Supplementary Information (ESI)

for

Label-free electrochemical detection of an *Entamoeba histolytica antigen* using cell-free yeast-scFv probes

Yadveer S. Grewal, Muhammad J. A. Shiddiky,* Sean A. Gray, Kris M. Weigel, Gerard A. Cangelosi, and Matt Trau*

Chemicals

All chemicals purchased from the Australian supplier's branch, unless otherwise stated. Lyophilized yeast-scFv, '350' and Jacob antigens were generated at Seattle Biomedical Research Institute, USA. Biotinylated anti-HA obtained from Sapphire Bioscience. Biotinylated BSA from Thermo scientific. Streptavidin from Invitrogen. PBS tablets from Astral Scientific. Potassium ferrocyanide, potassium ferricyanide, and potassium chloride from Sigma Aldrich. Protease Inhibitor EDTA-free cocktail tablets from Roche. Glycerol from Ajax Finechem.

Determination of the surface area of the electrodes

Gold macrodisk (diameter = 3 mm) working electrodes were purchased from CH Instrument (Austin, USA). Prior to electrochemical experiment, the electrodes were cleaned physically with 0.1 micron alumina, sonicated in acetone for 20 min, and chemically with piranha solution ($H_2SO4:H_2O_2$; 3:1) for 30 seconds to remove any organic impurities and finally electrochemically in 0.5 M H_2SO_4 until characteristic gold electrode profiles were achieved. The effective working area of the electrodes were determined under linear sweep voltammetric conditions for the one-electron reduction of $K_3[Fe(CN)_6]$ [1.0 mM in water (0.5 M KCl)] and use of the Randles-Sevcik relationship.[1]

$$i_p = 0.4463nF(nF/RT)^{1/2}AD^{1/2}\nu^{1/2}C \text{ .... (1)}$$

where i_p is the peak current (A), n (=1) is the number of electrons transferred, A is the effective area of the electrode(cm^2), D is the diffusion coefficient of $[Fe(CN)_6]^{3-}$ (taken to be $7.60 \times 10^{-6} cm^2 s^{-1}$), C is the concentration (mol cm^{-3}), ν is the scan rate (Vs^{-1}), and other symbols have their usual meanings.

Generation of cell-free yeast-scFv fragments

Whole yeast cells expressing scFv on their surface (yeast-scFv), were selected for their binding towards the *E. histolytica* antigen EHI_115350,[2] called '350' in this report. After confirmation of specific antigen binding, the cells were lyophilized for long-term storage as described. [2] Lyophilized yeast were disrupted with a mortar and pestle into a fine powder, then 10mL of PBS, 5% glycerol and protease inhibitor cocktail were added. The sample was centrifuged at 500 rpm for 2 minutes at 15ºC to remove whole yeast. The processed lysates (supernatant) were stored at 4ºC until use.

Dynamic light scattering sizing of fragments

To assure that the yeast-scFv fragments were small enough to be useable in the bioassay, DLS measurements (Fig. S1) (Malvern Zetasizer series) were taken of the yeast-scFv fragments after filtration through a 0.1µm filter (Millipore). The DLS data confirmed that fragments existed in the sub 100nm size range, and lacked detectable whole-cell yeast-scFv.

