**Electronic Supplementary Information (ESI)**

# DNA Detection by SERS: Hybridisation Parameters and the Potential for Asymmetric PCR

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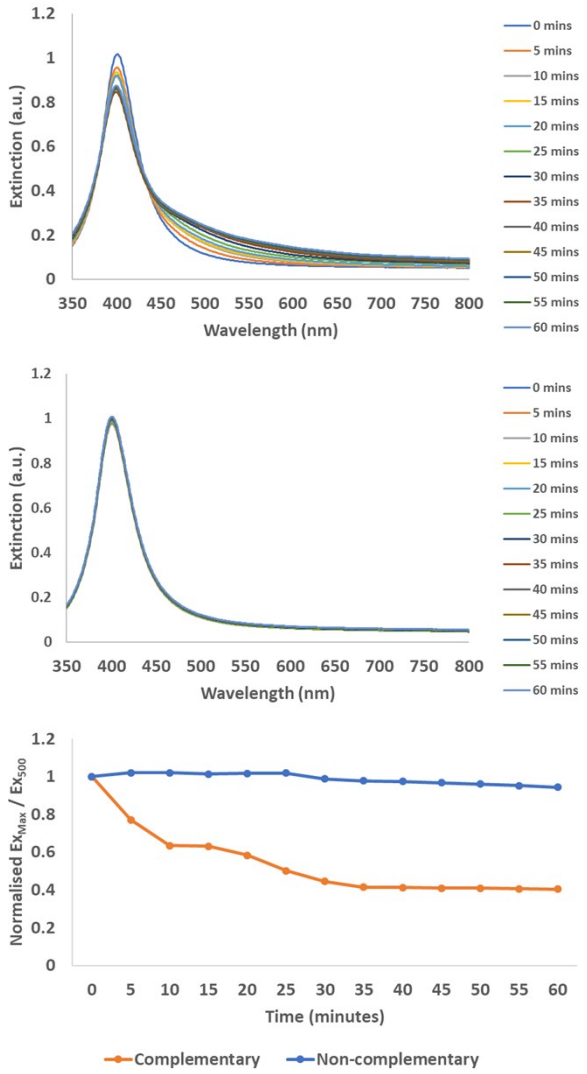
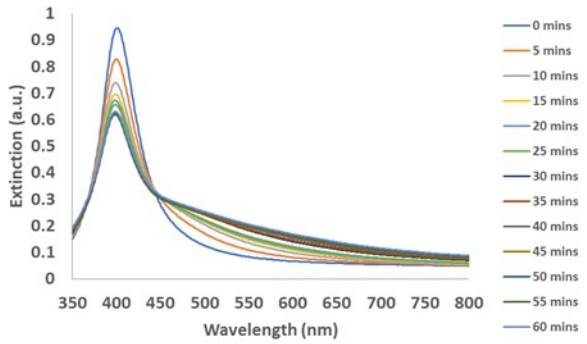
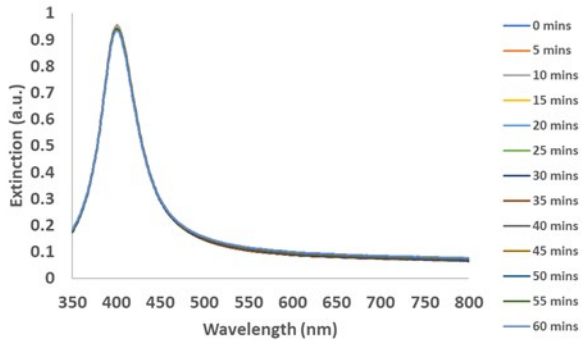



**Figure S1.** UV-Vis kinetics: Conjugates (10 pM) hybridised to synthetic target (10 nM) in the head-to-head orientation using 0.3 M PBS with 3 % PEG 6000 at 45 °C for 1 hour. A. Addition of 100-base complementary target. B. Addition of a 100-base non-complementary sequence. C. Normalised ratio of maximum extinction (nm) / 500 nm extinction values at 5-minute intervals for both complementary and non-complementary DNA. Maximum extinction / 500 nm data normalised to extinction maximum at 0 minutes.



