

Supporting information

# Beyond PAINs: Chemotype Sensitivity of Protein Methyltransferases in Screens

Cen Gao,<sup>†</sup> Brandon Margolis,<sup>†</sup> John M. Strelow,<sup>†</sup> Lewis R. Vidler,<sup>‡</sup> Mary M. Mader<sup>†\*</sup>

<sup>†</sup>Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285, United States

<sup>‡</sup>Research and Development, Eli Lilly and Company Ltd., Sunninghill Road, Windlesham, Surrey GU20 6PH, United Kingdom

\*Corresponding author: [mader@lilly.com](mailto:mader@lilly.com)

Figure S1 Statistics of number of compounds screened in these 13 assays and number of times each compounds screened.

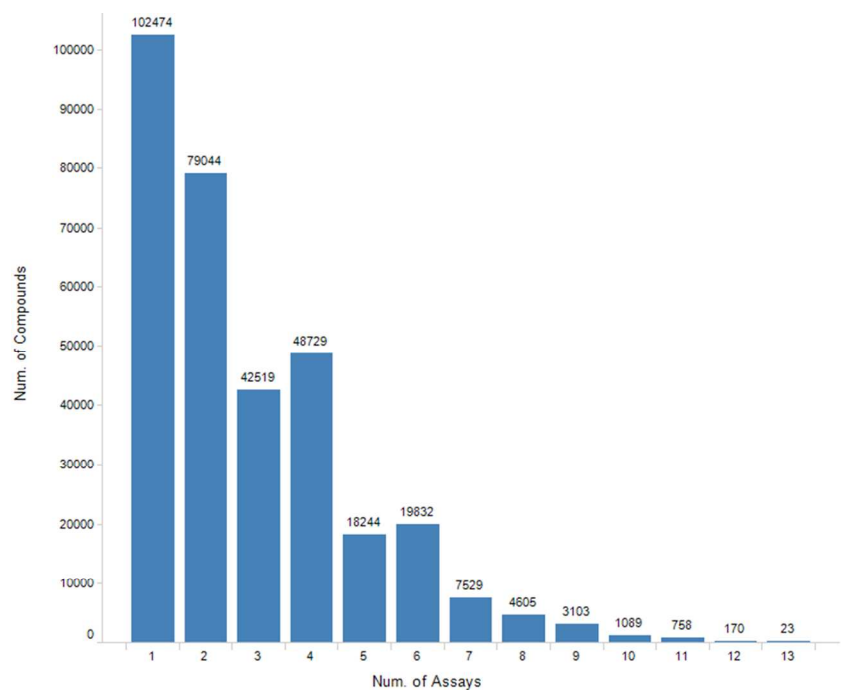


Figure S2 Number of times compounds are active. Active is defined as > 50% inhibition in single point assay.

