Fluoroalkyl and Alkyl Chains Have Similar Hydrophobicities in Binding to the ‘‘Hydrophobic Wall’’ of Carbonic Anhydrase

Supporting Information

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Purification of HCA.

Wild-type HCA II was purified from the crude cell lysate in two steps. First, the lysate was added to agarose resin that was functionalized with aminomethyl-benzenesulfonamide, washed with Triton-X (0.1%) and with Triton-X (0.1%) and sodium sulfate (500 mM), and eluted with sodium azide (400 – 600 mM). Second, this eluate was purified further by size-exclusion chromatography on Superdex 75 using 10 mM sodium phosphate, pH 7. 6 as running buffer. The concentration of the pooled fractions of HCA II (100-500 μM) was measured by UV-Vis spectroscopy. The purified protein was diluted to 50 μM in buffer (10 mM sodium phosphate, pH 7.6) and used for ITC experiments.

Isothermal Titration Calorimetry.

Sulfonamides were dissolved in DMSO at a concentration ~20 mM. These solutions were diluted to ~2.0 μM in sodium phosphate (10 mM, pH = 7.6), mixed, and used for ITC experiments.

Titration experiments were conducted at 298.15 K, using an Auto VP-ITC instrument (MicroCal). The titration of an HCA II sample (20 μM) into a solution of sulfonamide ligand (~ 2.0 μM) comprised 10 injections (initially one injection of 1 μL followed by nine injections of 29 μL per injection, with a 400-second interval between injections). The initial data point from the 1 μL injection was deleted from the integrated data to minimize the effect of diffusive mixing that occurs during equilibration of the instrument.

Crystal Growth and Ligand Soaking Experiments.

Aliquots of HCA II (purified as described in the text) were concentrated to 200 – 800 μM in 50 mM Tris-SO_4 buffer (pH = 7.6). These solutions were mixed in equal volume with crystallization buffer (1.14 – 1.25 M sodium citrate, pH = 7.8) and suspended on glass slides over 1.0 mL of crystallization buffer in the well of a 24-well plate at 4°C. Crystals typically grew within one week to typical sizes of 250 x 200 x 100 μm, and were stored at 4°C until needed. Soaking experiments were performed as described in the main text.

X-ray Diffraction Experiments.

Crystals of HCA II soaked with ligand were harvested with cryoloops, flash frozen in liquid nitrogen, and shipped to the National Synchrotron Light Source. Data were collected on an ADSC Quantum Q315 CCD detector at beamlines X25 and X29 in collaboration with the Mail-Program. The dataset were indexed and scaled using HKL2000.[1]

Solution of Crystal Structures.

Diffraction data were analyzed using the CCP4i suite of crystallography software.[2] To obtain phase information for each data set, we used Phaser[3] for molecular replacement with a previously reported structure of native HCA II (PDBID: 2ILI), from which we removed the atoms of water and zinc. After one round of restrained refinement using REFMAC,[4] we used OMIT to generate a weighted difference electron density map (OMIT map). For every data set, visual inspection showed contiguous electron density (at ~1σ on the OMIT map) for residues 3 – 259, and well-defined electron density for the ligands in the active site. We added the atoms of

the ligand and refined them locally. We added molecules of water in 4 – 6 cycles (with 5 – 10 intervening rounds of restrained refinement) to the peaks in the difference map that were >5σ.

Figure S1. Dependence of $\Delta G°_b$ for binding of benzenesulfonamide ligands ($H_2NO_2SC_6H_4$-$CONHCH_2(CX_2)_nCX_3$, X = H, F) on surface area (top) and volume of ligands (bottom).

