Mapping the conformational energy landscape of Abl kinase using ClyA nanopore tweezers

Fanjun Li, Monifa A. Fahie, Kaitlyn Gilliam, Ryan Pham, and Min Chen

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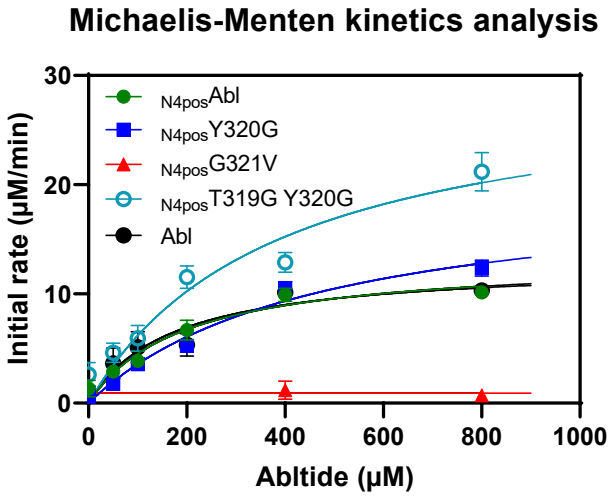
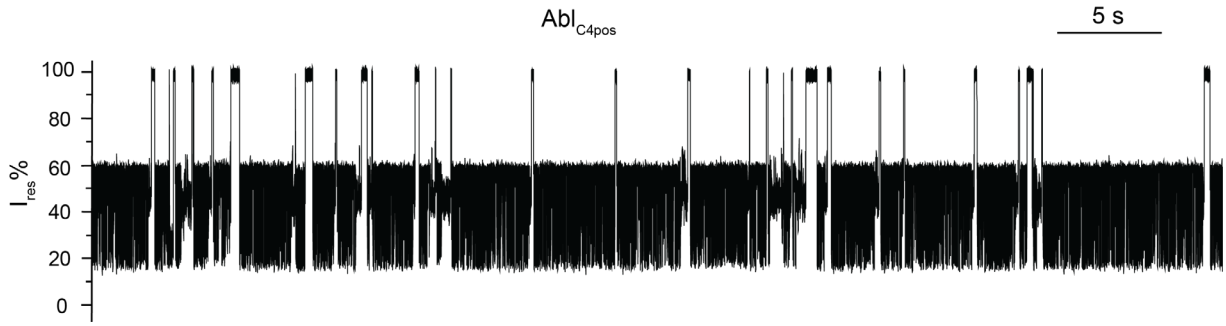
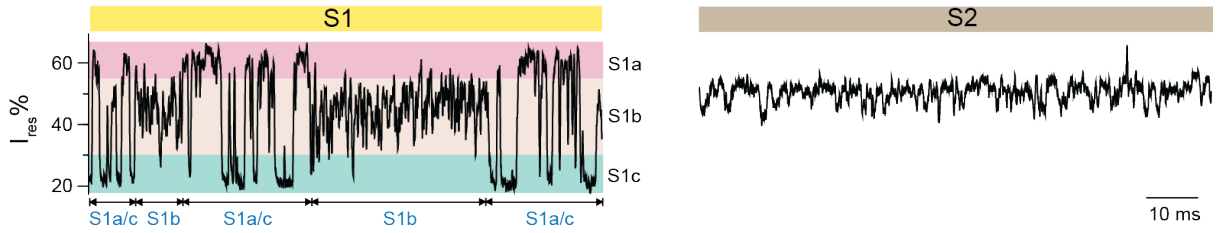


