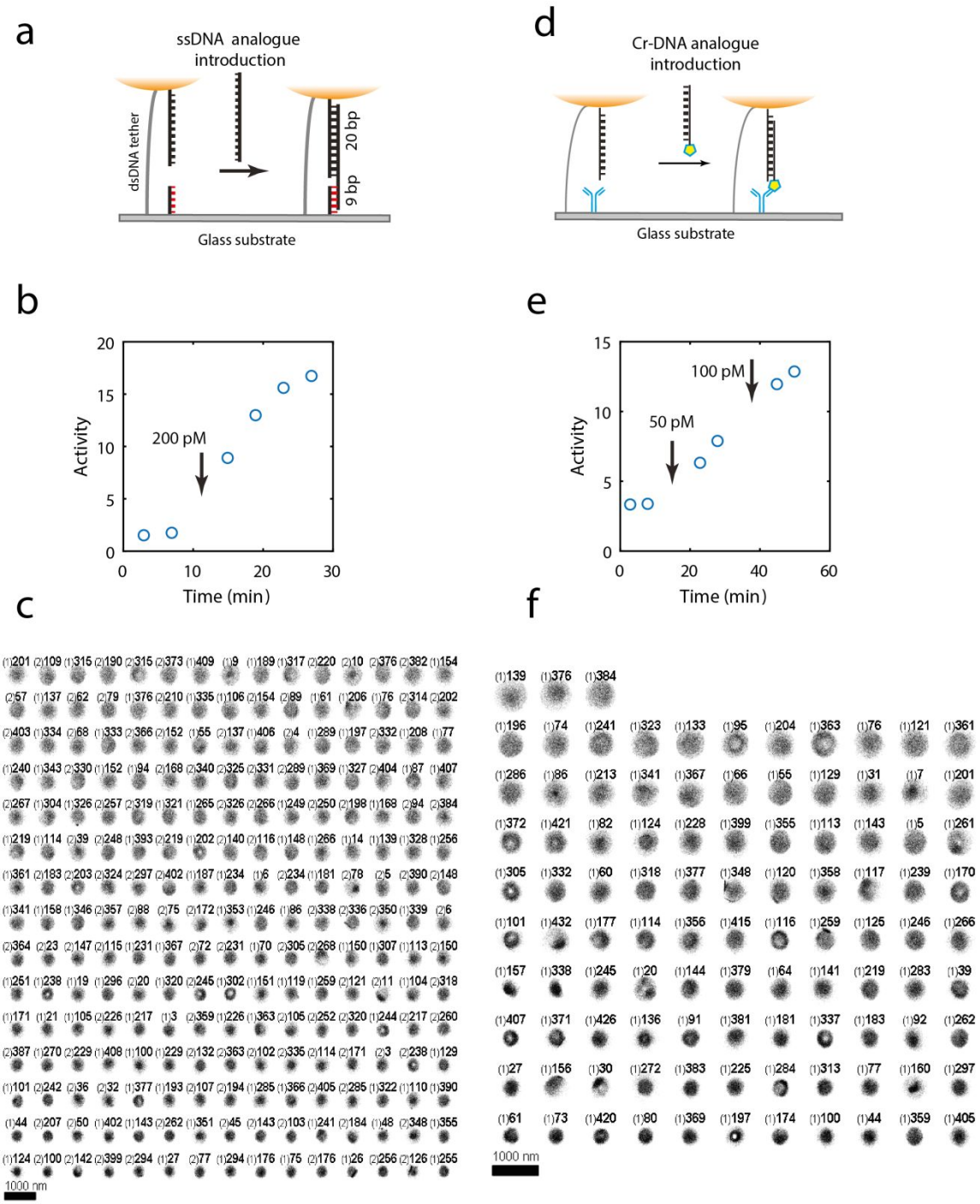
Continuous small-molecule monitoring with a digital single particle switch

Junhong Yan[1,3 *], Laura van Smeden[1,3], Maarten Merkx[1,3], Peter Zijlstra[2,3], Menno Prins[1,2,3,*]

