

Lipodisks integrated with weak affinity chromatography enable fragment screening of integral membrane proteins

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Supporting information

Lipodisks Integrated with Weak Affinity Chromatography Enable Fragment Screening of Integral Membrane Proteins

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Table S-1: Lipid concentration and protein content of the samples at different stages during the lipodisk preparation process.

<i>Lipodisks in solution</i>				
	Lipodisks with AQP1			Lipodisks without AQP1
Preparation step	Lipid concentration ^[a] (mM)		AQP1:Lipid mol:mol ratio ^[b]	Lipid concentration ^[a] (mM)
Solubilization	11.73		1:243	5
Gel filtration (detergent depletion)	Fraction 1	0.81	1:1256	0.24
	Fraction 2	1.07	1:571	
	Fraction 3	0.19	1:635	
	Fraction 4	0.05	1:414	
Ultrafiltration (concentration)	Fraction 1	19.7	1:267	33.5
	Fraction 2	33.3	1:333	
	Fraction 3	4.3	1:1177	
	Fraction 4	10.6	1:2227	
<i>Lipodisks immobilized on silica</i>				
	Lipodisks with AQP1 (fraction 1 only)			Lipodisks without AQP1
	Lipid / silica ratio (nmol mg ⁻¹) ^[c]	AQP1 / silica ratio (pmol mg ⁻¹) ^[d]	Lipid / silica ratio (nmol mg ⁻¹) ^[c]	
After immobilization	22.4	50.1	10.6	
Before packing^e	15.7	7.0	10.6	
After packing and HPLC experiments^e	13.6	9.4	10.6	

^[a] Determined by phosphorus analysis according to the method by Murphy and Riley¹ with the modifications described by Paraskova et al².

^[b] Protein concentration determined by the dot-it-spot-it³ assay.

^[c] Silica amount determined gravimetrically.

^[d] Indirectly immobilized AQP1 (solely via the lipodisks). Determined by dot-it-spot-it³.

^[e] Performed on another batch of AQP1 silica, without fractionation of the lipodisk slurry. Only one batch was used for the control lipodisk silica.

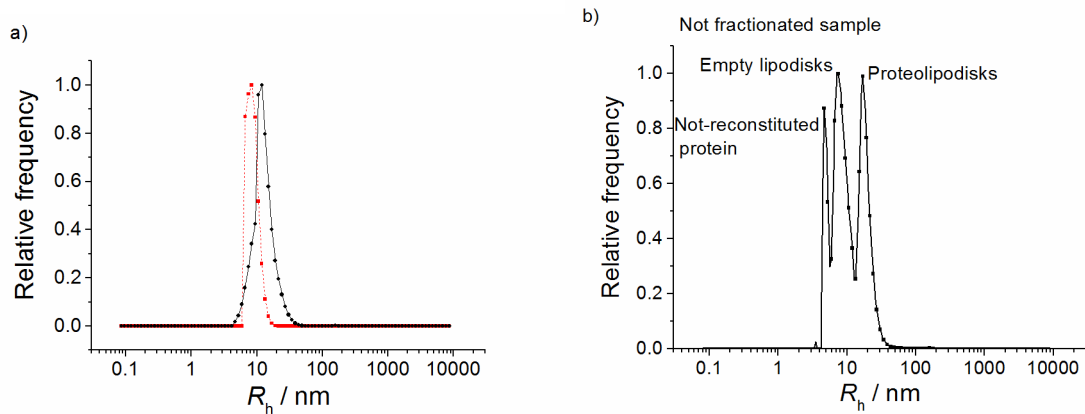


Figure S-1: DLS results (number weighted) obtained a) after the concentration of fraction 1 of lipodisks containing human AQP1 (solid black line and symbols) and of control lipodisks (without protein, dashed red line and symbols), and b) from a non-fractionated sample (corresponding to the Cryo-TEM picture in Figure 2 in the main text). R_h represents the hydrodynamic radius. The results show that the most abundant particle population in the concentrated fraction 1 of the lipodisks prepared in the presence of protein has an average hydrodynamic radius of 13 ± 0.3 nm. These particles are likely to correspond to protein-containing lipodisks, in agreement with the Cryo-TEM observations. The wide tail on the left hand side of the distribution may correspond to empty disks, which are significantly smaller than proteolipodisks. In the absence of protein, a single population of empty lipodisks with a rather homogeneous particle size distribution is obtained (8.58 ± 0.2 nm). If no fractionation of the proteolipodisk sample is performed, significant amounts of empty lipodisks and small particles are observed in the final suspension. The latter particles most likely correspond to detergent stabilized protein. Given that these particles are not observed in fraction 1, it is safe to assume that all of the protein in this fraction is reconstituted into lipodisks.

