Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Single Molecule Based SNP Detection Using Designed DNA Carrier and Solid-State Nanopores

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S1. Nanopore fabrication

We use glass nanopores fabricated with a laser-assisted puller (Sutter Instrument, USA). The pulling program and quartz capillaries used are as described previously.[1, 2] The magnitude of the double-stranded DNA current drop is -0.134 ± 0.013 nA where the error is the standard deviation. The distribution of double-stranded DNA (dsDNA) current drop level is shown in Figure S1. The dsDNA level from each nanopore is used for setting the threshold to determine the presence of the central peak in events. This is also reflects the distribution of pore diameters and shapes.

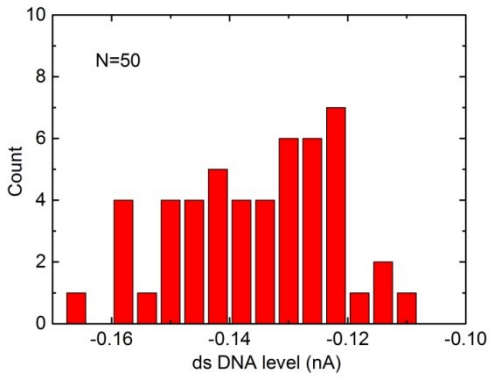

Figure S1. Distribution of dsDNA translocation ionic current drops for 50 nanopore used in this study.

S2. DNA carrier synthesis and preparation

The DNA carrier is hybridized by a linearized 7.2 kb m13mp18 virus genome (New England Biolab) and 190 designed 38 bases long oligonucleotides (Integrated DNA Technology) as shown in Figure 1c. Details of the synthesis has been described previously.[3, 4]

DNA and protein concentrations were measured by the absorbance using a Nanodrop 2000 (Thermo Scientific) before any dilution. DNA carriers were incubated with wild type or mutant strands for 2 hours in 100 mM NaCl, 10 mM $MgCl_2$ buffered with TE (pH~8). Before the nanopore measurement, the sample is diluted by adding the same volume of 8 M LiCl, 100 mM NaCl, 10 mM $MgCl_2$ buffered with TE (pH~7.5). 4 nM monovalent streptavidin was

added after the incubations. Monovalent streptavidin[5] used in this work is generously provided by the Howarth Lab, Oxford University.

S3. Experimental setup

All nanopore measurements were carried out at 600 mV applied voltage. Ionic current signals were acquired using an Axopatch 200B with sampling frequency of 250 kHz and filtered at 49.9 kHz with an external 8-pole Bessel filter. Fluorescence data was collected at room temperature (20 °C) on a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, USA).

S4. Data analysis

The translocation events detected include the signals from DNA carriers in linear or folded confirmations, free streptavidin molecules and small amount of short DNA fragments from the sample preparation. Only the full length, unfolded DNA carrier translocation events are considered. The event selection is achieved by analyzing the event charge deficit of each event above a threshold and in addition an ionic current amplitude threshold at the beginning of each event. This process was described in detail previously.[3] A searching window of 25% of the event duration and thresholds of 1.4 or 1.35 times the dsDNA current drop level[4] are set to determine the presence of the central peak in each event. The threshold factor of 1.35 is used for comparable bigger nanopore with the double-stranded DNA current drop above -0.13 nA.

S5. Fluorescent-based structure validation for SNP detection

Sequences of designed strands F, Q, M and W, as listed in Table S1, were used in bulk fluorescent-based measurements. Strand F and Q was hybridized in 100 mM NaCl, 10 mM $MgCl_2$ buffered with TE (pH~8) using the following program: Heat up to 88 °C followed by cooling down to room temperature over 40 minutes. 100nM strand M and W were incubated

with hybridized FQ, in the same concentration and buffer condition for 1 hour before the measurements. Fluorescent intensity was read at the excitation wavelength of 495 nm and emission wavelength of 528 nm. The kinetics measurement was done in 4 M LiCl, 100 mM NaCl, 10 mM $MgCl_2$ buffered with TE (pH~7.6). Strand M and W were mixed with FQ and immediately started for a ~2 hours of recording. 3 repeats were measured for each sample. Figure S2 shows the summarised histogram and the example spectra are shown in Figure 2b.

Table 1. Sequences of oligonucleotides used in the DNA carrier design and structure validation with fluorescent-based and electrophoresis assays. The color scheme is the same as in Figure 1c.

