Supporting Information

Extracting the Shape and Size of Biomolecules Attached to a Surface as Suspended Discrete Nano-Particles

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AFM measurements

Atomic force microscopy was performed using an Agilent Technologies (Santa Clara, CA) 5500 microscope. Measurements were always made under liquid conditions in PBS buffer at room temperature using Olympus rectangular silicon nitride cantilevers (RC800PSA, 200 x 20 μm^2) with a spring constant of 0.05 N/m, an estimated tip radius of 20 nm, and a resonance frequency in the liquid cell of approximately 27 kHz. Scanning rates were kept close to 1 Hz. All images contain 1024 pixels x 1024 pixels and were first-order flattened using Picoimage software from Agilent. The gold substrates imaged were the same ones used for the QCM measurements.

DNA sequences and manipulation

DNA anchor-sequences for streptavidin immobilization

All biotinylated DNA strands were synthesized by Eurogentec S.A, Belgium, while all non-biotinylated DNA strands by the Microchemistry laboratory, IMBB-FORTH-Hellas. All the oligonucleotides were synthesized at the 200nmol scale and purified by high-performance liquid chromatography (HPLC). The sequences of the DNA molecules used for the SAv binding experiments are shown in Table S1. In addition to the presence of a biotin molecule at the 5' end of the surface attached strand, a second biotin was also incorporated for SAv binding. The second biotin was linked either at the 3'-end of the surface-attached strand or at the 5'-end of the complementary strand; both types of molecules showed the same capability for SAv binding, and were therefore used without preference in the experiments. Below are the sequences of 21, 50 and 76 nt DNAs used to anchor SAv to the device surface. The presence of the second biotin molecule (attached either at the 3′ end of the surface attached probe or the complementary strand) is not depicted in the sequence.

21 nt: *Surface attached strand: 5' –biotin-TAGAGCTCCCTTCAATCCAAA-3'* *Complementary strand: 5'- TTT GGA TTG AAG GGA GCT CTA -3'*
50 nt: *Surface attached strand: 5'-biotin-ATTCAGAGAGGAGGAGAGAGCGGTGCGGTAGGAGAGAGAGAGGAGGATC-3'* *Complementary strand: 5'-GATCCTCCTCTCTCTCTCCTACCGCACCGC TCTCTCCT CCTCTCTGAATT-3'*
76 nt: *Surface attached strand: 5'-biotin-CCAAAGCGAGCAAGAGAATCCCAGGACAGAAAGGTAAAGCTCCCTCCCTCAAG TTGACAAAAATCTCACCCC ACCA-3'* *Complementary strand: 5'biotin- TGGTGGGGTGAGATTTTTGTCAACTTGAGGGAGGGAGCTTTACCTTTCTGTCCTG GGATTCTCTTGCTCGCTTTGG-3'*

Table S1.Sequences of 21, 50 and 76 nt DNAs used to anchor SAv to the device surface. The presence of the second biotin molecule (attached either at the 3' end of the surface attached probe or the complementary strand) is not depicted in the sequence.

DNA anchor-sequences for the 47bp DNA immobilization

Single stranded DNA molecules biotinylated at their 5'-end and with a variable length of 15, 21, 35, 50, 85 nucleotides (Eurogentec S.A, Belgium), depicted as molecule (A) in Figure S2, are given in Table S2; single stranded DNAs (Microchemistry laboratory, IMBB-FORTH-Hellas) employing a strand complementary to each one of the above sequences followed by a 50nt sequence (depicted as molecule (B) in Figure S2), are shown in Table S3. The sequence of the 47 nt DNA (molecule (D) in Figure S2) which is complementary to the upper part of the ss-overhang of molecule (C) is not shown.

15nt	*5'-biotin-AGATTCGTTTGGTGG-3'*
21nt	*5' -biotin-TAGAGCTCCCTTCAATCCAAA-3'*
35nt	*5'-biotin-TATAATGAACTGCGACTCTCAGATTCGTTTGGTGG-3'*
50nt	*5'-biotin-ATTCAGAGAGGAGGAGAGAGCGGTGCGGTAGGAGAGAGAGA GGAGGATC - 3'*
85nt	*5'-biotin-ATGCACAATTAGATTCGTTTGGTGGATAGATCGTCGTAAGCGC TGGGTACTATAATGAACTGCGACTCTCAGATTCGTTTGGTGG-3'*

Table S2. Biotinylated DNA sequences used for molecule (A) in Figure S2.

	Variable length (to be hybridized with sequences shown on Table S2)	**50 nt sequence** (3nt linker + 47 nt)	
(15+50) nt	CCACCAAACGAATCT	CCT	CGTTCGCGACGCGAGG CTGGTTCAACTTCCCCA TTCATTGATATATT
(21+50) nt	TTTGGATTGAAGGGAGCTCTA		
(35+50) nt	CCACCAAACGAATCTGAGAGTCGCAGTTCATTATA		
(50+50) nt	GATCCTCCTCTCTCTCCTACCGCACCGCTCTCTC CTCCTCTCTGAATT		
(85+50) nt	CCACCAAACGAATCTGAGAGTCGCAGTTCATTATA GTACCCAGCGCTTACGACGATCTATCCACCAAACG AATCTAATTGTGCAT		

Table S3. DNA sequences used for molecule (B) in Figure S2.

