

Supplementary Information

Cryo-EM structure-based selection of computed ligand poses enables design of MTA-synergic PRMT5 inhibitors of better potency

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List of supplementary items:

Supplementary Figures 1-9

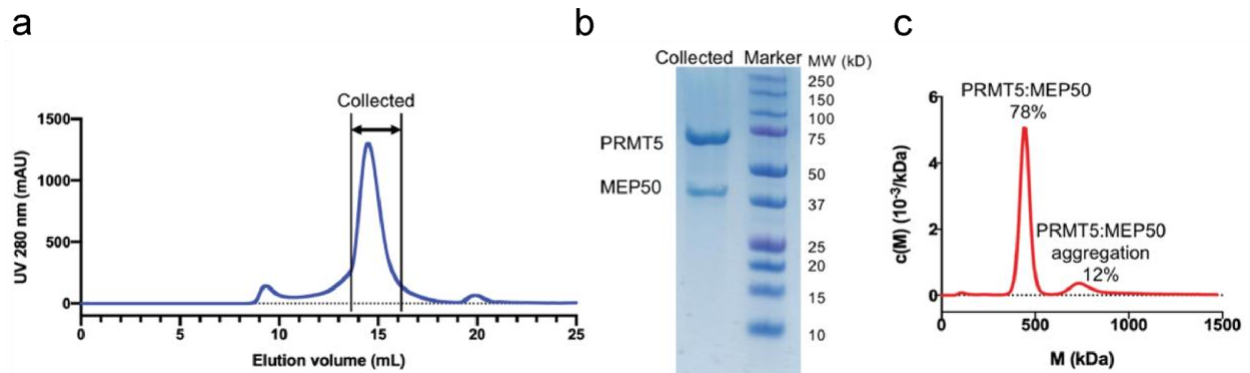
Supplementary Tables 1-7

Supplementary References

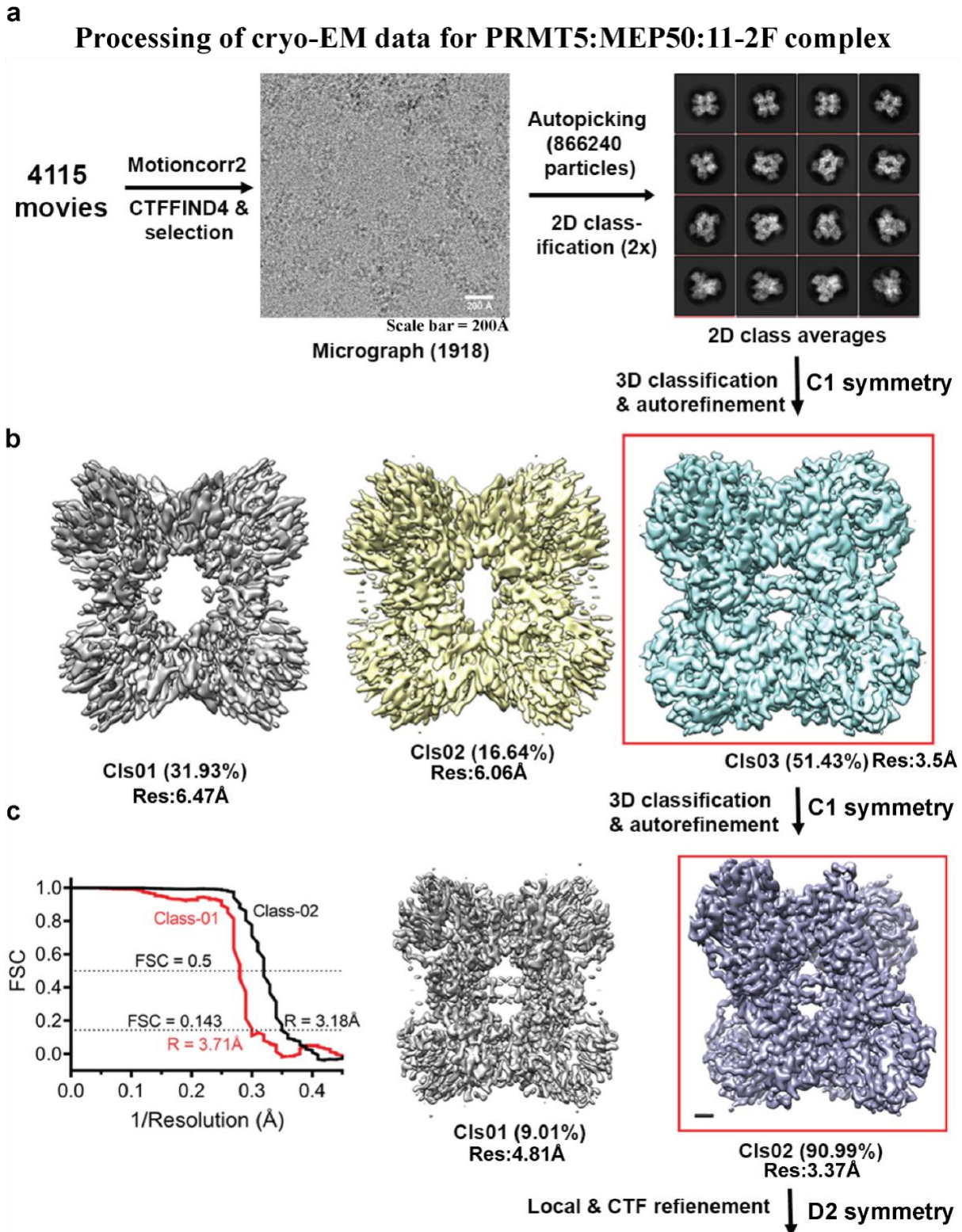
Supplementary Figures and legends:

Supplementary Figure 1. PRMT5:MEP50 complex purification and characterization.

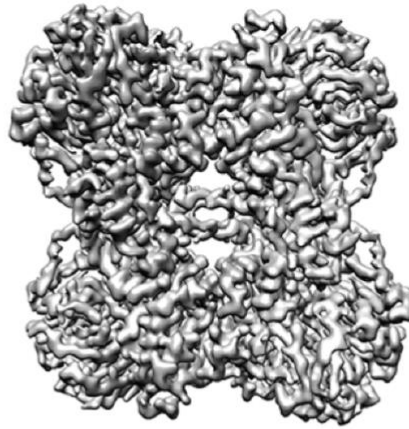
a). Size exclusion chromatography of the PRMT5:MEP50 complex after anti-FLAG affinity column. Fractions at the indicated area were collected and concentrated. **b).** An SDS-PAGE gel is shown for the collected fractions of PRMT5:MEP50 with a purity greater than 95%. **c).** Sedimentation velocity analytical ultracentrifugation (SV-AUC). The PRMT5:MEP50 complex has an estimated mass of 445 kDa.



