Supporting Information

Estimation of Protein-Ligand Unbinding Kinetics Using Non-Equilibrium Targeted Molecular Dynamics Simulations

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Table S1. Analytical data for compounds **1j** and **2j**.

#	LC-MS	^{1}H NMR
1j	M+H [m/z] 287.08	1H NMR (250 MHz, DMSO-d6) δ 9.43 (s, 1H), 9.41 (s, 1H), 7.67 (d, J = 1.8 Hz, 1H), 7.54 – 7.48 (m, 1H), 7.45 – 7.33 (m, 3H), 6.71 (d, J = 8.3 Hz, 1H), 6.41 (d, J = 1.8 Hz, 1H), 6.23 (d, J = 2.3 Hz, 1H), 6.09 (dd, J = 8.4, 2.4 Hz, 1H).
2j	M+H [m/z] 415.90	1H NMR (500 MHz, DMSO-d6) δ 7.71 (dd, J = 8.9, 2.2 Hz, 1H), 7.65 – 7.60 (m, 2H), 7.50 (d, J = 8.9 Hz, 1H), 7.47 – 7.44 (m, 1H), 7.36 – 7.31 (m, 1H), 7.31 – 7.26 (m, 1H), 7.26 – 7.23 (m, 1H), 7.21 – 7.01 (m, 2H), 4.99 (s, 2H), 4.67 (s, 2H), 3.00 – 2.93 (m, 2H), 2.70 – 2.63 (m, 2H), 2.35 – 2.28 (m, 2H), 1.84 – 1.69 (m, 2H), 0.85 (t, J = 7.2 Hz, 3H).

Table S2. Data collection and refinement statistics

	2d
Data collection	
Space group	I222
Cell dimensions	
a, b, c (Å)	66.46 90.7 98.53
α, β, γ (°)	90.00, 90.00, 90;00
Resolution (Å)	66.73-1.33 (1.33-1.40)
Nr. observations	245593
Unique reflections	63093 (4299)
Redundancy	4.6 (2.5)
Completeness (%)	91.8 (90.1)
R_{merge} *(%)*[d]	5.2 (38.7)
I/σ(I)	14.8 (1.8)
Refinement	
Resolution (Å)	18.73-1.33
R_{work} *(%)*	17.4
R_{free} *(%)*	18.9
Model composition (No. of atoms)	
Protein	1638
Ligand	33
solvent	354
PDB ID	5LRL

Table S3. Statistics for ligand pulling via path 1 and path 2 (see Figure S3). Errors indicate the 1σ confidence level from bootstrap analysis (see Methods).

compound	<W> along path 1 / kJ/mol	<W> along path 2 / kJ/mol
1b	577 ± 11	974 ± 31
2a	550 ± 19	659 ± 16
2aa	508 ± 15	718 ± 27

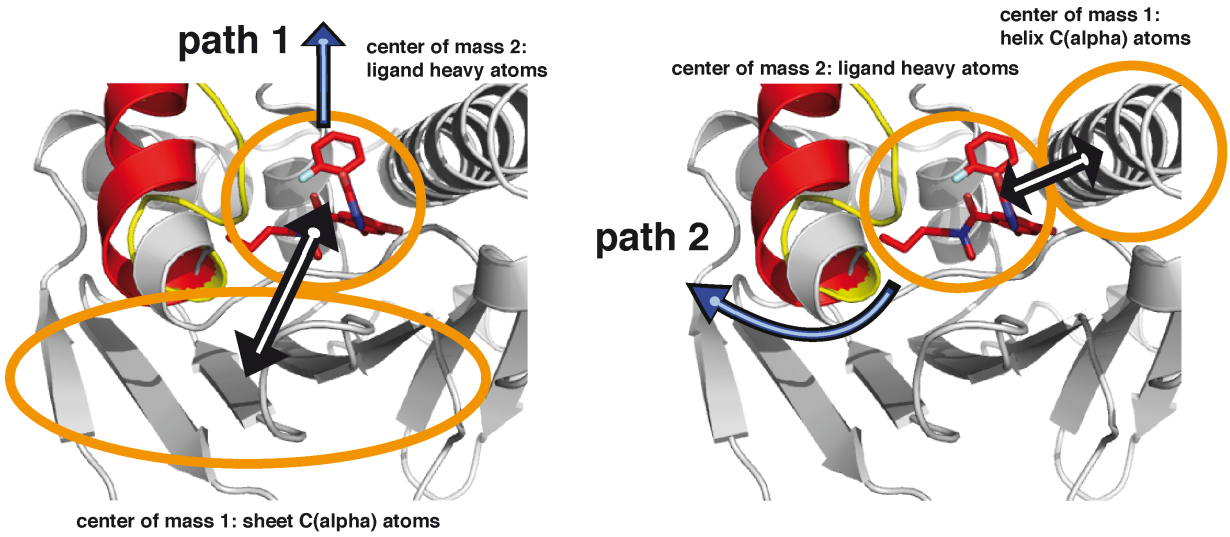


Figure S1: Definition of reaction coordinates in TMD runs.

