Supporting Information

Chemical Functionalization of Germanium with Dextran Brushes for Immobilization of Proteins Revealed by Attenuated Total Reflection Fourier Transform Infrared Difference Spectroscopy

Jonas Schartner[1], Nina Hoeck[1], Jörn Güldenhaupt[1], Laven Mavarani[1], Andreas Nabers[1], Klaus Gerwert[1], Carsten Kötting[1,*]

[1]Department of Biophysics, Faculty of Biology and Biotechnology, Ruhr-University Bochum, Germany.

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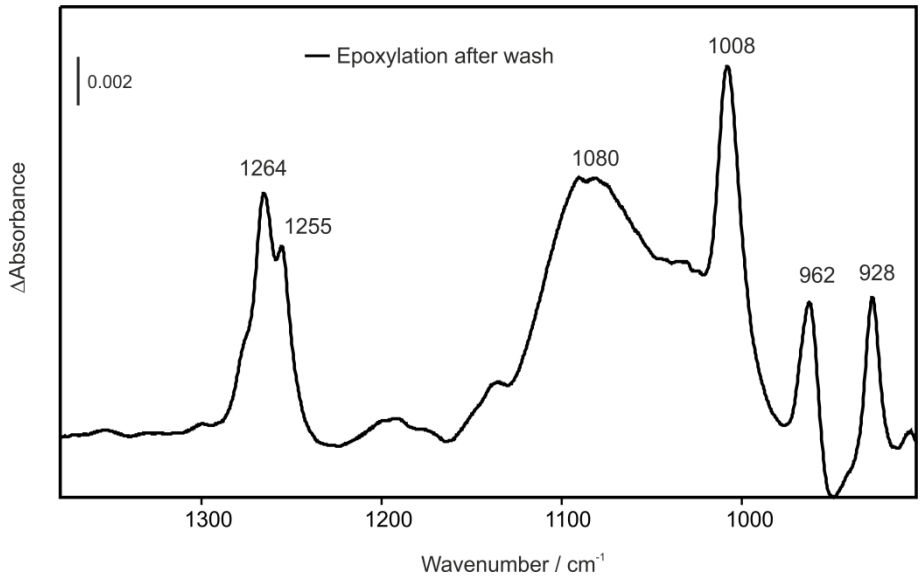


Figure S1: At first the germanium surface was epoxylated with epichlorohydrin. The surface was washed with water and ethanol. The obtained spectrum confirms the successful activation of germanium.

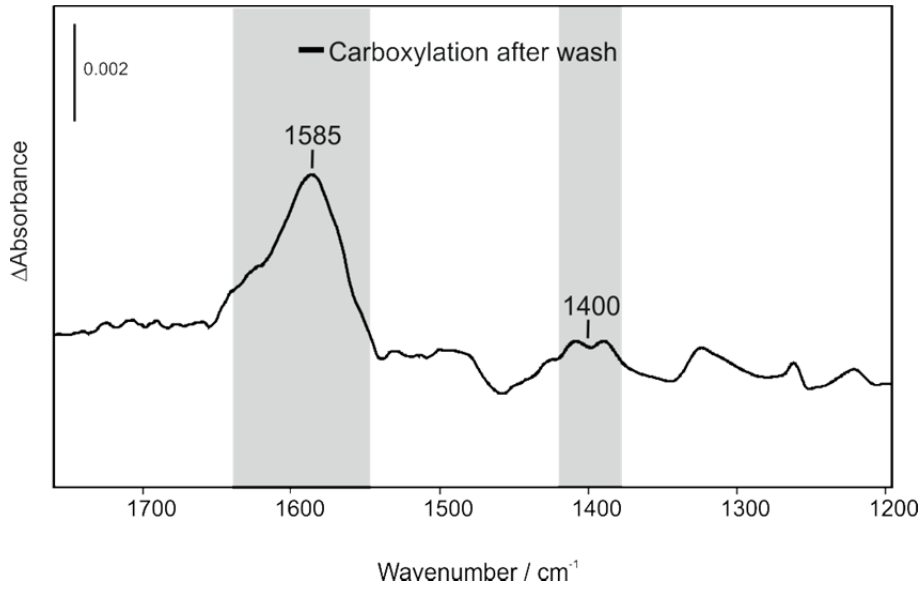


Figure S2: The immobilized dextran-brush was treated with bromoacetic acid and a carboxylated surface was obtained. After washing with water (pH 7) the characteristic bands for carboxylic acids remained.

