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Tuning the Emission Properties of a Fluorescent Polymer using a Polymer Microarray Approach – Identification of an Optothermo Responsive Polymer

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Polymer microarrays were prepared using inkjet printing mixtures of acrylate monomers each with a common fluorescent fluorene co-polymer. Fluorescent analysis of each of the features on the array allowed identification of polymers that could tune the fluorescence under a variety of insults. The "hit" polymers were made into beads via reverse suspension polymerization their fluorescence properties analyzed.

Conjugated fluorescentpolymers,[1]have attracted huge amounts ofinterest because oftheir application inlight emitting diodes,[2] optoelectronic devices [3]and biosensors,[4] in part due to their tunable electrical and optical properties while possessingattractive mechanical properties and processing characteristics.[5]

Since the electrons are conjugated along the backbone, the properties of theconjugated fluorescent polymer dyes are highly sensitive to minor external structural perturbations and local electron density changes that occur upon binding to other molecules.[6]It has been demonstrated that the chiral conformation of a polythiophene derivative can be manipulated by a "wrapping polymer" and its colour controlled,[7]while the fluorescence of conjugated polymers, such as polythiophene derivatives or fluorophores such as rhodamine,when grafted onto thermo-sensitive polymers can be switched on and off by altering the temperature.[8]Recently,conjugated fluorescent polymershave become candidatesto replacemolecular dyes and quantum dots for various fluorescence based biomedical-imaging applications,[9]while being used as fluorescent enhancers.

Compared tomolecular dyes, conjugated fluorescent polymers have advantages in terms of brightness of emission, high extinction coefficients and goodphoto-stabilities,[10]which makethem suitable for various fluorescence imaging tasks.In additionconjugated fluorescent polymersarebiologically compatible with lower toxicities[11]than quantum dots.[12]Another approach to generate highly intense fluorescent signals is to incorporate them into particles, thuspolymer beads loaded with fluorophoresare widely usedin many biomedical applications, including as standards and calibrants, and for cell tracking and labeling.[13]Such fluorescent polymer beadsthus providea powerful platform forvisualizingbiological structures from the anatomical to the cellular and the monitoring ofdynamic physiological processes.

Here polymers used for manipulating fluorescent polymers were identified using a high-throughput approach, with polymer microarrays applied to the identification ofpolymer targets that could control the properties of conjugated polymerswith monomers chosen according to the literature.[7,8a]The polymer microarrayswereconstructed from monomer collectionsto produce large numbers of polymerson a glass slideby inkjet printing of monomers, cross-linkers and photo-initiators and subsequent *in situ* polymerization initiated by UV light. This technique enables the polymer composition and the ratio of each monomer to be easily varied, resulting in arrays with large numbers of polymer features.[14]Such polymer microarrays have previously been used for the rapid identification of synthetic polymers with specific biological functions,[15] with the interactionsbetween cells and hundreds to thousands of individual polymers beingsimultaneously probed.[16,17]The interaction between conjugated fluorescent polymersand a matrix of polyacrylates/acrylamides wasthus envisaged as a means of tuning and controlling thefluorescence emission of the polymer, with a library approach allowing much greater chemical space to be explored than hashithertobeen possible. Here polymer microarray technology was used for thediscovery of polymers that would not only promote the optical stabilization of conjugated fluorescent polymers, but

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also allow the properties of the dye to be tuned, physically and thermally. Eleven acrylates and acrylamides were used for microarray preparation (Table 1, ESI Methods)

Table 1 Monomers used for polymer microarray preparation*

Labels.	Abbreviation	Monomers
A	HEMA	2-Hydroxyethyl methacrylate
B	EGDMA	Ethylene glycol dimethacrylate
C	DMAEMA	Dimethylaminoethyl methacrylate
D	DMOBAA	Diacetone acrylamide
E	NIPAA	N-Isopropyl acrylamide
F	CHMA	Cyclohexyl methacrylate
G	DMC	Methacrylatoethyltrimethyl ammonium chloride
H	CEA	2-Carboxyethyl acrylate
I	DEAA	N,N-Diethylacrylamide
J	AAm	Acrylamide
K	HPOAA	2-Hydroxy-3-phenoxypropyl acrylate

*: Polymersare coded by the monomer composition and their ratios. For instance, the copolymer prepared from 15 drops of HEMA and 5 drops of EGMA is coded as A15B5

The fabrication of polymer microarrays was achieved using aninkjet printing approach as has been described in detailelsewhere.[18] During fabrication of the array a solution of the dye(0.005 wt%) was added to each polymer (Fig.1, Fig.S1 and S3, ESI†)which was generated from solutions of the two monomers, the cross-linker N,N'-methylene-bis(acrylamide) (MBA)solution and the photoinitiator 1-hydroxycyclohexyl phenyl ketone.Across each line of the microarray two monomer solutions were printed in varying ratios designed as 20/0, 15/5, 10/10, 5/15, 0/20 (based on the number of drops of monomers printed)with 4 replicates for each ratio, with a common level of cross-linker (12.7 wt%) for each polymer. The glass slides were exposed to UV light (365nm) for 30 min to initiate *in situ*polymerization after printing.