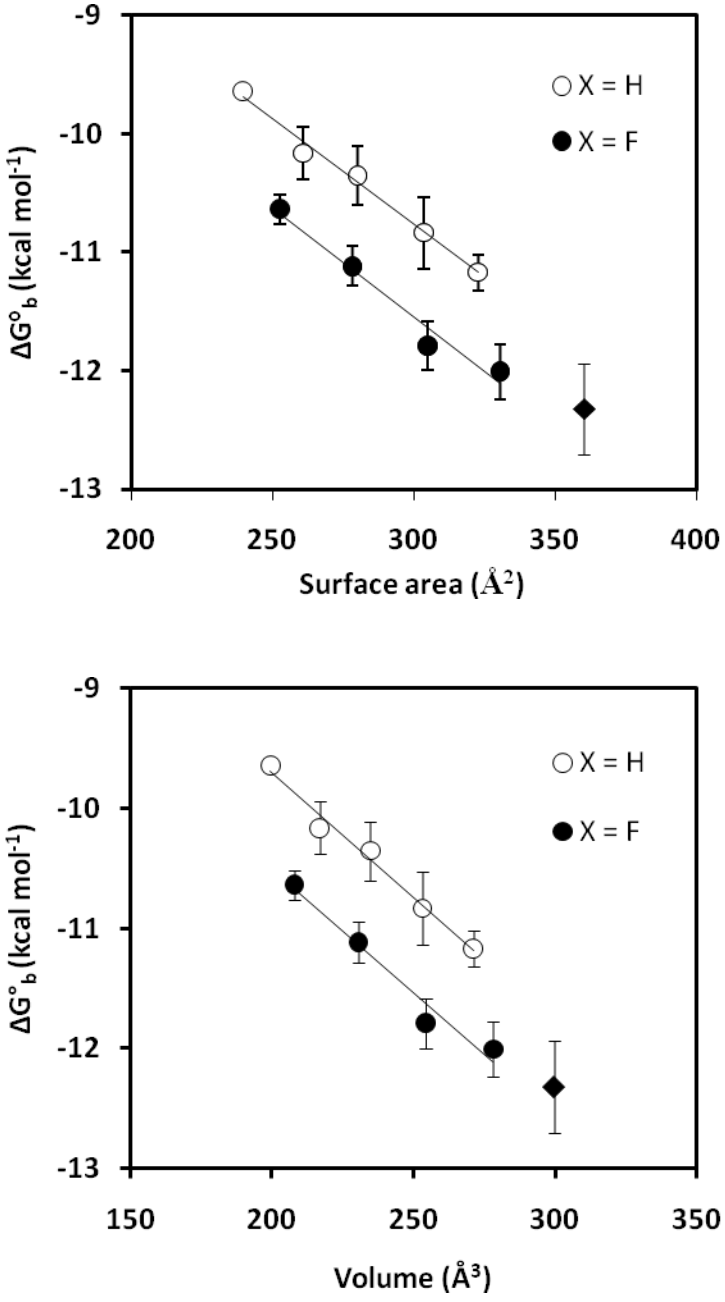


Figure S2. Dependence of $\Delta H°_b$ for binding of benzenesulfonamide ligands ($H_2NO_2SC_6H_4$-$CONHCH_2(CX_2)_nCX_3$, X = H, F) on surface area (top) and volume of ligands (bottom).

