Turning the Heat on Conjugated Polyelectrolytes: An Off-On ratiometric Nanothermometer

Ghinwa H. Darwish, Ali Koubeissi, Tharallah Shoker, Samir Abou Shaheen, Pierre Karam*

Department of Chemistry, American University of Beirut, P.O. Box 11-0236, Beirut, Lebanon

*To whom correspondence should be addressed:
Phone: 961-1-350000 Ext 3989 **Fax:** 961-1-350000 Ext 3970 **Email:** pierre.karam@aub.edu.lb

This section includes:

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- *Methods*
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- *Emission Spectra of PPE-CO$_2$-7 in 45% Methanol*
- *Emission Spectra of PPE-CO$_2$ 7 Solution At Different Temperatures*
- *Emission of PPE-CO$_2$ 7 + PVP*
- *Thermal Response Of PPE-CO$_2$ 7 -108 + PVP*
- *Thermal Response in Different solvents*
- *Nanothermometer Reversibility*
- *PPE-CO$_2$-7/PVP Photostability*

I-   MATERIALS:

Tetrakis(triphenylphosphine)palladium(0), 4-Iodobenzotrifluoride (**5**), Tetrabutylammonium hydroxide solution 1.0 M in methanol, bromoacetic acid, 1-dodecanol, Phosphoric acid, Carbon tetrachloride , polyvinylpyrrolidone (Mw = 55000) and Tetrabutylammonium fluoride solution 1.0 M in THF were purchased from Sigma Aldrish. Trimethylsilylacetylene, Tetrahydrofuran (Extra Dry over Molecular Sieve), Triethylamine (were purified by distillation), Magnesium Sulfate, Sodium bisulfite, Iodine, Copper(I) iodide, 1,4-Diethynylbenzene (**4**), Hydroquinone, 1,4-dioxane and [Bis(trifluoroacetoxy)iodo]benzene were purchased from Across.

II-   SYNTHESIS OF PPE-CO$_2$-7:

