Electronic Supplementary Information

Copper binding properties of metformin – QCM-D, XPS and nanobeads agglomeration

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S1. Experimental Section

Material and Regents

L-cysteine was ordered from Merck KGaA (Darmstadt, Germany). Reagent grade 1,1-Dimethylbiguanide hydrochloride (metformin), Na_2HPO_4, NaH_2PO_4, NaOH, HNO_3, $Cu(NO_3)_2$, 2-(N-morpholino)ethanesulfonic acid and ethanol used in the experiments were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA) and used without further purification. Streptavidin coated multicore magnetic nanobeads (MNBs) with diameter of 100 nm was purchased from Micromod (Rostock, Germany). The Thermo Scientific Ez-link NHS-LC-LC-Biotin and Ez-link HPDP biotin were obtained from Life technologies (Europe BV filial Denmark). All solutions were prepared using degassed ultrapure water (18.2 MΩ·cm) from a Milli-Q® purification system (Millipore Corporation, Billerica, MA, USA). Buffer solution used in this work was 50 mM Na_2HPO_4 + NaH_2PO_4 (pH= 6 for QCM and XPS, pH=7.3 for biotinylated and MNBs modification) and 50mM 2-(N-morpholino)ethanesulfonic acid (pH=6). The pH was adjusted with either NaOH or HNO_3.

L-cysteine modified chips/crystal preparation

Before the modification of L-cysteine, the gold chips/crystals were treated by UV ozone for 20 min. Then the gold chips were cleaned by piranha solution for 6 min and the gold coated crystals were placed in a 5:1:1 mixture of milliQ water, ammonia (25%) and hydrogen peroxide (30 %) solution for 5 min at 75 ^oC. The cleaned chips/crystals were rinsed thoroughly with milliQ water and pure ethanol, and dried by nitrogen gas. Subsequently, the chips and crystals were immersed into 2.5 mM L-cysteine solution with 50 mM buffer solution (pH= 6) for 3h. Before the measurements, all the chips/crystals were rinsed completely with buffer solution and milliQ water.

L-cysteine modified magnetic nanobeads preparation

For synthesis of a 500 µL batch of 0.7 mM NHS biotinylated L-cysteine mix was prepared by mixing 53 µL 4 mg/mL(7 mM) Ez-link NHS-LC-LC-Biotin with 447 µL of 16.5 mM L-cysteine in PBS buffer (pH=7.3) followed by 2 hours incubation at room temperature. To get 198 µL of MNBs (200 nm BNF-Starch streptavidin, 1 mg/mL of solid content), 100 µL of MNBs (100 nm BNF-Starch streptavidin, 10 mg/mL of solid content, 6×10^{12} beads/mL) was washed in 100 µL of PBS buffer (pH= 7.3) two times, followed by taking 20 µL MNBs (10 mg/mL) into 178 µL PBS buffer (pH=7.3). 2 µL of 0.7 mM NHS biotinylated L-cysteine was added to 198 µL of the bead suspension (1 mg/mL) for 1 h incubation.

QCM-D Experiments

All binding processes were in-situ monitored by using a Q-Sense E4 QCM-D instrument (Q-Sense AB, Sweden) with four temperatures controlled flow modules. In each flow module, an Au coated (300 nm) AT-cut quartz crystals (fundamental frequency 4.95 MHz) from Biolin Scientific (Stockholm, Sweden) was placed.

Before each measurement, the crystals were allowed to equilibrate in buffer solution until a stable baseline was achieved. The QCM-D data simultaneously measured in frequency and energy dissipation changes were obtained at 7 overtones.

For investigating the L-cysteine modification, crystals were mounted into the module immediately after cleaning. The QCM-D measurements were performed at 22 ^{o}C with a flow rate of 100 µL/min. All experiments were carried out in phosphate buffer. A 2.5 mM L-cysteine with buffer solution was injected into the QCM-D chamber for on-line self-assembly on the gold coated crystal for 2h.

