Supporting Information

Probing biomolecular interactions using surface enhanced Raman spectroscopy: label-free protein detection using a G-quadruplex DNA aptamer.

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Reagents. All reagents and proteins were purchased from Sigma and were used without further purification, except human thrombin which was additionally purified by gel filtration chromatography. Nanoshells were purchased from Nanospectra Biosciences Inc, Houston Texas. All buffers were prepared in deionized water and autoclaved afterwards. Nucleic acid aptamers were synthesised and RP-HPLC purified by Eurogentec Ltd. (Southampton, UK). The following oligonucleotide sequences were used:

Oligo nucleotide sequences used:

Thrombin binding aptamer (TBA):

HS-$(CH_2)_6$-TTT TTT GGT TGG TGT GGT TGG

Control-sequence thrombin binding aptamer (csTBA):

HS-$(CH_2)_6$-TTT TTT TTG GTT GTG TTG GTT

G-quadruplex forming aptamer[1]:

HS-$(CH_2)_6$-TTT TTT GGT GGT GGG GGG GGT TGG TAG GGT GTC TTC

Preparation of mixed monolayer Sensor Surface.

Preparation of thiol modified aptamer solution. Dithiol bound nucleotides (100 μM, 5 uL) were diluted to 100 uL in water and treated with Tris(carboxyethyl)phosphine (TCEP) (1 mg) for 30 min at RT, gently agitated and purified using a G-25 MicroSpin^{TM} size exclusion column purchased from Amersham Biosciences (Buckinghamshire, UK). The aptamer was then diluted to a concentration of 10 μM in bindingbuffer (BB) (Tris-HCl 50 mM, NaCl 140mM, $MgCl_2$ 10 mM, KCl 10 mM, pH 7.4), heated to 95 °C for 5 min, cooled down to 72 °C for 15 min for hairpin loop formation and afterwards cooled down to RT over 30 min.

Preparation of NS substrate: NS solutions with a concentration of 8 x 10^9 particles/ml were placed in 1 ul spots on aminopropyl triethoxy silanized (APTES) glass slides and left until dry. Excess particles, which were not physisorbed on the surface were washed off with copious amounts of ddH_2O.

Particles formed self-assembled layered aggregates of 0.5 – 5 μm (Figure 1b and Figure S2).

Functionalisation of fixed NS aggregates: NS aggregate spots on the APTES glass slides were incubated with 15 μL of TBA (10 μM) solution in BB placed on the surface. This was left for reaction with the surface for 18 h at 30 °C with slight shaking in a Thermomixer (Fischer Scientific UK, Leicestershire) using SecureSeal adhesive 8 well sheets (Sigma, UK), excess oligos were removed afterwards by rinsing the slide with copious amounts of binding buffer. To prevent non-specific interactions, the spots were incubated with a solution of mercapto-hexanol (MCH) (1mM, 15 μL) in binding buffer for 30 min and rinsed afterwards again with BB.

Incubation experiments with thrombin and control proteins: The mixed monolayer modified nanoshell spots were incubated with thrombin solution (50 μL) in BB for 30 min. Measurements were taken with the buffer and protein solutions left on during the measurements, to prevent disruption of any aptamer target interactions. After measurements, the bound protein was denatured and displaced by washing 2 times with 100 μL 8M urea and also with the following buffer (Tris-HCl 50 mM, $CaCl_2$ 200mM, EDTA 1 μM, pH 7.0). To recover aptamer binding properties the sensing spot was washed with BB before fresh thrombin solution was applied again.

Raman instrumentation and measurements: In this work was used a Renishaw InVia system connected to a 785 nm diode laser. Measurements were conducted with a 50x Olympus water objective (NA: 0.8) immersed into the buffer solution and a laser power of 0.03±0.02 mW at the sample with an estimated focal area of 0.5 μm. Spectra were taken of different nanoparticle aggregates with an acquisition time of 10 sec and the displayed spectra are averages of 10 spectra at the same spot (aggregate).