Supplementary Figure 2. Processing cryo-EM data of the PRMT5: MEP50/ 11-2F/ MTA complex. The final map has an overall nominal resolution of 3.14 Å (FSC=0.143). The presentations were prepared in Chimera¹ and PyMOL².

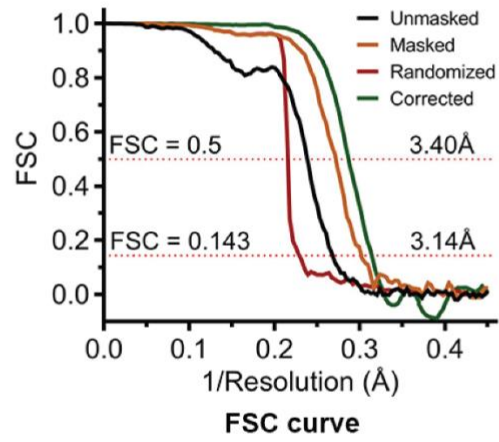


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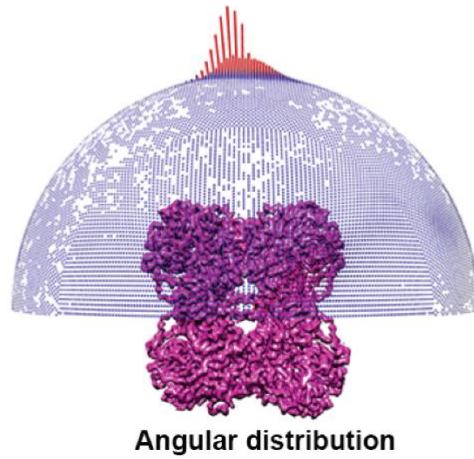


Final map (207392 particles)
(Res = 3.14Å, D2 symmetry)

e

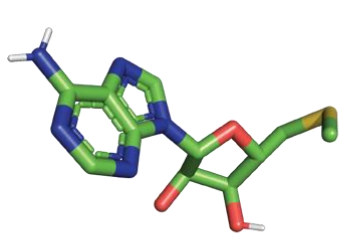


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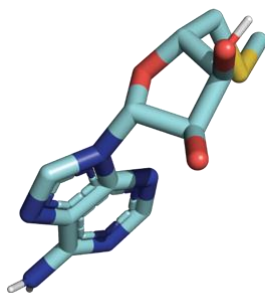


Supplementary Figure 3. Top poses of different ligands from computational analysis

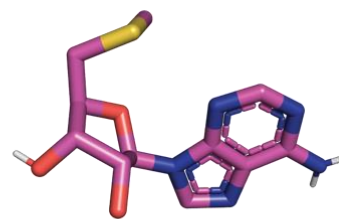
a). Top 8 poses of MTA. In each of the 2,000 runs, 25 million seed positions were used as starting points for movements and energy minimization. The top poses from 2,000 runs were clustered based on their RMSD. The best ones of the resultant 8 clusters are showed.



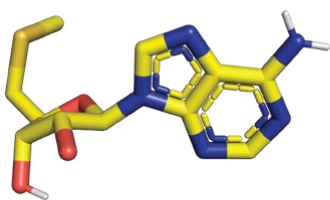
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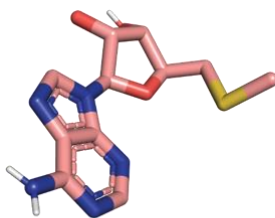
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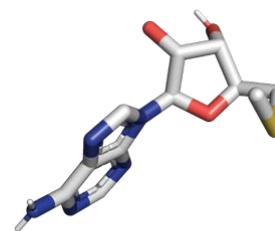
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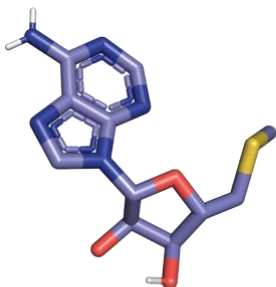
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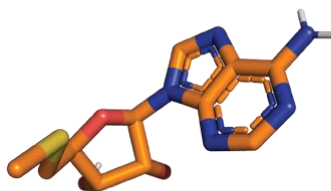
Cluster 5



Cluster 6

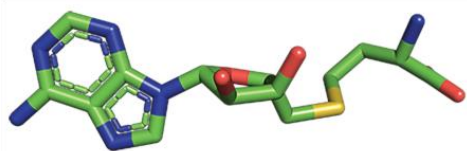


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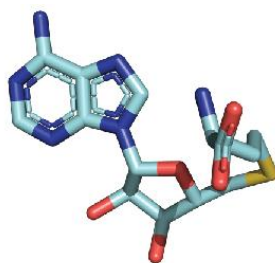


Cluster 8

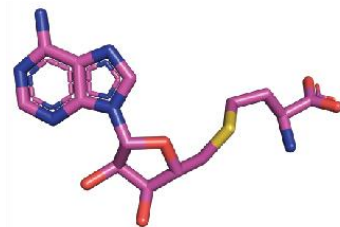
b). Top 10 poses of SAH came from analysis as explained in a.



