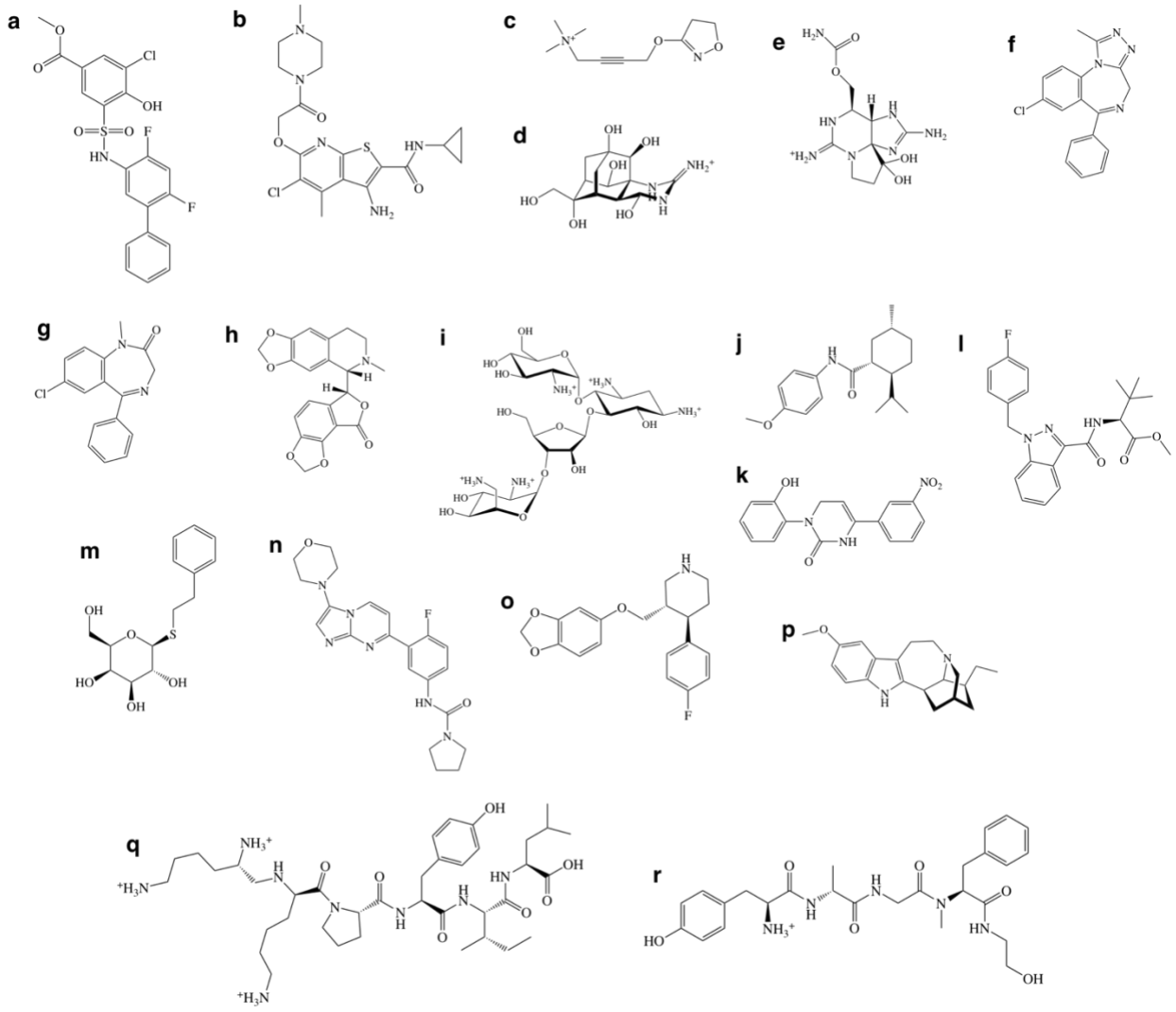

Figure S1: Ligands docked in this work; related to Figures 2, 3, 4, 5, 6, and 8.

