Table S1. Experimental binding free energy converted from IC_{50} values (kcal/mol).

System name	Exp IC_{50} (nM)	ΔG_{exp} (kcal/mol)
T790M	3.48	-11.53
T790M/L792F	10.04	-10.90
T790M/L792Y	33.04	-10.20
T790M/L792H	90.43	-9.60

ΔG_{exp}: Experimental binding free energies are calculated from IC_{50} using the following relationship: $\Delta G_{binding} = RT\ln K_{dissociated} = RT\ln(IC_{50} + 0.5C_{enzyme}) \approx RT\ln IC_{50}$, where R is ideal gas constant, T is temperature in K (298 K is used in this paper), C_{enzyme} is the concentration of enzyme, which is a very small number after equilibration and can be omitted in most cases, and IC_{50} values refer to this paper[1].

Table S2. PMF value calculated on each window (kcal/mol).

windows	T790M	T790M/L791F	T790M/L791Y	T790M/L791H
0	0.00	0.00	0.00	0.00
1	0.12	0.10	0.38	0.81
2	0.88	0.76	1.35	1.94
3	2.21	1.86	2.64	3.29
4	3.82	3.35	3.57	4.09
5	5.32	4.99	3.99	4.21
6	6.56	5.64	4.43	4.20
7	7.09	5.77	4.71	4.15
8	7.31	6.02	4.91	4.12
9	7.52	6.22	5.25	4.20
10	7.63	6.17	5.49	4.41
11	7.73	6.13	5.66	4.62
12	7.94	6.11	5.98	4.77
13	8.01	6.04	6.20	5.07
14	7.88	6.12	6.34	5.51
15	7.89	6.52	6.58	5.91
16	8.16	7.07	6.94	6.24
17	8.73	7.43	7.36	6.44
18	9.52	7.58	7.73	6.39
19	9.86	7.83	8.04	6.32
20	9.78	7.81	8.32	6.29
21	9.72	7.63	8.44	6.44
22	9.82	7.72	8.36	6.74
23	10.07	8.00	8.21	6.88
24	10.19	8.35	8.11	6.91
25	10.26	8.71	8.08	6.99
26	10.49	8.94	8.21	7.24
27	10.71	9.03	8.45	7.50
28	10.89	9.06	8.48	7.57
29	11.13	9.15	8.33	7.57
30	11.45	9.39	8.36	7.69
31	11.67	9.48	8.48	7.74
32	11.78	9.45	8.55	7.73
33	11.69	9.48	8.64	7.98
34	11.43	9.56	8.87	8.49
35	11.39	9.64	9.04	8.89
36	11.42	9.71	9.16	8.96
37	11.31	9.80	9.29	9.02
38	11.30	9.81	9.28	9.04
39	11.41	9.71	9.29	8.94

