Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

A. Supporting Figures

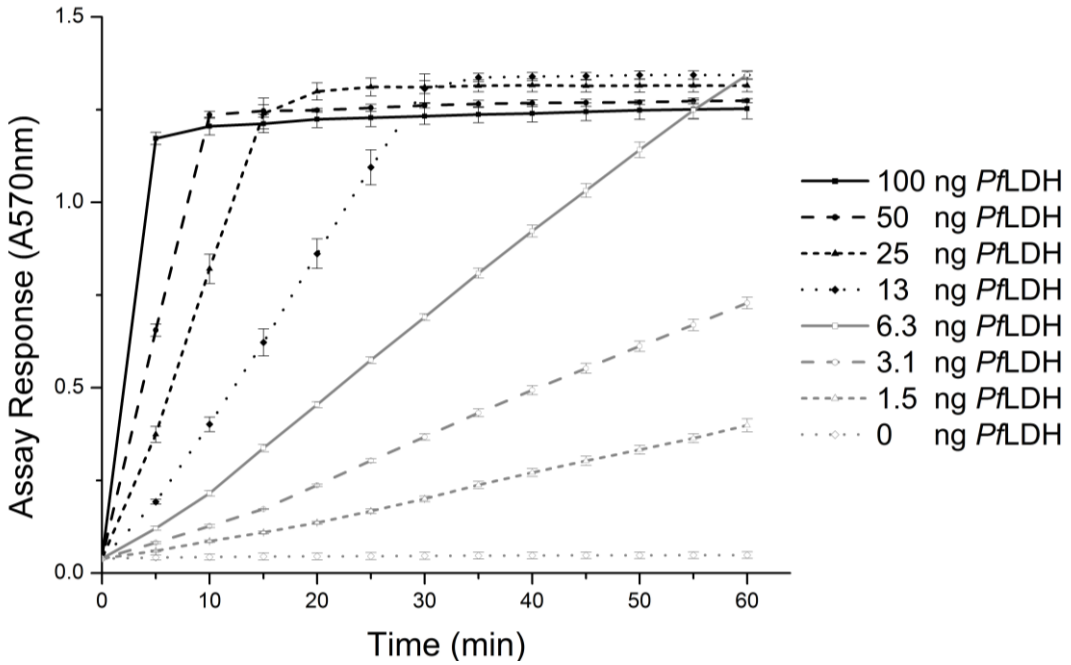

Fig. S1 Assay response over time using different amounts of free *Pf*LDH in L-lactate/NTB solution. The reaction shows good linearity until substrate depletion, causing signal saturation.

