Supplementary Materials:

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

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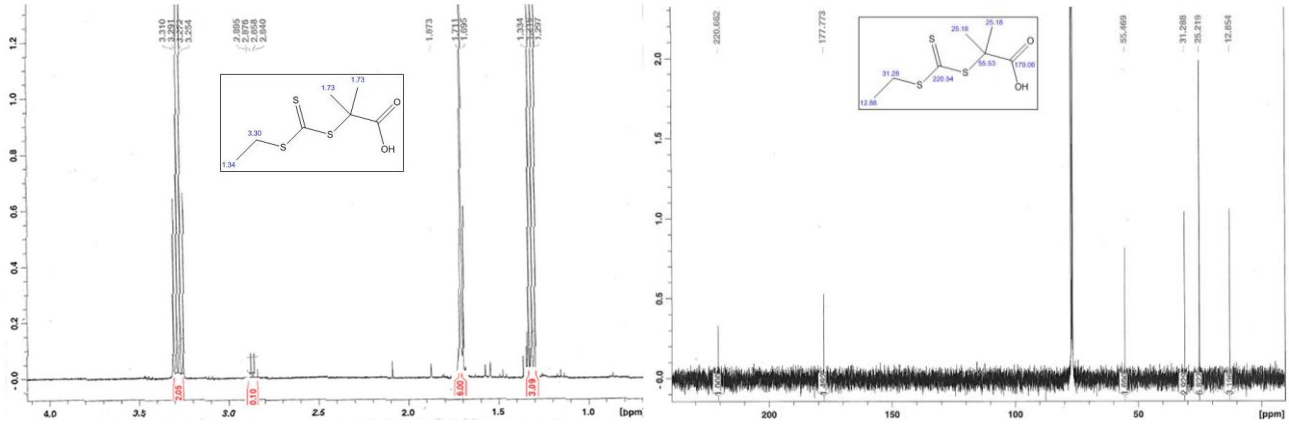
Experimental Methods

Materials

3-mercaptopropyl trimethoxysilane (MPS) was purchased from Lancaster (UK). Fluorescent dyes were purchased from ATTO-TEC (Siegem, Germany). All solvents; acetone, chloroform, ethanol (EtOH), tetrahydrofuran (THF), dimethylformamide (DMF) and toluene were analytical grade. Fluorescein-labeled bovine serum albumin (BSA) and Immunoglobulin Gamma (IgG) were purchased from Sigma Aldrich and Streptavidin Phycoerthrin (SAPE) was purchased from Invitrogen. All other reagents were purchased from Sigma Aldrich of the highest purity available and used as received. 2-ethyl sulfonyl thiocarbonyl 2-methyl propionic acid (EMP) chain transfer agent (CTA) was synthesized according to the procedure outlined in Convertine *et al.*[1] Successful synthesis of EMP was confirmed by ^{1}H and ^{13}C NMR (300MHz, $CDCl_3$) δ (ppm): 3.30 (q, 2H), 1.73 (s, 6H), 1.34 (t, 3H) and 220.5 (C=S), 178 (COOH), 55.5 (S***C***$(CH_3)_2$), 31.3 (SCH_2), 25.2 (C(***C***$H_3)_2$), 12.9 (CH_2CH_3), as shown if Figure 1S.



(A) (B)

Figure 1S: ^{1}H and ^{13}C NMR spectra (A and B, respectively) of *S*-Ethyl-*S'*(α,α'-dimethyl-α"-acetic acid)trithiocarbonate (EMP) with the structure and predicted chemical shifts inset.

Particle synthesis, optical encoding and RAFT CTA modification. Organosilica particles were synthesized from MPS according to the protocol outlined in Miller *et al.*[2] In brief, 7 mL of MPS was hydrolyzed in acidified water overnight followed by condensation with triethylamine (TEA) for 30 minutes. Particles of ~4.50 μm in diameter with ~10% polydispersity, as determined by optical microscopy, were optically encoded through the covalent incorporation of increasing concentrations of maleimide functionalized Atto-Tec 620 or a combination of Atto-Tec 620 and Atto-Tec 550 fluorescent dyes to create 5 and 41 unique particle populations, respectively. Particles were then amine surface modified by reacting with 10 v/w% of 3-aminopropyl trimethoxysilane and TEA in EtOH for 2 hrs under constant agitation. EMP was covalently immobilized on amine modified particles by activating EMP with equal molar amounts of HBtU and DIPEA in anhydrous DMF and reacting with the amine modified particles overnight.

