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Supplementary Information for:

Biomimetic non-fouling surfaces: extending the concepts

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Materials

CuCl, CuBr, $CuBr_2$, 2,2'-dipyridyl (BiPy), 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane (Me_4Cyclam), 2-bromo-2methylpropanoyl bromide, triethylamine, and 4-(dimethylamino) pyridine were purchased from Sigma-Aldrich. *N*-[3-(dimethylamino)propyl] acrylamide (DMAPA, 98%) and β-propiolactone (90%) were from TCI Europe and Serva Electrophoresis GmbH, respectively. Monomer (2-hydroxyethyl methacrylate (HEMA)) and macromonomers (oligo(ethylene glycol) methyl ether methacrylate M_n 300 (MeOEGMA) and oligo(ethylene glycol) methacrylate M_n 526 (HOEGMA)) inhibited with 900 ppm of hydroquinone monomethyl ether were from Aldrich. The inhibitor was removed by passing through a basic alumina column immediately before the polymerization experiment.

Human serum albumin (HSA, 99% by electrophoresis), fibrinogen from human blood plasma (Fbg) and pooled human blood plasma (HBP) were from Sigma. *Solvents:* Ethanol (99.8%), tetrahydrofuran (THF, 99.5%) and methanol (99.6%) were purchased from Lachner. Phosphate buffered saline, pH 7.4 (PBS), and TRIS buffer, 50 mM, pH 7.2, were from Sigma-Aldrich.

Synthesis of carboxybetaine acrylamide:

(3-Acryloylamino-propyl)-(2-carboxy-ethyl)-dimethyl-ammonium (CBAA) was synthesized by the modified procedure published earlier.[1] DMAPA (7.8 g, 55 mmol) was dissolved in 100 mL of anhydrous THF and cooled to 0 ºC. Subsequently, β-propiolactone (5.0 g, 69 mmol) was dissolved in 40 mL of THF and added under nitrogen. The reaction was allowed to proceed for 24 hours at 4 ºC. The white precipitate was washed with dry THF and ether. Yield: 80 %. ^{1}H NMR (Bruker 250 MHz in D_2O: 6.32 (t, 1H, CHH=C*H*), 6.2 (t, 1H, CH*H*=CH), 5.87 (t, 1H, C*H*H=CH), 3.66 (t, 2H, N-CH_2-CH_2-COO), 3.48 (m, 4H, NH-CH_2-CH_2-CH_2), 3.17 (s, 6H, N-$(CH_3)_2$), 2.75 (t, 2H, CH_2-COO).

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Measurement of non-specific protein adsorption

On gold-coated BK7 glass SPR chips: The irreversible fouling from biological media on chips coated with the antifouling films was measured using Surface Plasmon Resonance (SPR) using an instrument based on the Kretschmann geometry and spectral interrogation developed at the Institute of Photonics and Electronic of the Academy of Sciences of the Czech Republic. This sensor is based on the spectroscopy of surface plasmons.[1, 2] The shift in the resonant wavelength is proportional to the refractive index change at the interface of the sensor and the tested medium. Broadband light from a halogen lamp (Mikropack) was collimated, polarized and coupled with the SPR sensing element using a prism. The SPR chip consist of a glass coated with an adhesion-promoting titanium film (thickness 2 nm) and a gold film (thickness 50 nm) which is interfaced with the prism using a Cargille matching oil (n_D = 1.5150 ± 0.0002). Upon the incidence on the gold film, the light beam excites a surface plasmon at a wavelength of 750 nm on four independent sensing spots. The reflected light is collected into four optical fibers and couple to an Ocean Optics spectrophotometer. The acquired spectra were analysed in real time with the software package SPR-UP developed in the Institute of Photonics (Czech Republic) that allowed the determination of the resonant wavelength for each sensing channel. The refractive index resolution was 3 x 10^{-7} RIU. A low volume (1 µL) flow-cell with four separate chambers facing each of the sensing spots was used. The sensor was equipped with a temperature controller which enables to stabilise the temperature in a range of 0.01 ºC.

