Structural insights into cubane-modified aptamer recognition of a malaria biomarker

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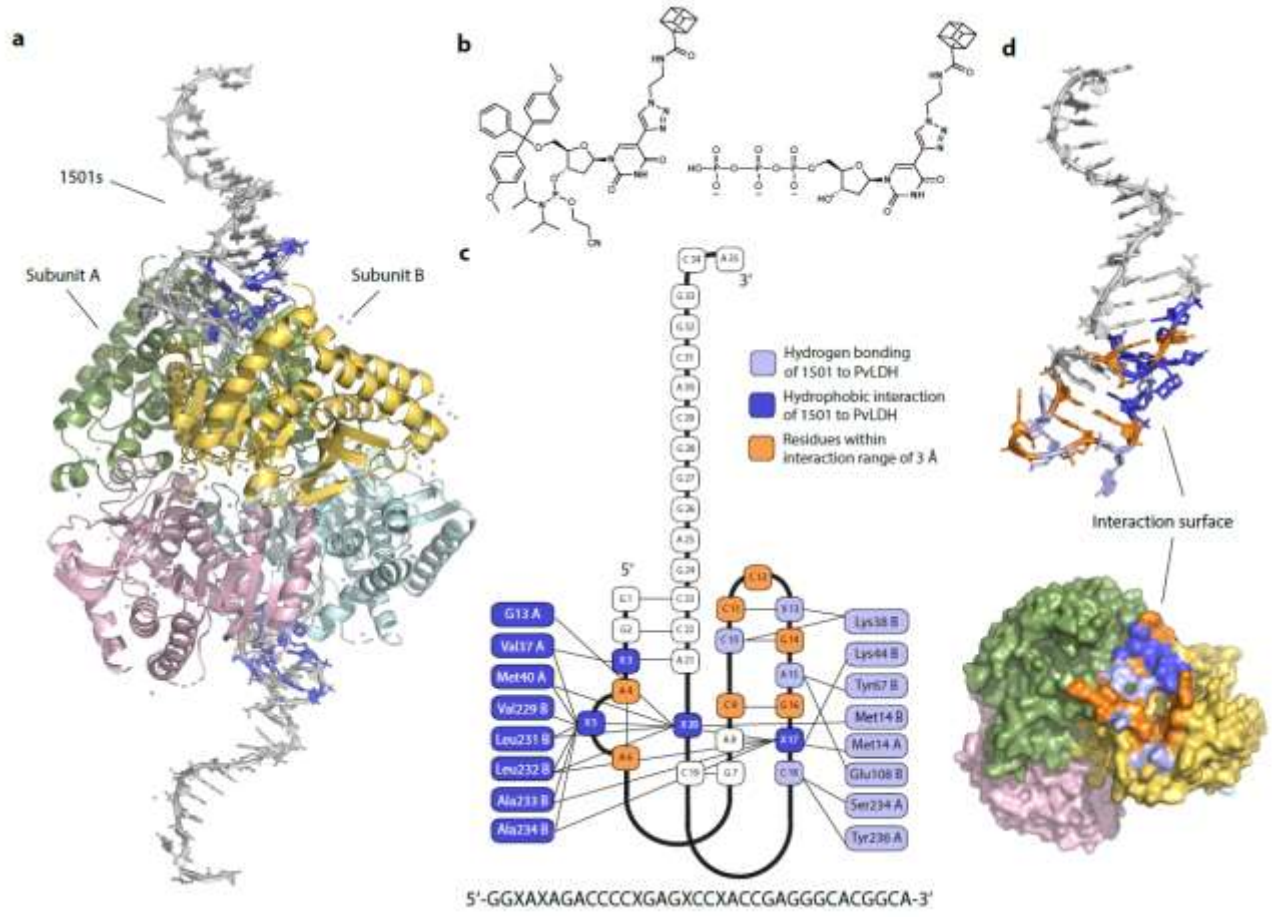

