

# Supplementary Information

## Activity-Enhanced DNAzyme for Design of Label-free Copper (II)

### Biosensor

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## 1 Polydopamine Nanoparticle Characterisation

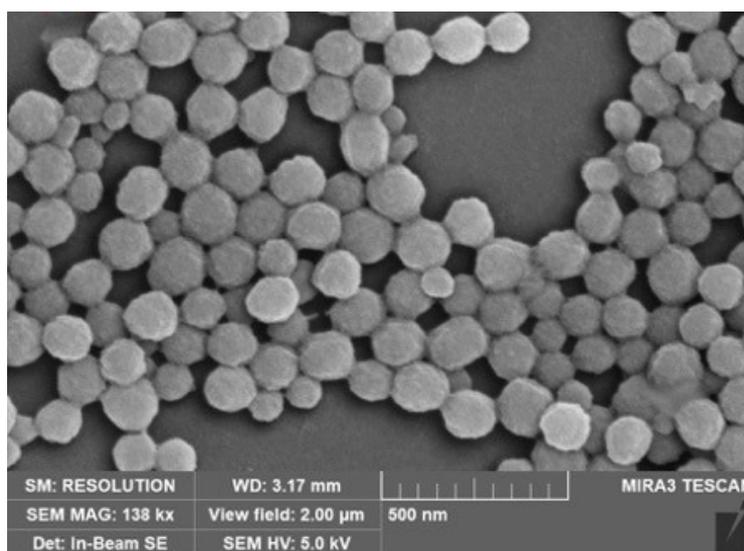
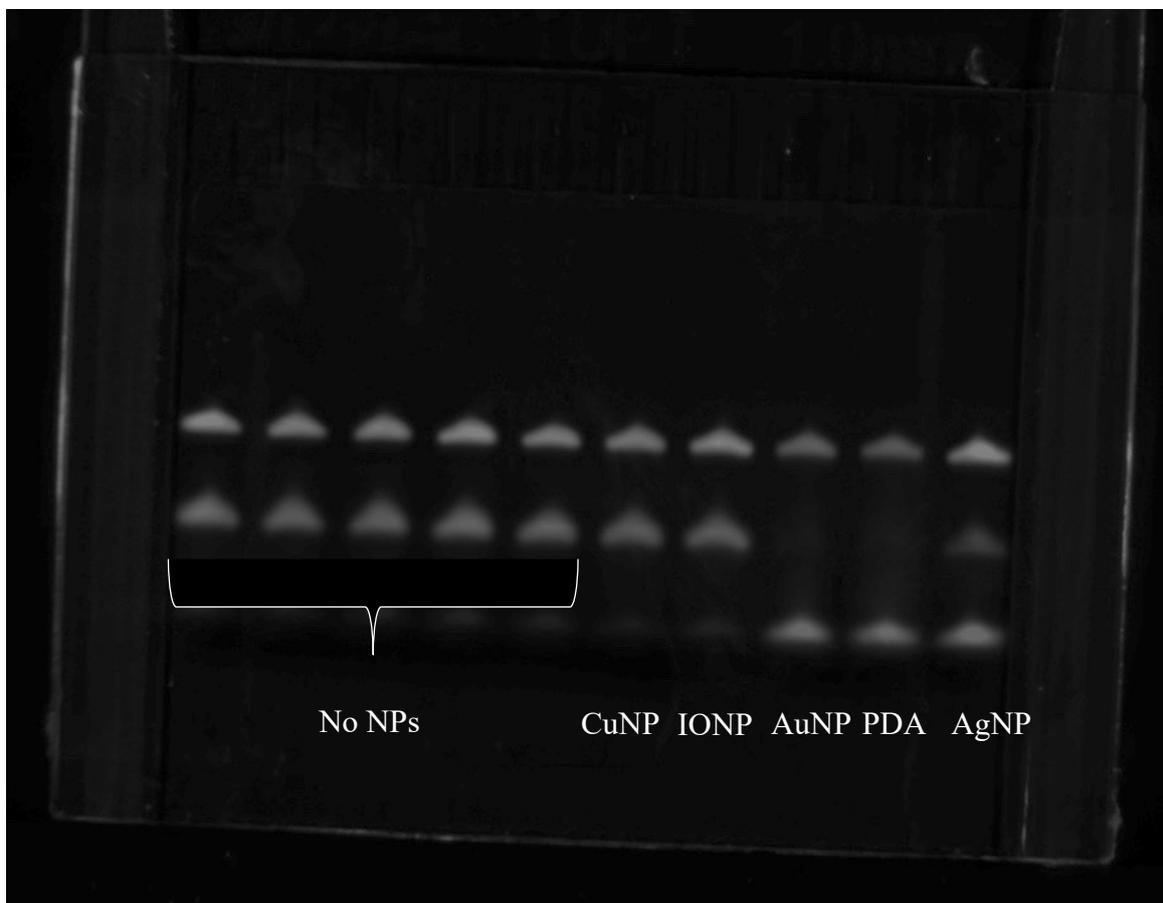