The tested solutions were driven for 15 min at 25 µL·min^{-1} by a peristaltic pump through four independent channels of a flow cell in which SPR responses were measured simultaneously. Typically, PBS was flowed until a stable baseline was achieved (approximately 15 min). Subsequently, it was replaced by the samples to be tested for 15 min and then replaced once

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again with PBS. The sensor response ($\Delta\lambda_{Res}$) was obtained as the difference between the baselines in PBS before and after the injection of the tested samples. The sensor response can be calibrated to the mass deposited at the surface of bound molecules. For this SPR working in the vicinity of λ_{Res} 750 nm a 1 nm SPR wavelength shift represents a surface coverage of 15 ng·cm^{-2}.[1, 3, 4] The limit of detection (LOD) was estimated as the sensor response equivalent to 3 standard deviations of the baseline noise. For this set-up the LOD corresponds to a SPR wavelength shift of 0.02 nm, *i.e.* a surface coverage of 0.3 ng·cm^{-2}.

The interaction of pooled and undiluted human fluids: blood plasma (HBP), model solutions of plasma proteins; human serum albumin (HSA, 5 mg·mL^{-1}) and fibrinogen (Fbg, 1 mg·mL^{-1}) in phosphate buffered saline (PBS, pH 7.4) were also studied for comparison. All the fouling studies were carried out in triplicate. Undetectable fouling, *i.e.* below the SPR detection limit (0.3 ng·cm^{-2}) was equated to zero.

Protein fouling

The SPR results on non-fouling properties of the polymer brushes grown from the PDA surface were verified by independent *ex situ* IRRAS and SE measurements on replica samples bearing polymer brushes with the same characteristics as the ones used in the *in situ* study. The IRRAS and SE characterized samples bearing polymer brushes of poly(HOEGMA), poly(MeOEGMA), poly(HEMA) and poly(CBAA) grown from the PDA anchor layer by SI-ATRP were immersed in PBS and HBP for the same time periods as in the SPR experiments, i.e. subsequent immersion in PBS, HBP and PBS for 15 min for each step. In order to remove the salt remains, the samples were washed with copious amounts of water and dried under stream of

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nitrogen. The samples were measured by IRRAS and SE once again to determine the amount of plasma deposits and compare them to the values determined for the bare gold surface.

Ex situ verification of SPR results by IRRAS

In the region 1800−1500 cm^{-1} the IRRAS spectrum of plasma deposits on the bare gold surface is dominated by two prominent amide I and amide II bands at 1667 and 1547 cm^{-1}, respectively. The spectra of deposits from HBP on the polymer brushes can be obtained by subtraction of the corresponding (HOEGMA), poly(MeOEGMA), poly(HEMA) and poly(CBAA) /PDA backgrounds from the IRRAS spectra obtained after the immersion (Figure 5). The comparison of the corresponding areas of the amide bands arising from the plasma deposits on polymer brushes with the ones obtained from the gold layer resulted in adsorbed amounts of 1.2 %, 5.2 %, 0.7 % and 1.0 % for poly(CBAA), poly(HEMA), poly(MeOEGMA) and poly(HOEGMA), respectively, from the plasma deposits on bare gold.

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Ex situ verification of SPR results by SE

Figures S1− S5 report the changes in SE spectra of bare gold and brushes of poly(HOEGMA), poly(MeOEGMA), poly(HEMA) and poly(CBAA) grown from PDA caused by adsorption of HBP. Essentially the same SE data are obtained for the as prepared polymer brushes and the ones exposed to HBP. The increase in layer thickness due to adsorbed plasma was 6.60 ± 0.66 nm on bare gold and 0.08 ± 0.05 nm, 0.18 ± 0.10 nm, 0.05 ± 0.03 nm and 0.11 ± 0.06 nm for poly(CBAA), poly(HEMA), poly(MeOEGMA) and poly(HOEGMA), respectively. The SE data on the non-fouling properties of the brushes, expressed as increase in ellipsometric thickness is in agreement with the SPR and IRRAS findings (Table S1).

