Supporting Information

Protein-Protein Interactions in Reversibly Assembled Nanopatterns

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EXPERIMENTAL

Materials. All inorganic salts and basic chemicals were p.a. grade and purchased from Roth or Merck. All experiments were carried out using water taken directly from a Synergy 185 unit or a MilliQ unit, both equipped with a UV lamp (Millipore). Streptavidin was purchased from SERVA Gmbh (Heidelberg, Germany). Quantum dots coated with streptavidin (Qdot®655 – streptavidin conjugate) were purchased from Invitrogen. The biotin disulfide ink and OEG disulfide ($-S(CH_2)_2(OCH_2CH_2)_6OCH_3)_2$ were purchased from Polypure AS (Oslo, Norway). Eicosane thiol (HSC_{20}) used in microcontact printing experiments was a generous gift from Prof. David Allara. The synthesis of OEG thiol $HS(CH_2)_{11}O(CH_2CH_2O)_7$-H.[1, 2] and biotin-trisNTA conjugate (^{BT}trisNTA)[3] has been described previously. All protein solutions were prepared in Hepes buffer, pH7.5, 20 mM Hepes, 150 mM NaCl, and 0.005 % Tween. The expression and purification of proteins MBP-His_{10}, ifnar2-His_{10}, IFNα2, and IFNα2-biotin conjugate has been described elsewhere[4-7].

Substrate preparation. Gold evaporation was performed in a Balzers UMS 500 P system by electron-beam deposition[8]. For dip-pen nanolithography (DPN) experiments, a 10 Å-thick titanium adhesion layer and a 120 Å-thick layer of gold were evaporated onto clean substrates cut from standard Si(100) wafers. For imaging SPR (iSPR) measurements, 300 Å of gold was evaporated onto cleaned SF10 glass substrates (Schott, Germany), pre-coated with 10 Å of titanium. The base pressure was below 10^{-9} mbar and the evaporation pressure for gold was always below 10^{-7} mbar. For both types of samples the gold evaporation rate was 5 Å/s and the titanium evaporation rate was 1 Å/s. Before

patterning, the prepared DPN gold chips were cleaned for 15 min at 85 °C in the SC-1 solution, i.e. a 5:1:1 mixture of water, 30 % hydrogen peroxide, and 25 % ammonia. The cleaned chips were rinsed in water, dried in a stream of nitrogen gas and immediately used for patterning. The chips for iSPR were washed in an ultrasonic bath for 5 min. in hexane, 5 min. in ethanol and dried in a stream of nitrogen gas. Before patterning they were also cleaned in the SC-1 solution at 85 °C for 2 min., rinsed in water and dried in a stream of nitrogen gas.

Dip pen nanolithography. Dip pen nanolithography (DPN) was carried out on two scanning probe microscopy platforms. A CP-II system equipped with a Nanolithography software package (Veeco, Santa Barbara, USA) was used to investigate the diffusion of ink as well as to prepare and analyze the functional patterns for the fluorescence experiments. A Dimension 3100 scanning probe microscope with a NanoMan software package (Veeco, Santa Barbara, USA) was employed to prepare and analyze samples for iSPR experiments. Before DPN printing, Veeco MLCT and DNP-S AFM tips were rinsed in ethanol and cleaned in SC-1 solution at 85 °C for 3 min., rinsed in MilliQ water and dried in a stream of nitrogen. Then the tips were inked twice in a 1 mM biotin disulfide ink solution in ethanol for 30 s. Typical conditions for DPN printing: 22 ^{o}C and 50 % - 60 % humidity, writing speed 0.01-0.1 µm/s for line and 60 s contact time for dot arrays, respectively. After the DPN patterning the surfaces were incubated overnight with 20 µM OEG thiol or OEG disulfide ethanol solutions, respectively. Subsequently, they were rinsed and washed in an ultrasonic bath in ethanol for 5 min, 1 to 3 times. In addition, for protein binding experiments, the samples were washed in an ultrasonic bath in water for another 5 min. After the last washing step they were blown dry with nitrogen gas.