a, NDI-091143/ATP citrate lyase. **b,** LY2119620/M2 receptor. **c,** Iperoxo/M2 receptor. **d,** Tetrodotoxin/NaVaPas. **e,** Saxitoxin/NaV1.7. **f,** Xanax/GABA$_A$. **g,** Valium/GABA$_A$. **h,** Biculine/GABA$_A$. **i,** Paromomycin/*leishmania* ribosome. **j,** Menthol analogue WS-12/TRPM8. **k,** Icilin/TRPM8. **l,** Fubinaca/cannabinoid type 1 receptor. **m,** PETG/beta-galactosidase. **n,** GSK3494245/*leishmania* 20S proteasome. **o,** Paroxetine/serotonin transporter. **p,** Ibogaine/serotonin transporter. **q,** DAMGO/μ-opioid receptor. **r,** JMV449/neurotensin type 1 receptor.

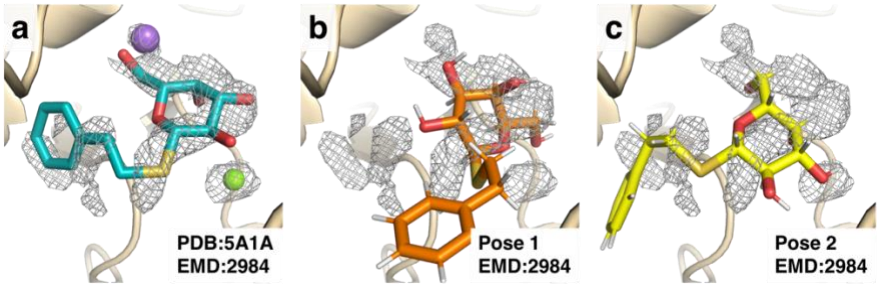

Figure S2: **PETG poses docked without EM map densities; related to Figure 2.**

a, Deposited structure PDB:5A1A PETG bound to beta-galactosidase, with displayed density from EMD:2984 (σ=2.0). The purple and green sphere correspond to a sodium and magnesium ion, respectively. **b,** Top pose for PETG from docking to PDB:5A1A without using the EM density, overlaid with EMD:2984 (σ=2.0). **c,** Another pose from docking to PDB:5A1A without density, overlaid with EMD:2984 (σ=2.0).

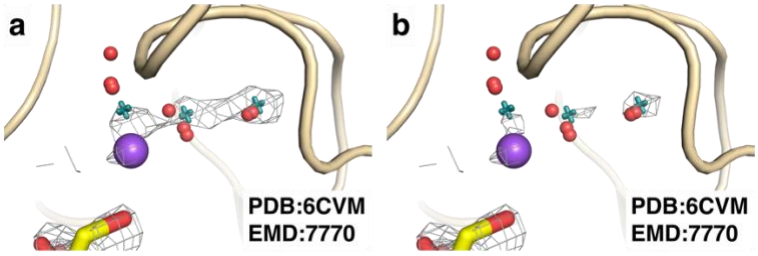

Figure S3: **Additional beta-galactosidase hydration sites predicted by JAWS, related to Figure 2, 3, and 4**

Predicted sites of hydration from triplicate JAWS simulations on beta-galactosidase are represented as red spheres, while locations of real-space refined waters are represented as teal crosses. The purple sphere corresponds to a sodium ion. **a, b,** Show density for EMD:7770 at two different contour levels (σ=1.5, 2.0 respectively).