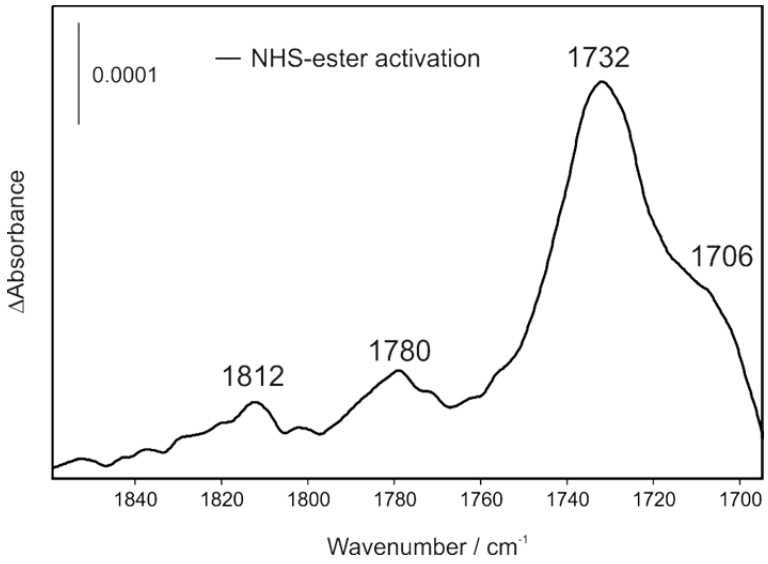


Figure S3: The carboxylated dextran-brush was incubated with EDC/NHS to form NHS-esters. The spectrum showed the typical bands for NHS-esters, which remained after rinsing the surface with water.

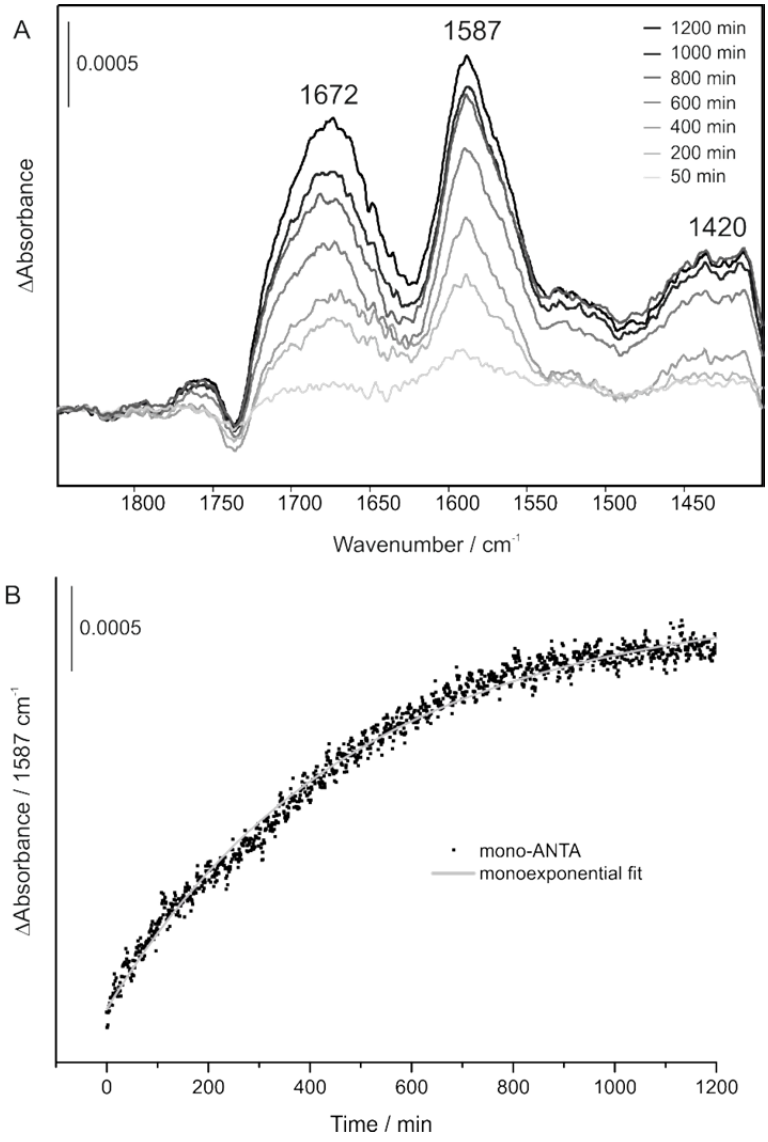


Figure S4: (A) Reaction of mono-ANTA with the NHS activated dextran. The formed peptide bond was characterized by the amide 1 and amide 2. (B) Kinetics of the reaction with mono-ANTA.