Table S-2: Compounds screened by WAC

No.	Name/ID	MW	SMILES ¹	Mixture
1	ST007539	156	<chem>OCc1c(C)nc(O)nc1O</chem>	1
2	ST001938	192	<chem>CCC(=O)Nc(ccc1)cc1C(=O)N</chem>	1
3	ST004826	197	<chem>CCOC(=O)CNC(=O)c1cccc1</chem>	1
4	ST012610	202	<chem>o1cccc1C(=O)Nc2cc(N)ccc2</chem>	1
5	ST009343	204	<chem>N#CCC(=O)NCc1ccc(cc1)OC</chem>	1
6	ST002193	216	<chem>NC(=O)c(c1)c(C)nc(c12)ccc(c2)OC</chem>	1
7	ST002443	242	<chem>COc(cc1)ccc1C(=O)NCc2cccnc2</chem>	1
8	ST005426	256	<chem>CCOc(cc1)ccc1C(=O)NCc2cccnc2</chem>	1
9	ST008659	268	<chem>N#CCN(CC#N)C(=O)c1c(Cl)ccc(Cl)c1</chem>	1
10	ST007450	279	<chem>c1cc(Cl)ccc1C(=O)CC(C(=O)O)n2ccnc2</chem>	1
11	ST013917	136	<chem>N#CC(C)(C)C(C#N)(C)C</chem>	2
12	ST013870	193	<chem>CCOC(=O)C(N)Cc1cccc1.Cl</chem>	2
13	ST020706	197	<chem>O=C(O)CNC(=O)c1c(F)cccc1</chem>	2
14	ST018517	204	<chem>CC(=O)Nc1c(Cl)cccc1Cl</chem>	2
15	ST012357	212	<chem>Cc1ccc(c(n1)N)/N=N/c2cccc2</chem>	2
16	ST013868	221	<chem>s1ccnc1/N=N/c2c(O)cc(O)cc2</chem>	2
17	ST019644	242	<chem>c1cccc(C(=O)O)c1C(=O)Nc2cccn2</chem>	2
18	ST016629	267	<chem>n1onc(NC=O)c1NC(=O)c2c(Cl)cccc2</chem>	2
19	ST007393	269	<chem>Cc1c(Cl)cc(cc1)NC(=O)CN2CCOCC2</chem>	2
20	ST019847	286	<chem>N#CCCN(C)CC(=O)Nc(c1)ccc(Cl)c1Cl</chem>	2
21	ST021132	151	<chem>NCCCOc1cccc1.Cl</chem>	3
22	ST025313	179	<chem>CCC(N)(C(=O)O)c1cccc1</chem>	3
23	ST026253	199	<chem>CC(=O)OCC(=O)NC1CCCC1</chem>	3
24	ST024947	201	<chem>O=C(O)C(N)Cc1cc(F)cc(F)c1</chem>	3
25	ST028992	218	<chem>O=C(O)CCc(n1)c(O)nc(c12)cccc2</chem>	3
26	ST024942	219	<chem>O=C(O)C(N)Cc1c(F)c(F)ccc1F</chem>	3
27	ST025563	246	<chem>[nH]1cnnc1NC(=O)COc(cc2)ccc2CC</chem>	3
28	ST025110	262	<chem>CC(=O)OCC(=O)Nc1c(Cl)cc(Cl)cc1</chem>	3
29	ST020960	271	<chem>c1ccc(F)cc1C(=O)Nc(c2)ccc(c23)[nH]c(=O)[nH]3</chem>	3
30	ST028998	278	<chem>O=C(O)Cn(c1=O)cnc2sc(c3c12)CC(C)CC3</chem>	3
31	ST035586	139	<chem>CC(N)c1c(C)n[nH]c1C.Cl</chem>	4
32	ST038822	191	<chem>COc(cc1)ccc1-c2c(N)non2</chem>	4
33	ST039860	201	<chem>CC(=O)c1cn(C(=O)C)c(c12)cccc2</chem>	4
34	ST039942	204	<chem>C1CC1C(=O)Nc(cc2)ccc2C(=O)N</chem>	4
35	ST039904	218	<chem>CC(=O)Nc(n1)sc1-c2cccc2</chem>	4

No.	Name/ID	MW	SMILES ¹	Mixture
36	ST032955	233	<chem>COC(=O)c1cc(on1)-c2c(OC)cccc2</chem>	4
37	ST034565	246	<chem>s1cccc1C(=O)Nc(cc2)ccc2C(=O)N</chem>	4
38	ST040330	249	<chem>CC(=O)c1ccc(cc1)NC(=O)CCC(=O)OC</chem>	4
39	ST040469	260	<chem>N#CC1=C(N)OC(C)=C(C(=O)C)C1c2cccs2</chem>	4
40	ST031339	261	<chem>CC(=O)Nc(ccc1)cc1C(=O)Nc2nccs2</chem>	4
41	ST040398	163	<chem>O=C1NC(CO1)c2cccc2</chem>	5
42	ST042788	211	<chem>COC(=O)CNC(=O)c1c(F)cccc1</chem>	5
43	ST042585	212	<chem>Cc1cc(ccc1)NC(=O)c2cccnc2</chem>	5
44	ST042625	226	<chem>c1cccc1CC(=O)NCc2ccncc2</chem>	5
45	ST034373	245	<chem>n1[nH]ccc1C(=O)Nc(c2C(=O)OC)cccc2</chem>	5
46	ST042214	248	<chem>c1cccc1C(=O)NCCCN2CCOCC2</chem>	5
47	ST042685	250	<chem>CN(C)CCCNC(=O)Cc1ccc(cc1)OC</chem>	5
48	ST041074	255	<chem>CC(=O)Nc(cc1)c(Cl)cc1C(=O)NCCC</chem>	5
49	ST043170	260	<chem>C1COCCN1C(=O)c2c(Cl)cccc2Cl</chem>	5
50	ST042979	265	<chem>O1CCCC1CNC(=O)c2cc(OC)cc(c2)OC</chem>	5
51	ST043348	207	<chem>CCOC(=O)C(=O)Nc(c1C)cccc1</chem>	6
52	ST047884	211	<chem>s1cccc1C(=O)Nc2nncs2</chem>	6
53	ST043356	215	<chem>CC1CN(CC(O1)C)C(=O)C(=O)OCC</chem>	6
54	ST048346	228	<chem>O=c1cc(C)oc(c12)c3c(oc2=O)cccc3</chem>	6
55	ST057435	240	<chem>CCOC(=O)c1c(N)sc(c12)CN(C)CC2</chem>	6
56	ST043370	254	<chem>C1CN(C)CCN1C(=O)C(=O)N2CCN(C)CC2</chem>	6
57	ST055034	262	<chem>O=C(O)CCC(=O)Nc(c1)ccc(Cl)c1Cl</chem>	6
58	ST044212	263	<chem>Cc1nnc(s1)NC(=O)C(C)Oc2cccc2</chem>	6
59	ST055117	266	<chem>N#Cc1ccc(cc1)NC(=O)c2c(C(=O)O)cccc2</chem>	6
60	ST052834	280	<chem>CCOC(=O)Cc1csc(n1)NC(=O)c2cccc2</chem>	6
61	ST055554	157	<chem>CC1(C)CC(O)C(C)CN1C</chem>	7
62	ST057195	167	<chem>O=C(O)COc(cc1)ccc1N</chem>	7
63	ST057330	174	<chem>n1ncn(C)c1-c2ccc(N)cc2</chem>	7
64	ST055641	218	<chem>O=C(O)C(N)Cc1cn(C)c(c12)cccc2</chem>	7
65	ST060177	228	<chem>c1cccc(O)c1C(=O)NCc2ccccn2</chem>	7
66	ST058710	234	<chem>C1CCCCN1C(=O)COc(cc2)ccc2N</chem>	7
67	ST055460	250	<chem>CCOC(=O)C(=O)Nc(n1)sc(c12)cccc2</chem>	7
68	ST055435	256	<chem>c1ncccc1C(=O)Nc(c2C(=O)OC)cccc2</chem>	7
69	ST058429	261	<chem>n1nncn1CCC(=O)Nc2c(OCC)cccc2</chem>	7
70	ST044057	262	<chem>Cc1cc(no1)NC(=O)c2cc(OC)cc(c2)OC</chem>	7
71	ST059084	99	<chem>O=C1CCNCC1.O.Cl</chem>	8
72	ST066714	190	<chem>[nH]1nnc1Cc2c(OC)cccc2</chem>	8

