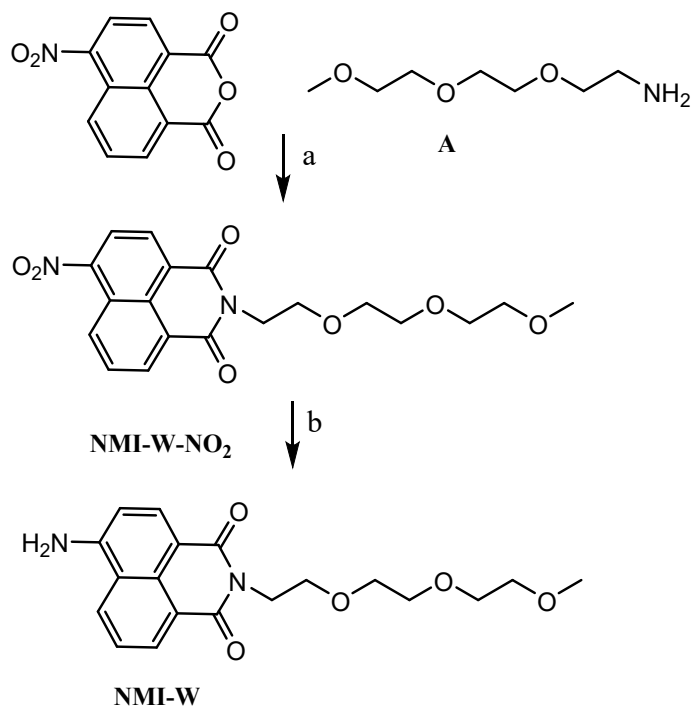
^{13}C -NMR (300 MHz, CDCl_3 , TMS): δ (ppm) = 163.33, 162.53, 149.56, 132.47, 129.86, 129.34, 129.12, 126.93, 123.90, 123.64, 122.95, 71.85, 70.50, 70.14, 67.74, 58.99, 38.92.

ESI-MS: m/z calculated for $[\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_7\text{H}] (\text{M} + \text{H})^+ = 389.1271$, Found-389.1331.

Compound NMI-W: First, the compound NMI-W-NO₂ (0.410g, 1.05 mmol) was dissolved into a minimum volume of ethyl acetate (35 mL) in a two-neck round bottle flask. Then the mixture was degassed for some time. Next Pd/C was cautiously added to the mixture and constantly stirred under H₂ atmosphere at room temperature for 24h. The Pd/C was removed by filtering with celite 545 and the brown crude product was obtained by evaporating ethyl acetate. After that, it was cleaned with ethyl acetate, yielding 89% pure yellow solid product. ^1H -NMR (300MHz, CDCl_3 , TMS): δ (ppm) = 8.44 (d, 1H), 8.15 (d, 1H), 8.00 (d, 1H), 7.50 (t, 1H), 6.64 (d, 1H), 6.03(s, broad, 2H) 4.42 (t, 2H), 3.91 (t, 2H), 3.79 (t, 2H), 3.67 (t, 2H), 3.62 (t, 2H), 3.51 (t, 2H), 3.33 (s, 3H).

^{13}C -NMR (300 MHz, CDCl_3 , TMS): δ (ppm) = 164.77, 164.04, 149.60, 133.74, 131.31, 129.57, 127.23, 124.57, 122.61, 119.78, 111.01, 109.28, 71.87, 70.62, 70.33, 70.09, 68.50, 58.92. 38.84

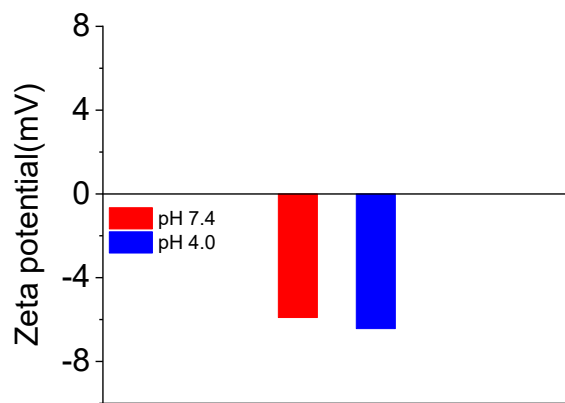
ESI-MS: m/z calculated for $[\text{C}_{19}\text{H}_{22}\text{N}_2\text{OH}] (\text{M} + \text{H})^+ = 359.1529$, Found-359.1884.



Reagents and conditions: a) Toluene, 100°C, 24h, 72%, b) Ethyl acetate, Pd/C, rt, 24h, 89%.

Scheme 1: Schematic representation of the synthesis of NMI-W.

Additional Figures



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Figure S1: Zeta potential of NMI-W at pH 7.4 and pH 4.0.

