Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Biological Evaluation of Native Streptococcal Competence Stimulating Peptides Reveals Potential Crosstalk Between *Streptococcus mitis* and *Streptococcus pneumoniae* and a New Scaffold for the Development of *S. pneumoniae* Quorum Sensing Modulators

Tahmina Ahmed Milly and Yftah Tal-Gan*

Department of Chemistry, University of Nevada, Reno, 1664 N. Virginia Street, Reno, Nevada 89557

Supporting Information.

* To whom correspondence should be addressed. ytalgan@unr.edu

Additional Experimental Details

Peptide Synthesis: 0.1 g of resin was first swelled by suspension in DMF for 15 min at room temperature and then drained. Deprotection of the Fmoc group was performed first using 5 mL of 20% piperidine in DMF (90 sec, 90 °C) followed by another 5 mL of 20% piperidine in DMF (90 sec, 90 °C). The resin was washed with DMF (3 x 5 mL) after each deprotection cycle. Coupling reactions were performed using 2.5 mL solution containing Fmoc-protected amino acid (5 equiv.), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU; 5 equiv.) and diisopropylethylamine (DIPEA; 5 equiv.). All amino acids were coupled for 20 min (30 W, 75 °C), except His. His was coupled for 10 min (0 W, 25 °C) then for 40 min (20 W, 50 °C). After the synthesis was completed the resin was washed with DMF (2 x 5 mL).

Cleavage: Upon completion of peptide synthesis, the resin containing the final peptide product 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 the protecting groups, by mixing the resin with 3 mL of 2.5% de-ionized water and 2.5% triisopropylsilane (TIPS) in trifluoroacetic acid (TFA) for 3 h with agitation. The cleaved peptide 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 in a freezer at -20 °C for 10 min. The pellet of the crude peptide was obtained by centrifuging the mixture at 3000 RPM for 5 min and the supernatant was removed to yield crude peptide, which was dissolved in 10 mL acetonitrile (ACN):water (1:1) and lyophilized before HPLC purification.

Peptide Verification with Mass Spectrometry: During purification, MALDI-TOF MS was used to verify the peaks containing the desired peptide. Samples were prepared using α-Cyano-4-hydroxycinammic acid as matrix and aliquots were taken directly from the preparative HPLC fractions. The exact masses of the peptides were obtained with a high resolution ESI-TOF MS for the final verification of the peptides. 8 - 30 μM stock solutions were prepared in acetonitrile (ACN):water (1:1). The instrument was calibrated before each run and an internal reference mass standard was used.

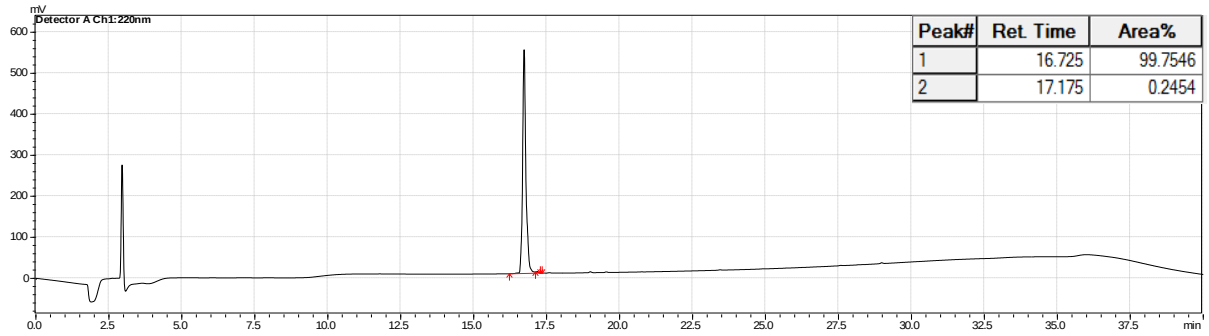
Beta-Galactosidase Assays: The ability of synthetic CSP analogues to activate the expression of *S. pneumoniae comX* was determined using indicated reporter strains grown in THY (pH 7.3). An initial activation screening was performed at a high concentration (10 μM) for all CSP analogues. 198 μL bacterial culture were placed in triplicate to a 96-well plate containing 2 μL of CSP peptides and incubated at 37 °C for 30 min. A total of 2 μL of 20 μM solution of *S. pneumoniae* CSP1 (200 nM final concentration) were added in triplicate and served as the positive control for the *S. pneumoniae* group I strain (D39pcomX::lacZ), while 2 μL of 100 μM solution of *S. pneumoniae* CSP2 (1000 nM final concentration) were added as the positive control for the *S. pneumoniae* group II strain (TIGR4pcomX::lacZ). These concentrations were chosen to afford full activation of the QS circuit, as determined from the dose-dependent curves created for the native *S. pneumoniae* CSPs.[2] Two μL dimethyl sulfoxide (DMSO) were added in triplicate and served as the negative control. After the incubation time (30 min) had elapsed, the absorbance at 600_{nm} was read. The cells were then lysed by incubating the culture for 30 min at 37 °C with 20 μL 0.1% Triton X-100 in water. In a new plate, 100 μL Z-buffer solution (60.2 mM Na_2HPO_4, 45.8 mM NaH_2PO_4, 10 mM KCl, and 1.0 mM $MgSO_4$ in 18 MΩ H_2O; pH was adjusted to 7.0 and the buffer was sterilized before use) containing 2-Nitrophenyl-Beta-D-galactopyranoside (ONPG) at a final concentration of 0.4 mg/mL were added, followed by 100 μL lysate, and the plate was incubated for 3 hours at 37 °C. After the incubation, the reaction was stopped by adding 50 μL of 1 M sodium carbonate solution, and the OD 420_{nm} and OD 550_{nm} were measured using a plate reader, allowing for the calculation of the activity in Miller units (see below). The results were reported as percent activation, which is the ratio between the Miller units of the analogue and that of the positive control.

$$Miller\ Unit = 1000 \times \frac{[Abs_{420} - (1.75 \times Abs_{550})]}{(t \times v \times Abs_{600})}$$

Abs_{420} is the absorbance of o-nitrophenol (ONP). Abs_{550} is the scatter from cell debris, which, when multiplied by 1.75 approximates the scatter observed at 420 nm. *t* is the duration of incubation with ONPG in minutes, *v* is volume of lysate in milliliters, and Abs_{600} reflects cell density.

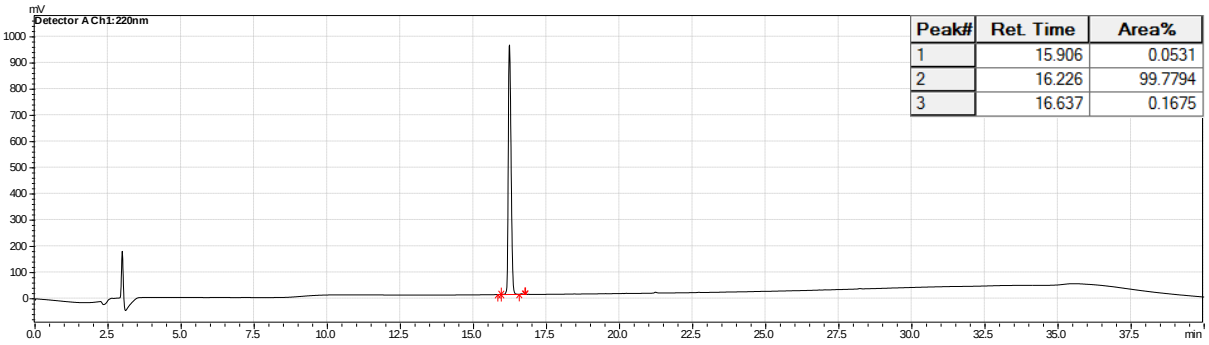
HPLC traces for CSP analogues

S. mitis-CSP-1



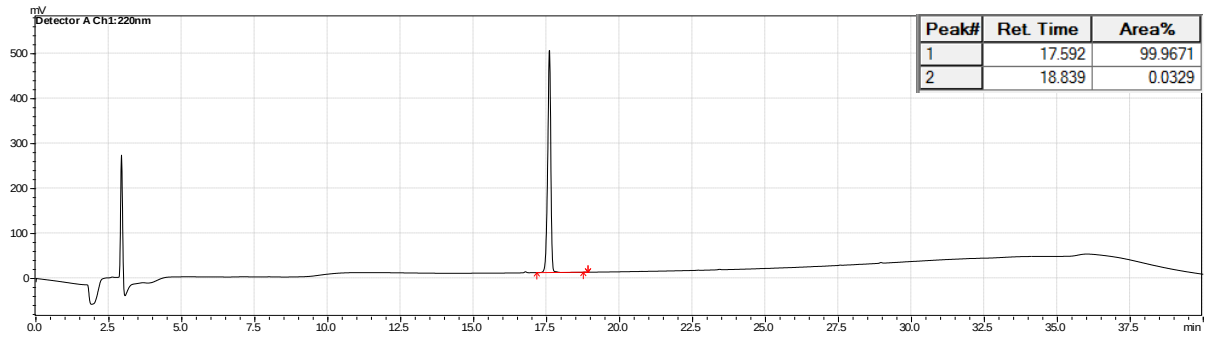
Peak#	Ret. Time	Area%
1	16.725	99.7546
2	17.175	0.2454

S. mitis-CSP-2



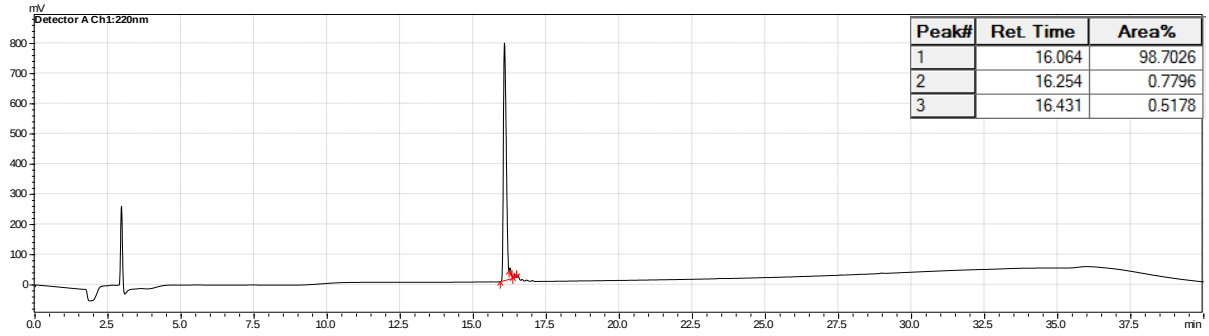
Peak#	Ret. Time	Area%
1	15.906	0.0531
2	16.226	99.7794
3	16.637	0.1675

S. oralis-CSP



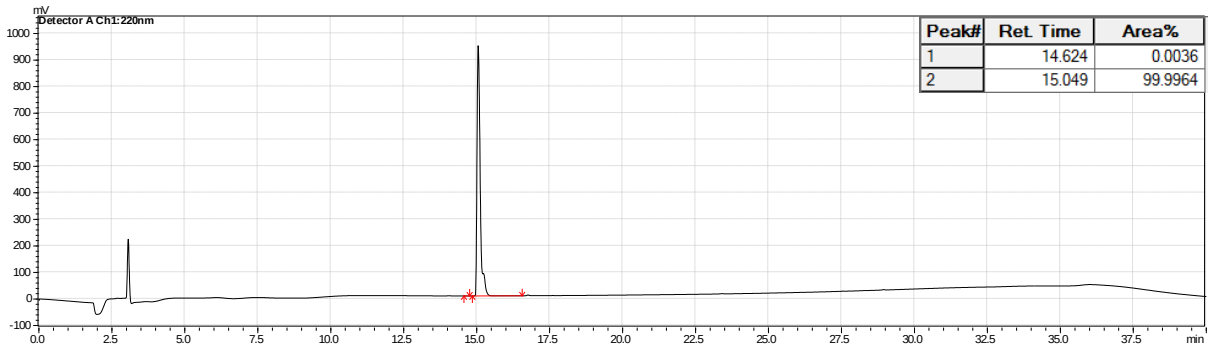
Peak#	Ret. Time	Area%
1	17.592	99.9671
2	18.839	0.0329

S. cristatus-CSP



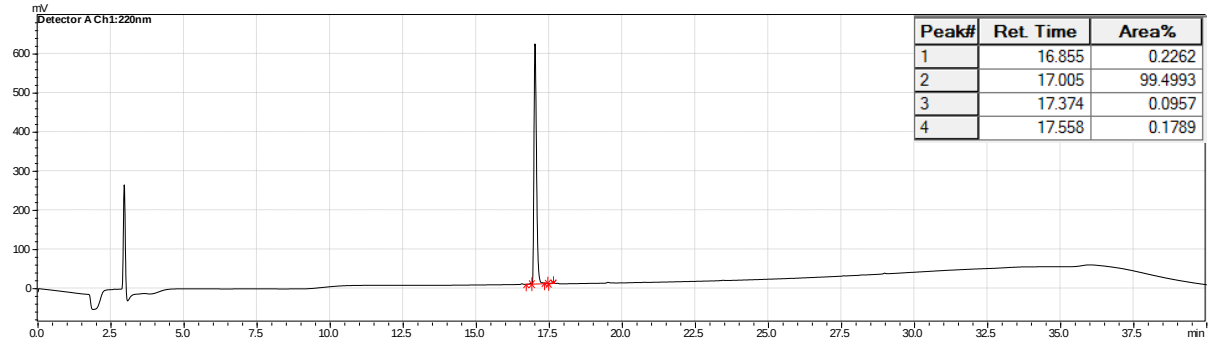
Peak#	Ret. Time	Area%
1	16.064	98.7026
2	16.254	0.7796
3	16.431	0.5178

S. oligofermentans-CSP



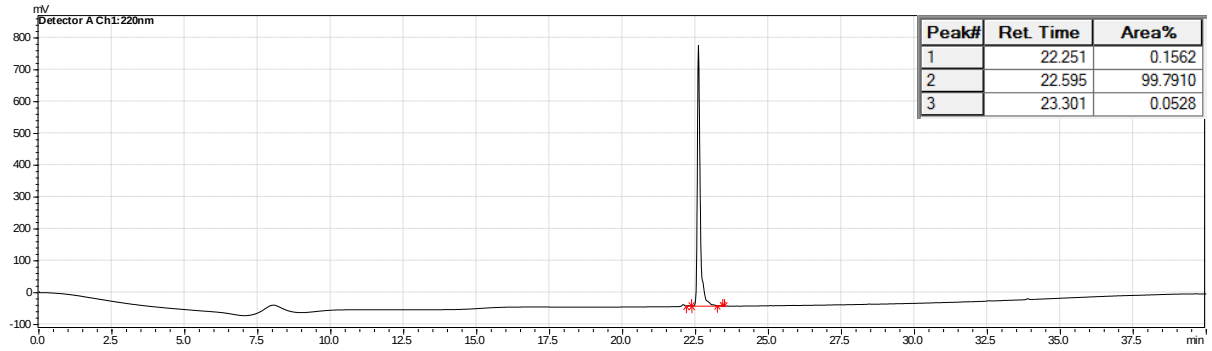
Peak#	Ret. Time	Area%
1	14.624	0.0036
2	15.049	99.9964

S. intermedius-CSP



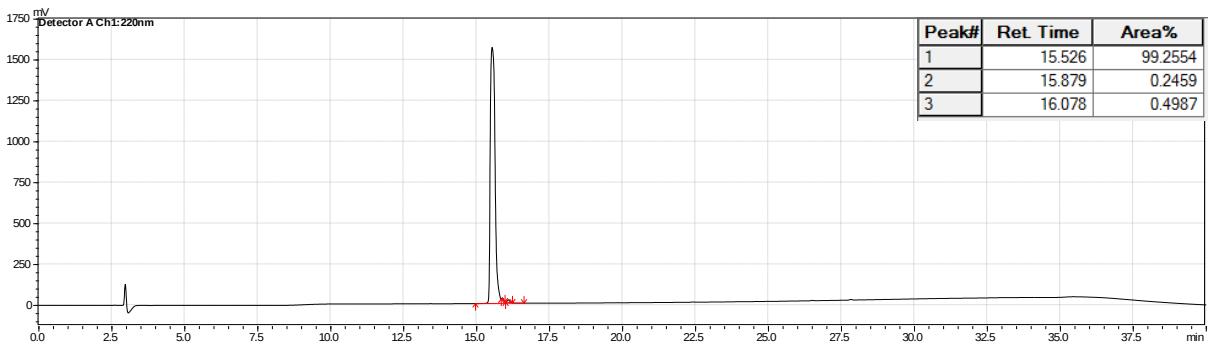
Peak#	Ret. Time	Area%
1	16.855	0.2262
2	17.005	99.4993
3	17.374	0.0957
4	17.558	0.1789

S. gordonii-CSP-1



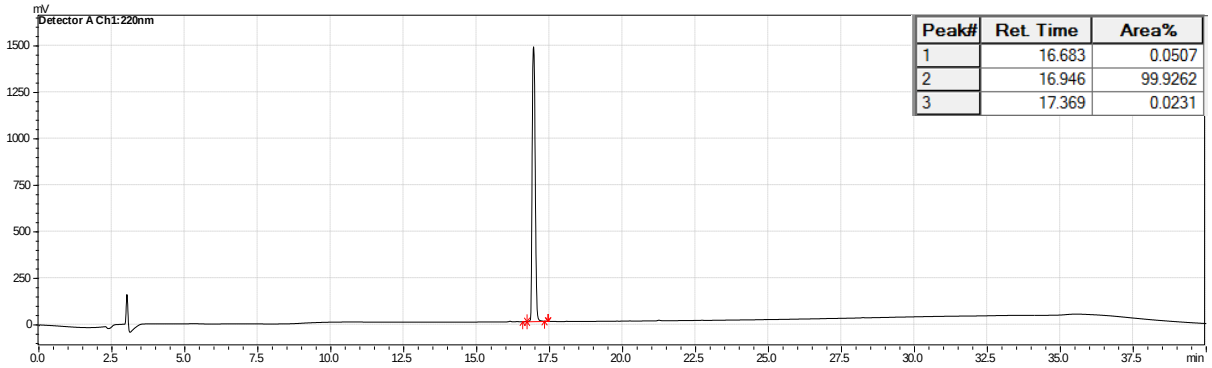
Peak#	Ret. Time	Area%
1	22.251	0.1562
2	22.595	99.7910
3	23.301	0.0528

S. gordonii-CSP-2



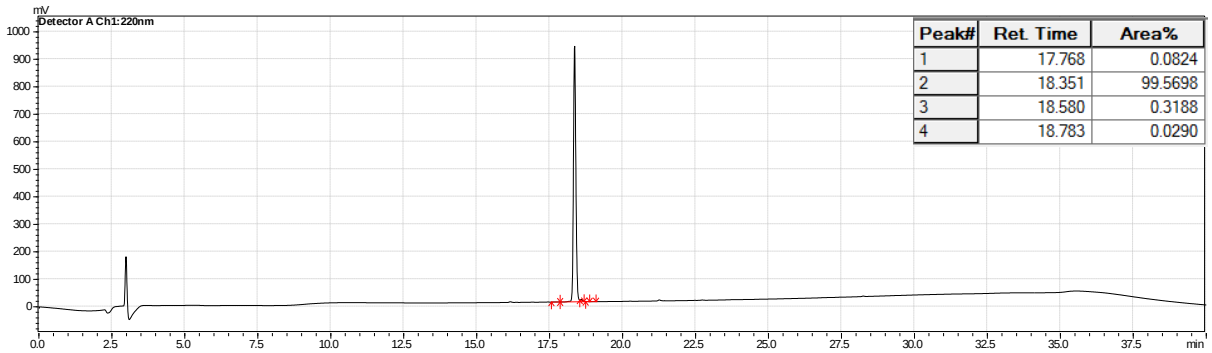
Peak#	Ret. Time	Area%
1	15.526	99.2554
2	15.879	0.2459
3	16.078	0.4987

S. gordonii challis-CSP



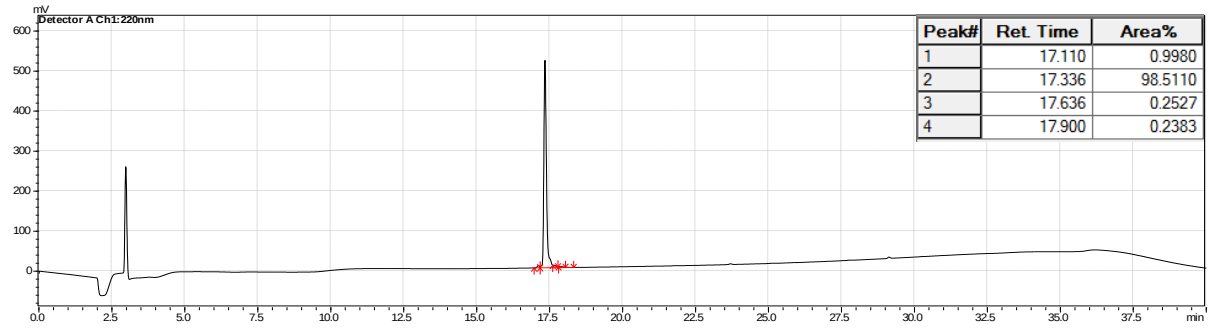
Peak#	Ret. Time	Area%
1	16.683	0.0507
2	16.946	99.9262
3	17.369	0.0231

S. sanguinis-CSP



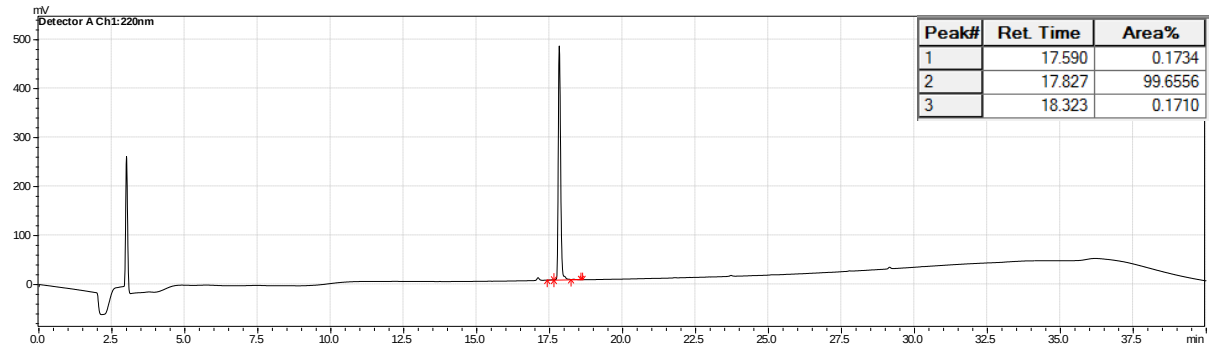
Peak#	Ret. Time	Area%
1	17.768	0.0824
2	18.351	99.5698
3	18.580	0.3188
4	18.783	0.0290