No.	Name/ID	MW	SMILES ¹	Mixture
73	ST060251	206	<chem>O=C(O)C1CCN(CC1)c2ccncc2.Cl</chem>	8
74	ST060990	220	<chem>CCC(=O)Nc(c1C)cc(cc1)NC(=O)C</chem>	8
75	ST066501	221	<chem>CCC(=O)NCCNC(=O)c1ccccc1</chem>	8
76	ST066271	229	<chem>CCOCCC(=O)Nc(nc1)ccc1Cl</chem>	8
77	ST065849	239	<chem>C=CCNC(=O)C(=O)Nc1cc(Cl)ccc1</chem>	8
78	ST058342	245	<chem>O=C(O)c1cnn(c12)c(ccn2)-c3cccs3</chem>	8
79	ST071904	257	<chem>CC(=O)Nc1c(C#N)sc(c12)nc3c(c2)CCC3</chem>	8
80	ST065071	276	<chem>O1CCCC1CNC(=O)C(=O)Nc(c2CC)cccc2</chem>	8
81	ST066818	177	<chem>CCNCC(C1)Oc(c12)cccc2</chem>	9
82	ST072153	193	<chem>O=C(O)c(c1)sc(c12)ccc(c2)N</chem>	9
83	ST069077	202	<chem>n1onc(c12)nc(C)c3c2[nH]ccc3=O</chem>	9
84	ST067028	226	<chem>CC(C)NC(=O)C(=O)NCc1cccs1</chem>	9
85	ST060179	228	<chem>c1cccc(O)c1C(=O)Nc(n2)cccc2C</chem>	9
86	ST066879	233	<chem>COC(=O)c1c(C(=O)OC)[nH]c(c12)cccc2</chem>	9
87	ST068836	251	<chem>c1cccc1Cn(c2=O)cnc(c23)ccc(c3)N</chem>	9
88	ST071056	256	<chem>C1COCCN1C(C(=O)O)c2ccc(Cl)cc2</chem>	9
89	ST071531	273	<chem>c1cccc1CNc(s2)nc(c23)nc([O-])cc3O</chem>	9
90	ST071626	277	<chem>Oc1cc(=O)[nH]c(c12)nc(s2)Nc3ccc(F)cc3</chem>	9
91	ST074974	128	<chem>NCC1CCN(C)CC1</chem>	10
92	ST073961	130	<chem>CC(=O)CCCOC=O</chem>	10
93	ST072514	139	<chem>CC(N)c1c(C)nn(C)c1</chem>	10
94	ST074933	169	<chem>CCC(N)C(=O)c1cccs1</chem>	10
95	ST081142	180	<chem>CC(=O)COc1ccc(cc1)OC</chem>	10
96	ST074965	187	<chem>n1c[nH]cc1C(=O)Nc2ccccc2</chem>	10
97	ST072532	216	<chem>O=C(O)CCn1ccnc1-c2ccccc2</chem>	10
98	ST072523	217	<chem>NCc(c1)ccc(OC)c1Cn2cccn2</chem>	10
99	ST075856	236	<chem>Nc1nc(O)c(C#N)c(c1C#N)-c2ccccc2</chem>	10
100	ST073768	246	<chem>CC(=O)N1CCC(=O)N(C(C)C)c(c12)cccc2</chem>	10
101	ST080885	169	<chem>O=C(O)Cn(nn1)cc1C(C)C</chem>	11
102	ST081136	172	<chem>OCCc1c(N)cccc1Cl</chem>	11
103	ST079407	173	<chem>Cc(n1)c(C)nc(c12)ccc(c2)N</chem>	11
104	ST079515	176	<chem>Cn1c(=O)c(=O)[nH]c(c12)cccc2</chem>	11
105	ST081354	180	<chem>COc1cccc(c12)sc(n2)N</chem>	11
106	ST079730	190	<chem>O=C(O)c1cc(ccc1)-c2nnn[nH]2</chem>	11
107	ST078480	191	<chem>CC(=O)Nc1c(cccc1)OCC=C</chem>	11
108	ST074499	216	<chem>c1ncccc1CNc(n2)[nH]c(=O)cc2C</chem>	11
109	ST079397	218	<chem>c1cnccc1C(=O)c2ccc(Cl)cc2</chem>	11

