Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Resonance Raman Scattering of Catalytic Beacons for DNA Detection

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Experimental

Materials

Haemin was purchased from Sigma Aldrich, UK as was buffer components Hepes, Sodium Chloride and Potassium Chloride. SureBlue™ TMB Microwell Peroxidase Substrate (1-component) was purchased from KPL, inc., USA. ABTS One Component HRP Microwell Substrate was purchased from SureModics IVD, USA.

Oligonucleotide Sequences

All DNA oligonucleotides were purchased from Eurofins MWG Operon, Germany.

PS2.M:

5'GTGGGTAGGGCGGGTTGG3'

Beacon:

5'GTGGGTAGGGCGGGTTGGATGACGTCTATCCATTTATGTCCAACCC3'

Target:

5'ACATAAATGGATAGACGTCAT 3'

Instrumentation

UV-Vis absorption spectra were carried out using a Cary 300 Bio UV-vis spectrometer. 500 µL sample volumes were analysed in 1 cm glass cuvettes and scanned from 200 nm to 800 nm. Raman spectra were collected using a Renishaw inVia microscope system with a HeNe laser (633 nm). Samples were analysed in 1.5 mL semi-micro disposable plastic cuvettes using a 20 x long working distance objective. 3 accumulations were taken, each with a time of 3 seconds, and 5 scans were taken of each sample. WiRE 2.0 software (Renishaw PLC) was used to run the analysis and spectra were baseline corrected using a sextic function and a Level and Zero levelling mode in Grams software.

Formation of DNAzymes

A 5mM haemin stock solution in DMSO was prepared and stored at 2 ºC. For initial

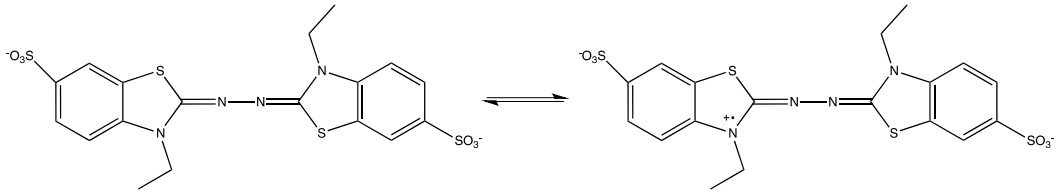
experiments, 200 µL of 1 µM PS2. M sequence was incubated with 40 µL of 5 µM haemin at 25ºC for 30 min in either 10 mM HEPES / 10 mM NaCl, pH 7.1 or 10 mM HEPES / 10mM KCl, pH 7.1. Substrate was added to 500 µL final volume and UV-vis and RRS measurements were taken after approximately 3 h. Subsequently, the DNAzyme and haemin concentration was reduced to 0.1 µM to prevent over oxidation of the substrates, and oxidation time was changed to 1 h for ABTS and 3.5 h for TMB based on the results from UV-Vis time studies. Additionally, the substrate was fixed at 200 µL for ABTS and 100 µL for TMB.

Catalytic Beacon Assay Protocol

50 µL of 1 µM beacon and 5 µL of 100 µM target were hybridised in 10 mM HEPES / 10 mM NaCl, pH 7.1, at 90 ºC for 10 min and 10 ºC for 10 min. 10 µL of 5 µM haemin was added and allowed to complex for 30 min at room temperature. 100 µL of TMB solution was added to a final volume of 500 µL and RRS measurements were taken approximately 3.5 h later using both centred (1400 cm^{-1}) and extended scans (200-2000 cm^{-1}). 3 accumulations were taken, each lasting 3 s and 5 scans of each sample were taken.

