Supplementary information

Protein adsorption on polymer brushes is a long-lived interfacial process

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Characterizing the PHEMA orthogonal gradients

The dry thickness of PHEMA brushes \((h)\) on orthogonal \(M-\sigma\) substrates can be deconvoluted into the contribution due to the brush molecular weight \((M)\) and grafting density \((\sigma)\). Specifically, \(M\) can be estimated from \(M \approx 1200h\), where \(M\) is in Daltons and \(h\) is in nanometers.1,2 This approximate relation has been obtained by growing chains simultaneously on small silica particles and on flat surfaces and determining \(M\) from polymers cleaved off the silica particles via size exclusion chromatography and \(h\) from polymers grown on flat surfaces via ellipsometry. Although this relationship has been found to be valid for a range of methacrylates and acrylates grown from BMPUS initiator layers deposited under identical conditions, it should be considered as an estimate only because it assumes that chains grown under confinement possess the same rate of polymerization as those polymerized in solution. The relationship also assumes that the rate of polymerization does not depend strongly on the density of the growing chains on the surface. In spite of these limitations, this relationship provides a very reasonable estimate for

\[ \sigma \approx 0.45 \text{ chains/nm}^2. \] The dry thickness in PHEMA orthogonal samples as well as the corresponding \( M \) and \( \sigma \) gradients for each sample reported in this study is shown in Figure S1. As apparent from the plots both \( M \) and \( \sigma \) vary gradually in two orthogonal directions. This justifies that brush length (or \( M \)) does not depend strongly on the density of the initiators on the surface.

**Adsorption of Lys from concentrated solutions**

We have also performed a complete set of adsorption experiments of protein adsorption onto PHEMA orthogonal substrate using Lys solutions having concentration of 1 mg/mol of Lys. The resultant adsorption isotherms are plotted in Figure S2. As apparent from the data, the amount of adsorbed Lys remains independent of the coverage of PHEMA on the substrate (\( \sigma N \)). While for 24 hrs adsorption the isotherms display the same shape as those reported earlier for the more dilute solutions, for longer adsorption times, the amount of Lys is seen to increase with increasing PHEMA coverage. This rather unexpected result is attributed to the complexity of the system, where protein-protein interactions are no longer negligible and as a result proteins adsorb onto the polymer-coated substrates in the form of multilayers. It is challenging to provide more insight into what governs protein adsorption under such high concentration without resorting to *in situ* monitoring of the process using some imaging technique, such as atomic force microscopy in solution.
Figure S1  Dry thickness of PHEMA as measured by ellipsometry (left panel) can be deconvoluted to obtain information about the position-dependent variation of PHEMA molecular weight ($M$, middle panel) and grafting density ($\sigma$, right panel). In all samples the variation of $M$ and $\sigma$ is orthogonal, as expected.
Figure S2  Dry thickness of lysozyme (Lys) as a function of the dry thickness of PHEMA on $M\sigma$ orthogonal PHEMA gradients after depositing Lys (concentration 1 mg Lys/ml solution, pH=10, phosphate buffered solution) for 24 (A), 54 (B), 72 (C), 72 (D), and 96 (E) hours. The legend in the upper left corner indicates the direction of increased PHEMA grafting density ($\sigma$) on the substrate.