

Supplementary Material

Oxidization increases the binding of EGCG to serum albumin revealed by kinetic data from label-free optical biosensor with reference channel

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Kinetic model equations

Homogeneous binding site model

The homogeneous binding site model supposes that between the analyte (A, in our case EGCG or ox. EGCG) and the ligand (L, in our case BSA) only one type of binding can occur, resulting in the ligand-analyte complex (LA). The process is reversible, involving association and dissociation, which are characterized by the kinetic constants k_a and k_d , respectively. The interaction of the ligand and analyte can be described with the following reaction scheme:



Based on the mass action law, the rate equations expressing the change of the concentration of L and LA can be written as:

$$\begin{aligned} \frac{d[L]}{dt} &= -(k_a[L][A] - k_d[LA]) \\ \frac{d[LA]}{dt} &= (k_a[L][A] - k_d[LA]) \end{aligned} \quad (\text{S2})$$

where t denotes the time, and $[L]$, $[A]$ as well as $[LA]$ are the concentration of the related components. The dissociation constant (K_d) that measures the strength of the L-A interaction can be determined by calculating the ratio k_a/k_d .

Heterogeneous binding site model

The heterogeneous binding site model can be used to model interacting L-A systems where L has two independent binding sites with different affinities to A.

Considering two types of binding sites which have different binding affinities (characterized by kinetic constants of k_{a1} , k_{d1} , k_{a2} and k_{d2}), one can write the following scheme which hypothesizes two different ligands, L_1 and L_2 .



The rate equations for the relevant components are:

$$\begin{aligned} \frac{d[L_1]}{dt} &= -(k_{a1}[L_1][A] - k_{d1}[L_1A]) \\ \frac{d[L_1A]}{dt} &= (k_{a1}[L_1][A] - k_{d1}[L_1A]) \\ \frac{d[L_2]}{dt} &= -(k_{a2}[L_2][A] - k_{d2}[L_2A]) \\ \frac{d[L_2A]}{dt} &= (k_{a2}[L_2][A] - k_{d2}[L_2A]) \end{aligned} \tag{S4}$$

Adsorption of EGCG to different type of surfaces

In order to characterize the adsorption characteristics of EGCG, we tested different type of chip surfaces using the GCI technique: PCP (thin polycarboxylate functionalization, quasi-planar surface, hydrophilic), PCP-L (quasi-planar polycarboxylate coating with lipid anchors, hydrophobic) as well as Ta_2O_5 surface (bare chip). As demonstrated in Fig. S1, we found that the EGCG can adsorbed at a high level to all of the examined surfaces.

Considering the reproducibility and variability of the measurements, we determined the standard deviations of data obtained on the bound BSA and EGCG amounts. Based on three repeated experiments performed on different PCP chips, the bound surface mass of BSA was determined to be 541 ± 22 $\mu\text{g}/\text{mm}^2$ (average \pm standard deviation), representing only a small variation in the

replicated measurements. Even using three types of surfaces (PCP, PCP-L and Ta₂O₅), relatively low value of standard deviation was obtained for EGCG binding, both on the measurement (*chl*) and reference (*ch2*) channel (1786 ± 44 pg/mm² on *chl* and 1562 ± 106 pg/mm² on *ch2*, respectively).

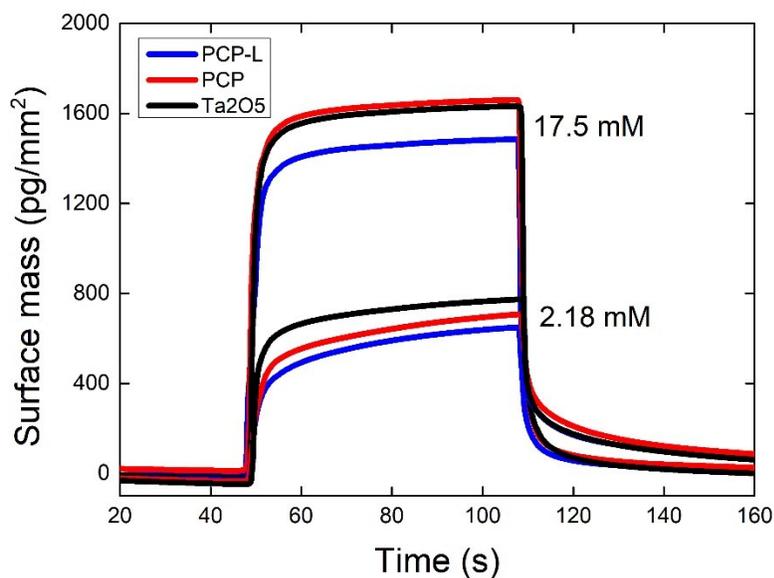


Figure S1. Kinetic surface mass curves representing the adsorption of EGCG to PCP-L, PCP and Ta₂O₅ surfaces at EGCG concentrations of 2.18 mM and 17.5 mM.

Kinetic measurement results demonstrating the performance of the Creoptix WAVE

To prove the performance of the novel GCI technique, we carried out reference measurements on the furosemide-carbonic anhydrase II (CAII) analyte-ligand pair, which is a widely used model system of small molecule binding kinetic experiments. Our measurement results are presented in Fig. S2.

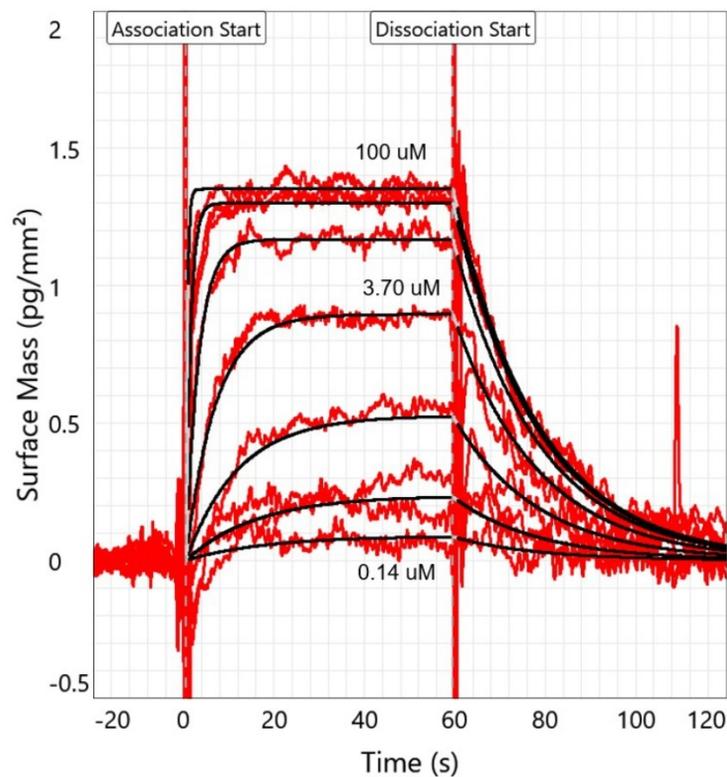


Figure S2. Kinetic measurement series performed to measure the binding characteristics of the small molecule furosemide (331 Da) to CAII protein. We fitted the obtained kinetic curves by the homogeneous binding site model and we determined a $K_d = 2.2 \mu\text{M}$ value. Measured concentration series: 100 μM , 33.3 μM , 11.1 μM , 3.70 μM , 1.24 μM , 0.41 μM , 0.14 μM (in the figure, only the 100 μM , 3.70 μM and 0.14 μM concentrations are marked by their values).