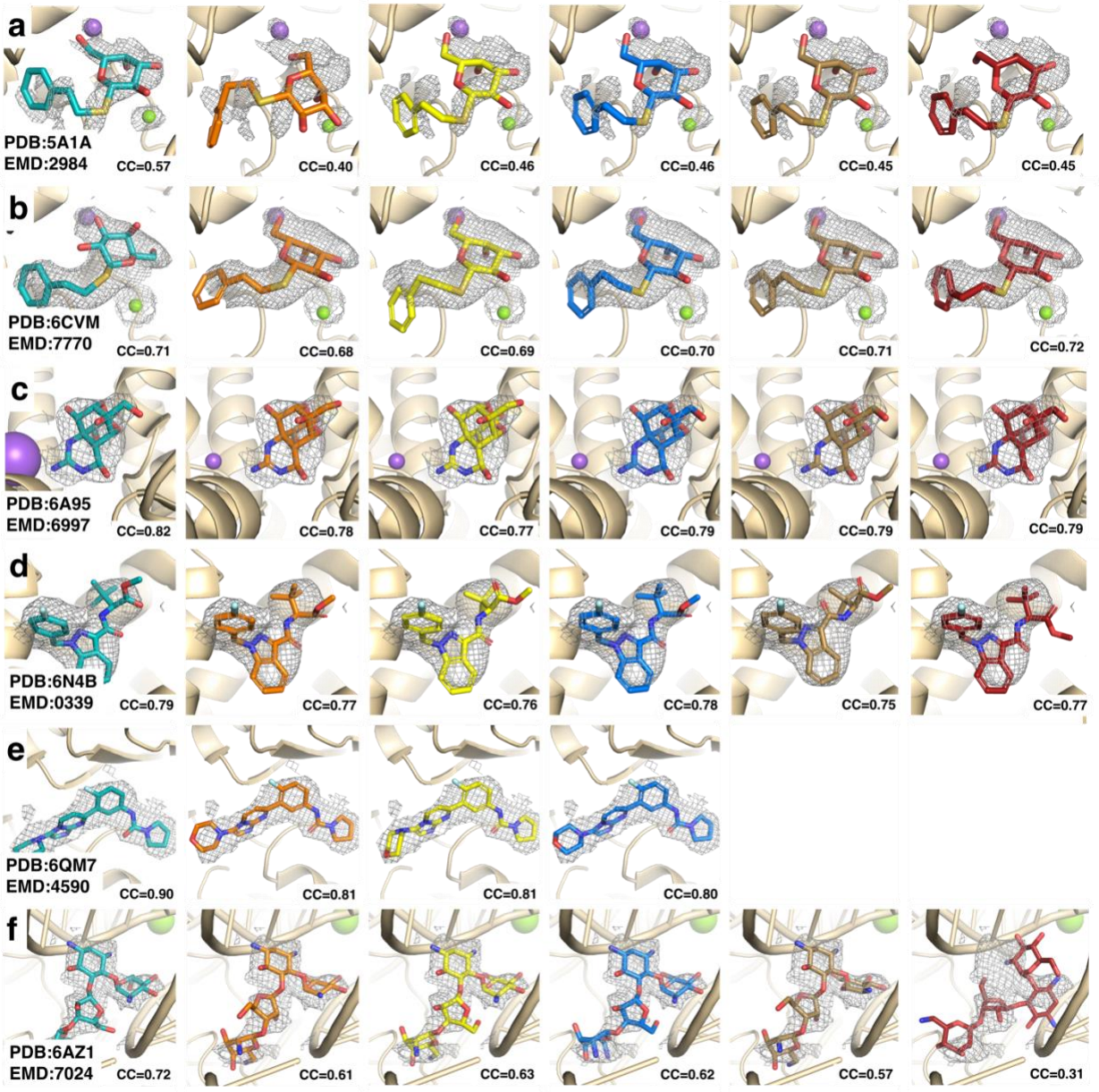

Figure S4: **Sub-3.0 Å cryoEM maps from this work, related to Figure 4, 5**

In all cases the deposited pose in the PDB is in teal while the orange, yellow, blue, brown, and red poses in the same row correspond to pose 1-5, respectively, in supplementary table 2. CC is the real space cross-correlation calculated without hydrogen atoms. **a,** PETG/beta-galactosidase (PDB:5A1A, EMD:2984 (σ=2.0)). **b,** PETG/beta-galactosidase (PDB:6CVM, EMD:7770 (σ=3.0)). **c,** Tetrodotoxin/NavPaS (PDB:6A95, EMD:6995 (σ=11.0)). **d,** Fubinaca/cannabinoid type 1 receptor (PDB:6N4B, EMD:0339 (σ=3.0)). **e,** GSK 3494245/*leishmania* 20S proteasome (PDB:6QM7, EMD:4590 (σ=3.0)). **f,** Paromomycin/*leishmania* ribosome (PDB:6AZ1, EMD:7024 (σ=1.0)).

