Dual-functionalized nanostructured bio-interfaces by click chemistry

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Materials: H_2N-PEG_{2000}-OCH_3 and H_2N-PEG_{3000}-alkyne are purchased from Iris Biotech (Marktredwitz, Germany), 5/6-carboxyrhodamine 110-PEG_3-azide and azide-PEG_3-biotin conjugate from Jena Bioscience (Jena, Germany) and HS-C_{11}-$(EG)_3$-NTA from ProChimia (Sopot, Poland). The peptides K(N_3)GGNGEPRGDTYRAYK(fluorescein)GG, cyc(RGDfE)K(N_3) and K(N_3)PHSRN are acquired from Peptide Specialty Laboratories (Heidelberg, Germany) and c[RGDfK(3-mercaptopropionyl-aminohexanoic acid)] from Peptides International (Louisville, USA). Cell culture supplies and reagents are purchased from Gibco (Carlsbad, USA). Rabbit anti-paxillin IgG is obtained from Abcam (Cambridge, UK), mouse anti-vinculin IgG from Sigma-Aldrich (St. Louis, USA), Alexa Fluor 488 goat anti-mouse

IgG and Alexa Fluor 647 goat anti-rabbit IgG from Invitrogen (Carlsbad, USA). Glass cover slides (20 x 20 mm^2, 24 x 24 mm^2), mole sieve (3 Å), tris(hydroxymethyl)aminomethane (Tris), methanol, ethanol, sodium dodecyl sufate pellets and Mowiol 4-88 are purchased from Carl Roth (Karlsruhe, Germany), ethyl acetate from Merck (Darmstadt, Germany) and ethylenediamine tetraacetate from Acros Organics (Geel, Belgium). All other chemicals are purchased from Sigma-Aldrich (St. Louis, USA). Milli-Q water is used.

Synthesis of 3-azidomethyl-5-iodopyridine: 1 eq. of 3-chloromethyl-5-iodopyridine (0.9 mmol) is dissolved in 5 mL dimethylformamide (DMF) and 10 eq. of sodiumazide (8.6 mmol) are added. The suspension is stirred for 24h at room temperature. The reaction mixture is extracted with water/ethyl acetate and the desired product is obtained from the dried organic phase by evaporation. ^{1}H-NMR [300MHz, $CDCl_3$] δ 8.81 (d, ^4J = 1.8 Hz, 1H, Ar*H*), 8.50 (d, ^4J = 1.6 Hz, 1H, Ar*H*), 8.01 (t, ^4J = 1.9 Hz, 1H, Ar*H*), 4.36 (s,2H; CH_2).

XPS measurements: X-ray photoelectron spectra are acquired with a Max 200 photoelectron spectrometer (Leybold-Heraeus, Cologne; Germany) equipped with a magnesium anode with a characteristic Kα radiation energy of 1253.6 eV. Emitted photoelectrons are collected perpendicular to the surface by a multichannel detector at constant pass energy of 96 eV and 48 eV for overview spectra and for detailed spectra respectively. Measurements are performed under UHV with a pressure lower than 10^{-8} mbar. Binding energies are calibrated with respect to C1s signal of ethylene glycol at 286.4 eV or to Si2p signal of the glass substrate at 103.4 eV. The N 1s XPS data is fitted using XPS Fit 4.1. The N1s signal after the click reaction is fitted for 4 chemically different N species (marked in red), in the fit the relative are under the N=**N**-N, C-**N**-N and C-**N**=C peaks is fixed to be 1:2:1 due to the chemical structure of the azide and the

relative areas under the peaks are computed 5.2, 10.4, 21.0, 5.2 from higher to lower binding energy

To determine if copper ions remain on the surface after the click reaction, the Cu XPS signal is recorded for the following PEG-alkyne monolayers: before the click reaction, subjected to click reaction conditions without any azide, functionalized with azide-cRGD, with azide-NTA[32] and with azide-NTA/Cu^{2+} complexes. Remaining copper on substrates coupled with azide-NTA is removed in an extra wash step with 25 mM EDTA in Tris-NaCl buffer (50 mM Tris (pH7.4), 300 mM NaCl). The NTA/ Cu^{2+} complex is formed by incubating the surface with 100 mM $CuSO_4$ in Tris-NaCl buffer, followed by washing with Tris-NaCl buffer.