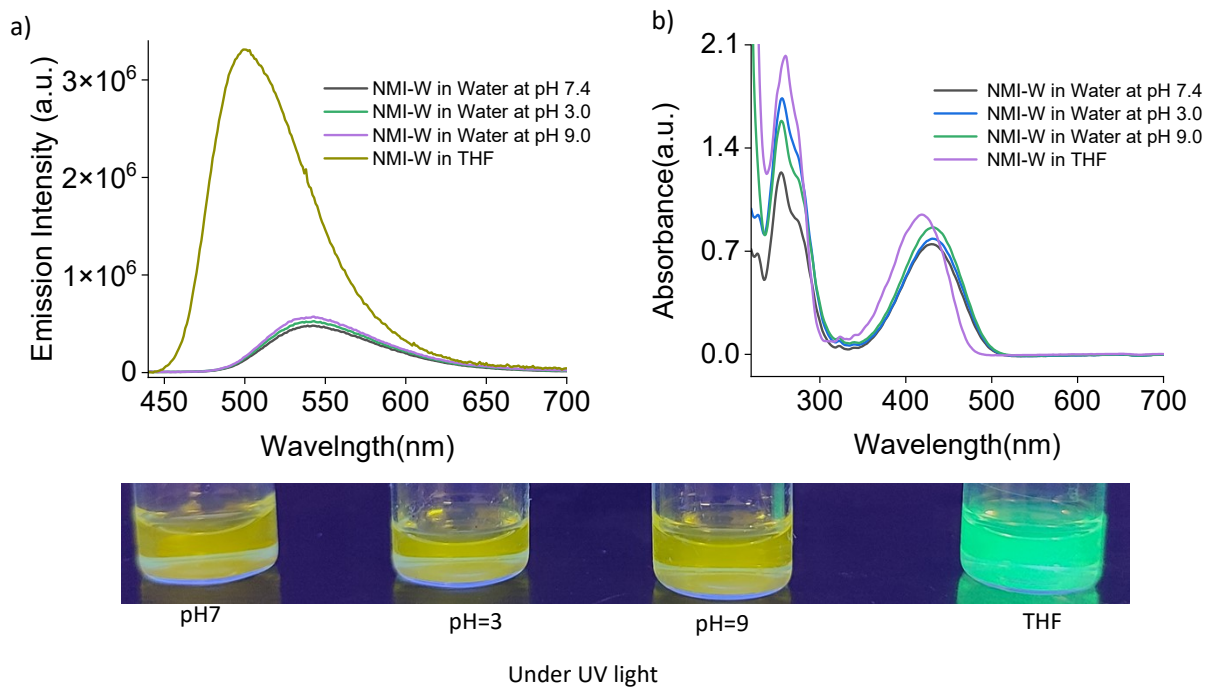


Figure S2: a) UV/Vis and b) Emission spectra to monitor the effect of pH on the self-assembly of NMI-W in aqueous medium. Below we represented corresponding solution picture under UV light.