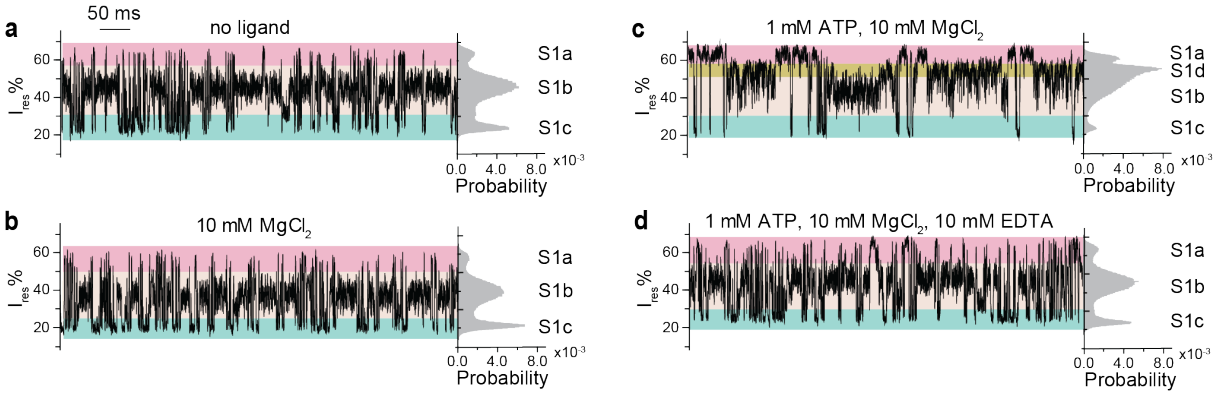
Supplementary Figure 1. Michaelis-Menten kinetics analysis of Abl kinases (Abl, $_{N4pos}$Abl, $_{N4pos}$G321V, $_{N4pos}$Y320G, and $_{N4pos}$T319G Y320G).



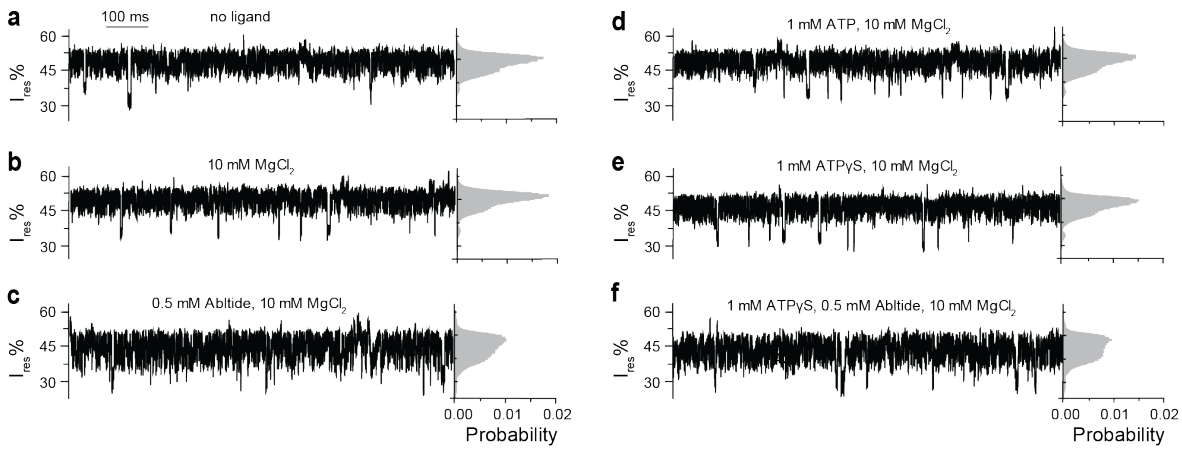
Supplementary Figure 2. A representative trace of Abl$_{C4pos}$ trapped in a ClyA-AS nanopore. The current traces were collected at −80 mV in 150 mM NaCl, 100 mM Tris-HCl, pH 7.5, with ~100 nM Abl$_{C4pos}$ kinase added to *cis* chamber, at 22 ºC.



