Supplementary Information

Amplification-Free, Sequence-Specific 16S rRNA Detection at 1 aM

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Calculations used to estimated the average number of target 16S rRNA molecules hybridized per PNA-bead conjugate and the distribution of hybridized RNA molecules per bead are presented below.

Estimation of nucleic acids (NAs) hybridized per bead

Based on published studies of equilibrium binding between NA and tethered PNA of similar length to that used here, the dissociation constant, K_D, was estimated to be ~10^{-9} M.[1, 2] This dissociation constant can be used to estimate the concentration of NA-PNA probe complex, x, that results from the hybridization of NA at an initial concentration, $[NA]_0$, with probe at an initial concentration, $[Probe]_0$,

$$K_D = \frac{[NA][Probe]}{x}$$

$$K_D = \frac{([NA]_0 - x)([Probe]_0 - x)}{x}$$

where $[NA]$ is the concentration of free nucleic acid at equilibrium, and $[Probe]$ is the concentration of unbound PNA probe at equilibrium. Once x is determined, it can be used to estimate the order-of-magnitude average number of NA molecules bound per PNA-bead conjugate. The calculation requires input of the average bead diameter, 820 nm; an order of magnitude estimate of PNA surface concentration on the beads, 10^{14}/cm^2 (see text); and a bead number concentration corresponding to a suspension of 1.26×10^6 beads in 600 μL (see text).

NA Concentration	Average NA per bead
10 fM	1
100 aM	0.01
1 aM	0.0001

Table S1. Calculated order-of-magnitude average number of nucleic acids hybridized per PNA-bead conjugate at equilibrium.

Table S1 shows that below 10 fM, the average number of hybridized nucleic acids per PNA-bead conjugate drops below one. In the attomolar range of interest, the average number of NAs per bead drops orders of magnitude below one indicating that the vast majority of beads do not have any bound nucleic acid.

Estimation of the distribution of NAs per bead using Poisson statistics

Poisson statistics may be used to estimate the probability of finding a certain number of hybridized target nucleic acids per PNA-bead conjugate. The overall defining equation may be expressed as

$$P_\mu(v) = e^{-\mu}\frac{\mu^v}{v!}$$

where μ is the average number of nucleic acid molecules per bead, v is the actual number of NAs on an individual bead, and $P_\mu(v)$ is the probability of finding a bead with v NAs hybridized. Using the dissociation constant calculations in the previous section and given a 1 aM solution of nucleic acid, 10^{-4} molecules are bound per bead on average (μ). Therefore, the probability of finding one NA on a bead is 0.0001 and the probability of finding two NAs on a bead is 5×10^{-9}. Since 1.26×10^6 beads were used in the 600 µl hybridization volume and 80 µl of the bead solution was injected, there were ~10 beads with one NA hybridized and essentially no beads with two NA for the experimental runs at 1 aM. Therefore, it appears that one bound NA per bead conjugate was sufficient to cause pore blockage at an applied potential of 1.5 V.

References

1. K. K. Jensen, H. Orum, P. E. Nielsen and B. Norden, *Biochemistry-Us*, 1997, **36**, 5072-5077.
2. A. De, S. Souchelnytskyi, A. van den Berg and E. T. Carlen, *Acs Appl Mater Inter*, 2013, **5**, 4607-4612.