S. mitis-CSP-2-I2M



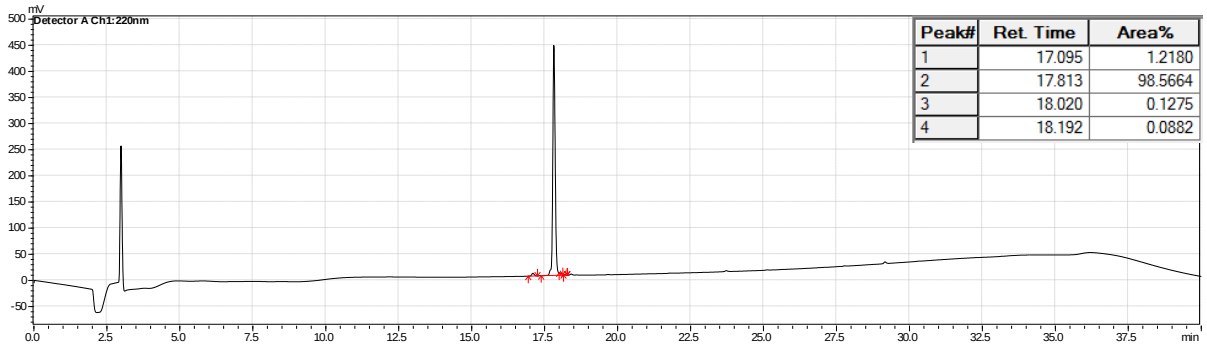
Peak#	Ret. Time	Area%
1	17.110	0.9980
2	17.336	98.5110
3	17.636	0.2527
4	17.900	0.2383

S. mitis-CSP-2-Q4L



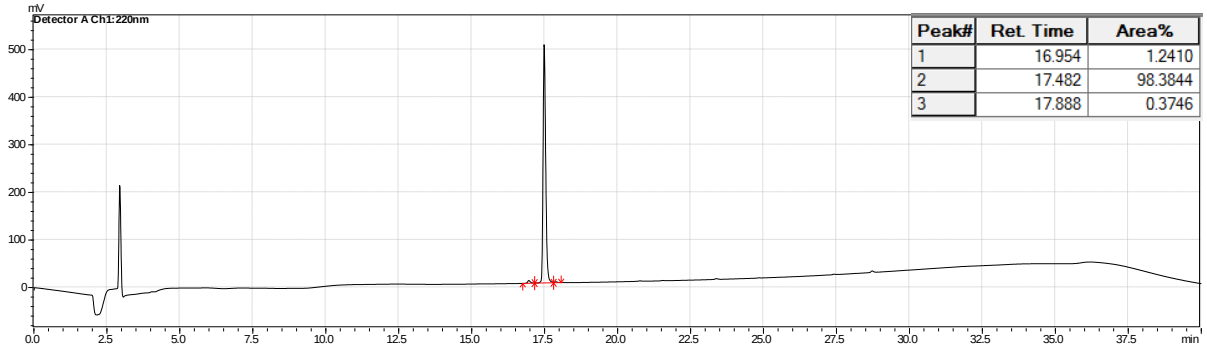
Peak#	Ret. Time	Area%
1	17.590	0.1734
2	17.827	99.6556
3	18.323	0.1710

S. mitis-CSP-2-N7F



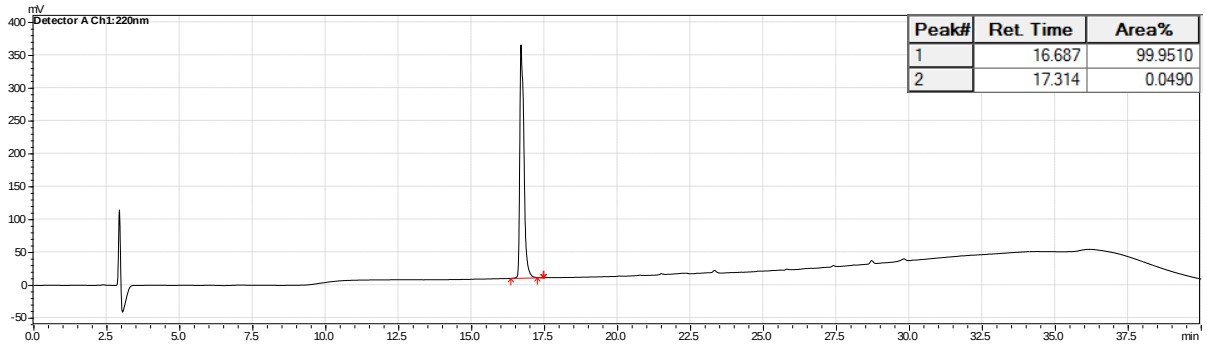
Peak#	Ret. Time	Area%
1	17.095	1.2180
2	17.813	98.5664
3	18.020	0.1275
4	18.192	0.0882

S. mitis-CSP-2-I8F



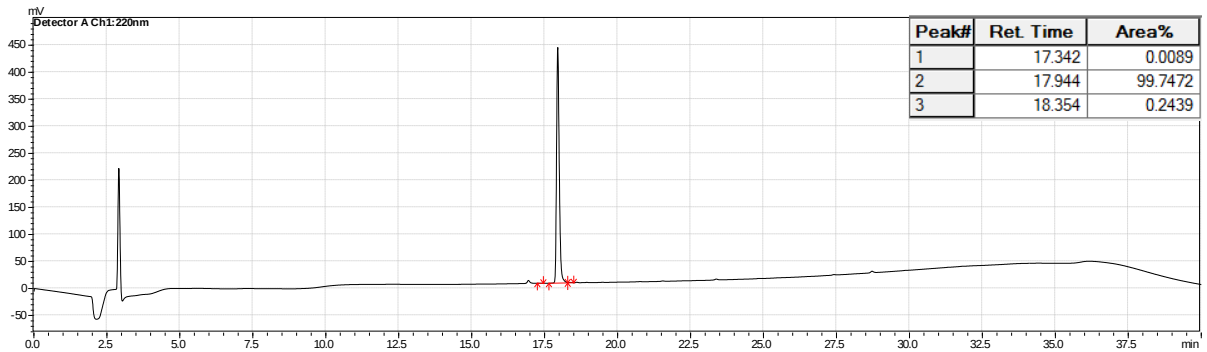
Peak#	Ret. Time	Area%
1	16.954	1.2410
2	17.482	98.3844
3	17.888	0.3746

S. mitis-CSP-2-F10D



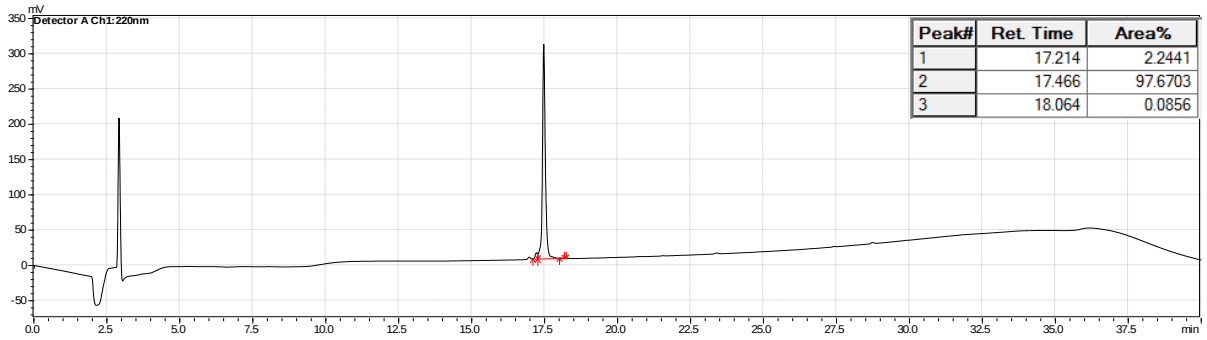
Peak#	Ret. Time	Area%
1	16.687	99.9510
2	17.314	0.0490

S. mitis-CSP-2-N11F



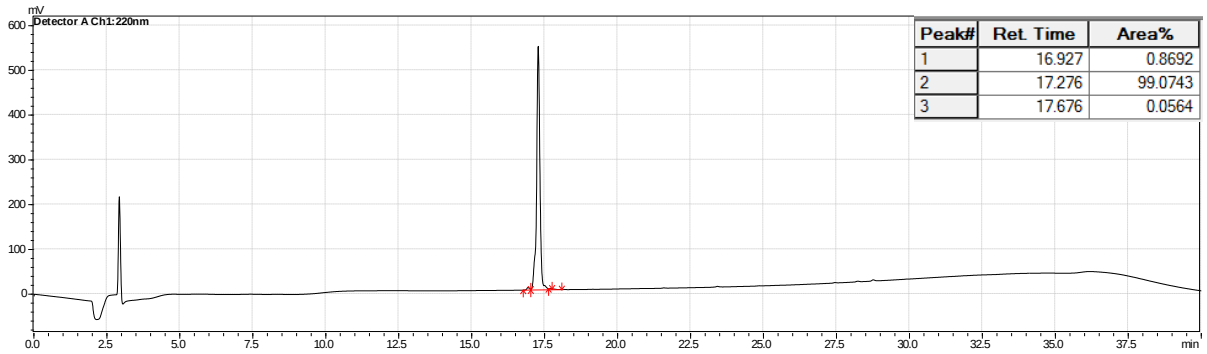
Peak#	Ret. Time	Area%
1	17.342	0.0089
2	17.944	99.7472
3	18.354	0.2439

S. mitis-CSP-2-F12I



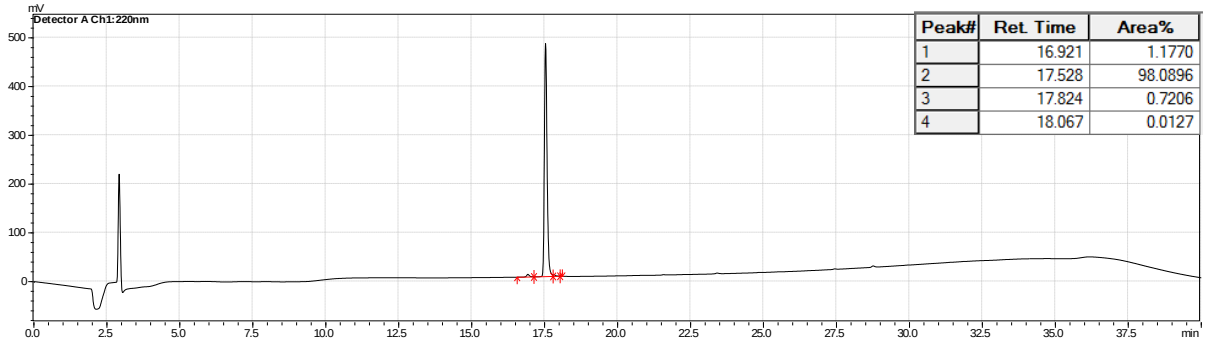
Peak#	Ret. Time	Area%
1	17.214	2.2441
2	17.466	97.6703
3	18.064	0.0856

S. mitis-CSP-2-F12L



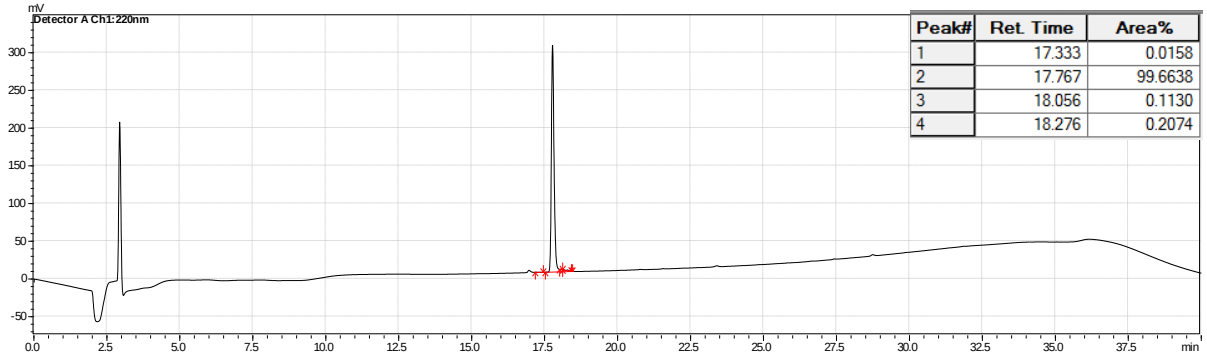
Peak#	Ret. Time	Area%
1	16.927	0.8692
2	17.276	99.0743
3	17.676	0.0564

S. mitis-CSP-2-N7I



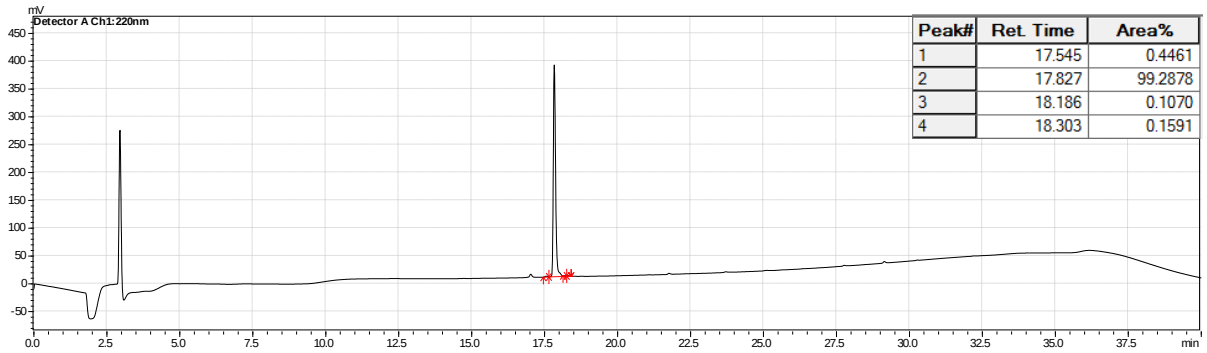
Peak#	Ret. Time	Area%
1	16.921	1.1770
2	17.528	98.0896
3	17.824	0.7206
4	18.067	0.0127

S. mitis-CSP-2-Q4I



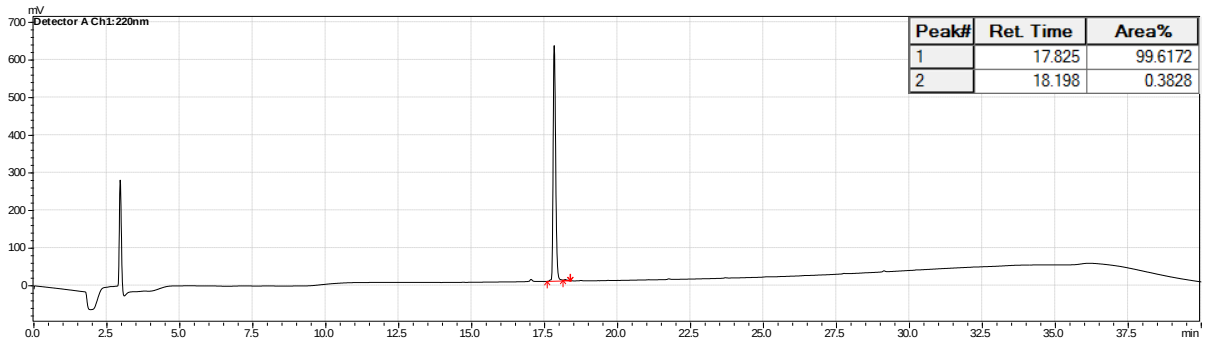
Peak#	Ret. Time	Area%
1	17.333	0.0158
2	17.767	99.6638
3	18.056	0.1130
4	18.276	0.2074

S. mitis-CSP-2-I2MQ4L



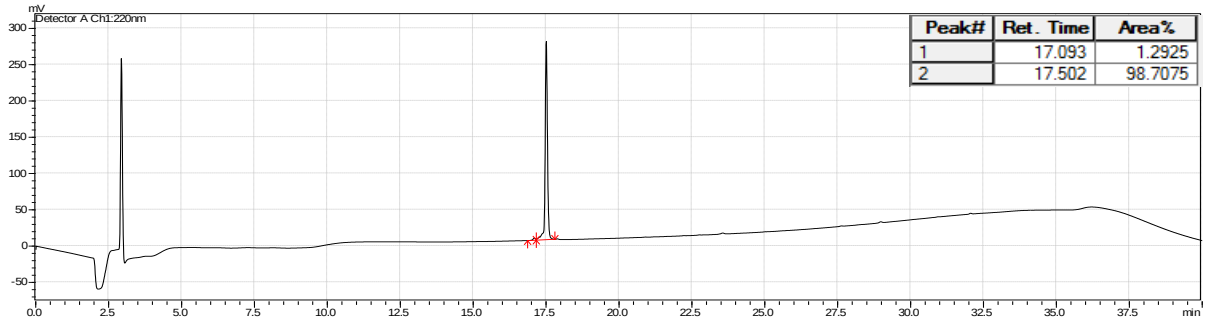
Peak#	Ret. Time	Area%
1	17.545	0.4461
2	17.827	99.2878
3	18.186	0.1070
4	18.303	0.1591

S. mitis-CSP-2-I2MN7F



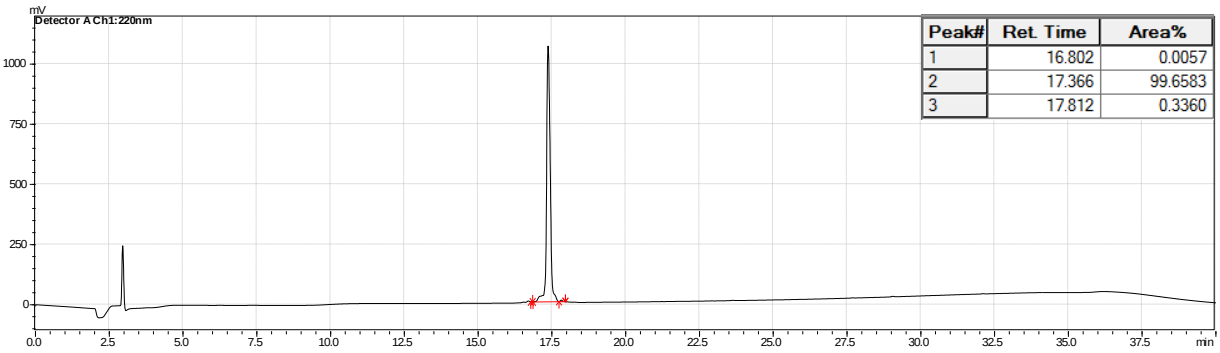
Peak#	Ret. Time	Area%
1	17.825	99.6172
2	18.198	0.3828

S. mitis-CSP-2-I2MN7I



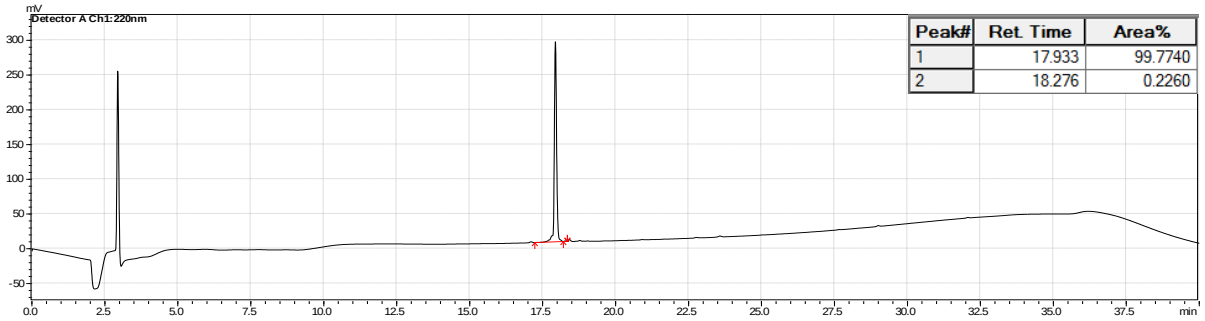
Peak#	Ret. Time	Area%
1	17.093	1.2925
2	17.502	98.7075

S. mitis-CSP-2-I2MI8F



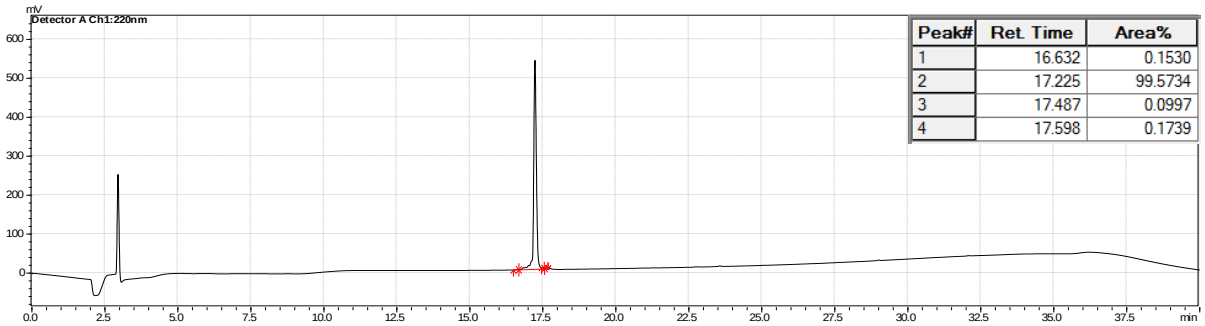
Peak#	Ret. Time	Area%
1	16.802	0.0057
2	17.366	99.6583
3	17.812	0.3360