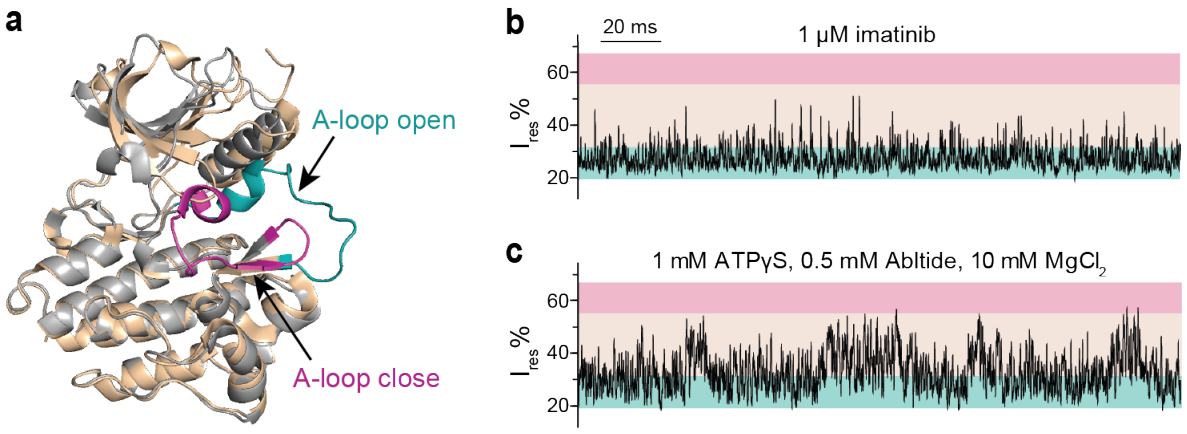
Supplementary Figure 3. Zoom in trace of S1 and S2 states from *apo* Abl WT trapping signal. Three sub-states were observed within S1, namely, S1a, S1b, and S1c. The state transition pathways were: S1↔S2, S1a/c↔S1b, and S1a↔S1c. The current traces were collected at −80 mV in the buffer 150 mM NaCl, 100 mM Tris-HCl, pH 7.5, with ~100 nM Abl kinase added to *cis* chamber, at 22 ºC.



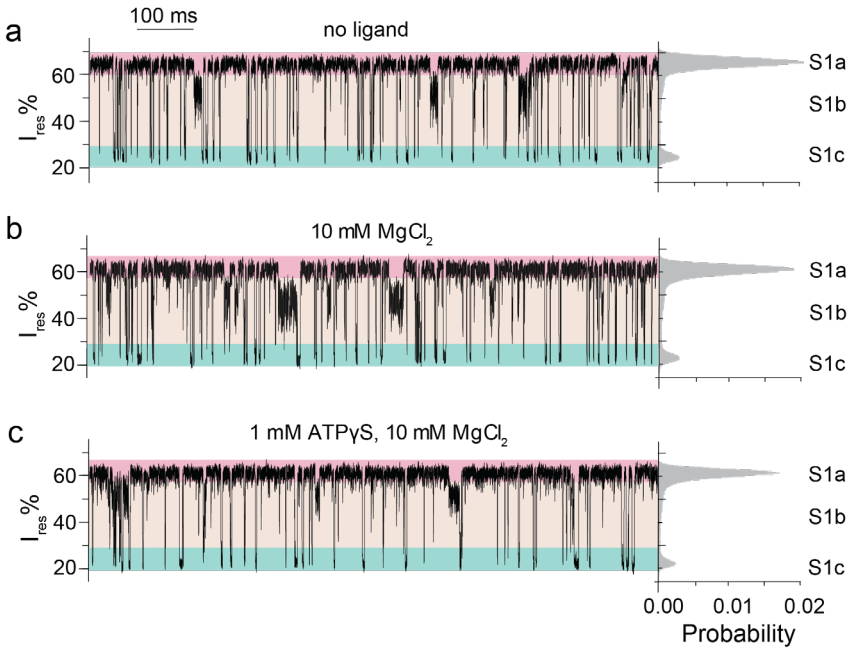
Supplementary Figure 4. Effect of $MgCl_2$ on ATP binding with $_{N4pos}$Abl. (**a-d**) Representative traces and corresponding histograms of S1 of *apo* $_{N4pos}$Abl (**a**) and $_{N4pos}$Abl in the presence of 10 mM $MgCl_2$ (**b**), 1 mM ATP and 10 mM $MgCl_2$ (**c**), 1 mM ATP, 10 mM $MgCl_2$ and 10 mM EDTA (**d**). The current traces were collected at −80 mV in the buffer 150 mM NaCl, 100 mM Tris-HCl, pH 7.5, at 22 ºC, with ~100 nM $_{N4pos}$WT and ligands added to *cis*.