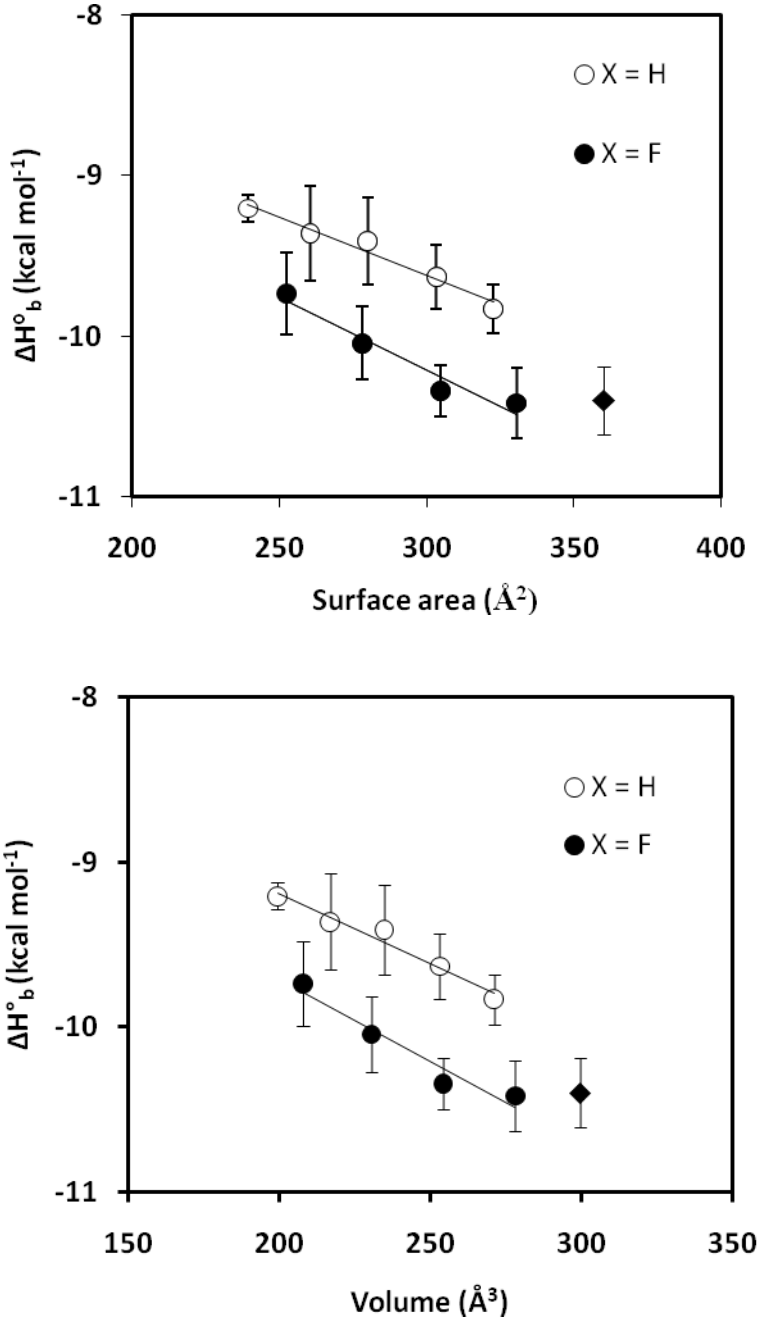
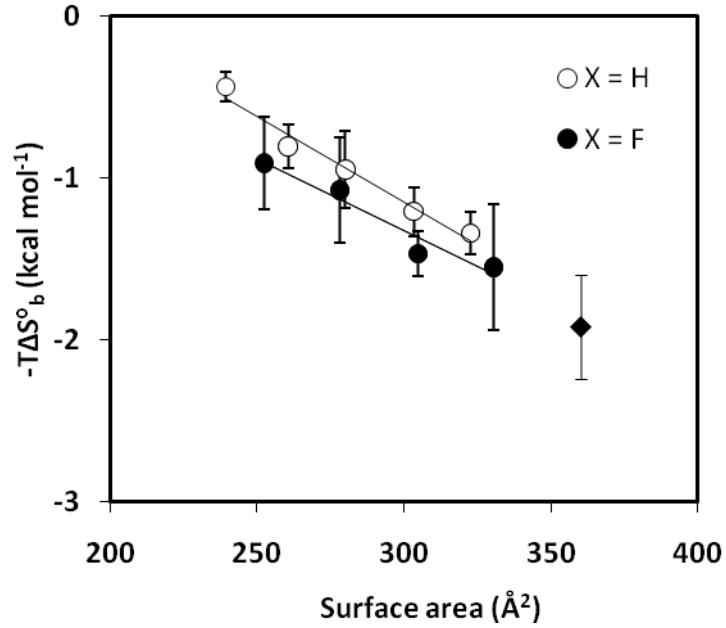


Figure S3. Dependence of $-T\Delta S°_b$ for binding of benzenesulfonamide ligands ($H_2NO_2SC_6H_4$-$CONHCH_2(CX_2)_nCX_3$, X = H, F) on surface area (top) and volume of ligands (bottom).