PPE-CO$_2$-7 was prepared according to a previously reported procedure [1] and the thesis work of Dr. Xiaoyong Zhao (University of Florida).[2] **Scheme 1** summarizes the synthesis of monomer **3**. **Scheme 2** summarizes the polymerization reaction. 4-Iodobenzotrifluoride (**5**) was incorporated at final molar ratio of 25% to yield a polymer with a **7** monomer repeat units following the previously published procedures. All reactions yields were comparable to the published values.
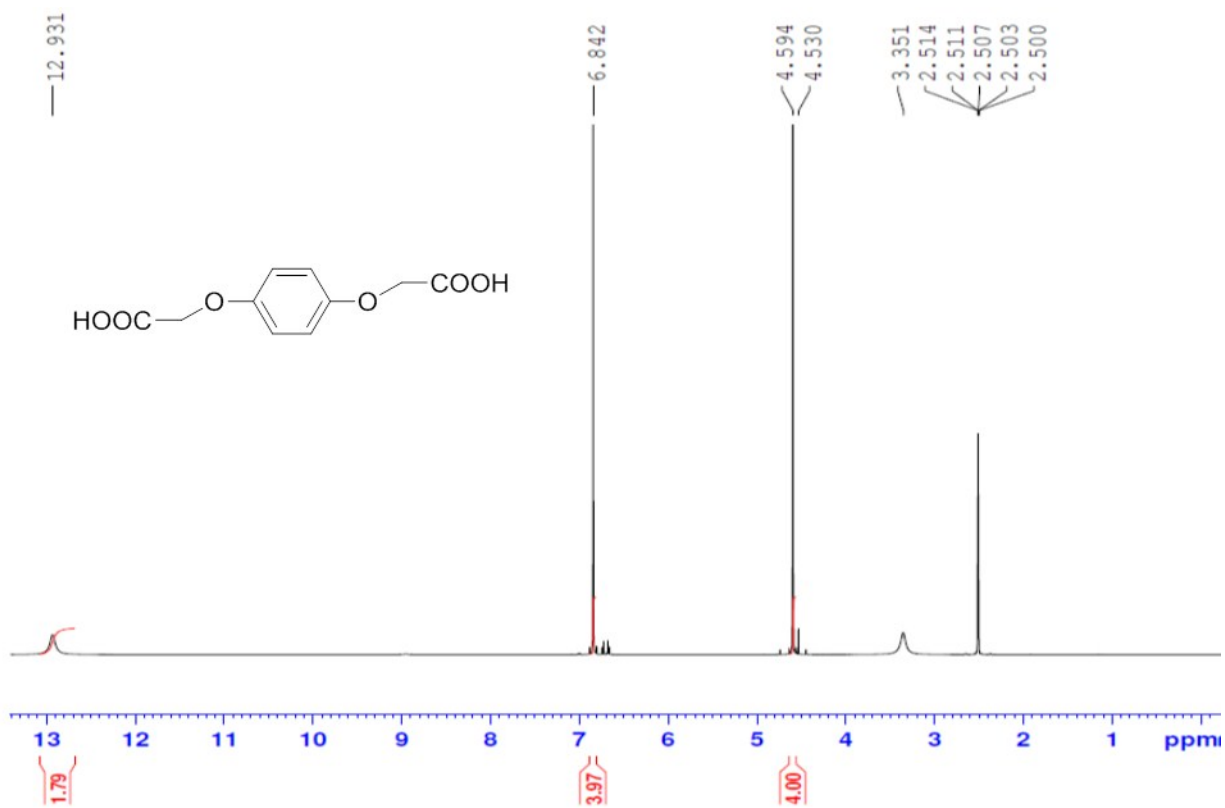
Scheme 1: Synthesis of monomer **3** starting from hydroquinone.
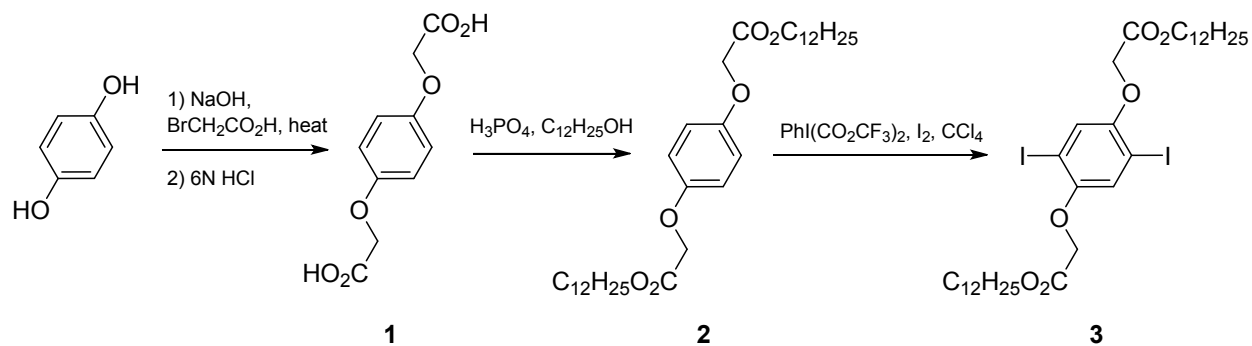
Scheme 2: Polymerization reaction starting from monomer **3**, **4** and **5**. The terminator concentration (**5**) was used at 25% molar ratio to lead a short conjugated polymer of 7 monomers on average.
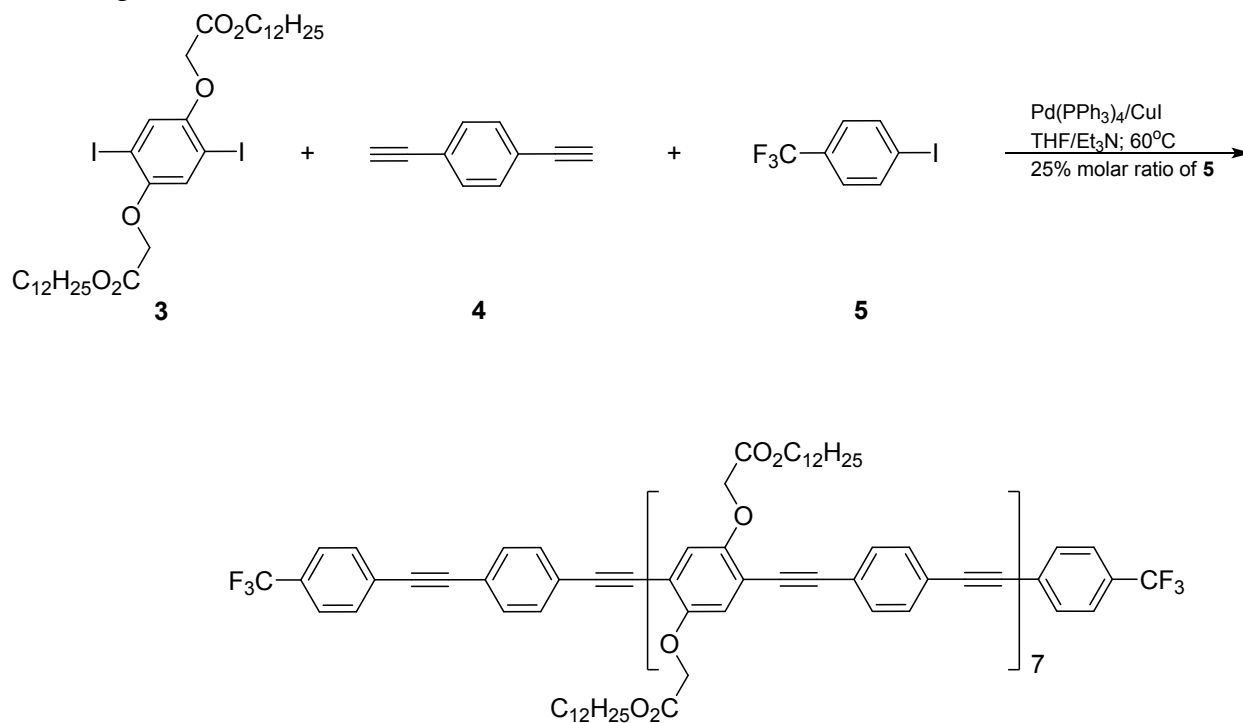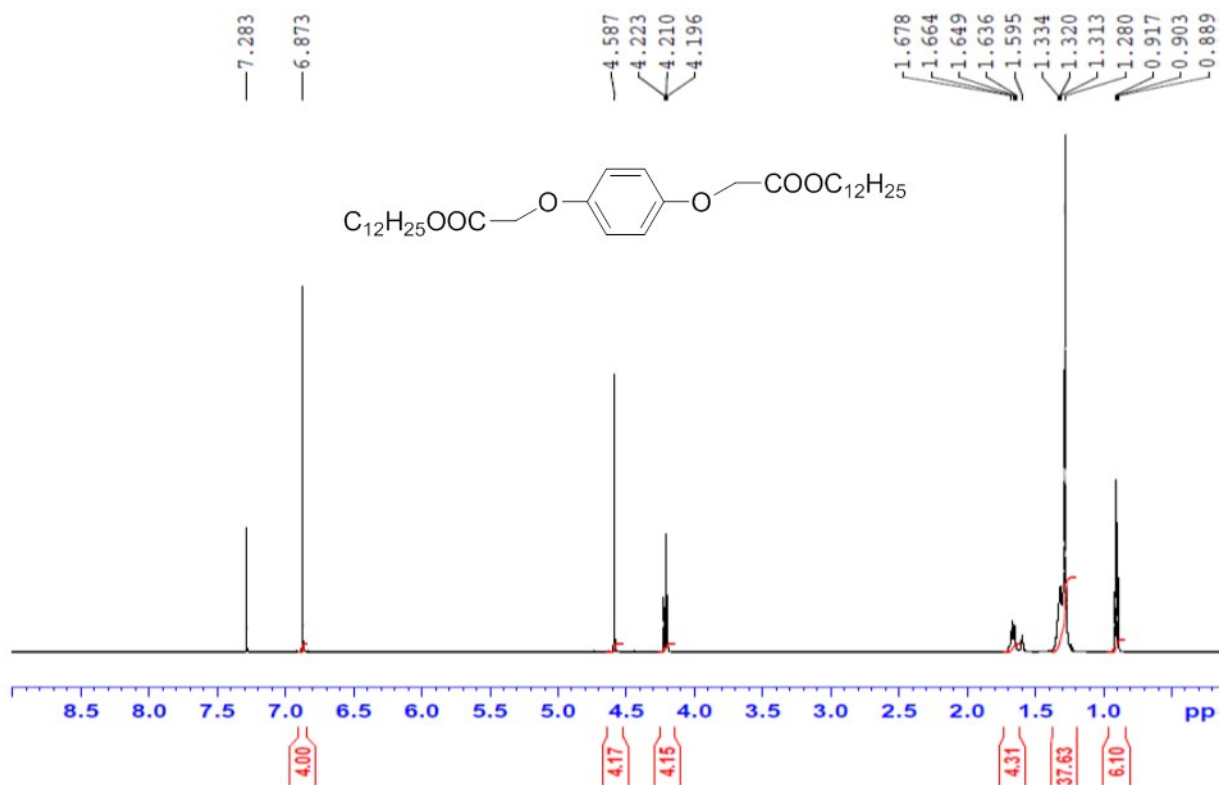
NMR spectra of the synthesized intermediates (**1**, **2** and **3**) and the final product:
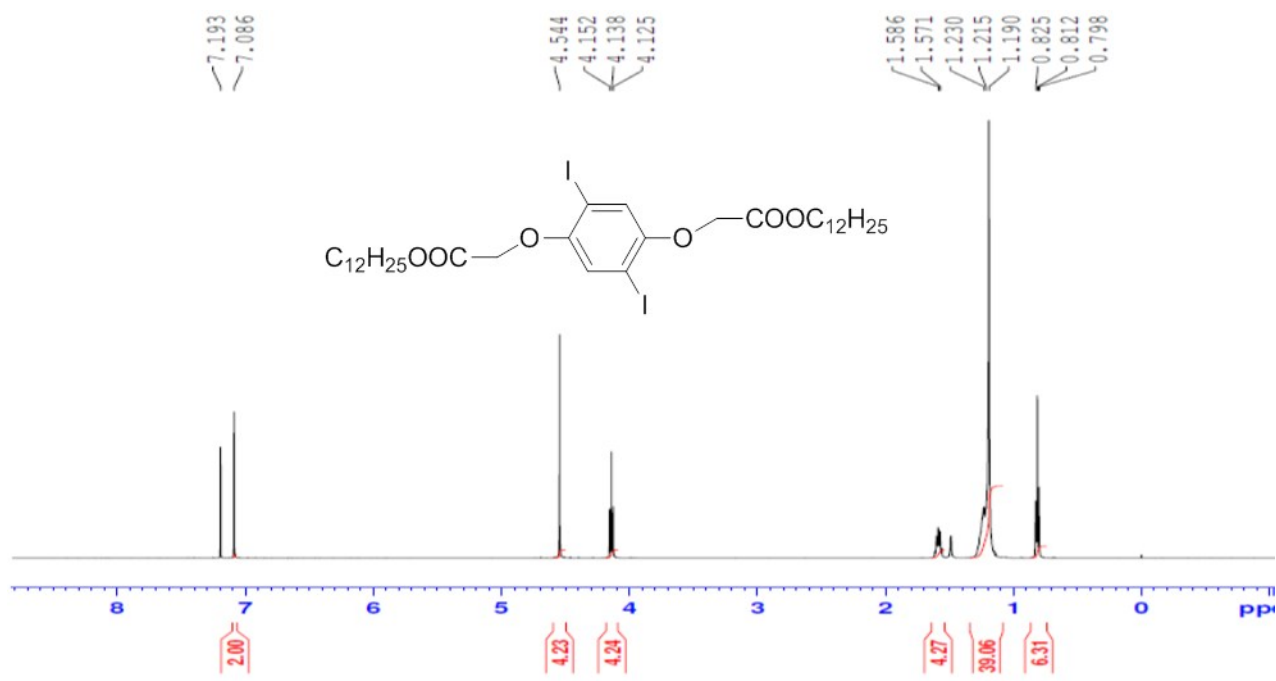2,2'-(1,4-Phenylenebis(oxy))diacetic acid (**1**):