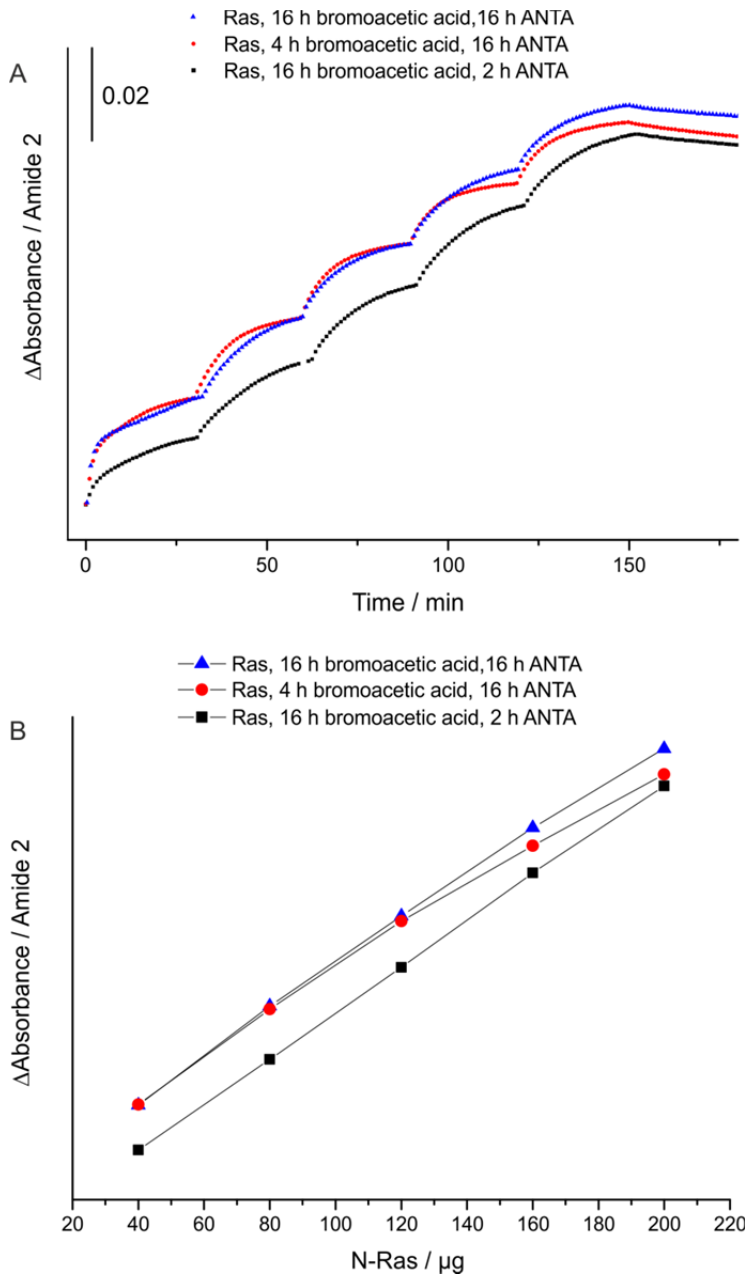


Figure S5: (A) Binding kinetics of N-Ras1-180 His-tag on a 70 kDa dextran surface with different carboxylation and ANTA reaction times. The binding kinetics were very similar and in all cases protein 3D layers were obtained. (B) Plot of the amide 2 absorbance against the amount of offered Ras showed that a 4 h carboxylation time (red) and an ANTA coupling overnight were favorable.