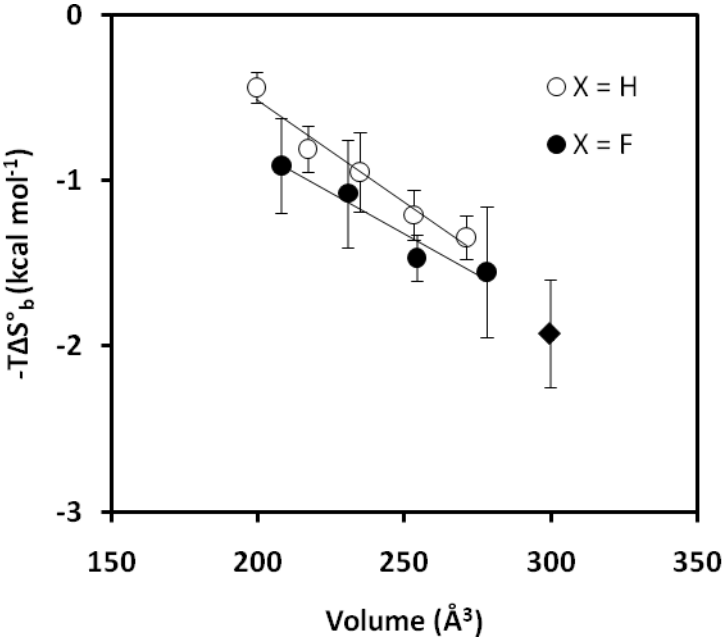


Figure S4. Electron density map shows ligand in the active site. The atoms of HCA and those of the ligands (R_H; n = 4) appear as stick representations, the Zn^{2+} appears as a silver sphere, and one water molecule appears as a red sphere. The mesh represents the electron density ($F_o - F_c$) derived from molecular replacement using the coordinates for a reported structure of the native protein (PDB: 2ILI). Electron density for the ligand appears dark blue and that for the protein light blue.

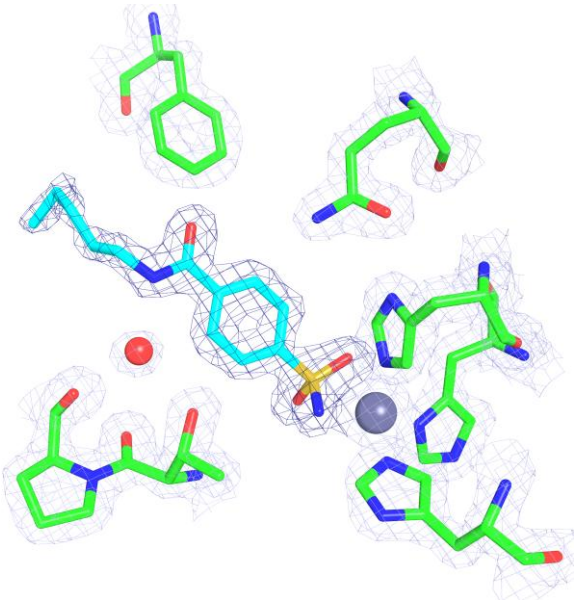


Figure S5. Comparing the ^{1}H NMR chemical shift of the amide hydrogen for R_H and R_F. Ligands exhibit a significant difference in the chemical shift between the two series ($\Delta\Delta H°_b$ = ~0.7 ppm), but the change in chemical shift across each series is negligible.