S. mitis-CSP-2-I2MN11F



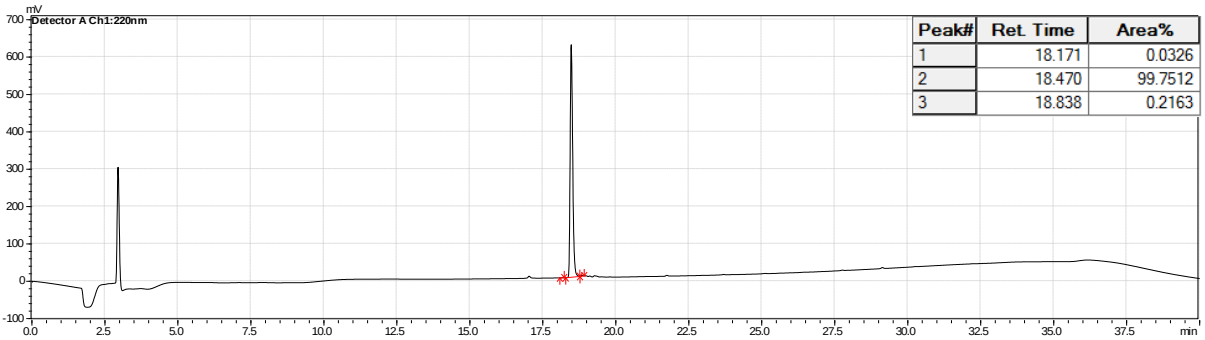
Peak#	Ret. Time	Area%
1	17.933	99.7740
2	18.276	0.2260

S. mitis-CSP-2-I2MF12L



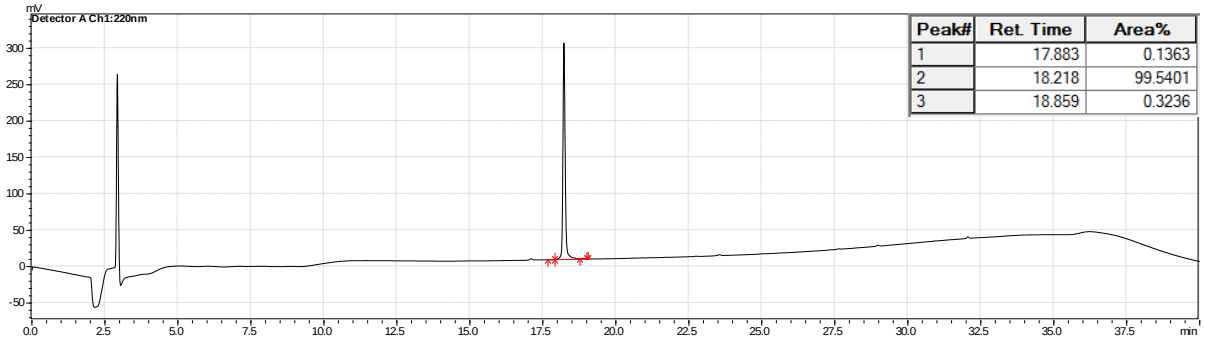
Peak#	Ret. Time	Area%
1	16.632	0.1530
2	17.225	99.5734
3	17.487	0.0997
4	17.598	0.1739

S. mitis-CSP-2-Q4LN7F



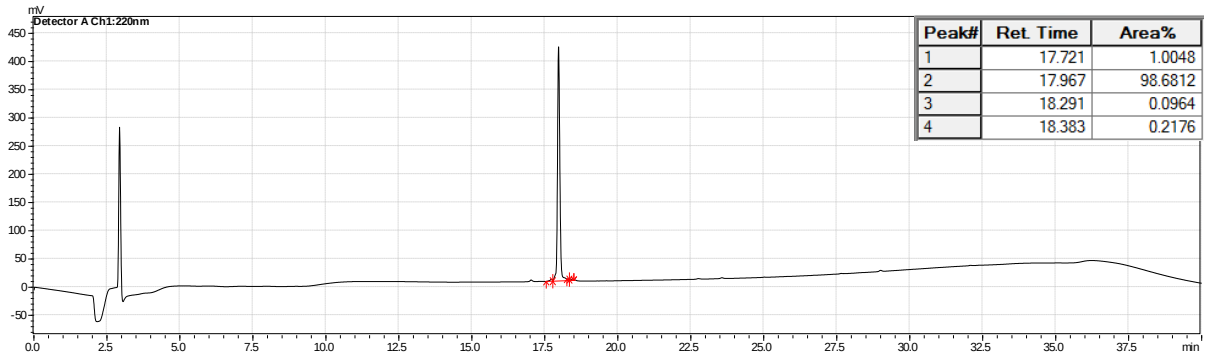
Peak#	Ret. Time	Area%
1	18.171	0.0326
2	18.470	99.7512
3	18.838	0.2163

S. mitis-CSP-2-Q4LN7I



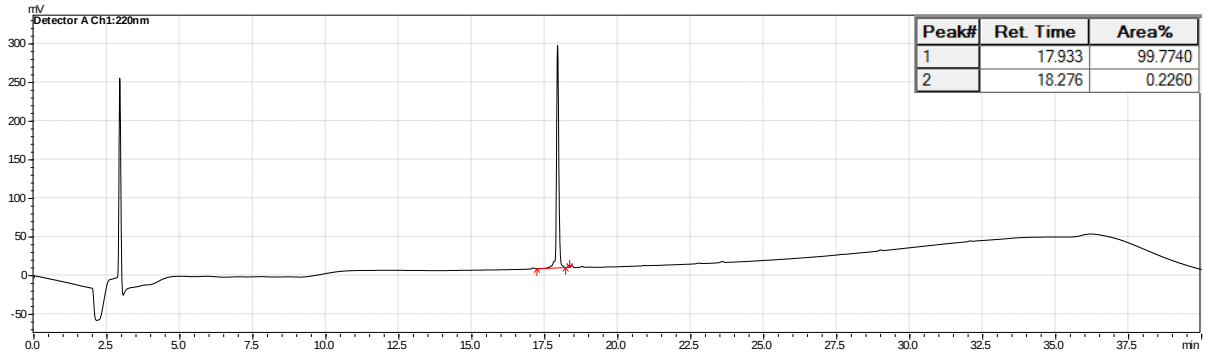
Peak#	Ret. Time	Area%
1	17.883	0.1363
2	18.218	99.5401
3	18.859	0.3236

S. mitis-CSP-2-Q4LI8F



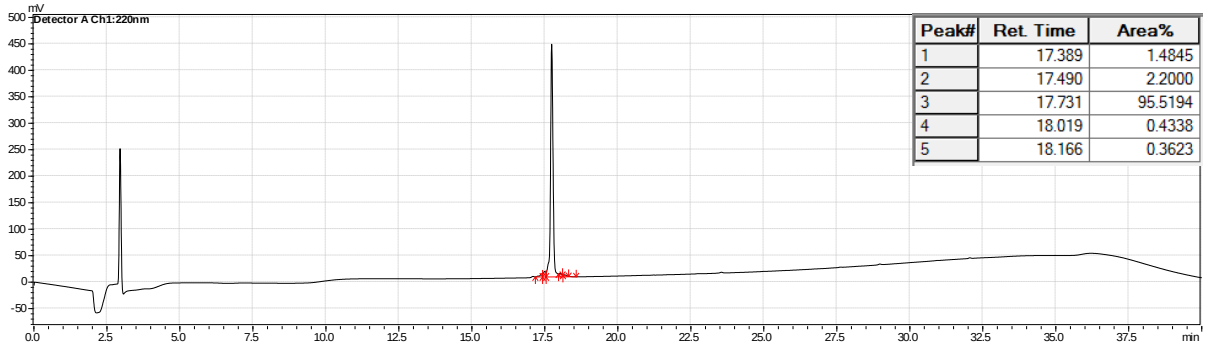
Peak#	Ret. Time	Area%
1	17.721	1.0048
2	17.967	98.6812
3	18.291	0.0964
4	18.383	0.2176

S. mitis-CSP-2-Q4LN11F



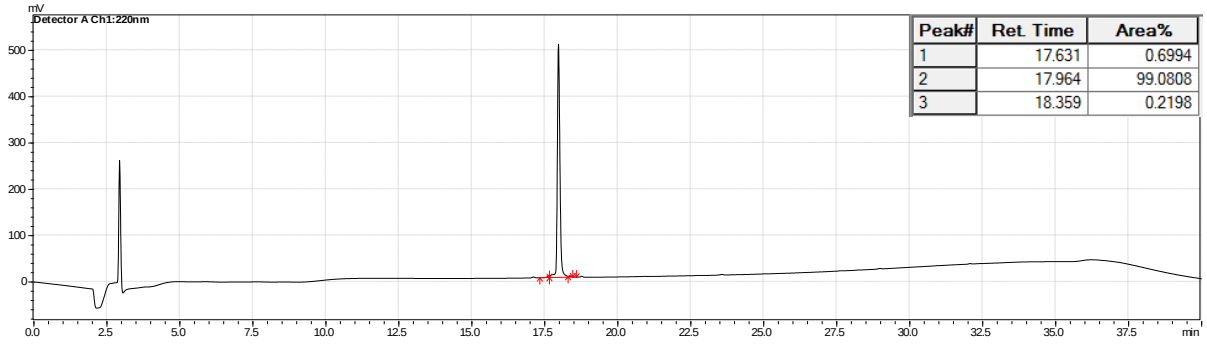
Peak#	Ret. Time	Area%
1	17.933	99.7740
2	18.276	0.2260

S. mitis-CSP-2-Q4LF12L



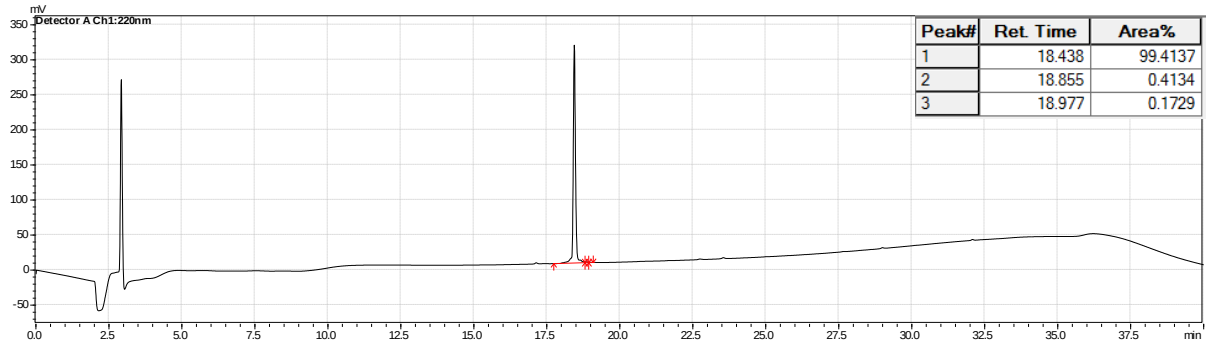
Peak#	Ret. Time	Area%
1	17.389	1.4845
2	17.490	2.2000
3	17.731	95.5194
4	18.019	0.4338
5	18.166	0.3623

S. mitis-CSP-2-N7FI8F



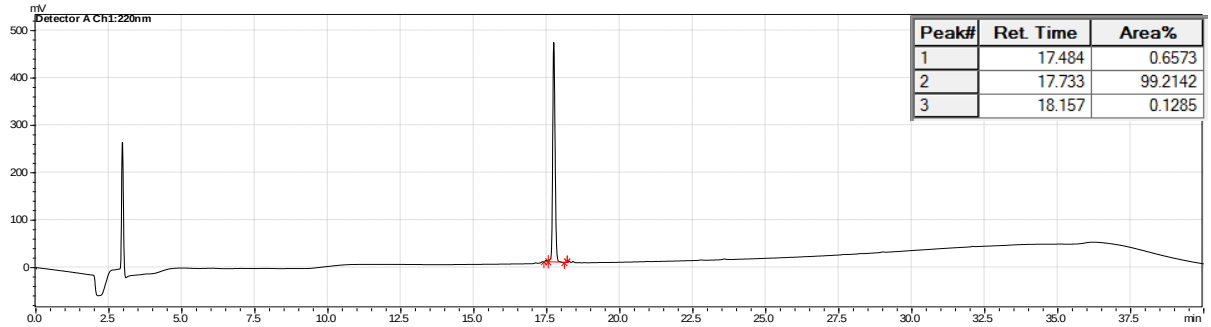
Peak#	Ret. Time	Area%
1	17.631	0.6994
2	17.964	99.0808
3	18.359	0.2198

S. mitis-CSP-2-N7FN11F



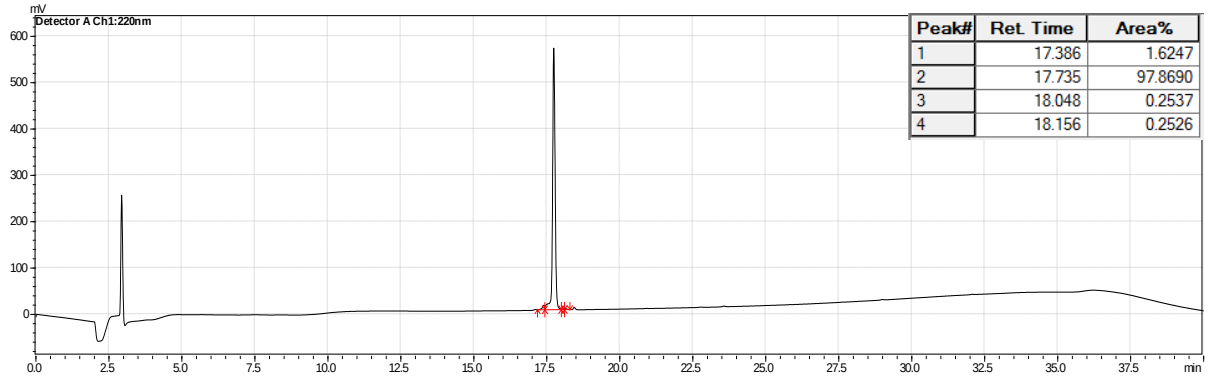
Peak#	Ret. Time	Area%
1	18.438	99.4137
2	18.855	0.4134
3	18.977	0.1729

S. mitis-CSP-2-N7FF12L



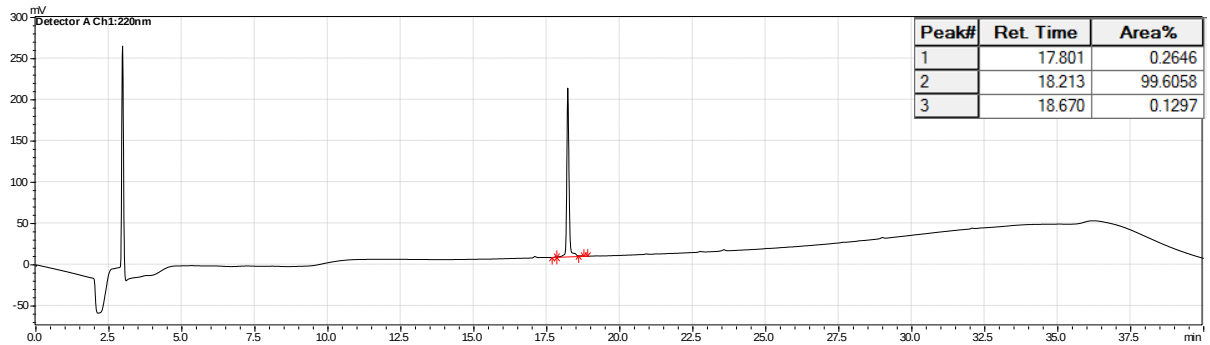
Peak#	Ret. Time	Area%
1	17.484	0.6573
2	17.733	99.2142
3	18.157	0.1285

S. mitis-CSP-2-N7II8F



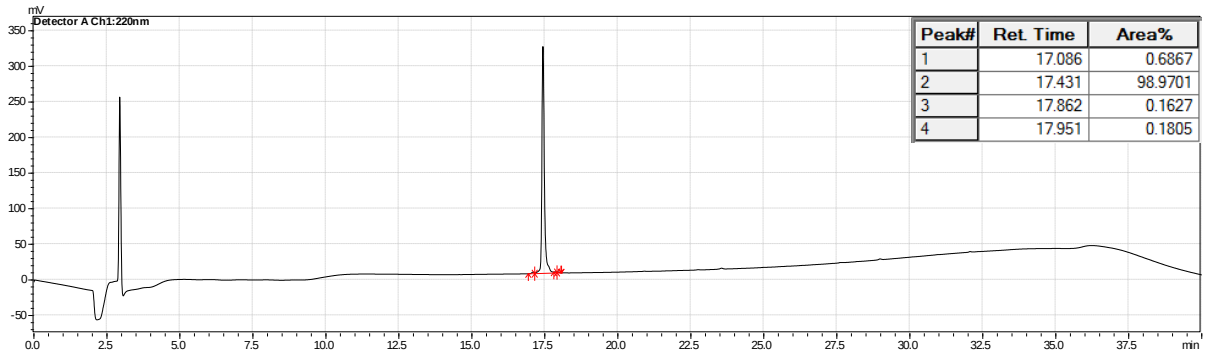
Peak#	Ret. Time	Area%
1	17.386	1.6247
2	17.735	97.8690
3	18.048	0.2537
4	18.156	0.2526

S. mitis-CSP-2-N7IN11F



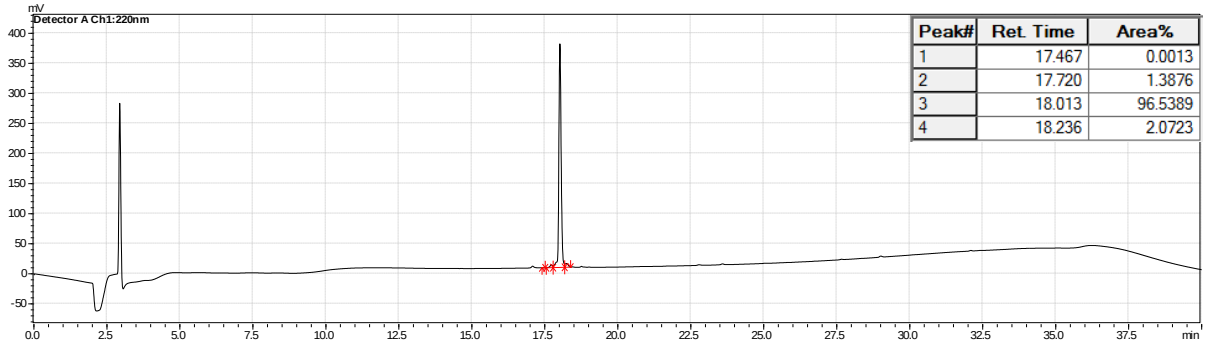
Peak#	Ret. Time	Area%
1	17.801	0.2646
2	18.213	99.6058
3	18.670	0.1297

S. mitis-CSP-2-N7IF12L



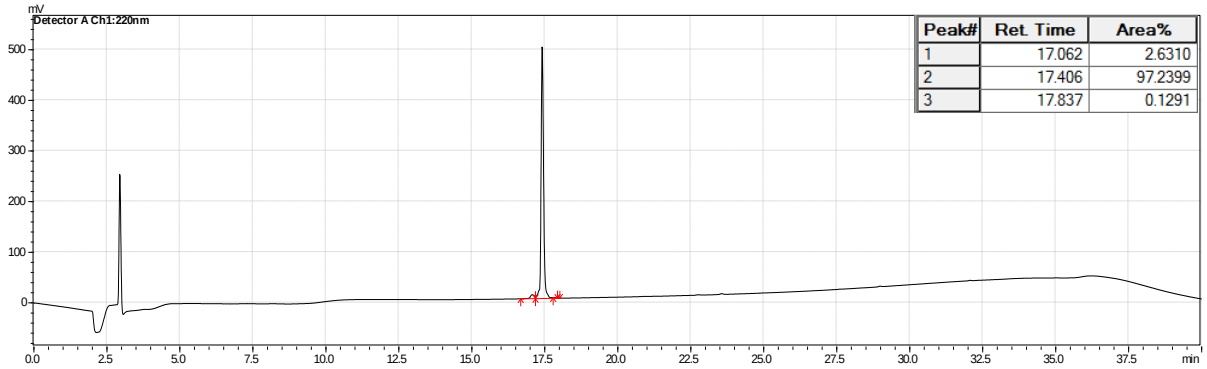
Peak#	Ret. Time	Area%
1	17.086	0.6867
2	17.431	98.9701
3	17.862	0.1627
4	17.951	0.1805

S. mitis-CSP-2-I8FN11F



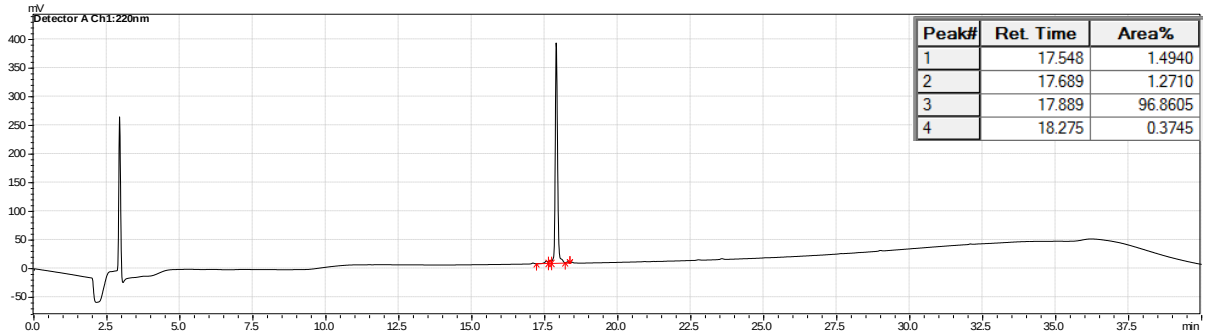
Peak#	Ret. Time	Area%
1	17.467	0.0013
2	17.720	1.3876
3	18.013	96.5389
4	18.236	2.0723

S. mitis-CSP-2-I8FF12L



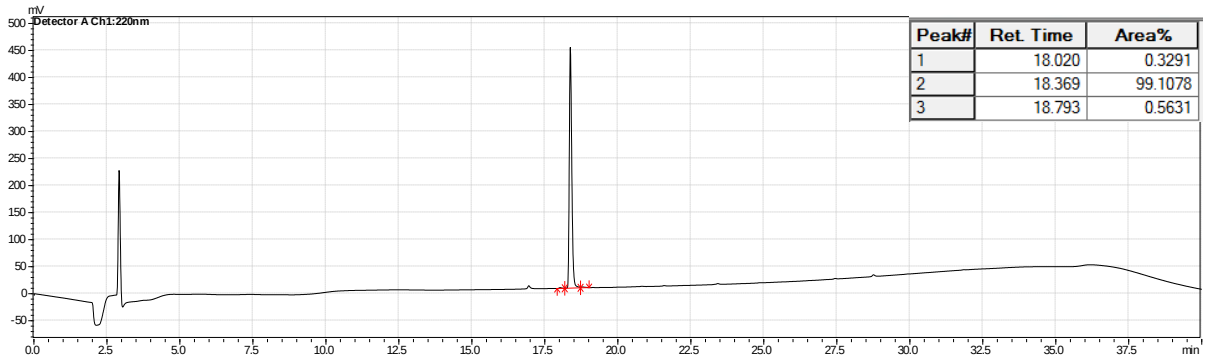
Peak#	Ret. Time	Area%
1	17.062	2.6310
2	17.406	97.2399
3	17.837	0.1291

