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Turning the heat on conjugated polyelectrolytes: an off–on ratiometric nanothermometer†

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We report a self-referenced ratiometric nanothermometer based on short conjugated polyelectrolytes. An amphiphilic macromolecule destabilizes the polymer π – π stacking and makes it possible to shift the equilibrium between the less emissive aggregated state (520 nm) and the brighter individual chain (450 nm) within 20.0 °C and 70.0 °C.

Temperature is a fundamental variable that controls and drives many chemical, biological and physical processes.^{1–4} Nowadays, there is a tremendous drive to explore and understand processes at the nanoscale level in many fields of science and technology.^{5–7} For instance, a nanotemperature probe will provide a powerful tool to unravel the biological functions induced or correlated with local cellular temperature fluctuations. Mapping localized hot spots in engineered nanothermoelectric devices is also of fundamental importance.⁸

Many promising approaches have been recently developed.^{5,6,9,10} Non-invasive methods, such as fluorescent or lifetime-based probes, are advantageous because they allow accurate signal readout with minimal interference with the system of interest.^{11–15} An ideal probe must remain stable over time to avoid frequent calibration and false readings. A fluorescence signal is, however, prone to many external factors which might compromise the thermometric response. Probes with ratiometric signals minimize errors produced by the light source fluctuations, the detector instability, and the optical setup, as well as being unaffected by changes in fluorophore concentration.^{16,17} They often consist of two subunits each with a different thermal response property. Any fluctuation in the excitation source is then eliminated by the signal ratio. However, fluorophores photobleach at different rates and are quenched by different species. We therefore searched for a material that satisfies two important criteria; (i) it must be a single component fluorescent

probe and (ii) it must possess a two distinct temperature dependent emission states.¹⁸

Conjugated polyelectrolytes (CPEs) are a class of polymers with high extinction coefficients and exceptional brightness.^{19–22} Their photophysical and photochemical properties are highly dependent on their microenvironment. Whether dissolved in hydrophobic or hydrophilic media, their fluorescence emission dramatically changes.^{23–25} In particular phenylene based polymers exhibit a pronounced spectral emission shift when going from the aggregated to the free chain.²⁶ As such, they have been employed as piezochromic probes²⁷ and membrane reporters.²⁸

Herein, we report on the use of short phenylene based conjugated polyelectrolytes to develop a single component reversible nanothermometer with a ratiometric readout, an excellent sensitivity and a wide linear range. For practical purposes, we will report the ratiometric signal readout as the ratio of the integrated intensity between 435 nm and 509.9 nm (I_{Blue}) and between 510 nm and 650 nm (I_{Red}).

In water, short poly(phenylene ethynylene) carboxylate (PPE-CO₂), driven by the π – π stacking and the hydrophobic-backbone interactions, aggregates. Subsequently, its fluorescence emission is mostly from excimer species leading to a broad, red-shifted and structureless spectra with a maximum emission at 520 nm and a quantum yield of 0.14 ± 0.04 (Fig. 1B).²⁶ In ethanol, PPE-CO₂ is well dissolved and mostly emits from disaggregated individual polymer chains with a well-structured emission band at 450 nm and a quantum yield of 0.62 ± 0.03 (Fig. 1B).²⁶ This distinct difference in the spectral signatures of the aggregated polymers, *versus* individual chains, satisfied our aforementioned criteria and made PPE-CO₂ an appealing material for us to test its thermal response. Poly(phenylene ethynylene) carboxylate with an average of 7 monomer repeating units was therefore prepared using Sonagashira coupling following previously reported procedures.^{26,29}

Fig. 2 summarizes the fluorescence intensity changes obtained for PPE-CO₂-7 upon controlled heating between 20.0 °C and 70.0 °C with a step increment of 5.0 °C. The fluorescence intensity increase was accompanied by a gradual blue-shift (from 520 nm to 485 nm) and a slight decrease

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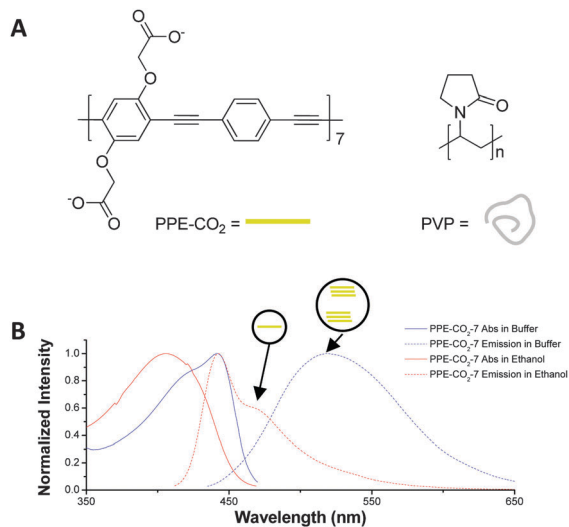


Fig. 1 (A) Schematic and chemical structure of the short anionic conjugated polyelectrolyte poly(phenylene ethynylene) carboxylate with an average of 7 monomer repeating units (PPE-CO₂-7) and the amphiphilic polymer polyvinylpyrrolidone (PVP). (B) Normalized absorption (solid) and emission (dashed) spectra of PPE-CO₂-7 in 10 mM HEPES and 150 mM NaCl (pH 7.0) buffer (blue) and ethanol (red) upon excitation at 420 nm.

