Structure−Activity Relationships (SARs) of the Competence Stimulating Peptide (CSP) in *Streptococcus mutans* Reveal Motifs Critical for Membrane Protease SepM Recognition and ComD Receptor Activation

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Supporting Information.

Additional experimental details S-2
HPLC traces for CSP analogs S-4
Primary reporter gene assay data S-30
Agonism dose response curves S-36
Complete tables of EC_{50} values for CSP analogs (SMCOM2) S-56
Comparison of results found from both reporter strains SAB249 and SMCOM2 ..... S-58
References S-74

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Additional experimental details

Resin loading. 0.1 g of Wang resin (0.94 mmol/g) was placed in a clean, dry round bottom flask with a minimal amount of dimethylformamide (DMF) to fully cover the resin, and the resin was allowed to swell at room temperature for 30 min. 0.94 mmol (10 equiv. relative to the overall loading of the resin) of Fmoc-protected amino acid were dissolved in dry dichloromethane (DCM) and placed on an ice bath. A few drops of DMF were added to aid complete dissolution when the amino acid had low solubility in DCM. A solution of 0.47 mmol (5 equiv. relative to the overall loading of the resin) diisopropylcarbodiimide (DIC) in dry DCM was then added dropwise to the amino acid solution. The mixture was stirred for 20 min on an ice bath and the DCM was removed from the mixture using a rotary evaporator. The residue was then dissolved with a minimal amount of DMF and the solution was added to the resin. 0.0094 mmol (0.1 equiv. relative to the overall loading of the resin) of dimethylaminopyridine (DMAP) were dissolved in DMF and the solution was added to the reaction mixture. The mixture was sealed and allowed to stand at room temperature for 2 h with occasional swirling. The resin was washed with DCM, then diethyl ether, followed by overnight drying under vacuum.

Loading test. Two small samples of the amino acid-loaded resin (approximately 5 mg each) were weighed and mixed with 3 mL of fresh 20% piperidine in DMF solution. This solution was stirred on a shaker for 2 h. The absorbance (290 nm) of the supernatants of the two piperidine-resin mixtures was measured by using a UV-Vis spectrophotometer to estimate the level of Fmoc removal, which correlates to first residue attachment. The first residue attachment was estimated from the following equations:

Equation 1 $$A\ (mmol/g) = B \times 1000/[1000 + (B\ \times (M - X))]$$

A is the theoretical substitution (mmol/g), B is the substitution of starting resin (mmol/g), and M is the molecular weight of target peptide with all protecting groups. X is 18 for hydroxymethyl-based resin.

Equation 2 $$B\ (mmol/g) = (Abs_{sample}\ \times\ N)/(mg\ of\ sample\ \times 1.75)$$

B is the experimental first residue attachment and the equation is based on $\varepsilon = 5253\ M^{-1}\ cm^{-1}$. N is the fold of dilution of the supernatant. $\frac{B}{A} \times 100\%$ is the percent yield of the loading. If the average of the percent yield of the two samples was greater than 70%, resin loading was deemed successful and the resin would be used for the synthesis of CSP analogs.

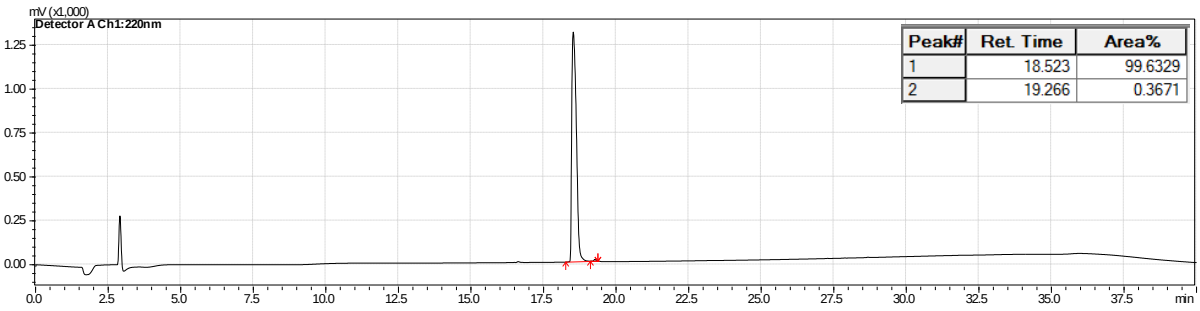
Synthesis. The resin (0.1 g) was first swelled by suspension in DCM for 30 min at room temperature and then drained. The resin was then washed with DMF (3 × 2 mL). To remove the Fmoc-protecting group, the resin was treated with piperidine (2 mL of 20% piperidine in DMF, 2 × 7 min) and washed with DMF (3 × 2 mL). To couple each amino acid, Fmoc-protected amino acids (2 equiv. relative to the overall loading of the resin) were dissolved in DMF (2 mL) and mixed with 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU; 2 equiv.) and diisopropylethylamine (DIPEA; 2 equiv.). The solution was allowed to pre-activate for 1 min prior to being added to the resin and agitated for 1 h at room temperature. After each coupling step, the resin was drained and washed with DMF (2 × 2 mL). This process was repeated until the desired peptide sequence was obtained. CSP and analogs were synthesized either manually or with the AAPPTec Focus-XC peptide synthesizer.

Cleavage. Upon completion of peptide synthesis, the resin was washed with diethyl ether (2 mL) and dried under nitrogen stream for 3 min before it was transferred into a 15 mL falcon tube. The peptide was cleaved from the resin, along with all of the protecting groups, by mixing the resin with 3 mL cleavage cocktail of

95% trifluoroacetic acid (TFA), 2.5% triisopropylsilane (TIPS), and 2.5% 18 MΩ water for 3 h with agitation. The resulting cleavage product solution was separated from the resin by filtration and the filtrate was transferred into a new 50 mL falcon tube. A cooled solution of diethyl ether:hexane (1:1, 45 mL, 0 °C) was added to the filtrate, and the peptide was allowed to precipitate for 10 min in a freezer at -20 °C. The mixture was centrifuged for 5 min at 3000 RPM and the supernatant was removed to yield crude peptide that was dissolved in 10 mL acetonitrile (ACN):water (1:1) and lyophilized before HPLC purification.

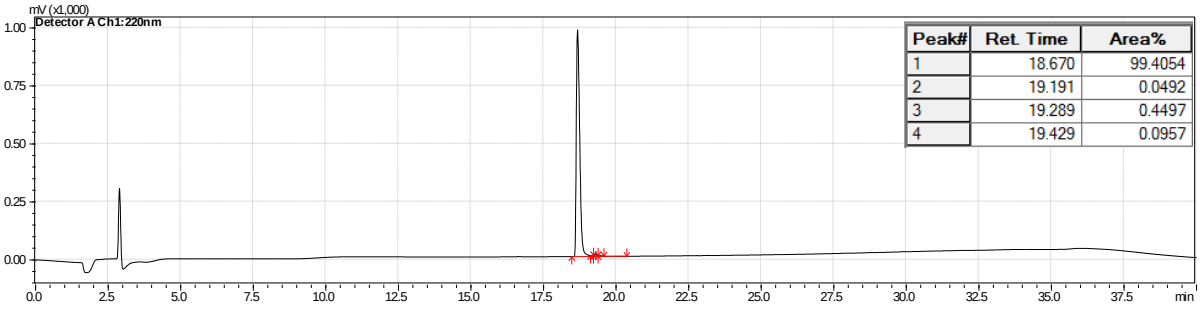
HPLC traces for CSP analogs

21-CSP



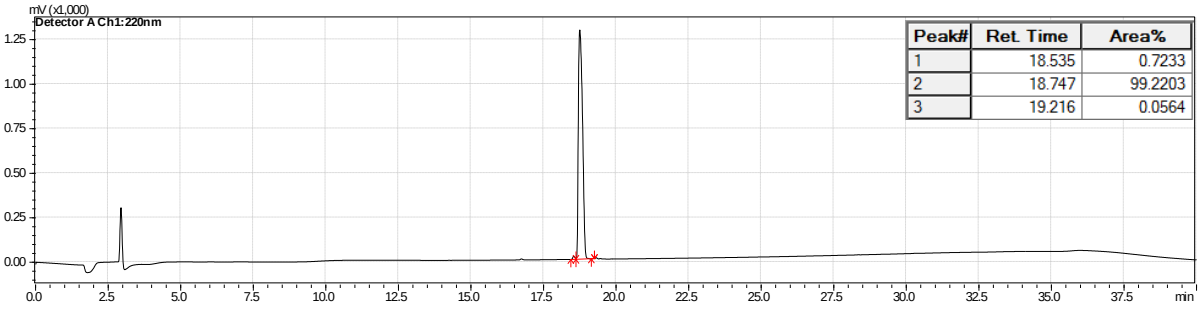
Peak#	Ret. Time	Area%
1	18.523	99.6329
2	19.266	0.3671

21-CSP-S1A



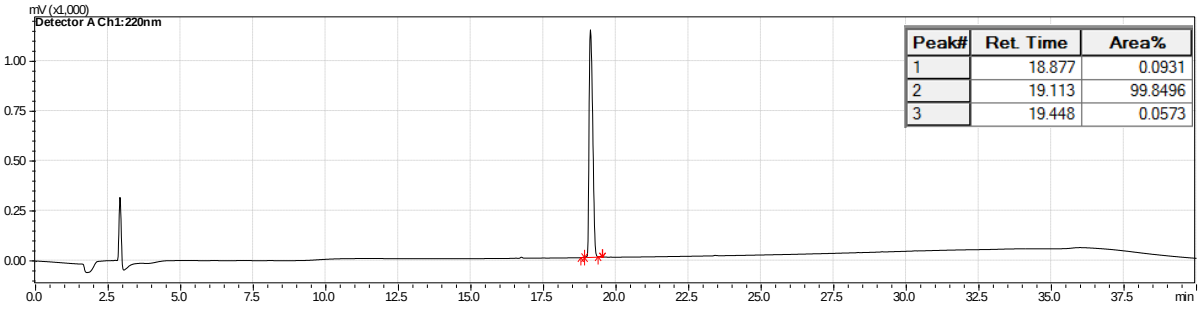
Peak#	Ret. Time	Area%
1	18.670	99.4054
2	19.191	0.0492
3	19.289	0.4497
4	19.429	0.0957

21-CSP-G2A



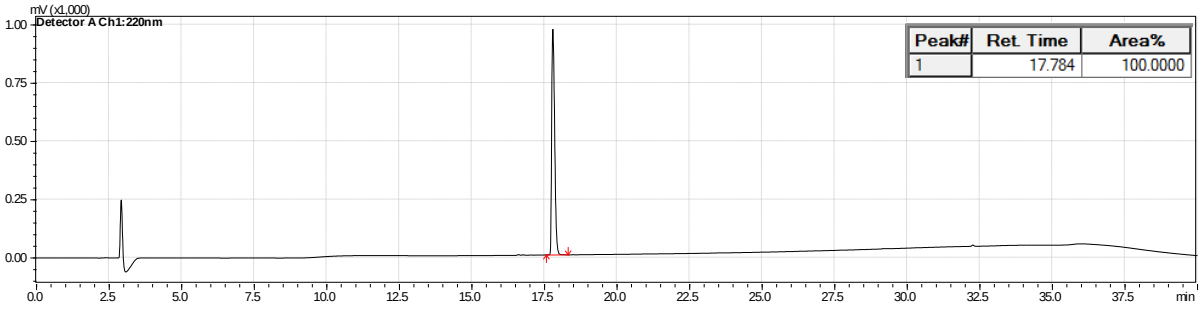
Peak#	Ret. Time	Area%
1	18.535	0.7233
2	18.747	99.2203
3	19.216	0.0564

21-CSP-S3A



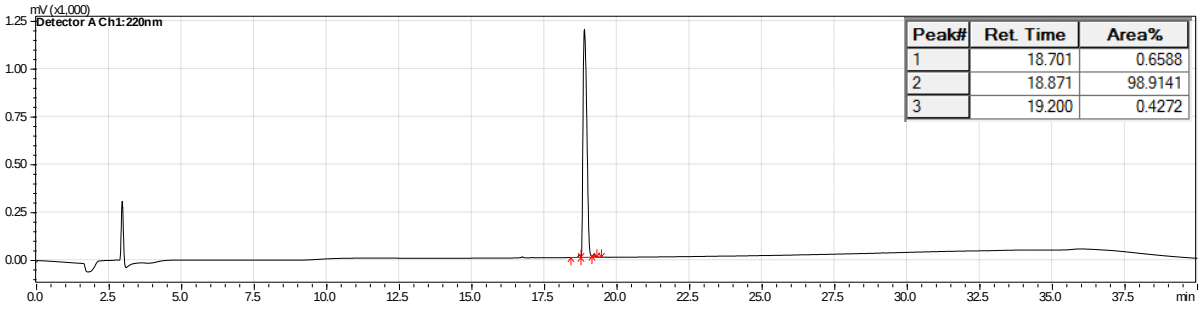
Peak#	Ret. Time	Area%
1	18.877	0.0931
2	19.113	99.8496
3	19.448	0.0573

21-CSP-L4A



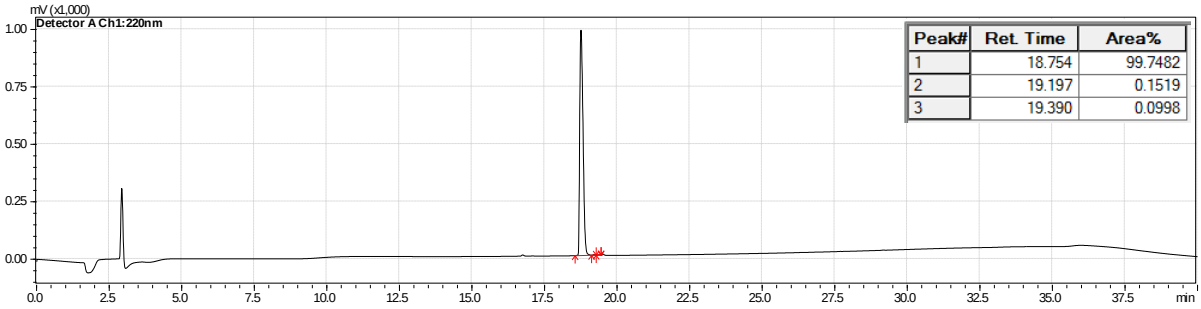
Peak#	Ret. Time	Area%
1	17.784	100.0000

21-CSP-S5A



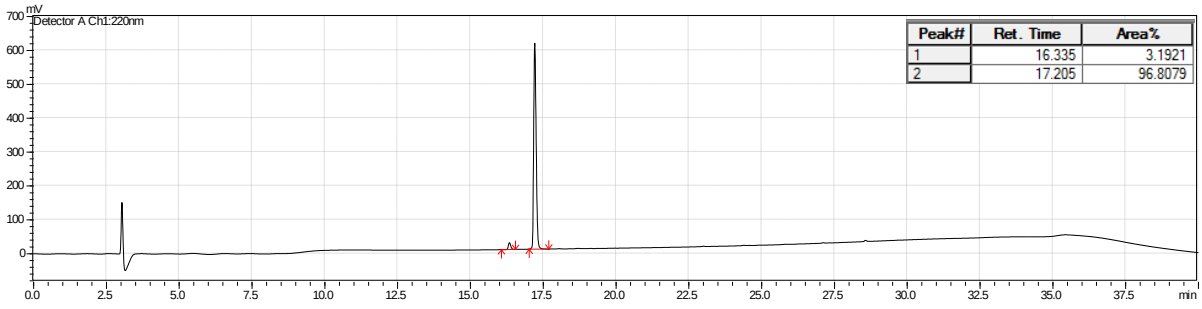
Peak#	Ret. Time	Area%
1	18.701	0.6588
2	18.871	98.9141
3	19.200	0.4272

21-CSP-T6A



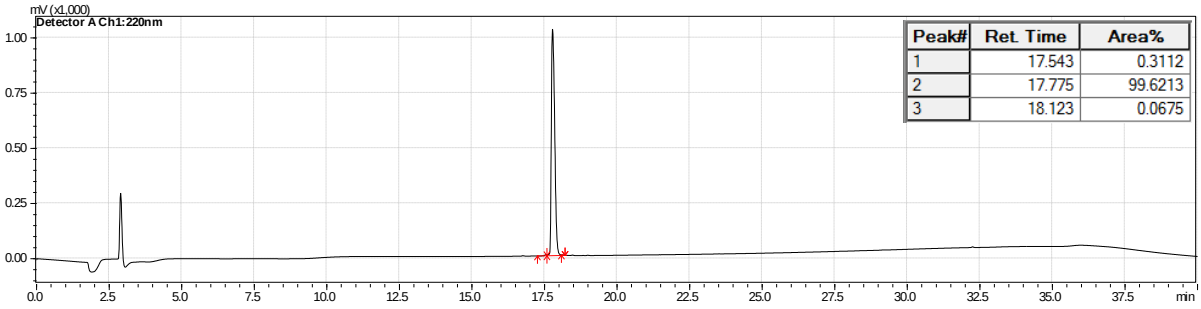
Peak#	Ret. Time	Area%
1	18.754	99.7482
2	19.197	0.1519
3	19.390	0.0998

21-CSP-F7A



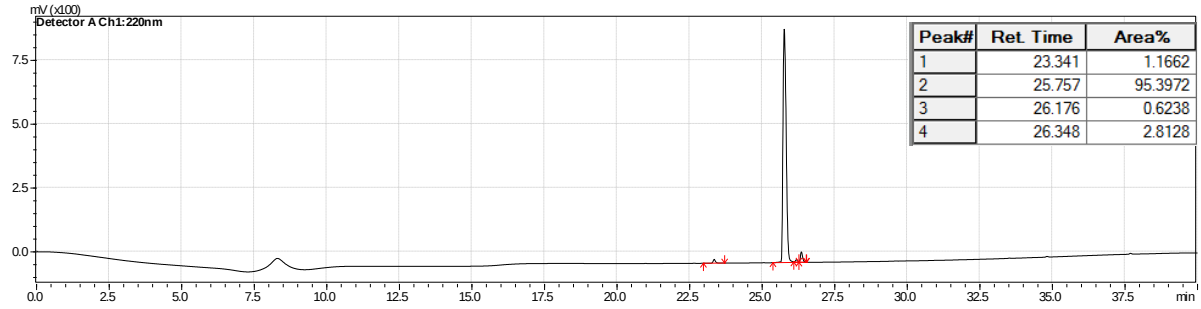
Peak#	Ret. Time	Area%
1	16.335	3.1921
2	17.205	96.8079

21-CSP-F8A



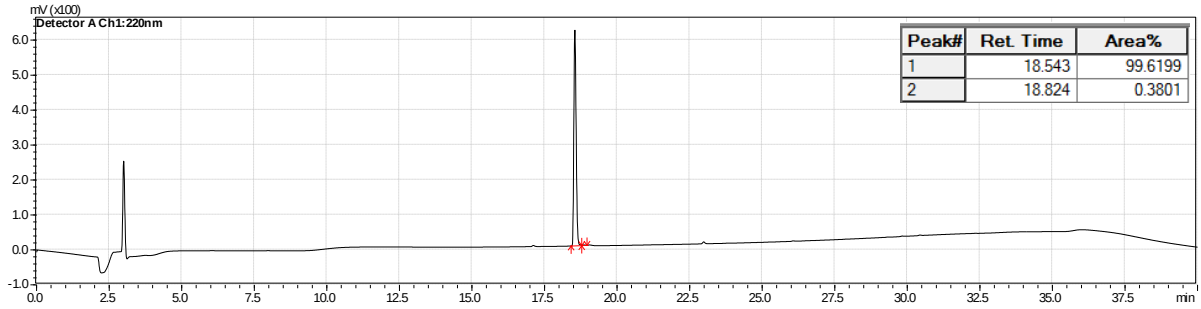
Peak#	Ret. Time	Area%
1	17.543	0.3112
2	17.775	99.6213
3	18.123	0.0675

21-CSP-R9A



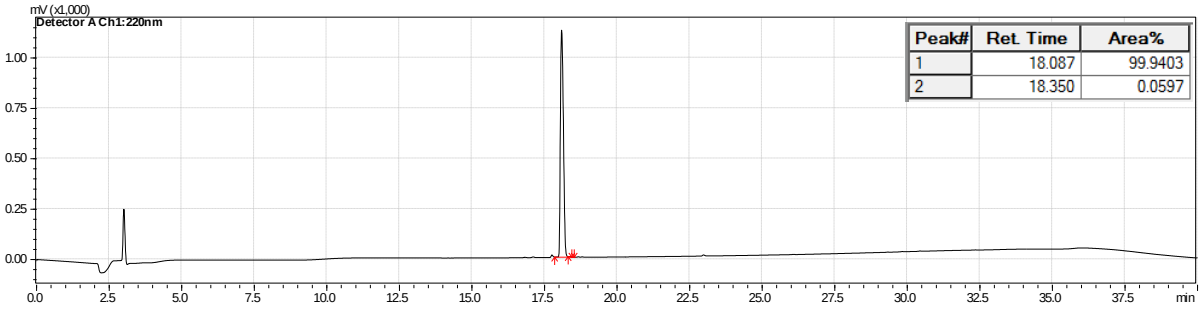
Peak#	Ret. Time	Area%
1	23.341	1.1662
2	25.757	95.3972
3	26.176	0.6238
4	26.348	2.8128

21-CSP-L10A



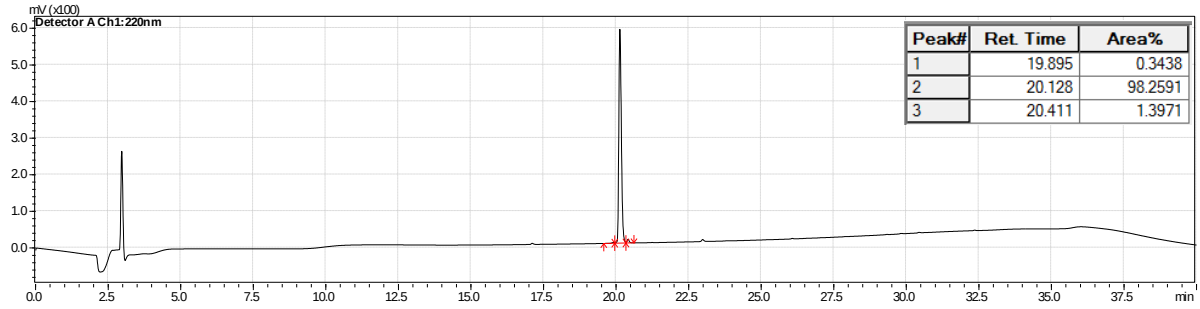
Peak#	Ret. Time	Area%
1	18.543	99.6199
2	18.824	0.3801

21-CSP-F11A



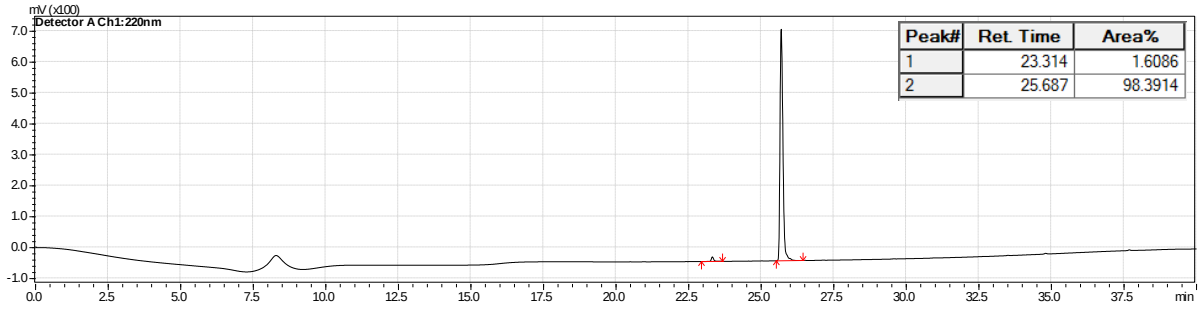
Peak#	Ret. Time	Area%
1	18.087	99.9403
2	18.350	0.0597

21-CSP-N12A



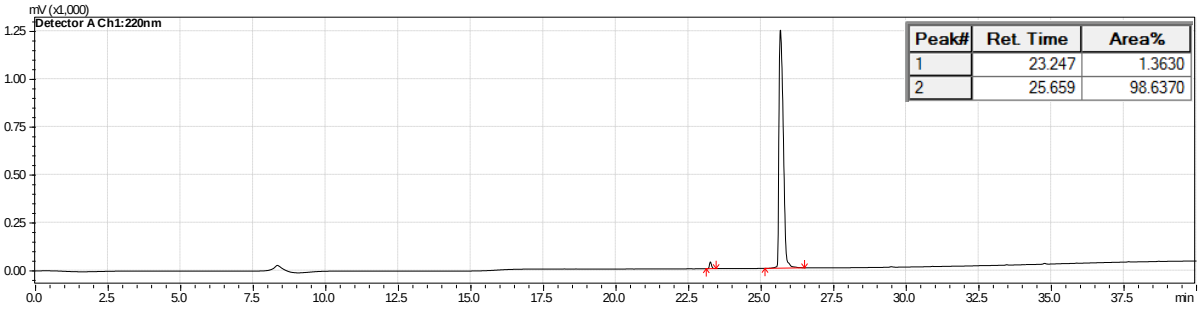
Peak#	Ret. Time	Area%
1	19.895	0.3438
2	20.128	98.2591
3	20.411	1.3971

21-CSP-R13A



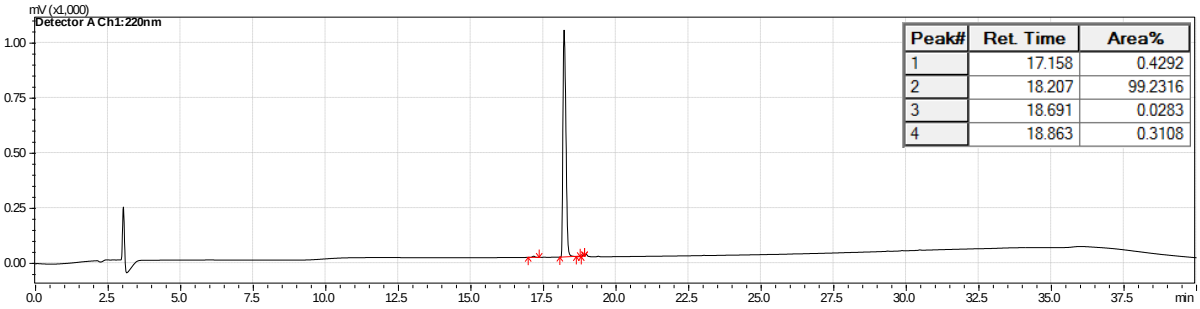
Peak#	Ret. Time	Area%
1	23.314	1.6086
2	25.687	98.3914

21-CSP-S14A



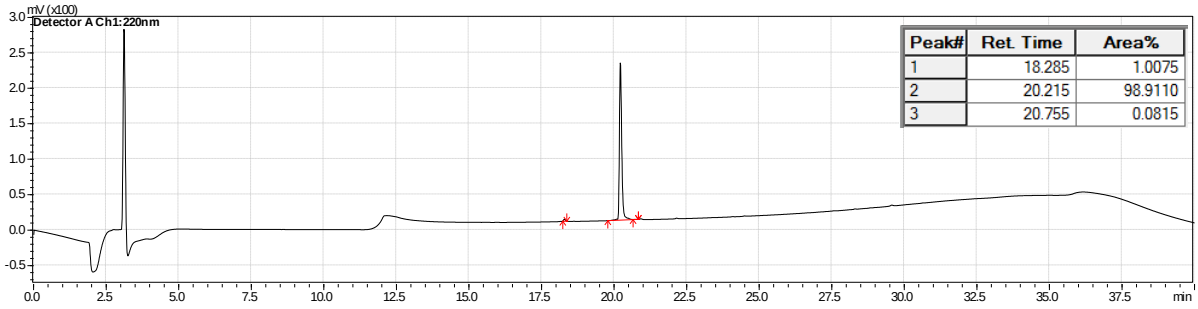
Peak#	Ret. Time	Area%
1	23.247	1.3630
2	25.659	98.6370

21-CSP-F15A



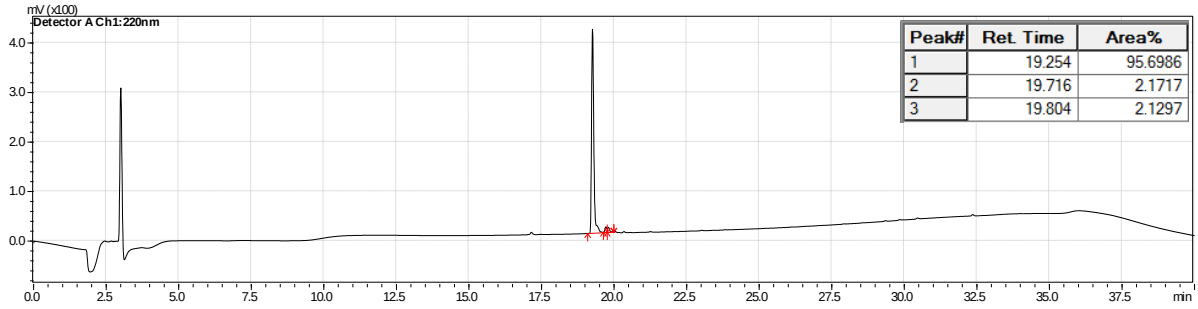
Peak#	Ret. Time	Area%
1	17.158	0.4292
2	18.207	99.2316
3	18.691	0.0283
4	18.863	0.3108

21-CSP-T16A



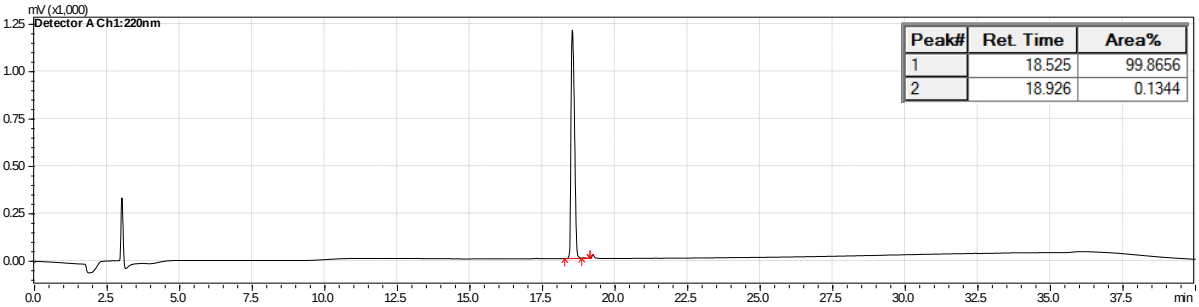
Peak#	Ret. Time	Area%
1	18.285	1.0075
2	20.215	98.9110
3	20.755	0.0815

21-CSP-Q17A



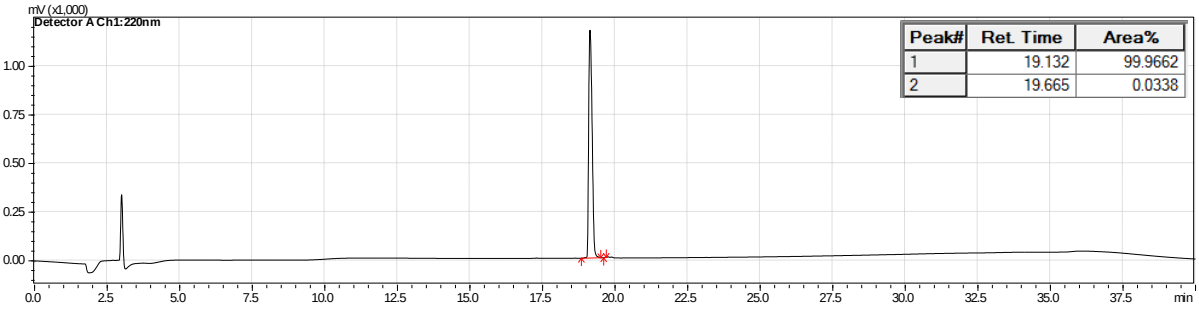
Peak#	Ret. Time	Area%
1	19.254	95.6986
2	19.716	2.1717
3	19.804	2.1297

21-CSP-L19A



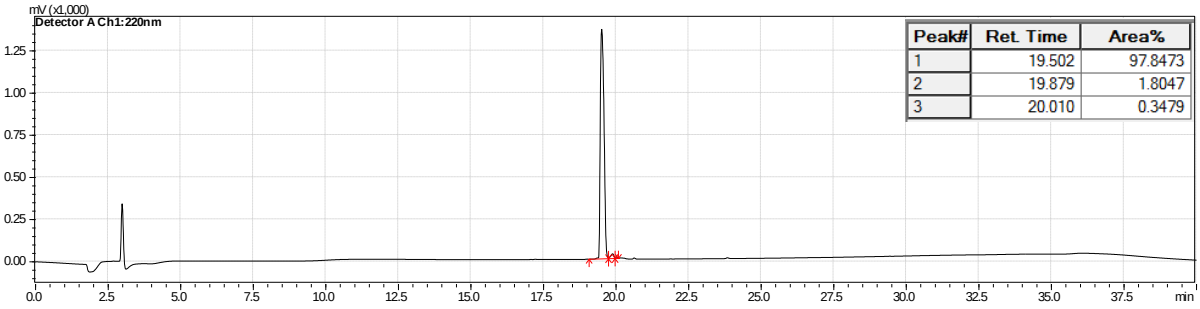
Peak#	Ret. Time	Area%
1	18.525	99.8656
2	18.926	0.1344

21-CSP-G20A



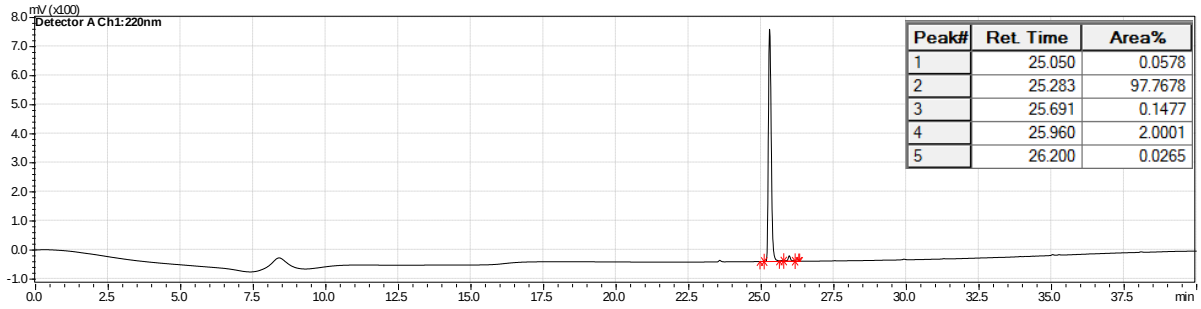
Peak#	Ret. Time	Area%
1	19.132	99.9662
2	19.665	0.0338

21-CSP-K21A



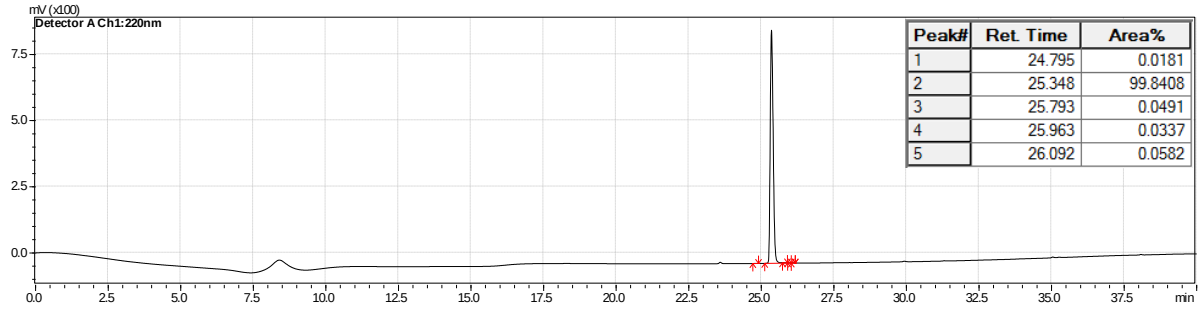
Peak#	Ret. Time	Area%
1	19.502	97.8473
2	19.879	1.8047
3	20.010	0.3479

21-CSP-s1



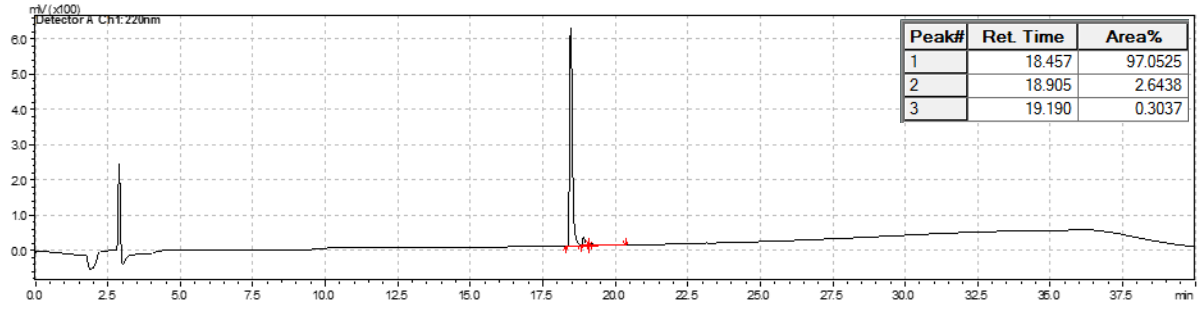
Peak#	Ret. Time	Area%
1	25.050	0.0578
2	25.283	97.7678
3	25.691	0.1477
4	25.960	2.0001
5	26.200	0.0265

21-CSP-s3



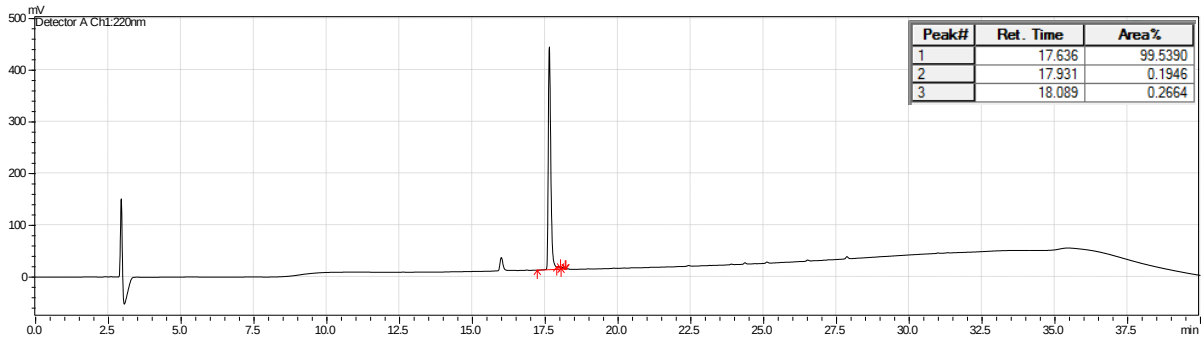
Peak#	Ret. Time	Area%
1	24.795	0.0181
2	25.348	99.8408
3	25.793	0.0491
4	25.963	0.0337
5	26.092	0.0582

21-CSP-l4



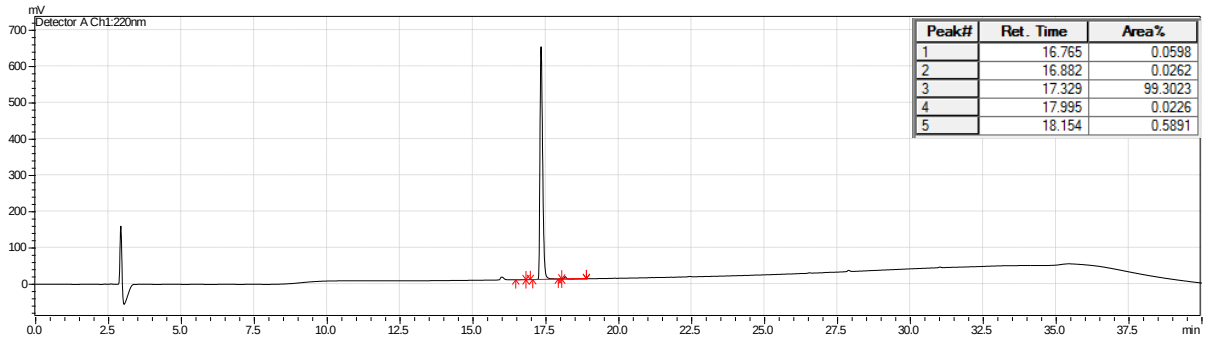
Peak#	Ret. Time	Area%
1	18.457	97.0525
2	18.905	2.6438
3	19.190	0.3037