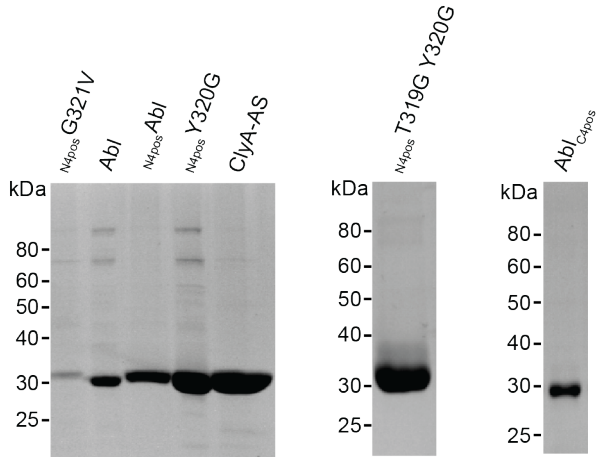
Supplementary Figure 5. Representative traces and corresponding histograms of S2 state during ligand interactions with $_{N4pos}$Abl. (**a-f**) Representative traces and corresponding histograms of S2 of *apo* $_{N4pos}$Abl (**a**) and $_{N4pos}$Abl in the presence of 10 mM $MgCl_2$ (**b**), 0.5mM Abltide and 10 mM $MgCl_2$ (**c**), 1 mM ATP and 10 mM $MgCl_2$ (**d**), 1 mM ATPγS and 10 mM $MgCl_2$ (**e**), 0.5mM Abltide, 1mM ATPγS and 10 mM $MgCl_2$ (**f**). Amino acid sequence of Abltide: KKGEAIYAAPFA. The current traces were collected at −80 mV in the buffer 150 mM NaCl, 100 mM Tris-HCl, pH 7.5, at 22ºC, with ~100 nM Abl and ligands added to *cis*.



Supplementary Figure 6. Comparison of $_{N4pos}$Abl binding with imatinib and $_{N4pos}$Abl binding with ATPγS, Abltide. (**a**) Superposition of imatinib bound Abl kinase (PDB: 2HYY, Abl in grey, A-loop in magenta) with ATP-peptide bound Abl kinase structures (PDB: 2G1T, Abl in wheat, A-loop in teal). (**b-c**) Representative traces of $_{N4pos}$Abl with 1μM imatinib (**b**) and 1mM ATPγS, 0.5mM Abltide and 10mM $MgCl_2$ (**c**). The current traces were collected at −80 mV in the buffer 150 mM NaCl, 100 mM Tris-HCl, pH 7.5, at 22 ºC, with ~100 nM $_{N4pos}$Abl and ligands added to *cis*.



Supplementary Figure 7. Interaction of ATPγS with $_{N4pos}$G321V. (**a-c**) Representative traces and corresponding histograms of S1 of *apo* $_{N4pos}$G321V (**a**), $_{N4pos}$G321V in the presence of 10 mM $MgCl_2$ (**b**), and $_{N4pos}$G321V in the presence of 1 mM ATPγS10 mM $MgCl_2$. The current traces were collected at −80 mV in the buffer 150 mM NaCl, 100 mM Tris-HCl, pH 7.5, at 22 ºC, with ~100 nM $_{N4pos}$G321V and ATPγS and $MgCl_2$ added to *cis*.