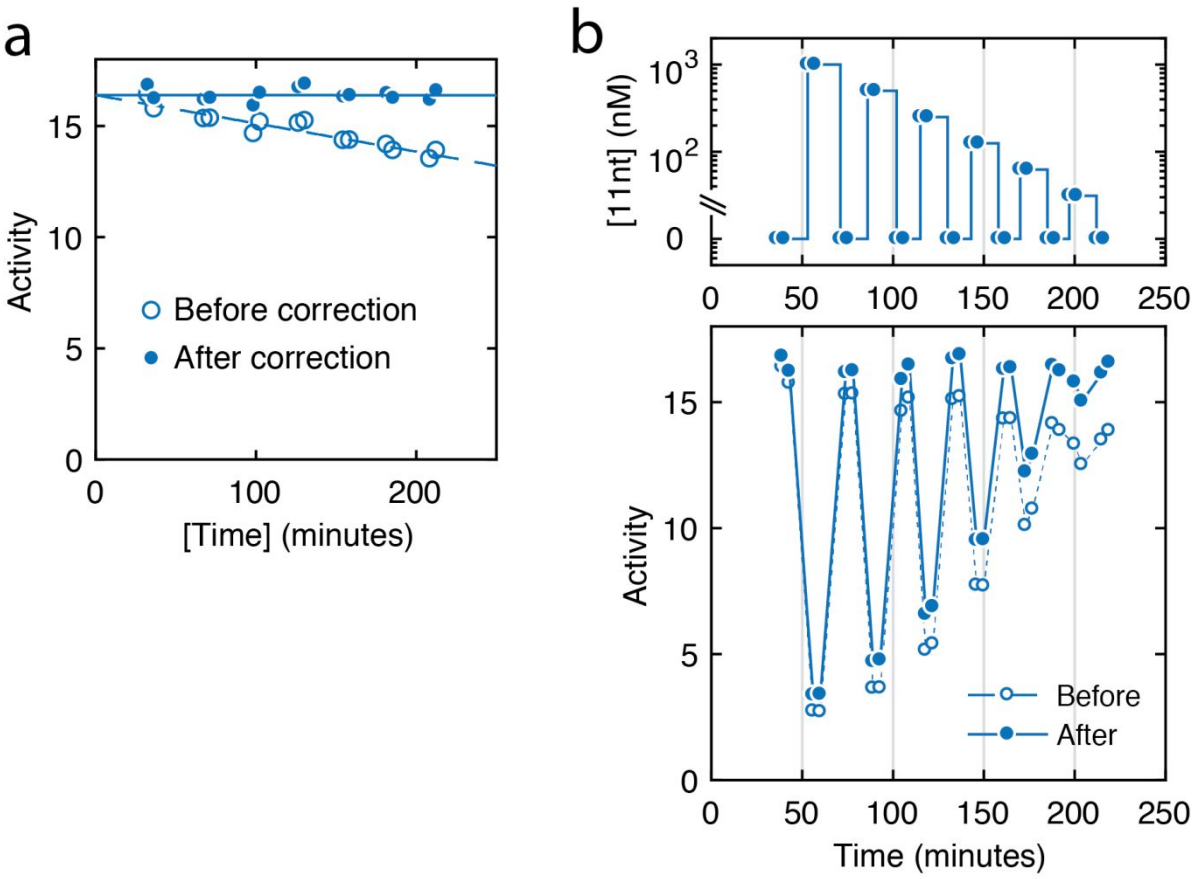
1. Dept. of Biomedical Engineering, Eindhoven University of Technology
2. Dept. of Applied Physics, Eindhoven University of Technology
3. Institute for Complex Molecular Systems (ICMS), Eindhoven University of Technology
*corresponding authors: junhongyan826@gmail.com, m.w.j.prins@tue.nl