21-CSP-s5



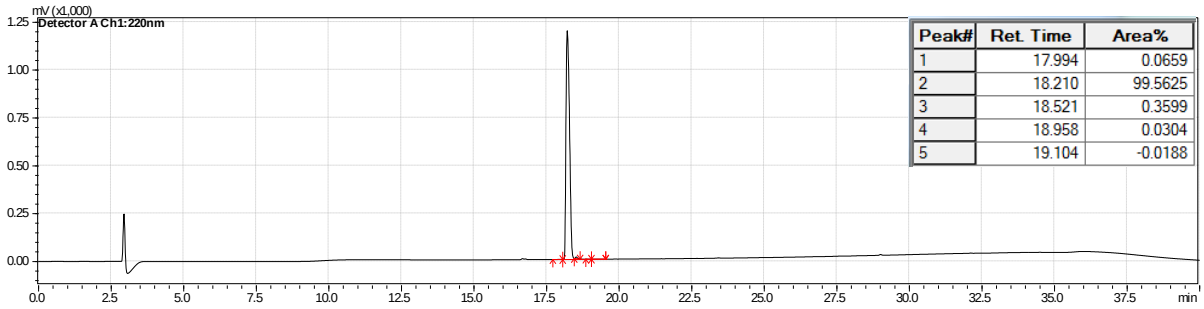
Peak#	Ret. Time	Area%
1	17.636	99.5390
2	17.931	0.1946
3	18.089	0.2664

21-CSP-t6



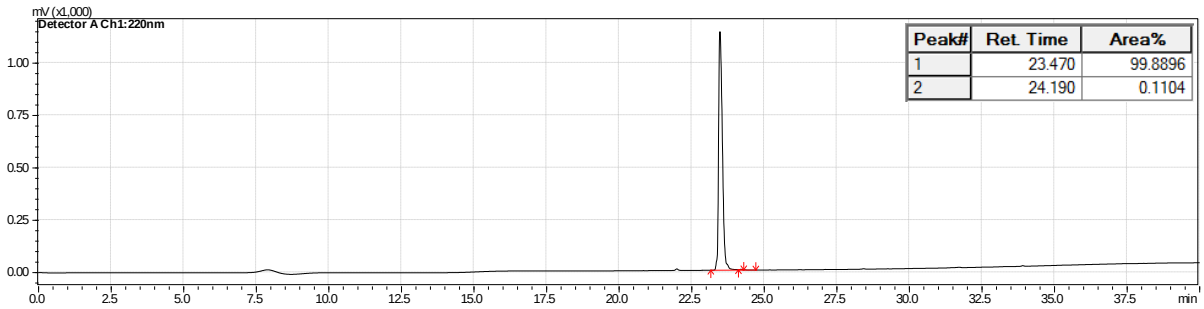
Peak#	Ret. Time	Area%
1	16.765	0.0598
2	16.882	0.0262
3	17.329	99.3023
4	17.995	0.0226
5	18.154	0.5891

21-CSP-f7



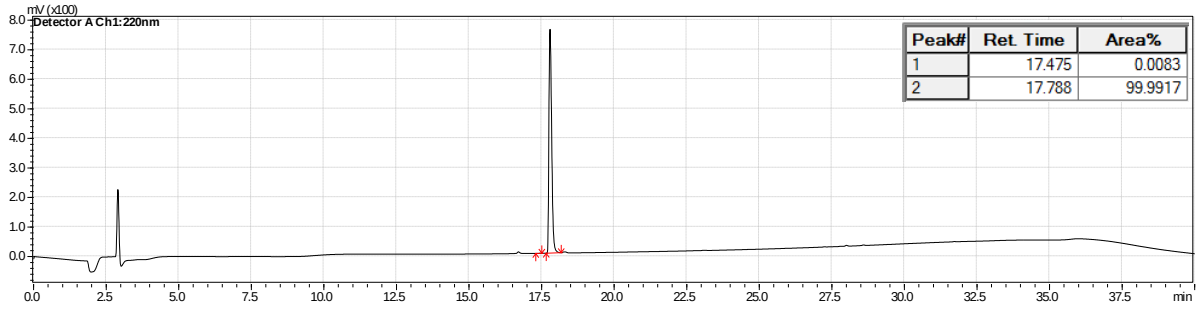
Peak#	Ret. Time	Area%
1	17.994	0.0659
2	18.210	99.5625
3	18.521	0.3599
4	18.958	0.0304
5	19.104	-0.0188

21-CSP-f8



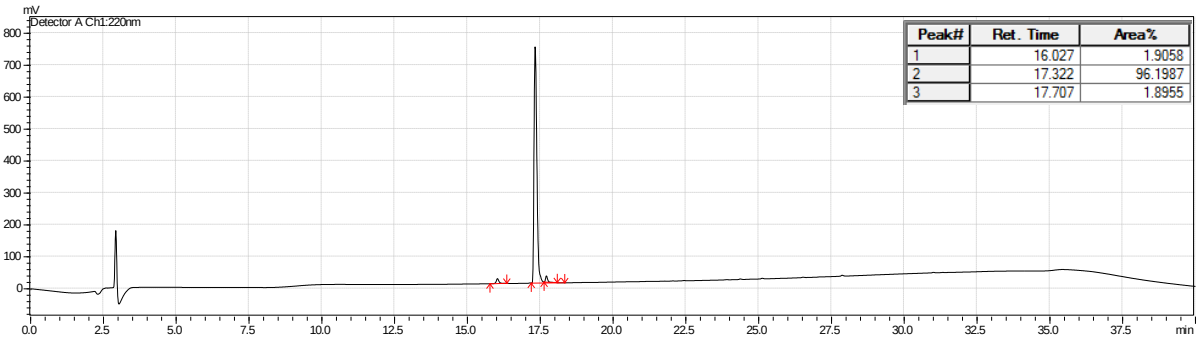
Peak#	Ret. Time	Area%
1	23.470	99.8896
2	24.190	0.1104

21-CSP-r9



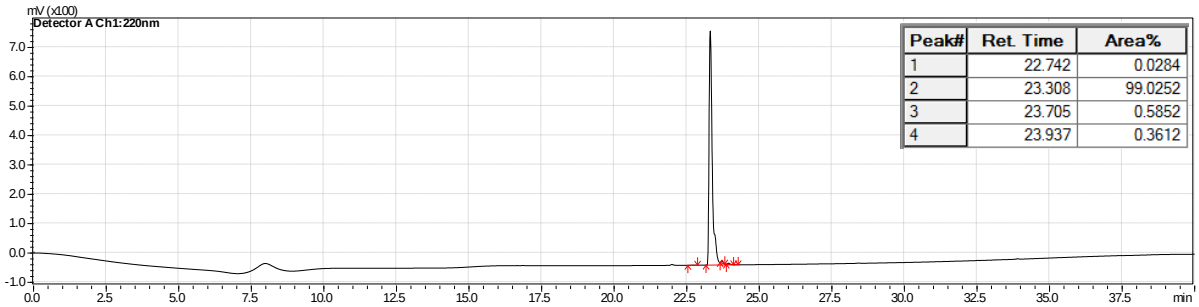
Peak#	Ret. Time	Area%
1	17.475	0.0083
2	17.788	99.9917

21-CSP-l10



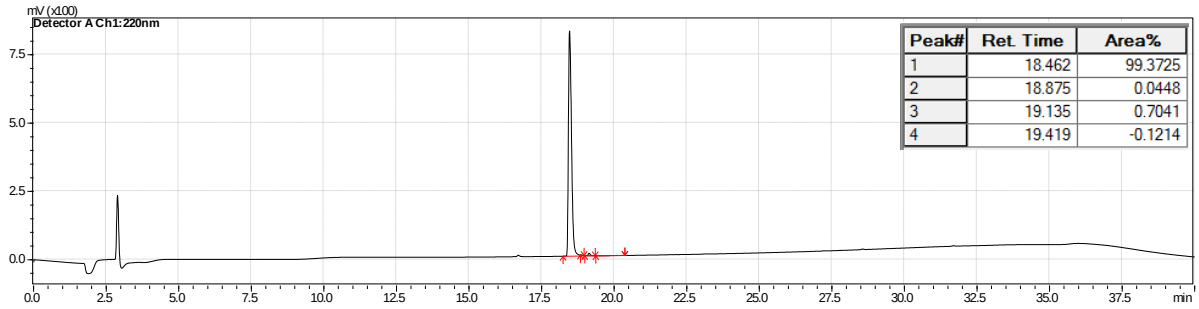
Peak#	Ret. Time	Area%
1	16.027	1.9058
2	17.322	96.1987
3	17.707	1.8955

21-CSP-f11



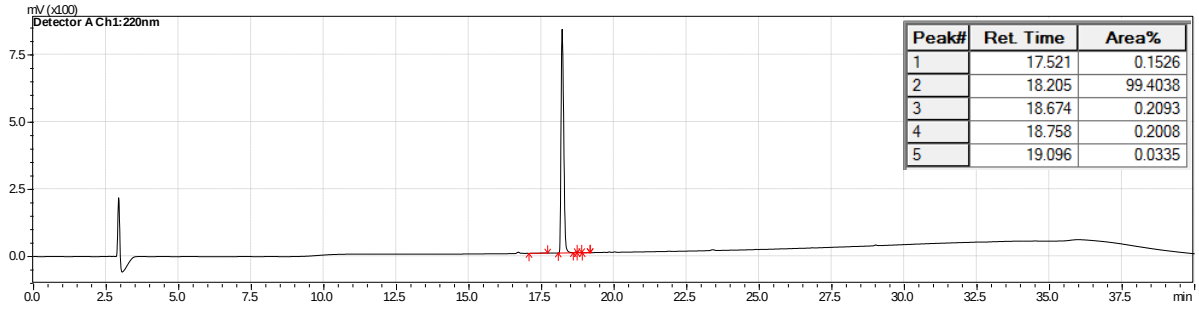
Peak#	Ret. Time	Area%
1	22.742	0.0284
2	23.308	99.0252
3	23.705	0.5852
4	23.937	0.3612

21-CSP-n12



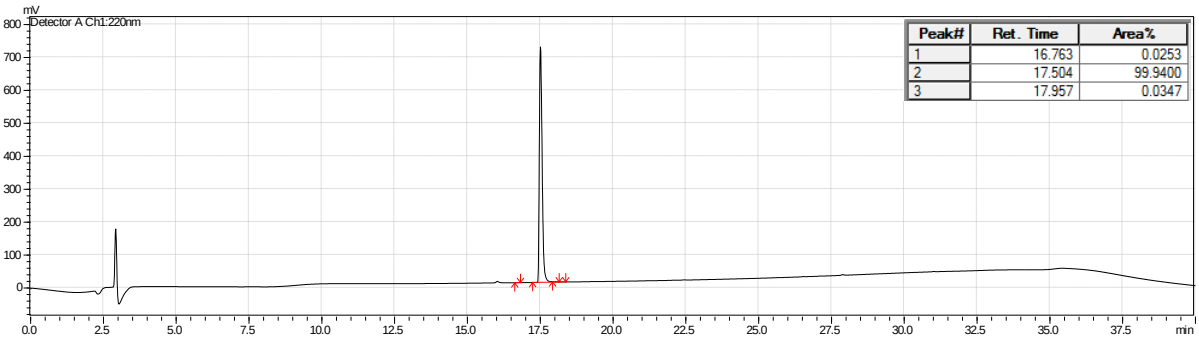
Peak#	Ret. Time	Area%
1	18.462	99.3725
2	18.875	0.0448
3	19.135	0.7041
4	19.419	-0.1214

21-CSP-r13



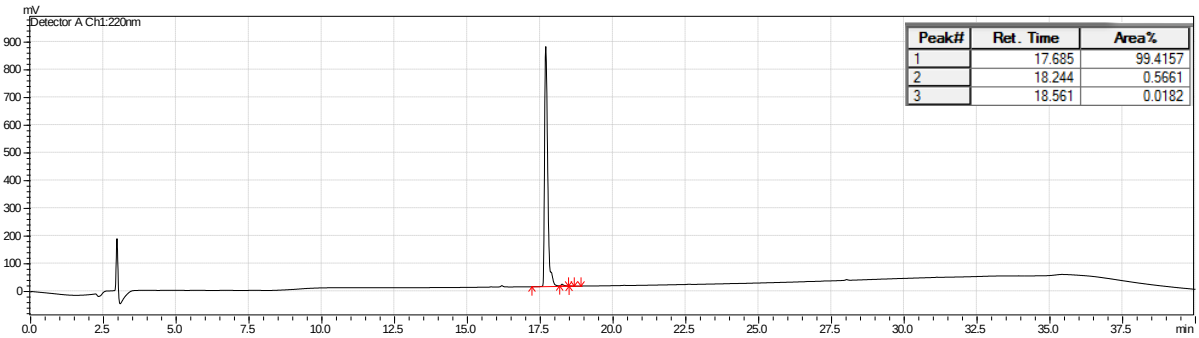
Peak#	Ret. Time	Area%
1	17.521	0.1526
2	18.205	99.4038
3	18.674	0.2093
4	18.758	0.2008
5	19.096	0.0335

21-CSP-s14



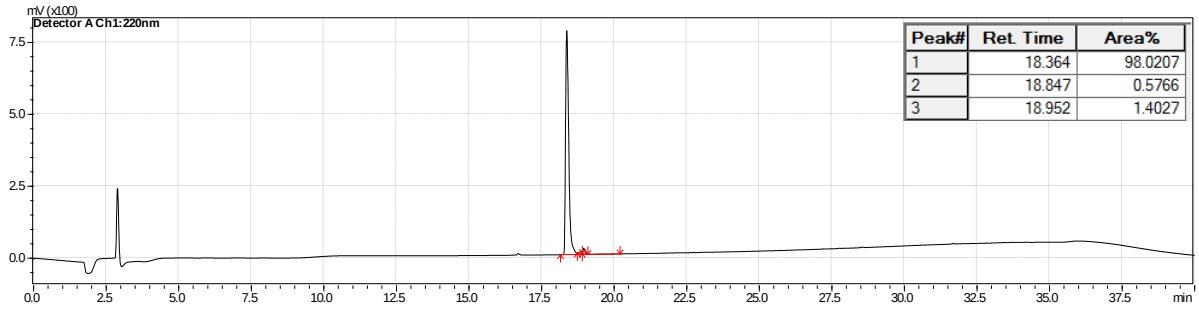
Peak#	Ret. Time	Area%
1	16.763	0.0253
2	17.504	99.9400
3	17.957	0.0347

21-CSP-f15



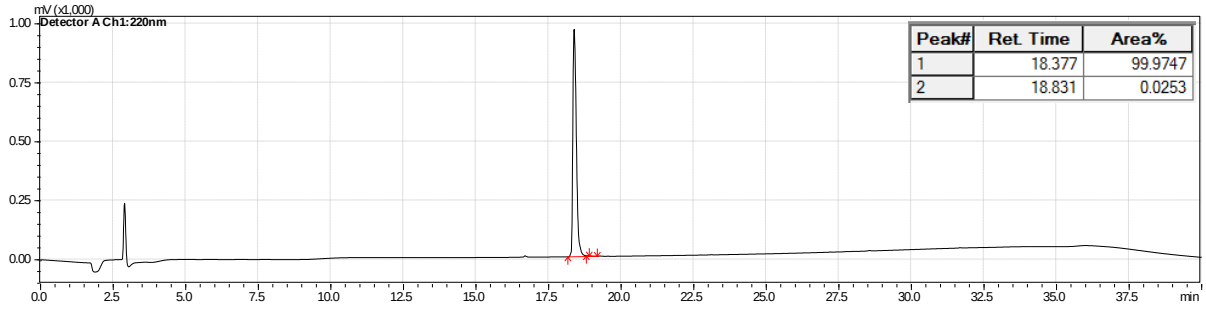
Peak#	Ret. Time	Area%
1	17.685	99.4157
2	18.244	0.5661
3	18.561	0.0182

21-CSP-t16



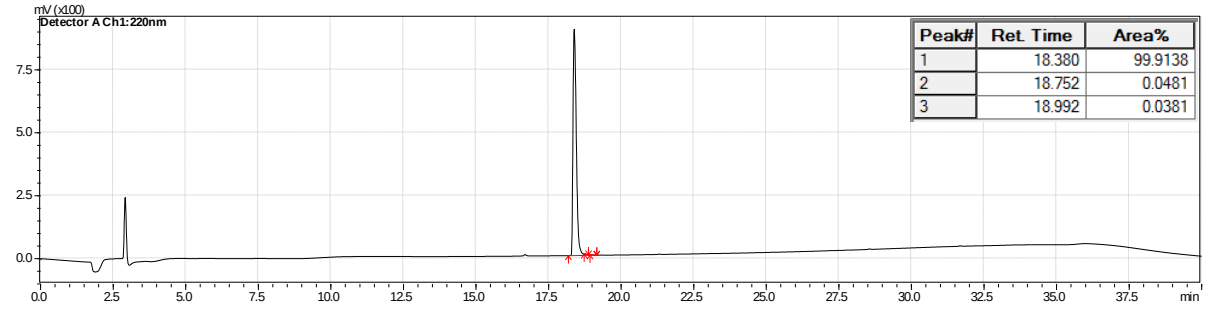
Peak#	Ret. Time	Area%
1	18.364	98.0207
2	18.847	0.5766
3	18.952	1.4027

21-CSP-q17



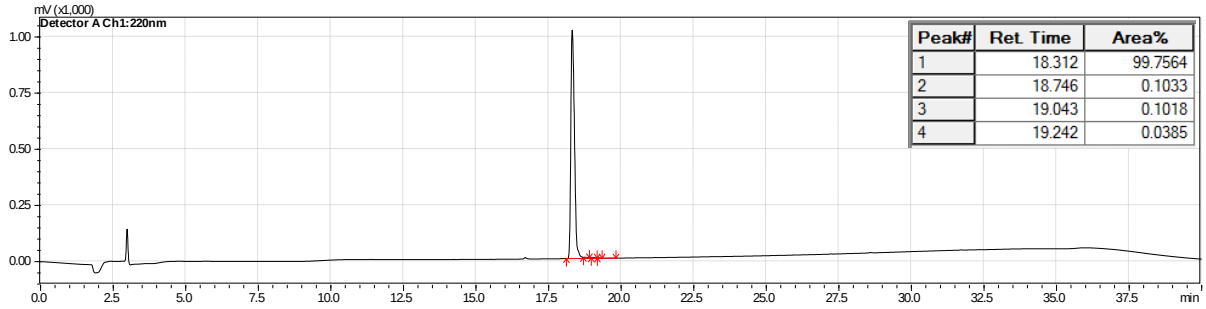
Peak#	Ret. Time	Area%
1	18.377	99.9747
2	18.831	0.0253

21-CSP-a18



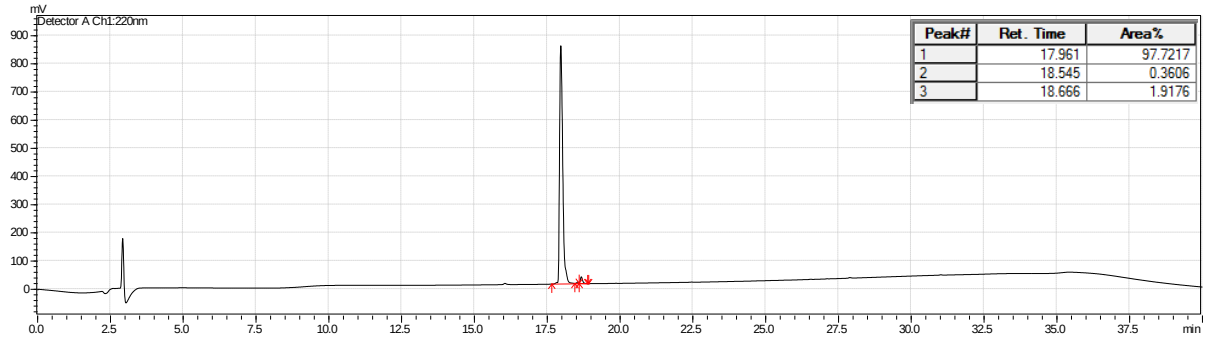
Peak#	Ret. Time	Area%
1	18.380	99.9138
2	18.752	0.0481
3	18.992	0.0381

21-CSP-l19



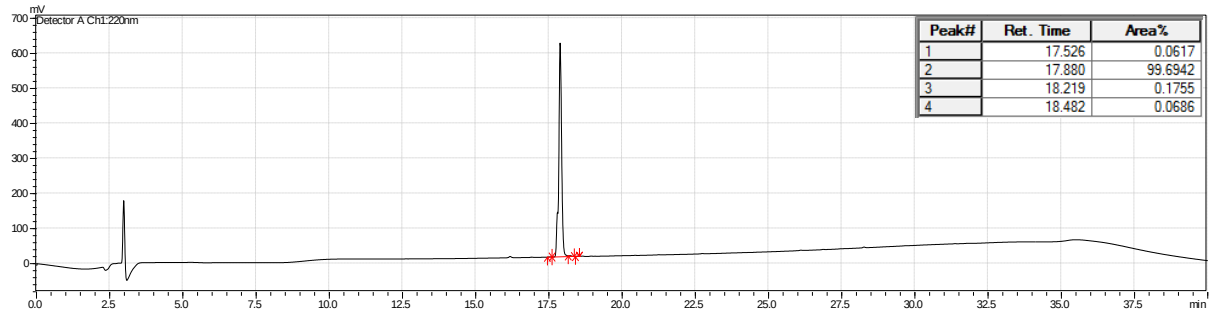
Peak#	Ret. Time	Area%
1	18.312	99.7564
2	18.746	0.1033
3	19.043	0.1018
4	19.242	0.0385

21-CSP-k21



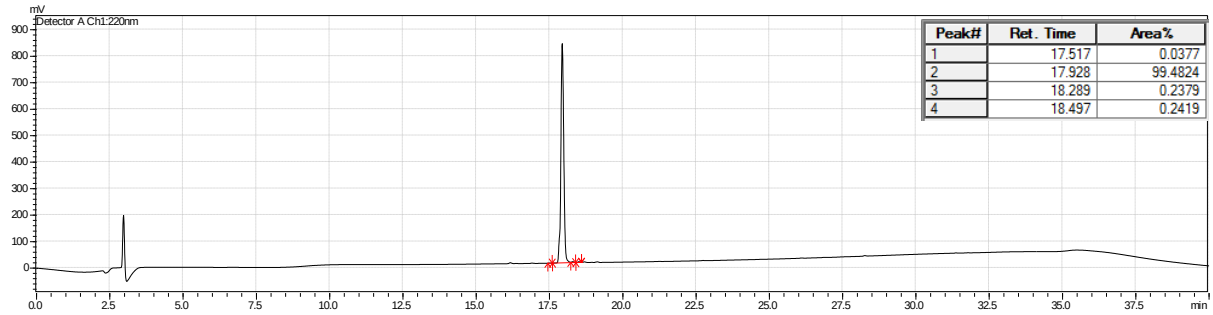
Peak#	Ret. Time	Area%
1	17.961	97.7217
2	18.545	0.3606
3	18.666	1.9176

18-CSP-S1A



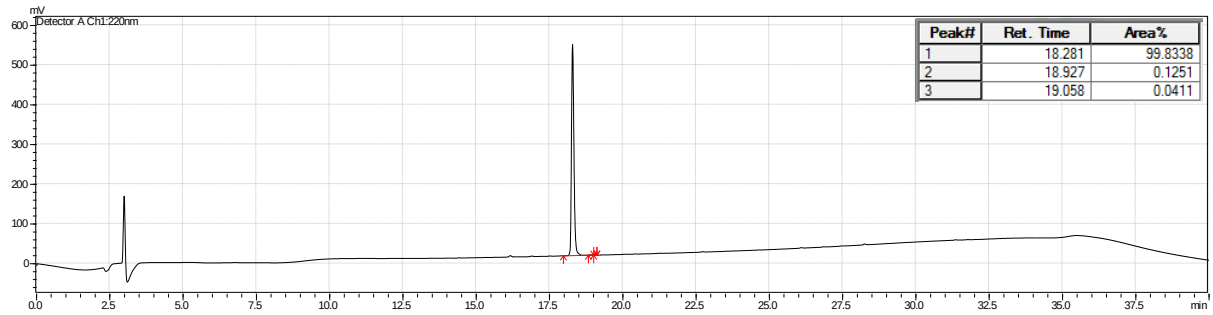
Peak#	Ret. Time	Area%
1	17.526	0.0617
2	17.880	99.6942
3	18.219	0.1755
4	18.482	0.0686

18-CSP-G2A



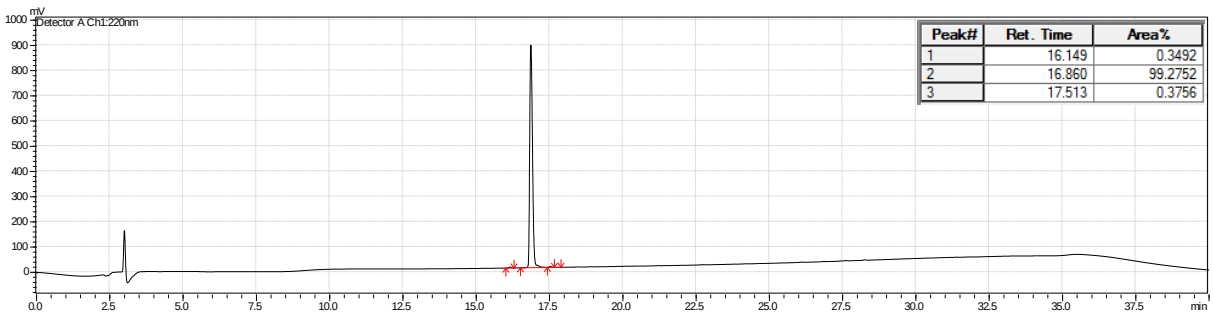
Peak#	Ret. Time	Area%
1	17.517	0.0377
2	17.928	99.4824
3	18.289	0.2379
4	18.497	0.2419

18-CSP-S3A



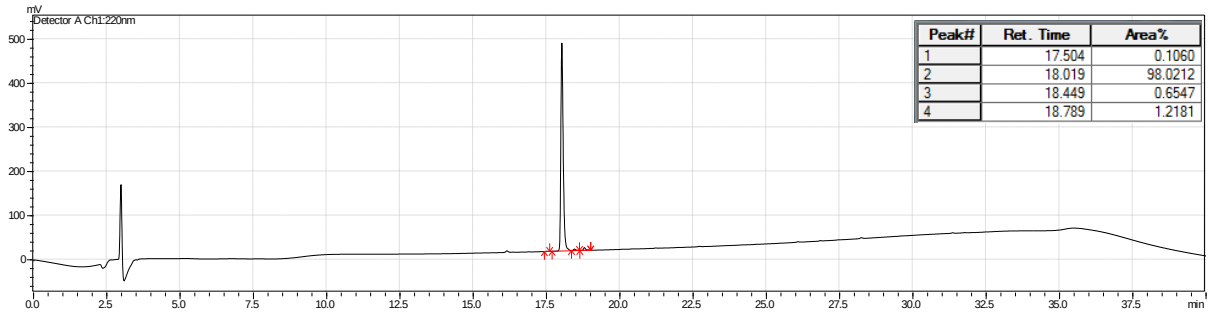
Peak#	Ret. Time	Area%
1	18.281	99.8338
2	18.927	0.1251
3	19.058	0.0411

18-CSP-L4A



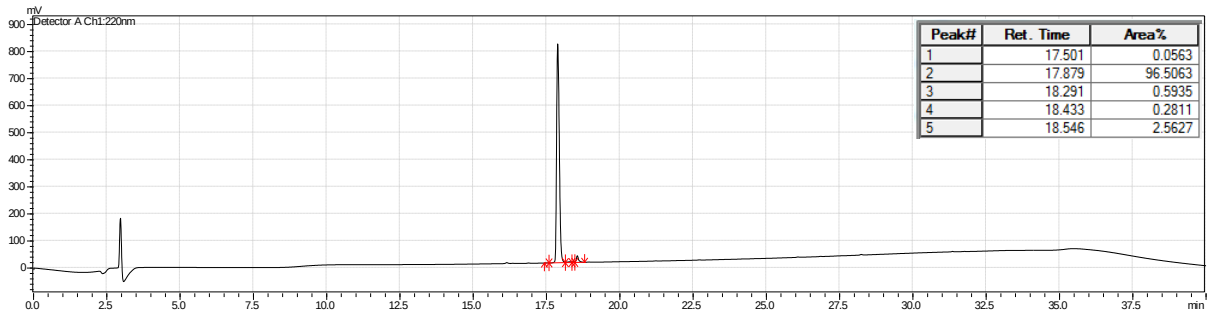
Peak#	Ret. Time	Area%
1	16.149	0.3492
2	16.860	99.2752
3	17.513	0.3756

18-CSP-S5A



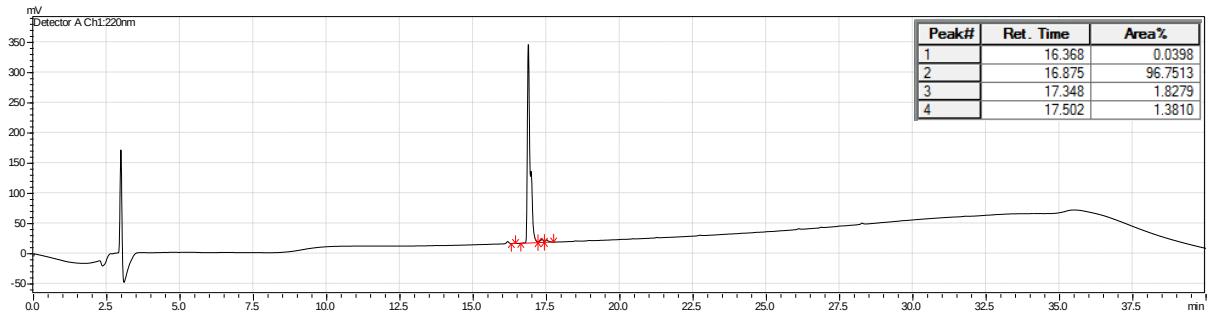
Peak#	Ret. Time	Area%
1	17.504	0.1060
2	18.019	98.0212
3	18.449	0.6547
4	18.789	1.2181

18-CSP-T6A



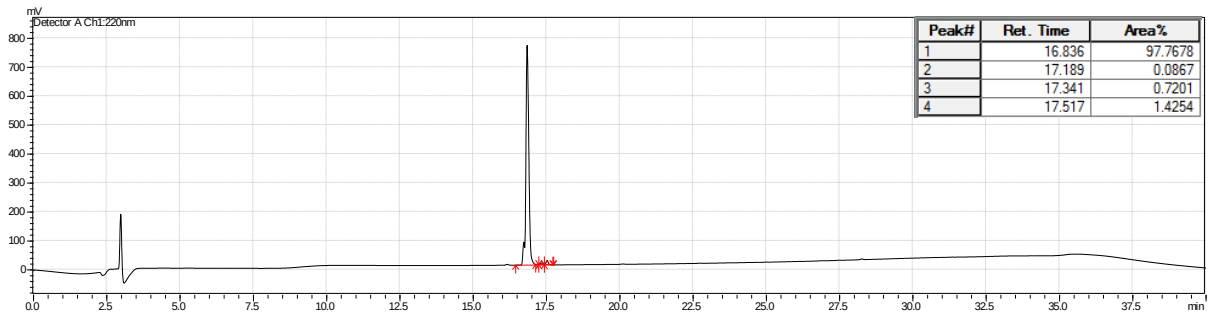
Peak#	Ret. Time	Area%
1	17.501	0.0563
2	17.879	96.5063
3	18.291	0.5935
4	18.433	0.2811
5	18.546	2.5627

18-CSP-F7A



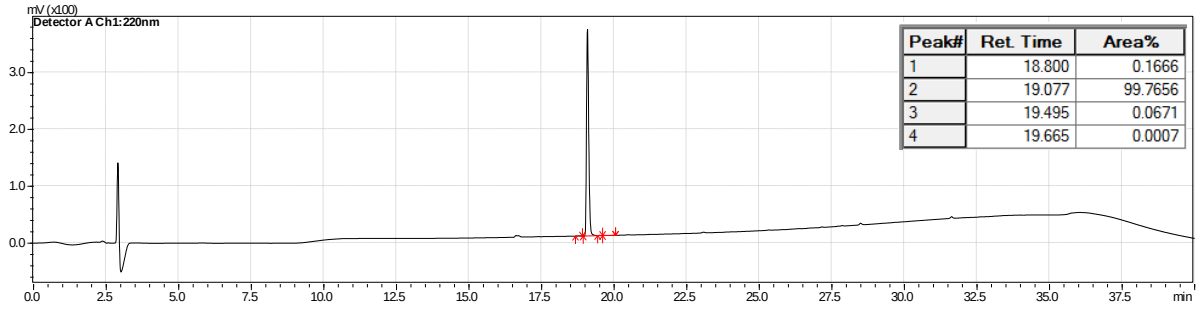
Peak#	Ret. Time	Area%
1	16.368	0.0398
2	16.875	96.7513
3	17.348	1.8279
4	17.502	1.3810

18-CSP-F8A



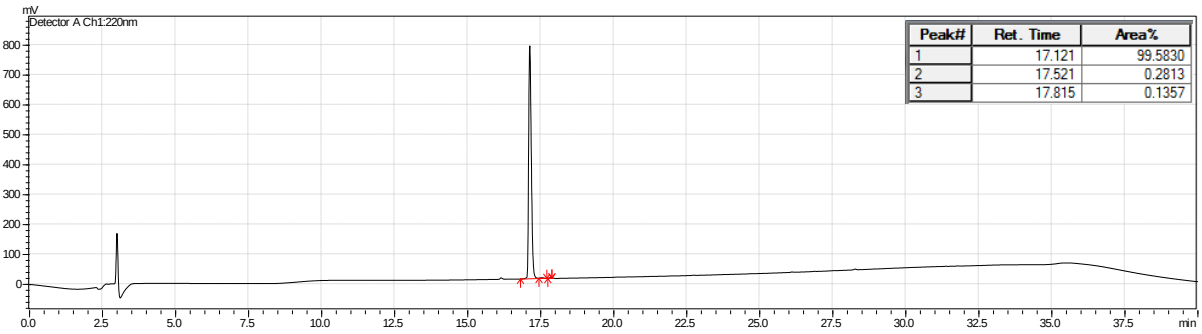
Peak#	Ret. Time	Area%
1	16.836	97.7678
2	17.189	0.0867
3	17.341	0.7201
4	17.517	1.4254

18-CSP-R9A



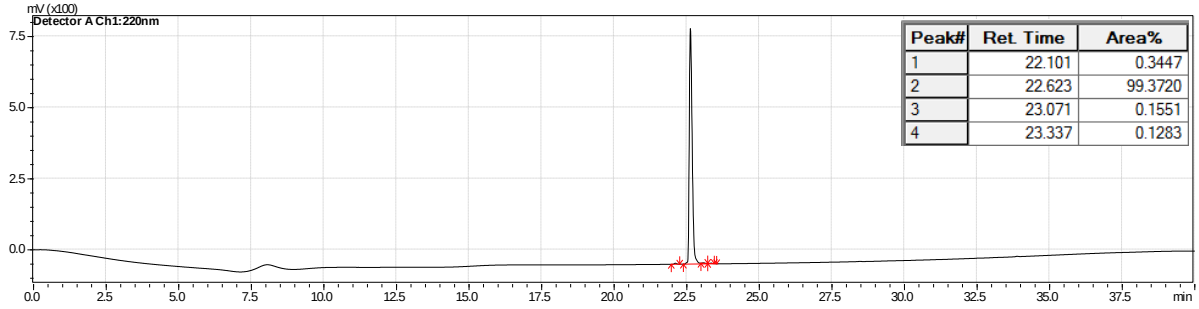
Peak#	Ret. Time	Area%
1	18.800	0.1666
2	19.077	99.7656
3	19.495	0.0671
4	19.665	0.0007

18-CSP-L10A



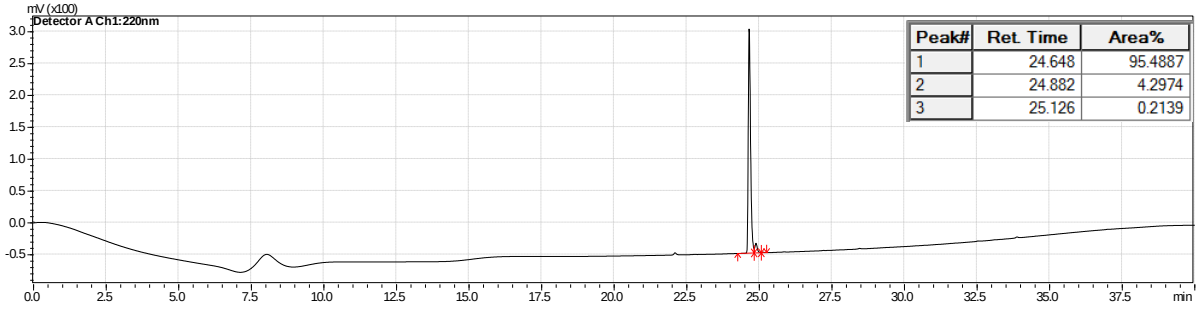
Peak#	Ret. Time	Area%
1	17.121	99.5830
2	17.521	0.2813
3	17.815	0.1357

18-CSP-F11A



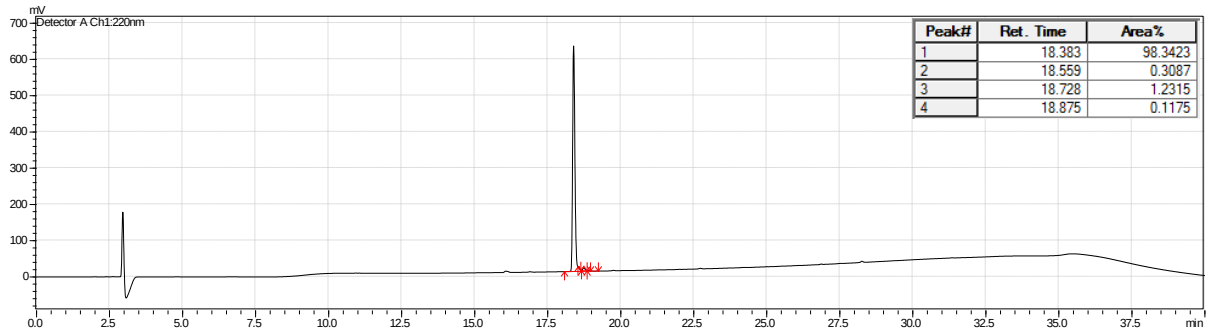
Peak#	Ret. Time	Area%
1	22.101	0.3447
2	22.623	99.3720
3	23.071	0.1551
4	23.337	0.1283

18-CSP-N12A



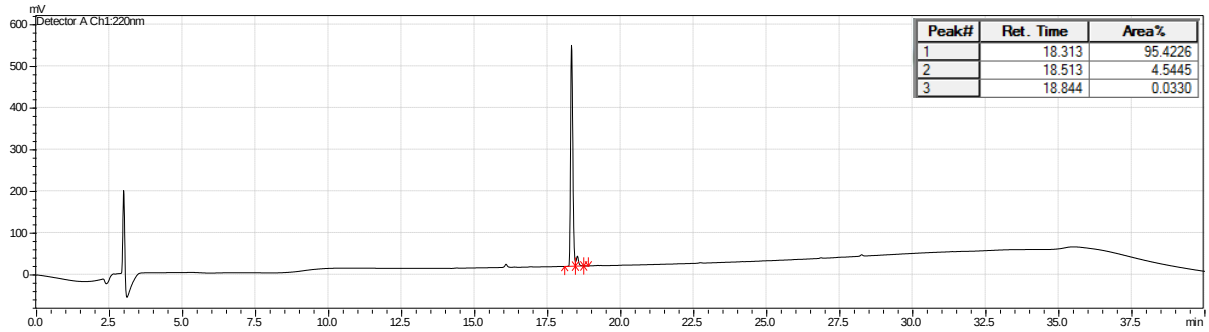
Peak#	Ret. Time	Area%
1	24.648	95.4887
2	24.882	4.2974
3	25.126	0.2139

18-CSP-R13A



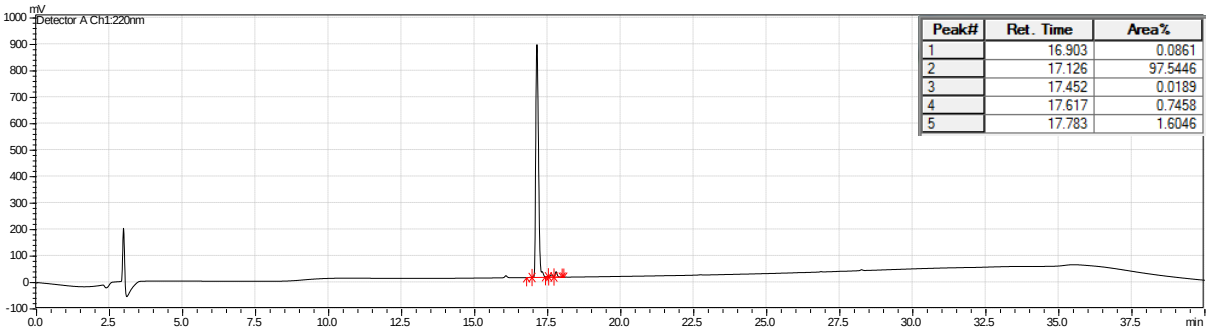
Peak#	Ret. Time	Area%
1	18.383	98.3423
2	18.559	0.3087
3	18.728	1.2315
4	18.875	0.1175