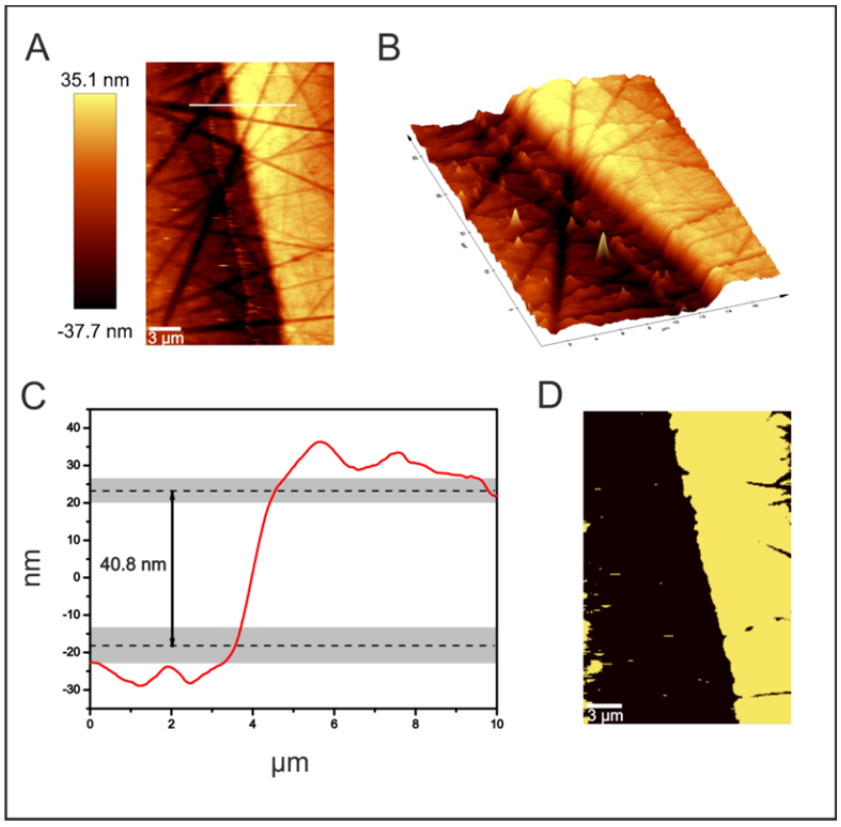


Figure S6: Characterization of a germanium crystal coated with a 500 kDa dextran (16 h carboxylated). (A) Topography of the substrate measured by AFM. The white line indicates the cross section shown in C. (B) 3D topographic image of the dextran coated surface. (C) Cross section through the unmodified and dextran coated germanium surface resulting in a thickness of 40.8 nm. (D) Image mask separating the dextran coated germanium surface from the uncoated surface.

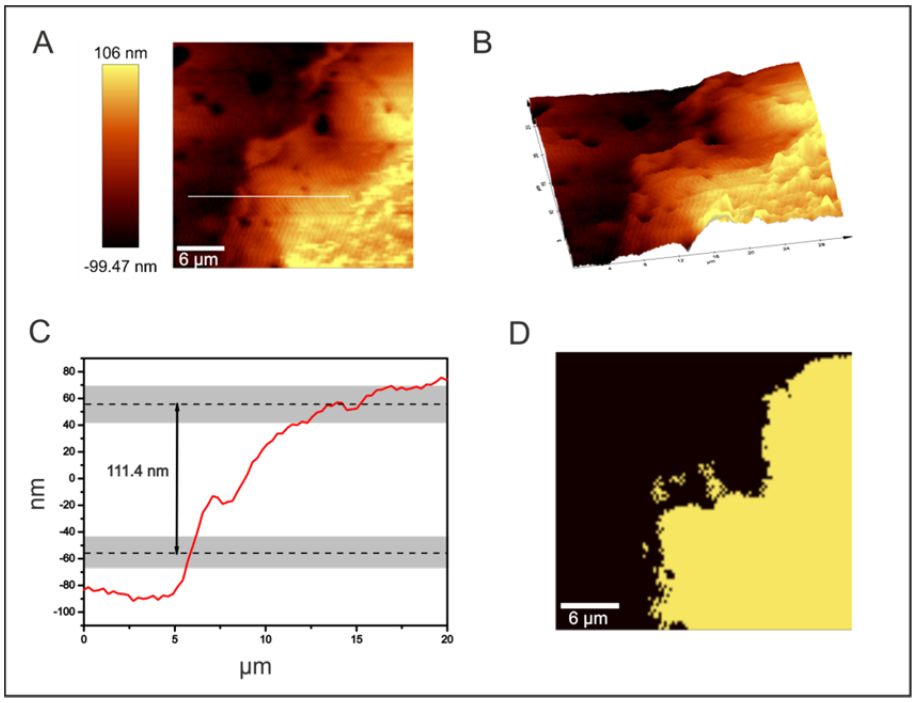


Figure S7: Characterization of a germanium crystal coated with a 70 kDa dextran (16 h carboxylated). (A) Topography of the substrate measured by AFM. The white line indicates the cross section shown in C. (B) 3D topographic image of the dextran coated surface. (C) Cross section through the unmodified and dextran coated germanium surface resulting in a thickness of 111.4 nm. (D) Image mask separating the dextran coated germanium surface from the uncoated surface.

