Electronic Supplementary information

Label-free electrochemical detection of malaria-infected red blood cells

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# Equal contribution

ST 1. Preparation of the electrode

A solution of 20% (vol/vol) citrate capped GNP in PBS was spread over the surface of the electrode and static potential of +0.7 V was applied for 10 minutes. Fig. S1 shows relative current variation with respect to time due to bulk electrodeposition of the citrate capped GNP over the surface.

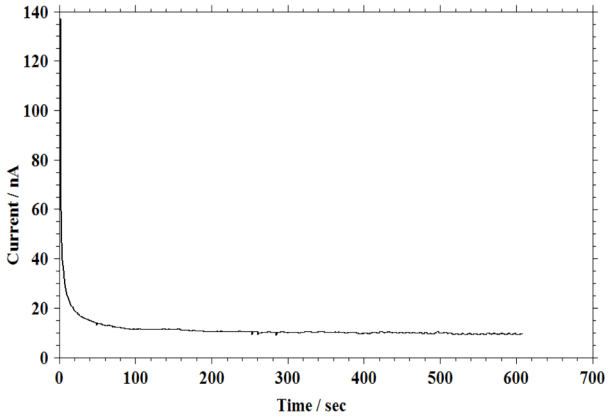


Fig. S1 Change in current with time during electrodeposition.

ST 2. Cyclic voltammetry and EIS scan response due to binding of *P. falciparum* infected cells to the modified surface.

The surface of the screen printed electrode was modified by electrodeposition of citrate capped GNP. MAbD2 in PBS was adsorbed on the surface of SPE/GNP electrode by overnight incubation. The CV (Fig. S2a) and EIS (Fig. S2b) scans of the bare electrode, electrode after GNP deposition (SPE/GNP)

and modified surface after antibody binding (SPE/GNP/MAb) were recorded in the redox couple. In CV, an increase in current was observed due to increased conductivity after GNP deposition. The current decreased after adsorption of MAbD2 on SPE/GNP. Further decrease in current was observed when cells were applied in increasing concentrations from 10^2 cells/mL to 10^7 cells/mL on SPE/GNP/MAbD2, but a small change in current was observed (Fig. S2a). Similarly in EIS scan, the impedance decreased after GNP deposition and increased again after Ab binding, showing the same trend as observed in CV. However in contrast to CV, the EIS results showed a wide variation in impedance and a well resolved Nyquist plot was observed at all the concentrations from 10^2 cells/mL to 10^7 cells/mL (Fig. S2b).

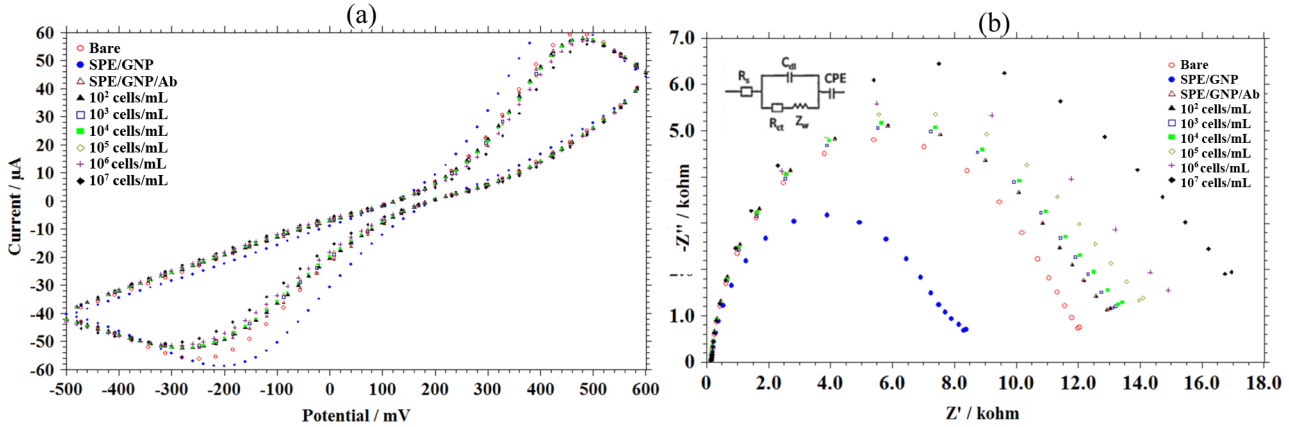


Fig. S2 (a) Cyclic voltammetry showing each steps of electrode modification. The CV scans for the binding of *P. falciparum* cells from 10^2 cells/mL to 10^7 cells/mL shows little variation in the current between each dilution. (b) EIS scans showing each steps of electrode modification. The scans due to binding of cells from 10^2 cells/mL to 10^7 cells/mL shows a well resolved Nyquist plot.

