Supporting information for: Diversity of long-lived intermediates along the binding pathway of Imatinib to Abl kinase revealed by MD simulations

Fabian Paul, Trayder Thomas, and Benoît Roux*

Department of Biochemistry and Molecular Biology, The University of Chicago, U.S.A.

E-mail: roux@uchicago.edu

Table S1: Events observed in the pulling simulations. Pathways are labeled as follows: H=through hydrophobic pocket, C=through cleft, P=under P loop, U=unphysical distortion of the N lobe. H-DFG=through hydrophobic pocket with DFG flip, C-DFG=through cleft with DFG flip. Three smallest values of non-equilibrium work are highlighted in red.

simulation	pathway	αC restraint	duration / ns	DFG flip	$W_{\text{non-eq}}$ (kcal mol^{-1})	max F (kcal mol^{-1}A^{-1})
fast1	U	n	40	n		
fast2	U	n	40	n		
fast3	H	n	40	n		
fast4	H	n	40	n		
fast5	H	n	40	n		
fast6	H	n	40	n		
fast7	U	n	40	n		
fast8	U	n	40	n		
fast9	U	n	40	n		
fast10	U	n	40	n		
fix1	H	y	40	n		
fix2	H	y	40	n		
fix3	H	y	40	n		
fix4	H	y	40	n		
fix5	H	y	40	n		
fix6	H	y	40	n		
slow1	U	n	400	n	54	40
slow2	C	n	400	n	33	37
slow3	C	n	400	n	51	36
slow4	U	n	400	n	55	39
slow5	H	n	400	n	49	42
slow6	C	n	400	n	43	38
slow7	C-DFG	n	400	y	56	38
slow8	H-DFG	n	400	y	55	41
slow9	H	n	400	n	56	40
slow10	U	n	400	n	49	35
slow11	U	n	400	n	51	37
slow12	H	n	400	n	36	39
slow13	H	n	400	n	56	37
slow14	H	n	400	n	36	34
slow15	H	n	400	n	54	39
directed1	P	n	400	n	77	42
directed2	P	n	400	n	55	43

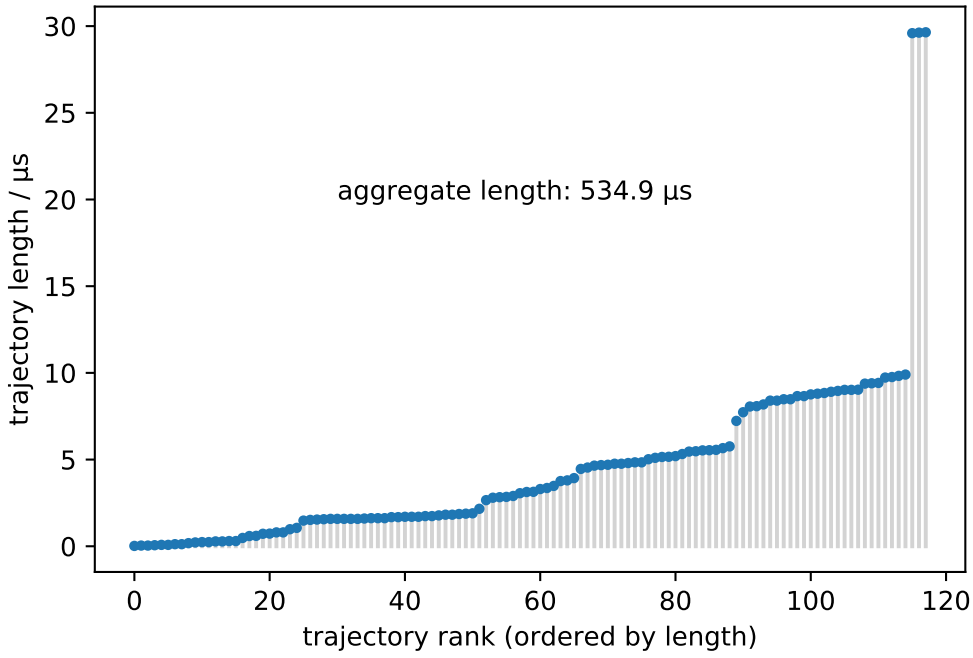

Figure S1: Lengths of the unbiased MD trajectories (sorted by length).

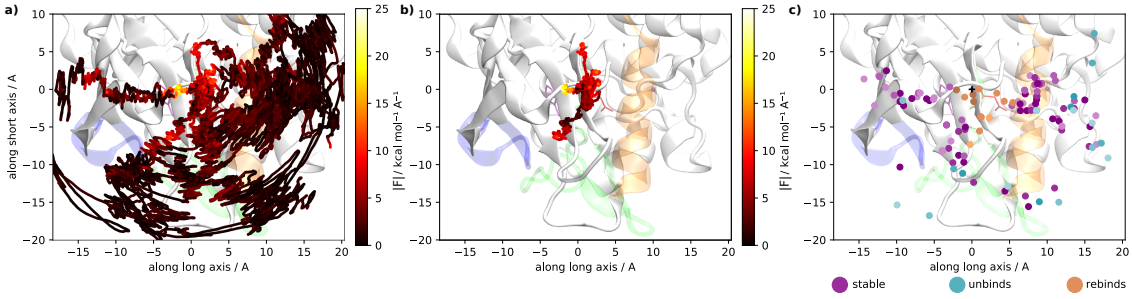

Figure S2: Same as main text Figure 4b but showing the magnitude of the pulling force instead of the non-equilibrium work. The shown forces were averaged with the same moving average filter as the quantities shown in main text Figure 4a,b. (a) shows the force along the whole pathways. (b) shows each pathways until it reaches the absolute maximum of the average force (on that pathway). (c) shows results from the unbiased simulations that were released after the pulling. The representation in (c) is mostly identical to that of main Figure 6a but disc sizes carries no meaning and discs are plotted at the location of the *initial* frame of every trajectory, thereby conveying a clearer picture of the boundaries of the rebinding and unbinding regions.