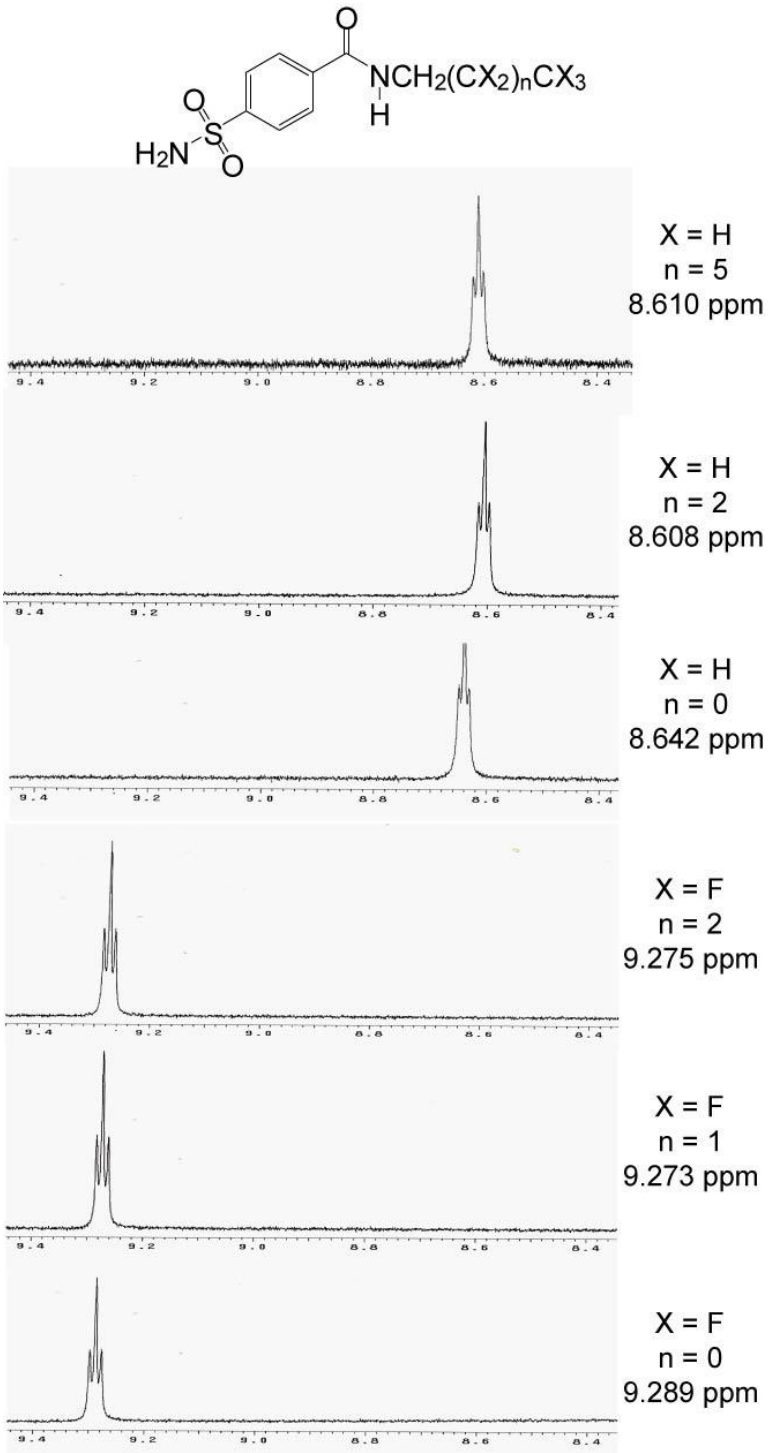


Figure S6. Individual images of the each of the ligands coordinated to the active site zinc ion. Structures for both R_H and R_F are presented from two different viewpoints.

