SPR Biosensing using a MUA/ Poly-L-lysine Platform for the Detection of 2,4-Dinitrophenol as Small Molecule Model System

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1. Characterization of the 2,4-dinitrophenol- protein conjugates

In order to evaluate the success in conjugation, UV- visible espectra were acquired with a spectrophotometer from Perkin Elmer (Waltham, MA, USA). Qualitative differences respect to DNBS reactive and to the carrier protein suggest successful DNP conjugation to the carrier protein. Absorption at 360 nm is due to DNP-εNH_2 lysine (BSA) covalent bond formation[1] and thus verifies the successful conjugation of 2,4- dinitrophenol to BSA. (Figure S1).

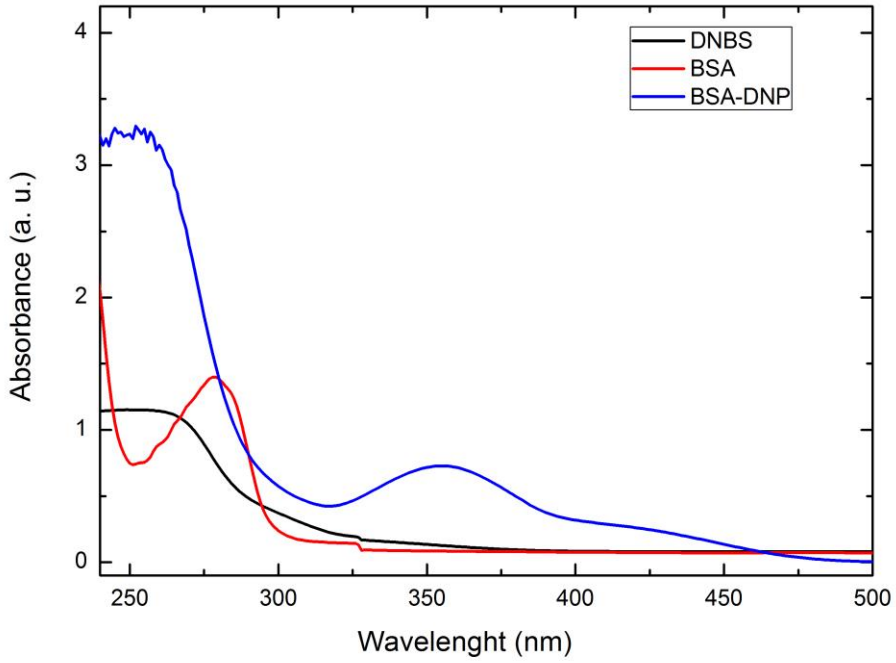


Figure S1. UV-vis spectra of BSA (red), BSA-DNP (blue) and DNBS reactive employed in conjugation (black) in PBS buffer solution. Absorption at 360 nm is due to DNP-εNH_2 lysine (BSA) covalent bond formation.

The degree of conjugation of BSA with DNP molecules was assessed by ultraviolet matrix assisted laser desorption-ionization mass spectrometry (UV–MALDI MS) performed at the Proteomics Core Facility CEQUIBIEM, at the University of Buenos Aires/CONICET (National Research Council) using an Ultraflex II (Bruker Daltonics) MALDI TOF. Spectra were analyzed with Flex Analysis Software (Bruker Daltonics) verifying, by mass difference, the attachment of 2,4-dinitrophenol to the carrier protein. Figure S2 shows a conjugate with 14 DNP molecules per carrier protein, although conjugates showed up to 25 DNP molecules per BSA.

