Supporting Information

Sequence-specific DNA detection at 10 fM by electromechanical signal transduction

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A. Zeta potential and mobility measurements

The zeta potential and electrophoretic mobility of the beads before PNA conjugation (-87 mV and -6.9x10^{-8} m^2/Vs, respectively), after PNA conjugation (-2.4 mV and -0.2x10^{-8} m^2/Vs, respectively) were measured with a Malvern Zetasizer.

Table S-1. After incubation with various DNA lengths and concentrations the zeta potential and electrophoretic mobility were measured and are listed below. In the instances where the instrument identified more than one maximum in the distribution of measurements, the values for each peak and the percentage of total area of that peak are listed.

	Target			Control		
[DNA]	Length	Zeta Potential (mV) and % area of peak	Mobility (10^{-8} m^2/Vs)	Length	Zeta Potential (mV) and % area of peak	Mobility (10^{-8} m^2/Vs)
1 pM	110	-34.6 / 97.5% 6.99 / 2.5%	-2.71 0.54	125	-8.58 / 100%	-0.67
	235	-41.6 / 98.6% -0.4 / 1.4%	-3.28 -0.03	184	-32.8 / 31.6% -4.61 / 68.4%	-2.53 -0.36
	419	-43.6 / 100%	-3.41	309	-29.6 / 80.8% 4.65 / 19.2%	-2.29 0.36
	1613	-66.6 / 2.5% -35.1 / 59.0% -3.31 / 38.5%	-5.22 -2.76 -0.26	1503	-34.8 / 27.3% -2.63 / 72.7%	-2.72 -0.21
100 fM	110	-63 / 1.4% -37.7 / 98.6%	-4.94 -2.96	125	-37.7 / 100%	-2.96
	235	-46.3 / 89.2% -5.89 / 10.8%	-3.60 -0.46	184	-35.7 / 100%	-2.78
	419	-43.6 / 98.9% -13.8 / 1.1%	-3.41 -1.08	309	-32.5 / 97.7% 6.75 / 2.3%	-2.53 0.52
	1613	-41.3 / 98.3% 0.01 / 1.7%	-3.21 0.01	1503	-31.2 / 97.9% 6.91 / 2.1%	-2.44 0.54
10 fM	110	-31.3 / 28.8% -18.1 / 22.1% -5.11 / 49.1%	-2.44 -1.42 -0.40	125	-27.7 / 73.9% -8.38 / 9.2% 2.18 / 16.9%	-2.14 -0.65 0.17
	235	-30.3 / 31% -11.3 / 28.6% 8.93 / 37.3%	-2.36 -0.88 0.69	184	-27.9 / 96.1% 7.7 / 3.9%	-2.15 0.60
	419	-38.6 / 52% -2.81 / 48%	-3.03 -0.22	309	-38.6 / 4.7% -21.3 / 94.7% 10.5 / 0.6%	-3.03 -1.65 0.81
	1613	-43.8 / 68.6% -14.7 / 13.7% 2.84 / 8.9%	-3.42 -1.15 0.22	1503	-44.6 / 17.3% -31.3 / 51% -1.95 / 31.5%	-3.49 -2.44 -0.15
10 fM	110	-45.5 / 100%	-3.55	125	-38.0 / 89.9% -5.8 / 10.1%	-3.00 -0.45
	235	-35.5 / 98.4% -4.35 / 1.6%	-2.77 0.34	184	-31.6 / 100%	-2.46
	419	-41.1 / 100%	-3.20	309	-37.9 / 98.9% -0.024 / 1.1%	-2.97 0
	1613	-44 / 95% -4.73 / 5.0%	-3.44 -0.37	1503	-34.1 / 100%	-2.66