^{1}**H NMR** (DMSO, δ_{ppm}): 12.93 (br. s, 2H), 6.84 (s, 4H), 4.59 (s, 4H).

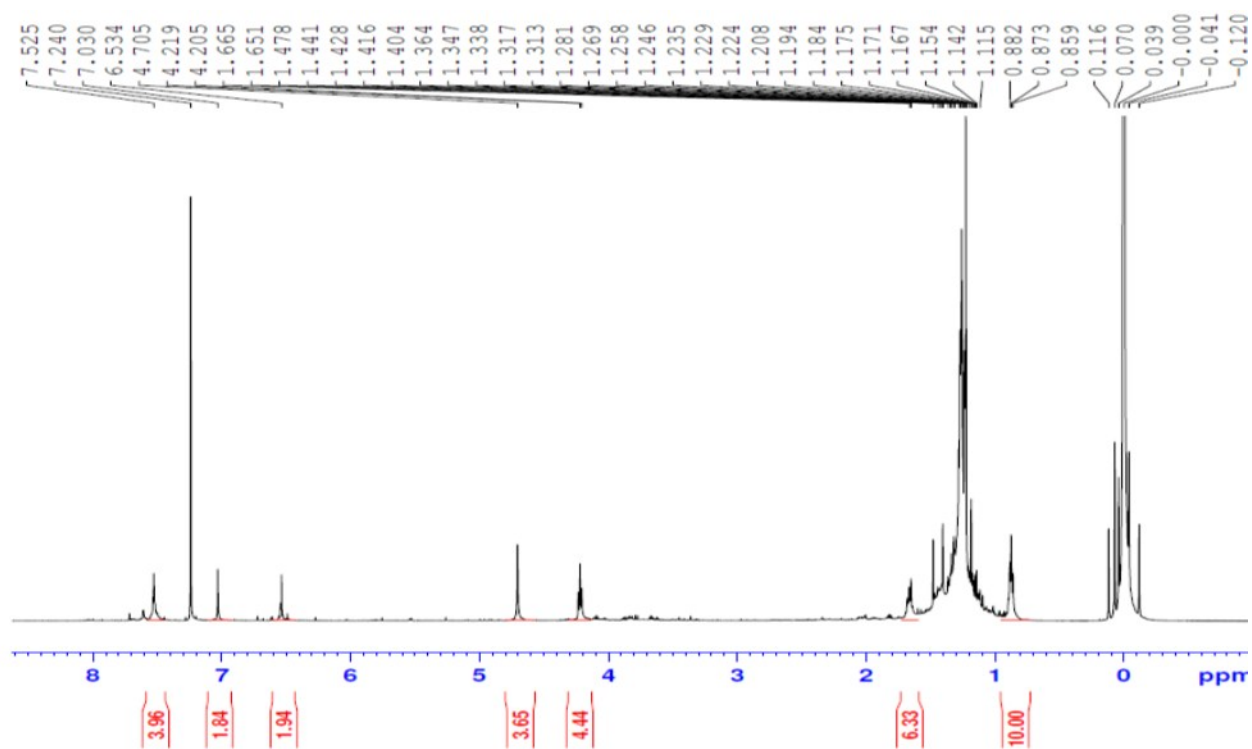Didodecyl 2,2'-(1,4-phenylenebis(oxy))diacetate **(2)**

¹H NMR (CDCl₃, δ$_{ppm}$): 6.87 (s, 4H), 4.58 (s, 4H), 4.21 (t, 4H), 1.67-1.63 (m, 4H), 1.33-1.28 (m, 36H), 0.90 (t, 6H)

Didodecyl 2,2'-(2,5-diiodo-1,4-phenylene)bis(oxy)diacetate **(3)**

¹H NMR (CDCl$_3$, δ$_{ppm}$): 7.08 (s, 2H), 4.54 (s, 4H), 4.13 (t, 4H), 1.58-1.57 (m, 4H), 1.23-1.19 (m, 36H), 0.81 (t, 6H).

^{1}**H NMR** (CDCl$_3$, δ_{ppm}): 7.52 (br. m, 4H), 7.03 (s, 2H), 4.70 (s, 4H), 4.22 (t, 4H), 1.65-0.85 (m).

III-   METHODS:

Absorption and Emission Spectrum: Steady-state fluorescence spectroscopy was carried out using a Thermo Scientific Lumina spectrophotometer. Absorption spectra were recorded using a Jasco V-570 UV−vis spectrophotometer in double-beam mode. For all steady-state absorption and emission experiments, the solutions were placed in 1 cm × 1 cm quartz cuvettes. Unless otherwise stated, the experiments were all performed in a 10mM HEPES buffer solution (pH 7.0) and 150 mM NaCl to mimic physiological pH.

Temperature Sensing Experiments: Temperature controlled experiments were performed using a T3 quantum northwest unit. To maintain a homogenous temperature, the solution was continuously stirred. Before each measurement solution was allowed to stabilize for 2 to 3 mins. Each experiment was done in at least three replicas.

Ratiometric Calculation: The reported nanothermometer displayed a clear isoemission point at 510 nm. To calculate the ratiometric signal readout, we defined two spectral regions; I_{Blue} and I_{Red}. I_{Blue} was calculated by integrating the signal between 435 and 509.9nm. I_{Red} was calculated by integrating the emission intensity between 510 nm and 650 nm. The ratiometric signal was then calculated by evaluating the I_{Blue}/I_{Red} ratio.

We opted to calculate the ratio as described above for a practical consideration. The nanothermometer calibration curve could therefore be easily translated into a microscopy setup. Long-path filter (510LP, for example) could be used to split the emission at 510 nm. Two detectors will record the integrated intensity and a ratiometric signal readout is therefore calculated.

Thermal relative sensitivity calculation:

In the absence of a physical model that can fully describe the ratiometric signal readout, the thermal response was fitted into a second order polynomial function. The relative sensitivity (S) was calculated using equation 1 (Brites *el al.*):[3]

$$(S = \frac{\frac{\partial Q}{\partial T}}{Q})$$ **(1)**

The maximum relative sensitivity is reported as the highest computed sensitivity within the studied range.