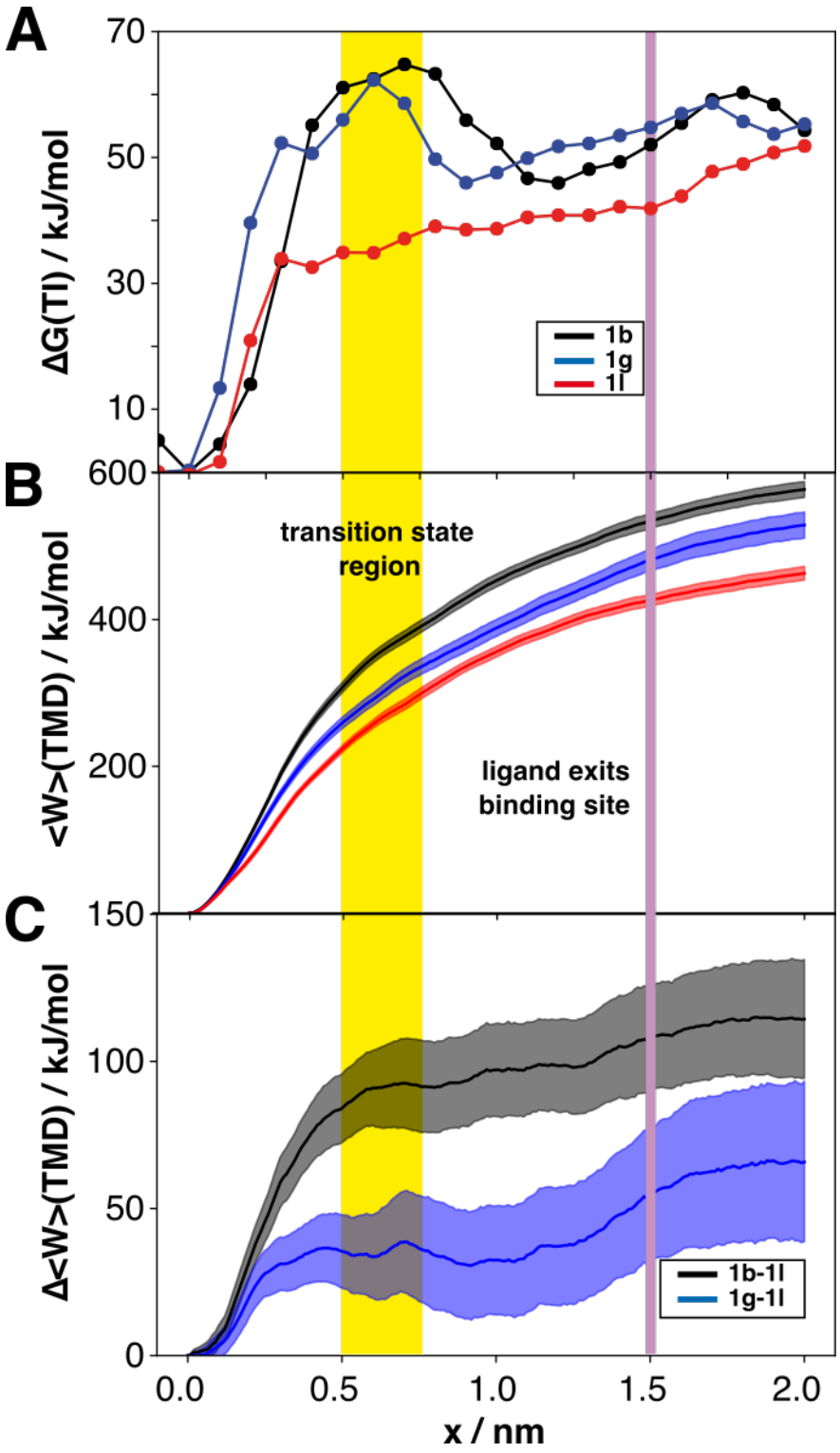
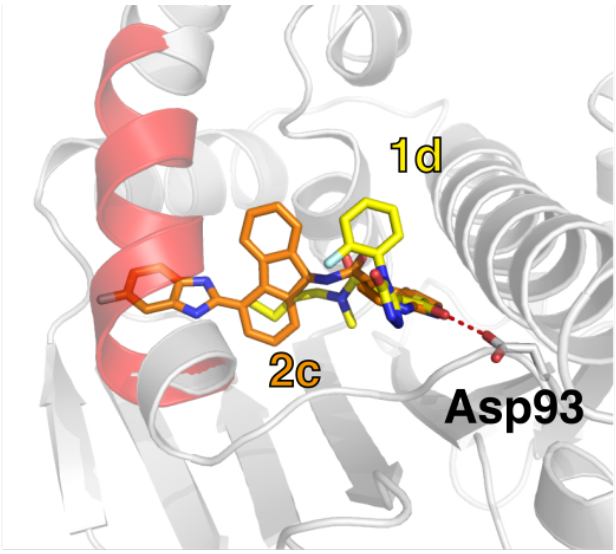
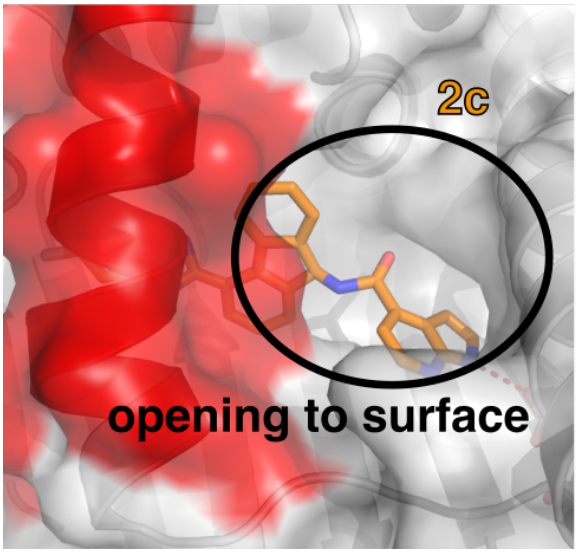


