Electronic Supplementary Information

Freeze the dynamicity: Charge transfer complexation assisted control over reaction pathway

Nilotpal Singha, Basab Kanti Das, Bapan Pramanik, Saurav Das and Debapratim Das*

Department of Chemistry, Indian Institute of Technology Guwahati, Assam 781039, India
Email: ddas@iitg.ac.in

General Information and materials: 1,4,5,8-naphthalenetetracarboxylic acid dianhydride (NDA), n-hexylamine were procured from SigmaAldrich (USA) and used without further purification. Rink amide MBHA resin and protected amino acids and coupling reagents were obtained from Novabiochem. HPLC-grade solvents were purchased from Spectrochem (India) and Fisher Scientific (India). To prepare samples, Milli-Q water with a conductivity of less than 2 μScm^{-1} was used. Chromatographic purifications were performed on a Luna 5 μm (C18) column (Phenomenex) using a Dionex Ultimate 3000 HPLC. UV-Visible spectra were recorded on a PerkinElmer Lambda 750 spectrometer, while fluorescence measurements were performed on Fluoromax 4 (Horiba, Japan) spectrophotometer. Standard 10 mm-path quartz cuvettes were used for all spectroscopic measurements. For FTIR, Nicolet™ iS™ 10 FTIR Spectrometer was used. ^{1}H NMR, ^{13}C NMR were recorded with a Bruker Ascend 400 MHz (Bruker, Coventry, UK) spectrometer and referenced to deuterated solvents. ESI-MS were performed with a Q-tof-Micro Quadrupole mass spectrophotometer (Micromass).

UV–Visible and Fluorescence Spectroscopic Studies: Super stock solutions of **PyKC**, **PyK^{D}C**, **Py-1-KC** and **NDI-1** were prepared in 5 mL volumetric flasks by weighing appropriate amounts of the compound and dissolving in water containing 1% TFA. These stock solutions were diluted to the concentrations required for the experiment.

FESEM: The freeze dried samples were casted on a carbon tape and the FESEM images were taken on a Gemini SEM 300 (Sigma Zeiss) instrument.

Powder XRD (PXRD): The PXRD of all the samples were measured on a Bruker D2 Phaser X-ray diffractometer (30 kV, 10 mA). The Bragg peak λ was extracted from the XRD data and the layer thickness d could be obtained according to the Bragg equation $d = \lambda / 2\sin\theta$, $\lambda = 0.15405$ nm.

Isothermal Titration Calorimetry (ITC): The formation constants and thermodynamic parameters for the inclusion complexes were determined via isothermal titration calorimetry using a Nano-ITC instrument from MicroCal. **PyKC** solution (0.1 mM, in water containing 1% TFA) was placed in the (volume = 200 mL). **NDI-1** solution in water containing 1% TFA (each injection, 0.5 μL, 2 mM) was injected from a 40 μL syringe at an interval of 2 min into the reaction cell with stirring at 298 K. The first data point was omitted from the data set for curve fitting. All solutions were degassed prior to titration. The data were fitted to a theoretical titration curve using software supplied by Microcal.

Density Functinal Theory (DFT): The M06 family of functions was chosen over other conventional DFT functions as they are proven to be more accurate toward geometries and energy calculations for a variety of dispersion-dominated systems like DNA base-pair stacks and D-A CT complexes.[1] HOMO and LUMO orbital energies were obtained using the density functional theory (DFT) at the B3LYP/6-31G(d,p) accuracy level using the Gaussian 09 package of programs.

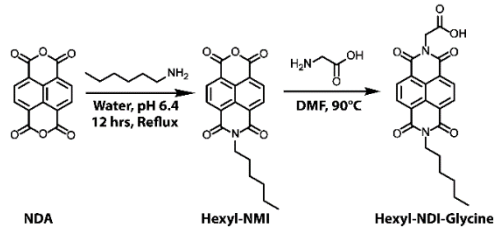
General procedure of hetero-dimerization using the new method:

The method described here is for **PyKC-NDI-1** pair.

In a typical experiment, 200 μL solutions of **PyKC** and **NDI-1** (both 1 mM) prepared in water (containing 1 % TFA) were mixed and incubated for 12h at room temperature. The generation of pink color indicates the formation of charge transfer complex. The solution was then dipped into liquid nitrogen and freeze-dried. 2 mL of cyclohexane was then added to the freeze dried solid and the sample was shaken at 100 rpm at room temperature. Cyclohexane was added to the sample at different time intervals to make up any loss due to evaporation. After the required time period, the samples were centrifuged, solvents were removed and the solid was dried under reduced pressure. The dried samples were then re-dissolved in water (containing 1 % TFA) and analysed using analytical HPLC. The % yield of the different dimers were calculated using calibration curves obtained from the pure samples.

For the preparation of **PyKC-NDI-1** heterodimer, the same process was used in a larger scale and the heterodimer was purified using semi-preparative HPLC and characterized using ESI-MS. ESI-MS calcd. for $[M+H]^+$, $C_{60}H_{71}N_{10}O_{10}S_2^+$: 1155.48, found 1155.48, and 578.24 $[M^{2+}]$.

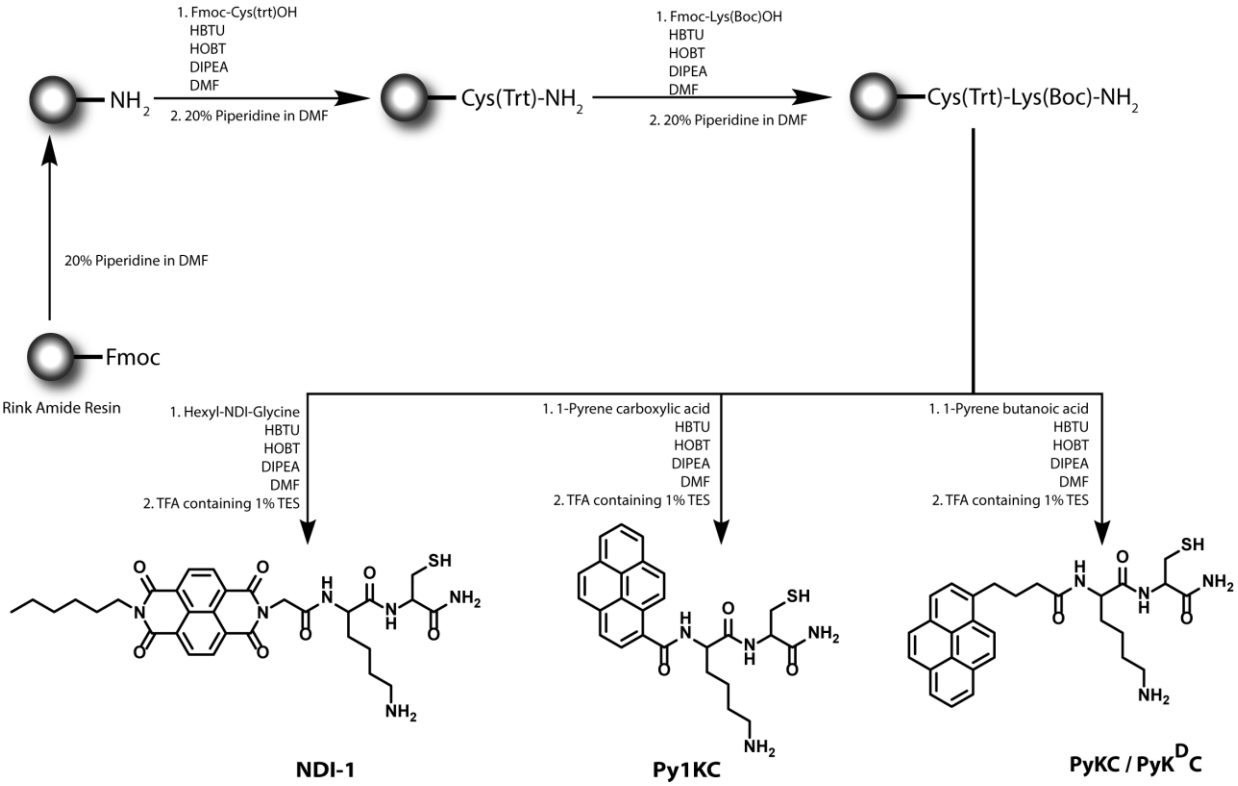
Synthesis: Hexyl-NDI-Glycine was synthesized according to Scheme **S1**.