No.	Name/ID	MW	SMILES ¹	Mixture
110	ST082361	222	<chem>CCOC(=O)c(c[nH]1)c(=O)n(c12)nc(n2)C</chem>	11
111	ST082325	142	<chem>O=C(O)c1ccc(s1)C</chem>	12
112	ST081121	169	<chem>N#Cc1c(N)ccc(c12)nccc2</chem>	12
113	ST084694	173	<chem>N#Cc1c(C)cc(C)n(c12)nnc2</chem>	12
114	ST084657	185	<chem>Oc1c(Cl)c(C)nn(c12)ncn2</chem>	12
115	ST084809	195	<chem>CCOC(=O)c(c(n1)N)n(c12)CCC2</chem>	12
116	ST081404	217	<chem>O=C1C(C)C(C)N(C1)Cc2ccccc2</chem>	12
117	ST082040	233	<chem>CC(C)(O)c1cc(no1)-c2ccc(cc2)OC</chem>	12
118	ST084648	237	<chem>CCC(=O)Nc(non1)c1-c(no2)nc2CC</chem>	12
119	ST083226	260	<chem>Nc(s1)nnc1CCN(CC2)Cc(c23)cccc3</chem>	12
120	ST084486	279	<chem>CCC(=O)Nc1c(N)sc(c12)nc(C)cc2COC</chem>	12
121	ST086491	162	<chem>O=C(O)c(c1)ccc(c12)[nH]cn2</chem>	13
122	ST085489	165	<chem>OCCNC(C)c1cccc1</chem>	13
123	ST086495	182	<chem>O=C(O)C1C(C(=O)O)C(C2)C=CC12</chem>	13
124	ST084814	184	<chem>CC(=O)c1cc(cs1)CC(=O)O</chem>	13
125	ST084965	200	<chem>c1ncccc1COc(c2N)cccc2</chem>	13
126	ST085529	209	<chem>C1CNCCC1C(O)c2ccc(F)cc2</chem>	13
127	ST086616	220	<chem>O=C(O)c(n1)nn(c2=O)c1[nH]c(c23)CCC3</chem>	13
128	ST086467	238	<chem>C1CCCN1CC(=O)Nc(o2)nnc2CC</chem>	13
129	ST086434	250	<chem>C1COCCN1CC(=O)Nc2c(OC)cccc2</chem>	13
130	ST085670	254	<chem>c1cccc(c12)oc(c(c2=O)O)-c3ccc(O)cc3</chem>	13
131	ST088216	125	<chem>CNCc1cc(C)[nH]n1</chem>	14
132	ST087933	129	<chem>O=C(O)c1nc(=O)[nH][nH]1</chem>	14
133	ST092304	142	<chem>CN1CC(=O)N(C)CC1=O</chem>	14
134	ST092310	166	<chem>O=C(O)c1cc(C)c(O)c(C)c1</chem>	14
135	ST088687	179	<chem>OCc(c1)ccc(c12)OCC(=O)N2</chem>	14
136	ST088579	192	<chem>CC(C)(C)NC(=O)c1c(N)cccc1</chem>	14
137	ST089337	214	<chem>CC(C)COC(=O)CC(C1=O)NCCN1</chem>	14
138	ST088714	245	<chem>n1[nH]c(C)c(c1C)NC(=O)c2cc(OC)ccc2</chem>	14
139	ST089346	256	<chem>CC(C)COC(=O)CC(C1=O)N(C(=O)C)CCN1</chem>	14
140	ST092546	276	<chem>C1COCCN1C(=O)c2cc(no2)-c3ccc(F)cc3</chem>	14
141	ST093059	130	<chem>Clc1ccc(O)nc1</chem>	15
142	ST092736	149	<chem>NC(=O)c1ccc(CC)cc1</chem>	15
143	ST092941	164	<chem>Cn1c(=O)oc(c12)cc(N)cc2</chem>	15
144	ST094925	178	<chem>c1ccc(O)cc1N2CCNCC2</chem>	15
145	ST000762	201	<chem>N=C(N)SCc1ccc(Cl)cc1</chem>	15
146	ST094844	202	<chem>c1cccc(c12)oc(n2)C3CCCN3</chem>	15