Figure S2: Free energy profile ΔG (A) and non-equilibrium work <W> (B) for compounds **1b**, **1g**, and **1l** calculated via thermodynamic integration and non-equilibrium TMD. C: differences of <W> referenced to **1l**. The shaded surfaces represent the 1σ level from bootstrap analysis (see Methods). The range in *x* which corresponds to the transition state region highlighted in yellow, distance at which the ligand exits the binding site highlighted in purple.





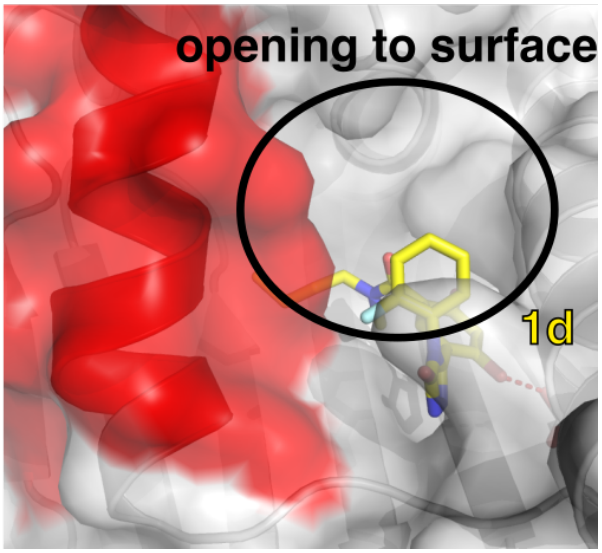


Figure S3: comparison of binding mode of resorcinol compound **1d** and N-heterocycle compound **2c**. Hydrogen bonds displayed as red dashes.