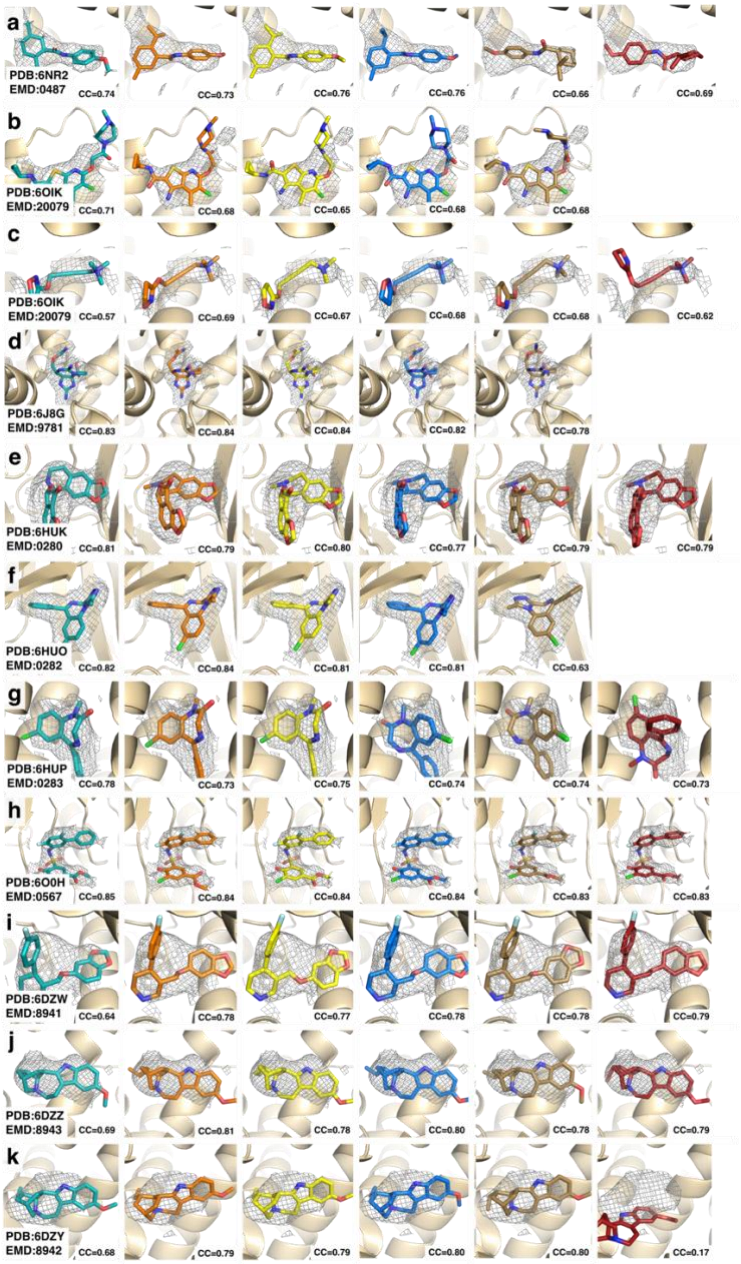

Figure S5: **3.0 Å - 4.5 Å maps from this work, related to Figure 6.**

In all cases the deposited pose in the PDB is in teal while the orange, yellow, blue, brown, and red poses in the same row correspond to pose 1-5, respectively, in supplementary table 2. CC is the real space cross-correlation calculated without hydrogen atoms. **a,** Menthol analogue WS-12/TRPM8 (PDB:6NR2, EMD:0487 (σ=3.0)). **b,** LY2119620/M2R (PDB:6OIK, EMD:20079 (σ=7.0)). **c,** Iperoxo/M2R (PDB:6OIK, EMD:20079 (σ=3.0)). **d,** Saxitoxin/Nav1.7 (PDB: 6J8G, EMD:9781 (σ=3.0)). **e,** Biculine/GABA$_A$ (PDB:6HUK, EMD:0280 (σ=5.0)). **f,** Xanax/GABA$_A$ (PDB:6HUO, EMD:0282 (σ=3.0)). **g,** Valium, GABA$_A$ (PDB:6HUP, EMD:0283 (σ=5.0)). **h,** NDI-091143/ATP Citrate Lyase (PDB:6O0H, EMD:0567 (σ=3.5)). **i,** Paroxetine/serotonin transporter (PDB:6DZW, EMD:8941 (σ=3.0)). **j,** Ibogaine/serotonin transporter (PDB:6DZZ, EMD:8943 (σ=1.4)). **k,** Ibogaine, serotonin transporter (PDB:6DZ, EMD:8942 (σ=4.0)).