S. mitis-CSP-2-N11FF12L



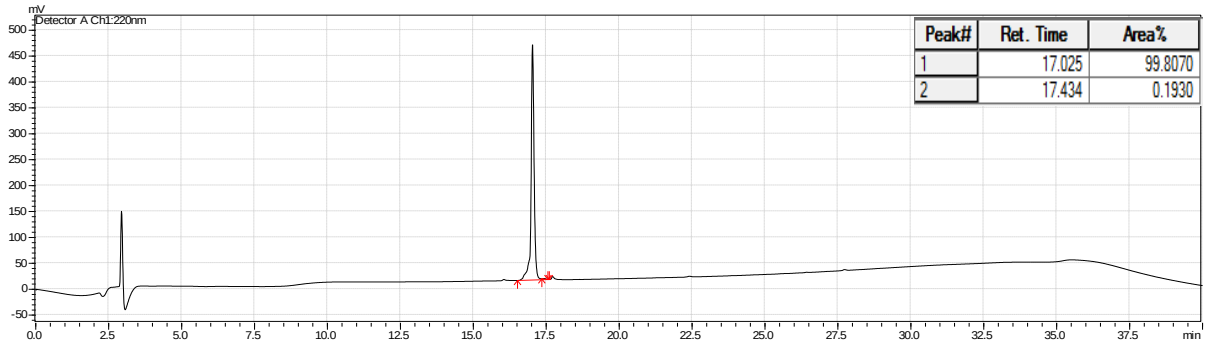
Peak#	Ret. Time	Area%
1	17.548	1.4940
2	17.689	1.2710
3	17.889	96.8605
4	18.275	0.3745

S. mitis-CSP-2-I2MQ4LN7F



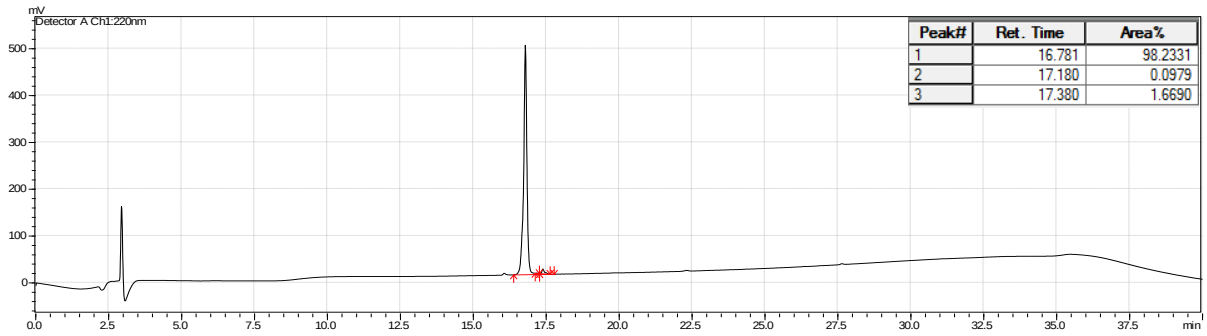
Peak#	Ret. Time	Area%
1	18.020	0.3291
2	18.369	99.1078
3	18.793	0.5631

S. mitis-CSP-2-I2MI8FN11F



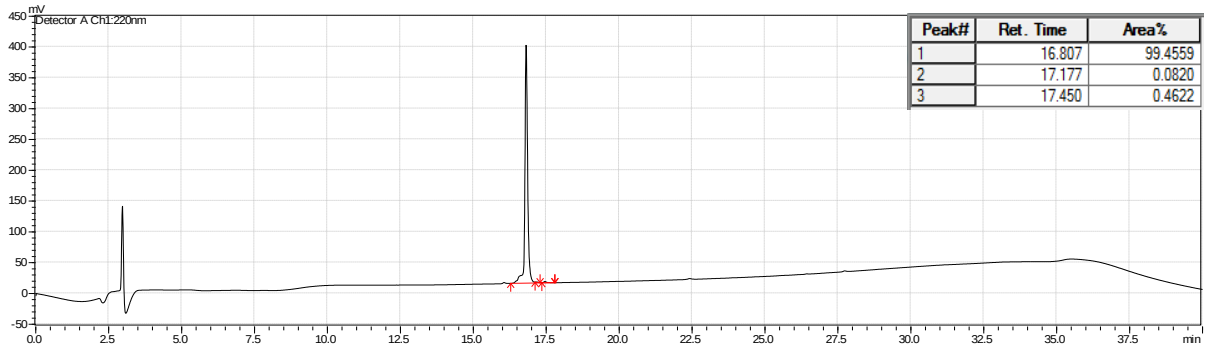
Peak#	Ret. Time	Area%
1	17.025	99.8070
2	17.434	0.1930

S. mitis-CSP-2-I2MN7FF12L



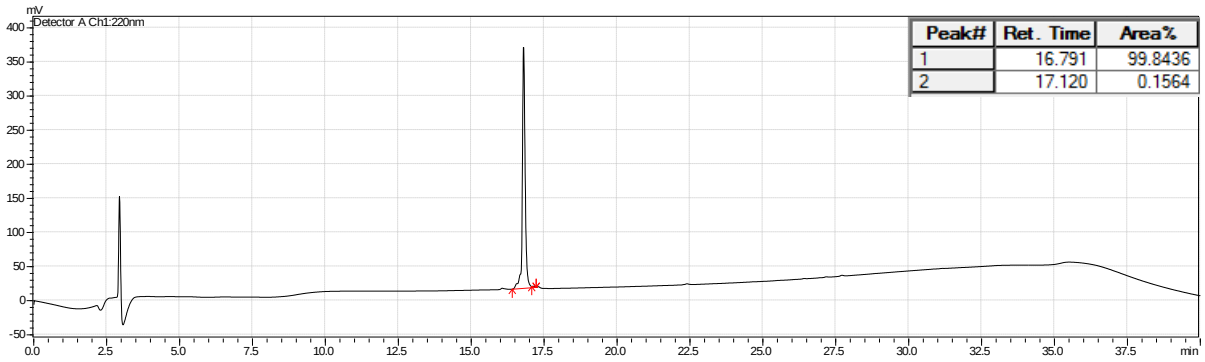
Peak#	Ret. Time	Area%
1	16.781	98.2331
2	17.180	0.0979
3	17.380	1.6690

S. mitis-CSP-2-I2MN7II8F



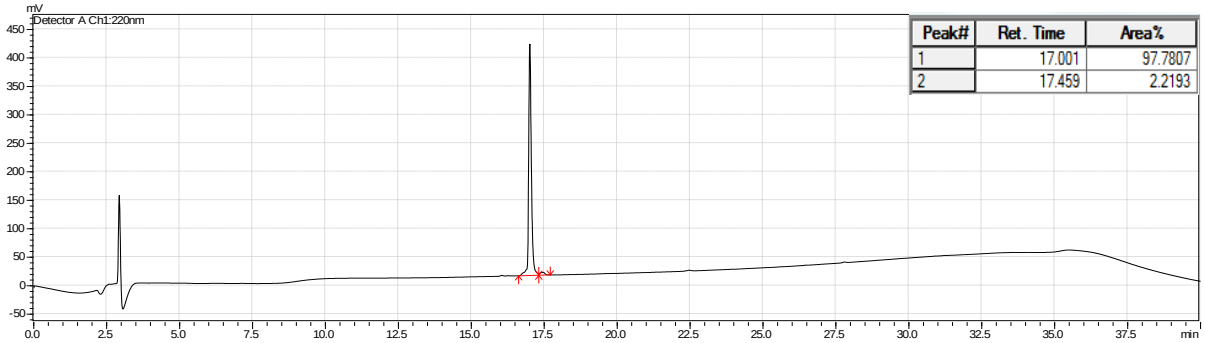
Peak#	Ret. Time	Area%
1	16.807	99.4559
2	17.177	0.0820
3	17.450	0.4622

S. mitis-CSP-2-I2MQ4LF12L



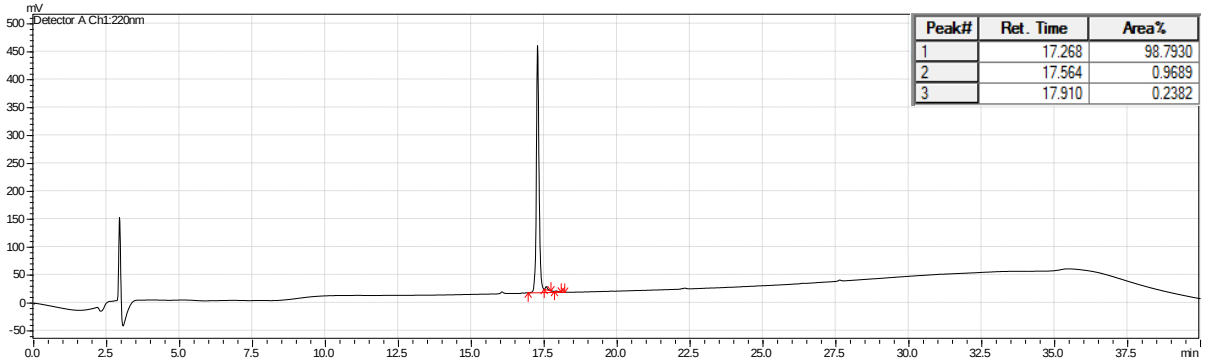
Peak#	Ret. Time	Area%
1	16.791	99.8436
2	17.120	0.1564

S. mitis-CSP-2-I2MQ4LI8F



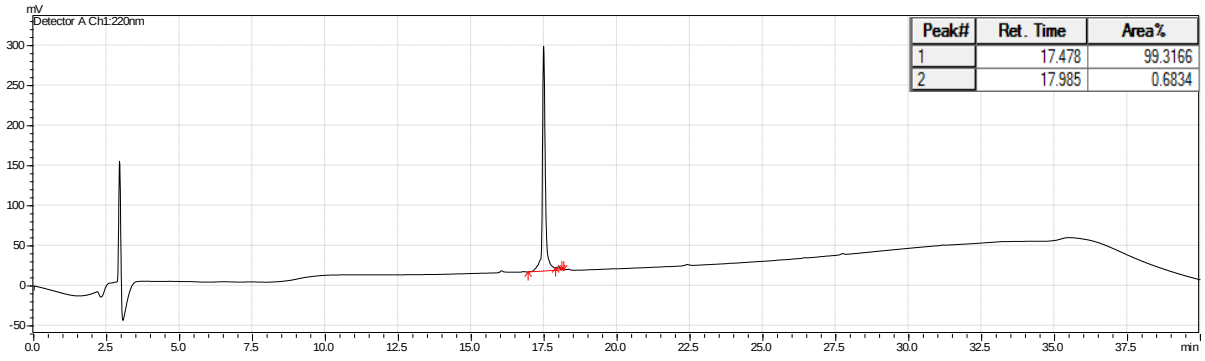
Peak#	Ret. Time	Area%
1	17.001	97.7807
2	17.459	2.2193

S. mitis-CSP-2-I2MQ4LN7I



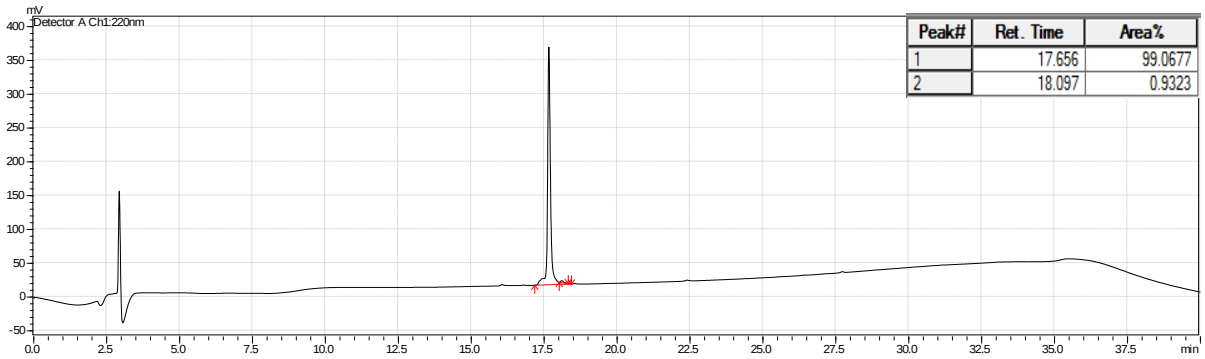
Peak#	Ret. Time	Area%
1	17.268	98.7930
2	17.564	0.9689
3	17.910	0.2382

S. mitis-CSP-2-I2MQ4LN11F



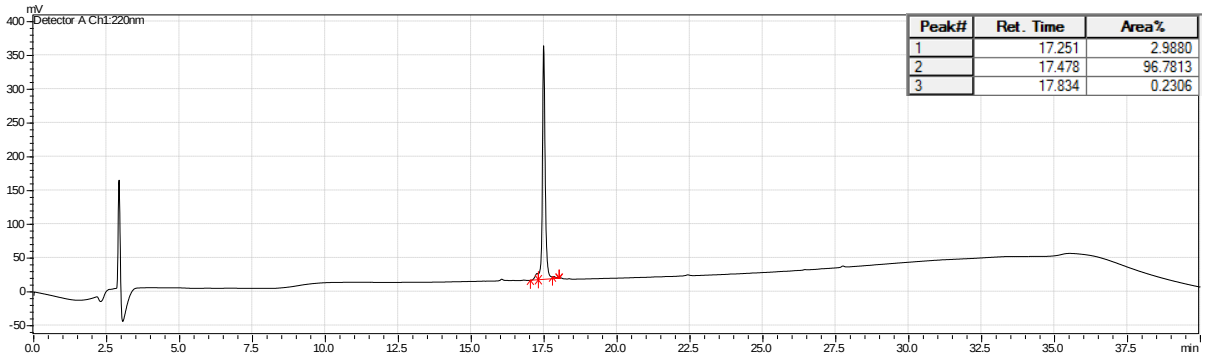
Peak#	Ret. Time	Area%
1	17.478	99.3166
2	17.985	0.6834

S. mitis-CSP-2-Q4LN7FI8F



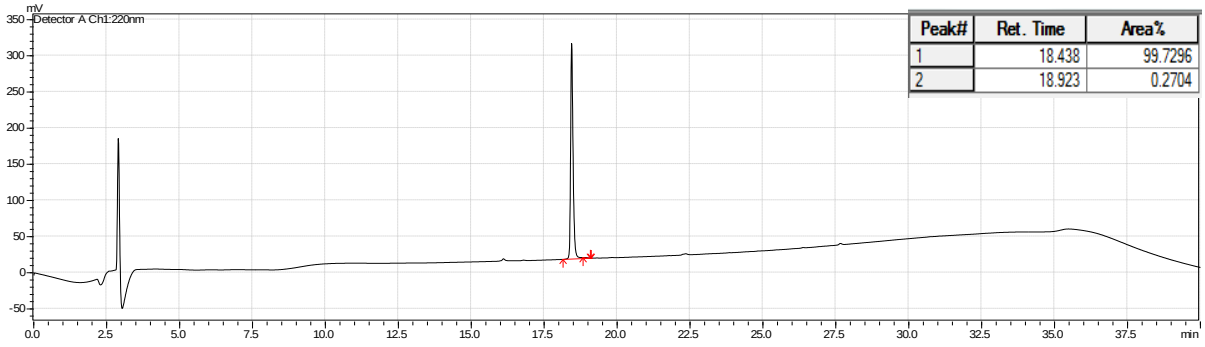
Peak#	Ret. Time	Area%
1	17.656	99.0677
2	18.097	0.9323

S. mitis-CSP-2-Q4LN7II8F



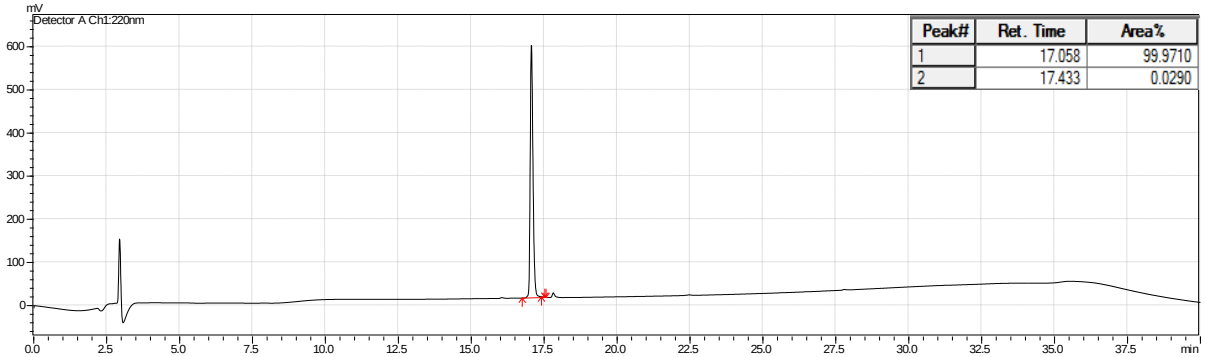
Peak#	Ret. Time	Area%
1	17.251	2.9880
2	17.478	96.7813
3	17.834	0.2306

S. mitis-CSP-2-Q4LN7FN11F



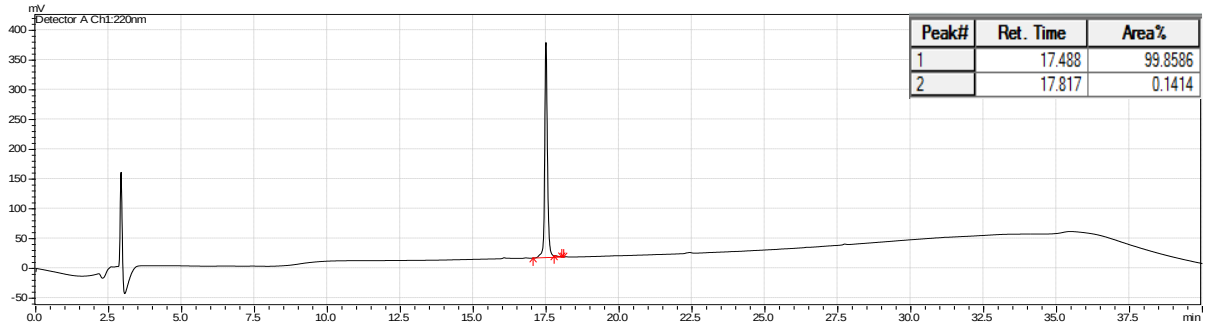
Peak#	Ret. Time	Area%
1	18.438	99.7296
2	18.923	0.2704

S. mitis-CSP-2-I2MN7FI8F



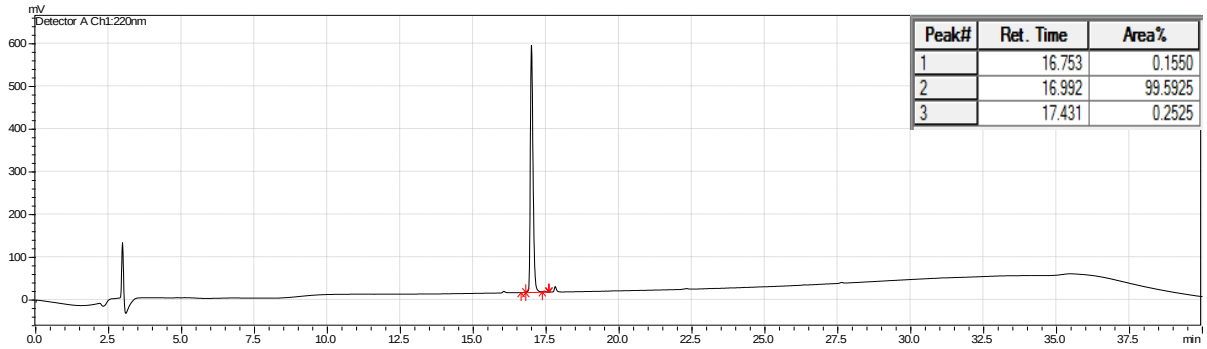
Peak#	Ret. Time	Area%
1	17.058	99.9710
2	17.433	0.0290

S. mitis-CSP-2-Q4LN7FF12L



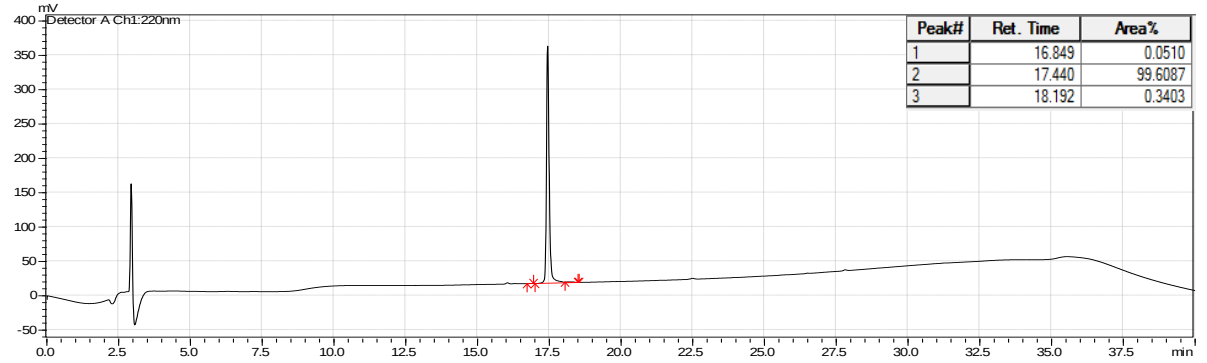
Peak#	Ret. Time	Area%
1	17.488	99.8586
2	17.817	0.1414

