Supporting Information

A peroxidase-active aptazyme as an isothermally-amplifiable label in an aptazyme-linked oligonucleotide assay for low-picomolar IgE detection†

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1) DNA Sequences (IDT, USA):

   G-quadruplex hemin aptamer with 5` hexynyl label.
   5` hexynyl -TGATGACGCTTCTGTATCTA AGT CCG **TGG GTA GGG CGG GTT GGG** GGT GAC T ATGGTGCCAGGCATAATCCAGG 3`

   IgE aptamer with 3` azide label
   5` AAAAAG GGG CAC GTT TAT CCG TCC CTC CTA GTG GCG TGC CCC-` azide 3`

   Reverse primer (limiting)
   CCTGGATTATGCCTGGCACCAT

   Forward primer (excess)
   TGATGACGCTTCTGTATCTA

2) Protocol for the copper catalyzed Huisgen 1,3-dipolar cycloaddition reaction
   A. Prepare 0.1 M CuBr and 0.1 M Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) in 3:1 DMSO/t-BuOH
   B. Mix
      - 35 µL of 5 µM 5` hexynyl G-quadruplex hemin aptamer
      - 35 µL of 5 µM 3` azide IgE aptamer
      - 30 µL 2:1 0.1M TBTA and 0.1M CuBr
   C. Incubate 2 hours at room temperature
   D. Purification on agarose gel. The DNA clean-up from the gel was done with a Zymoclean™ Gel DNA Recovery Kit (Zymo Research, USA)

3) Agarose gel

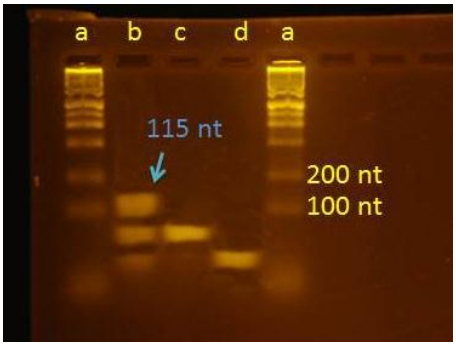

Fig. S 1 Agarose gel showing a ssDNA ladder (a) the bifunctional aptazyme (b, 115 nt) the hexynyl G-quadruplex hemin aptamer (c, 74 nt) and the IgE aptamer (d, 41 nt)

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4) Optimum concentration of hemin in ALONA

To determine the optimal concentration of hemin needed for the ALONA, we made several 4-point calibration curves (DNAzyme: 10 nM, 20 nM, 50 nM, 100 nM) using different concentrations of hemin. We compared the slopes of the linear calibration curves. 100 nM of hemin resulted in the steepest slope; hence this concentration was used in all subsequent ALONAs.

