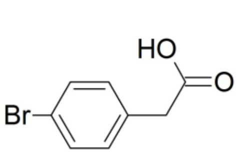
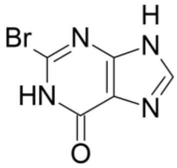
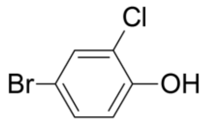
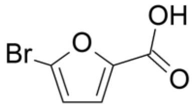
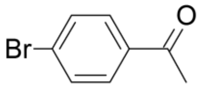
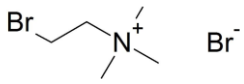
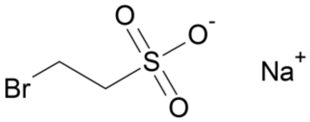
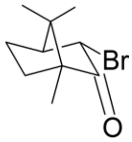
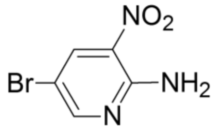
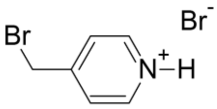
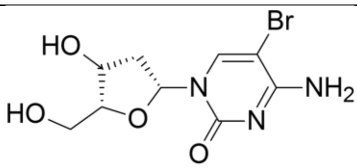
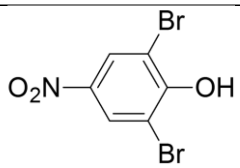
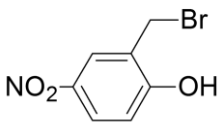
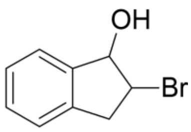
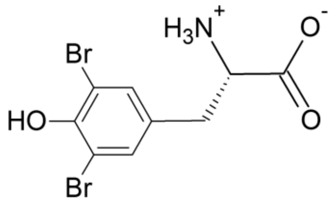
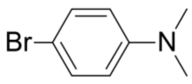
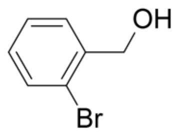
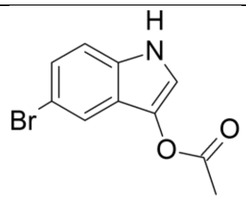
Supplementary Material

Supplementary Table S1. Compound cocktails A to F were dissolved in the cryo-buffer for the final concentrations given.