Scheme S1: Synthetic scheme for **Hexyl-NDI-Glycine.**

Hexyl-NDI-Glycine: Hexyl-NDI-Glycine was prepared according to our previously reported procedure following the steps mentioned in Scheme S1.[2]

PyKC, PyK^{D}C, Py1KC and **NDI-1** were prepared following the routes shown in Scheme **S2.**



Scheme S2: Synthetic routes for **PyKC, Py1KC** and **NDGKC.**

General synthesis of the peptides: The peptides were synthesized on Rink amide MBHA resin using standard Fmoc (9-fluorenylmethoxycarbonyl) solid phase peptide synthesis (SPPS) method. In a typical coupling, 3 equiv. of protected amino acid (with respect to the loading of the resin), 3 equiv. of HBTU, and 6 equiv. of DIPEA were taken in 5 mL of DMF (for 0.1 mmol scale with respect to the resin loading) and stirred for 5 minutes prior to addition of the mixture to the resin. The reaction mixture was shaken for 60 min and the resin was washed several times with DMF. The Fmoc-deprotection was achieved by treatment of the resin with 20% piperidine (5 mL, 5 minutes, three times) followed by thorough washing of the resin with DMF. The Fmoc-deprotection and coupling steps were repeated until the designed peptide sequence was obtained. After the final Fmoc-deprotection, the peptide loaded resin was washed several times with DMF followed by DCM and dried under reduced pressure. The dried resin was then treated with a mixture of 95% trifluoroacetic acid (TFA) in DCM containing 1% triethylsilane (TES) and stirred for 1 h. The resin was finally washed with DCM several times. The cleavage cocktail and the washings combined were concentrated to a minimum volume on a rotary

evaporator. The cleaved peptide was then precipitated from cold dry ether, centrifuged and lyophilized to get the crude peptide. For **PyK^{D}C**, Fmoc-D-Cys(Trt)-OH was coupled as the first amino acid of the sequence followed by similar procedure as mentioned above. Purification was done in a semi-preparative HPLC using a Luna 5 μm (C18) column (Phenomenex) with a programme of acetonitrile and water starting at 5% acetonitrile to reach 30% after 5 min and continued to reach 100% at 40 min.

Characterisation of NDI-1: Yield = 70%. ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 8.74 – 8.66 (m, 4H), 8.62 – 8.52 (br, 1H), 7.99 (br, J = 8.1 Hz, 1H), 7.63 (s, 2H), 7.36 (s, 1H), 4.84 – 4.70 (m, 2H), 4.33 (tt, J = 7.9, 4.5 Hz, 2H), 4.07 (t, J = 7.5 Hz, 2H), 2.78 (s, 5H), 2.24 (s, 1H), 1.76 – 1.63 (m, 2H), 1.37 (s, 4H), 1.38 – 1.27 (m, 1H), 1.31 (s, 4H), 0.91 – 0.83 (m, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 171.87, 171.79, 163.06, 162.99, 131.17, 130.94, 127.16, 126.75, 126.35, 55.29, 52.92, 43.12, 31.41, 27.79, 27.03, 26.62, 26.51, 22.55, 22.43, 14.37. Mass (ESI-MS): m/z calcd. for $C_{31}H_{38}N_6O_7S$ $[M+H]^+$, 639.29; found 639.25.

Synthesis of PyKC, Py1KC, PyK^{D}C: The peptides were previously reported by us, and the same protocol was followed for this work.[3]

Characterisation of PyK^{D}C, Py1KC, PyKC and intermediates for **NDI-1:** As the **PyK^{D}C**,[3] **Py1KC**,[3] **PyKC**[3] and intermediates for **NDI-1**[2] were previously reported by us, the characterization data are not provided here.

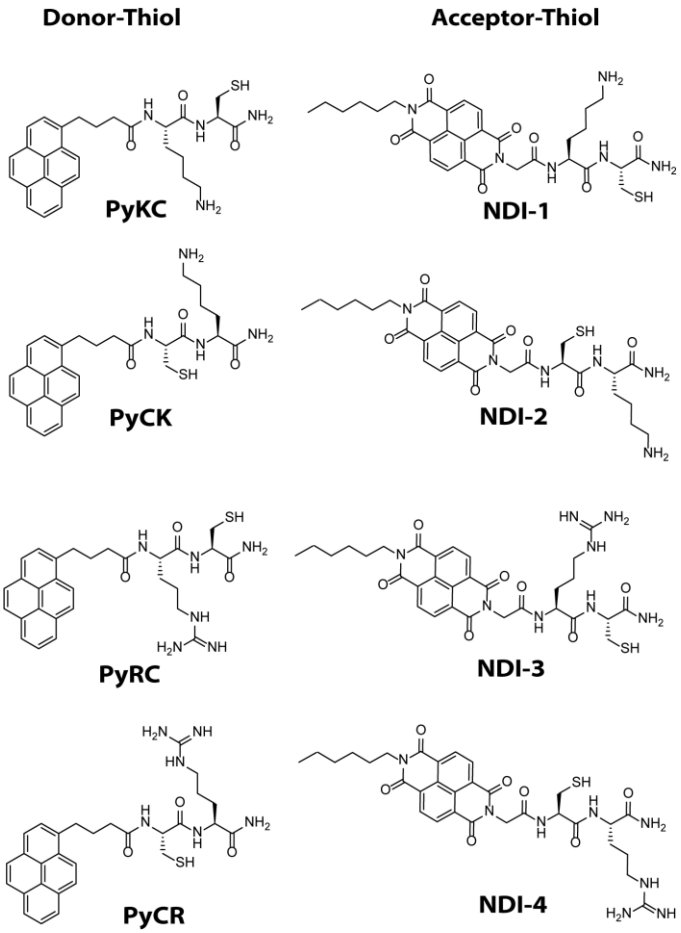
PyKC-dimer: The **PyKC**-dimer was prepared by incubating **PyKC** solution in pH 8 buffer for 72 h followed by lyophilisation and purity check by analytical HPLC and ESI-MS. No further purification was needed and the yield was more than 99.5%. Calibration plots were made by injecting samples of different concentration and their respective peak areas from analytical HPLC. ESI-MS calcd. for $[M+H]^+$, $C_{58}H_{66}N_8O_6S_2^+$: 1035.4580, found: 1035.4576, and 518.2320 $[M^{2+}]$.

