Lab on a Chip

Supplementary Material (ESI) for Lab on a Chip
This journal is (c) The Royal Society of Chemistry 2011

Dynamic Article Links ►

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

SUPPORTING MATERIAL

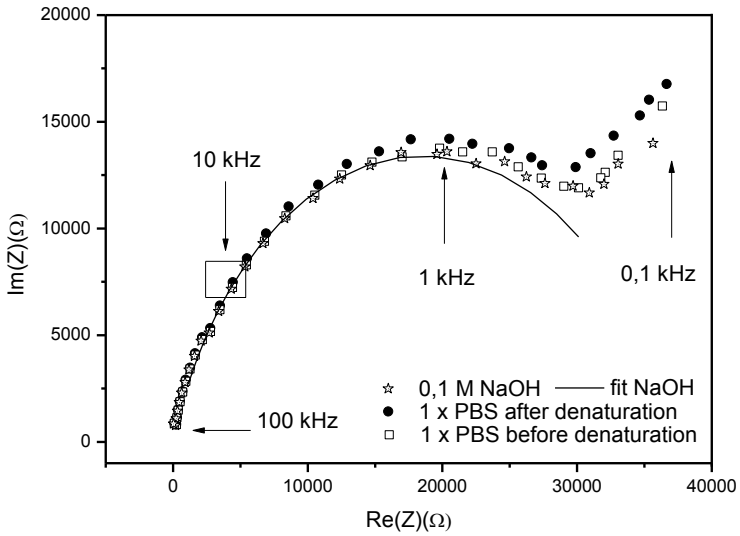
Rapid assessment of the stability of DNA duplexes by impedimetric real-time monitoring of chemically induced denaturation

B. van Grinsven[a], N. Vandenbon[b], L. Grieten[a], M. Murib[a], S.D. Janssens[a], K. Haenen[a,c], E. Schneider[d], S. Ingebrandt[d], M.J. Schöning[e], V. Vermeeren[b], M. Ameloot[b], L. Michiels[b], R. Thoelen[a,f], W. De Ceuninck[a,c], and P. Wagner[a,c]

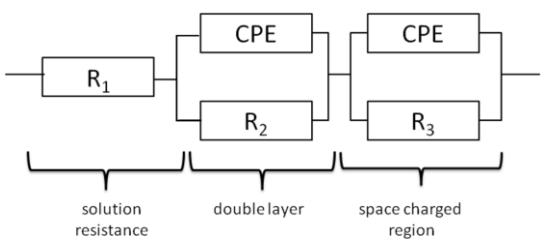
Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Supporting information

Nyquist plots of the impedance spectra



This Nyquist plot shows the impedance data of # D1, see Figure 2, in the full frequency range from 100 Hz to 100 kHz before denaturation (sensor cell filled with 1 × PBS), after denaturation (cell filled with 0.1 M NaOH), and after refilling with 1 × PBS. The 10 kHz point, the frequency used to extract the denaturation dynamics, is indicated by an open box. The high frequency part of all plots can be simulated with a three-element circuit consisting of a solution resistance R_1 in series with a complex resistance describing the biological and the topmost DNA layer. This layer can be represented by a resistor R_2 and a constant-phase element CPE with an almost capacitive behaviour (n = 0.80). The Nyquist plot indicates that there is a second semi circle in the low-frequency domain, but the data, not extending below 100 Hz, do not allow for a reliable fitting. A complete scheme, taking into account also the space- charge region to model the second semi circle, is supposed to correspond to the circuit below.



The fit parameters obtained for the three elements R_1, CPE and n, and R_2 are summarized in the following table. Only data points in the frequency range between 1 kHz and 100 kHz were taken into account. Although there is no marked difference for the situation in 1 × PBS buffer before and after denaturation, we suppose that the sensor effect is mainly related to the capacitive constant-phase properties of the electrode surface.

	1 x PBS before denaturation	1 x PBS after denaturation	0.1 M NaOH	Error (%)
R_1 (Ω)	142	142	140	2.5
CPE (nSs^n)	21.9	24.1	23.9	2.2
n	0.8	0.79	0.79	1.0
R_2 (kΩ)	39.3	38.0	37.1	1.2

Chemical agents and buffer solutions

The probe ssDNA molecules were purchased from Eurogentec (Seraing, Belgium), and were modified at the 5' end with a NH_2-modifier (36 bp: 5'-NH_2-C_6H_{12}-AAA-AAA-ACC-CCT-GCA-GCC-CAT-GTA-TAC-CCC-CGA-ACC-3'). The target ssDNA molecules bought from Invitrogen (Merelbeke, Belgium), were modified at the 5'-end with an Alexa Fluor® 488 label. Target ssDNA contains a sequence that is either completely complementary to the probe ssDNA (29 bp : 5'-Alexa 488- C_6H_{12}-GGT-TCG-GGG-GTA-TAC-ATG-GGC-TGC-AGG-GG-3'), carries a 1-base mismatch to the probe ssDNA on to distinct locations (29 bp: 5'-Alexa 488- C_6H_{12}-GGT-TCG-GGG-GTA-TAC-ATG-GGC-TCC-AGG-GG-3' and 29 bp: 5' -Alexa 488-C_6H_{12}-GGT-TCG-GGG-CTA-TAC-ATG-GGC-TGC-AGG-GG-3'), or complementary random (29 bp: 5'-Alexa 488- C_6H_{12}-TCA-AAT-TGC-CAG-AAC-AAC-TAC-TGA-CTG-AA-3'). Sodium dodecyl sulfate (SDS) was obtained from VWR International (Zaventem, Belgium). 10-Undecenoic acid (UDA) was bought from Sigma-Aldrich (Bornem, Belgium). EDC and 2-[N-morpholino]-ethanesulphonicacid (MES) were purchased from Perbio Science (Erembodegem, Belgium). Sodiumhydroxide (NaOH) was acquired from Merck (Overijse, Belgium). Phosphate buffered saline (PBS) (1.29 M NaCl, 0.05 M Na_2HPO_4*$2H_2O$, 0.015 M KH_2PO_4, pH 7.2) sodium chloride/ sodium citrate (SSC) buffer (3 M NaCl, 0.3 M $C_6H_8O_7$*3Na, pH7.5) and hybridization buffer (0.75 M NaCl, 0.075 M $C_6H_8O_7$*3Na, 0.1% BSA, 0.1% Ficoll, 0.1% C_6H_9NO, 5% dextran sulphate, 5 mM Na_2HPO_4, 0,1% SDS, 50µg/L herring sperm DNA) were homemade.