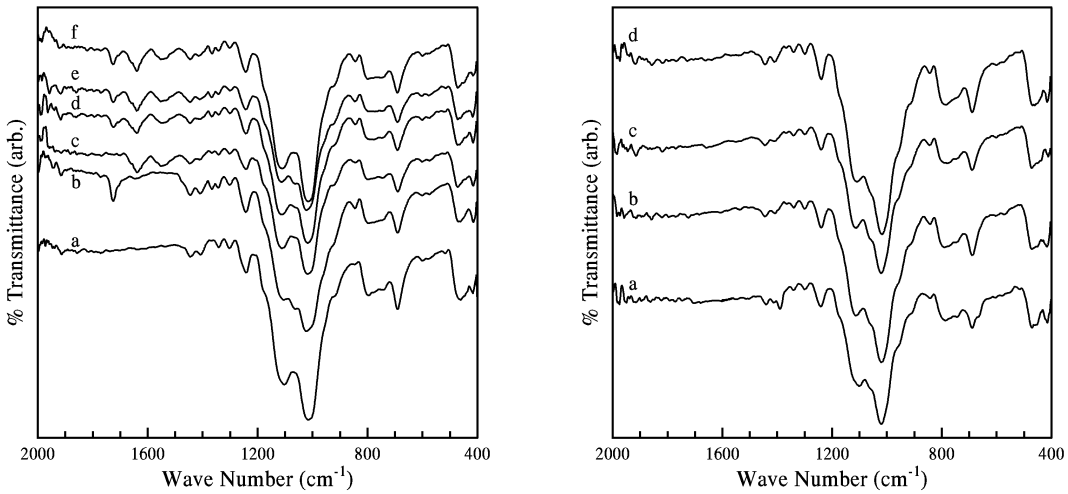
On particle polymerization and characterization; FTIR, XPS, and TGA. Four uniquely encoded EMP-particle populations were modified with polymers created from tert-butyl acrylate (tBA) and N-isopropylacrylamide (NiPAM) monomers. The reaction conditions and structures of the grafted polymers and copolymers formed are given in Table 1S. All polymerization reactions were undertaken using 30 mg of EMP-particles of a single population mixed with 4 x 10^{-3} moles of monomer and 1 x 10^{-6} moles of 2,2'-azobis(isobutyronitrile) (AIBN) initiator in THF with a total volume of 2 ml. The reaction flask was purged with argon for 30 minutes prior to heating to 55° C for 2 hrs under an argon atmosphere. Unreacted monomer and free polymer was removed by centrifugation and washing the particles in THF repeatedly (6 times). The tert-butyl group of tBA was removed by reacting the particles with a solution of 95% TFA, 2.5% TIPS and 2.5% water for 1 hr.

Table 1S – Polymerization Conditions

Particle Name	Polymer structure	Monomer and quantity, mg		Reaction duration, hours		AIBN, mg
		Block-1	Block-2	Block-1	Block-2	
Poly A	Poly(tBA)	tBA, 512	--	2	--	0.16
Poly B	Poly(tBA-*co*-NIPAM)*	tBA, 256	NIPAM, 226	2	--	0.16
Poly C	Poly(tBA-*block*-*co*-NIPAM)	tBA, 512	NIPAM, 452	1	1	0.16
Poly D	Poly(NIPAM-*block*-*co*-tBA)	NIPAM, 452	tBA, 512	1	1	0.16

* Random copolymer of tBA and NIPAM.

Polymer layers were characterized by Fourier Transform Infra-Red (FTIR) spectroscopy, X-ray photoelectron spectroscopy (XPS) and Thermo-gravimetric Analysis (TGA) using a Nicolet 6700, Kratos Axis ULTRA and Mettler Toledo TGA LF 1600, respectively. FTIR of polymer coated particles were done in attenuated total reflection (ATR) mode. Each sample was scanned between 4000 cm^{-1} and 400 cm^{-1} at a resolution of 4 cm^{-1} and an average of 100 scans were used for analysis. XPS survey and high resolution C 1s spectra were conducted using pass energies of 160 and 20 eV, resolution of 1.0 and 0.3 eV, and an average of 1 and 3 scans, respectively.