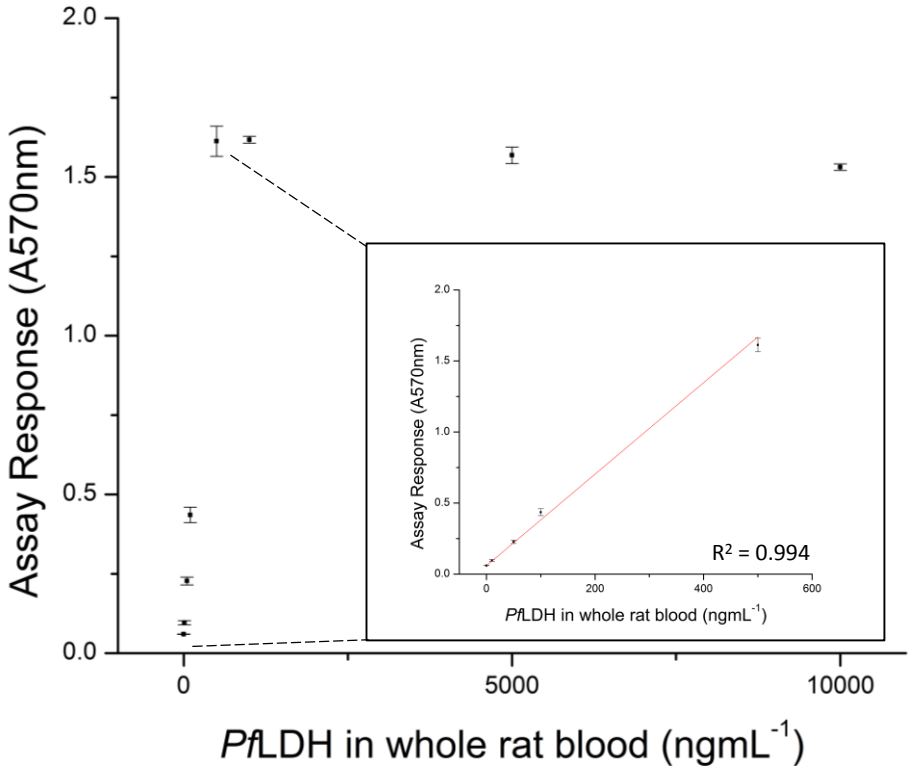

Fig. S2 APTEC assay response for detecting exogenous recombinant *Pf*LDH in whole rat blood samples using bound 2008s aptamers, non-binding DNA and DNA-free wells.

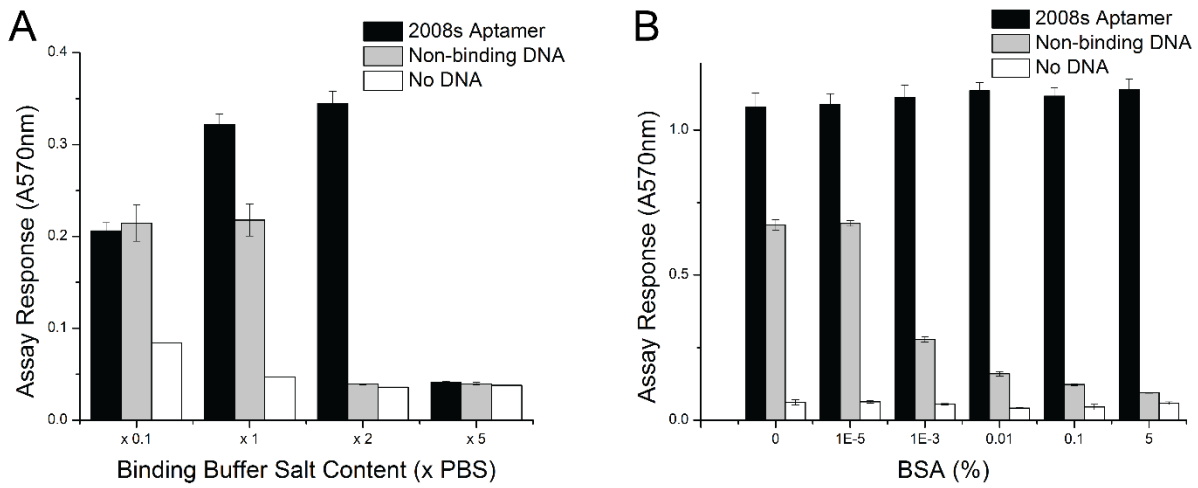

Fig. S3 Effect of binding buffer on non-specific ionic interaction in the APTEC assay including wells with non-binding DNA immobilized and DNA-free controls. (a) Effect of salt content on aptamer binding capability and non-specific interactions when detecting 100 ngmL^{-1} *Pf*LDH in different PBS strength buffers. 2 x PBS was chosen as the optimal buffer. (b) Effect of increasing BSA on capture ability and non-specific interaction of the assay for 250 ngmL^{-1} *Pf*LDH. Increasing BSA content in the binding buffer out-competes *Pf*LDH for non-specific ionic interaction with the DNA molecules, improving specificity.

