

Strongly Stretched Protein Resistant Poly(ethylene glycol) Brushes Prepared by Grafting-To

*Gustav Emilsson, Rafael L. Schoch, Laurent Feuz, Fredrik Höök, Roderick Y. H. Lim and Andreas B. Dahlin.**

1: Department of Applied Physics, Chalmers University of Technology, Göteborg, Sweden.

2: Biozentrum and the Swiss Nanoscience Institute, University of Basel, Switzerland.

* Corresponding author: adahlin@chalmers.se

Polymer brush theory

The end to end distance r of a single linear homopolymer in a good solvent is a random variable. The expected value of r , denoted R , is well described by the Flory radius:

$$R_F = aN^{3/5} \quad (\text{S1})$$

The exponent $3/5$ arises from balancing the excluded volume entropy (self-avoidance) and the conformational entropy (coil stretching). In Equation S1 a is the statistical step length in an idealized freely jointed chain. Importantly, the chemical monomer length in real polymers is significantly shorter than the value corresponding to a step in a random walk because covalent bonds are generally not that flexible. If we still let N represent the degree of polymerization (the number of chemical monomers), Equation S1 needs to be rescaled to be valid. This can be achieved by replacing a with the length along the chain that actually corresponds to a random walk step, i.e. the Kuhn length b . Also, N needs to be replaced with the number of Kuhn lengths, which is equal to the contour length aN divided by b . N is simply the molecular weight M divided by the weight of one monomer (44 g mol^{-1} for PEG). In summary, to accurately calculate coil size one must have information about both the contour length of the chain and its flexibility. Values of a and b can change with the physiochemical environment (mainly solvent, ionic strength, pH and temperature).

For coils end-grafted to a planar surface with Γ coils per area, there is significant stretching when Γ is higher than R_F^{-2} . Assuming that the end to end distance of the coils in the brush is equal to the brush height h and that each coil in the brush occupies a volume of h/Γ , the free energy G of a coil can be written as:

$$\frac{G(h)}{k_B T} = \frac{3h^2}{2Na^2} + \frac{\Gamma N^2 a^3}{h} + \text{constant} \quad (\text{S2})$$

The “constant” is constant in the sense that it does not depend on h . Equation S2 is analogous to the free energy of a coil in solution in the sense that the terms represent conformational entropy and excluded volume entropy. Differentiating and solving for the value of h that minimizes G gives:

$$H = \left[\frac{\Gamma}{3} \right]^{1/3} a^{5/3} N \quad (\text{S3})$$

Models such as Equation S3 (the Flory argument in solution transferred to a surface) are usually considered as decent approximations of brush height if the coils are in the “strongly stretched” regime.[S1] When using Equation S3, it should be rescaled to use Kuhn steps as described for the Flory radius ($a \rightarrow b$ and $N \rightarrow aN/b$), which results in the expression given in the main text. Equation S3 is thus based on the exact same principles as Equation S1 but holds for polymers on a surface. Thus, when calculating H one can use the same values for monomer size a and Kuhn length b as those for a polymer in solution (as long as it is the same solution in the same physiochemical environment).

Although Equation S3 is considered to be a quite good model to determine brush height, it does not give a realistic monomer density profile $C(z)$. In fact, the Flory argument treats the polymer as a homogenous “gas” of monomers and predicts a constant monomer concentration $C = \Gamma N/H$ along with distance from the surface, which then suddenly drops to zero at $z = H$. A more realistic density profile is that of a parabolic function[S1] which in essence can be expressed as:

$$C(z) = A - Bz^2 \quad (\text{S4})$$

To generate the plots in the main text, the parameters A and B were determined from the condition from Milner:[S1]

$$C(1.3H) = 0 \quad (\text{S5})$$

And that the total amount of monomers must be the same as for the Flory step function:

$$\int_0^{1.3H} C(z) dz = \Gamma N \quad (\text{S6})$$

SPR theory

The SPR reflectivity spectrum can be modelled by Fresnel equations using a transfer matrix formalism. In brief, the reflectivity from an arbitrary layer of isotropic and non-magnetic thin films can be calculated as long as thickness and refractive index are known for each film. There is much literature available on this topic (see e.g. our work[S2] and the webpage <http://www.adahlin.com/> for a MATLAB implementation) and no further details will be given here.