40	11.58	9.73	9.29	8.94

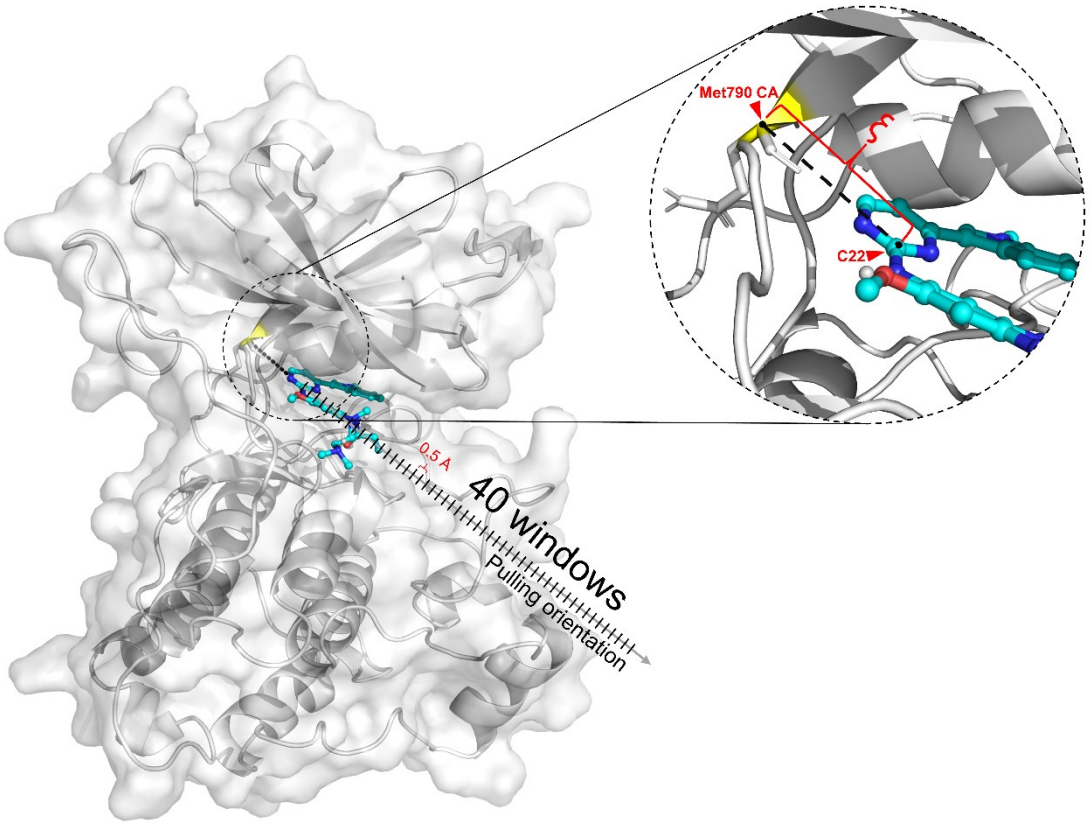

Figure S1. The computational modelling of the EGFR-ligand unbinding path estimated using the US approach. The inhibitor was pulled along the reaction coordinate ξ. The reaction coordinate ξ was defined by the actual distance of CA_{Met790}-$C22_{Osimertinib}$

