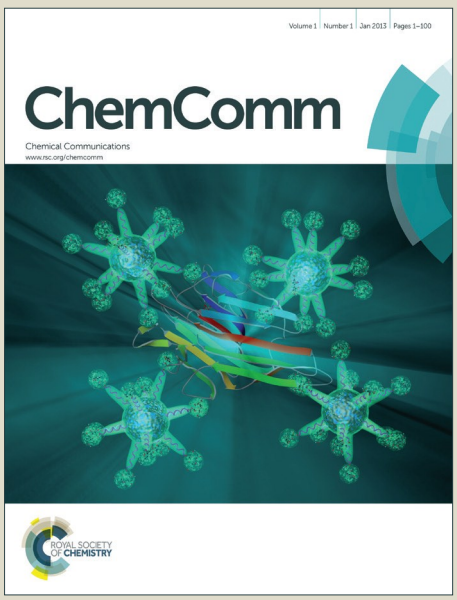
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

COMMUNICATION

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

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Guirong Wang[a], Zongquan Duan[a], Yang Sheng[a], Kevin Neumann[b], Linhong Deng[d], Jian Li[a]†, Mark Bradley[b], Rong Zhang[a,c]†

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 fluorescent polymers,[1] have attracted huge amounts of interest because of their application in light emitting diodes,[2] optoelectronic devices[3] and biosensors,[4] in part due to their tunable electrical and optical properties while possessing attractive mechanical properties and processing characteristics.[5] Due to electron conjugation along the backbone, the properties of the conjugated fluorescent polymer dyes are highly sensitive to minor external structural perturbations and local electron density changes that occur upon binding to other molecules and solvation effects.[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 modulated by altering the temperature.[8] Recently, conjugated fluorescent polymers have become candidates to replace molecular dyes and quantum dots for various fluorescence based biomedical-imaging applications,[9] while being used as fluorescent enhancers.

Compared to molecular dyes, conjugated fluorescent polymers have advantages in terms of brightness of emission, high extinction coefficients and good photo-stabilities,[10] which make them suitable for various fluorescence imaging tasks. In addition conjugated fluorescent polymers are biologically compatible, often with lower toxicities[11] than quantum dots.[12] Another approach to generate highly intense fluorescent signals is to incorporate them into particles, thus polymer beads loaded with fluorophores are widely used as standards and calibrants, and for cell tracking and labeling.[13]

Here polymer microarrays were applied to the identification of polymers that could control or manipulating the fluorescent properties of conjugated polymers. This high-throughput approach, used monomers[7,8a] that included a diversity of the functional groups (hydroxyls, amines and carboxylic acids) as well as hydrophobic moieties (e.g. cyclohexyl methacrylate) as well as incorporating known thermo-responsive monomers (e.g. N-Isopropyl acrylamide).

The polymer microarrays were constructed to 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 was thus 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,[10b]but also allow the properties of the dye to be tuned, physically and thermally. Eleven acrylates and acrylamides were used for preparation of the microarray (Table 1, ESI Methods).

[a] *School of Materials Science&Engineering, Changzhou University, Changzhou 213164, Jiangsu, China.rzhang@cczu.edu.cn, lijian@cczu.edu.cn*
[b] *School of Chemistry, EaStCHEM, University of Edinburgh, Joseph Black Building, West Mains Road, Edinburgh, EH9 3JJ, UK*
[c] *Jiangsu Collaborative Innovation Centre of Photovoltaic Science and Engineering, Changzhou University, Changzhou 213164, Jiangsu, China.*
[d] *Institute of Biomedical Engineering and Health Sciences, Changzhou University, Changzhou 213164, Jiangsu, China.*
† They both are corresponding authors.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

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

*: Polymers synthesized are 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) and the photoinitiator 1-hydroxycyclohexyl phenyl ketone.Across each line of the microarray two monomer solutions were printed in varying ratios designated 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 (365 nm) for 30 min to initiate *in situ* polymerization after printing.

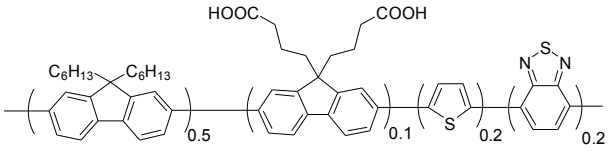


Fig. 1. The structure of the conjugated fluorescent polymer used in this study. This was synthesized using Suzuki cross-coupling chemistries.

