Supporting Information

# Acoustic methodology for selecting highly dissipative probes for ultrasensitive DNA detection

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**PROCEDURES**

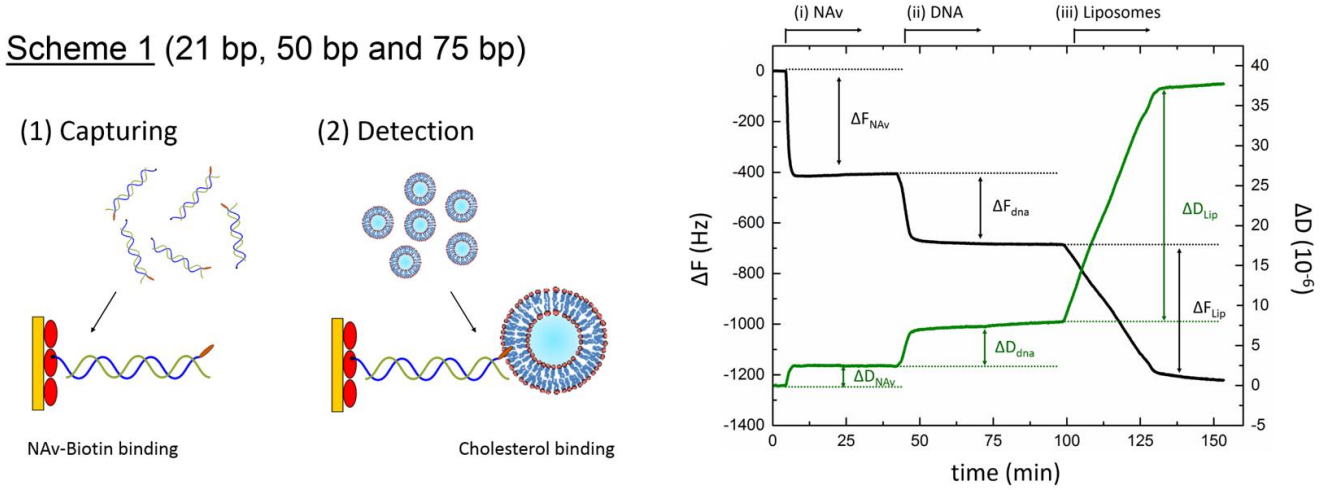


**Figure S1. Measurement of the acoustic ratio of DNA and liposomes at the plateau.** Scheme 1 gives a schematic representation of the biorecognition surface, as this was created and followed in real time through acoustic measurements (right graph). Specifically, a continuous flow of PBS (0.01 M $Na_2HPO_4$, 0.0018 M $KH_2PO4$, 0.0027 M KCl and 0.137 M NaCl, pH 7.4, at 25 °C; tablets from Sigma-Aldrich) was pumped through the QCM-D chamber and the acoustic signal was allowed to equilibrate prior to the first addition. An initial protein layer made of neutravidin (NAv) was formed directly on gold upon injection of 200 µL of NAv solution at 0.2 mg/mL in PBS. After rinsing with PBS, 200 µL of 5'biotin/3'cholesterol-modified DNA solutions of various lengths and sequences (see Tables S1 and S2) and at various concentrations (from 10 nM to 500 nM) were pumped through the chamber and the DNA surface binding was recorded leading to $\Delta F_{dna}$ from ~5 Hz to ~300 Hz. Finally, a suspension of liposomes was added within the concentration range of 0.025-0.200 mg/ml; changes in frequency ($\Delta F_{dna}$ and $\Delta F_{Lip}$) and dissipation ($\Delta D_{dna}$ and $\Delta D_{Lip}$) were calculated at the plateau for the DNA and liposomes additions and, subsequently, used to derive the corresponding acoustic ratio ($\Delta D_{dna}/\Delta F_{dna}$ and $\Delta D_{Lip}/\Delta F_{Lip}$).