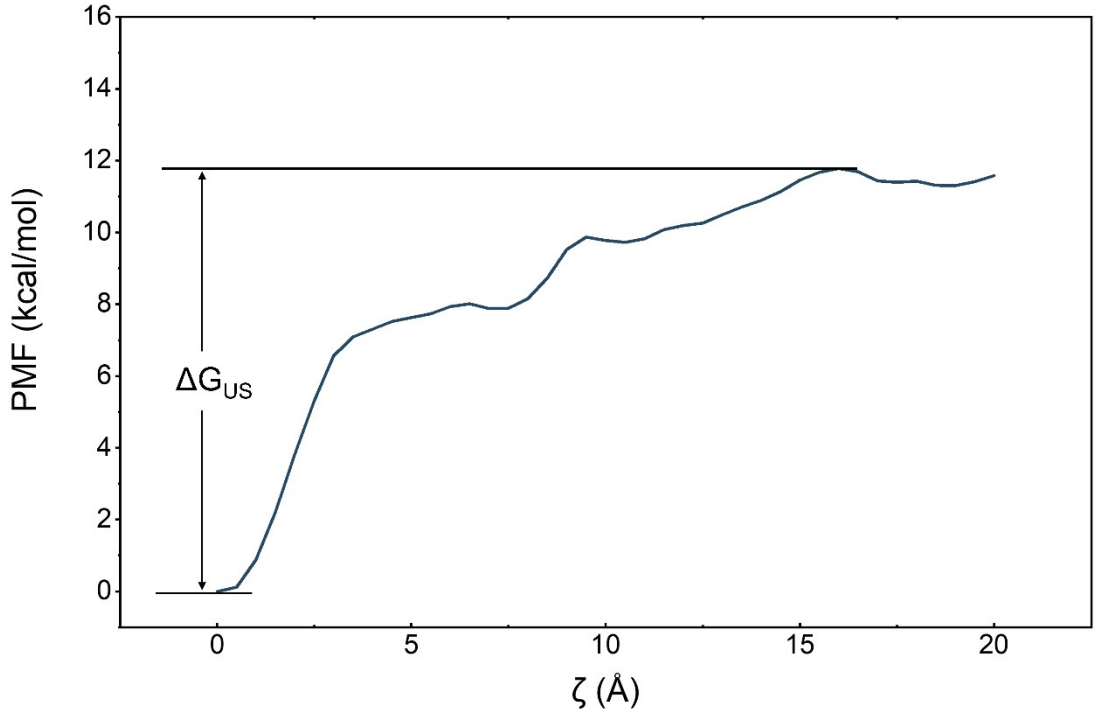

Figure S2. The free energy profile of the AZD9291/EGFR T790M system was obtained via WHAM analysis. The binding free energy ΔG_{US} was described as the difference between the lowest and largest PMF values.

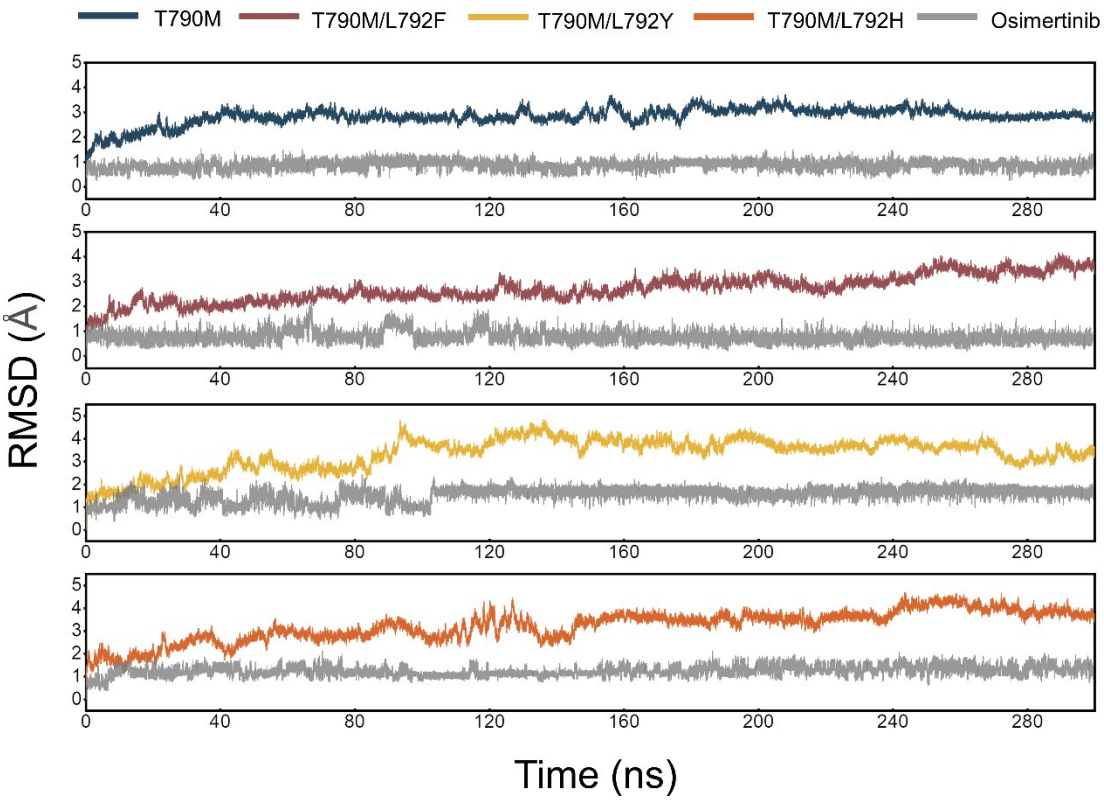

Figure S3. Backbone (CA, C, N atoms) RMSDs are shown as a function of time for EGFR mutants and Osimertinib during 300ns molecular dynamics simulation, using the first frame of the production trajectory as reference.