NDI-1-dimer: The disulphide linked dimer of **NDI-1** was prepared by incubating **NDI-1** solution in pH 8 buffer for 72 h followed by lyophilisation and purity check by analytical HPLC and ESI-MS. No further purification was needed and the yield was more than 99%. Calibration plots were made by injecting samples of different concentration and their respective peak areas from analytical HPLC. ESI-MS calcd. for $[M+H]^+$, $C_{62}H_{75}N_{12}O_{14}S_2^+$: 1275.50, found: 1275.49, and 638.25 $[M^{2+}]$.

For **PyK^{D}C-NDI-1 heterodimer** and **Py1KC-NDI-1 heterodimer**, the donor and acceptor solutions were mixed to maintain the molar ratio of 1:1 followed by changing the pH to 8 by addition of NaOH solution and the mixtures were incubated at room temperature for 72h. The mixtures were analysed by analytical HPLC and the peaks corresponding to the heterodimers were identified by analysing the ESI-MS of the fractions. These two dimers were not isolated for calibration curves. However, the % conversions during the reactions using the presented methodology were calculated from the disappearance of the starting materials.

CONTROL EXPERIMENTS

1. **To check the disulphide formation in aqueous 1% TFA solution,** both **PyKC** and **NDI-1** were dissolved in aqueous 1% TFA solution separately as well as in 1:1 molar ratio. All three samples were incubated at room temperature for 24h and then analysed by HPLC and ESI-MS. No detectable dimerization was obtained in any of these three solutions. Based on these results, the initial solutions of the monomers were prepared in aqueous 1% TFA solutions to prevent any dimerization.
2. **To check any dimerization in cyclohexane without lyophilisation of the CT complexes,** both as synthesized powdered **PyKC** and **NDI-1** were suspended in cyclohexane separately as well as in 1:1 molar ratio. All three samples were incubated at room temperature for 24h and then centrifuged, solids were dried and dissolved in 1% TFA solutions. These solutions were analysed by HPLC and ESI-MS. No detectable dimerization was obtained in any of these three solutions.
3. **To check the solubility of the building blocks in Cyclohexane,** both **PyKC** and **NDI-1** were suspended (10 mg) in cyclohexane/hexane (10 mL) and the suspensions were sonicated for 30 minutes. After that the suspensions were centrifuged at 12000 rpm for 10 mins. 100 μL of the supernatant cyclohexane samples were pipetted out, dried and to that 50 mL of water was added and the solutions were analysed by HPLC and ESI-MS. No trace of the monomers were found in any case suggesting insolubility of the monomers in cyclohexane.



Scheme S3. Chemical structures of different NDI and Pyrene containing thiols used for DFT calculations to find the most suitable D-A pair for the study.

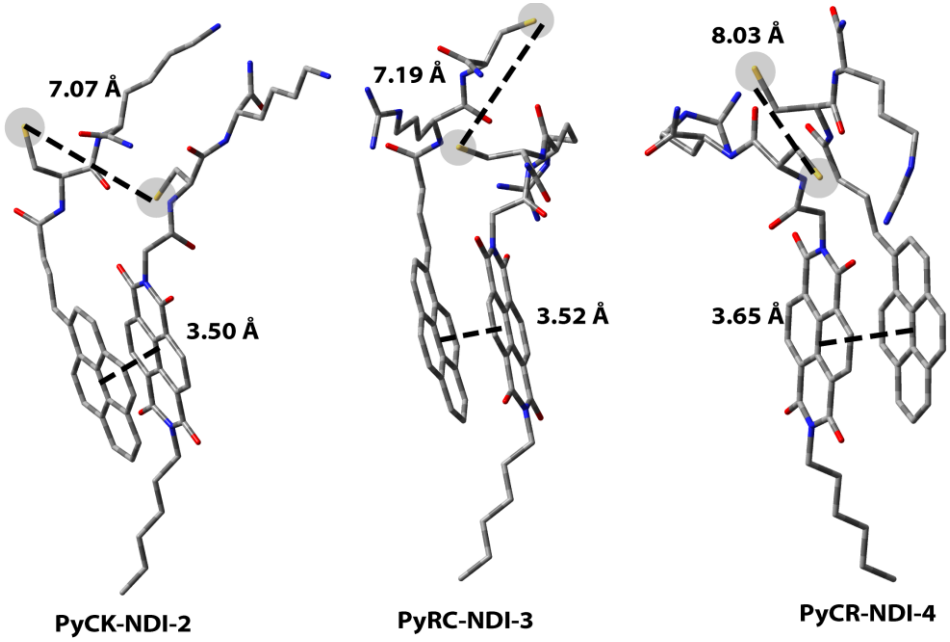


Figure S1. Energy minimized structures of different D-A pairs (from Scheme S3) showing the distances between the –SH groups and between π-rings of the donor and acceptor.