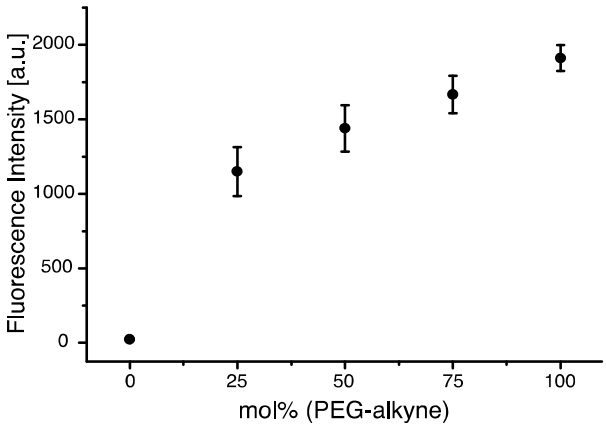

Figure S1. Fluorescence intensity on surfaces of different PEG-alkyne and PEG_{2000} compositions modified with small molecule fluorophores by click reaction. Each data point represents the mean ± standard deviation of at least three independent experiments.

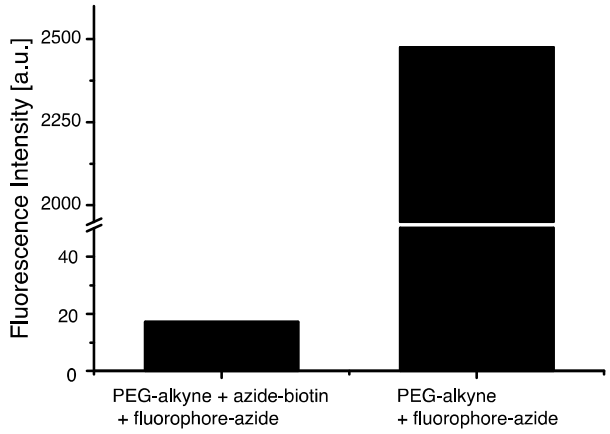

Figure S2. Fluorescence intensity measurements on PEG-alkyne coated surfaces modified successively with biotin and a small molecule fluorophore by copper(I) catalyzed azide alkyne cycloaddition or only with a small molecule fluorophore.