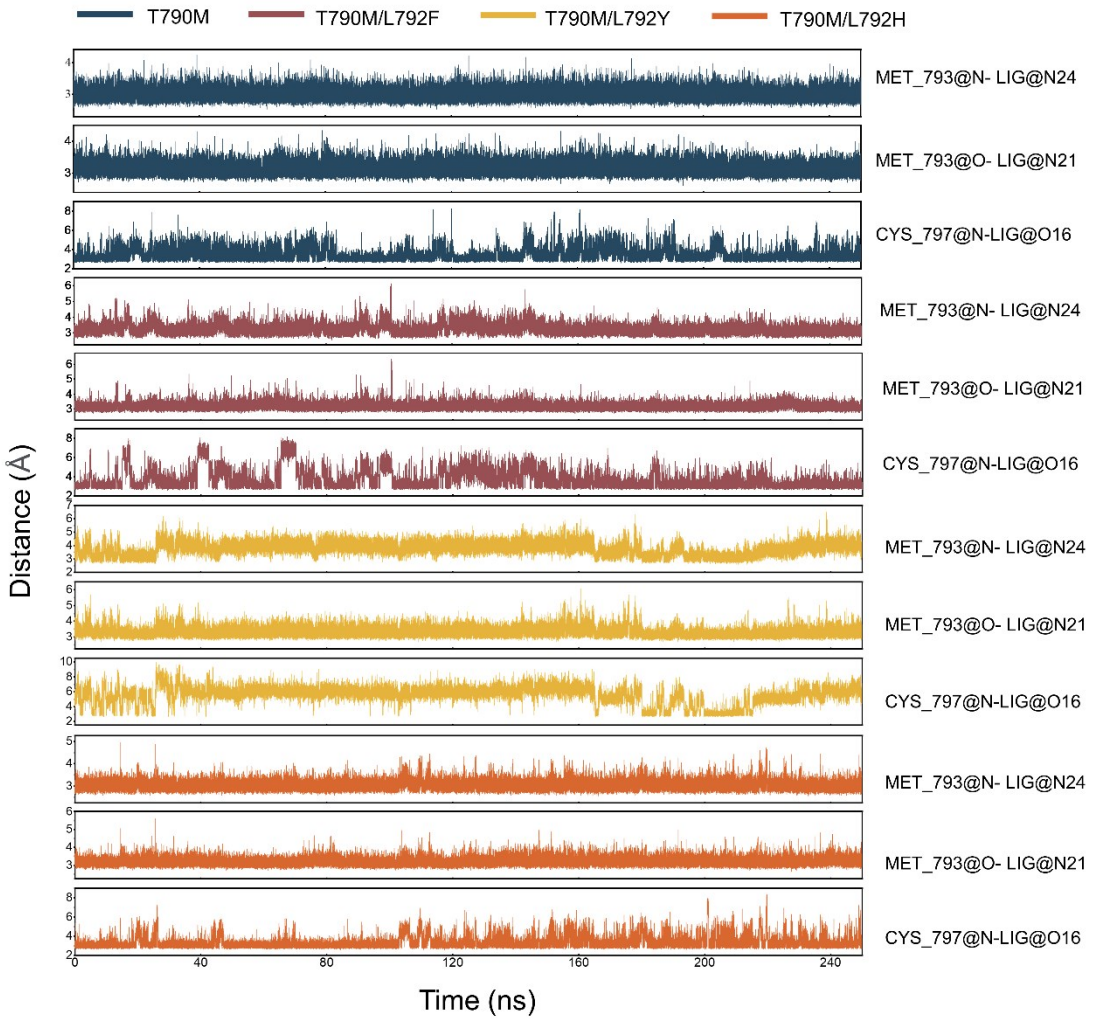

Figure S4. The distance for the hydrogen bonds between Osimertinib and EGFR as a function of time. Here we mainly consider the distance of hydrogen bonds MET_793@N-LIG@N24, MET_793@O-LIG@N21, and CYS_797@N-LIG@O16.