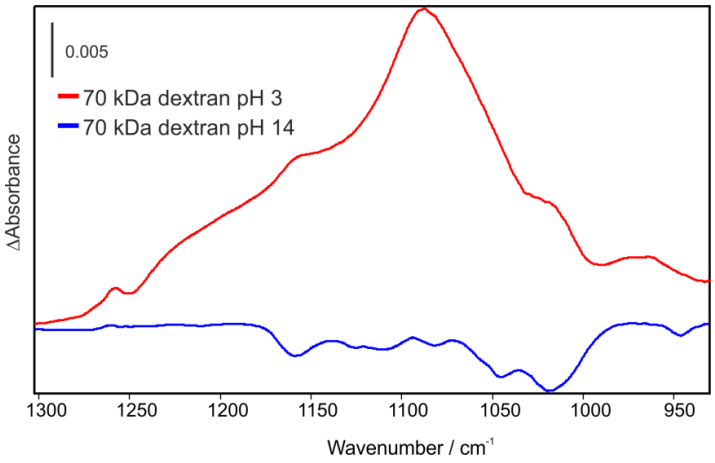


Figure S8: Analysis of the 70 kDa dextran under acidic pH 3 (red) and basic pH 14 (blue). The positive bands were induced by shrinking of the layer due to the protonation of the dextran. Negative bands (blue) were caused by a growing of the dextran-brush, which was induced by the charge repulsion caused by deprotonation of the hydroxyl-groups.

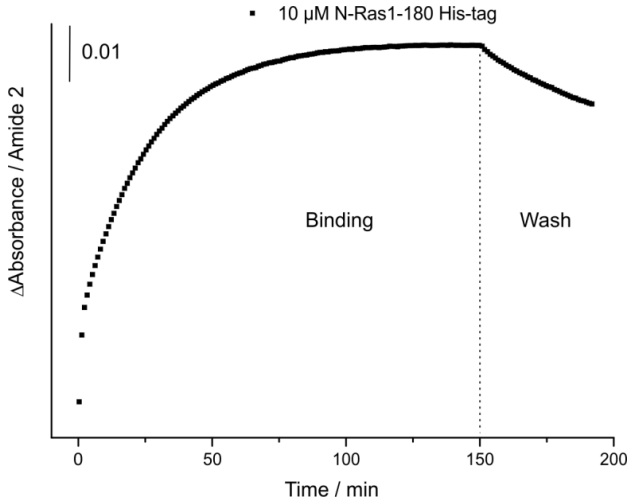


Figure S9: Binding kinetics of 10 µM N-Ras1-180 His-tag on a 70 kDa dextran surface (mono-Ni-NTA).