Epifluorescence microscopy. Fluorescent images were acquired with an Olympus BX51 upright microscope equipped with a 40x, NA 0.8 water immersion objective and a Peltier-cooled F-View II CCD camera (Olympus). Image acquisition and analysis was done using an analySIS software (Olympus Soft Imaging Solutions GmbH, Münster, Germany). For the fluorescence assays, samples were first placed into a Petri dish. A drop of buffer was put onto the sample and kept for 5 min to precondition the surface. For visualization of the biotin-terminated DPN patterns on gold, a 2 nM solution of streptavidin-quantum dot conjugate in buffer was applied to the surface for 5 min, followed by rinsing with buffer using a pipette. Care was taken to avoid exposure of the patterned surface to air during the rinsing procedure.

The assembly of ^{BT}trisNTA compound and the visualization of receptor-ligand interaction on the biotin-terminated DPN array spots was carried out by adding the reagents in the following sequence: streptavidin (100 nM), ^{BT}trisNTA preloaded with Ni(II) ions[3] (100 nM), ifnar2-H_{10}-EC receptor (100 nM), IFNα2-biotin ligand and streptavidin coated quantum dot mixture (25 nM and 5 nM respectively). The incubation time in each step was 5 min. In between the steps, the sample was rinsed with buffer. In His-tagged protein binding reversibility test, the trisNTA groups were reloaded with Ni(II) ions by adding 10 mM $NiCl_2$ solution in buffer. Note, that in control experiments performed without Ni(II) ions, ifnar2-His_{10} or IFNα2-biotin, respectively, no fluorescence signal was detected in the array spots.

Imaging ellipsometry and SPR. For iSPR studies the gold surface was pre-structured before the DPN printing. Namely, microcontact printing (μCP) with eicosanethiol (HSC_{20}) was carried out to create a defined working area for DPN and also to generate a

reference surface for the quantitative analysis of protein adsorption to the DPN patterns (see below). A poly(dimethylsiloxane) (PDMS) stamp used for μCP had a surface relief consisting of 20 μm protruding frames, which separated 50×50 μm^2 recessed areas. The production of the PDMS stamps has been described in detail elsewhere[9]. Before use, the stamp was thoroughly rinsed in ethanol and dried in a stream of nitrogen gas. The stamp was inked with 0.2 mM solution of HSC_{20} in ethanol for 30 s. Subsequently, the stamp was carefully dried in a stream of nitrogen, and then it was applied to the gold surface for 2 min using a pair tweezers.

Before and directly after μCP the gold surface was analyzed in an EP^3-SE imaging null-ellipsometer equipped with a laser emitting at 532 nm wavelength (Nanofilm, Göttingen, Germany). For data acquisition and analyzis the software included with the instrument was used. The thickness measurements were performed at a 60 ° angle of incidence. The complex refractive index of gold film was measured immediately after the sample cleaning procedure. From the measured Δ map of the μCP sample, the thickness of the HSC_{20} SAM grid was recalculated using the three layer model, were the refractive index n of the organic layer on gold was assumed to be 1.5. Subsequently, the DPN patterning and backfilling of the surface was carried out as described above. After this fabrication process, the samples were incubated with 1 mg/ml BSA solution in buffer, rinsed with MilliQ water, blown dry with nitrogen gas and analyzed again in imaging ellipsometry using the same three layers optical model as for HSC_{20}. No adsorption of BSA in the μCP squares was detected considering the measurement error of the method, whereas the BSA adsorbed to the μCP hydrophobic grid consisting of HSC_{20}. The calculated ellipsometric thickness difference between the hydrophobic HSC_{20} grid coated with BSA and the OEG

disulfide region was 2 nm. This thickness is equivalent to 2.4 ng mm^{-2} of protein[10, 11]. In our protein adsorption kinetics measurements, this thickness difference was used as a reference: in iSPR it corresponds to a change in the Δ value between the BSA/ HSC_{20} and OEG disulfide regions.