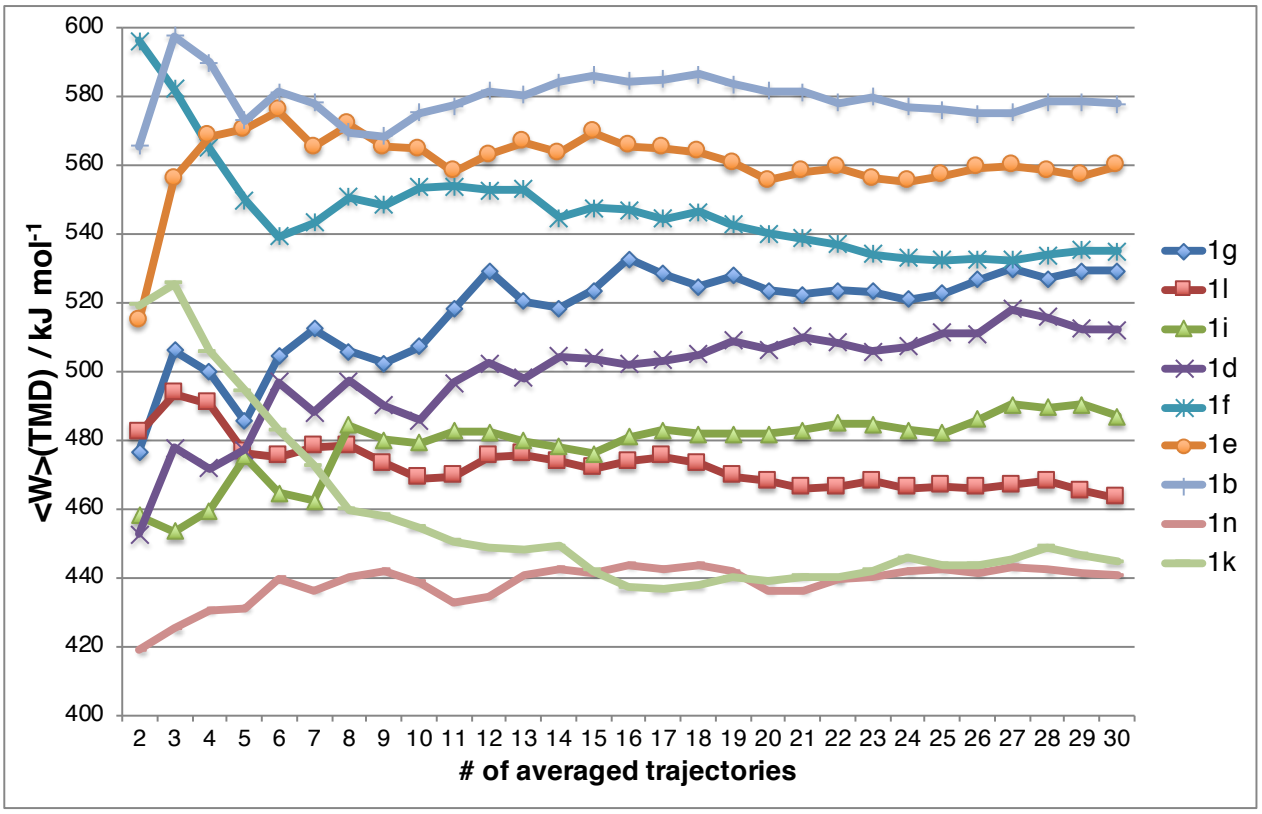


Figure S4: Convergence of non-equilibrium work <W> in dependence to the number of averaged trajectories for group **1** helix binding compounds. The work appears to be converged after ca. 25 averaged trajectories.

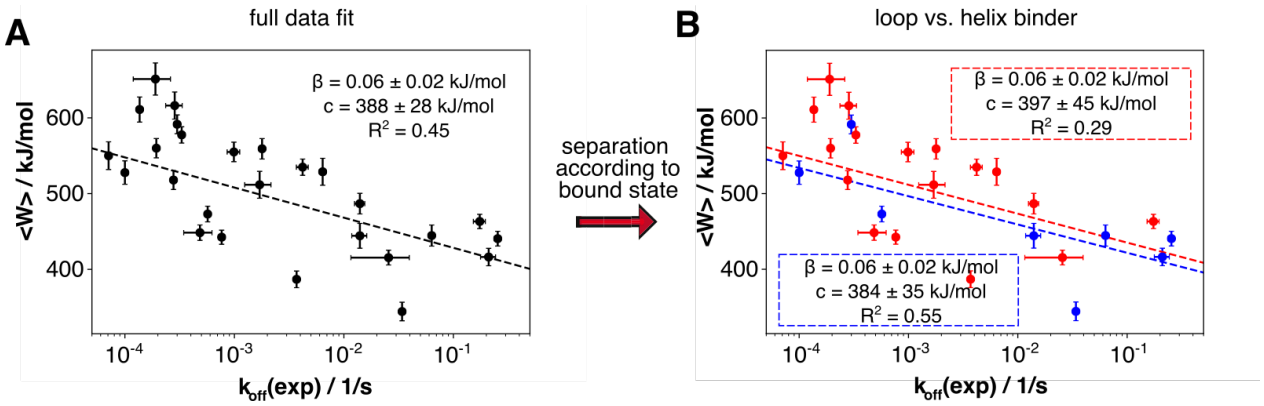


Figure S5: Model building for rationalization of non-equilibrium work and kinetic data. Vertical error bars indicate the 1σ level from bootstrap analysis (see Methods), horizontal error bars indicate the standard error of the mean (SEM) for N=2-4 measurements. A: fit to full data set, i.e., all compounds. B: separation into helix- (red) and loop-binding compounds (blue).