Refractivity of PEG

To determine the refractive index increment for PEG, different concentrations of non-thiolated, PEG with an average molecular weight of 8 kDa were measured in SPR. These data also verified that there was no detectable interaction between PEG and Au in the absence of a thiol group. (There was only an immediate signal and the baseline was recovered upon rinsing.) The angular shift, at a wavelength of 785 nm, was converted into a change in refractive index using the specified bulk sensitivity for the instrument of 105 degrees/RI (also verified by us experimentally). The refractive index increment for PEG is given by the slope of the straight line fitted to the refractive index versus concentration (Fig. S1).

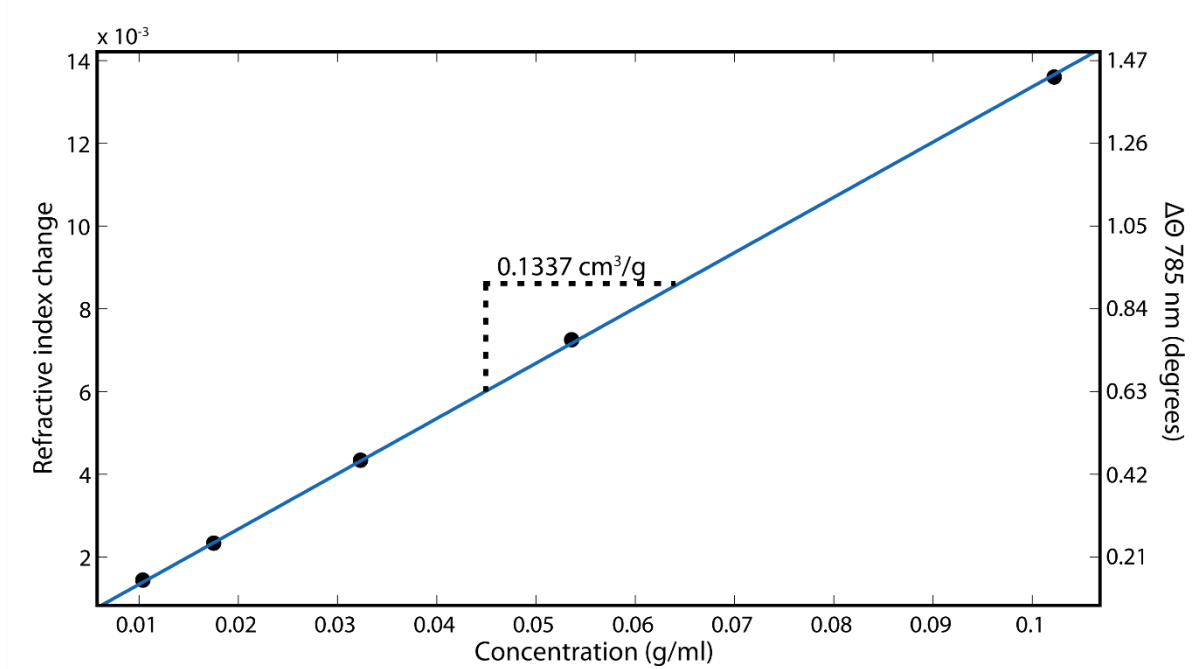


Figure S1. SPR response for different concentrations of non-thiolated PEG (8 kDa). The signals are due to the bulk refractive index change. The refractivity of PEG at 785 nm is determined from the slope of the linear fit.

Determination of wet brush height

The theory for determining brush height by non-invasive probes in SPR has been described previously.[S3] The thickness of the polymer brush is given by:

$$d = \frac{\delta}{2} \ln \left(\frac{R_{\text{ref}}}{R} \right) + d_{\text{ref}} \quad (\text{S7})$$

Here R is the response of the SPR instrument and δ is the decay length of the evanescent field. The reference OEG coating has an approximate thickness of $d_{\text{ref}} = 2$ nm and any error in this value clearly does not influence the brush thickness determination much when it is on the order of tens of nm. The effective decay length of the field is a somewhat more important source of error, which is also evident from Equation S7. We calculated an effective field decay length of 368 nm using

Fresnel models for simulating dielectric coatings of different thickness. The value was verified using the previously presented iterative procedure associated with the non-invasive probe method.[S3] The measured heights and calculated refractive index values of the polymer brushes were plotted in a so called “decay length map”[S3] (Fig. S2). The generation of the map was done with the appropriate parameters for the SPR instrument used. Especially the fact that the measurements were done with 785 nm incident light needs to be taken into account. All permittivity parameters were obtained by fitting experimental spectra to Fresnel equations.