IV-   EMISSION SPECTRA OF PPE-CO$_2$-7 in 45% METHANOL

Upon addition of methanol to final percent volume of 45, a shift in the emission spectra was observed with a maximum emission at 485 nm. This peak was attributed to a quazi-distabilized state of the PPE-CO$_2$ -7 aggregates.
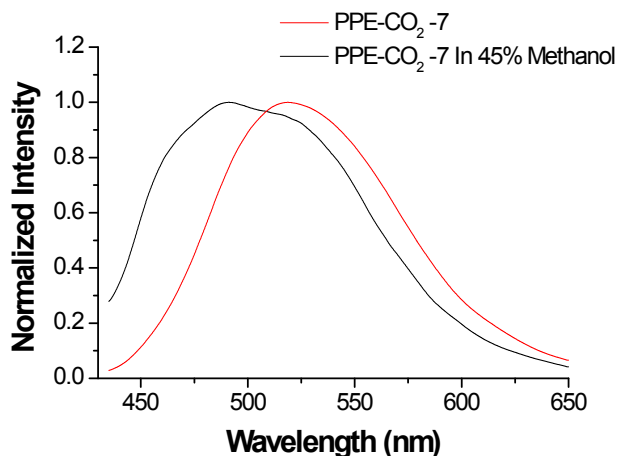
Figure S1: Emission spectra of PPE-CO$_2$ -7 in water and 45% methanol. It shows the destabilized state at 485 nm.

V-   EMISSION SPECTRA OF PPE-CO$_2$ 7 SOLUTION AT DIFFERENT TEMPERATURES:

A solution of 5 µg/ml of PPE-CO$_2$ -7 in a buffer solution (pH=7.0, 10 mM HEPES and 150 mM NaCl) was subjected to a control heating between 20.0 ^{0}C and 70.0 ^{0}C with a step increment of 5.0 ^{0}C. The time length of each experiment was an hour and half and the total time was 15 hours. The fluorescence intensity changes were acquired upon excitation of the solution at 420 nm. Below, we summarize the fluorescence intensity changes for all 10 runs:
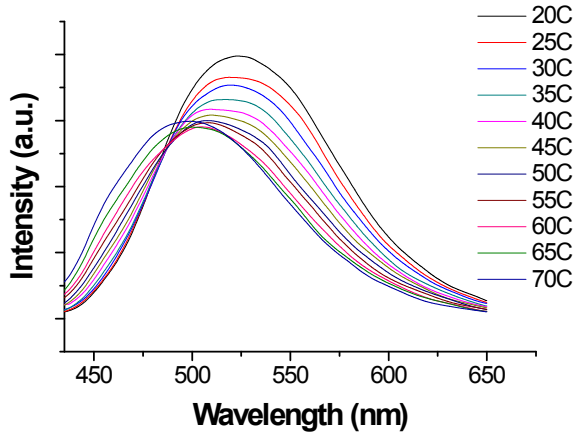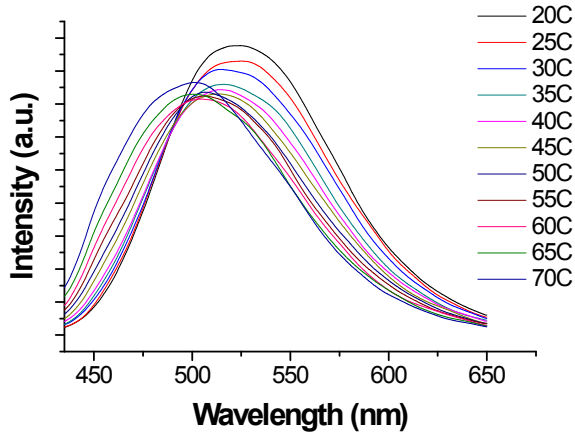
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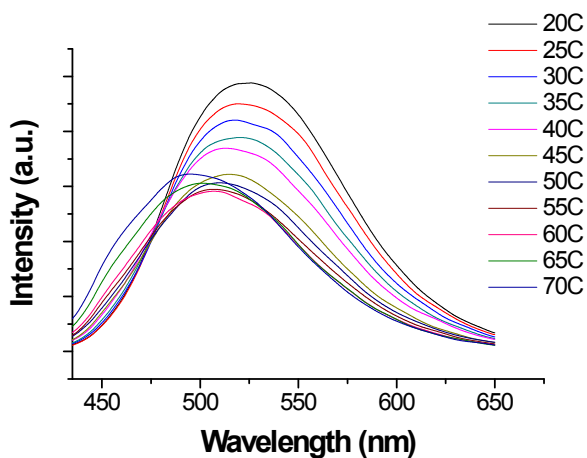
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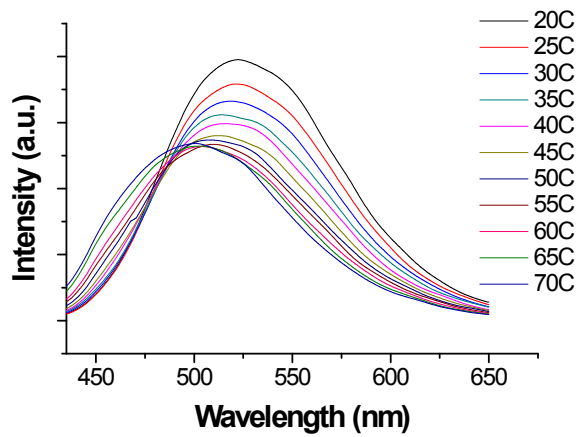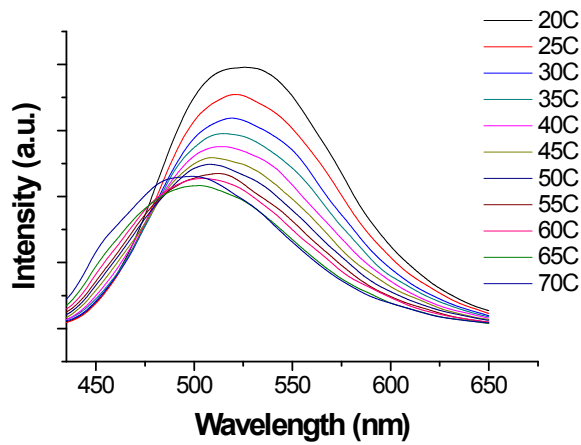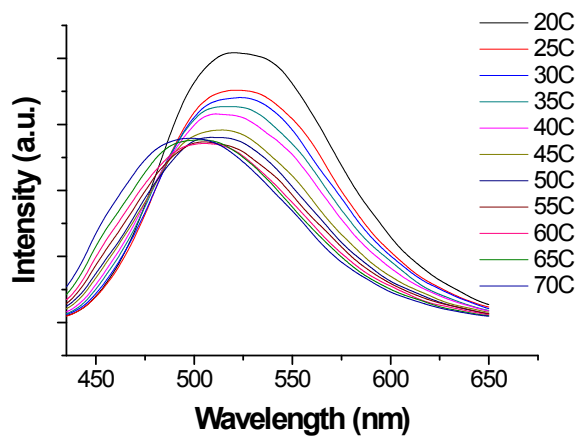
Sample 3:

Sample 4:
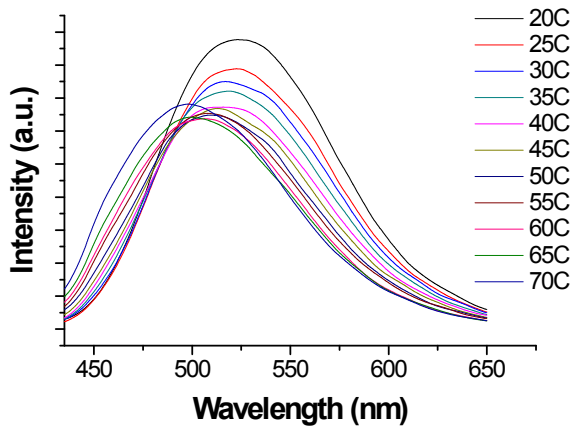
Sample 5:
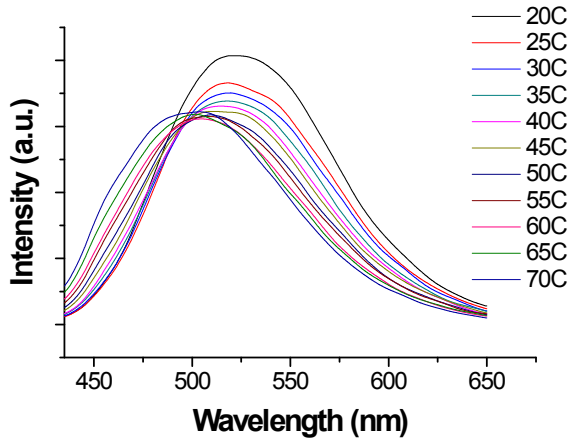
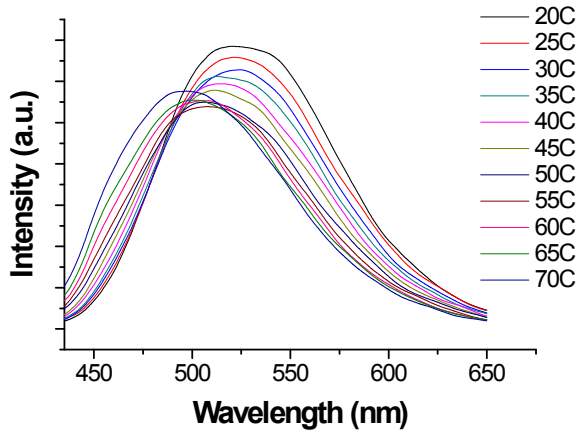
Sample 6:

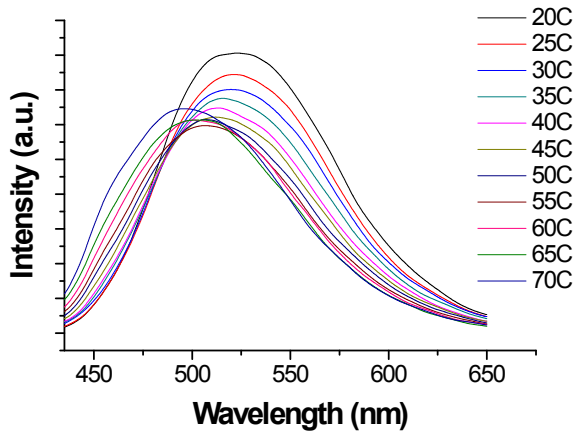

Sample 7:

Sample 8:

Sample 9:

Sample 10:

VI- EMISSION OF PPE-CO$_2$ 7 + PVP

Our laboratory has extensively studied the effect of polyvinylpyrrolidone (PVP) an amphiphilic macromolecule on the photophysical properties of an anionic conjugated polyelectrolyte. We reported on the ability of the amphiphilic macromolecule to disaggregate the CPE backbone and therefore enhance its fluorescence emission. Surprisingly, the amphiphilic macromolecule provided an external shield to protect the polymer from destruction catalyzed by collision events.

Based on our previous findings and considering the aim to partially destabilize the PPE-CO2-7 aggregates, we tested the potential effect of PVP on the CPE emission. Incremental amounts of PVP were added to a solution of PPE-CO$_2$-7.

Figure S2 shows the fluorescence emission spectra of PPE-CO$_2$-7 (5 μg/ml) in the absence and presence of PVP (Mw 55,000) at 750 μg/ml concentration upon excitation at 420 nm at 20 ˚C. The PVP concentration represents the value at which the fluorescence enhancement reached saturation. A 1.5 fold increase in fluorescence emission is observed with the appearance of a new peak at 485 nm. We attributed the new peak to be the result of the partial disaggregation of PPE-CO$_2$-7 in solution.
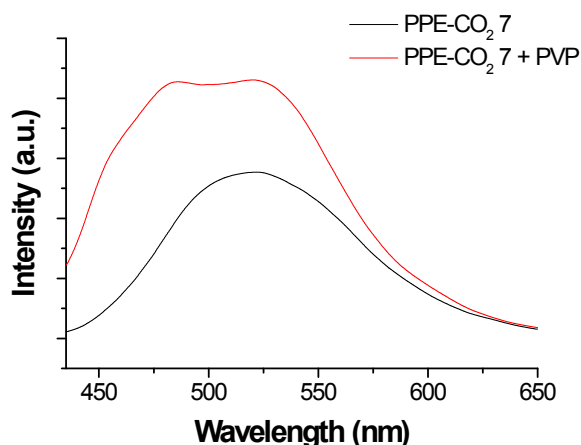

Figure S2. Fluorescence emission spectra of PPE-CO$_2$- 7 (5 μg/ml) and PPE-CO$_2$- 7+ PVP at **1:10** (PPE-CO$_2$ -7: PVP) in polymer ratio in buffer (10mM HEPES and 150 mM NaCl, pH=7.0) acquired upon excitation at 420 nm.

Below we summarize the emission spectra of all the PPE-CO$_2$- 7+ PVP samples in buffer (10mM HEPES and 150 mM NaCl, pH=7.0) acquired between 20.0 ^{0}C and 70.0 ^{0}C at 5.0 ^{0}C interval upon excitation at 420 nm. The time length of each experiment was an hour and half and the total time was 15 hours.