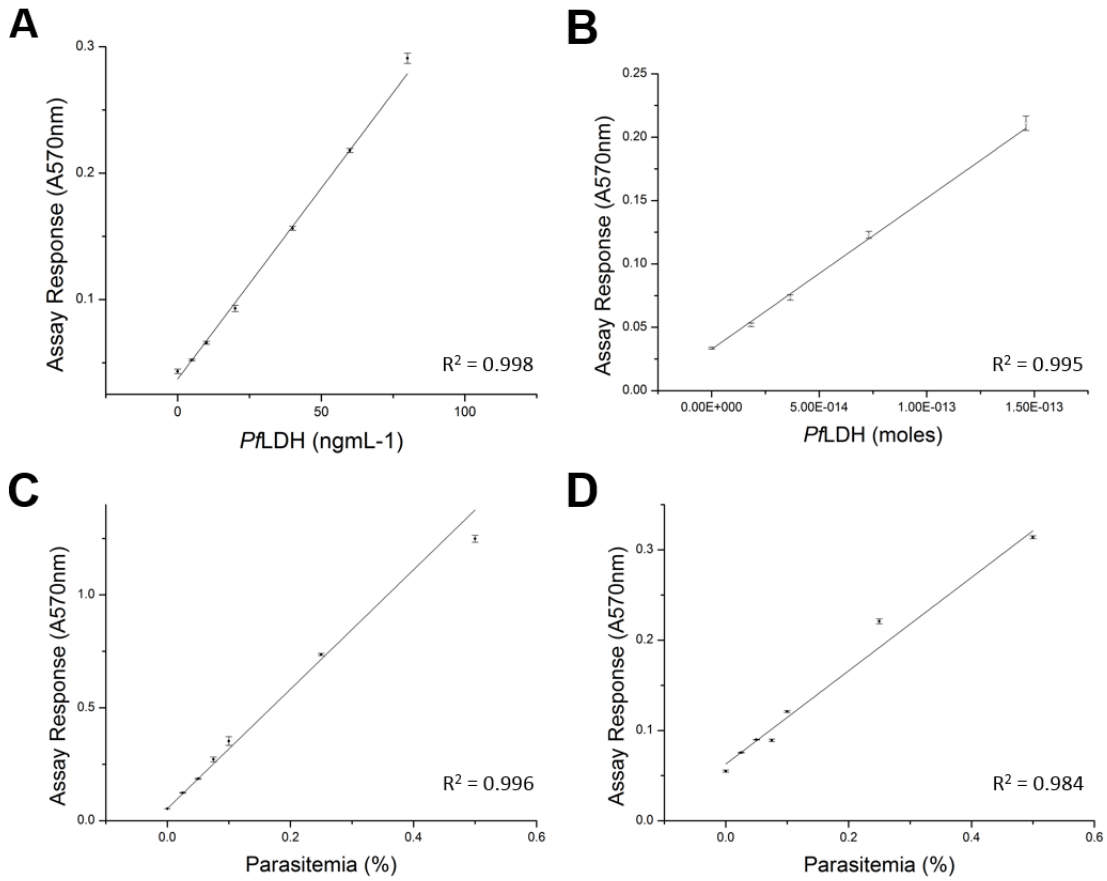

Fig. S4 The response calibration curves used to calculate LODs. LOD was defined as 3σ/m where σ is the standard deviation and m is the gradient of the curve, calculated using OriginPro linear regression analysis. (a) APTEC assay with *Pf*LDH in 5% BSA with 0.5 µgmL^{-1} hLDHb in PBST (0.005% Tween-20). (b) Recombinant *Pf*LDH diluted in PBS tested with L-lactate/NTB development reagent in solution. (c) APTEC assay with asynchronous 3D7 parasite cultures Electronic made up to 40% haematocrit using human serum. (d) APTEC assay with synchronised ring stage 3D7 parasites made up to 40% haematocrit using human serum.

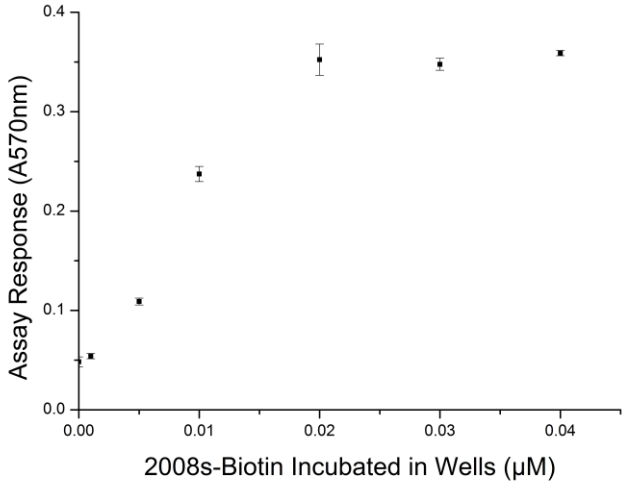

Fig. S5 Optimization of aptamer density in the streptavidin-coated 96-well plates. 100 ngmL^{-1} *Pf*LDH in 2 x PBS buffer was tested using the APTEC protocol in each well to investigate the capture ability of different aptamer densities. For the semi-quantitative APTEC assay, wells were incubated with 2 µM, 0.0125 µM and 0.0075 µM to represent high, medium and low aptamer density test wells.