S. mitis-CSP-2-N7FI8FF12L



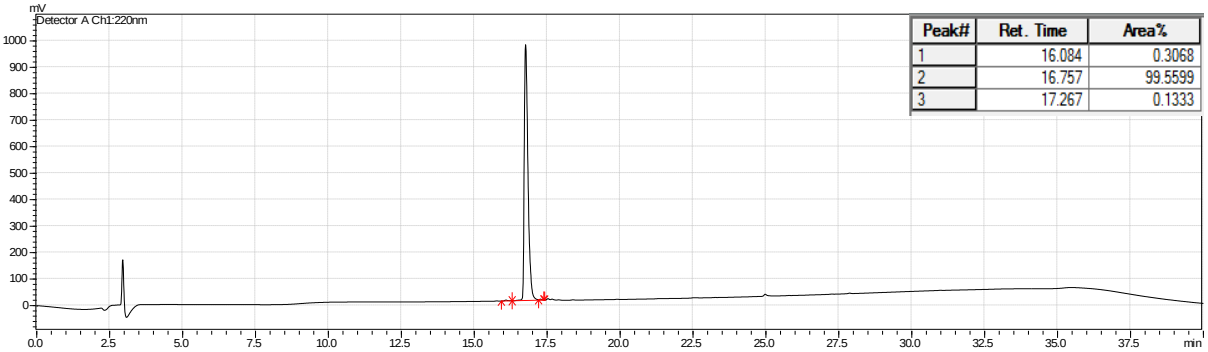
Peak#	Ret. Time	Area%
1	16.753	0.1550
2	16.992	99.5925
3	17.431	0.2525

S. mitis-CSP-2-N7II8FN11F



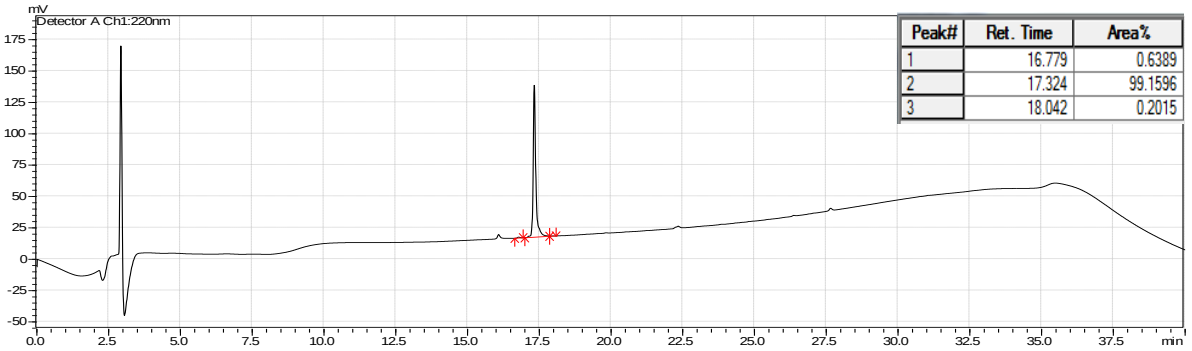
Peak#	Ret. Time	Area%
1	16.849	0.0510
2	17.440	99.6087
3	18.192	0.3403

S. mitis-CSP-2-N7II8FF12L



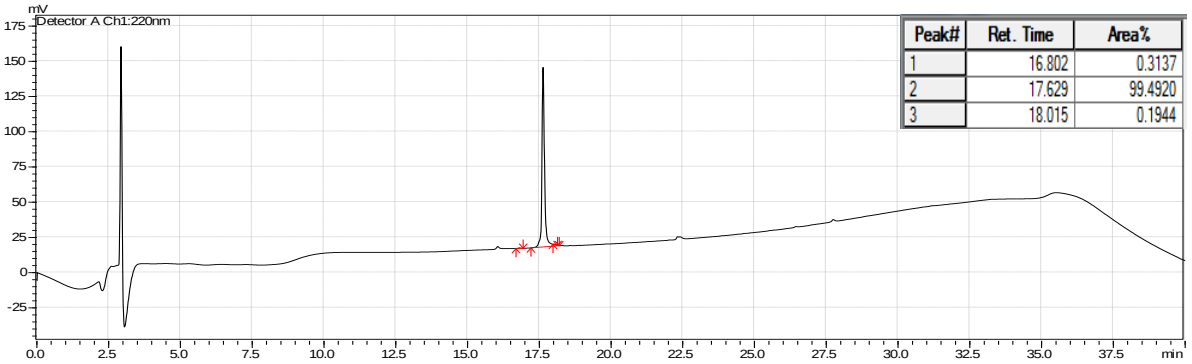
Peak#	Ret. Time	Area%
1	16.084	0.3068
2	16.757	99.5599
3	17.267	0.1333

S. mitis-CSP-2-I2MN7IN11F



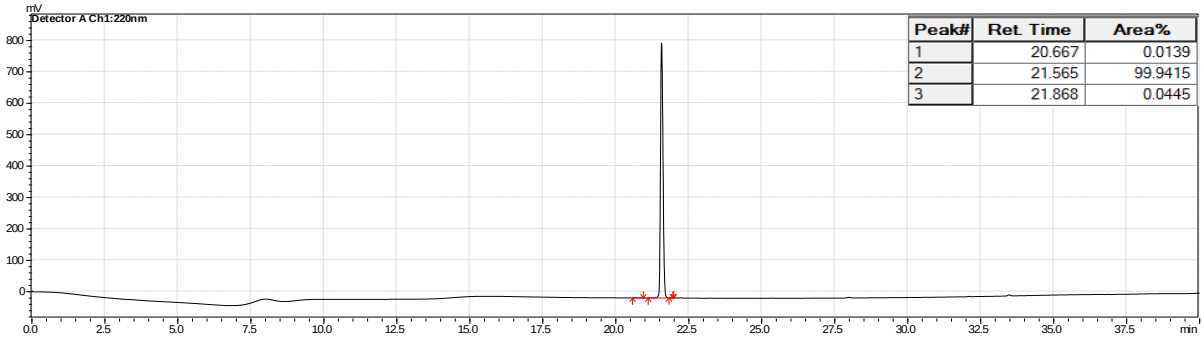
Peak#	Ret. Time	Area%
1	16.779	0.6389
2	17.324	99.1596
3	18.042	0.2015

S. mitis-CSP-2-N7FI8FN11F



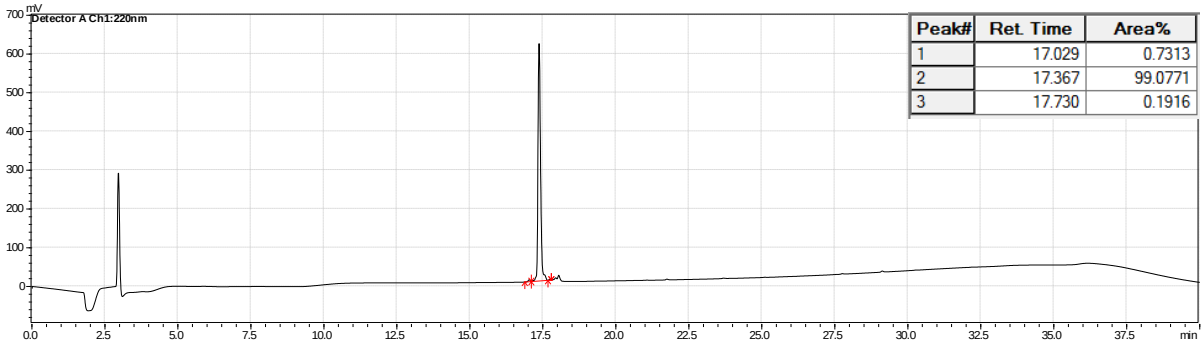
Peak#	Ret. Time	Area%
1	16.802	0.3137
2	17.629	99.4920
3	18.015	0.1944

S. mitis-CSP-2-E1A



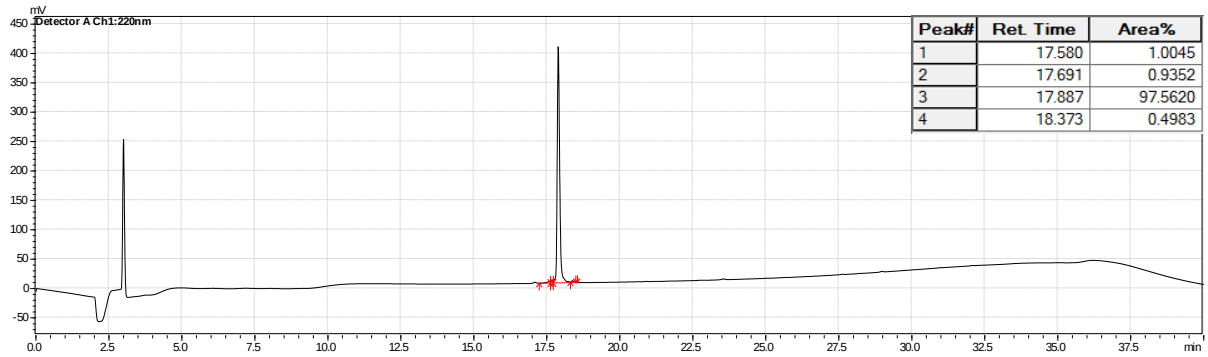
Peak#	Ret. Time	Area%
1	20.667	0.0139
2	21.565	99.9415
3	21.868	0.0445

S. mitis-CSP-2-E1AI2M



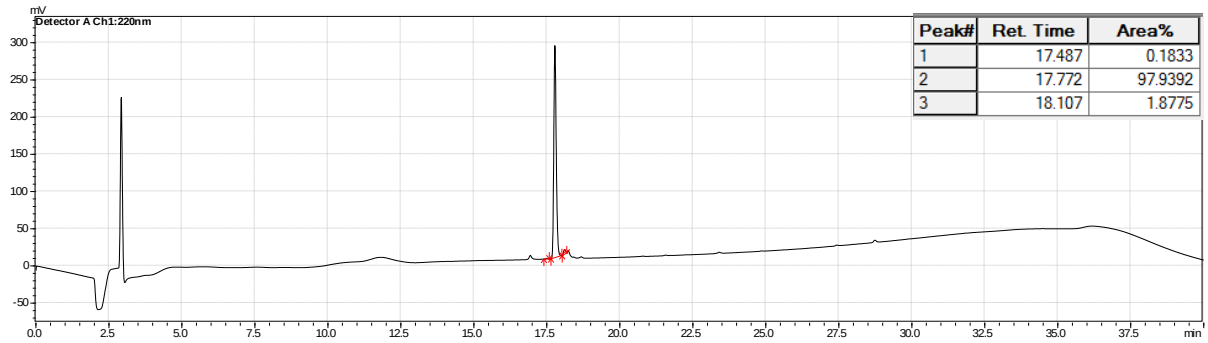
Peak#	Ret. Time	Area%
1	17.029	0.7313
2	17.367	99.0771
3	17.730	0.1916

S. mitis-CSP-2-E1AN11F



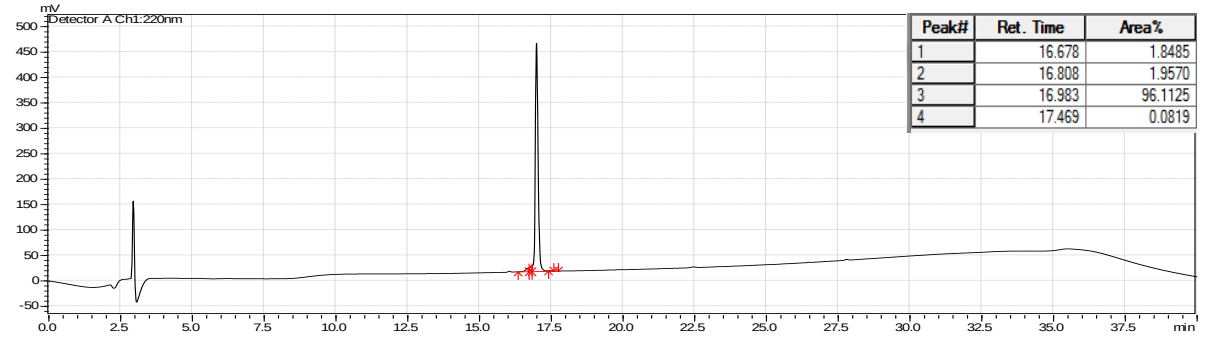
Peak#	Ret. Time	Area%
1	17.580	1.0045
2	17.691	0.9352
3	17.887	97.5620
4	18.373	0.4983

S. mitis-CSP-2-E1AI2MQ4L



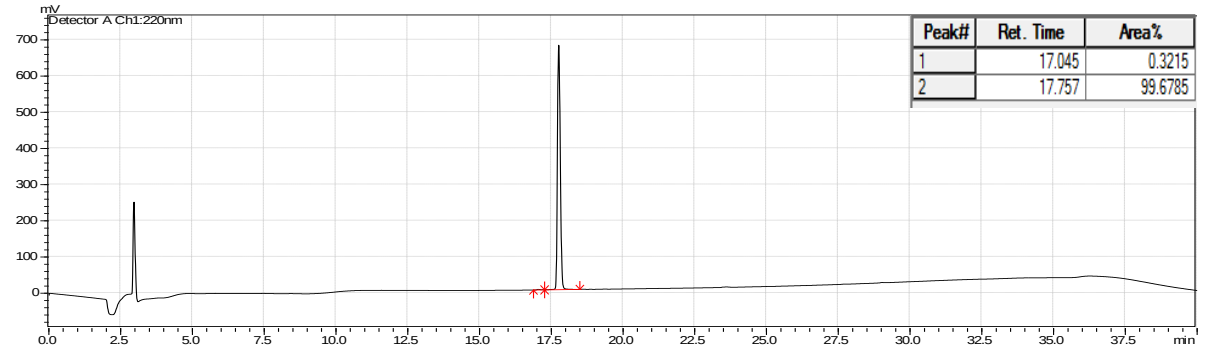
Peak#	Ret. Time	Area%
1	17.487	0.1833
2	17.772	97.9392
3	18.107	1.8775

S. mitis-CSP-2-E1AN11FF12L



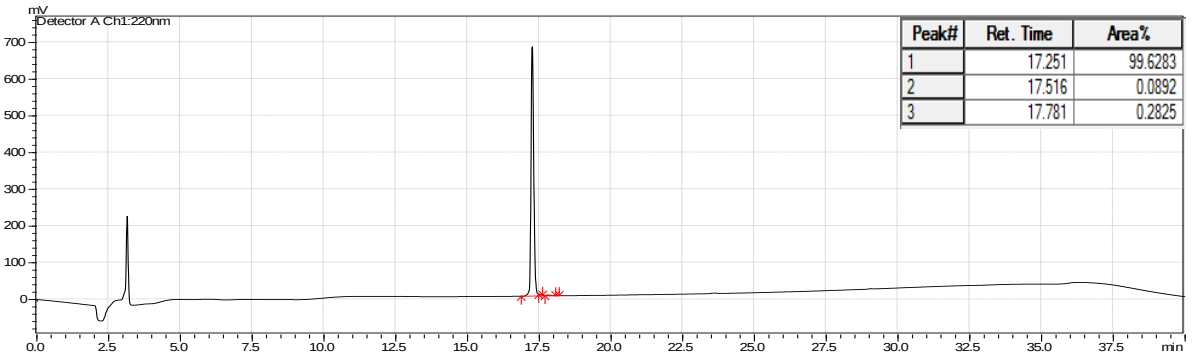
Peak#	Ret. Time	Area%
1	16.678	1.8485
2	16.808	1.9570
3	16.983	96.1125
4	17.469	0.0819

S. mitis-CSP-2-E1AN7II8F



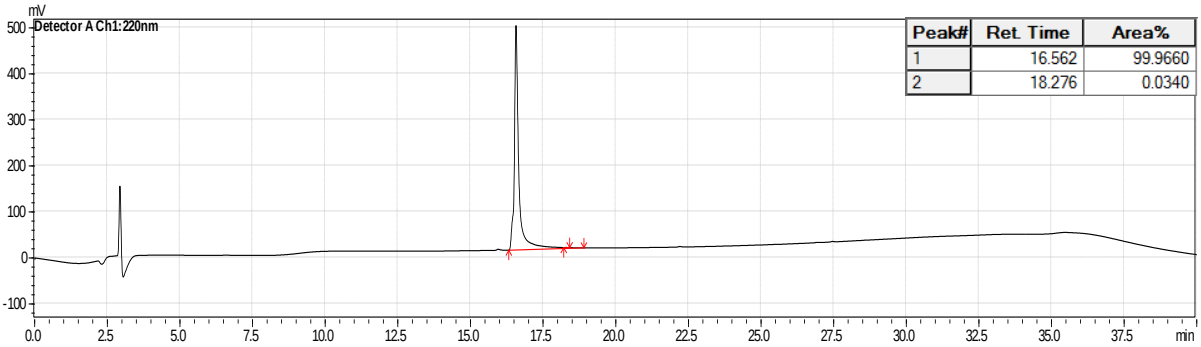
Peak#	Ret. Time	Area%
1	17.045	0.3215
2	17.757	99.6785

S. mitis-CSP-2-E1AI2MF12L



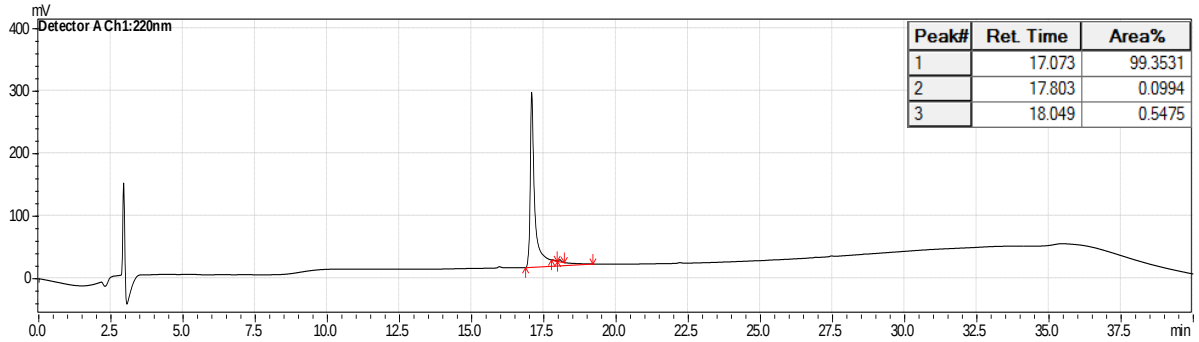
Peak#	Ret. Time	Area%
1	17.251	99.6283
2	17.516	0.0892
3	17.781	0.2825

S. mitis-CSP-2-E1AI2MI8F



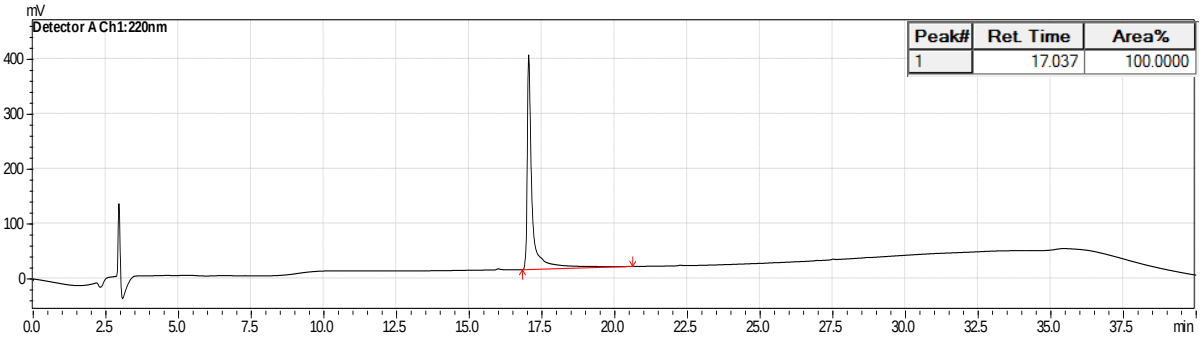
Peak#	Ret. Time	Area%
1	16.562	99.9660
2	18.276	0.0340

S. mitis-CSP-2-E1AI2MI8FN11F



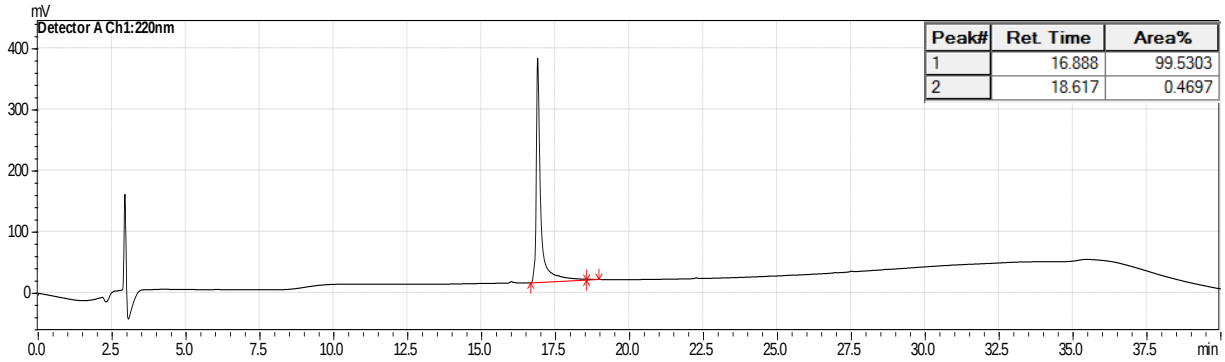
Peak#	Ret. Time	Area%
1	17.073	99.3531
2	17.803	0.0994
3	18.049	0.5475

S. mitis-CSP-2-E1AI2MQ4LI8F

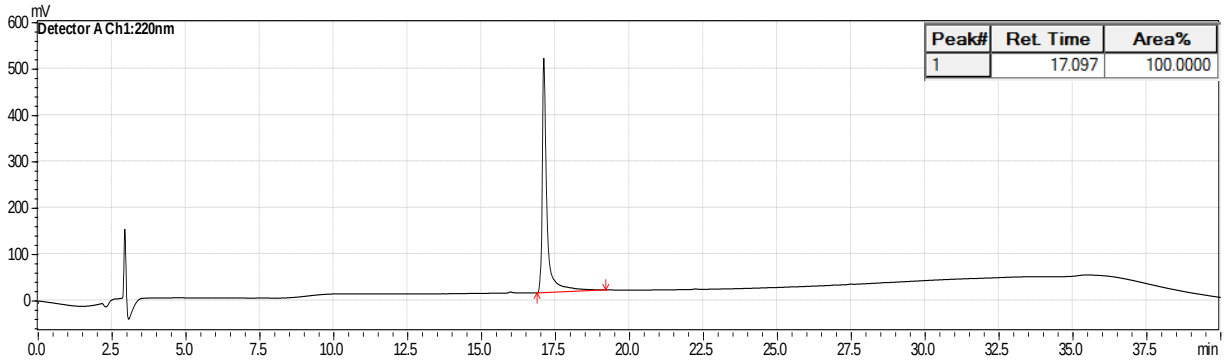


Peak#	Ret. Time	Area%
1	17.037	100.0000

S. mitis-CSP-2-E1AI2MN7II8F



S. mitis-CSP-2-E1AI2MN7FI8F



MS and HPLC data for CSP analogues

Table S-1. MS and HPLC data for the synthetic native CSPs.