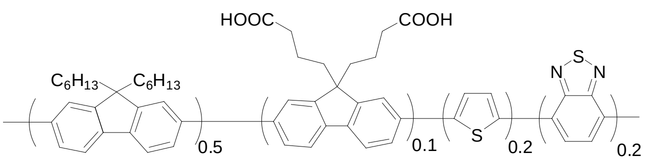

Fig. 1 The structure of the conjugated fluorescent polymer used in this study, which was synthesized using Suzuki cross-coupling chemistries.

Following microarray fabrication the array was screened, with fluorescent images of each polymer featurecapturedusingan inverted fluorescent microscope (excitation 350-370 nm) witha 20× objective (Fig. S2, ESI†). When the conjugated fluorescent polymers were immobilized within the polymers, the colour of polymers became cyan in colour indicating that the polymers interacted with the dye and affected its fluorescent emission.The fluorescent intensity of each spot was analyzed and compared to the starting conjugated fluorescent polymer allowing nine polymers to be identified that either increasedor decreased the fluorescent intensities, as well as those that appeared tototally quench thedye'sfluorescence.

Fluorescent images of each polymer spot on the array (20×55 spots in total) were analyzed with Image J(Table S1, ESI†).[18] The relative fluorescent intensity was used for comparison among the features and wascalculated using following equation (1):

$$I_r = \frac{I_{fp} - I_p}{I_f} \quad (1)$$

Where, I_{fp} is the absolute fluorescent intensity of a polymer spot with the immobilizedconjugated fluorescent polymer, I_p is the auto-fluorescence intensity of the polymer spot without the dye; I_f is the fluorescent intensity of the dye.

Thus the relative fluorescent intensities of 1100 polymer spotswere calculated and are given in descending order (Fig.2a).Three polymer candidates that increased the relative fluorescent intensity and three candidates where the fluorescent intensities were reduced after immobilization and three that appeared to have quenched or lost the dye were chosen for theprinting of the secondary arrays for further confirmation (Fig.2b). These 9 polymers were used for the preparation of a secondary array with the fabrication of 20 replicates for each of the polymers (Table 2). The printing process was the same as above. Features with a negative I_r represented host polymers that had suppressed thedye'sfluorescence and were not studied further.

Table 2 Polymer candidates used for preparation of the secondary microarray

I_r	$I_r>1$	$0<I_r<1$	$I_r\approx0$
Polymer candidates	A10G10	E5G15	F15I5
	A5B15	E5H15	F5I15
	E15G5	B15J5	A15K5

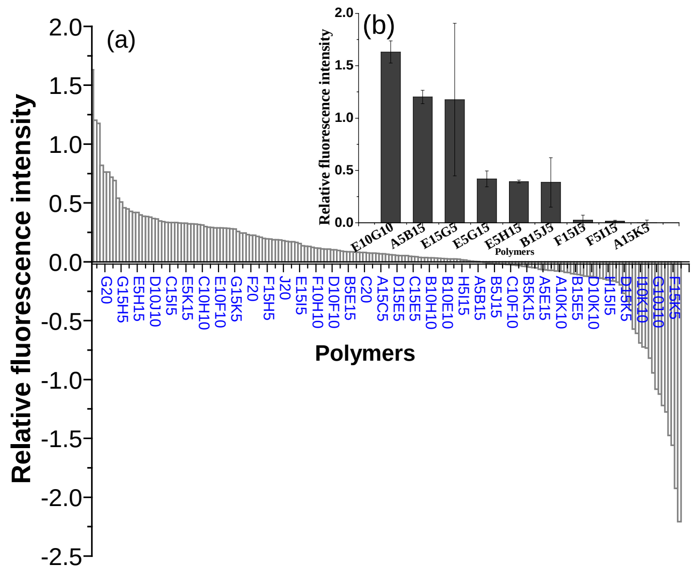

Fig.2 Fluorescent intensity analysis of the polymer microarray: (a) The relative fluorescent intensities of the spots on theprimary polymer microarray (dataare means of quadruplicate

polymersofeach combination).STDEVs were calculated (see SI) butare not shown here for clarity); (b) The insert shows the 9 polymers taken forward, with three polymersshowing increased fluorescent intensity; three with reducedfluorescence intensity and three with no fluorescence(The error bars are STDEV, n=4).