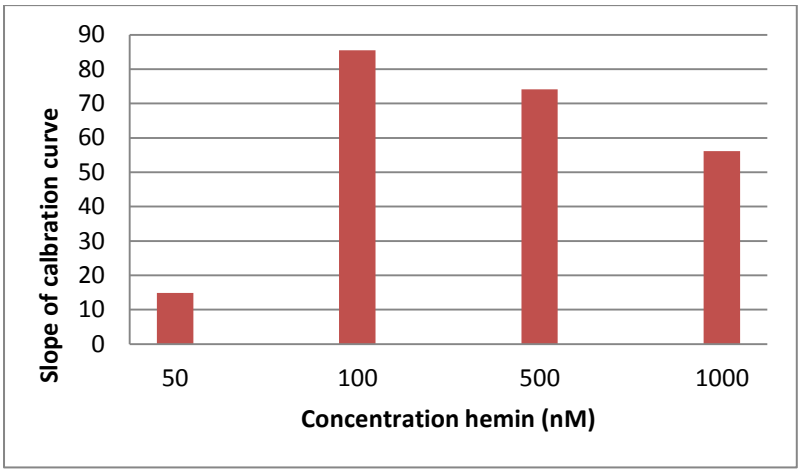

Fig. S 2 Slopes of the calibration curve for different concentrations of hemin

5) ALONA protocol

All synthesized oligonucleotides were purchased from Integrated DNA Technologies (USA). All general chemicals and the antibodies were purchased from Sigma-Aldrich (USA) unless stated otherwise. A scheme can be found in Figure 1b. In short; Monoclonal IgE-antibodies were immobilized through adsorption on the wall of polystyrene PCR tubes. Non-fat dry milk (NFDM) was used as blocking agent to prevent non-specific IgE adsorption. The samples (50 µL) were prepared in PBS buffer with IgE concentrations ranging from 1 to 10^4 pM. The binding reaction was stopped after 15 min. Thereafter the samples were rinsed 3 times with PBS containing 0.01% Tween using an automatic plate washer (Tecan, USA). Then, 50 µL of 0.1 nM bifunctional aptamer was added to each sample. After 15 min., the samples were washed again three times with the plate washer. Next, isothermal asymmetric HDA was performed using the helicase enzyme (one step IsoAmp®III Universal tHDA Kit, BioHelix, USA) at 62°C for 35 min. The amplification was done in a total volume of 50.0 µl (H_2O *31.5 µl, 10X Annealing buffer II *5.0 µl MgSO4 (100 mM)*2.0 µl, NaCl (500 mM)* 4.0 µl, IsoAmp® dNTP Solution* 3.5 µl, Forward Primer (50 µM)* 1 µl, Reverse Primer (2.5 µM)* 1 µl, IsoAmp® III Enzyme Mix* 2.0 µl). Finally, 20 µL of the amplified sample was mixed with 20 µL of 100 nM hemin in HEPES buffer (Ph 7.4) containing 0.1% Triton X-100 and 2% DMSO. After 5 min, 40 µL of a two-fold concentrated ready-to-use ABTS solution (Life technologies, USA) was added to initiate and monitor the enzymatic oxidation

of ABTS, creating a green colored solution. The results were both observed by eye and also measured with a spectrophotometer (Infinite M200 PRO, Tecan, US) at 415 nm.

6) Linear after the exponential amplification

Asymmetrical amplification of the bifunctional aptamer with a Taqman probe (/56-FAM/TGA CTA TGG /ZEN/TGC CAG GCA TA/3IABkFQ/, IDT, USA). Using Agilent Brilliant II QPCR Master Mix. Reverse primer (limiting) concentration: 50 nM, Excess primer concentration 1 µM.

PCR protocol:

- 10 min at 95˚C
- 45 cycli:
  - 10 s at 95 ˚C
  - 20 s at 52 ˚C
  - 20 s at 75 ˚C
- 4 ˚C

Figure S3 illustrates that a linear amplification enables the use of an endpoint measurement after 35 min of isothermal PCR.

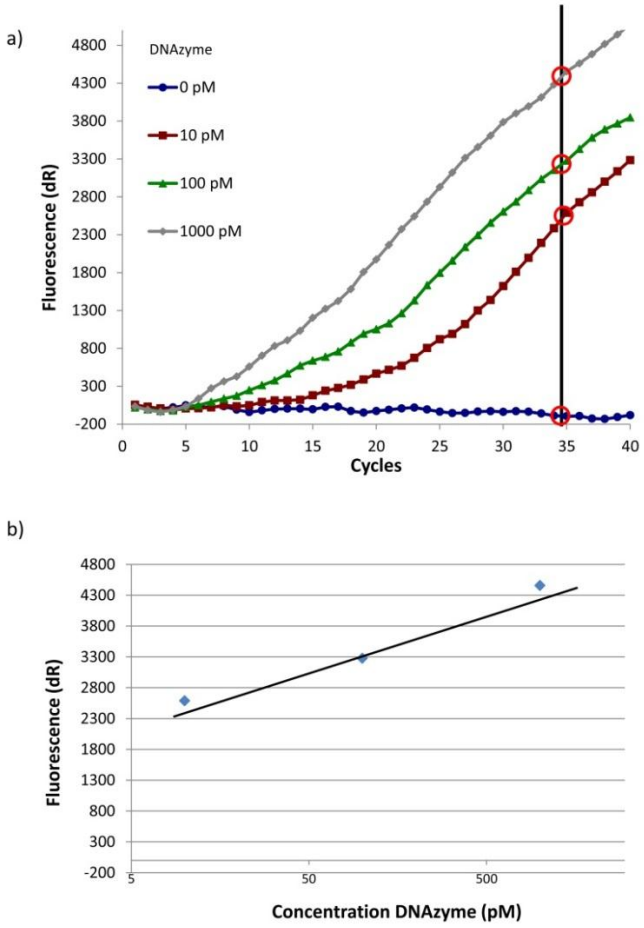

Fig S3 a) Asymmetrical amplification with a Taqman probe. b) Fluorescence intensity after 35 min of asymmetrical amplification, for given input DNA, derived from the circles in fig. S3a