Electronic Supplementary Information

Chemically Fueled Dynamic Switching Between Assembly-Encoded Emissions

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Materials and Instruments

2-Chlorotrilyl Chloride Resin, protected amino acids and 2-(1h-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) were purchased from BLD Pharma, China. All other reagents, chemicals and solvents were procured from Sigma-Aldrich, TCI, Spectrochem and SRL India. All components were used without further purification unless otherwise indicated. The peptides were synthesized employing standard Solid Phase Peptide Synthesis (SPPS) strategy using 9-Fluorenylmethyloxycarbonyl (Fmoc) chemistry. All the samples for the experiments were prepared using Milli-Q water with a conductivity of less than 2 μS cm^{-1}. Peptides were purified by Waters HPLC system equipped with UV-Vis detector using Atlantis® T3 C18 5 μm, 10 x 250 mm semi-prep HPLC column. Analytical HPLC was carried out in an Agilent 1260 Infinity II system using a YMC Triart C18 5 μm, 4.6 x 250 mm analytical column. High-resolution mass spectra (HRMS) were recorded using 1290 Infinity II UPLC System with Agilent 6545 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) and UHPLC-QTOF-HRMS from Agilent, Model: G6546A. ^{1}H-NMR and ^{13}C-NMR were acquired using a JEOL 400 YH instrument (400 MHz). Emission spectra were recorded on a Fluoromax Plus spectrophotometer whereas Absorption spectra were acquired on an Agilent Cary Series spectrometer utilizing standard 10 mm and 1 mm path quartz cuvettes. For the FTIR experiment, a Parkin Elmer Spectrum FT-IR spectrometer was used. Circular Dichroism (CD) spectra were acquired in a Jasco J-1500 CD spectrometer using a 2 mm path quartz cuvette. Fluorescence lifetimes were measured using Picosecond Time-resolved and Steady State Luminescence Spectrometer on an Edinburg Instruments Lifespec II & FSP 920 instrument using pulsed diode laser with λ_{ex}= 375 nm. Powder XRD data were recorded on a Malvern PANalytical Empyrean Series III. SEM and TEM images were captured on a ZEISS Gemini SEM 360 instrument and JEOL 2100F microscope respectively.

Synthesis

Synthetic routes of building block
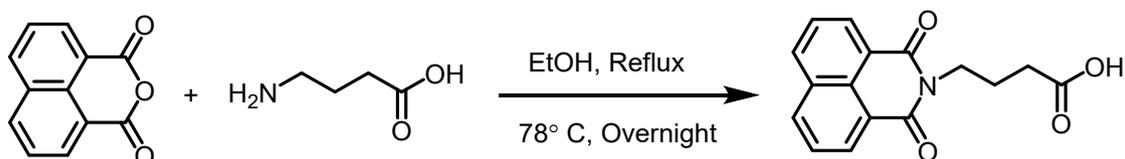
**Scheme S1.** Synthesis of **NI-GABA-OH**

NI-GABA-OH was synthesized using a previously published protocol with slight modification.[1] In a typical procedure, 2g (10.1 mmol) 1,8-Naphthalic anhydride and 1.35g (1.3 eqv. 13.1 mmol.) 4-aminobutyric acid were taken in a 100 mL round bottom flask and 15 mL ethanol was added. The reaction mixture was heated at 80 °C for 10 hours with constant stirring. After cooling to ambient temperature, the reaction mixture was diluted with 50 mL of cold water and vacuum filtered followed by repetitive washing with water to afford a white-colored product. Finally, the product was dried under vacuum. Yield: 74% (2.11g).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 12.01 (s, 1H), 8.45-8.39 (m, 4H), 7.85-7.81 (t, J = 7.8 Hz, 2H), 4.09-4.05 (t, J = 6.8 Hz, 2H), 2.32-2.29 (t, J = 7.4 Hz, 2H), 1.92-1.85 (m, 2H).

^{13}C-NMR (101 MHz, DMSO-d_6) δ: 173.9, 163.3, 134.1, 131.2, 130.4, 127.3, 127.0, 121.9, 31.3, 30.4, 22.9.

MS-ESI (HRMS): m/z: calculated 283.0845 for $C_{31}H_{38}N_4O_9$, found: 284.0924[M+H]$^+$

Synthetic route of peptides
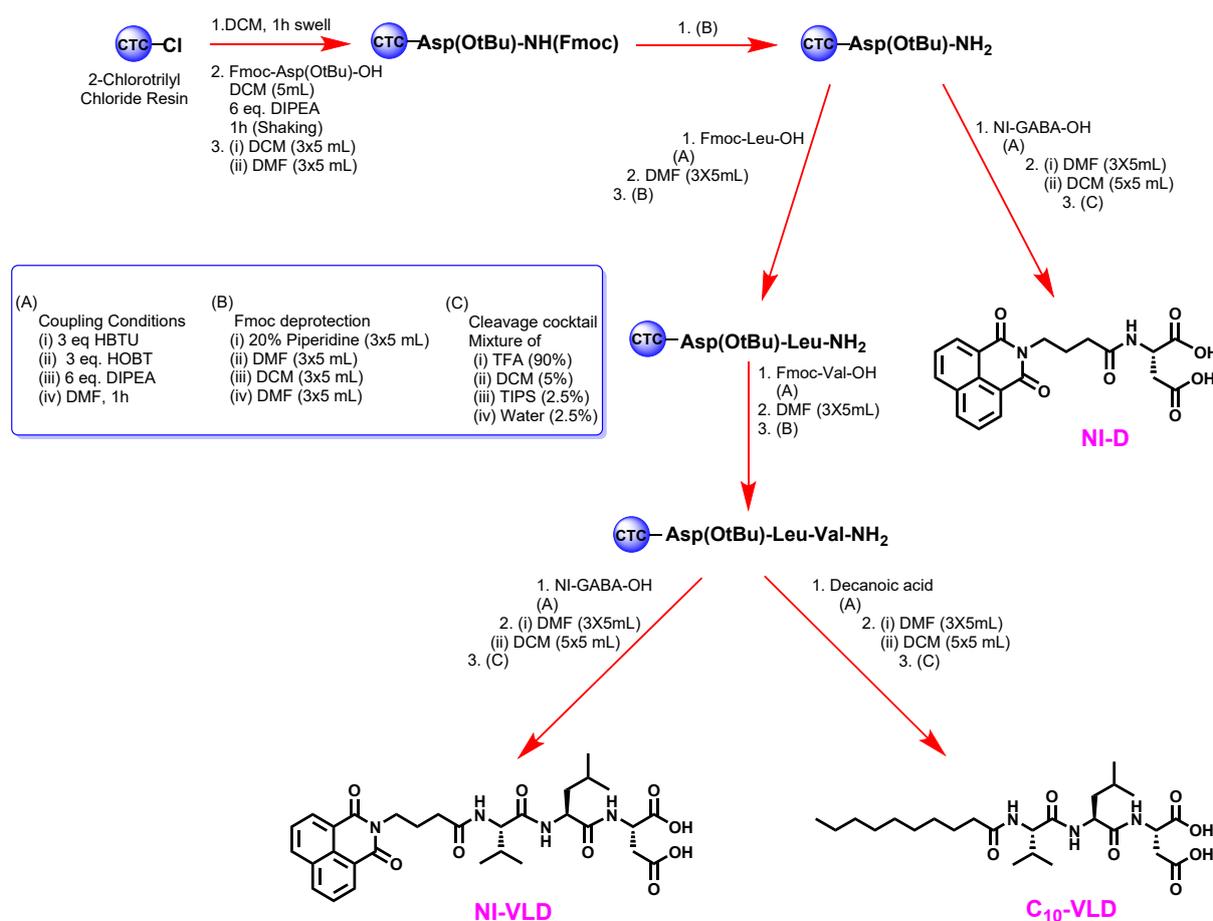
Scheme S2. Solid Phase Peptide Synthesis

All the peptides (**NI-VLD**, **C$_{10}$-VLD** and **NI-D**) were synthesized by standard 'Fmoc' solid-phase peptide synthesis strategy employing 2-Chlorotrilyl Chloride Resin (2-CTC resin) as the solid support. The loading capacity of 2-CTC resin was 1.082 mmol/g. Prior to the synthesis, the resin was appropriately dried under reduced pressure. The first coupling was carried out in DCM employing Fmoc-Asp(OtBu)-OH (3 eqv.) and DIPEA (6 eqv.) followed by washing and 'Fmoc' deprotection. The peptide chain elongation at the N-terminus was carried out by coupling the appropriate moiety (3 eqv.) under standard conditions employing HBTU (3 eqv.) and DIPEA (6 eqv.) in the presence of HOBT (3 eqv.) in DMF. The final peptides were cleaved from the resin using a cleaving cocktail of TFA-DCM-TIPS-Water (90:5:2.5:2.5). The cleaved peptides were precipitated from cold dry diethyl ether followed by purification using semi-prep HPLC (acetonitrile/water having 0.1% TFA system as the eluent, in a gradient of 10 to 80 % acetonitrile over 20 min) followed by lyophilization to afford the pure peptides.

Characterization of the peptides

NI-VLD: **^{1}HNMR** (400 MHz, DMSO-d_6) δ: 8.51-8.46 (m, 4H), 8.04 (s, 1H), 7.92-7.83 (m, 4H), 4.52-4.46 (m, 1H), 4.34-4.28 (m, 1H), 4.14-4.06 (m, 3H), 2.67-2.53 (m, 2H), 2.29-2.20 (m, 2H), 1.98-1.84 (m, 3H), 1.63-1.55 (m, 1H), 1.45-1.41 (t, J = 7.6 Hz, 2H), 0.85-0.79 (m, 12H)

^{13}C-NMR (101 MHz, DMSO-d_6) δ: 172.61, 171.96, 171.87, 171.60, 171.03, 163.56, 158.81, 158.50, 134.37, 131.37, 130.80, 127.48, 127.29, 122.18, 118.66, 115.69, 57.75, 50.75, 48.62, 40.69, 40.43, 36.58, 32.98, 30.31, 24.20, 24.07, 23.11, 21.46, 19.34, 18.14.

MS-ESI (HRMS): m/z: calculated 610.2639 for C$_{31}$H$_{38}$N$_4$O$_9$, found: 611.2646 [M+H]$^+$

C$_{10}$-VLD: **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 8.11 (s, 1H), 7.92 (s, 1H), 7.82 (s, 1H), 4.52-4.47 (m, 1H), 4.36-4.30 (m, 1H), 4.15-4.11 (m, 1H), 2.68-2.53 (m, 2H), 2.19-2.07 (m, 2H), 1.98-1.89 (m, 1H), 1.64-1.54 (m, 1H), 1.49-1.38 (m, 4H), 1.28-1.17 (m, 12H), 0.99-0.97 (m, 1H), 0.87-0.79 (m, 14H).

^{13}C-NMR (101 MHz, DMSO-d_6) δ: 172.32, 172.27, 171.75, 171.69, 171.02, 57.64, 50.64, 48.79, 40.92, 35.15, 31.33, 30.26, 28.98, 28.86, 28.72, 28.60, 25.49, 24.04, 23.12, 22.15, 21.53, 19.35, 18.25, 17.90, 14.02.

MS-ESI (HRMS): m/z calculated 499.3258 for C$_{25}$H$_{45}$N$_3$O$_7$, found: 500.3486 [M+H]$^+$

NI-D: **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 8.51-8.45 (m, 4H), 8.17 (s, 1H), 7.89-7.86 (t, J = 7.8 Hz, 2H), 4.50-4.45 (dd, 1H), 4.08-4.05 (t, J = 7.2 Hz, 2H), 2.67-2.53 (m, 2H), 2.22-2.18 (t, J = 8.4 Hz, 2H), 1.89-1.82 (m, 2H)

^{13}C-NMR (101 MHz, DMSO-d$_6$) δ: 172.57, 171.75, 171.49, 163.54, 134.34, 131.36, 130.79, 127.47, 127.28, 122.17, 48.50, 40.60, 36.12, 32.90, 23.97.

MS-ESI (HRMS): m/z calculated 398.1114 for $C_{20}H_{18}N_2O_7$, found $[M+H]^+$: 399.1285

Methods

Sample preparation

Stock solutions of all the peptides were prepared by dissolving the solid peptide in DMSO to make the final concentration as 100 mM. A 500 mM MES buffer of pH 6 was prepared. Stock solutions of 1 M EDC were prepared by dissolving EDC in MQ water, freshly every time prior to the experiments. Working solutions at different compositions of aqueous buffer in DMSO (represented as f_a) were prepared by adding the required amount of stock solution to DMSO and then diluted with MES buffer of pH 6, making the final concentration of buffer 50 mM (except for pure DMSO case, i.e. f_a= 0%). For instance, **Agg-1** was prepared by adding 20 μL of **NI-VLD** stock (100 mM in DMSO) to 80 μL DMSO and then 100 μL MES buffer (500 mM) and 800 μL Milli-Q water were added, followed by thorough mixing. The final concentration of **NI-VLD** was 2 mM in f_a= 90%. The mixture was incubated at room temperature for 1 hr before conducting experiments. The **Agg-1** to **Agg-2** dynamic assembly cycle was initiated by adding the required amount of 1 M freshly prepared EDC stock to the **Agg-1** (2 mM).

Absorption spectra and Turbidity measurement

The absorption spectra of **NI-VLD** at different solvent compositions (f_a= 0 to 90%) were measured using an Agilent Cary Series spectrometer utilizing standard 10 mm or 1 mm path quartz cuvettes at room temperature. The transient turbidity experiments of reaction cycles were measured by recording the change in absorbance at 500 nm (where no absorbance peak appeared from the sample) as a function of time after adding EDC to **Agg-1** at room temperature. The final EDC concentrations were 2.5 mM, 5 mM, 7.5 mM and 10 mM.