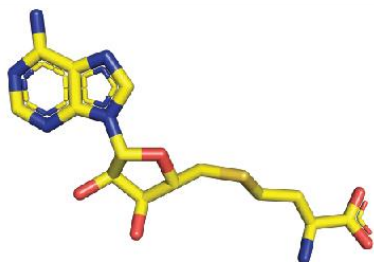
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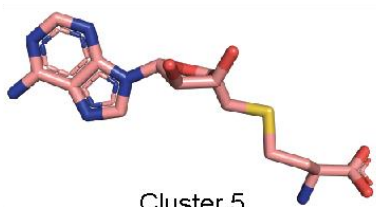
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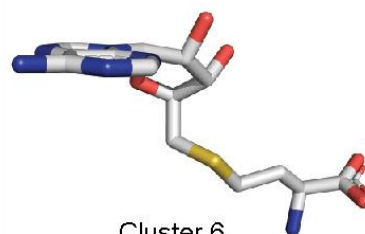
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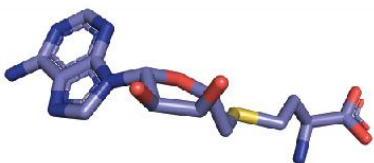
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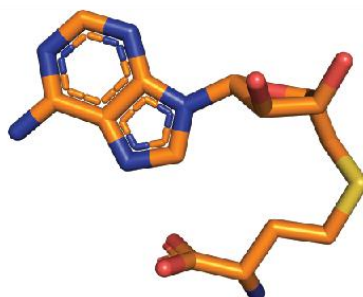
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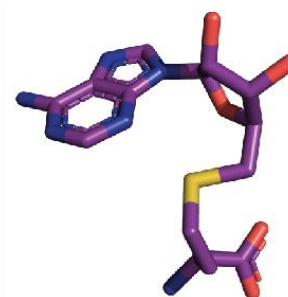
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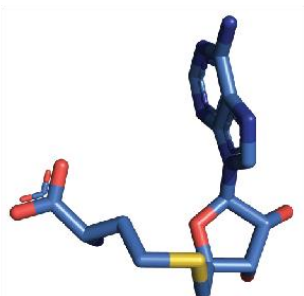
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Cluster 8

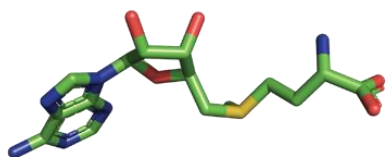


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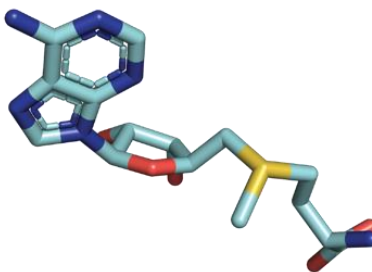


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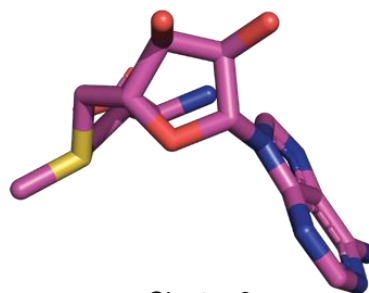
c). Top 10 poses of SAM.



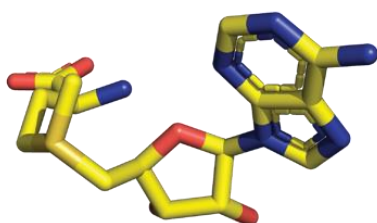
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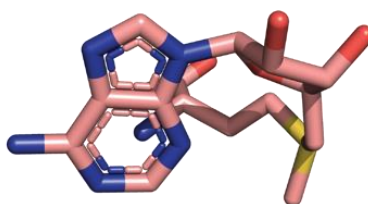
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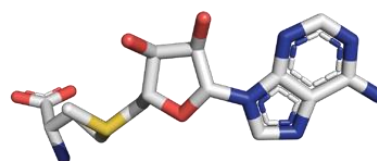
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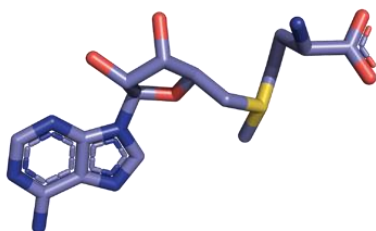
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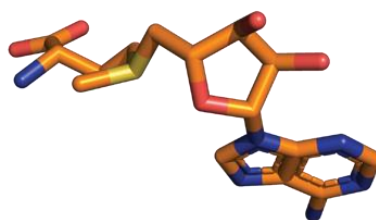
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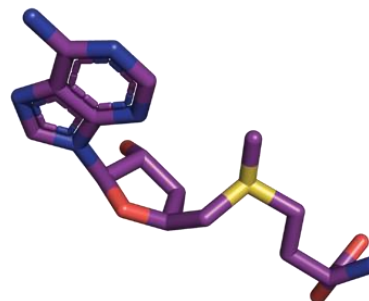
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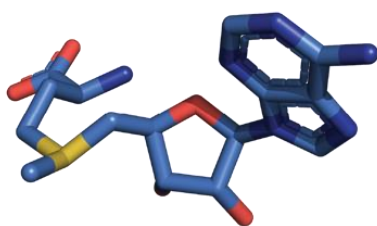
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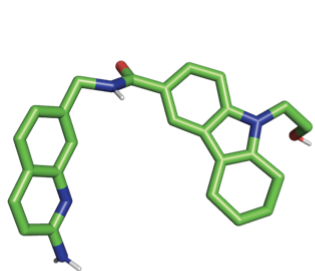


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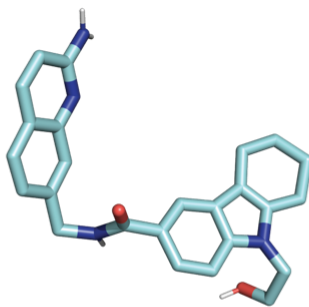


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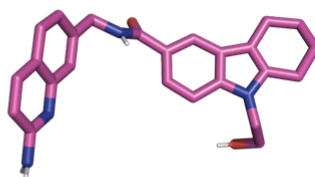
d). Top 10 poses of 11-2F.



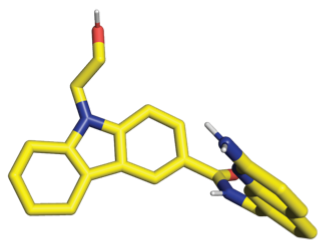
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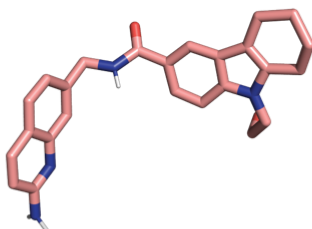
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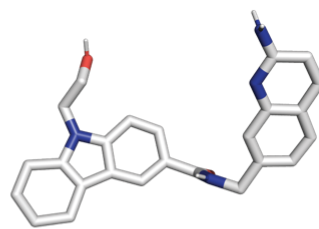
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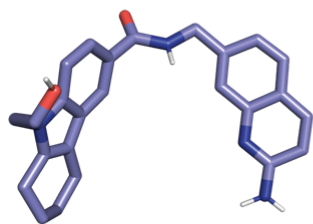
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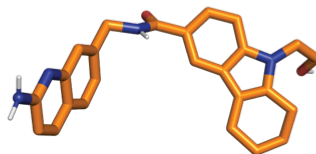
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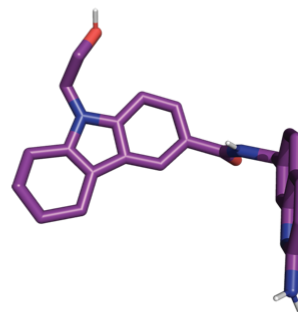
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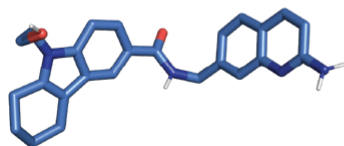
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Cluster 8