Oligo		Sequence (5' to 3')	Modification
M	JAK2 Mutant	**AATTATGGAGTATGTTTCTG**TGGAGACG[6]	None
W	JAK2 Wild type	**AATTATGGAGTATGTGTCTG**TGGAGACG[6]	None
S95F	On carrier probe	**CAGAAACATACTCCATAATT**TTT GAAATTATTCATTAAAGGTGAATTATCACCGTCACCGA	None
F	Fluorescent probe	**CAGAAACATACTCCATAATT**	3'FAM
Q	Quencher probe (on carrier signal probe)	**AATTATGGAGTATG**	5'Iowa Black, 3'Biotin

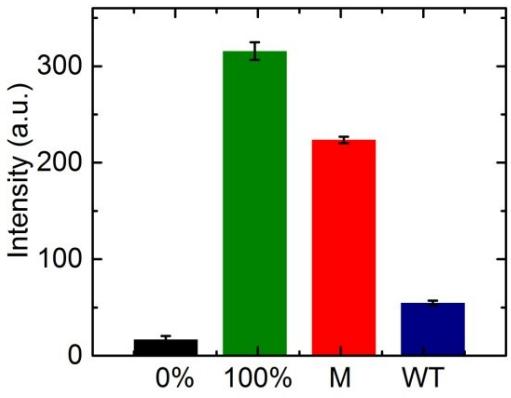

Figure S2. Histogram of peak fluorescent intensity obtained in each sample condition. Errors are standard deviation.

S6. Gel electrophoresis assays

Designed strand F, Q, M and W, as listed in Table 1, were again used in the bulk gel electrophoresis assay. 1.67 μM solutions of strand M and W were incubated with hybridized FQ at 1 to 1 ratio for 1 hour before the measurements. Incubations were carried out in 100 mM NaCl, 10 mM $MgCl_2$ buffered with TE (pH~8). 15% PAGE was run at 110 V with 1 time TBE buffer.

S7. Example kinetics results from the 2 hours incubated measurements

The target concentration dependence was measured after 2 hours incubation which was considered as the end of the reaction. The occupied fractions in 30 events groups are plotted in Figure S3 as examples. Each data set is from one nanopore over the recording time of around 30 minutes. The plot in green shows the occupied fraction verses time of JAK2 carrier incubated with 1 time mutant strand after 2 hours of incubation while the orange plot shows results with 10 times mutant strand in the same conditions. In both of the two experiments, the occupied fraction has reached the stable level indicating the end of the reaction.

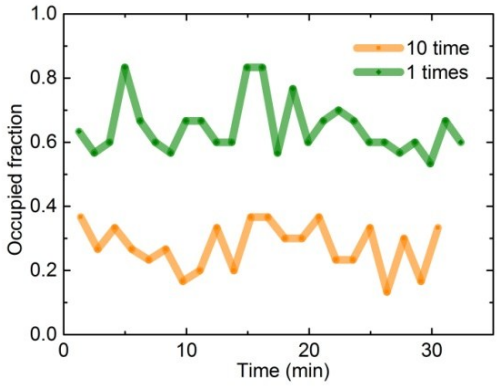

Figure S3. Kinetics results from 2 typical nanopores shoeing that a stable occupied fraction is obtained after 2 hours of incubation.

S8. Secondary current drop and time duration analysis

To further confirm the secondary current drop signal is not missing due to the limit of the setup, a scatter plot of time duration versus current drop amplitude of the secondary current drop is shown in Figure S4, together with current drop histograms of 0% and 100% control

translocation events. For each event, only the central 25% of event duration is considered in this analysis. A threshold of 50 pA is set to define the presence of the secondary peak. In the 'fully occupied' 0% control measurement, 786 out of 824 linear translocation events contain a secondary peak, equal to 95% occupancy in this example. Though a bandwidth limit is indicated at the cutoff of ~ 40 µs, the high occupancy (~90%) demonstrates that the sampling and filtering setting used is adequate for the sensing purpose. Furthermore, a clear discrimination is shown by the two populations of current drop amplitude from the two control measurements. A small peak from the 0% control is overlapping with the 100% control, representing the ~ 10% missing signal which is mainly due to the impurity of the modified DNA oligo. [4] The outliers that show bigger current drop amplitudes and longer time duration is assumed coming from unspecific DNA nanopore interaction.