B. Materials and Methods

Enzymes, chemicals and other materials. Biotinylated 2008s aptamer (5'-biotin-CTG GGC GGT AGA ACC ATA GTG ACC CAG CCG TCT AC-3') and the non-binding aptamer control (5'-biotin-GTC TAT CCG ATG CAG ACC CCT TCG GTC CTG CCC TC-3') were obtained from Integrated DNA Technologies. Streptavidin-coated 96-well plates preblocked with SuperBlock were purchased from ThermoScientific. Phosphate buffered saline (PBS) was prepared from commercially available tablets from Oxoid Ltd. Sybr® Safe and magnetic Dynabeads® MyOne™ Streptavidin T1 were purchased from Invitrogen. OptiMAL-IT tests were purchased from Bio-Rad. All other chemicals were purchased from Sigma-Aldrich. Human blood was obtained from blood donors at the Australian Red Cross Blood Service. Malaria patient samples were obtained from the Victorian Infectious Diseases Reference Laboratory, Melbourne and rat blood from the Department of Pharmacology, The University of Hong Kong, Hong Kong. Recombinant *Pf*LDH, hLDHa and hLDHb were expressed in *E. coli* BL21 and purified by HisTrap chromatography (GE Healthcare) as detailed previously.[1]

Parasite culture. *P. falciparum* 3D7 parasites were cultured in RPMI media with 0.5% Albumax and 0.2% sodium bicarbonate at 4% hematocrit as detailed previously.[2] Cultures were maintained below 10% parasitemia and synchronized by sorbitol treatment as required. Parasitemia was calculated by using flow cytometry: the culture was diluted 1:10 in PBS and stained with Sybr® Safe (Invitrogen) for 30 min then analyzed using fluorescent (FITC setting) flow cytometry (BD FACSVerse™). Parasitemia was calculated by comparing total RBC number to number of RBCs with parasite DNA present. Blood samples with various 3D7 parasitemia were prepared by diluting 3D7 infected RBCs in additional RBCs and heat inactivated human sera (30 min, 55 °C) to a total 40% hematocrit. Parasites per µL values were calculated by averaging RBC counts of samples using a haemacytometer and relating them to parasitemia values.

Aptamer functionalization of 96-well plates. Aptamers were immobilised onto streptavidin coated 96-well plates as follows: plates were washed 3 x with PBST (0.1% Tween-20), incubated with 100 µL of 2 µM biotinylated aptamer in PBS for 2 h followed by 3 x PBST washes. Plates could be used immediately for the assay or stored at 4 °C for later use.

Aptamer functionalization of magnetic beads. Aptamers were immobilized onto magnetic beads as follows: 0.5 mg streptavidin coated beads were incubated with 200 µL of 2.5 µM biotinylated aptamer in PBS for 1 h and then washed 3 x PBST by magnetic separation before resuspension in 50 µL PBS. Beads were stored at 4 °C.

L-lactate/NTB solution preparation. L-lactate/NTB development reagent was prepared immediately before use and required 12 mL L-lactate buffer (0.2 M sodium L-lactate, 100 mM Tris HCl, 0.2% Triton X-100, pH 9.1) 158 µL NAD^+ solution (50 $mgmL^{-1}$ in H_2O), 48µL NTB solution (25 $mgmL^{-1}$ in H_2O) and 25 µL PES solution (5 $mgmL^{-1}$ in H_2O). L-lactate buffer was stored at 4 °C and other stock solutions at -20 °C for up to 2 months.

L-lactate/NTB solution incubation with LDH isoforms. For solution phase assays without aptamer-capture steps, 20 µL of samples (*Pf*LDH, hLDHa, hLDHb) were incubated with 120 µL L-lactate/NTB solution in triplicate in the dark for 45 min before adding 5% acetic acid to stop the reaction. Scanning absorbance (400 – 700 nm) was recorded.