XPS Measurements

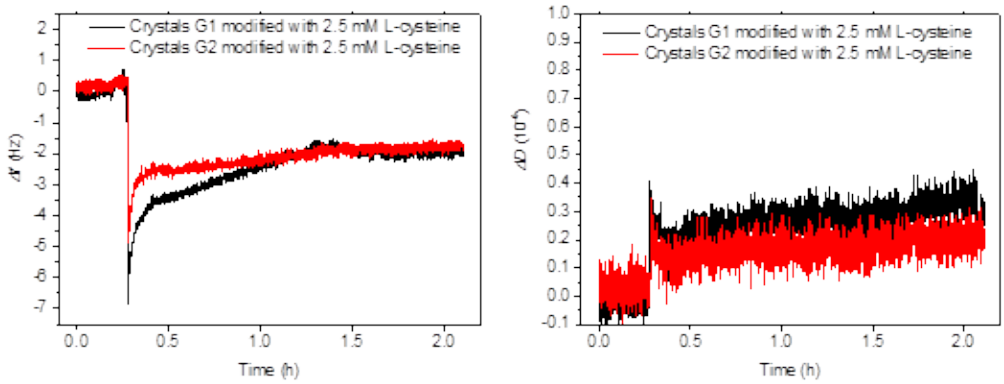
X-ray photoelectron spectra were obtained with a ThermoScientific XPS that focused monochromatic Al-K-Alpha X-rays (1486 eV) on the sample. Spot size was approximately 400 µm and pass energy was 50 eV for scans. Self-assembled monolayers of L-cysteine were prepared as described above on 1 cm^2 gold coated silicon chips. S 2p and Cu 2p spectra were collected and the binding energies were referenced to Au ($4f_{7/2}$) at 84 eV.

Nanoparticle agglomeration Measurements

All optomagnetic measurements were performed on the home-made setup of a Blu-ray optical pick up unit (OPU) as the single optical excitation and sensing element combined with a transparent disc as microfluidic cartridge.[1, 2] The discs were fabricated in PMMA and bonded using pressure sensitive adhesive (PSA). 30µl of the sample volume was required for each optomagnetic measurement. Under excitation by a weak alternating magnetic field, the magnetization of the MNBs changes due to their physical rotation. In the time domain, the MNBs response is the frequency-dependent amplitude of the magnetization. In the frequency domain, the MNBs response is recorded by the complex magnetic susceptibility with in-phase signal $V_2^{'}$and out-of-phase signal $V_2^{''}$. The more free MNBs will induce the higher amplitudes of $V_2^{'}$ and $V_2^{''}$.

The L-cysteine modified MNBs (1 mg/mL) were incubated with 500 µM $Cu(NO_3)_2$ in 50 mM MES buffer (pH=6) (volume ratio 1:1) for 30s. This incubation facilitated the bonding between copper and L-cysteine which caused the formation of L-cysteine-MNB-copper clusters. Next, the samples were further incubated with 0.1mM, 1mM, 10 mM, 30mM and 60mM of metformin in 50 mM MES buffer (pH=6) (volume ratio 2:1) separately. The addition of metformin facilitated the breakage of L-cysteine-copper bonds leading to the breakage of the previously formed clusters and the release of single MNBs. Finally, the sample was loaded in the disc followed by 5 min magnetic incubation right before the measurement. Optomagnetic measurement using the Blu-ray OPU was performed in the disc before and after the addition of metformin in order to quantify the effect of different concentrations of Metformin on extracting copper from L-cysteine-copper complex.