Supplementary Information

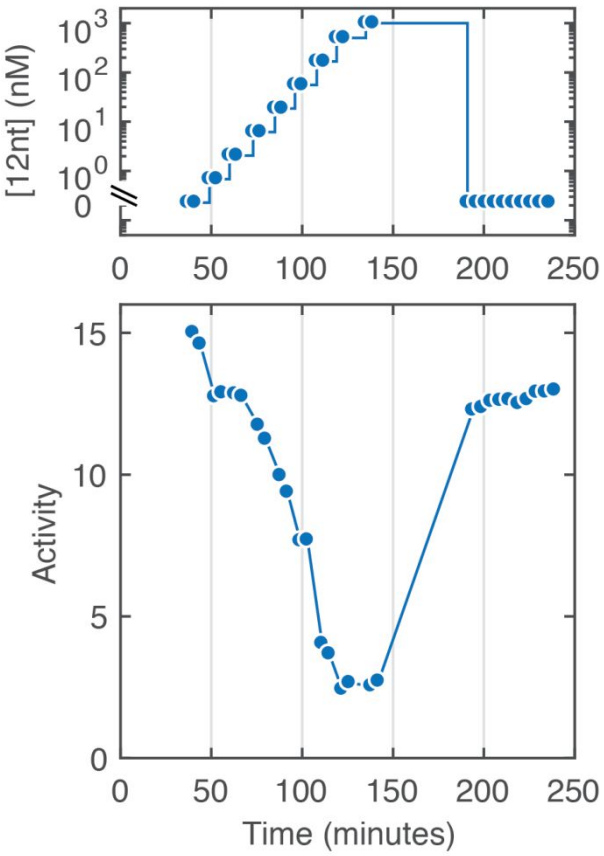
Supplementary Figures and Tables



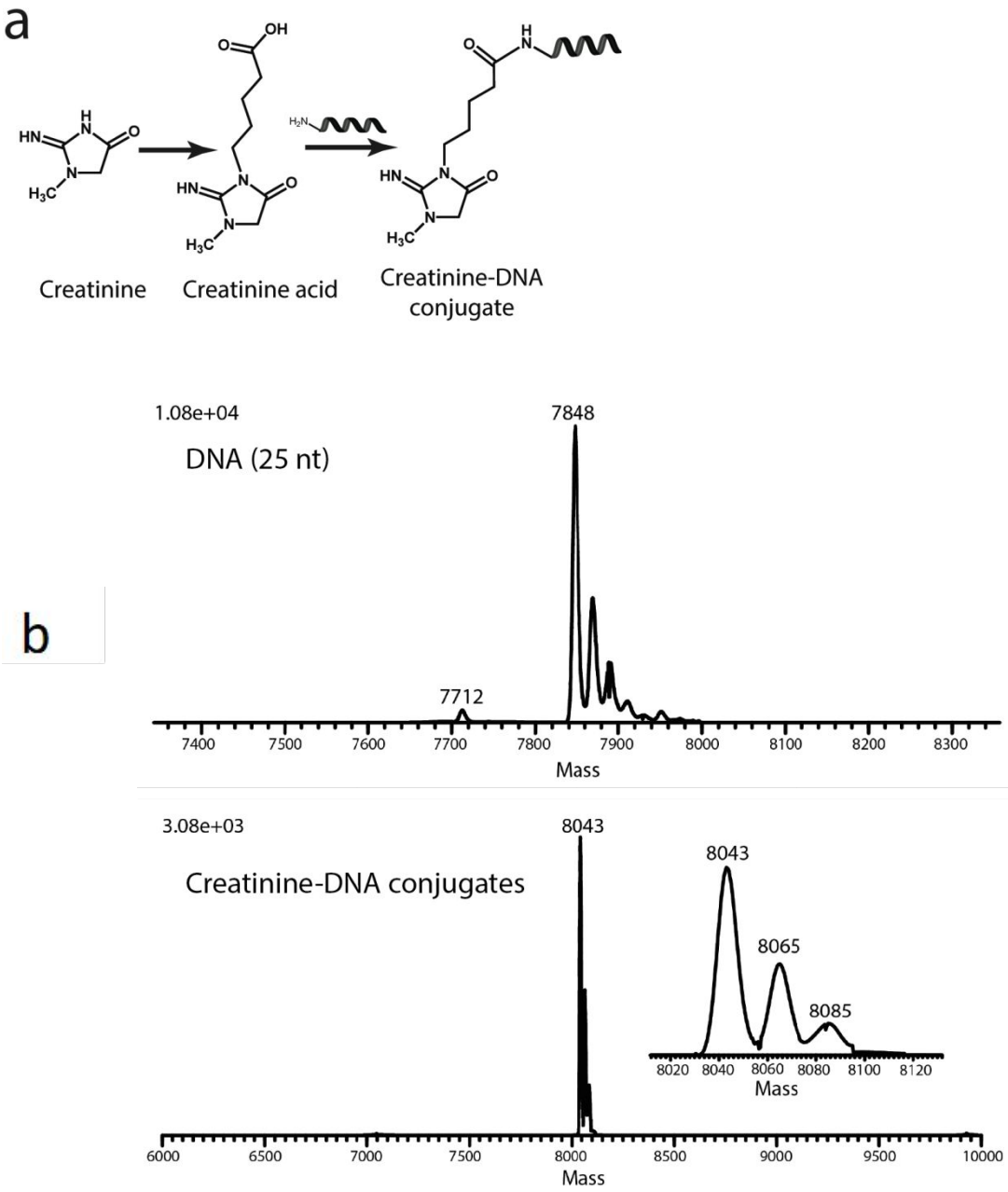
Supplementary Figure S1. Sensor preparations for the BPM competition assays, for measuring short ssDNA (a-c) and creatinine (d-f). (a,d) Schematic illustration of analogue introduction before BPM competition assay. ssDNA-analogue (a) and creatinine-DNA analogue (d) both have a 20-nt region that hybridizes to the capture oligo on the particle. The ssDNA analogue has a 9-nt overhang that can hybridize to the detection oligo on the glass substrate (red). The cr-DNA analogue can bind to the creatinine antibody immobilized on the glass substrate (blue). (b, e) Activity before and after analogue introduction. Black arrow indicates the time when analogue was introduced into the flow cell (200 pM for the ssDNA analogue in b, 50 pM and 100 pM for cr-DNA analogue in e). (c,f) Motion patterns of tethered particles (78% of the total tracked particles) before introduction of analogue.