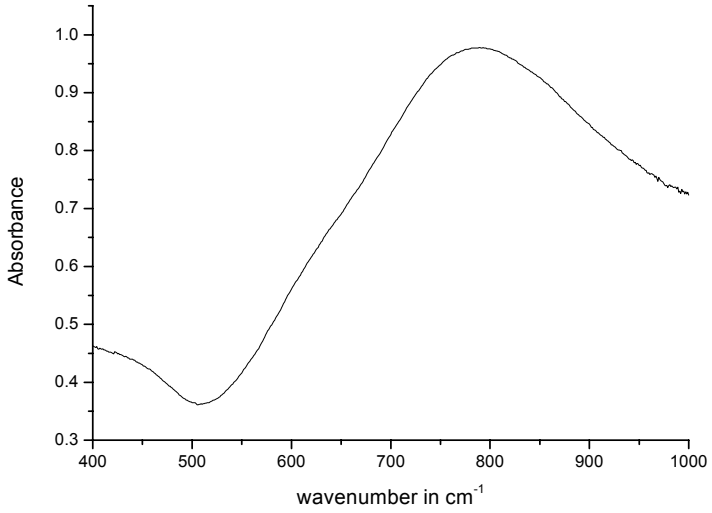
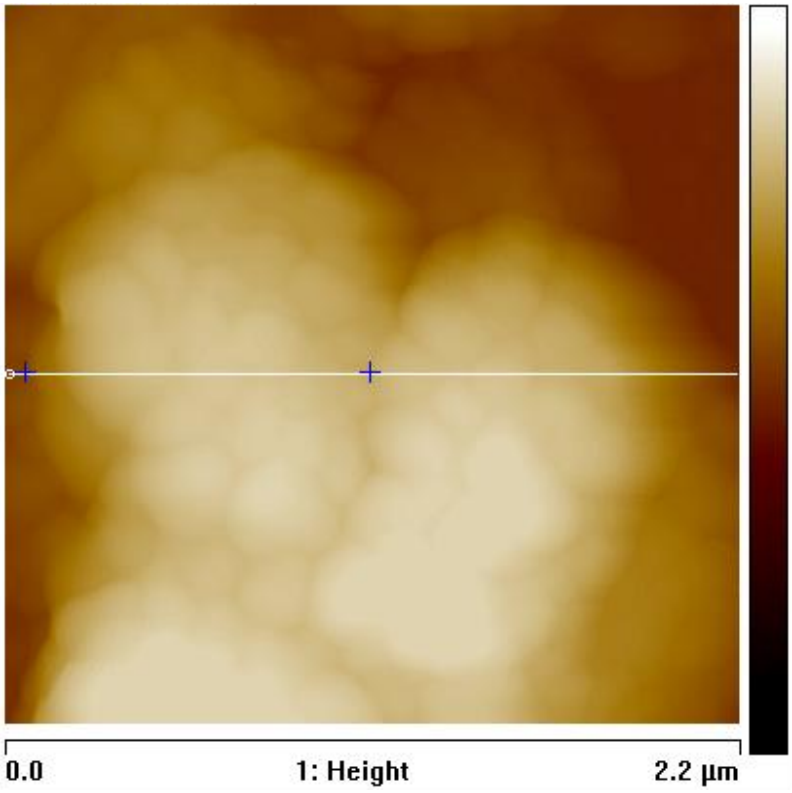


Figure S1: UV-Vis spectrum of gold nanoshells with a concentration of 8x 10^9 NS/ml in water in a range between 400 and 1000 nm. The absorbance maximum lies at 780 nm.

a)



b)

