Supplementary Information for

Evaluating Tumor-Associated Activity of Extracellular Sulfatase by Analyzing Naturally-Occurring Substrate in Tumor Microenvironment of Hepatocellular Carcinoma

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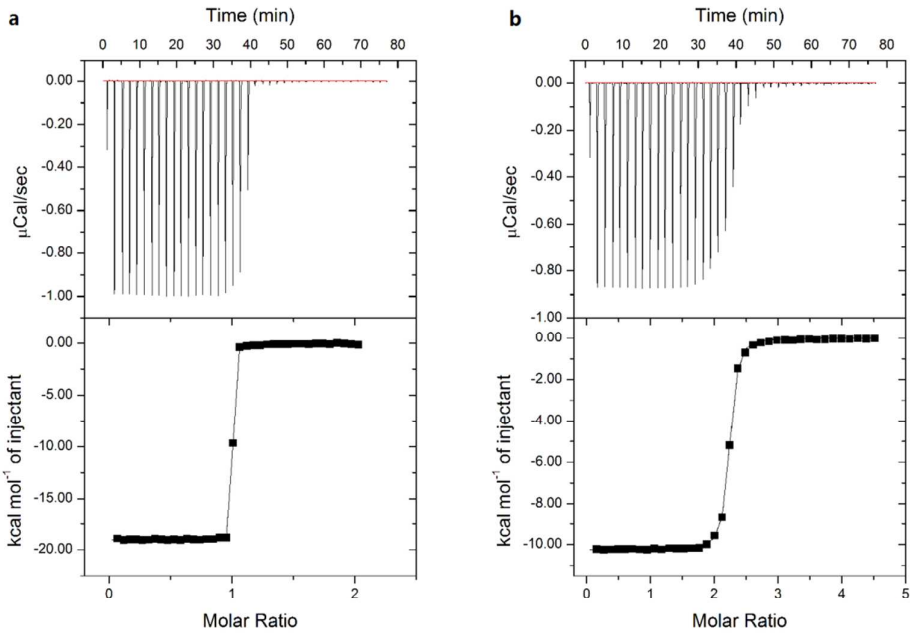

Figure S1. Isothermal titration calorimetry (ITC) data obtained by titrating 0.5 mM of the peptide probe into 0.05 mM of perlecan core protein (a), and by titrating 0.5 mM purified HS into 0.05 mM probe peptide. The solution adopted is 50 mM TBS, pH 7.5. The top row displays the raw data of power versus time. The bottom row is the corresponding data by integrating enthalpy values versus the molar ratio of titrant: titrand. These data are fit using Origin 7.0 software, the resulted fitting curve is also shown in the lower row.

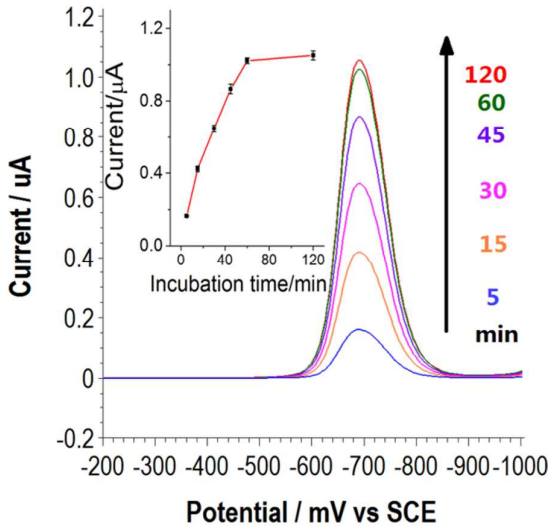

Figure S2. Optimization of the incubation time with the cellular sample. DPVs of HS cathodic stripping are recorded in detecting the ECM fraction of the cellular sample diluted to 3.5×10^5cell/mL, following the route a~f in Scheme 1, the time for incubating with the sample is varied as indicated on the graph. Inset shows the peak currents as a function of the incubation time, and error bars show the standard deviation (n=3).

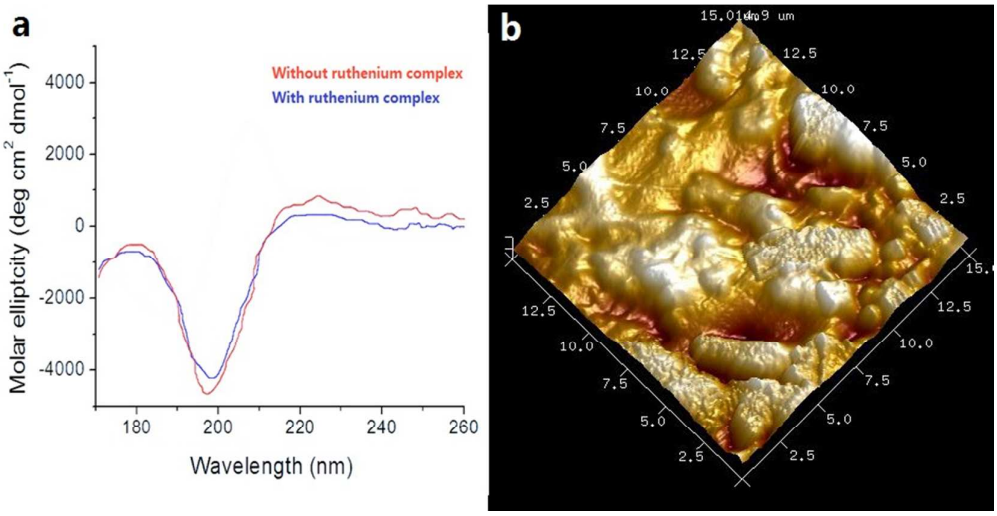

Figure S3. (a) The effects on the conformation of the peptide probe upon the interaction with the ruthenium complex. Circular dichroism (CD) spectra were obtained using a JASCO J-750 circular dichroism spectrometer. (b) Atomic force microscopic characterization of the biosensing surface after binding of the perlecan from the cellular sample.