Supplementary Figure 8. 12% SDS gels of SDS-PAGE for ClyA-AS and Abl kinases (Abl, $_{N4pos}$Abl, Abl$_{C4pos}$, $_{N4pos}$G321V, $_{N4pos}$Y320G, and $_{N4pos}$T319G Y320G).

Supplementary Table 1. Michaelis-Menten kinetics analysis of Abl kinases (Abl, $_{N4pos}$Abl, $_{N4pos}$Y320G, $_{N4pos}$T319G Y320G and $_{N4pos}$G321V).

Abl kinase	K_m (μM)	V_{max} (μM/min)	k_{cat} (min^{-1})	k_{cat}/K_m ($min^{-1}μM^{-1}$)
Abl	167.6 ± 47.7	12.8 ± 1.3	426.7 ± 43.0	2.5 ± 0.9
$_{N4pos}$Abl	191.1 ± 39.9	13.3 ± 1.1	443.7± 35.1	2.3 ± 0.9
$_{N4pos}$Y320G	459.5 ± 93.5	20.2 ± 2.1	674.3 ± 68.8	1.5 ± 0.7
$_{N4pos}$T319G Y320G	382.9 ± 106.0	29.8 ± 3.9	993.3 ± 130.0	2.6 ± 1.2
$_{N4pos}$G321V	N/A	N/A	N/A	N/A

Supplementary Table 2. Dwell time of *apo* Abl kinases trapping and their S1, S2 states.

Abl kinase	$\tau_{trapping}$ (s)	τ_{S1} (s)	τ_{S2} (s)
Abl	0.06 ± 0.03	ND	ND
Abl_{C4pos}	1.52 ± 0.13	ND	ND
$_{N4pos}$Abl	20.72 ± 2.83	0.55 ± 0.07	12.25 ± 3.16
$_{N4pos}$Y320G	16.00 ± 2.46	0.45 ± 0.06	5.94 ± 0.41
$_{N4pos}$T319G Y320G	6.57 ± 1.83	0.20 ± 0.03	4.00 ± 0.95
$_{N4pos}$G321V	22.07 ± 5.19	0.79*	24.46*

The average τ and standard deviation were determined from three experiments. * Indicates that the data was fitted after combining events from different experiments. For $_{N4pos}$G321V, 231 events were used for τ_{S1} fitting, 222 events were used for τ_{S2} fitting.

Supplementary Table 3. Characteristics of *apo* $_{N4pos}$Abl and $_{N4pos}$G321V current signals.

State	State population % (*P*)		Residual Current (I_{res}%)		State Dwell time (τ)	
	$_{N4pos}$Abl	$_{N4pos}$G321V	$_{N4pos}$Abl	$_{N4pos}$G321V	$_{N4pos}$Abl	$_{N4pos}$G321V
S1	4.6 ± 1.5	3.1*	19~69	21~69	0.55 ± 0.07 s	0.79 s*
S2	95.4 ± 1.5	96.9*	38~58	40~59	12.25 ± 3.16 s	24.46 s*
S1a	0.8 ± 0.1	2.6 ± 0.1	58~69	60~69	1.1 ± 0.1 ms	9.1 ± 0.5 ms
S1b	2.8 ± 0.9	0.2 ± 0.1	31~58	30~60	10.2 ± 0.2 ms	6.2 ± 0.3 ms
S1c	1.0 ± 0.1	0.4 ± 0.1	19~31	21~30	1.3 ± 0.2 ms	1.4 ± 0.2 ms
S1a/c	1.8 ± 0.1	3.0 ± 0.1	19~69	21~69	6.5 ± 0.4 ms	104.3 ± 1.0 ms

Average state population, dwell time and standard deviation were determined from at least three independent pore recordings. ***** Indicates that the data was fitted after combining events from different experiments. For $_{N4pos}$G321V, 231 events were used for τ_{S1} fitting, 222 events were used for τ_{S2} fitting, P_{S1} and P_{S2} were calculated by τ_{S1} and τ_{S2} described in Method section.