After this analysis the samples were mounted into an iSPR cell and they were used for measurements of protein adsorption kinetics using imaging iSPR operating in ellipsometric mode (the same EP^3-SE imaging null-ellipsometer). Imaging SPR measurements were carried out at a 532 nm wavelength laser light and at an angle of incidence of 62 °. Imaging SPR cell was built in the Kretschmann configuration[12] and it consisted of a 60 ° angle SF10 prism, a holder for the prism and a flow cell with ~10 µl volume. A typical flow rate during the injections was 10 µl/s.

The kinetics of IFNα2 binding to immobilized ifnar2 was analyzed using a 1:1 interaction model including parameterization of mass transport as implemented in the BIAevaluation 3.1 software (Biacore AB, Sweden). Association and dissociation kinetics were fitted simultaneously with the rate constants of association (k_a), dissociation (k_d) and mass transfer (k_t) as variable parameters. The maximal amplitude R_{max} was estimated from the binding curve (saturation of all binding sites is achieved in the experiment due to the high ligand concentration), and the background signal R_I was kept at 0 (the binding curves were background-corrected).

RESULTS

Figures S1 and S4 show AFM images of patterns of lines printed on two different gold samples by DPN with the biotin disulfide ink. Figures S2, S3 and S5, respectively, show iSPR images and protein binding kinetics obtained from the same two samples. Figure S3 shows an iSPR image and two additional protein binding/interaction kinetics obtained from the same sample as in Figures S1 and S2 after approximately 24 h of storage in the flow cell filled with buffer. Figure S6 shows the result of the analysis of an iSPR kinetics of IFNα2 binding to ifnar2 immobilized in microscopic domains formed by multivalent chelator-terminated self-assembled monolayers on gold.[13] This kinetics was measured using the same iSPR instrument and the same fluidics system as in the present study.

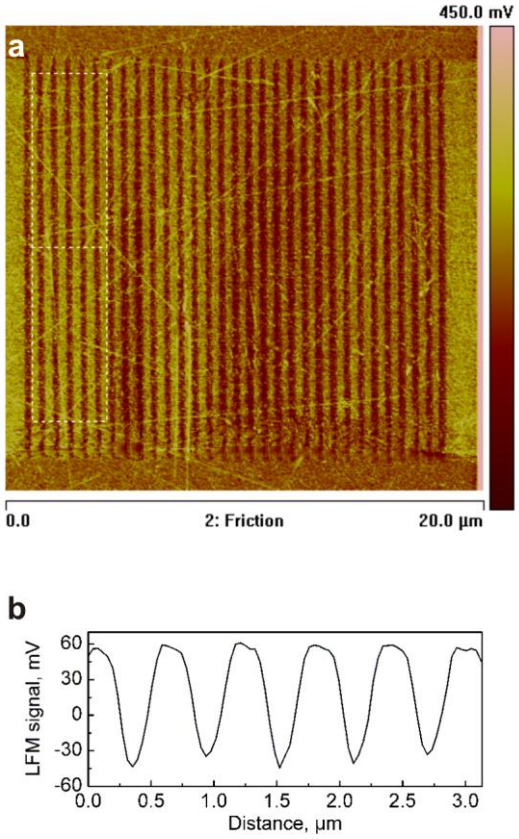

Figure S1. Contact mode atomic force microscopy (AFM) of a pattern of lines on gold obtained by dip-pen nanolithography with biotin-disulfide as ink. a) Lateral force (friction) image of lines, which were fabricated at a 0.2 µm/s writing speed on a gold-coated SF10 glass substrate. The AFM image was obtained by scanning the pattern at a rate 200 µm/s with the same tip immediately after the DPN fabrication. The observed scratches are features of SF10 substrates due to glass polishing in the manufacturing process. b) Integrated line widths measured in the frame shown in a. The sample was used for further assembly of ^{BT}trisNTA compound and, subsequently, to immobilize MBP-His_{10} as shown in Figure S2.