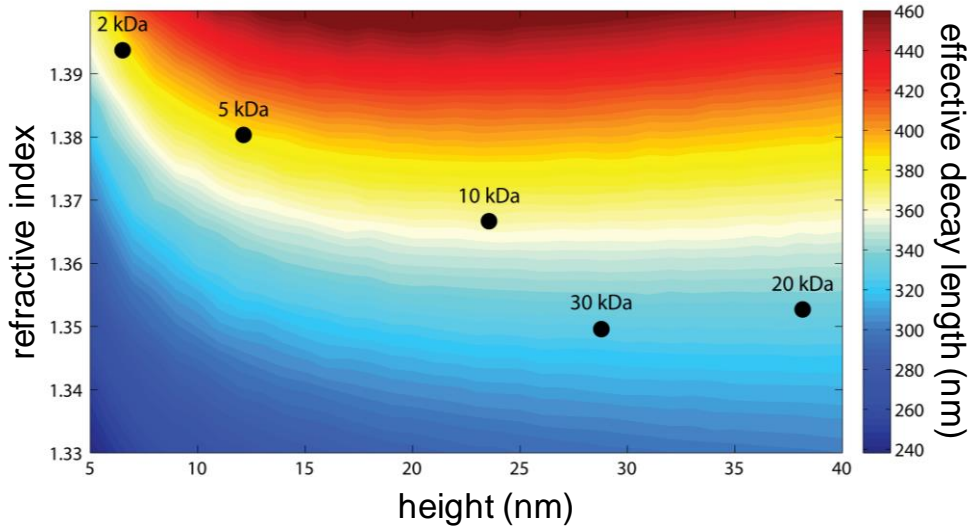


Figure S2. Effective “decay length map” showing how the estimated decay length of 368 nm represents a good average for the PEG brushes since they fall on approximately the same line (maximum error of ~10%).

Quantification of serum adsorption

When quantifying the response from serum adsorption, one can utilize a linear relation between surface coverage and SPR signal which appears when the thickness d is much smaller than δ . We give a simple proof here. The general relation between surface coverage Γ (here mass per area) and signal $\Delta\theta$ for adsorption directly on the surface is:[S4]

$$\Gamma = \frac{d\Delta\theta}{S_0 b \left[1 - \exp\left(-\frac{2d}{\delta}\right) \right]} \quad (\text{S8})$$

Here S_0 is the bulk sensitivity (angular shift per refractive index increment) and b is the increase in refractive index with concentration (0.184 cm³/g is the accepted average value for proteins). A Taylor expansion of $\exp(-2d/\delta)$ around $d = 0$ gives $\exp(-2d/\delta) \approx 1 - 2d/\delta + 4d^2/\delta^2$ which means that:

$$\Gamma = \frac{\Delta\theta}{S_0 b} \times \frac{d}{1 - 1 + \frac{2d}{\delta} - \frac{4d^2}{\delta^2}} = \frac{\Delta\theta}{S_0 b} \times \frac{1}{\frac{2}{\delta} - \frac{4d}{\delta^2}} \quad (\text{S9})$$

When $d \rightarrow 0$ the result is:

$$\Gamma = \frac{\Delta\theta\delta}{2S_0 b} \quad (\text{S10})$$

Hence d is no longer relevant when much smaller than δ , so that only the field decay length is needed. For quantifying the serum response using Equation S10 we simply estimated δ from the field distribution of the surface plasmon (Fig. S3). The program used to calculate the field is described in literature[S5] and available at <http://www.adahlin.com/> with a MATLAB implementation. The experimentally determined parameters for the Cr and Au layers (thickness and permittivity) were used, resulting in $\delta = 218$ nm.