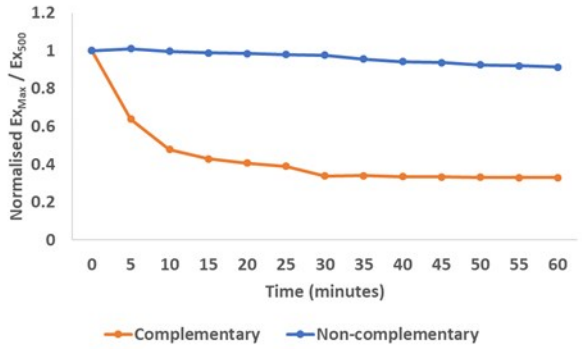




**Figure S2.** UV-Vis kinetics: Conjugates (10 pM) hybridised to synthetic target (10 nM) in the head-to-head orientation using 0.3 M PBS with 5 % PEG 6000 at 45 °C for 1 hour. A. Addition of 100-base complementary target. B. Addition of a 100-base non-complementary sequence. C. Normalised ratio of maximum extinction (nm) / 500 nm extinction values at 5-minute intervals for both complementary and non-complementary DNA. Maximum extinction / 500 nm data normalised to extinction maximum at 0 minutes.

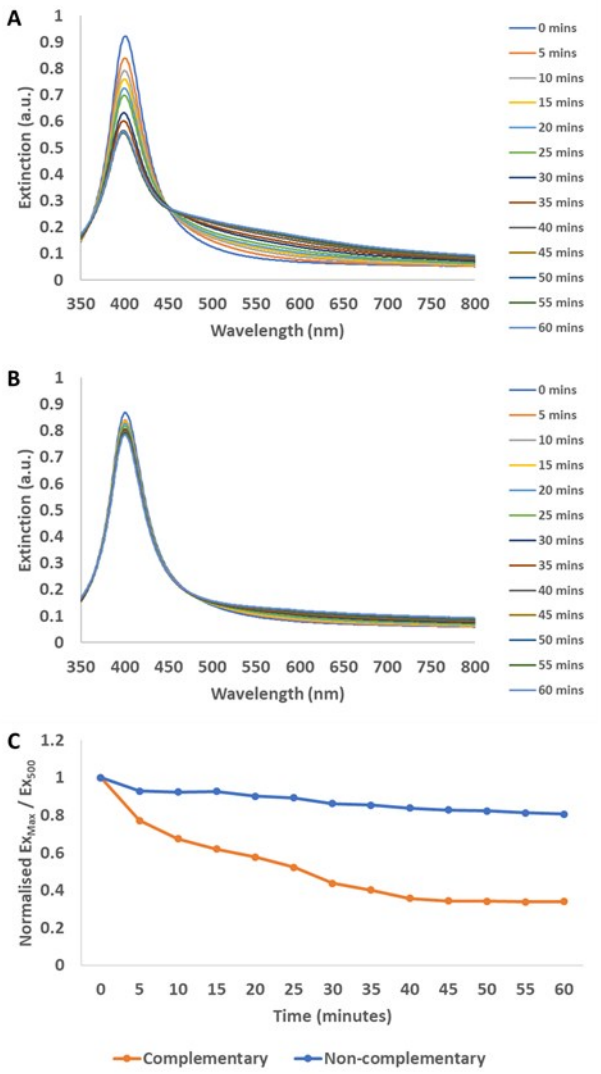



**Figure S3.** UV-Vis kinetics: Conjugates (10 pM) hybridised to synthetic target (10 nM) in the head-to-head orientation using 0.3 M PBS with 7 % PEG 6000 at 45 °C for 1 hour. A. Addition of 100-base complementary target. B. Addition of a 100-base non-complementary sequence. C. Normalised ratio of maximum extinction (nm) / 500 nm extinction values at 5-minute intervals for both complementary and non-complementary DNA. Maximum extinction / 500 nm data normalised to extinction maximum at 0 minutes.