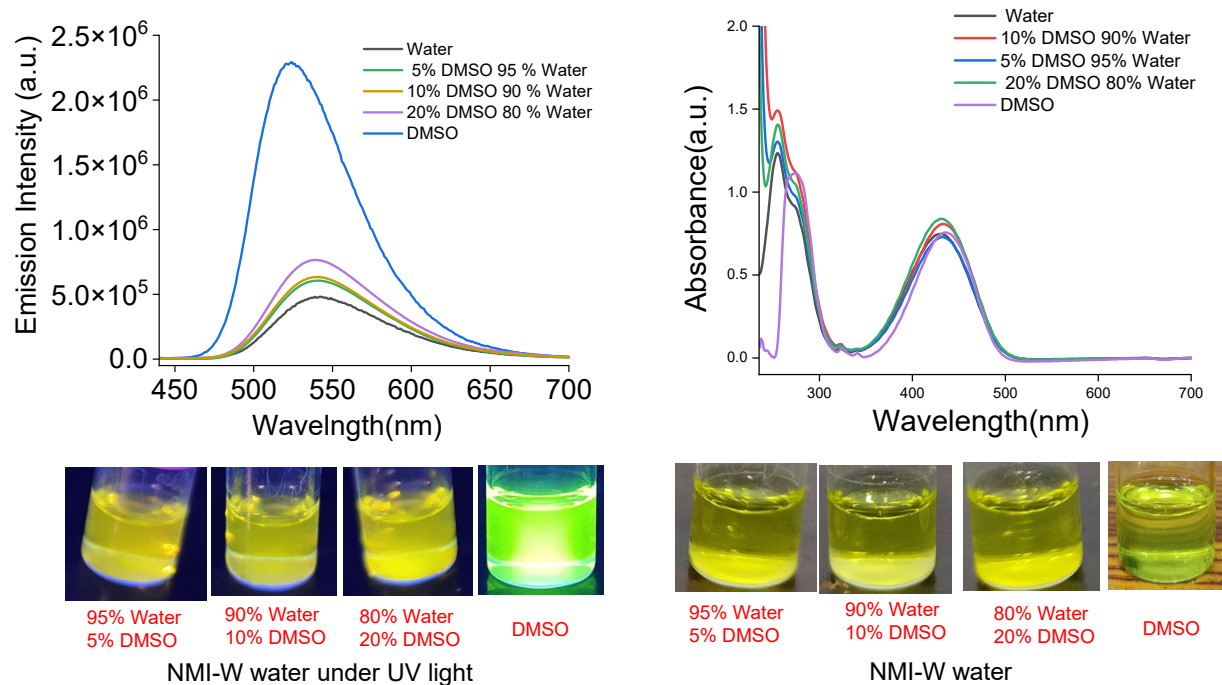


Figure S3: a) Emission spectra and b) UV/Vis spectra to examine the effect of co-solvent DMSO in aqueous solution of NMI-W (Concentration-0.1mM) c) Images in normal light d) Images under UV light.

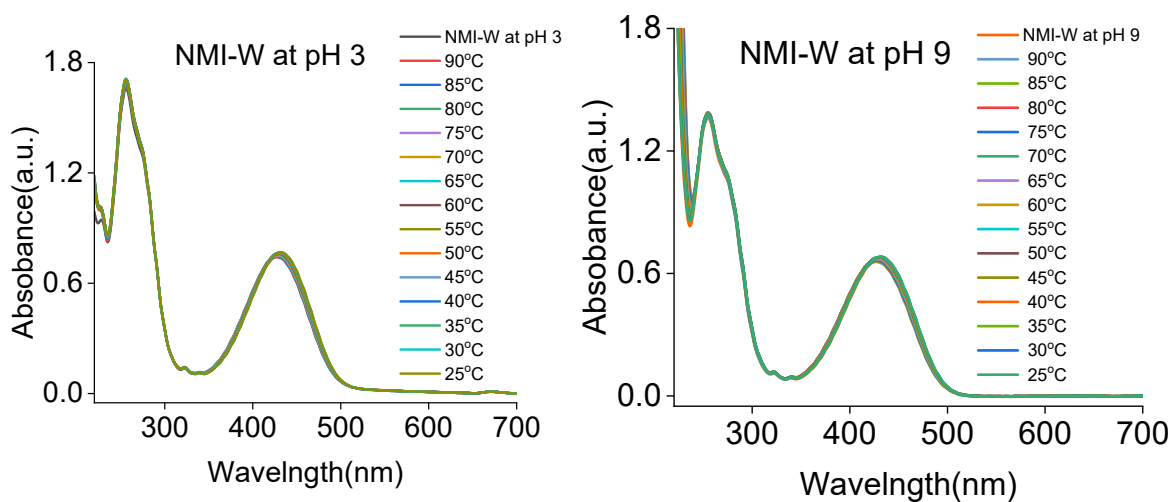


Figure S4: The UV/ Vis spectra to monitor the effect of temperature on the self-assembly of NMI-W at different pH. a) NMI-W at pH-3. b) NMI-W pH-9. Concentration of each aqueous solution=0.1mM.