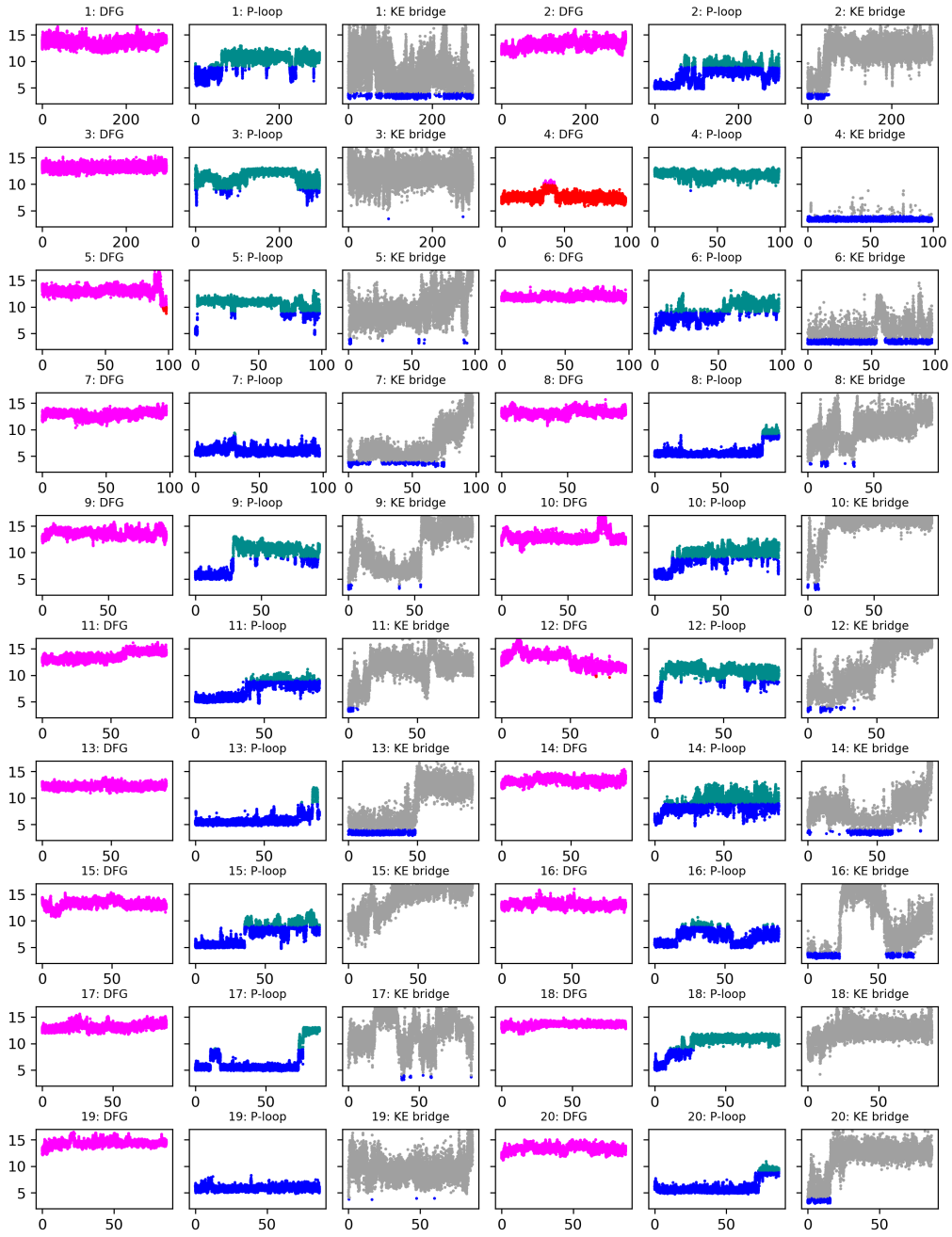

Figure S3: Status of the DFG motif, P-loop, and Lys271-Glu286 salt bridge during the longest 20 unbiased MD simulation trajectories. The first and fourth columns show the value $(d_3 + d_4)/2$ as a function of time. Time points with $(d_3+d_4)/2 > 10$A are shown in magenta ($\approx$ DFG out), time points with $(d_3+d_4)/2 < 10$A are shown in red ($\approx$ DFG in). The second and fifth column show the value $(d_1+d_2)/2$ as a function of time. Points with $(d_1+d_2)/2 > 9$A are shown in cyan ($\approx$ extended P-loop), time points with $(d_1+d_2)/2 < 9$A are shown in blue ($\approx$ kinked P-loop). The third and sixth columns show is the value d_6 as a function of time. Time points with $d_6 > 4$A are shown in gray ($\approx$ broken salt bridge), time points with $d_6 < 4$A are shown in blue ($\approx$ formed salt bridge). Continued in next figures.