Compound Name	Calc. EM MH_2^{2+}	Obs. EM MH_2^{2+}	Purity (%)
S. mitis-CSP-1	1089.6404	1089.6442	≥ 99
S. mitis-CSP-2	1077.5808	1077.5850	≥ 99
S. cristatus-CSP	896.0720	896.0753	≥ 98
S. intermedius-CSP	952.9974	953.0012	≥ 99
S. oralis-CSP	1101.1281	1101.1232	≥ 99
S. oligofermentans-CSP	883.5474	883.5482	≥ 99
S. gordonii-CSP-1	1395.2448	1395.2455	≥ 99
S. gordonii-CSP-2	1241.2105	1241.2152	≥ 99
S. gordonii challis-CSP	1247.6808	1247.6844	≥ 99
S. sanguinis-CSP	885.9668	885.9653	≥ 99

EM = Exact Mass. See methods above.

Table S-2. MS and HPLC data for *S. mitis*-CSP-2-point modification analogues.

Compound Name	Calc. EM MH_2^{2+}	Obs. EM MH_2^{2+}	Purity (%)
S. mitis-CSP-2-I2M	1086.5590	1086.5602	≥ 98
S. mitis-CSP-2-Q4L	1070.0936	1070.0942	≥ 99
S. mitis-CSP-2-N7F	729.7315*	729.7332*	≥ 98
S. mitis-CSP-2-I8F	1094.5730	1094.5764	≥ 98
S. mitis-CSP-2-F10D	1061.5601	1061.5640	≥ 99
S. mitis-CSP-2-N11F	1094.0936	1094.0977	≥ 99
S. mitis-CSP-2-F12I	1060.5886	1060.5867	≥ 97
S. mitis-CSP-2-F12L	1060.5886	1060.5921	≥ 99
S. mitis-CSP-2-N7I	1077.1014	1077.0982	≥ 98
S. mitis-CSP-2-Q4I	1070.0936	1070.0964	≥ 99

EM = Exact Mass. See methods above, *MH_3^{3+}.

Table S-3. MS and HPLC data for *S. mitis*-CSP-2-multiple modification analogues.

Compound Name	Calc. EM MH_2^{2+}	Obs. EM MH_2^{2+}	Purity (%)
S. mitis-CSP-2-I2MQ4L	1079.0718	1079.0687	≥ 99
S. mitis-CSP-2-I2MN7F	1103.0718	1103.0717	≥ 99
S. mitis-CSP-2-I2MN7I	1086.0796	1086.0803	≥ 99
S. mitis-CSP-2-I2MI8F	1103.5512	1103.5481	≥ 99
S. mitis-CSP-2-I2MN11F	1103.0718	1103.0748	≥ 99
S. mitis-CSP-2-I2MF12L	1069.5668	1069.5640	≥ 99
S. mitis-CSP-2-Q4LN7F	724.7400*	724.7381*	≥ 99
S. mitis-CSP-2-Q4LN7I	1069.6142	1069.6135	≥ 99
S. mitis-CSP-2-Q4LI8F	1087.0858	1087.0833	≥ 98
S. mitis-CSP-2-Q4LN11F	1086.6063	1086.6083	≥ 99
S. mitis-CSP-2-Q4LF12L	1053.1014	1053.0997	≥ 95
S. mitis-CSP-2-N7FI8F	1111.0858	1111.0843	≥ 99
S. mitis-CSP-2-N7FN11F	1110.6063	1110.6092	≥ 99
S. mitis-CSP-2-N7FF12L	1077.1014	1077.0977	≥ 99
S. mitis-CSP-2-N7II8F	1094.0936	1094.0988	≥ 97
S. mitis-CSP-2-N7IN11F	1093.6142	1093.6111	≥ 99
S. mitis-CSP-2-N7IF12L	1060.1092	1060.1133	≥ 98
S. mitis-CSP-2-I8FN11F	1111.0858	1111.0847	≥ 96
S. mitis-CSP-2-I8FF12L	1077.5808	1077.5810	≥ 97
S. mitis-CSP-2-N11FF12L	1077.1014	1077.1060	≥ 96
S. mitis-CSP-2-I2MQ4LN7F	1095.5845	1095.5869	≥ 99
S. mitis-CSP-2-I2MI8FN11F	1120.0640	1120.0617	≥ 99
S. mitis-CSP-2-I2MN7FF12L	1086.0796	1086.0753	≥ 98
S. mitis-CSP-2-I2MN7II8F	1103.0718	1103.0679	≥ 99
S. mitis-CSP-2-I2MQ4LF12L	1062.0796	1062.0760	≥ 99
S. mitis-CSP-2-I2MQ4LI8F	1096.0639	1096.0586	≥ 97
S. mitis-CSP-2-I2MQ4LN7I	1078.5923	1078.5886	≥ 98
S. mitis-CSP-2-I2MQ4LN11F	1095.5845	1095.5807	≥ 99
S. mitis-CSP-2-Q4LN7FI8F	1103.5985	1103.5936	≥ 99
S. mitis-CSP-2-Q4LN7II8F	1086.6063	1086.6027	≥ 96
S. mitis-CSP-2-Q4LN7FN11F	1103.1191	1103.1189	≥ 99
S. mitis-CSP-2-I2MN7FI8F	1120.0640	1120.0586	≥ 99
S. mitis-CSP-2-Q4LN7FF12L	1069.6142	1069.6119	≥ 99
S. mitis-CSP-2-N7FI8FF12L	1094.0936	1094.0899	≥ 99
S. mitis-CSP-2-N7II8FN11F	1110.6063	1110.6049	≥ 99
S. mitis-CSP-2-N7II8FF12L	1077.1014	1077.0976	≥ 98
S. mitis-CSP-2-I2MN7IN11F	1102.5924	1102.5881	≥ 99
S. mitis 2 CSP-N7FI8FN11F	1127.5985	1127.6010	≥ 99

EM = Exact Mass. See methods above, $^{*}\mathbf{MH_3^{3+}}$.

Table S-4. MS and HPLC data for *S. mitis*-CSP-2-E1A modification analogues.

Compound Name	**Calc. EM MH_2^{2+}**	**Obs. EM MH_2^{2+}**	**Purity (%)**
S. mitis-CSP-2-E1A	1048.5781	1048.5754	≥ 99
S. mitis-CSP-2-E1AI2M	705.3733*	705.3763*	≥ 99
S. mitis-CSP-2-E1AN11F	1065.0908	1065.0892	≥ 97
S. mitis-CSP-2-E1AI2MQ4L	1050.0690	1050.0645	≥ 97
S. mitis-CSP-2-E1AN11FF12L	1048.0987	1048.0947	≥ 96
S. mitis-CSP-2-E1AN7II8F	1065.0908	1065.0857	≥ 99
S. mitis-CSP-2-E1AI2MF12L	1040.5641	1040.5611	≥ 99
S. mitis-CSP-2-E1AI2MI8F	1074.5484	1074.5499	≥ 99
S. mitis-CSP-2-E1AI2MI8FN11F	1091.0612	1091.0647	≥ 99
S. mitis-CSP-2-E1AI2MQ4LI8F	1067.0612	1067.0613	≥ 99
S. mitis-CSP-2-E1AI2MN7II8F	1074.069	1074.0735	≥ 99
S. mitis-CSP-2-E1AI2MN7FI8F	1091.0612	1091.0608	≥ 99

EM = Exact Mass. See methods above, $^{*}\mathbf{MH_3^{3+}}$.

Primary reporter gene assay data

***S. pneumoniae* D39pcomX::lacZ (ComD1)**

Agonism assays were performed at 10 µM concentration of synthetic CSP. *S. pneumoniae* CSP1 was used as the positive control (100%) while DMSO as the negative control (0%). Percent (%) ComD1 activation was measured by normalizing the Miller units obtained for each peptide to that of the native CSP1. All peptides were screened in triplicate over three separate trials. Error bars indicate standard error of the mean of nine values.

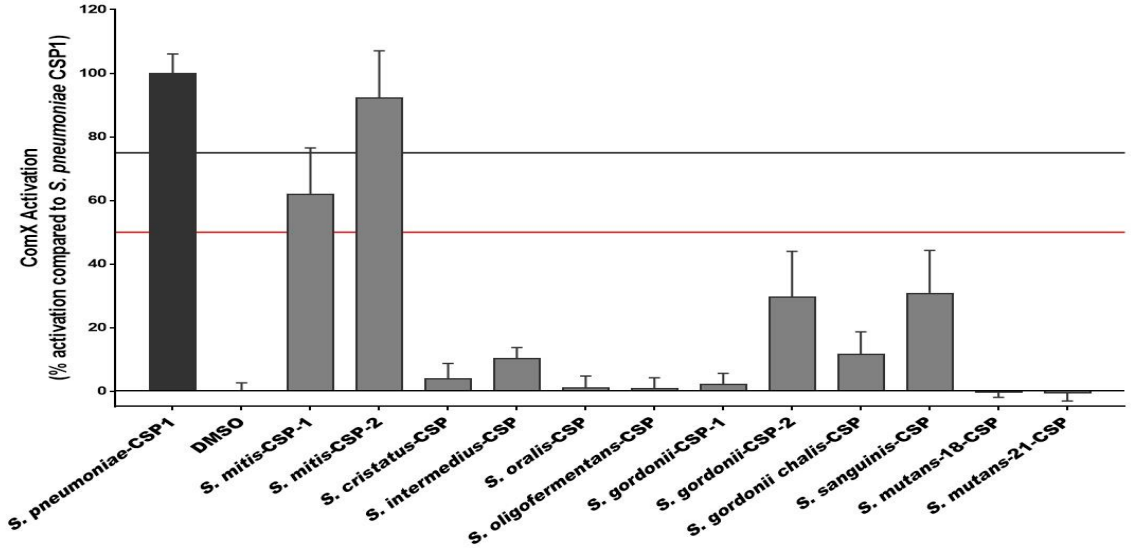


Figure S-1. Primary agonism screening assay data for the synthetic *Streptococci* native CSP pheromones. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

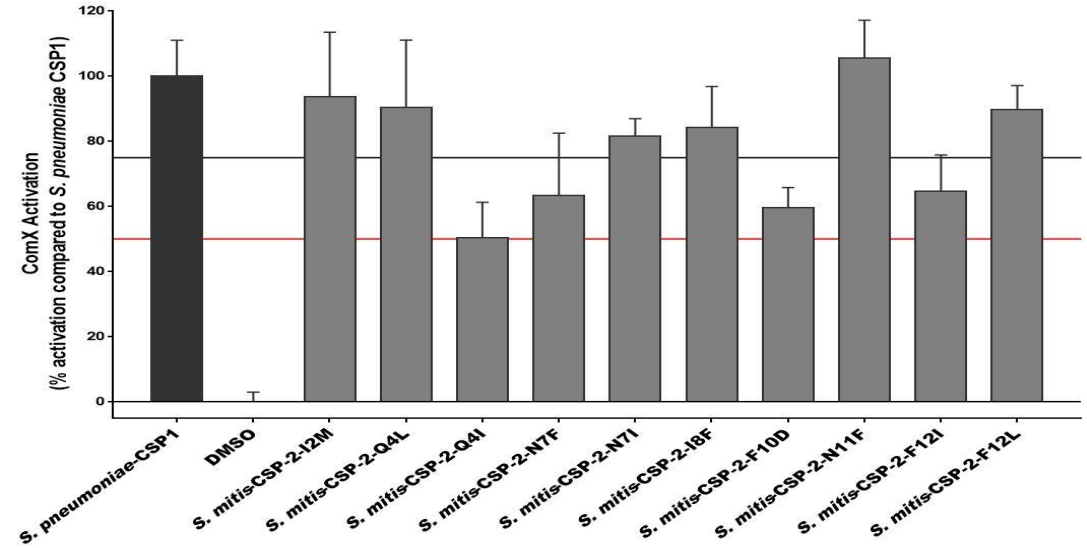


Figure S-2. Primary agonism screening assay data for the *S. mitis*-CSP-2-point modification analogues. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} values.

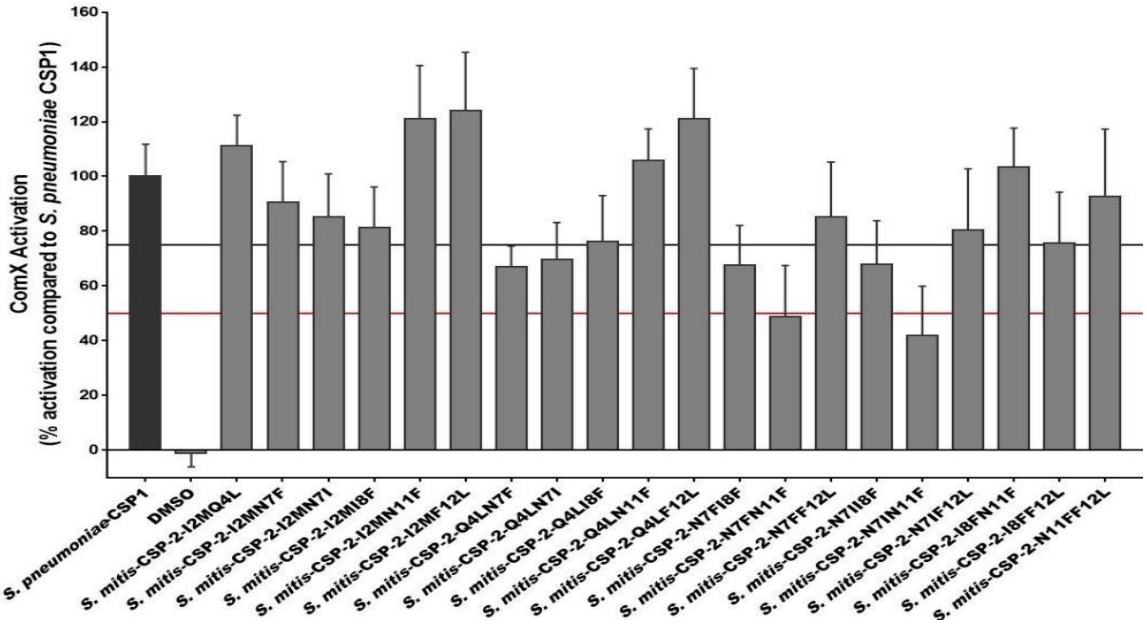


Figure S-3. Primary agonism screening assay data for the *S. mitis*-CSP-2 double modification analogues. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

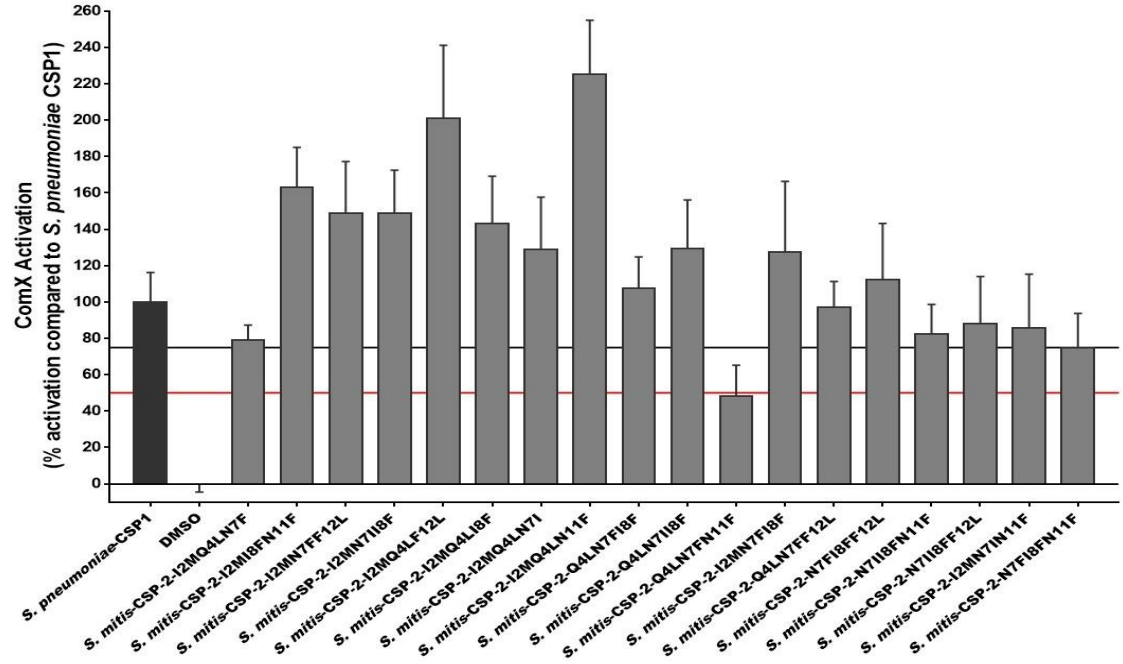


Figure S-4. Primary agonism screening assay data for the *S. mitis*-CSP-2 triple modification analogues. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

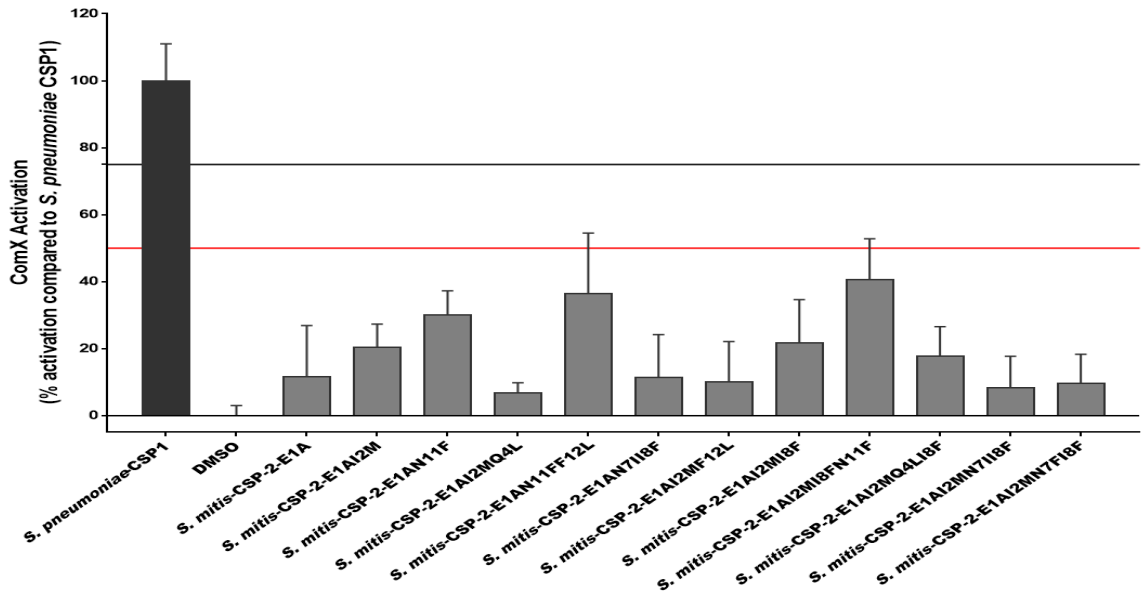


Figure S-5. Primary agonism screening assay data for the *S. mitis*-CSP-2-E1A modification analogues. None of the peptides exhibited activation of the *S. pneumoniae* ComD1 receptor and peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

Antagonism assays were performed at 10 μM concentration of peptides against 50 nM concentration of *S. pneumoniae* CSP1. *S. pneumoniae* CSP1 (50 nM) was used as the positive control (100%) while DMSO as the negative control (0%). Percent (%) *comX* activation was measured by normalizing the Miller units obtained for each peptide to that of CSP1. All peptides were screened in triplicate over three separate trials. Error bars indicate standard error of the mean of nine values.

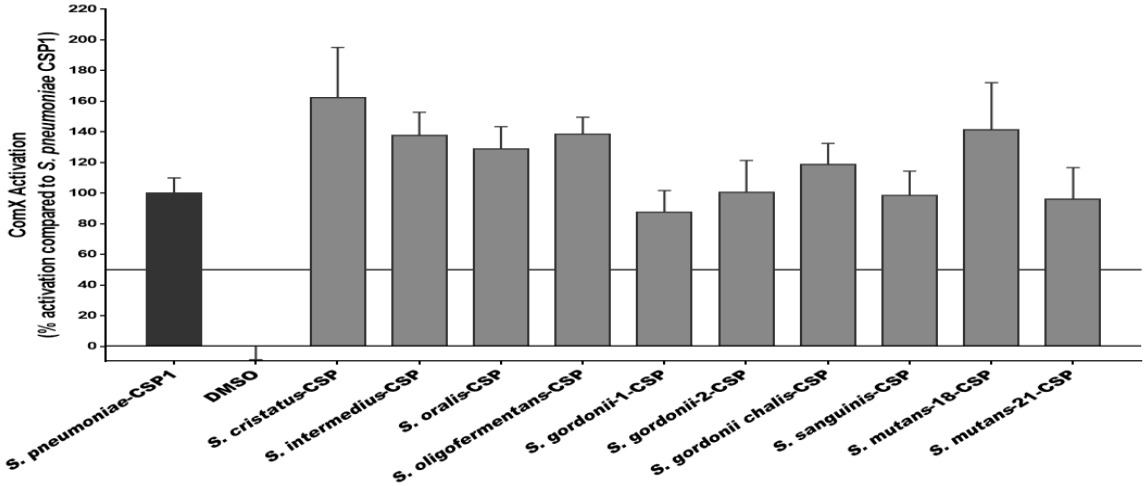


Figure S-6. Primary antagonism screening assay data for the synthetic *Streptococci* native CSP pheromones. None of the peptides exhibited inhibition of the *S. pneumoniae* ComD1 receptor.