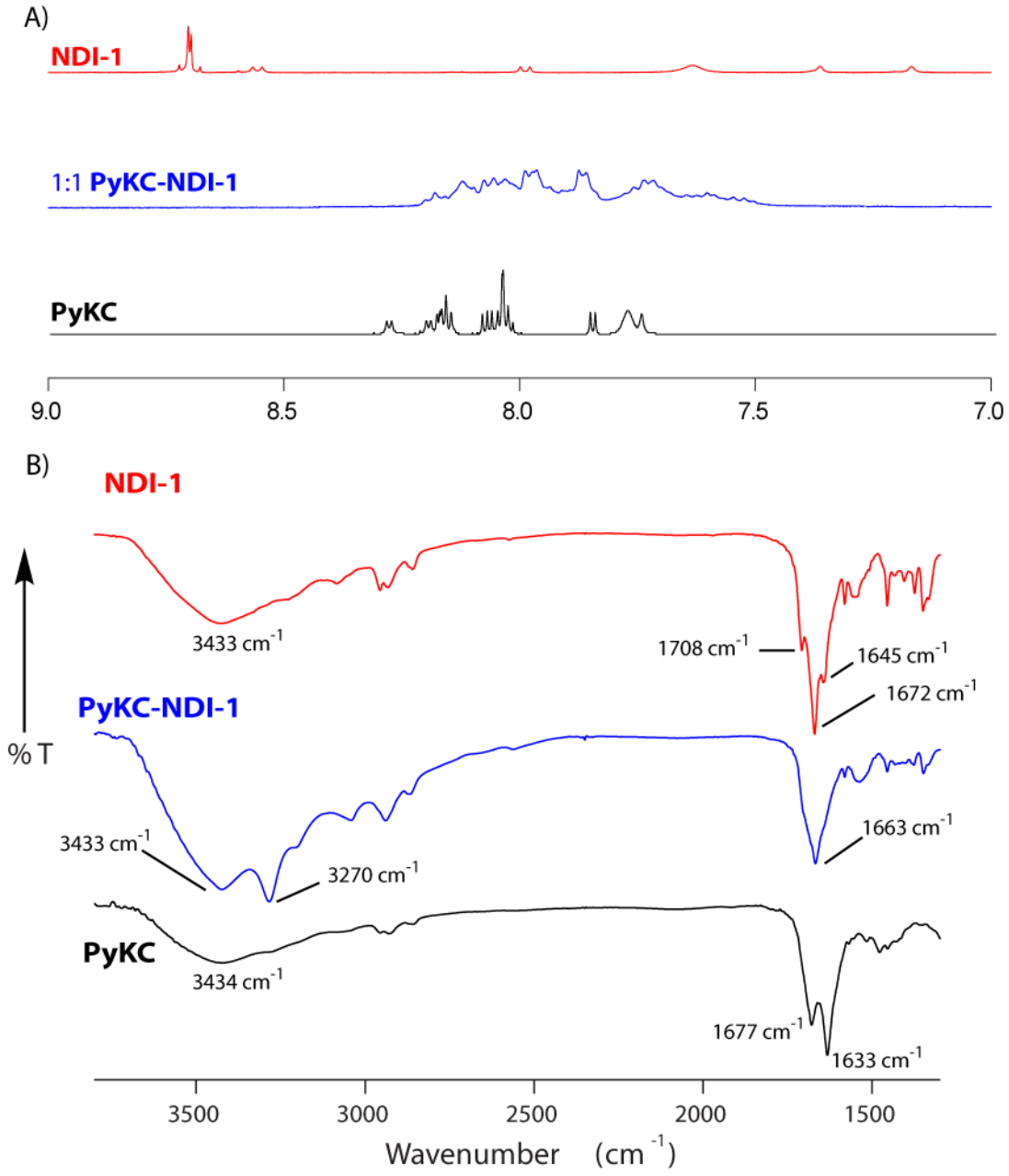


Figure S2. A)^{1}H NMR spectra (in 10% DMSO-d_6 in D_2O) of **NDI-1**, **PyKC** and a 1:1 mixture of these two compounds showing the up-field shift of aromatic protons of both compounds upon mixing. (Since, the pyrene protons of **PyKC** as well as the NH/NH_2 protons do not appear in D_2O, we took the help of 10% DMSO-d_6). B) FTIR spectra of **PyKC**, **NDI-1** and CT complex of **PyKC** and **NDI-1.**

