

Electronic Supporting Information

Physical Immobilization of Antibodies in Densely Grafted Polymer Brushes via Spot-Drying: Towards Optimal Protein Loading

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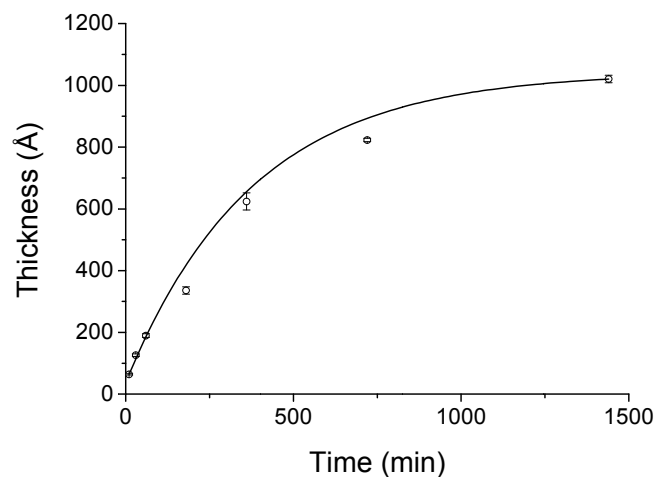


Fig. S1 Ellipsometric thickness of polymer brush as a function of time. Error bars represent standard deviation ($n = 5$).

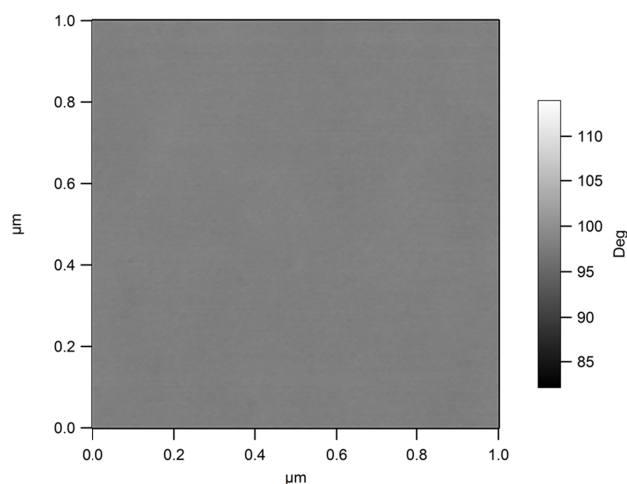


Fig. S2 AFM phase retrace of a representative pristine POEGMA grown to a thickness of ~ 60 nm.

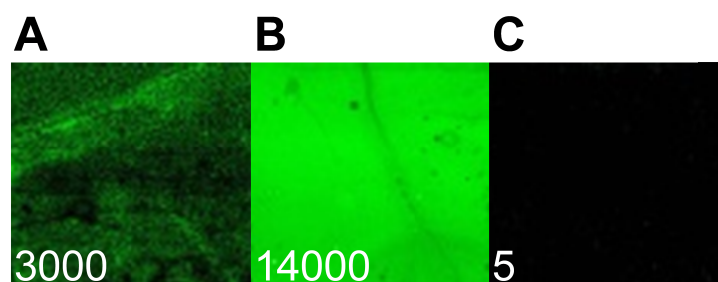


Fig. S3 A comparison of fluorescence intensities of an adsorbed layer of AlexaFluor 546-goat anti-human IgG on (A) bare, (B) aminopropyltriethoxysilane- and (C) POEGMA-treated glass substrates. The substrates were fully immersed in a solution of $1 \mu\text{g}/\text{mL}$ AlexaFluor 546-goat anti-human IgG in PBS buffer. The numbers at the bottom left of each scan represents the average fluorescence signal intensities expressed in arbitrary units.

Sample Calculations.

Below are sample calculations of the size scales presented in Table 1 based on polymer dry thickness of 6.40 nm. The expressions are obtained from Kim et. al.⁴⁶ and numbers in parenthesis represent the equation numbers as appeared in their paper.

Definition of parameters.

In their paper,

h is defined as brush height in a fully-stretched conformation.

Since spot drying involves the removal of macroscopic bulk water, the system technically undergoes a transition from a fully wet to fully dry state. Here, our approximations are based on the case where protein-brush interactions are assumed to occur just when the top of the brush is starting to dry up (i.e. we assume the evaporation of bulk water to be vertically homogeneous). At this point, based on the water-barrier model, the underlying layer is effectively still water-laden (i.e. brush is almost fully-stretched). We therefore take this into account and approximate $h = 1.5 \times$ dry thickness obtained via ellipsometry. The correction factor 1.5 is based on the report that pristine POEGMA thickness can increase by ~50% due to swelling in Milli-Q water.²⁴ Therefore for thickness of 6.40 nm:

$$h \cong 1.5 \times 6.40 = 9.60 \text{ nm}$$

a is defined as monomer size.

This was calculated from bulk density of the polymer (1.105 g cm^{-3} as stated by manufacturer).

$$a = \left(\frac{360 \times 10000000^3}{1.105 \times 6.022 \times 10^{23}} \right)^{\frac{1}{3}} = 0.8148 \text{ nm}$$

σ is defined as the graft density.

Since the initiator is deposited undiluted with other non-ATRP active moieties, we hereby assume the brushes formed were prepared at maximal grafting density. Hence, this was calculated based on maximum area, a^2 , which can be occupied by the monomer. However, the initiation step in surface-initiated ATRP is not 100% efficient. Based on Sumerlin, B. S., D. Neugebauer, et al. (2005). "Initiation Efficiency in the Synthesis of Molecular Brushes by Grafting from via Atom Transfer Radical Polymerization." *Macromolecules* **38**(3): 702-708, we will consider ~ 40% efficiency for our system. Therefore we include a correction factor of 0.4 in our calculation of σ .

$$\therefore \sigma \cong 0.4 \times \frac{1}{a^2} = 0.4 \times \frac{1}{0.8148^2} \cong 0.6025 \text{ chains/nm}^2$$

N is defined as the monomer units per polymer.

$$h = \sigma N a^3$$
$$\therefore N = \frac{h}{\sigma a^3} \cong \frac{9.60}{0.6025 \times 0.8148^3} = 29.455$$

Calculation of $\bar{\xi}_{blob}$ according to equation (7).

$$\bar{\xi}_{blob} \cong \frac{8Na^2}{h} \cong \frac{1}{\sigma a}$$
$$\therefore \bar{\xi}_{blob} \cong \frac{1}{0.6025 \times 0.8148} = 2.037 \text{ nm}$$

Calculation of ξ_{surf} according to equation (8).

$$\xi_{surf} \cong \left(\frac{8Na}{3\pi^2\sigma} \right)^{\frac{1}{3}}$$
$$\therefore \xi_{surf} \cong \left(\frac{8 \times 29.455 \times 0.8148}{3\pi^2 \times 0.6025} \right)^{\frac{1}{3}} = 2.208 \text{ nm}$$

Calculation of b^* according to equation (18).

$$b^* = \frac{2^{\frac{1}{3}}}{\pi} \times a \left(\frac{Na}{h} \right)^{\frac{2}{3}} = \frac{2^{\frac{1}{3}}}{\pi} \times 0.8148 \left(\frac{29.455 \times 0.8148}{9.60} \right)^{\frac{2}{3}} = 0.601 \text{ nm}$$

Calculation of b_{max} according to equation (21).

$$b_{max} = \left(\frac{Na^2}{2\pi^3\sigma} \right)^{\frac{1}{4}} \sim N^{\frac{1}{4}} = N^{\frac{1}{4}} = 2.330 \text{ nm}$$