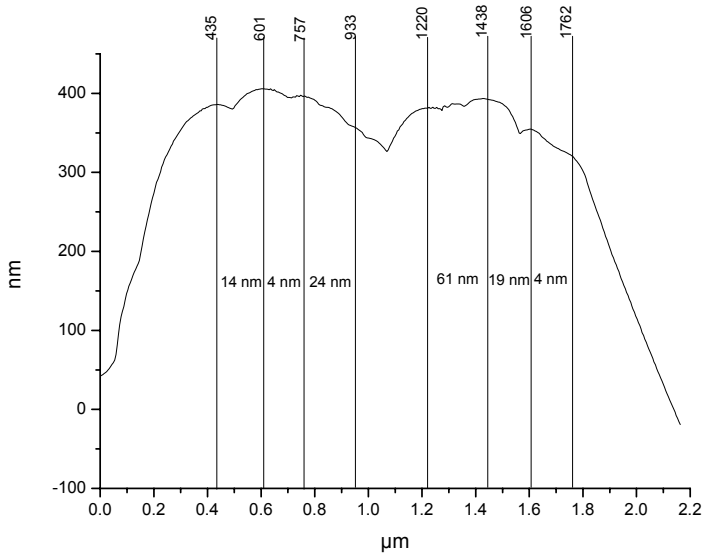


Figure S2: a) AFM measurement used for the determination of aggregate structure and particle distances b) Graph shows one representative histogram of the AFM measurement cross section of the above NS aggregate. Separation between particles was estimated by measuring the distance between NS

peaks and subtracting the NS diameter. We found separations between 0-25 ± 12 nm.

