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Supplementary Information

Potentiometric sensing of nucleic acids using chemically modified nanopores

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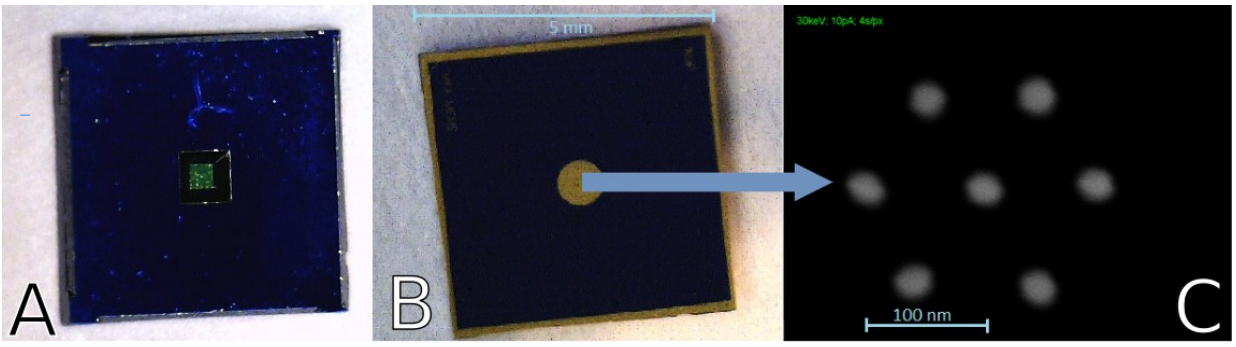

Figure S1. Optical and scanning electron microscopic images of a nanopore chip used in this study (A). The reference side of the chip, i.e., the side from which the Si layer (blue) was etched until reaching the SiN_x layer (the smaller rectangular area in the middle of the chip). The pores were milled by FIB from this side, thus it features the larger entrances of the conically-shaped nanopores. (B) The sample side of the chip covered with the gold layer (yellow disk) comprising the smaller entrance of the nanopores. (C) SEM image of the array indicating the smallest constriction of the 7 nanopores forming the array.

The sequence specific net charge of the PNA probe and the nucleic acid strand were calculated based on the nucleotide (and lysine in case of PNA) content as well as the nucleotide pK-s[1, 2] with the use of the Henderson-Hasselbalch equation.

$$Q_{DNA/RNA} = -(N_b - 1) + \sum_N \frac{(q_- + q_+ \cdot 10^{pK_N - pH})}{10^{pK_N - pH} + 1} \qquad \text{Eq. S1}$$

$$Q_{PNA} = 2 + \sum_{N} \frac{(q_{-} + q_{+} \cdot 10^{pK_N - pH})}{10^{pK_N - pH} + 1} \qquad \text{Eq. S2}$$

$Q_{DNA/RNA}$ and Q_{PNA} are the charges of the DNA/RNA and PNA strands, where a DNA/RNA strand with base number N_b have $(N_b - 1)$ phosphate groups each bearing a negative charge on pH 4.2, q_- and q_+ are the charges of the deprotonated and protonated forms of the corresponding pK and summation is performed on every N nucleotide. In case of PNA strands the backbone is uncharged and the lysine at the N-terminal has a charge of +2 on pH 4.2.

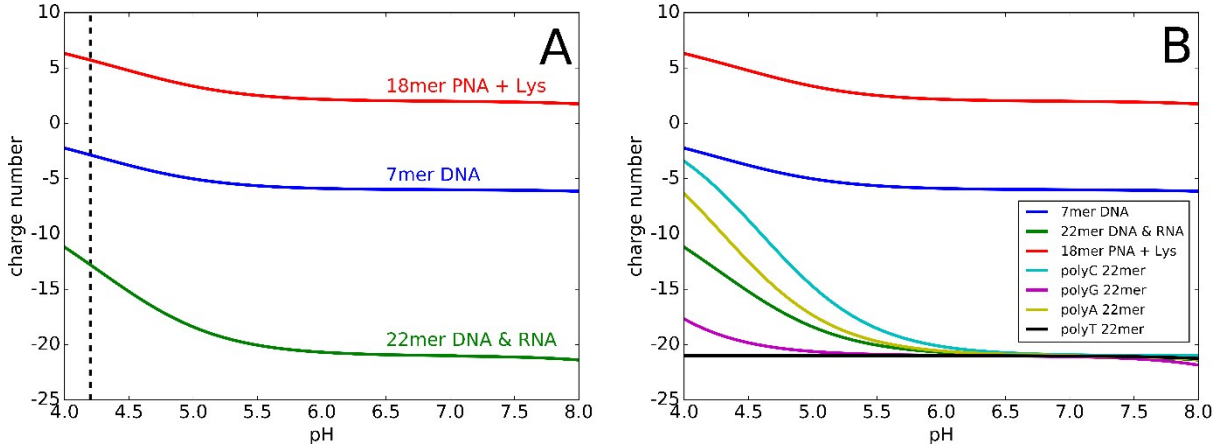

Figure S2. pH dependent net charge of nucleic acids (A) Net charges of the nucleic acids used in this study (the vertical dashed line indicates the values at pH 4.2). (B) Extended comparison of the pH dependent charge number of nucleic acids used in this study and 22-mer A, T, G, and C homooligomers.