Sample 1:
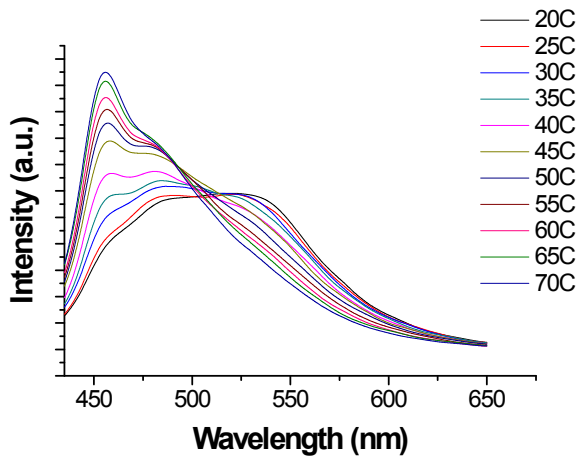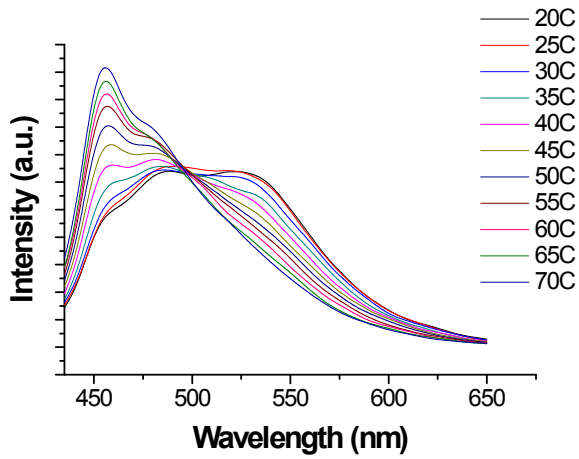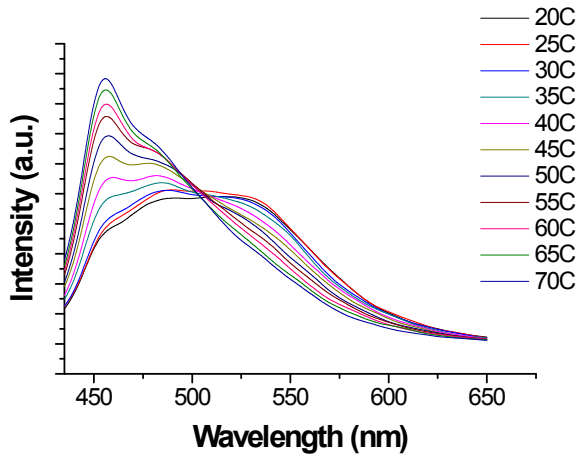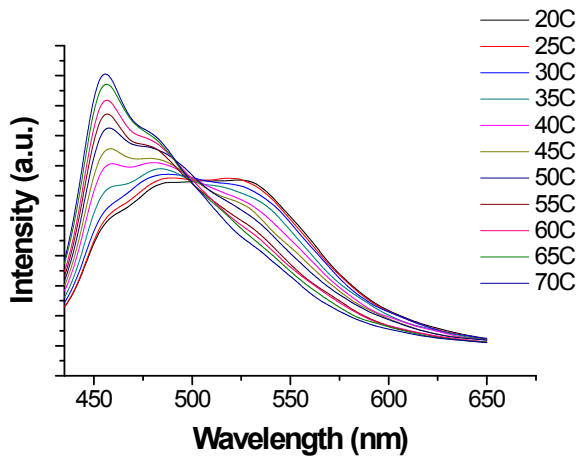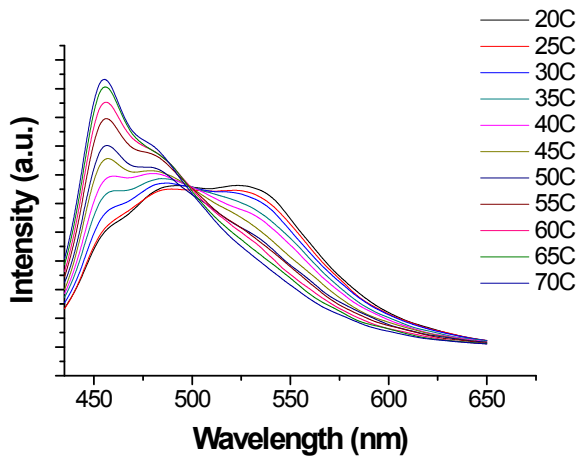

Sample 2:
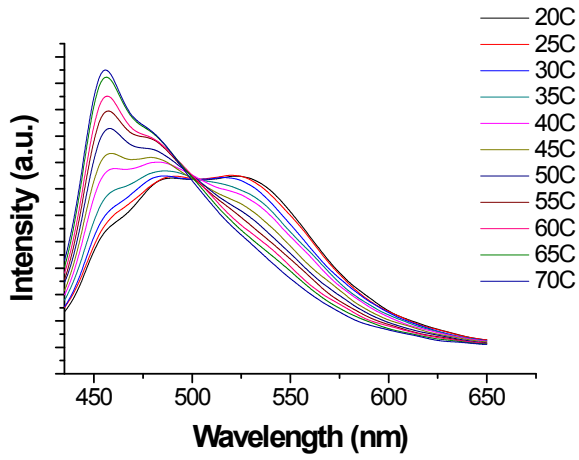
Sample 3:

Sample 4:

Sample 5:
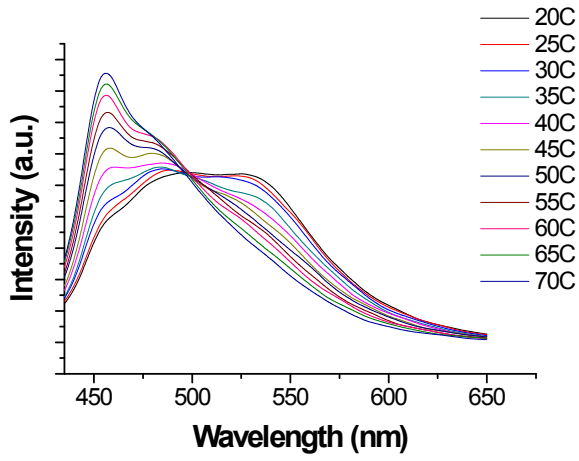
Sample 6:
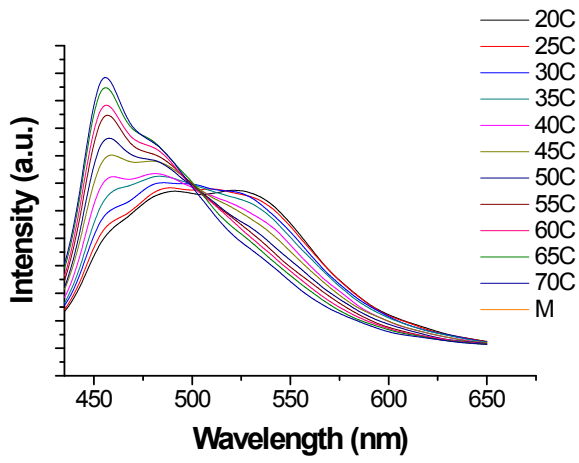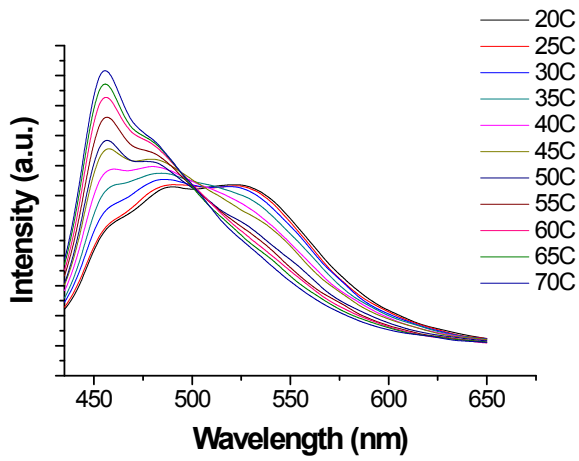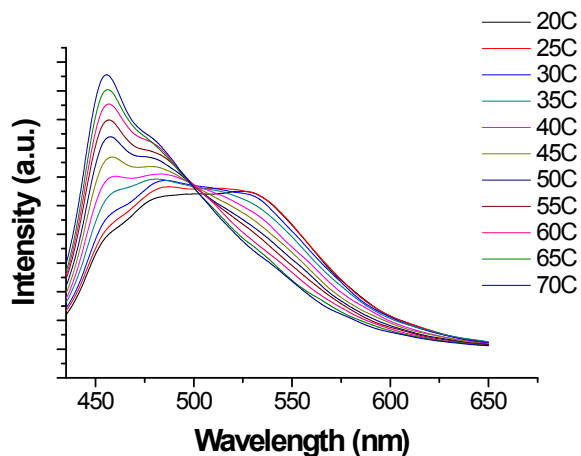
Sample 7:
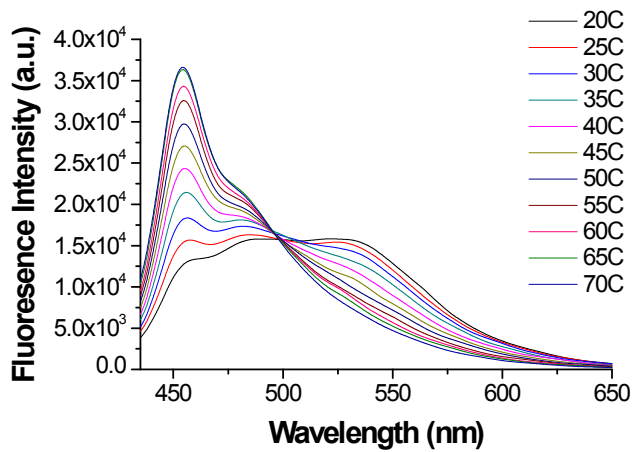