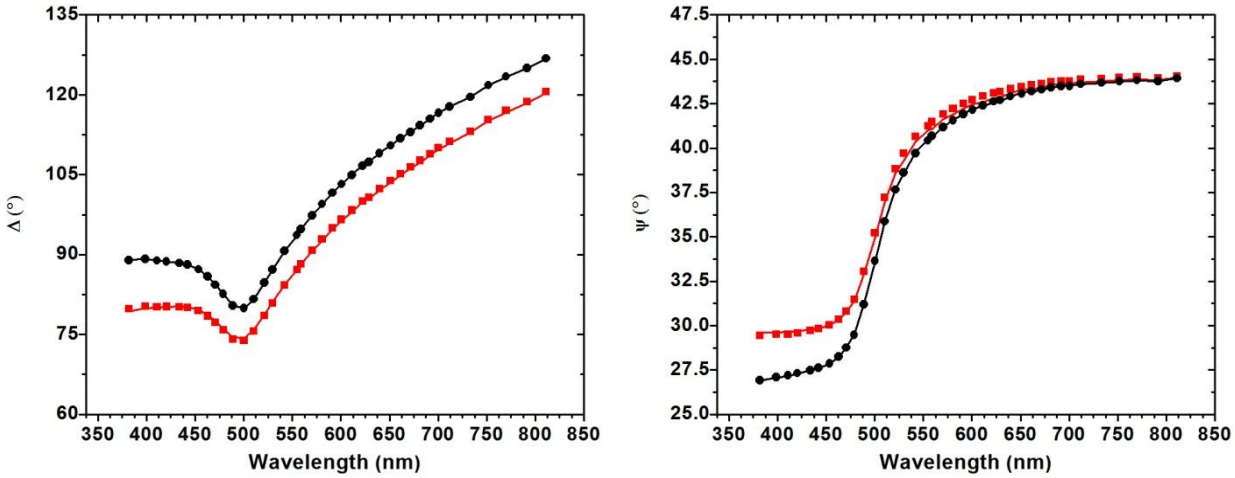

Figure S 1. Change in Δ and Ψ ellipsometric data of bare Au (●) caused by adsorption of HBP (■). The curves are the corresponding fits (MSE ~ 1.1 (Mean-Squared Error)) to the measured data resulting in formation of 6.6 ± 0.7 nm thick protein deposit on the gold surface.

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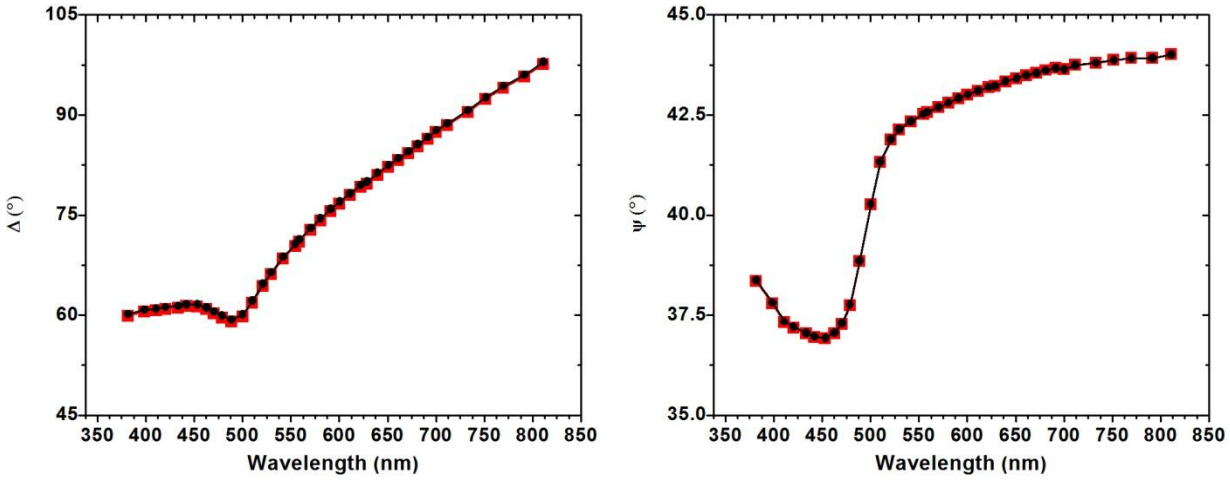

Figure S 2. Change in Δ and Ψ ellipsometric data of poly(CBAA) brush (17.4 ± 1.1 nm) grown from PDA anchor layer (13.0 ± 2.0 nm) by SI-ATRP (●) caused by adsorption of HBP (■). The curves are the corresponding fits (MSE ~ 1.5) to the measured data resulting in formation of 0.08 ± 0.05 nm thick protein deposit on the poly(CBAA) brush.