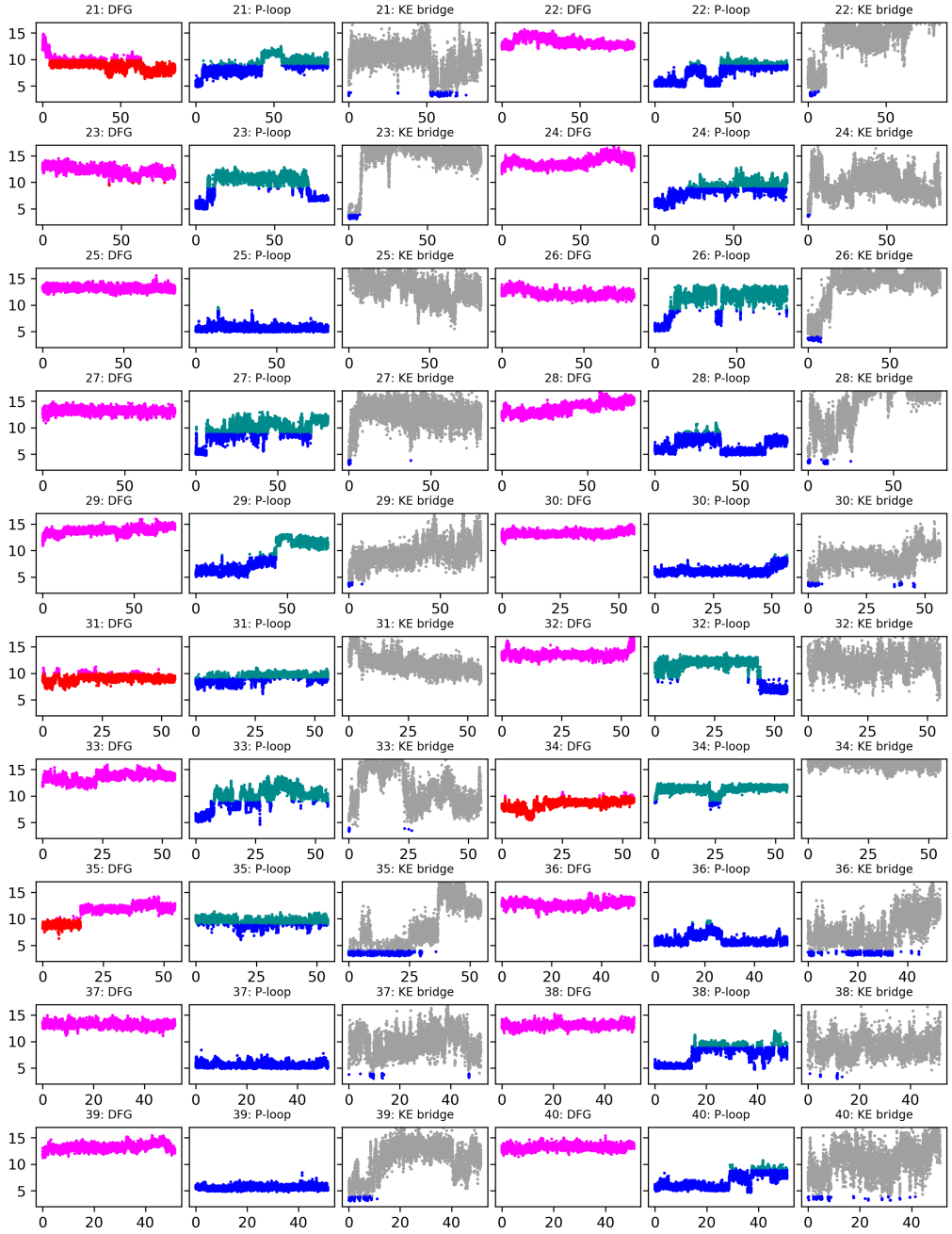

Figure S4: Continued from previous figure.

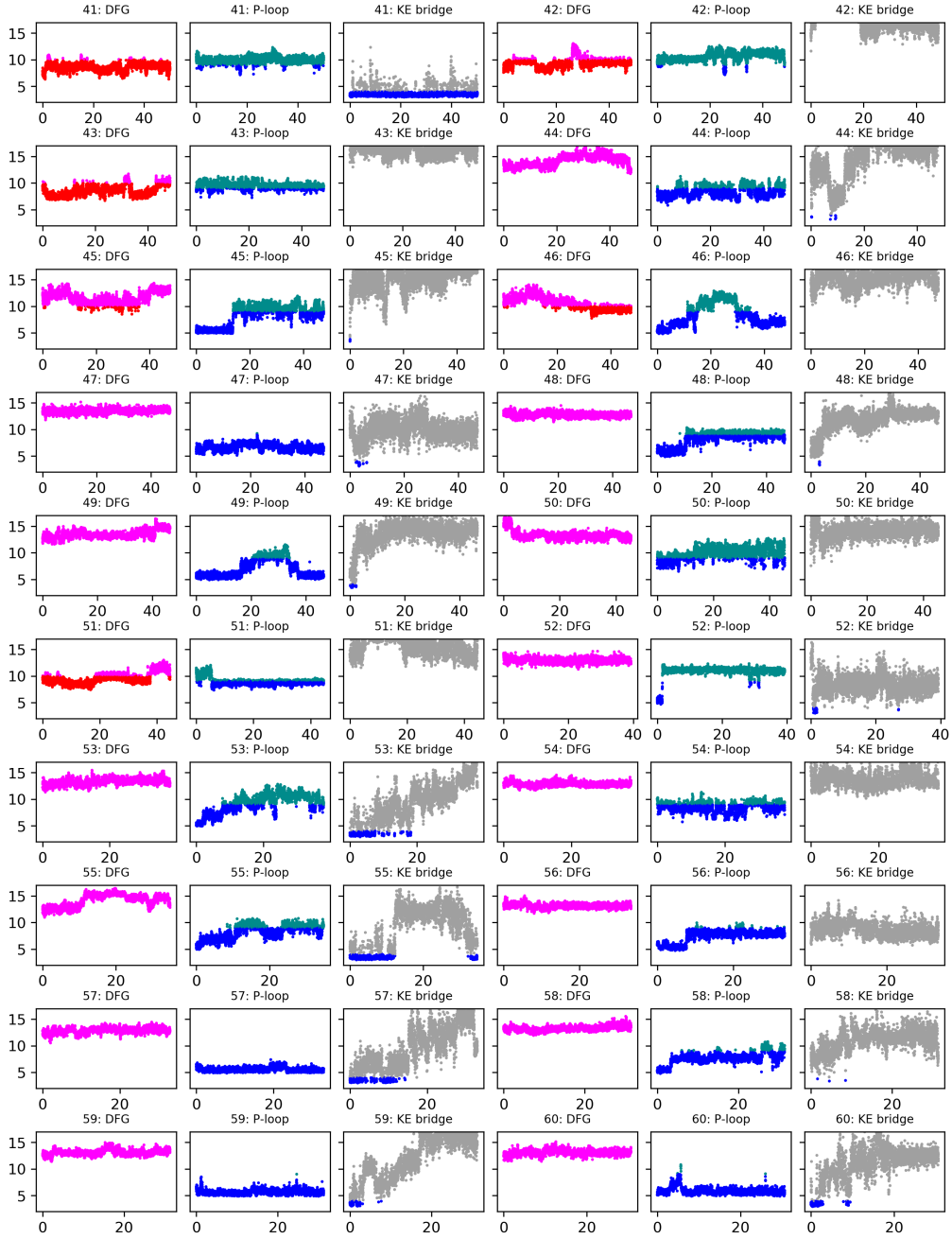

Figure S5: Continued from previous figure.