(A) (B)

Figure 2S: FTIR spectra of polymers grafted on (A) MPMS-EMP particles and (B) MPMS particles. (a) MPMS-EMP coupled particles in A and MPMS particles in B, (b) Poly A, (c) poly(NIPAM), (d) Poly B, (e) Poly C and (f) Poly D.

The band at 1730 cm^{-1} for ester groups and the bands at 1640 cm^{-1} (amide I) and 1550 cm^{-1} (amide II) for amide groups in samples of polymer grafted on EMP coupled particles suggests that presence

of the polymers. However, these bands are absent in the APS coated particles suggesting the absence of these polymers.

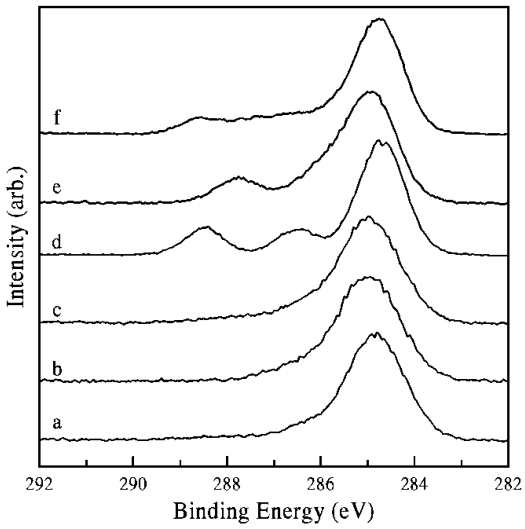


Figure 3S: XPS C1s spectra of (a) MPMS particles, (b) poly(tBA) polymerization with MPMS particles, (c) MPMS–EMP particles, (d) Poly–A grafted MPMS-EMP particles, (e) poly(NIPAM) grafted MPMS-EMP particles, (f) Poly–B grafted MPMS-EMP particles.

The grafting ratio of polymers on the particles was measured by TGA using Equation 1. About 8 mg of dried samples were heated to 800° C at a heating rate of 10° C/min under reactive gas (air) atmosphere.

$$G_r = \frac{W_{PB}^r - W_{Sub}^r}{W_P^r - W_{PB}^r} \qquad (1S)$$

-where, G_r is the grafting ratio, W_{PB}^r, W_P^r and W_{Sub}^r are the residual weight fraction of surface modified particles, pure polymer and unmodified particles, respectively, after heating to 800° C. The grafting ratio is calculated using Equation 1S, which is different from the equation used in the literature (Equation 2S).[3]

$$G_r = \frac{W_{PB}^l}{1-W_{PB}^l} - \frac{W_{Sub}^l}{1-W_{Sub}^l} \qquad (2S)$$

-where, W^l represents the fraction weight lost. This equation may be modified in terms of residual weight fractions after heating to 800 ºC and given in Equation 3S.

$$G_r = \frac{1-W_{PB}^r}{W_{PB}^r} - \frac{1-W_{Sub}^r}{W_{Sub}^r} \Rightarrow \frac{W_{Sub}^r - W_{PB}^r}{W_{PB}^r \cdot W_{Sub}^r} \Rightarrow \frac{1}{W_{PB}^r} - \frac{1}{W_{Sub}^r} \qquad (3S)$$

Clearly, Equations 1S and 3S are different and the latter tend to over estimate the grafting ratio, depending on the weight loss observed in the substrate, which is close to 40 wt.% in the organosilica particles used in this study (Figure 4S).