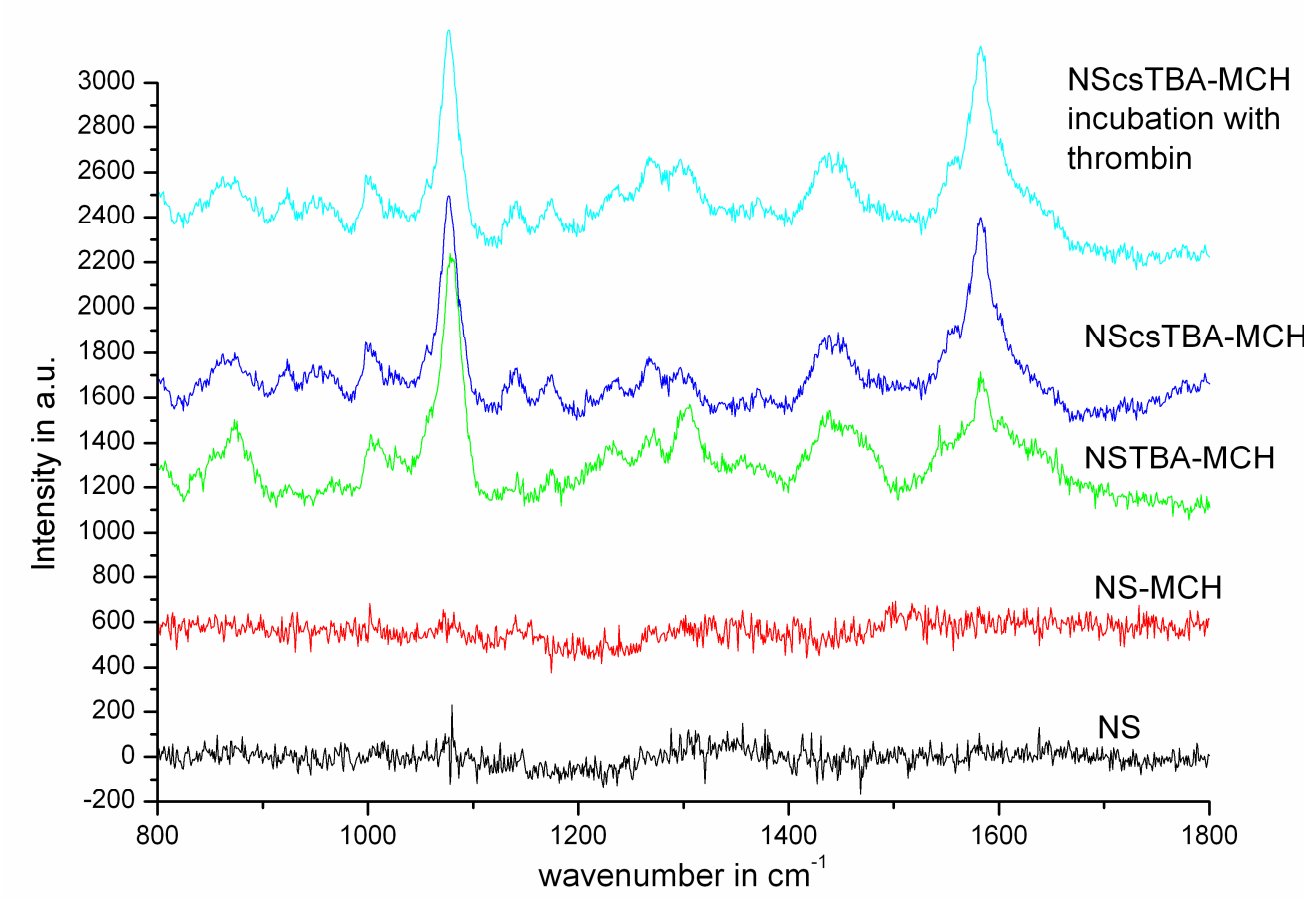


Figure S3: SERS spectra of control sequence aptamer (csTBA) and csTBA incubated for 30 min with 100 nM of thrombin in binding buffer. Also shown are SERS spectra of NS with mercapto-hexanol and the spectra for the synthesized sensor (NSTBA-MCH).

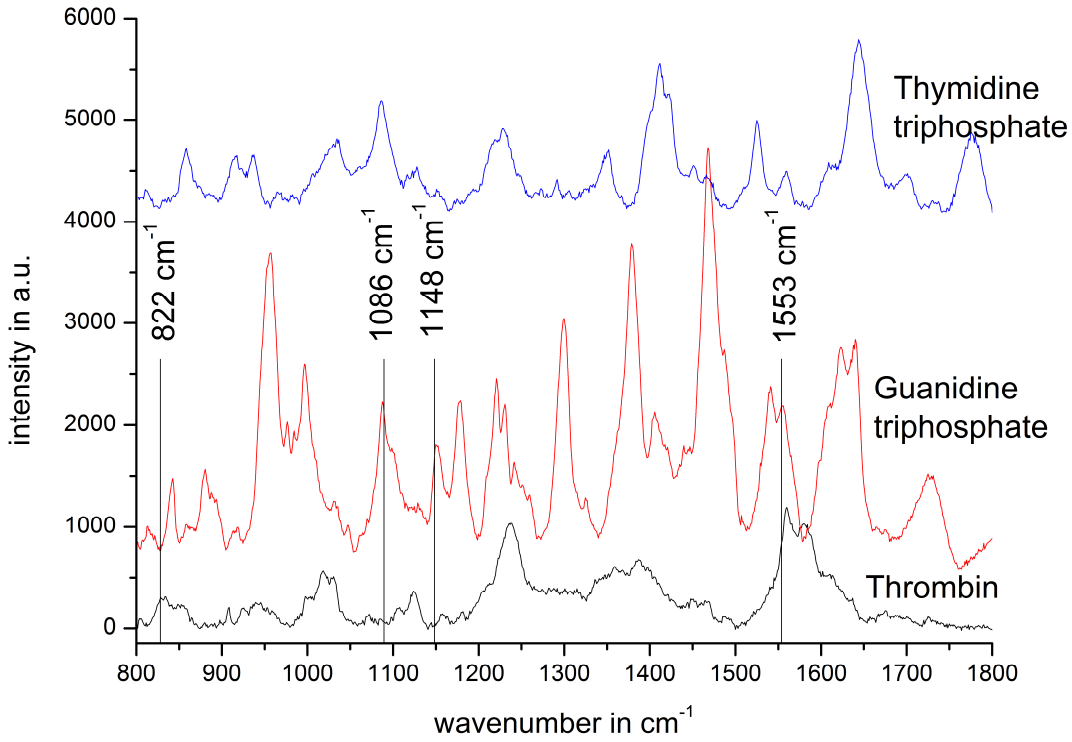


Figure S4: Above Graph shows control measurements of the 3 possible components appearing in the spectra of the sensor. Thymidinetriphosphate (10 mM), Guanidinetriphosphate (10 mM) and human α Thrombin (1 mM) were left overnight to adhere to bare aggregated NS. The spectra were acquired similar to the spectra of the sensor in buffer at ~30 μW of laser power. The spectrum for Thrombin corresponds well to some of the smaller peaks formed on binding of thrombin to TBA (Fig. 2). The GTP and TTP spectra also correspond well with Figure 2.