No.	Name/ID	MW	SMILES ¹	Mixture
147	ST092858	204	<chem>O=C(O)c1cccn(c12)c(=O)cc(n2)C</chem>	15
148	ST092952	207	<chem>COC(=O)c(cc1)cc(c12)OCC(=O)N2</chem>	15
149	ST094899	214	<chem>n1cccc1C(=O)C(O)c2cccn2</chem>	15
150	ST094878	231	<chem>Cc(n1)oc(c1C#N)NCCCN2ccnc2</chem>	15
151	ST002754	115	<chem>Cc1c(N)no[n+1][O-]</chem>	16
152	ST006406	195	<chem>NCCCNc1ccc([N+][O-])=O)cc1</chem>	16
153	ST021500	215	<chem>O=C(O)C1CSC(N1)c2cccs2</chem>	16
154	ST024756	225	<chem>O1CCN(C1=O)\N=C\c2ccc(o2)[N+][O-]=O</chem>	16
155	ST002776	226	<chem>[O-][n+1]onc(c1C)/N=N/c(c2C)no[n+2][O-]</chem>	16
156	ST006408	230	<chem>NCCCNc1c([N+][O-])=O)cc(Cl)cc1</chem>	16
157	ST019864	240	<chem>CS(=O)(=O)c1ccc(s1)S(=O)(=O)C</chem>	16
158	ST013456	247	<chem>c1oncc1-c(c2[N+][O-])=O)n[nH]c2-c3cnoc3</chem>	16
159	ST016476	272	<chem>NC(=S)N/N=C(\C)c1ccc(Br)cc1</chem>	16
160	ST024130	278	<chem>C1C(Br)C(Br)CS1(=O)=O</chem>	16
161	ST037923	148	<chem>c1cc(C)n(c12)CCN=C2C</chem>	17
162	ST043640	166	<chem>CC(C)NS(=O)(=O)N(C)C</chem>	17
163	ST041068	200	<chem>OCC(Br)([N+][O-])=O)CO</chem>	17
164	ST043313	202	<chem>C1COCCN1C(=O)NCCCO</chem>	17
165	ST042454	241	<chem>O1CCCC1CNS(=O)(=O)c2ccccc2</chem>	17
166	ST036363	260	<chem>O\N=C\c1c(OC)cc(c(Br)c1)OC</chem>	17
167	ST044520	263	<chem>O=C1C=CC(=O)N1c2c(F)c(F)c(F)c2F</chem>	17
168	ST043757	264	<chem>CCOC(=O)C1CCN(CC1)S(=O)(=O)N(C)C</chem>	17
169	ST029575	270	<chem>o1cccc1/C=c2\c(=O)[nH]c/c(=O)[nH]2)=C\c3ccco3</chem>	17
170	ST049194	276	<chem>[O-][N+](=O)c(c1)ccc(c1C(F)(F)F)N2CCOCC2</chem>	17
171	ST068470	163	<chem>Cn(n1)cc(c1C)-c2ccno2</chem>	18
172	ST065680	182	<chem>CC12C(C)(C)C(CC1)C(=O)OC2=O</chem>	18
173	ST057427	208	<chem>[O-][N+](=O)c1ccc(cc1)N2CCOCC2</chem>	18
174	ST060476	210	<chem>[O-][N+](=O)c(c1)c(Cl)cc(c12)nccn2</chem>	18
175	ST059739	224	<chem>c1nccn1-c(c2[N+][O-])=O)ccc(Cl)c2</chem>	18
176	ST056172	233	<chem>O=C(O)CS(=O)c([nH]n1)c([N+][O-])=O)c1C</chem>	18
177	ST066977	251	<chem>CS(=O)(=O)Nc(nc1)ccc1Br</chem>	18
178	ST068634	277	<chem>[O-][N+](=O)c1cnn(c1)CSc(n2)nn(c23)cccn3</chem>	18
179	ST068678	280	<chem>o1cccc1CNC(=S)Nc2cnn(c2)COCC</chem>	18
180	ST057090	287	<chem>OCC1C(O)C(O)C(O1)n(cn2)c(c23)ncnc3Cl</chem>	18
181	ST083950	161	<chem>CCC1([N+][O-])=O)COCOC1</chem>	19
182	ST084494	180	<chem>N#CC1=C(N)ONC(=O)N1CC=C</chem>	19
183	ST081624	191	<chem>O=C(O)c1ccc(o1)Br</chem>	19

No.	Name/ID	MW	SMILES ¹	Mixture
184	ST083788	202	<chem>C=CCN1CCC(=O)Nc(c12)cccc2</chem>	19
185	ST082297	215	<chem>C1CC1C(=O)CSc(s2)nnc2N</chem>	19
186	ST081237	216	<chem>NCCOc1ccc(Br)cc1</chem>	19
187	ST084053	225	<chem>O/N=C(N)\c1nc(N(C)C)nc(n1)N(C)C</chem>	19
188	ST084863	252	<chem>C1COCCN1Cc2nc(no2)-c3c(N)non3</chem>	19
189	ST081261	253	<chem>n1nncn1-c(c2C)c(C)ccc2S(=O)(=O)N</chem>	19
190	ST081188	265	<chem>FC(F)(F)COCCNc(nc1)ccc1[N+][[O-]]=O</chem>	19
191	ST092683	174	<chem>c1cccc(c12)ccnc2[N+][[O-]]=O</chem>	20
192	ST094801	175	<chem>O=C(O)CC(C1=O)SC(=O)N1</chem>	20
193	ST093057	181	<chem>CSc1nc(N)nc(c12)nc[nH]2</chem>	20
194	ST086484	195	<chem>Cc(c1)sc(c12)nc(C)n(c2=O)N</chem>	20
195	ST092679	196	<chem>N#CCn(c1=O)c(=O)n(C)c(=O)n1C</chem>	20
196	ST024163	201	<chem>CNC(=S)Nc1ccc(Cl)cc1</chem>	20
197	ST088456	219	<chem>O1COc(c12)ccc(c2)N3CCCCC3=O</chem>	20
198	ST089003	222	<chem>CCOC(=O)c(c1C)oc(c12)CCCC2=O</chem>	20
199	ST085645	230	<chem>CCCNC(=O)OCC12N(COC1)COC2</chem>	20
200	ST088463	285	<chem>CS(=O)(=O)N(CC1)CCN1c(cc2)ccc2[N+][[O-]]=O</chem>	20

¹SMILES: Simplified Molecular-Input Line-Entry System

Table S-3: Results from the chromatography experiments. The identified hit is highlighted in blue.