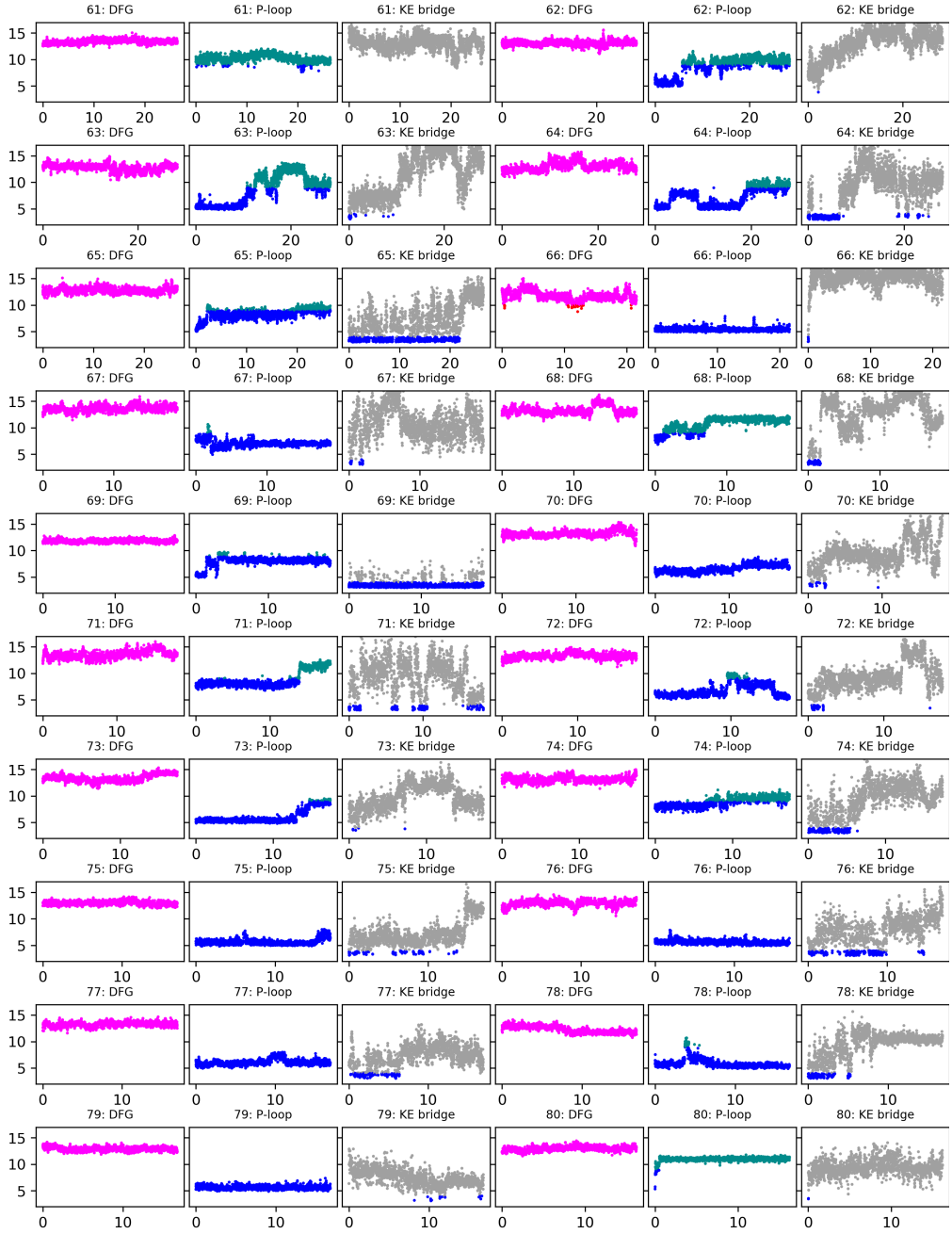

Figure S6: Continued from previous figure.

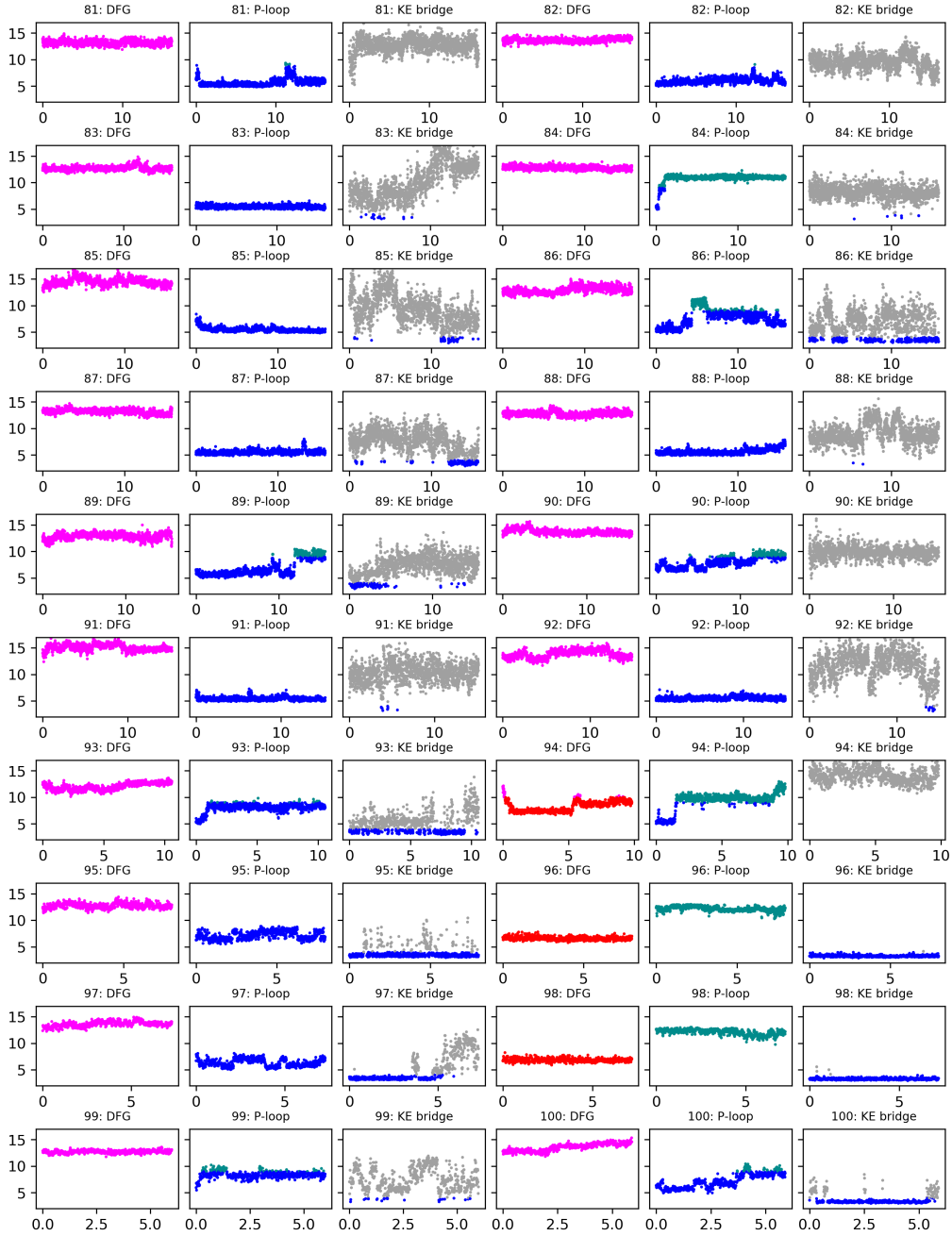

Figure S7: Continued from previous figure.

