Published in final edited form as:

Chem Commun (Camb). 2011 September 14; 47(34): 9687–9689. doi:10.1039/c1cc13571f.

Development of Encoded Particle-Polymer Arrays for the Accelerated Screening of Antifouling Layers

Darby Kozak, Prakash Kithva, Jacinda Bax, Peter P.T. Surawski, Michael J. Montero, and Matt Trau

Australian Institute for Bioengineering and Nanotechnology, University of Queensland, Brisbane, Australia, 4072. Fax: 61 7 334 63973; Tel: 61 7 334 64173

Darby Kozak: d.kozak@uq.edu.au; Matt Trau: m.trau@uq.edu.au

Abstract

A multiplexed screening methodology for the rapid development of antifouling polymer surfaces is presented. An array of protein resistant polymer layers with high grafting ($>100 \text{ mg/m}^2$) were polymerized on optically encoded particles. Multiplexed analysis showed a 97% reduction in nonspecific protein adsorption for all polymer layers created.

A chief limitation to the performance and lifespan of materials used in biological applications are their susceptibility to ‘biofouling’, i.e. the nonspecific adsorption of biomolecules on their surface. Nonspecific protein adsorption can impair industrial equipment, induce inflammation, facilitate the rejection of biomedical implants and nanoparticles,¹ and reduce the signal-to-noise ratio of biosensors.² Thus, considerable focus and several strategies have been put forward to prevent nonspecific protein adsorption.³ Currently, the most effective approach is to coat the surface with an ‘antifouling’ polymer layer.

Screening antifouling polymer layers has been to date slow, often laborious and must be tuned on a case-by-case basis. This process was recently given a high throughput boost with the creation of 96-well⁴ and polymer-spot microarrays.⁵ Using UV initiated polymerization techniques, these 2-dimensional polymer arrays have been used to rapidly create and screen protein adsorption on polymer libraries composed of 66 homopolymer⁴ and 576 unique random copolymers.^{5b}

Encoded particles have recently generated interest as a next generation of higher throughput array platform to 2-dimensional spot coordinate arrays.⁶ Particle library size, i.e., number of unique particle populations, scales to the power of encoding parameters (e.g., number of fluorophores or light scattering features) used⁷ with current libraries approaching several hundred.⁸ Encoded particles have been used previously as genomic,⁹ proteomic,¹⁰ and antifouling assays.¹¹

Herein, we present a methodology to rapidly create and screen libraries of antifouling polymer layers. To do this we have modified an expandable library of optically encoded organosilica particles, which are stable to organic synthesis conditions,¹⁰ with a reversible addition-fragmentation chain transfer (RAFT) agent. This gives rise to surface initiated ‘on-particle’ polymerization, as shown in Fig. 1. Living radical polymerization techniques such as RAFT enables greater control over polymer composition, architecture and molecular weight, thereby increasing the diversity and number of polymer materials possible.¹¹

Therefore, library based analysis techniques, such as demonstrated in this study, presents a high throughput means of examining potential biological applications of these new polymer materials.

Organosilica particles of $4.5 \pm 0.5 \mu\text{m}$ in diameter were synthesized from 3-mercaptopropyl trimethoxysilane (MPMS).¹² These particles were then optically encoded with increasing concentrations of covalently incorporated fluorescent dyes (Atto-Tech 550 and 620). Fig. 2 shows the flow cytometry readout of a library containing 41 unique populations. In this study 5 unique populations of the library were used to demonstrate our proof of concept experiment.

Encoded particles were coated with 3-amino propyl trimethoxysilane and then [2-ethyl sulfonyl thiocarbonyl] 2-methyl propionic acid (EMP)¹³ RAFT agent was coupled to the thiol and/or amine surface groups using standard HBTU coupling chemistry. RAFT polymerization from the surface (experimental details, ESI[†]), via the R-group approach, produced well-defined polymers of 1. poly(tBA) (**Poly-A**), 2. poly(tBA-co-NIPAM) (**Poly-B**) random copolymer, 3. poly(tBA-block-NIPAM) (**Poly-C**) and 4. poly(NIPAM-block-tBA) (**Poly-D**) block copolymers, resulting in an encoded particle polymer library. Mixing the particles together ‘multiplexing’ and in combination with particle-by-particle flow cytometric analysis enables high throughput screening of many polymer surfaces in a single sample tube. Acrylic acid and NIPAM monomers were chosen as they are utilised in generating polymer materials for biological and biosensor applications.^{3a}