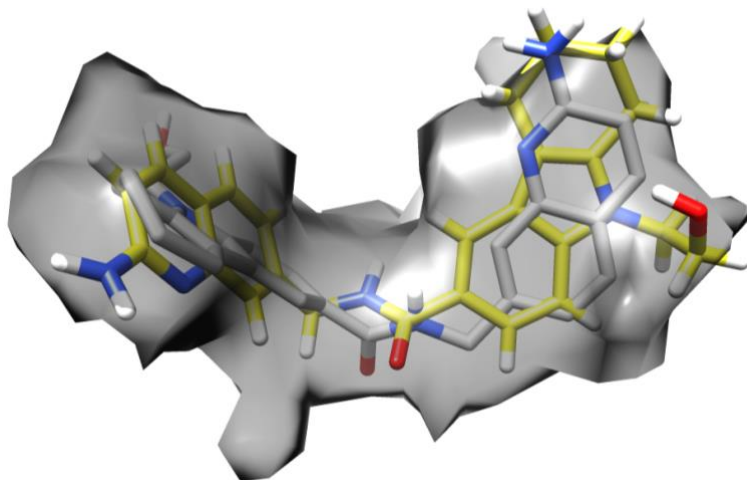


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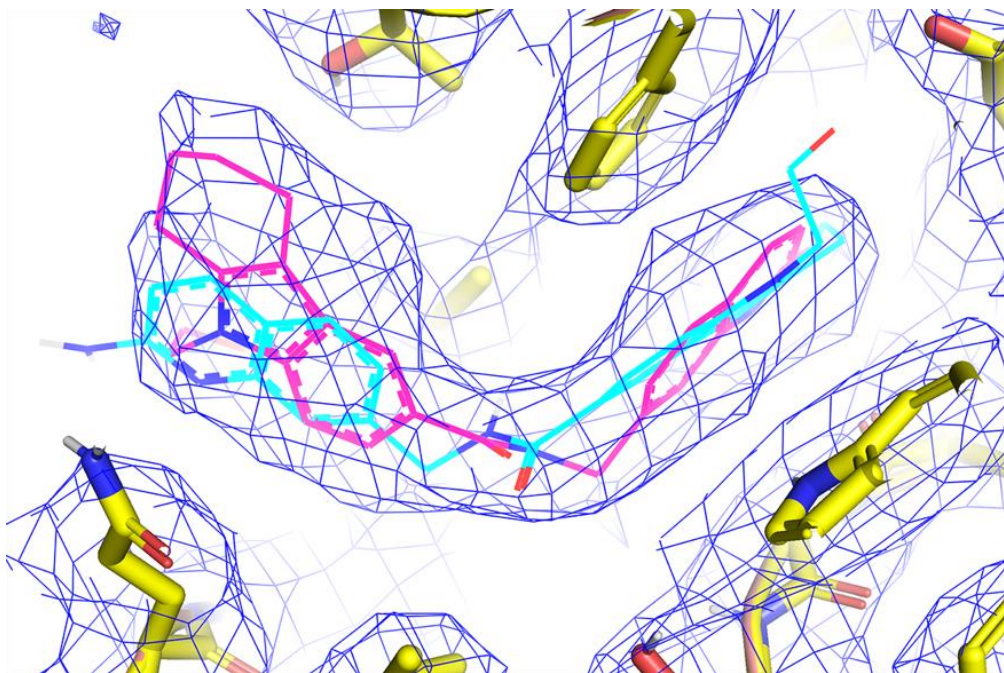


Cluster 10

e). Two opposite orientations of 11-2F differ in docking energy. The refined pose in yellow and the other pose representing cluster 4 (white) are fitted into the cryo-EM density map of the ligand (semitransparent grey). The two had their head and tail parts in opposite arrangements and were not distinguished reliably by fitting ligand atoms into the cryo-EM density at the current 3.1 Å resolution.