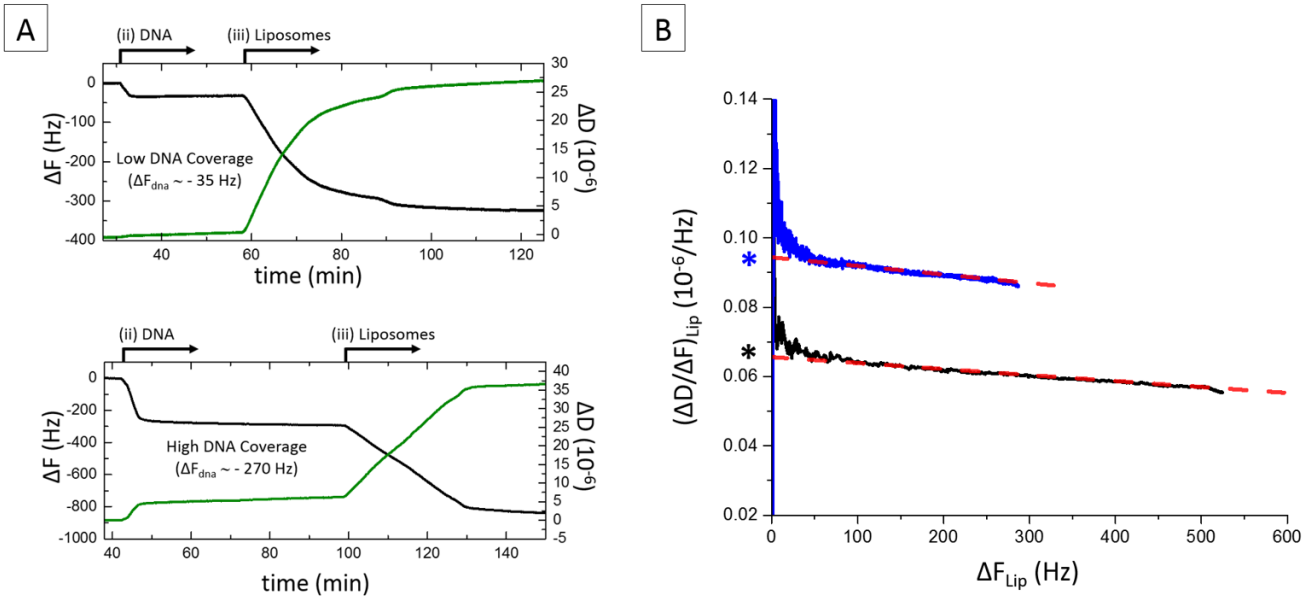


**Figure S2. Measurement of dissipation capacity (DC) of liposomes, i.e. lim $(\Delta D/\Delta F)_{Lip}$ when $\Delta F_{Lip} \rightarrow 0$, from real-time acoustic sensogram; effect of DNA surface coverage.** Following the same experimental procedure described above, real time graphs were derived upon initial addition of various DNA surface coverages. Panel (A) shows examples of a low (top ~50 nM) and high (bottom ~400 nM) concentration of 50 bp DNA leading to $\Delta F_{DNA}$ ~ -35 Hz and $\Delta F_{DNA}$ ~ -270 Hz respectively. Following the addition of liposomes, changes in frequency $\Delta F_{Lip}$ and dissipation $\Delta D_{Lip}$ were recorded as a function of time, and used to calculate the corresponding ratio $(\Delta D/\Delta F)_{Lip}$. Panel (B) depicts plots of $(\Delta D/\Delta F)_{Lipo}$ against the equivalent amount of liposomes on the surface $\Delta F^{t}_{Lipo}$; the blue and black lines correspond to the low and high DNA coverages. Dashed red lines were used to obtain the DC of liposomes as the intercepts for $\Delta F_{Lipo} \rightarrow 0$ (marked with an asterisk *).