Dynamic Light Scattering (DLS)

The particle size of **Agg-1** (2 mM, f_a= 90%) was measured at 298 K using Zetasizer Nano-ZS90 (Malvern) at room temperature. A He-Ne LASER (λ= 632.8 nm) was used as an excitation source for dynamic light scattering.

Emission Spectra

Emission spectra of different **NI-VLD** samples were recorded by exciting at 365 nm. For the dynamic emission cycle, the spectra were recorded by exciting at the same wave length just after the addition

of EDC and the intensities at 408 nm, 490 nm and ratios of 490 nm to 408 nm were plotted as a function of time. Moreover, emission intensities were recorded for repetitive dynamic cycles until the intensities came back close to the initial values after each EDC batch addition. For temperature-dependent emission spectra, the sample temperature was varied from 30° C to 70° C to and fro at a rate of change temperature 1° C/min.

For the Thioflavin T (ThT) assay, 10 μM ThT was used as the fluorescent probe for all experiments, using an excitation wavelength of 450 nm and an emission wavelength of 482 nm. Similarly for the Nile Red assay, 2 μM final probe concentration was used where the excitation wavelength was fixed at 552 nm and emission at 617 nm.

Time-dependent NMR for dynamic Agg-1 to Agg-2 cycle

A 600 μL of 2 mM **NI-VLD** was prepared in aqueous buffer/DMSO (f_a= 90%, all in the deuterated solvent) and taken in a NMR tube for a typical reaction cycle. The reaction cycle was initiated by adding 6 μL freshly prepared EDC (1 M in D_2O) and the spectra were recorded at different time intervals.

Confocal Fluorescence Microscopy

Confocal fluorescence microscopy was performed on a Leica STELLARIS 5 Confocal Microscope. Nile Red was used as a fluorescent probe. A 2 mM **NI-VLD** solution was prepared (**Agg-1**) as previously mentioned and during the sample preparation Nile red was added and incubated for 1 hr. Following that, 50 μL of this sample was deposited on a glass slide and covered with a 12 mm diameter coverslip and the image was taken. In another slide, a 100 μL sample was placed (**Agg-1**) and fueled with 10 mM EDC, covered similarly and the image was recorded at around 7 min after the addition of EDC (to provide time to form **Agg-2** with maximum cyclic anhydride population). The samples were excited with a 552 nm laser and imaged at 580-660 nm.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy Study of the samples was done in a ZEISS Gemini SEM 360, Germany. SEM samples were prepared by drop casting method on aluminium coated glass slides. In a typical procedure, 10 μL **Agg-1** was drop-cast and air dried prior to imaging. However, for **Agg-2**, 10 mM EDC was added to **Agg-1**, and after 7 mins (to get the maximum population of cyclic anhydride) of reaction, 10 μL sample was drop cast and immediately freeze-dried (to arrest the reaction kinetics) and then imaged.[2]

Transmission Electron Microscopy (TEM)

TEM study was conducted by casting the sample on carbon coated Cu grid (300 mesh Cu grid with thick carbon film from Pacific Grid Tech, USA). In a typical process, a 5 μL sample was drop-cast on the grid and allowed to absorb for 30 sec, followed by bloating the excess sample with tissue paper and air drying. However, for **Agg-2,** the sample was freeze-dried (around 7 min after the addition of EDC to **Agg-1)**, instead of air drying before imaging.[2]

Circular Dichroism

CD Spectra of samples were recorded using a JASCO J-1500 CD Spectrometer, equipped with a Peltier temperature Control. The data were collected using a 2 mm path length cuvette at a scan rate of 100 nm min^{-1} with 0.5 nm intervals and 2 nm bandwidth. In a typical experiment, 2 mM **NI-VLD** (**Agg-1**) was prepared and to it EDC (10 mM final conc.) was added and the CD spectra were recorded at different time intervals. Additionally, CD kinetics at a fixed wavelength (370 nm) was also recorded after initiating the **Agg-1** to **Agg-2** temporal and dynamic cycle. Prior to every CD experiment, baseline correction using comparable reference solutions was carried out.

Powder X-ray diffraction (**PXRD) experiment**

PXRD data were collected on Malvern PANalytical Empyrean Series III, using Cu -Kα radiation (wavelength 1.540598 Å) at a 45 kV voltage and a 40mA current. Data were interpreted using High Score Plus software. The layer thickness d was calculated according to the Bragg equation: $d = \lambda/2 \sin \theta$, $\lambda = 1.540598$ Å).[3] For PXRD experiments, lyophilized powders of a) **Agg-1** and b) **Agg-2** were used. At this point, it is worth mentioning that, to avoid XRD peaks of MES buffer, we conducted the formation of **Agg-1** and **Agg-2** by adjusting the pH with aqueous NaOH solution.

Fourier-Transform Infrared Spectroscopy (FT-IR)

FTIR spectra were recorded on a Parkin Elmer Spectrum FT-IR Spectrometer using a Diamond ATR from Parkin Elmer. For the FT-IR measurement **NI-VLD**, MES buffer and EDC were all prepared in deuterated solvents described in the sample preparation section. 10 μL of **Agg-1** and **Agg-2** (7 min after the addition of EDC to **Agg-1**) and **NI-VLD** (in pure DMSO-d_6) were applied to the sample holder and transmittances were recorded.

Analysis of the reaction kinetics by HPLC

The kinetics of the chemical reaction cycle was monitored over time employing analytical HPLC (Agilent 1260 Infinity II system using a YMC Triart C18 5 μm, 4.6 X 250 mm analytical column). In a typical method, 5 mL of 2 mM **NI-VLD** sample was prepared as mentioned in the sample preparation section. The reaction cycle was initiated by adding a solution of EDC (10 mM as the final concentration). At different time intervals, 125 μL reaction mixtures were aliquoted and added to 250 μL of 20 mM benzylamine solution which acts as a quencher and irreversibly converts the cyclic anhydride into monoamides.[4] These quenched samples were diluted by adding 125 μL acetonitrile and mixed well. Following that, the diluted samples were filtered and injected into HPLC. The eluent we employed was a mixture of 0.1% TFA in acetonitrile and 0.1% TFA in water and utilizing a linear gradient of acetonitrile: water from 2: 98 to 98:2, all the compounds in the reaction cycle were well resolved. The concentrations of the unreacted **NI-VLD** at different time intervals were calculated by using a calibration curves of **NI-VLD**. From the concentration of **NI-VLD** present at a particular time, we calculated the **NI-VLD** consumed at that time which we considered as the indirect measure of anhydride formation in the reaction cycle over time. Measurements were performed at 25 °C, with a flow rate of 1mL/min at a data collection rate 2.0 Hz, and the UV detector's wave lengths were kept as 220, 254 and 345 nm.

For the Calibration curve, a series of **NI-VLD** solutions were prepared having a concentration range 0.1 mM to 1 mM and injected in the analytical HPLC. The chromatogram was recorded at 345 nm wavelength for each of the samples. Then area under the curves was calculated and plotted against the concentrations. Thereby a straight line was obtained having a slope and intercept. Using this calibration curve, unknown concentrations of **NI-VLD** at different time points during the reaction were calculated. Briefly, the area of the diluted aliquots of the reaction time points was obtained from the chromatograms which were put in the calibration curve (straight line equation) and corresponding concentrations were calculated. Finally, after multiplying with the dilution factor, the concentrations of **NI-VLD** during the reaction course were calculated.

Time-lapse photography

Photograph of the reaction cycle in a cuvette under UV Light and Normal Light

500 μL of 2 mM **NI-VLD** precursor solution was taken into a 1 mL quartz cuvette of path length 10 mm or 1 mm, and 10 mM EDC was added. The reaction cycle was observed under both UV light (365 nm) and normal light. At different time intervals, the reaction cycle was captured using a camera. Under UV, the cyan color of **Agg-1** solution became bluish after 3 minutes of the EDC addition, most intense

after 7 minutes, and reappearance of the cyan color was observed after ca 120 min. Under normal light, the formation of the turbid assembly was noticed, which was most prominent at around 7 min and became clear after ca. 60 min. A similar experiment was done for the **NI-D** which showed cyan color, however, on EDC addition, no turbidity in normal light or formation of bluish color was seen under UV light. Another control molecule, **C$_{10}$-VLD**, showed turbidity on EDC fueling under normal light.

Transient pattern formation in 96 well plate

A pattern of 3 letters "**DST**" was generated by filling 200 μL **NI-VLD** solution and imaged under UV light (365 nm) showing cyan color. The letter '**S**' was fueled with 10 mM EDC solution, and cyan to bright blue emission was observed. After ca 120 min of fueling, the cyan color reappeared.