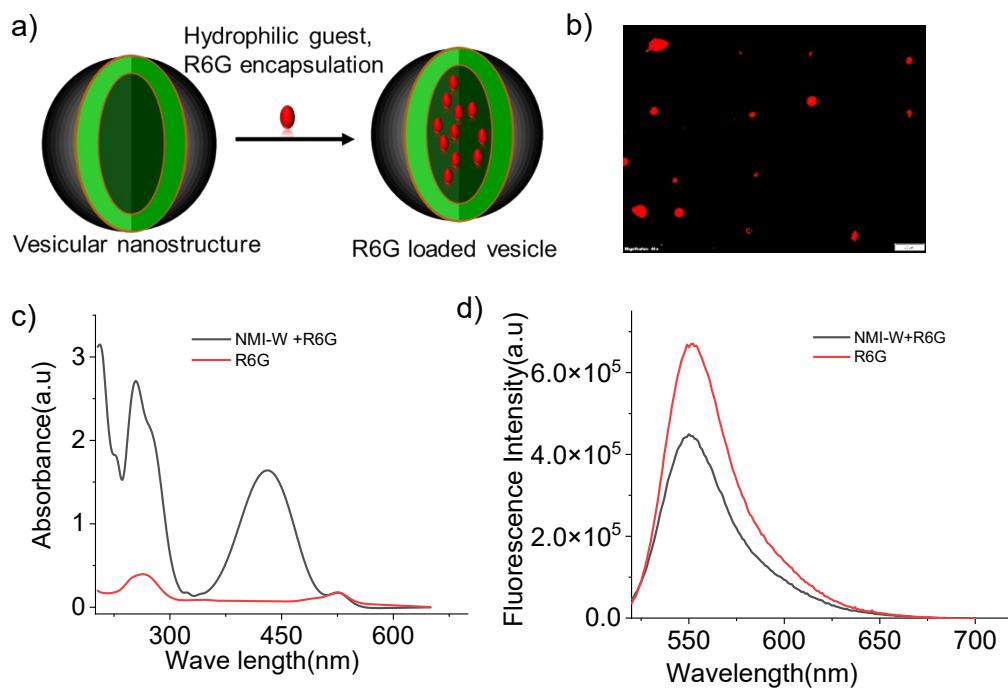


Figure S5: a) Schematic illustration of NMI-W with hydrophilic guest R6G encapsulation. b) OPM image of R6G encapsulated NMI-W. c) UV-Vis spectra of NMI-W with R6G and without R6G. d) Monitoring emission spectra to reveal the R6G dye inside the vesicular aggregates. $\lambda_{\text{ex}} = 515 \text{ nm}$, $\lambda_{\text{em}} = 550 \text{ nm}$

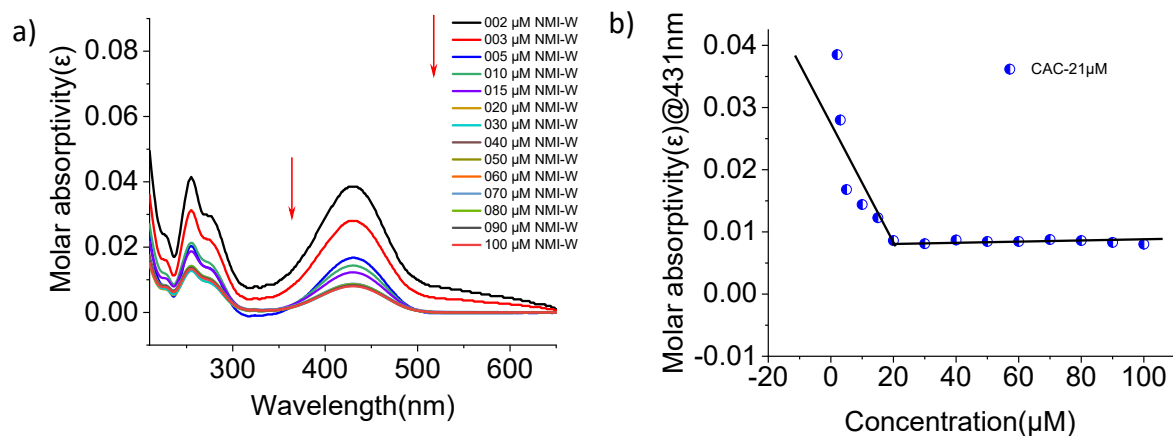


Figure S6: a) Concentration variable UV-vis spectra of NMI-W b) Measurement of CAC from molar absorptivity@431nm vs Concentration plot.