ST 3. Impedance scan of *P. falciparum* infected cells with control MAb.

To rule out the possibility of nonspecific binding of the *P. falciparum* infected cells to the modified surface, the electrode was prepared with negative control antibody (MAbA3E1 raised against human TNF α). Cell concentrations ranging from 10^2 cells/mL to 10^7 cells/mL were incubated on SPE/GNP/MAbA3E1, and the impedance measurements were done in 5 mM ferricyanide/ferrocyanide couple at pH 7.4 in PBS buffer in the frequency range from 1 Hz to 100 kHz. No change was observed in the impedance (Fig S3).

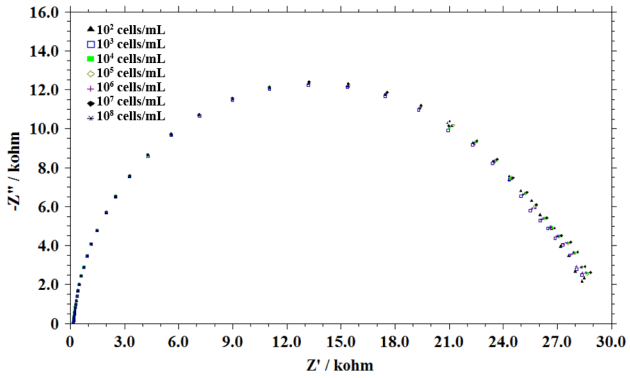


Fig. S3 Impedance scan of *P. falciparum* infected cells on SPE/GNP/control MAb showing no change in impedance with the increase in cell numbers.

ST 4. Change in EIS spectra due to IRBC and NRBC binding.

The change in impedance as a result of binding of MAbD2 on *P. falciparum* infected and non-infected cells were recorded on the modified electrode surface (SPE/GNP/MAbD2). Cells were incubated with a concentration of 10^2 cells/mL to 10^7 cells/mL on SPE/GNP/MAbD2 and the impedance measurements were recorded in 5 mM ferricyanide/ferrocyanide couple at pH 7.4 in PBS buffer in the frequency range from 1 Hz to 100 kHz. Randles equivalent circuit used for fitting the data showed a linear relationship between R_{ct} and the logarithm of number of cells (Fig. S4).

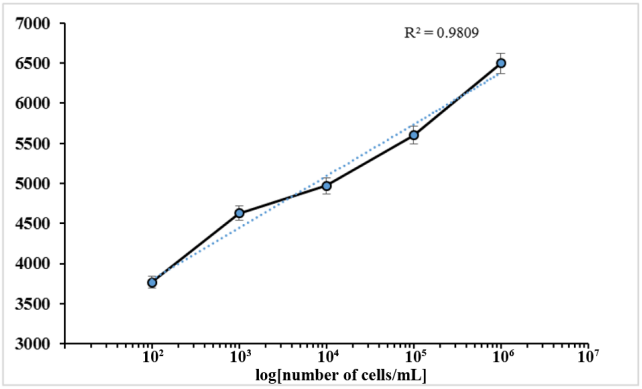
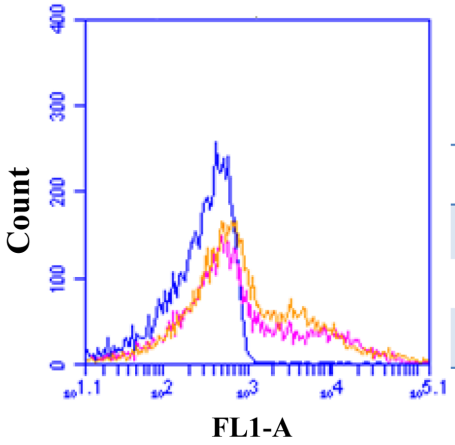


Fig. S4 Plot representing linear fit of R_{ct} vs. log [number of *P. falciparum* infected cells] with R squared value of 0.9809 showing linear relationship.

ST 5. Binding of MabD2 with ring, trophozoite and schizont stages.

FACS analysis was done by using ethidium bromide (EtBr) to stain the the infected cells. EtBr in PBS was added to 500 uL of the cell suspension (10^7 cells/mL) to obtain a final concentration of 1µg/mL and incubated for 30 minutes at room temperature on a rotospin in dark. After washing, for surface staining, the test and control antibodies in 1% BSA/PBS at a concentration of 15 µg/mL was added to

the cell suspension and incubated at RT for 90 minutes followed by washing twice with PBS. The cells were than incubated in Alexa 488 labelled secondary antibody (1:200 vol/vol) for an hour followed by 2 times washing in PBS. The cells were then suspended in PBS for FACS analysis. RBC population was gated based on FSC and SSC. Acquisition time was set to obtain a count of 10000 ethidium bromide positive cells. Binding of MAbD2 was observed with the trophozoites and the schizont stage while no binding was observed in case of the ring stage.