Generation of Self-erasable Message

A 2% agarose gel was prepared by dissolving 12 mg of extra pure agarose powder in 600 μL of a 2 mM **NI-VLD** solution (f_a= 90%), and then the solution was stirred and heated at a constant temperature of 60°C inside a microwave oven for 30 seconds. The hot solution was then transferred into a borosilicate glass petri dish measuring 25 mm in diameter. After 10 minutes of cooling down the solution to room temperature, a semi-solid gel was formed which fluoresced cyan color under UV light. A 50 mM EDC solution was applied as an ink to write a message over the gel. The concentration of EDC tuned the lifetime of the message, and the message was rewritten on a single gel matrix. Similarly, the message was written on another gel using 15 mM and 25 mM EDC solutions, resulting in less visibility and a shorter lifetime than the 50 mM case.
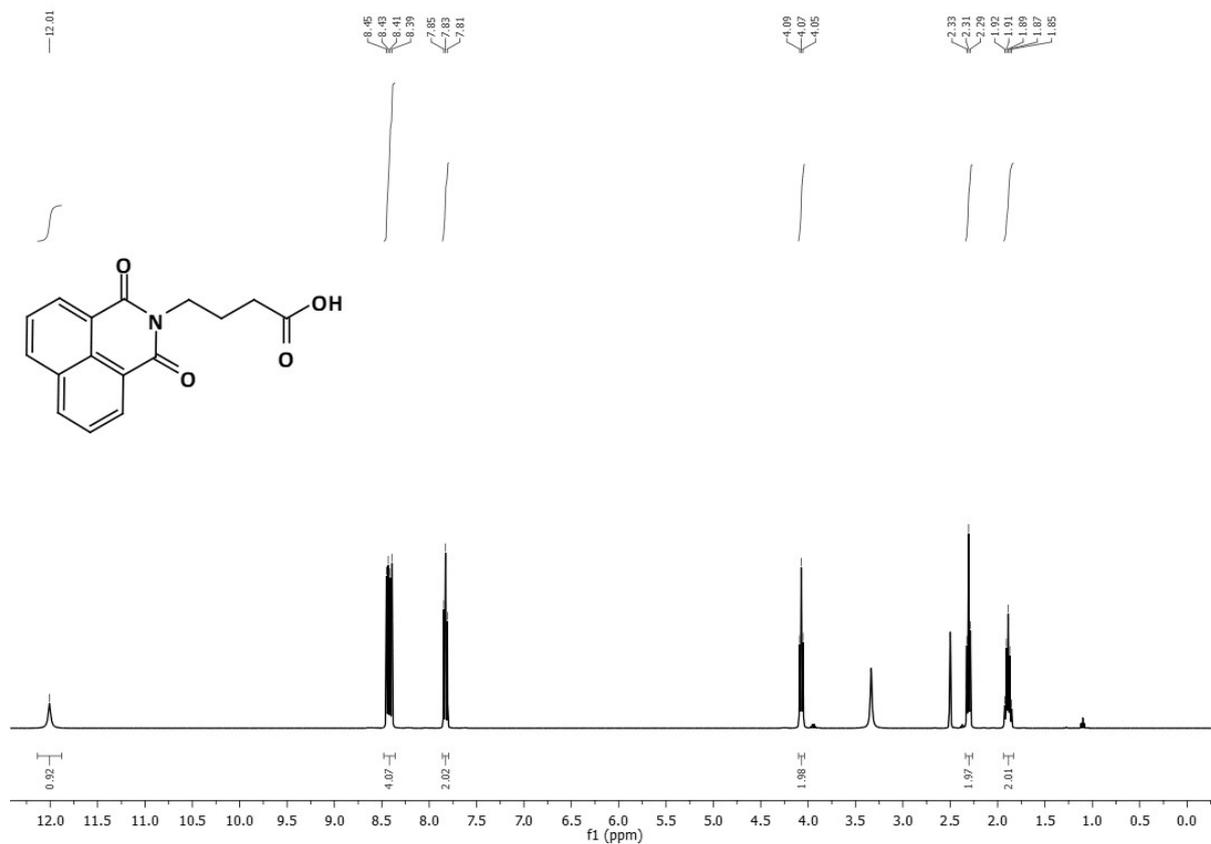
Figure S1: ^{1}H-NMR spectrum of **NI-GABA-OH** in DMSO-d_6.
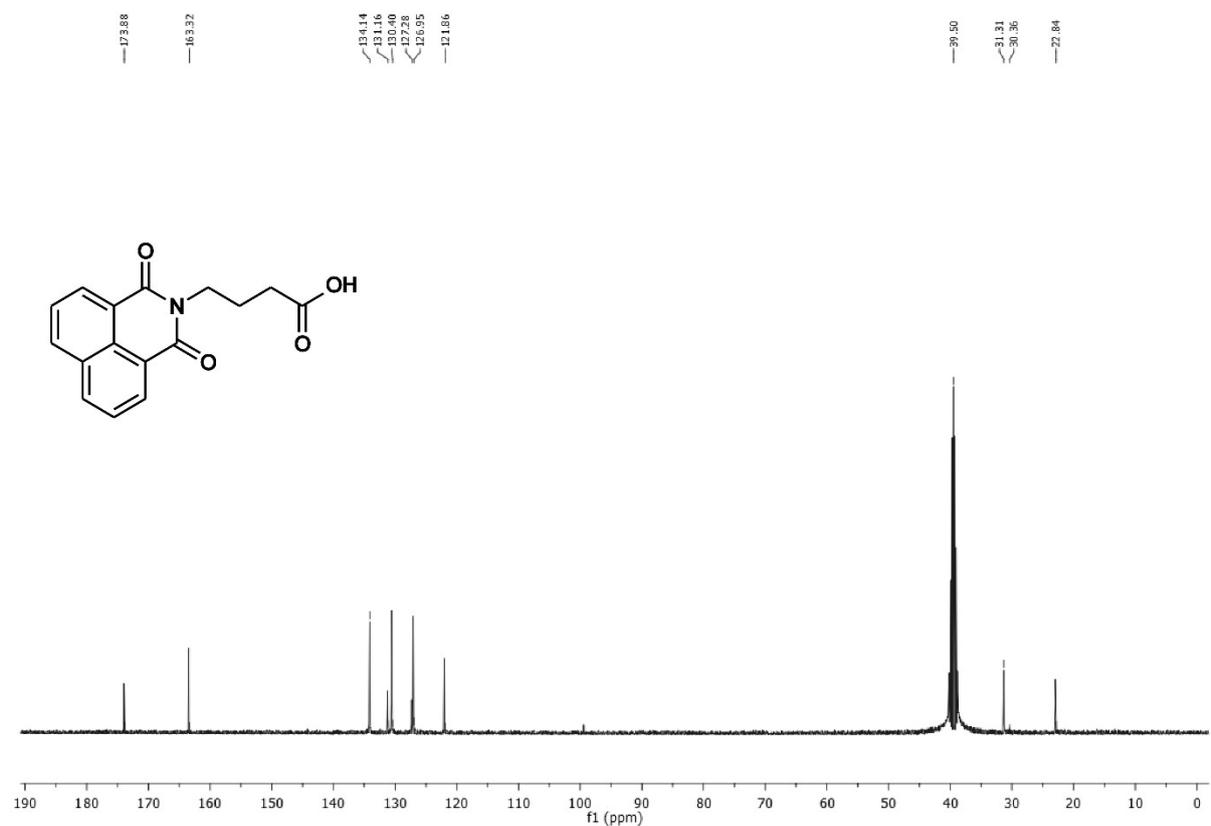
Figure S2: ^{13}C-NMR spectrum of **NI-GABA-OH** in DMSO-d_6.
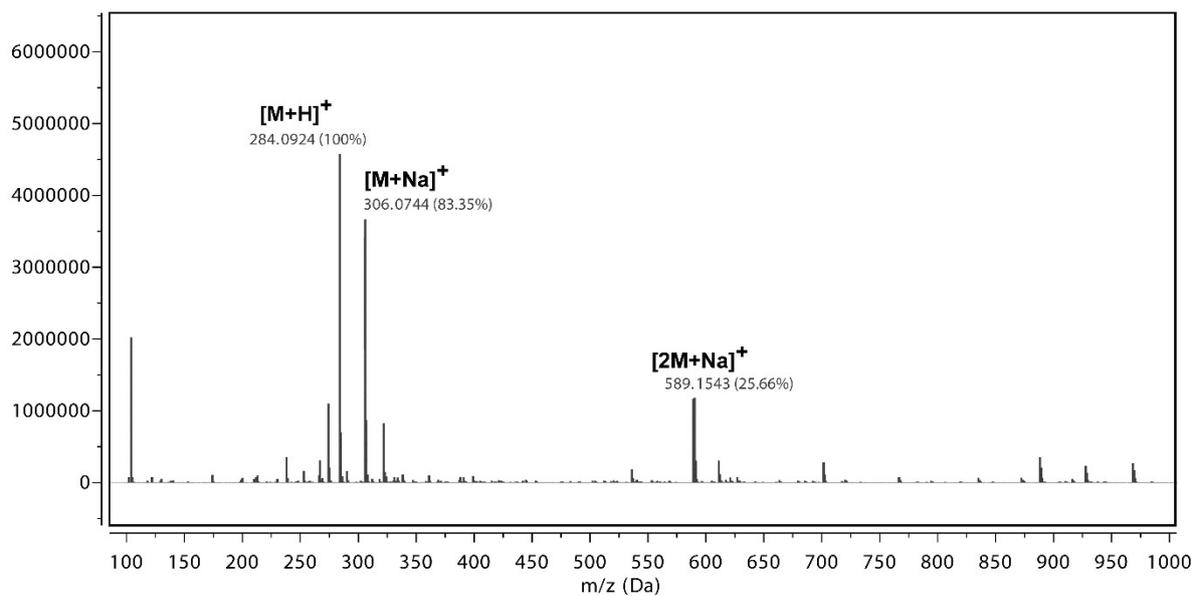
Figure S3: HRMS of **NI-GABA-OH**
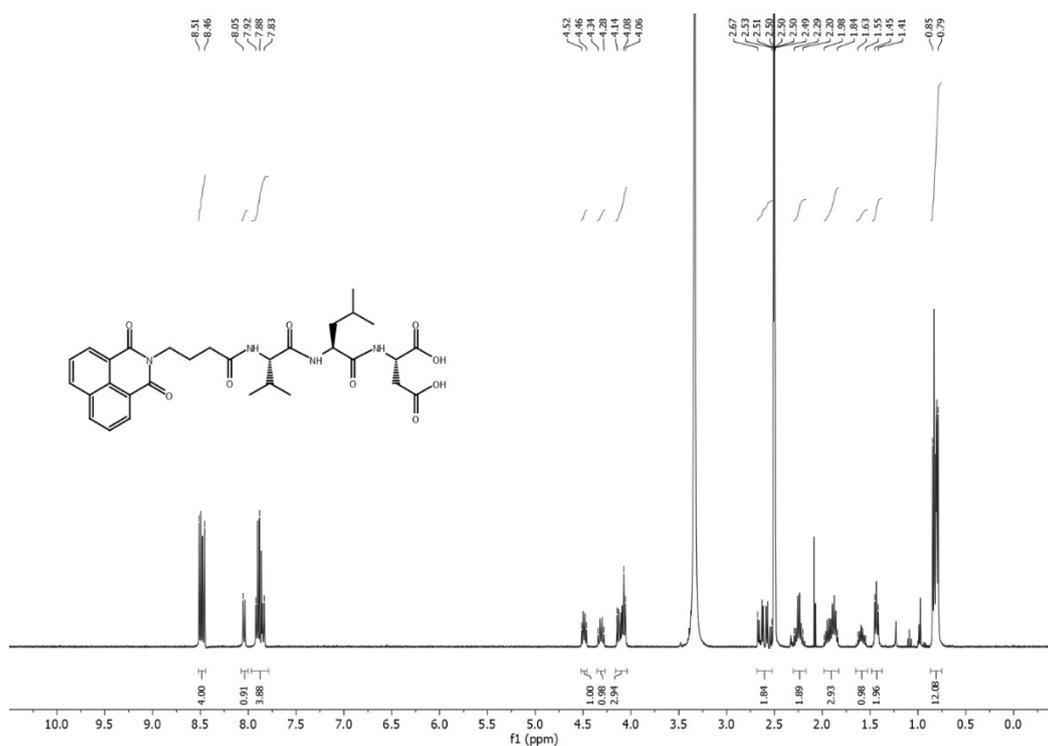
Figure S4: ^{1}H-NMR spectrum of **NI-VLD** in DMSO-d_6.

Figure S5: ^{13}C-NMR spectrum of **NI-VLD** in DMSO-d_6.