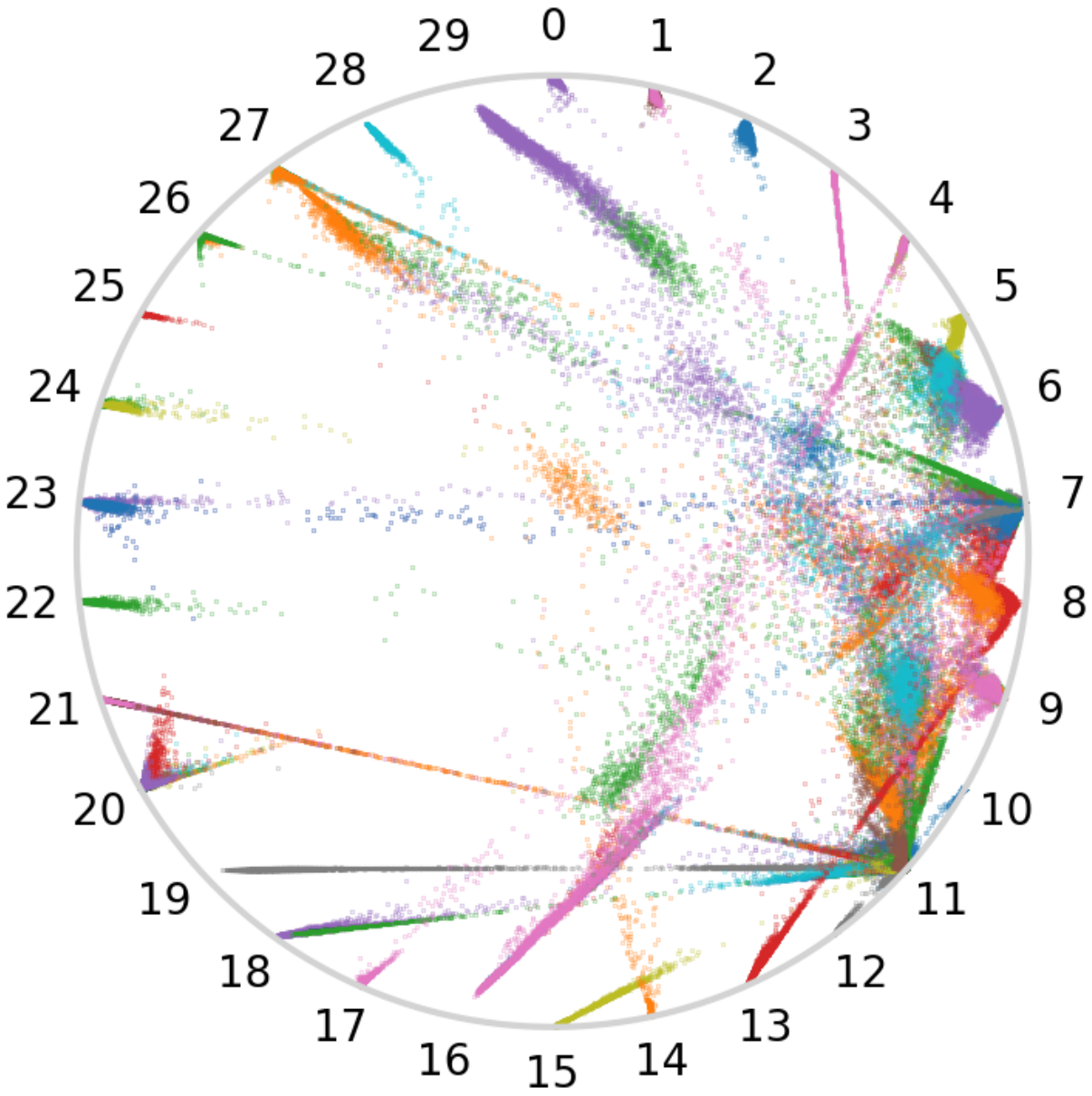

Figure S8: Circular plot of metastable memberships that were computed with the VAMPnet (see separation plots analysis for details and for our definition of circular plots). a) shows a (circular) scatter plot where all points from the same trajectory are shown in the same color (colors repeat).