Figure 1. Structure of PvLDH **in complex with cubamer a,** Crystal structure of tetrameric PvLDH binds to two cubamers. The four subunits of PvLDH are coloured in yellow, green, cyan and pink. The two 1501s cubamers are coloured in grey and the cubane modification coloured in blue. **b,** Protected cubane-modified deoxyuridine phosphoramidite (left) and cubane-modified deoxyuridine triphosphate (right). **c,** Details of interactions between 1501s and PvLDH. Light blue indicates hydrogen bonding interactions between cubamer and protein. Blue indicates hydrophobic interactions between cubamer and protein. Orange indicates residues within interaction range of 3 Å. Connected nucleotides are within 3.8 Å. Below, the sequence of the 1501s cubamer is shown with **X** representing cubane-modified deoxyuridine. **d,** Buried surface of PvLDH-1501s complex. We pinpointed by different colours the regions of interactions between the monomers A and B of PvLDH and a 1501 cubamer.

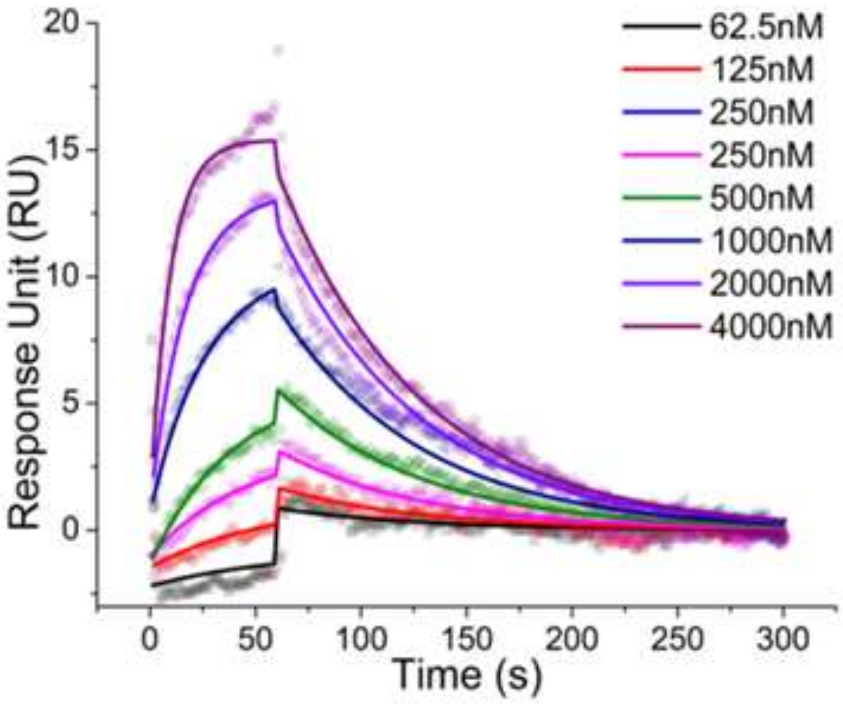

Figure 2. Determination of binding affinity of cubamer towards PvLDH**.** Surface plasmon resonance (Biacore) analysis for the interaction between 1501s and PvLDH. PvLDH was immobilised to a Ni-NTA chip, then the response was observed at multiple concentration of 1501s as shown in the figure. Raw data are shown background with the best fit overlaid in the thick solid line. The association (k_{on}), dissociation (k_{off}) and the equilibrium constant (K_D) were 2.2 x 10^4 $M^{-1}s^{-1}$, 1.4 x 10^{-2} s^{-1} and 670 nM, respectively.