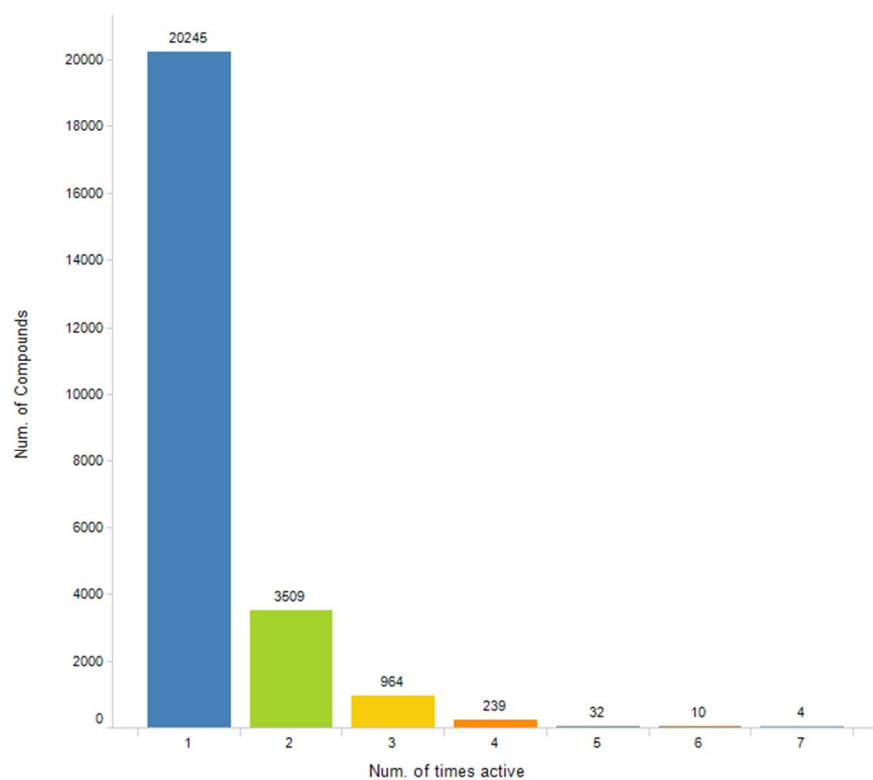


Table S1. Physicochemical properties of compounds screened in different assays.

Assay	Molecular Weight	AlogP	Hbond Acceptor	Hbond Donor	Rotatable bonds	PSA (Å)
EZH2 (w.t.)	369±54	2.9±1.3	3.6±1.4	1.0±0.9	5±2	84±29
EZH2 (A677G)	369±53	3.0±1.2	3.5±1.4	1.0±0.8	5±2	83±28
EZH2 (Y641N)	367±54	3.0±1.2	3.5±1.4	1.0±0.8	5±2	83±28
PRMT5	339±70	3.0±1.6	3.1±1.5	0.9±0.9	5±2	75±32
PRMT6	392±33	3.0±1.1	3.8±1.4	1.1±0.8	6±2	89±28
SETD8	358±69	3.1±1.5	3.3±1.5	1.0±0.9	5±2	78±31
SUV420H1	366±56	2.7±1.4	3.8±1.6	1.2±1.0	5±2	89±32
SMYD2	338±71	3.0±1.6	3.1±1.5	0.9±0.9	5±2	75±32
NHR A	361±90	3.5±2.0	2.9±1.5	0.8±0.9	5±3	79±33
PDE A	333±93	3.2±1.7	2.9±1.6	0.9±0.9	4±3	72±34
PDE B	356±83	3.3±1.6	3.0±1.5	1.0±0.9	5±3	74±33
PDE C	333±93	3.2±1.7	2.9±1.6	0.9±0.9	4±3	72±34
PDE D	358±83	3.3±1.6	3.1±1.5	1.0±0.9	5±3	76±33

Table S2. Lilly historical assay profile for 285 frequent hitters from current analysis

Compound ID	Lilly historical			Current analysis	
	# of assay tested	# of assay active	# of protein family active	# of assay tested	# of assay active
1	111	0	0	4	4
2	185	1	1	4	4
3	45	1	1	4	4
4	56	2	1	4	4
5	131	2	1	4	4
6	99	2	1	4	4
7	51	2	2	4	4
8	60	2	1	4	4
9	91	3	1	4	4
10	142	4	3	4	4
11	47	4	2	4	4
12	157	5	2	4	4
13	51	5	3	4	4
14	105	5	2	4	4
15	461	6	3	4	4
16	284	6	3	4	4
17	58	7	2	4	4
18	62	7	4	4	4
19	109	7	2	4	4

20	302	8	2	4	4
21	207	8	2	4	4
22	111	8	3	4	4
23	62	8	2	4	4
24	338	9	5	4	4
25	352	10	4	4	4
26	364	10	4	4	4
27	137	10	3	4	4
28	103	10	5	4	4
29	106	10	4	4	4
30	145	11	2	4	4
31	338	12	4	4	4
32	151	12	4	4	4
33	267	12	6	4	4
34	516	12	5	4	4
35	74	12	3	4	4
36	472	13	4	4	4
37	76	13	2	4	4
38	115	14	2	4	4
39	496	14	5	4	4
40	441	14	4	4	4
41	488	14	6	4	4
42	365	14	6	4	4
43	622	15	5	4	4
44	523	15	4	4	4
45	78	15	4	4	4
46	91	15	2	4	4
47	387	16	3	4	4
48	359	16	5	4	4
49	78	16	4	4	4
50	453	17	5	4	4
51	428	18	4	4	4
52	185	18	3	4	4
53	828	20	6	4	4
54	376	20	6	4	4
55	365	20	5	4	4
56	409	20	6	4	4
57	361	20	4	4	4
58	351	20	6	4	4
59	519	21	7	4	4
60	133	22	4	4	4