Figure S 1 – SEM image of polydopamine nanoparticles made using polymerisation of dopamine under alkaline conditions

## 2. Nanoparticle Screening Raw Gel

Figure S2 shows the raw gel presented in the main text in figure 2.



*Figure S 2 – 12% urea PAGE gel of nanoparticle screening assay. All assays contained 12.5 $\mu$ M CuCl<sub>2</sub> and were run for one hour before quenching with 8M urea quenching buffer. The top row of blots are F8 DNAzyme, the middle row is uncleaved substrate, and the bottom row is cleaved substrate.*

## 3. The role of citrate in rate enhancement of DNA-cleaving DNAzyme

Figure S3 shows the activity (percentage cleaved) of F8-DNAzyme in the presence of different concentrations of citrate ions (0, 2, 4, 6, 8, 10mM) with 12.5 $\mu$ M copper (II) and a control with 10mM citrate and no copper (II) . The F8-substrate complex was reacted in 10mM HEPES, 15mM NaCl and 12.5  $\mu$ M CuCl<sub>2</sub> along with the citrate for 1 hour at 37°C. The reaction was then quenched using 8M urea quenching buffer. Samples were analysed using 15% Urea-

PAGE gel run at 45V for 120 minutes. Gels were imaged using Syngene fluorescent camera and quantified using ImageJ Gel analysis software.