DNA hybridization and amplification

Hybridized DNA molecules (21bp and 50bp) were produced by mixing the two strands in a ratio of 1:10 for (biotinylated strand):(complementary non biotinylated strand) and 1:1 for two complementary biotinylated strands in PBS buffer; in both cases the solution was kept at 92°C for 5 min in a thermocycler (peqSTAR 2X, peqlab, Germany) and then at room temperature for one hour before use.

The 76 bp DNA used for the SAv detection was produced by PCR amplification in a thermo-cycler (peqSTAR 2X, peqlab, Germany) using the KAPA 2G Fast Hot Start PCR amplification kit (KAPA Biosystems, USA) and 20 ng of human genomic DNA for template (Clontech, USA). The primers used were the following: *forward 5'–biotin–CCAAAGCGAGCAAGAGGATCTC–3'* and *reverse 5–biotin'–TGGTGGGGTGAGATTTTTGTC–3'* (Eurogentec S.A, Belgium); 10 pmoles of each primer were added in each reaction. After amplification, DNA was purified using the Nucleospin Gel and PCR clean-up kit (Macherey-Nagel, Germany) following manufacturer instructions.

Acoustic measurements

i) SAv on Au

After equilibration of the baseline with buffer, 20 μg/mL of SAv (provided in a lyophilized form, ≥ 13 units/mg; Sigma Aldrich, USA) in PBS (PBS: 0.01 M Na_2HPO_4, 0.0018 M KH_2PO_4, 0.0027 M KCl and 0.137 M NaCl, pH 7.4, at 25 °C; tablets from Sigma Aldrich, USA) was added to the clean device surface, followed by buffer rinse.

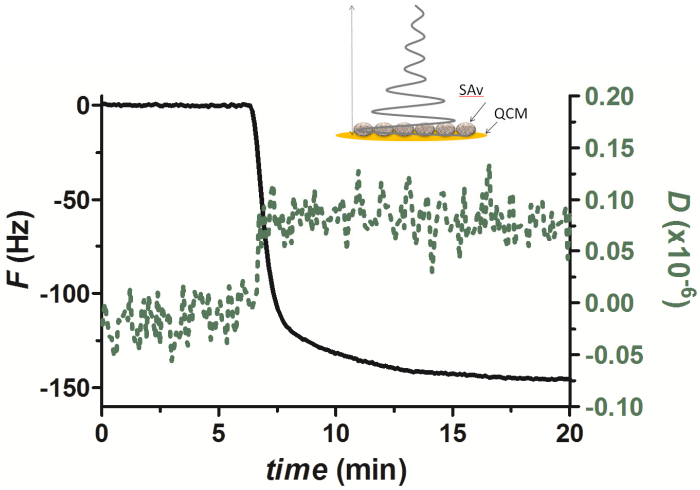

Figure S1. Real time acoustic data of frequency and energy dissipation response during the physisorption of 20 μg/mL of SAv in PBS buffer on the Au-coated QCM surface.

ii) SAv on DNA

Neutravidin adsorption on gold involved the addition of 200 μg/mL of the protein (Invitrogen-Life technologies, ThermoFisher Scientific, USA) in PBS until both signals (F and D) reached equilibrium. Following PBS rinse, each one of the double stranded DNA molecules shown in Table S1 were pumped over the device surface in a concentration range varying between 1 and 50 pmol in 200 μL PBS, followed again by PBS rinse. Finally, a solution of 20 μg/ml of SAv in PBS was pumped over each DNA-modified surface and the biosensor was rinsed with PBS in order to remove any non-bound molecules.

iii) Detection of 47bp DNA

The procedure used for the measurement of the ΔD/ΔF acoustic ratio of the 47bp DNA is shown in Figure S2. A solution of DNA molecule (C) (produced after solution-based hybridization of DNAs (A) and (B), shown in Tables S1 and S2) at a concentration range varying from 1 to 50 pmol in 200 μL of PBS was pumped over the NAv-modified surface and left to adsorb until equilibrium was reached. After PBS rinse, a 47 nt DNA (D), complementary to the upper part of the ss overhang of molecule (C), was added at a concentration that was ten times higher than that of molecule (C).