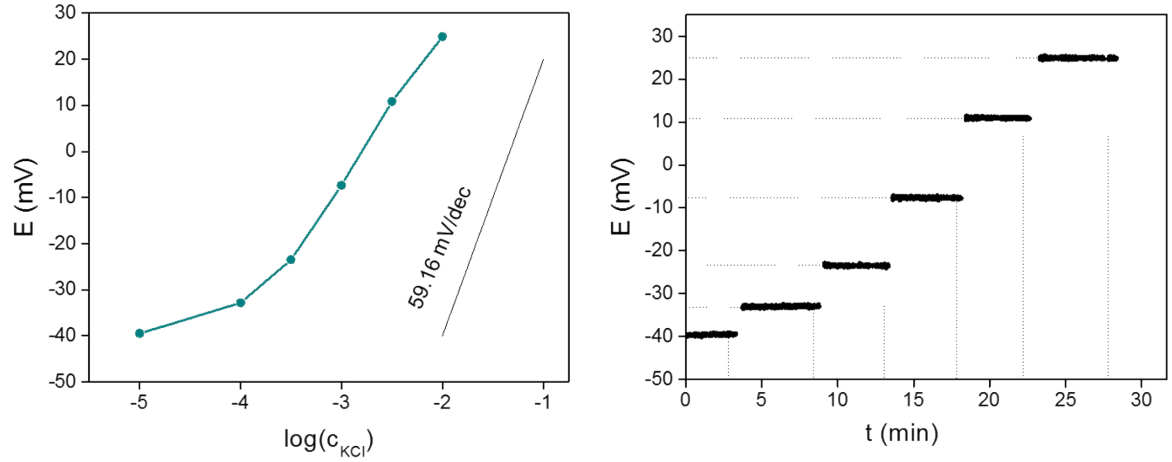

Figure S3. Typical potential responses for KCl of the PNA-functionalized 26.5 nm diameter nanopore array upon NA hybridization (A) and the respective potential-time traces. The deviation from the linearity at low KCL concentration is due to the lack of selectivity (H^+-interference), while at higher

concentrations due to breakdown of the permselectivity (the high ionic strength decreases the Debye length).

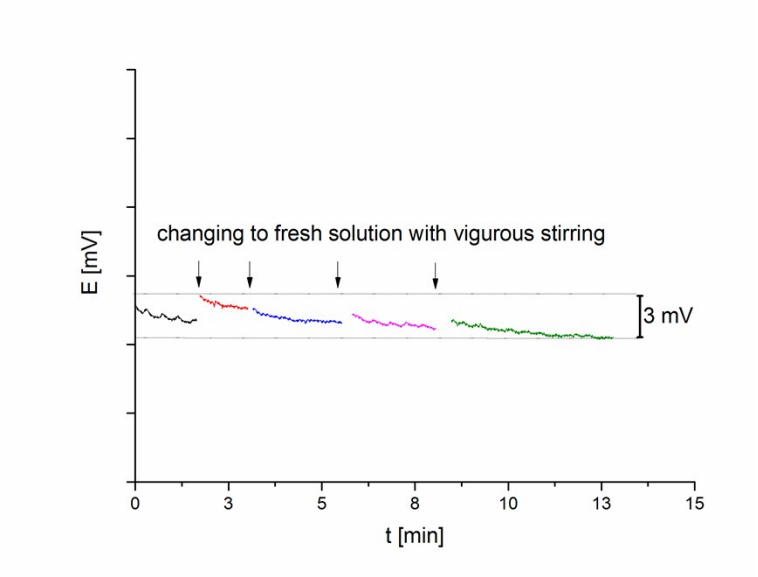

Figure S4. Potential stability upon 5 consecutive washing steps with KCl solution of a PNA-modified nanopore array comprising 30 nm diameter pores after incubation with 5 μM NA. For each washing step 10 mL fresh solution was used under vigorous stirring and the potential measured.

References

S1. *CRC Handbook of Chemistry and Physics, 90th Edition*, CRC Press, Boca Raton, FL., 2009.

S2. V. Verdolino, R. Cammi, B. H. Munk and H. B. Schlegel, *The Journal of Physical Chemistry B*, 2008, **112**, 16860-16873.