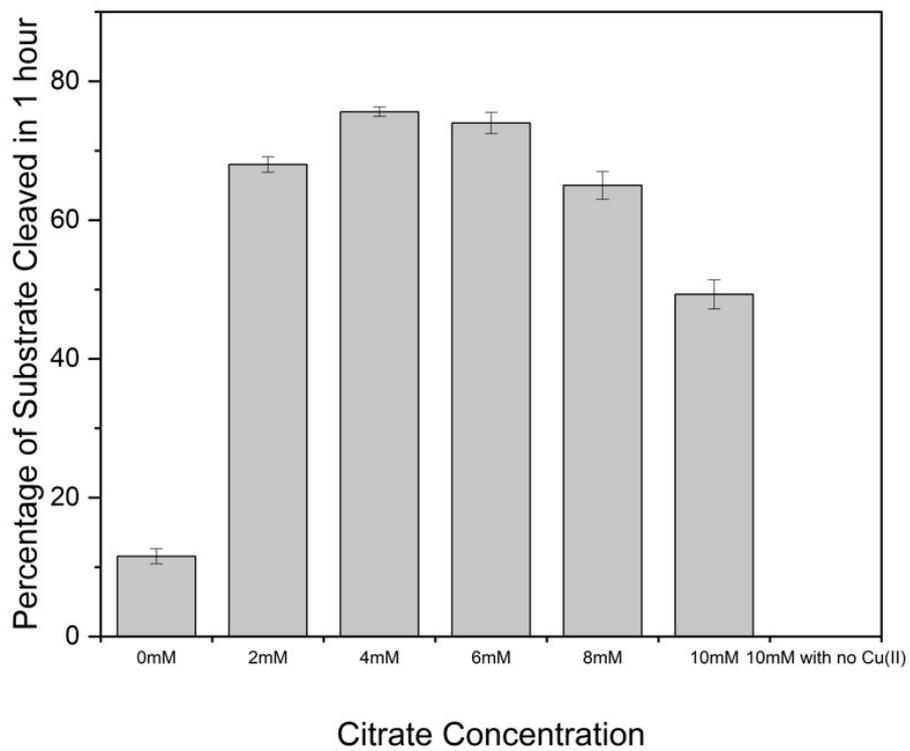


Figure S 3 - Effect of citrate ions on the cleavage performance of F8 DNzyme.

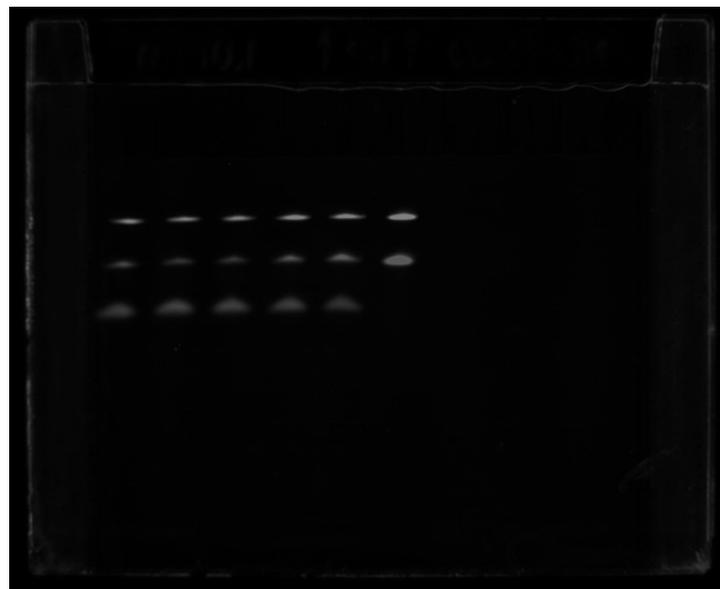


Figure S 4 – Raw PAGE gel data for the effect of the citrate ions on DNA cleavage in presence of DNzyme . Top row is F8 DNzyme, second row is un-cleaved substrate, bottom row is cleaved substrate. Columns from left to right: (1) 2mM citrate, (2) 4mM citrate, (3) 6mM citrate, (4) 8mM citrate, (5) 10mM citrate, (6)10mM citrate with no copper (II).

## 4. Production of H<sub>2</sub>O<sub>2</sub> by PDA NPs

Figure S5 shows the percentage of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) generated by polydopamine nanoparticles (PDA NPs) at different concentrations (0.01mg/mL and 0.1mg/mL). Fluorescent H<sub>2</sub>O<sub>2</sub> assay was purchased from Sigma Aldrich and values were calculated from a standard curve using stock 0, 0.01, 0.03, 0.1, 0.3, 1, 3 and 10μM H<sub>2</sub>O<sub>2</sub> solutions.