Mix	IDNUMBER	AQP1-lipodisk column		Control-lipodisk column			Specific retention time
		Average adjusted retention time (min)	Standard deviation (min)	Average adjusted retention time (min)	Standard deviation (min)	Corrected retention time (average*2.113)	
1	ST007539						
1	ST001938	0.002	0.028	-0.001	0.019	-0.001	0.003
1	ST004826	0.554	0.154	0.804	0.093	1.699	-1.145
1	ST012610	0.553	0.057	0.777	0.003	1.642	-1.089
1	ST009343	0.092	0.002	0.098	0.013	0.206	-0.114
1	ST002193	0.153	0.001	0.135	0.001	0.285	-0.132
1	ST002443	0.216	0.010	0.203	0.010	0.428	-0.212
1	ST005426	0.334	0.036	0.010	0.034	0.021	0.313
1	ST008659	-0.007	0.010	-0.002	0.013	-0.005	-0.002
1	ST007450	0.013	0.007	0.022	0.016	0.046	-0.033
2	ST013917	0.025	0.012	0.347	0.109	0.733	-0.708
2	ST013870	0.055	0.143	0.537	0.303	1.134	-1.079
2	ST020706	Not detectable		0.190	0.026	NA	NA
2	ST018517	0.446	0.127	0.766	0.004	1.619	-1.172
2	ST012357	0.483	0.029	0.010	0.000	0.021	0.463
2	ST013868	4.313	0.069	0.426	0.038	0.899	3.413
2	ST019644	-0.002	0.005	0.025	0.030	0.052	-0.054
2	ST016629	0.196	0.005	0.161	0.002	0.340	-0.144
2	ST007393	0.638	0.008	0.491	0.001	1.037	-0.399
2	ST019847	4.309	0.025	3.027	0.169	6.397	-2.087
3	ST021132	0.226	0.358	0.503	0.370	1.063	-0.837
3	ST025313	0.154	0.211	0.023	0.002	0.049	0.105
3	ST026253	0.030	NA	0.485	0.123	1.023	-0.993
3	ST024947	0.045	0.005	0.052	0.028	0.110	-0.065

Mix	IDNUMBER	AQP1-lipodisk column		Control-lipodisk column			Specific retention time
		Average adjusted retention time (min)	Standard deviation (min)	Average adjusted retention time (min)	Standard deviation (min)	Corrected retention time (average*2.113)	
	3 ST028992			Not detectable			
	3 ST024942	0.578	NA	0.394	0.007	0.831	-0.253
	3 ST025563	0.059	NA	0.607	0.058	1.281	-1.222
	3 ST025110	0.027	NA	0.008	0.008	0.017	-0.010
	3 ST020960	0.036	0.010	-0.001	0.004	-0.001	0.037
	3 ST028998	0.290	0.037	0.094	0.004	0.199	0.091
	4 ST035586	0.171	0.010	0.222	0.016	0.469	-0.297
	4 ST038822	0.657	0.003	0.608	0.020	1.285	-0.628
	4 ST039860	0.041	0.117	-0.058	0.002	-0.123	0.164
	4 ST039942			Not detectable			
	4 ST039904	0.492	0.082	0.693	0.131	1.465	-0.973
	4 ST032955	0.401	0.008	0.426	0.123	0.900	-0.500
	4 ST034565	-0.010	NA	-0.38	0.533	0	0.010
	4 ST040330			Not detectable			
	4 ST040469	0.243	0.349	0.689	0.035	1.455	-1.213
	4 ST031339	0.495	0.007	0.506	0.018	1.070	-0.574
	5 ST040398	0.147	0.041	0.140	0.016	0.296	-0.149
	5 ST042788	0.575	0.122	0.925	0.257	1.956	-1.380
	5 ST042585			Not detectable			
	5 ST042625	0.115	0.002	0.090	0.011	0.191	-0.076
	5 ST034373	1.920	0.028	1.695	0.043	3.581	-1.661
	5 ST042214	0.231	0.009	0.261	0.005	0.551	-0.319
	5 ST042685	0.208	0.015	0.275	0.019	0.582	-0.374
	5 ST041074	0.139	0.024	0.085	0.031	0.180	-0.041
	5 ST043170	-0.016	0.042	0.884	0.070	1.869	-1.885
	5 ST042979	0.027	0.007	0.020	0.013	0.042	-0.015
	6 ST043348	0.325	0.218	-0.079	0.002	-0.168	0.492

Mix	IDNUMBER	AQP1-lipodisk column		Control-lipodisk column			Specific retention time
		Average adjusted retention time (min)	Standard deviation (min)	Average adjusted retention time (min)	Standard deviation (min)	Corrected retention time (average*2.113)	
6	ST047884	0.221	0.017	0.244	0.027	0.516	-0.295
6	ST043356	0.075	0.088	0.048	0.033	0.102	-0.027
6	ST048346	0.452	0.027	-0.034	0.047	-0.073	0.525
6	ST057435	1.169	0.018	1.170	0.012	2.472	-1.303
6	ST043370	0.709	0.036	0.815	0.024	1.721	-1.012
6	ST055034	-0.001	0.008	0.018	NA	0.038	-0.037
6	ST044212	0.290	0.007	0.352	0.034	0.745	-0.455
6	ST055117	0.053	0.039	0.029	0.015	0.061	-0.008
6	ST052834	0.196	0.357	-0.073	0.001	-0.154	0.350
7	ST055554	Not detectable					
7	ST057195	-0.031	0.013	0.010	0.013	0.021	-0.052
7	ST057330	0.040	0.066	0.049	0.003	0.104	-0.064
7	ST055641	Not detectable					
7	ST060177	0.394	0.020	0.274	0.021	0.578	-0.184
7	ST058710	0.113	0.007	0.078	0.023	0.164	-0.051
7	ST055460	--0.012	NA	0.012	NA	0.025	-0.037
7	ST055435	0.001	0.032	0.048	0.003	0.101	-0.100
7	ST058429	0.094	0.006	0.093	0.017	0.197	-0.103
7	ST044057	0.773	0.023	0.740	0.007	1.563	-0.791
8	ST059084	0.126	0.018	0.121	0.005	0.255	-0.130
8	ST066714	0.082	0.015	0.107	0.001	0.226	-0.144
8	ST060251	0.038	0.047	0.032	0.003	0.067	-0.030
8	ST060990	0.052	0.038	0.040	0.006	0.085	-0.034
8	ST066501	0.059	0.080	0.092	0.005	0.194	-0.135
8	ST066271	0.141	0.024	0.157	0.052	0.333	-0.192
8	ST065849	-0.019	0.051	Not detectable	NA	NA	NA
8	ST058342	0.055	0.040	0.021	0.008	0.044	0.011