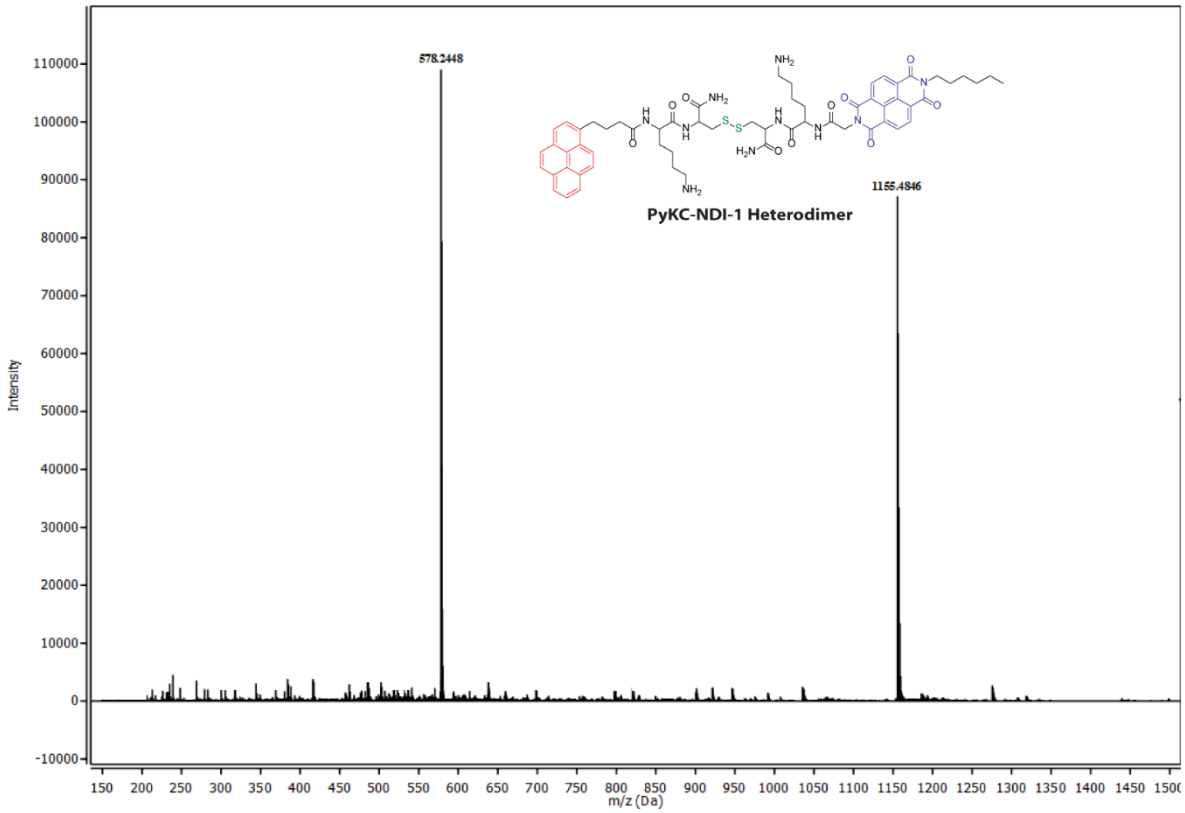


Figure S3. ESI MS of the **PyKC-NDI-1 heterodimer.**

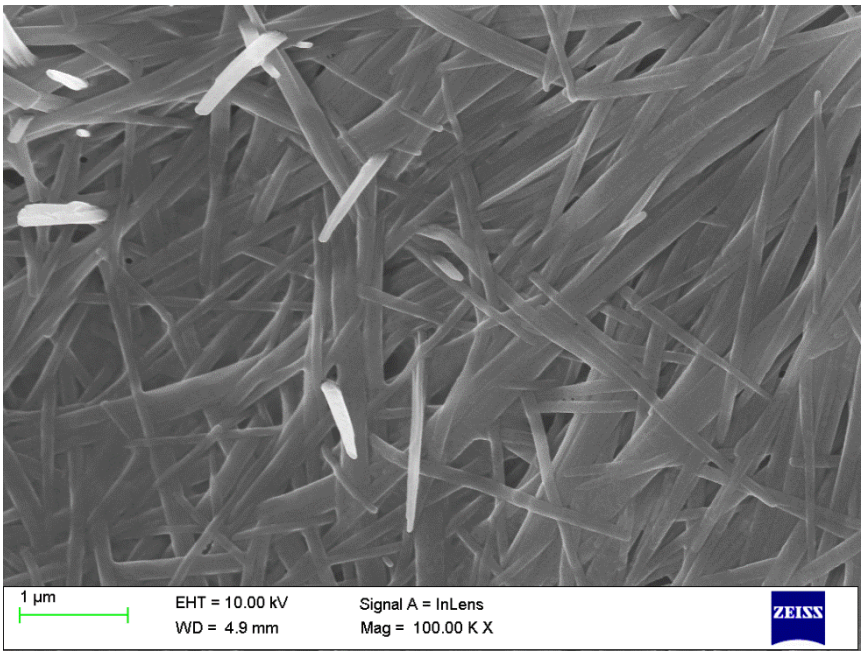


Figure S4. FESEM image of the lyophilized solid of a 1:1 mixture of **PyKC** and **NDI-1** after incubating the solid in cyclohexane for 24h.

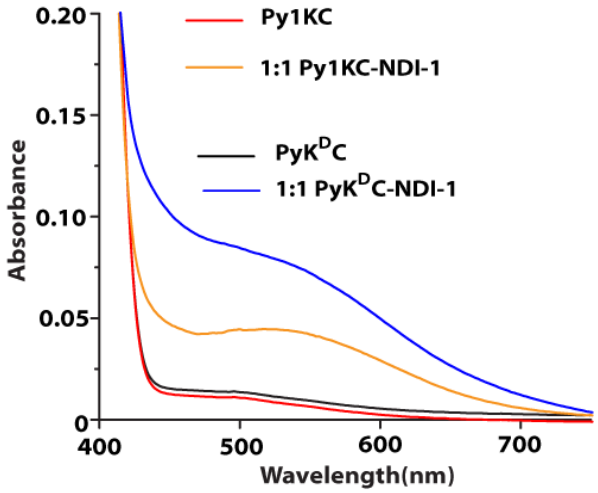


Figure S5. UV-Visible spectra of **PyK^{D}C** (1 mM) or **Py1KC** in presence increasing amounts of **NDI-1** showing the appearance of CT-band.

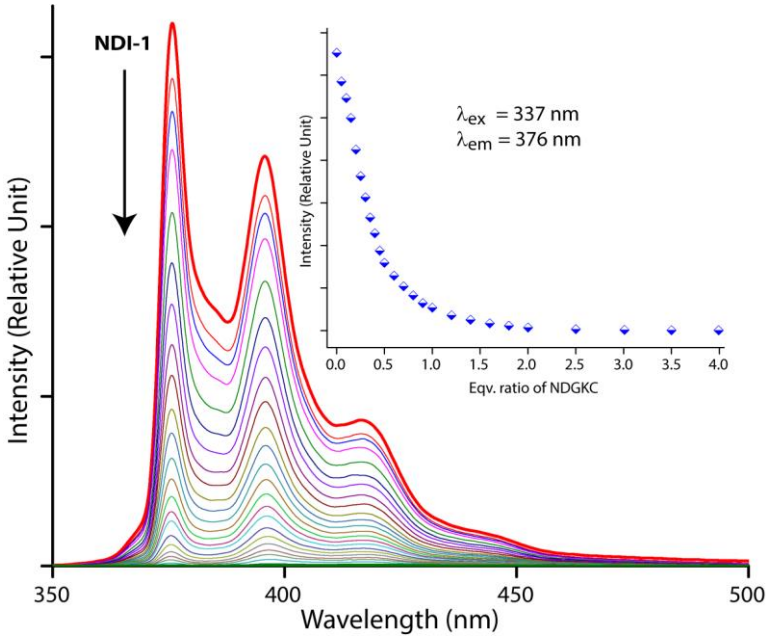


Figure S6. Emission spectra of **PyK^{D}C** when titrated with **NDI-1** showing the quenching of the emission. Inset: the changes in emission intensity at 376 nm against the molar ratio of **PyK^{D}C** and **NDI-1**.

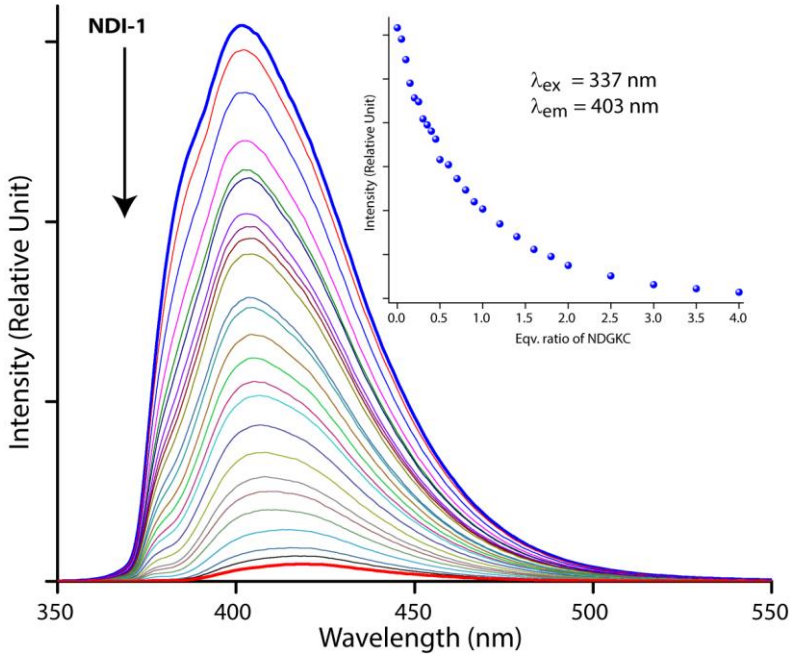


Figure S7. Emission spectra of **Py1KC** when titrated with **NDI-1** showing the quenching of the emission. Inset: the changes in emission intensity at 403 nm against the molar ratio of **Py1KC** and **NDI-1**. The broad and structure-less profile could be a result of exciplex formation.