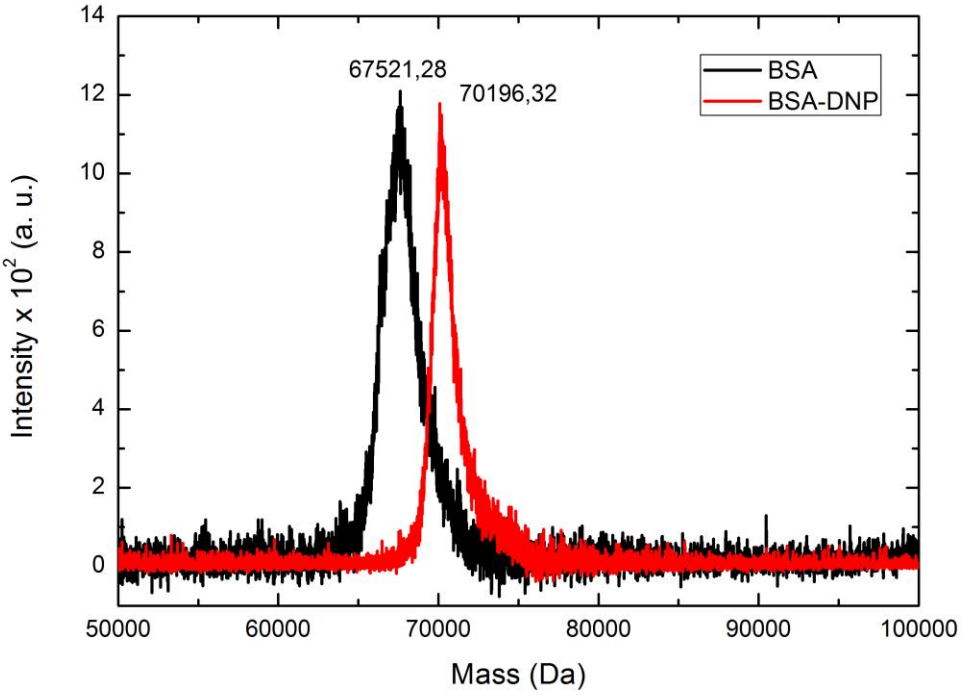


Figure S2. MALDI-TOF spectra of BSA (black) and BSA-DNP conjugate (red). Mass difference yields 14 DNP molecules per BSA (conjugates showed up to 25 DNP molecules per BSA).

2. SPR curve fitting for PLL thickness determination

SPR curves were fitted with WinSpall 3.02 software based on the Fresnel equations and recursion formalism, freely available from Res-Tec GmbH. The two wavelength method employed is based on the fact that refractive index (RI) wavelength dependency can be approximated as linear for ultrathin films (thickness d < 50 nm). First, SPR curves of gold substrate were simulated in order to obtain the effective parameters of the thickness and dielectric constant of bare gold in Milli-Q® water (Fig. S3) for both wavelengths (670 and 785 nm). Once the parameters were obtained, an additional layer for MUA-PLL was included with RI 1,52[2] and the fitting was performed until a d value was reached. This values were 2.95 ± 0.02 nm at 670 nm and 3.06 ± 0.09 nm at 785 nm, we calculated an average of 3.0 ± 0.1 nm to report.