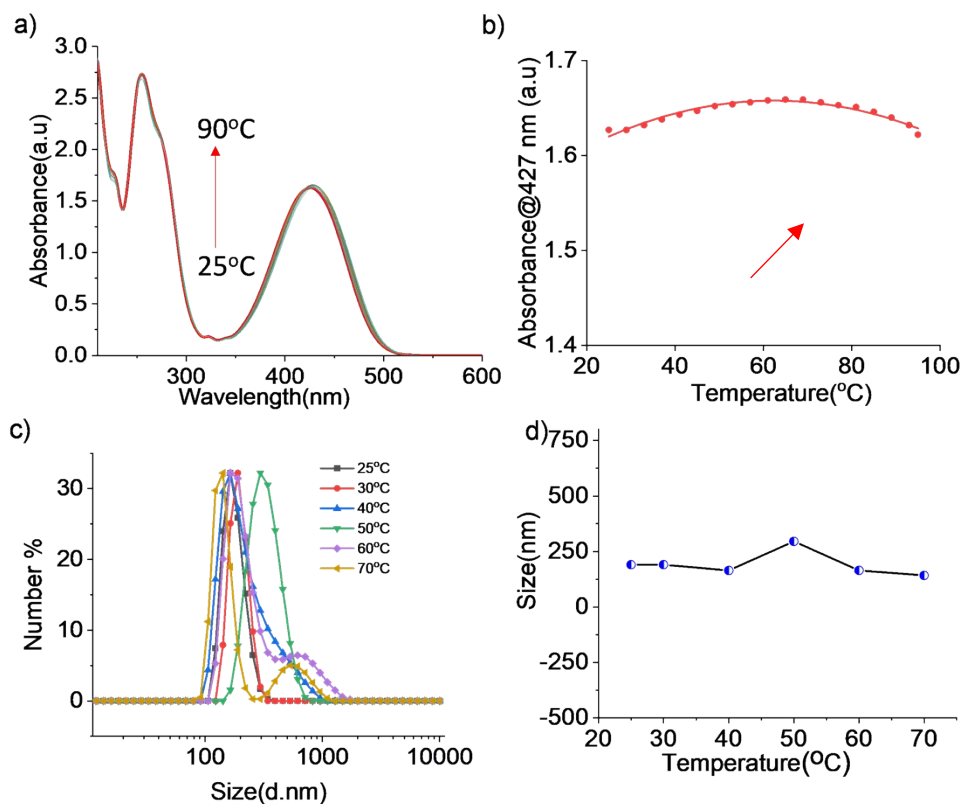


Figure S7: a) The UV-Vis spectra of self-assembled vesicular nanostructure of NMI-W at variable temperature. b) Monitoring thermal stability of vesicular nanostructure @427 nm wavelength. c) Temperature variable DLS plot d) probing thermal stability of self-assembled nanostructure by temperature variable DLS plot.