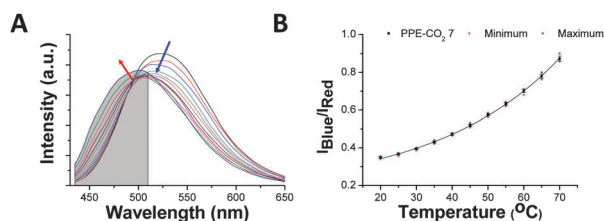


Fig. 2 (A) Fluorescence emission spectra of PPE-CO₂-7 (5 μg ml⁻¹) in 10 mM HEPES and 150 mM NaCl (pH 7.0) buffer acquired between 20.0 °C and 70.0 °C at 5.0 °C intervals upon excitation at 420 nm. (B) Ratio of the integrated signal before (dark area in A; I_{Blue}) and after (light area in A; I_{Red}) 510 nm versus temperature. The error bars represent the standard deviation of ten independent measurements. The maximum and minimum represent the lowest and highest recorded value at each measured temperature. Fitting (B) into a second degree polynomial function gave a relative sensitivity of 0.99% at 20.0 °C and maximum relative sensitivity of 2.06% at 55.0 °C.

followed by an increase in the emission intensity. Plotting the ratio of the integrated signals versus the applied temperature gave an exponentially changing thermal response with a relative calculated sensitivity of 0.99% at 20.0 °C and a maximum relative sensitivity of 2.06% at 55.0 °C. Even though promising, the fluorescence shift was not significant hence limiting its practical applications. The experiment also revealed that the CPE, even at temperatures as high as 70.0 °C, did not achieve a disaggregated state as we have originally aimed for. We therefore attributed the observed peak at 485 nm to a quasi-destabilized state of the PPE-CO₂-7 aggregates (Fig. S1, ESI[†]).

We next searched for a material that would destabilize the strong π - π interactions but not completely dissolve the conjugated polyelectrolyte backbone at room temperature. In fact, our laboratory has previously exploited the extraordinary amphiphilic properties of polyvinylpyrrolidone (PVP)

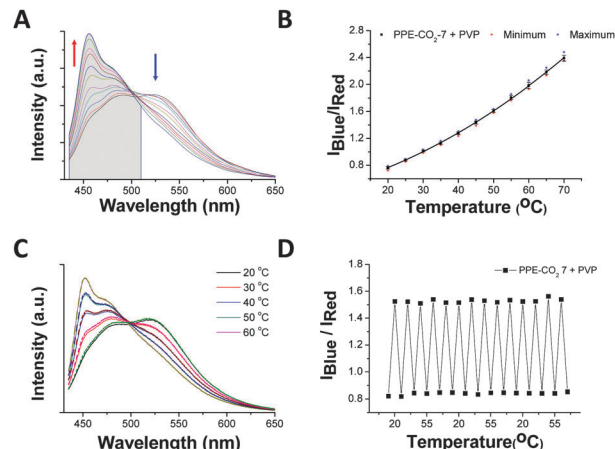


Fig. 3 (A) Fluorescence emission spectrum of PPE-CO₂-7 in complexation with PVP acquired at different temperatures between 20.0 °C with a 5.0 °C increment. (B) Integrated intensity ratio before (dark area in A; I_{Blue}) and after (light area in A; I_{Red}) the isoemissive point at 510 nm versus temperature. The thermal response was fitted into a second degree polynomial function which gave a maximum relative sensitivity of 2.78% at 20.0 °C. The error bars represent the standard deviation of ten independent measurements. The maximum and minimum represent the lowest and highest recorded values at each measured temperature. (C) Fluorescence emission spectrum of PPE-CO₂-7 in complexation with PVP during heating and cooling cycles at 20.0, 30.0, 40.0, 50.0 and 60.0 °C. (D) Cycling a solution of PPE-CO₂-7: PVP between 20.0 °C and 55.0 °C. The lines connecting the experimental points are for visual aid. All experiments were performed in 10 mM HEPES and 150 mM NaCl (pH 7.0) buffer.

to break down the strong hydrophobic backbone interactions in an anionic conjugated polyelectrolyte.³⁰ To this end, PPE-CO₂-7 was mixed with PVP ($M_w = 55\ 000$) at an optimized concentration of 1 : 10 (CPE : PVP) polymer ratio. At room temperature, the CPE fluorescence emission increased by 1.5 fold in the presence of PVP (Fig. S2, ESI[†]). In addition to the previously reported peak at 520 nm, a new blue-shifted maximum emission centered at 485 nm was observed coinciding with the previously observed emission peak reported when PPE-CO₂-7 solution was heated to 70.0 °C and attributed to a quasi-destabilized state.