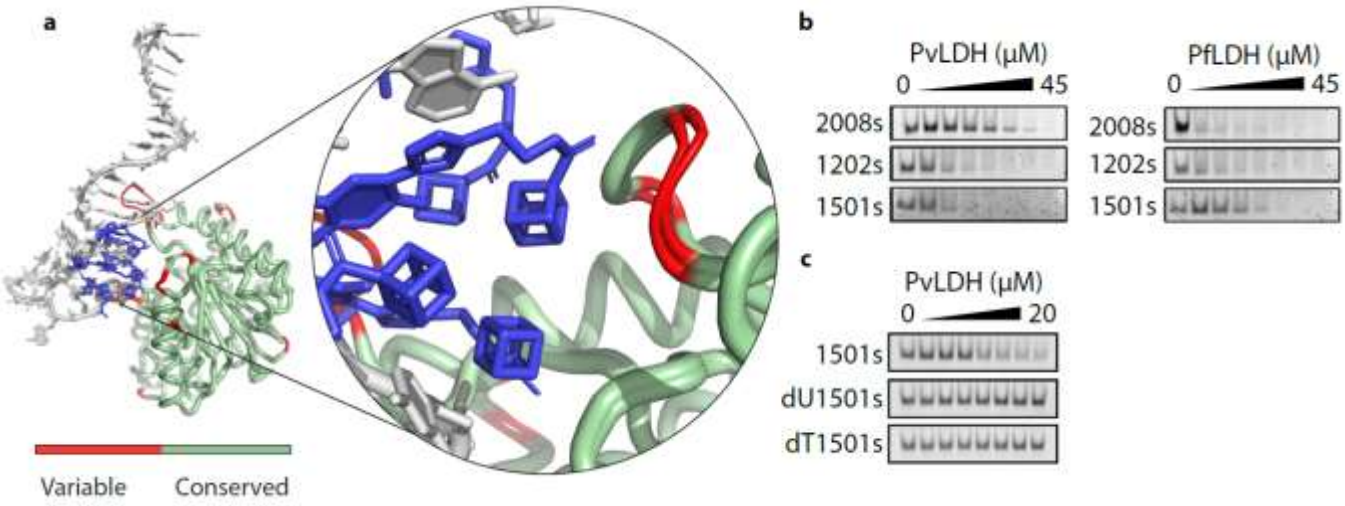

Figure 3. The cubamer discriminates PvLDH **from *Pf*LDH through binding to a variable region.** **a,** Superimposition of PvLDH and *Pf*LDH shows they are highly conserved with some difference on the variable region. The variable region is coloured in red, whereas the conserved region is in light green. The insert shows the cubanes on the modified nucleotides interact with the variable region. **b,** Comparing the affinity of 1501s to PvLDH and *Pf*LDH with the control of *Pf*LDH specific aptamer, 2008s, and pan-LDH specific aptamer, 1202s. EMSA analysis showed 1501s is highly specific to PvLDH. **c,** EMSA analysis showed cubanes on modified triphosphates involved in the binding between 1501s and PvLDH. With the replaced cubane modified triphosphate by dU^{C}TP, dU1501s, and by dTTP, dT1501s, the affinity of the cubamers are significantly decreased.