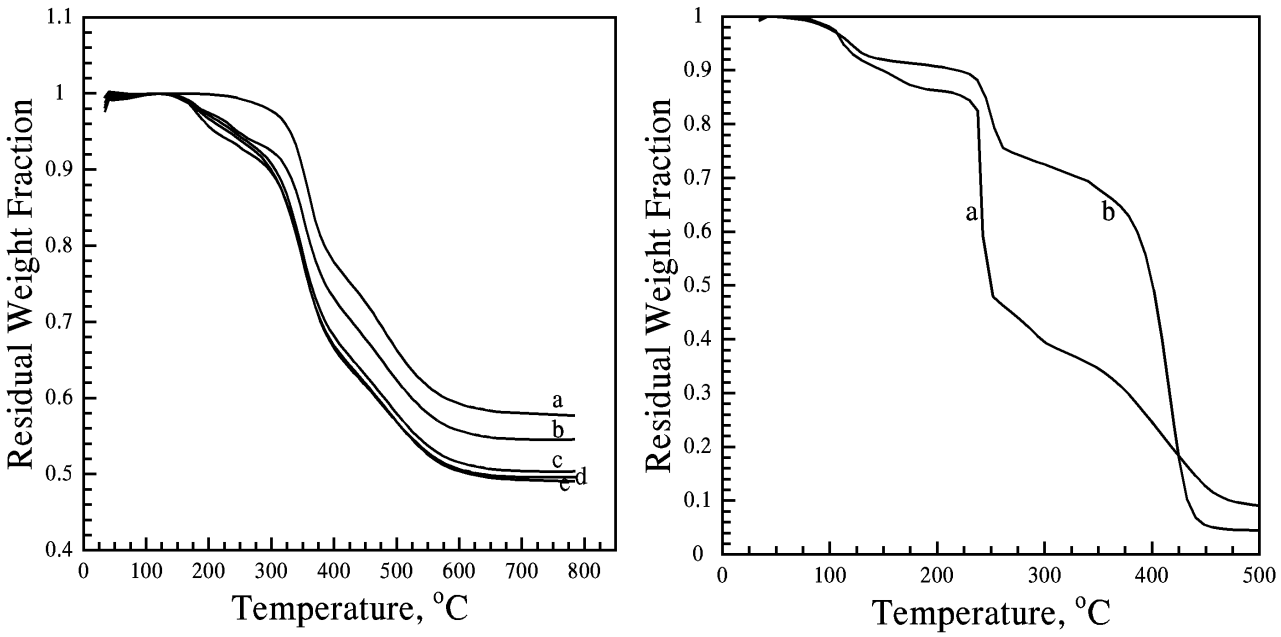


Figure 4S: TGA thermograms of (A) polymer grafted EMP coupled particles – (a) EMP coupled particles, (b) Poly A, (c) Poly B, (d) Poly C and (e) Poly D grafted particles and (B) pure polymers – (a) poly(tBA) and (b) poly(tBA-*co*-NIPAM).

The Equation 1S is derived as follows: The weight of the surface modified particles is equal to that of the polymer attached and the unmodified particles at room temperature and also at 800 °C.

$$W_{PB} = W_P + W_{Sub} \quad \text{and} \quad W_{PB}^T = W_P^T + W_{Sub}^T \qquad (4S)$$

This follows, $$\frac{W_{PB}^T}{W_{PB}} = \frac{W_P^T + W_{Sub}^T}{W_{PB}} \Rightarrow \frac{\frac{W_P^T}{W_P} \cdot W_P + \frac{W_{Sub}^T}{W_{Sub}} \cdot W_{Sub}}{W_{PB}}$$

$$\Rightarrow W_{PB}^r = \frac{W_P^r \cdot W_P + W_{Sub}^r \cdot W_{Sub}}{W_P + W_{Sub}} \Rightarrow W_P^r \cdot \frac{W_P}{W_{PB}} + W_{Sub}^r \cdot \frac{W_{Sub}}{W_{PB}} \Rightarrow W_P^r \cdot W_P^f + W_{Sub}^r \cdot W_{Sub}^f \qquad (5S)$$

-where, W_P^f and W_{Sub}^f are the weight fractions of polymer and unmodified particles, respectively, with the relationship $W_P^f + W_{Sub}^f = 1$. Equation 5S can be rearranged to give Equation 6S, which measures the weight fractions of the components in the surface modified particles.