10 fM	110	-34 / 95.3% 1.35 / 3.9%	-2.66 0.11	125	-30.1 / 100%	-2.35
	235	-34.5 / 96.1% 8.55 / 3.9%	-2.70 0.66	184	-36.3 / 97.3% 2.9 / 2.7%	-2.84 0.22
	419	-40.5 / 92.6% -13.3 / 7.4%	-3.16 -1.04	309	-31 / 73.3% 6.44 / 26.7%	-2.42 0.50
	1613	-40.7 / 86.1% -13.7 / 10.6% 0.53 / 4.3%	-3.20 -1.07 0.04	1503	-30.1 / 100%	-2.35
1 fM	110	-35.4 / 0.1% -5 / 99.9%	-2.77 -0.39	125	-0.27 / 100%	-0.02
	235	-26.6 / 27.6% -11 / 47.5% 3.13 / 24.8%	-2.05 -0.86 0.24	184	-14.3 / 49.5% 4.7 / 50.5%	-1.11 0.36
	419	-33.8 / 11.5% -19.7 / 30.9% -2.42 / 57.5%	-2.65 -1.55 -0.19	309	-35 / 16.7% -13.6 / 57.9% 9.71 / 23.6%	-2.75 -1.10 0.75
	1613	-18.1 / 74.8% 5.8 / 25.2%	-1.42 0.45	1503	-35.5 / 25.3% -18.2 / 6.5% 0.418 / 68.2%	-2.77 -1.43 0.03

For the PNA-beads incubated with 10 fM of 1613-mer target DNA and 30 pM of 1503-mer control DNA, the zeta potentials measured were -36.5mV (46.2%) and -22.5mV (53.8%), with corresponding mobilities of -2.86×10^{-8} m^2/Vs and -1.74×10^{-8} m^2/Vs, respectively. Control experiments, in which PNA-beads were incubated only with 30 pM of 1503-mer control DNA, yielded zeta potential of -21.7 mV (100%) and mobility of -1.68×10^{-8} m^2/Vs.

B. Measurement results for Target and Control DNA from 1 pM to 10 fM

I_{open} is the current measured in the capillary with no obstruction, I_{block} is the current measured with an obstructing bead present, $R_{open} = 25\ V/I_{open}$, $\Delta R = R_{open} - V/I_{block}$. A Permanent block is noted if the block persisted beyond 60 sec, and was reversible when the sign of the applied voltage was reversed.

1 pM Target

110-mer Target

No capillary blockades observed.

Table S-2. 235-mer Target

	I_{open} (µA)	I_{block} (µA)	$(I_o - I_b)/I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.518	-0.436	0.158	4.83E+07	9.10E+06	0.200	Permanent
	-0.527	-0.462	0.123	4.74E+07	6.70E+06	0.141	Permanent
	-0.559	-0.488	0.127	4.47E+07	6.51E+06	0.145	Permanent
	-0.577	-0.495	0.142	4.33E+07	7.20E+06	0.200	Permanent
	-0.585	-0.522	0.108	4.27E+07	5.20E+06	0.121	Permanent
Average	-0.553	-0.481	0.132	4.53E+07	6.91E+06	0.152	
Std.Dev.	0.027	0.029	0.017	2.20E+06	1.26E+06	0.022	

Table S-3. 419-mer Target

	I_{open} (µA)	I_{block} (µA)	$(I_o - I_b)/I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.617	-0.546	0.115	4.11E+07	5.30E+06	0.130	Permanent
	-0.612	-0.512	0.163	4.20E+07	7.98E+06	0.200	Permanent
	-0.609	-0.516	0.153	4.11E+07	7.40E+06	0.180	Permanent
	-0.62	-0.529	0.147	4.03E+07	6.94E+06	0.172	Permanent
Average	-0.615	-0.526	0.144	4.20E+07	6.90E+06	0.169	
Std.Dev.	0.004	0.013	0.018	2.83E+05	1.01E+06	0.024	

Table S-4. 1613-mer Target

	I_{open} (µA)	I_{block} (µA)	$(I_o - I_b)/I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.576	-0.512	0.111	4.34E+07	5.43E+06	0.125	Permanent
	-0.53	-0.450	0.151	4.72E+07	8.39E+06	0.178	Permanent
	-0.524	-0.447	0.147	4.77E+07	8.22E+06	0.172	Permanent
	-0.523	-0.439	0.161	4.78E+07	9.15E+06	0.191	Permanent
Average	-0.538	-0.462	0.142	4.65E+07	7.79E+06	0.167	
Std.Dev.	0.022	0.029	0.019	1.82E+06	1.41E+06	0.025	

1 pM Control

125-mer Control

No capillary blockades detected.