Figure S6: HPLC Chromatogram of **NI-VLD**

13
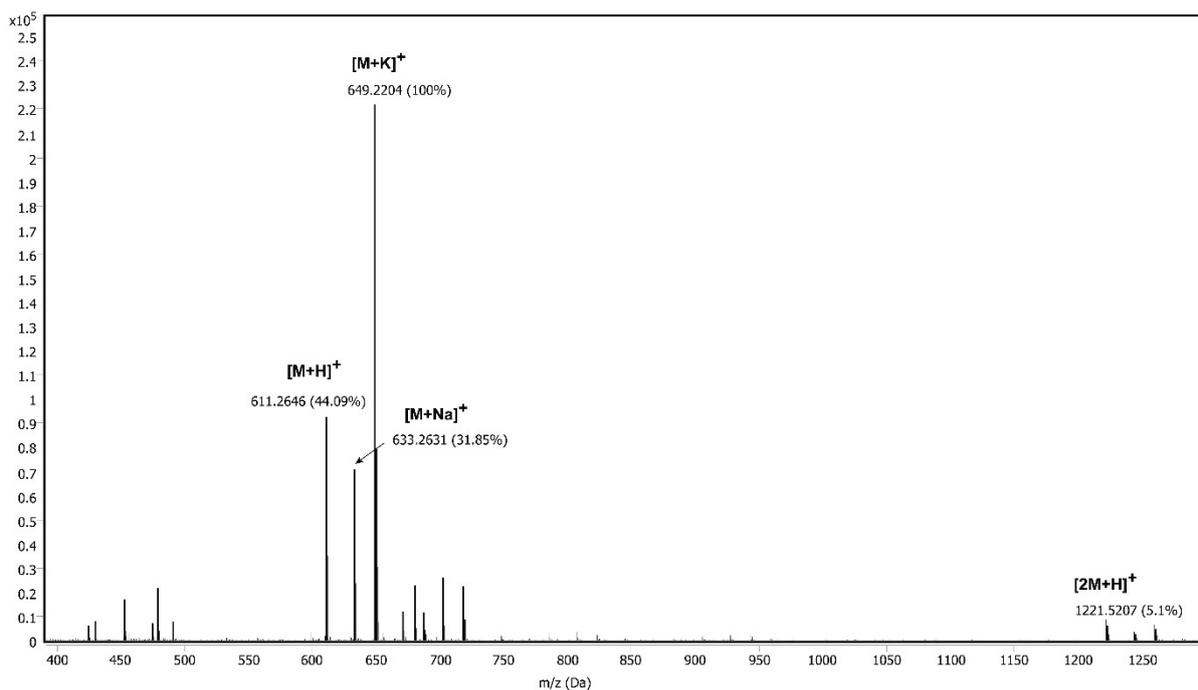
Figure S7: HRMS of **NI-VLD**.
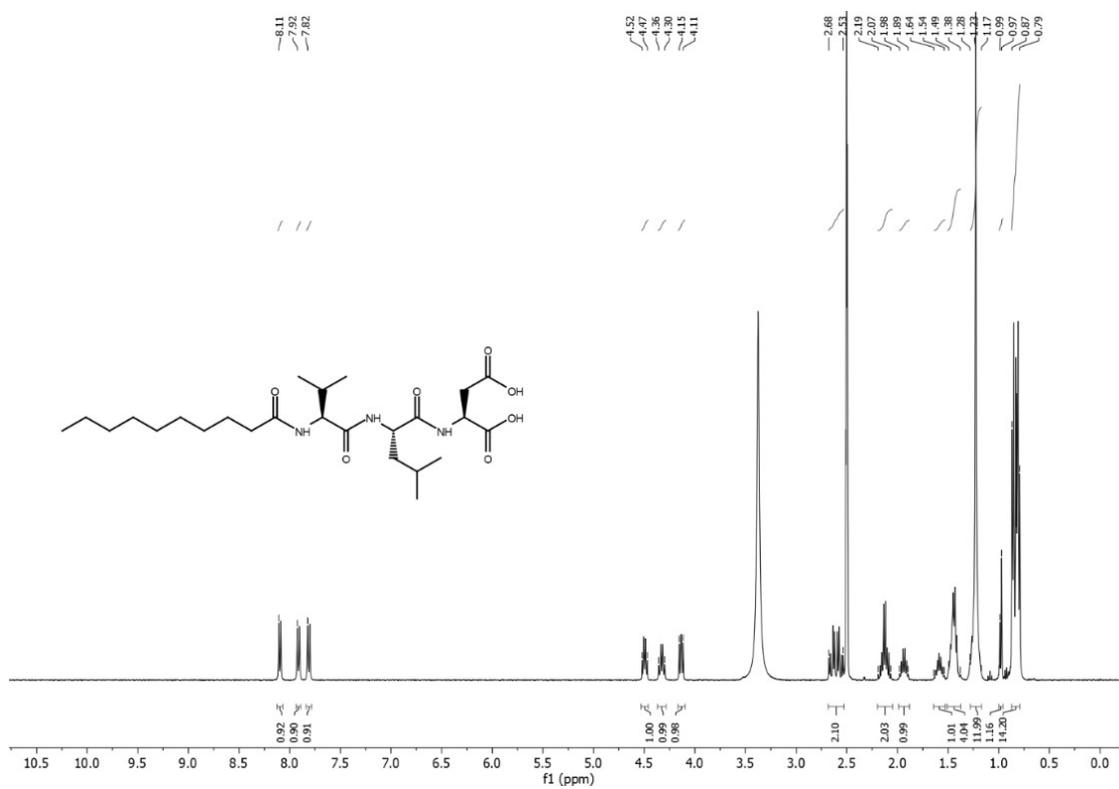
Figure S8: ^{1}H-NMR spectrum of **C$_{10}$-VLD** in DMSO-d_6.
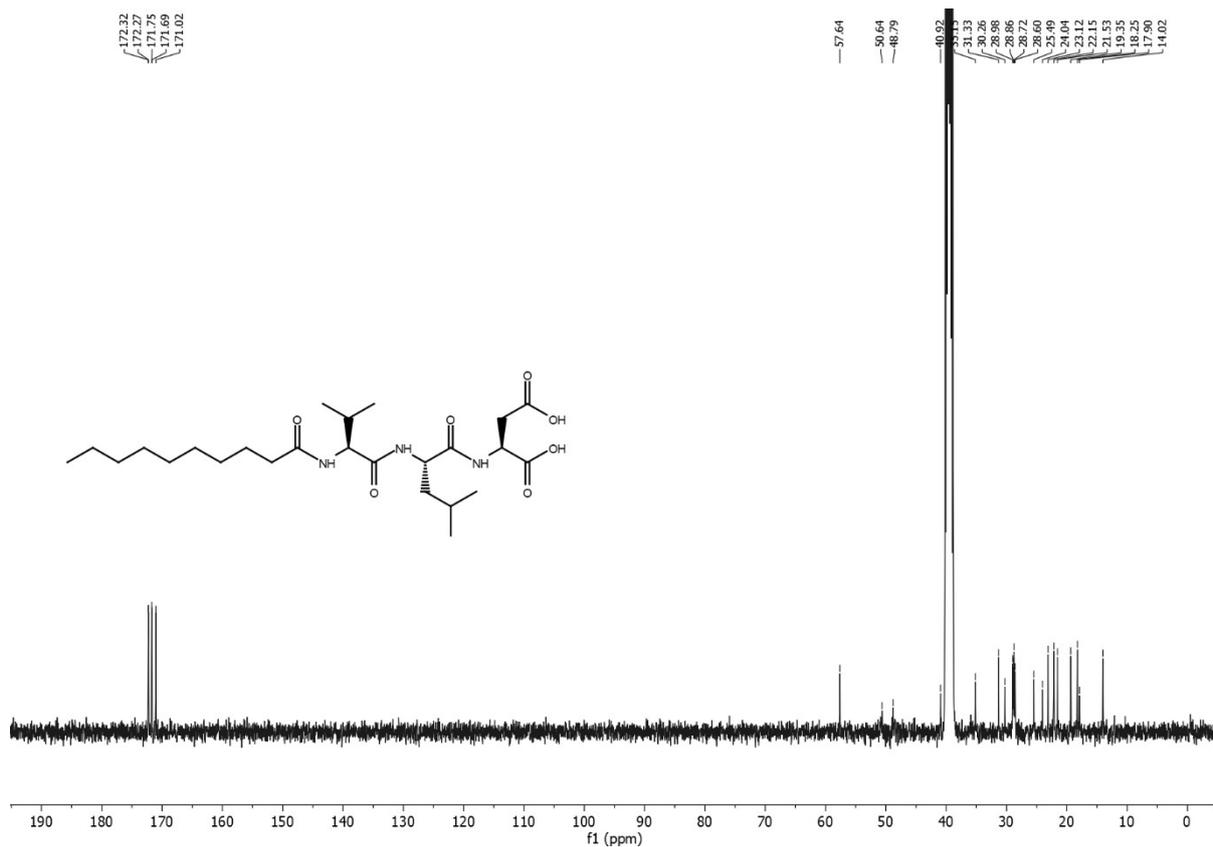
Figure S9: ^{13}C-NMR spectrum of **C$_{10}$-VLD** in DMSO-d_6.
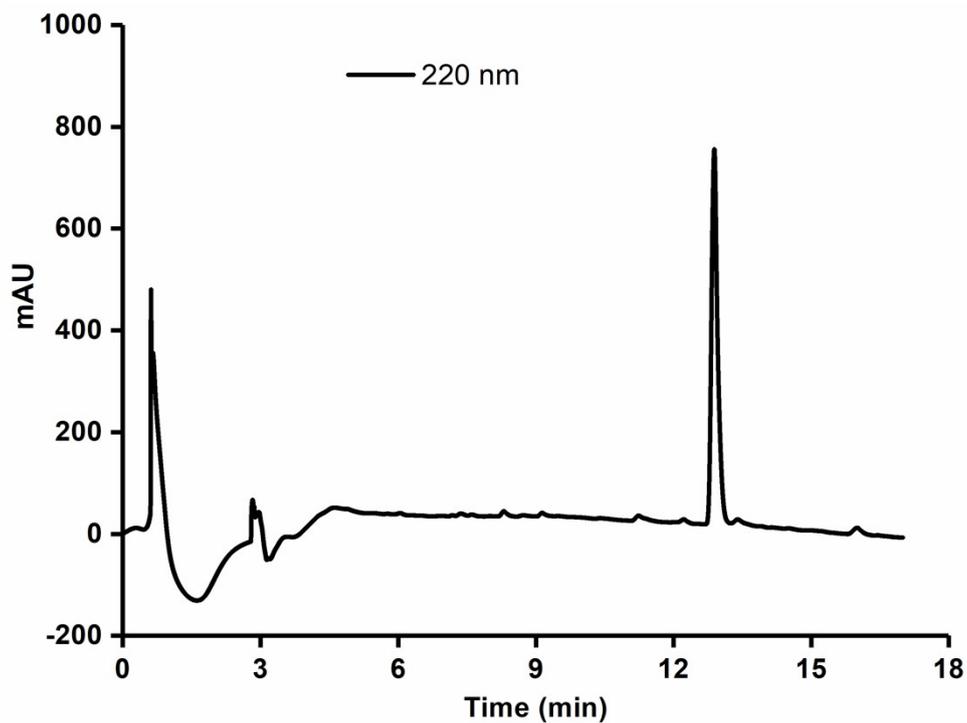
Figure S10: HPLC Chromatogram of **C$_{10}$-VLD** measured at room temperature.
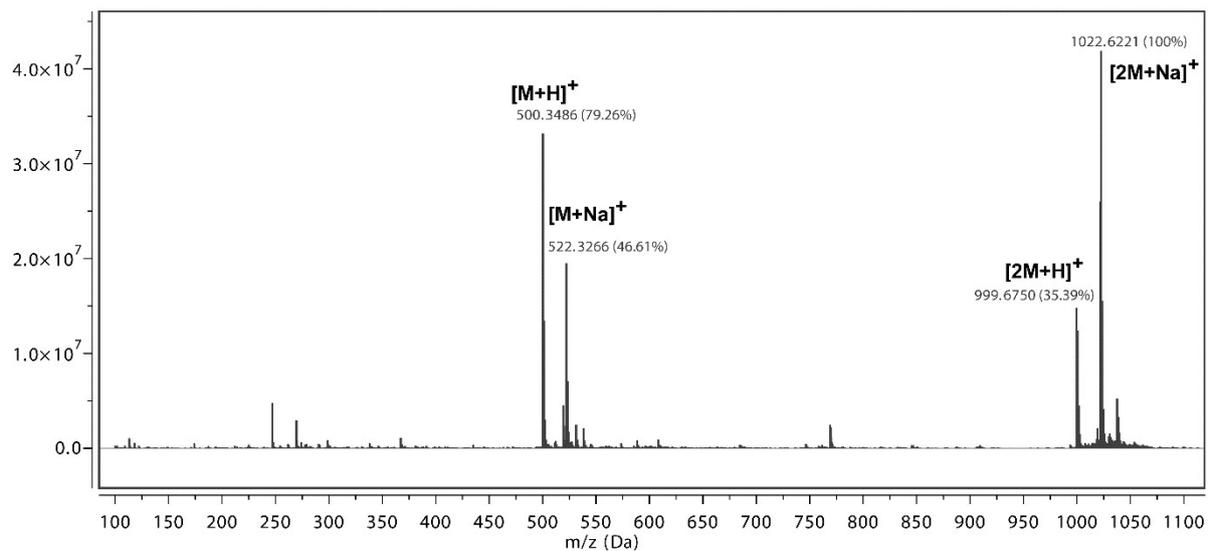
Figure S11: HRMS of **C₁₀-VLD**.
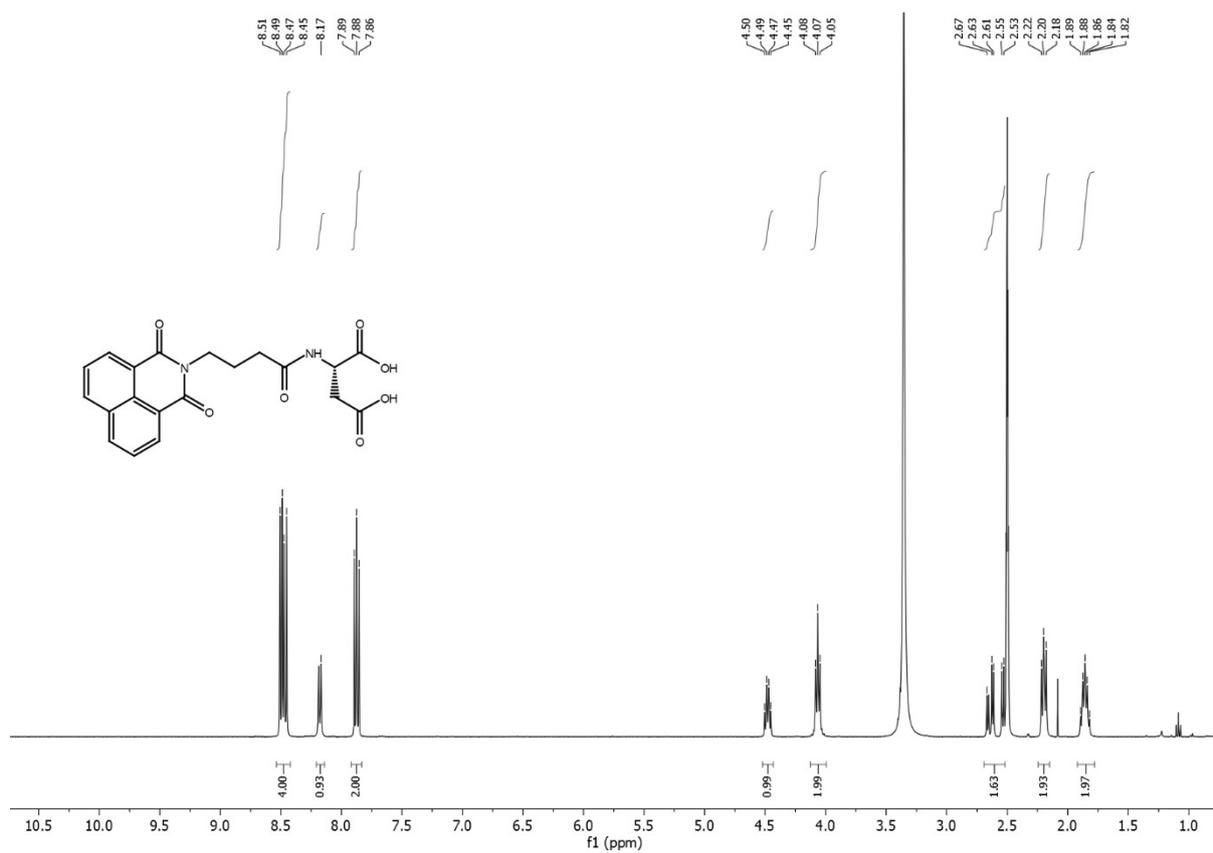
Figure S12: ¹H-NMR spectrum of **NI-D** in DMSO-d_6.

Figure S13: ^{13}C-NMR spectrum of **NI-D** in DMSO-d_6.