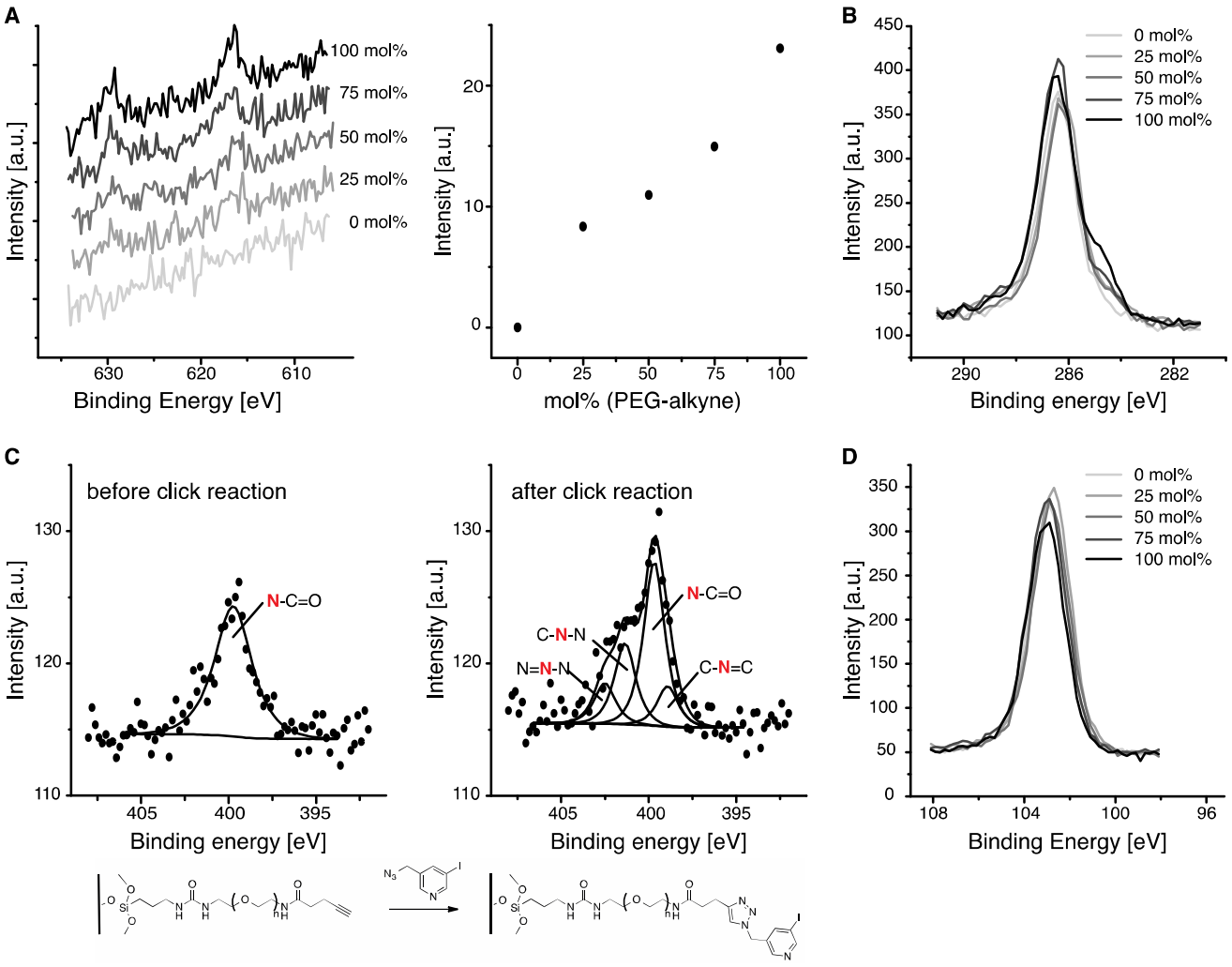

Figure S3. XPS measurement. (A) I 5d XPS signals of surfaces coupled with an iodine containing small molecule at various mol% of PEG-alkyne and the area under the I 5d signal vs. the various mol% of PEG-alkyne. I 5d XPS signals are shown with an offset. (B) C 1s XPS signals on surfaces used in (A). (C) N 1s XPS signals and fits of a pure PEG-alkyne surfaces before and after the click reaction with the iodine containing small molecule. The N1s signal after the click reaction is fitted for 4 chemically different N species (marked in red), the relative are under the N=**N**-N, C-**N**-N and C-**N**=C peaks is fixed to be 1:2:1 due to the chemical structure of the azide and the relative areas under the peaks is 5.2, 10.4, 21.0, 5.2 from higher to lower binding energy. (D) Si 2p XPS signals of surfaces in (A).

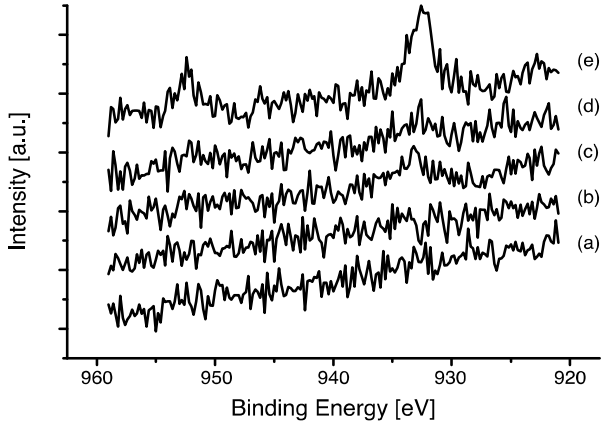

Figure S4. XPS signal of the Cu 2p region of glass substrates (a) coated with PEG-alkyne and (b) additionally subjected to click reaction conditions without any azide respectively (c) clicked with an azide-cRGD conjugate or (d)-(e) clicked with an azide-NTA conjugate. (e) The addition of copper(II) ions to NTA modified substrates results in a NTA/Cu^{2+} complex formation. XPS signals from different probes are shown with an offset.

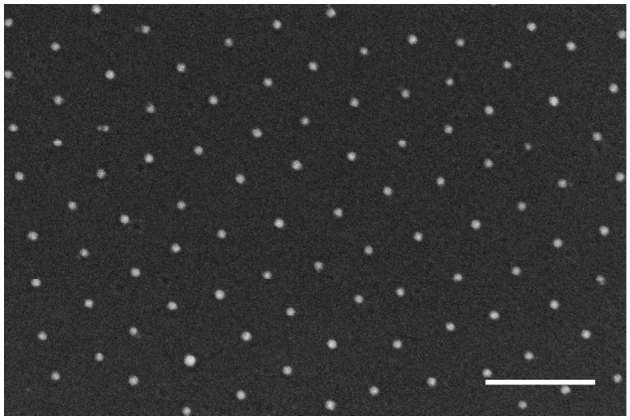

Figure S5. SEM image of gold nanoparticles on glass surfaces with a spacing of 100 nm. The scale bar is 200 nm.