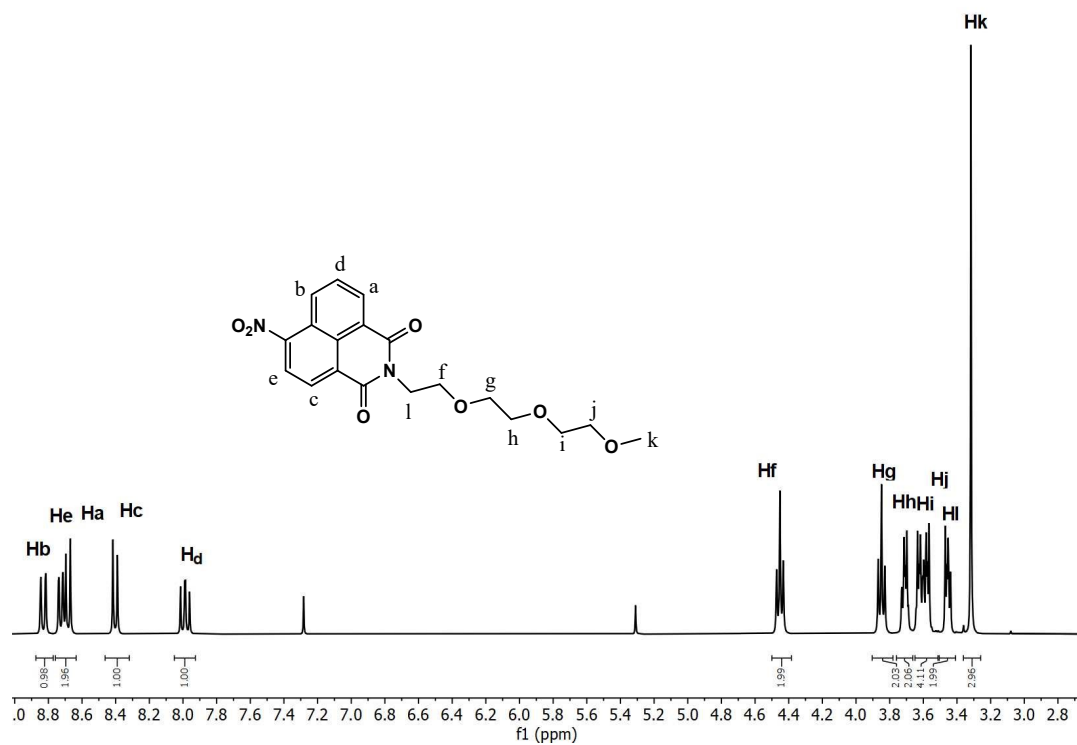


Figure S8: ¹H- NMR spectrum of Compound NMI-W-NO₂. Solvent-CDCl₃

SM-NM1-WNO2.2.fid

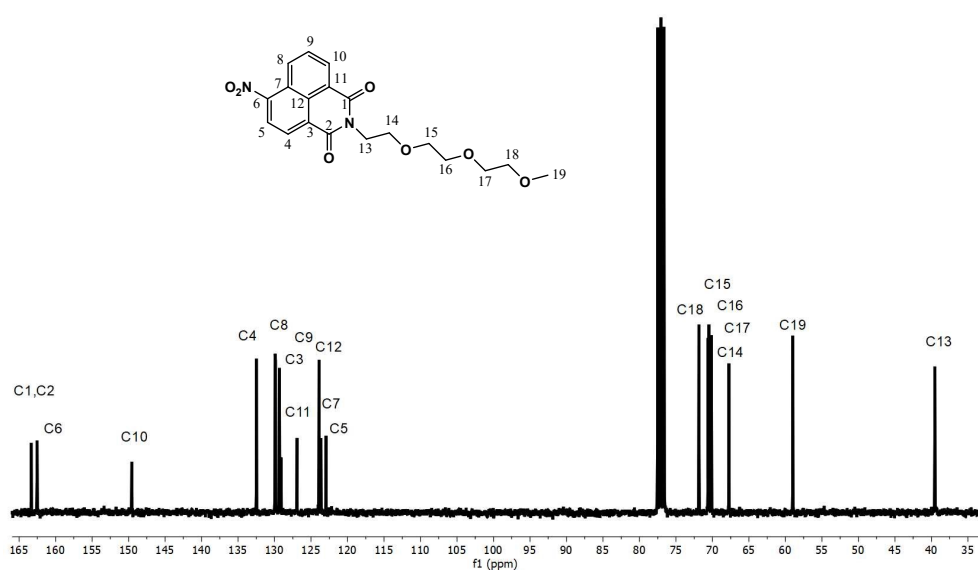


Figure S9: ¹³C- NMR spectrum of Compound NMI-W-NO₂. Solvent-CDCl₃

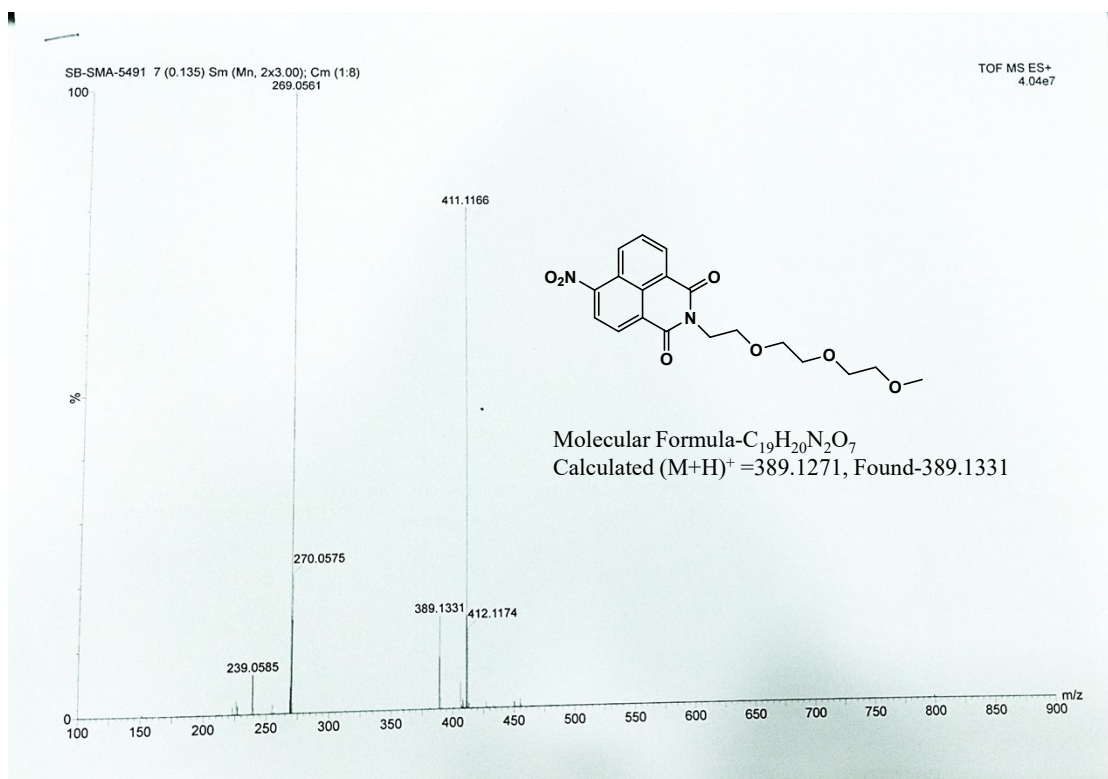


Figure S10: ESI-MS spectrum of Compound NMI-W-NO2.