Mix	IDNUMBER	AQP1-lipodisk column		Control-lipodisk column			Specific retention time
		Average adjusted retention time (min)	Standard deviation (min)	Average adjusted retention time (min)	Standard deviation (min)	Corrected retention time (average*2.113)	
8	ST071904	0.368	0.018	0.282	0.014	0.595	-0.227
8	ST065071	0.197	0.041	0.176	0.009	0.371	-0.174
9	ST066818	0.349	0.001	0.371	0.001	0.783	-0.435
9	ST072153	0.033	0.035	0.038	0.007	0.079	-0.046
9	ST069077	0.076	0.005	0.062	0.009	0.132	-0.055
9	ST067028	0.066	0.005	0.041	0.010	0.086	-0.020
9	ST060179	2.353	0.019	2.095	0.006	4.427	-2.075
9	ST066879	1.219	0.023	1.099	0.006	2.323	-1.104
9	ST068836	0.513	0.001	0.477	0.008	1.009	-0.496
9	ST071056	-0.006	0.005	1.085	0.034	2.292	-2.298
9	ST071531	0.500	0.002	0.498	0.041	1.051	-0.552
9	ST071626		Not detectable				
10	ST074974	-0.007	0.003	0.022	0.009	0.046	-0.053
10	ST073961	-0.014	0.004	0.017	0.006	0.036	-0.050
10	ST072514	0.160	0.004	0.199	0.016	0.420	-0.261
10	ST074933	0.226	0.020	0.528	0.178	1.115	-0.889
10	ST081142	-0.002	0.002	0.021	0.001	0.045	-0.047
10	ST074965	0.246	0.007	0.237	0.006	0.502	-0.255
10	ST072532	-0.008	0.004	0.018	0.005	0.037	-0.045
10	ST072523	0.027	0.023	0.013	0.006	0.028	-0.001
10	ST075856	0.405	0.006	0.275	0.004	0.581	-0.176
10	ST073768	0.082	0.007	0.043	0.003	0.090	-0.008
11	ST080885	-0.017	0.002	-0.003	0.003	-0.005	-0.012
11	ST081136	0.200	0.001	0.198	0.004	0.418	-0.218
11	ST079407	0.178	0.005	0.184	0.004	0.389	-0.211
11	ST079515	0.268	0.207	0.387	0.173	0.817	-0.549
11	ST081354	0.360	0.007	0.322	0.003	0.679	-0.320

Mix	IDNUMBER	AQP1-lipodisk column		Control-lipodisk column			Specific retention time
		Average adjusted retention time (min)	Standard deviation (min)	Average adjusted retention time (min)	Standard deviation (min)	Corrected retention time (average*2.113)	
11	ST079730	-0.002	0.000	0.009	0.006	0.019	-0.020
11	ST078480	0.103	0.002	0.111	0.010	0.234	-0.130
11	ST074499	0.091	0.003	0.097	0.013	0.206	-0.115
11	ST079397	0.085	0.006	0.079	0.032	0.167	-0.083
11	ST082361	0.005	0.004	0.019	0.007	0.039	-0.035
12	ST082325	0.822	0.145	1.264	0.107	2.671	-1.850
12	ST081121	0.355	0.004	0.332	0.002	0.701	-0.345
12	ST084694	0.065	0.049	0.085	0.036	0.179	-0.113
12	ST084657	0.006	0.003	0.022	0.049	0.047	-0.042
12	ST084809	0.065	0.020	0.082	0.049	0.173	-0.109
12	ST081404	0.230	0.000	0.224	0.005	0.473	-0.242
12	ST082040	0.748	0.001	0.668	0.008	1.411	-0.663
12	ST084648	0.016	0.005	0.010	0.045	0.022	-0.006
12	ST083226	0.369	0.001	0.397	0.008	0.839	-0.471
12	ST084486	0.007	0.004	0.032	NA	0.068	-0.061
13	ST086491	-0.015	0.001	-0.007	0.002	-0.014	-0.001
13	ST085489	0.171	0.003	0.200	0.001	0.422	-0.251
13	ST086495	0.008	0.005	0.058	0.003	0.123	-0.115
13	ST084814	-0.017	0.000	0.019	0.007	0.041	-0.057
13	ST084965	0.173	0.001	0.206	0.003	0.436	-0.263
13	ST085529	0.230	0.001	0.241	0.002	0.508	-0.278
13	ST086616	0.080	0.028	0.035	0.030	0.075	0.005
13	ST086467	0.002	0.005	0.014	0.002	0.029	-0.028
13	ST086434	0.101	0.000	0.098	0.001	0.206	-0.105
13	ST085670	0.009	0.005	0.036	0.001	0.076	-0.067
14	ST088216	0.189	0.002	0.233	0.001	0.493	-0.304
14	ST087933	-0.014	0.011	0.005	0.009	0.011	-0.024