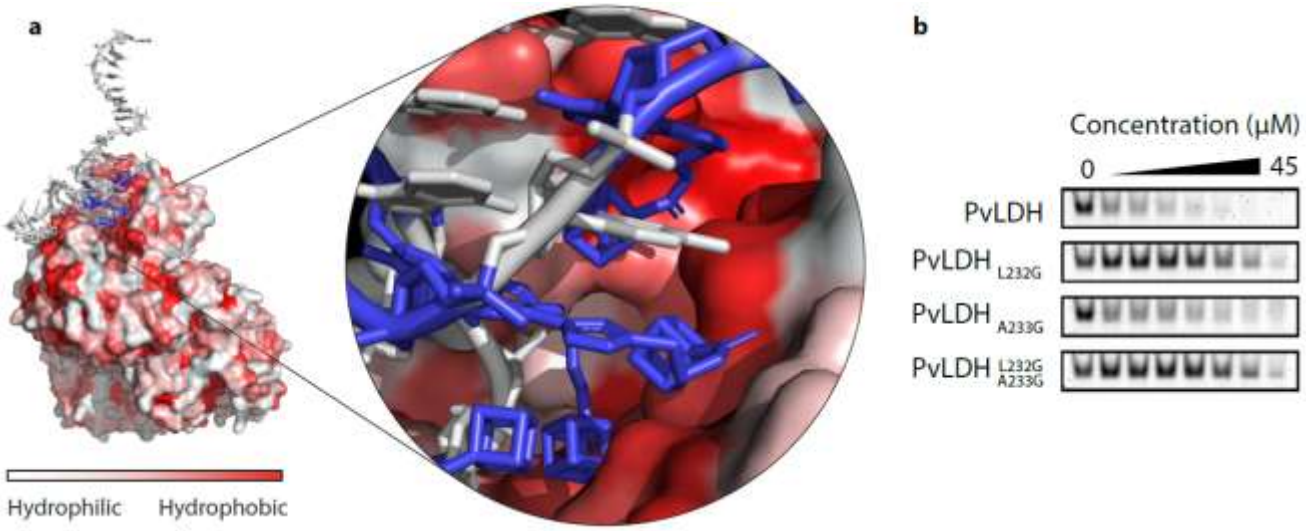

Figure 4. The cubamer interacts with PvLDH **by hydrophobic interactions. a,** Structure of PvLDH-1501s complex shows the cubane modified nucleotides, T1, T5, T20, T13 and T17 formed a hydrophobic cluster. The hydrophobic surface of PvLDH was illustrated with a colour code according to the polarity of the respective amino acids. Hydrophobic amino acids were coloured in red whereas hydrophilic residues are in white. **b,** EMSA reveals the binding of PvLDH and 1501s along with amino acid side chains. The affinities of 1501s to three mutants of PvLDH, $PvLDH_{L232G}$, $PvLDH_{A233G}$ $PvLDH_{L232G/A233G}$, and the wide-type PvLDH were estimated. Result indicates the affinities among all the mutants are significantly decreased by mutating the hydrophobic amino acids to glycine.

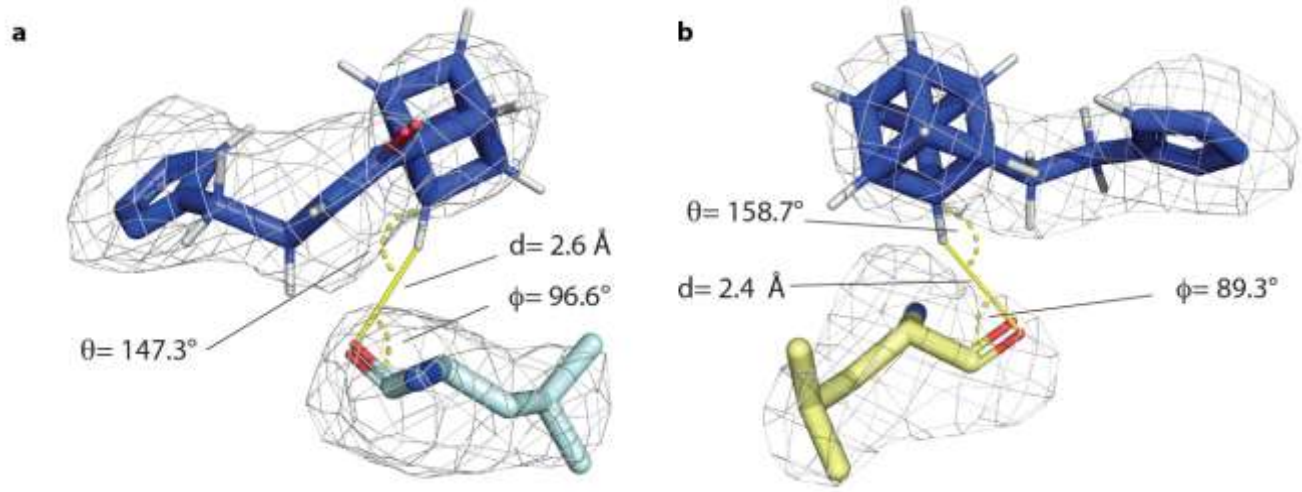

Figure 5. A unique hydrogen bond of the cubamer with PvLDH. Close up views of the interaction between a dU^C unit (T5) of the two cubamers 1501s with PvLDH (Leu232) showing the respective angles and bond lengths of the unique C-H(2) ···O hydrogen bond. The grey mesh corresponds to a Sigma-A weighted mFo-DFc difference map (contoured at 3 σ), in which Leu232 and the cubane moiety of T5 were omitted from the model before map calculation.