$$W_P^f = \frac{W_{PB}^r - W_{Sub}^r}{W_P^r - W_{Sub}^r} \qquad \text{(6S-a)}$$

$$W_{Sub}^f = 1 - W_P^f = \frac{W_P^r - W_{PB}^r}{W_P^r - W_{Sub}^r} \qquad \text{(6S-b)}$$

By dividing, Equation 6S-a by Equation 6S-b, we get equation to calculate the grafting ratio shown in Equation 1S.

$$G_r = \frac{W_P}{W_{Sub}} = \frac{W_P^f}{W_{Sub}^f} = \frac{W_{PB}^r - W_{Sub}^r}{W_P^r - W_{PB}^r}$$

Multiplexed high throughput flow cytometric readout of non-specific protein adsorption. Flow cytometry adsorption measurements were made on a DakoCytomation MoFlo™ with three laser excitation lines and 6 optical parameters, excluding forward and side scatter. The laser specifications were a Coherent Innova 90C Argon ion laser (emission: 366 nm, power: 50 mW) with corresponding 450 ± 32 nm and 530 ± 20 nm detectors, a iCyt Visionary Bioscience Inc. (emission: 488 nm, power: 100 mW) with corresponding 530 ± 20 nm and 580 ± 10 nm detectors, and a 635 nm diode laser at 12 mW power with corresponding 670 ± 15 nm and 710 ± 10 nm.

Fluorescently tagged FITC bovine immunoglobulin gamma (FITC-IgG, ~150 kDa, p*I* 8.3-8.9), FITC bovine serum albumin (FITC-BSA, ~66 kDa, p*I* ~4.7) and streptavidin phycoerythrin (SAPE, ~300 kDa, p*I* ~*5*) purchased from Sigma Aldrich were dispersed in phosphate buffer saline (PBS) pH 7.4, 140 mM NaCl, 10mM phosphate buffer. Non-specific adsorption of SAPE and FITC tagged BSA and IgG were conducted by incubating 90µL of increasing concentrations (0.5 to 200 µg/mL) of protein with a 10µL mixed suspension of particles containing ~20,000 of each of the five encoded particle sets. Samples were incubated at 20 ^{o}C under constant agitation for 2hrs and then washed in

100uL of PBS for flow cytometric analysis. A minimum of 500 particles of each set were recorded and analyzed using Summit 4.0 software.

Chatelier et al.[4] first proposed an isoparametric methodology to obtain the adsorption parameters of K (adsorption equilibrium coefficient) and Γ (adsorbed amount) from fluorescence histograms generated by flow cytometry. This methodology is based on the conservation of adsorbate (e.g. fluorescent protein) mass in the system, being $\Gamma[A] = C_o - C_{eq}$, where [A] is the adsorbent (e.g. particles) concentration, C_o and C_{eq} are the original and equilibrium adsorbate concentration, respectively. Kozak et al.[5] modified and simplified this methodology using typical relationship of high affinity Langmuir like (Type I) non-specific adsorption of proteins on hydrophobic surfaces. At very dilute conditions these isotherms can be approximated by Henry's Law, $\Gamma = KC_{eq}$ and C_{eq} becomes negligible for very high affinity adsorption (e.g. K goes to infinity). Therefore, by eliminating C_{eq} term from the conservation of mass equation gives rise to,

$$\Gamma = \frac{C_o}{[A]} \propto MFI \qquad (7S)$$

If the adsorbate is homogenously tagged with a fluorophore, Γ is typically linearly proportional to the mean fluorescence intensity (MFI) of the adsorbent particle at C_o concentrations below monolayer coverage as illustrated in Figure 5S at concentrations less than 0.0025 mg/mL. This relationship can then be used to generate a flow cytometric calibration curve. Quantitative adsorption isotherms generated by the outlined flow cytoemtric methodology have shown good correlation to independent solution depletion measurements for BSA,[5] IgG,[5] fibrinogen,[5] protein G[6] and SAPE.[7]