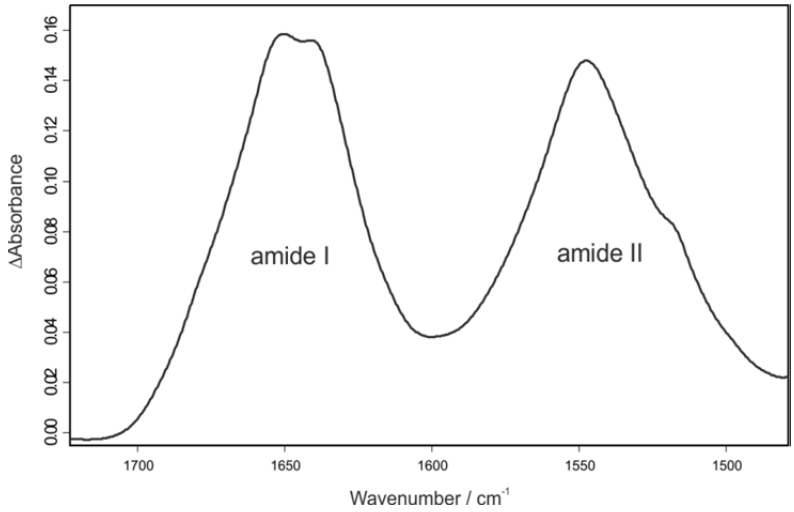
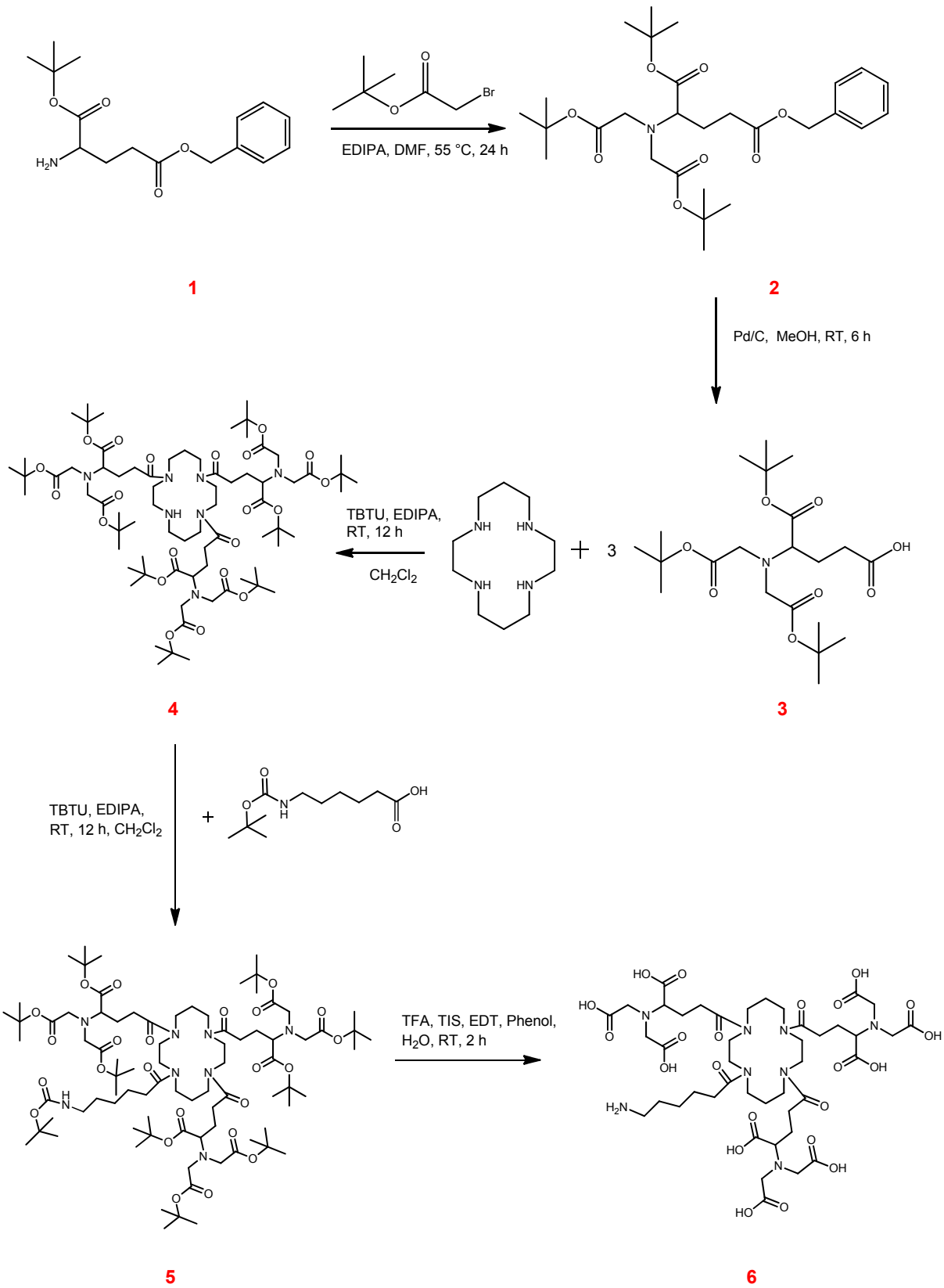


Figure S10: FT-IR spectrum of immobilized Ras on a 70 kDa dextran-brush (mono-Ni-NTA). An absorbance of 145 mOD was observed after offering 400 µg of protein.



Scheme S1: Organic synthesis of tris-ANTA based on Lata et al..[1] Used chemicals were N,N-Diisopropylethylamine (EDIPA), Dimethylformamide (DMF), Palladium on carbon (Pd/C), Methanol (MeOH), O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate (TBTU), Trifluoroacetic acid (TFA), Triisopropylsilane (TIS), 1,2-Ethanedithiol (EDT).

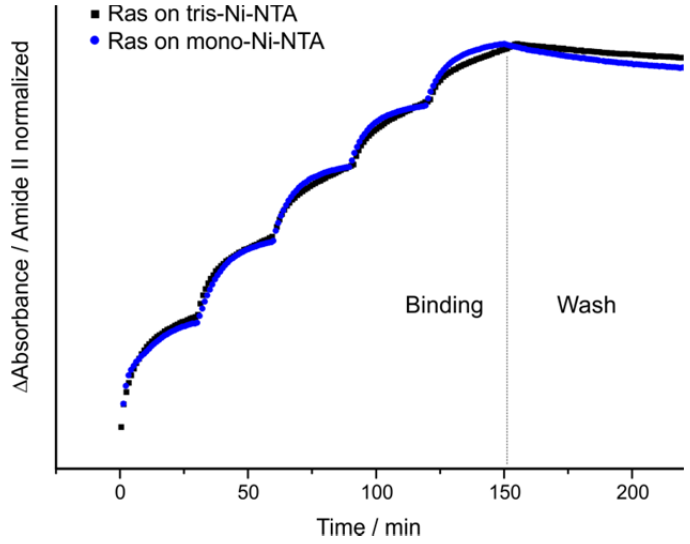


Figure S11: Comparison of the Ras immobilization on mono-Ni-NTA dextran (blue) with tris-Ni-NTA dextran (black). The protein stability was slightly increased.