Sample 8:

Sample 9:

Sample 10:

VII- THERMAL RESPONSE OF PPE-CO$_2$ -108 + PVP

PPE-CO$_2$ with longer polymer chain was prepared and its thermal response was tested between 20.0 ^{0}C and 70.0 ^{0}C when complexed with PVP. The ratiometric thermal response was plotted and a maximum relative sensitivity of 3.97% was calculated at 20.0 ^{0}C (Figure S3)
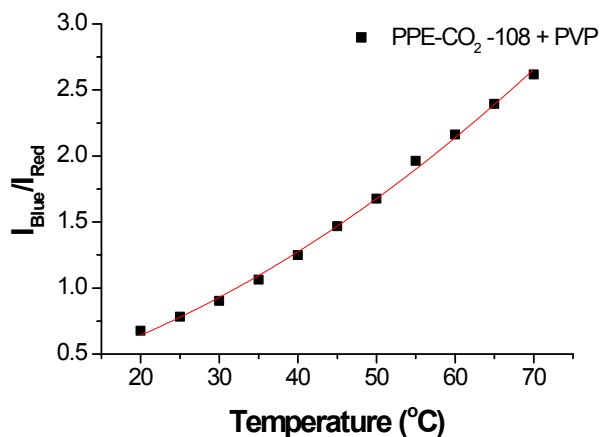
Figure S3: Fluorescence emission spectra of PPE-CO$_2$ -108 (5 µg/ml) in complexation with PVP in 10mM HEPES and 150 mM NaCl (pH 7.0) buffer acquired between 20.0 ^{0}C and 70.0 ^{0}C at 5.0 ^{0}C interval upon excitation at 420 nm. Ratio of the integrated signal before (I$_{Blue}$) and after (I$_{Red}$) 460 nm versus temperature is also shown.

VIII- THERMAL RESPONSE IN DIFFERENTSOLVENTS:

The thermal sensitivity is highly dependent on the polymer solubility and the shift between its aggregated and disaggregated state. To probe this effect, we have evaluated the thermal response of PPE-CO$_2$ -7 in different solvents. In 45 % methanol solution, where the PPE-CO$_2$ -7 was shown to be quazi- disaggregated, the solution was subjected to a control heating between 20.0 ^{0}C and 70.0 ^{0}C with a step increment of 5.0 ^{0}C. The ratiometric thermal response was plotted and a maximum relative sensitivity of 2.84% was calculated at 20.0 ^{0}C (Figure S4).
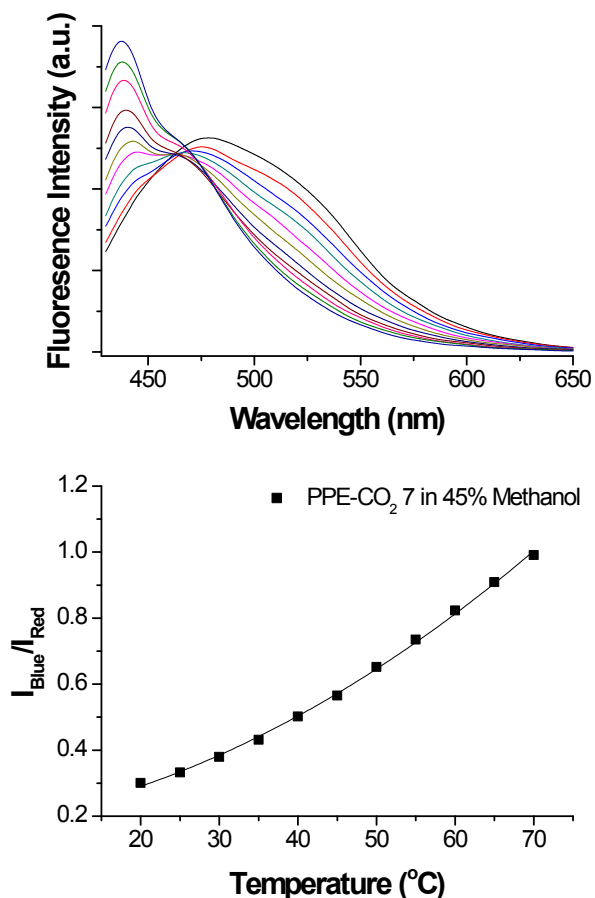
Figure S4: Fluorescence emission spectra of PPE-CO_2 -7 (5 µg/ml) in 45% methanol 55% 10mM HEPES and 150 mM NaCl (pH 7.0) buffer acquired between 20.0 ^{0}C and 70.0 ^{0}C at 5.0 ^{0}C interval upon excitation at 420 nm. Ratio of the integrated signal before (I_{Blue}) and after (I_{Red}) 460 nm versus temperature is also shown.

Similarly, the thermal response of PPE-CO_2 -7 was tested in pure ethanol. The fluorescent intensity decreased with increasing temperature. The thermal reponse was therefore calculated as the ratio of I_0/I and plotted against the temperature. A maximum relative sensitivity of 0.80% at 20.0 ^{0}C was calculated (Figure S5).
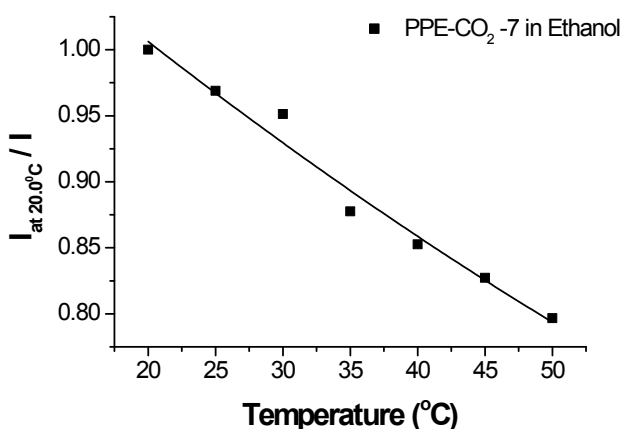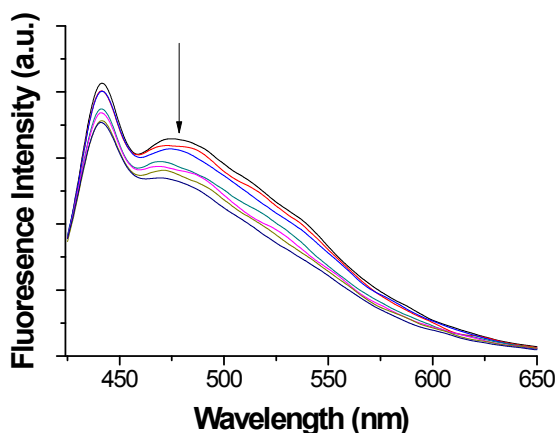
Figure S5: Fluorescence emission spectra of PPE-CO$_2$ -7 (5 μg/ml) in Ethanol acquired between 20.0 ^{0}C and 50.0 ^{0}C at 5.0 ^{0}C interval upon excitation at 420 nm. The fluorescence emission is shown to decrease with increasing temperature.