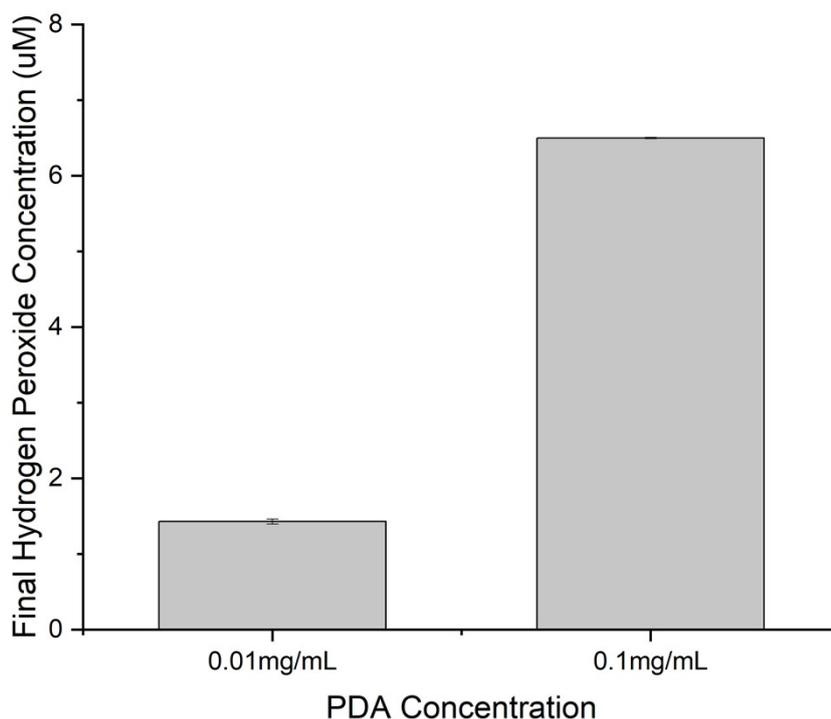
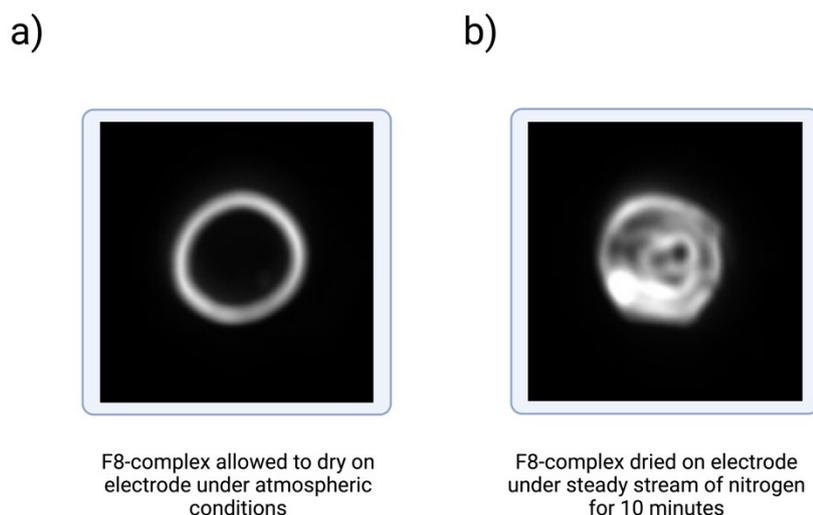


Figure S 5 – Hydrogen peroxide generated from 0.01mg/mL of 140nm PDA NPs in water.

## 5. Electrode Modification – Drying DNAzyme

Figure S5(a) shows the sensing electrode after cy3-DNAzyme droplet drying under atmospheric conditions. 10μL of pre-annealed F8-Substrate complex in 10mM HEPES and 15mM NaCl was allowed to dry at room temperature. The image was taken with a fluorescent camera from Syngene using 543nm excitation and 580nm emission. A distinct coffee ring could be observed with little signal from the centre of the electrode. S5(b) shows an electrode

prepared with the same procedure as in S5(a), however, the electrode was dried under a steady and gentle stream of nitrogen for 10 minutes until completely dry. The coffee ring effect could not be observed when imaging with the same conditions as in S5(a).