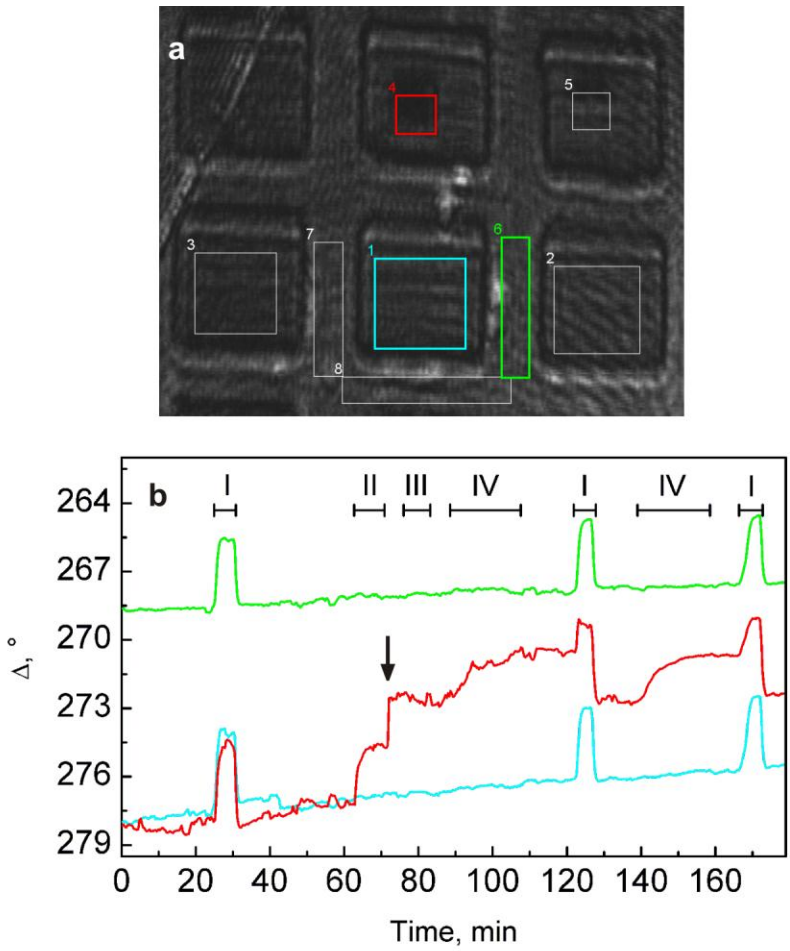

Figure S2. Imaging SPR (iSPR) analysis of the assembly of ^{BT}trisNTA and reversible adsorption of MBP-His_{10} on the fabricated pattern of biotin lines shown in Figure S1. a) SPR images showing the hydrophobic HSC_{20} grid separating 50×50 μm^2 working areas after adsorption of BSA. The three colored frames indicate the ROIs for recording of protein binding kinetics: red-pattern of DPN lines, blue-OEG SAM, green- BSA on the HSC_{20} grid. b) Unmodified SPR binding curves recorded from the three ROIs shown in a. The arrow indicates a necessary adjustment of the ROI. The injections are: I - 250 mM imidazole, II – a 300 nM streptavidin and 750 nM ^{BT}trisNTA mixture, III - 1 μM ^{BT}trisNTA, IV - 500 nM MBP-His_{10}.