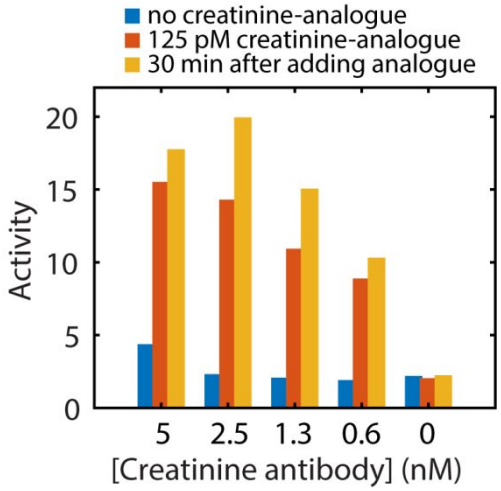
Supplementary Figure S2. Correction of activity drift over time. The 11nt competition assay is shown as an example. (a) The time dependence of the blank activity of the sensor, i.e. when buffer with no analyte was aspirated. The data points were measured in between different concentrations of analyte, in an experiment as in panel b. The data show that the blank activity signal reduces linearly over time (open circles). The underlying process could be a loss of functional biomolecules immobilized on the particle or glass substrate. A linear fit of the activity allows a drift correction of the sensor signal: $A_{corr}(t) = A(t).\frac{A_{blank}(0)}{A_{blank}(t)}$. The corrected data points are depicted as solid blue dots. (b) Corrected activity for continuous measurement of 11-nt over time.



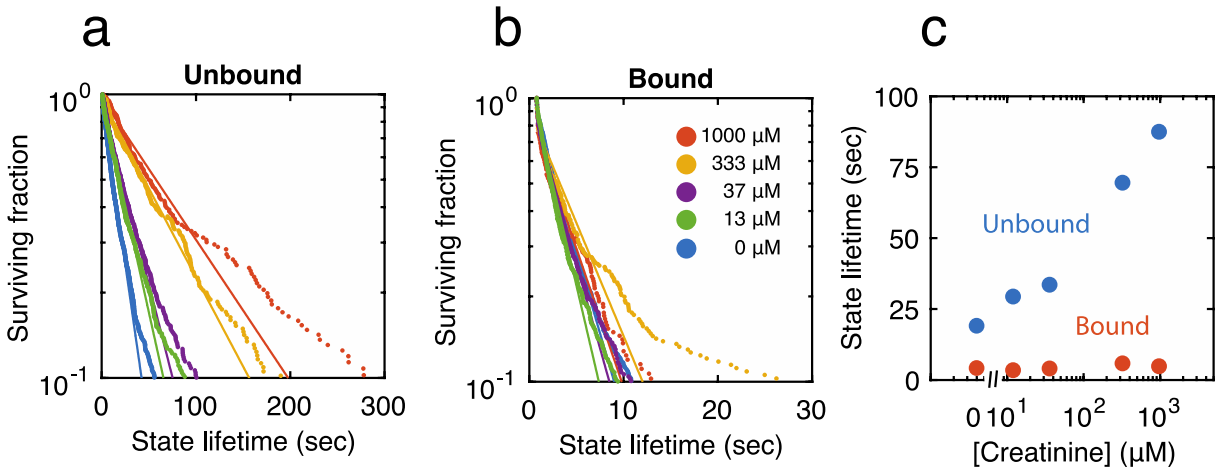
Supplementary Figure S3. Particle switching activity as a function of time with 12-nt oligonucleotide. Top graph: Different concentrations of analyte were aspirated into the flow cell and thereafter buffer without analyte. Bottom graph: Measured activity of the sensor resulting from measurement data of 3 min duration.