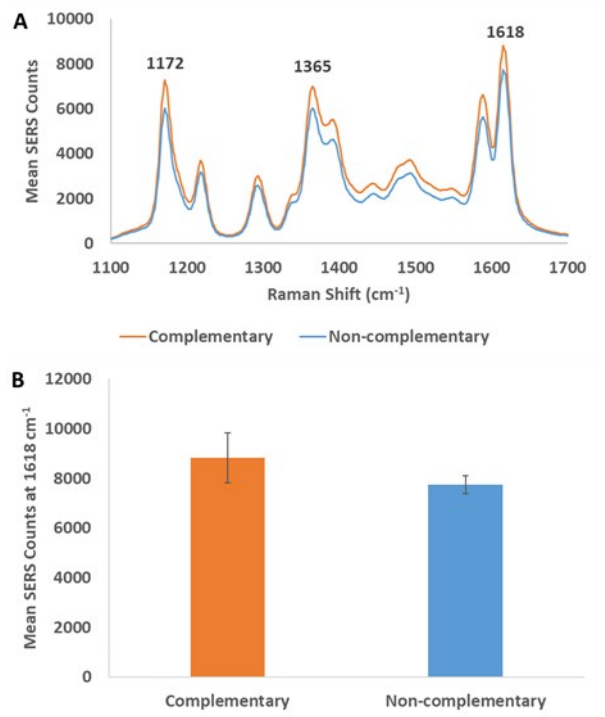



**Figure S4.** SERS analysis comparing probe orientations: Conjugates (10 pM) hybridised to synthetic target (10 nM) using 0.3 M PBS with 5 % PEG 6000 at 45 °C for 1 hour. A. Mean SERS spectra in tail-to-tail orientation (1 second integration time). B. Tail-to-tail orientation: On-to-off ratio of 1: 1 with error bars representative of standard deviation. Data accumulated using a 532 nm laser wavelength and 10 % (5 mW) laser power.

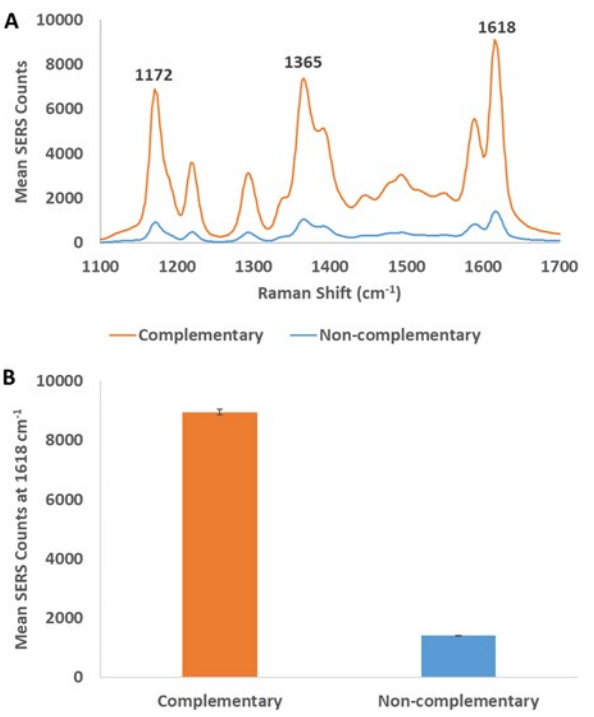



**Figure S5.** SERS analysis comparing probe orientations: Conjugates (10 pM) hybridised to synthetic target (10 nM) using 0.3 M PBS with 5 % PEG 6000 at 45 °C for 1 hour. A. Mean SERS spectral in head-to-tail orientation (0.1 second integration time). B. Head-to-tail orientation: On-to-off ratio of 6: 1 with error bars representative of standard deviation. Data accumulated using a 532 nm laser wavelength and 10 % (5 mW) laser power.