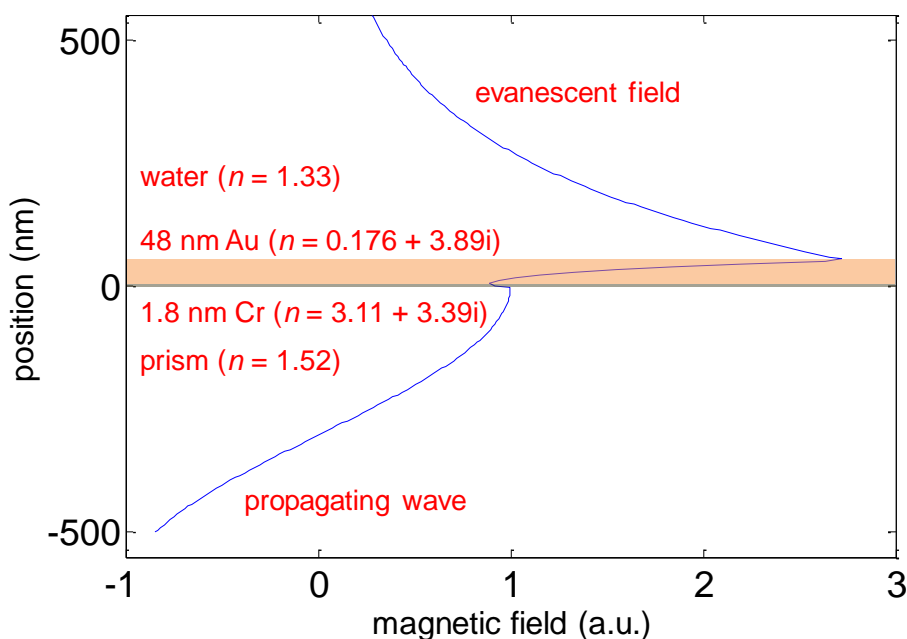


Figure S3. Transverse magnetic field of surface plasmon at a vacuum wavelength of 670 nm in two finite metal films with parameters as specified in the plot. The field is not decaying into the prism because the mode exchanges energy at this side (excitation through Kretschmann configuration). The field in the other semi-infinite medium is evanescent, showing an exponential decay with a decay length of 218 nm.

Multiple serum injections

To test long-term inertness of the PEG brushes, repeated serum injections were conducted in the same manner as described in the main text. In between each injection the surface was rinsed with PBS for 20 minutes (5 μ L/min). The shifts were taken as the difference in angle before the first injection and after rinsing for 10 minutes (Fig. S4). As can be seen, the uncertainty in these experiments is high since the signals are very small and the baseline drift comes into play over such a long times (over 10 h). We attempted to compensate for the instrumental drift by a linear correction for the baseline. The result is that a weak increase in the serum response can be seen

after multiple injections but the effect is not strong and the brushes remain inert for the typical timescale of most experiments.

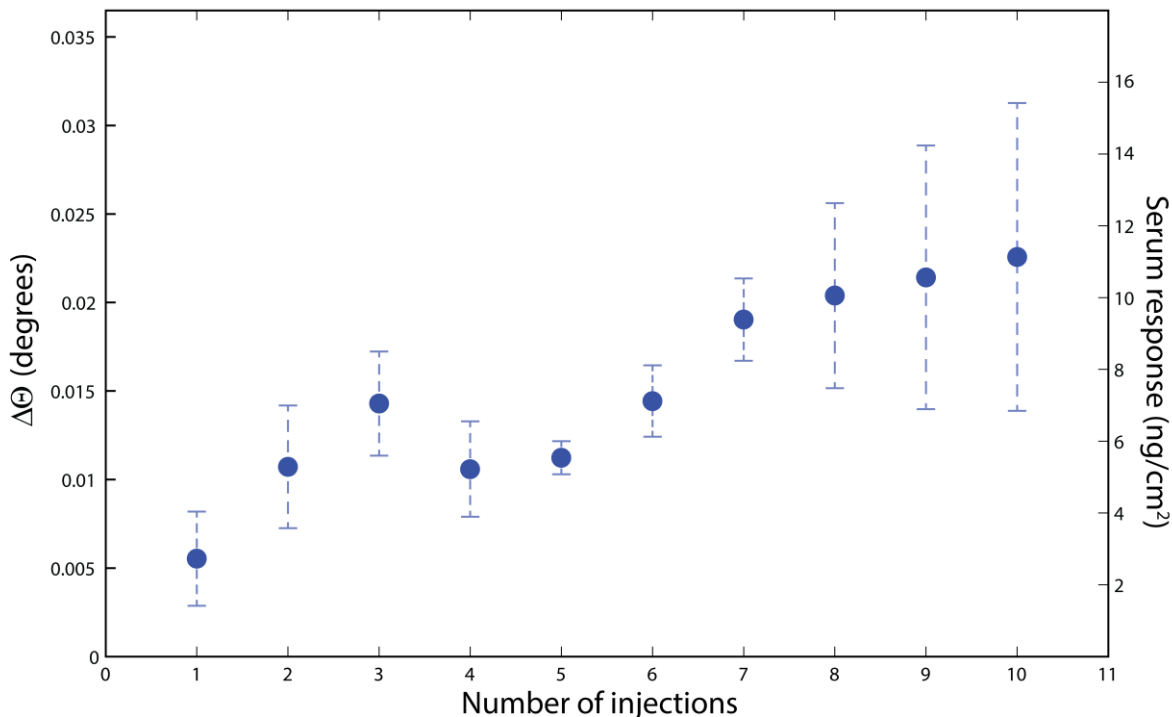


Figure S4. Total response to repeated serum injections for -OH terminated 10 kDa PEG. Each injection is 50 min long and in between a 20 min rinse with PBS was performed.