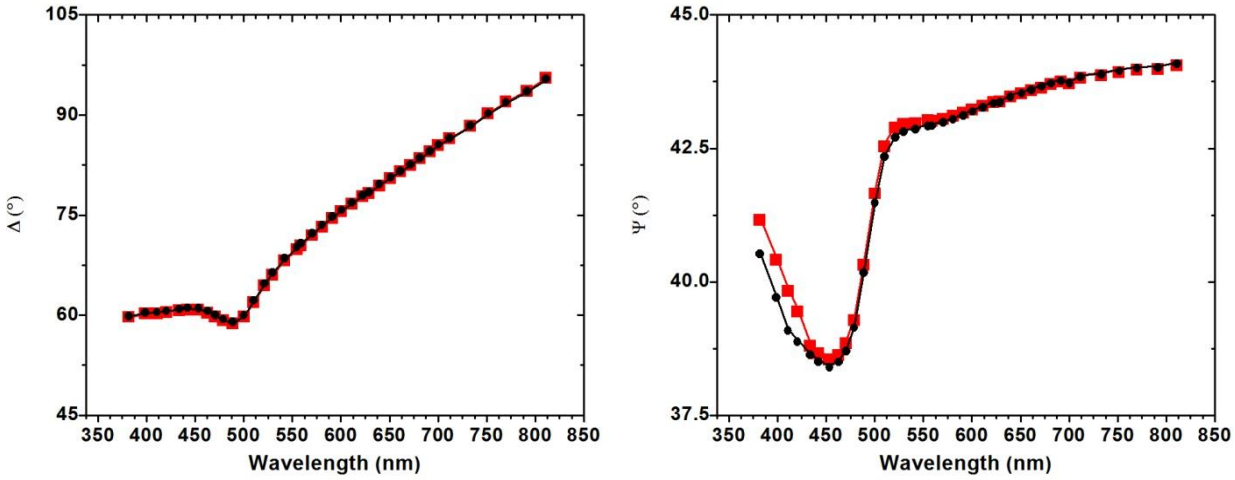

Figure S 3. Change in Δ and Ψ ellipsometric data of poly(HEMA) brush (18.0 ± 2.1 nm) grown from PDA anchor layer (13.0 ± 2.0 nm) by SI-ATRP (●) caused by adsorption of HBP (■). The curves are the corresponding fits (MSE ~ 1.7) to the measured data resulting in formation of 0.18 ± 0.08 nm thick protein deposit on the poly(HEMA) brush.

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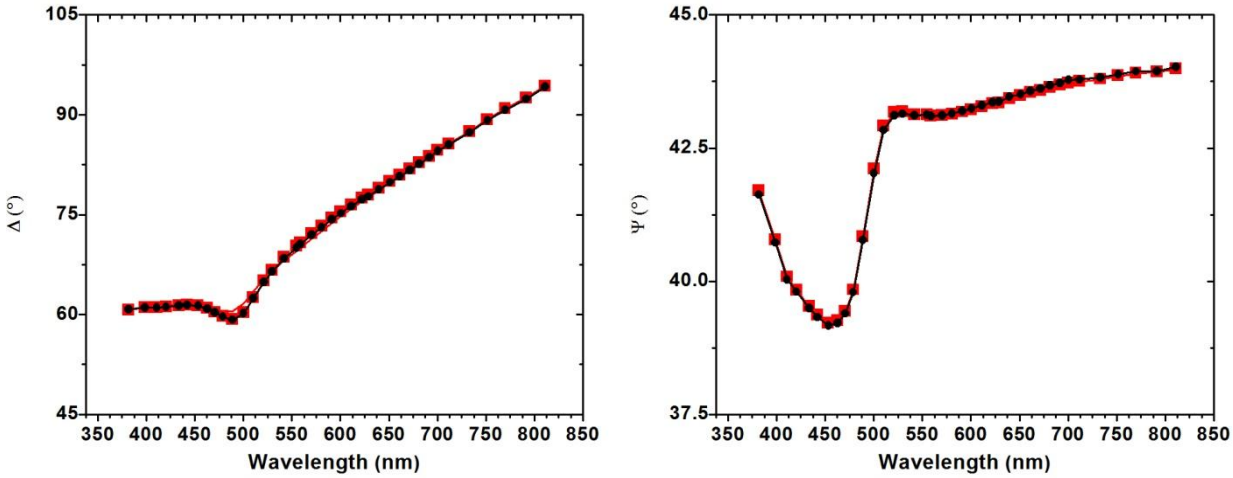