61	352	23	5	4	4
62	344	23	5	4	4
63	463	23	7	4	4
64	455	23	6	4	4
65	447	23	4	4	4
66	177	24	6	4	4
67	522	24	6	4	4
68	366	24	7	4	4
69	78	24	1	4	4
70	594	25	5	4	4
71	396	25	8	4	4
72	393	26	9	4	4
73	454	27	6	4	4
74	428	27	7	4	4
75	453	27	5	4	4
76	90	27	6	4	4
77	495	28	7	4	4
78	233	30	3	4	4
79	408	30	7	4	4
80	452	30	6	4	4
81	162	30	3	4	4
82	386	31	8	4	4
83	475	31	6	4	4
84	182	31	7	4	4
85	519	35	6	4	4
86	462	38	6	4	4
87	507	39	5	4	4
88	844	40	4	4	4
89	503	40	5	4	4
90	462	40	6	4	4
91	258	42	8	4	4
92	445	43	9	4	4
93	305	44	4	4	4
94	438	45	9	4	4
95	503	49	8	4	4
96	289	49	3	4	4
97	362	51	9	4	4
98	472	54	7	4	4
99	398	55	8	4	4
100	480	56	8	4	4
101	385	56	8	4	4

102	544	75	7	4	4
103	645	190	9	4	4
104	611	217	2	4	4
105	118	3	2	5	5
106	150	4	3	5	4
107	171	4	3	5	4
108	119	5	2	5	4
109	183	5	3	5	4
110	167	7	2	5	5
111	396	8	5	5	4
112	208	10	6	5	4
113	114	10	2	5	4
114	328	14	3	5	4
115	596	17	7	5	4
116	551	17	4	5	4
117	402	19	6	5	4
118	387	19	8	5	4
119	311	20	6	5	4
120	209	21	2	5	4
121	363	22	7	5	4
122	284	23	5	5	4
123	380	24	6	5	4
124	461	24	3	5	5
125	495	25	7	5	4
126	335	26	7	5	4
127	518	28	4	5	4
128	278	29	7	5	4
129	366	33	6	5	4
130	494	36	8	5	4
131	361	37	9	5	4
132	685	38	5	5	4
133	676	42	9	5	4
134	474	44	9	5	4
135	292	47	5	5	5
136	615	48	7	5	4
137	450	48	6	5	4
138	315	49	2	5	4
139	460	66	11	5	5
140	430	71	9	5	4
141	439	82	8	5	4
142	154	0	0	6	4

143	266	1	1	6	4
144	189	1	1	6	4
145	213	1	1	6	5
146	144	1	1	6	4
147	209	2	1	6	5
148	152	2	2	6	4
149	133	2	1	6	4
150	301	3	2	6	5
151	173	3	2	6	4
152	238	4	1	6	5
153	166	4	2	6	4
154	147	4	2	6	4
155	251	5	3	6	4
156	172	5	2	6	4
157	157	5	2	6	4
158	293	6	4	6	4
159	103	6	2	6	4
160	791	7	3	6	4
161	307	7	3	6	4
162	179	7	4	6	4
163	211	7	2	6	6
164	196	7	4	6	5
165	169	8	2	6	5
166	360	10	4	6	6
167	179	10	2	6	4
168	337	11	6	6	4
169	186	12	2	6	4
170	203	12	5	6	4
171	142	12	3	6	4
172	235	13	6	6	4
173	216	14	4	6	4
174	291	15	3	6	4
175	508	15	6	6	4
176	218	16	5	6	5
177	573	17	4	6	4
178	304	18	4	6	4
179	544	20	6	6	4
180	590	20	8	6	4
181	309	20	4	6	4
182	370	21	5	6	4
183	424	22	6	6	5