Supplementary Table 4. Occupancy of S1 sub-states of $_{N4pos}$Abl under different conditions.

State occupancy (%)	S1a/S1	S1b/S1	S1c/S1	S1d/S1
No ligand	17.4 ± 2.1	60.9 ± 2.1	21.7 ± 3.8	0
10mM $MgCl_2$	13.3 ± 2.6	62.1 ± 2.9	25.0 ± 3.2	0
1mM ATP, 10mM $MgCl_2$	19.3 ± 2.2	36.3 ± 3.0	4.5 ± 0.1	39.8 ± 2.1
1mM ATPγS, 10mM $MgCl_2$	30.0 ± 2.0	42.4 ± 3.5	3.4 ± 1.1	24.1 ± 1.6
0.5mM Abltide, 10mM $MgCl_2$	2.4 ± 0.3	93.6 ± 0.3	4.0 ± 0.6	0
1mM ATPγS, 0.5mM Abltide 10mM $MgCl_2$	4.1 ± 0.5	58.0 ± 2.0	37.9 ± 2.5	0

Supplementary Table 5. Kinetic and thermodynamic parameters for *apo* $_{N4pos}$Abl

State transition rate constant		Free energy difference between states	
$k_{S1\rightarrow S2}$, s^{-1}	1.86 ±0.24	$\Delta G_{S1, S2}$, $KJ*mol^{-1}$	7.57±0.91
$k_{S2\rightarrow S1}$, s^{-1}	0.09 ± 0.02		
$k_{S1a/c\rightarrow S1b}$, s^{-1}	154.1 ± 9.6	$\Delta G_{S1a/c, S1b}$, $KJ*mol^{-1}$	1.11 ± 0.17
$k_{S1b\rightarrow S1a/c}$, s^{-1}	97.9 ± 1.5		
$k_{S1a\rightarrow S1c}$, s^{-1}	974.0 ± 142.0	$\Delta G_{S1a, S1c}$, $KJ*mol^{-1}$	0.42 ± 0.21
$k_{S1c\rightarrow S1a}$, s^{-1}	824.0 ± 141.5		

Supplementary Table 6. Primers for cloning.

Application	Primer name	Sequence (5' to 3')
Insert KRKKSGG to the N-terminus of Abl WT, generating $_{N4pos}$Abl	N4pos_F	AAACGTAAGAAAAGCGGAGGTTCCCCCAACT ACGACAAGTGGGAG
	N4pos_R	ACCTCCGCTTTTCTTACGTTTTGCATTGGATT GGAAGTACAGG
Insert GGSKKRK to the C-terminus of Abl WT, generating Abl$_{C4pos}$	C4pos_F	GGAGGTAGCAAGAAACGCAAATAATAACATT GGAAGTGGATAACG
	C4pos_R	TTTGCGTTTCTTGCTACCTCCTTTCCCCAGCT CCTTTTCCACTTC
Insert Y320G to $_{N4pos}$Abl, generating $_{N4pos}$Y320G	Y320G_F	GTTCATGACCGGAGGGAACCTCCTGGACTAC
	Y320G_R	GAGGTTCCCTCCGGTCATGAACTCAGTGATG AT
Insert G321V to $_{N4pos}$Abl, generating $_{N4pos}$G321V	G321V_F	CATGACCTACGTGAACCTCCTGGACTACC
	G321V_R	GTTCACGTAGGTCATGAACTCAGTGATGATA TAG
Insert T319G Y320G to $_{N4pos}$Abl, generating $_{N4pos}$T319G Y320G	T319G Y320G_F	GAGTTCATGGGCGGAGGGAACCTCCTGGAC
	T319G Y320G_R	TCCGCCCATGAACTCAGTGATGATATAGAAC