*Figure S 6 - (A) Coffee ring effect from drying 10 $\mu$ L of F8-DNAzyme-Substrate onto gold screen printed electrode. (B) Removal of coffee ring effect when drying 10 $\mu$ L of F8-DNAzyme-Substrate onto gold screen printed electrode under steady stream of nitrogen gas at room temperature for 10 minutes*

## 6. Electrode Modification – PDA Film Formation

Figure S6(a) and S6(b) shows a camera image of the electrode before and after exposure to 10mM tris-HCl pH 8.5 with 5mg/mL of Dopamine-HCl for 120 minutes. Darkening of the surface could be observed with the naked eye.

Figure S7 shows the impedance spectroscopy scan for electrodes with and without exposure to 10mM tris-HCl pH 8.5 with 5mg/mL of Dopamine-HCl for 120 minutes and washing 3 times. Impedance of the electrode was increased indicating the formation of insulating PDA layer.

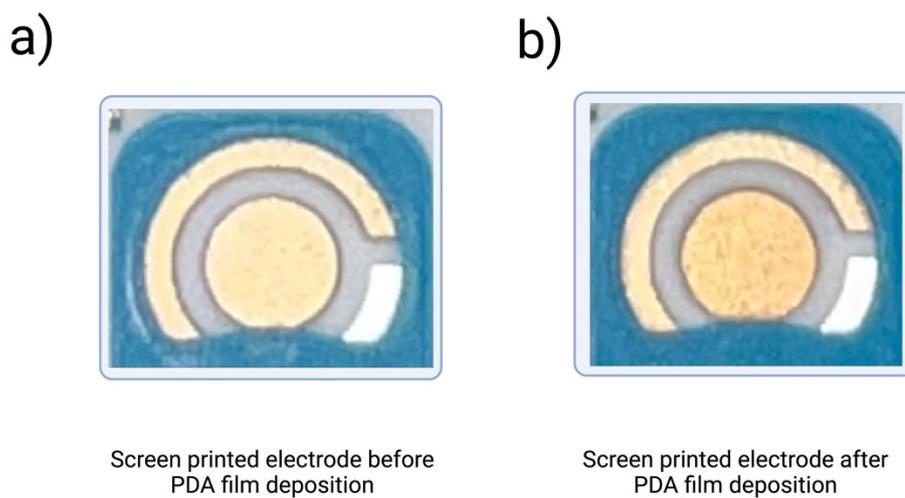


Figure S 7 - (A) Camera image of screen-printed electrode before PDA film deposition. (B) Camera image of screen-printed electrode demonstrating a darker sensing electrode observed by naked eye after PDA film deposition.