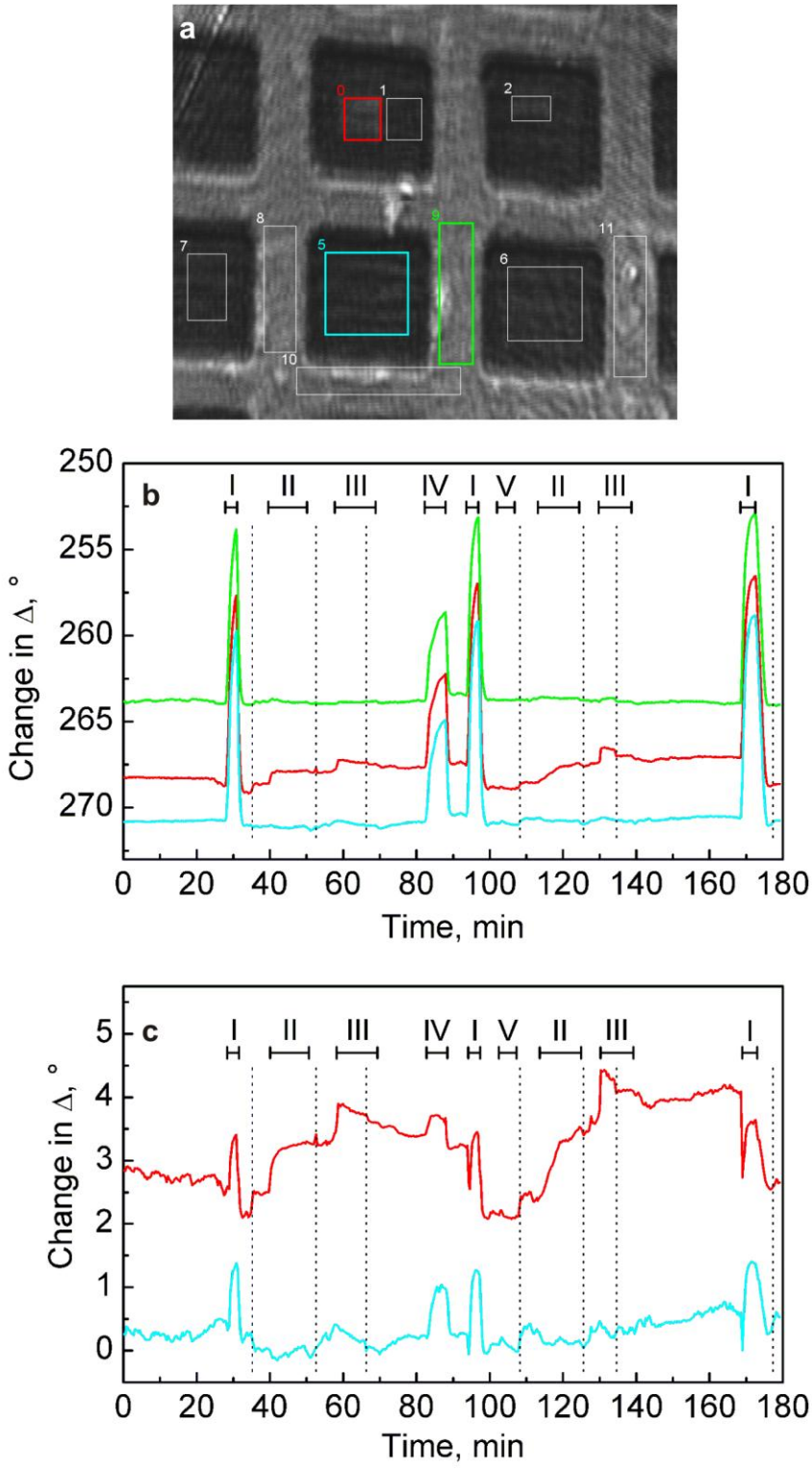

Figure S3. Imaging SPR (iSPR) analysis of the same pattern shown in Figures S1 and S2 and after approximately 24 h of storage in the flow cell filled with buffer. a) SPR image

