Supplemental Data for manuscript entitled, "Microfluidic Means of Achieving Attomolar Detection Limits with Molecular Beacon Probes", Article Ref.: **B819605B**.

Time Course Measurements of DNA Adsorption to the PDMS Device

Fluorescence correlation spectroscopy (FCS) was used to monitor the concentration of small Cy5-labelled Oligonucleotides (5'-Cy5-CTCCATCGAGATTTCA-3') within the closed ~4 nL detection chamber. FCS measurements were carried out with the same custom-built, confocal fluorescence spectroscope described above. The output electronic signal was fed into a correlator (ALV-5000/EPP, ALV-GmbH) for computing autocorrelation functions. Analysis of the autocorrelation curve was carried out with a least squares fit based on the Levenberg-Marquardt algorithm within Origin 7.0 (OriginLab) and the following analytical model,[S1]

Equation S.2.3: $$G(\tau) = \frac{1}{N_p(1+\frac{4D\tau}{\varpi_1^2})(1+\frac{4D\tau}{\varpi_2^2})^{1/2}}$$

where N_p is the average number of fluorophores diffusing in the optical probe volume, τ is lag time, D is the diffusion coefficient of the fluorescent molecule, and G is the autocorrelation function arising from fluorescence fluctuations due to translational diffusion. The average number of moleulces within the optical focal volume (N_p), as measured by FCS, for 5 nM oligonucleotide samples are shown in Figure S1 over a 3 hour time course within the 4 nL rotary chamber. The figure shows no measureable decease in concentration over that time, showing additional evidence that adsorption of the small oligonucleotides to PDMS remains negligible over long time periods. Autocorrelation curves were collected over 60 second periods and then the laser shutter was shut to avoid significant photobleaching of the samples.

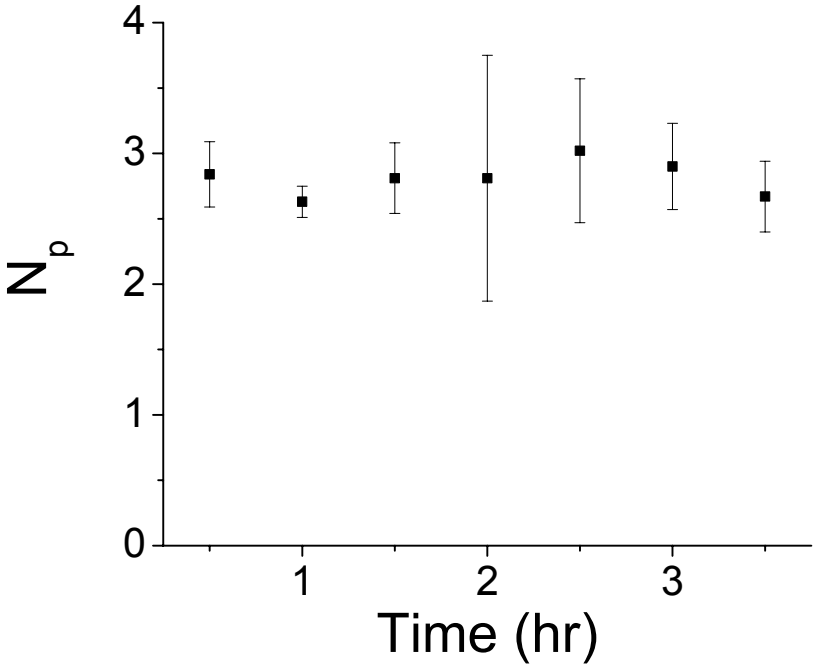

Fig. S1 Average number of oligonucleotides within the optical focal volume over time for a 5 nM sample, as measured by FCS. Samples were loaded into the ~4 nL rotary chamber, closed off, and then monitored for over three hours. Time points are the mean of three separate measurements ± standard error.

While FCS provides an efficient way to monitor oligonucleotide concentrations over long time periods, these measurements are limited to nM concentrations. Therefore, figure S1 does not show a direct comparison to the experiments in this report, which dealt with extremely dilute solutions. Figure S2 is meant to show a more

direct comparison, where 1 pM concentrations of the same Cy5-labelled oligonucleotide were loaded into the 1 meter long evaporation coil and fluorescent peak counts were monitored both at the beginning (inlet) and end (1 meter point) of the microchannel. In this case, molecules travel the same distance through the microchannel as in the evaporation experiments within this report, and DNA adsorption should be detectable as an appreciable drop in fluorescent peak counts from the inlet to the 1 meter point. The 1 meter point measurements were taken just after the loaded sample front passed, minimizing any effects of side-wall passivation. Then, the inlet measurements were taken for comparison. Clearly, figure S2 shows no drop in fluorescent burst counts across the 1 meter channels, showing further evidence that DNA adsorption remains negligible during experiments.

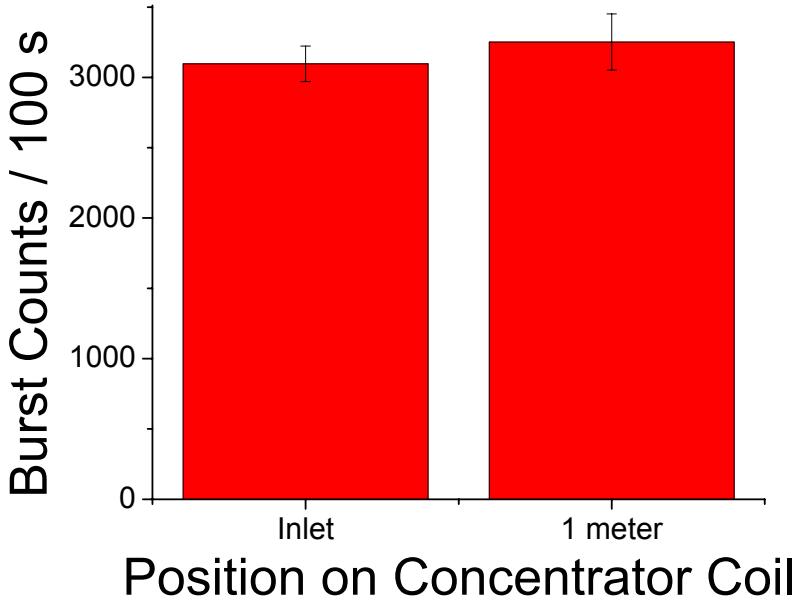

Fig. S2 Fluorescent burst measured at the inlet and end of the 1 meter evaporation coil during constant flow rate 1 pM oligonucleotide samples. Measurements are the mean of 3 separate measurements ±standard error.

Supplemental Section References

S1. M. Gosch, H. Blom, J. Holm, T. Heino and R. Rigler, *Anal. Chem.,* 2000, **72**, 3260-3265.