The fluorescent images of the spots of the secondary array were analyzed (Fig. 3) and showed good agreementwith theprimary array screen.

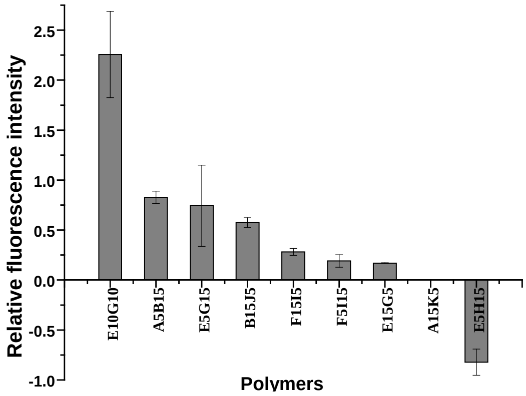

Fig.3 The fluorescent intensities of the polymers as fabricatedon the secondary array. The fluorescent images of the polymer features were captured and the fluorescent intensitiesanalyzed using Image J and equation (1).(The error bars are STDEV, n=20)

This array was screened and analyzed and 5 combinations were identified to take forward for further examination.The three polymer combinations that enhanced the fluorescent intensity of the conjugated fluorescent polymer (E10G10, A5B15 and E5G15) and another two E15G5 and A15K5, that had no or limited fluorescence were up-scaled to produce polymer beads with the entrapped conjugated fluorescent polymer (Scheme S1, Fig. S4, ESI†, Methods). The fluorescent beads were characterized at different temperatures (25℃, 35℃, 45℃ and 55℃) and compared tosolutions of the conjugated fluorescent polymers.

From the fluorescent images, the beads are fluorescent corresponding to the emission light of the dye with wavelength of 500-700 nm when λ_{Ex}= 450 nm (Fig. 4). Quantitative fluorescent analysis showed that the fluorescent intensity of A5B15-A was some 2.5 times larger than that of the conjugated fluorescent polymer, while the fluorescent intensities of E5G15-A and E15G5-A were similar to that of the dye. A15K5-A was only the third of the fluorescent intensity of the pure dye. The fluorescence emission peaks of the immobilized dyeshifted 20-40nm to shorter wavelengths. It indicates that the main chains of the embedded-conjugated fluorescent polymer may have been twisted or strained to interrupt their conjugation during thepolymerization process, resulting in a blue shift for the emission wavelength.[19]

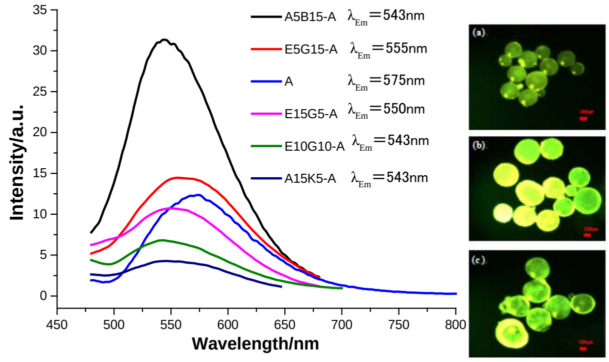

Fig.4 Left) The fluorescent intensity of the fluorescent conjugated polymer (A) and polymer beads with the immobilized dye, A5B15-A, E5G15-A, E15G5-A, E10G10-A and A15K5-A respectively. The fluorescent dye and beads were suspended in (PEG-400:H2O=2:1) and analyzedat 25℃ with λ_{Ex}= 450 nm. The concentration of the dye was estimated according to the dye immobilized in the beads during fabrication (see Fig. S5, ESI†).Right) Fluorescent images of the polymer beads.(a), (b) and (c) polymer beads A15K5-A, A5B15-A and E15G5-A respectively.

The influence of temperature on the fluorescence intensity of the conjugated fluorescent polymerwas investigated and showed negligible effects on theintensityof the emission peak as thetemperature increasedfrom 25 to 60°C (Fig. S6, ESI†).However, the fluorescent intensity reduced when the dye was immobilized in the polymers A5B15, E15G5 (Fig. S7, S8, ESI†) and it dropped dramatically when the conjugated fluorescent polymerwas embedded in the polymer E5G15 with the fluorescence intensity dropping from 14.5 to 6.5 RFU when the temperature increased from 25 to 60°C(Fig. 5), which was recovered when cooled down to 25°C again. Thus E5G15-polymer beads exhibited thermo-fluorescence - fluorescence that could be switched on and off by just by altering the temperature over many cycles (Fig. 6).