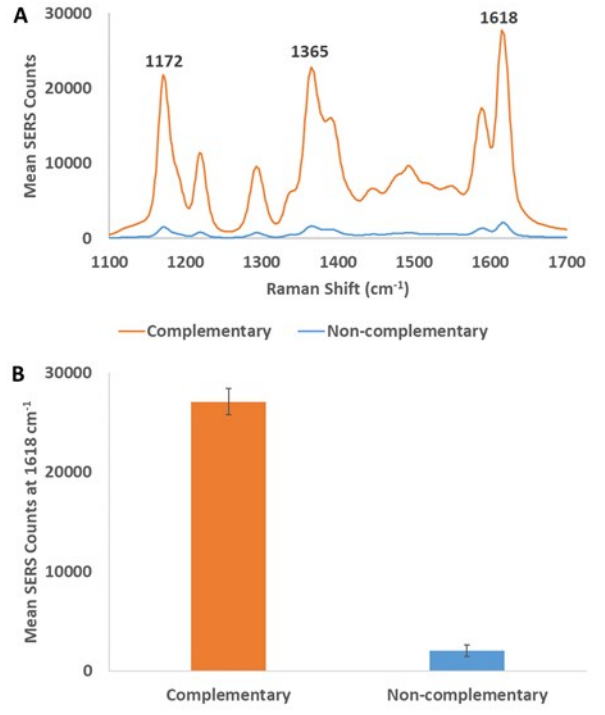



**Figure S6.** SERS analysis comparing probe orientations: Conjugates (10 pM) hybridised to synthetic target (10 nM) using 0.3 M PBS with 5 % PEG 6000 at 45 °C for 1 hour. A. Mean SERS spectra in head-to-head orientation (0.1 second integration time). B. Head-to-head orientation: On-to-off ratio of 13: 1 with error bars representative of standard deviation. Data accumulated using a 532 nm laser wavelength and 10 % (5 mW) laser power.

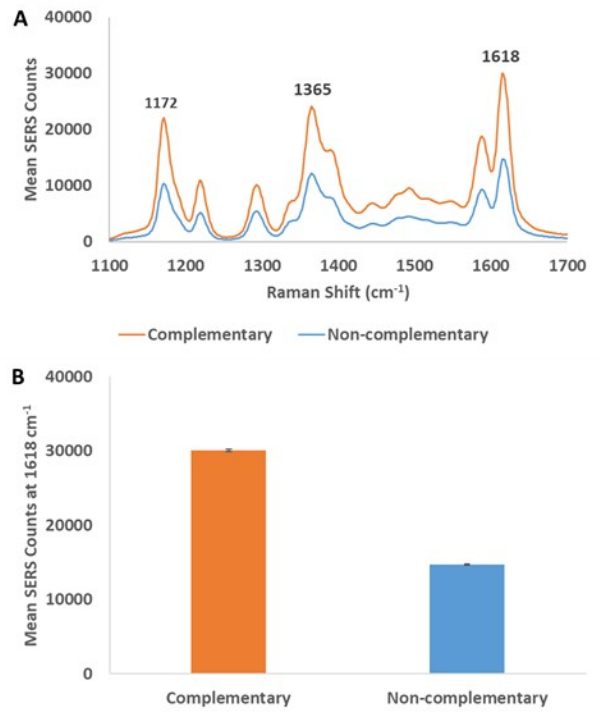



**Figure S7.** SERS analysis comparing PEG molecular weight: Conjugates (10 pM) hybridised to synthetic target (10 nM) in head-to-head orientation using 0.3 M PBS at 45 °C for 1 hour. A. Mean SERS spectra with addition of 5% PEG 2000 (0.05 second integration time). B. 5 % PEG 2000: On-to-off ratio of 2: 1 with error bars representative of standard deviation. Data accumulated using a 532 nm laser wavelength and 10 % (5 mW) laser power.