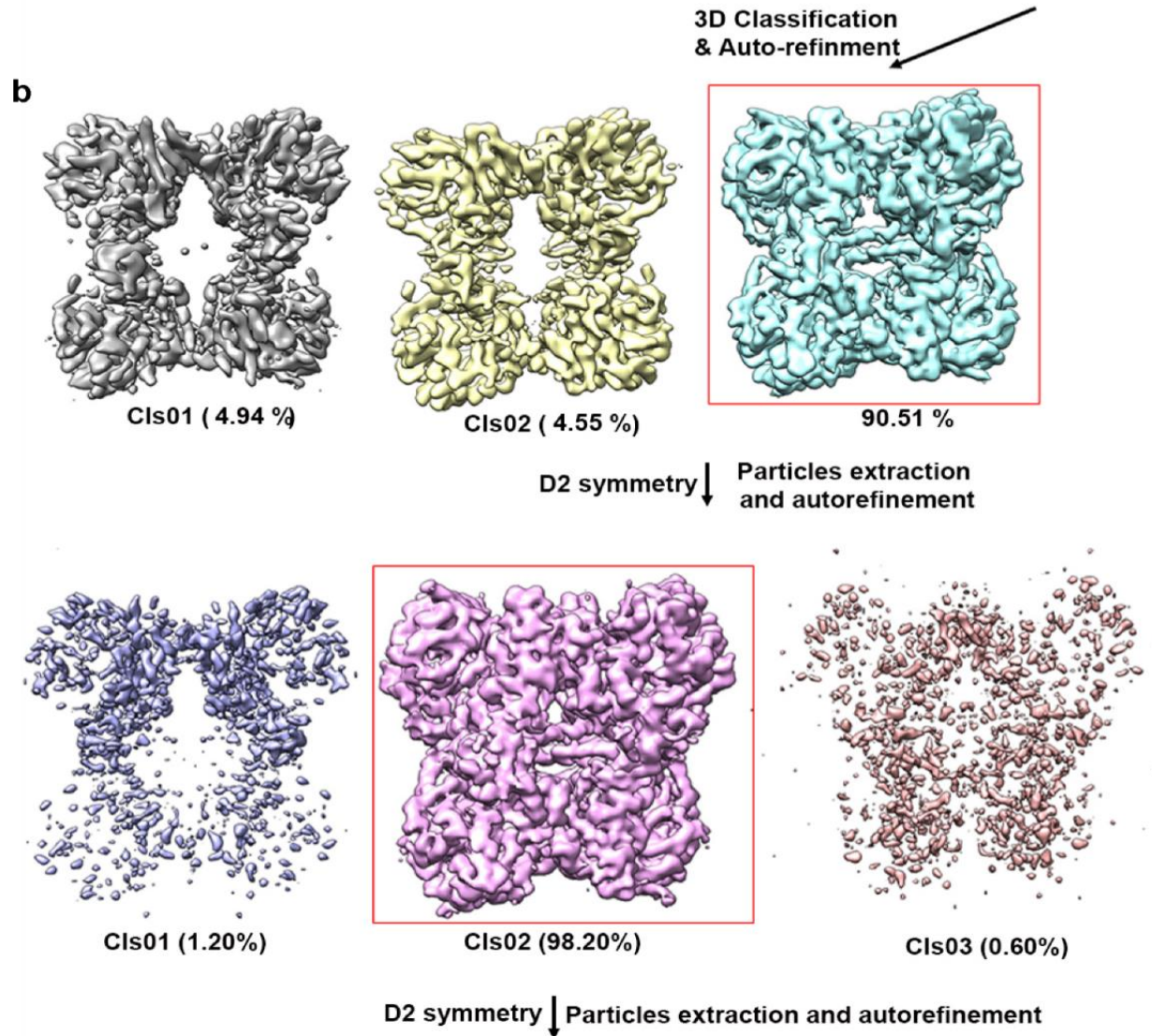
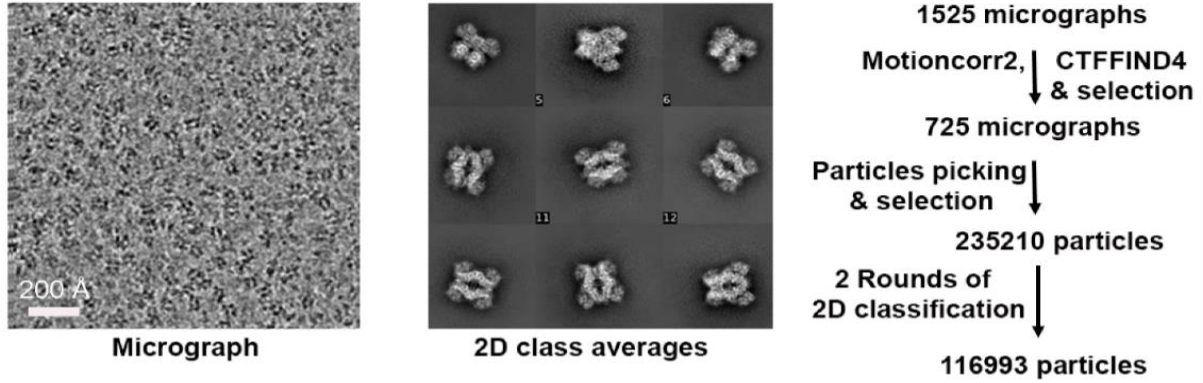


f). Two opposite orientations of 11-2F inside the binding pocket. The residues in the binding pocket (blue mesh) did not change significantly to accommodate the two poses. The refinement statistics in PHENIX for PRMT5 and ligands with 11-2F in two opposite orientations in the binding pocket (cyan vs. pink) were the same as in Table 1.



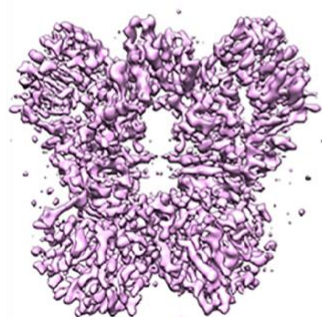
Supplementary Figure 4. Processing cryo-EM data of the *apo* PRMT5: MEP50 complex. The final map is of 3.2 Å (FSC = 0.143). Presentations were prepared in Chimera¹ and PyMOL².

a Processing of cryo-EM data for PRMT5:MEP50

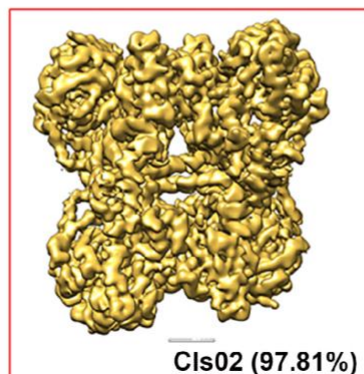


C

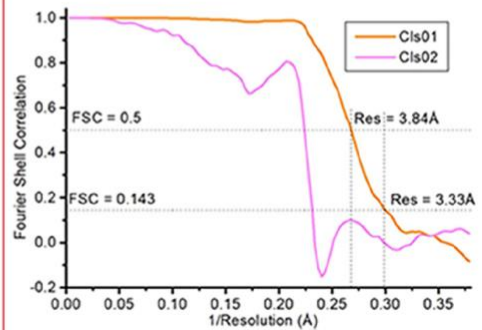
D2 symmetry ↓ Particles extraction and autorefinement



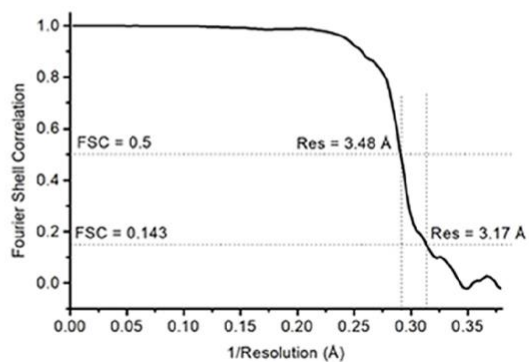
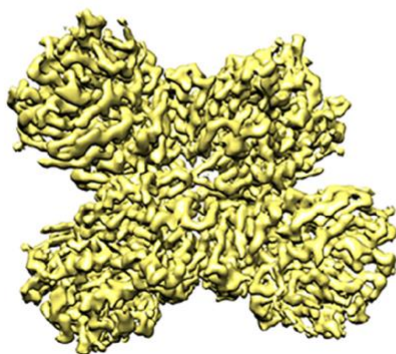
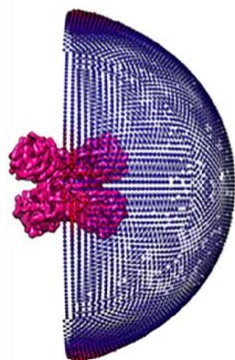
CIs01 (2.19 %)