Particles took on a visually discernable yellow hue after EMP attachment (MPMS-EMP) as well as giving rise to a marginal increase in the sulphur to silicon ratio (from 0.69 to 0.75) as measured by XPS (Table 1). FTIR (Fig. 2S-A, ESI[†]) and thermogravimetric analysis (TGA) of the MPMS-EMP particles, however, were not sensitive enough to measure EMP attachment indicating low (surface limited) concentrations. The presence and polymerization via coupled EMP was confirmed by successful polymer grafting on the MPMS-EMP particles and no detectable polymer on MPMS particles without attached EMP, illustrated by the lack of characteristic polymer ester peaks in both the FTIR (Fig. 2S-B, ESI[†]) and XPS C1s spectra (Fig. 3S-b, ESI[†]).

As all of the polymer grafted particles contained tBA, they all displayed the characteristic FTIR ester group absorption band at 1730 cm^{-1} (Fig. 2S-A) and corresponding XPS C1s peak shifts (Fig. 3S, ESI[†]) at 286.5 eV (O-C) and 288.5 eV (O=C-O). NIPAM in Poly-B, Poly-C and Poly-D gave rise to FTIR amide group bands at 1640 cm^{-1} (amide I, C=O) and at 1550 cm^{-1} (amide II, N-H) and an XPS C1s peak shift at 288 eV (O=C-N). The ‘living’ radical nature of the immobilised EMP RAFT agent was further demonstrated by the successful block copolymer synthesis (Poly-C and Poly-D).¹² The position of the NIPAM block in the copolymer layers could also be inferred from the elemental percentage of nitrogen as measured by XPS (Table 1). Both Poly-B and Poly-C (a block-copolymer which had the NIPAM block furthest from the surface) gave rise to similar amount of N, ~ 5.9%. In contrast Poly-D, which had the NIPAM block closest to the surface, showed a much lower amount of N, being 2.6%.

All of the polymerized surface layers created (Poly A B, C and D) exhibited very high polymer grafting (hundreds of mg/m^2) as measured by TGA (Table 1). The grafted amount of polymer was calculated from the residual weight fraction from the TGA thermograms between 0–800 °C (Fig. 4S, ESI[†]). MPMS-EMP particles, without a grafted polymer layer,

[†]Electronic ESI (ESI) available: [polymerization methods, NMR spectra of EMP, FTIR and XPS spectra of polymer grafted particles, detailed TGA and flow cytometry calculations, IgG, BSA and SAPE adsorption isotherms]. See DOI: 10.1039/b000000x/

exhibited major weight loss after heating beyond 350 °C in contrast to polymer grafted particles which started to degrade around 150 °C. High polymer grafting calculated by TGA was supported by XPS results that the Si and S silica substrate signal went from ~13% and ~10%, respectively, to below 1% after polymerization. This reduction is proportional to the thickness of the grafted polymer layer^{11b} and indicates that the polymer layer thickness was close to the maximum penetration depth of XPS analysis being, ~10 nm.

Polymer chain grafting density (i.e. number of polymer chains/nm²) is typically considered to be imperative to antifouling efficacy. Polymer chains can be cleaved from silica particles by dissolving the particles in hydrofluoric acid (HF).¹⁶ However, in this study HF dissolution of MPMS particles produced a non-volatile fluorinated silane-polymer oil, making polymer isolation difficult. Therefore, only Poly-A, the poly(tBA), was able to be cleaved and analysed by size exclusion chromatography. The molecular weight (M_n) of surface bound poly(tBA) was 44,000 g/mol with a polydispersity index (PDI) of 1.2. Poly-A grafting density therefore was 1.89 $\mu\text{mol}/\text{m}^2$ or approximately 1.14 chains/nm². This grafting density is similar to the highest achieved via previous 'grafting-to' of pre-polymerized RAFT-based polymers onto gold nanoparticles.¹⁷

Polyacrylic acid (PAA) and polyNIPAM (PNIPAM) are interesting antifouling layers as changing solution pH, salt concentration and temperature can 'switch' their antifouling properties.^{3a, 18} Previous high throughput screening by Zhou et al⁴ identified methacrylic acid, chemically similar to acrylic acid, as the 6th best monomer of 66 tested for creating a protein resistant polymer layer. Protein adsorption however is complex and varies depending upon the protein and solution properties i.e. adsorption increases near the isoelectric point (pI) of a protein.¹⁹

Protein adsorption was measured by multiplexing the polymer grafted particles and MPMS-EMP control enabling the simultaneous and comparative screening of the 5 surfaces. This reduces the number of experimental samples, inter-sample variation, and screening time required. Equal concentrations of each particle population were mixed together and incubated with fluorescently tagged immunoglobulin gamma (IgG, ~150 kDa, pI ~8.5), bovine serum albumin (BSA, ~66 kDa, pI ~4.7) or streptavidin phycoerythrin (SAPE, ~300 kDa, pI ~5) in phosphate buffered saline (150 mM, pH 7.4) at 20 °C under constant agitation for 2 hrs. These proteins are commonly employed in biosensors as surface blocking agents, biomolecule capture probes and fluorescent readouts.