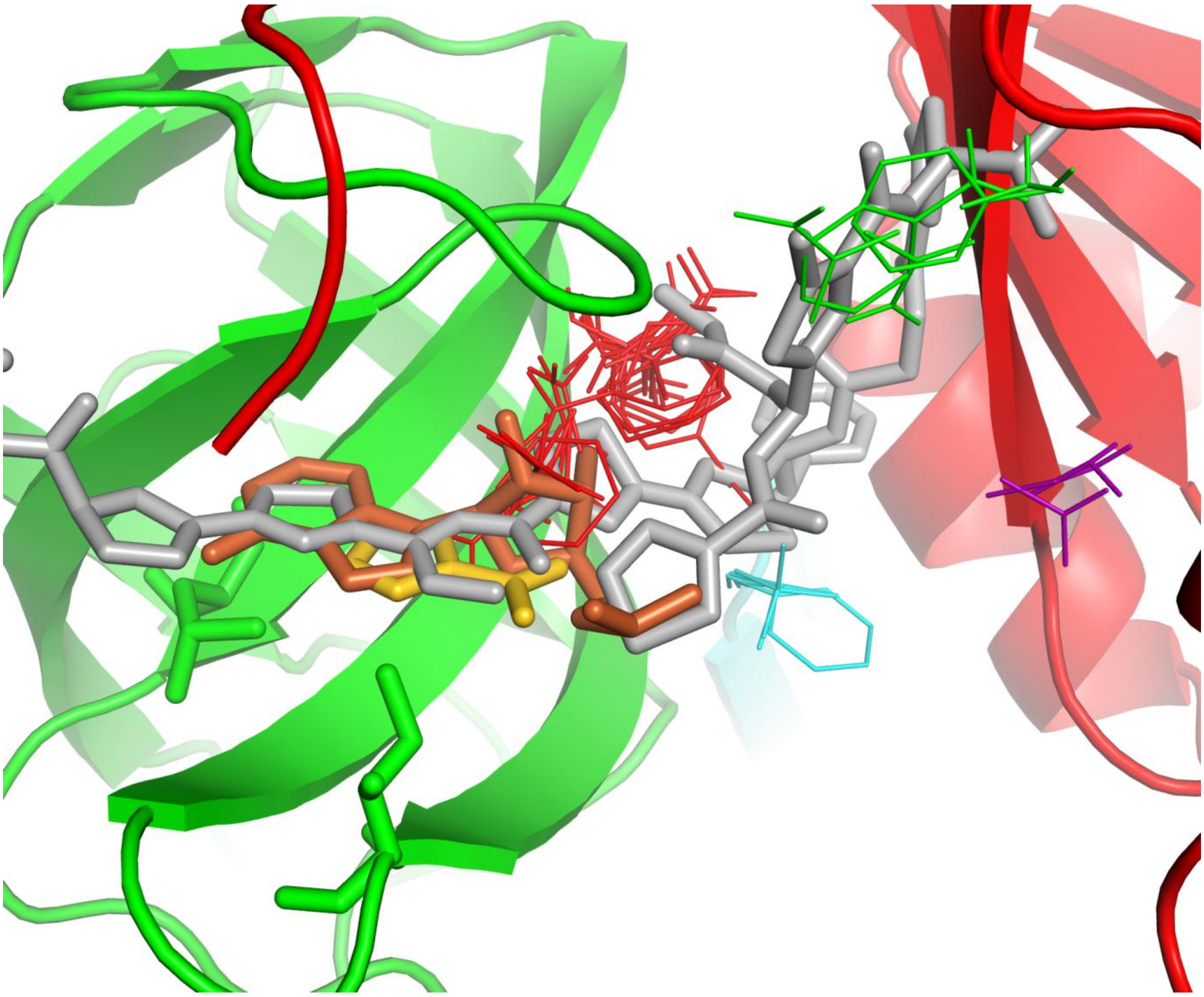
A			
	4-Bromophenylacetic acid	4-Bromoisoquinoline	4-Bromo-2-chlorophenol
	215.04 Da 4.5 mM	215.01 Da 7.5 mM	207.45 Da 4.5 mM
B			
	5-Bromo-2-furancarboxylic acid	4-Bromoacetophenone	Bromocholine
	190.98 Da 7.5 mM	199.04 Da 7.5 mM	246.97 Da 5.0 mM
C			
	2-Bromoethanesulfonic acid sodium	(+)-3-Bromocamphor	2-Amino-3-nitro-5-bromopyridine
	211.01 Da 5.0 mM	231.13 Da 5.0 mM	218.01 Da 5.0 mM
D			
	4-Bromomethylpyridine hydrobromide	5-Bromodeoxy cytidine	2.6-Dibromo-4-nitrophenol
	252.93 Da 6.0 mM	306.11 Da 5.0 mM	296.90 Da 5.0 mM
E			
	2-Hydroxy-5-nitrobenzyl bromide	2-Bromo-1-indanol	3.5-Dibromo-L-tyrosine
	232.03 Da 6.0 mM	213.07 Da 5.0 mM	338.98 Da 4.5 mM