Table S-5. 184-mer control

I_{open} (μA)	I_{block} (μA)	$(I_o - I_b)/ I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
-0.535	-0.458	0.158	4.8E+07	9.10E+06	0.20	7
-0.543	-0.421	0.123	4.7E+07	6.70E+06	0.141	5

419-mer Control

No capillary blockades detected.

Table S-6. 1503-mer Control

	I_{open} (μA)	I_{block} (μA)	$(I_o - I_b)/ I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.608	-0.406	0.332	4.11E+07	2.05E+07	0.498	Permanent
	-0.624	-0.575	0.079	4.01E+07	3.41E+06	0.085	29
	-0.636	-0.523	0.177	3.93E+07	8.49E+06	0.216	16
Average	-0.623	-0.501	0.196	4.02E+07	1.08E+07	0.266	
Std.Dev.	0.011	0.071	0.104	7.42E+05	7.14E+06	0.172	

100 fM Target

Table S-7. 110-mer Target

I_{open} (μA)	I_{block} (μA)	$(I_o - I_b)/ I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
-0.610	-0.475	0.222	4.09E+07	1.20E+07	0.286	4
-0.615	-0.550	0.105	4.07E+07	4.78E+06	0.117	16

Table S-8. 235-mer Target

	I_{open} (μA)	I_{block} (μA)	$(I_o - I_b)/ I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.574	-0.485	0.155	4.36E+07	7.99E+06	0.184	Permanent
	-0.552	-0.479	0.132	4.53E+07	6.90E+06	0.152	Permanent
	-0.583	-0.500	0.143	4.29E+07	7.15E+06	0.167	Permanent
Average	-0.570	-0.488	0.143	4.39E+07	7.35E+06	0.168	
Std.Dev.	0.013	0.009	0.009	1.02E+06	4.67E+05	0.013	

Table S-9. 419-mer Target

	I_{open} (μA)	I_{block} (μA)	$(I_o - I_b)/I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.572	-0.353	0.383	4.37E+07	2.71E+07	0.621	Permanent
	-0.574	-0.358	0.376	4.36E+07	2.63E+07	0.603	Permanent
	-0.576	-0.485	0.158	4.34E+07	8.14E+06	0.188	Permanent
	-0.571	-0.356	0.377	4.38E+07	2.64E+07	0.604	Permanent
	-0.581	-0.4191	0.279	4.303E+07	1.66E+07	0.386	Permanent
Average	-0.575	-0.394	0.315	4.35E+07	2.09E+07	0.480	
Std.Dev.	0.003	0.052	0.087	2.65E+05	7.48E+06	0.170	

Table S-10. 1613-mer Target

	I_{open} (μA)	I_{block} (μA)	$(I_o - I_b)/I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.676	-0.600	0.112	3.70E+07	4.67E+06	0.126	Permanent
	-0.671	-0.599	0.107	3.73E+07	4.45E+06	0.119	Permanent
	-0.639	-0.571	0.106	3.91E+07	4.66E+06	0.119	Permanent
	-0.636	-0.535	0.159	3.93E+07	7.43E+06	0.189	Permanent
	-0.613	-0.488	0.203	4.08E+07	1.04E+07	0.255	Permanent
Average	-0.647	-0.559	0.137	3.87E+07	6.32E+06	0.162	
Std.Dev.	0.023	0.043	0.038	1.41E+06	2.32E+06	0.054	

100 fM Control

No capillary blockades detected for the 125-mer, 184-mer, 309-mer, and 1503-mer control DNA.

10 fM Target

Experiment 1

110-mer and 235-mer target

No capillary blockades detected.

Table S-11. 419-mer Target

	I_{open} (μA)	I_{block} (μA)	$(I_o - I_b)/I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.597	-0.478	0.199	4.19E+07	1.04E+07	0.249	2
	-0.574	-0.486	0.153	4.36E+07	7.89E+07	0.181	2
	-0.583	-0.465	0.202	4.29E+07	1.09E+07	0.254	Permanent
	-0.579	-0.475	0.180	4.32E+07	9.45E+06	0.219	Irreversible
Average	-0.583	-0.476	0.184	4.29E+07	9.66E+06	0.226	
Std.Dev.	0.009	0.008	0.020	6.23E+05	1.147E+06	0.029	