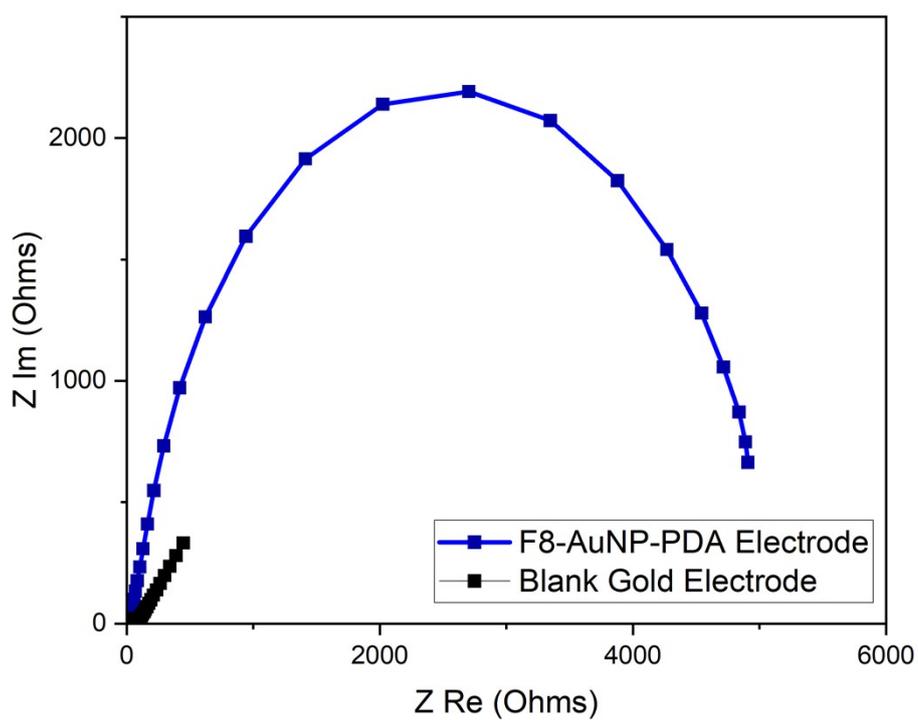


Figure S 8 – Impedance Spectroscopy traces for gold screen printed electrodes in 5 mM ferri/ferrocyanide redox medium. (black data points) show impedance scan for a blank electrode, (blue data points) show impedance scan for functionalised F8-AuNP-PDA electrode.

## 7 Sensor Performance

Figure S9 shows the DPV curves for F8-DNAzyme-PDA electrode with and without 0.01mg/mL 20nm citrate capped gold nanoparticles. Both measurements were taken after 15 minutes in 100 $\mu$ M Cu (II) in 1-times PBS.

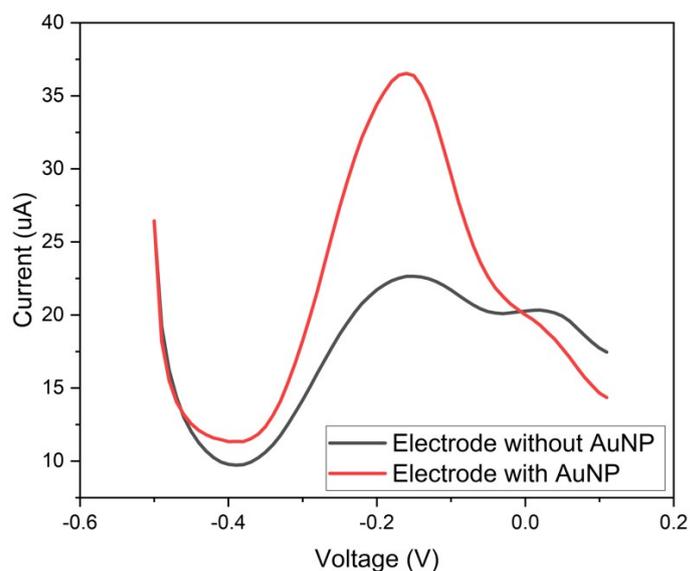


Figure S 9 - DPV response curves for F8-DNAzyme-PDA-AuNP Electrode and F8-DNAzyme-PDA electrode.

Figure S10 shows the sensor stability over time.

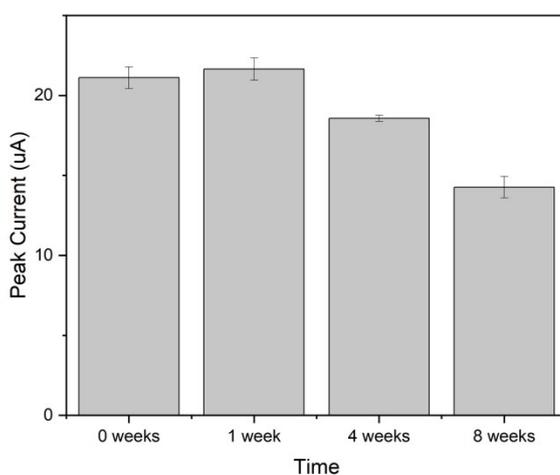


Figure S10 – Sensor Performance over a time frame of 8 weeks. Sensors were stored in a dark cupboard under normal atmospheric conditions.