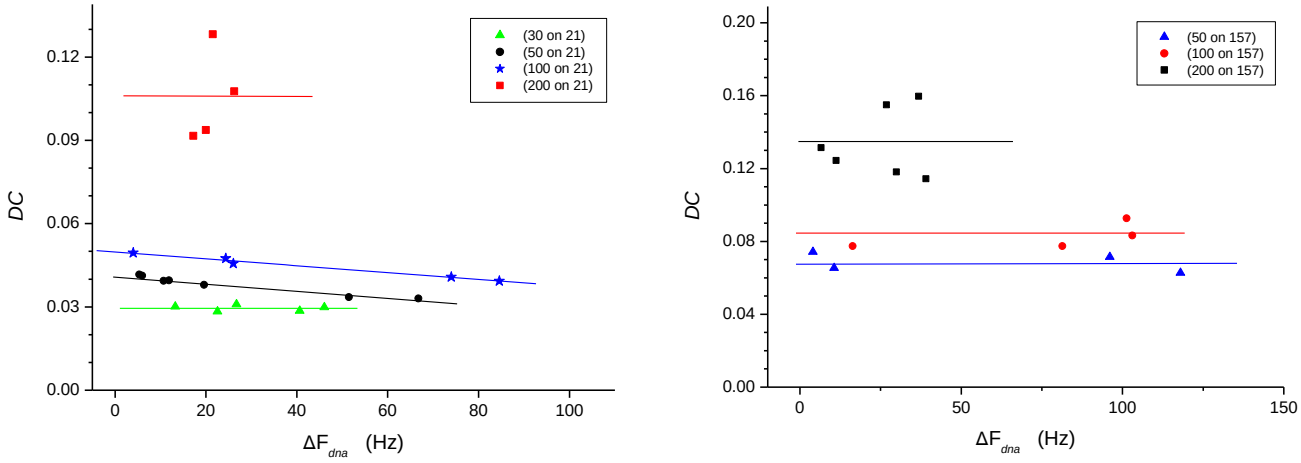


**Figure S3. Dissipation capacity of liposomes anchored on a 21 and 157 bp dsDNA.** From real time graphs, similar to those shown in Fig. S2, the DC ($10^{-6}$/Hz) value of liposomes (30, 50, 100 and 200 nm) anchored to 21 and 157 bp dsDNAs were calculated at $\Delta F_{Lip} \rightarrow 0$. Graphs shown on the left and right represent plots of the calculated DC values as a function of the amount of surface-bound DNA. The dependency of the DC to $\Delta F_{Lip}$ observed in some cases, is attributed to attachment through various points.