Time response of L-lactate/NTB solution with *Pf*LDH. 120 µL of L-lactate/NTB solution was added to 20 µL of *Pf*LDH samples in triplicate of varying concentration in PBS. Absorbance at 570 nm was recorded every 5 min for an hour.

Long-term L-lactate/NTB solution stability investigation. L-lactate/NTB solution was made up freshly as described previously and kept covered from the light at 22 °C for 11 days. Each, or every other day, an aliquot of the solution was removed and stored at -20 °C. Once all samples were collected, they were thawed and 70 µL of each sample incubated with 15 µL of 0.5 $µgmL^{-1}$ *Pf*LDH in PBS in triplicate for 45 min. 100 µL 5% acetic acid was then added and absorbance taken at 570 nm.

APTEC assay protocol. 100 µL of sample was added to each aptamer-decorated well in triplicate for 1 h followed by washing with 5 x PBST. 120 µL of the pre-prepared L-lactate/NTB solution was added and left to incubate for 45 min with mild shaking in the dark. 100 µL acetic acid (5%) was added to stop the reaction and the absorbance at 570 nm was recorded. For recombinant LDH samples, unless stated otherwise the binding buffer was either 2 x PBS or for the LOD calculation 5% BSA in PBST (0.005% Tween-20) with 0.5 $µgmL^{-1}$ hLDHb. For *Pf*LDH-spiked whole rat blood samples, 3D7 cultures and patient samples, 50 µL of whole blood sample was mixed with 50 µL PBS (0.5% Triton X-100) to allow for RBC lysis prior to incubation. For patient samples, a positive result was defined as a response $\geq$ 0.1 absorbance units at 570 nm.

APTEC regeneration. A shortened APTEC protocol was used for regeneration experiments. 100 µL of 1 $µgmL^{-1}$ *Pf*LDH or hLDHb in 2 x PBS was incubated for 20 min in triplicate in the 2008s decorated wells. Wells were then washed with 3 x PBST and 100 µL L-lactate/NTB solution added and incubated in the dark for 20 min before recording the absorbance at 570 nm (T values). No stop reagent was used but instead the wells were washed with 200 µL elution buffer (7 M urea, 0.1 M sodium citrate, 10 mM EDTA pH 7.3) and then 3 x PBST. 100 µL L-lactate/NTB solution was added again for 20 min and absorbance taken at 570 nm to ensure *Pf*LDH elution from the aptamers (R values). The process was repeated for 6 cycles.

Semi-quantitative APTEC assay. To prepare high, medium and low aptamer density wells, 2 µM, 0.0125 µM and 0.0075 µM of 2008s-biotin in PBS were respectively incubated in streptavidin-coated wells as described previously. Different concentrations of *Pf*LDH in 2 x PBS buffer were assayed in the low, medium and high aptamer density wells using the APTEC protocol described previously. After color development, positive or negative results were assigned to each well by visual assessment and correlated to a threshold absorbance value of 0.15 units at 570 nm.

APTEC assay protocol – magnetic beads. 1 µL of aptamer decorated magnetic beads were incubated in 10 µL sample (2 x PBS binding buffer) for 20 min, washed with 3 x PBST then incubated with 20 µL L-lactate/NTB solution for 20 min followed by 10 µL stop solution (5% acetic acid).

Limit of detection (LOD) calculations. Limit of detection (LOD) values were calculated by linear regression (OriginPro). LOD was defined as $3\sigma/m$ where σ is the standard deviation and m is the gradient of the response curve. Each LOD value was calculated in 2 or 3 separate experiments and averaged.

C. Ethics

Ethics approval for this study was obtained from the Melbourne Health Human Research Ethics Committee. Permission for a waiver of consent was granted due to the use of patient samples from a pre-existing, de-identified tissue bank.

D. References

1 Y. W. Cheung, J. Kwok, A. W. Law, R. M. Watt, M. Kotaka and J. A. Tanner, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 15967.

2 K. E. M. Persson, C. T. Lee, K. Marsh and J. G. Beeson, *J. Clin. Microbiol.*, 2006, **44**, 1665.