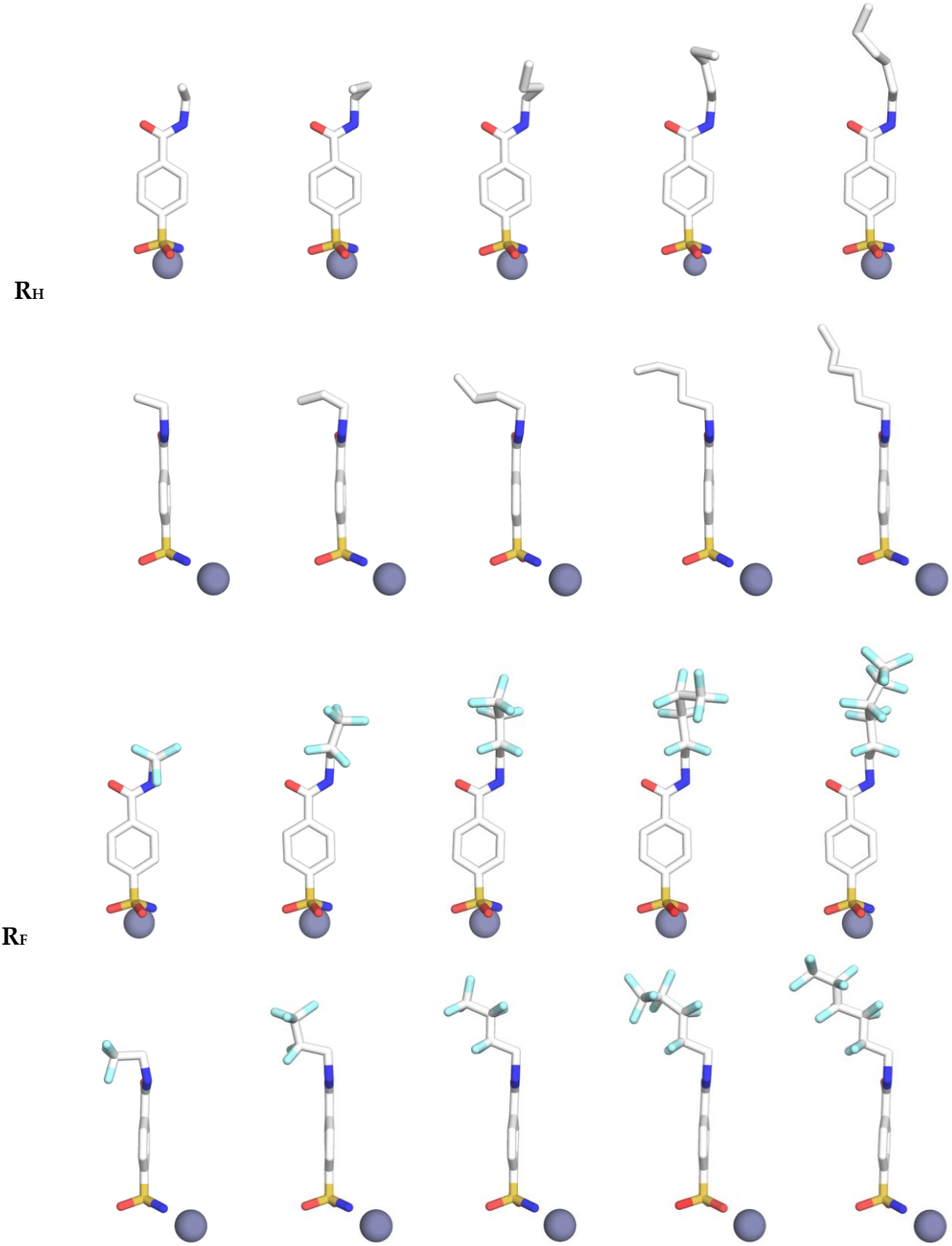
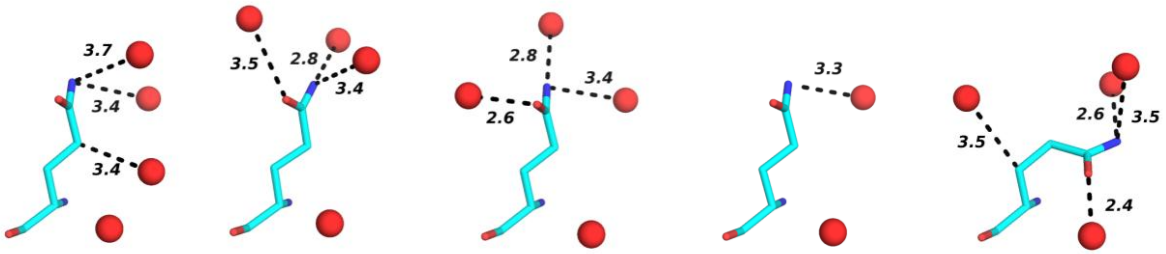


Figure S7. Crystallographically defined water molecules contacting Gln136 in structures with fluorinated ligands.



n = 0 n = 1 n = 2 n = 3 n = 4

Table S1. Crystallographic data.