showing the 50×50 μm^2 working areas defined by the hydrophobic HSC_{20} grid blocked with BSA. The three colored frames indicate the ROIs for recording of protein binding kinetics: red - pattern of DPN lines, blue - OEG SAM, green - BSA on the HSC_{20} grid. b) Unmodified SPR binding curves showing two additional cycles of reversible adsorption of ifnar2-His_{10}/ IFNα2 recorded after approximately 24 h after the MBP-His_{10} immobilization experiments shown in Figure S2. The color of each binding curve corresponds to the ROI shown in a. c) Corrected binding curves obtained by subtracting the original shown in b with reference signal. The dotted lines indicates the points when the acquisition of the binding curve was temporarily stopped to record SPR images (only the first image is shown in a). The injections are: I – 1 M imidazole, II – 500 nM ifnar2-His_{10}, III - 1 μM IFNα2, IV - 200 nM EDTA, V – 10 mM $NiCl_2$. Bearing in mind a surface filling factor of 0.7 (obtained from Figure S1), and assuming a stable layer of BSA, which in this experiment corresponds to a 6.97° change in Δ, the amount of ifnar2-His_{10} immobilized in each cycle is ~0.4 ng mm^{-2}.

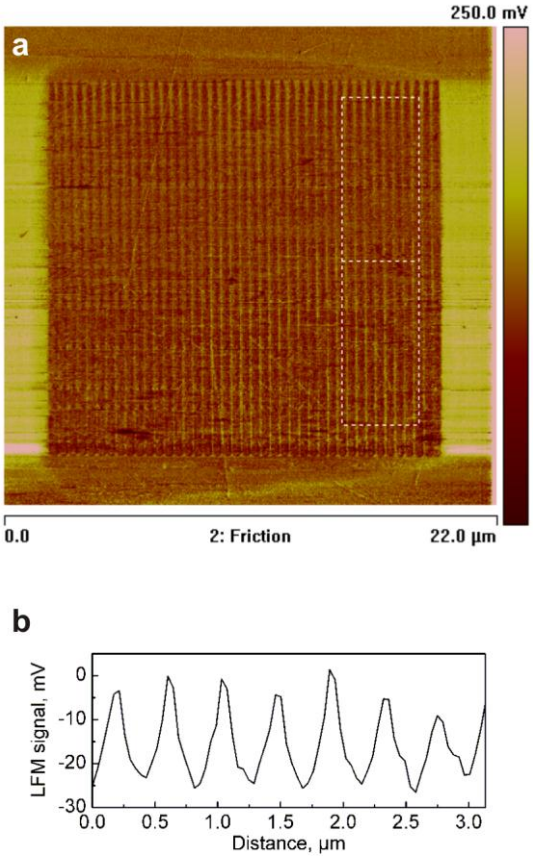

Figure S4. Contact mode atomic force microscopy (AFM) of a pattern of lines on gold obtained by dip-pen nanolithography with the biotin-disulfide as ink. a) Lateral force (friction) image b) integrated line thicknesses measured close to the frame shown in a. The sample was prepared under identical conditions as that shown in Figure S1 (except the writing speed was 0.3 µm/s). This sample was used for ifnar2-His_{10}/ IFNα2 binding experiments, Figure S4.