18-CSP-S14A



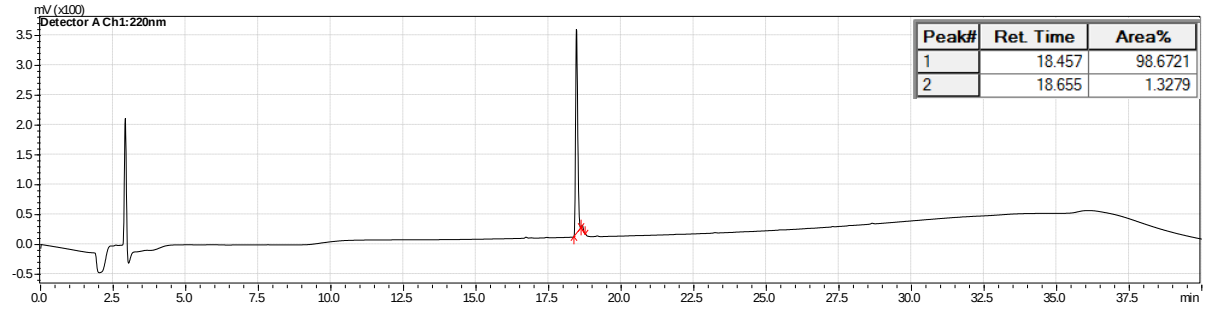
Peak#	Ret. Time	Area%
1	18.313	95.4226
2	18.513	4.5445
3	18.844	0.0330

18-CSP-F15A



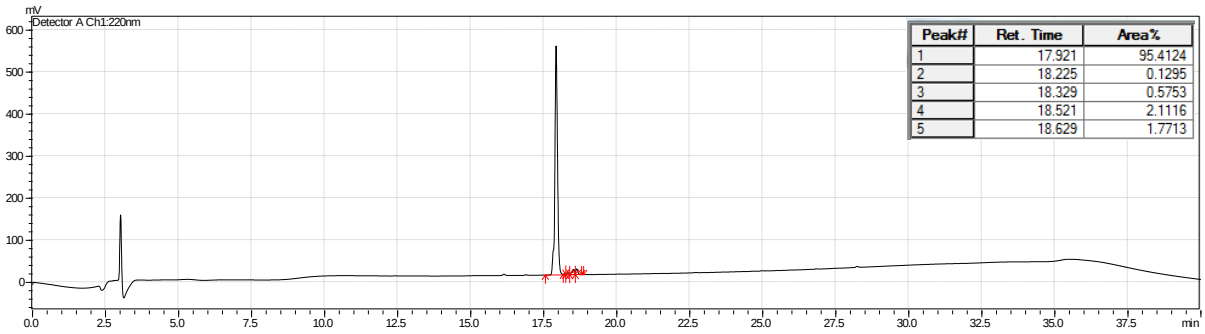
Peak#	Ret. Time	Area%
1	16.903	0.0861
2	17.126	97.5446
3	17.452	0.0189
4	17.617	0.7458
5	17.783	1.6046

18-CSP-T16A



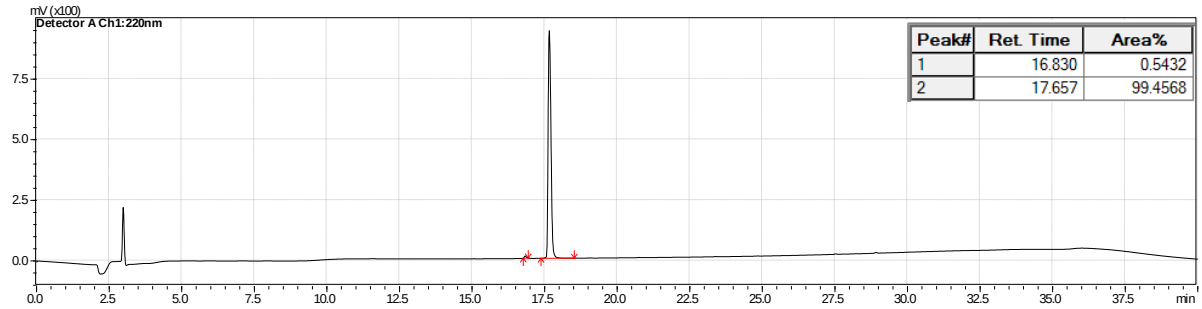
Peak#	Ret. Time	Area%
1	18.457	98.6721
2	18.655	1.3279

18-CSP-Q17A



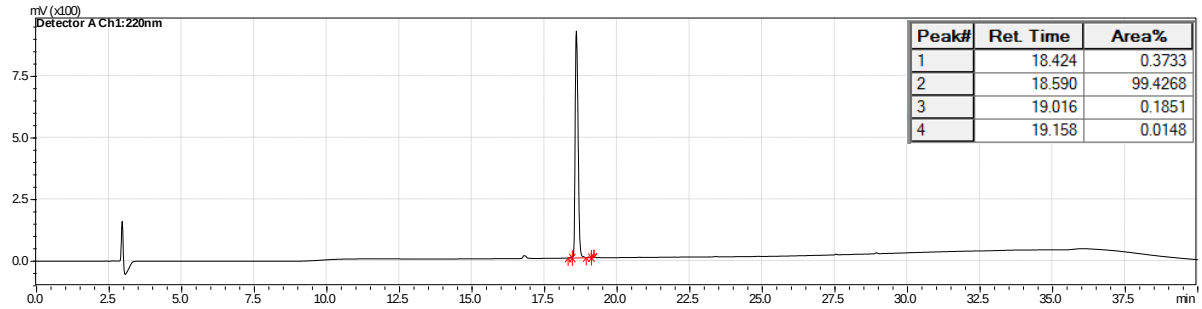
Peak#	Ret. Time	Area%
1	17.921	95.4124
2	18.225	0.1295
3	18.329	0.5753
4	18.521	2.1116
5	18.629	1.7713

18-CSP-s1



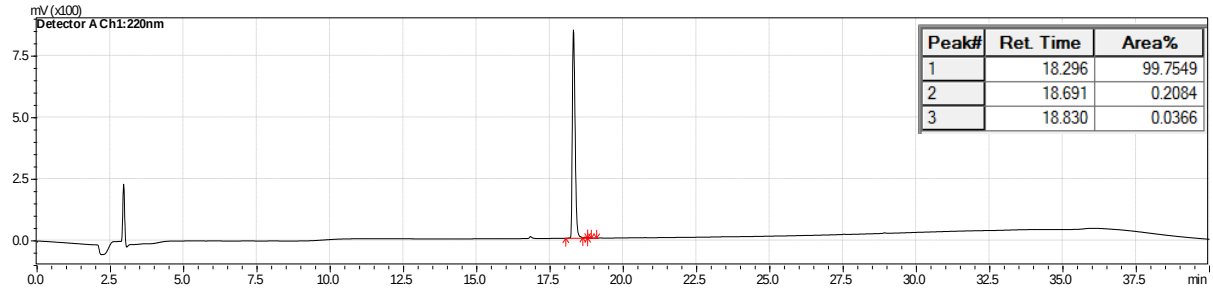
Peak#	Ret. Time	Area%
1	16.830	0.5432
2	17.657	99.4568

18-CSP-s3



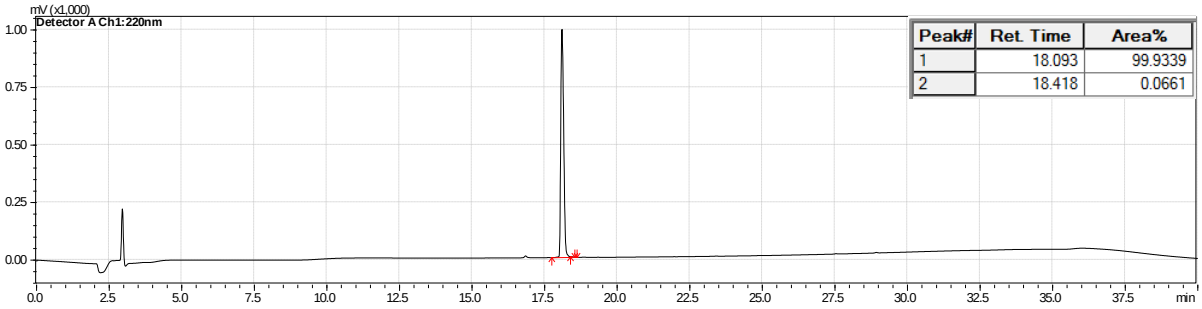
Peak#	Ret. Time	Area%
1	18.424	0.3733
2	18.590	99.4268
3	19.016	0.1851
4	19.158	0.0148

18-CSP-l4



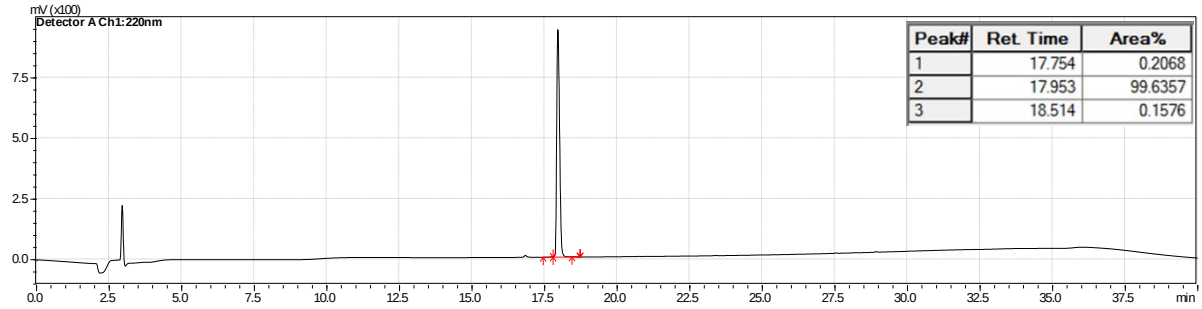
Peak#	Ret. Time	Area%
1	18.296	99.7549
2	18.691	0.2084
3	18.830	0.0366

18-CSP-s5



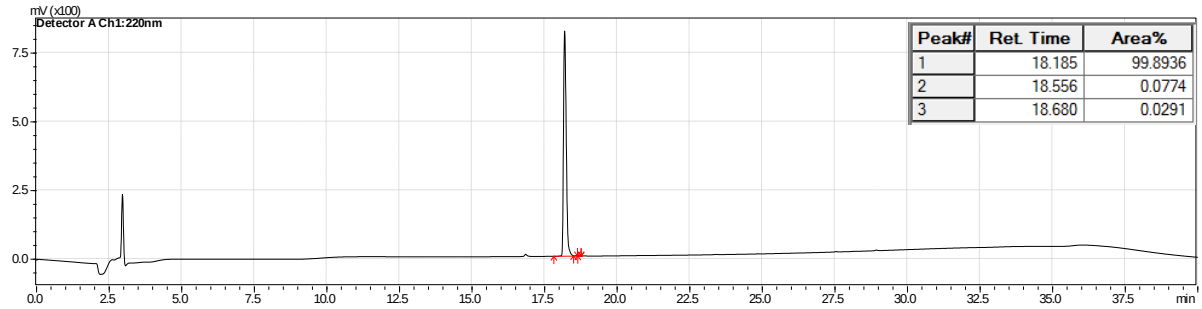
Peak#	Ret. Time	Area%
1	18.093	99.9339
2	18.418	0.0661

18-CSP-t6



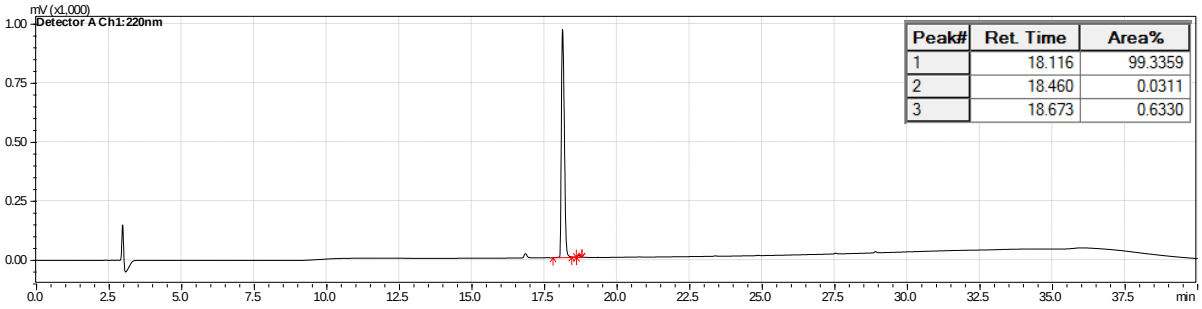
Peak#	Ret. Time	Area%
1	17.754	0.2068
2	17.953	99.6357
3	18.514	0.1576

18-CSP-f7



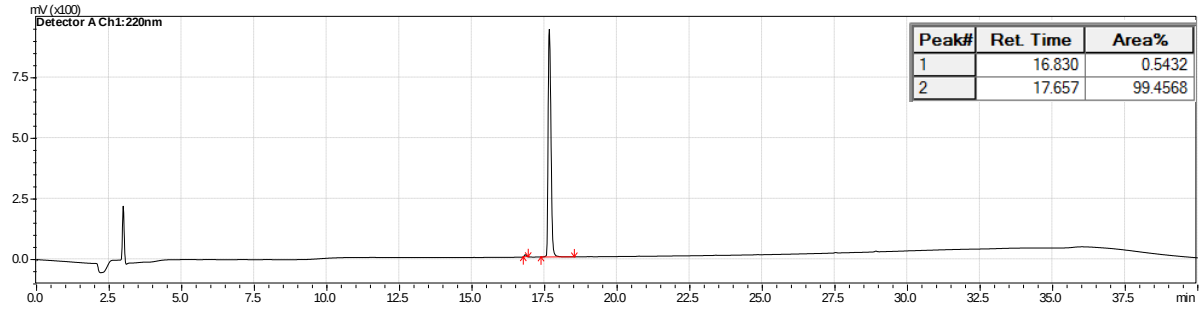
Peak#	Ret. Time	Area%
1	18.185	99.8936
2	18.556	0.0774
3	18.680	0.0291

18-CSP-f8



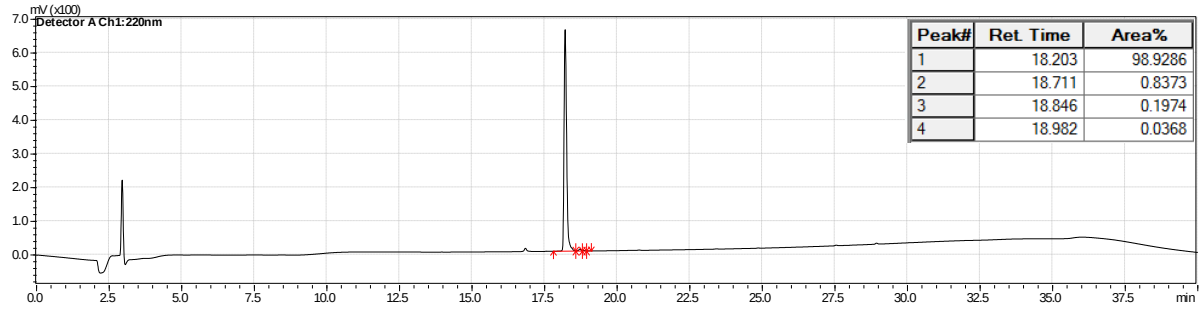
Peak#	Ret. Time	Area%
1	18.116	99.3359
2	18.460	0.0311
3	18.673	0.6330

18-CSP-r9



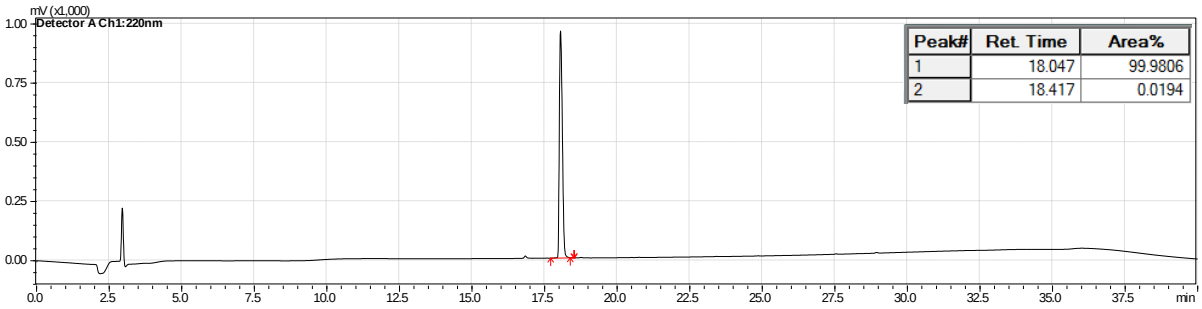
Peak#	Ret. Time	Area%
1	16.830	0.5432
2	17.657	99.4568

18-CSP-l10



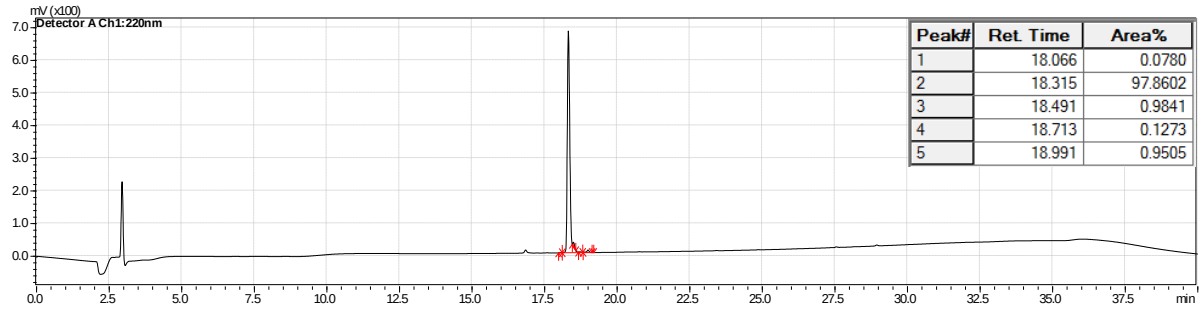
Peak#	Ret. Time	Area%
1	18.203	98.9286
2	18.711	0.8373
3	18.846	0.1974
4	18.982	0.0368

18-CSP-f11



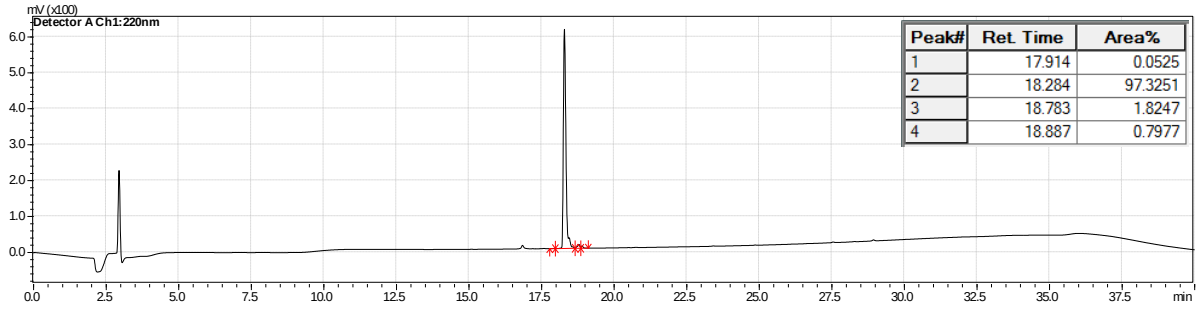
Peak#	Ret. Time	Area%
1	18.047	99.9806
2	18.417	0.0194

18-CSP-n12



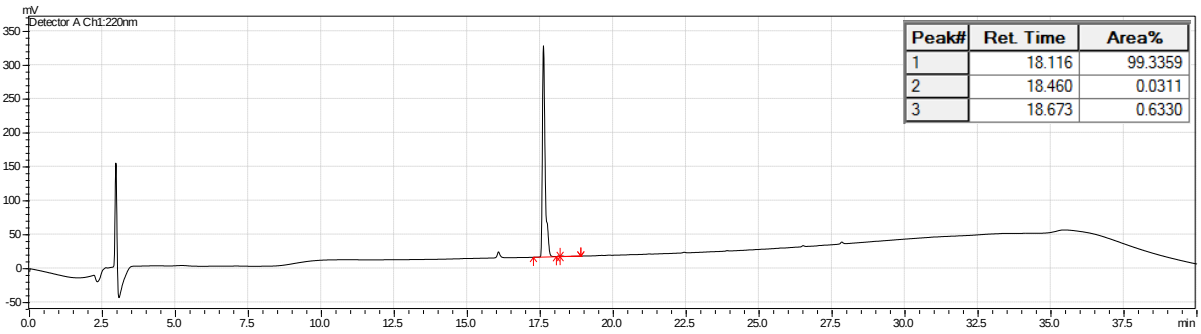
Peak#	Ret. Time	Area%
1	18.066	0.0780
2	18.315	97.8602
3	18.491	0.9841
4	18.713	0.1273
5	18.991	0.9505

18-CSP-r13



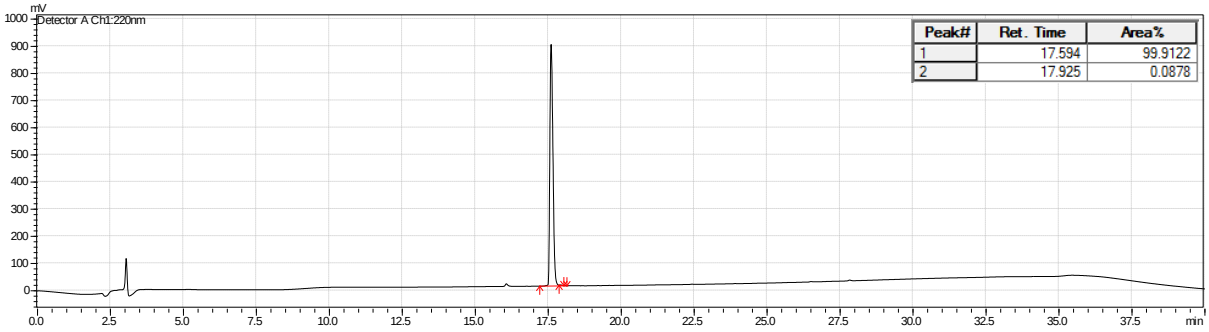
Peak#	Ret. Time	Area%
1	17.914	0.0525
2	18.284	97.3251
3	18.783	1.8247
4	18.887	0.7977

18-CSP-s14



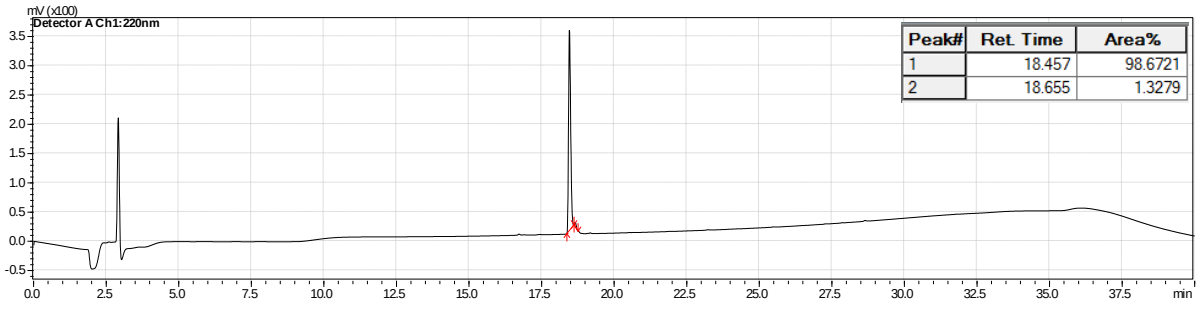
Peak#	Ret. Time	Area%
1	18.116	99.3359
2	18.460	0.0311
3	18.673	0.6330

18-CSP-f15



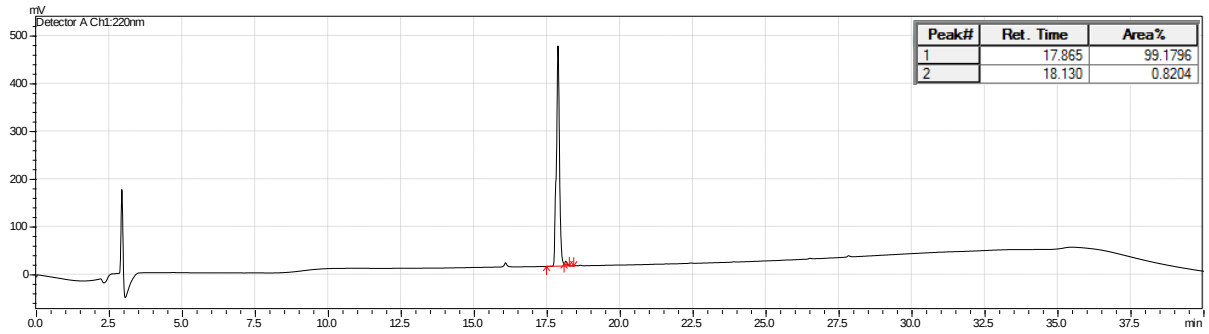
Peak#	Ret. Time	Area%
1	17.594	99.9122
2	17.925	0.0878

18-CSP-t16

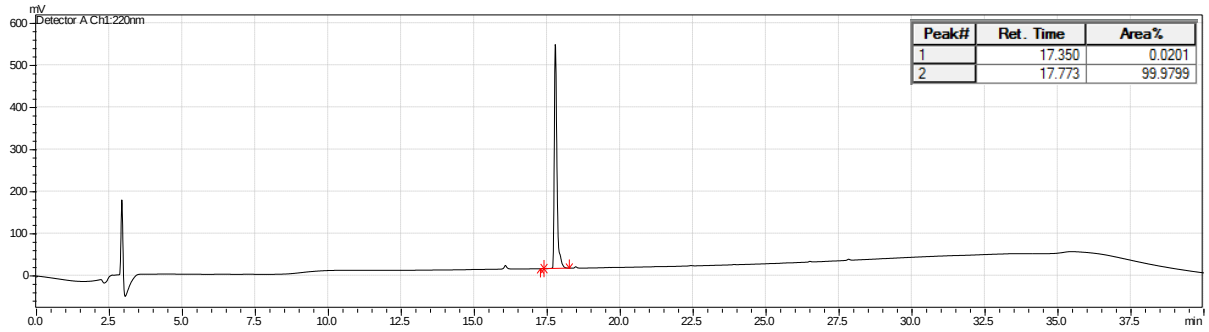


Peak#	Ret. Time	Area%
1	18.457	98.6721
2	18.655	1.3279

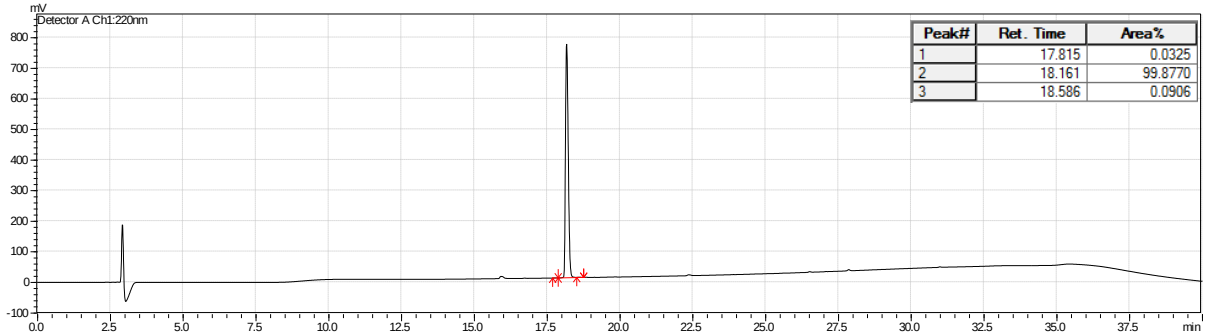
18-CSP-q17



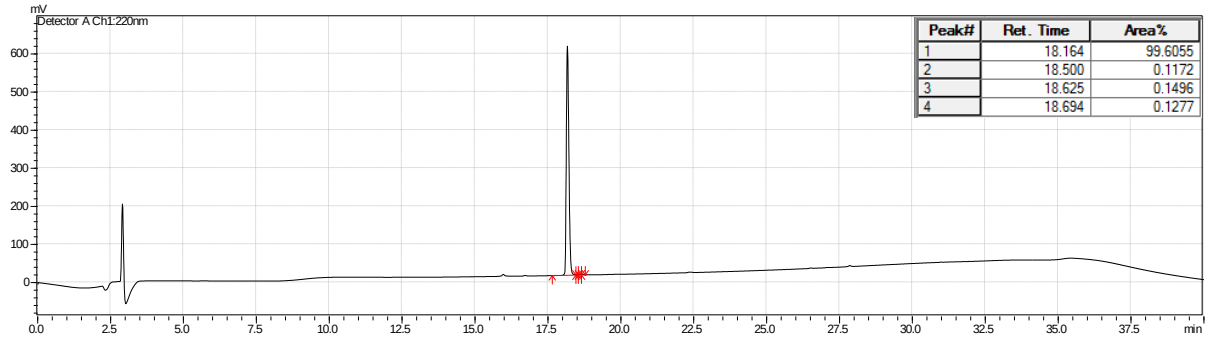
18-CSP-a18



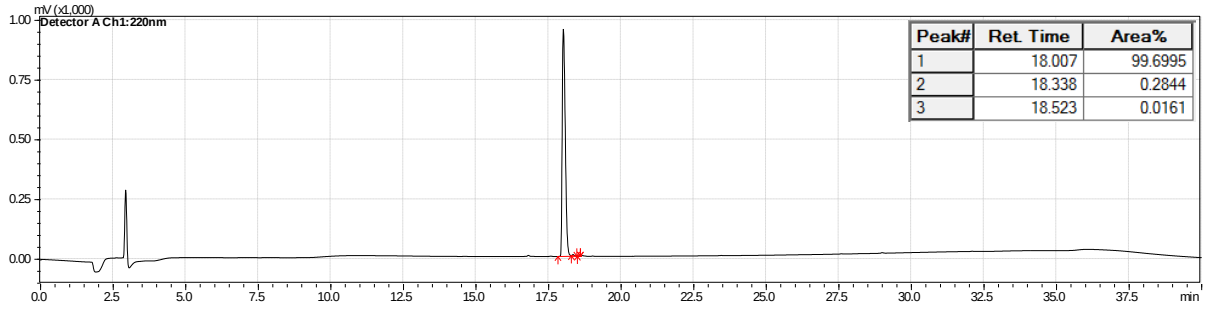
21-CSP-des-S1



21-CSP-des-S1G2

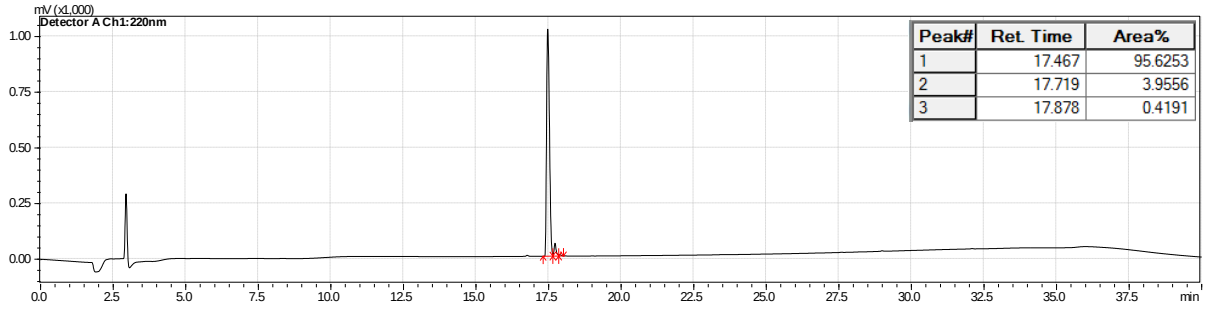


21-CSP-des-S1G2S3



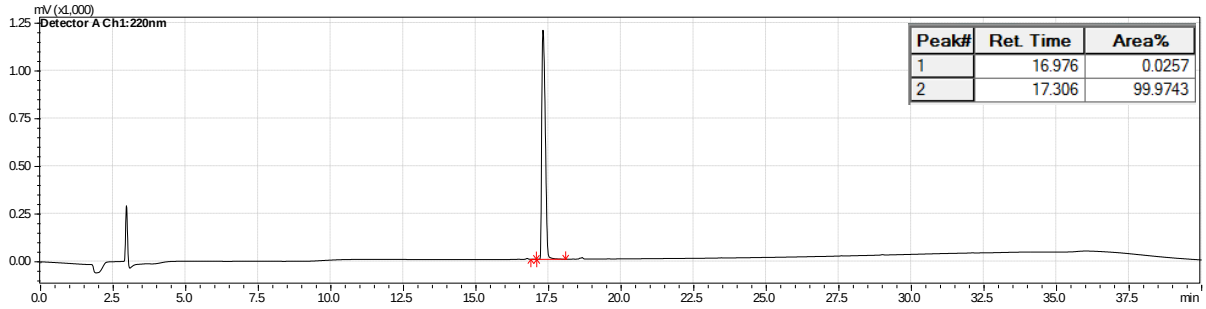
Peak#	Ret. Time	Area%
1	18.007	99.6995
2	18.338	0.2844
3	18.523	0.0161

21-CSP-des-S1G2S3L4



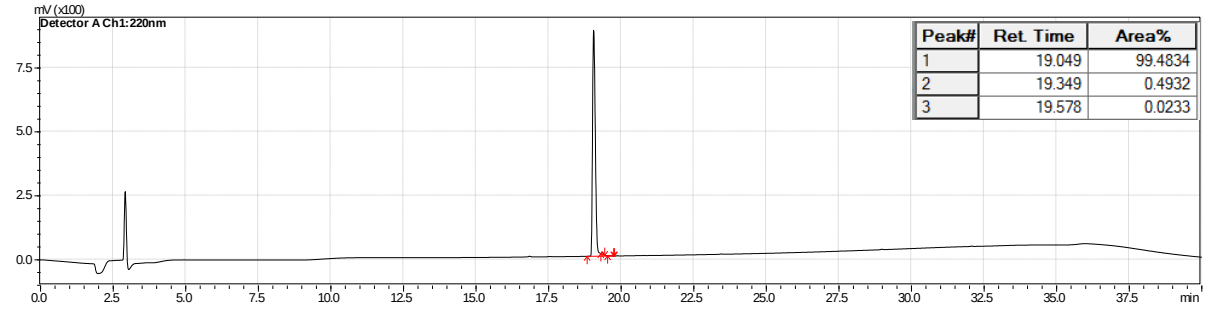
Peak#	Ret. Time	Area%
1	17.467	95.6253
2	17.719	3.9556
3	17.878	0.4191

21-CSP-des-S1G2S3L4S5



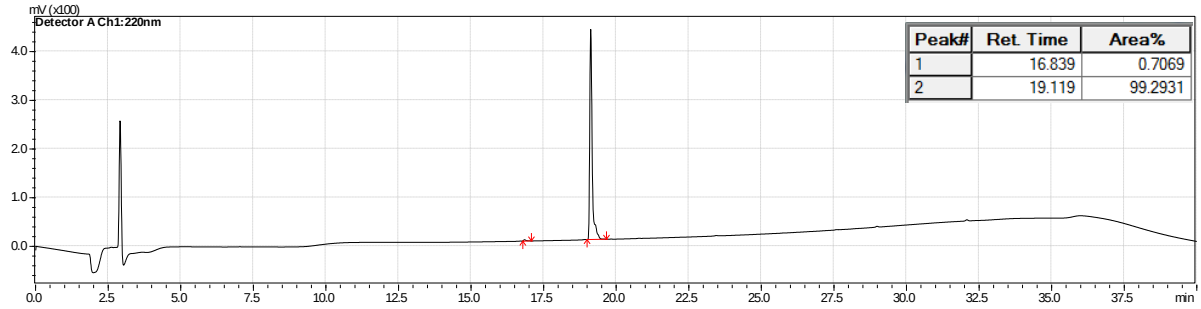
Peak#	Ret. Time	Area%
1	16.976	0.0257
2	17.306	99.9743

21-CSP-des-K21

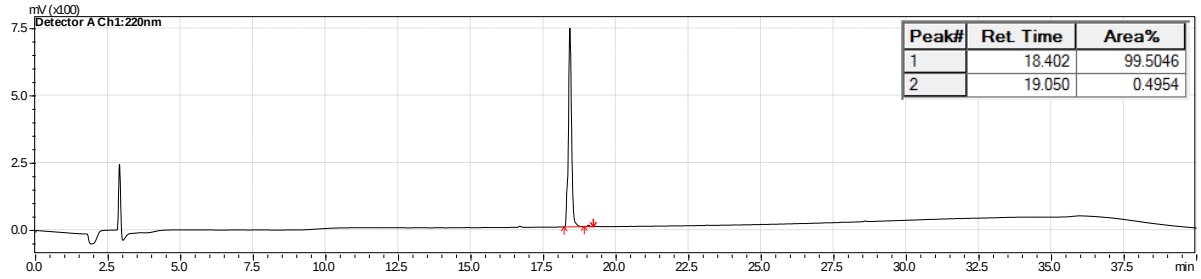


Peak#	Ret. Time	Area%
1	19.049	99.4834
2	19.349	0.4932
3	19.578	0.0233

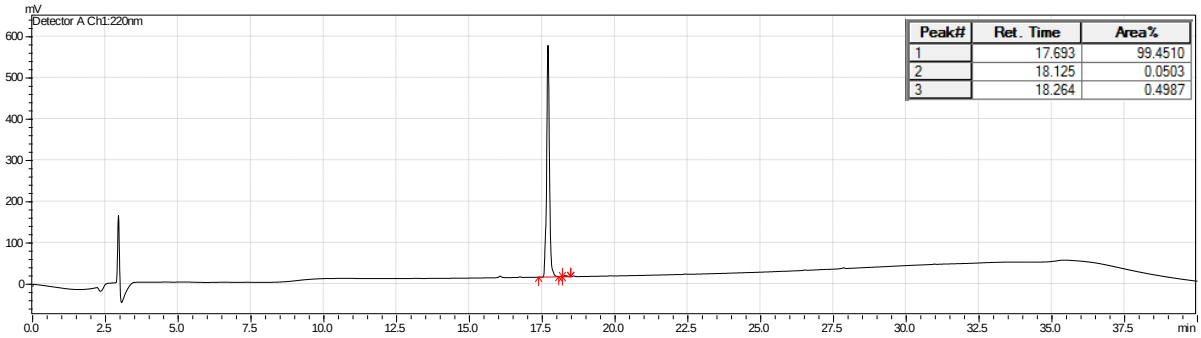
21-CSP-des-G20K21



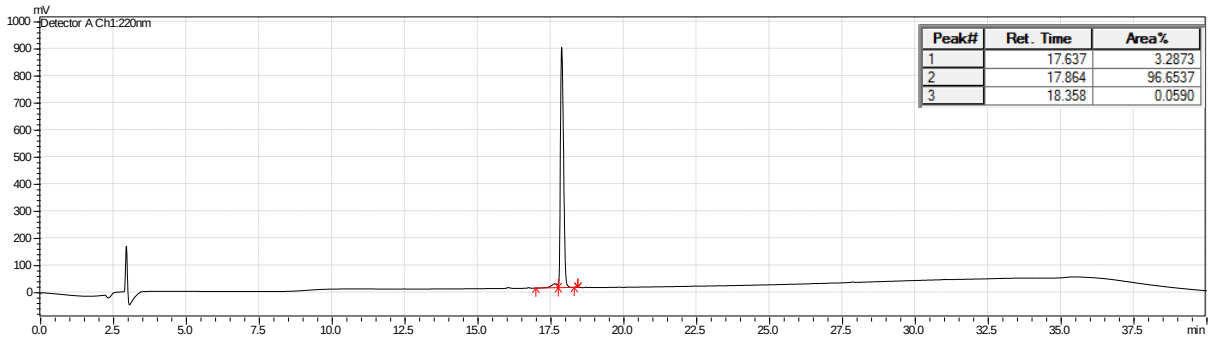
21-CSP-des-L19G20K21 (18-CSP)