F

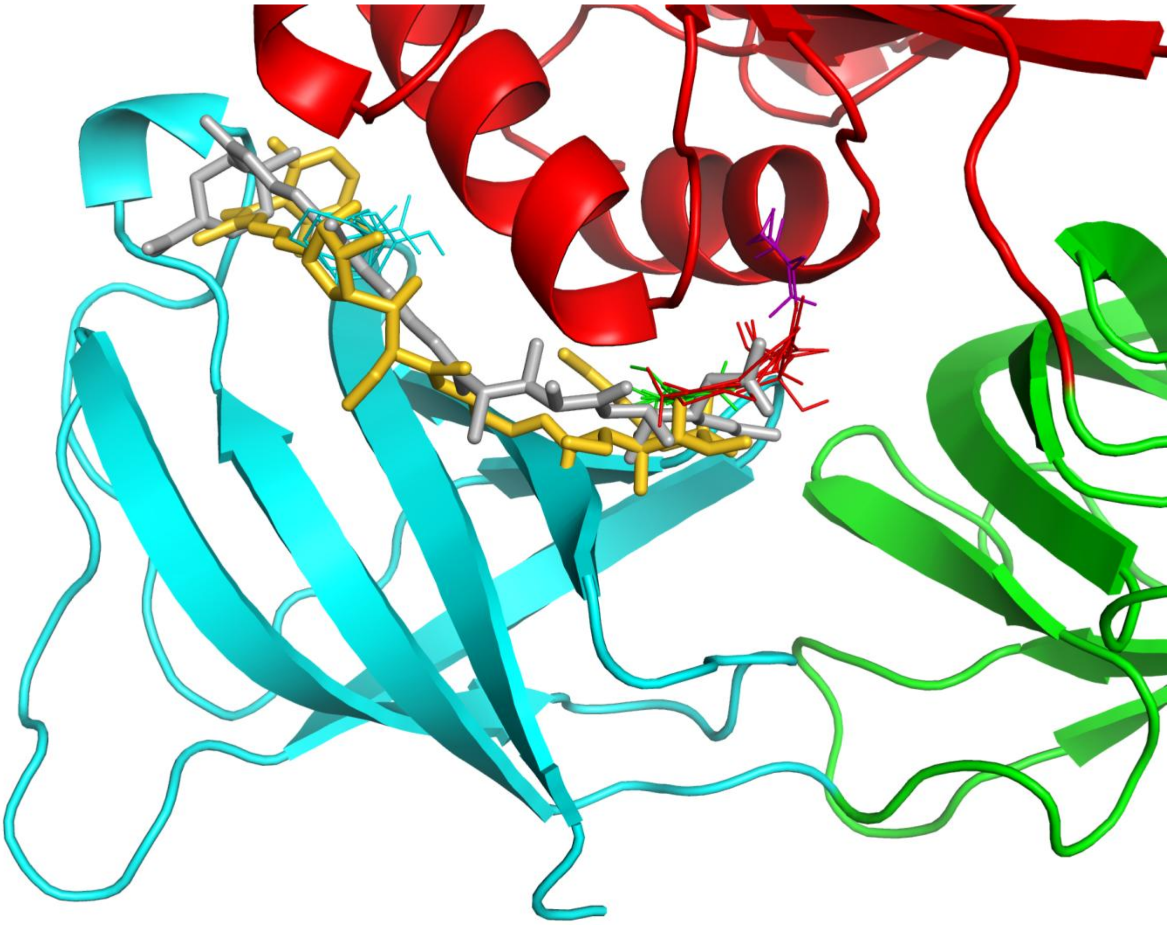
		
4-Bromo-dimethylaniline	2-Bromobenzyl alcohol	3-Acetoxy-5-bromoindole
200.08 Da 5.0 mM	187.03 Da 5.0 mM	254.08 Da 5.0 mM

Supplementary Table S2. Values in parentheses are for the highest resolution bin. Each data set was collected from individual, single crystals. From 'Anom. completeness' to 'DelAnom correlation between half-sets' statistics are reported from AIMLESS. All other statistics are reported from Phenix.