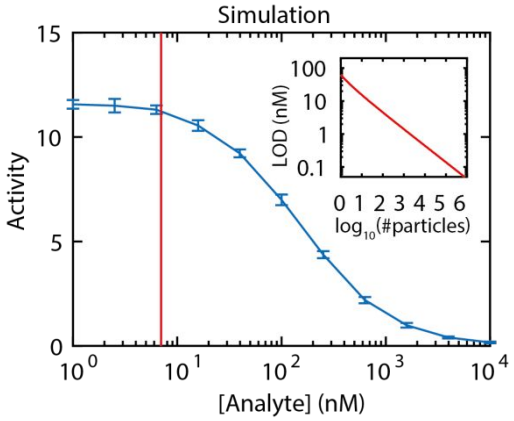
Supplementary Figure S4. (a) Synthesis of creatinine conjugate. Creatinine was converted to creatinine acid and then conjugated with amine-modified DNA via EDC/HOBt/DIPEA chemistry. (b) Deconvoluted m/z spectra obtained with MagTran 1.03 of 25-nt ssDNA (top graph, 7848 Da) and creatinine-DNA (with an addition of 195 Da, compared to the DNA, bottom graph, 8043 Da). Additionally observed peaks are sodium adducts.



Supplementary Figure S5. Titration of creatinine antibody on the glass substrate for the creatinine competition assay. Different amounts of biotinylated anti-creatinine antibody were immobilized on the streptavidin glass substrate. Activity was recorded before adding analogue, after adding 125 pM of creatinine analogue, and 30 min after adding the analogue. Measurement duration was 5 minutes.



Supplementary Figure S6. Survival curves of unbound (a) and bound (b) state lifetimes for different creatinine concentrations in the creatinine competition assay of Fig. 2. Concentrations are indicated in panel b. The curves were fitted with a single exponential lifetime (solid lines). (c) Dependence of the lifetimes of the unbound (blue) and bound (orange) states on analyte concentration.



Supplementary Figure S7. Simulated dose-response curve. Averaged over 100 particles that were each measured for 3 minutes. Error bars are the standard deviation of the number of events obtained from 25 independent simulations. The vertical red line (6 nM) indicates the 3σ limit-of-detection (LOD). The inset shows the simulated LOD versus number of particles in a 3 minute measurement.

Details of the simulation are given in the Materials and Methods section. The fractional occupancy of detection molecules was modelled by a Langmuir isotherm with an equilibrium binding constant that matches the experimental value (i.e. 100 nM in case of the 11-nt ssDNA experiment). We generated 3-minute time traces for a range of analyte concentrations and determined the mean activity averaged over 100 particles, resulting in the dose-response curve above. The red indicates the limit of detection (LOD), determined by the concentration where the signal deviates by three times the standard deviation from the signal at zero concentration.

An expression for the LOD can be derived using the generalized fit equation of the dose-response curve:

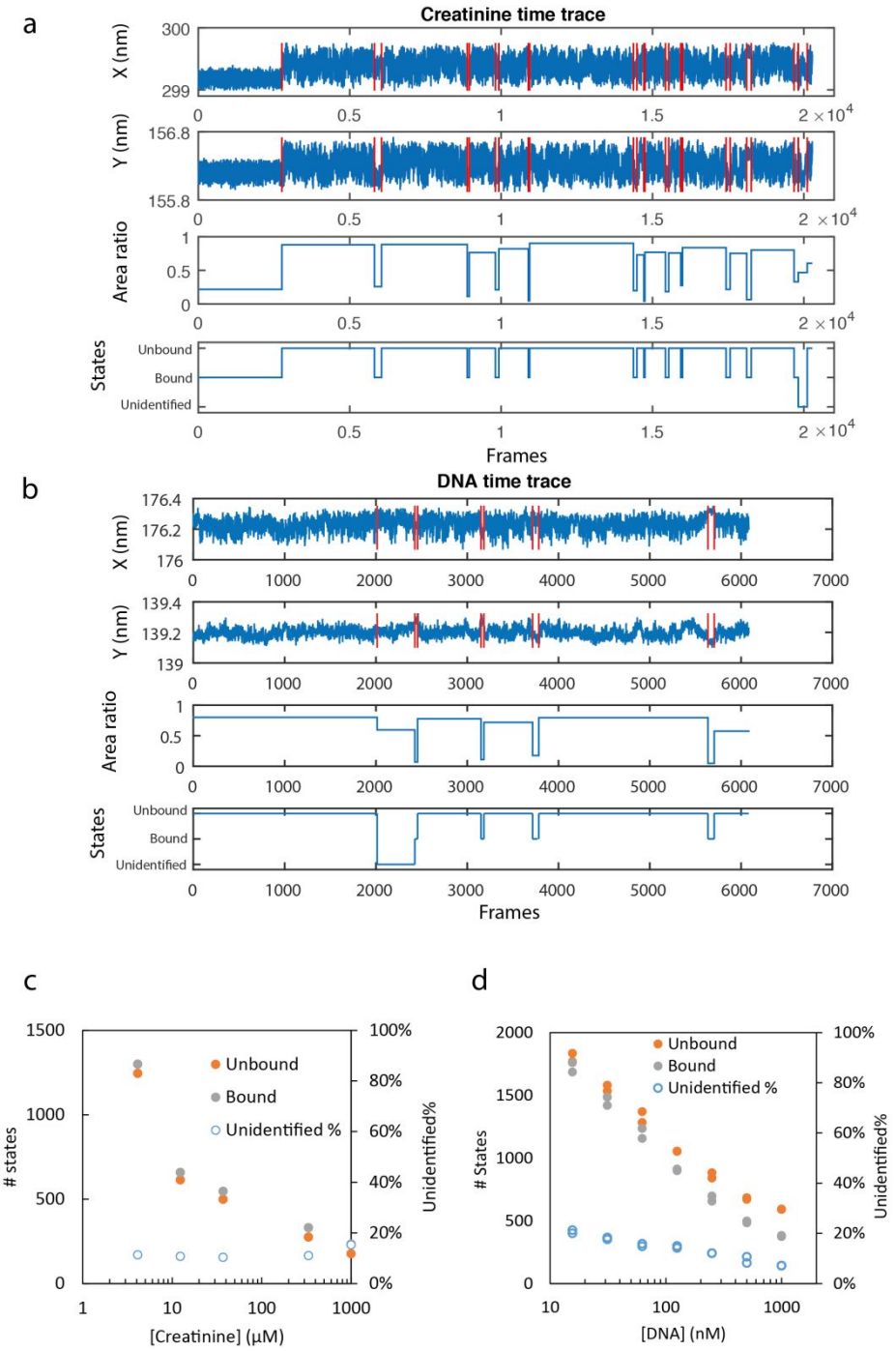
$$A = A_{min} + \frac{A_{max} - A_{min}}{1 + \frac{C}{IC_{50}}}$$

Here, the blank signal is A_{max}, the activity measured at zero analyte concentration, and the standard deviation is the CV times A_{max}. Thus the LOD can be expressed as:

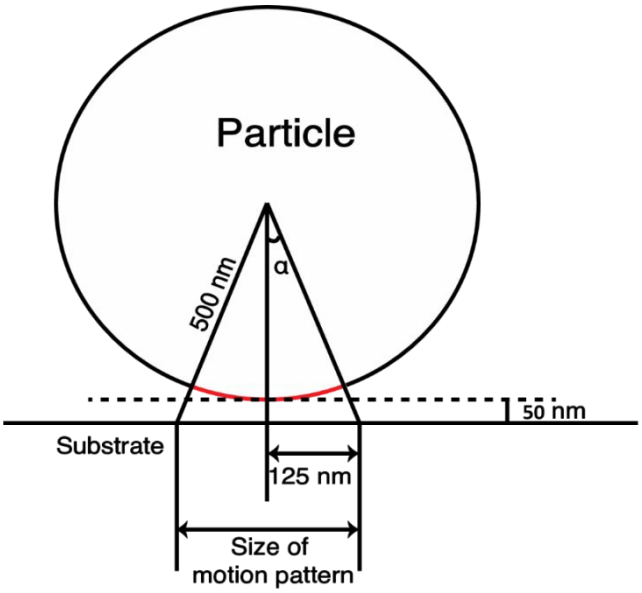
$$LOD = \frac{IC_{50}}{\frac{1}{3 \cdot CV} - 1}$$