Figures

(a)



(b)

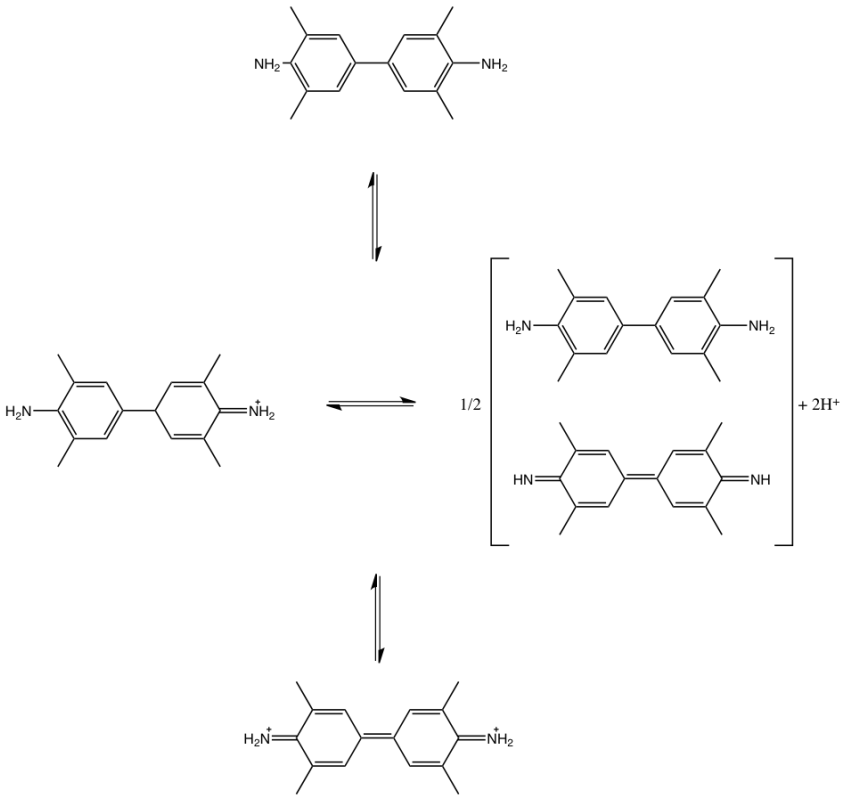


Fig. S1 Schematic representation of the oxidation of (a) ABTS and (b) TMB

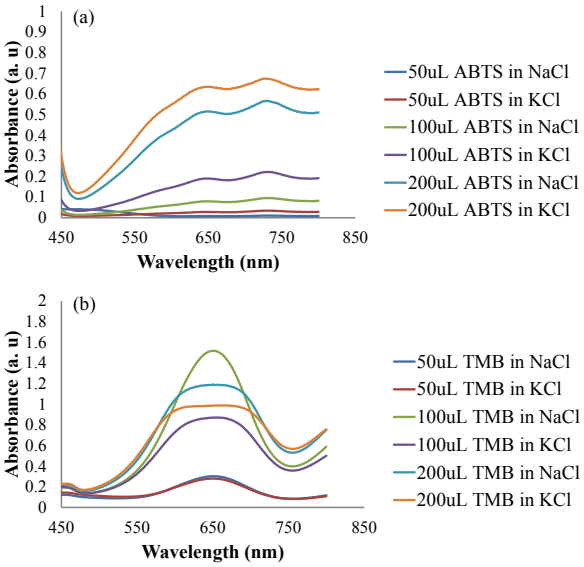


Fig. S2 Investigation of the effect of buffer and substrate concentration on absorbance for (a) ABTS and (b) TMB. 0.4 μM of DNAzyme and 0.4 μM of haemin were used along with either 10 mM HEPES / 10 mM KCl, pH 7.1 or 10 mM HEPES / 10 mM NaCl, pH 7.1 with varying amounts of substrate. Samples were analysed by UV-Vis spectroscopy 3 h after substrate addition.