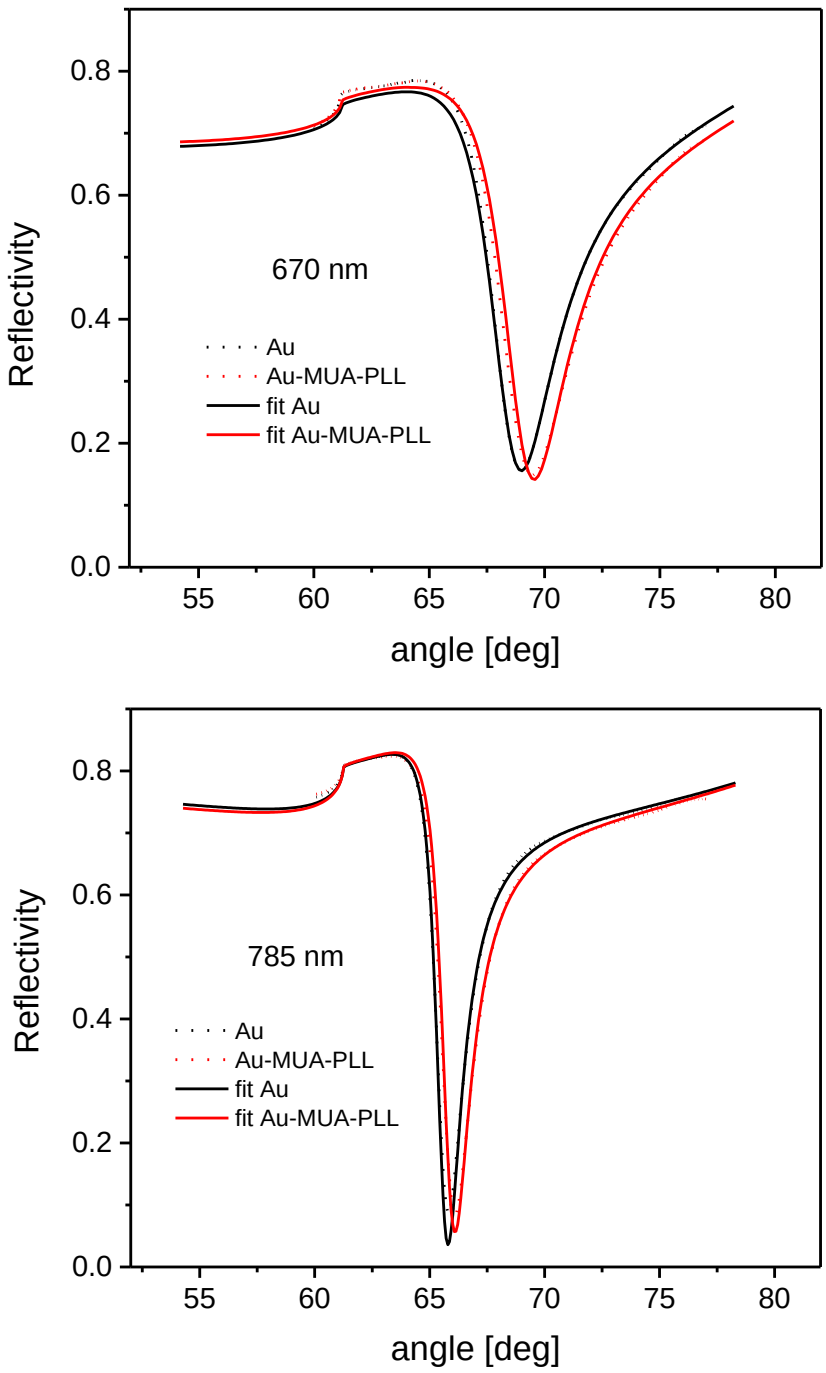
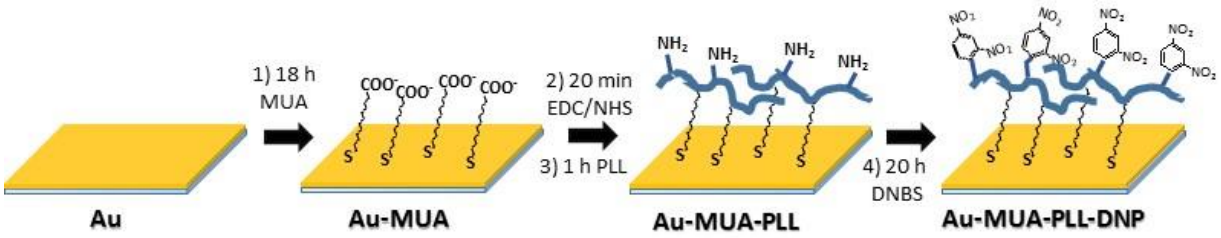


Figure S3. SPR curves fitting with Winspall at 670 nm (top) and 785 nm (bottom). Experimental data is shown in dotted line and fitting in straight line. Black and red curves correspond to bare gold and Au-MUA-PLL substrates respectively

3. Construction of Au-MUA-PLL-DNP platform



Scheme S1: Illustration of the different steps involved in the construction of the Au-MUA-PLL-DNP platform.