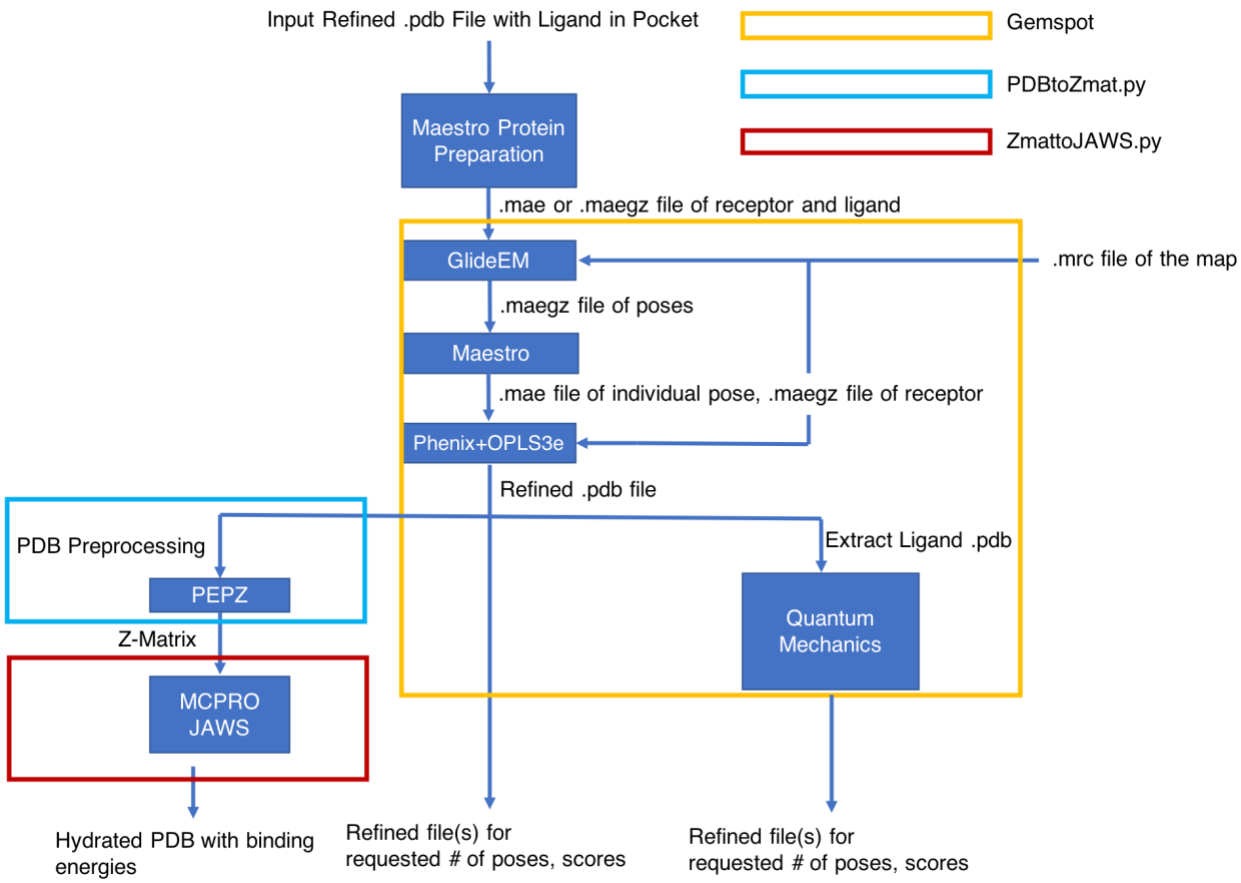

Figure S6: GemSpot Usage Diagram, related to Figure 1. Commands to call the various scripts are as follows:
Gemspot pipeline:

$SCHRODINGER/run glideEM20-1.py AAA.mae -ligand_asl “res.ptype BBB” -map CCC.mrc -mode exhaustive -docking_option FACEDEN=1 -docking_option FACRF=1 -docking_option POSES_PER_LIG=5 -phenix_opls3e_refine only_ligands -calc_jaguar_pose_energy -NOJOBID -resolution DDD -jaguar_restraint_strength -write_local_region -write_region_distance_cutoff 24 -write_region_min_molecule_size 50 -DEBUG -SAVE

Where AAA is the name of the system file, BBB the unique residue name, CCC the map name, and DDD the resolution of the map.