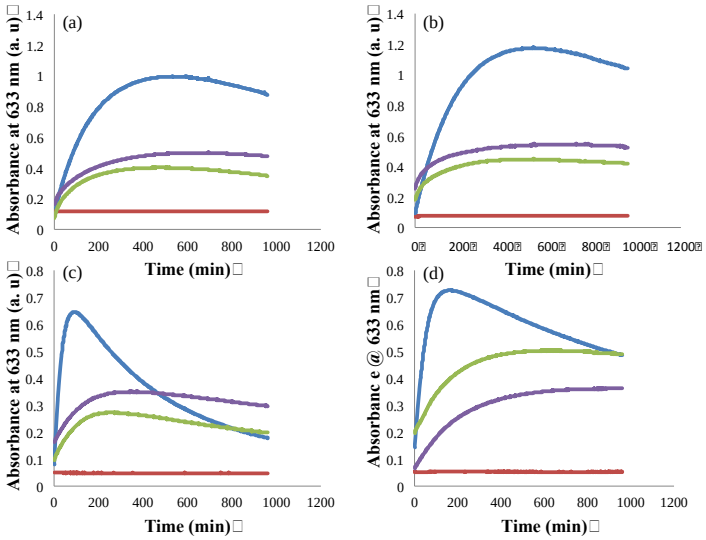


Fig. S3 UV-Vis time studies to assess the optimum oxidation time before analysis. 0.1 μM DNAzyme and 0.1 μM haemin were complexed and then measurements taken at 633 nm every 5 min for 960 min after the addition of substrate. (a) 100 μL of TMB in 10 mM HEPES / 10 mM NaCl, pH 7.1. (b) 100 μL of TMB in 10 mM HEPES / 10 mM KCl, pH 7.1. (c) 200 μL of ABTS in 10 mM HEPES / 10 mM NaCl, pH 7.1. (d) 200 μL of ABTS in 10 mM HEPES / 10 mM KCl, pH 7.1. All components of the DNAzyme (blue) along with DNAzyme only (red), haemin only (green) and Nonsense DNA (purple) were analysed.

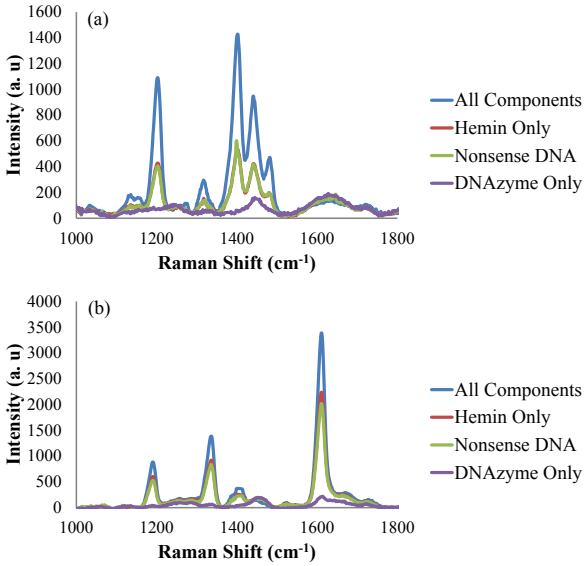


Fig. S4 Resonance Raman spectra of (a) 200 µL ABTS in 10 mM HEPES / 10 mM KCl, pH 7.1 and (b) 200 µL TMB in 10 mM HEPES / 10 mM NaCl, pH 7.1 after interaction with 0.1 µM DNAzyme and 0.1 µM haemin. A laser excitation wavelength of 633 nm was used for both substrates, which were analysed after 1 h and 3.5 h for ABTS and TMB, respectively. Centred scans (1400 cm^{-1}) were used in this study with 3 accumulations each lasting 3 s. 5 replicate samples of each were prepared and scanned 5 times. The average was taken to obtain the spectra shown.