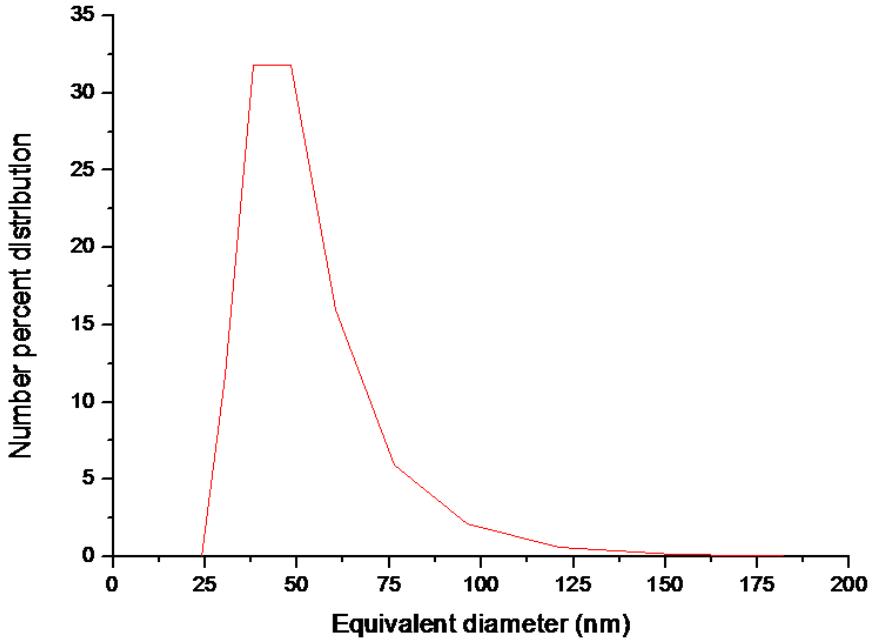

Fig. S1 DLS of yeast-scFv after 100nm filter. Analysis performed on Malvern Zetasizer 3000 HSa using multimodal algorithm.

Assay protocol

Au disk electrodes were functionalised with BSA by incubating them for 45 min in biotinylated BSA solution (100 µg/mL) and incubated on a thermoshaker set to 25ºC at 300 rpm. The thermoshaker and these settings (time and temperature) were used in all subsequent steps. The electrodes were washed with 1XPBS (pH 7.4, 137mM sodium chloride, 2mM potassium chloride, 10mM phosphate buffer) after each incubation step. The electrode was incubated in 400 µl of streptavidin (100 ug/mL) solution for 45 minutes. These electrodes were treated with 100 ug/mL of biotinylated HA antibody solution at room temperature for another 45 min, leading to immobilization of the anti-HA on the electrode surface. Finally the cell-free yeast-scFv reagent diluted 1/10 in PBS was allowed to bind for 45 minutes before washing with PBS. The sensing electrodes were then incubated in 400 µL of antigen solution at varying concentrations to complete the immunoreactions.

Electrochemical procedure

All electrochemical experiments were conducted at room temperature (25 ± 1°C) in a standard three-electrode electrochemical cell arrangement using an electrochemical analyzer CHI 650D (CH Instruments, Austin, TX), where the electrochemical cell consisted of a Au sensor as a working electrode, a Pt wire counter electrode, and a Ag/AgCl (3 M NaCl) reference electrode. Electrochemical signals were measured in a 10 mM phosphate buffer solution (pH 7.4) containing 2.5 mM $[Fe(CN)_6]^{3-}$/ $[Fe(CN)_6]^{4-}$ (1:1) and 0.1 M KCl. Differential pulse voltammetric (DPV) signals were obtained with a potential step of 5 mV, pulse amplitude of 50 mV, pulse with 50 ms, and a pulse period of 100 ms.The EIS spectra were recorded in 10 mM phosphate buffer solution (pH 7.4) containing 2.5 mM $[Fe(CN)_6]^{3-}$/ $[Fe(CN)_6]^{4-}$ (1:1) and 0.1 M KCl using an alternating current voltage of 10 mV, with the frequency range of 0.1 Hz – 100 kHz.

The faradic current generated by the $K_3[Fe(CN)_6]/K_2[Fe(CN)_6]$ probe accounts on the presence of a protein. The current changes corresponding to target antigen binding to the antibody was calculated as follows:

% Decrease of peak current = $(I_{before} - I_{after})/ I_{before} \times 100$ .... (2)

where I_{before} = mean current at zero target concentration (*e.g.*, current generated by the bio BSA/streptavidin/bio anti-HA/yeast-scFv layer), I_{after} = mean current at any concentration of target antigen.

References

1. A. J. Bard and L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*, John Wiley & Sons, 2000.
2. S. A. Gray, K. M. Weigel, I. K. M. Ali, A. A. Lakey, J. Capalungan, G. J. Domingo, and G. A. Cangelosi, *PLoS ONE*, 2012, **7**, e32042.