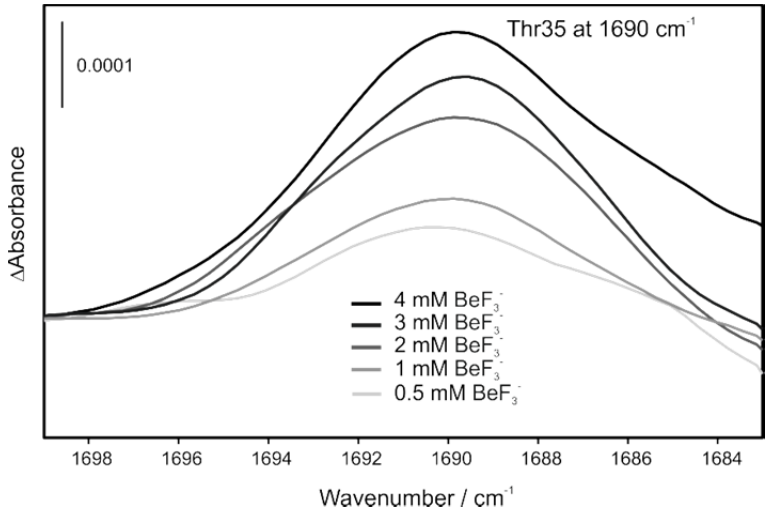


Figure S12: BeF_x-titration of the amino acid Thr35 of Ras. The spectra were additionally baseline corrected in the analyzed region to isolate the absorbance changes of Thr35.

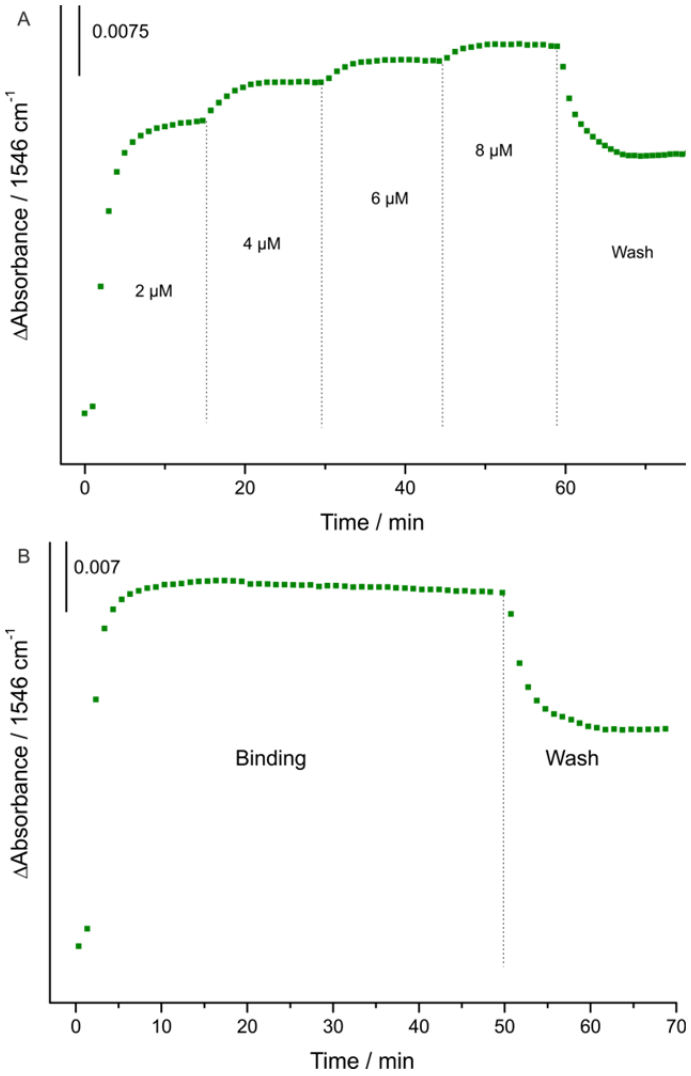


Figure S13: Binding kinetics of GFP with His-tag on a 70 kDa dextran surface functionalized with tris-ANTA. (A) The GFP concentration was successive increased (total concentration 8 µM). (B) A 4 µM GFP solution was employed and the binding monitored over time.

Table S1: Ratio of GFP and mCherry during the immobilization on a 70 kDa dextran surface functionalized with tris-ANTA.