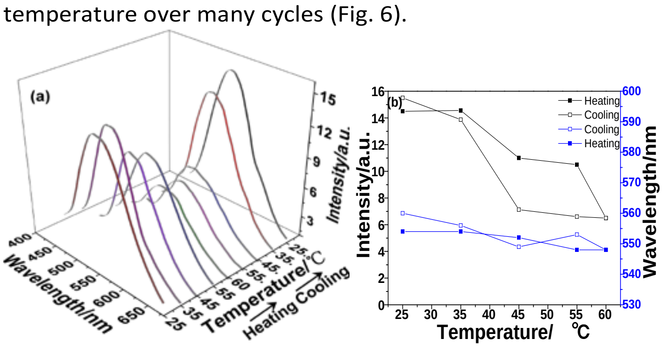

Fig.5 Fluorescence intensity change inpolymer beads made from E5G15-A following the temperature rise from 25to 60°C and then cooling to 25°Cmeasured with anλ_{Ex}=450nm(a) and (b) corresponding fluorescence intensities and emission wavelengths at various temperatures.

The temperature dependence of the intensity of the fluorescent beads could be the result of the 'on/off' switching of the aggregation-caused quenching(ACQ)[20] of the dye immobilized in the host polymer under different temperatures

(Video S1 and S2, ESI†) because the shift of the emission peak was very small, less than 10 nm in wavelength (Fig 5b).PNIPAA is a well-known thermo-responsive polymer, therefore the host polymer consisting of monomer NIPAA has the possibility to trigger the fluorescence switching of beads by polymer shrinking at high temperature and swelling at room temperature.The temperature-dependent thickness alteration of the host polymer was examined using a rheometer as the temperature changing from 10 to 55ºC and cooled down to 10ºC again under constant compressive forces (2 or 4kPa).During the measurement, an oscillatory shear stress was imposed on the polymer at a frequency of1Hz to obtain a correspondingoscillatory shear strain. Extrapolation to zero compressive force showed that the relative thickness of the hydrogel polymers reduced when they were heated from 10 to 55 ºCshrinkingsome 4% in thickness and increased for 1-2% when the hydrogels were cooled down to 10 ºC again (Fig.6b, c).

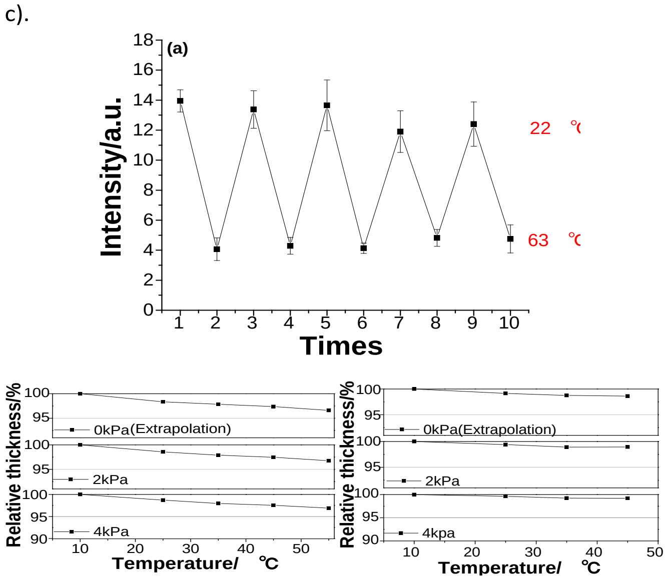

Fig.6(a) Switching cycles of the fluorescence intensity of the E5G15-A polymer beads in PEG400/H2O (2/1) triggered with temperature changes between 22 and 63ºC(λ_{Ex}= 450 nm) (error bars are STDEV, n=5). (b) Change in relative thickness of the E5G15 polymer as a function of temperature in response to actual (2 and 4 kPa) and extrapolated (0kPa) compressive forces as the temperature was raised from 10 to 55ºC and (c) the temperature reduced from 55 to 10ºC.

In conclusion polymer microarrays were fabricated with polymers immobilizing a fluorescent polymer. Upon screening of the arrays, it was found that copolymers of specific combinations (of acrylates and acrylamides) could affect the fluorescence of the dye. For example the polymer HEMA/EGDMA (1/3) enhanced the fluorescence of the dye 2.5 fold while polymer HEMA/HPOAA (3/1) reduced its fluorescence to 1/3. Host polymer NIPAA/DMC(1/3)was thermo-responsive, with switch-on and off of the fluorescence of the immobilized dye because of ACQ.The work demonstrates that the microarray approach based on fluorescent polymers could identify host polymers, manipulating the fluorescence of conjugated polymers for various applications such as labeling and imaging of tissues, smart sensors, switching devices, molecular logic gates and other electronic device.