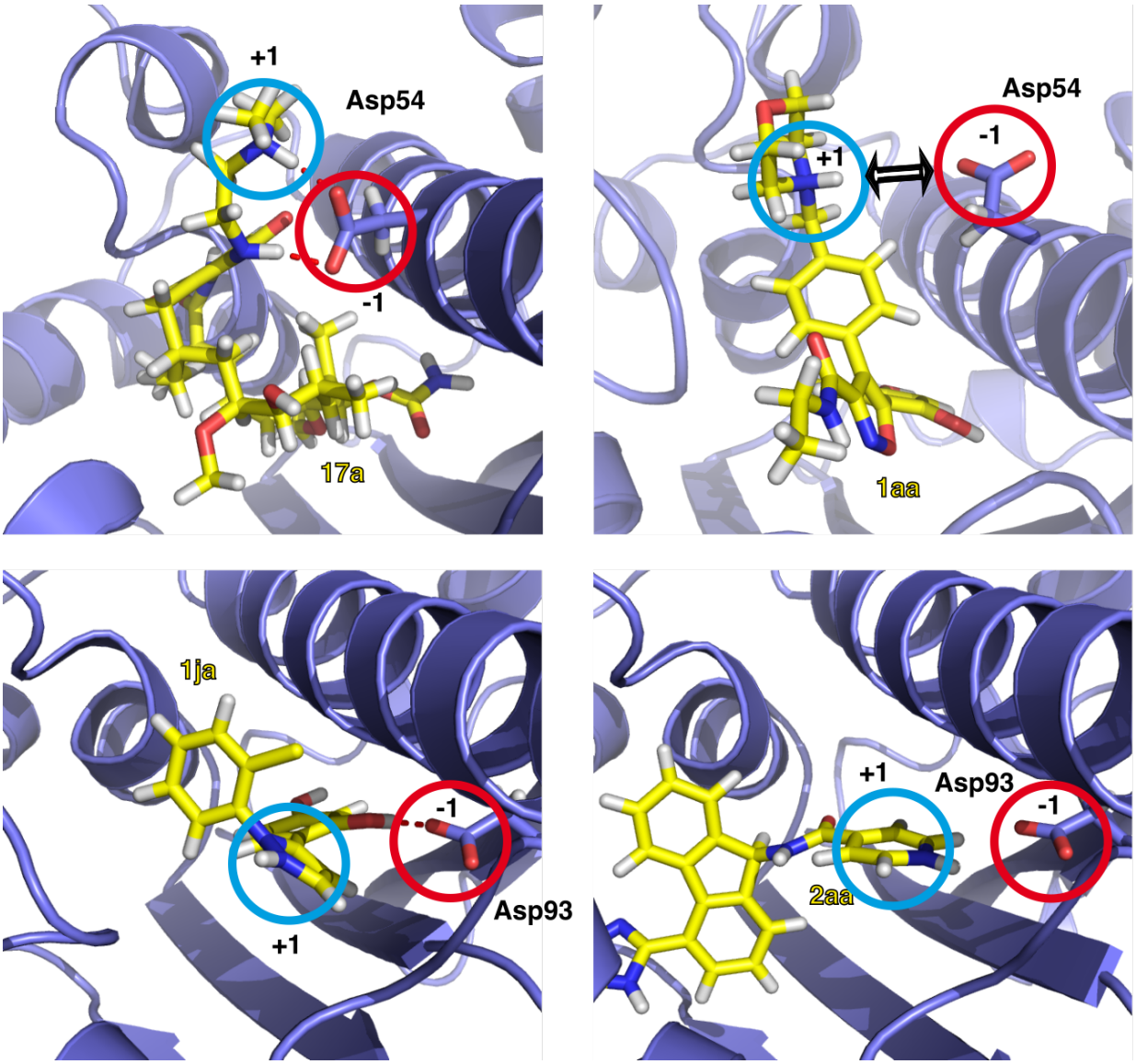


Figure S6. Charge interaction of compounds **17a**, **1aa**, **1ja** and **2aa** with Hsp90. Hydrogen bonds displayed as red dashes.