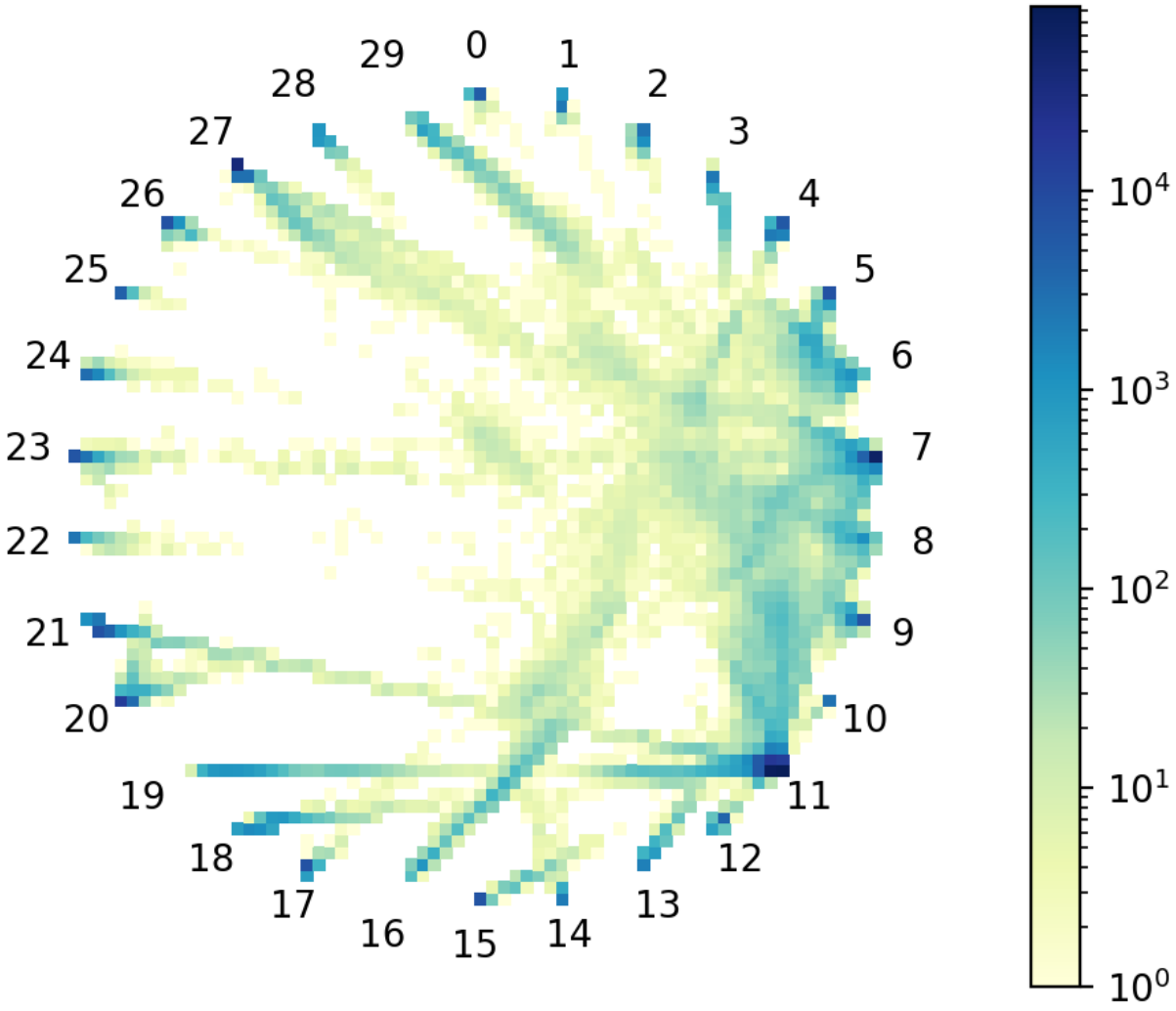

Figure S9: The same data as 2-D histogram. The plots show that for most states the metastable membership reaches it's maximal possible value of 1, which indicates that the chosen number of metastable states is reasonable (Röblitz, S.; Weber, M. Adv. Data. Anal. Classif. 2013, 7, 147–179). There are no major populations in the transition regions, which is also indicative of a meaningful metastable clustering.

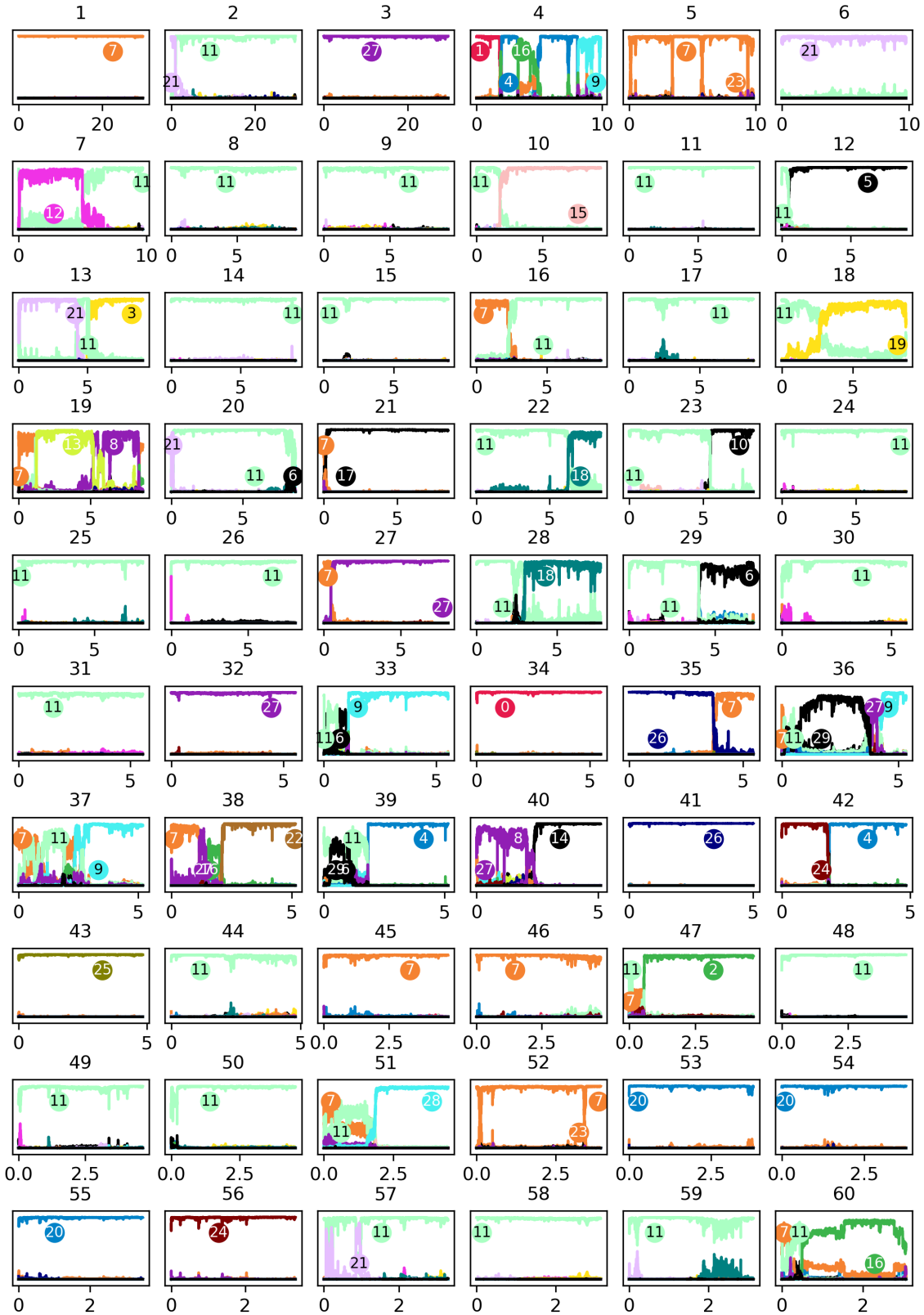

Figure S10: Metastable memberships for the 30 metastable states that were computed with the VAMPnet as a function of time. The membership in each metastable states is rendered in a different colors. The indices of metastable states visited in each trajectory are shown as circled numbers. Continued in next figure.