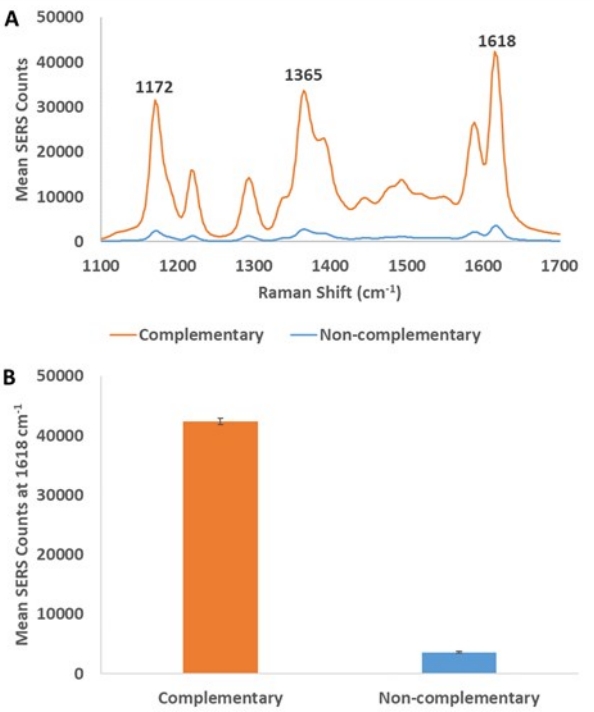



**Figure S8.** SERS analysis comparing PEG molecular weight: Conjugates (10 pM) hybridised to synthetic target (10 nM) in head-to-head orientation using 0.3 M PBS at 45 °C for 1 hour. A. Mean SERS spectra with addition of 5 % PEG 6000 (0.1 second integration time). B. 5 % PEG 6000: On-to-off ratio of 12: 1 with error bars representative of standard deviation. Data accumulated using a 532 nm laser wavelength and 10 % (5 mW) laser power.

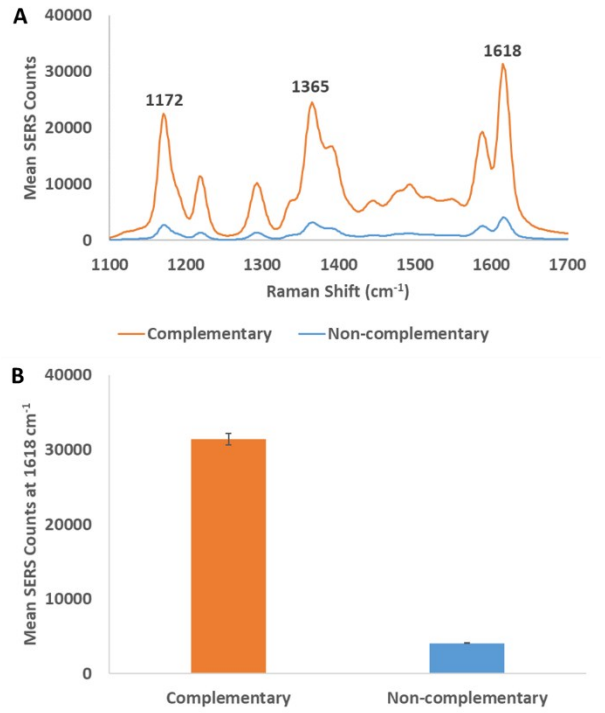



**Figure S9.** SERS analysis comparing PEG molecular weight: Conjugates (10 pM) hybridised to synthetic target (10 nM) in head-to-head orientation using 0.3 M PBS at 45 °C for 1 hour. A. Mean SERS spectra with addition of 5 % PEG 10000 (0.05 second integration time). B. 5 % PEG 10000: On-to-off ratio of 8:1 with error bars representative of standard deviation. Data accumulated using a 532 nm laser wavelength and 10 % (5 mW) laser power.