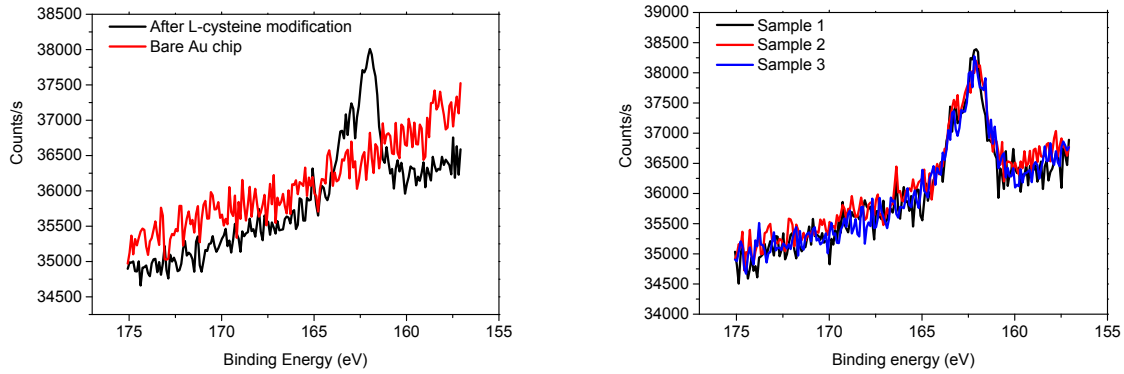
S2. Characterization of L-cysteine functionalization by QCM-D and XPS



(a) (b)

Fig. S2-1 QCM-D frequency (a) and dissipation (b) data (overtone 7) for characterizing the self-assemble L-cysteine monolayer.

To characterize the qualification of self-assembled monolayers of L-cysteine, the changes in frequency and dissipation data upon the modification are recorded simultaneously. As shown in Fig. S2-1, a characteristic two-phase behavior in frequency reflects the initial adsorption of L-cysteine, which is followed by the formation of monolayer. The low dissipation shift confirms that a flat and thin layer is formed on the surface, being consistent with the measurements on other chips.



(a) (b)

Fig. S2-2 The XPS spectrum of S 2p on gold chips: (a) before and after 2 hours L-Cysteine modification; (b) after 2h L-cysteine modification on different chips.

Prior to experiments for each film, the film homogeneity was confirmed by measuring S 2p spectra at three different points on the sample. Fig. S2-2 (a) shows the S 2p spectrum before and after L-cysteine modification. The comparison of S 2p from three different chips is shown in Fig. S2-2 (b).

S3. The effect of X-ray irradiation on the concentration of Cu(II)

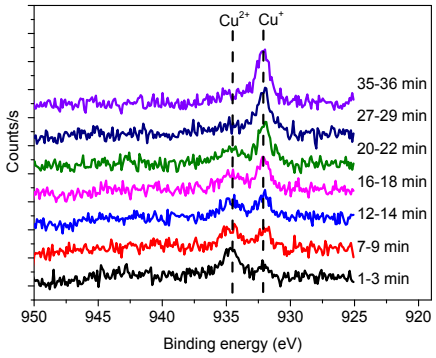


Fig. S3-1 Series of spectra of Cu 2P from an L-cysteine copper complex monolayer on gold chip showing the autoreduction process from Cu(II) to Cu(I) with X-ray irradiation time increasing. Each spectrum was acquired during 50s.

To characterize X-ray induced effect and influence of concentration of Cu(II) on the copper oxidation state in L-cysteine copper complex, XPS spectra were collected at different X-ray exposure time (Fig. S3-1). An increase in the Cu(I) peak is observed as the X-ray irradiation time is increased. And X-ray induced reduction results from an interaction of the low energy electrons with the material.[3, 4]

Reference

1. M. Donolato, P. Antunes, R. S. Bejhed, T. Zardán Gómez de la Torre, F. W. Østerberg, M. Strömberg, M. Nilsson, M. Strømme, P. Svedlindh, M. F. Hansen and P. Vavassori, *Anal. Chem.*, 2015, **87**, 1622-1629.

2. M. Donolato, P. Antunes, T. Z. G. de la Torre, E.-T. Hwu, C.-H. Chen, R. Burger, G. Rizzi, F. G. Bosco, M. Strømme, A. Boisen and M. F. Hansen, *Biosensors and Bioelectronics*, 2015, **67**, 649-655.

3. M. Brust, P. M. Blass and A. J. Bard, *Langmuir*, 1997, **13**, 5602-5607.

4. J. J. Yang, T. Regier, J. J. Dynes, J. Wang, J. Y. Shi, D. Peak, Y. D. Zhao, T. D. Hu, Y. X. Chen and J. S. Tse, *Anal. Chem.*, 2011, **83**, 7856-7862.