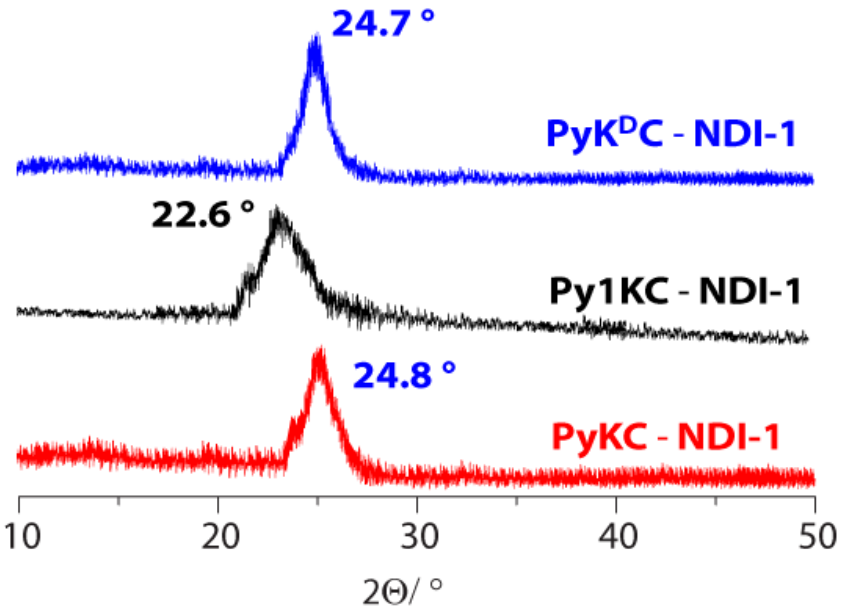


Figure S8. PXRD data of lyophilized 1:1 mixtures of **PyKC**+ **NDI-1**, **PyK^{D}C**+ **NDI-1**, and **Py1KC**+**NDI-1** measured at room temperature.

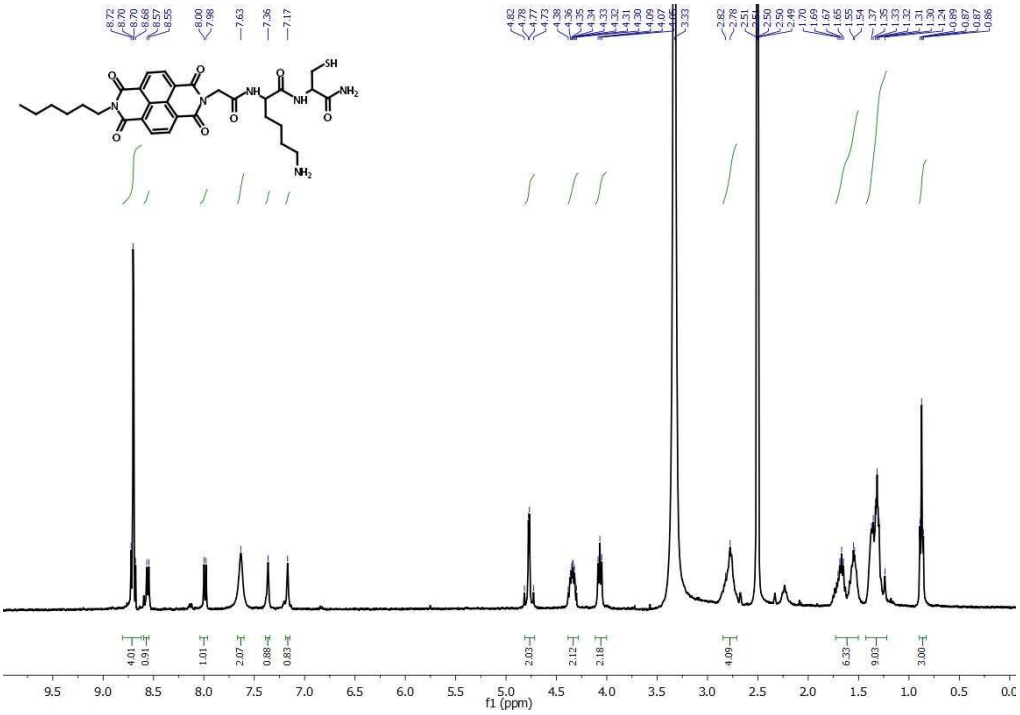


Figure S9. ^{1}H NMR spectra of **NDI-1** in DMSO-d_6.