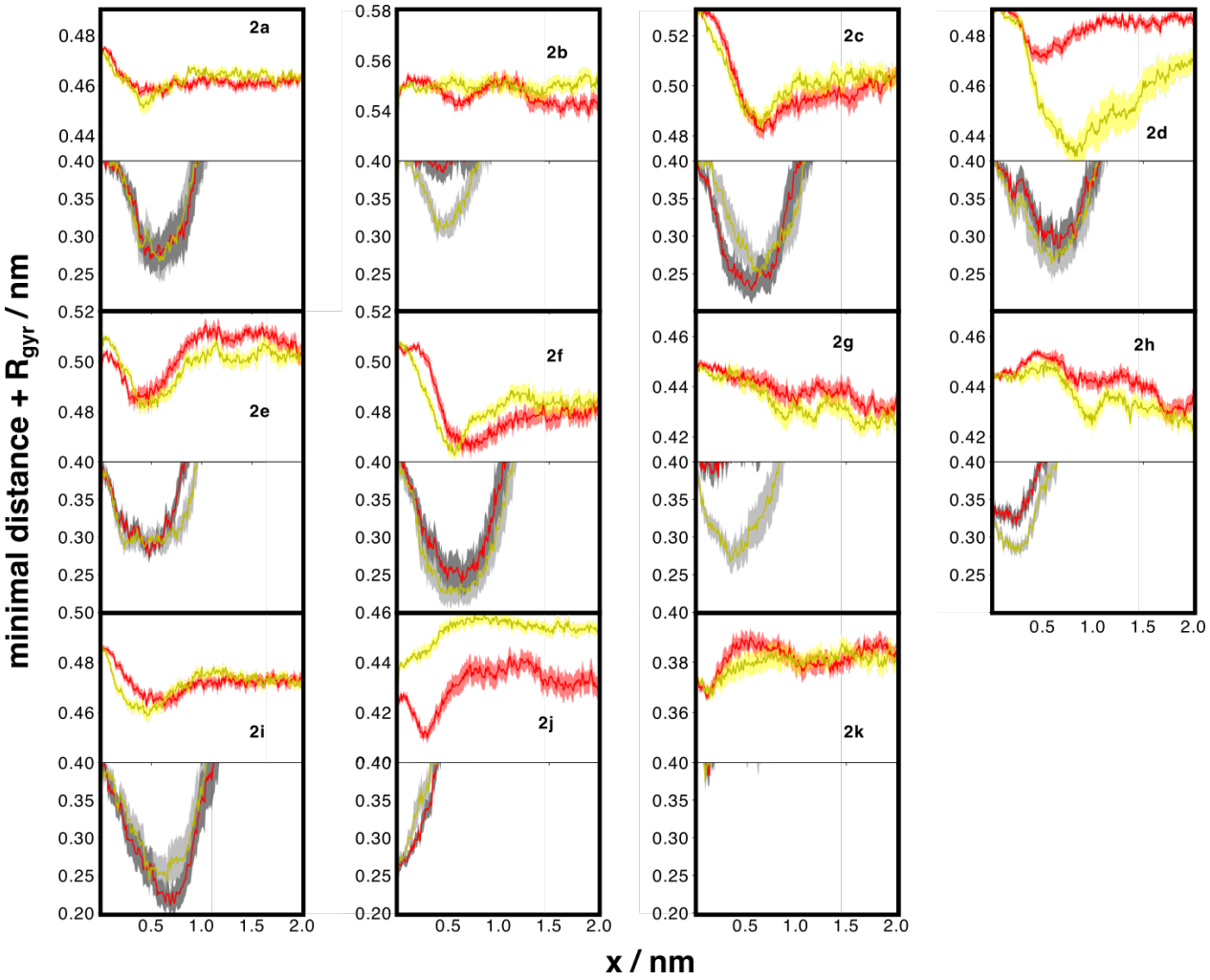


Figure S7. Electrostatic facilitation in group **2f** compounds. Radius of gyration and minimal distances between ligand and Asp102 as average of N=30 simulations. Trajectory means as lines, 1σ error level from bootstrap analysis (see Methods) as shaded area. Uncharged ligand **2f** in red, protonated form **2fa** in yellow. Radii shaded in red and yellow, respectively, minimal distances in dark and light gray, respectively.