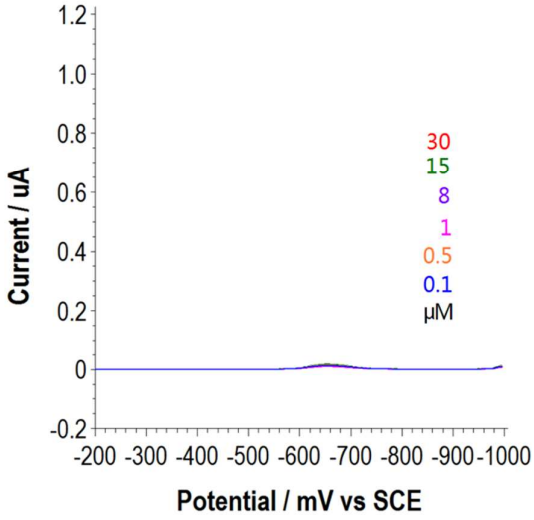

Figure S4. Controls following the same procedure as in Figure 2b, except that after the complex cation catalyzed photo-cleavage of the surface immobilized HS, the electrode, after thorough rinsing, is transferred to another solution containing no fragmented HS generated by the cleavage.

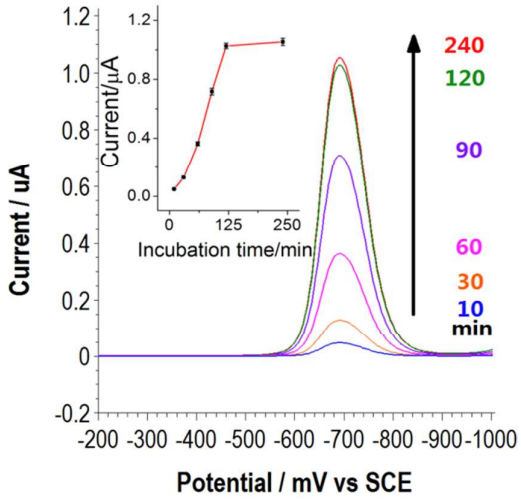

Figure S5. Optimization of the incubation time with the complex cation. Experimental details are the same as in Figure S2, except that the time for incubating with the complex cation is varied as indicated on the graph. Inset is also similar to the inset of Figure S2.

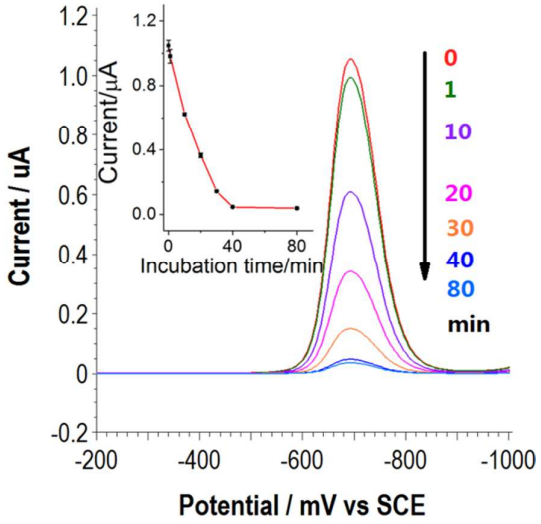

Figure S6. Optimization of the incubation time with sulf2. Experimental details are the same as in Figure S2, except that before the incubation with the complex cation, the electrode is incubated with 100 pM sulf2 for different time as indicated on the graph. Inset is also similar to the inset of Figure S2.

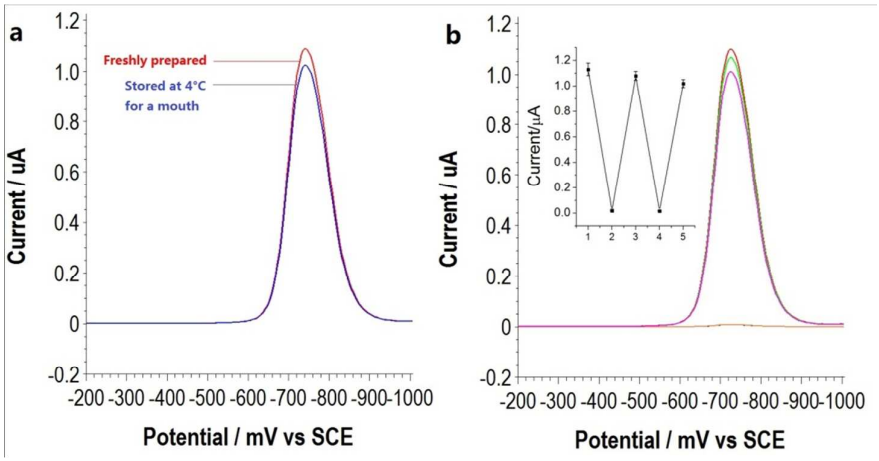

Figure S7. The stability and reproducibility of the proposed method. (a) The signal responses for detecting 3.5×10^5cell/mL cellular sample using peptide modified electrodes freshly prepared or that had been stored at 4 ℃ for one mouth. (b) Similar experiments carried repetitively after the peptide modified electrode is regenerated using SDS for several times. Experimental details are the same as in Figure S2, inset is also similar to the inset of Figure S2.