4. Binding curves for single and mixed MUA SAMs

Three independent experiments (i.e. different sensor surfaces) obtained by injection of solutions at known concentrations of anti-DNP antibodies on single MUA monolayers covered with BSA-DNP is shown in Fig. S3. Each point indicates a mean value of SPR signals from one to four times of repeated measurements of a sample at the specified concentration. The parabolic curve in Fig. S3 was obtained by non-linear fit function of statistical software, Origin^{TM} (USA) and Michaelis Menten equation was used as a fitting mode: R = Rmax*[Ab]/(KD + [Ab]), where R represents the analyte SPR response being Rmax the maximum, [Ab] is the concentration of anti-DNP antibodies and KD is the dissociation constant of anti-DNP antibodies from the BSA-DNP coated surface. Based on this result, the linear working region of the curve was estimated to be from 8 nM to 400 nM (showed in Figure S4 inset). In this region, the antibody concentration is far from the saturation zone were the binding curve turns flat and, at the same time, the SPR signal is high enough to enable a competitive inhibition assay.

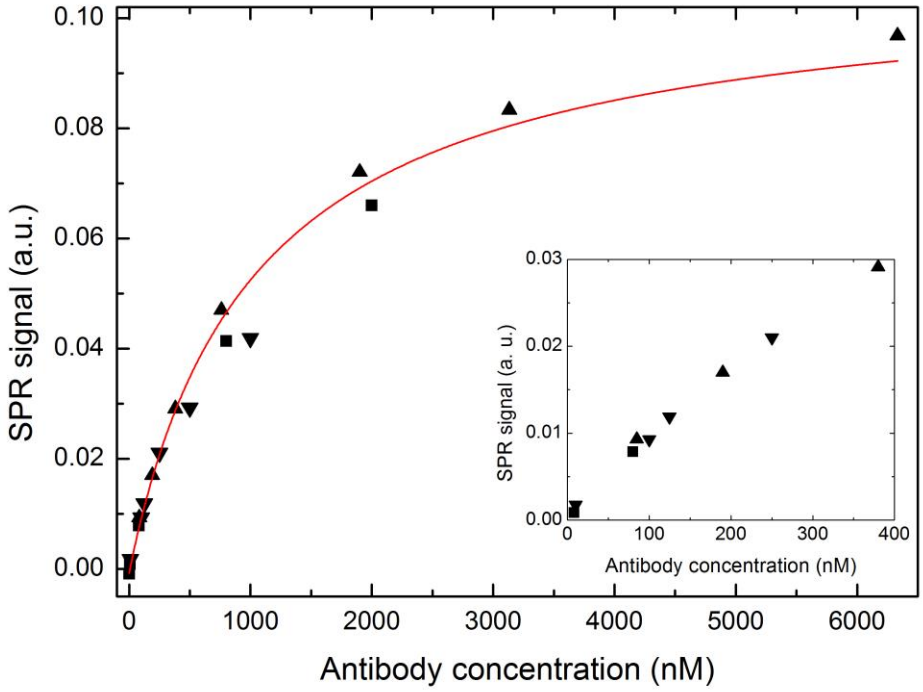


Figure S4. SPR binding isotherms of BSA-DNP covalently linked to MUA monolayer and anti-DNP in solution. Squares, up and down triangles correspond to independent experiments. Inset shows the linear working region.

The SPR response of the MUA-DTT monolayers bound to BSA-DNP was evaluated through the injection of anti-DNP solutions in concentrations which range from 0.67 to 190 nM. The binding curves together with the SPR signal of single MUA bound to BSA-DNP platforms are displayed in Figure S5. The experimental points were fitted to Michaelis Menten (squares) and to linear regression (circles and triangles) equations. Although the same proportion of MUA and DTT solutions was employed to prepare the self-assembled mixed monolayers on Au slides, their behavior as SPR substrates after the derivatization with BSA-DNP was quite different. It has been previously reported that mixed SAMs could exhibit different surface structures, like interdigitated domains of both thiols, or domains of different sizes of each of them on Au surfaces.[3, 4] Since this phenomena is surface-defect dependent, it is reasonable that these mixed thiol

monolayers Au surfaces offer different linking sites to BSA-DNP conjugates. Although the different behavior of each substrate in separate experiments, mixed thiols SAMs surfaces showed equal (triangles) or better (circles and squares) SPR responses compared to the same working region of single MUA SAMs (non-filled triangles).