GlideEM:

$SCHRODINGER/run redock_with_emden.py AAA.mae -ligand_asl "res.ptype " -map CCC.mrc -SAVE -JOBNAME 6o72dock3 -faceden 1 -facrf 1

Where AAA is the name of the system file, BBB the unique residue name, and CCC the map name

PHENIX/OPLS3e:

$SCHRODINGER/run run_phenix_real_space_refine.py AAA.mae -receptor EEE.maegz -map CCC.mrc -force_field_region "res.ptype CCC" -JOBNAME 6o6rtest -resolution DDD -NOJOBID -phenix_refine_option output.model_format=pdb

Where AAA is the name of the ligand file, BBB the unique residue name, CCC the map name, DDD the resolution of the map, and EEE the name of the receptor file.

Table S1

All systems studied in this work, sorted by resolution, related to Figures 2, 3, 4, 5, 6, 7, and 8.

Name	PDB Code	Resolution	Biomolecule	Ligand
Beta-Galactosidase	6CVM	1.9	Protein	Phenethyl β-d-thiogalactoside
Beta-Galactosidase	5A1A	2.2	Protein	Phenethyl β-d-thiogalactoside
NavPaS	6A95	2.6	Membrane Protein	Tetrodotoxin
Leishmania Ribosome Small Subunit	6AZ1	2.7	RNA/Protein	Paromomycin
Leishmania 20S Proteosome	6QM7	2.8	Protein	GSK3494245
Cannabinoid Receptor 1	6N4B	3.0	Membrane Protein	Fubinaca
Nav1.7	6J8G	3.2	Membrane Protein	Saxitoxin
$GABA_A$ Receptor	6HUO	3.26	Membrane Protein	Xanax
TRPM8	6NR3	3.4	Membrane Protein	Icilin
Mu Opioid Receptor	6DDF	3.5	Membrane Protein	DAMGO
$GABA_A$ Receptor	6HUP	3.58	Membrane Protein	Valium
Muscarinic Receptor 2	6OIK	3.6	Membrane Protein	Iperoxo
Muscarinic Receptor 2	6OIK	3.6	Membrane Protein	LY2119620
Serotonin Transporter	6DZZ	3.6	Membrane Protein	Ibogaine
ATP Citrate Lyase	6O0H	3.67	Protein	NDI-091143
$GABA_A$ Receptor	6HUK	3.69	Membrane Protein	Biculine
TRPM8	6NR2	4.0	Membrane Protein	Menthol Analogue WS-12
Serotonin Transporter	6DZY	4.1	Membrane Protein	Ibogaine
Serotonin Transporter	6DZW	4.3	Membrane Protein	Paroxetine

Table S2

Docking and refinement results for the systems studied in this work, related to Figures 2, 3, 4, 5, 6, 7, and 8.