Table S-12. 1613-mer Target

	I_{open} (μA)	I_{block} (μA)	$(I_o - I_b)/I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.638	-0.542	0.150	3.92E+07	6.93E+06	0.177	Permanent
	-0.654	-0.518	0.208	3.82E+07	1.00E+07	0.263	Permanent
	-0.603	-0.527	0.126	4.15E+07	5.98E+06	0.144	Permanent
	-0.602	-0.525	0.128	4.15E+07	6.09E+06	0.147	Permanent
	-0.598	-0.524	0.124	4.18E+07	5.90E+06	0.141	Permanent
Average	-0.619	-0.527	0.147	4.04E+07	6.99E+06	0.174	
Std.Dev.	0.023	0.008	0.032	1.45E+06	1.57E+06	0.046	

Experiment 2

Table S-13. 110-mer Target

	I_{open} (μA)	I_{block} (μA)	$(I_o - I_b)/I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.514	-0.446	0.132	4.86E+07	7.42E+06	0.152	43
	-0.531	-0.451	0.151	4.71E+07	8.35E+06	0.177	Permanent
	-0.543	-0.422	0.223	4.60E+07	1.32E+07	0.287	Permanent
Average	-0.529	-0.440	0.169	4.73E+07	9.66E+06	0.206	
Std.Dev.	0.012	0.013	0.039	1.07E+06	2.54E+06	0.058	

235-mer and 419-mer Target

No capillary blockades detected.

Table S-14. 1613-mer Target

	I_{open} (μA)	I_{block} (μA)	$(I_o - I_b)/I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.622	-0.548	0.119	4.02E+07	5.43E+06	0.135	Permanent
	-0.614	-0.454	0.261	4.07E+07	1.43E+07	0.352	Permanent
	-0.628	-0.457	0.272	3.98E+07	1.49E+07	0.374	Permanent
	-0.6143	-0.463	0.246	4.07E+07	1.33E+07	0.326	Permanent
	-0.597	-0.454	0.240	4.19E+07	1.32E+07	0.315	Permanent
Average	-0.615	-0.475	0.228	4.07E+07	1.22E+07	0.301	
Std.Dev.	0.010	0.037	0.055	6.97E+05	3.46E+06	0.085	

Experiment 3

110-mer and 235-mer Target

No capillary blockades detected.

Table S-15. 419-mer Target

	I_{open} (μA)	I_{block} (μA)	$(I_o - I_b)/ I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.64	-0.532	0.169	3.91E+07	7.93E+06	0.203	Permanent
	-0.628	-0.536	0.146	3.98E+07	6.83E+06	0.172	Permanent
	-0.614	-0.530	0.136	4.07E+07	6.43E+06	0.158	Permanent
Average	-0.627	-0.533	0.151	3.99E+07	7.07E+06	0.178	
Std.Dev.	0.011	0.002	0.013	6.74E+05	6.33E+05	0.019	

Table S-16. 1613-mer Target

	I_{open} (μA)	I_{block} (μA)	$(I_o - I_b)/ I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.626	-0.506	0.192	3.99E+07	9.47E+06	0.237	Permanent
	-0.632	-0.544	0.139	3.96E+07	6.40E+06	0.162	Permanent
	-0.629	-0.534	0.151	3.97E+07	7.07E+06	0.178	Permanent
	-0.643	-0.547	0.149	3.89E+07	6.82E+06	0.176	Permanent
Average	-0.633	-0.533	0.158	3.95E+07	7.44E+06	0.188	
Std.Dev.	0.006	0.016	0.020	3.98E+05	1.2E+06	0.029	

10 fM Control

Experiment 1

125-mer, 184-mer, and 309-mer control

No capillary blockades detected.

Table S-17. 1503-mer Control

	I_{open} (μA)	I_{block} (μA)	$(I_o - I_b)/ I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.560	-0.484	0.135	4.47E+07	6.98E+06	0.156	20
	-0.592	-0.425	0.283	4.22E+07	1.66E+07	0.394	37
	-0.587	-0.477	0.188	4.26E+07	9.83E+06	0.231	21
Average	-0.580	-0.462	0.202	4.31E+07	1.11E+07	0.260	
Std.Dev.	0.014	0.026	0.061	1.08E+06	4.05E+06	0.099	

Experiment 2

Table S-18. 125-mer Control

I_{open} (μA)	I_{block} (μA)	$(I_o - I_b)/ I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
-0.557	-0.465	0.165	4.48E+07	8.96E+06	0.201	2

184-mer and 309-mer Control

No capillary blockades detected.