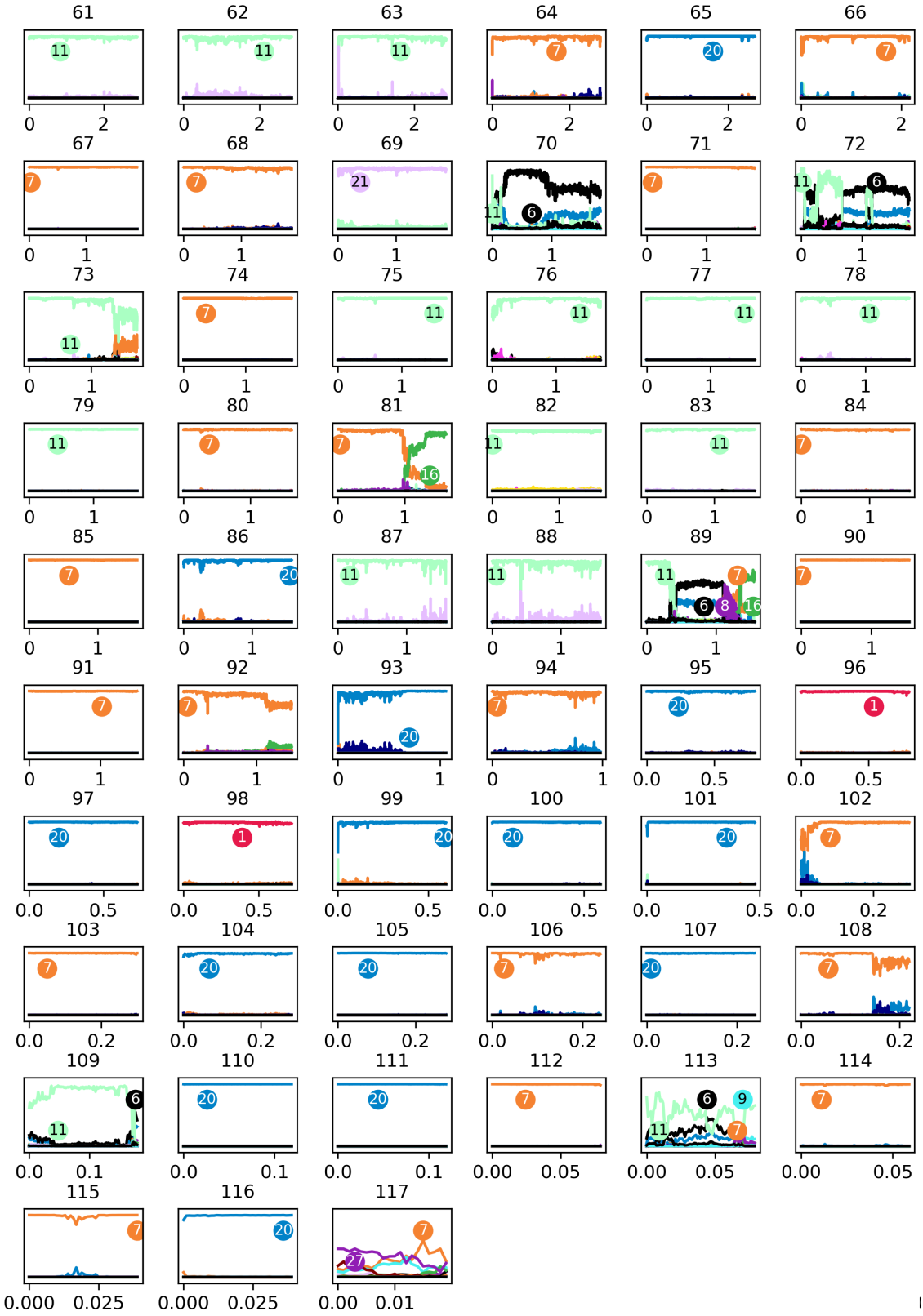

Figure S11: Continued from previous figure.