CIs02 (97.81%)



D2 symmetry ↓ CTF & local refinement



Supplementary Figure 5. Sequence alignment of PRMT1 - 7 focusing on their catalytic domains. Key residues conserved among the catalytic domains are highlighted (*, :).

hPRMT7	LHDKDRNVKYYQGIRAAV---SRVKDRGQKALVLDIGTGTGLLSMM---AVTAG-ADF	89
hPRMT5	EKDPIKYSQYQQAIIYKCLLDRVPEEEKDTNVQVLMVLGAGRGPLVNASLRAAKQADRRIK	387
hPRMT1	LKDEVRTLTYRNSMFHNR-----HLFKDKVWLDVGSSTGILCMF----AAKAG-ARK	113
hPRMT3	LKDKIRTESYRDFIYQNP-----HIFKDKVWLDVGCSTGILSMF----AAKAG-AKK	280
rPRMT4	MQDYVRTGTYQRAILQNH-----TDFKDKIVLDVGCSTGILSFF----AAQAG-ARK	210
hPRMT2	LADQPRTTKYH-SVILQNK-----ESLTDKVIDVGCSTGIISLF----CAHYARPRA	163
hPRMT6	IADRVRTDAYRLGILRNW-----AALRGKTVLDVAGAGTILSIF----CAQAG-ARR	107
	* : * : : : * * * * :	
hPRMT7	CYAIIEVFKPMADAIVKIVEKNGFSDKIKVINKHSTEVTVGPEGDMPCRANILVTELFDETE	149
hPRMT5	LYAVEK-NPNAVVTLENWQFEWGSQVTVVSSDMREWVAP-----EKADIIVSELLGSGF	440
hPRMT1	VIGIEC-SSISDYAVKIVKANKLDHVVTIIKGVVEEVELPV----EKVDIIISEWMGYC	167
hPRMT3	VLGVDQ-SEILYQAMDIIIRLNKLEDITITLIKGIKEEVLHPV----EKVDVIISEWMGYF	334
rPRMT4	IYAVEA-STMAQHAEVLVKSNLTDRIVVIPGKVEEVSVP-----EQVDIIISEPMGYM	263
hPRMT2	VYAVEA-SEMAQHTGQLVVLQNGFADIIITVYQQKVEDVWLP-----EKVDVIVSEWMGTC	216
hPRMT6	VYAVEA-SAIWQQAREVVRFNGLIEDRVHVLPGPVETVELP-----EQVDIVSEWMGYG	160
	::: . : : : : : : : : : : : * :	
hPRMT7	LIGEGALPSYEHARRHLVEENCEAVPHRATVYAQLVESGRMWSW---NKLFPPIHVQTSLG	206
hPRMT5	ADNELSPE-CLDGAQHFLKDDGVSIPEYTSFLAPISSSKLYNEVRACREKDRDP--EAQ	497
hPRMT1	LFYESMLNTVLYARDKWLAPDGLIFPDRATLYVTAIEDRQYKDY---KIHWWENV---YG	221
hPRMT3	LLFESMLDSVLYAKNKYLAKGGSVYPDICTISLVAVSDVNKHAD---RIAFWDDV---YG	388
rPRMT4	LFNERMLESYLHAK-KYLKPSGNMFPITIGDVHLAPFTDEQLYMEQFTKANFWYQPS-FHG	321
hPRMT2	LLFESMIESILYARDAWLKEDGVIWPTMAALHLVPCASADKDYRS---KVLFDWNA---YE	270
hPRMT6	LLHESMLSSVLHARTKWLKEGGLLLPASAELEIAPISDQ-MLEW---RLGFWSQVKQHYG	216
	* . : . *	
hPRMT7	-----EQVIVPPVDVESC-----GAPSVCDIQLNQVSPADFTVLSVLPVPMFSI	250
hPRMT5	FEMPYV-----VRLHNFHQLSAPQPCFTFVSHPN--RDPMIDN----NRYC	536
hPRMT1	FDMSCIKDVAIKEPL-----VDVDPKQ-LVTNACLIKEVDI--YTVKVED---LTFTS	269
hPRMT3	FKMSCMKKAVIPEAV-----VEVLDPKT-LISEPCGIKHIDC--HTTSISD---LEFSS	436
rPRMT4	VDLSALRGAADVFRQPVVDTFDIRI---LMAKSV---KYTV--NFLEAKEGDLHRIEI	373
hPRMT2	FNLSALKSLAVKEFFSKPKY-NHILKPED-CLSEPCTILQLDM--RTVQIS--DLETLRG	324
hPRMT6	VDMSCLEGFATRCLMGHSEIVVQGLSGED-VLARPQRFAQLEL--SRAGLEQELEAGVGG	273
hPRMT7	DFSKQVSSSAACHSRRFEPLTSGRAQVVLSSWWDIEMDPEGKIKCTMAPFWAHSDEPE-EMQ	309
hPRMT5	TLE-----FPVEVNTVLHGAGYFETVLYQD-----ITLSIRPETHSP	574
hPRMT1	PFC-----LQVKRNDYVHALVAYFNIEFT-R----CH-KRTGFSTSP---S	307
hPRMT3	DFT-----LKITRTSMCTAIAGYFDIYFEKN---CH-NRVVFSTGPF---S	475
rPRMT4	PFK-----FHMLHSGLVHGLAFWFDVAFVIGS-----I-MTVWLSTAPT---E	411
hPRMT2	ELR-----FDIRKAGTLHGFTAWFSVHFQSLQ--EGQ-PPQVLSSTGPF---H	365
hPRMT6	RFR-----CSCYGSAPMHGFAIWFQVTFPGG---ESE-KPLVLSSTSPF---H	313
	: . : : :	
hPRMT7	WRDHMMQCVYFLPQEEPVVQGSALYLVA-----HHDDYCVWYSLQRTSPEKNERVRQM	362
hPRMT5	GMFSWFFILFPIKQPIVREGQTCVRFWRCSNS-----KKVWYEWAVTAPVCSAIHNP-	628
hPRMT1	PYTHWKQTVFYMEDYLTVKTGEEIFGTIGMRPNAKNNRDLDFIDLDFKQGLCELSCST-	366
hPRMT3	TKTHWKQTVFLLEKPFVSKAGEALKGKVTVHKNNKDPKSLVTLTLNNSSTQTYGLQ----	531
rPRMT4	PLTHWYQVRCLFQSPLFAKAGDTLSGTCLLIANKRQSYDISIVAQVDQT---GSKSSNLL	468
hPRMT2	PTTHWKQTLFMMDDPVVHTGDVVTGSVVLQRNPVWRRHMSVALSWAVTSRQDPTSQKVG	425
hPRMT6	PATHWKQALLYLNEPVQVEQDQDVSGETLLPSRDNPRRLRVLLRYKVGQDEEKTQDFAM	373
	* : . . . :	

Supplementary Figure 6. Top 10 poses of 11-9F from computational analysis.