System	Pose	CC	Deposited Pose CC	Glide Density Score	Glide Docking Score	QM Pose Strain	GlideEM CPU Time (Minutes: Seconds)	OPLS3e/Phenix Refinement Time (Minutes: Seconds)	Pipeline CPU Time (Hours: Minutes: Seconds)	Traditional Glide CPU Time (Minutes: Seconds)	Traditional Glide CC Post-Refinement
Bgal	1	0.40	0.57	-3.4	-5.8	5.7	25:45	32:41	32:36:41	24:46	0.32
PETG	2	0.46		-3.7	-5.4	2.5		34:21			0.32
5A1A	3	0.46		-3.6	-5.3	2.7		34:04			0.49
	4	0.45		-3.8	-5.1	2.3		34:12			0.50
	5	0.45		-3.7	-5.3	0.0		35:09			0.50
Bgal	1	0.68	0.71	-6.5	-8.0	1.0	25:43	34:43	30:28:09	27:55	0.75
PETG	2	0.69		-7.3	-7.6	0.6		34:29			0.75
6CVM	3	0.70		-7.7	-7.2	0.5		34:47			0.73
	4	0.71		-7.8	-7.4	0.6		34:55			0.59
	5	0.72		-6.9	-7.5	0.0		35:05			
NavPaS	1	0.78	0.82	-2.8	-5.2	5.6	05:01	29:28	17:06:59	03:23	0.77
Tetrodotoxin	2	0.77		-2.8	-5.3	4.4		29:45			0.77
6A95	3	0.79		-2.8	-5.4	0.0		30:40			0.76
	4	0.79		-2.8	-5.2	0.0		30:37			0.78
	5	0.79		-2.8	-5.0	0.8		29:35			0.74
TRPM8	1	0.74	0.74	-0.69	-5.35	1.6	01:42	58:29	13:04:48	01:10	0.55
Menthol Analogue WS-12	2	0.68		-0.62	-4.93	0.9		53:12			0.73
6NR2	3	0.73		-0.92	-3.7	0.0		48:49			0.72
	4	0.66		-0.76	-4.7	0.1		49:43			0.71
	5	0.70		-0.67	-4.2	1.3		49:11			0.71
TRPM8	1	0.75	0.67	-1.9	-6.4	0.0	01:36	49:22	07:04:08	01:05	0.75
Icilin	2	0.79		-2.4	-5.7	2.2		52:08			0.75
6NR3	3	0.79		-2.3	-6.4	1.0		50:34			0.78
	4	0.78		-2.0	-6.3			48:51			0.77
	5	0.81		-1.8	-6.5			55:04			0.78
Cannabinoid Receptor 1	1	0.77	0.79	-10.0	-9.5	0.0	02:47	21:22	14:40:00	01:55	0.74
Fubinaca	2	0.76		-9.4	-9.2	0.1		23:55			0.75
6N4B	3	0.78		-9.5	-9.6	0.0		25:19			0.74
	4	0.75		-9.9	-9.6	6.8		21:08			0.74
	5	0.77		-9.2	-9.2			19:19			
GABA(A)	1	0.80	0.82	-3.4	-7.4	0.0	06:39	17:07		05:10	0.80
Xanax	2	0.74		-4.0	-8.3	0.0		17:00			0.70
6HUO											0.76
GABA(A)	1	0.77	0.81	-4.8	-6.7	0.0	06:44	16:42		05:32	0.77
Biculine	2	0.80		-5.6	-8.0	0.1		16:20			0.73
6HUK	3	0.75		-5.2	-8.5	0.1		16:36			0.76
GABA(A)	1	0.76	0.78	-2.2	-8.5		06:41	16:11	09:47:32	05:26	0.75
Valium	2	0.75		-2.3	-7.2			16:15			0.75