X, n	**H, 0**	**H, 1**	**H, 2**	**H, 3**	**H, 4**	**F, 0**	**F, 1**	**F, 2**	**F, 3**	**F, 4**
Unit cell[a] (Å)	42.37, 41.45, 72.09	42.00, 41.18, 71.45	42.61, 41.87, 72.35	42.30, 41.63, 72.30	42.22, 41.54, 72.18	42.55, 41.72, 72.34	42.58, 41.78, 72.35	42.41, 41.70, 72.36	42.28, 41.68, 72.33	42.37, 41.88, 72.56
(°)	90.00, 104.54, 90.00	90.00, 104.51, 90.00	90.00, 103.37, 90.00	90.00, 104.52, 90.00	90.00, 104.56, 90.00	90.00, 104.20, 90.00	90.00, 103.58, 90.00	90.00, 104.81, 90.00	90.00, 104.66, 90.00	90.00, 104.65, 90.00
Resolution Range[b] (Å)	35.62-1.20 (1.22-1.19)	20.98-1.15 (1.19-1.16)	21.26-1.80 (1.85-1.80)	39.98-1.65 (1.69-1.65)	30.63-1.70 (1.75-1.70)	23.38-1.40 (1.43-1.39)	20.02-1.60 (1.64-1.60)	30.78-1.37 (1.41-1.37)	35.81-1.51 (1.55-1.51)	35.10-1.80 (1.84-1.80)
Reflections (#)	72206 (5133)	79221 (5376)	20971 (1502)	25854 (1287)	25463 (1865)	41793 (1369)	27856 (1642)	51504 (2950)	36181 (2588)	21145 (1484)
Completeness (%)	100 (100)	95.00 (91.33)	100 (100)	92.13 (61.56)	99.81 (99.19)	100 (100)	100 (100)	92.30 (83.30)	98.92 (96.50)	96.00 (91.52)
R_{factor}	0.133 (0.215)	0.124 (0.184)	0.138 (0.124)	0.148 (0.34)	0.130 (0.206)	0.131 (0.361)	0.165 (0.352)	0.138 (0.244)	0.127 (0.177)	0.128 (0.183)
R_{free}	0.168 (0.227)	0.152 (0.221)	0.206 (0.225)	0.202 (0.487)	0.186 (0.326)	0.170 (0.393)	0.240 (0.400)	0.183 (0.307)	0.177 (0.265)	0.203 (0.353)
Bond Length	0.026	0.028	0.023	0.024	0.024	0.025	0.023	0.025	0.025	0.023
Angles	2.275	2.312	1.984	1.807	1.94	2.063	2.005	2.138	2.089	1.956
PDBID	3RYV	3RYY	3RZ0	3RZ5	3RZ8	3RYJ	3RYX	3RYZ	3RZ1	3RZ7

[a] All crystals belonged to the $P2_1$ space group. [b]Values in parentheses represent those for the highest resolution shell.

References.

1. Otwinowski Z.; Minor W., "Processing of X-ray Diffraction Data Collectedin Oscillation Mode", Methods in Enzymology, Macromolecular Crystallography, part A, **1997**, *276*, 307-326.

2. Collaborative Computational Project, Acta Cryst., **1994**, D50, 760-76.

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4. McCoy A. J.; Grosse-Kunstleve R. W.; Adams P. D.; Winn M. D.; Storoni L. C.; Read R. J. J. Appl. Cryst. **2007**, *40*, 658-674.