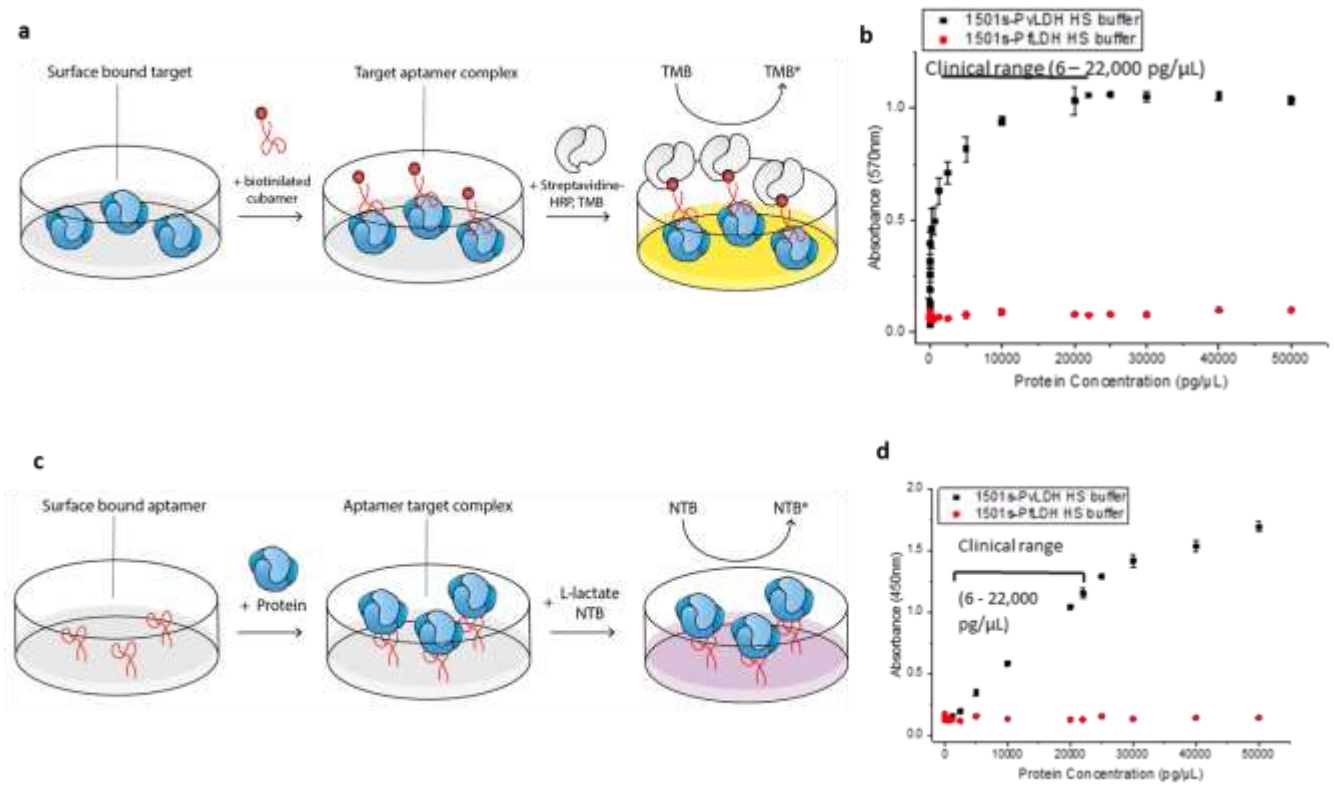

Figure 6. Determination of affinity and specificity of the 1501s cubamer against pLDHs in human serum (HS) buffer by Enzyme linked oligonucleotide assay (ELONA) and aptamer-tethered enzyme capture (APTEC) assay. (a) Diagram illustrating the ELONA. Target pLDHs are immobilized onto a microtiter plate, then biotinylated cubamers are added to recognize pLDHs. Streptavidin-HRP is added to recognize the biotin-end of the cubamer, and TMB solution is used to allow for colour development. **(b)** The response of the ELONA against clinical range of PvLDH and PfLDH in human serum (HS) buffer. The LOD of 1501s-PvLDH in the ELONA assay was determined to be 1.05 ng·µL^{-1} (Fig. S29) **(c)** Schematic diagram illustrating the APTEC assay. Biotinylated cubamers are immobilized onto streptavidin-coated wells, then pLDHs are added and captured by the cubamer. L-lactate/NTB solution is then added for colour development. **(d)** The response of the APTEC assay against clinical range of PvLDH and PfLDH in HS buffer. The LOD of 1501s-PvLDH in the APTEC assay was determined to be 3.34 pgµL^{-1} (Fig. S29). Measurements were done in triplicate, reported in standard error (SE).