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 were interacting with the dye, affecting its fluorescent emission. Fluorescent images of each polymer spot on the array (20×55 spots in total) were analyzed with Image J (Table S1, ESI†)[18] and the relative fluorescent intensities calculated 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 immobilized conjugated fluorescent polymer, I_p is the auto-fluorescence intensity of the polymer spot without the dye andI_f is the fluorescent intensity of the dye alone. The relative fluorescent intensities of 1100 polymer features were calculated and are given in descending order in Fig.2a.

Three "hit" polymer where chosen on the basis of displaying the largest increases in relative fluorescent intensity, while three control polymers were selected that showed no fluorescence (either because the dye was totally quenched or was not incorporated ($I_r \approx 0$)). In addition 3 polymers were selected which displayed similar values to the dye feature alone. Features with a negative I_r represented host polymers that had suppressed the dye's fluorescence or had very high auto-fluorescence and were not studied further. These 9 selected polymers were used for printing of the secondary arrays for further confirmation (Fig.2b) with 20 replicate features printed for each of the polymers (Table 2).

Table 2 Polymer candidates selected 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

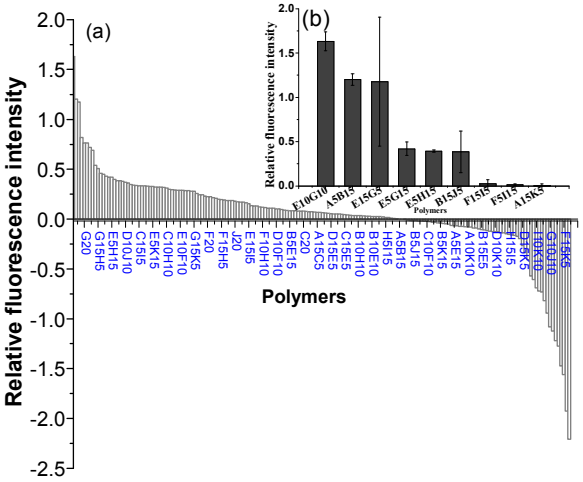


Fig.2 Fluorescent intensity analysis of the polymer microarray: (a) The relative fluorescent intensities of the individual polymers on the primary polymer microarray (data are the average of four polymer features (STDEVs are given in the SI butare not shown here for clarity); (b) The insert shows the 9 polymers taken forward, with three polymers showing increased fluorescent intensity; three with fluorescence intensity similar as the dye feature alone and three with an I_r of zero (The error bars are STDEV, n=4).

Fluorescent analysis of the spots of the secondary array were analyzed (Fig. 3) and showed good agreement with the primary array screen.

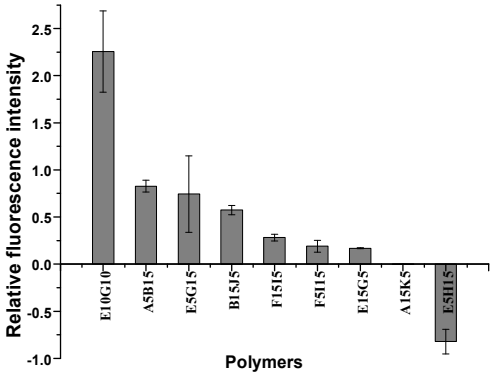


Fig.3. The fluorescent intensities of the polymers as fabricatedon the secondary array. The fluorescent images of the polymer features were captured and the fluorescent intensities analyzed using Image J a equation (1) (The error bars are STDEV, n=20)

This array was screened and analyzed and 5 combinations monomers were identified to take forward for furth examination. The three polymer combinations that exhibit enhancement of the fluorescent intensity of the conjugat fluorescent polymer (E10G10, A5B15 and E5G15) and tv (E15G5 and A15K5), that had no or limited fluorescence we up-scaled to produce polymer beads with entrapped conjugat fluorescent polymer (Scheme S1, Fig. S4, ESI†, Methods). T fluorescent beads were characterized at different temperatures (25, 35, 45 and 55 °C) and compared to solutions of the conjugated fluorescent polymers.

The beads were fluorescent with emission at 500-700 nm upon excitation (λ_{Ex}) at 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 a third as intense of the pure dye. The fluorescence emission peaks of the immobilized dye also shifted some 20-40 nm to shorter wavelengths.