Table S-19. 1503-mer Control

	I_{open} (µA)	I_{block} (µA)	$(I_o - I_b)/ I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.614	-0.497	0.191	4.07E+07	9.60E+06	0.236	14
	-0.622	-0.406	0.347	4.02E+07	2.14E+07	0.532	2
	-0.589	-0.487	0.173	4.24E+07	8.89E+06	0.209	10
Average	-0.608	-0.463	0.237	4.11E+07	1.33E+07	0.326	
Std.Dev.	0.014	0.041	0.078	9.64E+05	5.73E+06	0.146	

Experiment 3

125-mer Control

No capillary blockades detected.

Table S-20. 184-mer Control

I_{open} (µA)	I_{block} (µA)	$(I_o - I_b)/ I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
-0.552	-0.483	0.123	4.53E+07	6.46E+06	0.143	2

309-mer Control

No capillary blockades detected.

Table S-21. 1503mer Control, Ex.3

I_{open} (µA)	I_{block} (µA)	$(I_o - I_b)/ I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
-0.581	-0.462	0.205	4.30E+07	1.11E+07	0.258	13
-0.572	-0.481	0.159	4.37E+07	8.27E+06	0.189	5

1 fM Target and Control

No permanent or transient blocks observed for any target or control DNA oligomer.

10 fM 1613-mer Target in presence of 30 pM 1503-mer control background

Table S-22.

	I_{open} (µA)	I_{block} (µA)	$(I_o - I_b)/ I_o$	R_{open} (Ω)	ΔR (Ω)	ΔR/R	Block duration (Sec)
	-0.565	-0.487	0.138	4.42E+07	7.09E+06	0.160	6
	-0.578	-0.464	0.197	4.33E+07	1.06E+07	0.246	4
	-0.596	-0.433	0.273	4.19E+07	1.58E+07	0.376	4
	-0.579	-0.478	0.174	4.32E+07	9.12E+06	0.211	Permanent
	-0.567	-0.440	0.224	4.41E+07	1.28E+07	0.289	Permanent
	-0.619	-0.475	0.233	4.04E+07	1.22E+07	0.303	3
	-0.623	-0.481	0.228	4.01E+07	1.19E+07	0.295	3
	-0.596	-0.481	0.193	4.19E+07	1.00E+07	0.239	8
	-0.589	-0.493	0.162	4.24E+07	8.23E+06	0.194	Permanent
	-0.587	-0.375	0.361	4.27E+07	2.41E+07	0.566	Permanent
	-0.614	-0.464	0.244	4.07E+07	1.32E+07	0.323	Permanent
	-0.564	-0.413	0.268	4.43E+07	1.62E+07	0.366	Permanent
	-0.625	-0.403	0.355	4.00E+07	2.20E+07	0.551	Permanent
	-0.625	-0.532	0.149	4.00E+07	6.99E+06	0.175	14
	-0.598	-0.484	0.191	4.18E+07	9.85E+06	0.236	7
	-0.597	-0.464	0.223	4.19E+07	1.20E+07	0.287	Permanent
	-0.634	-0.458	0.278	3.94E+07	1.52E+07	0.384	Permanent
	-0.581	-0.486	0.164	4.30E+07	8.41E+06	0.195	Permanent
	-0.592	-0.415	0.299	4.22E+07	1.80E+07	0.427	Permanent
Average	-0.596	-0.459	0.229	4.02E+07	1.28E+07	0.307	
Std.Dev.	0.021	0.040	0.063	1.48E+06	4.64E+06	0.113	

No permanent or transient blocks observed for 30pM 1503-mer control DNA measured alone.

C. Poisson distribution estimating amount of beads bound to different DNA counts for different DNA concentration and total bead count

Table S-23. Poisson distribution calculation for 10 fM DNA concentration (2.1x10^6 beads).

DNA concentration	Number of target DNA	Number of beads	Average # of target DNA per bead (μ)	# of bound DNA (ν)	Percent of beads bound to ν target DNA	#of beads with ν bound DNA
10fM	6.02x10^5	2.1x10^6	0.287	0	75.07619%	1576600
				1	21.52184%	451958
				2	3.084797%	64780
				3	0.29477%	6190
				4	0.021125%	443
				5	0.00121%	25
				6	0.00006%	1

Table S-24. Poisson distribution calculation for 1 fM DNA concentration (2.1x10^6 beads).