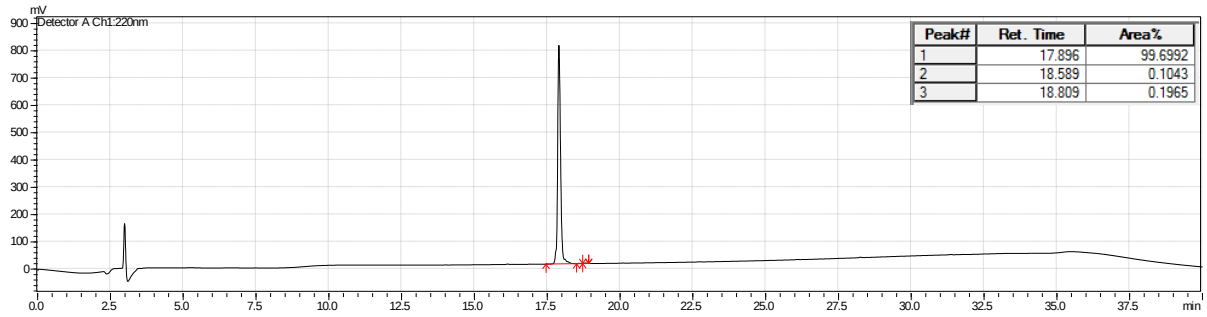
18-CSP-des-A18



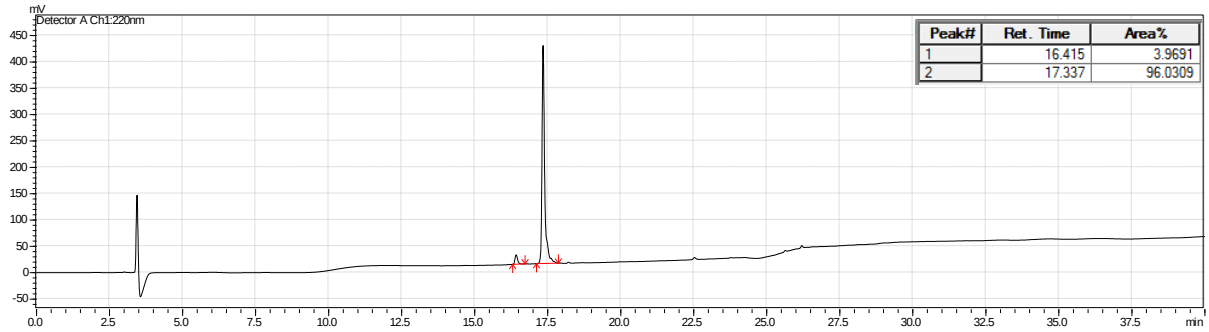
18-CSP-des-Q17A18



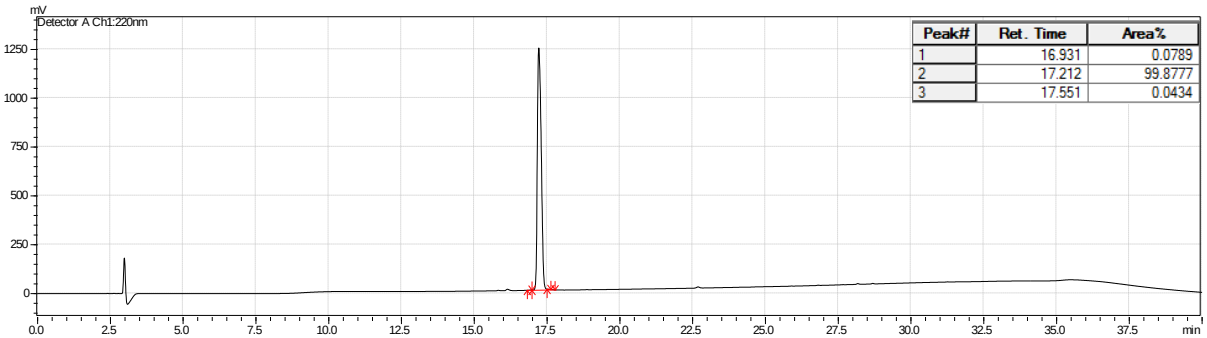
18-CSP-des-S1



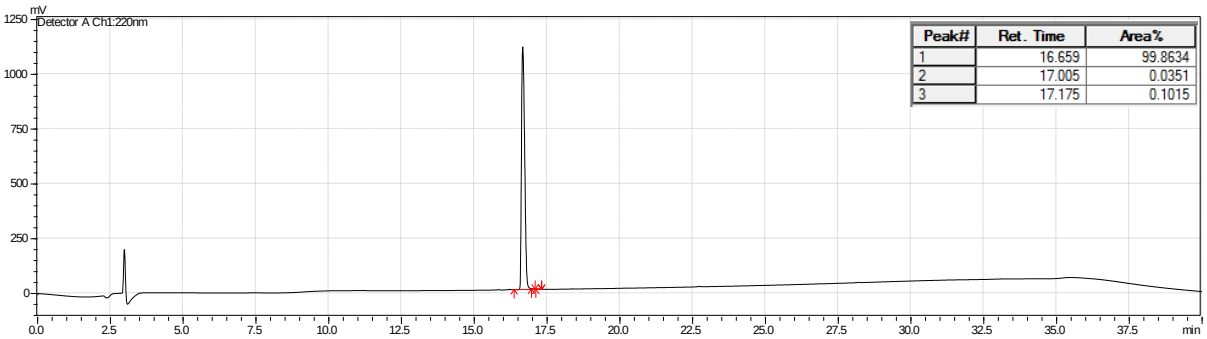
18-CSP-des-S1G2



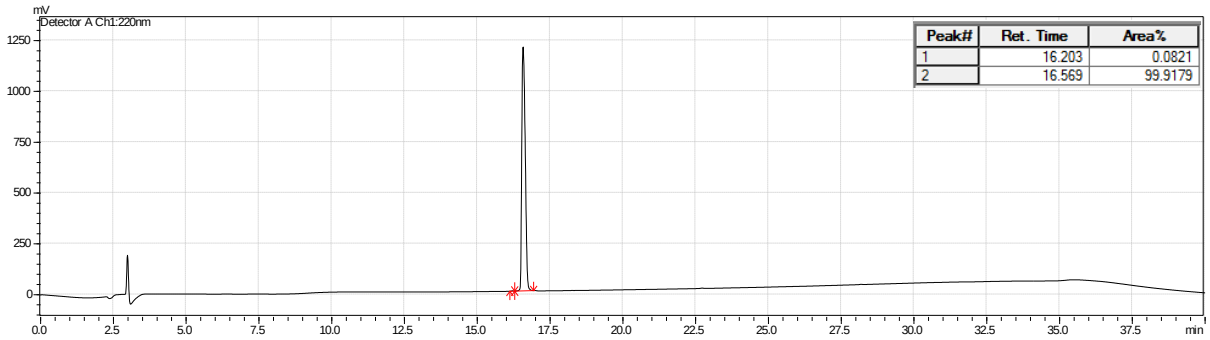
18-CSP-des-S1G2S3



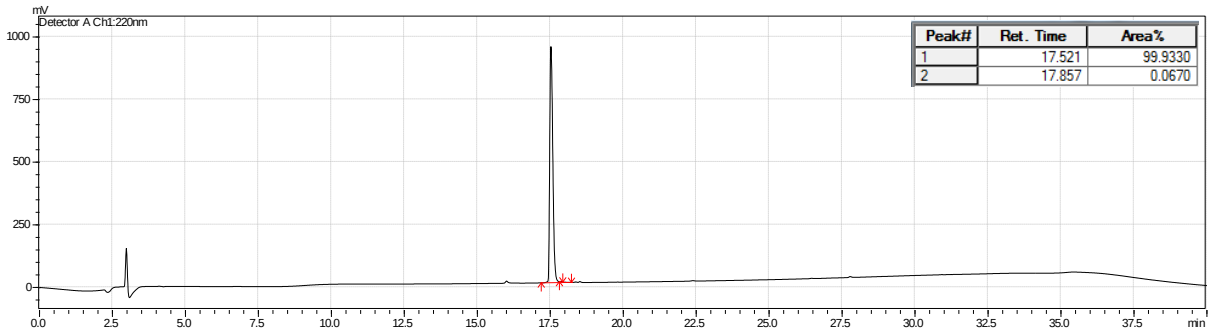
18-CSP-des-S1G2S3L4



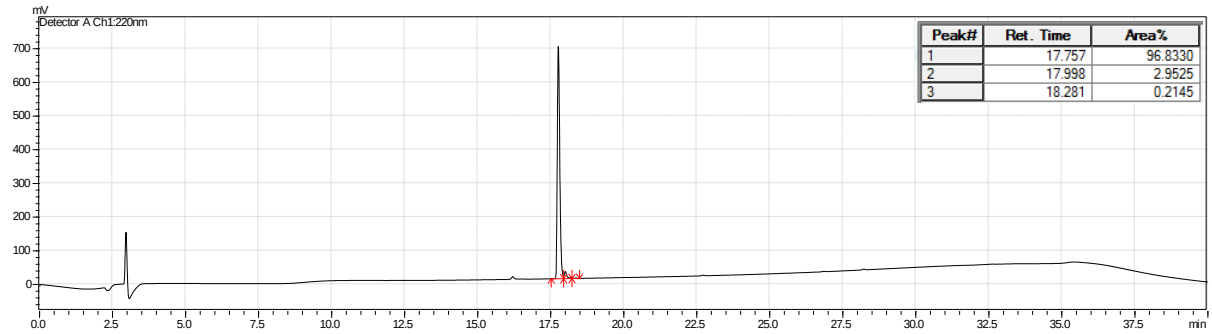
18-CSP-des-S1G2S3L4S5



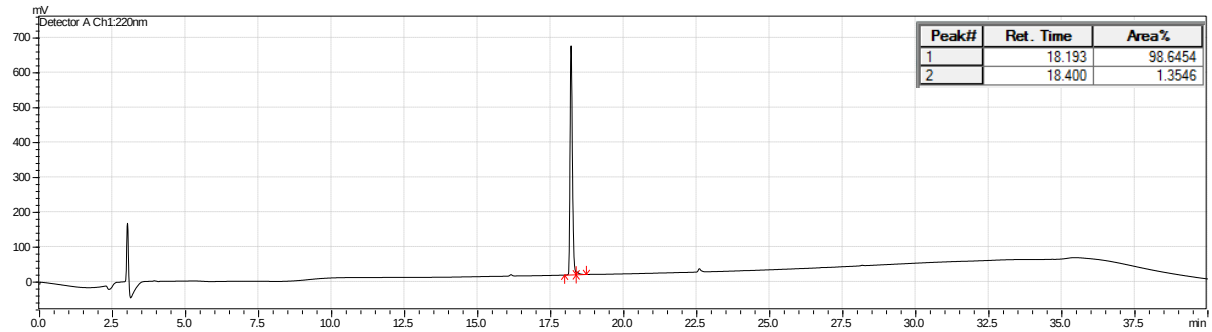
18-CSP-s1s3l4



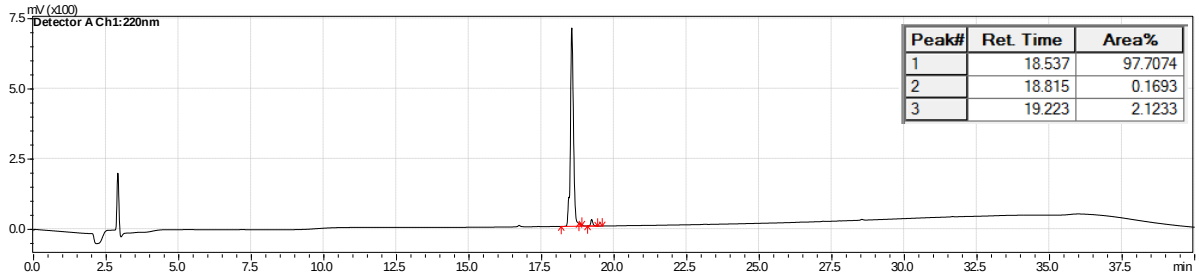
18-CSP-s1s3l4L10AR13AS14A



18-CSP-S1AL10AR13AS14A

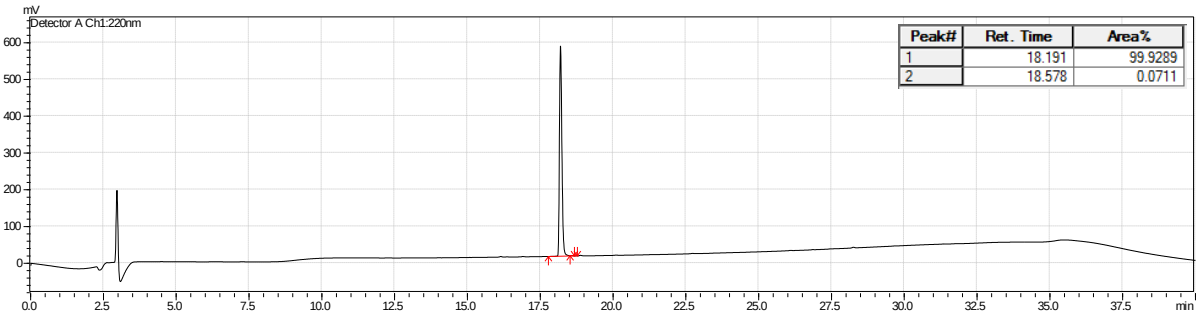


18-CSP-S3T



Peak#	Ret. Time	Area%
1	18.537	97.7074
2	18.815	0.1693
3	19.223	2.1233

21-CSP-S3T



Peak#	Ret. Time	Area%
1	18.191	99.9289
2	18.578	0.0711

MS and HPLC data for CSP analogs

Table S-1. MS and HPLC data for 21-CSP alanine & D-amino acid scanning analogs.

Compound Name	Calc. EM MH_2^{2+}	Obs. EM MH_2^{2+}	Purity (%)	Compound Name	Calc. EM MH_2^{2+}	Obs. EM MH_2^{2+}	Purity (%)
21-CSP	1183.1280	1183.1260	>99				
21-CSP-S1A	1175.6291	1175.6294	>99	21-CSP-s1	1183.1280	1183.1249	>97
21-CSP-G2A	1190.1358	1190.1329	>99				
21-CSP-S3A	1175.6291	1175.6287	>99	21-CSP-s3	1183.1280	1183.1296	>99
21-CSP-L4A	1162.1045	1162.1029	>99	21-CSP-l4	1183.1280	1183.1330	>97
21-CSP-S5A	1175.6291	1175.6292	>98	21-CSP-s5	1183.1280	1183.1324	>99
21-CSP-T6A	1168.1230	1168.1203	>99	21-CSP-t6	1183.1280	1183.1332	>99
21-CSP-F7A	1145.1124	1145.1091	>96	21-CSP-f7	1183.1280	1183.1329	>99
21-CSP-F8A	1145.1124	1145.1103	>99	21-CSP-f8	1183.1280	1183.1300	>99
21-CSP-R9A	1140.5960	1140.5929	>95	21-CSP-r9	1183.1280	1183.1296	>99
21-CSP-L10A	1162.1045	1162.1024	>99	21-CSP-l10	1183.1280	1183.1325	>96
21-CSP-F11A	1145.1124	1145.1099	>99	21-CSP-f11	1183.1280	1183.1322	>99
21-CSP-N12A	1161.6251	1161.6229	>98	21-CSP-n12	1183.1280	1183.1294	>99
21-CSP-R13A	1140.5960	1140.5932	>98	21-CSP-r13	1183.1280	1183.1282	>99
21-CSP-S14A	1175.6291	1175.6281	>98	21-CSP-s14	1183.1280	1183.1287	>99
21-CSP-F15A	1145.1124	1145.1095	>98	21-CSP-f15	1183.1280	1183.1284	>99
21-CSP-T16A	1168.1230	1168.1195	>98	21-CSP-t16	1183.1280	1183.1288	>98
21-CSP-Q17A	1154.6175	1154.6138	>95	21-CSP-q17	1183.1280	1183.1257	>99
				21-CSP-a18	1183.1280	1183.1301	>99
21-CSP-L19A	1162.1045	1162.1008	>99	21-CSP-l19	1183.1280	1183.1300	>99
21-CSP-G20A	1190.1358	1190.1330	>99				
21-CSP-K21A	1154.5991	1154.5965	>97	21-CSP-k21	1183.1280	1183.1258	>97

EM = Exact Mass. See main text for methods.

Table S-2. MS and HPLC data for 21-CSP and 18-CSP truncated analogs.

Compound Name	Calc. EM MH_2^{2+}	Obs. EM MH_2^{2+}	Purity (%)
21-CSP-des-S1	1139.6120	1139.6116	>99
21-CSP-des-S1G2	1111.1013	1111.1006	>99
21-CSP-des-S1G2S3	1067.5853	1067.5871	>99
21-CSP-des-S1G2S3L4	1011.0432	1011.0430	>95
21-CSP-des-S1G2S3L4S5	967.5272	967.5232	>99
21-CSP-des-K21	1119.0805	1119.0827	>99
21-CSP-des-G20K21	1090.5698	1090.5706	>99
18-CSP-des-S1	990.5118	990.5131	>99
18-CSP-des-S1G2	962.0010	961.9970	>96
18-CSP-des-S1G2S3	918.4850	918.4848	>99
18-CSP-des-S1G2S3L4	861.4415	861.4402	>99
18-CSP-des-S1G2S3L4S5	817.9255	817.9263	>99
18-CSP-des-A18	998.5092	998.5065	>99
18-CSP-des-Q17A18	934.4799	934.4802	>96

EM = Exact Mass. See main text for methods.

Table S-3. MS and HPLC data for 18-CSP alanine & D-amino acid scanning analogs.

Compound Name	Calc. EM MH_2^{2+}	Obs. EM MH_2^{2+}	Purity (%)	Compound Name	Calc. EM MH_2^{2+}	Obs. EM MH_2^{2+}	Purity (%)
18-CSP	1034.0278	1034.0261	>99				
18-CSP-S1A	1026.0303	1026.0315	>99	18-CSP-s1	1034.0278	1034.0305	>99
18-CSP-G2A	1041.0356	1041.0354	>99				
18-CSP-S3A	1026.0303	1026.0330	>99	18-CSP-s3	1034.0278	1034.0323	>99
18-CSP-L4A	1013.0043	1013.0085	>99	18-CSP-l4	1034.0278	1034.0284	>99
18-CSP-S5A	1026.0303	1026.0289	>98	18-CSP-s5	1034.0278	1034.0299	>99
18-CSP-T6A	1019.0225	1019.0255	>96	18-CSP-t6	1034.0278	1034.0287	>99
18-CSP-F7A	996.0121	996.0167	>96	18-CSP-f7	1034.0278	1034.0304	>99
18-CSP-F8A	996.0121	996.0125	>97	18-CSP-f8	1034.0278	1034.0313	>96
18-CSP-R9A	991.4958	991.4975	>99	18-CSP-r9	1034.0278	1034.0317	>99
18-CSP-L10A	1013.0043	1013.0057	>99	18-CSP-l10	1034.0278	1034.0283	>98
18-CSP-F11A	996.0121	996.0125	>99	18-CSP-f11	1034.0278	1034.0295	>99
18-CSP-N12A	1012.5249	1012.5214	>95	18-CSP-n12	1034.0278	1034.0302	>97
18-CSP-R13A	991.4958	991.4958	>98	18-CSP-r13	1034.0278	1034.0299	>97
18-CSP-S14A	1026.0303	1026.0318	>95	18-CSP-s14	1034.0278	1034.0296	>99
18-CSP-F15A	996.0121	996.0110	>97	18-CSP-f15	1034.0278	1034.0298	>99
18-CSP-T16A	1019.0225	1019.0230	>98	18-CSP-t16	1034.0278	1034.0300	>98
18-CSP-Q17A	1005.5170	1005.5187	>95	18-CSP-q17	1034.0278	1034.0303	>99
				18-CSP-a18	1034.0278	1034.0307	>99

EM = Exact Mass. See main text for methods.

Table S-4. MS and HPLC data for 21-CSP and 18-CSP multiple mutation analogs.

Compound Name	Calc. EM MH_2^{2+}	Obs. EM MH_2^{2+}	Purity (%)
18-CSP-S3T	1041.0356	1041.0364	>97
21-CSP-S3T	1190.1358	1190.1328	>99
18-CSP-s1s3l4	1034.0278	1034.0262	>99
18-CSP-s1s3l4L10AR13AS14A	962.4746	962.4786	>96
18-CSP-S1AL10AR13AS14A	954.4774	954.4801	>98

EM = Exact Mass. See main text for methods.

Primary reporter gene assay data

***S. mutans* SAB249 (pCipB::*lacZ*)**

Agonism assays were performed at 10 μM concentration. 21-CSP was used as the positive control (100%) with DMSO as the negative control (0%). Percent (%) ComD activation was measured by normalizing the miller units obtained for each peptide to that of 21-CSP. All peptides were screened in triplicates over three separate trials. Error bars indicate standard error of the mean of nine values.

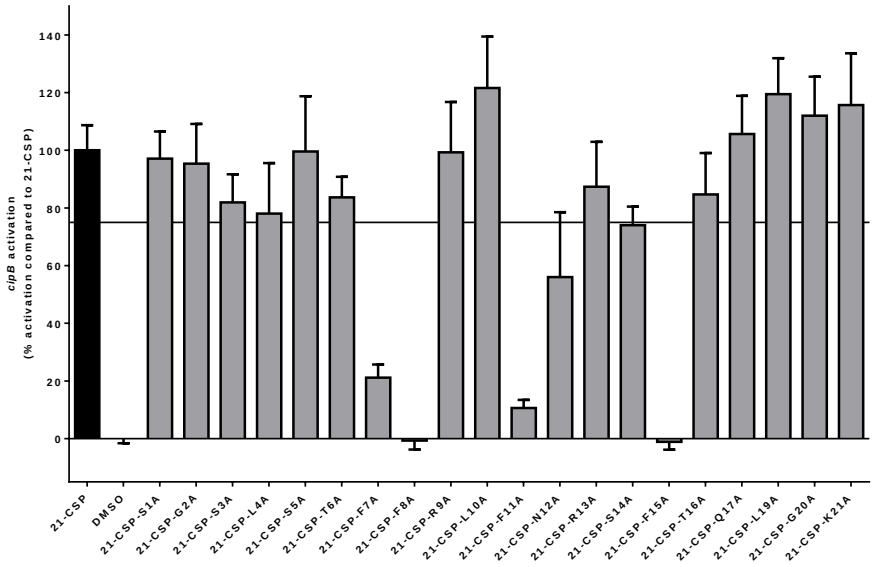


Figure S-1. Primary agonism screening assay data for the 21-CSP alanine scan library. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

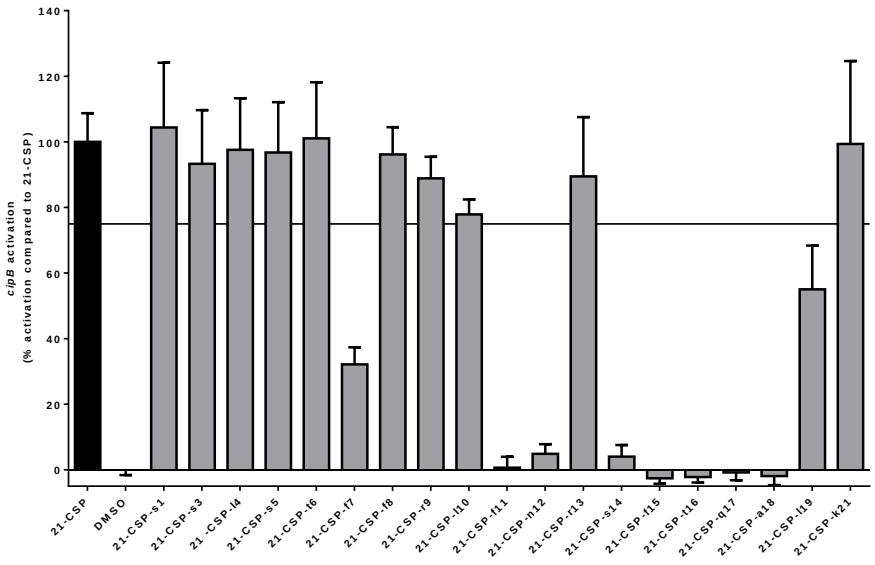


Figure S-2. Primary agonism screening assay data for the 21-CSP D-amino acid scan library. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

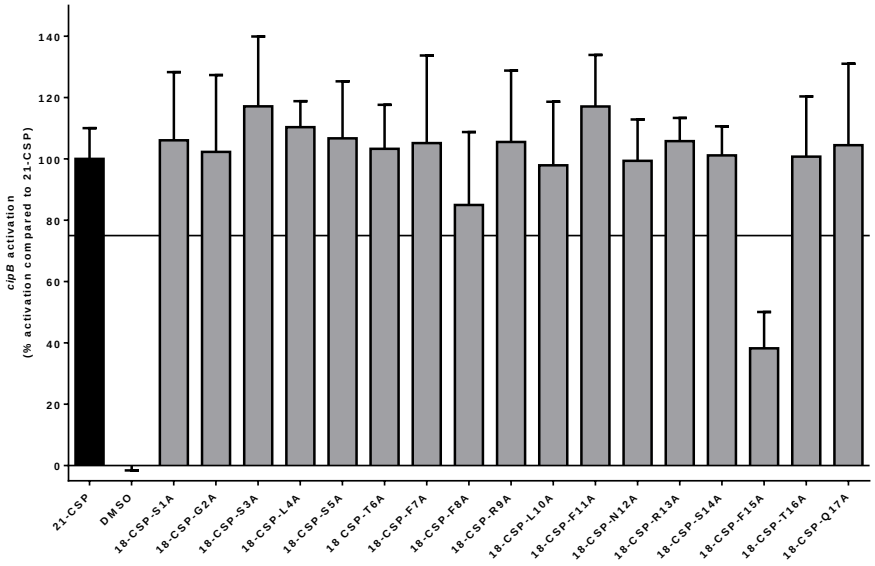


Figure S-3. Primary agonism screening assay data for the 18-CSP alanine scan library. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

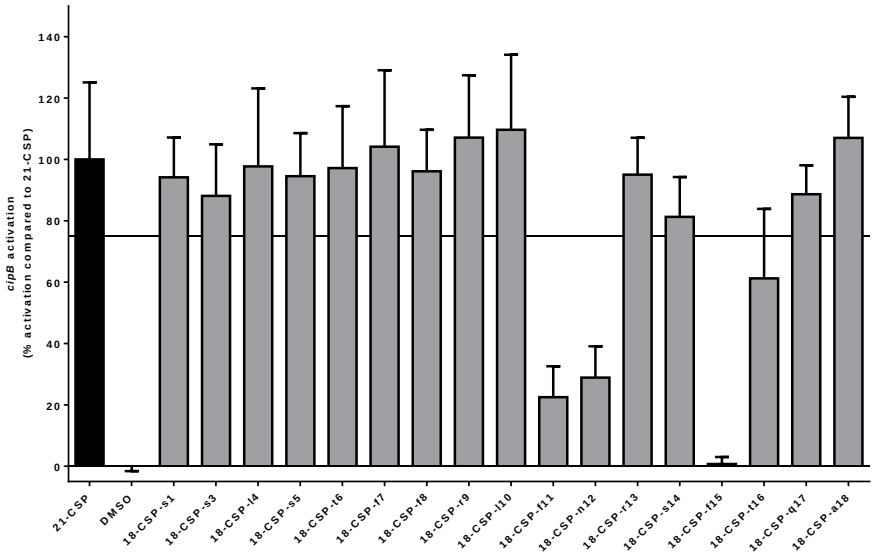


Figure S-4. Primary agonism screening assay data for the 18-CSP D-amino acid scan library. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

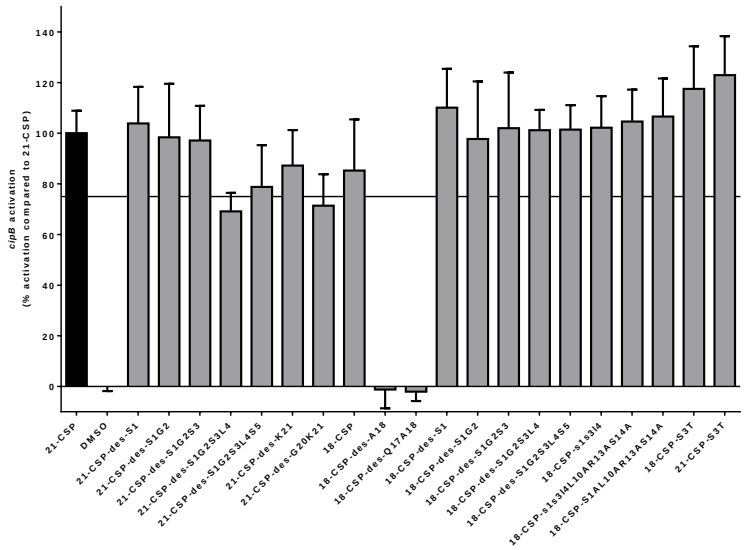


Figure S-5. Primary agonism screening assay data for the 21-CSP & 18-CSP truncated peptides and second-generation peptide analogs. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

Antagonism assays were performed at 10 μM concentration of peptides against 1000 nM concentration of 21-CSP. 21-CSP (1000 nM) was used as the positive control (100%) with DMSO as the negative control (0%). Percent (%) ComD activation was measured by normalizing the miller units obtained for each peptide to that of 21-CSP. All peptides were screened in triplicates over three separate trials. Error bars indicate standard error of the mean of nine values.

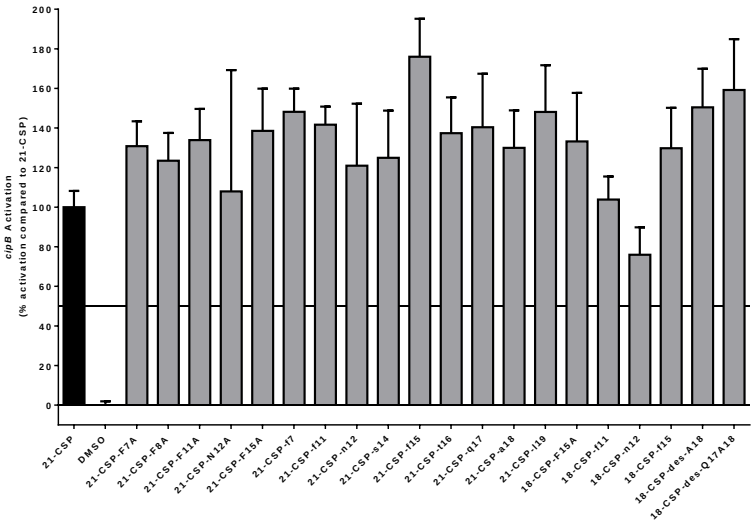


Figure S-6. Primary antagonism screening assay data for the 21-CSP and 18-CSP alanine, D-amino acid scan libraries and truncated peptides.

***S. mutans* SMCOM2 (Δ*comC*, pcomX::*lacZ*)**

Agonism assays were performed at 10 μM concentration. 21-CSP was used as the positive control (100%) with DMSO as the negative control (0%). Percent (%) ComD activation was measured by normalizing the miller units obtained for each peptide to that of 21-CSP. All peptides were screened in triplicates over three separate trials. Error bars indicate standard error of the mean of nine values.

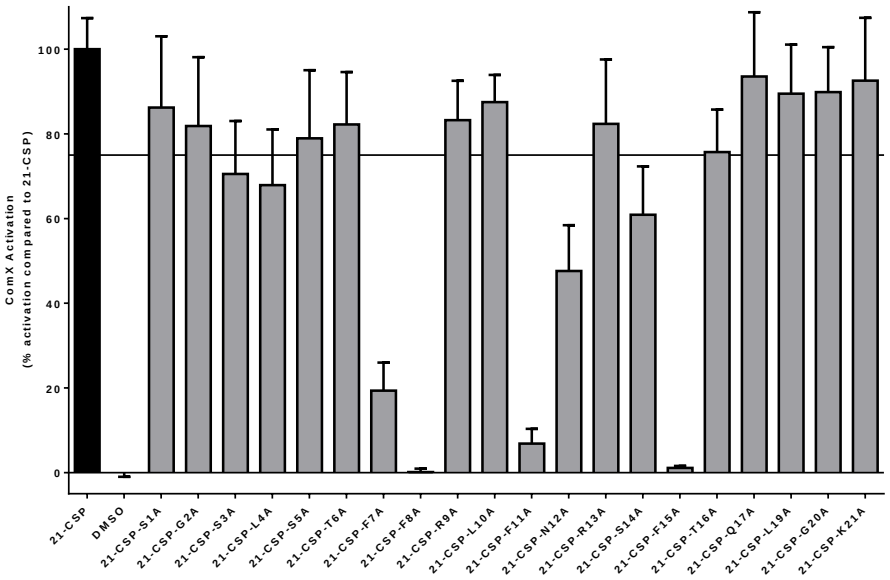


Figure S-7. Primary agonism screening assay data for the 21-CSP alanine scan library. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

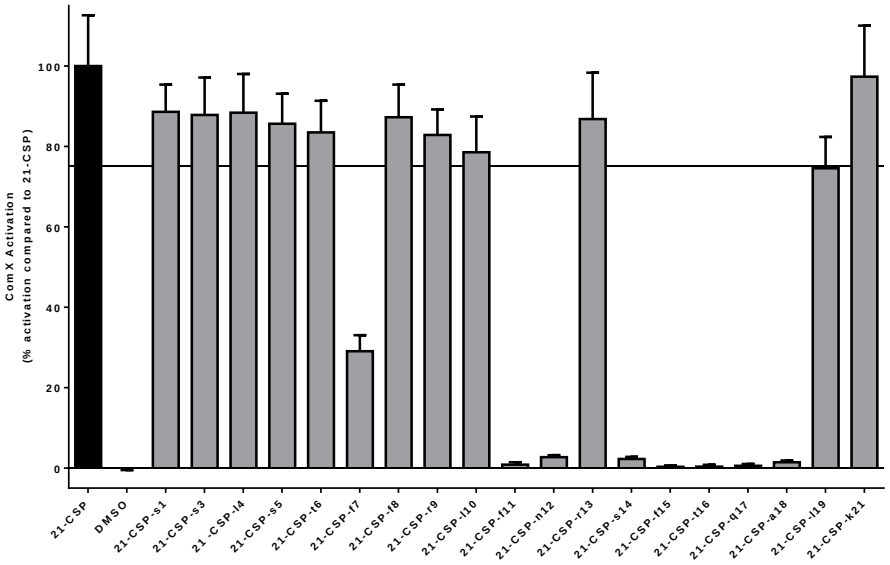


Figure S-8. Primary agonism screening assay data for the 21-CSP D-amino acid scan library. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

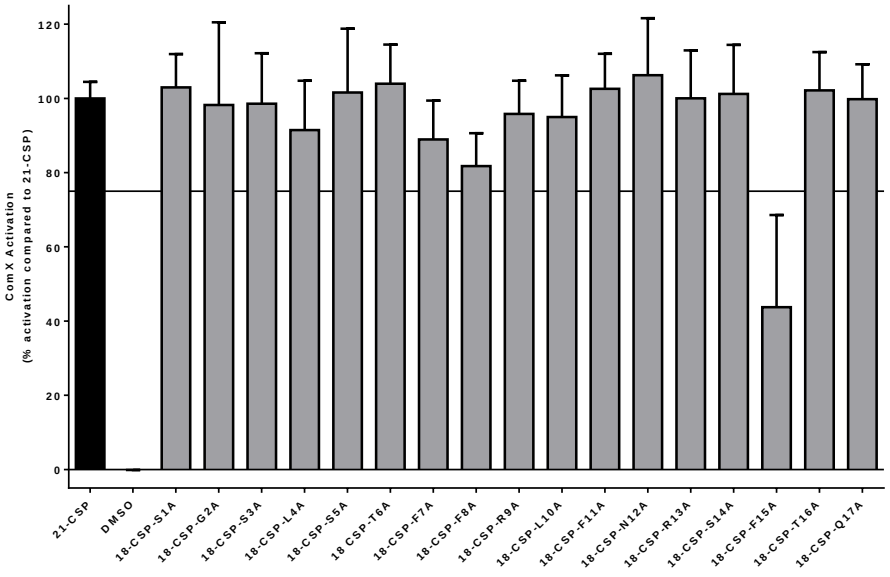


Figure S-9. Primary agonism screening assay data for the 18-CSP alanine scan library. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

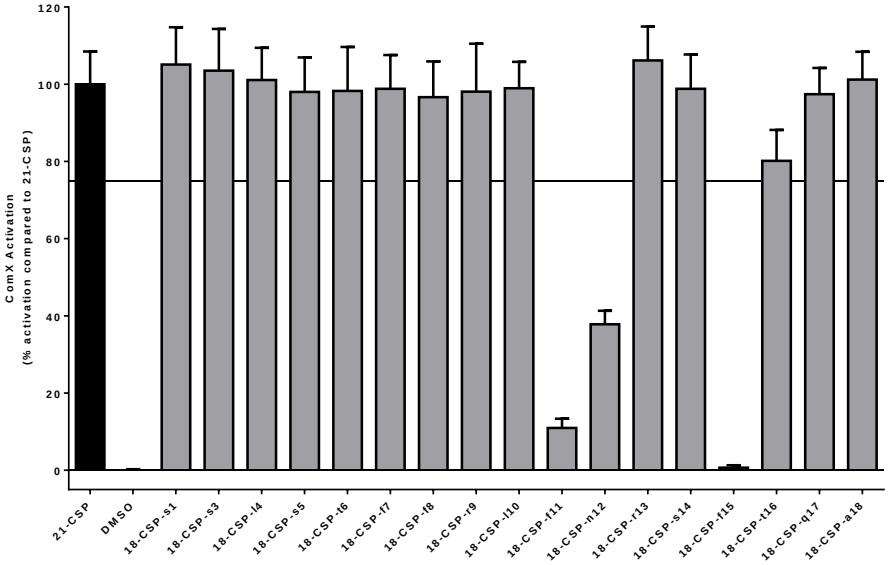


Figure S-10. Primary agonism screening assay data for the 18-CSP D-amino acid scan library. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

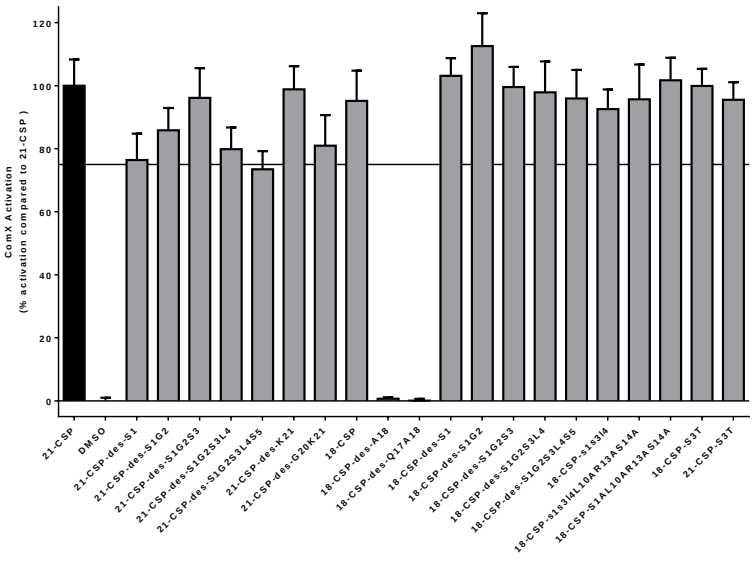


Figure S-11. Primary agonism screening assay data for the 21-CSP & 18-CSP truncated peptides and second-generation peptide analogs. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

Antagonism assays were performed at 10 μM concentration of peptides against 350 nM concentration of 21-CSP. 21-CSP (350 nM) was used as the positive control (100%) with DMSO as the negative control (0%). Percent (%) ComD activation was measured by normalizing the miller units obtained for each peptide to that of 21-CSP. All peptides were screened in triplicates over three separate trials. Error bars indicate standard error of the mean of nine values.

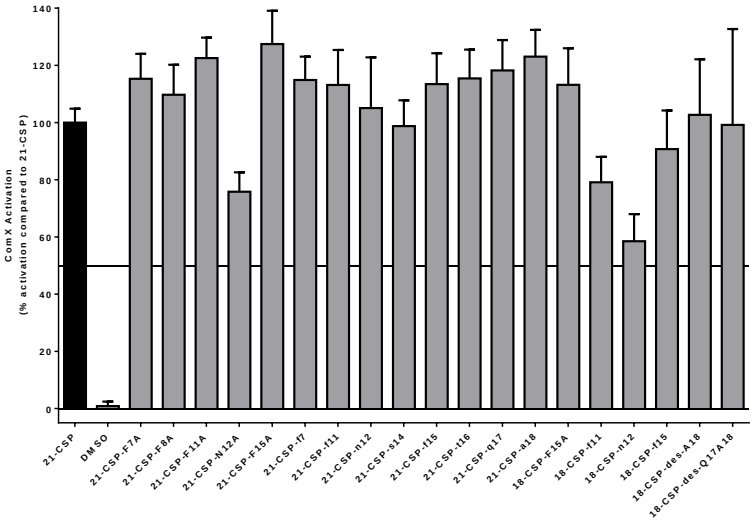


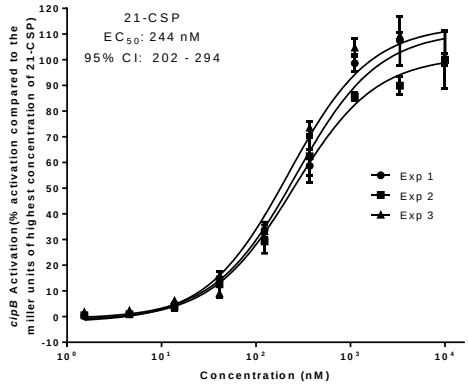
Figure S-12. Primary antagonism screening assay data for the 21-CSP and 18-CSP alanine, D-amino acid scan libraries and truncated peptides.

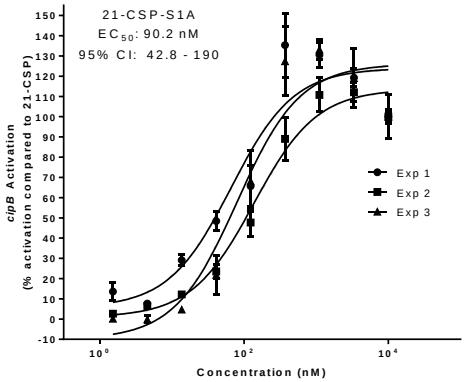
Agonism dose response curves

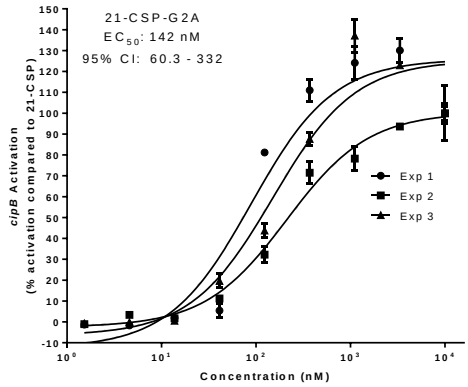
Both the 21-CSP and 18-CSP analogs were screened over varying concentrations in the two indicated *S. mutans* beta-galactosidase reporter strains (SAB249 and SMCOM2). Each dose response experiment was performed in triplicate on three separate occasions (i.e., experiments (Exp.) #1-3; shown for each peptide below). Error bars indicate standard error of the mean of triplicate values. In each plot the peptide as well as its EC_{50} value and 95% confidence interval values (95% CI) are indicated at top left.