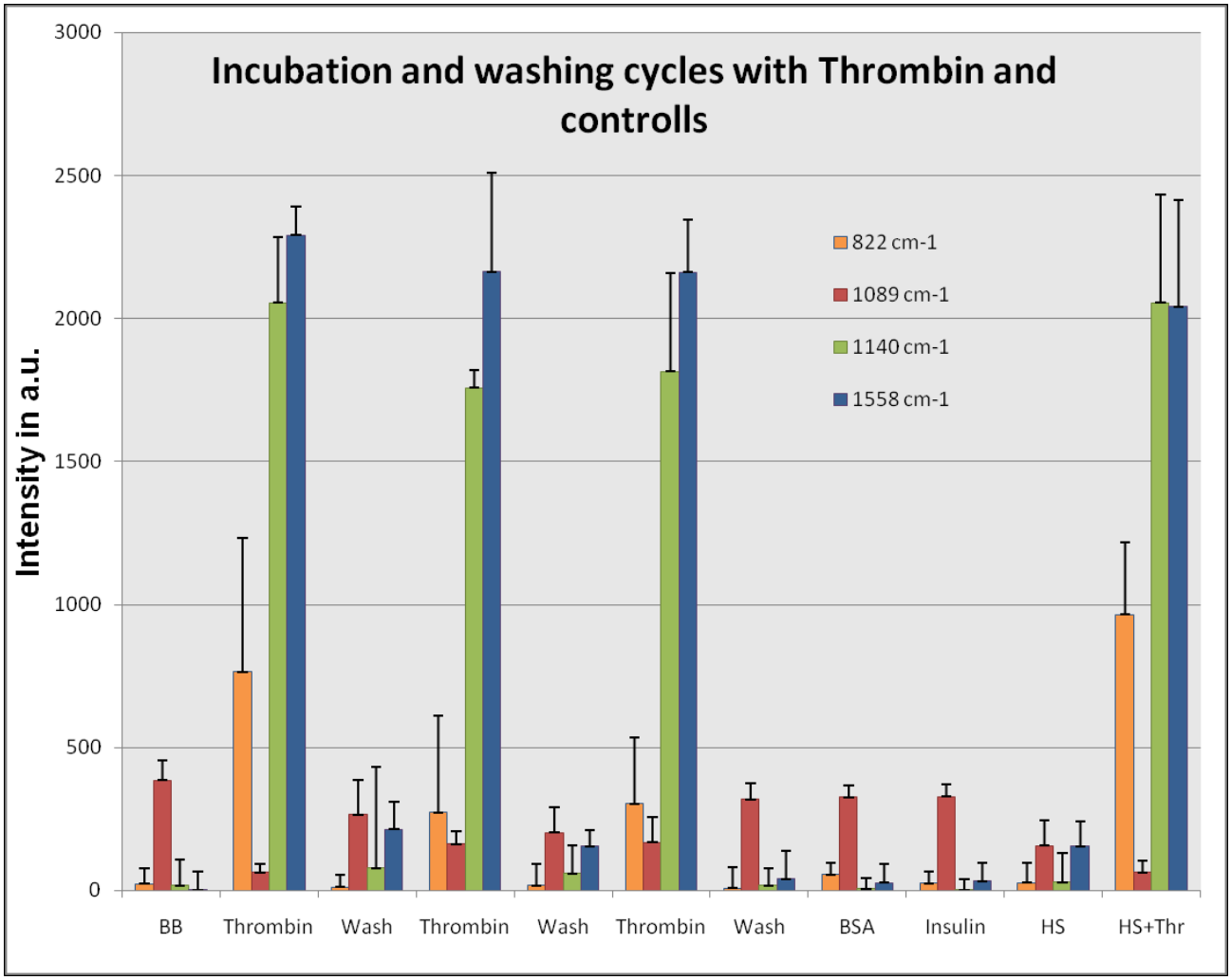


Figure S5: Above Graph shows the data of Figure for 4a presented with errors. Errors depict one standard deviation of 10 spectra.

References

(1) Yoshida, W.; Mochizuki, E.; Takase, M.; Hasegawa, H.; Morita, Y.; Yamazaki, H.; Sode, K.; Ikebukuro, K. *Biosen. and Bioelectron.* **2009**, *24*, 1116-1120.