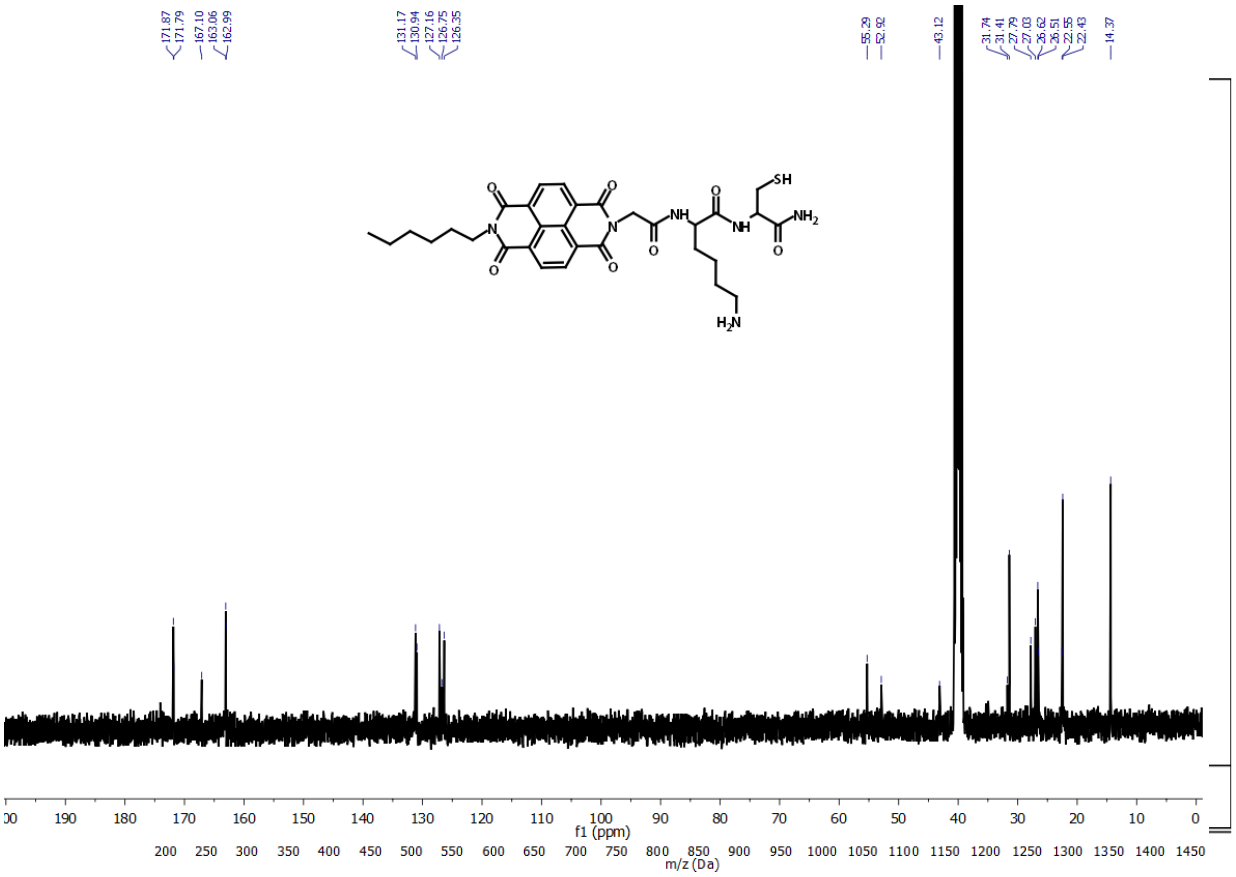


Figure S10. ^{13}C NMR spectra of **NDI-1** in DMSO-d_6.

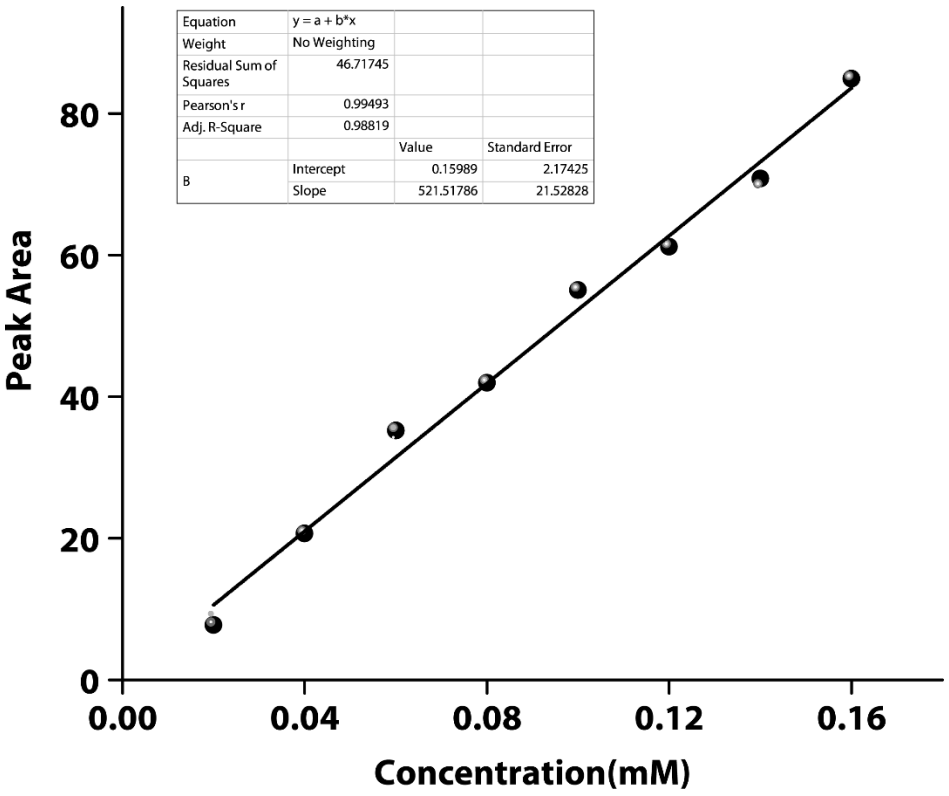


Figure S11. Calibration plot of PyKC-dimer.

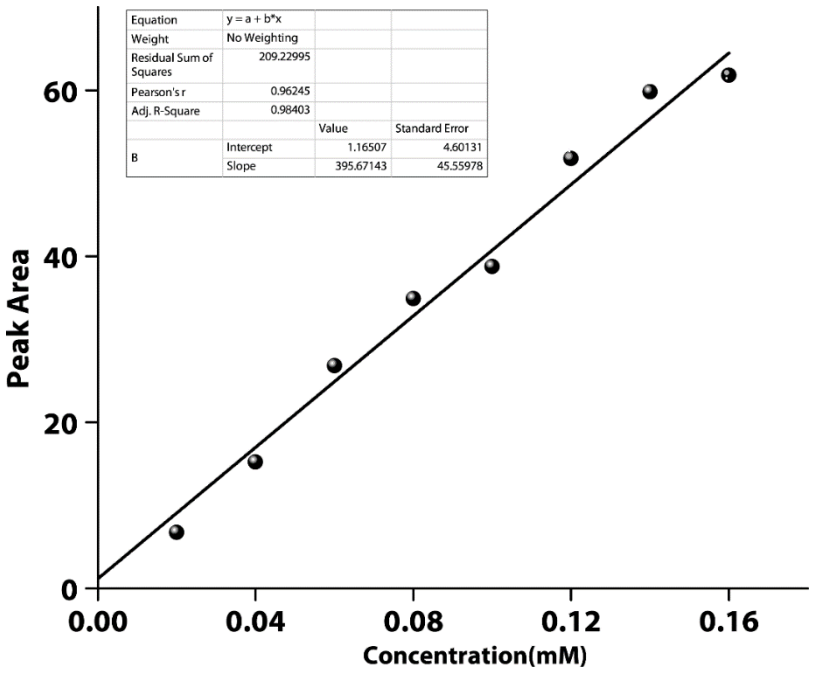


Figure S12. Calibration plot of NDI-1 dimer.