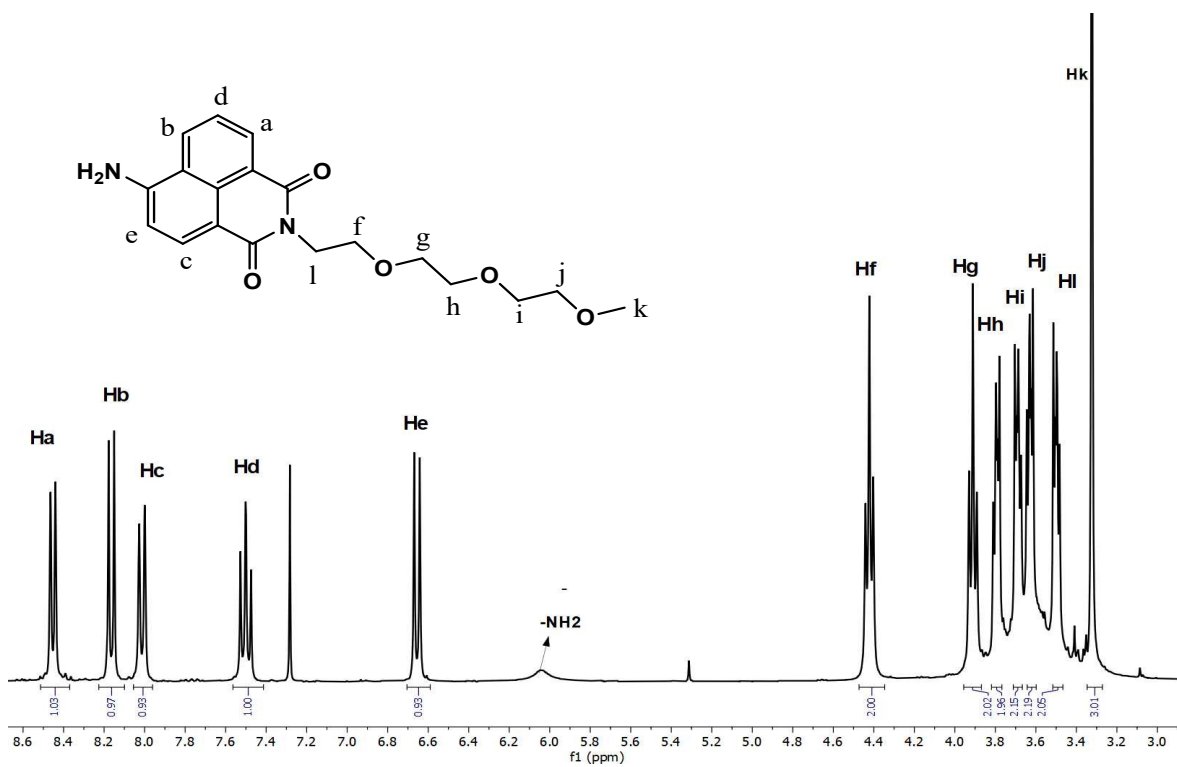


Figure S11: ¹H- NMR spectrum of Compound NMI-W. Solvent-CDCl₃

MA-NMI-W1.2.fid

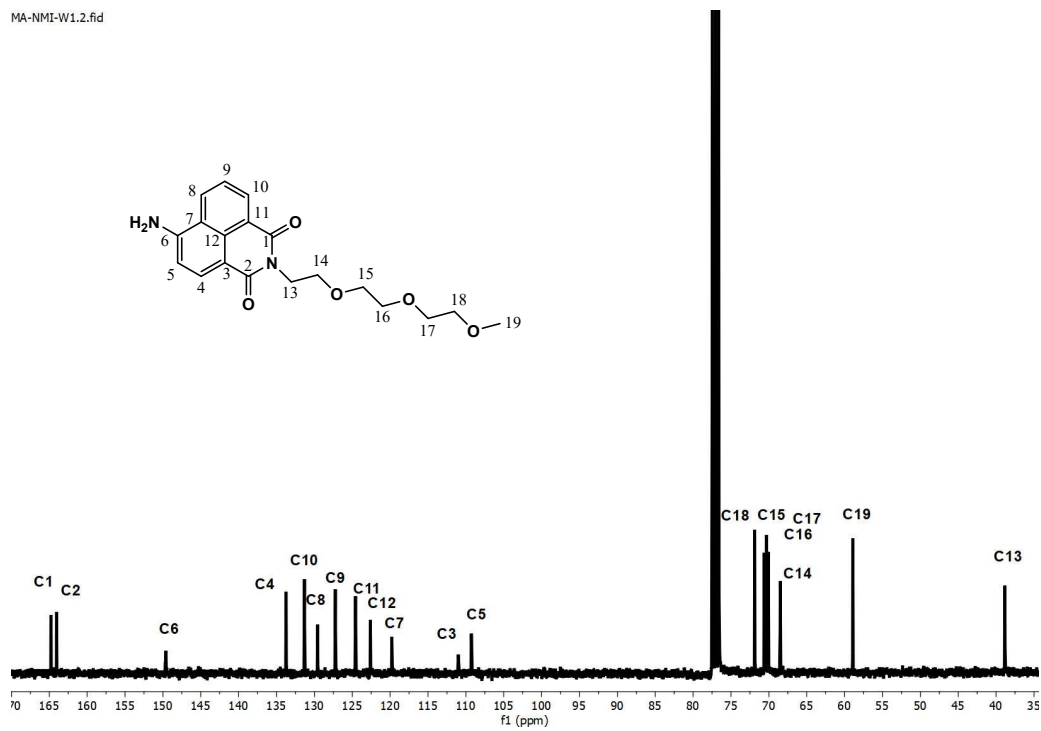


Figure S12: ¹³C- NMR spectrum of Compound NMI-W. Solvent-CDCl₃

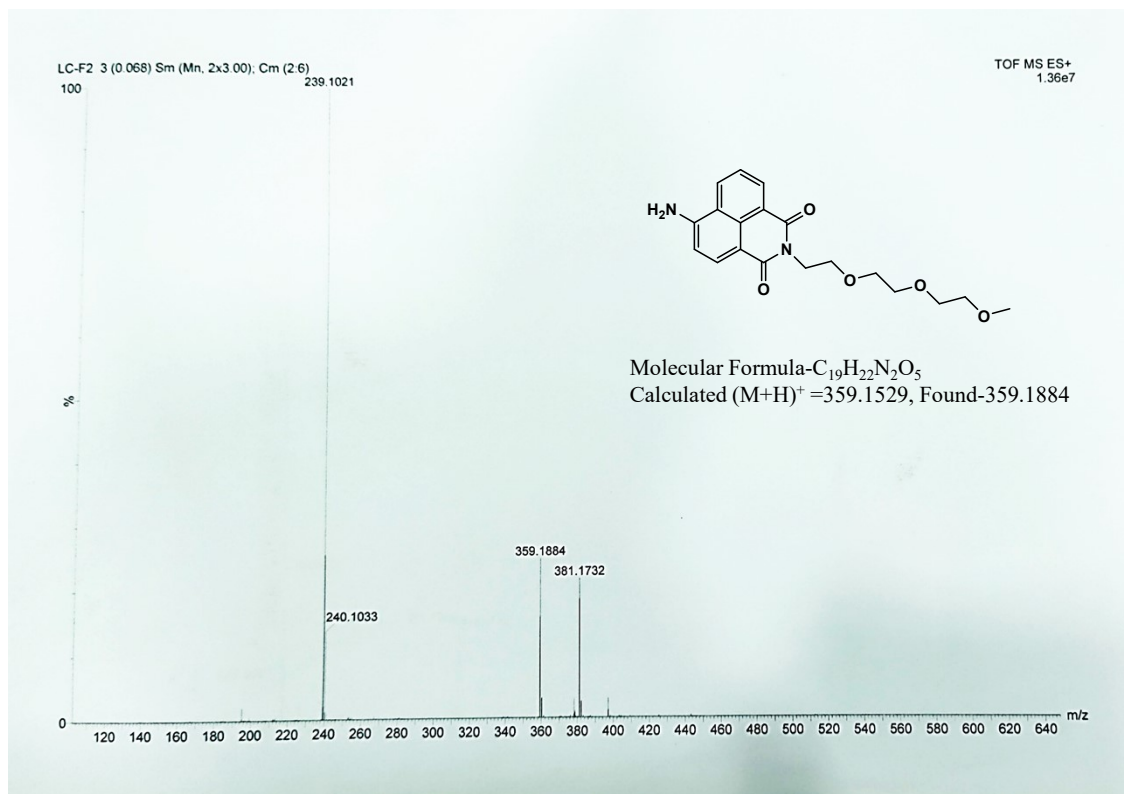


Figure S13: ESI-MS spectrum of Compound NMI-W.

Molecular Dynamics (MD) Study: Here, single NMI molecule was energy-minimized using molecular mechanics using Desmond (version 2020.1). A random distribution of the 5 NMI molecules was allowed for the MD study. The Desmond, a GPU-accelerated program, was utilized for running molecular dynamics simulations on the Linux operating system. We employed the explicit solvent model with SPC water molecules² and the OPLS-2005 force field³ to prepare the systems. To reduce the energy of each system, we used a cubic boundary solvation box with dimensions of 10Å x 10Å x 10Å. Subsequently, each system was neutralized by adding an appropriate amount of Na⁺ or Cl⁻ ions. Additionally, an extra 0.15 M NaCl solution was introduced to each system to mimic physiological conditions.