where the CV is the coefficient of variation of the blank signal. The CV can be reduced by increasing the number of recorded switching events (see Fig. 4f). This can be done by increasing the density of analogue and detection molecules so that the rate of particle binding to the glass substrate is increased, or by increasing the field of view to monitor more particles.

The inset shows that the LOD can be reduced to well below 1 nM by observing more than 1000 particles. Error bars are the standard deviation of the number of events obtained from 25 independent simulations.



Supplementary Figure S8. Switching state recognition from detected events. Events from particle switching were detected according to the description in the Materials and Methods section. Two particles from creatinine sensor (a) and 11 nt DNA sensor (b) are shown as examples, with the x,y trajectory of the center of the particle over the entire measurement. Events are shown as vertical red lines. The calculated area ratio, which is the convex hull area of each state divided by the convex hull area of the total measurement frames, is shown below. State attributions, bound, unbound and unidentified, are shown as a function of time. The number of bound and unbound states as a function of analyte concentration, from the creatinine (c) and 11-nt DNA measurement (d) in Figure 2, using an area ratio neighbor comparision. Unidentified states (Unidentified %) are indicated as percentage of unidentified states versus the total number of states.



Supplementary Figure S9. Estimation of number of accesible molecules on the particle. The particle has 1 μm diameter and the tether length is around 50 nm. The radius of the motion pattern observed in the experiments is around 250 nm, which defines the maximum contact with the glass substrate. The angle that the particle can rotate when it reaches its maximum contact with the glass substrate is α, which is around 12.6°. The part of the surface on the particle close to the substrate that created the motion pattern, indicated red in the graph, is considered the accessible area. This area is around 1% of the total surface area of the particle. The analogue is provided to the flow cell at a concentration of 250 pM (note: incubation of detection molecule on the substrate occurs at a concentration of 100 nM, which is about 400 times higher). We usually observe about 400 particles per field of view of 400 μm × 400 μm. The flow cell depth is 500 μm, therefore the volume of the field of view is 0.08 mm^3. With this information we can estimate that the maximum number of analogue molecules on each particle is 30,000 and the maximum number of accessible molecules is around 300.

Supplementary Table S1. Summary of analytical performance of the competition assays

	Analyte (concentration)		
	11nt (nM)	12nt (nM)	Creatinine (μM)
IC_{50}	$(1.5\pm0.5)\times10^{2}$	$(0.5\pm0.1)\times10^{2}$	$(2.5\pm0.7)\times10^{1}$
Blank Activity:			
# Measurements	14	11	18
Average	16.4	13.0	13.3
Standard deviation	0.3	0.9	1.2
LOD	18.2	15.0	13.2
Dynamic range	31-1000	2-500	13-1000
R^2	0.98	0.97	0.98

IC_{50} is the analyte concentration causing a 50% inhibition of the activity, fitted with the Hill equation in the dose-response curve. Blank activity is the sensor activity with no analyte present; the number of measurements, the average, and the standard deviation of the data shown in Fig. 2. The LOD, the limit of detection, is the concentration extrapolated from the dose-response curve fit with activity that is 3 times the standard deviation below the blank activity. The dynamic range is the linear part of the dose-response curve where the R^2 of the linear fit of the dose-response curve (lin-log scale) is larger than 0.95.