DNA concentration	Number of target DNA	Number of beads	Average # of target DNA per bead (μ)	# of bound DNA (ν)	Percent of beads bound to ν target DNA	#of beads with ν bound DNA
1fM	6.02x10^4	2.1x10^6	0.0287	0	97.17403%	2040654
				1	2.78566%	58498
				2	0.03993%	838
				3	0.00038%	8

Table S-25. Poisson distribution calculation for 1 fM DNA concentration (2.1x10^5 beads).

DNA concentration	Number of target DNA	Number of beads	Average # of target DNA per bead (μ)	# of bound DNA (ν)	Percent of beads bound to ν target DNA	#of beads with ν bound DNA
1fM	6.02x10^4	2.1x10^5	0.287	0	75.07619%	157660
				1	21.52184%	45195
				2	3.08479%	6478
				3	0.29477%	619
				4	0.02113%	44
				5	0.00121%	2

Table S-26. Poisson distribution calculation for 1 fM DNA concentration (2.1x10^4 beads).

DNA concentration	Number of target	Number of beads	Average # of target DNA	# of bound	Percent of beads bound to ν target	#of beads with ν

	DNA		per bead (μ)	DNA (ν)	DNA	bound DNA
1fM	$6.02x10^4$	$2.1x10^4$	2.87	0	5.68882%	1194
				1	16.30796%	3424
				2	23.37475%	4908
				3	22.33587%	4690
				4	16.00737%	3361
				5	9.17759%	1927
				6	4.38483%	920
				7	1.79569%	377
				8	0.643457%	135
				9	0.204953%	43
				10	0.058753%	12
				11	0.015311%	3
				12	0.0036577%	1

D. Calculation of equilibrium DNA-PNA binding

Assuming a first order association between DNA in solution and PNA groups on the bead surfaces

$$PNA + DNA \rightleftarrows PNA \cdot DNA$$

$$K_{eq} = \frac{[PNA \cdot DNA]}{[PNA][DNA]} = \frac{[PNA \cdot DNA]}{([PNA]_{tot} - [PNA \cdot DNA])([DNA]_{tot} - [PNA \cdot DNA])}$$

Defining the DNA bound fraction: $f_{bound} \equiv \frac{[PNA \cdot DNA]}{[DNA]_{tot}}$

$$\frac{1}{K_{eq}} = ([PNA]_{tot} - f_{bound}[DNA]_{tot})\left(\frac{1}{f_{bound}} - 1\right)$$

$$f_{bound} = \frac{[PNA]_{tot} + [DNA]_{tot} + \frac{1}{K_{eq}} \pm \sqrt{\left([PNA]_{tot} + [DNA]_{tot} + \frac{1}{K_{eq}}\right)^2 - 4[PNA]_{tot}[DNA]_{tot}}}{2[DNA]_{tot}}$$

For carboxylic acid modified polystyrene beads, there are ~10^7 carboxylic acid groups on the surface. (Thomson, D.; Dimitrov, K.; Cooper, M. *Analyst* **2011**, 136, 1599-1607.)

Conservatively estimating that only 10% of these groups are functionalized, we have 10^6 PNA molecules per bead. In the experiments described in the manuscript, 2.1x10^6 beads were used in a 100 µL reaction volume, giving a PNA concentration of 35 nM. Using $K_{eq} = 10^9$ M^{-1} (Park et al. Biointerphases 1(4), 113-122 (2006)), which applies to surface-bound PNA and a low concentration of bound DNA, we find 97.2% for the bound fraction for the target DNA concentrations ($[DNA]_{tot}$) tested.

Note that for free 12-mer PNA, the binding constant to DNA is expected to be in the 10^{13}-10^{14} M^{-1} range (Ratilainen et al. Biochemistry, 39, 7781-7791 (2000)). If the value of K_{eq} in our case is closer to this range, the fraction of bound DNA would be substantially higher. Thus, our use of the Poisson distribution to calculate the distribution of target DNA molecules among beads, which assumes that all DNA is bound, likely gives meaningful estimates.