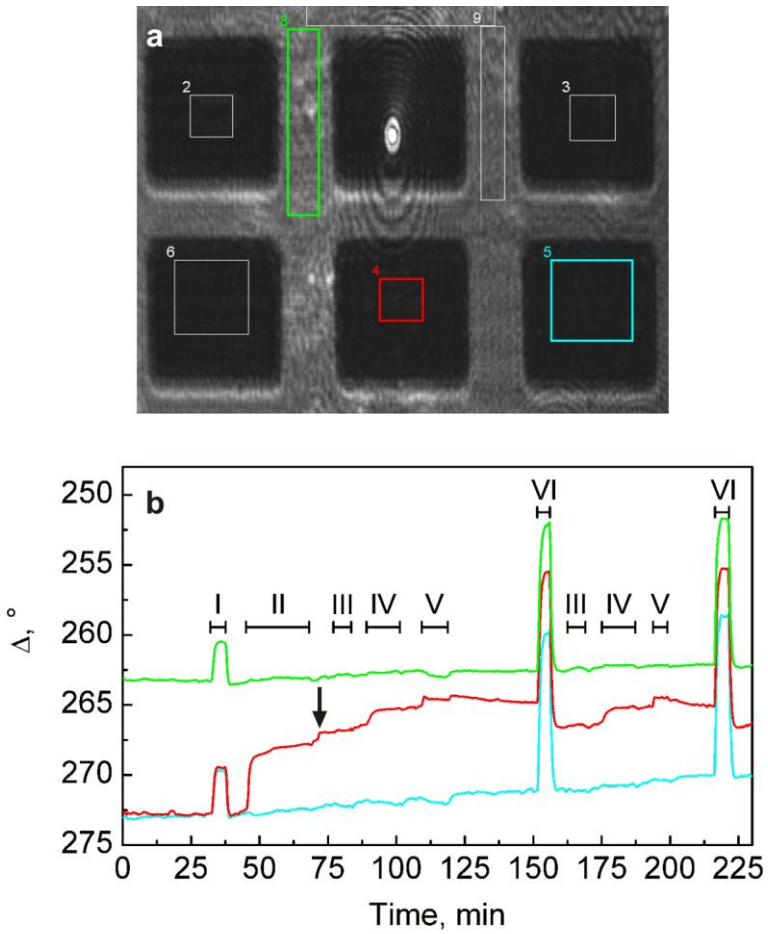

Figure S5. Imaging SPR (iSPR) analysis of the assembly of ^{BT}trisNTA and reversible adsorption of ifnar2-His_{10}/ IFNα2 on the DPN-fabricated biotin lines shown in Figure S4. a) SPR image showing the 50×50 μm^2 working areas with the hydrophobic HSC_{20} grid after immobilization of BSA. The three colored frames indicate the ROIs for recording of protein binding kinetics: red - pattern of DPN lines, blue - OEG SAM, green - BSA on the HSC_{20} grid. b) Unmodified SPR binding curves recorded from the three ROIs shown in a. The arrow indicates a necessary adjustment of the ROI. The injections are: I - 250 mM imidazole, II - 300 nM streptavidin and 750 nM ^{BT}trisNTA mixture, III - 1 μM ^{BT}trisNTA, IV - 500 nM ifnar2-His_{10}, V - 1 μM IFNα2, VI - 1M imidazole.

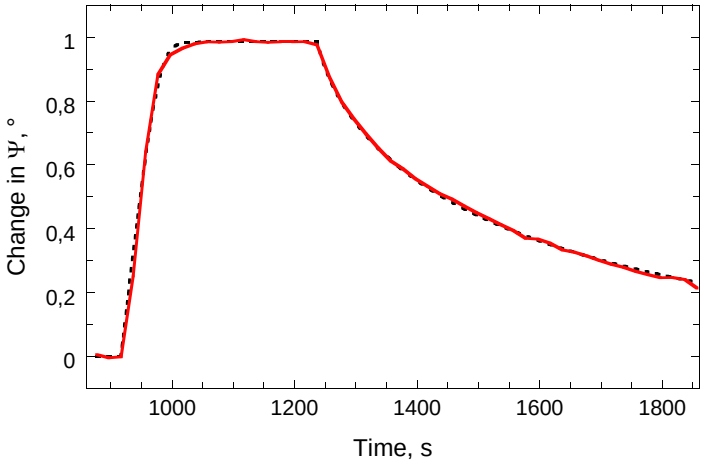

Figure S6. Modeling of the ifnar2-His_{10}/ IFNα2 interaction kinetics previously measured in micropatterns formed by multivalent chelator-terminated self-assembled monolayer [13]. The experimental data (red solid line) was obtained by employing the same iSPR system as in the present study. The curve fitting (black dashed line) yielded an association rate constant k_a of ~4×10^5 $M^{-1}s^{-1}$ and a dissociation rate constant of ~1×10^{-2} s^{-1}.

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