Sample	Count	MFI
Ring	10000	259.79
Trophozoite	10000	5915.94
Schizont	10000	5483.49

Fig. S5 Flow cytometric analysis of binding of MAbD2 with the IRBC with the ring, trophozoite and the schizont stage of the parasite. The MFI values of the negative control IgG antibody with all the three stages (ring, trophozoite and schizont) were between 250 to 400.

ST 6. Change in EIS spectra due to the binding of the ring and the trophozoite stage cells.

Impedance spectra recorded for the binding of the trophozoite cells to MAbD2 showed a linear increase in the impedance with the increase in cell dilution from 10^2 cells to 10^7 cells (Fig. S6 a and b) while no change was observed with the ring stage cells (Fig. S6c).

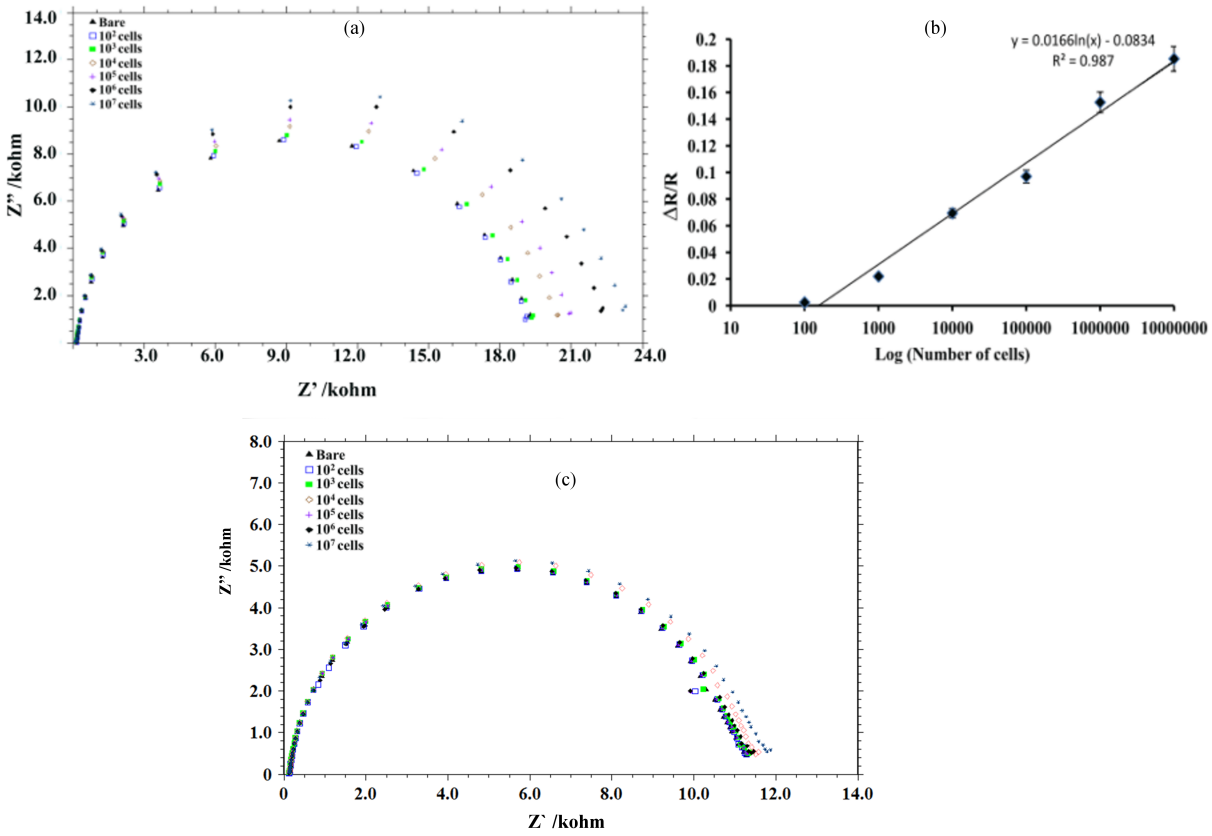


Fig. S6 a & c. Nyquist plots showing the change in EIS spectra with different number of *P. falciparum* infected cells at the trophozoite stage and ring stage on modified electrode surface (SPE/GNP/MAbD2). The measurements were recorded in 5 mM ferricyanide/ferrocyanide couple at pH 7.4 in PBS buffer in the frequency range from 1 Hz to 100 kHz. (b) Plot representing linear fit of R_{ct} vs. log [number of *P. falciparum* infected cells] with R squared value of 0.987 showing linear relationship.