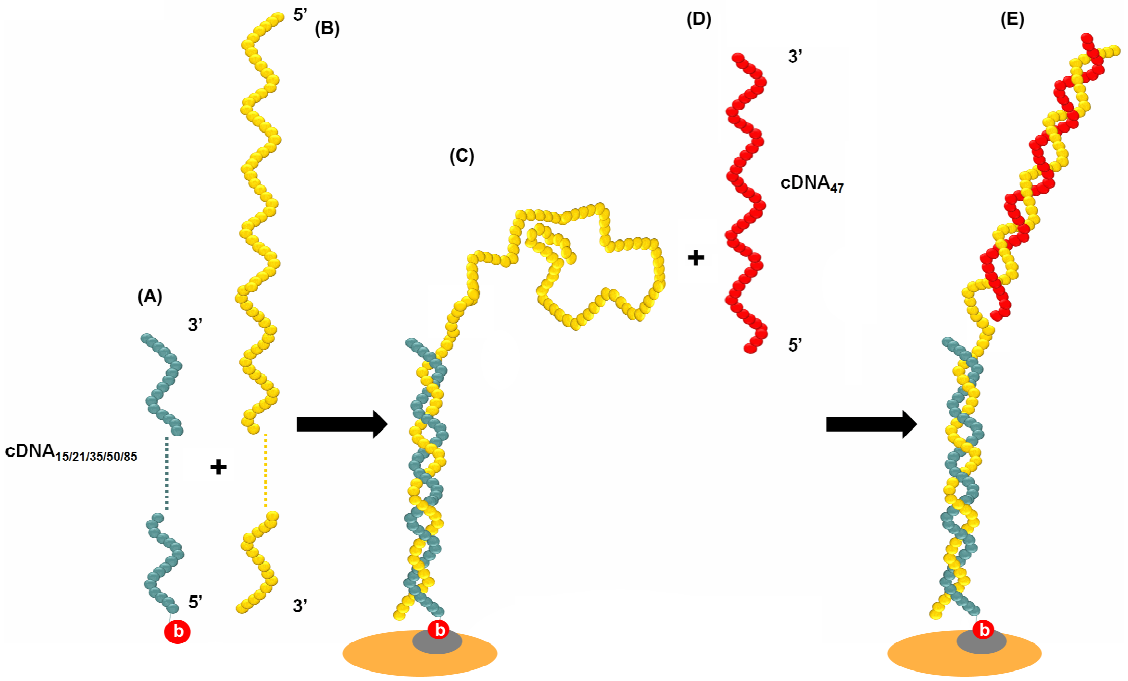

Figure S2. Schematic representation of the procedure used for anchoring the 47bp DNA to the device surface. Biotinylated DNA (C), produced after solution-based hybridization of molecules (A) and (B), is attached to the NAv-modified QCM surface, followed by addition of a 47nt DNA (D) which hybridizes with the ss overhang to produce molecule (E).

The real time frequency and amplitude plots of each binding step are shown in Figure S3(a) for the (50+50) nt DNA. The D versus F plot (Figure S3(b)) obtained during the addition of the 47 nt DNA (D) was used to calculate an acoustic ratio of $(0.0291\pm0.0003)\times10^{-6}$/Hz. Similar graphs, obtained for all DNA sequences given in Tables S2 and S3, were used to calculate the corresponding acoustic ratio in each case.

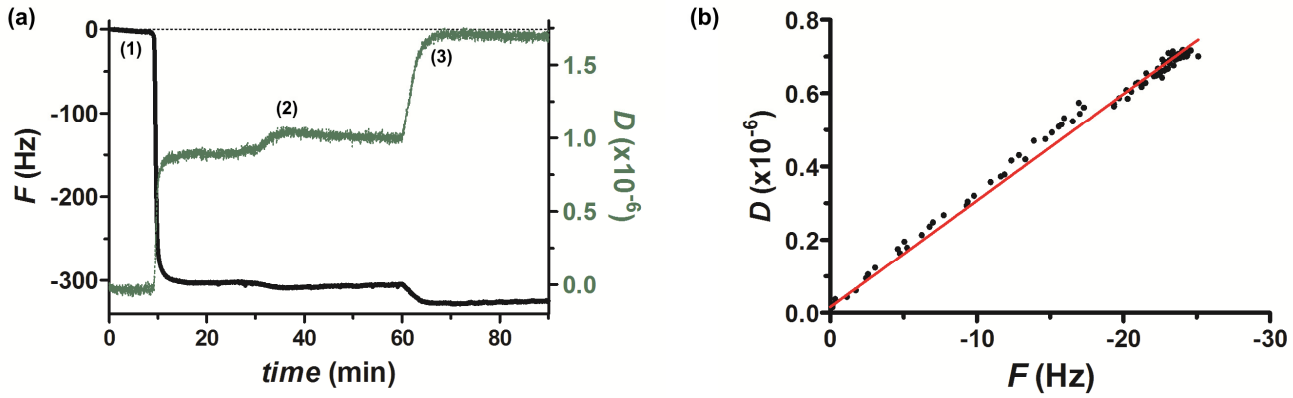

Figure S3. (a) Real-time plots obtained during the binding of a DNA strand on the device surface consisting of the following steps: (1) NAv binding; (2) binding of the (50+50) DNA; and (3) hybridization of the 47 nt DNA; **(b)** D versus F plot of step (3) was used to calculate the corresponding acoustic ratio.