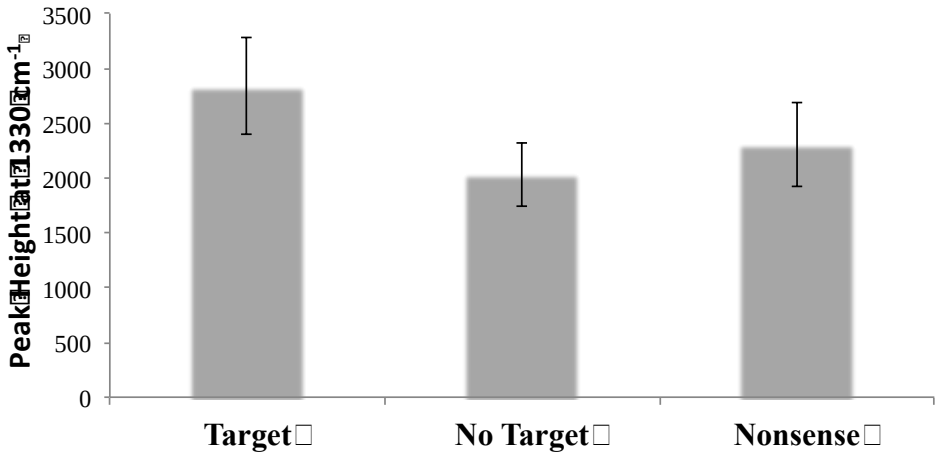


Fig. S5 0.1 µM Beacon and 10 nM target were hybridised in 10 mM HEPES / 10 mM NaCl, pH 7.1 and 0.1 µM haemin added and allowed to complex for 30 min. 100 µL TMB was added and left to react for 3 h before analysis. Peak intensities were obtained at 1608 cm^{-1} by subtracting the intensity given by haemin alone from that obtained in the presence of target DNA. A laser excitation wavelength of 633 nm was used with centred scans (1400 cm^{-1}) and each of the 3 accumulations lasting 3 s. 5 replicate samples of each were prepared and scanned 5 times. The average was taken to obtain the results shown.

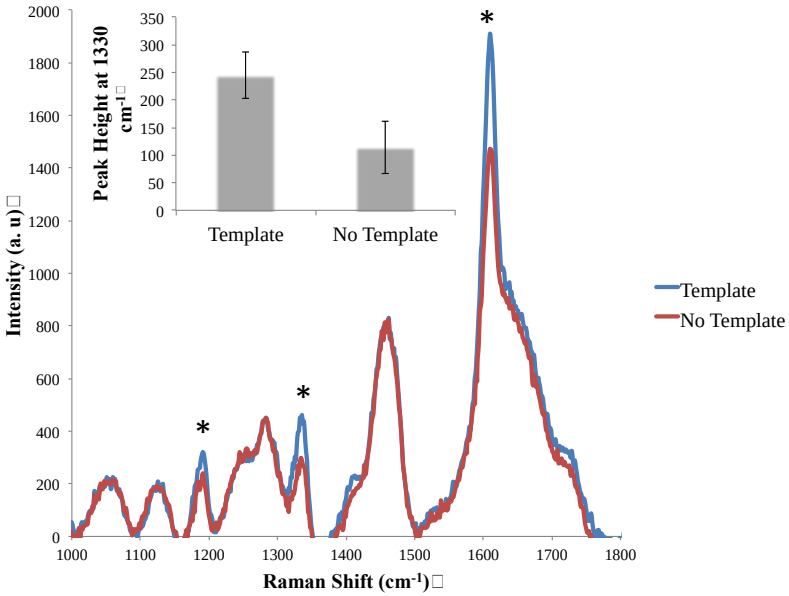


Fig. S6 5 μL of post PCR DNA was allowed to hybridise with 0.1 μM beacon in 10 mM HEPES / 10 mM NaCl, pH 7.1 before adding 10 nM haemin and allowing the DNAzyme to form for 30 min. 40 μL TMB was added to a final volume of 200 μL and analysed after 3 h. A laser excitation wavelength of 633 nm was used with centred scans (1400 cm^{-1}) and each of the 3 accumulations lasting 3 s.