Time	GFP / %	mCherry / %
Binding 5 min	47	53
Binding 30 min	51	49
Binding 60 min	56	44
Binding 90 min	60	40
Binding 105 min	65	35
After washing (2 h total)	65	35

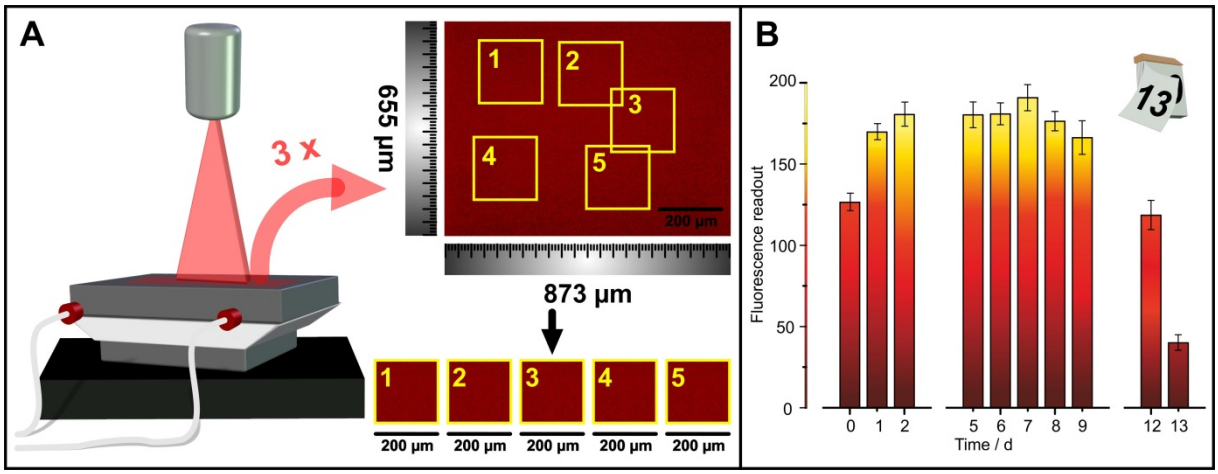


Figure S13: (A) Fluorescence microscopy of immobilized mCherry on a 70 kDa dextran surface functionalized with mono-ANTA. (B) Fluorescence intensity observed over days (stored at 4 °C, dark).

Calculation of the surface concentration for Ras:

The surface concentration of a Ras monolayer was deduced from the literature.[2] The measured surface area is 3 cm^2.

(1) $$n(Ras\_monolayer) = 20\frac{pmol}{cm^2} \times 3cm^2 = 60\,pmol$$

(2) $$m(Ras\_monolayer) = 60\,pmol \times 20000\frac{g}{mol} = 1.3\mu g$$

(3) $$m(Ras\_dextran) = 435\,pmol \times 20000\frac{g}{mol} = 8.7\mu g$$

Calculation of the surface concentration for mCherry and GFP:

The surface concentration of all mCherry present in the 3D-layer projected to the surface was calculated using formulas from the literature.[3] Equation 4 is the projection of the whole protein amount to the surface.

(4) $$n(mCherry\_GFP\_dextran) = 22\frac{pmol}{cm^2} \times 3cm^2 = 66\,pmol$$

(5) $$c(mCherry\_GFP\_dextran) = \frac{66\,pmol}{0.0003m^2 \times 0.0000001m}l = 2.2\frac{mol}{m^3}$$

The average distance is the cubic root of the volume per molecule:

(6) $$d(mCherry\_GFP\_dextran) = \sqrt[3]{\frac{1}{2.2\frac{mol}{m^3} \times N_A}} = \sqrt[3]{\frac{1}{1.32 \times 10^{24}}}m = 9nm$$

References:

(1) Lata, S., Reichel, A., Brock, R., Tampé, R., and Piehler, J. (2005) High-Affinity Adaptors for Switchable Recognition of Histidine-Tagged Proteins. *J. Am. Chem. Soc. 127*, 10205–10215.

(2) Schartner, J., Güldenhaupt, J., Mei, B., Rögner, M., Muhler, M., Gerwert, K., and Kötting, C. (2013) Universal method for protein immobilization on chemically functionalized germanium investigated by ATR-FTIR difference spectroscopy. *J. Am. Chem. Soc. 135*, 4079–4087.
(3) Schartner, J., Gavriljuk, K., Nabers, A., Weide, P., Muhler, M., Gerwert, K., and Kötting, C. (2014) Immobilization of Proteins in their Physiological Active State at Functionalized Thiol Monolayers on ATR-Germanium Crystals. *ChemBioChem 15*, 2529–2534.