Mix	IDNUMBER	AQP1-lipodisk column		Control-lipodisk column			Specific retention time
		Average adjusted retention time (min)	Standard deviation (min)	Average adjusted retention time (min)	Standard deviation (min)	Corrected retention time (average*2.113)	
14	ST092304	1.503	0.090	1.323	0.033	2.796	-1.294
14	ST092310	-0.004	0.017	0.011	0.027	0.023	-0.027
14	ST088687	-0.001	0.003	0.020	0.007	0.042	-0.043
14	ST088579	0.096	0.008	0.042	0.016	0.090	0.006
14	ST089337	0.074	0.006	0.041	0.023	0.087	-0.013
14	ST088714	0.121	0.001	0.136	0.000	0.288	-0.168
14	ST089346	-0.001	0.004	0.018	0.039	0.038	-0.039
14	ST092546	0.448	0.001	0.368	0.010	0.777	-0.329
15	ST093059	0.103	0.006	0.089	0.031	0.187	-0.085
15	ST092736	0.025	0.009	-0.082	0.000	-0.174	0.198
15	ST092941	-0.007	0.016	0.007	0.008	0.014	-0.021
15	ST094925	0.040	0.010	0.019	0.012	0.040	0.001
15	ST000762	1.625	0.074	1.708	0.007	3.608	-1.984
15	ST094844	0.426	0.009	0.452	0.012	0.954	-0.528
15	ST092858	-0.049	0.005	-0.053	0.002	-0.113	0.063
15	ST092952			Not detectable			
15	ST094899	0.448	0.113	0.806	0.001	1.702	-1.254
15	ST094878	0.226	0.006	0.266	0.018	0.562	-0.336
16	ST002754	0.003	0.018	0.034	0.022	0.072	-0.069
16	ST006406	1.233	0.030	1.068	0.015	2.257	-1.023
16	ST021500	0.002	0.011	0.017	0.039	0.035	-0.034
16	ST024756	0.045	0.040	0.061	0.001	0.129	-0.084
16	ST002776	0.030	0.015	0.059	0.013	0.125	-0.095
16	ST006408	0.088	0.203	0.126	0.250	0.265	-0.178
16	ST019864	-0.047	0.004	-0.063	0.005	-0.133	0.086
16	ST013456	1.046	0.001	0.910	0.012	1.923	-0.877
16	ST016476	-0.001	0.010	0.014	0.038	0.029	-0.030

Mix	IDNUMBER	AQP1-lipodisk column		Control-lipodisk column			Specific retention time
		Average adjusted retention time (min)	Standard deviation (min)	Average adjusted retention time (min)	Standard deviation (min)	Corrected retention time (average*2.113)	
	16 ST024130	0.020	0.013	0.012	0.005	0.026	-0.006
	17 ST037923	0.459	0.003	0.493	0.004	1.042	-0.583
	17 ST043640	0.107	0.001	0.098	0.004	0.207	-0.100
	17 ST041068	Not detectable					
	17 ST043313	-0.079	0.0004	Not detectable	NA	NA	NA
	17 ST042454	0.107	0.005	0.102	0.001	0.215	-0.108
	17 ST036363	1.856	0.005	Not detectable	NA	NA	NA
	17 ST044520	0.123	0.009	0.106	0.013	0.223	-0.100
	17 ST043757	0.144	0.041	0.112	0.022	0.236	-0.092
	17 ST029575	-0.012	0.015	0.010	0.007	0.022	-0.034
	17 ST049194	Not detectable					
	18 ST068470	0.154	0.005	0.176	0.004	0.372	-0.218
	18 ST065680	0.108	0.003	0.521	0.049	1.102	-0.993
	18 ST057427	0.027	NA	0.027	0.008	0.057	-0.030
	18 ST060476	0.270	0.007	0.301	0.046	0.637	-0.367
	18 ST059739	0.219	0.005	0.205	0.008	0.433	-0.215
	18 ST056172	-0.014	0.020	0.003	0.001	0.006	-0.020
	18 ST066977	0.063	0.028	0.049	0.002	0.104	-0.041
	18 ST068634	0.072	0.051	0.086	0.011	0.182	-0.110
	18 ST068678	0.222	0.006	0.224	0.001	0.474	-0.252
	18 ST057090	-0.009	0.010	0.027	0.004	0.056	-0.065
	19 ST083950	0.090	0.127	-0.057	0.003	-0.121	0.211
	19 ST084494	0.113	0.001	0.152	0.009	0.322	-0.209
	19 ST081624	0.014	0.030	0.018	0.011	0.038	-0.025
	19 ST083788	0.182	0.005	0.289	0.052	0.610	-0.428
	19 ST082297	0.049	0.005	0.056	0.004	0.119	-0.070

Mix	IDNUMBER	AQP1-lipodisk column		Control-lipodisk column			Specific retention time
		Average adjusted retention time (min)	Standard deviation (min)	Average adjusted retention time (min)	Standard deviation (min)	Corrected retention time (average*2.113)	
19	ST081237	0.073	0.038	0.047	0.032	0.099	-0.026
19	ST084053	0.118	0.001	0.108	0.015	0.227	-0.109
19	ST084863	-0.003	0.010	-0.001	0.000	-0.003	0.000
19	ST081261	0.118	0.007	0.119	0.003	0.251	-0.133
19	ST081188	0.418	0.004	0.376	0.004	0.795	-0.377
20	ST092683	-0.098	0.002	Not detectable	NA	NA	NA
20	ST094801	-0.015	0.002	0.018	0.026	0.038	-0.054
20	ST093057	0.107	0.002	0.138	0.030	0.292	-0.185
20	ST086484	0.134	0.009	0.139	0.002	0.293	-0.159
20	ST092679	1.004	0.019	0.708	1.145	1.497	-0.493
20	ST024163	0.619	0.021	0.510	0.046	1.077	-0.458
20	ST088456	0.097	0.019	0.071	0.019	0.149	-0.052
20	ST089003	0.259	0.012	0.539	0.086	1.139	-0.880
20	ST085645	0.666	0.046	1.006	0.007	2.126	-1.460
20	ST088463	0.500	0.004	0.415	0.005	0.877	-0.378
	7027127	24.203	0.316	30.428	1.215	64.294	-40.091

NA: not applicable, when one or more of duplicate samples were not detectable.

References

- 1 J. Murphy, and J.P. Riley, A modified single solution method for the determination of phosphate in natural waters, *Analytica Chimica Acta*, 1962, **27**, 31-36.
- 2 J.V. Paraskova, E. Rydin, and P.J.R. Sjöberg, Extraction and quantification of phosphorus derived from DNA and lipids in environmental samples, *Talanta*, 2013, **115**, 336-341.
- 3 Method « Dot it Spot it, in, 2014.