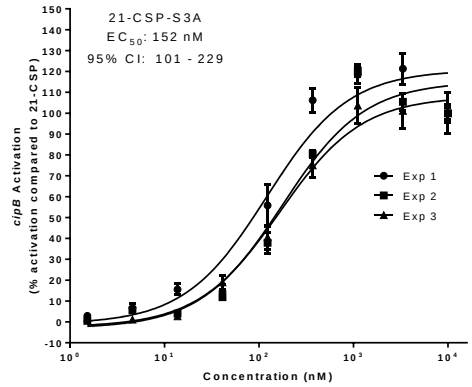
S. mutans SAB249 (pCipB::*lacZ*)

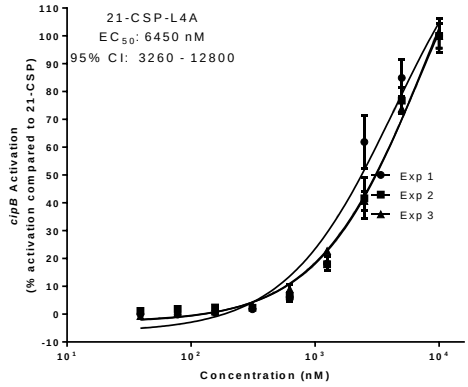
Activation dose response curves (21-CSP)

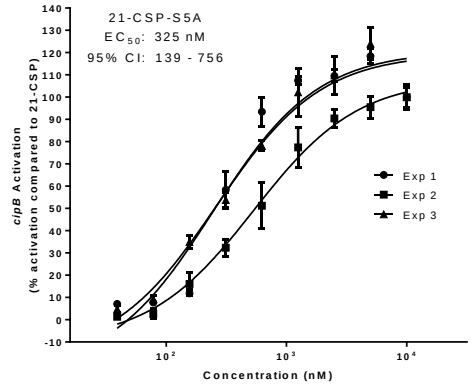


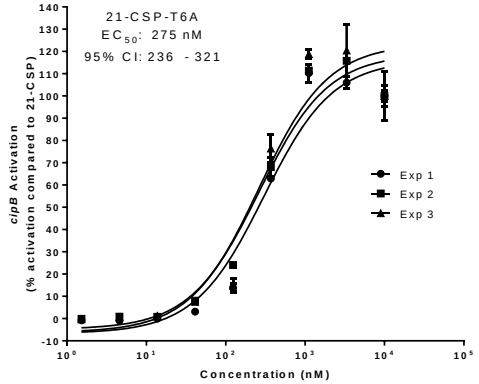


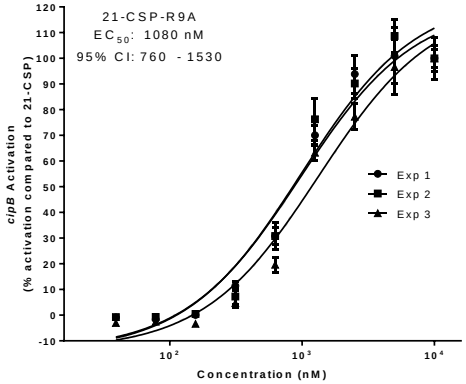


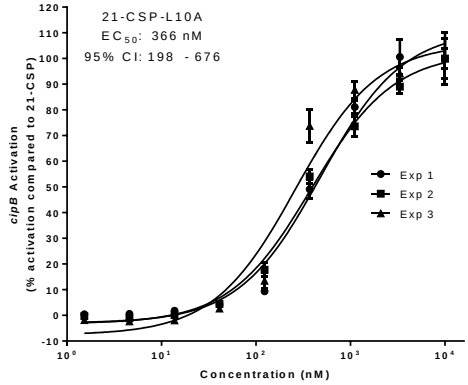


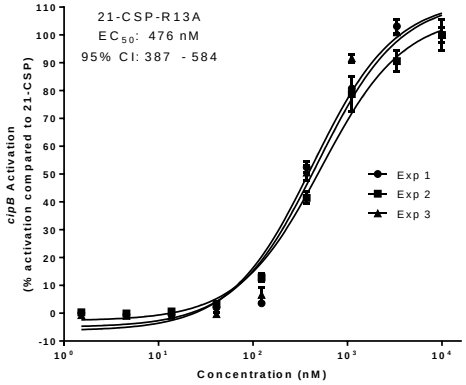


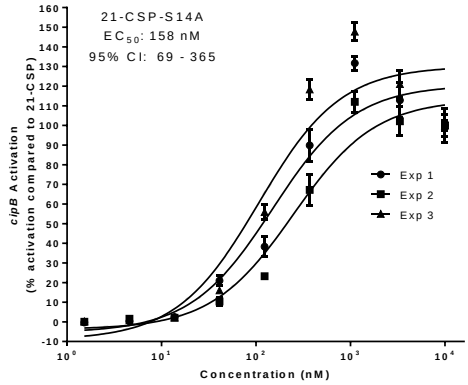


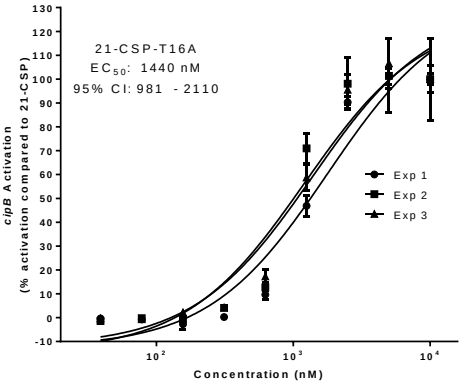

21-CSP-T6A
EC50: 275 nM
95% CI: 236 - 321
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

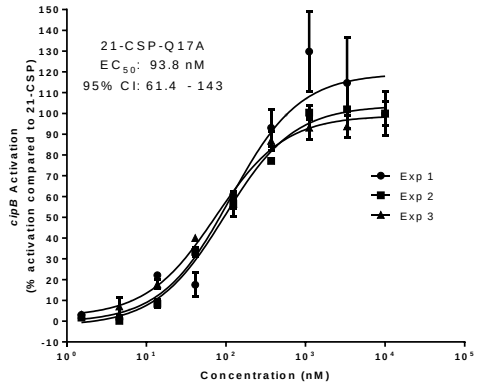

21-CSP-R9A
EC50: 1080 nM
95% CI: 760 - 1530
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

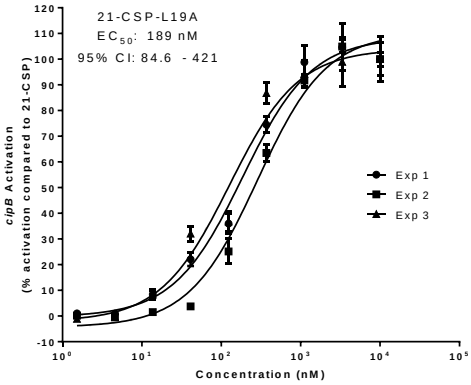

21-CSP-L10A
EC50: 366 nM
95% CI: 198 - 676
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

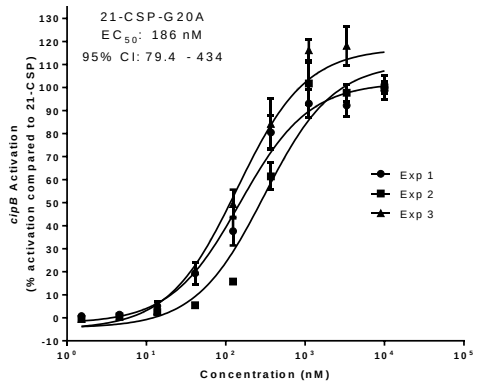

21-CSP-R13A
EC50: 476 nM
95% CI: 387 - 584
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

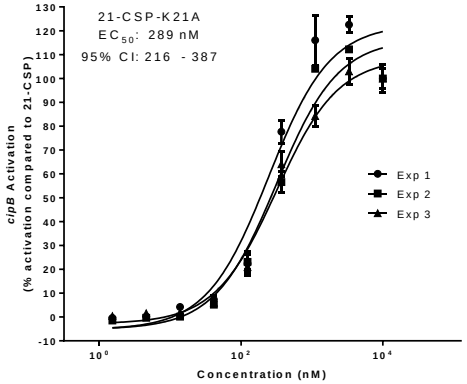

21-CSP-S14A
EC50: 158 nM
95% CI: 69 - 365
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

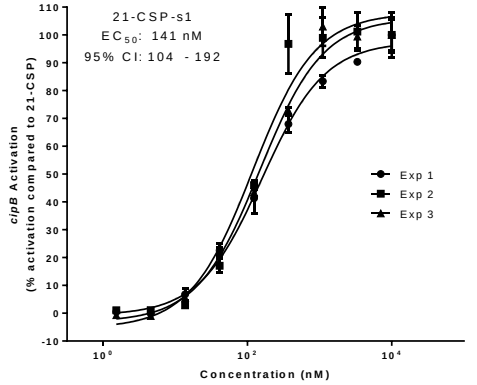

21-CSP-T16A
EC50: 1440 nM
95% CI: 981 - 2110
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

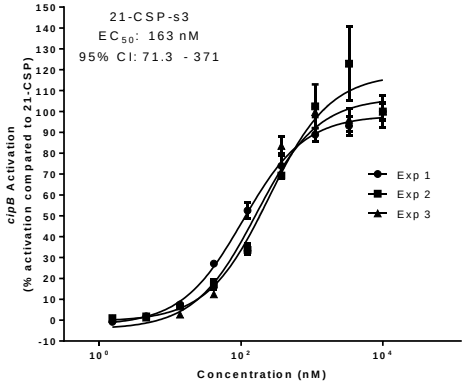

21-CSP-Q17A
EC50: 93.8 nM
95% CI: 61.4 - 143
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

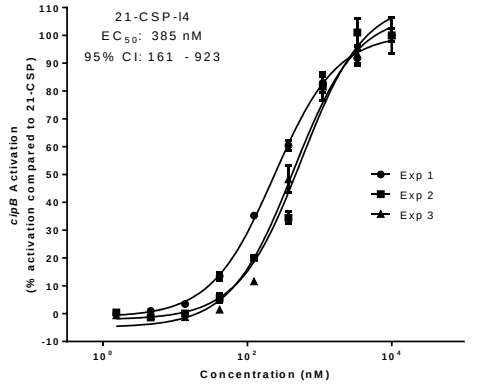

21-CSP-L19A
EC50: 189 nM
95% CI: 84.6 - 421
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

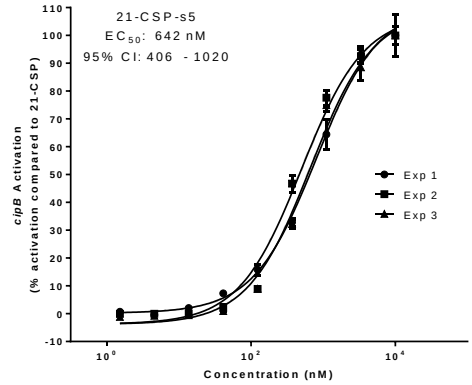

21-CSP-G20A
EC50: 186 nM
95% CI: 79.4 - 434
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

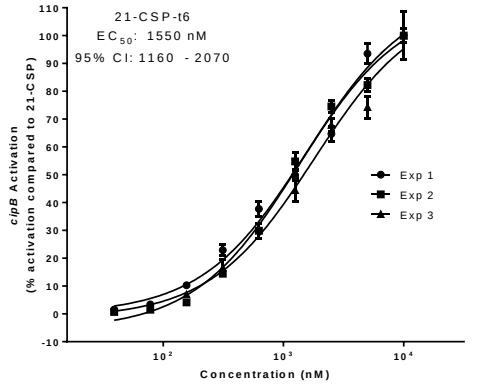

21-CSP-K21A
EC50: 289 nM
95% CI: 216 - 387
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

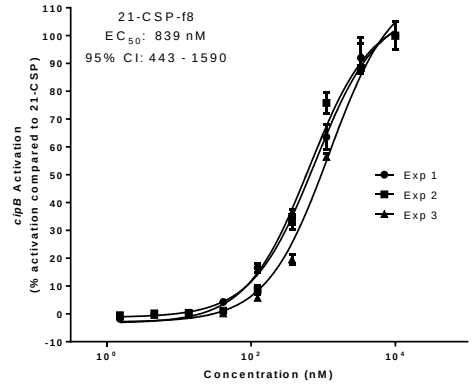

21-CSP-s1
EC50: 141 nM
95% CI: 104 - 192
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

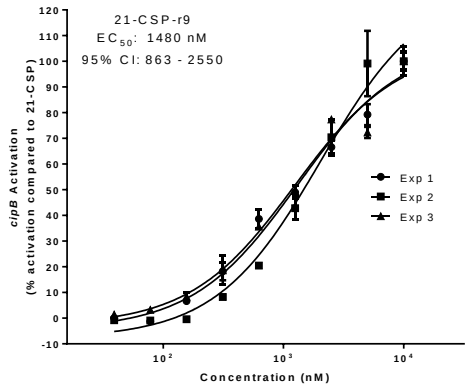

21-CSP-s3
EC50: 163 nM
95% CI: 71.3 - 371
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

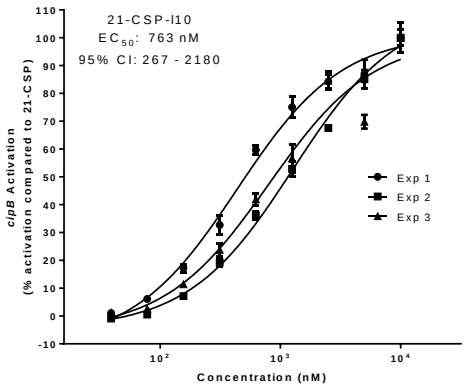

21-CSP-l4
EC50: 385 nM
95% CI: 161 - 923
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

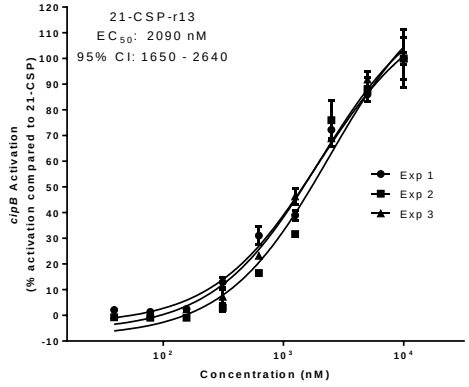

21-CSP-s5
EC50: 642 nM
95% CI: 406 - 1020
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

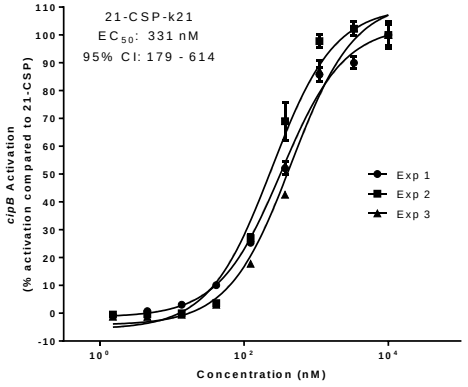

21-CSP-t6
EC50: 1550 nM
95% CI: 1160 - 2070
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

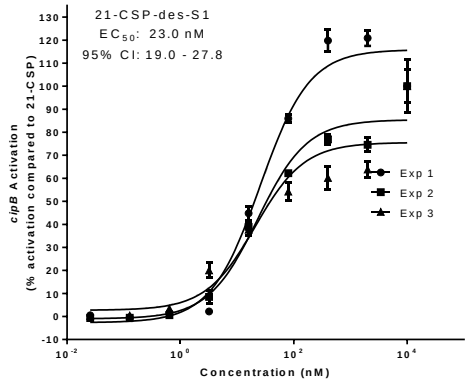

21-CSP-f8
EC50: 839 nM
95% CI: 443 - 1590
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

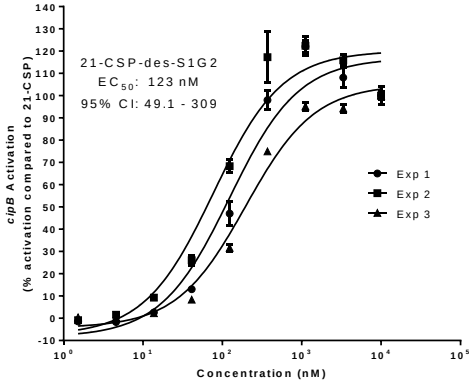

21-CSP-r9
EC50: 1480 nM
95% CI: 863 - 2550
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

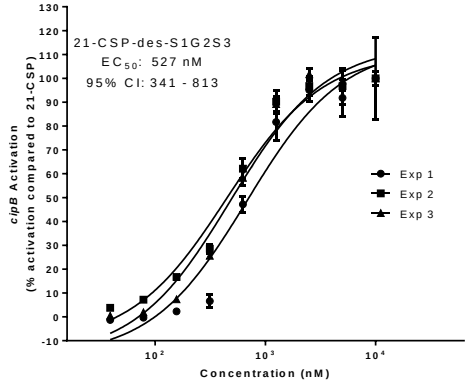

21-CSP-l10
EC50: 763 nM
95% CI: 267 - 2180
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

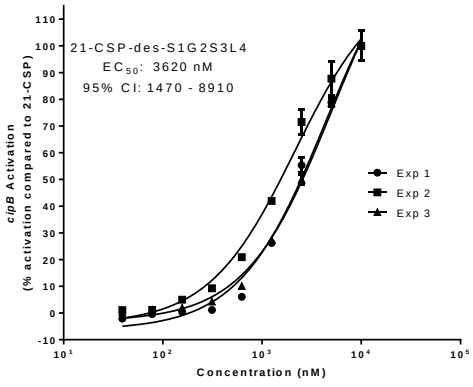

21-CSP-r13
EC50: 2090 nM
95% CI: 1650 - 2640
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

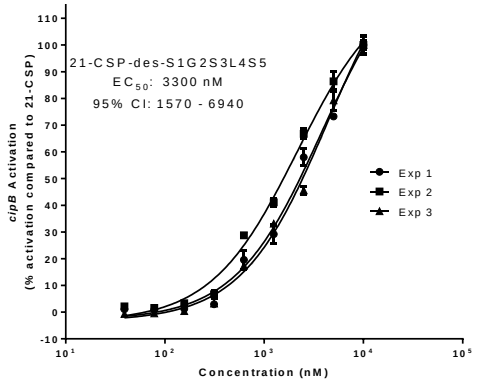

21-CSP-k21
EC50: 331 nM
95% CI: 179 - 614
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

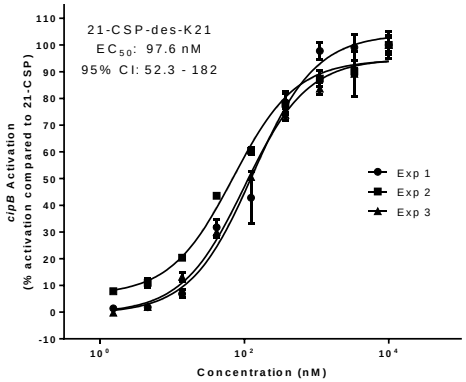

21-CSP-des-S1
EC50: 23.0 nM
95% CI: 19.0 - 27.8
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

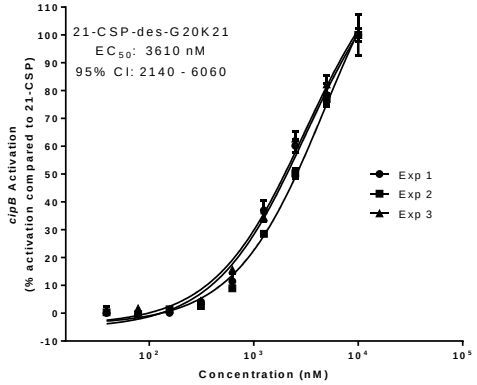

21-CSP-des-S1G2
EC50: 123 nM
95% CI: 49.1 - 309
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

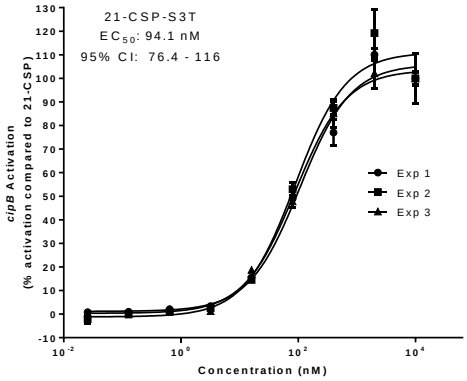

21-CSP-des-S1G2S3
EC50: 527 nM
95% CI: 341 - 813
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3


21-CSP-des-S1G2S3L4
EC50: 3620 nM
95% CI: 1470 - 8910
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3


21-CSP-des-S1G2S3L4S5
EC50: 3300 nM
95% CI: 1570 - 6940
Exp 1
Exp 2
Exp 3
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)

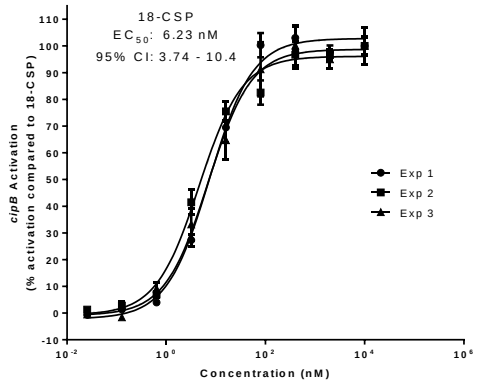

21-CSP-des-K21
EC50: 97.6 nM
95% CI: 52.3 - 182
Exp 1
Exp 2
Exp 3
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)

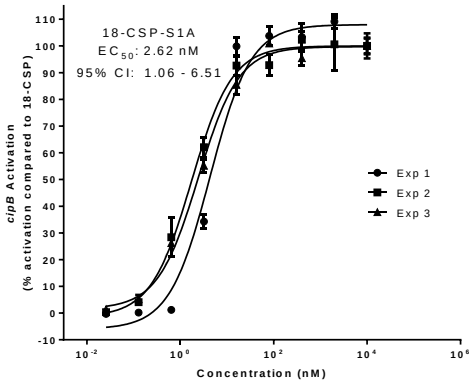

21-CSP-des-G20K21
EC50: 3610 nM
95% CI: 2140 - 6060
Exp 1
Exp 2
Exp 3
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)

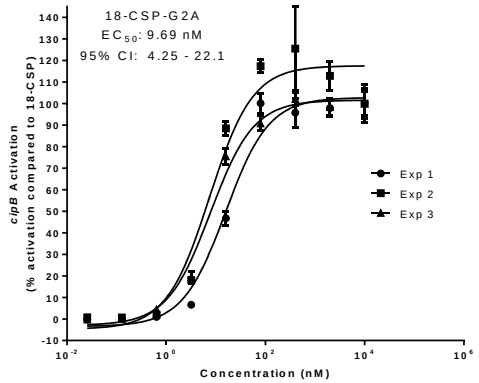

21-CSP-S3T
EC50: 94.1 nM
95% CI: 76.4 - 116
Exp 1
Exp 2
Exp 3
cipB Activation
(% activation compared to 21-CSP)
Concentration (nM)

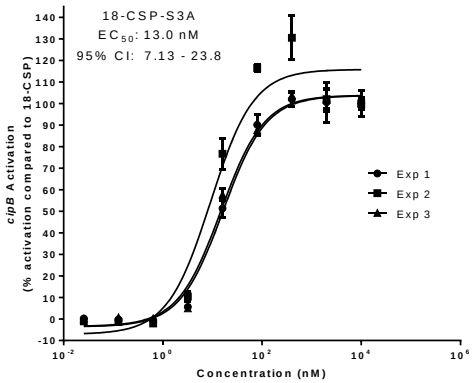
***S. mutans* SAB249 (pCipB::*lacZ*)**

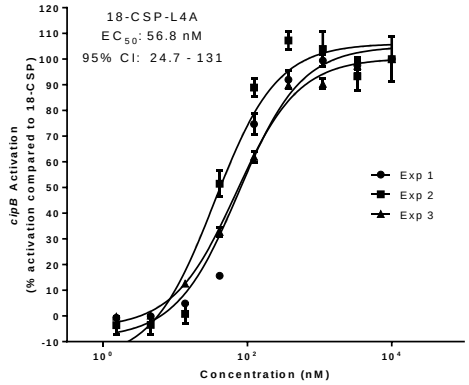
Activation dose response curves (18-CSP)

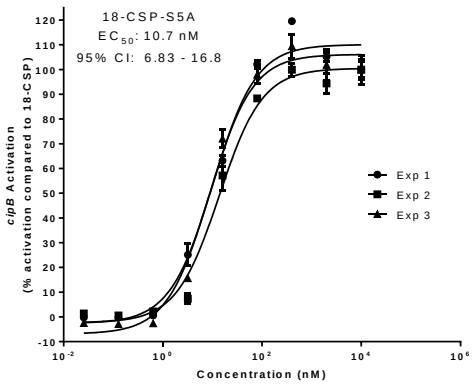


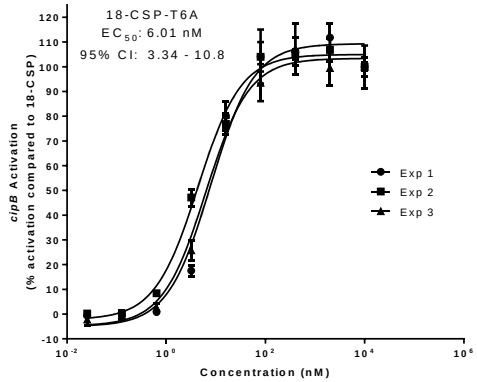


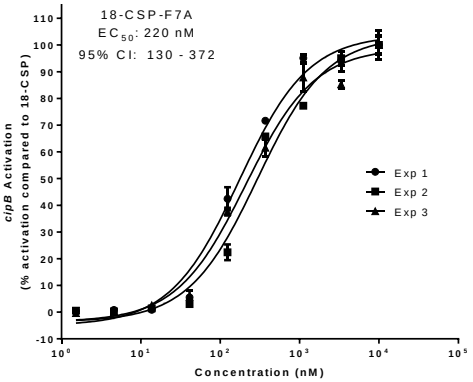


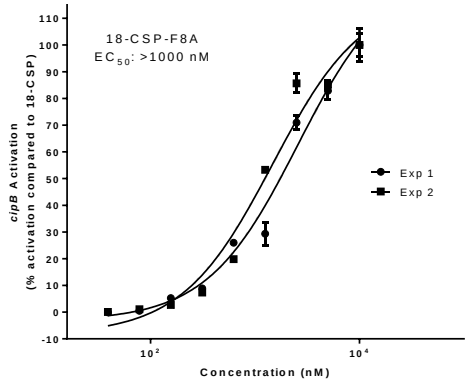


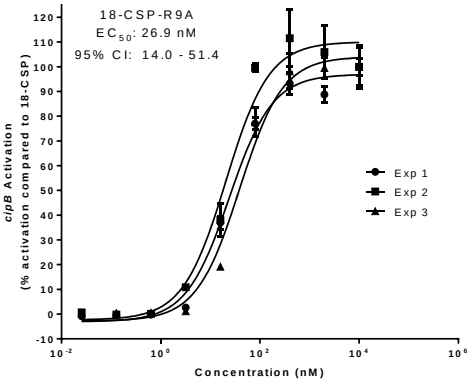


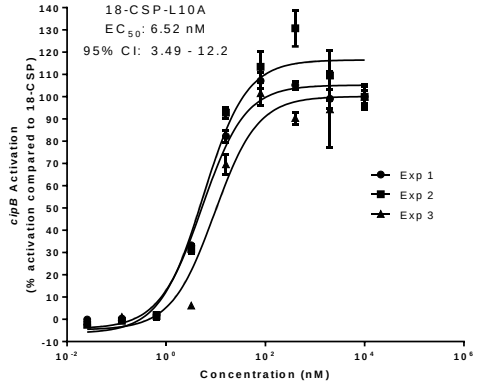


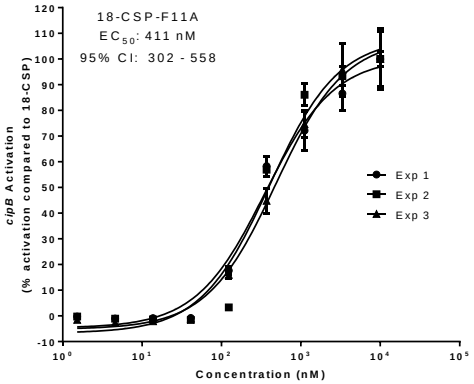

18-CSP-T6A
EC50: 6.01 nM
95% CI: 3.34 - 10.8
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

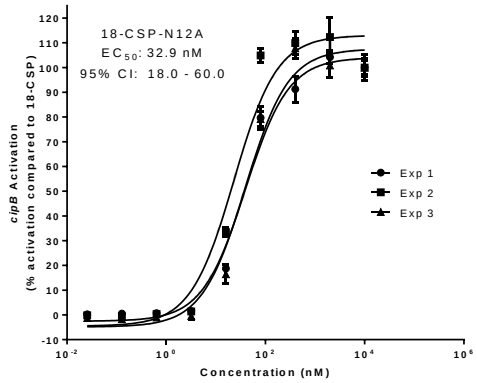

18-CSP-F7A
EC50: 220 nM
95% CI: 130 - 372
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

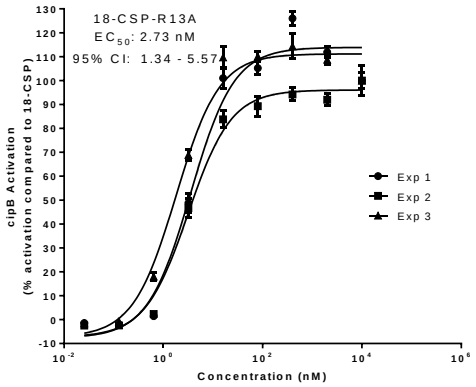

18-CSP-F8A
EC50: >1000 nM
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2

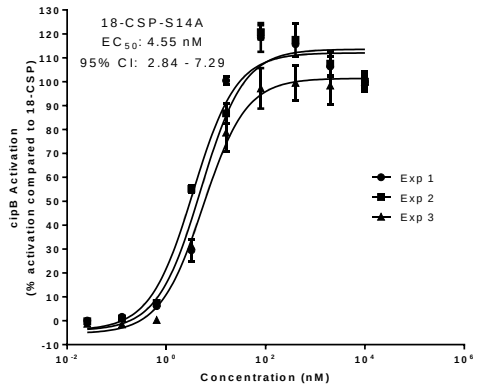

18-CSP-R9A
EC50: 26.9 nM
95% CI: 14.0 - 51.4
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

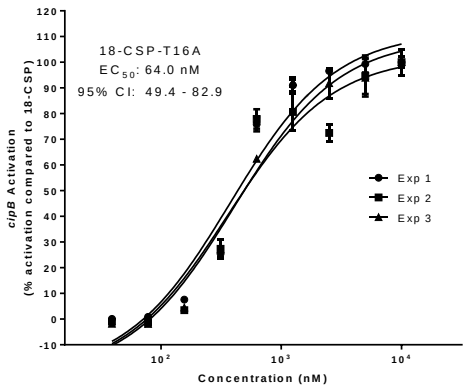

18-CSP-L10A
EC50: 6.52 nM
95% CI: 3.49 - 12.2
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

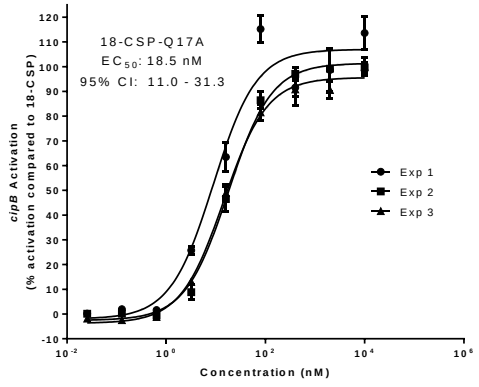

18-CSP-F11A
EC50: 411 nM
95% CI: 302 - 558
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

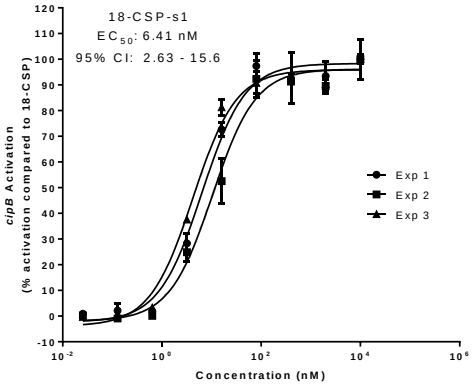

18-CSP-N12A
EC50: 32.9 nM
95% CI: 18.0 - 60.0
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

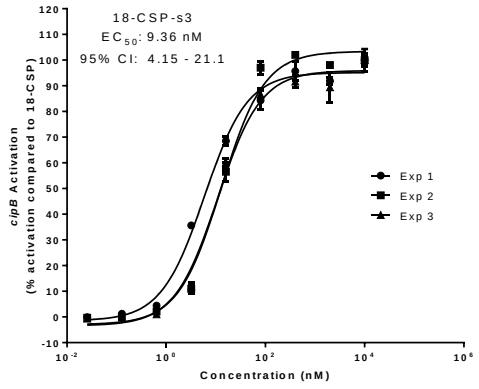

18-CSP-R13A
EC50: 2.73 nM
95% CI: 1.34 - 5.57
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

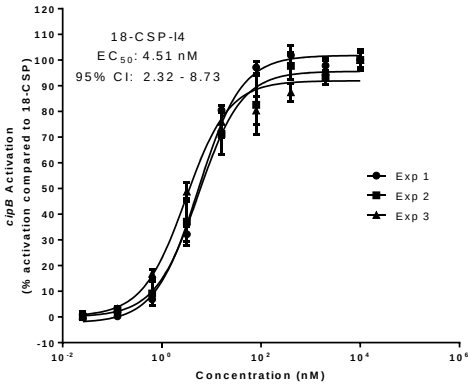

18-CSP-S14A
EC50: 4.55 nM
95% CI: 2.84 - 7.29
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

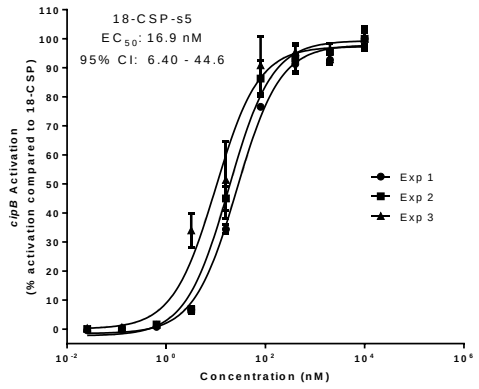

18-CSP-T16A
EC50: 64.0 nM
95% CI: 49.4 - 82.9
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

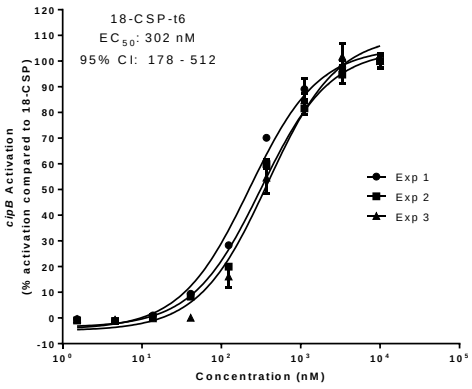

18-CSP-Q17A
EC50: 18.5 nM
95% CI: 11.0 - 31.3
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

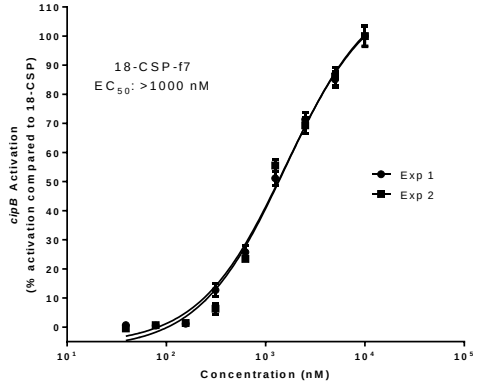

18-CSP-s1
EC50: 6.41 nM
95% CI: 2.63 - 15.6
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

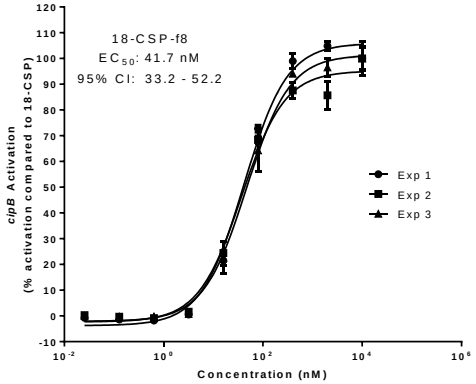

18-CSP-s3
EC50: 9.36 nM
95% CI: 4.15 - 21.1
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

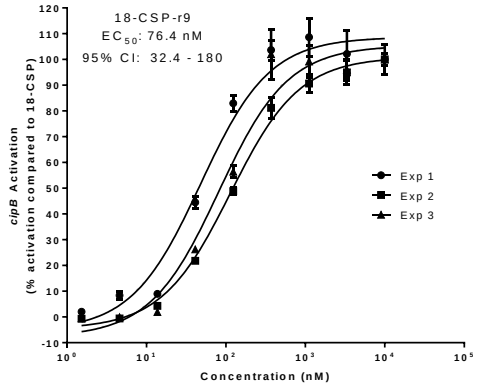

18-CSP-l4
EC50: 4.51 nM
95% CI: 2.32 - 8.73
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

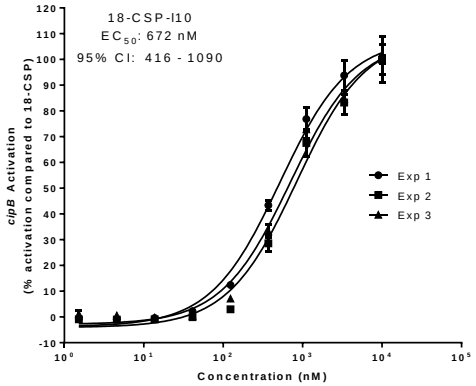

18-CSP-s5
EC50: 16.9 nM
95% CI: 6.40 - 44.6
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

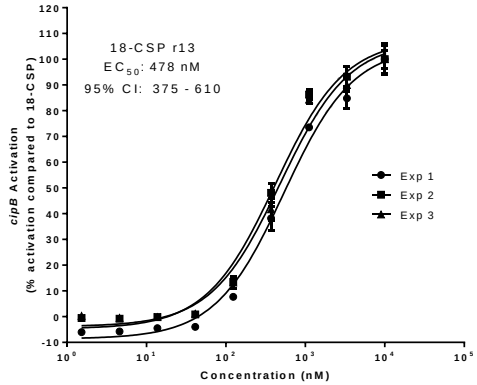

18-CSP-t6
EC50: 302 nM
95% CI: 178 - 512
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

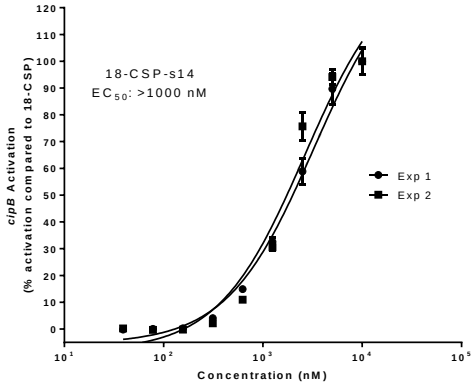

18-CSP-f7
EC50: >1000 nM
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2

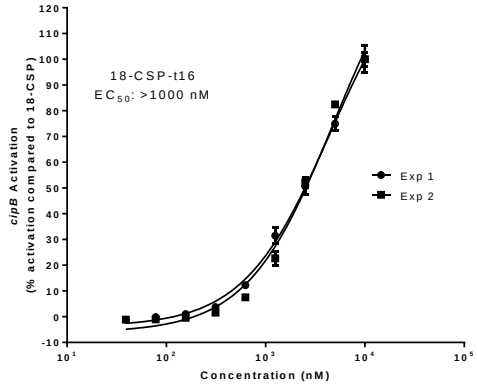

18-CSP-f8
EC50: 41.7 nM
95% CI: 33.2 - 52.2
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

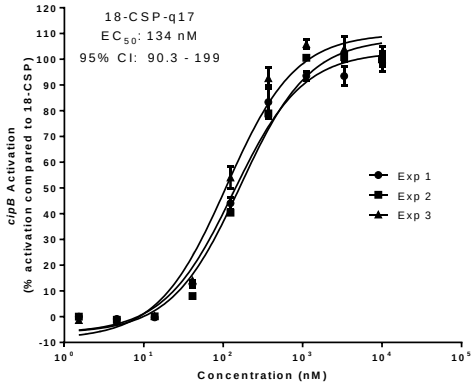

18-CSP-r9
EC50: 76.4 nM
95% CI: 32.4 - 180
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