Figure S 4. Change in Δ and Ψ ellipsometric data of poly(MeOEGMA) brush (18.4 ±0.8 nm) grown from PDA anchor layer (13.0 ± 2.0 nm) by SI-ATRP (●) caused by adsorption of HBP (■).The curves are the corresponding fits (MSE ~ 1.7) to the measured data resulting in formation of 0.05 ± 0.03 nm thick protein deposit on the poly(MeOEGMA) brush.

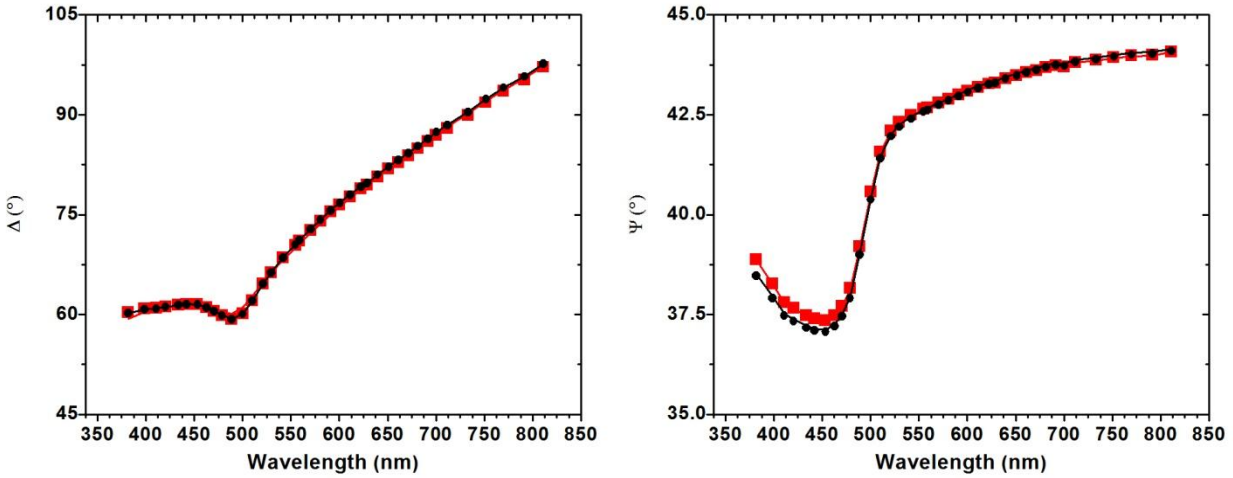

Figure S 5. Change in Δ and Ψ ellipsometric data of poly(HOEGMA) brush (20.1 ± 1.1 nm) grown from PDA anchor layer (13.0 ± 2.0 nm) by SI-ATRP (●) caused by adsorption of HBP (■). The curves are the corresponding fits (MSE ~ 2.2) to the measured data resulting in formation of 0.11 ± 0.06 nm thick protein deposit on the poly(HOEGMA) brush.

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Table S1. Comparison of non-fouling properties of poly(CBAA), poly(HEMA), poly(MeOEGMA) and poly(HOEGMA) brushes formed from the PDA surface by SI-ATRP as determined by SPR, IRRAS and SE. Adsorption of HBP to bare gold is taken as a reference.

Technique	SPR	IRRAS	SE	
Units	[%]	[%]	[nm]	[%]
Bare Au	100	100	6.60 ± 0.66	100
CBAA	0.5	1.2	0.08 ± 0.05	1.2
HEMA	3.7	5.2	0.18 ± 0.08	2.7
MeOEGMA	0.0	0.7	0.05 ± 0.03	0.8
HOEGMA	1.0	1.0	0.11 ± 0.06	1.7

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