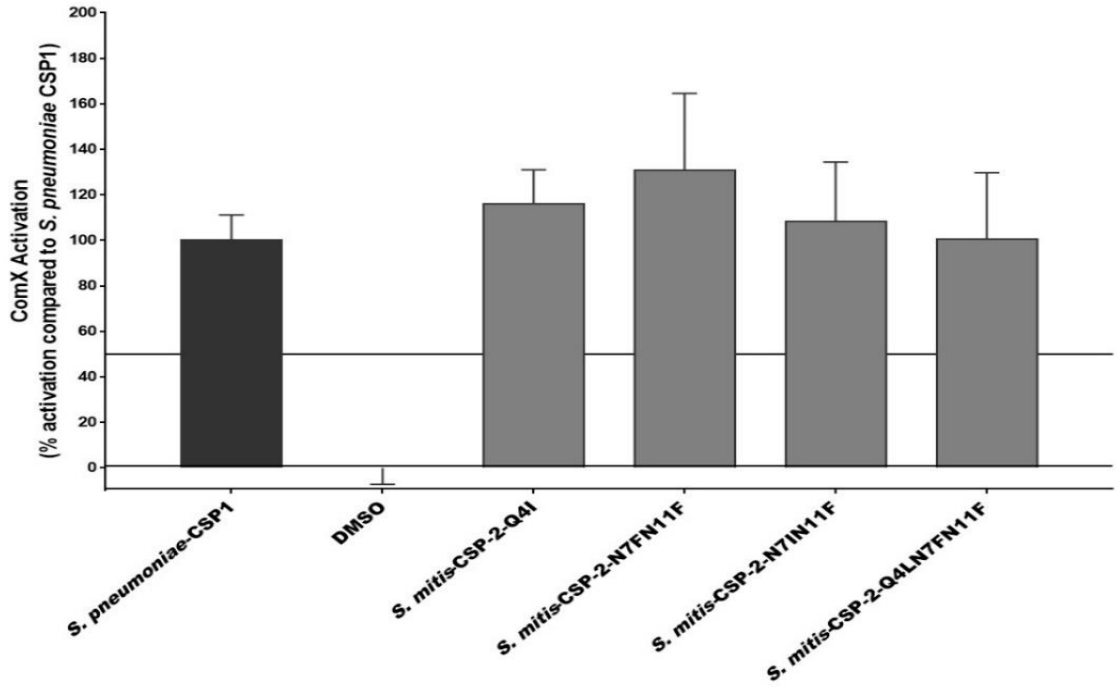


Figure S-7. Primary antagonism screening assay data for the *S. mitis*-CSP-2 point and multiple modification analogues. None of the peptides exhibited inhibition of the *S. pneumoniae* ComD1 receptor.

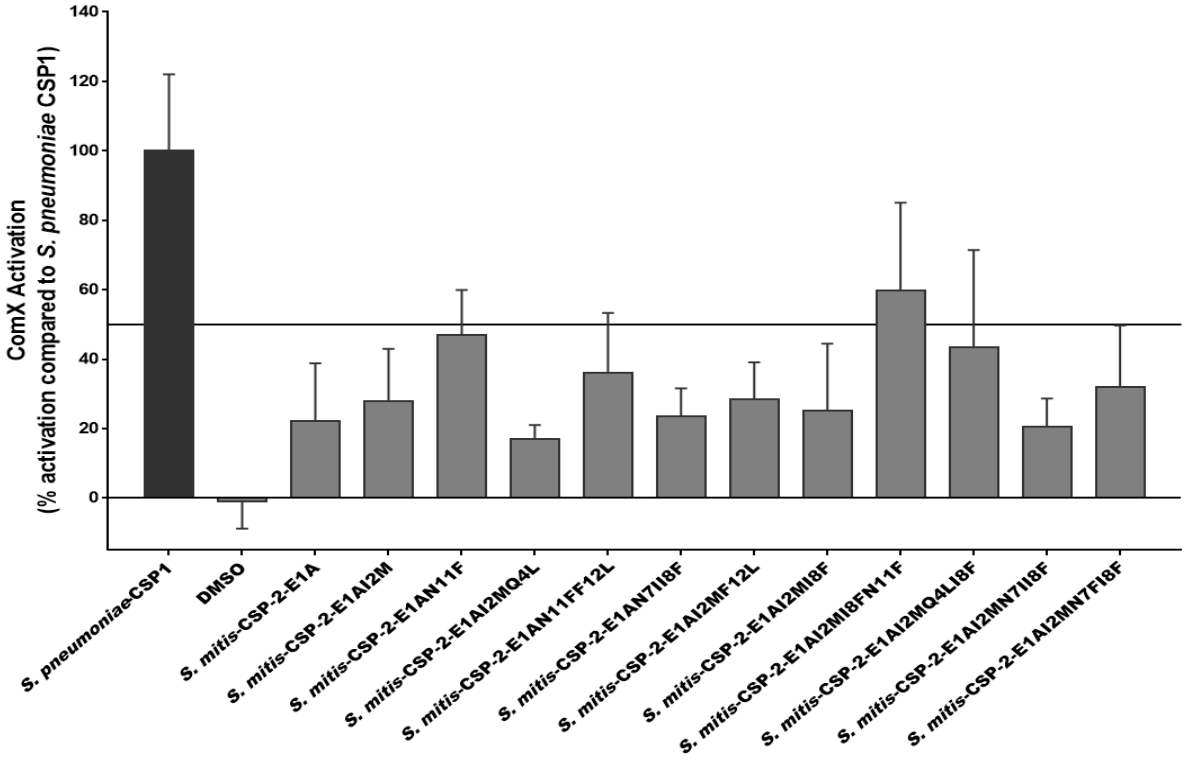


Figure S-8. Primary antagonism screening assay data for the *S. mitis*-CSP-2-E1A modification analogues. Peptides that exhibited less than 50% activation were further evaluated to determine their IC_{50}.

***S. pneumoniae* TIGR4pcomX::lacZ (ComD2)**

Agonism assays were performed at 10 μM concentration. *S. pneumoniae* CSP2 was used as the positive control (100%) while DMSO as the negative control (0%). Percent (%) *comX* activation was measured by normalizing the Miller units obtained for each peptide to that of CSP2. All peptides were screened in triplicate over three separate trials. Error bars indicate standard error of the mean of nine values.

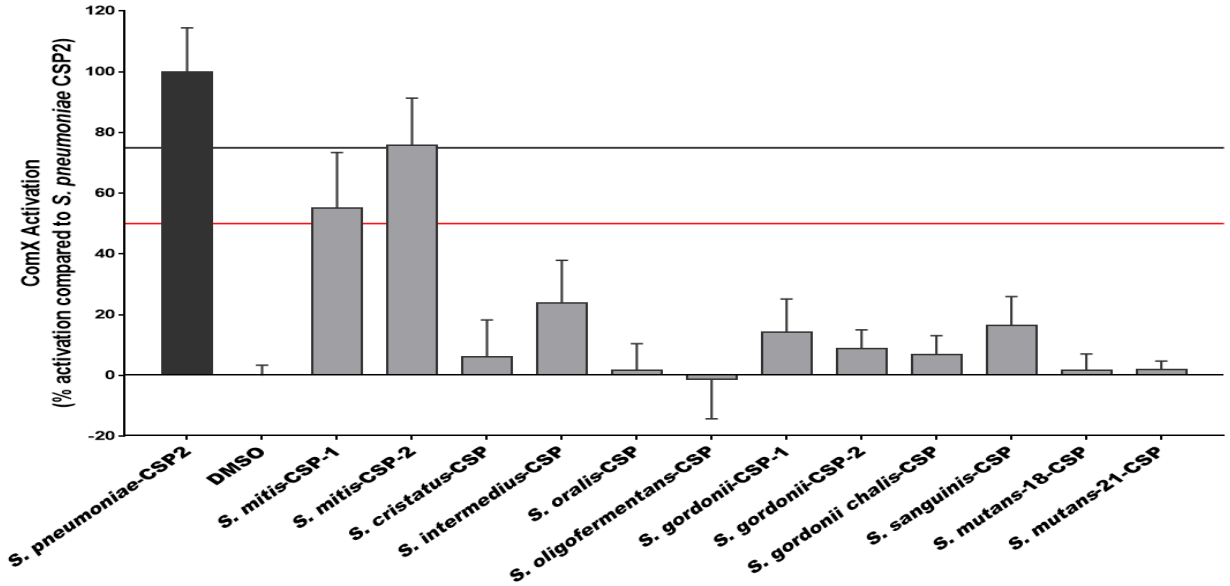


Figure S-9. Primary agonism screening assay data for the synthetic *Streptococci* native CSP pheromones. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

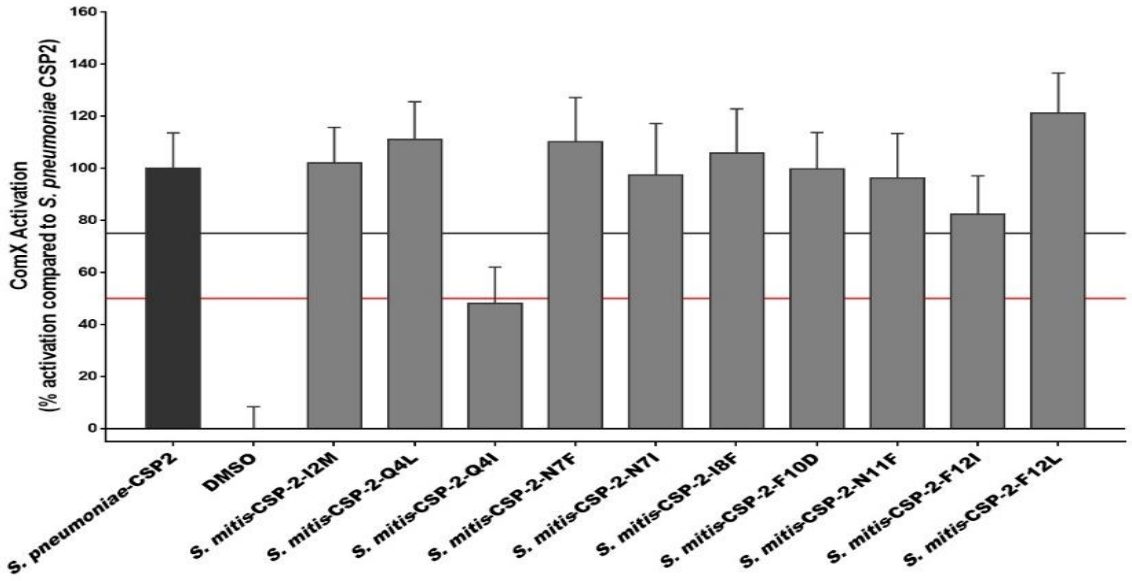


Figure S-10. Primary agonism screening assay data for the *S. mitis*-CSP-2-point modification analogues. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

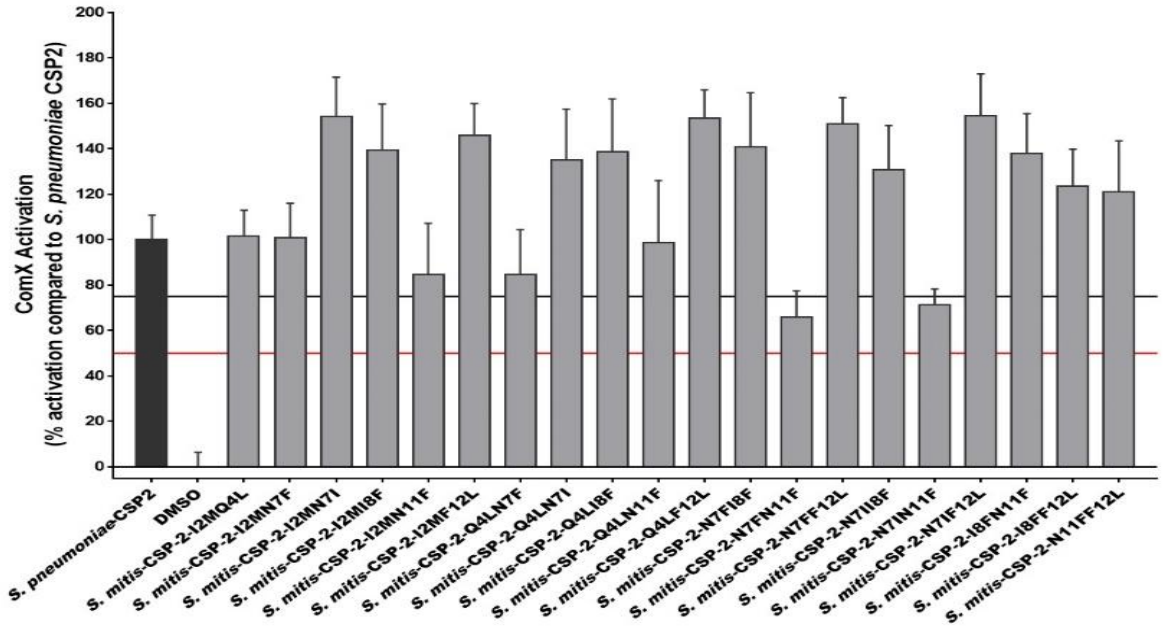


Figure S-11. Primary agonism screening assay data for the *S. mitis*-CSP-2 double modification analogues. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} values.

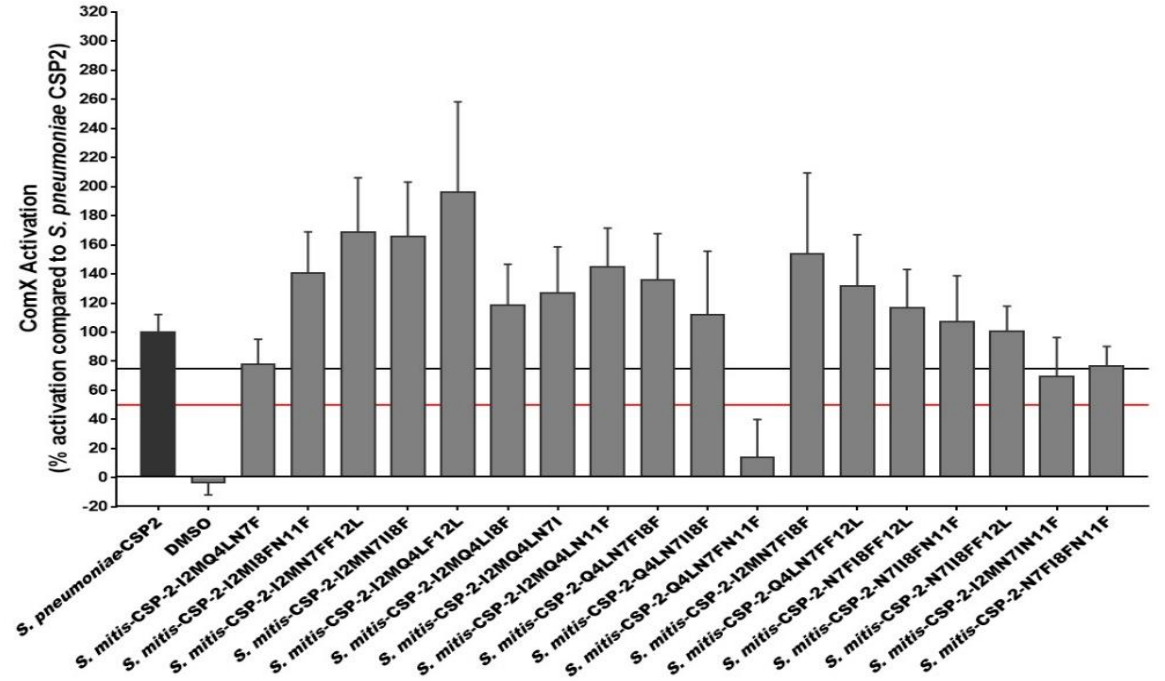


Figure S-12. Primary agonism screening assay data for the *S. mitis*-CSP-2 triple modification analogues. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

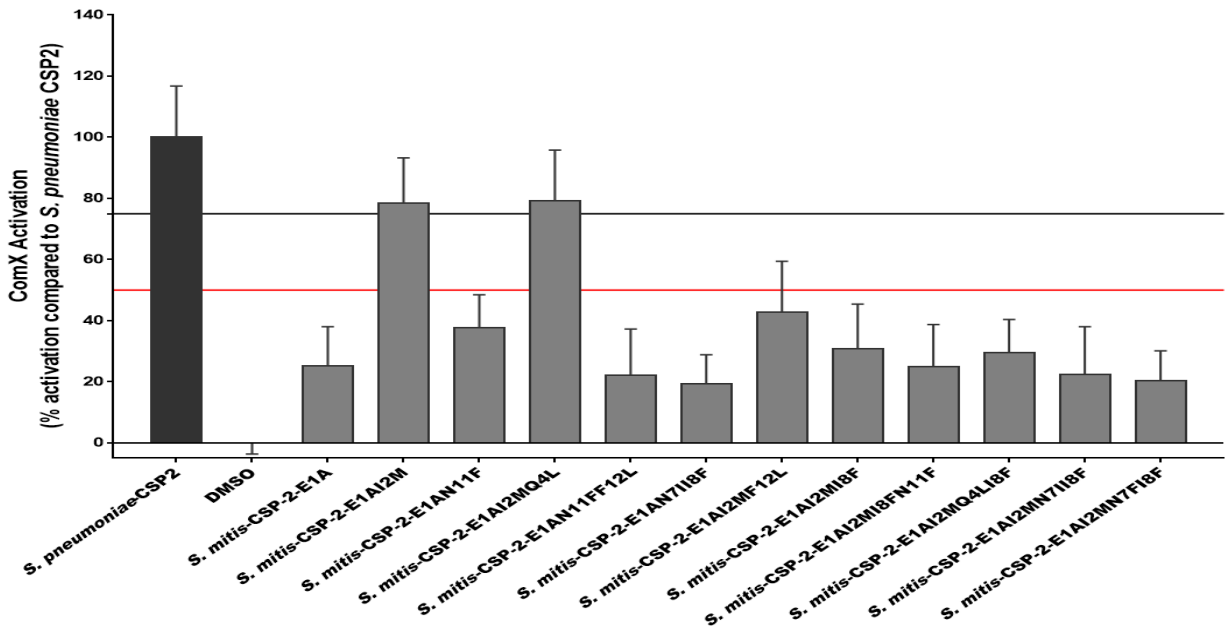


Figure S-13. Primary agonism screening assay data for the *S. mitis*-CSP-2-E1A modification analogues. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

Antagonism assays were performed at 10 μM concentration of peptides against 250 nM concentration of *S. pneumoniae* CSP2. *S. pneumoniae* CSP2 (250 nM) was used as the positive control (100%) while DMSO as the negative control (0%). Percent (%) *comX* activation was measured by normalizing the Miller units obtained for each peptide to that of CSP2. All peptides were screened in triplicate over three separate trials. Error bars indicate standard error of the mean of nine values.

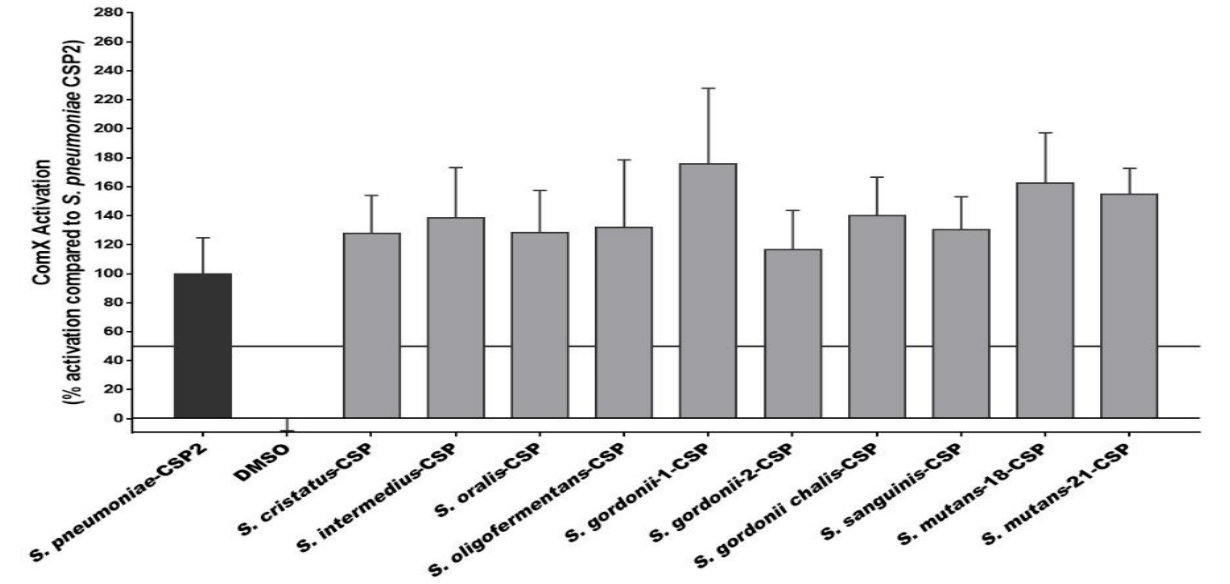


Figure S-14. Primary antagonism screening assay data for the synthetic *Streptococci* native CSP pheromones. None of the peptides exhibited inhibition of the *S. pneumoniae* ComD2 receptor.

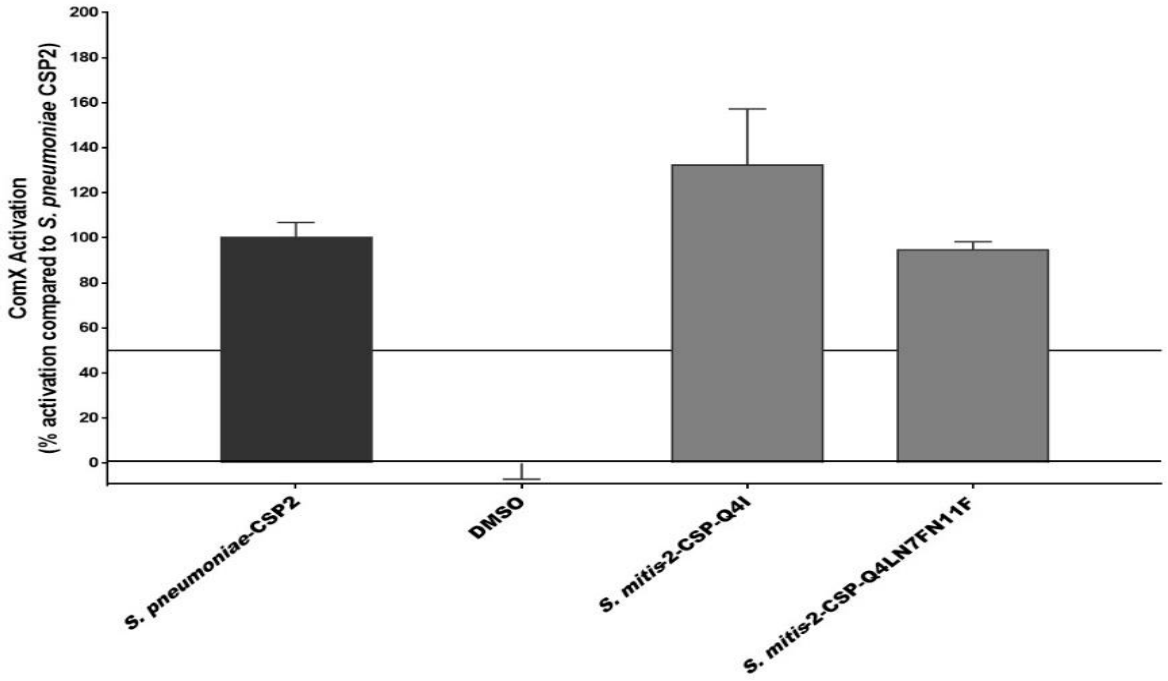


Figure S-15. Primary antagonism screening assay data for the *S. mitis*-CSP-2 point and multiple modification analogues. None of the peptides exhibited inhibition of the *S. pneumoniae* ComD2 receptor.