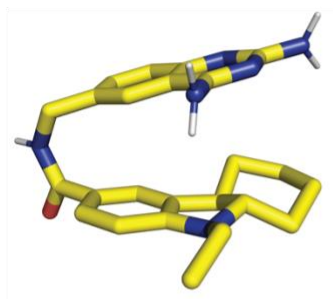
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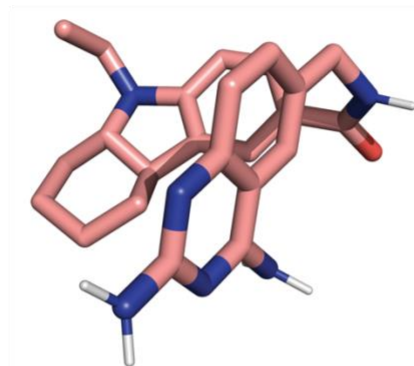
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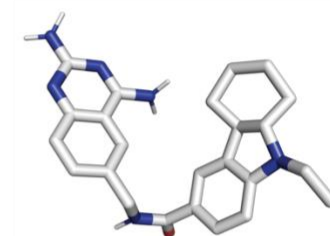
Cluster 3



Cluster 4



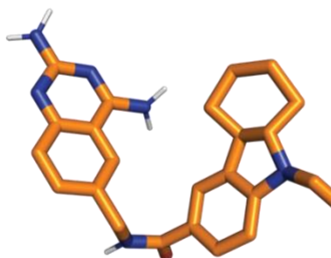
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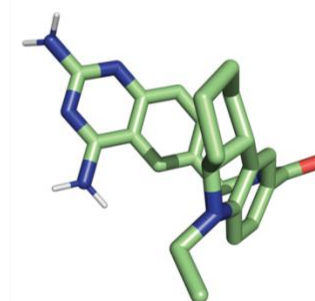
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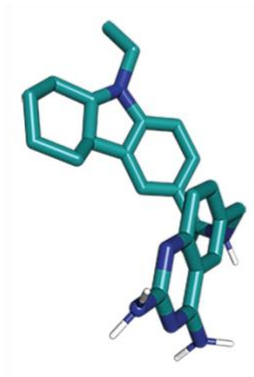
Cluster 7



Cluster 8

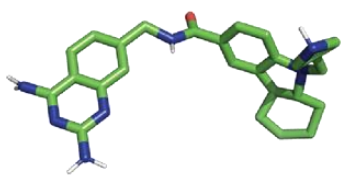


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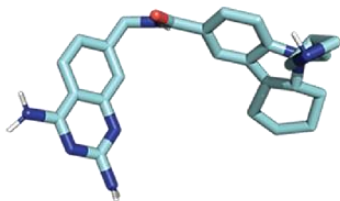


Cluster 10

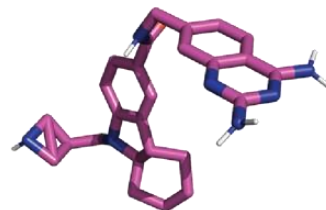
Supplementary Figure 7. Top 10 poses of HWIem2104 from computational analysis.



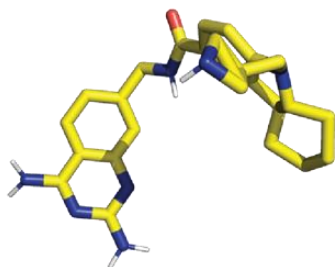
Cluster 1



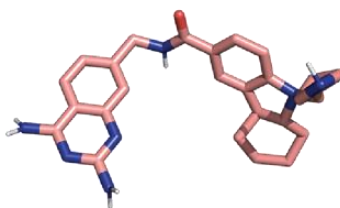
Cluster 2



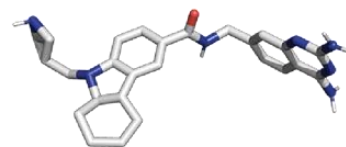
Cluster 3



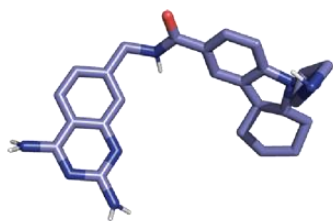
Cluster 4



Cluster 5



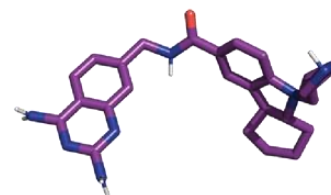
Cluster 6



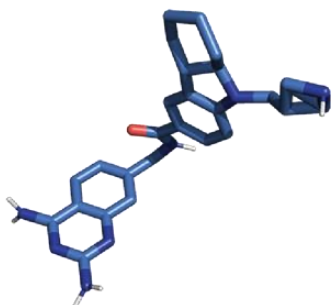
Cluster 7



Cluster 8

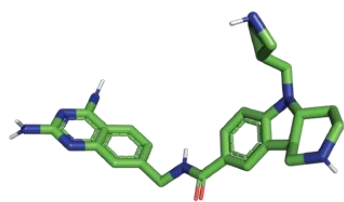


Cluster 9

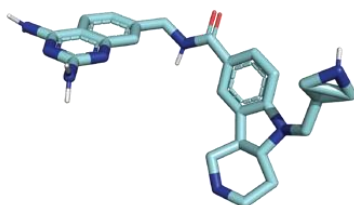


Cluster 10

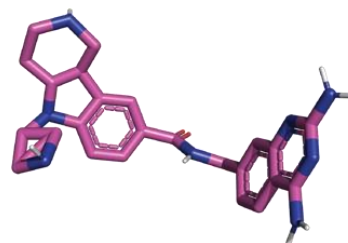
Supplementary Figure 8. Top 10 poses of HWIem2109 from computational analysis.