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Notes and references

1 C. L. Zhu,L. B. Liu,Q. Yang,F. T. Lv,S. Wang, *Chem.Rev*.,2012,**112**(8),4687-4735.
2 Y. T. Tao,C. L. Yang,J. G. Qin, *Chem. Soc. Rev.*,2011,**40**(5),2943-2970.
3 A. Facchetti, *Chem. Mater.*,2011, **23**(3), 733-758.
4X. L. Feng,L. B. Liu,S. Wang,D. B. Zhu, *Chem.Soc.Rev*.,2010,**39**(7),2411-2419.
5C. F. Wu, D. T. Chiu, *Angew.Chem. Int. Edit*.,2013,**52**(11), 3086-3109.
6A.Alvarez, J. M. Costa-Fernández, R. Pereiro,A. Sanz-Medel, A. Salinas-Castillo, *Trend.Anal.Chem*., 2011, **30**(9), 1513-1525.
7T. Shiraki, A. Dawn, Y. Tsuchiya and S.Shinkai, *J. Am. Chem. Soc*,. 2010, **132**, 13928–13935.
8(a) S. Das, D. P. Chatterjee, S. Samanta and A. K. Nandi, *RSC Adv.*, 2013, **3**, 17540–17550; (b)E. Bat,E. W. Lin , S. Saxer, H. D. Maynard, *Macromol. Rapid Commun*.,2014, **35**, 1260–1265.
9L. H. Feng,C. L. Zhu,H. X. Yuan,L. Liu, F. Lv, S.Wang, *Chem. Soc. Rev*.,2013,**42**(16), 6620-6633.
10C. F.Wu, C.Szymanski , J. McNeill, *Langmuir*,2006, **22**(7), 2956-2960.
11 N. K. Guimard, N. Gomez, C. E. Schmidt, *Prog. Polym.Sci.*, 2007, **32**(8-9), 876–921.
12A. M. Derfus, W. C. W. Chan, S. N. Bhatia, *Nano Lett*., 2004, **4**(1), 11–18.
13(a) K. Li, R. Y.Zhan, S. S. Feng, B. Liu, *Anal.Chem*., 2011, **83**(6), 2125-2132; (b)A. J.C.Kuehne,D. A.Weitz, *Chem. Commun*.,2011, **47**, 12379-12381.
14(a) D. G. Anderson, S. Levenberg, R.Langer, *Nat. Biotechnol*., 2004, **22**, 863–866; (b)H. Mizomoto, The synthesis and screening of polymer libraries using a high throughput approach. PhD Thesis, 2004, University of Southampton; (c)G. Tourniaire, J. Collins, S. Campbell, H. Mizomoto, S. Ogawa, J. F. Thaburet, M. Bradley, *Chem. Commun*., 2006, **20**, 2118–2120
15 A. Liberski,R. Zhang, M. Bradley, *Chem. Commun*.,2009, **3**, 334-336.
16 A. Hansen, H. K. Mjoseng, R. Zhang, M. Kalloudis, V. Koutsos, P. A. de Souza and M. Bradley, *Adv. Healthc.Mater*., 2014, **3**(6), 848-853.
17(a) R. Zhang., H. K.Mjoseng, M. A. Hoeve, N. G. Bauer, S. Pells, R.Besseling, S.Velugotla, G. Tourniaire, R. E. Kishen, Y .Tsenkina, , C. Armit, C. R. E. Duffy, M. Helfen, F. Edenhofer, P. A. de Sousa, M. Bradley,*Nat. Commun.,* 2013, **4**,1335–1345; (b) R. Zhang, A. Liberski, R. Sanchez-Martin, M. Bradley,*Biomaterials*,2009,**30**(31), 6193-6201; (c)C. R. E. Duffy, R. Zhang., S. E. How, A. Lilienkampf, P. A. De Sousa, M. Bradley,*Biomaterials*, 2014, **35**, 5998–6005.
18S.I. Anderson, *Tissue Engineering Using Ceramics and Polymers* (2nd Edition), 2014, 196-223.
19B. L. Lucht, W. B. Euler, O. J. Gregory, *Polym.Prepr*., 2002, **43**(1), 59-60.
20K. Li, D. Ding, Q. L. Zhao, J. Z. Sun, B. Z. Tang, B. Liu, *Sci. China Chem*., 2013, **56**(9), 1228-1233.