Nonspecific adsorption gives rise to an increase in the mean fluorescence intensity (MFI) of a particle relative to the protein fluorophore tag. As shown in Fig. 3, the optical encoding signature of the 5 individual particles are read from the X & Y (Optical Encoding MFI 1 and 2, respectively) coordinates and the amount of adsorbed fluorescent protein is proportional the vertical shift in the Z-axis (MFI of Protein Adsorption). Utilising more encoding parameters enables larger libraries or even multi-component adsorption analysis. Calibrating the MFI signal enables the adsorbed amount of protein to be calculated.^{11a} Previous studies have shown good correlation between flow cytometric readout and solution depletion measurements for BSA, IgG, SAPE, Protein G and fibrinogen adsorption on organosilica particles.¹¹

All of the generated polymer surfaces in this study displayed a reduction in nonspecific protein adsorption for the three proteins examined. All three proteins had similar adsorption curves with increasing protein concentration [0 to 200 $\mu\text{g}/\text{mL}$] (Fig. 4S, ESI[†]). The MPMS-EMP particles always had the greatest adsorbed amount of protein and followed the expected Type I, monolayer limited, adsorption curve.

Prior to deprotection of the tert-butyl group, the hydrophobic poly(tBA) coating of the Poly-A particle set gave rise to adsorbed amounts lower than that of the blank EMP beads. After deprotection of the tert-butyl group the resulting PAA and acrylic acid-NIPAM block and random copolymers all reduced protein adsorption by more than 97% at 100 µg/mL of protein (Table 2). The random copolymer Poly-B appeared to provide the greatest antifouling however this was within the experimental error of the other polymers tested (Poly-A, Poly-C and Poly-D). The high reduction in the nonspecific adsorption is thought to be due to the high polymer grafting density as well as their hydrophilic and antifouling nature at the adsorption conditions used being, high salt (150mM), pH 7.4, and 20 °C.^{3a, 18}

In summary, the combination of optically encoded particles and surface initiated RAFT polymerisation produced a polymer library with high grafting densities. This approach represents a simple and fully expandable system for the high throughput multiplexed screening of protein resistant polymers. Screening these layers as a multiplexed assay against nonspecific protein adsorption indicated that PAA and its copolymers with PNIPAM were highly effective at preventing protein adsorption.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Queensland Smart State National and International Research Alliances Program (RM2007001266) and the National Institutes of Health (U01 AI082186-01).

Notes and references

1. (a) Anderson JM, Rodriguez A, Chang DT. *Seminars in Immunology*. 2008; 20:86–100. [PubMed: 18162407] (b) Dobrovolskaia MA, McNeil SE. *Nat Nanotechnol*. 2007; 2:469–478. [PubMed: 18654343]
2. Chen A, Kozak D, Battersby BJ, Forrest RM, Scholler N, Urban N, Trau M. *Langmuir*. 2009; 25:13510–13515. [PubMed: 19928944]
3. (a) Banerjee I, Pangule RC, Kane RS. *Adv Mater*. 2011; 23:690–718. [PubMed: 20886559] (b) Magin CM, Cooper SP, Brennan AB. *Materials Today*. 2010; 13:36–44.
4. Zhou M, Liu H, Venkiteshwaran A, Kilduff J, Anderson DG, Langer R, Belfort G. *J Mater Chem*. 2011; 21:693–704.
5. (a) Anderson DG, Levenberg S, Langer R. *Nat Biotechnol*. 2004; 22:863–866. [PubMed: 15195101] (b) Taylor M, Urquhart AJ, Anderson DG, Williams PM, Langer R, Alexander MR, Davies MC. *Macromol Rapid Commun*. 2008; 29:1298–1302.
6. Cederquist KB, Dean SL, Keating CD. *Wiley Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology*. 2010; 2:578–600. [PubMed: 20890960]
7. Battersby BJ, Lawrie GA, Johnston APR, Trau M. *Chem Commun*. 2002:1435–1441.
8. Vignali DAA. *J Immunol Methods*. 2000; 243:243–255. [PubMed: 10986418]
9. (a) Corrie S, Sova P, Lawrie G, Battersby B, Kiviat N, Trau M. *Mol BioSyst*. 2009; 5:262–268. [PubMed: 19225617] (b) Corrie SR, Sova P, Feng QH, Blair T, Kiviat NB, Trau M. *Analyst*. 2011; 136:688–691. [PubMed: 21103519]
10. Marcon L, Kozak D, Battersby BJ, Chappell KJ, Fairlie DP, Young P, Trau M. *Anal Biochem*. 2008; 376:151–153. [PubMed: 18312847]
11. (a) Kozak D, Chen A, Trau M. *Langmuir*. 2008; 24:1204–1211. [PubMed: 18067330] (b) Chen A, Kozak D, Battersby BJ, Trau M. *Biofouling*. 2008; 24:267–273. [PubMed: 18589493] (c) Kozak D, Chen A, Bax J, Trau M. *Biofouling*. 2011; 27:497–503. [PubMed: 21614699]
12. Barbey R, Lavanant L, Paripovic D, Schuwer N, Sugnaux C, Tugulu S, Klok HA. *Chem Rev*. 2009; 109:5437–5527. [PubMed: 19845393]