184	266	22	6	6	4
185	455	25	5	6	4
186	770	30	6	6	4
187	306	31	8	6	4
188	547	31	6	6	5
189	241	31	7	6	5
190	389	33	5	6	4
191	352	34	4	6	5
192	483	36	8	6	4
193	246	45	3	6	4
194	318	47	8	6	4
195	605	49	7	6	4
196	474	50	9	6	4
197	689	50	7	6	6
198	862	66	7	6	4
199	747	100	8	6	4
200	151	1	1	7	4
201	301	2	2	7	4
202	55	6	1	7	5
203	347	7	5	7	4
204	365	7	2	7	4
205	119	8	2	7	4
206	482	9	5	7	4
207	297	9	3	7	4
208	211	9	2	7	4
209	208	9	2	7	4
210	505	14	6	7	4
211	320	14	6	7	4
212	292	16	5	7	4
213	538	17	6	7	4
214	419	19	8	7	4
215	594	20	6	7	4
216	299	26	5	7	5
217	542	28	7	7	4
218	541	29	7	7	4
219	326	32	4	7	4
220	563	36	5	7	4
221	656	41	8	7	4
222	574	44	7	7	5
223	459	58	3	7	4
224	883	59	6	7	4



225	509	61	8	7	4
226	698	79	8	7	4
227	320	1	1	8	4
228	322	4	3	8	4
229	337	5	2	8	5
230	127	7	4	8	4
231	353	7	2	8	4
232	201	7	3	8	4
233	384	8	5	8	5
234	194	8	2	8	4
235	303	9	3	8	4
236	321	12	6	8	4
237	328	12	6	8	4
238	497	14	6	8	5
239	283	15	3	8	5
240	667	16	7	8	4
241	405	18	7	8	4
242	459	19	6	8	4
243	529	22	5	8	6
244	594	23	6	8	6
245	244	24	8	8	6
246	467	38	9	8	4
247	359	41	6	8	6
248	303	46	7	8	5
249	522	54	9	8	4
250	611	59	8	8	4
251	356	2	2	9	4
252	331	5	3	9	5
253	348	7	3	9	4
254	389	9	5	9	4
255	398	10	4	9	7
256	442	11	5	9	4
257	479	14	6	9	4
258	311	15	5	9	4
259	324	16	6	9	6
260	189	16	4	9	5
261	641	18	5	9	4
262	403	26	7	9	4
263	454	26	6	9	5
264	472	35	6	9	4
265	718	39	10	9	4

266	919	40	7	9	4
267	328	47	6	9	6
268	987	102	11	9	7
269	442	5	4	10	5
270	332	5	4	10	4
271	375	10	4	10	5
272	569	12	4	10	4
273	485	13	7	10	5
274	334	25	5	10	4
275	611	26	3	10	4
276	691	28	6	10	7
277	384	29	6	10	5
278	760	49	5	10	6
279	646	81	6	10	4
280	699	97	7	10	5
281	376	11	5	11	4
282	398	14	8	11	4
283	607	26	7	11	4
284	567	30	8	11	4
285	702	32	5	12	7

Table S3 PAINS substructures that are prone to the set of 13 SPA assays. Category name and definition are directly taken from Table S6-S8 of the original PAINS publication.<sup>1</sup>

PAINS Rules	hit rate	# of times active	# of time tested
<i>het_pyridiniums_A(39)</i>	0.81	35	43
<i>amino_acridine_A(46)</i>	0.54	38	70
<i>azo_A(324)</i>	0.35	42	120
<i>quinone_A(370)</i>	0.35	54	156
<i>ene_cyano_B(7)</i>	0.33	11	33
<i>pyrrole_E(5)</i>	0.31	16	52
<i>ene_one_hal(17)</i>	0.26	10	38
<i>hzone_phenol_B(215)</i>	0.25	13	53
<i>ene_five_het_C(85)</i>	0.24	9	37
<i>anil_alk_ene(51)</i>	0.24	82	341
<i>ene_rhod_A(235)</i>	0.23	92	397

#### References:

1. Baell, J. B., & Holloway, G. A. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J. of Med. Chem.*, **2010**, 53(7), 2719–40.