IX-   NANOTHERMOMETER REVERSIBILITY:

In addition to the reported cycling experiment that was shown in Figure 3D in the main manuscript, the temperature response of four different samples were tested during a heating run between 20.0 and 70.0 ˚C with 5.0 ˚C temperature increment. The integrated ratio was also measured during a cooling run for the same set of samples at 60.0, 50.0, 40.0, 30.0 and 20.0 ˚C. Figure S.6 summarizes our findings where an outstanding repeatability is reported for the tested temperatures. It reveals the lack of hysteresis which is a common problem with polymer based nanothermometers.

The percentage repeatability was calculated following the below equation:

$$\% \ Repeatability = \left(1 - \frac{Ratiometric_{Heating} - Ratiometric_{Cooling}}{Ratiometric_{Heating}}\right) x \ 100$$
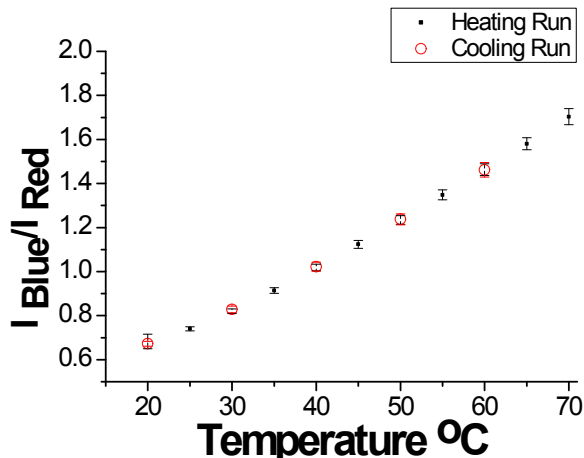
Figure S6. Integrated intensity ratio before and after the observed isoemissive point at 510 nm versus temperature. The sample was heated between 20 ˚C and 70 ˚C at 5 ˚C increment and then cooled down at 10 ˚C step decrement. The error bars represent the standard deviation of four independent measurements. All Experiments are done with PPE-CO$_2$- 7 (5 µg/ml) and PVP (750 µg/ml) in 10 mM HEPES pH7.0 acquired upon excitation at 420 nm.

X-   PHOTOSTABILITY OF PPE-CO$_2$ -7 IN THE PRESENCE OF PVP:

As we have mentioned earlier, Our group has previously reported on the exceptional photostability that PVP provides to anionic conjugated polyelectrolytes (MPS-PPV) by protecting their backbone from induced simultaneous rupture of polymer chains. We therefore assed any potential effect of PVP on the PPE-CO$_2$- 7 photostability. Upon continues illumination at 420 nm, the polymer emission was recorded at 520 in the presence and absence of PVP. The addition of PVP provided substantial stability. After 450 seconds of continues illumination, the pristine polymer has already lost 10 % of its initial intensity, on the other hand, in the presence of PVP, the loss was only about 3%.
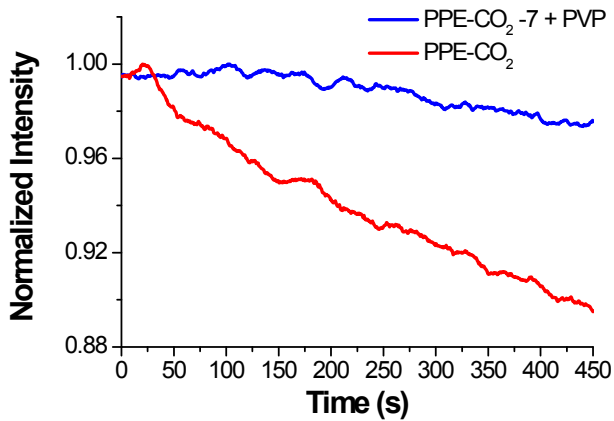
Figure S7. Intensity versus time trajectories of PPE-CO$_2$- 7 (5 μg/ml) (Red) and PPE-CO$_2$- 7+ PVP at 1:10 (PPE-CO$_2$ -7: PVP) polymer ratio (Blue) in buffer (10mM HEPES and 150 mM NaCl, pH 7.0) acquired upon excitation at 420 nm.

To show the effect of photobleaching on the probe sensitivity, we photodegraded a solution of PPE-CO$_2$-7 + PVP for 30 min till it lost 30% of its original intensity. Then a temperature sensing experiment was performed on the photobleached sample. The reported absolute sensitivity was calculated to be equal to 0.027 ^{0}C^{-1}. It is slightly different from the average reported value earlier (0.031^{0}C^{-1}) but the probe nevertheless retained a substantial sensitivity.
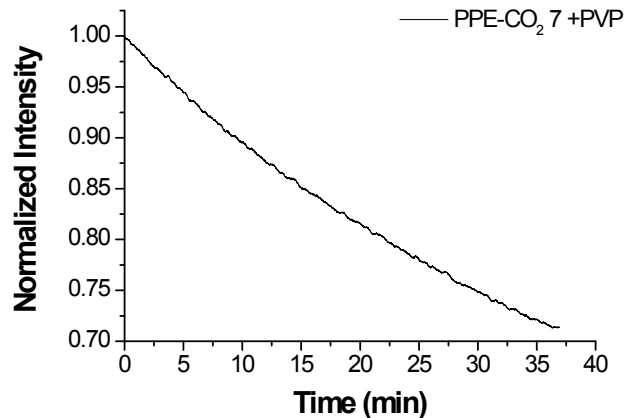

Figure S8: Normalized intensity change of a solution of PPE-CO$_2$ 7 + PVP upon continues excitation at 420 nm in HEPES buffer solution.
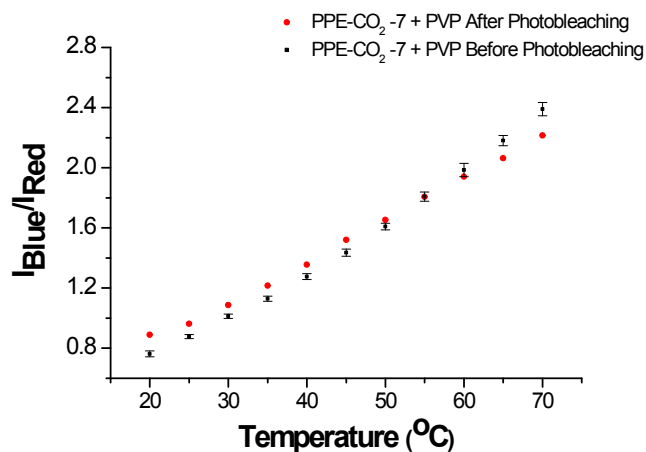
Figure S9 : Ratiometric change of PPE-CO$_2$ 7 + PVP before (■) and after (●) photobleaching.

References:

1.   Zhao, X.; Jiang, H.; Schanze, K. S. Polymer Chain Length Dependence of Amplified Fluorescence Quenching in Conjugated Polyelectrolytes. *Macromolecules* 2008, 41, 3422-3428.
2.   Zhao, X. Conjugated polyelectrolytes based on poly (arylene ethynylene): Synthesis, solution photophysics and applications to sensors and solar cells. University of Florida, 2007.
3.   Brites, C. D.; Lima, P. P.; Silva, N. J.; Millán, A.; Amaral, V. S.; Palacio, F.; Carlos, L. D. Thermometry at the nanoscale. *Nanoscale* 2012, 4, 4799-4829.