The prepared systems underwent relaxation using the default method in Desmond. Firstly, the systems were equilibrated for 500 ps at 10 K using an NVT ensemble. This was followed by a short equilibration and minimization run for 100 ns at 300 K and 1.01325 bar pressure using an NPT ensemble.

The NPT ensemble⁴ was set up using the Nose-How over chain coupling method. Throughout all simulations, the temperature was maintained at 300 K, the relaxation period was set to 1.0 ps, and the pressure was set to 1 bar. A time step of 2 fs was employed. Pressure was controlled using the Martyna-Tobias-Klein chain coupling scheme barostat⁵ method with a relaxation time of 2 ps. Long-range electrostatic interactions were calculated using the particle mesh Ewald technique with a fixed coulomb interaction radius of 9.0 Å.⁶

Bonded forces for each trajectory were calculated using the RESPA integrator at a time step of 2 fs. Lastly, simulation interaction graphs were analyzed using Maestro, a Desmond GUI program. The simulation-derived data including root-mean-square deviation (RMSD), root-mean-square

fluctuation (RMSF), number of hydrogen bonds, and radius of gyration can be used to predict the stability of NMI self-assembly.

1. M.R. Molla; P. Prasad; S. Thayumanavan. *J. Am. Chem. Soc.* **2015**, *137*, 23, 7286–7289.
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