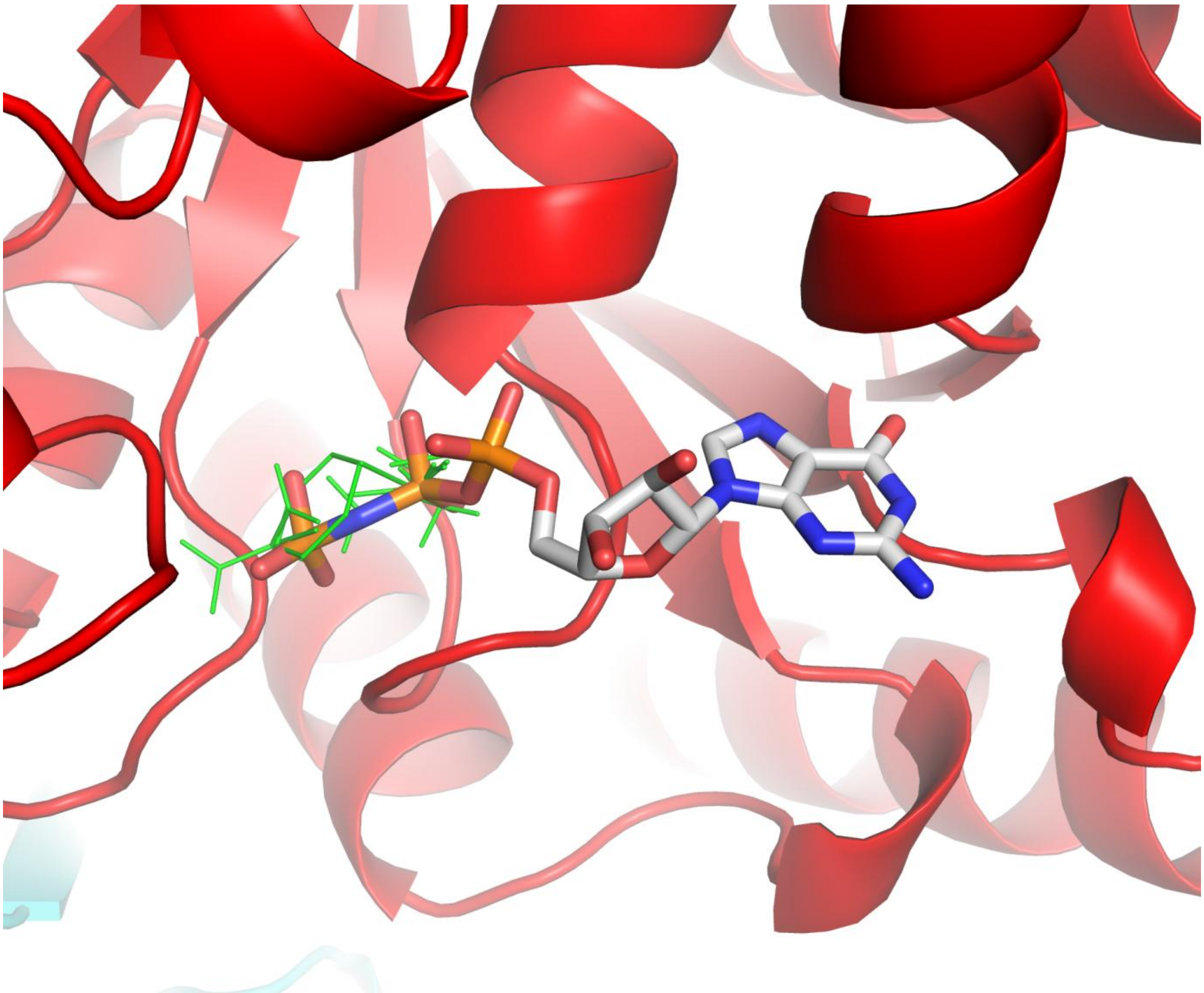
	Cocktail A	Cocktail C	Cocktail D	Cocktail E	Cocktail F
PDB code	4LBV	4LBW	4LBY	4LBZ	4LC0
Space group	C2	C2	C2	C2	C2
Wavelength (Å)	0.9000	0.9000	1.5418	1.5418	1.5418
Temp. of collection (K)	100	100	100	100	100
Images / degrees per image	315 / 0.5	360 / 0.5	180 / 1	200 / 1	180 / 1
Resolution range	28.08-2.03 (2.102-2.029)	30.82-1.741 (1.803- 1.741)	33.35-2.692 (2.788- 2.691)	28.26-2.223 (2.303-2.223)	30.8-2.221 (2.301-2.221)
Unit cell	*a*=147.38 *b*=98.61 *c*=39.78 β=95.97°	*a*=147.22 *b*=98.49 *c*=39.70 β=95.68°	*a*=147.48 *b*=98.38 *c*=39.65 β=95.85°	*a*=146.44 *b*=98.4 *c*=39.68 β=95.93°	*a*=146.83 *b*=98.44 *c*=39.70 β=95.79°
Total reflections	122415 (12256)	218723 (21803)	58421 (5744)	111797 (10380)	100226 (8757)
Unique reflections	36477 (3665)	57510 (5749)	15614 (1543)	27415 (2673)	27510 (2585)
Completeness (%)	99.77 (99.78)	99.86 (99.86)	99.73 (99.74)	99.60 (98.09)	99.25 (94.38)
Multiplicity	3.3 (3.3)	3.8 (3.8)	3.7 (3.7)	4.1 (3.9)	3.7 (3.4)
Anom. completeness (%)	97.6 (98.0)	97.9 (98.7)	95.1 (94.8)	96.5 (93)	90.3 (74.2)