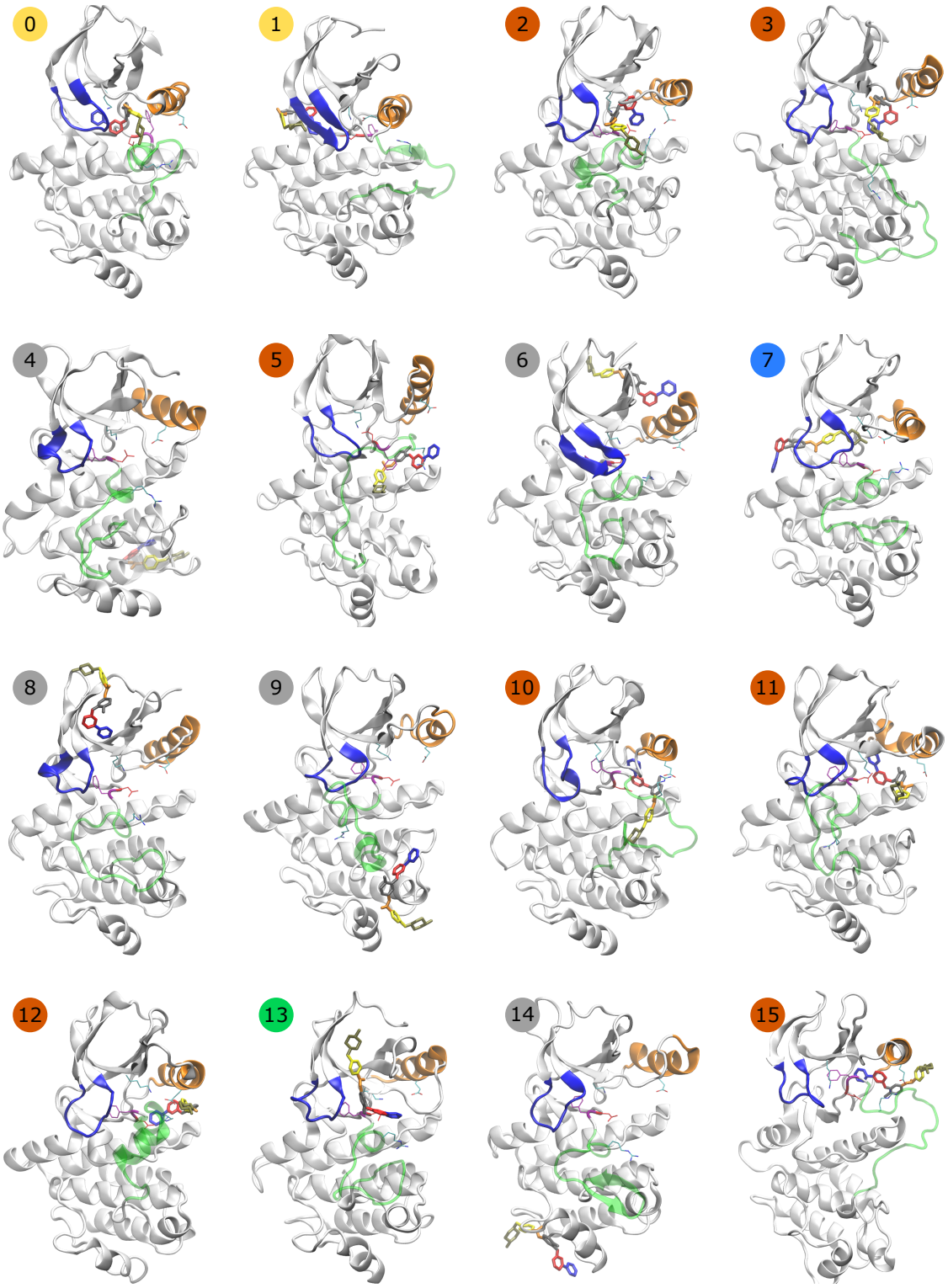

Figure S12: Representative structures for each metastable states. Shown structures are magnified uncropped version of the structures shown main text Fig. 8. The color of the disc surrounding the state number encodes the broad category of imatinib binding: in orange states imatinib is bound in the hydrophobic pocket; in blue states imatinib is bound under the P-loop; in green states imatinib is bound in front of the cleft; yellow states are DFG-in; in gray states imatinib binds on the surface of Abl. (Continued in Fig. S13.)

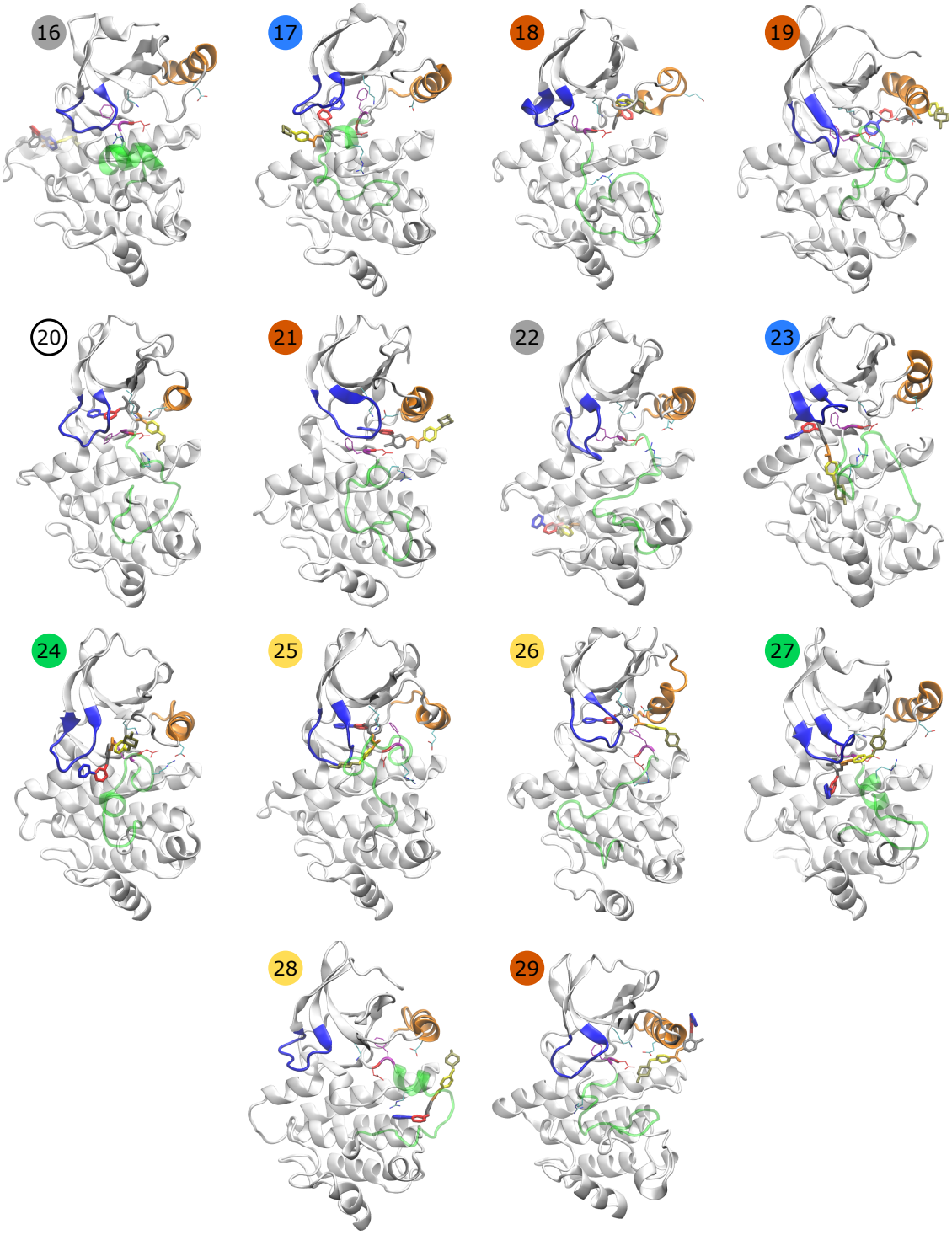

Figure S13: Continued from Fig. S12. State 20 is the X-ray-like state.