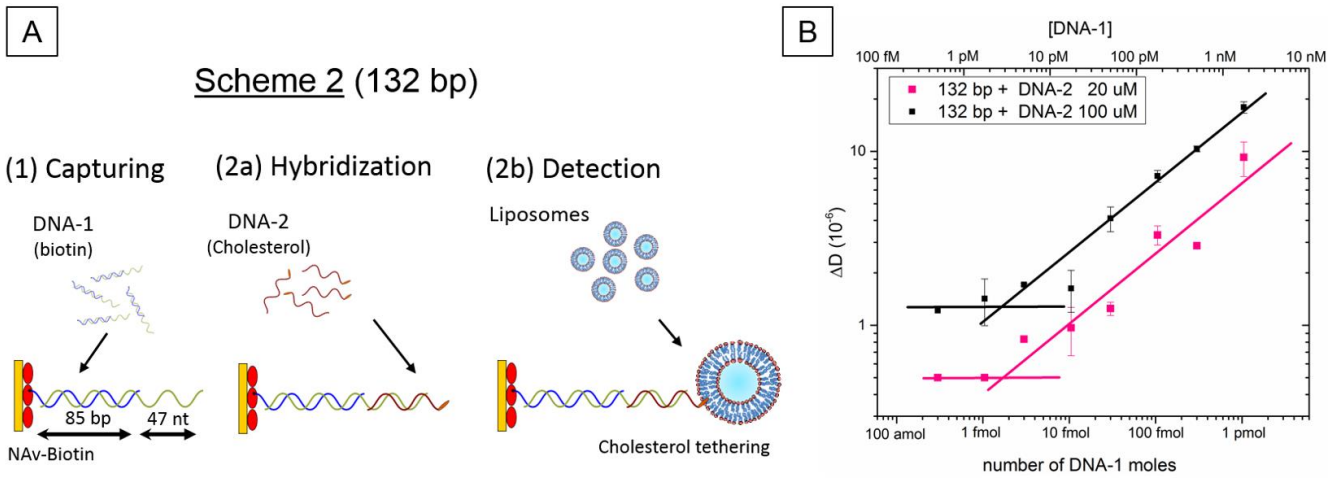


**Fig. S4. Ultrasensitive detection of 132 bp dsDNA.** A different approach was followed during ultrasensitive DNA detection of the 132 cholesterol-modified DNA. A two-step surface hybridization scheme was followed where the cholesterol was introduced at a second stage. (A) depicts the detection scheme consisting of: (1) capturing the DNA-1 construct formed in solution by directly hybridization, where DNA-1 comprises a double stranded region of 85bp as well as a ss target sequence of 47 nt, followed by the addition of first (2a) a ssDNA-2 consisting of 47 nt complementary to target sequence bearing a cholesterol group, and second (2b) the injection of liposomes. Panel (B) shows the dissipation change ($\Delta D$ in $10^{-6}$) registered upon injection and binding of liposomes of 200 nm for different concentrations of DNA-1. Different hybridization conditions for the ss target sequence (DNA-2) used in step (2a) led to a similar LoD ~ 2 pM. For the preparation of DNA-1, the two strands shown in Table S2 were mixed in a 10:1 molar concentration and hybridized following the protocol described in the "Preparation of DNA fragments" part of the experimental section of the manuscript.

**MATERIALS**

**Table S1.** DNA sequences of molecules used in Scheme 1.

| **21 bp:** | |
|---|---|
| **Double labeled 21nt strand** | 5’-biotin-TAG AGC TCC CTT CAA TCC AAA-cholesterol TEG-3’ |
| **Complementary 21nt strand** | 5’- TTT GGA TTG AAG GGA GCT CTA-3’ |
| **50 bp:** | |
| **Double labeled 50nt strand** | 5’-biotin-AAT TCA GAG AGG AGG AGA GAG CGG TGC GGT AGG AGA GAG AGA GGA GGA TC-cholesterol TEG-3’ |
| **Complementary 50nt strand** | 5’-GAT CCT CCT CTC TCT CTC CTA CCG CAC CGC TCT CTC CTC CTC TCT GAA TT-3’ |
| **75 bp:** | |
| **Double labeled 75nt strand** | 5’-biotin-CCA CCA AAC GTT TCG GCG AGA AGC AGG CCA TTA TCG CCG GCA TGG CGG CCG ACG CGC TGG GCT ACG TCT TGC TGG-cholesterol TEG-3’ |
| **Complementary 75nt strand** | 5’- CCA GCA AGA CGT AGC CCA GCG CGT CGG CCG CCA TGC CGG CGA TAA TGG CCT GCT TCT CGC CGA AAC GTT TGG TGG-3’ |

**Table S2.** DNA sequences of molecules used in Scheme 2.

| **132 bp:** | |
|---|---|
| **DNA-1:** | |
| **Double labeled 85nt strand** | 5'-ATG CAC AAT TAG ATT CGT TTG GTG GAT AGA TCG TCG TAA GCG CTG GGT ACT ATA ATG AAC TGC GAC TCT CAG ATT CGT TT GGT GG-biotin-3' |
| **Complementary 132nt strand** | 5'-CCA CCA AAC GAA TCT GAG AGT CGC AGT TCA TTA TAG TAC CCA GCG CTT ACG ACG ATC TAT CCA CCA AAC GAA TCT AAT TGT GCA TCG TTC GCG ACG CGA GGC TGG TTC AAC TTC CCC ATT CAT TGA TAT ATT -3' |
| **DNA-2:** | |
| **47nt hybridization strand** | 5'-cholesteryl TEG-AAT ATA TCA ATG AAT GGG GAA GTT GAA CCA GCC TCG CGT CGC GAA CG-3' |