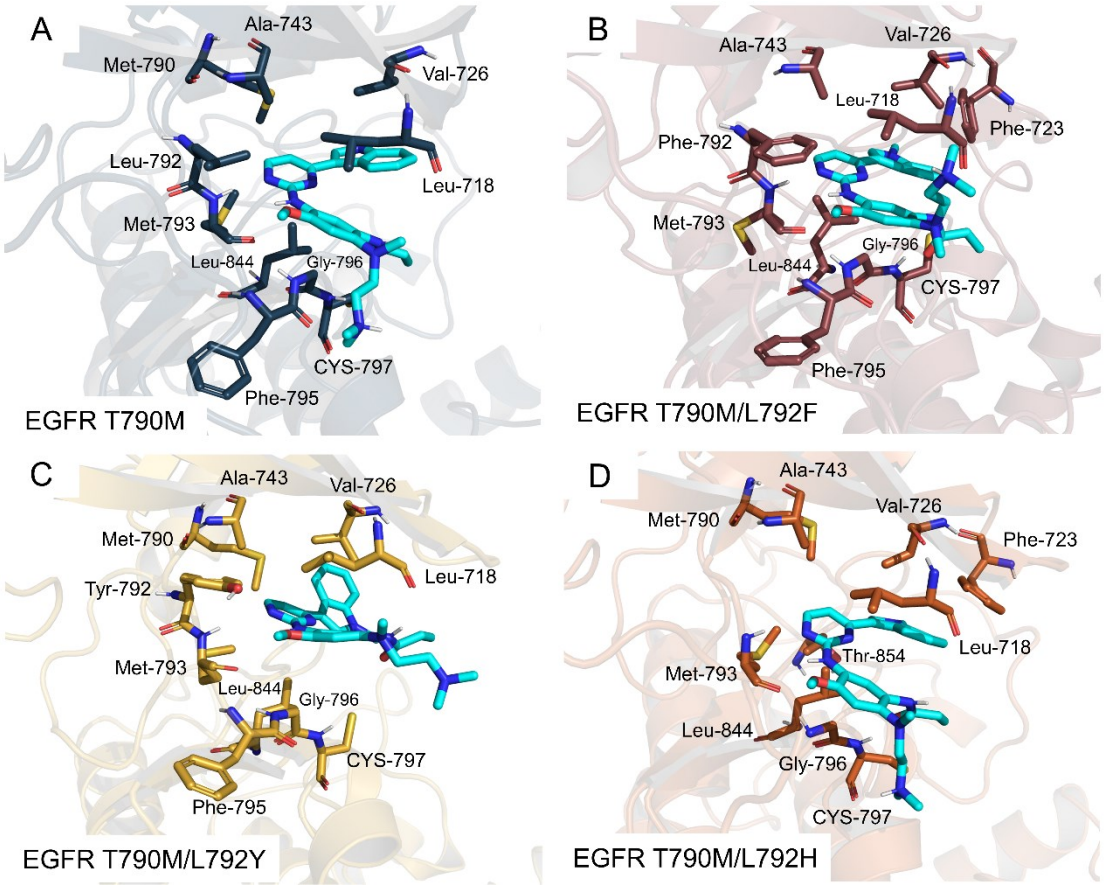

Figure S5. Structure analysis of Osimertinib binding to the ATP-binding pocket of EGFR mutants.

References

[1] Z. Yang, N. Yang, Q. Ou, Y. Xiang, T. Jiang, X. Wu, H. Bao, X. Tong, X. Wang, Y.W. Shao, Y. Liu, Y. Wang, C. Zhou, Investigating Novel Resistance Mechanisms to Third-Generation EGFR Tyrosine Kinase Inhibitor Osimertinib in Non-Small Cell Lung Cancer Patients, Clin Cancer Res 24(13) (2018) 3097-3107.