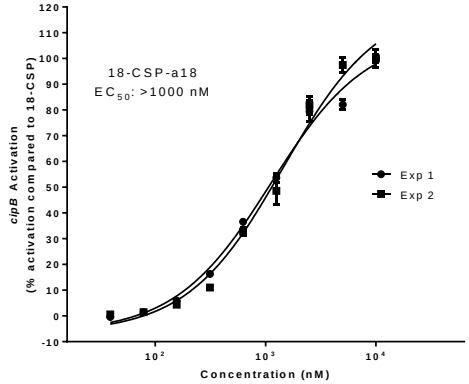

18-CSP-l10
EC50: 672 nM
95% CI: 416 - 1090
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

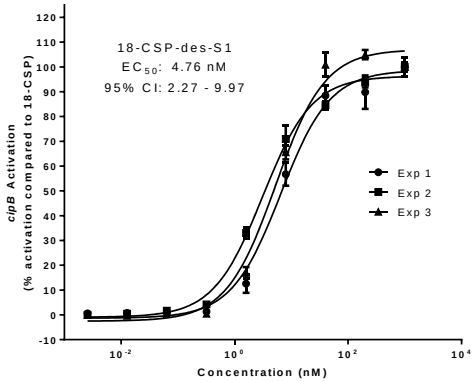

18-CSP r13
EC50: 478 nM
95% CI: 375 - 610
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

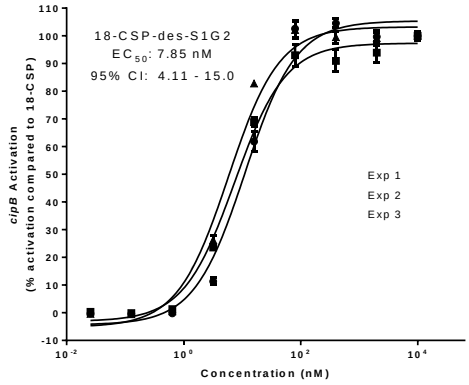

18-CSP-s14
EC50: >1000 nM
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2

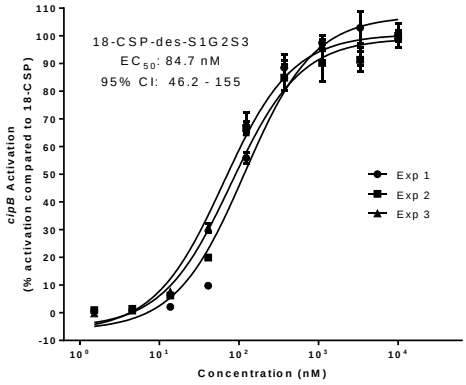

18-CSP-t16
EC50: >1000 nM
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2

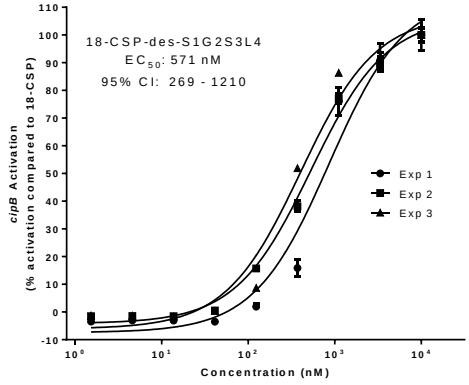

18-CSP-q17
EC50: 134 nM
95% CI: 90.3 - 199
cipB Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

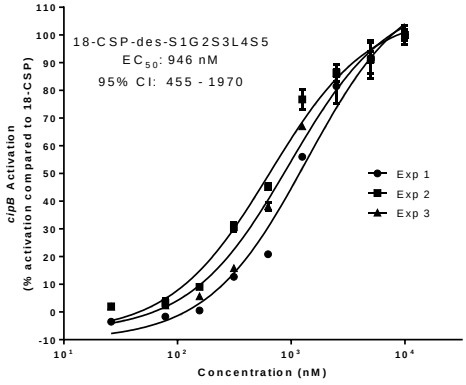

18-CSP-a18
EC50: >1000 nM
Exp 1
Exp 2
cipB Activation (% activation compared to 18-CSP)
Concentration (nM)

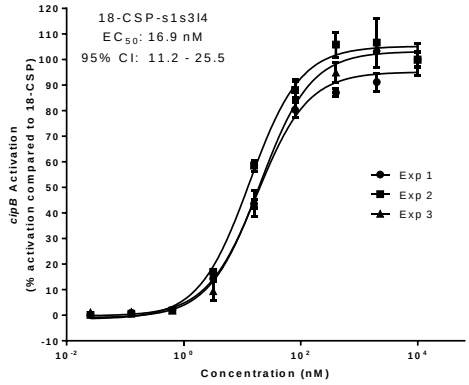

18-CSP-des-S1
EC50: 4.76 nM
95% CI: 2.27 - 9.97
Exp 1
Exp 2
Exp 3
cipB Activation (% activation compared to 18-CSP)
Concentration (nM)

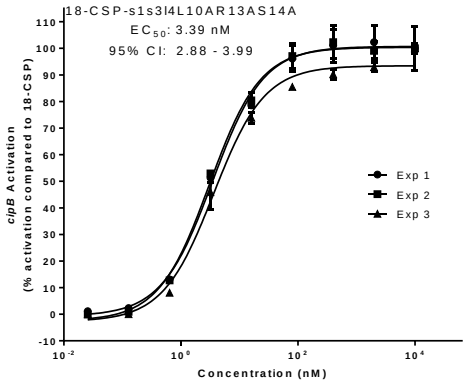

18-CSP-des-S1G2
EC50: 7.85 nM
95% CI: 4.11 - 15.0
Exp 1
Exp 2
Exp 3
cipB Activation (% activation compared to 18-CSP)
Concentration (nM)

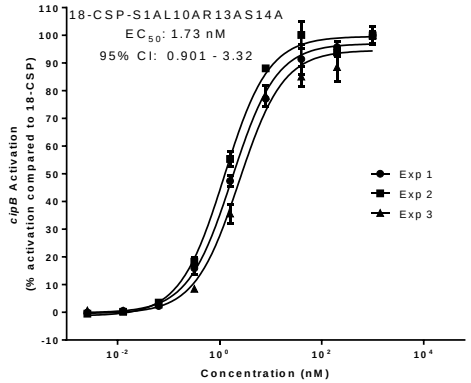

18-CSP-des-S1G2S3
EC50: 84.7 nM
95% CI: 46.2 - 155
Exp 1
Exp 2
Exp 3
cipB Activation (% activation compared to 18-CSP)
Concentration (nM)

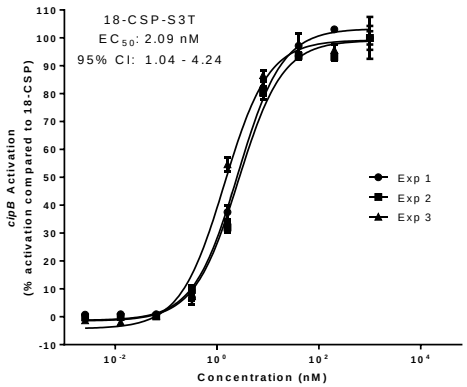

18-CSP-des-S1G2S3L4
EC50: 571 nM
95% CI: 269 - 1210
Exp 1
Exp 2
Exp 3
cipB Activation (% activation compared to 18-CSP)
Concentration (nM)


18-CSP-des-S1G2S3L4S5
EC50: 946 nM
95% CI: 455 - 1970
Exp 1
Exp 2
Exp 3
cipB Activation (% activation compared to 18-CSP)
Concentration (nM)


18-CSP-s1s3l4
EC50: 16.9 nM
95% CI: 11.2 - 25.5
Exp 1
Exp 2
Exp 3
cipB Activation (% activation compared to 18-CSP)
Concentration (nM)

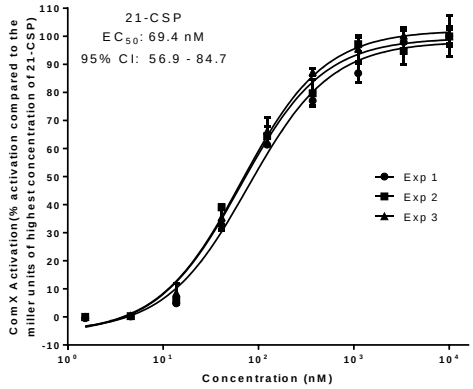

18-CSP-s1s3l4L10AR13AS14A
EC50: 3.39 nM
95% CI: 2.88 - 3.99
Exp 1
Exp 2
Exp 3
cipB Activation (% activation compared to 18-CSP)
Concentration (nM)

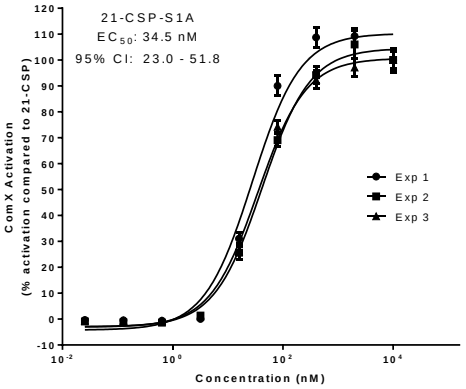


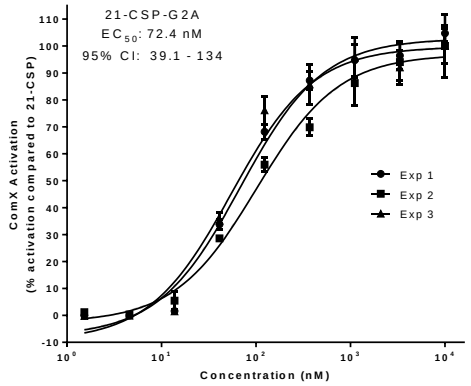


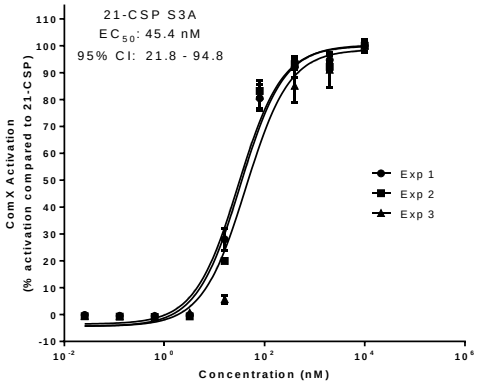
***S. mutans* SMCOM2 (Δ*comC*, pcomX::*lacZ*)**

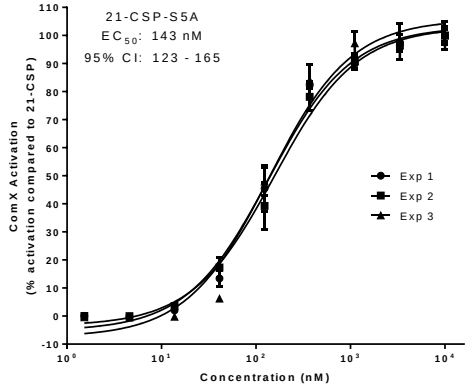
Activation dose response curves (21-CSP)

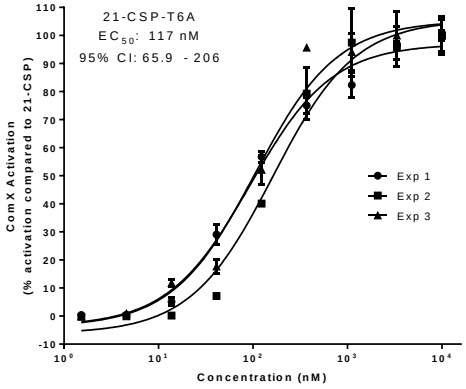


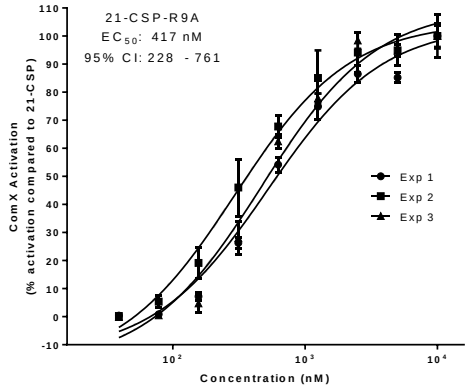


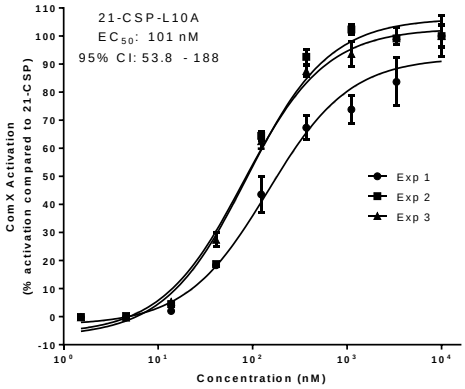


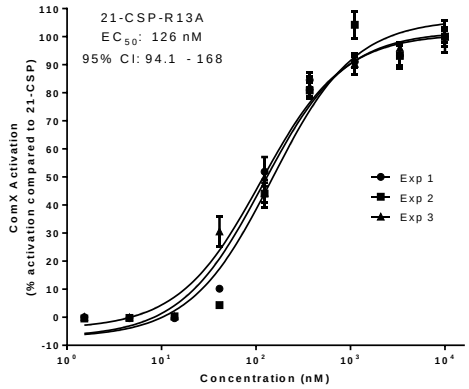


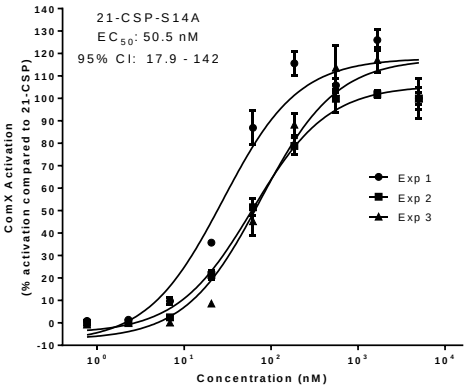

21-CSP-S5A
EC50: 143 nM
95% CI: 123 - 165
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

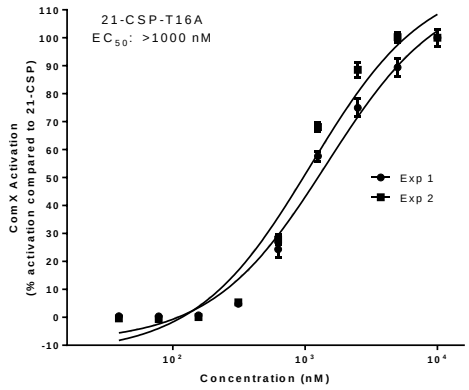

21-CSP-T6A
EC50: 117 nM
95% CI: 65.9 - 206
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

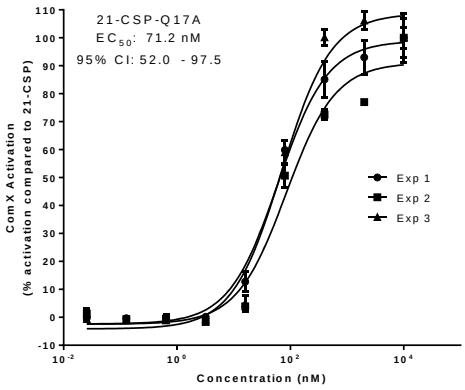

21-CSP-R9A
EC50: 417 nM
95% CI: 228 - 761
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

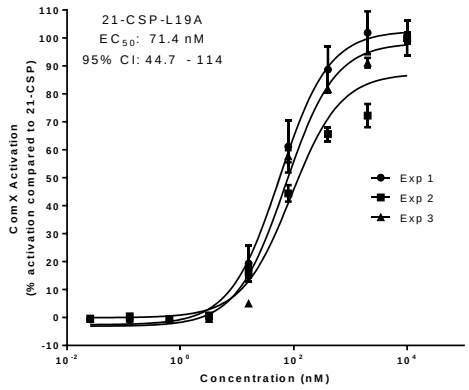

21-CSP-L10A
EC50: 101 nM
95% CI: 53.8 - 188
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

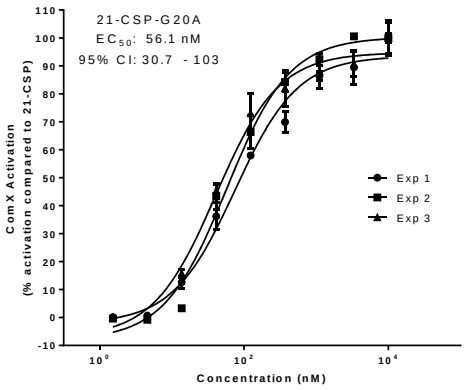

21-CSP-R13A
EC50: 126 nM
95% CI: 94.1 - 168
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

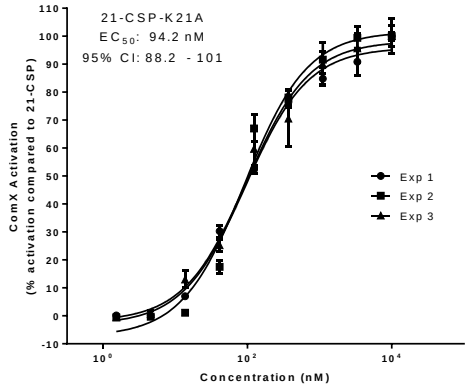

21-CSP-S14A
EC50: 50.5 nM
95% CI: 17.9 - 142
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

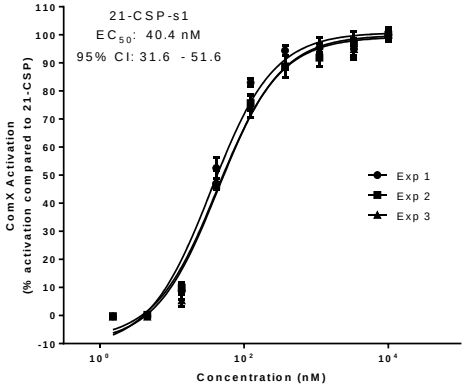

21-CSP-T16A
EC50: >1000 nM
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2

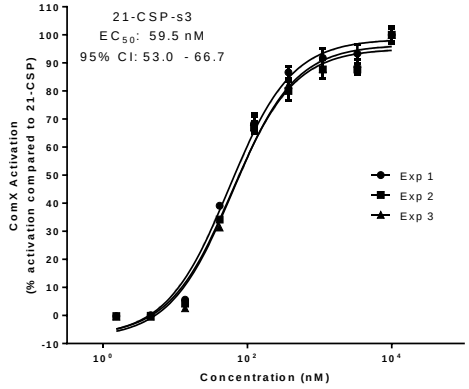

21-CSP-Q17A
EC50: 71.2 nM
95% CI: 52.0 - 97.5
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

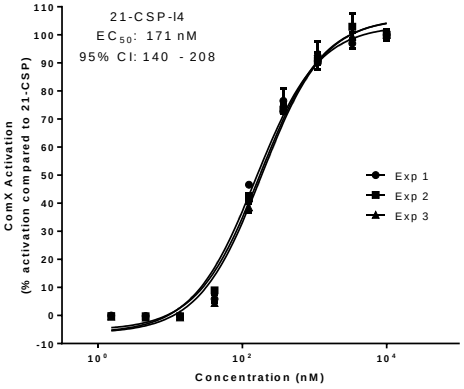

21-CSP-L19A
EC50: 71.4 nM
95% CI: 44.7 - 114
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

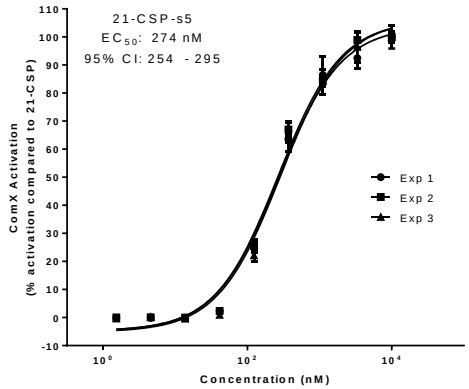

21-CSP-G20A
EC50: 56.1 nM
95% CI: 30.7 - 103
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

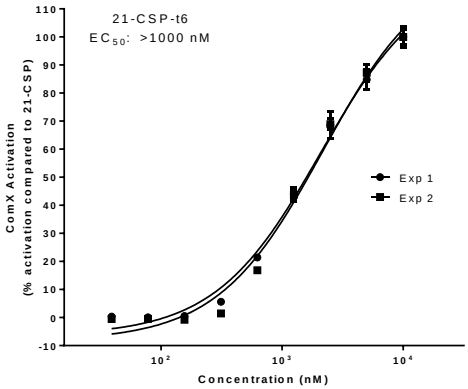

21-CSP-K21A
EC50: 94.2 nM
95% CI: 88.2 - 101
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

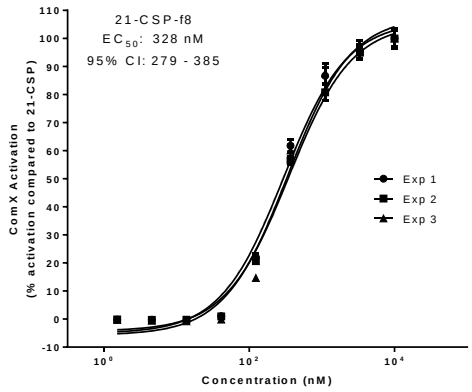

21-CSP-s1
EC50: 40.4 nM
95% CI: 31.6 - 51.6
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

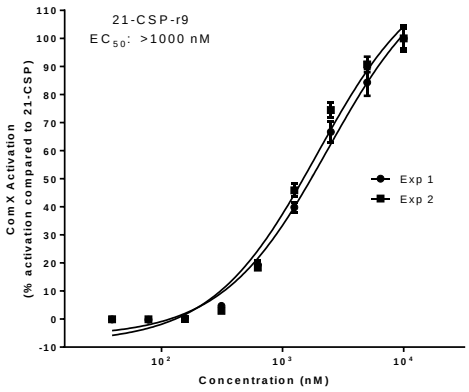

21-CSP-s3
EC50: 59.5 nM
95% CI: 53.0 - 66.7
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

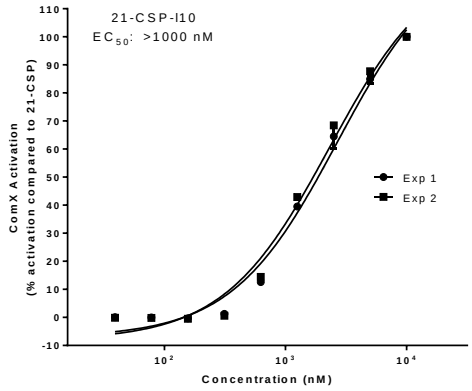

21-CSP-l4
EC50: 171 nM
95% CI: 140 - 208
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

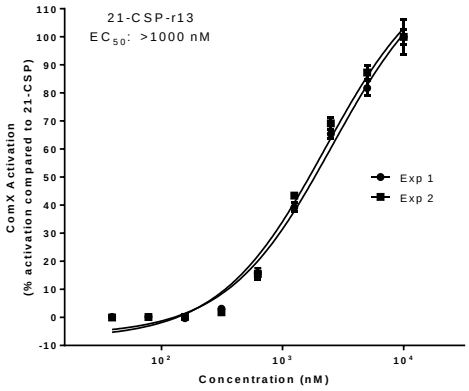

21-CSP-s5
EC50: 274 nM
95% CI: 254 - 295
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

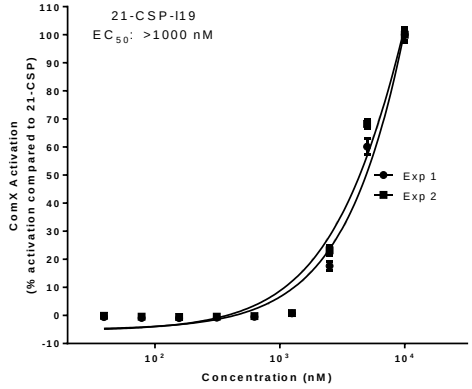

21-CSP-t6
EC50: >1000 nM
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2

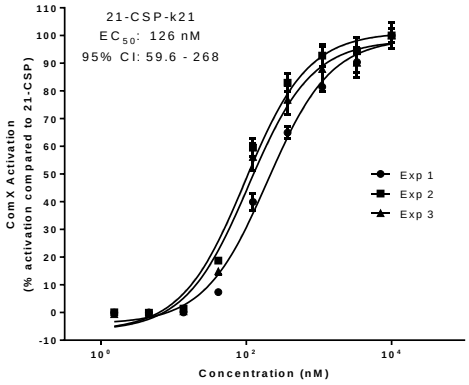

21-CSP-f8
EC50: 328 nM
95% CI: 279 - 385
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

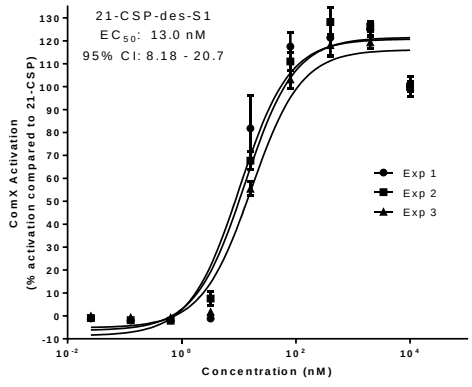

21-CSP-r9
EC50: >1000 nM
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2

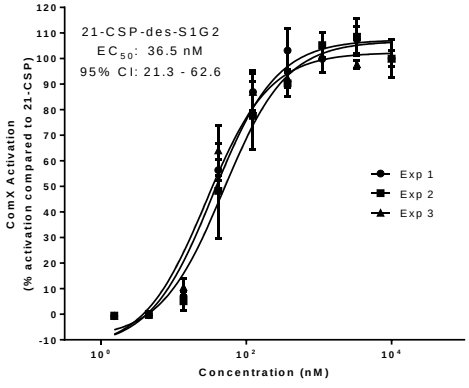

21-CSP-l10
EC50: >1000 nM
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2

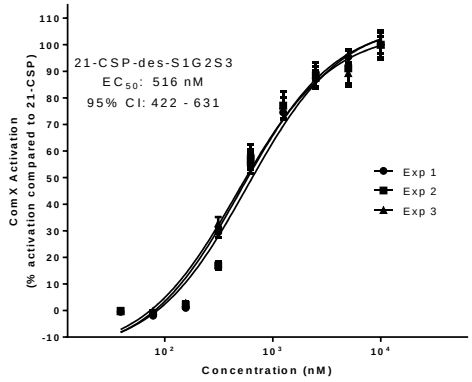

21-CSP-r13
EC50: >1000 nM
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2

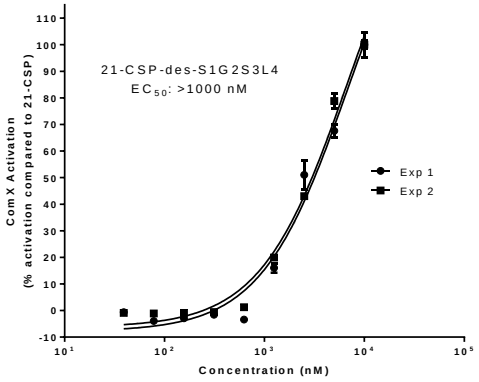

21-CSP-l19
EC50: >1000 nM
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2

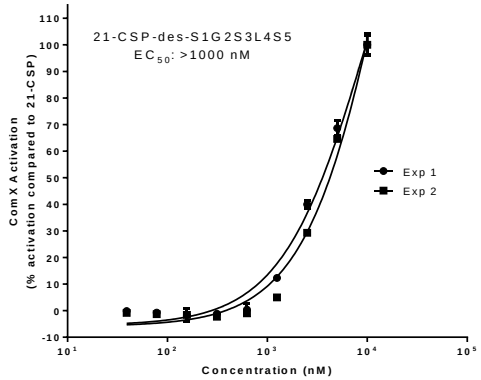

21-CSP-k21
EC50: 126 nM
95% CI: 59.6 - 268
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

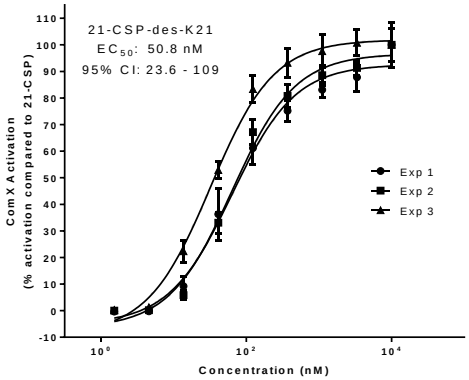

21-CSP-des-S1
EC50: 13.0 nM
95% CI: 8.18 - 20.7
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

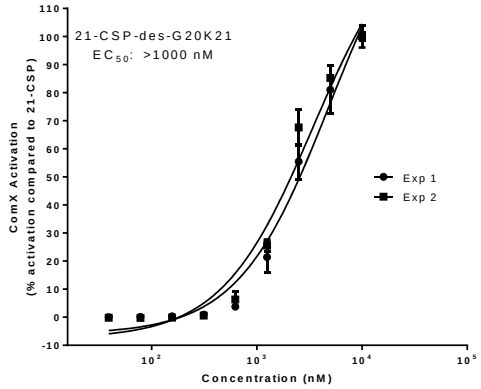

21-CSP-des-S1G2
EC50: 36.5 nM
95% CI: 21.3 - 62.6
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

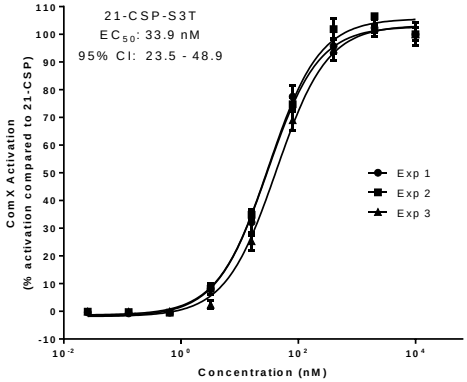

21-CSP-des-S1G2S3
EC50: 516 nM
95% CI: 422 - 631
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)


21-CSP-des-S1G2S3L4
EC50: >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)


21-CSP-des-S1G2S3L4S5
EC50: >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)

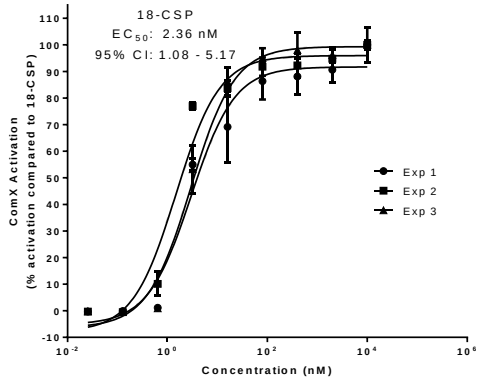

21-CSP-des-K21
EC50: 50.8 nM
95% CI: 23.6 - 109
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)

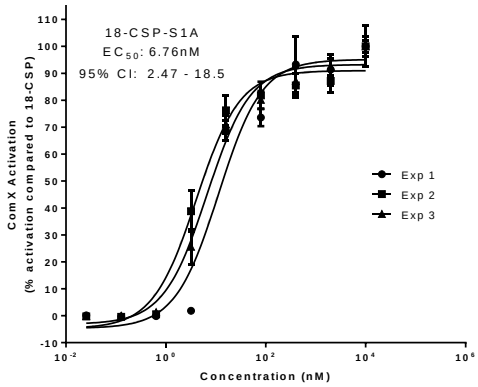

21-CSP-des-G20K21
EC50: >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)

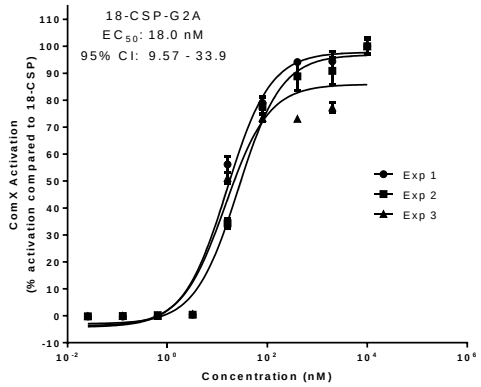

21-CSP-S3T
EC50: 33.9 nM
95% CI: 23.5 - 48.9
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to 21-CSP)
Concentration (nM)

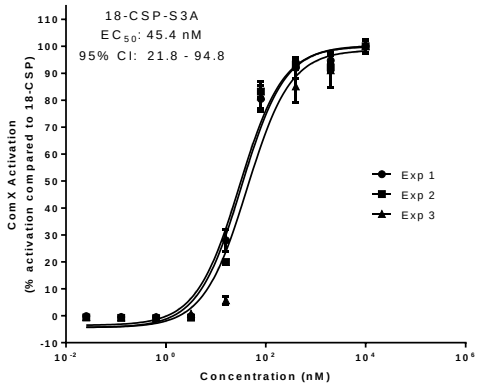
***S. mutans* SMCOM2 (Δ*comC*, pcomX::*lacZ*)**

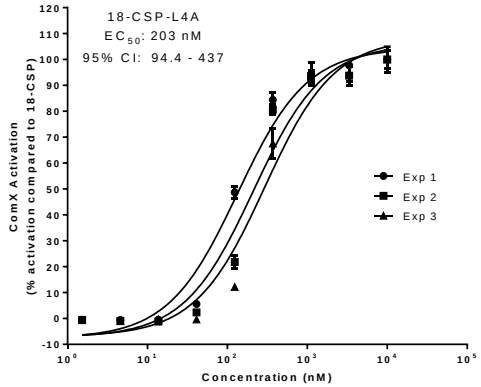
Activation dose response curves (18-CSP)

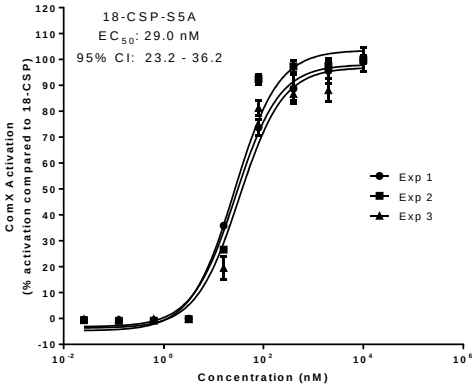


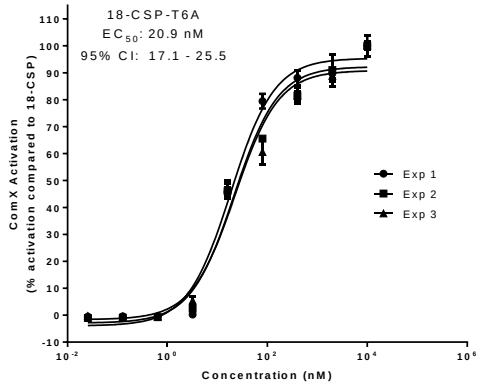


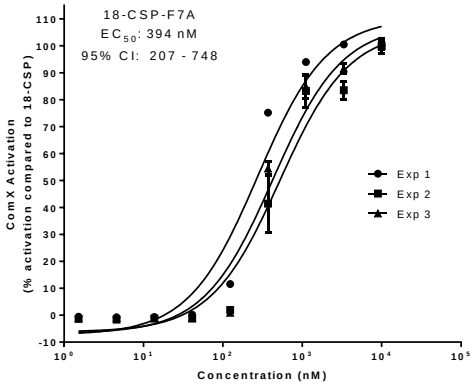


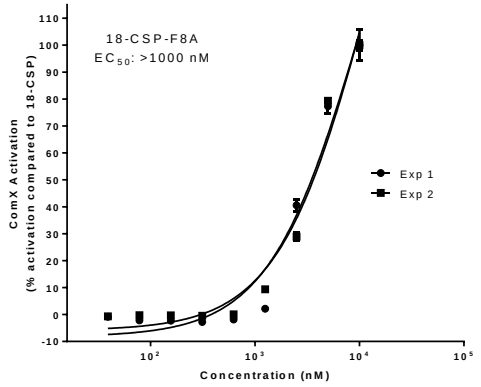


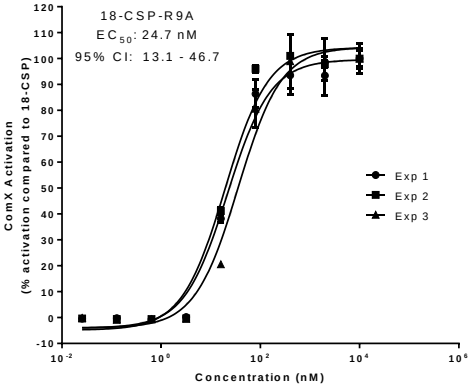


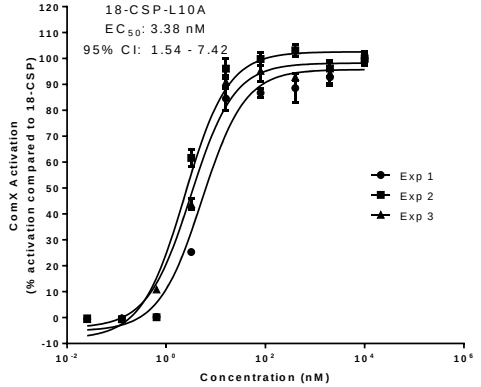


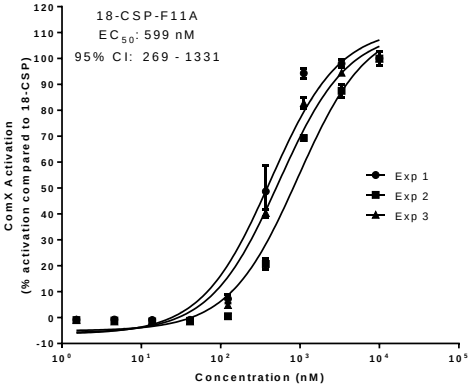

18-CSP-T6A
EC50: 20.9 nM
95% CI: 17.1 - 25.5
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

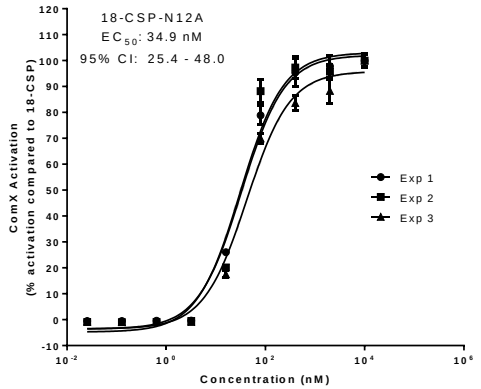

18-CSP-F7A
EC50: 394 nM
95% CI: 207 - 748
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

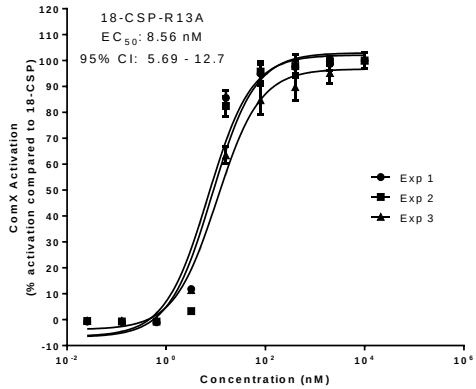

18-CSP-F8A
EC50: >1000 nM
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2

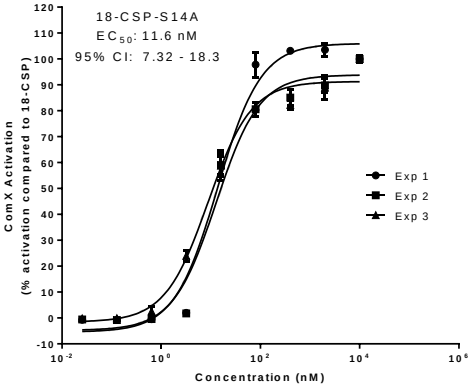

18-CSP-R9A
EC50: 24.7 nM
95% CI: 13.1 - 46.7
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

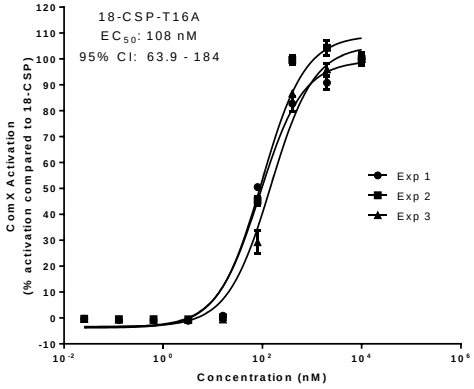

18-CSP-L10A
EC50: 3.38 nM
95% CI: 1.54 - 7.42
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

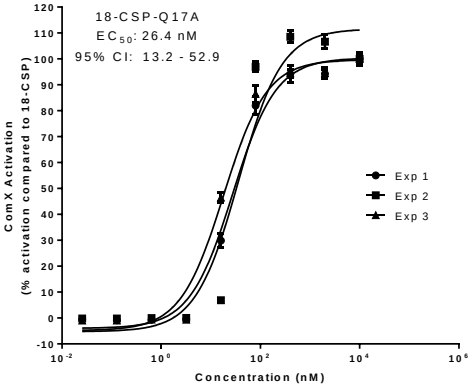

18-CSP-F11A
EC50: 599 nM
95% CI: 269 - 1331
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

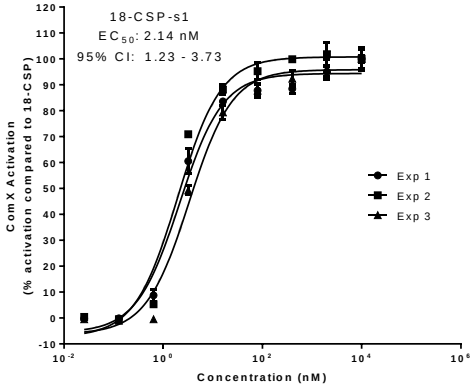

18-CSP-N12A
EC50: 34.9 nM
95% CI: 25.4 - 48.0
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