Anom. multiplicity	1.7 (1.6)	1.8 (1.8)	1.8 (1.8)	2.0 (1.8)	1.7 (1.4)
*R*merge (within I+/I-, %)	5.6 (44.8)	3.8 (56.6)	9.4 (52.6)	8.8 (55.7)	5.3 (19.2)
*R*merge (all I+/I-, %)	7.3 (53.3)	5.3 (69.8)	11.5 (63.9)	11.9 (62.9)	8.1 (23.7)
DelAnom correlation between half-sets	0.150 (0.037)	0.400 (0.011)	0.065 (0.042)	0.349 (0.029)	0.547 (0.173)
$I/\sigma(I)$	14.43 (2.71)	17.56 (2.16)	12.65 (2.41)	13.78 (2.54)	14.25 (5.46)
CC½	0.997 (0.768)	0.998 (0.716)	0.993 (0.692)	0.995 (0.732)	0.993 (0.917)
CC*	0.999 (0.932)	1.000 (0.914)	0.998 (0.904)	0.999 (0.92)	0.998 (0.978)
*R*work / *R*free (%)	16.15 / 19.30	17.05 / 19.43	15.59 / 20.73	16.40 / 21.25	16.07 / 20.05
RMS deviation from ideal					
Bonds (Å)	0.008	0.007	0.008	0.008	0.008
Angles (°)	1.17	1.14	1.16	1.14	1.16
Average *B*-factors ($Å^2$)					
Protein	32.30	37.20	41.40	38.90	39.30
Solvent	36.90	44.40	34.90	43.80	41.70
Ligands	54.00	45.80	54.40	52.90	56.60
Wilson *B*-factor	25.48	24.79	38.21	29.90	29.32
Ramachandran (%)					
Favoured	98.0	98.0	97.0	99.0	99.0
Outliers	0.0	0.0	0.0	0.0	0.0