13. Miller CR, Vogel R, Surawski PPT, Jack KS, Corrie SR, Trau M. *Langmuir*. 2005; 21:9733–9740. [PubMed: 16207060]
14. Convertine AJ, Lokitz BS, Vasileva Y, Myrick LJ, Scales CW, Lowe AB, McCormick CL. *Macromolecules*. 2006; 39:1724–1730.
15. Szleifer I. *Biophys J*. 1997; 72:595–612. [PubMed: 9017189]
16. Li CZ, Benicewicz BC. *Macromolecules*. 2005; 38:5929–5936.
17. Liang M, Lin IC, Whittaker MR, Minchin RF, Monteiro MJ, Toth I. *ACS Nano*. 2010; 4:403–413. [PubMed: 19947583]
18. de Vos WM, Biesheuvel PM, de Keizer A, Kleijn JM, Stuart MAC. *Langmuir*. 2008; 24:6575–6584. [PubMed: 18507422]
19. Malmsten, M. *Biopolymers at Interfaces*. M. Dekker; 2003.

Fig. 1.
RAFT modification and on-particle polymer synthesis.

Fig. 2.
Fluorescent image and corresponding flow cytometric optical 2D micrograph of 41 optically encoded particle populations.

Fig. 3. Flow cytometric readout of individual encoded particles before (black dots) and after (red dots) incubation with fluorescent protein. The middle population of the polymer library with greatest difference between black and red dots corresponds to MPMS-EMP particles without grafted polymer.

Elemental composition of particle surface and grafted amount of polymer before and after polymerization as calculated by XPS and TGA thermograms.

Table 1

Material	XPS Elemental composition (%)					Residual weight fraction	Grafted amount* (mg/m ²)
	Si	S	N	O	C		
MPMS	15.0	10.4	2.7	23.9	48.0	--	--
Poly-A**	13.6	7.8	2.9	21.9	53.8	--	--
MPMS-EMP	12.6	9.4	2.2	21.6	54.1	0.577	--
Poly-A	0.6	0.5	0.0	20.8	78.1	0.538	87
Poly-B	0.6	0.2	5.8	16.0	77.4	0.504	161
Poly-C	0.8	0.3	6.0	15.1	77.9	0.492	190
Poly-D	1.1	1.0	2.6	18.2	77.1	0.491	192

* Particle surface area is 1 m²/g as determined by BET.

** tBA grown on MPMS particles without coupled EMP.

Table 2

Multiplexed analysis of protein adsorption at 100 $\mu\text{g/mL}$ on 5 polymer grafted particle surfaces.

Material	BSA (mg/m^2)	IgG (mg/m^2)	SAPE (mg/m^2)
MPMS-EMP	2.0 ± 0.1	4.2 ± 0.25	2.0 ± 0.09
Poly(tBA)	0.78 ± 0.08	2.7 ± 0.47	0.53 ± 0.09
Poly-A	0.03 ± 0.01	0.03 ± 0.02	0.02 ± 0.01
Poly-B	0.02 ± 0.01	0.03 ± 0.02	0.01 ± 0.01
Poly-C	0.04 ± 0.01	0.10 ± 0.04	0.03 ± 0.01
Poly-D	0.05 ± 0.01	0.09 ± 0.06	0.01 ± 0.01

*
Average and standard deviation of three measurements.