The prepared hybrid complex was subjected to a gradual increase in temperature between 20.0 °C and 70.0 °C with a step increment of 5.0 °C (Fig. 3A). As a result, the emission peak at 520 nm decreased concomitantly with an increase in the individual chain polymer emission peak at 450 nm. We ascribe the observed temperature dependence on fluorescence emission intensity to the interconversion between the aggregated and the individual polymer chains. We speculate that upon addition of PVP, PPE-CO₂-7 aggregates are initially destabilized by the amphiphilic polymer and are further disaggregated due to the increased vibrational and rotational freedom of the macromolecules at higher temperatures. Subsequently, fluorescence from individual polymer chains gives rise to the distinctive and brighter peak at 450 nm.

We then compared the fluorescence intensities coming from individual and aggregated polymers by integrating the intensity before and after the isoemissive point. The ratiometric variation was plotted against the change in temperature. A maximum relative

sensitivity of 2.78% at 20.0 °C was computed (Fig. 3B). This value is substantially larger than previously reported fluorescent ratiometric probes.^{17,31} The ratiometric response was reproducible with small standard deviation calculated from ten runs. The isoemissive point observed at 510 nm clearly reflects the two state models that govern this system. In addition, the substantial spectral shift in emission from 520 nm to 450 nm makes the material ideal for implementation in microscopy imaging setups. Light could be hence split into two imaging channels to accurately map temperatures within diffraction limited spots. PPE-CO₂ with a longer polymer chain (108 repeating units) was also prepared and tested, in complexation with PVP, over the same temperature range. Upon plotting the ratiometric thermal response *versus* temperature, a maximum relative sensitivity of 3.97% was obtained at 20.0 °C (Fig. S3, ESI†). The results show that irrespective of the polymer chain length, the PPE-CO₂/PVP complex has an excellent thermal sensitivity.

A common limitation in polymer-based thermometers is hysteresis which adversely affects the probe performance; different output signals could be recorded for the same temperature value during a cycle of heating and cooling.³² In our case, the ratiometric response was independent of the cycling direction with a signal repeatability between 98.5% and 99.8%, at 20.0 °C and 70.0 °C, respectively (Fig. S6, ESI†). As shown in Fig. 3C, the solid lines are those obtained during the heating run, and the dashed lines denote the spectra obtained during the cooling run. A careful look reveals that the emission intensity slightly increases for the 20.0, 30.0, 40.0 and 50.0 °C during the cooling run possibly due to polymer-CPE restructuring, but the ratiometric nature of the probe minimizes the adversity of this effect (Fig. S6, ESI†). At 60.0 °C, the cooling and heating runs overlap.

We next investigated the probe stability by performing multiple testing runs. We recorded spectra at 20.0 °C and 55.0 °C (Fig. 3D). Over the 14 cycles, the probe ratiometric response showed high reversibility with only 2.5% deviation from the average at 20.0 °C and 2.1% at 55.0 °C revealing the lack of hysteresis and a great stability over the two hours and half of the experiment.

The signal of most previously reported fluorescent-based nanothermoeters decreases with the increase of temperature.^{7,16,23} In sensing, these probes are generally undesirable as the photo-destruction of the probe over time might be mistaken for an increase in temperature. In our case, the ratiometric signal increases with temperature providing an off-on like sensor. It is worth mentioning that the addition of PVP provided an enhanced photostability when compared to pristine PPE-CO₂-7 (Fig. S7, ESI†) and retained its sensitivity even after losing 30% of its original intensity (Fig. S8 and S9, ESI†). This makes the prepared system appealing for long term imaging applications.

The change in fluorescence emission was even visible to the naked eye when the sample was observed under a UV lamp at 20.0 °C and 70.0 °C (Fig. 4).

In conclusion, we have successfully prepared a fluorescent-based temperature probe that shows a self-referenced ratiometric change in intensity between 20.0 °C and 70.0 °C with a maximum

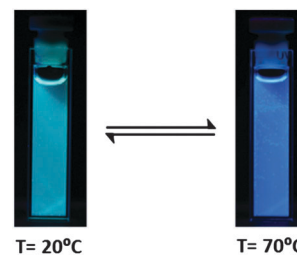


Fig. 4 Photographic images acquired using a DSLR Nikon 5100 camera (shutter speed = 40 ms) for a CPE solution prepared using PVP ($M_w = 55\,000$) at a 1:10 (PPE-CO₂-7:PVP) polymer ratio illuminated with a UV lamp at 20 °C and 70 °C.

relative sensitivity of 2.78% at 20.0 °C. The shift in the fluorescence emission was significant to allow practical microscopy imaging at the nanoscale level. The probe signal was highly reproducible with no hysteresis and most importantly an off-on signal response. We believe that this strategy can be easily extended to other conjugated polyelectrolyte systems. We are currently exploiting the effect of different PVP molecular weights as well as the effect of the conjugated polyelectrolyte length.

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