Figure S14: HPLC Chromatogram of **NI-D** measured at room temperature.
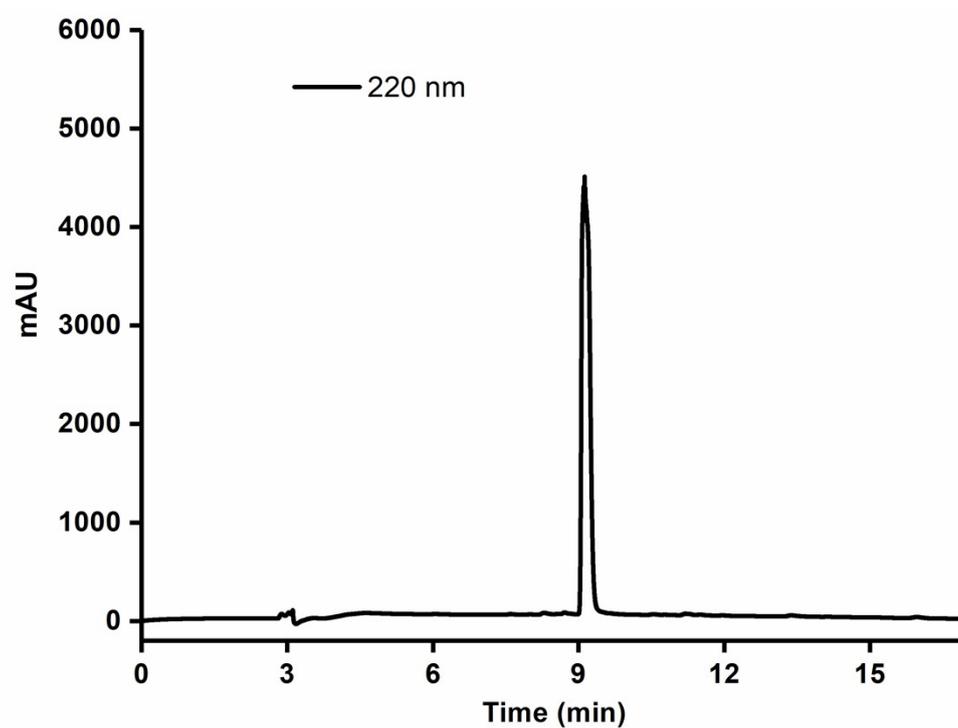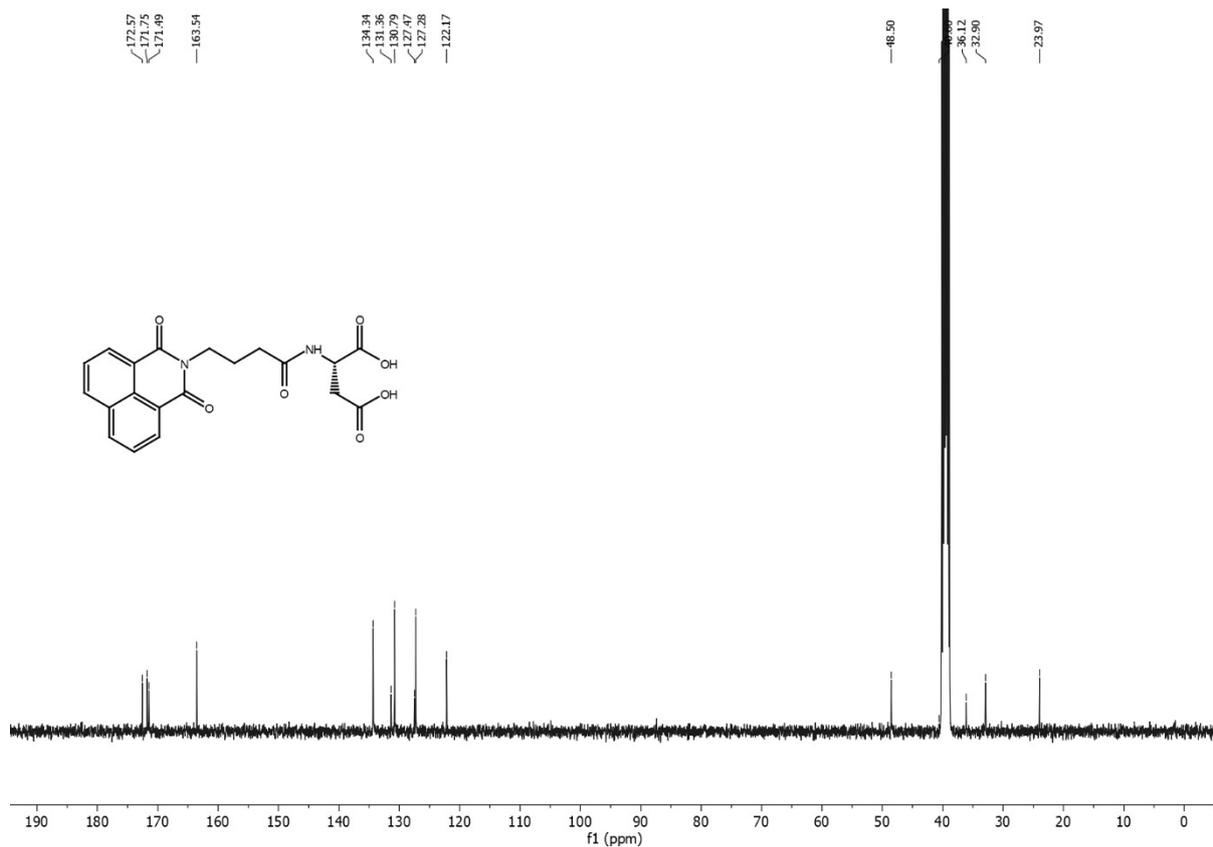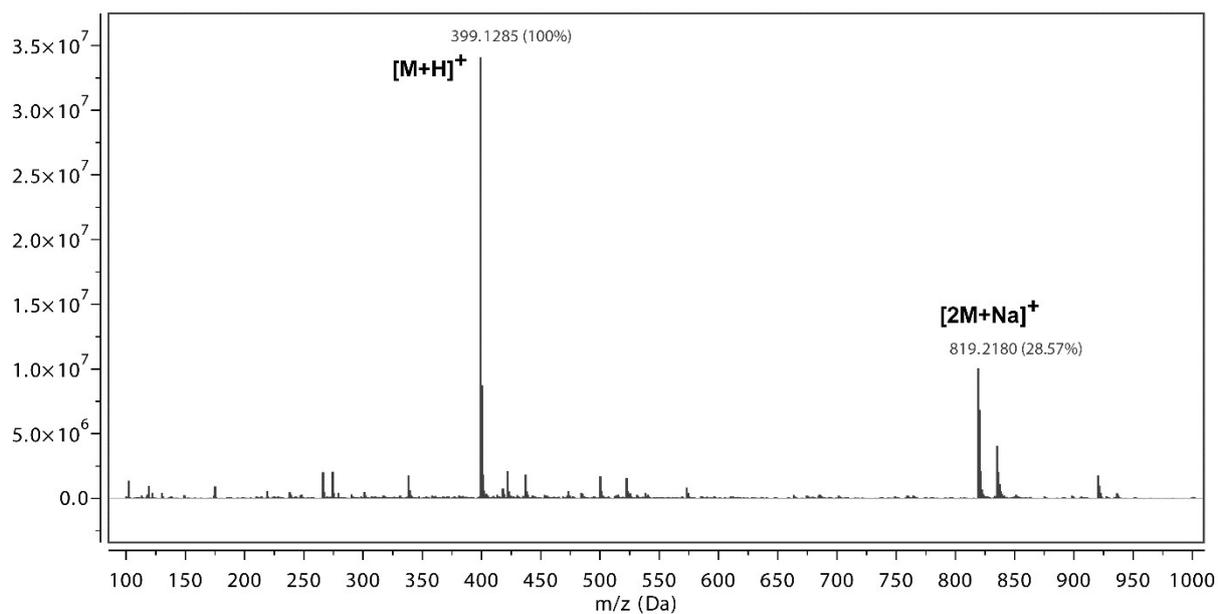
Figure S15: HRMS of **NI-D**
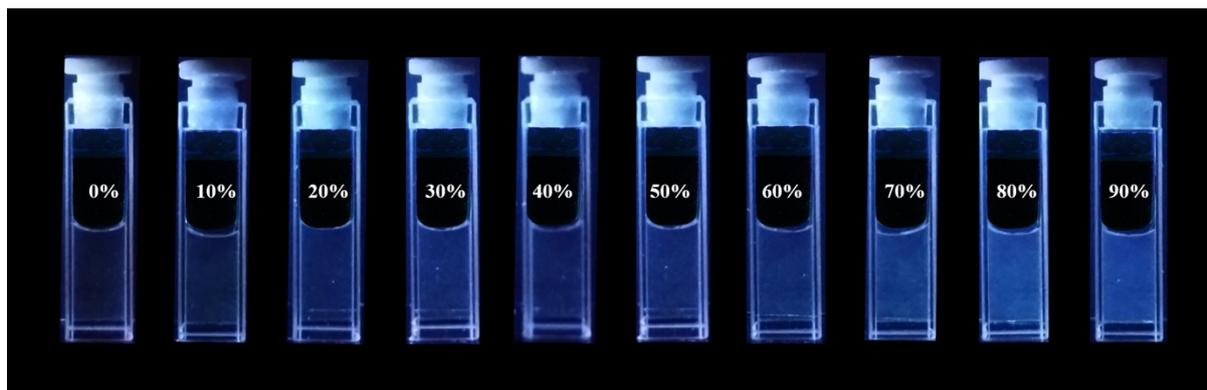
Figure S16: Photograph of **NI-VLD** (50 x 10^{-3} mM) with an increasing percentage of f_a (proportion of aqueous buffer in DMSO where the final buffer concentration is 50 mM except for the first one) captured under a UV lamp (365 nm).
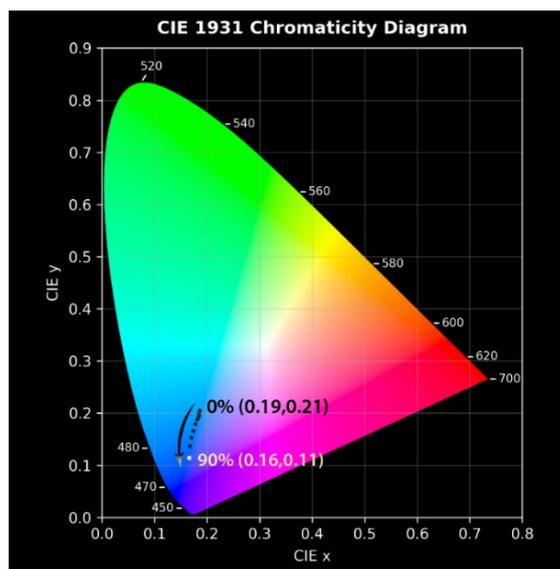
Figure S17: CIE plot of the fluorescence spectra of **NI-VLD** (50 x 10^{-3} mM) with increasing proportion of aqueous buffer in DMSO (f_a= 0 to 90%).
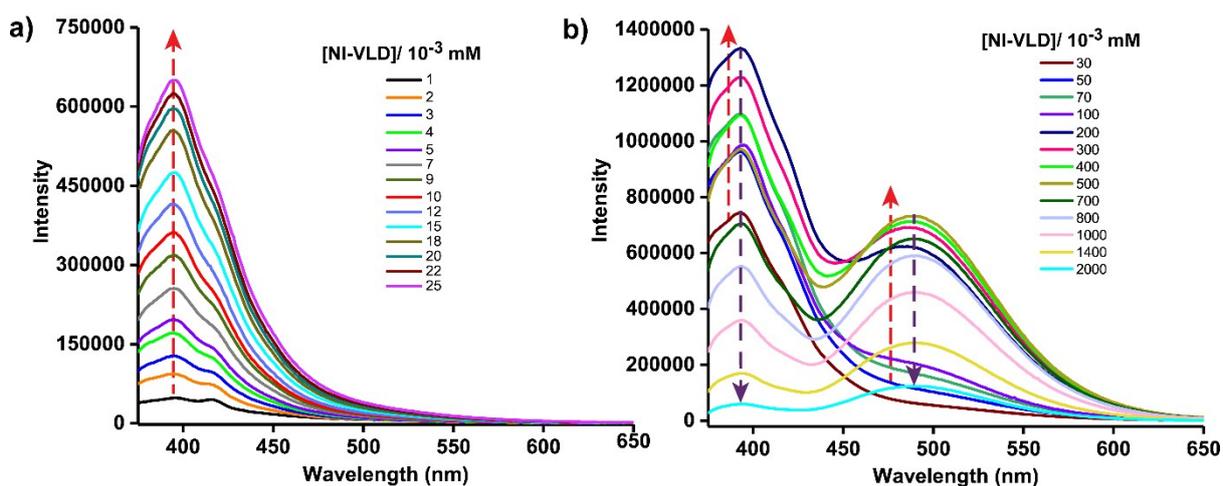
Figure S18: Concentration-dependent emission spectra of **NI-VLD a)** within the low concentration range and **b)** within the high concentration range. Red arrows indicate the increase of intensity with concentration increase, whereas the magenta arrows indicate the decrease of intensity with an increase in concentration. Solvent composition f_a= 90%, λ_{ex}= 365 nm.
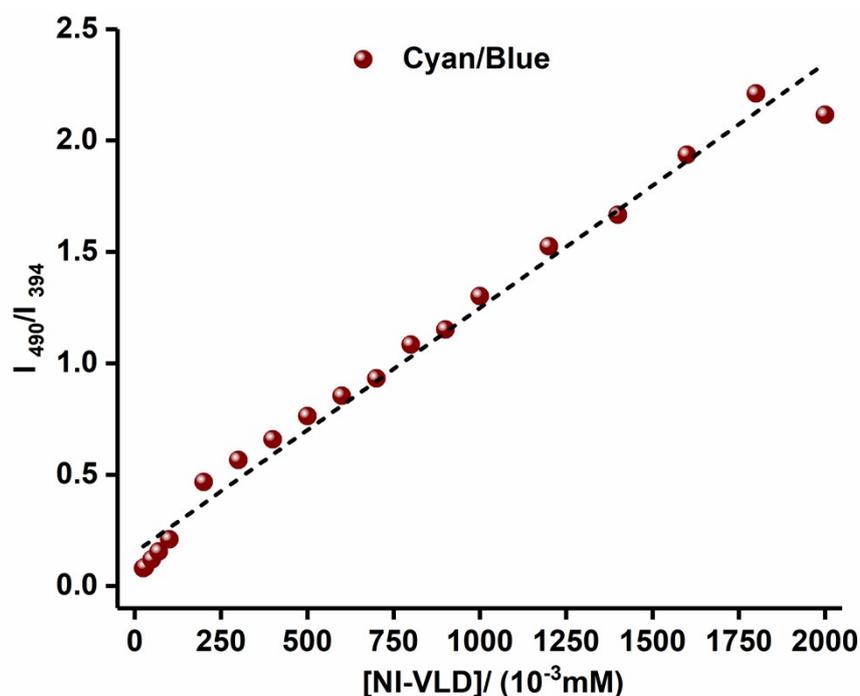
Figure S19: Plot of the ratio of the emission intensities at 490 nm to 394 nm as a function of **NI-VLD** concentrations (λ_{ex}= 365 nm and solvent composition f_a= 90% (aqueous buffer in DMSO, pH 6).
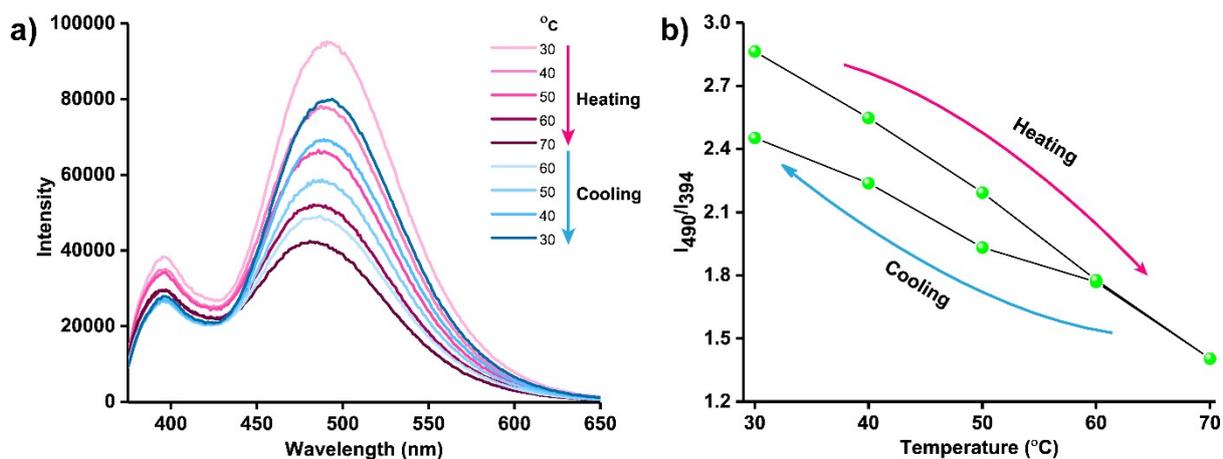