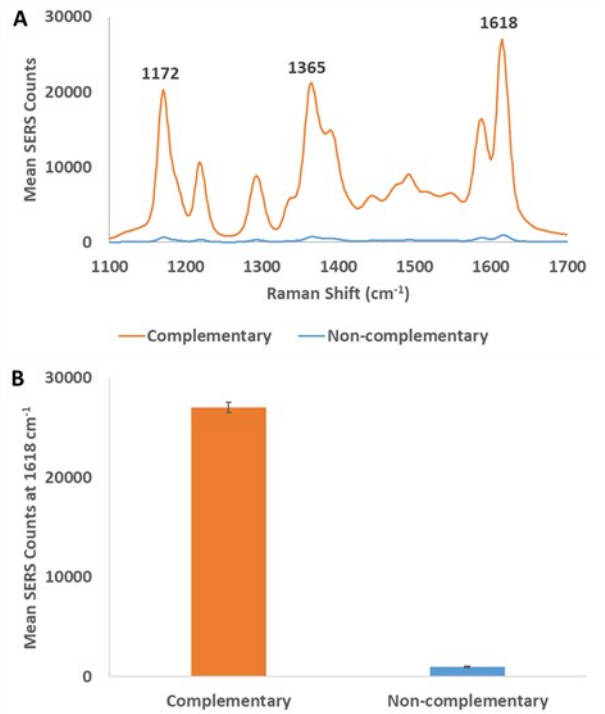



**Figure S10.** SERS analysis with addition of 0.1 % Tween 20: Conjugates (10 pM) hybridised to synthetic target (10 nM) in head-to-head orientation using 0.3 M PBS with 5 % PEG 10000 and 0.1 % Tween 20 at 45 °C for 1 hour. A. Mean (N=3) SERS spectra (0.05 s accumulation time). B. On-to-off ratio of 28: 1 with error bars representative of standard deviation. Data accumulated using a 532 nm laser wavelength and 10 % (5 mW) laser power.

**Table S1. Target and Probe Sequences**

| |
|---|
| ***Candida krusei* 18S rRNA 100 base synthetic target sequence:** |
| 5’ – TCC AGC TCC AAT AGC GTA TAT TAA AGT TGT TGC AGT TAA AAA GCT CGT AGT TGA ACT TTG GGC CTG GGC GGA CGG TCT ACC TAT GGT AAG CAC TGT TGC G – 3’ |
| ***Candida krusei* 18S rRNA probe 1 sequence:** |
| 5’ – CGC AAC AGT GCT TAC CAT – 3’ |
| ***Candida krusei* 18S rRNA probe 2 sequence:** |
| 5’ – AGG TAG ACC GTC CGC CCA – 3’ |
| ***Candida krusei* 18S rRNA asymmetric PCR target sequence:** |
| 5’ – GAC AAT ATA TAA CGA TAC AGG GCC TTT GGT CTT GTA ATT GGA ATG AGT ACA ATG TAA ATA CCT TAA CGA GGA ACA ATT GGA GGG CAA GTC TGG TGC CAG CAG CCG CGG TAA TTC CAG CTC CAA TAG CGT ATA TTA AAG TTG TTG CAG TTA AAA AGC TCG TAG TTG AAC TTT GGG CCT GGG CGG ACG GTC TAC CTA TGG TAA GCA CTG TTG CGG CCG GGT CTT TCC TTC TGG CTA GCC CTC GGG CGA ACC AGG ACG ATT ACT TTG AGG AAA TTA GAG TGT TCA AAG CAG GCC TTT GCT CGG ATA TAT TAG CAT GGA ATA ATA GAA TAG GAC GCA TGG TTC TAT TTT GTT GGT TTC TAG GAC CAT CGT AAT GAT TAA TAG GGA CG – 3’ |
| ***Candida krusei* 18S rRNA probe 1 sequence:** |
| 5’ – GCC GCA ACA GTG CTT ACC – 3’ |
| ***Candida krusei* 18S rRNA probe 2 sequence:** |
| 5’ – ATA GGT AGA CCG TCC GCC – 3’ |