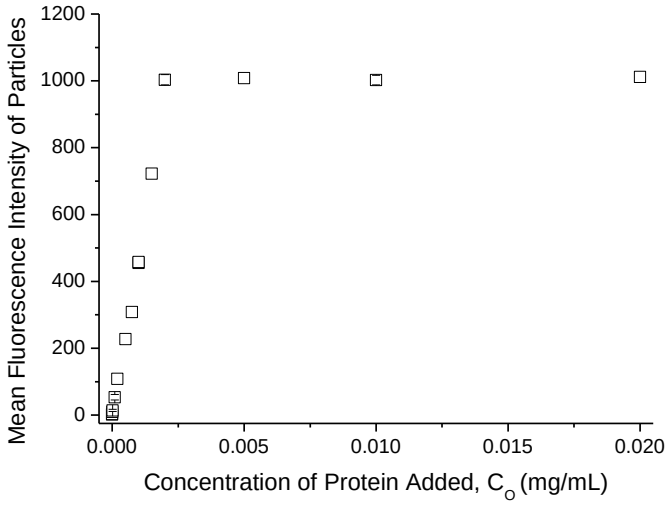
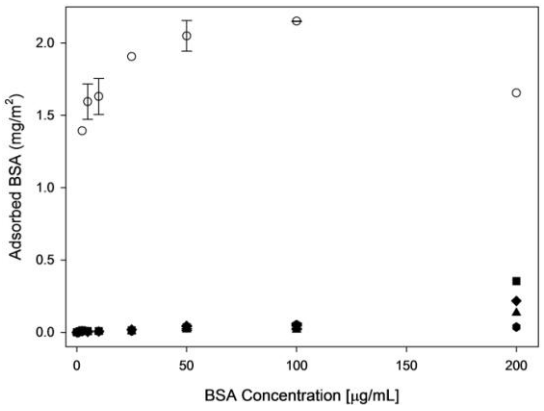
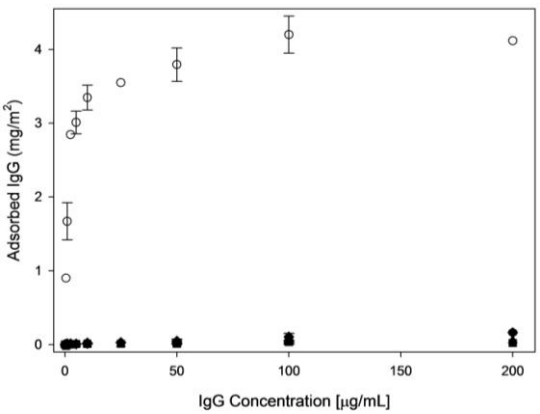


Figure 5S. Mean Fluorescence Intensity of particles incubated with increasing concentrations of fluorescent IgG.

Adsorption isotherms for BSA, IgG and SAPE, as shown in Figure 6S, gave rise to similar trends on the multiplexed polymer library. EMP modified particles always had the greatest adsorbed amount of protein and followed an expected Type I, monolayer limited, adsorption curve. We have previously observed similar high affinity monolayer limited protein (BSA, IgG and fibrinogen) adsorption on APS modified organosilica particles.[5] In contrast no significant protein adsorption was observed for any of the particles modified with acrylic acid and acrylic acid-NiPAM copolymers. Isotherm data points and errors at 1, 5, 10, 50 and 100 μg/mL are the average and standard deviation of three independent samples.





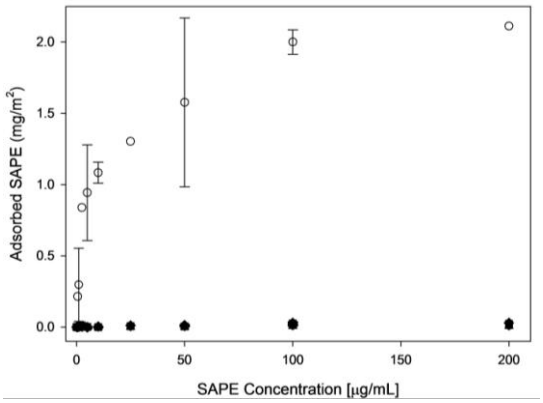


Figure 6S. Adsorption of A) BSA, B) IgG and C) SAPE on (○) EMP-particles, (■) Poly A, (▲) Poly B, (♦) Poly C, and (●) Poly D. Error bars represent the standard deviation of three independent measurements.

References

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