Figure S20: a) Temperature-dependent fluorescence emission spectra of **NI-VLD** demonstrating the drastic but reversible change of excimer emission with heating-cooling cycle, **b)** Plot of the ratio of the emission intensities at 490 nm to 394 nm of **NI-VLD** showing thermos-responsive nature of **Agg-1**. [**NI-VLD**]= 2 mM, λ_{ex}= 365 nm, temperature variation rate 1°C/min, solvent composition f_a= 90%.
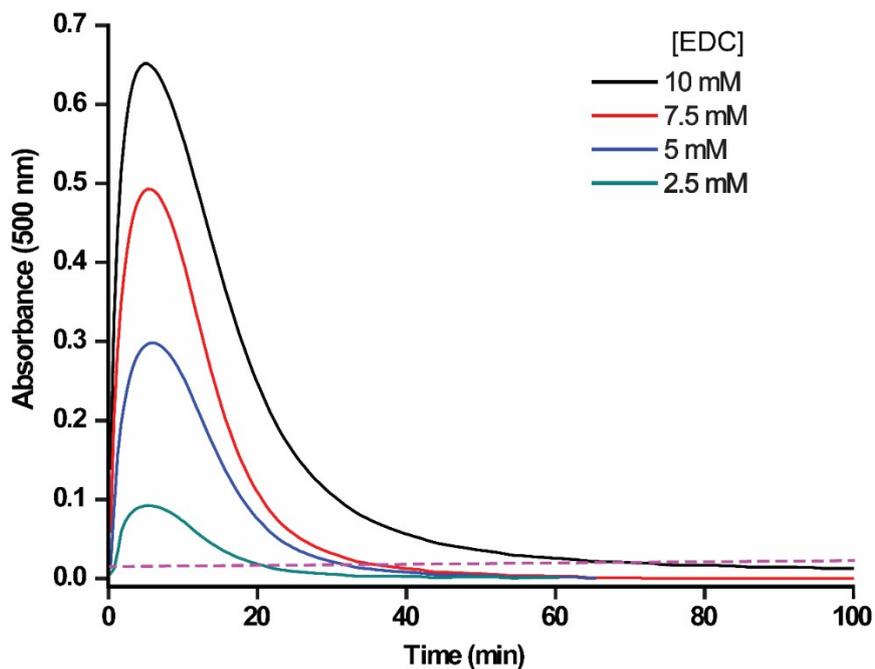
Figure S21: Turbidity measurement by recording the absorbance at 500 nm wavelength as a time function after the addition of different concentrations of EDC to **Agg-1**. [**NI-VLD**]= 2 mM, solvent composition f_a= 90%.
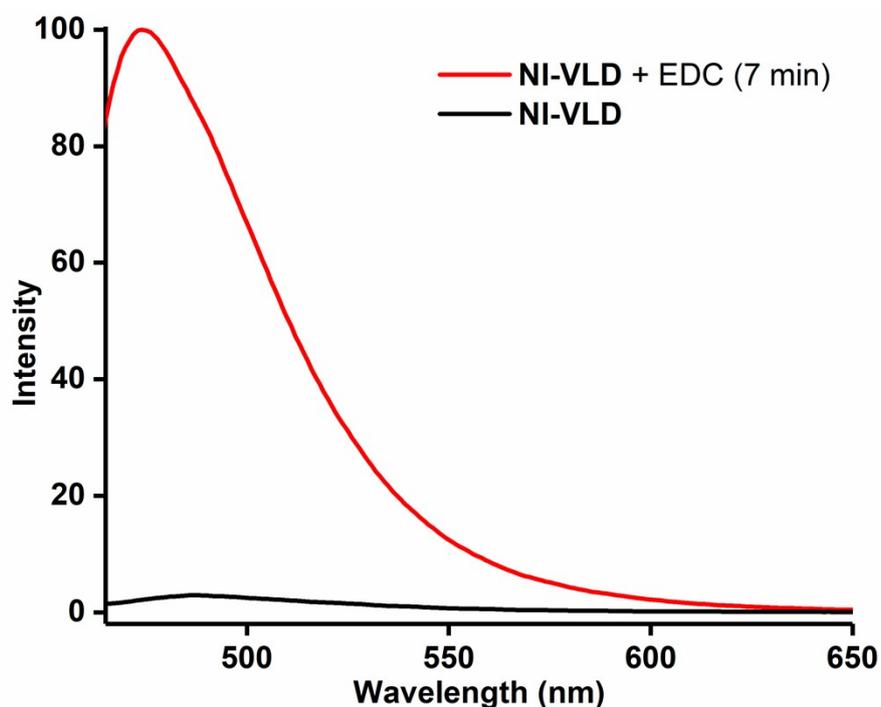
Figure S22: ThT assay of **NI-VLD**. Emission intensity before the addition of EDC (black) and after 7 min of EDC addition to **NI-VLD**. [**NI-VLD**]= 2mM, [ThT]= 10 μM, and [EDC]= 10 mM, solvent composition f_a= 90%.
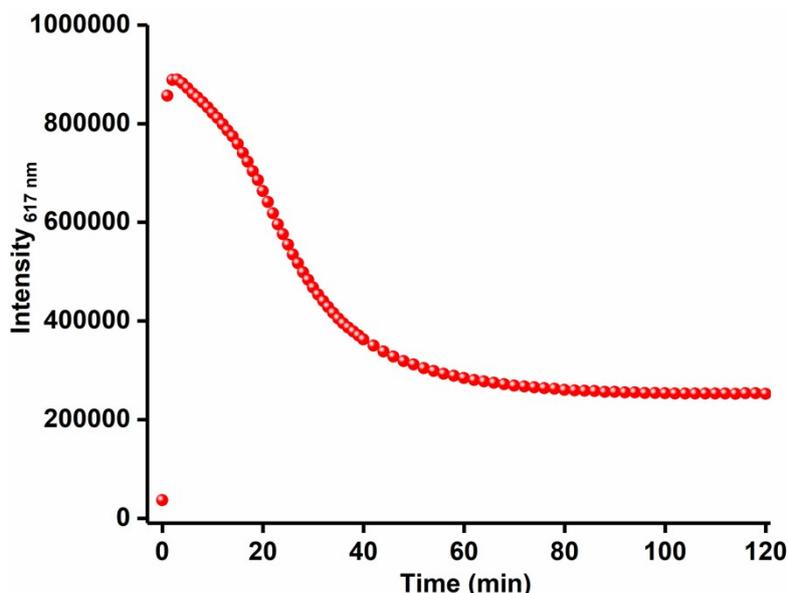
Figure S23: Transient assembly formation after the addition of EDC to **Agg-1**, assayed by Nile Red. [**NI-VLD**]= 2 mM, [Nile Red]= 2 μM, [EDC]= 10 mM, λ_{ex}= 550 nm, λ_{em}= 617 nm, solvent composition f_a= 90%.
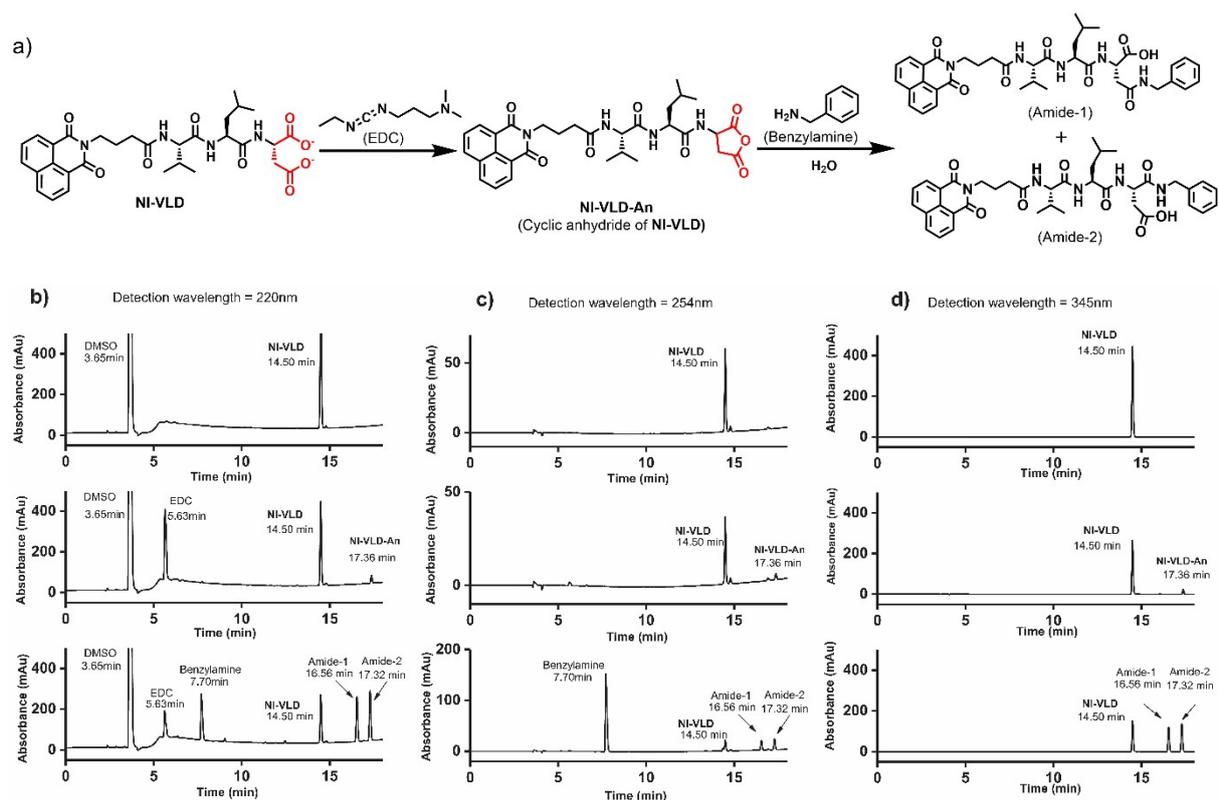
Figure S24: a) Reaction scheme showing the formation of cyclic anhydride (**NI-VLD-An**), and quenching of this cyclic anhydride by benzylamine to form Amide-1 and Amide-2, **b)** to **d)** HPLC chromatographic stacks of pure **NI-VLD**, after addition of EDC and the benzylamine quenched reaction cycle respectively, followed at different wavelengths **b)** 220 nm, **c)** 254 nm and **d)** 345 nm. For quenching, the sample was aliquoted after 7 min of EDC addition to **NI-VLD** and mixed thoroughly with benzylamine followed by HPLC injection.