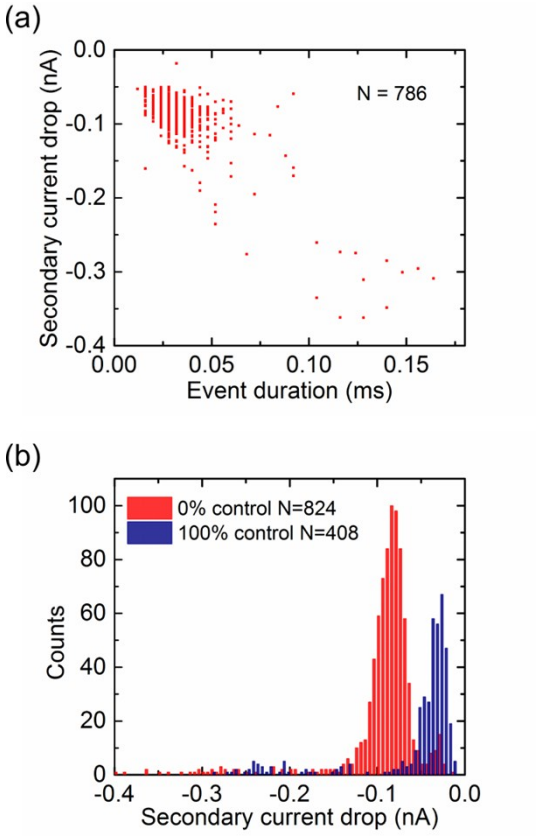

Figure S4. Analysis of secondary current drop. (a) Scatter plot of secondary current drop amplitude versus time duration. (b) Comparison of secondary current drop amplitude between 0% control and 100% control translocations. Data used in this figure are from Nanopore No. 1 for 100% control and Nanopore No. 8 for 0% control as listed in Table S3.

S9. Time dependence and example events of folded DNA carrier translocation

Data shown in Figure 5a are from 41 nanopores that are used for the concentration dependence measurements. The peak fraction is 0.30 ± 0.0096 as fitted by a Gauss function. The fraction of linear translocation events is plotted as a function of the event number of each nanopore used in the kinetics measurements. The total event number is used as the time axis since the event frequency can be assumed constant with the same DNA carrier concentration. The results do not show any clear trend during the 2 hours of recording since the linear event fractions are stable in the same level as in Figure S5a, where the data come from 41 individual measurements for ~30 minutes each. Therefore, we believe the percentage of linear events does not change along the recording time.

Though only event of DNA carrier translocating in linear formation is considered in the data analysis, there are a variety of folded states observed in the measurement. Several examples are shown in Figure S6. While (a) is the standard linear event, (b) to (e) show DNA carrier folded partially (b, c, d) or fully in half (e). The current drop level of single dsDNA and double dsDNA level can be easily recognized regardless of the folded proportion in the structure.

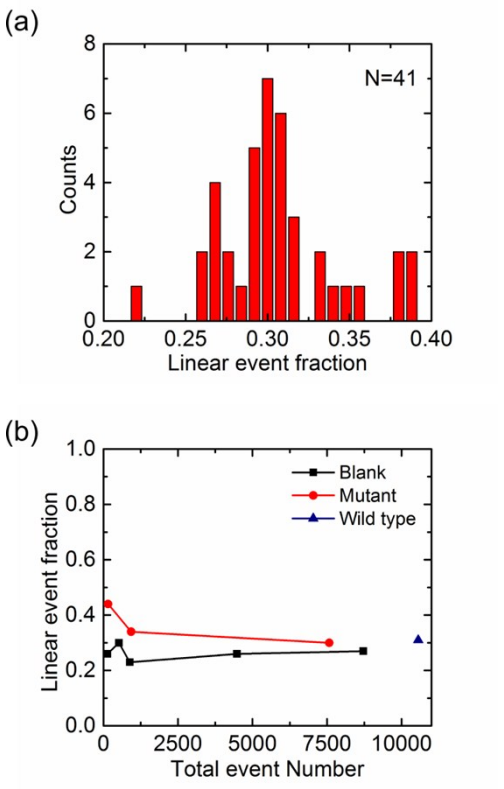

Figure S5. (a) Fraction of linear translocation events in of DNA carrier translocation events for 41 nanopores used here. (b) Linear event fraction as a function of total event number (equivalent to time) over the course of a displacement experiment.