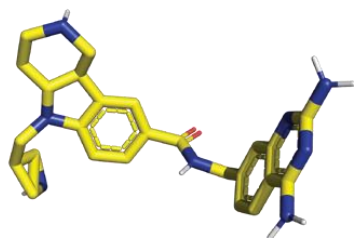
Cluster 1



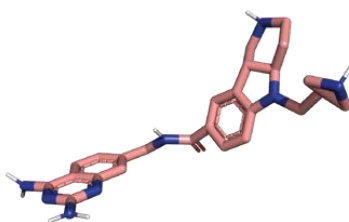
Cluster 2



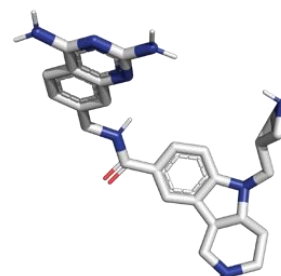
Cluster 3



Cluster 4



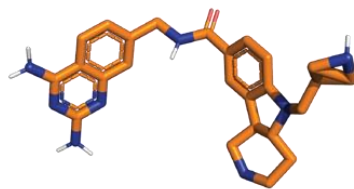
Cluster 5



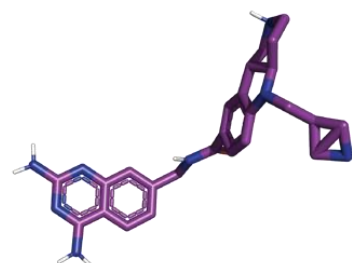
Cluster 6



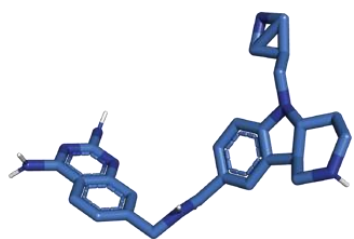
Cluster 7



Cluster 8



Cluster 9



Cluster 10

Supplementary Tables 1- 7:

Supplementary Table 1. Clusters of energy-minimized poses for MTA

Clus-ter Rank	Lowest Binding Energy	Run	Mean Binding Energy	Num in Clus
1	-6.63	1556	-6.03	1781
2	-5.95	241	-5.39	104
3	-5.62	1994	-5.54	48
4	-5.49	1083	-5.45	32
5	-5.21	1555	-5.17	4
6	-5.18	107	-5.18	1
7	-5.06	526	-4.94	24
8	-4.97	837	-4.97	6

Supplementary Table 2. Clusters of energy-minimized poses for SAH

Clus-ter Rank	Lowest Binding Energy	Run	Mean Binding Energy	Num in Clus
1	-8.80	1101	-8.13	346
2	-8.64	1726	-7.88	163
3	-8.46	9	-7.91	177
4	-8.30	694	-7.82	151
5	-8.28	496	-7.69	227
6	-7.82	353	-7.36	162
7	-7.78	1750	-7.53	24
8	-7.64	656	-7.33	15
9	-7.59	520	-6.97	241
10	-7.52	1639	-6.81	39

Supplementary Table 3. Clusters of energy-minimized poses for SAM

Cluster Rank	Lowest Binding Energy	Run	Mean Binding Energy	Number in Cluster
1	-8.65	1187	-8.22	112
2	-8.55	1520	-8.26	128
3	-8.12	276	-8.15	197
4	-7.99	1993	-7.93	256
5	-7.80	1899	-7.73	373
6	-7.80	653	-7.72	3
7	-7.75	1018	-7.71	50
8	-7.38	129	-7.17	30
9	-7.27	1644	-7.53	22
10	-7.17	1824	-7.51	3

Supplementary Table 4. Clusters of energy-minimized poses for 11-2F

Cluster Rank	Lowest Binding Energy	Run	Mean Binding Energy	Number in Cluster
1	-10.99	301	-10.18	49
2	-10.92	825	-10.43	70
3	-10.38	875	-9.72	10
4	-10.38	1353	-9.68	540
5	-10.36	234	-9.68	24
6	-10.21	512	-9.74	173
7	-10.13	304	-9.67	20
8	-10.10	1757	-9.28	11
9	-9.92	1551	-9.37	22
10	-9.89	217	-9.28	114

Supplementary Table 5: Clusters of energy-minimized poses for 11-9F

Cluster Rank	Lowest Binding Energy	Run	Mean Binding Energy	Number in Cluster
1	-10.95	1852	-10.43	1120
2	-10.80	42	-10.47	93
3	-10.64	1780	-10.58	4
4	-10.59	1386	-10.24	3
5	-10.25	626	-9.84	20
6	-10.09	68	-9.86	10
7	-10.08	1179	-9.93	20
8	-10.07	110	-9.33	17
9	-10.03	18	-9.84	23
10	-10.02	1457	-9.88	14

Supplementary Table 6. Clusters of energy-minimized poses for HWIem2104

Cluster Rank	Lowest Binding Energy	Run	Mean Binding Energy	Number in Cluster
1	-12.67	968	-11.98	1117
2	-12.64	1222	-11.54	160
3	-11.60	6	-10.62	284
4	-11.29	531	-10.17	4
5	-11.24	394	-10.75	14
6	-11.23	23	-10.57	5
7	-10.93	1715	-10.93	1
8	-10.82	446	-10.53	34
9	-10.63	1067	-10.31	15
10	-10.23	1373	-10.03	10

Supplementary Table 7. Clusters of energy-minimized poses for HWIem2109

Clus-ter Rank	Lowest Binding Energy	Run	Mean Binding Energy	Num in Clus
1	-12.26	725	-11.56	420
2	-11.69	879	-11.06	290
3	-10.98	1254	-10.48	911
4	-10.83	631	-10.14	44
5	-10.28	1034	-9.81	58
6	-10.27	1291	-9.72	21
7	-10.24	1561	-9.96	173
8	-10.22	169	-10.22	1
9	-10.21	939	-9.86	6
10	-10.12	754	-10.12	1

Supplementary References:

- 1 Pettersen, E. F. *et al.* UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* **25**, 1605-1612, doi:10.1002/jcc.20084 (2004).
- 2 The PyMOL Molecular Graphics System. v. 2.0 (Schrodinger, LLC, 2020).