Table S1: The synthesized peptides and other components of the reaction cycle.

Sl. No	Name	Structure	Exact Mass (gmol^{-1})	Mass found (gmol^{-1})
1	NI-GABA-OH		283.0845 $C_{16}H_{13}NO_4$	284.0924 [M+H]$^+$
2	NI-VLD		610.2639 $C_{31}H_{38}N_4O_9$	611.2646 [M+H]$^+$
3	C$_{10}$-VLD		499.3258 $C_{25}H_{45}N_3O_7$	500.3486 [M+H]$^+$
4	NI-D		398.1114 $C_{20}H_{18}N_2O_7$	399.1285 [M+H]$^+$
5	NI-VLD-An		592.2533 $C_{31}H_{36}N_4O_8$	593.2612 [M+H]$^+$
6	Amide 1 and Amide 2		699.3268 $C_{38}H_{45}N_5O_8$	700.3353 [M+H]$^+$ 722.3168 [M+Na]$^+$
7	EDC		155.14 $C_8H_{17}N_3$	156.15 [M+H]$^+$
8	Benzylamine		107.07 C_7H_9N	-
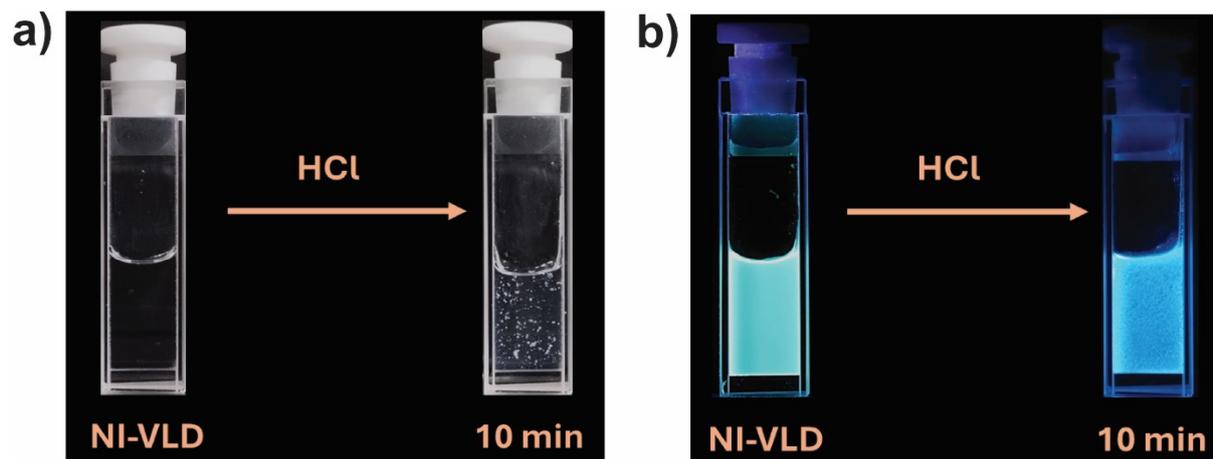
Figure S25: Adding HCl to **Agg-1 a)** creates turbidity, visualized under normal light and **b)** changes emission color, visualized under UV light (365 nm). The final pH of the system was 4, [**Agg-1**]= 2 mM.
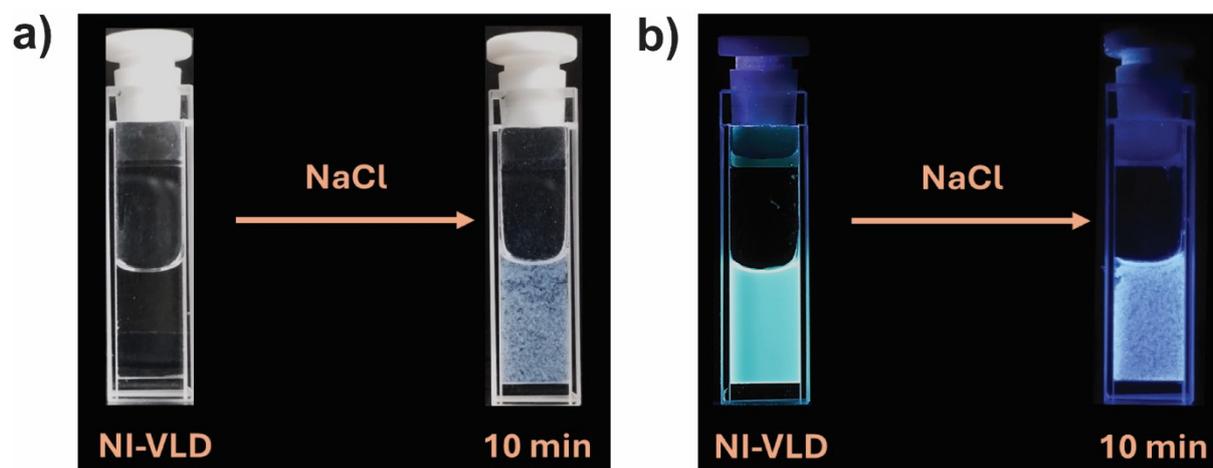
Figure S26: Adding 3N NaCl to **Agg-1** creates **a)** turbidity, visualized under normal light and **b)** changes emission color, visualized under UV light (365 nm). [**Agg-1**]= 2 mM.
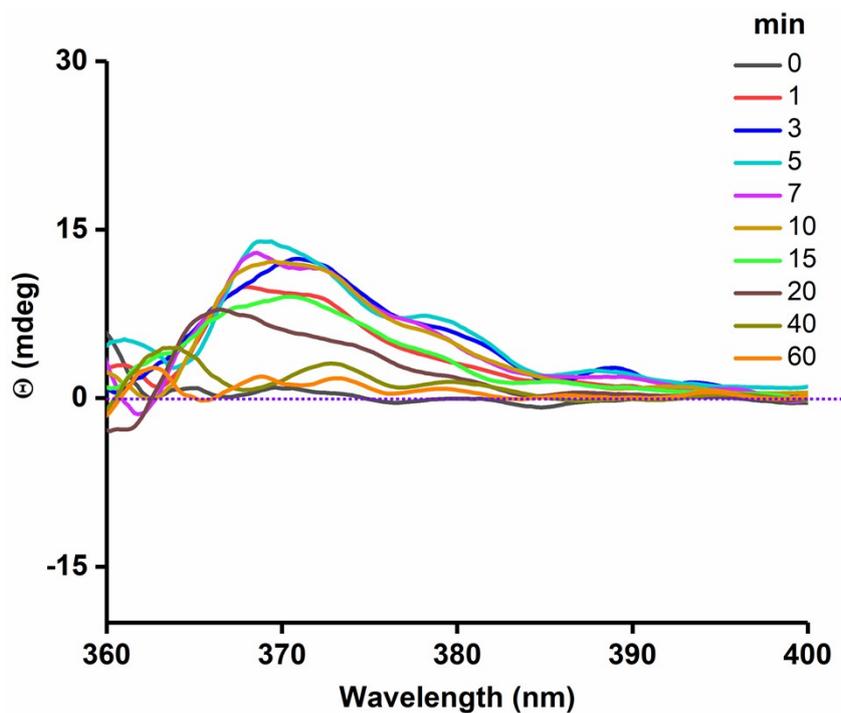
Figure S27: Time-dependent CD spectra of **Agg-1** after adding EDC, demonstrating the generation of transient chirality. [**NI-VLD**]= 2 mM, [EDC]= 10 mM, solvent composition f_a= 90%.
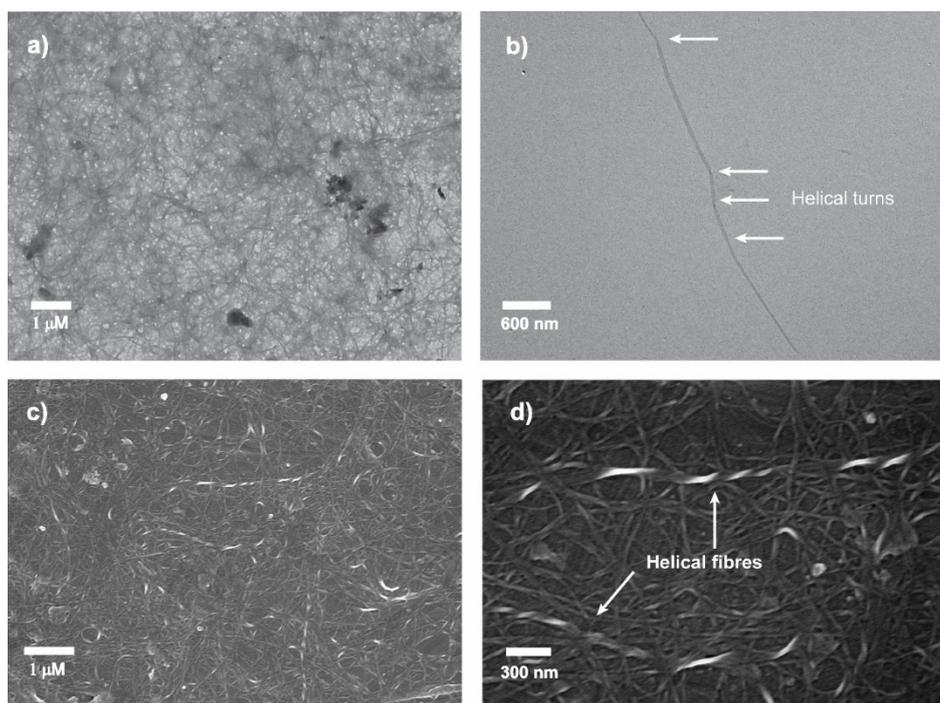
Figure S28: a) and **b)** TEM images of **Agg-2** at low and high magnifications respectively, **c)** and **d)** SEM images of **Agg-2** at low and high magnifications respectively. White arrows in **b)** and **d)** indicate the helical nature of the fibers. Note that, for sample preparation, the dynamicity of the EDC-fueled reaction was arrested by freezing the samples in liquid N_2 followed by lyophilization. For the current case, the **Agg-2** was cast after 7 min of EDC addition to **Agg-1**.
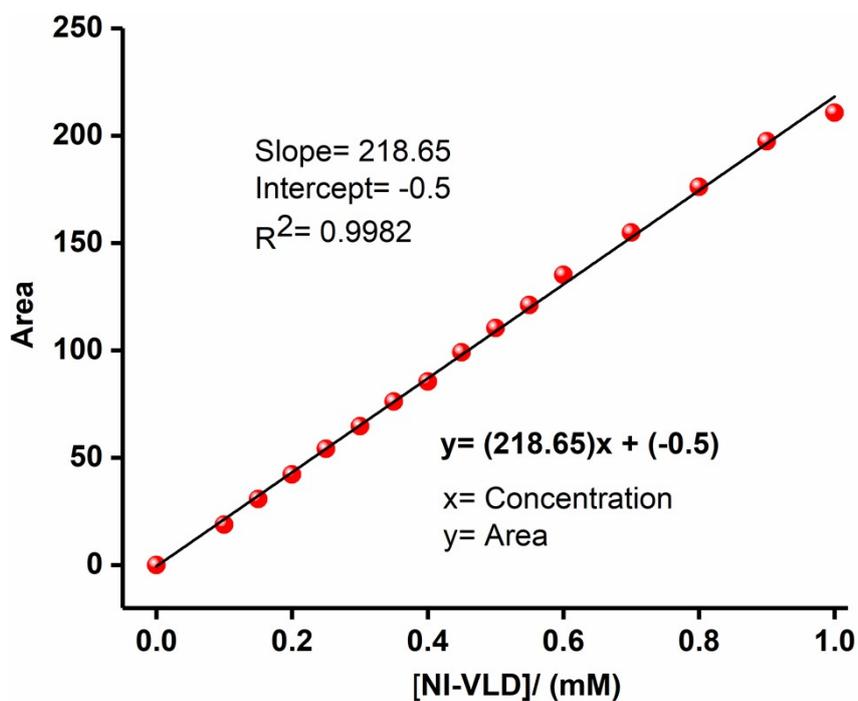
Figure S29: HPLC Calibration Curve of **NI-VLD**.
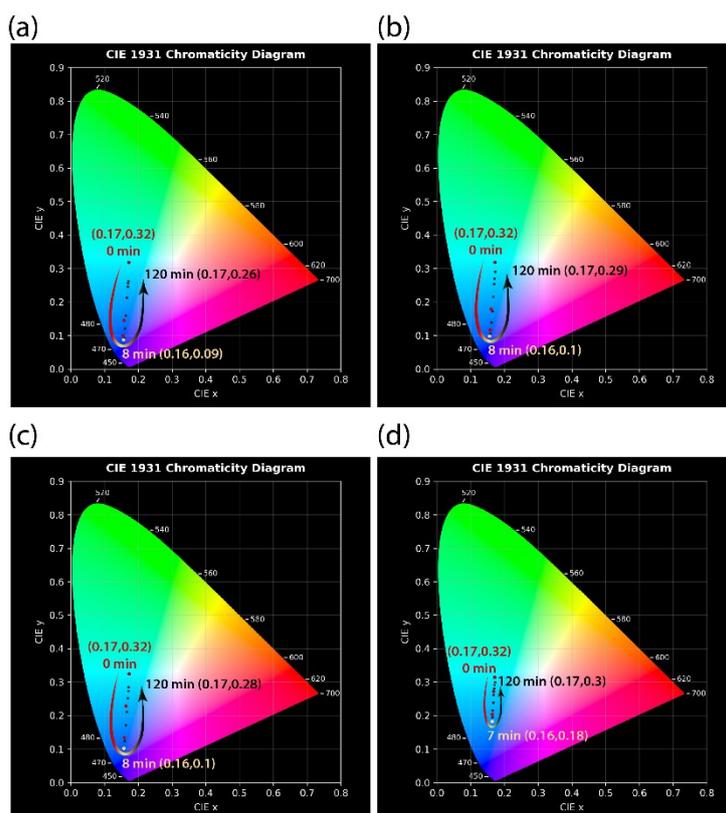
Figure S30: CIE 1931 chromaticity diagram of dynamic emissive system fueled with different concentrations of EDC: a) 7.5 mM, b) 5 mM, c) 2,5 mM and d) 1 mM. [**NI-VLD**]= 2 mM, λ_{ex}= 365 nm and solvent composition f_a= 90%. For all the cases, the total measurement time was kept fixed as 120 min.
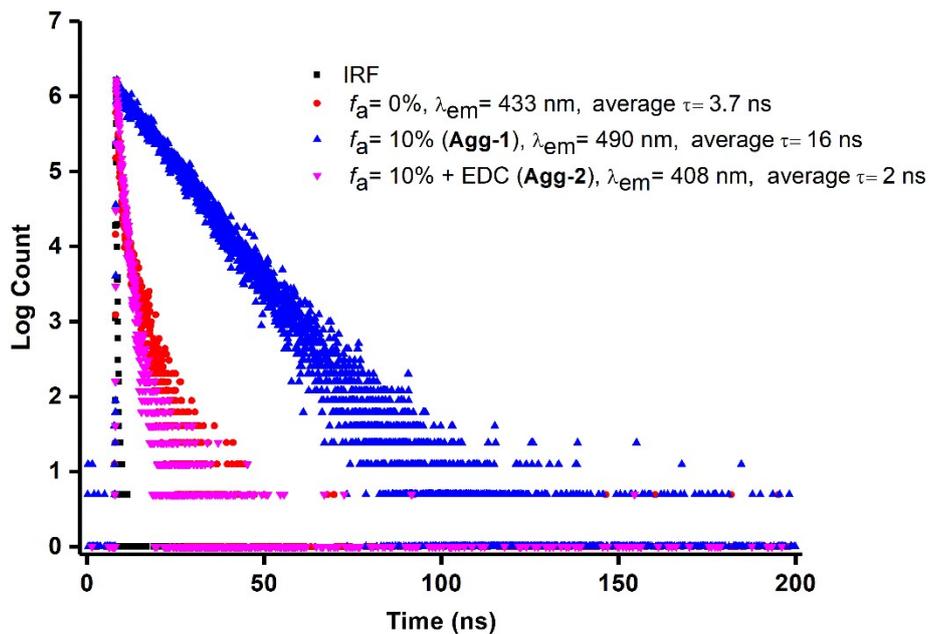
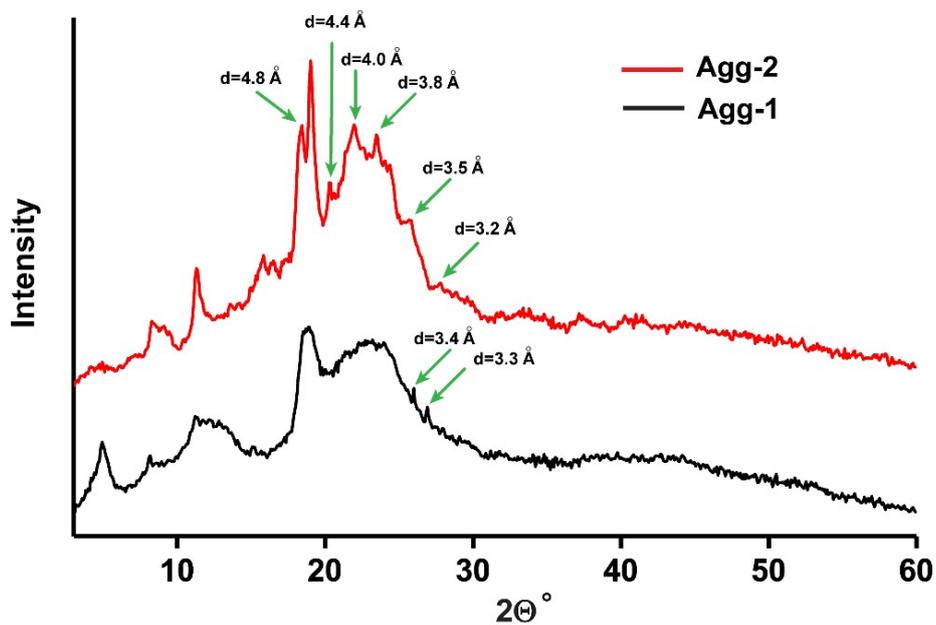**Figure S31:** Fluorescence decay profile of **NI-VLD** at different states acquired at their corresponding λ_{em} when $\lambda_{ex}= 375$ nm.