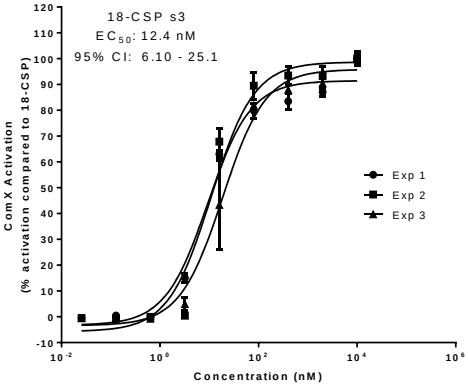

18-CSP-R13A
EC50: 8.56 nM
95% CI: 5.69 - 12.7
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

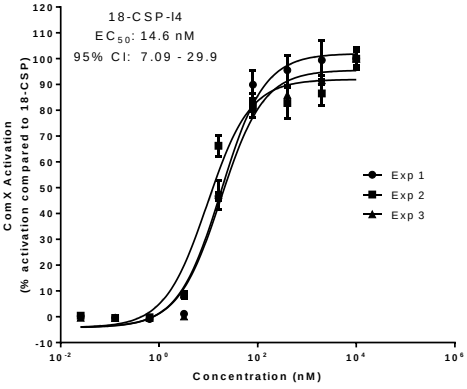

18-CSP-S14A
EC50: 11.6 nM
95% CI: 7.32 - 18.3
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

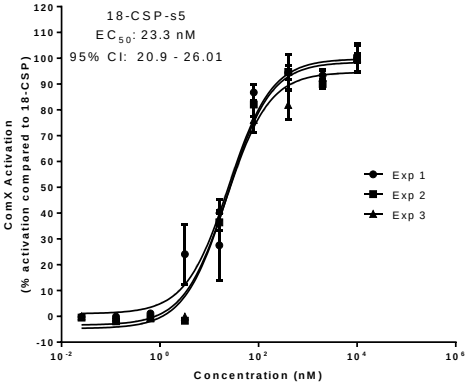

18-CSP-T16A
EC50: 108 nM
95% CI: 63.9 - 184
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

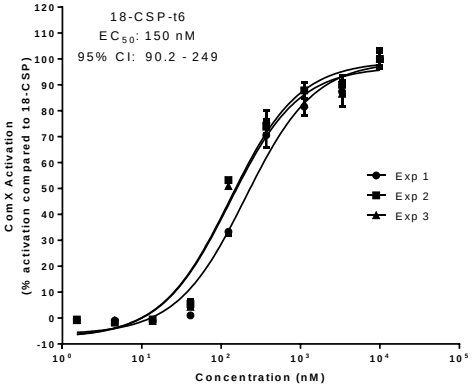

18-CSP-Q17A
EC50: 26.4 nM
95% CI: 13.2 - 52.9
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

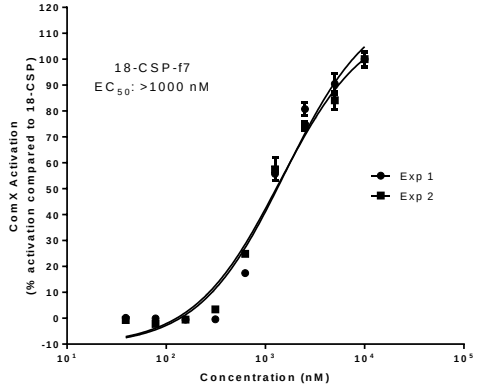

18-CSP-s1
EC50: 2.14 nM
95% CI: 1.23 - 3.73
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

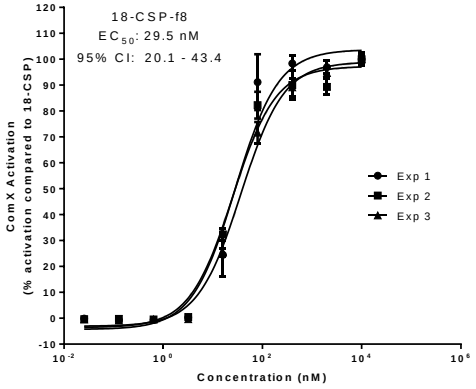

18-CSP s3
EC50: 12.4 nM
95% CI: 6.10 - 25.1
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

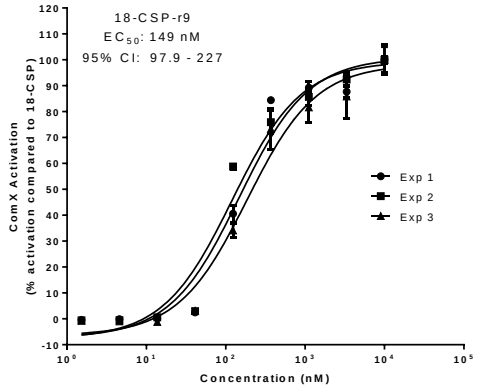

18-CSP-l4
EC50: 14.6 nM
95% CI: 7.09 - 29.9
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

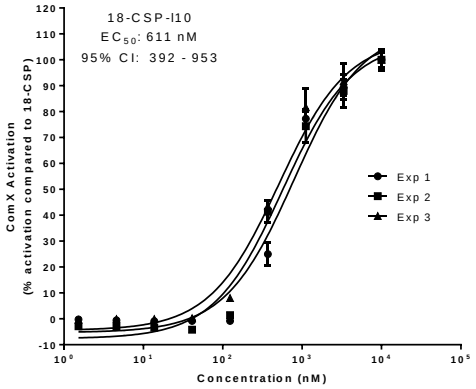

18-CSP-s5
EC50: 23.3 nM
95% CI: 20.9 - 26.01
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

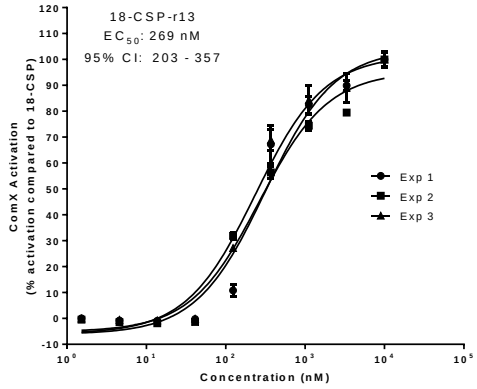

18-CSP-t6
EC50: 150 nM
95% CI: 90.2 - 249
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

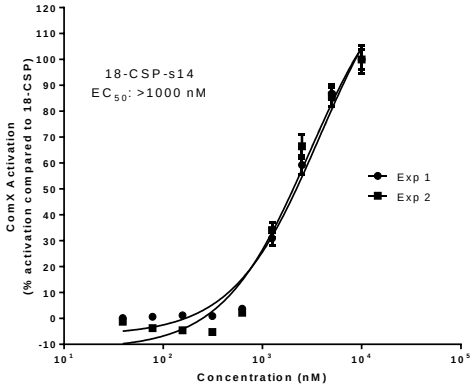

18-CSP-f7
EC50: >1000 nM
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2

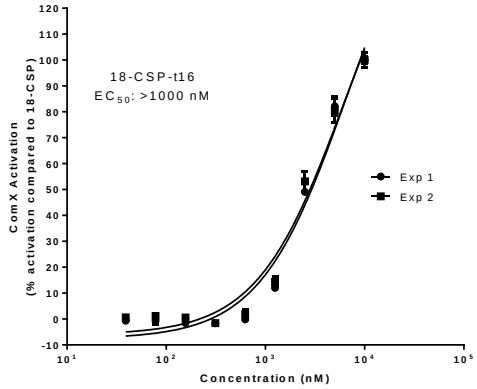

18-CSP-f8
EC50: 29.5 nM
95% CI: 20.1 - 43.4
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

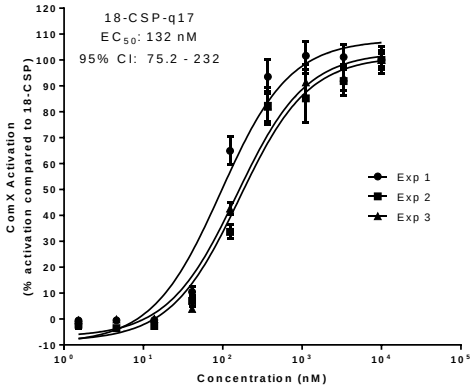

18-CSP-r9
EC50: 149 nM
95% CI: 97.9 - 227
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

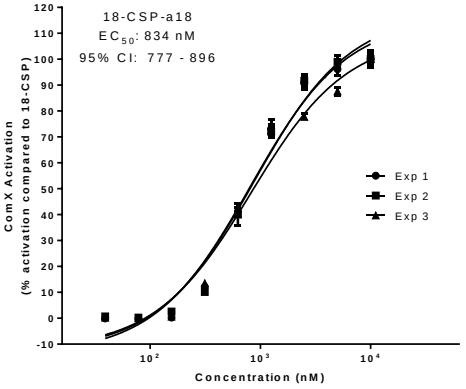

18-CSP-l10
EC50: 611 nM
95% CI: 392 - 953
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

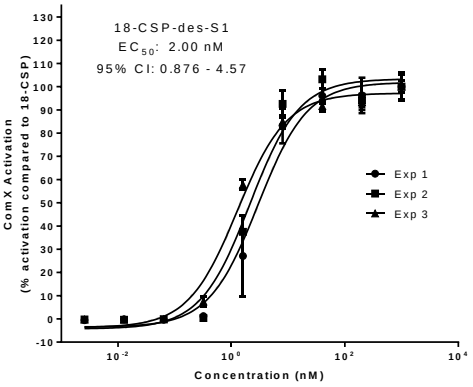

18-CSP-r13
EC50: 269 nM
95% CI: 203 - 357
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

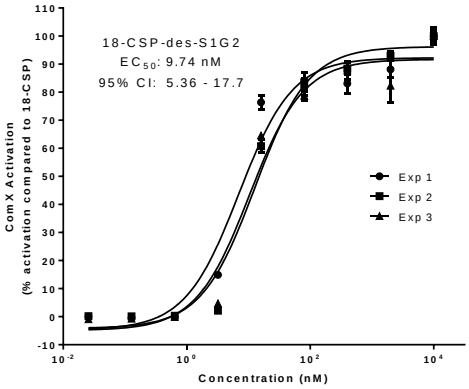

18-CSP-s14
EC50: >1000 nM
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2

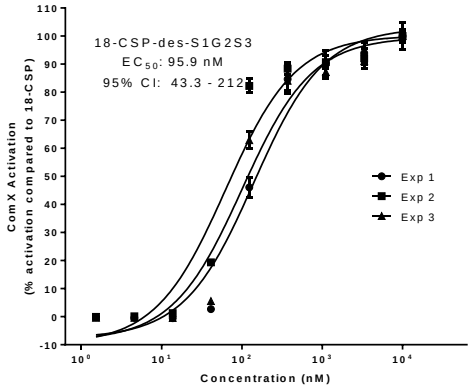

18-CSP-t16
EC50: >1000 nM
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2

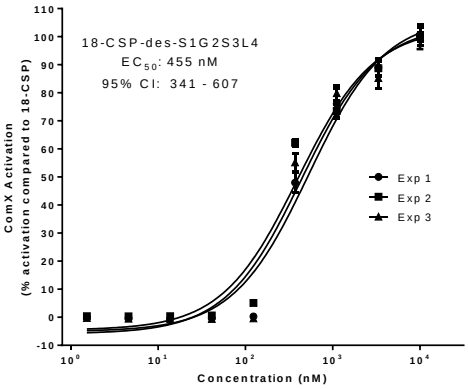

18-CSP-q17
EC50: 132 nM
95% CI: 75.2 - 232
ComX Activation
(% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

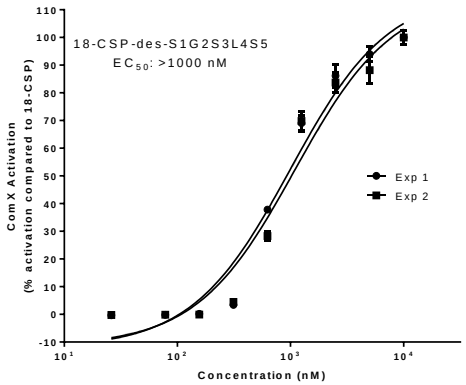

18-CSP-a18
EC50: 834 nM
95% CI: 777 - 896
ComX Activation (% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

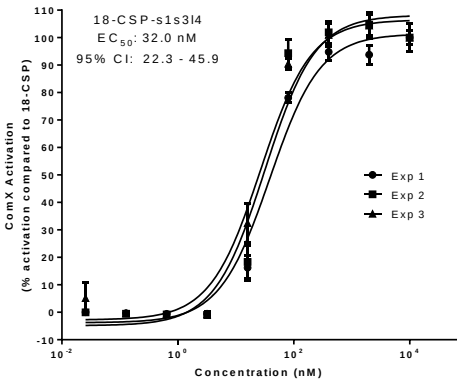

18-CSP-des-S1
EC50: 2.00 nM
95% CI: 0.876 - 4.57
ComX Activation (% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

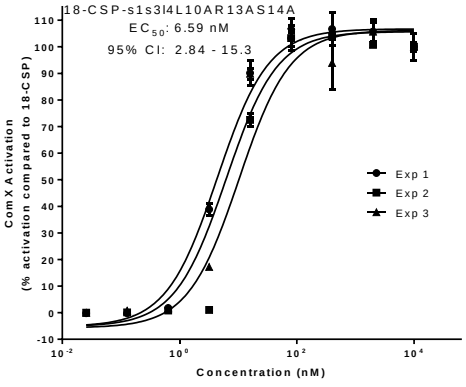

18-CSP-des-S1G2
EC50: 9.74 nM
95% CI: 5.36 - 17.7
ComX Activation (% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

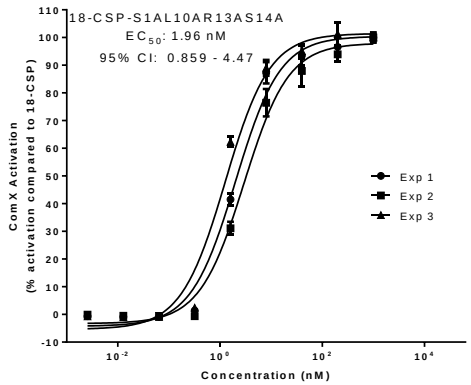

18-CSP-des-S1G2S3
EC50: 95.9 nM
95% CI: 43.3 - 212
ComX Activation (% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3

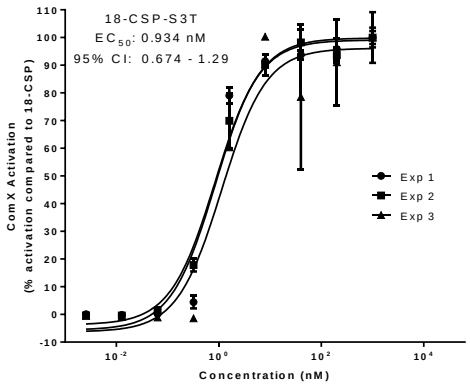

18-CSP-des-S1G2S3L4
EC50: 455 nM
95% CI: 341 - 607
ComX Activation (% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3


18-CSP-des-S1G2S3L4S5
EC50: >1000 nM
ComX Activation (% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2


18-CSP-s1s3l4
EC50: 32.0 nM
95% CI: 22.3 - 45.9
ComX Activation (% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3


18-CSP-s1s3l4L10AR13AS14A
EC50: 6.59 nM
95% CI: 2.84 - 15.3
ComX Activation (% activation compared to 18-CSP)
Concentration (nM)
Exp 1
Exp 2
Exp 3





Complete tables of EC_{50} values for CSP analogs (SMCOM2)

Table S-5. EC_{50} values of the alanine scan of 21-CSP and 18-CSP against the *S. mutans* SMCOM2[a]

Name	EC_{50} (nM)[b]	95% CI[c]	Fold change[d]	Name	EC_{50} (nM)[b]	95% CI[c]	Fold change[d]
21-CSP	69	57 – 85		18-CSP	2.4	1.1 – 5.2	
21-CSP-S1A	35	23 – 52	0.51	18-CSP-S1A	6.8	2.5 – 19	2.8
21-CSP-G2A	72	39 – 130	1.0	18-CSP-G2A	18	9.6 – 34	7.5
21-CSP-S3A	58	31 – 110	0.84	18-CSP-S3A	45	22 – 95	19
21-CSP-L4A	--[e]	--	--	18-CSP-L4A	200	94 – 440	83
21-CSP-S5A	140	120 – 170	2.0	18-CSP-S5A	29	23 – 36	12
21-CSP-T6A	120	66 – 210	1.7	18-CSP-T6A	21	17 – 25.5	8.8
21-CSP-F7A	--[e]	--	--	18-CSP-F7A	390	210 – 750	163
21-CSP-F8A	--[e]	--	--	18-CSP-F8A	>1,000	--	--
21-CSP-R9A	420	230 – 760	6.1	18-CSP-R9A	25	13 – 47	10
21-CSP-L10A	100	54 – 190	1.5	18-CSP-L10A	3.4	1.5 – 7.4	1.4
21-CSP-F11A	--[e]	--	--	18-CSP-F11A	600	270 – 1300	250
21-CSP-N12A	--[e]	--	--	18-CSP-N12A	35	25 – 48	15
21-CSP-R13A	130	94 –170	1.9	18-CSP-R13A	8.6	5.7 – 13	3.6
21-CSP-S14A	51	18 – 140	0.74	18-CSP-S14A	12	7.3 – 18	5.0
21-CSP-F15A	--[e]	--	--	18-CSP-F15A	--[e]	--	--
21-CSP-T16A	>1,000	--	--	18-CSP-T16A	110	64 – 180	46
21-CSP-Q17A	71	52 – 98	1.0	18-CSP-Q17A	26	13 – 53	11
21-CSP-L19A	71	45 – 110	1.0				
21-CSP-G20A	56	31 – 100	0.81				
21-CSP-K21A	94	88 – 100	1.4				

[a] See experimental section for details on reporter strain and methods. See supporting information for primary screening assay results and plots of agonism dose response curves. All assays were performed in triplicate. [b] EC_{50} values determined by testing peptides over a range of concentrations. [c] 95% confidence interval. [d] Ratio where each analog's EC_{50} is divided by native CSP EC_{50}; a value <1 indicates a better activator than the parent CSP. [e] EC_{50} not determined due to the analog's low induction in primary agonism screening assay.

Table S-6. EC_{50} values of the D-amino acid scan of 21-CSP and 18-CSP against the *S. mutans* SMCOM2[a]

Name	EC_{50} (nM)[b]	95% CI[c]	Fold change[d]	Name	EC_{50} (nM)[b]	95% CI[c]	Fold change[d]
21-CSP	69	57 – 85		18-CSP	2.4	1.1 – 5.2	
21-CSP-s1	40	32 – 52	0.58	18-CSP-s1	2.1	1.2 – 3.7	0.88
21-CSP-s3	60	53 – 67	0.87	18-CSP-s3	12	6.1 – 25	5.0
21-CSP-l4	170	140 – 210	2.5	18-CSP-l4	15	7.1 – 30	6.3
21-CSP-s5	270	250 – 300	3.9	18-CSP-s5	23	21 – 26	9.6
21-CSP-t6	>1,000	--	--	18-CSP-t6	150	90 – 250	63
21-CSP-f7	--[e]	--	--	18-CSP-f7	>1,000	--	--
21-CSP-f8	330	280 – 390	4.8	18-CSP-f8	30	20 – 43	13
21-CSP-r9	>1,000	--	--	18-CSP-r9	150	98 – 230	63
21-CSP-l10	>1,000	--	--	18-CSP-l10	610	390 – 950	254
21-CSP-f11	--[e]	--	--	18-CSP-f11	--[e]	--	--
21-CSP-n12	--[e]	--	--	18-CSP-n12	--[e]	--	--
21-CSP-r13	>1,000	--	--	18-CSP-r13	270	200 – 360	113
21-CSP-s14	--[e]	--	--	18-CSP-s14	>1,000	--	--
21-CSP-f15	--[e]	--	--	18-CSP-f15	--[e]	--	--
21-CSP-t16	--[e]	--	--	18-CSP-t16	>1,000	--	--
21-CSP-q17	--[e]	--	--	18-CSP-q17	130	75 – 230	54
21-CSP-a18	--[e]	--	--	18-CSP-a18	830	780 – 900	346
21-CSP-l19	>1,000	--	--				
21-CSP-k21	130	60 – 270	1.9				

[a] See experimental section for details on the reporter strain and methods. See supporting information for primary screening assay results and plots of agonism dose response curves. All assays performed in triplicate. [b] EC_{50} values determined by testing peptides over a range of concentrations. [c] 95% confidence interval. [d] Ratio where each analog's EC_{50} is divided by native CSP EC_{50}; a value <1 indicates a better activator than the parent CSP. [e] EC_{50} not determined due to the analog's low induction in primary agonism screening assay.

Table S-7. EC_{50} Values of the *2nd* generation Analogs of 21-CSP and 18-CSP against the *S. mutans* SMCOM2[a]

Name	EC_{50} (nM)[b]	95% CI[c]	Fold change[d]	Name	EC_{50} (nM)[b]	95% CI[c]	Fold change[d]
21-CSP	69	57 – 85		18-CSP	2.4	1.1 – 5.2	
21-CSP-des-S1	13	8.2 – 21	0.19	18-CSP-des-S1	2.0	0.88 – 4.6	0.83
21-CSP-des-S1G2	37	21 – 63	0.53	18-CSP-des-S1G2	9.7	5.4 – 18	4.0
21-CSP-des-S1G2S3	520	420 – 630	7.5	18-CSP-des-S1G2S3	96	43 – 210	40
21-CSP-des-S1G2S3L4	>1,000	--	--	18-CSP-des-S1G2S3L4	460	340 – 610	192
21-CSP-des-S1G2S3L4S5	>1,000	--	--	18-CSP-des-S1G2S3L4S5	>1,000	--	--
21-CSP-des-K21	51	24 – 110	0.74	18-CSP-des-A18	--[e]	--	--
21-CSP-des-G20K21	>1,000	--	--	18-CSP-des-Q17A18	--[e]	--	--
21-CSP-S3T	34	24 – 49	0.49	18-CSP-S3T	0.93	0.67 – 1.3	0.39
				18-CSP-s1s3l4	32	22 – 46	13
				18-CSP-s1s3l4L10AR13AS14A	6.6	2.8 – 15	2.8
				18-CSP-S1AL10AR13AS14A	2.0	0.86 – 4.5	0.83

[a] See experimental section for details on the reporter strain and methods. See supporting information for primary screening assay results and plots of agonism dose response curves. All assays performed in triplicate. [b] EC_{50} values determined by testing peptides over a range of concentrations. [c] 95% confidence interval. [d] Ratio where each analog's EC_{50} is divided by native CSP EC_{50}; a value <1 indicates a better activator than the parent CSP. [e] EC_{50} not determined due to the analog's low induction in primary agonism screening assay.

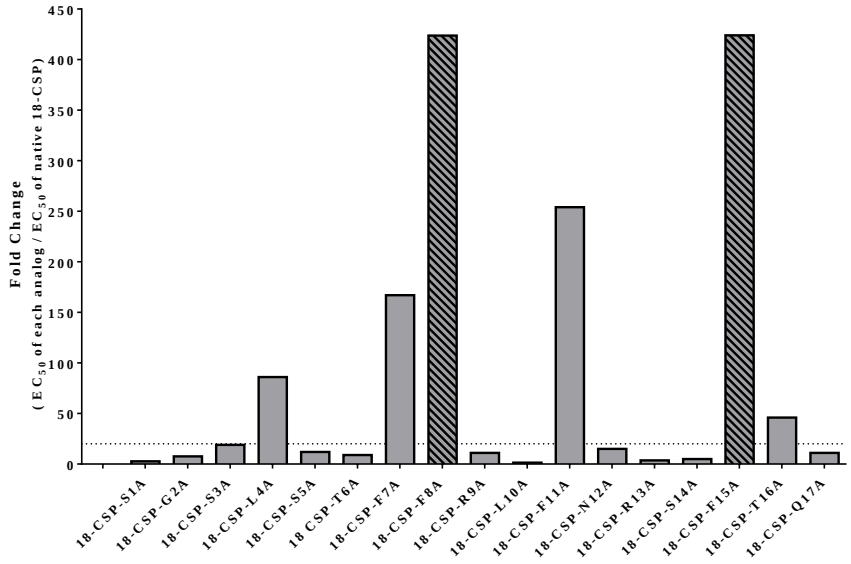


Figure S-13. Plot of the ratio of 18-CSP alanine analogs EC_{50} values divided by the EC_{50} value of 18-CSP in the SMCOM2 strain. The plot reveals a periodic sharp reduction in activity every 3-4 residues along the peptide sequence, consistent with an α-helix conformation. Striped bars represent analogs with EC_{50} values >1,000 nM, thus the EC_{50} ratio is >425. >20-fold decrease in potency was considered significant (dashed line).

Comparison of results found from both reporter strains SAB249 and SMCOM2

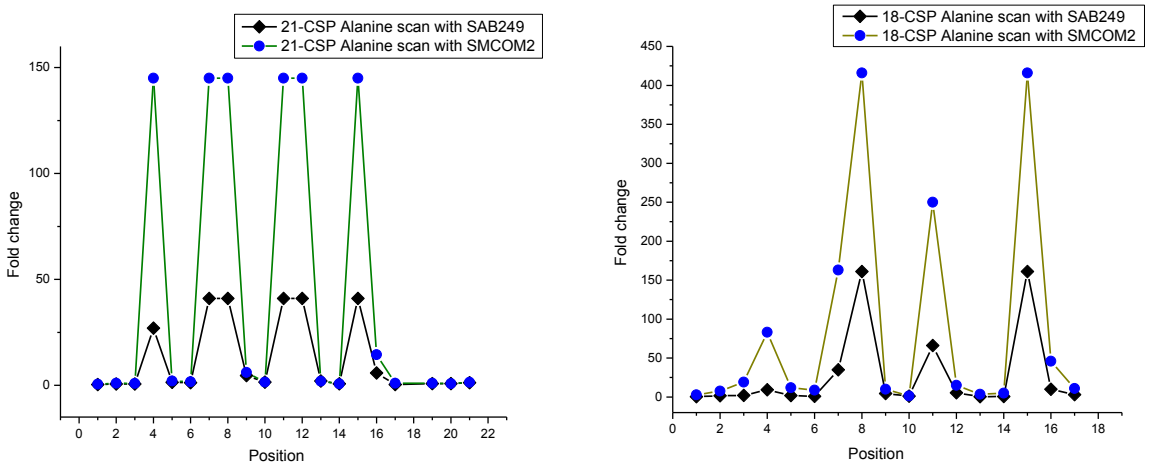


Figure S-14. Comparison of alanine scan results of 21-CSP and 18-CSP with both reporter strains SAB249 and SMCOM2. These results showed a similar trend in changing activity with both reporter systems.

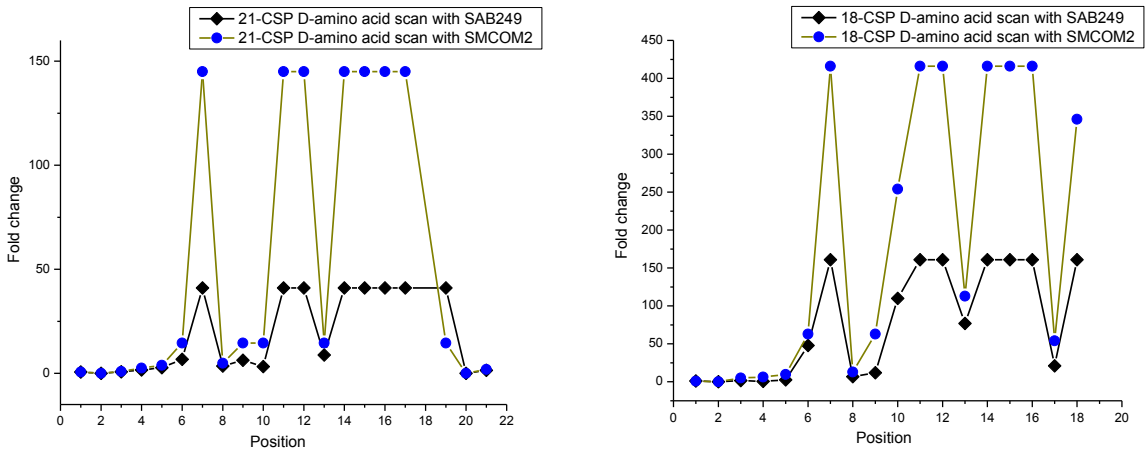


Figure S-15. Comparison of D-amino acid scan results of 21-CSP and 18-CSP with both reporter strains SAB249 and SMCOM2. These results showed a similar trend in changing activity with both reporter systems.

Circular dichroism (CD) spectra

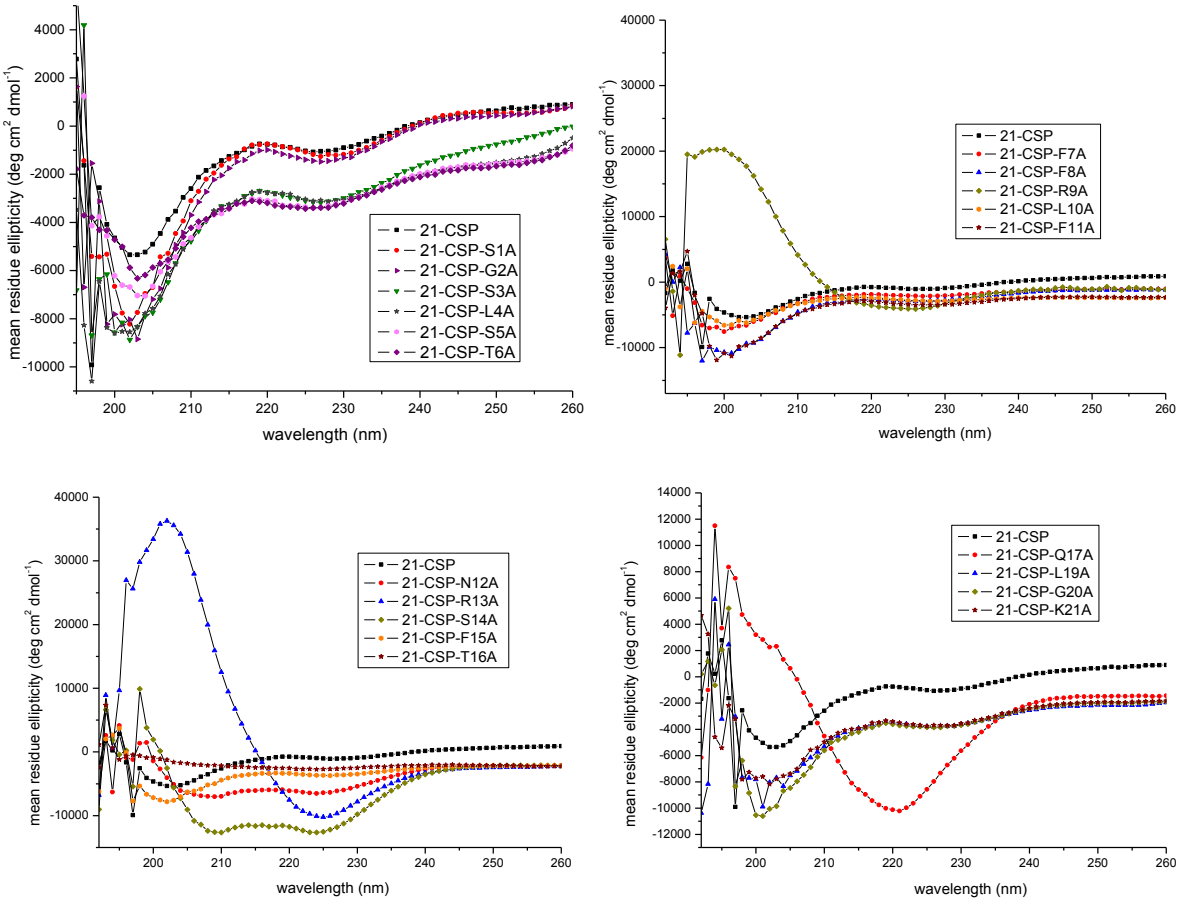


Figure S-16. CD spectra of the 21-CSP alanine scan library in aqueous solution (PBS, pH 7.4). All the measurements were performed with a peptide concentration of 200 µM. 21-CSP was added as a control. Most peptides exhibit a random coil pattern, while 21-CSP-R9A and 21-CSP-R13A exhibit a β-turn pattern, 21-CSP-Q17A exhibits a β-sheet pattern, and 21-CSP-S14A exhibits an α-helix pattern.

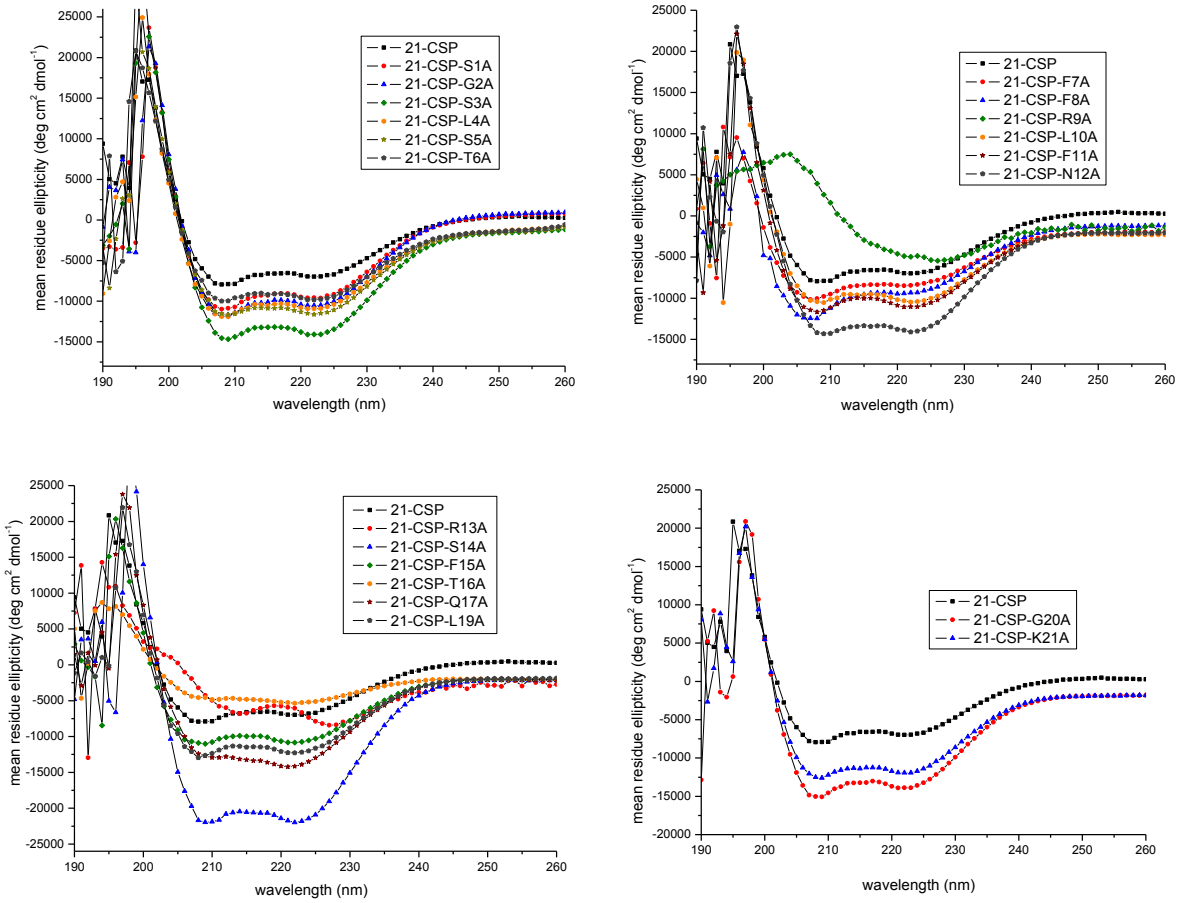


Figure S-17. CD spectra of the 21-CSP alanine scan library in membrane mimicking conditions (20% TFE: 80% PBS, pH 7.4). All the measurements were performed with a peptide concentration of 200 µM. 21-CSP was added as a control. Peptides exhibit varying degrees of an α-helix pattern. Measurements of 21-CSP-R9A and 21-CSP-R13A were performed with a peptide concentration of 100 µM. 21-CSP-R9A exhibits a distorted β-sheet pattern and 21-CSP-R13A exhibits a distorted α-helix (mixed with β-sheets and β-turns) pattern.[1]

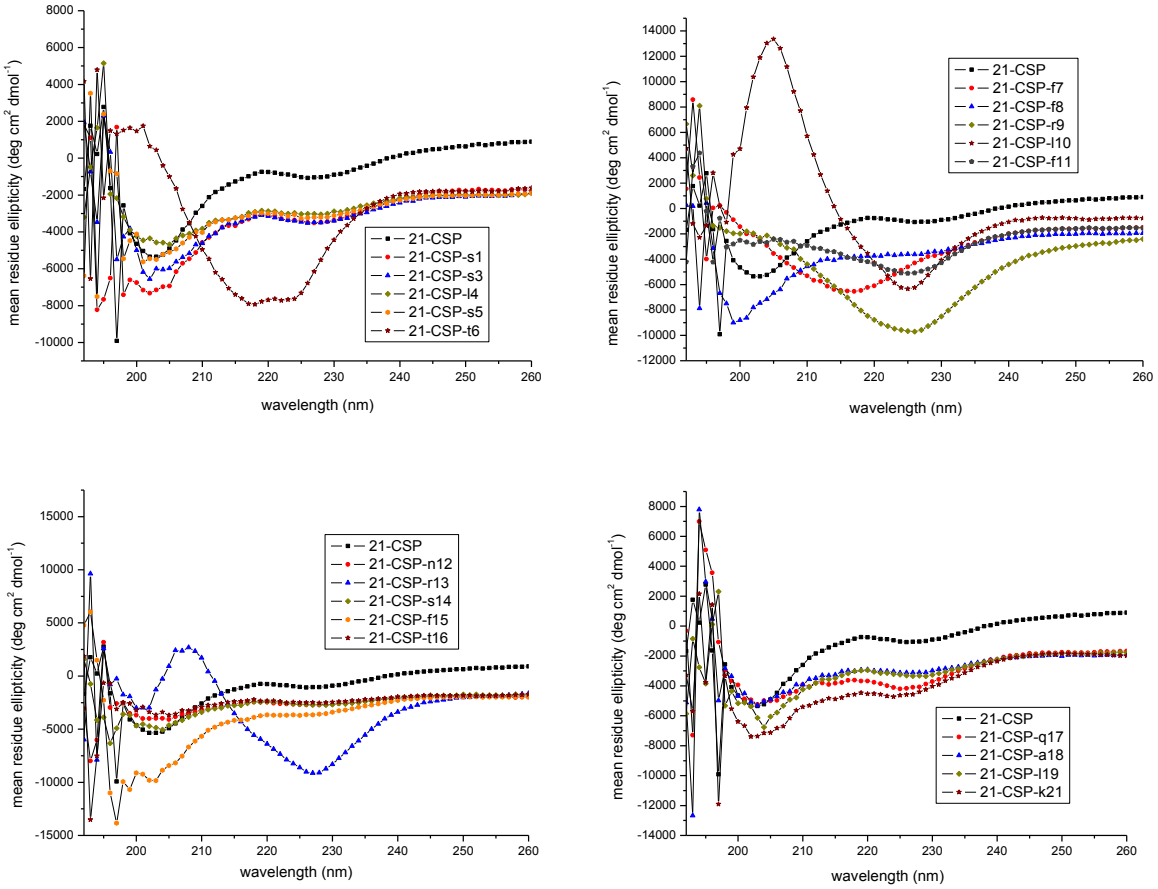


Figure S-18. CD spectra of the 21-CSP D-amino acid scan library in aqueous solution (PBS, pH 7.4). All the measurements were performed with a peptide concentration of 200 μM. 21-CSP was added as a control. Most peptides exhibit a random coil pattern, while 21-CSP-t6 exhibits an α-helix pattern, 21-CSP-r9 and 21-CSP-f11 exhibit a β-sheet pattern, and 21-CSP-l10 and 21-CSP-r13 exhibit a β-turn pattern.

