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Application Note

Nanolever temperature stability
Standard DNA nanolever

Key words: Temperature stability

The oligodeoxynucleotides used as standard nanolevers have been selected to maintain their double stranded conformation even at high temperatures and can be used up to 70°C in T50 buffer and up to 75°C in T20/5 buffer.

When performing measurements with proteins at variable temperatures it is crucial that the employed DNA nanolevers remain intact and do not denature.

The intrinsic melting temperature of the standard nanolever sequences A…F is above 70°C in most buffers. Figure 1 exemplarily shows DNA melting curves of nanolever sequence B measured by the UV absorption method in solution. Melting temperatures T_M were analyzed by evaluating the inflection points of the absorbance curves and are listed in Table 1.

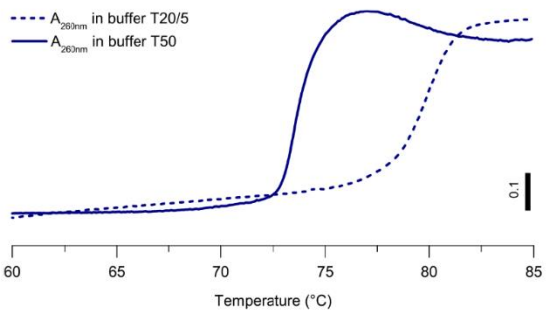

Figure 1 | Solution measurement of the DNA melting transition of standard nanolever sequence B by UV hypochromism. The absorbance at 260 nm increases as the DNA denatures and its conformation changes from double- to single-stranded.

Table 1 | DNA melting temperatures (T_M) of nanolever sequence B

Buffer	T_M (°C)
T50 50 mM NaCl, 10 mM Tris, pH 7.4	73.5
T20/5 20 mM NaCl, 5 mM MgCl2, 10 mM Tris, pH 7.4	79.8

In order to evaluate whether the nanolevers would denature during a temperature cycle that is typically applied for the investigation of protein melting curves, the following test was performed:

(1) An S2 chip was inserted into a DRX 2400 instrument and single stranded (ss) DNA nanolevers were prepared by chemical denaturation under basic pH.

(2) The ssDNA layer was hybridized with complementary sequences in solution to double stranded nanolevers and the hybridization kinetics were recorded.

(3) The chip temperature was ramped up to 75°C and subsequently cooled down to 25°C.

(4) A second hybridization with fresh complementary sequences was attempted to

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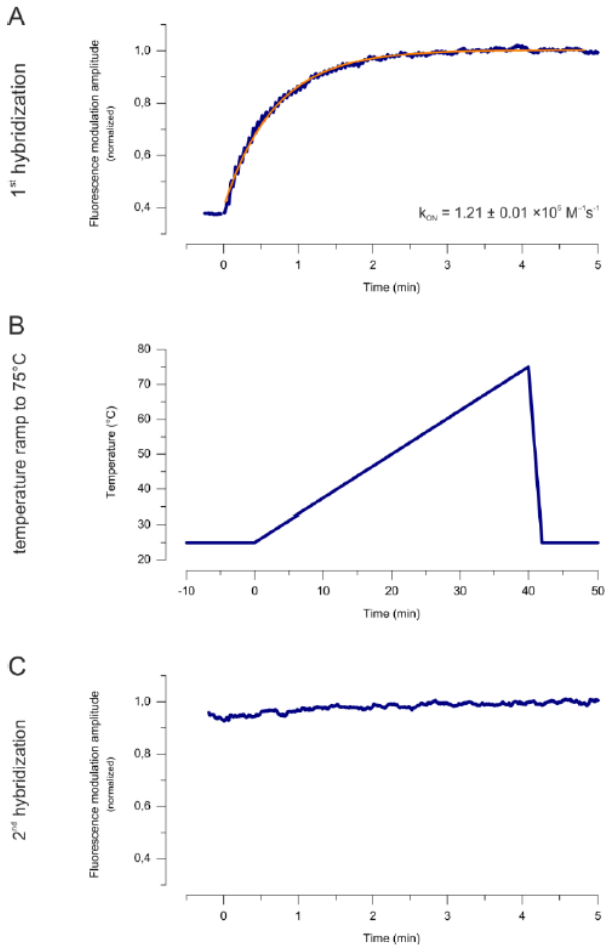

Figure 2 | **Thermal stability test** of DNA nanolever duplexes from 25°C to 75°C.
(A) A chip with a single stranded DNA layer of sequence B is incubated with a solution of complementary (cB) sequences at t = 0 min (200 nM in buffer T50). The hybridization of solute cB to surface-tethered single strands results in the formation of double-stranded nanolevers on the surface within app. 3 minutes. This is observable as a pronounced increase in the fluorescence modulation amplitude, because rigid DNA duplexes can be manipulated more efficiently than flexible single strands by the applied electric field (switching potentials ±0.4 V, 0.2 Hz). The hybridization time course can be fitted with very good agreement by a single exponential function, which yields the association rate constant $k_{ON} = (1.21 \pm 0.01) \times 10^5\ M^{-1}s^{-1}$.
(B) After the first hybridization step, the double stranded DNA layer is subjected to thermal stress by ramping the temperature up to 75°C within 40 min in T20/5 buffer.
(C) After cooling to 25°C, the DNA nanolevers are again incubated with a solution of single stranded cB sequences (200 nM in T50 buffer).

check if a fraction of the nanolevers has been denatured to single strands.
If a significant fraction of the nanolevers on the surface had been denatured as a result of the previous temperature cycle to 75°C, a hybridization curve as in (A) should be observable. This is not the case, which confirms that the nanolevers have maintained their double stranded conformation, i.e., the duplexes are still intact.

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Dynamic Biosensors GmbH I January 2014