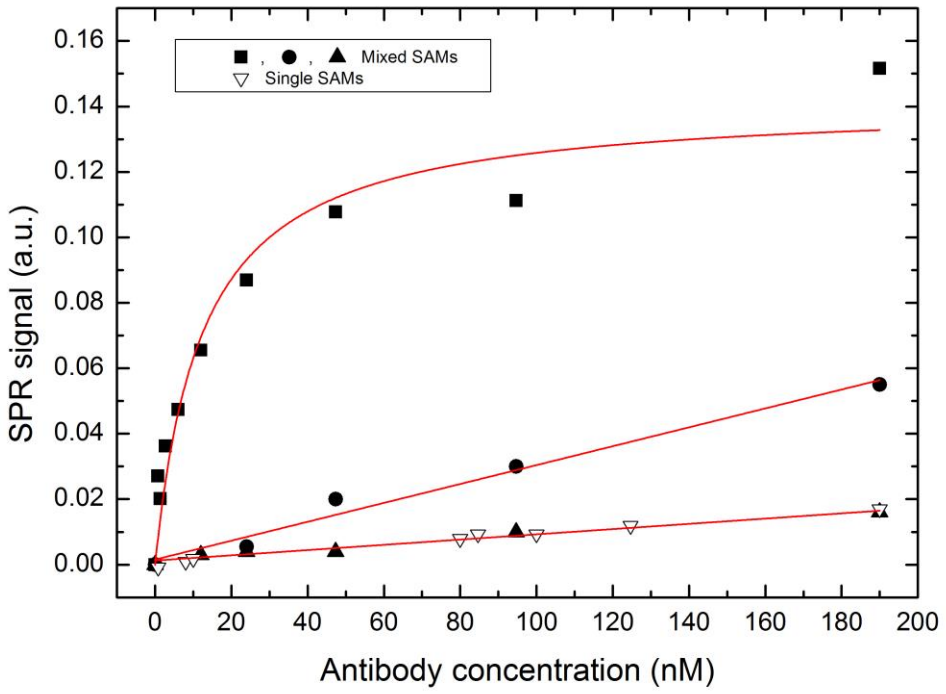


Figure S5. SPR binding isotherms of BSA-DNP covalently linked to MUA (non-filled triangles) or to mixed MUA/DTT (black) monolayers and soluble anti-DNP. Different symbols correspond to independent experiments. Mixed SAMs show equal (triangles) or better (circles and squares) SPR responses compared to the same working region of single MUA monolayers.

References:

[1] M.E. Carsten, H.N. Eisen, The Interaction of Dinitrobenzene Derivatives with Bovine Serum Albumin1,2, J. Am. Chem. Soc., 75 (1953) 4451-4456.

[2] C.E. Jordon, B.L. Frey, S. Kornguth, R.M. Corn, Characterization of poly-L-lysine adsorption onto alkanethiol-modified gold surfaces with polarization-modulation Fourier transform infrared spectroscopy and surface plasmon resonance measurements, Langmuir, 10 (1994) 3642-3648.

[3] J.C. Love, L.A. Estroff, J.K. Kriebel, R.G. Nuzzo, G.M. Whitesides, Self-Assembled Monolayers of Thiolates on Metals as a Form of Nanotechnology, Chem.Rev., 105 (2005) 1103-1170.
[4] A. Ulman, Formation and Structure of Self-Assembled Monolayers, Chem.Rev., 96 (1996) 1533-1554.