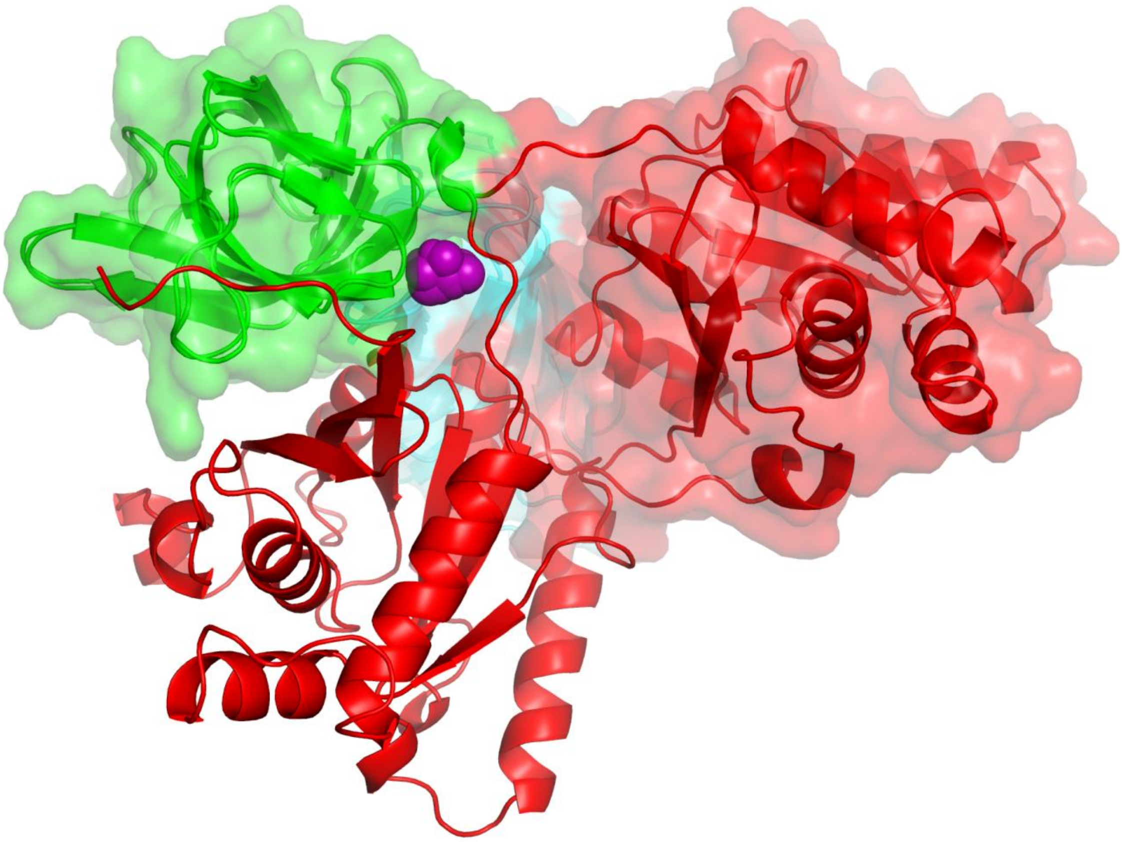
Supplementary Fig. S1. FTMap results overlaid with known ligands of EF-Tu. BFA in yellow sticks (4H9G), GE2270 A in grey sticks (2C77), adenosine in brown sticks (1TTT). Clusters of organic solvents docked by FTMap are shown as lines. Red is the 1st cluster, green is the 6th cluster, cyan is the 7th cluster, purple is the 10th cluster. The structure is 1EFT coloured as before, domain 1 in red, domain 2 in green, domain 3 in cyan. Ile231 and Leu289 are shown in sticks in the 1EFT rotamers that block the hydrophobic site. GE2270 A clashes with domain 1 from 1EFT because it induces domain movement.



Supplementary Fig. S2. Kirromycin (aka aurodox), 1HA3, in yellow and enacyloxin, 2BVN, in grey. 2nd cluster is red, the 3rd cluster is green, the 5th cluster is cyan, and the 8th cluster is purple. Structures are superposed on the red domain 1. In 1HA3 and 2BVN the area occupied by cluster 8 is filled with protein because of a conformational change. In 1EFT the area between cluster 3 and 5 is filled with protein.



Supplementary Fig. S3. Cluster 4 in green lines occupying the site where the beta and gamma phosphates of GDPNP bind.



Supplementary Fig. S4. The structures are 1EFT in complex with GDPNP and 1TUI in complex with GDP coloured as before, but 1TUI is shown with a transparent surface. Cluster 9 is located in the hinge region between domains 1 and 2 and shown as purple spheres for the sake of clarity.