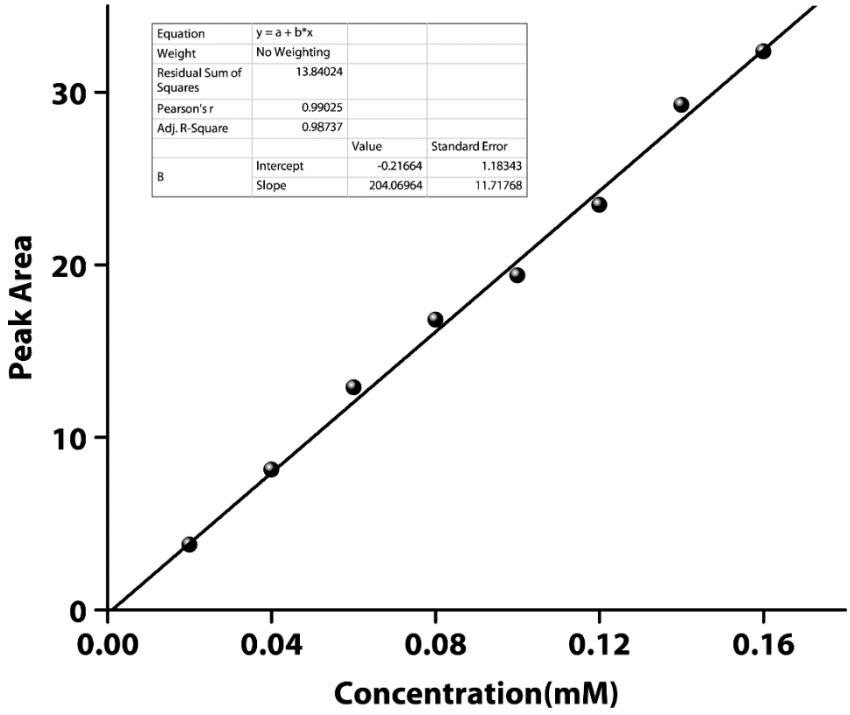


Figure S13. Calibration plot of **PyKC-NDI-1 heterodimer.**

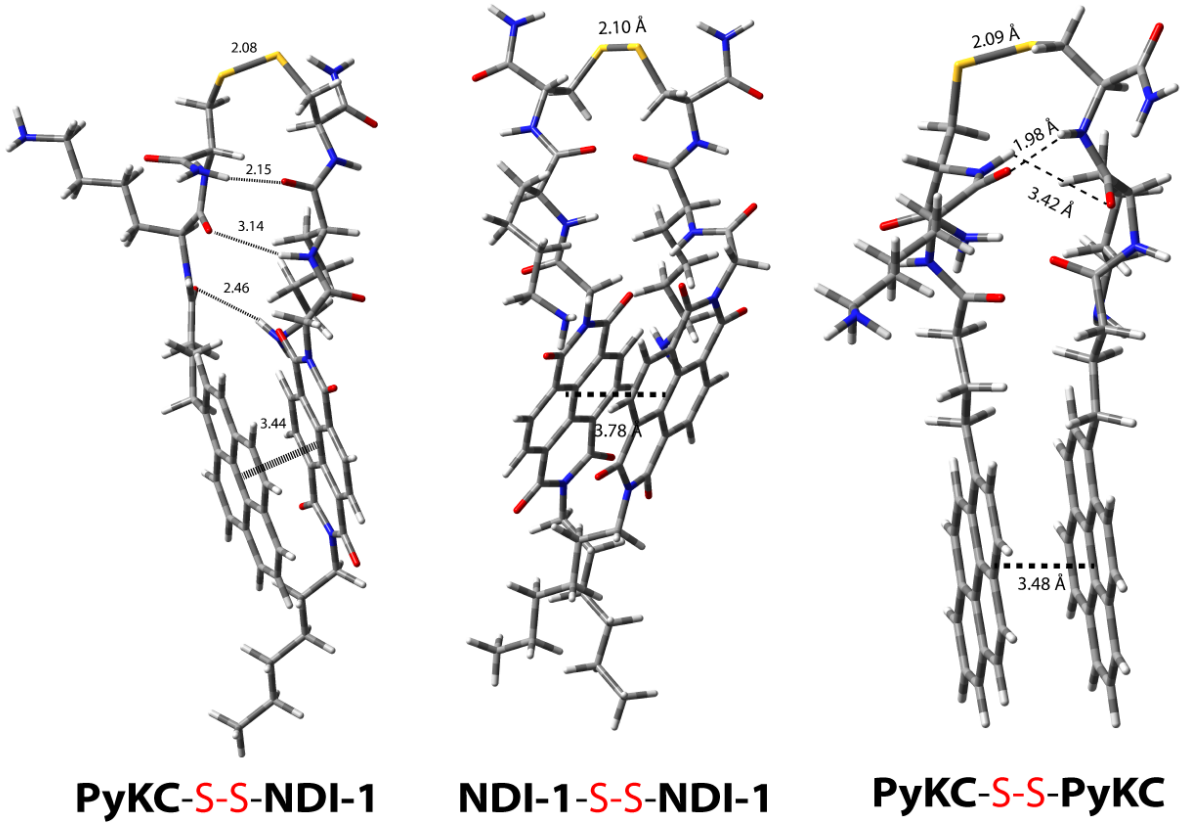


Figure S14. Energy minimized structures of different disulphide linked homo and heterodimers of **PyKC** and **NDI-1** as obtained from DFT calculations showing the distance between π-rings of the donor and acceptor and probable hydrogen bonding sites.

Table S1. Retention times of different molecules when eluted with a gradient of acetonitrile in water starting from 5% acetonitrile to reach 30% after 5 min and continued to reach 100% at 40 min on a a Luna 5 μm (C18) column (Phenomenex) at a flow rate of 1 mL/min using Dionex Ultimate 3000 analytical HPLC.

Compound	R_T (min)
PyKC /PyK^DC	14.8
NDI-1	18.9
PyKC-dimer/ PyK^DC-dimer	17.4
NDI-1-Dimer	20.9
PyKC-NDI-1 Heterodimer/ PyK^DC-NDI-1 Heterodimer	16.9
Py1KC	15.4
Py1KC-Dimer	18.0
Py1KC-NDI-1 Heterodimer	17.2

Table S2. The distance between the SH groups and π-planes as obtained from the energy minimized CT complexes using DFT calculations.

D-A pair	*SH – SH* distance (Å)	π-plane distance (Å)
PyKC – NDI-1	4.07	3.51
PyCK– NDI-2	7.07	3.50
PyRC– NDI-3	7.19	3.52
PyCR– NDI-4	8.03	3.65
PyK^DC– NDI-1	10.60	3.60
Py1KC – NDI-1	7.85	4.02

Table S3. Different reaction parameters optimised for the presented methodology involving **PYKC** and **NDI-1** (Amounts of lyophilized powder and solvents used were similar in each case)

Reaction condition/solvent	Oxygen Solubility (mol dm^{-3})	Starting concentrations of the donor and acceptor thiols before lyophilization	Time (h)	% heterodimer	% conversion
Aqueous solution	1.22×10^{-3} [a]	1 mM	24	48.9	100
Aerial	-	1 mM	20	98.5	5
Cyclohexane	1.28×10^{-3} [b]	1 mM	20	98.2	100
Cyclohexane	1.28×10^{-3} [b]	0.1 mM	20	98.1	100
Cyclohexane	1.28×10^{-3} [b]	0.01 mM	20	45.5	20
Hexane	1.25×10^{-3} [b]	1 mM	20	98.0	100
Benzene	8.20×10^{-4} [b]	1 mM	20	97.5	71
DCM	7.09×10^{-4} [b]	1 mM	20	97.8	56

[a] at 298 K and 101.3 kPa;[4] [b] at 298.2 K and 101.3 kPa.[5]

References:

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2. Singha, N.; Gupta, P.; Pramanik, B.; Ahmed, S.; Dasgupta, A.; Ukil, A.; Das, D. *Biomacromolecules* **2017**, *18*, 3630.
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5. Sato, T.; Hamada, Y.; Sumikawa, M.; Araki, S.; Yamamoto, H. *Ind. Eng. Chem. Res.* **2014**, *53*, 19331.