Figure S32: XRD data of lyophilized **Agg-1** (black) and **Agg-2** (red). The green arrows indicate *d* spacing.
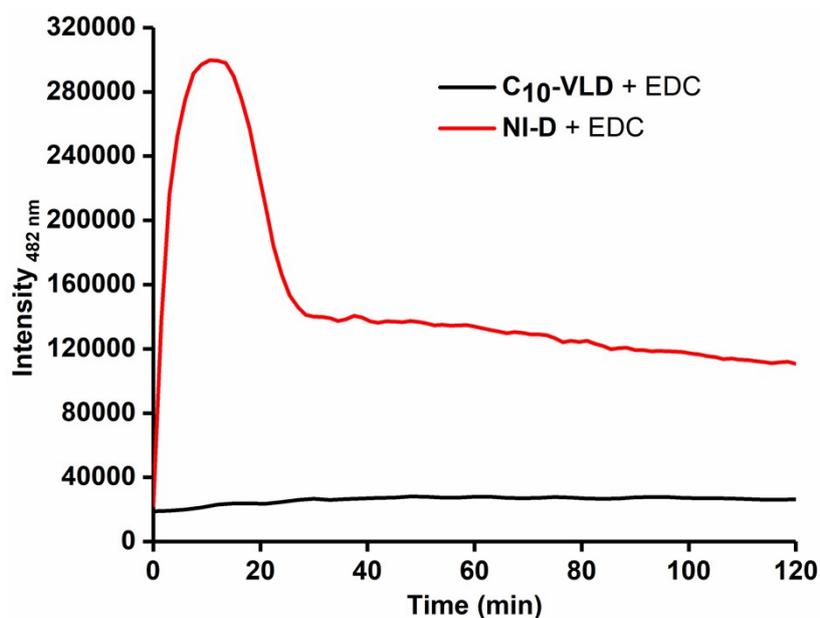
Figure S33: ThT emission kinetic assay of **C$_{10}$-VLD** and **NI-D** after the addition of EDC. [**C$_{10}$-VLD**]= [**NI-D**]= 2 mM, [ThT]= 10 µM, [EDC]= 10 mM, λ_{ex}= 450 nm, λ_{em}= 482 nm, solvent composition f_a= 90%.
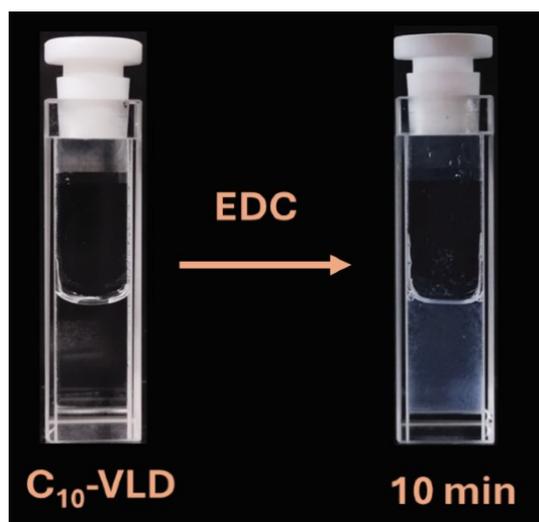

Figure S34: Visual assessment of turbidity of **C$_{10}$-VLD** when fueled with EDC, demonstrating macroscopic assembly formation. [**C$_{10}$-VLD**]= 2 mM, [EDC]= 10 mM, solvent composition f_a= 90%.
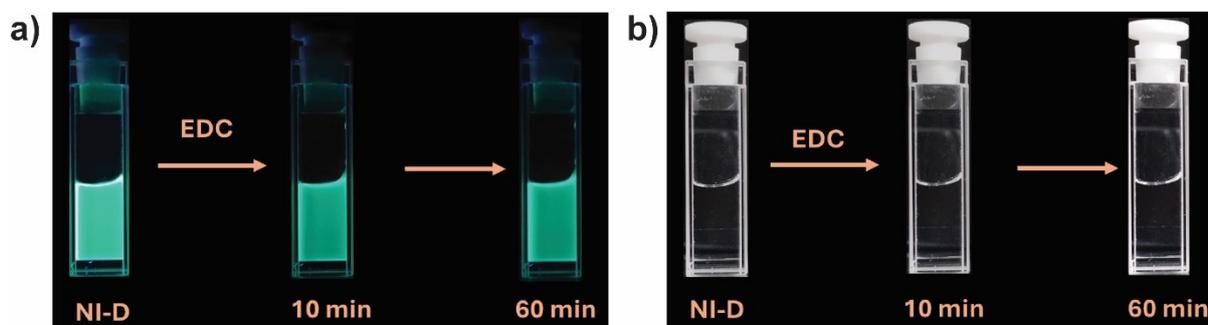

Figure S35: The addition of EDC to **NI-D** solution, neither changes **a)** emission color nor creates **b)** visual turbidity. [**NI-D**]= 2mM, [EDC]= 10 mM, solvent composition f_a= 90%.
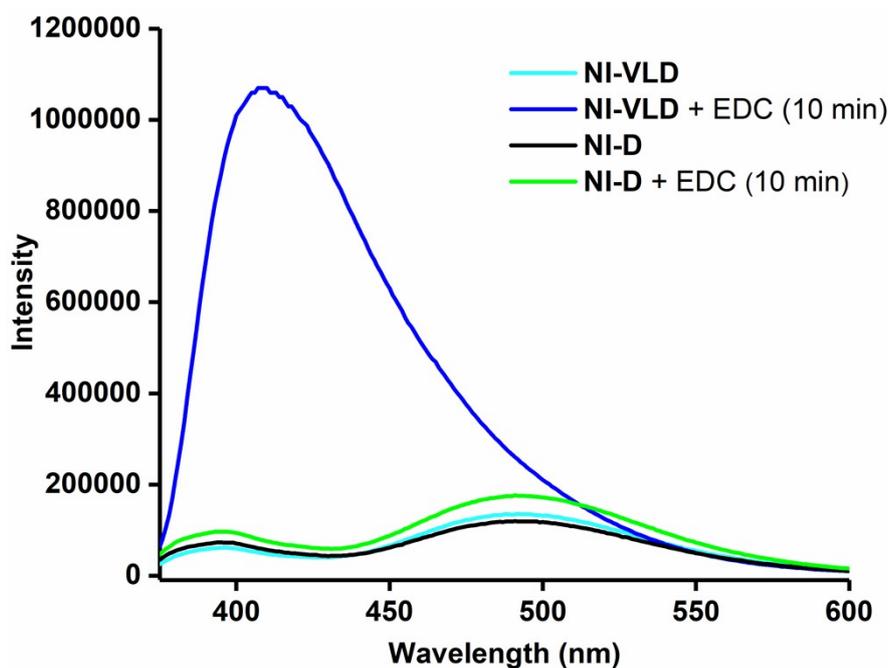
Figure S36: Comparison of emission spectra of **NI-D** (2 mM) and **NI-VLD** (2 mM) before and after 10 min of EDC (10 mM) addition. [**NI-D**]= [**NI-VLD**]= 2 mM, [EDC]= 10 mM, λ_{ex}= 365 nm and solvent composition f_a= 90%.
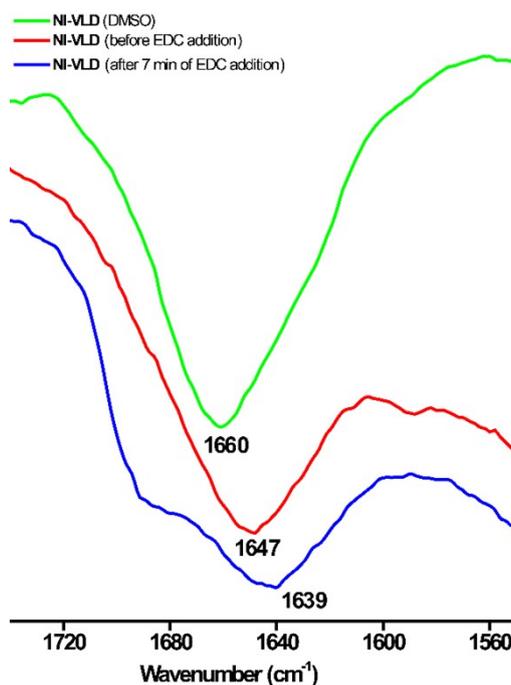

Figure S37: Normalized FTIR spectra (ATR mode) of **NI-VLD** in different states: in DMSO (green), **Agg-1** (red) and **Agg-2** (blue, after 7 min of addition of EDC to **Agg-1**). Only the amide-I region is shown. Normalization was done from 1750 to 1650 cm^{-1} region. [**NI-VLD**]= 2 mM, [EDC]= 10 mM. All the solvents used were deuterated.
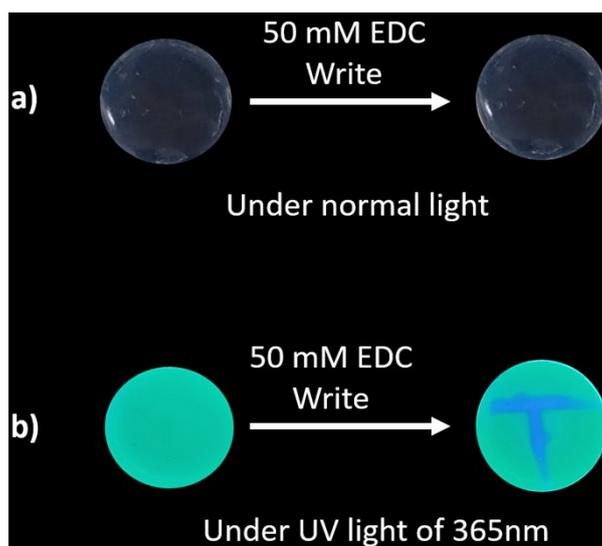
Figure S38: Visualization of written message on the **NI-VLD** entrapped-agarose gel using EDC as ink **a)** under normal light showing no message and **b)** under UV light of 365 nm showing a message. [**NI-VLD**]= 2 mM, [EDC]= 50 mM, f_a= 90%.
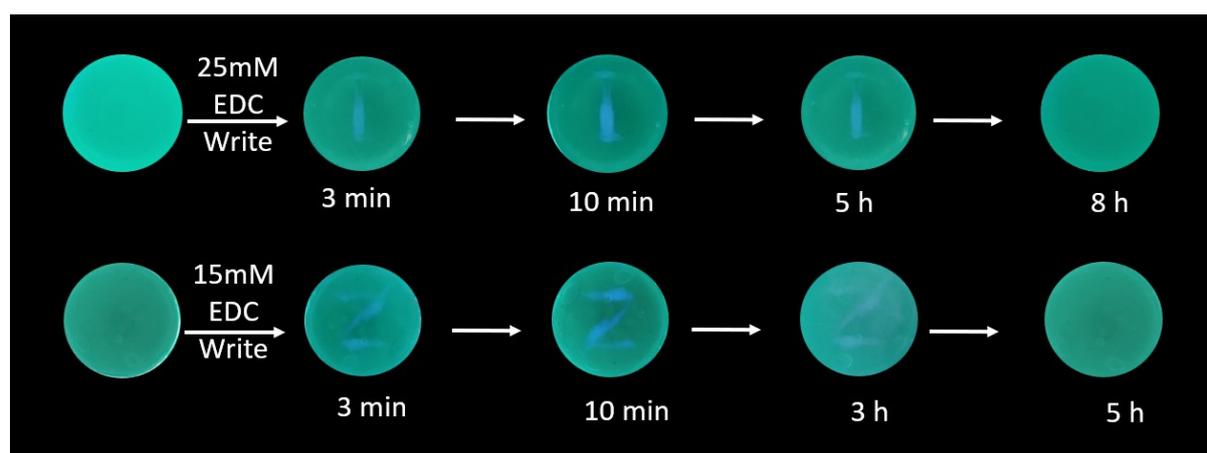

Figure S39: Variation of ink (EDC) concentration on writing a message on the **NI-VLD**-entrapped agarose gel. The concentration of EDC could manipulate the lifetime of the message. [**NI-VLD**]= 2 mM, [EDC]= 15 and 25 mM, f_a= 90%.

References

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