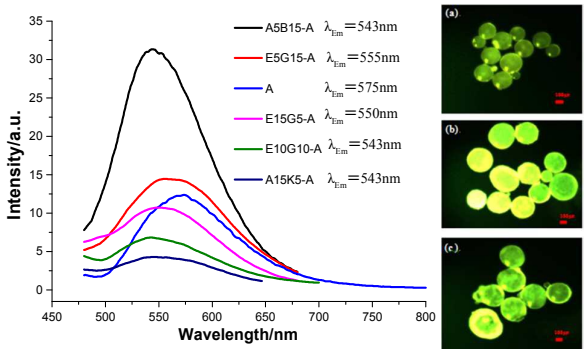


Fig.4 Left) The fluorescent intensities of the conjugated fluorescent polymer (A) with and without polymer bead entrapment (A5B15-A, E5G15-A, E15G5-A, E10G10-A and A15K5-A) prepared. The fluorescent dye and beads were suspended in PEG-400:H_2O (2:1) and analyzed at 25 °C with λ_{Ex}= 450 nm. The concentration of the dye in the beads was estimated according to the amount of dye immobilized into the beads during polymerization (see Fig. S5, ESI†). Right) Fluorescent images of the polymer beads: (a), (b) and (c) polymer beads composed of 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 as thetemperature increasedfrom 25 to 60 °C (Fig. S6, ESI†). However, the fluorescent intensity reduced when the dye was immobilized in the polymers A5B15 and E15G5 (Fig. S7, S8, ESI†) and it dropped dramatically when embedded in the polymer E5G15 with the fluorescence intensity dropping some 2.2 fold when the temperature increased from 25 to 60 °C (Fig. 5), and recovered upon cooling. Thus E5G15-polymer beads exhibited thermo-fluorescence - fluorescence that could be switched on and off many cycles (Fig. 6).

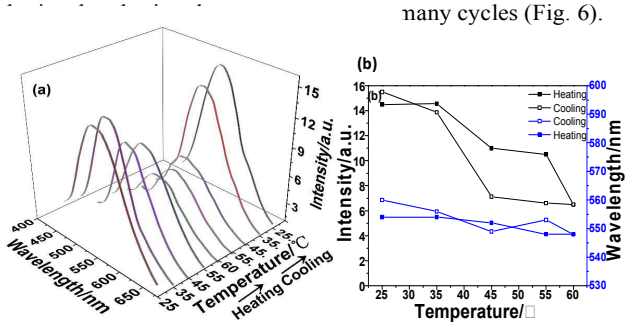
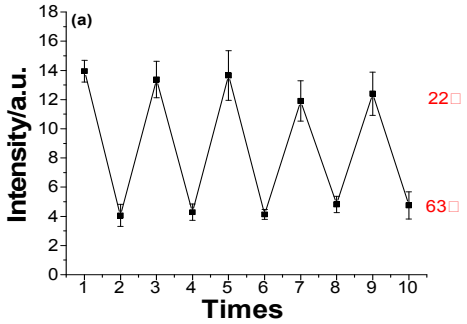


Fig.5 Fluorescence intensity change inpolymer beads made from E5G15-A following the temperature rise from 25 to 60 °C and then cooling to 25 °C with an λ_{Ex}=450 nm (a) and (b) corresponding fluorescence intensities and emission wavelengths at various temperatures.

The temperature-dependent thickness alteration of the host polymer was examined using a rheometer heating and cooling between 10 and 55 °C under constant compressive forces (2 or 4 kPa). During the measurement, an oscillatory shear stress was imposed on the polymer at a frequency of 1Hz 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 °C shrinking some 4% in thickness and increasing some 1-2% when the hydrogels were cooled down to 10 °C again (Fig.6b, c).