Depletion layer thickness

The dimensions of the channels in the SPR instrument are $\sim 2 \times 5 \times 0.1$ ($W \times L \times H$) mm³. The measurement area is ~ 2 mm from the inlet. We use this as the length of the sensor in the calculation since binding occurs up till this region of interest. As an example we calculate the Peclet numbers and depletion layer thickness for various diffusion constants. The formalism is given in the references.[S6] For 10 kDa PEG in water at room temperature the diffusion constant is ~ 72 $\mu\text{m}^2/\text{s}$, [S7] which can act as a rule of thumb. In the solvent used for the grafting (0.9 M Na₂SO₄) all of the diffusion constants should be larger since the diffusion constant scales inversely with

coil radius. The flow rate is $Q = 15 \mu\text{L}/\text{min}$. The Peclet number is $\text{Pe} = Q/[DW]$ with $\lambda = L/H = 20$. The shear Peclet number is then $\text{PeS} = 6\lambda^2\text{Pe}$ and the depletion layer thickness is given by $\delta = L/\text{PeS}^{1/3}$. Fig. S5 shows the depletion layer thickness in the SPR flow cell for different diffusion constants. Importantly, the curve in Fig. S5 represents a “worst case scenario” of mass transport as the bottleneck since it is assumed that molecules bind instantly when reaching the surface. Even so, the depletion layer thickness is only 10% of the channel height, suggesting little influence from mass transport in the binding kinetics.

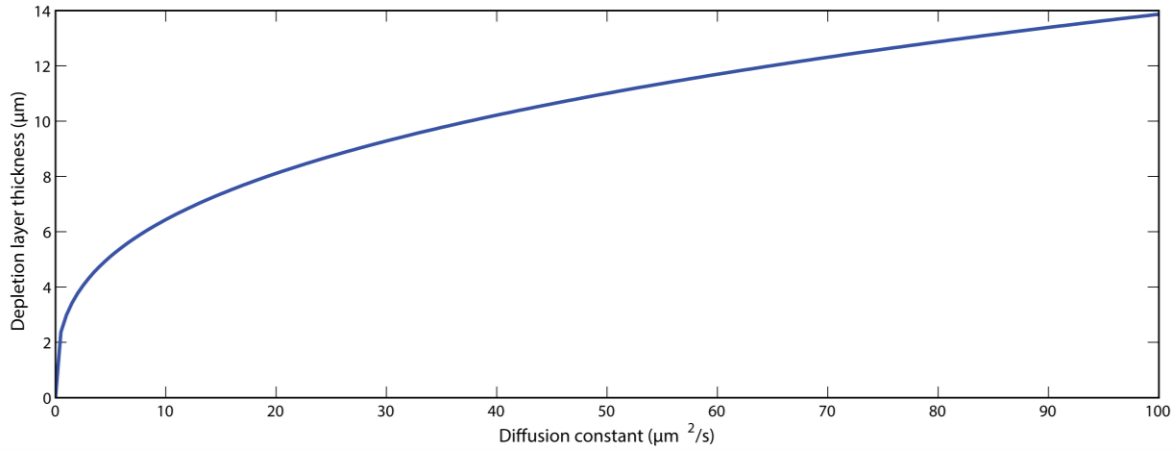


Figure S5. The depletion layer thickness for the flow cell used for different diffusion constants.

References

- S1 Milner, S. T., Polymer brushes. *Science* 1991, 251 (4996), 905-914.
- S2 Junesch, J., T. Sannomiya, and A.B. Dahlin, Optical properties of nanohole arrays in metal–dielectric double films prepared by mask-on-metal colloidal lithography. *ACS Nano* 2012, 6 (11), 10405-10415.
- S3 Schoch, R.L. and R.Y.H. Lim, Non-interacting molecules as innate structural probes in surface plasmon resonance. *Langmuir* 2013, 29 (12), 4068-4076.
- S4 Jung, L.S., et al., Quantitative interpretation of the response of surface plasmon resonance sensors to adsorbed films. *Langmuir* 1998, 14 (19), 5636-5648.
- S5 Dahlin, A.B., et al., Plasmonic nanopores in metal-insulator-metal films. *Advanced Optical Materials* 2014, 2 (6), 556-564.
- S6 Squires, T.M., et al., Making it stick: convection, reaction and diffusion in surface-based biosensors. *Nature Biotechnology* 2008, 26 (4), 417-426.
- S7 Mark, J. E., Polymer data handbook. Oxford University Press: New York, 1999.