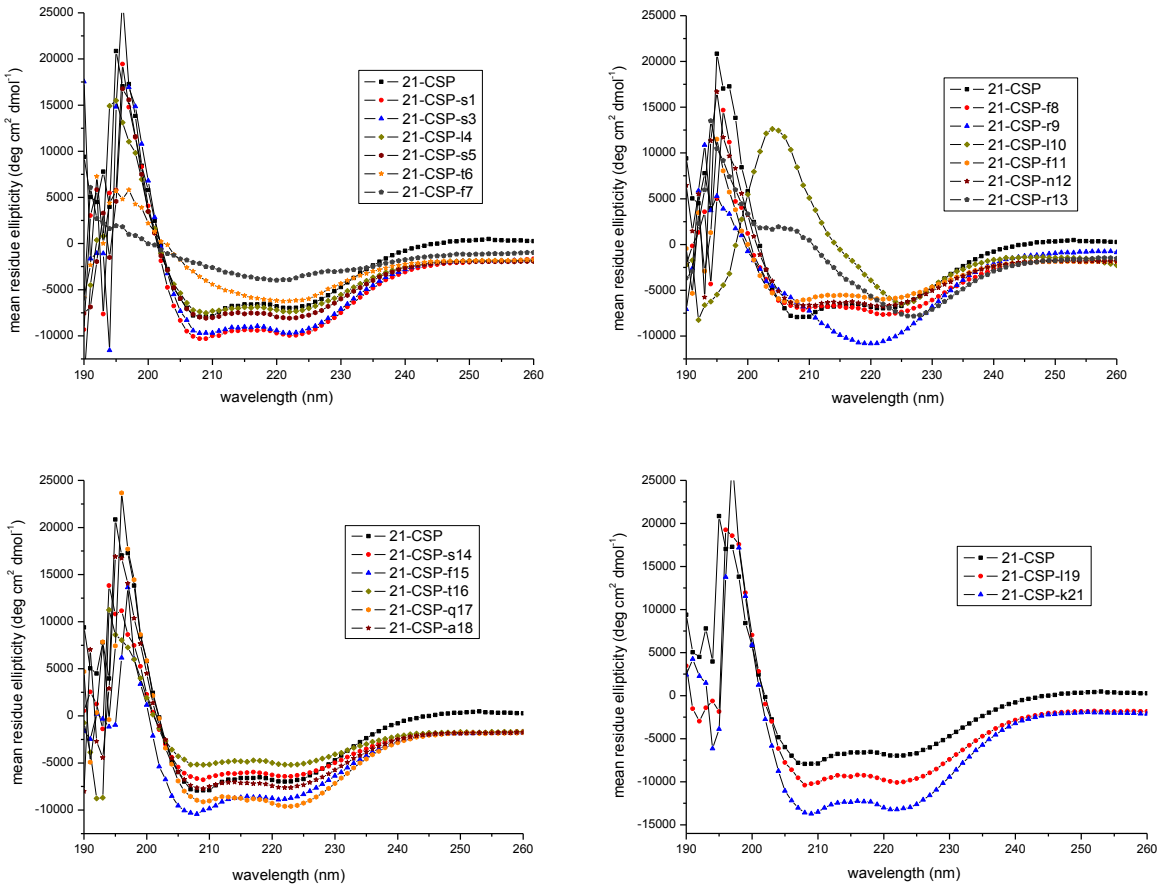


Figure S-19. CD spectra of the 21-CSP D-amino acid scan library in membrane mimicking conditions (20% TFE: 80% PBS, pH 7.4). All the measurements were performed with a peptide concentration of 200 µM. 21-CSP was added as a control. Most Peptides exhibit varying degrees of an α-helix pattern, while 21-CSP-t6 and 21-CSP-r9 exhibit a β-sheet pattern, 21-CSP-f7 exhibits a distorted β-sheet (mixed with α-helix) pattern, and 21-CSP-l10 and 21-CSP-r13 exhibit a β-turn pattern.

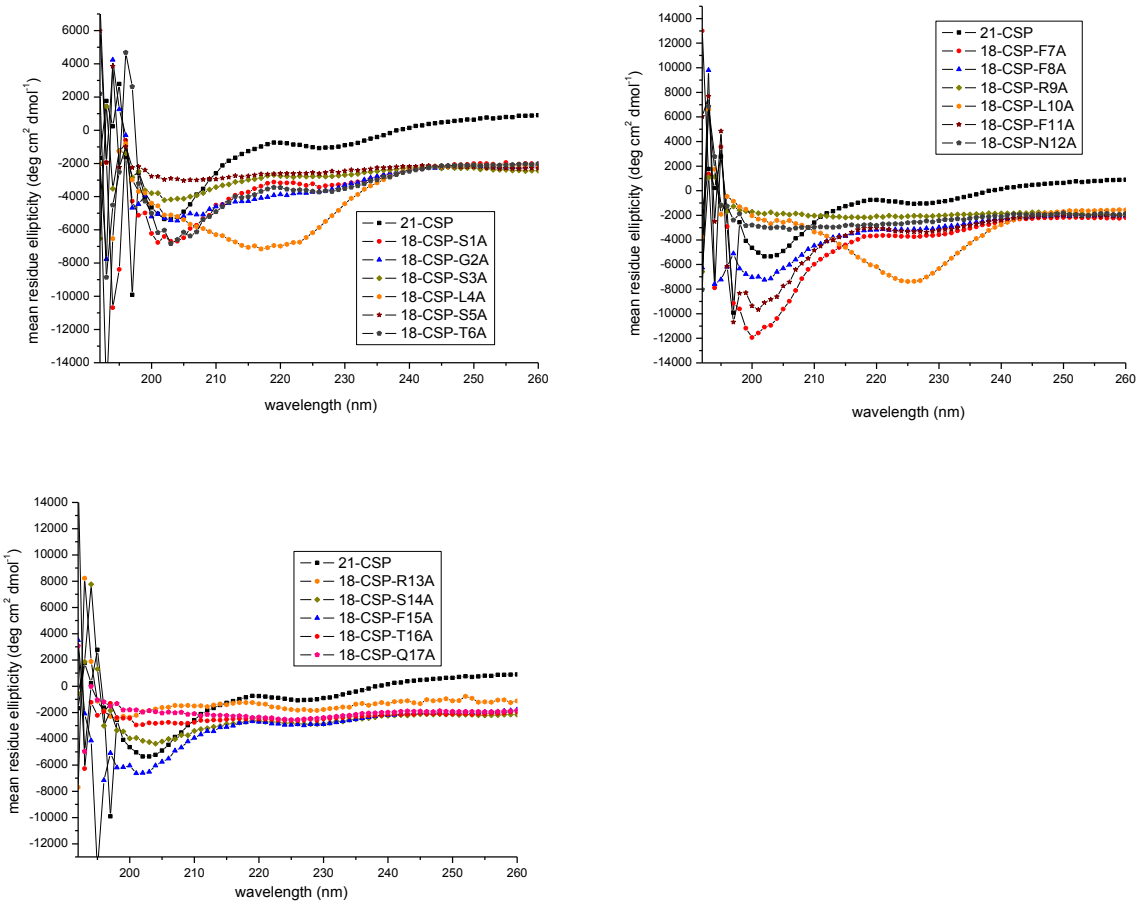


Figure S-20. CD spectra of the 18-CSP alanine scan library in aqueous solution (PBS, pH 7.4). All the measurements were performed with a peptide concentration of 200 µM. Measurements of 18-CSP-R9A and 18-CSP-R13A were performed with a peptide concentration of 100 µM as these peptides showed low solubility in PBS. 21-CSP was added as a control. Most peptides exhibit a random coil pattern, while few peptides exhibit a β-sheet pattern.

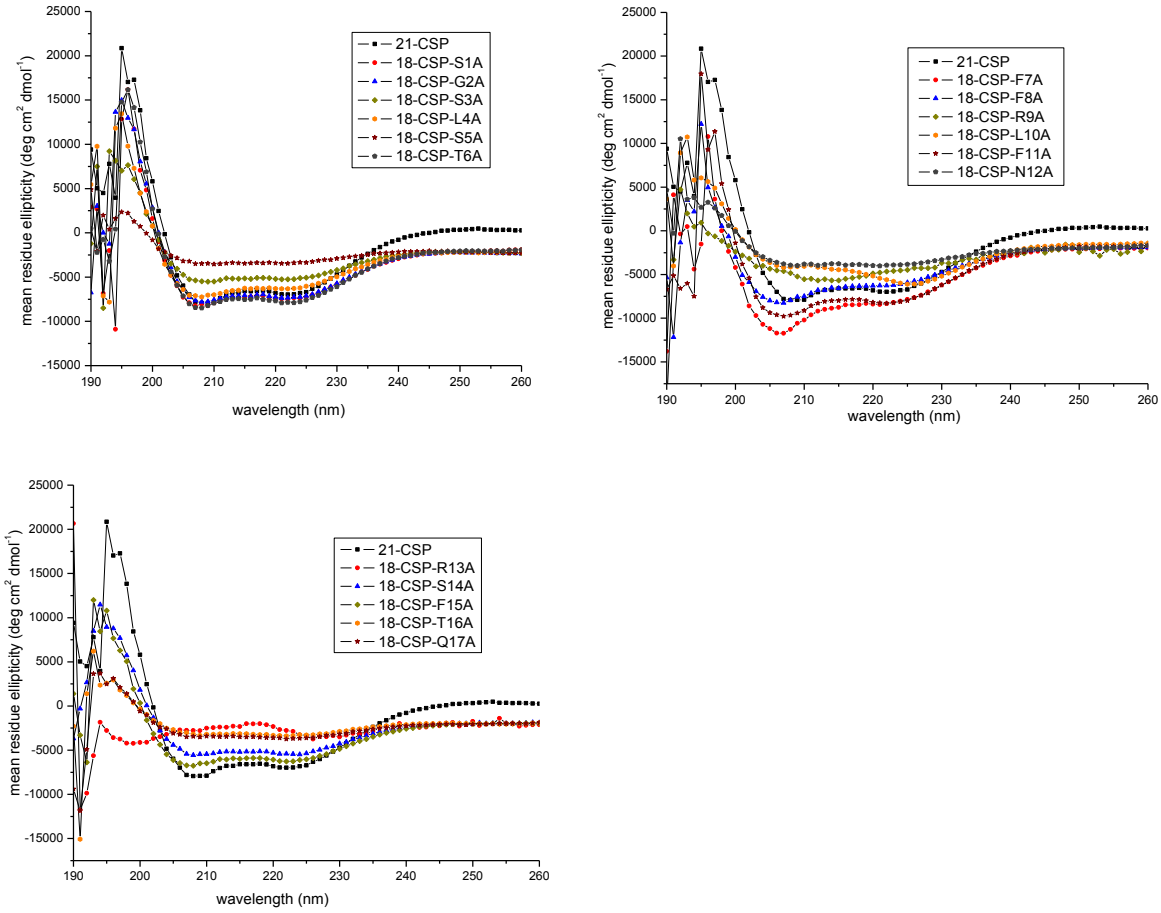


Figure S-21. CD spectra of the 18-CSP alanine scan library in membrane mimicking conditions (20% TFE: 80% PBS, pH 7.4). All the measurements were performed with a peptide concentration of 200 µM. Measurements of 18-CSP-R9A and 18-CSP-R13A were performed with a peptide concentration of 100 µM as these peptides showed low solubility in PBS. 21-CSP was added as a control. Peptides exhibit varying degrees of an α-helix pattern, while 18-CSP-L10A exhibits a distorted α-helix (mixed with β-sheet) pattern, 18-CSP-R9A exhibits a distorted α-helix (mixed with β-sheets) pattern, and 18-CSP-R13A exhibits a distorted α-helix (mixed with β-turns) pattern.

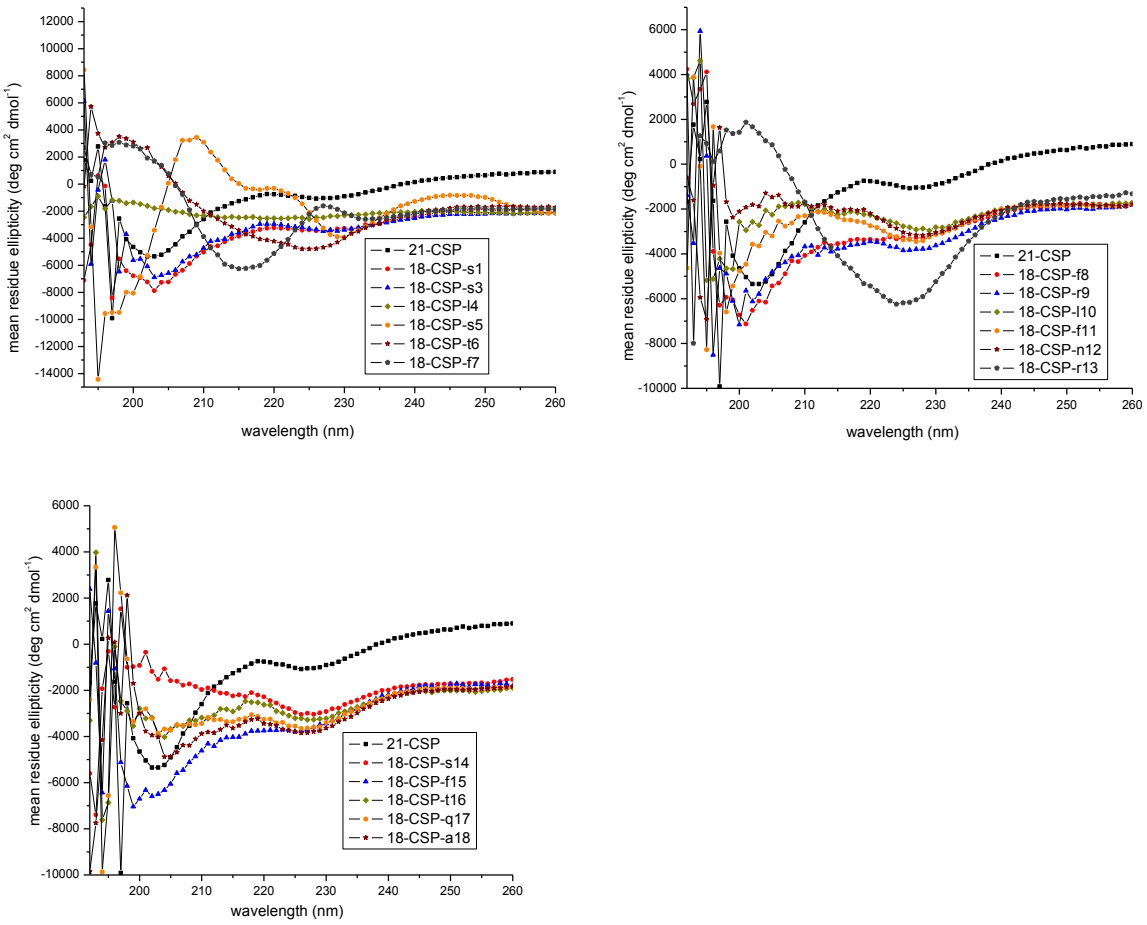


Figure S-22. CD spectra of the 18-CSP D-amino acid scan library in aqueous solution (PBS, pH 7.4). All the measurements were performed with a peptide concentration of 200 µM. 21-CSP was added as a control. Most peptides exhibit a random coil pattern, while few peptides exhibit a β-sheet pattern.

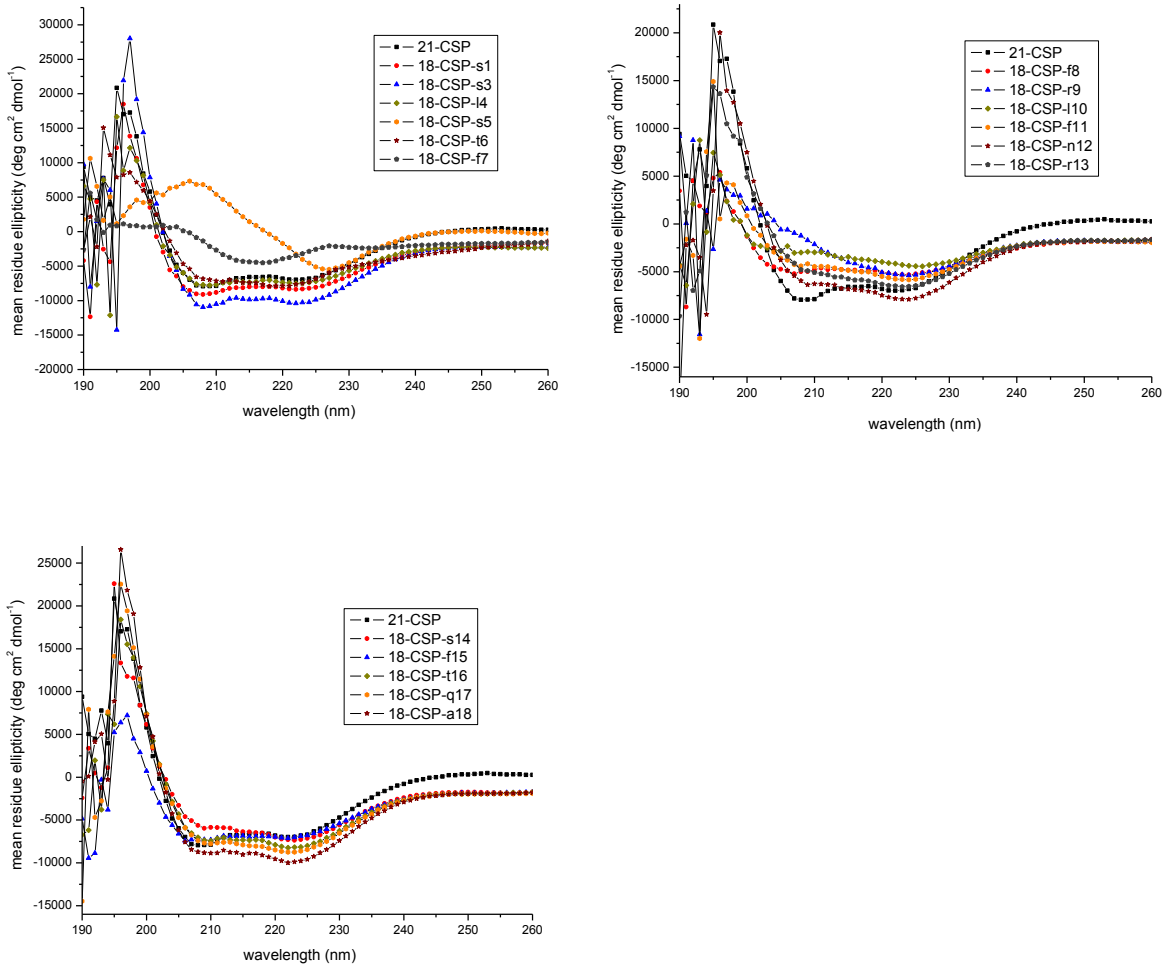


Figure S-23. CD spectra of the 18-CSP D-amino acid scan library in membrane mimicking conditions (20% TFE: 80% PBS, pH 7.4). All the measurements were performed with a peptide concentration of 200 µM. 21-CSP was added as a control. Most peptides exhibit varying degrees of an α-helix pattern, while 18-CSP-s5 exhibits a β-turn pattern, and 18-CSP-f7 and 18-CSP-r9 exhibit a β-sheet pattern.

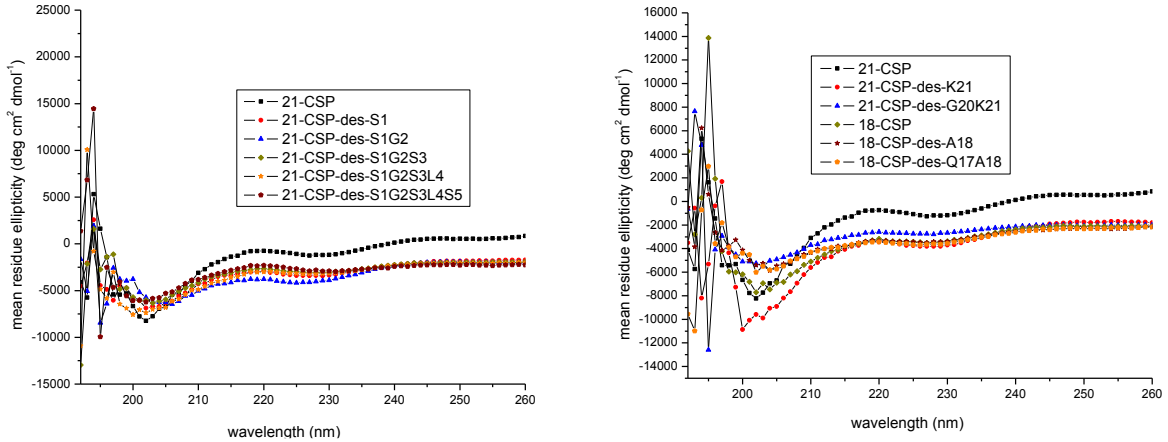


Figure S-24. CD spectra of the 21-CSP and 18-CSP truncated analogs in aqueous solution (PBS, pH 7.4). All the measurements were performed with a peptide concentration of 200 µM. 21-CSP was added as a control. All peptides exhibit a random coil pattern.

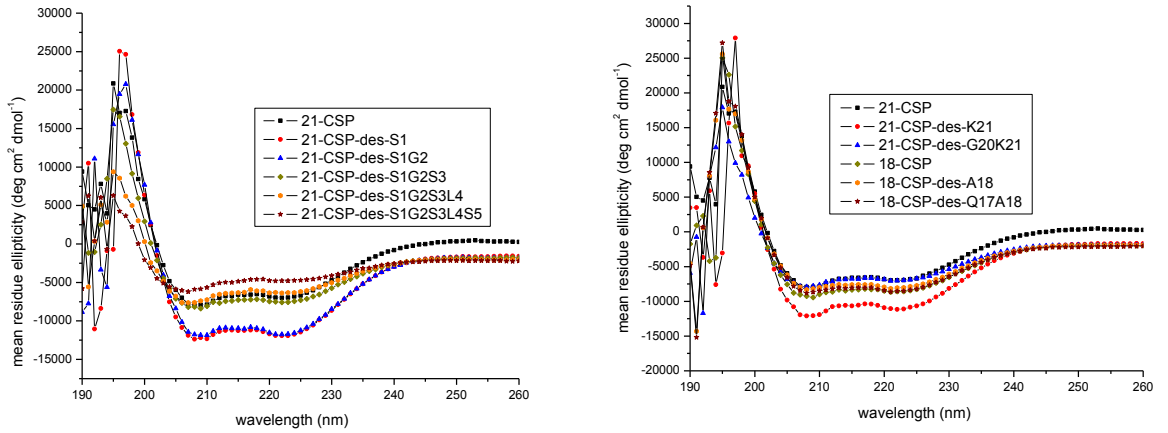


Figure S-25. CD spectra of the 21-CSP and 18-CSP truncated analogs in membrane mimicking conditions (20% TFE: 80% PBS, pH 7.4). All the measurements were performed with a peptide concentration of 200 µM. 21-CSP was added as a control. Peptides exhibit varying degrees of an α-helix pattern.

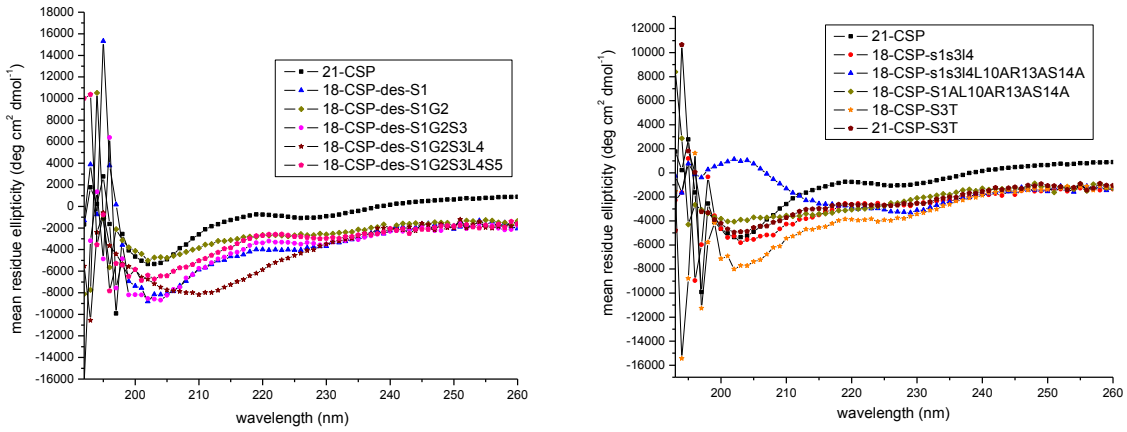


Figure S-26. CD spectra of 18-CSP truncated and 2nd generation CSP analogs in aqueous solution (PBS, pH 7.4). All the measurements were performed with a peptide concentration of 200 µM. 21-CSP was added as a control. Most peptides exhibit a random coil pattern, while 18-CSP-S1G2S3L4 exhibits a β-sheet pattern and 18-CSP-s1s3l4L10AR13AS14A exhibits a distorted β-sheet pattern.

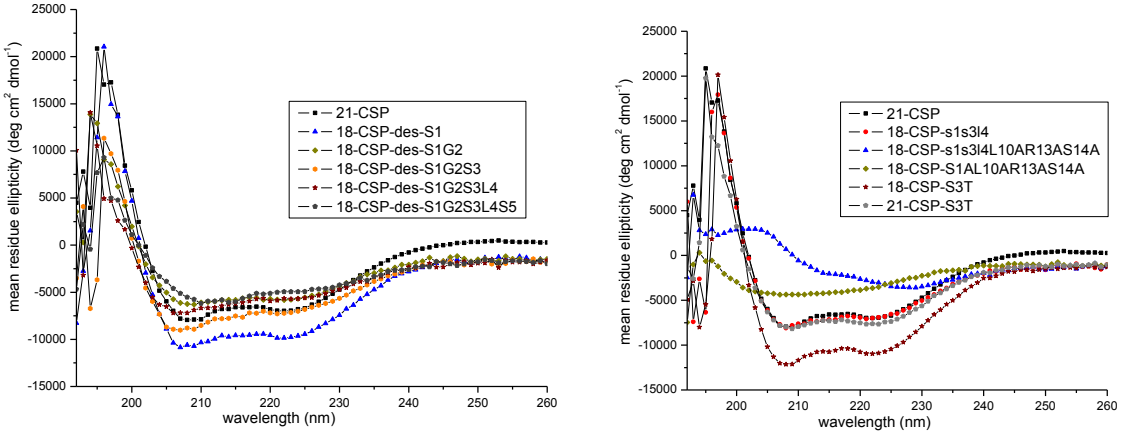


Figure S-27. CD spectra of 18-CSP truncated and 2nd generation CSP analogs in membrane mimicking conditions (20% TFE: 80% PBS, pH 7.4). All the measurements were performed with a peptide concentration of 200 µM. 21-CSP was added as a control. Most peptides exhibit varying degrees of an α-helix pattern, while 18-CSP-s1s3l4L10AR13AS14A exhibits a distorted β-sheet (mixed with β-turn) pattern, and 18-CSP-S1AL10AR13AS14A exhibits a distorted α-helix (mixed with β-sheet) pattern.

Table S-8. EC_{50} values (SAB249) and percent helicity (in 20% TFE) data for 21-CSP alanine & D-amino acid scanning analogs.

Compound Name	EC_{50} (nM)	Helicity (%)	Compound Name	EC_{50} (nM)	Helicity (%)
21-CSP	240	18.0%			
21-CSP-S1A	90	24.7%	21-CSP-s1	140	25.7%
21-CSP-G2A	140	27.2%	--		
21-CSP-S3A	150	36.3%	21-CSP-s3	160	25.0%
21-CSP-L4A	6500	28.2%	21-CSP-l4	390	19.0%
21-CSP-S5A	330	30.0%	21-CSP-s5	640	20.9%
21-CSP-T6A	280	25.4%	21-CSP-t6	1600	β-sheet
21-CSP-F7A	--	21.8%	21-CSP-f7	--	Distorted β-sheet
21-CSP-F8A	--	24.1%	21-CSP-f8	840	19.8%
21-CSP-R9A	1100	Distorted β-sheet	21-CSP-r9	1500	β-sheet
21-CSP-L10A	370	27.0%	21-CSP-l10	760	β-turn
21-CSP-F11A	--	28.5%	21-CSP-f11	--	15.4%
21-CSP-N12A	--	36.4%	21-CSP-n12	--	17.5%
21-CSP-R13A	480	Distorted α-helix	21-CSP-r13	2100	β-turn
21-CSP-S14A	160	56.7%	21-CSP-s14	--	16.6%
21-CSP-F15A	--	28.0%	21-CSP-f15	--	22.8%
21-CSP-T16A	1400	13.8%	21-CSP-t16	--	13.4%
21-CSP-Q17A	94	36.5%	21-CSP-q17	--	24.8%
--			21-CSP-a18	--	19.7%
21-CSP-L19A	190	31.7%	21-CSP-l19	--	26.0%
21-CSP-G20A	190	35.9%	--		
21-CSP-K21A	290	30.8%	21-CSP-k21	330	34.1%

Table S-9. EC_{50} values (SAB249) and percent helicity (in 20% TFE) data for 18-CSP alanine & D-amino acid scanning analogs.

Compound Name	EC_{50} (nM)	Helicity (%)	Compound Name	EC_{50} (nM)	Helicity (%)
18-CSP	6.2	22.6%			
18-CSP-S1A	2.6	19.8%	18-CSP-s1	6.4	22.1%
18-CSP-G2A	9.7	19.4%	--		
18-CSP-S3A	13	13.9%	18-CSP-s3	9.4	27.5%
18-CSP-L4A	57	16.7%	18-CSP-l4	4.5	19.9%
18-CSP-S5A	11	9.13%	18-CSP-s5	17	β-turn
18-CSP-T6A	6.0	20.7%	18-CSP-t6	300	20.3%
18-CSP-F7A	220	22.0%	18-CSP-f7	>1,000	β-sheet
18-CSP-F8A	>1,000	16.6%	18-CSP-f8	42	13.4%
18-CSP-R9A	27	Distorted α-helix	18-CSP-r9	76	β-sheet
18-CSP-L10A	6.5	Distorted α-helix	18-CSP-l10	670	10.9%
18-CSP-F11A	410	21.9%	18-CSP-f11	--	15.2%
18-CSP-N12A	33	10.4%	18-CSP-n12	--	20.7%
18-CSP-R13A	2.7	Distorted α-helix	18-CSP-r13	480	17.1%
18-CSP-S14A	4.5	14.3%	18-CSP-s14	>1,000	19.3%
18-CSP-F15A	--	16.6%	18-CSP-f15	--	18.8%
18-CSP-T16A	64	8.90%	18-CSP-t16	>1,000	21.8%
18-CSP-Q17A	19	9.90%	18-CSP-q17	130	23.1%
--			18-CSP-a18	>1,000	26.4%

Table S-10. EC_{50} values (SAB249) and percent helicity (in 20% TFE) data for 21-CSP and 18-CSP truncated & 2nd generation analogs.

Compound Name	EC_{50} (nM)	Helicity (%)
21-CSP-des-S1	23	31.0%
21-CSP-des-S1G2	120	30.8%
21-CSP-des-S1G2S3	530	20.0%
21-CSP-des-S1G2S3L4	3600	16.9%
21-CSP-des-S1G2S3L4S5	3300	12.9%
21-CSP-des-K21	98	28.9%
21-CSP-des-G20K21	3600	18.4%
18-CSP-des-S1	4.8	26.3%
18-CSP-des-S1G2	7.9	15.5%
18-CSP-des-S1G2S3	85	19.7%
18-CSP-des-S1G2S3L4	570	15.7%
18-CSP-des-S1G2S3L4S5	950	13.4%
18-CSP-des-A18	--	21.4%
18-CSP-des-Q17A18	--	23.0%
18-CSP-s1s3l4	17	18.4%
18-CSP-s1s3l4L10AR13AS14A	3.4	Distorted β-sheet
18-CSP-S1AL10AR13AS14A	1.7	Distorted α-helix
18-CSP-S3T	2.1	28.9%
21-CSP-S3T	94	19.7%

Table S-11. EC_{50} values (SAB249) and estimated secondary structure contents (in 20% TFE) data for 21-CSP alanine scan analogs.

Compound Name	EC_{50} (nM)	Estimated secondary structure contents (%)[a]				
		α-helix	Antiparallel β-sheet	Parallel β-sheet	β-turn	Others
21-CSP	240	27.1	16.4	0.0	9.7	46.7
21-CSP-S1A	90	34.2	17.0	0.0	5.8	43.1
21-CSP-G2A	140	37.3	19.1	0.0	7.4	36.2
21-CSP-S3A	150	38.0	9.5	0.0	5.1	47.4
21-CSP-L4A	6500	32.9	5.3	0.0	8.5	53.4
21-CSP-S5A	330	34.3	5.5	0.0	8.1	52.1
21-CSP-T6A	280	30.0	5.9	0.9	10.3	52.9
21-CSP-F7A	--	22.0	10.1	3.1	14.8	50.1
21-CSP-F8A	--	24.9	11.5	1.9	12.0	49.8
21-CSP-R9A	1100	4.1	28.6	19.3	14.6	33.5
21-CSP-L10A	370	32.8	17.6	0.0	10.5	39.1
21-CSP-F11A	--	29.7	11.1	0.0	7.7	51.5
21-CSP-N12A	--	33.4	9.7	0.0	7.6	49.3
21-CSP-R13A	480	13.8	9.1	17.9	19.6	39.6
21-CSP-S14A	160	41.9	25.5	23.2	1.7	7.7
21-CSP-F15A	--	30.1	14.3	0.0	9.5	46.1
21-CSP-T16A	1400	10.2	12.0	15.3	15.2	47.3
21-CSP-Q17A	94	39.3	17.5	0.0	5.9	37.3
--						
21-CSP-L19A	190	38.9	16.8	0.0	7.5	36.8
21-CSP-G20A	190	37.8	12.5	0.0	6.6	43.0
21-CSP-K21A	290	36.1	13.1	0.0	9.6	41.2

[a] Secondary structure contents were calculated using the BeStSel[1] (Beta Structure Selection) method.

Table S-12. EC_{50} values (SAB249) and estimated secondary structure contents (in 20% TFE) data for 21-CSP D-amino acid scan analogs.

Compound Name	EC_{50} (nM)	Estimated secondary structure contents (%)[a]				
		***α*-helix**	**Antiparallel *β*-sheet**	**Parallel *β*-sheet**	***β*-turn**	**Others**
21-CSP	240	27.1	16.4	0.0	9.7	46.7
21-CSP-s1	140	26.6	9.7	0.7	10.9	52.1
--						
21-CSP-s3	160	30.8	27.0	0.0	9.1	33.2
21-CSP-l4	390	13.1	4.7	14.5	10.8	56.9
21-CSP-s5	640	25.7	8.7	3.9	10.0	51.6
21-CSP-t6	1600	11.0	3.8	16.9	17.2	51.1
21-CSP-f7	--	3.0	20.5	14.4	16.4	45.8
21-CSP-f8	840	23.2	9.3	5.6	14.4	47.7
21-CSP-r9	1500	2.4	1.4	18.1	15.2	62.8
21-CSP-l10	760	0.0	65.7	0.0	24.2	10.2
21-CSP-f11	--	15.3	9.8	10.7	15.2	49.0
21-CSP-n12	--	19.9	13.4	8.3	13.5	45.0
21-CSP-r13	2100	5.7	4.4	14.8	22.2	52.9
21-CSP-s14	--	15.1	7.4	13.5	14.8	49.2
21-CSP-f15	--	26.1	14.3	0.0	12.7	46.8
21-CSP-t16	--	18.4	13.3	7.8	12.7	47.8
21-CSP-q17	--	30.5	10.1	0.0	10.2	49.2
21-CSP-a18	--	25.8	7.1	4.1	10.9	52.1
21-CSP-l19	--	35.0	18.0	0.0	8.5	38.4
--						
21-CSP-k21	330	43.9	19.1	0.0	7.1	29.9

[a] Secondary structure contents were calculated using the BeStSel[1] (Beta Structure Selection) method.

Table S-13. EC_{50} values (SAB249) and estimated secondary structure contents (in 20% TFE) data for 18-CSP alanine scan analogs.

Compound Name	EC_{50} (nM)	Estimated secondary structure contents (%)[a]				
		***α*-helix**	**Antiparallel *β*-sheet**	**Parallel *β*-sheet**	***β*-turn**	**Others**
18-CSP	6.2	27.1	6.8	0.0	9.1	57.0
18-CSP-S1A	2.6	21.1	16.2	0.0	12.4	50.3
18-CSP-G2A	9.7	15.4	6.1	13.3	13.5	51.7
18-CSP-S3A	13	10.5	6.2	16.1	17.6	49.6
18-CSP-L4A	57	18.1	16.9	4.7	14.7	45.6
18-CSP-S5A	11	8.6	22.2	11.2	15.5	42.5
18-CSP-T6A	6.0	24.2	9.7	0.0	11.2	54.9
18-CSP-F7A	220	19.2	8.3	8.3	12.5	51.7
18-CSP-F8A	>1,000	10.9	4.0	14.6	14.3	56.2
18-CSP-R9A	27	10.2	4.3	13.3	18.0	54.1
18-CSP-L10A	6.5	7.0	9.6	16.0	18.5	48.9
18-CSP-F11A	410	20.7	10.7	0.0	11.1	57.5
18-CSP-N12A	33	6.3	20.1	11.6	16.1	45.8
18-CSP-R13A	2.7	3.3	53.1	0.0	16.4	27.2
18-CSP-S14A	4.5	9.5	2.3	14.8	16.2	57.1
18-CSP-F15A	--	10.9	5.9	15.3	16.7	51.2
18-CSP-T16A	64	11.7	14.0	12.0	15.9	46.5
18-CSP-Q17A	19	13.7	5.7	13.3	15.8	51.4

[a] Secondary structure contents were calculated using the BeStSel[1] (Beta Structure Selection) method.

Table S-14. EC_{50} values (SAB249) and estimated secondary structure contents (in 20% TFE) data for 18-CSP D-amino acid scan analogs.

Compound Name	EC_{50} (nM)	Estimated secondary structure contents (%)[a]				
		***α*-helix**	**Antiparallel *β*-sheet**	**Parallel *β*-sheet**	***β*-turn**	**Others**
18-CSP	6.2	27.1	6.8	0.0	9.1	57.0
18-CSP-s1	6.4	24.2	9.1	0.0	9.9	56.8
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18-CSP-s3	9.4	43.6	17.3	0.0	7.2	31.9
18-CSP-l4	4.5	21.5	21.5	0.0	13.3	43.7
18-CSP-s5	17	0.0	47.5	0.0	25.3	27.2
18-CSP-t6	300	8.1	0.0	22.2	16.5	53.2
18-CSP-f7	>1,000	1.3	28.2	14.7	13.6	42.2
18-CSP-f8	42	10.5	11.7	13.4	18.4	46.1
18-CSP-r9	76	4.2	51.7	1.7	14.1	28.3
18-CSP-l10	670	9.7	9.7	14.0	17.8	48.8
18-CSP-f11	--	13.3	7.0	12.5	17.1	50.1
18-CSP-n12	--	20.5	10.9	5.1	10.6	52.9
18-CSP-r13	480	19.7	7.7	11.4	12.7	48.5
18-CSP-s14	>1,000	23.4	8.4	8.9	12.5	46.8
18-CSP-f15	--	18.7	12.0	5.4	13.3	50.5
18-CSP-t16	>1,000	26.8	7.8	5.5	10.0	49.9
18-CSP-q17	130	27.5	6.9	6.1	9.6	50.0
18-CSP-a18	>1,000	33.4	8.7	0.0	8.1	49.8

[a] Secondary structure contents were calculated using the BeStSel[1] (Beta Structure Selection) method.

Table S-15. EC_{50} values (SAB249) and estimated secondary structure contents (in 20% TFE) data for 21-CSP and 18-CSP truncated & 2nd generation analogs.

Compound Name	EC_{50} (nM)	Estimated secondary structure contents (%)[a]				
		***α*-helix**	**Antiparallel *β*-sheet**	**Parallel *β*-sheet**	***β*-turn**	**Others**
21-CSP	240	27.1	16.4	0.0	9.7	46.7
21-CSP-des-S1	23	40.3	19.9	0.0	5.9	33.8
21-CSP-des-S1G2	120	32.6	9.8	0.0	7.6	50.1
21-CSP-des-S1G2S3	530	23.7	9.5	3.9	11.5	51.4
21-CSP-des-S1G2S3L4	3600	16.1	13.5	9.1	14.6	46.8
21-CSP-des-S1G2S3L4S5	3300	10.4	5.0	16.3	18.5	49.8
21-CSP-des-K21	98	42.0	16.6	0.0	8.4	33.1
21-CSP-des-G20K21	3600	16.7	5.2	11.3	11.8	55.0
18-CSP-des-S1	4.8	29.6	12.6	0.0	7.5	50.2
18-CSP-des-S1G2	7.9	15.1	5.6	11.3	15.3	52.6
18-CSP-des-S1G2S3	85	21.5	15.0	0.8	13.0	49.7
18-CSP-des-S1G2S3L4	570	9.3	2.5	14.5	17.9	55.8
18-CSP-des-S1G2S3L4S5	950	17.9	14.8	10.1	14.5	42.6
18-CSP-des-A18	--	14.2	7.8	14.7	13.7	49.6
18-CSP-des-Q17A18	--	23.7	4.8	11.8	9.7	50.0
18-CSP-s1s3l4	17	27.2	22.2	0.0	9.9	40.7
18-CSP-s1s3l4L10AR13AS14A	3.4	3.4	36.8	11.7	15.2	32.9
18-CSP-S1AL10AR13AS14A	1.7	13.2	12.5	10.3	15.6	48.4
18-CSP-S3T	2.1	38.8	25.7	0.0	7.0	28.6
21-CSP-S3T	94	30.1	10.2	0.0	11.4	48.3

[a] Secondary structure contents were calculated using the BeStSel[1] (Beta Structure Selection) method.

References

[1] Micsonai, A., Wien, F., Kernya, L., Lee, Y. H., Goto, Y., Réfrégiers, M., and Kardos, J. (2015) Accurate secondary structure prediction and fold recognition for circular dichroism spectroscopy, *Proc. Natl. Acad. Sci. U. S. A. 112*, E3095-3103 DOI 10.1073/pnas.1500851112.