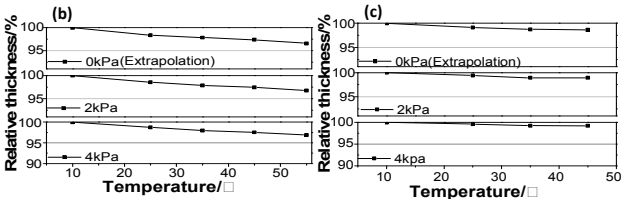


Fig.6: (a) Switching cycles of the fluorescence intensity of the E5G15-A polymer beads in PEG-400/H_2O (2/1) triggered with temperature changes between 22 and 63 °C (λ_{Ex}= 450 nm) (Error bars are STDEV, n=5). (b) Change in relative thickness of the E5G15 polymer as a function of temperature in response to actual (2 and 4 kPa) and extrapolated (0 kPa) compressive forces as the temperature was raised from 10 to 55 °C and (c) the temperature reduced from 55 to 10 °C.

In conclusion polymer microarrays were fabricated with polymers entrapping 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. We believe that the chains of the embedded-conjugated fluorescent polymer may have become twisted or strained during the entrapment/polymerization process, thus interrupting their conjugation and resulting in the observed blue shift in the emission wavelength.[19] Host polymer NIPAA/DMC (1/3) beads exhibited thermo-fluorescence presumably due to the incorporation of N-isopropyl acrylamide. PNIPAA is a well-known thermo-responsive polymer, therefore the host polymer has the possibility to trigger changes in bead fluorescence by alterations of polymer hydrophilicity/ hydrophobicity. We believe that the temperature dependence of the intensity could thus be the result of aggregation quenching (ACQ)[20] of the dye immobilized in the host polymer under different temperatures (Video S1 and S2, ESI†) since the shift of the emission peak was very small (less than 10 nm in wavelength (Fig 5b)), as well as alterations in solvation. 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 labelling and imaging of tissues, smart sensors, switching devices, molecular logic gates and other electronic device.

We gratefully acknowledge the support from the National Natural Science Foundation of China (21374012, 11532003), Jiangsu Province for support under the distinguished professorship program, the Priority Academic Program Development (PAPD) of Jiangsu Higher Education Institutions, and the Jiangsu Collaborative Innovation Centre of Photovoltaic Science and Engineering.

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) K. Watanabe , H. Hayasaka , T. Miyashita , K. Ueda and K. Akagi, *Adv. Funct. Mater.* 2015, **25**, 2794–280; (b) A. Facchetti, *Chem. Mater.*, 2011, **23**(3), 733-758.
4 X. L. Feng, L. B. Liu, S. Wang, D. B. Zhu, *Chem. Soc. Rev*., 2010, **39**(7), 2411-2419.
5 C. F. Wu, D. T. Chiu, *Angew. Chem. Int. Edit*., 2013, **52**(11), 3086-3109.
6 A. Alvarez, J. M. Costa-Fernández, R. Pereiro, A. Sanz-Medel, A. Salinas-Castillo, *Trend. Anal. Chem*., 2011, **30**(9), 1513-1525.
7 T. 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.
9 (a) Y. Q. Huang, C. X. Song, H. C. Li, R. Zhang, R. C. Jiang, X. F. Liu, G. W. Zhang, Q. L. Fan, L. H. Wang and W. Huang, *ACS Appl. Mater. Interfaces*, 2015, **7**, 21529−21537; (b) L. H. Feng, C. L. Zhu, H. X. Yuan, L. Liu, F. Lv, S. Wang, *Chem. Soc. Rev*., 2013, **42**(16), 6620-6633.
10 (a) C. F. Wu, C. Szymanski, J. McNeill, *Langmuir*, 2006, **22**(7), 2956-2960. (b) C. J. Yang, H. Liu, Y. D. Zhang, Z. G. Xu, X. C. Wang, B. Caoand M. F. Wang, *Biomacromolecules*, 2016, **17**, 1673−1683.
11 N. K. Guimard, N. Gomez, C. E. Schmidt, *Prog. Polym.Sci.*, 2007, **32**(8-9), 876–921.
12 (a) F. T. Lv, T. Qiu, L. B. Liu, J. M. Ying and S. Wang, *Small*, 2016, **12**(6), 696–705; (b) A. 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.
18 S.I. Anderson, *Tissue Engneering Using Ceramics and Polymers* (2nd Edition), 2014, 196-223.
19 B. L. Lucht, W. B. Euler, O. J. Gregory, *Polym. Prepr*., 2002, **43**(1), 59-60.
20 K. Li, D. Ding, Q. L. Zhao, J. Z. Sun, B. Z. Tang, B. Liu, *Sci. China Chem*., 2013, **56**(9), 1228-1233.