Supplementary Table S2. Measurement data of 11-nt DNA competition assay with 10 replicates and calculated CV

	Activity (3 min)																	
~ 300 particles																		
[Analyte] (nM)	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	**No. of Events**	Mean	SD	σ_{Mean}	σ_{SD}	**CV (%)**	$\sigma_{CV/CV}$ (%)	σ_{CV}
0	9.73	10.40	9.98	9.78	9.07	9.55	9.47	9.10	8.77	8.45	**3121**	9.43	0.59	0.18	0.14	**6.2**	23.9	0.015
1	8.45	8.38	8.38	8.53	8.29	8.66	8.57	8.62	8.32	8.00	**2888**	8.42	0.19	0.06	0.05	**2.3**	25.9	0.006
10	6.86	6.38	6.48	6.43	6.29	6.15	5.94	5.95	5.95	6.07	**2001**	6.25	0.30	0.09	0.07	**4.8**	23.6	0.011
100	4.10	3.95	4.10	4.10	4.12	4.02	3.68	3.88	3.63	3.66	**1279**	3.92	0.20	0.06	0.04	**5.1**	20.1	0.010
1000	1.77	1.71	1.79	1.57	1.51	1.59	1.63	1.44	1.70	1.66	**521**	1.64	0.11	0.03	0.02	**6.8**	18.0	0.012
30 particles																		
[Analyte] (nM)	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	**No. of Events**	Mean	SD	σ_{Mean}	σ_{SD}	**CV (%)**	$\sigma_{CV/CV}$ (%)	σ_{CV}
0.1	12.37	13.83	12.90	12.03	11.07	11.57	11.13	10.80	8.80	9.00	**341**	11.35	1.58	0.50	0.38	**14.0**	24.4	0.034
1	7.47	10.50	9.90	9.67	8.57	8.47	8.10	7.90	7.73	7.90	**259**	8.62	1.04	0.33	0.25	**12.1**	24.4	0.029
10	6.33	4.97	5.60	6.43	6.43	6.63	6.27	6.83	6.70	5.40	**185**	6.16	0.62	0.20	0.15	**10.1**	24.4	0.025
100	2.93	3.07	3.20	3.87	3.53	4.47	2.30	2.37	2.63	2.13	**92**	3.05	0.74	0.24	0.18	**24.4**	25.4	0.062
1000	1.60	1.13	1.27	1.67	1.10	0.97	0.93	1.00	1.03	1.20	**36**	1.19	0.26	0.08	0.06	**21.5**	24.4	0.052

M1-M10: 10 replicate measurements; SD: standard deviation; CV: coefficient of variation; The uncertainty of CV (σ_{CV}) is calculated with propagation of uncertainty from the uncertainty of the mean ($\sigma_{mean,}$ standard error of the mean) and the uncertainty of standard deviation (σ_{SD}) which is the error of fitting the measurement replicates into a normal distribution. Around 300 particles were found in the field of view. To show data with higher CV%, 30 particles were included in the analysis to obtain less events.

Supplementary Table S3. Oligonucleotides sequences and modification

Oligonucleotide name	Length	5' --> 3' Sequence and modification
DNA Intermediate	36	GTA GAC ATC CAA CCT GAC TAC GTG AGT AAT AAT GCG
Particle binder 20nt	20	TAG TCA GGT TGG ATG TCT AC - 3'Biotin
Competitor11nt	15	AAA AGC ATT ATT ACT
Competitor12nt	16	AAA AGC ATT ATT ACT C
Detection oligo docking	31	GCA GTC ACG TTC TCG AAT CGA ACA TTA TTA C
Docking strand 20nt	20	CGA TTC GAG AAC GTG ACT GC - 3'Biotin
Short arm	25	GTC ACC GAT GAA ACT GTC TAC AGA C - 3'Amine
Long arm	50	GTA GAC ATC CAA CCT GAC TAG CTG AGT CTG TAG ACA GTT TCA TCG GTG AC
Tether capture	40	5'Biotin - GTG CTC CGA CAG CCG CAT CCA TGA CAG AGT GCG GTG CGA G
Main Tether	166	5'Biotion - CGC TGC TGC GCC GCT CGA GCG GCA CGT CGC AGC CGA TTG GTG CAA TGG CCT GGC TCC GGT GTC GTC GCG CTC ATC TGC TGC ACG CAT CGC TAG TAC ACA CGT CGA CAT ACT AAC GCG GCA GGG TTG CTC GCA CCG CAC TCT GTC ATG GAT GCG GCT GTC GGA GCA C
End tether	120	CAA CCC TGC CGC GTT AGT ATG TCG ACG TGT GTA CTA GCG ATG CGT GCA GCA GAT GAG CGC GAC GAC ACC GGA GCC AGG CCA TTG CAC CAA TCG GCT GCG ACG TGC CGC TCG AGC GGC GCA GCA GCG
polyT	16	5'Biotion - TTT TTT TTT TTT TTT T