6HUP	3	0.77		-2.3	-6.5			16:18			0.67
	4	0.74		-1.6	-7.5			16:31			0.73
	5	0.76		-2.3	-7.0			16:22			0.75
Muscarinic Receptor 2	1	0.67	0.71	-11.0	-7.3	0.0	02:55	11:54		02:10	0.65
LY2119620	2	0.62		-11.4	-7.1	3.7		11:58			0.62
6OIK	3	0.61		-11.0	-8.5	3.8		12:16			0.65
	4	0.69		-11.3	-8.1	0.6		12:00			
	5	0.67		-11.0	-7.3	3.6					
Muscarinic Receptor 2	1	0.76	0.57	-0.7	-8.4	3.0	02:32	15:09		02:01	0.71
Iperoxo	2	0.72		-0.5	-8.2			15:28			0.70
6OIK	3	0.74		-0.6	-8.0			15:38			0.72
	4	0.73		-0.74	-7.8	2.7		15:35			0.71
	5	0.70		-0.72	-8.0	0.0		12:06			0.74
ATP-Citrate Lyase	1	0.85	0.85	-9.3	-665.7		23:04	25:06		22:03	0.85
NDI-091143	2	0.84		-8.8	-643.4			26:45			0.85
6O0H	3	0.84		-8.7	-680.7			24:41			0.82
											0.82
Nav1.7	1	0.80	0.83	-2.1	-7.8	0.0	4:50	21:38	09:33:47	03:41	0.67
Saxitoxin	2	0.81		-2.7	-7.1	1.2		21:56			0.82
6J8G	3	0.81		-2.5	-6.5	1.9		21:37			0.67
											0.65
Leishmania 20S Proteasome	1	0.81	0.90	-3.2	-7.2	0.0	70:55	59:41		67:45	0.80
GSK3494245	2	0.81		-3.2	-6.9	1.5		59:10			0.80
6QM7	3	0.80		-3.3	-7.1	1.6		59:06			0.76
											0.51
											0.72
Serotonin Transporter	1	0.79	0.64	-2.3	-7.7	1.0	02:50	25:28		01:53	0.77
Paroxetine	2	0.76		-2.5	-8.0	0.0		24:02			0.77
6DZW	3	0.79		-2.3	-7.5	1.1		23:52			0.78
	4	0.77		-2.1	-7.8	0.8		24:17			0.79
											0.52
Serotonin Transporter	1	0.77	0.68	-4.5	-7.8	0.0	02:35	23:53		01:43	0.76
Ibogaine	2	0.76		-4.4	-8.1	0.8		27:50			0.77`
6DZY	3	0.77		-4.4	-8.1	3.2		22:19			0.78
	4	0.78		-4.1	-8.1	0.0		27:54			0.74
Serotonin Transporter	1	0.74	0.69	-10.1	-7.5	0.7	02:39	22:15		01:26	0.77
Ibogaine	2	0.75		-10.0	-7.1	0.6		22:36			0.77
6DZZ	3	0.74		-9.5	-5.9	0.0		22:12			0.53
	4	0.75		-9.9	-6.8	0.9		22:15			0.55
											0.54
Leishmania Ribosome	1	0.70	0.72	-5.8	-40645		10:20	33:25		04:56	0.44
Paromomycin	2	0.68		-5.4	-40687			37:03			0.64
6AZ1	3	0.65		-5.4	-41242			33:58			0.48
	4	0.66		-4.7	-41279			33:32			0.41
	5	0.39		-4.3	-41017			33:28			0.47

Leishmania Ribosome	1	0.74		-5.8	-52301		10:29	39:18			
Paromomycin	2	0.77		-5.5	-51518			36:13			
15k particles	3	0.76		-4.2	-52797			40:26			
	4	0.77		-0.8	-52075			35:31			
	5	0.76		0.9	-51741			39:37			
Leishmania Ribosome	1	0.80		-6.4	-52255		10:27	38:33			
Paromomycin	2	0.79		-5.7	-51587			38:50			
5k particles	3	0.81		-5.6	-51189			36:01			
	4	0.80		-5.7	-51376			36:38			
	5	0.82		-1.3	-52117			38:47			
Leishmania Ribosome	1	0.89		-5.6	-43357		10:03	33:48			
Paromomycin	2	0.89		-4.5	-46328			36:48			
2.5k particles	3	0.89		-2.4	-46599			37:55			
	4	0.89		2.2	-43985			37:48			
	5	0.89		2.9	-43743			37:55			
Mu Opioid Receptor											
DAMGO	1	0.67		-5.5	-73.1		54:22:33	14:44			
6DDE	2	0.66		-5.5	-84.3			15:05			
	3	0.63		-5.7	-95.2			14:36			
	4	0.61		-5.7	-88.4			15:26			
	5	0.59		-5.5	-74.8			14:01			
	6	0.58		-5.5	-69.9			14:36			
	7	0.57		-5.4	-82.4			14:57			
	8	0.55		-5.5	-82.4			14:17			
Neurotensin Receptor											
JMV449	1	0.69		-8.2	-10.7		37:20:52	21:57			
6OS9	2	0.69		-8.0	-12.6			21:33			
	3	0.69		-8.7	-12.2			20:44			
	4	0.69		-7.7	-10.5			20:22			
	5	0.68		-7.9	-7.4			20:24			
	6	0.68		-8.2	-9.0			21:10			
	7	0.68		-8.0	-10.0			21:31			

Glide and Emodel correspond to the Glide score and Emodel described in the methods section. CC is the cross correlation between the ligand model and the map. Timing information represents the cpu time on a single core of an Intel Core i7-8700 @ 3.2 GHz.