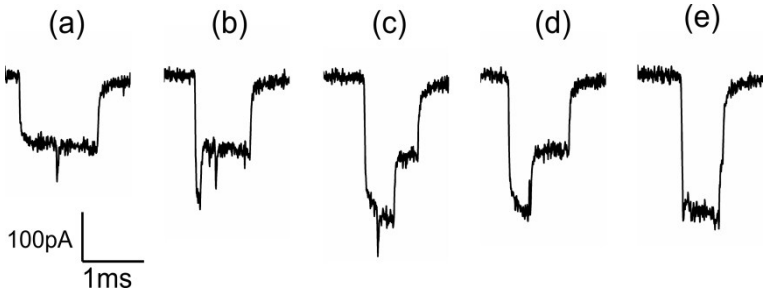

Figure S6. Example events of DNA carrier translocation in different folding states. (a) Linear event with bound probe, (b) partly folded event with bound probe in linear part, (c) folded event with bound probe in folded part, (d) partly folded event with no probe, (c) completely folded event with no probe signal.

S10. Control measurement of bleaching levels in the bulk fluorescence assay

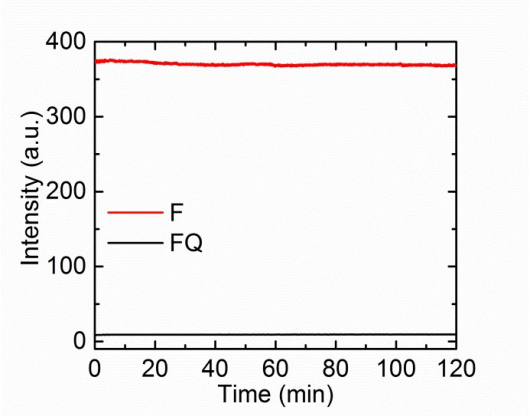

Figure S7. Control measurements to assess bleaching levels in the bulk fluorescence assay. The fluorescence intensity is almost unchanged over the course of our 2 hours long measurement.

S11. Statistics of JAK2 DNA carrier translocation events

Detailed statistics of translocation event numbers and nanopore information is listed in Table S2 and Table S3.

Table S2. Statistics of JAK2 carrier kinetics measurements.

Kinetics sample	Nanopores No.	dsDNA current drop amplitude (nA)	All carrier No.	Unfolded carrier No.
Blank	01	-0.141	131	34
	02	-0.159	390	118
	03	-0.129	362	82
	04	-0.140	3596	943
	05	-0.132	4238	1151
Mutant	06	-0.151	156	68
	07	-0.147	773	262
	08	-0.157	6646	2007

Wild type	09	-0.130	10561	3260

Table S3. Statistics of JAK2 carrier translocation measurement for target concentration dependence.

Sample group	Nanopores No.	dsDNA current drop amplitude (nA)	All carrier No.	Unfolded carrier No.	Occupied carrier No.	Occupied fraction
0% control	01	-0.153	2377	824	736	0.89
	02	-0.129	194	75	64	0.85
	03	-0.157	2511	899	791	0.88
	04	-0.132	687	203	164	0.81
	05	-0.141	131	977	691	0.71
	06	-0.159	390	118	108	0.92
	07	-0.165	4600	1233	948	0.77
100% control	08	-0.139	1588	408	41	0.10
	09	-0.132	137	38	5	0.13
	10	-0.124	1297	390	78	0.20
10 times wild type	11	-0.146	5638	1518	1068	0.70
	12	-0.146	184	71	58	0.82
	13	-0.150	323	122	96	0.79
7.5 times wild type	14	-0.122	459	234	203	0.87
	15	-0.137	78	26	22	0.85
	16	-0.118	1022	304	221	0.73
5 times wild type	17	-0.127	1439	442	364	0.82
	18	-0.141	916	247	182	0.74
	19	-0.148	625	197	163	0.83
3 times wild type	20	-0.125	1055	309	267	0.86
	21	-0.142	1077	301	255	0.85
	22	-0.150	777	246	221	0.90
1 time wild type	23	-0.128	990	308	276	0.90
	24	-0.124	1250	336	281	0.84
	25	-0.140	945	277	248	0.90
10 time mutant	26	-0.126	462	133	40	0.30
	27	-0.125	2443	728	185	0.25
	28	-0.121	1439	448	90	0.20
7.5 times mutant	29	-0.134	152	52	23	0.44
	30	-0.136	276	105	46	0.44
	31	-0.123	3270	992	247	0.25
5 times mutant	32	-0.115	919	276	139	0.50
	33	-0.155	1924	592	286	0.48
	34	-0.112	1359	422	202	0.48
	35	-0.146	353	79	37	0.47
3 times mutant	36	-0.138	1888	575	344	0.60
	37	-0.124	1871	545	302	0.55
	38	-0.127	1416	442	228	0.52
1 time mutant	39	-0.131	2784	818	457	0.56
	40	-0.121	2183	652	445	0.68
	41	-0.123	2405	808	539	0.67

Reference

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