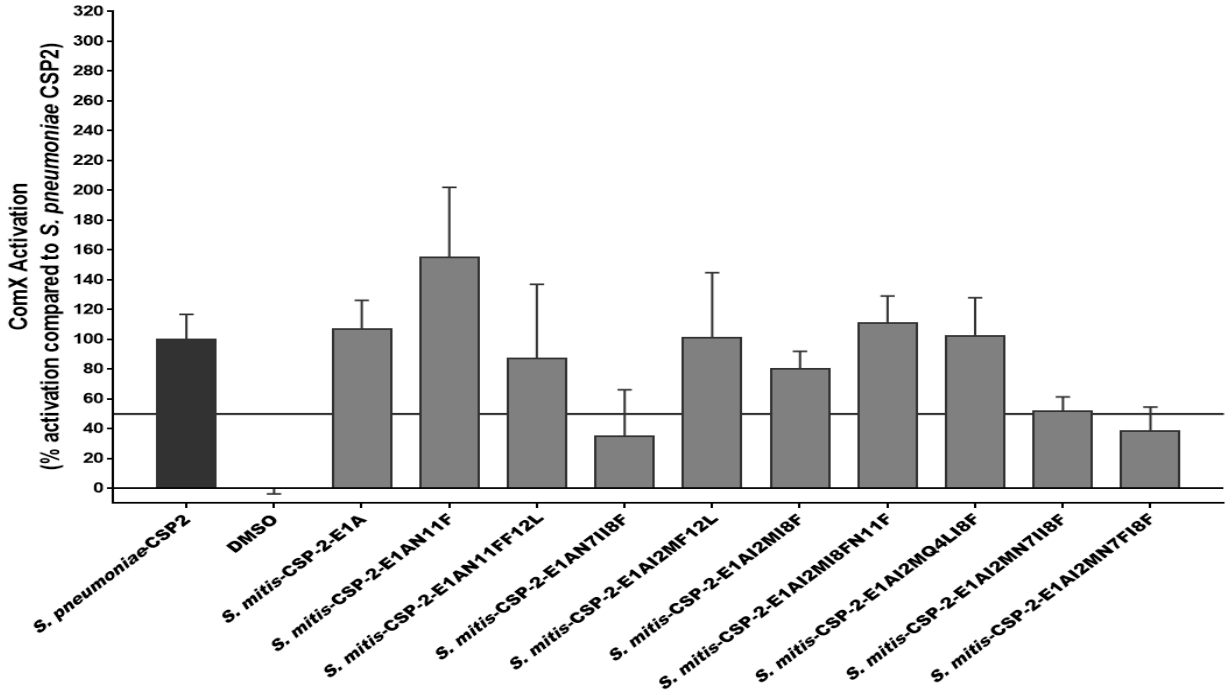


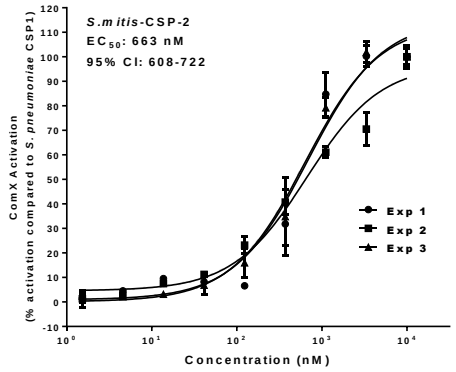
Figure S-16. Primary antagonism screening assay data for the *S. mitis*-CSP-2-E1A modification analogues. Peptides that exhibited less than 50% activation were further evaluated to determine their IC_{50}.

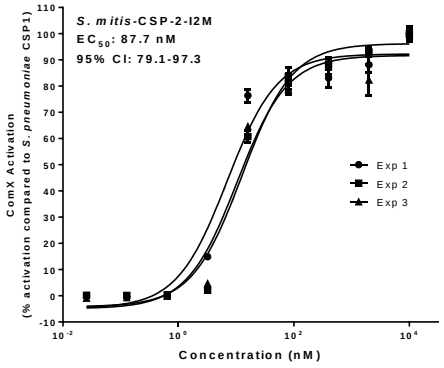
Agonism and antagonism dose response curves

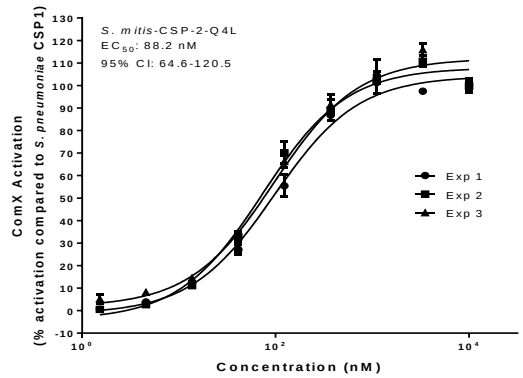
S. mitis-CSP-2 analogues were tested to determine their EC_{50} or IC_{50} values over varying concentrations in the two indicated *S. pneumoniae* beta-galactosidase reporter strains. Each dose response experiment was performed in triplicate on three separate occasions (i.e., experiments (Exp.) #1-3; 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} or IC_{50} value (in nM) and 95% confidence interval (95% CI) values (in nM), are indicated at top left.

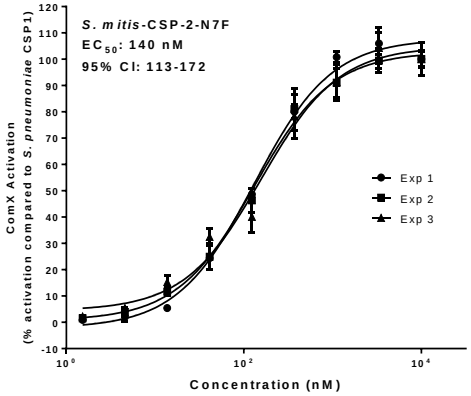
S. pneumoniae D39 pcomX::lacZ (ComD1)

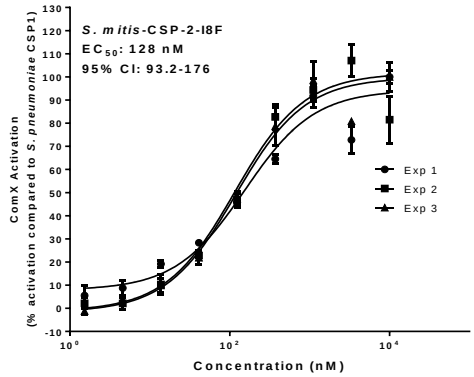
Activation dose response curves

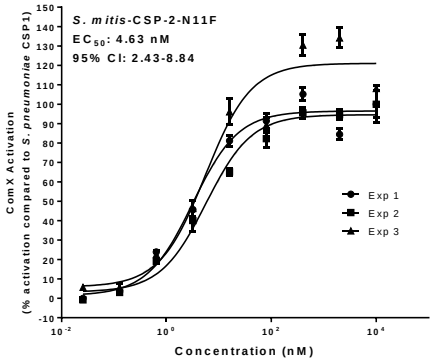


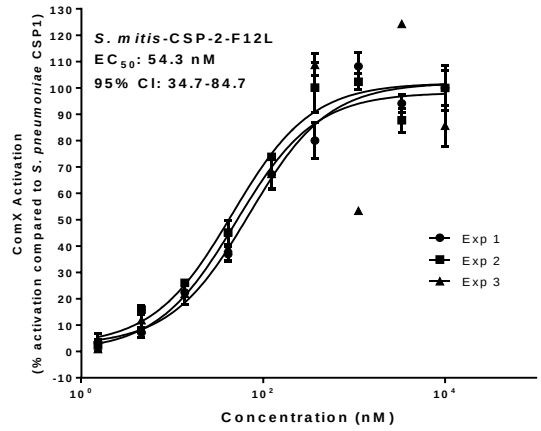


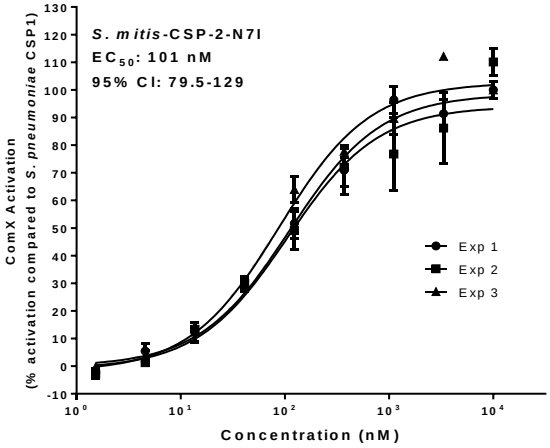


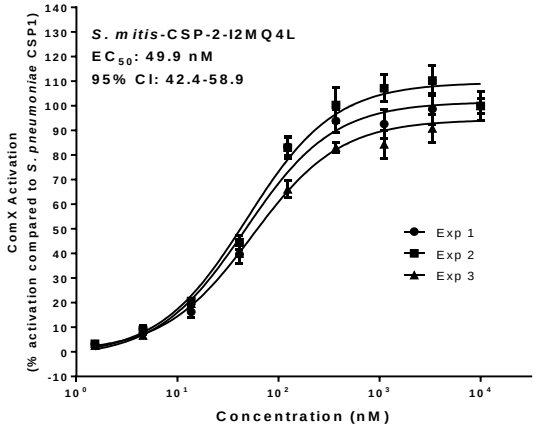


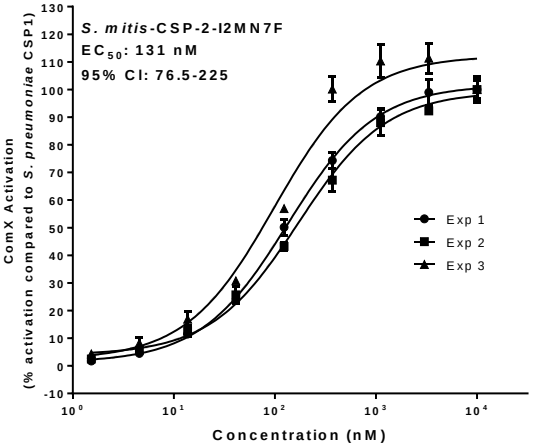


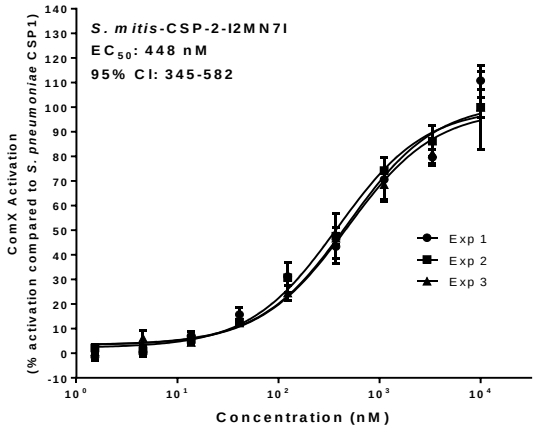


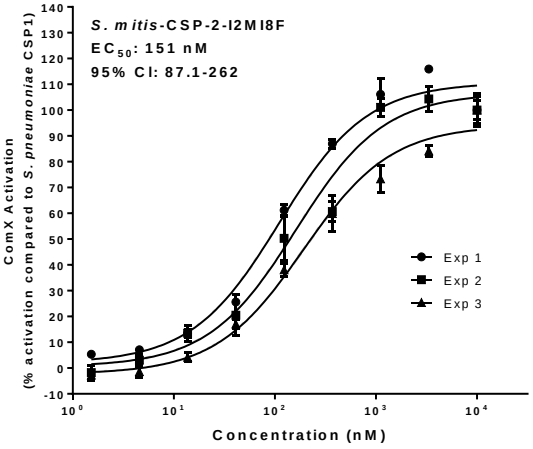

S. mitis-CSP-2-F12L
EC50: 54.3 nM
95% CI: 34.7-84.7
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

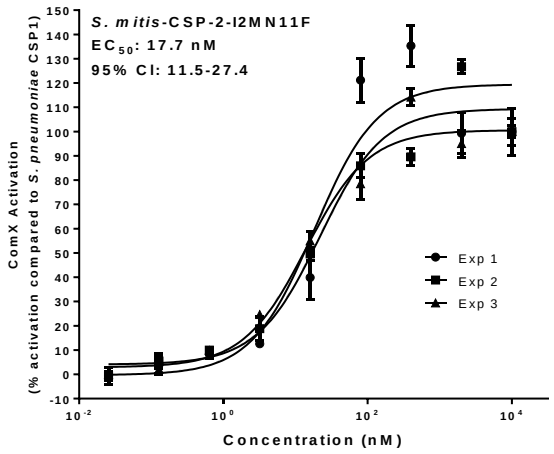

S. mitis-CSP-2-N7I
EC50: 101 nM
95% CI: 79.5-129
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

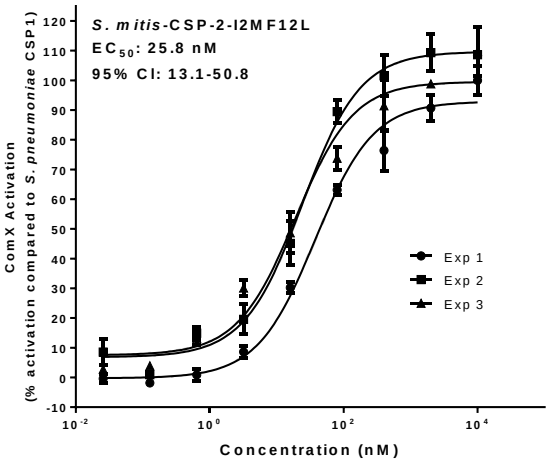

S. mitis-CSP-2-I2MQ4L
EC50: 49.9 nM
95% CI: 42.4-58.9
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

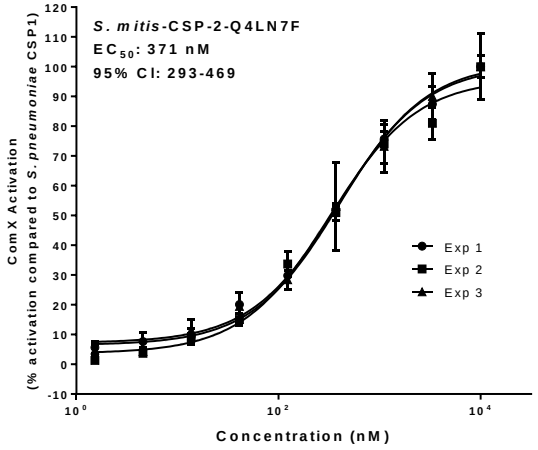

S. mitis-CSP-2-I2MN7F
EC50: 131 nM
95% CI: 76.5-225
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

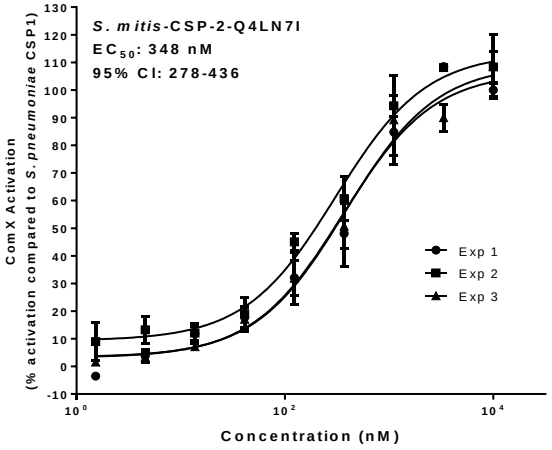

S. mitis-CSP-2-I2MN7I
EC50: 448 nM
95% CI: 345-582
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

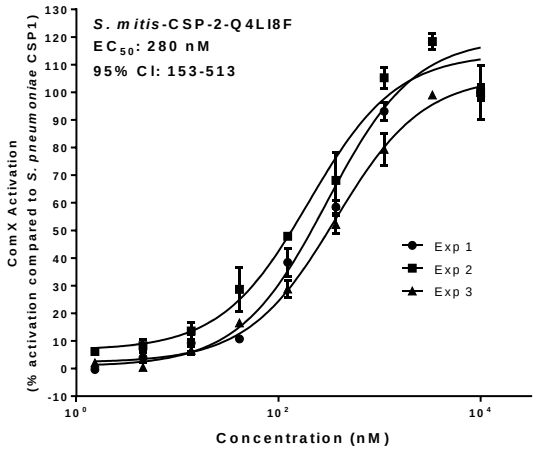

S. mitis-CSP-2-I2MI8F
EC50: 151 nM
95% CI: 87.1-262
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

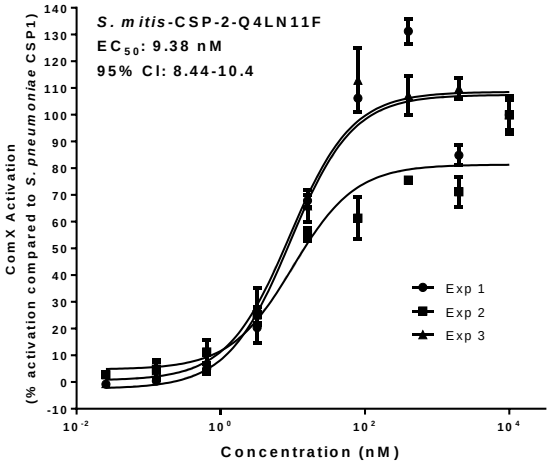

S. mitis-CSP-2-I2MN11F
EC50: 17.7 nM
95% CI: 11.5-27.4
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

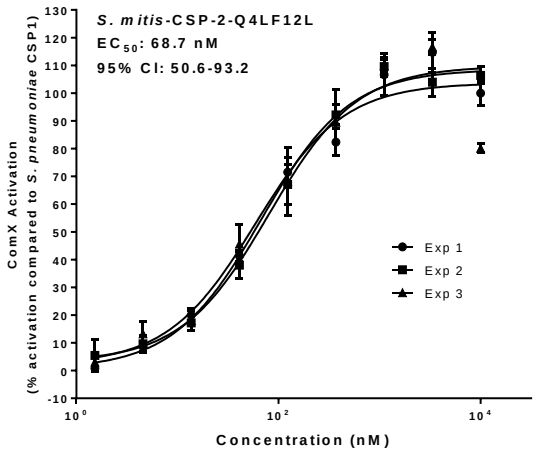

S. mitis-CSP-2-I2MF12L
EC50: 25.8 nM
95% CI: 13.1-50.8
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

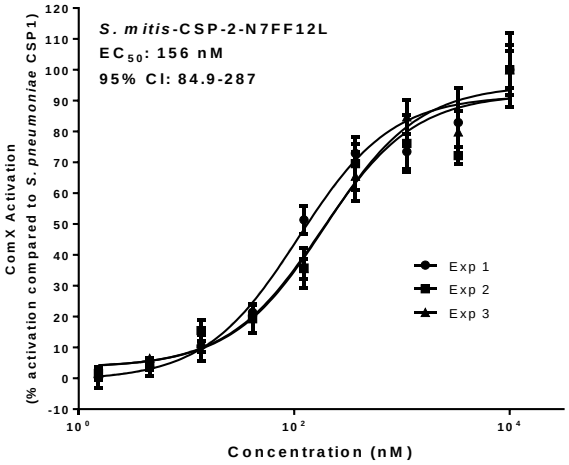

S. mitis-CSP-2-Q4LN7F
EC50: 371 nM
95% CI: 293-469
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

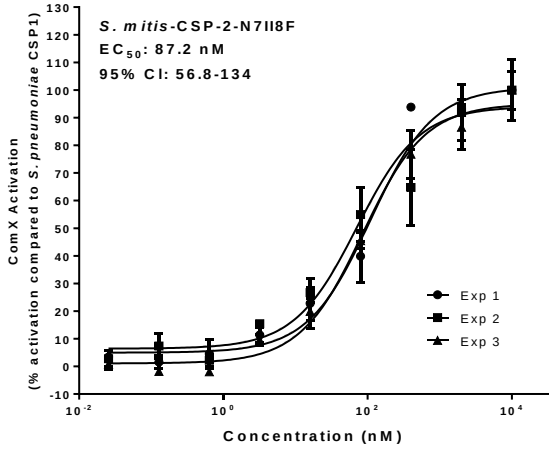

S. mitis-CSP-2-Q4LN7I
EC50: 348 nM
95% CI: 278-436
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

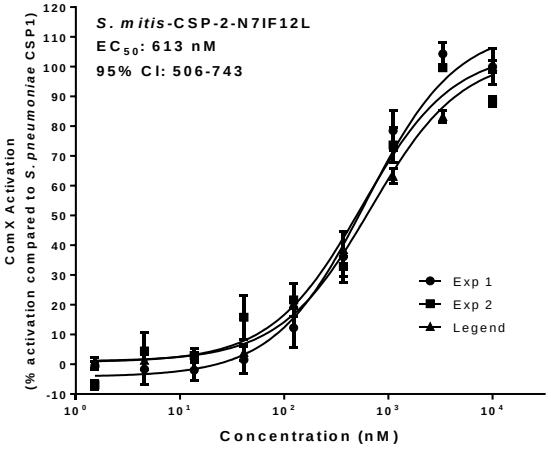

S. mitis-CSP-2-Q4LI8F
EC50: 280 nM
95% CI: 153-513
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

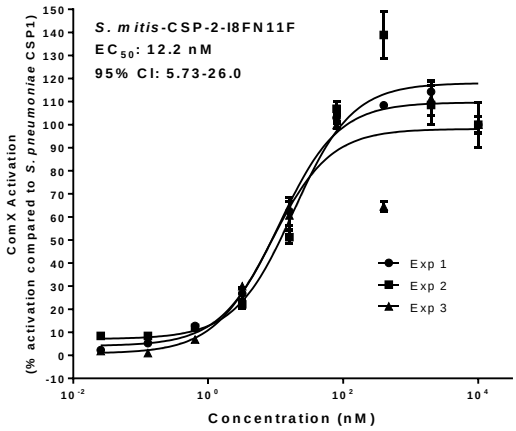

S. mitis-CSP-2-Q4LN11F
EC50: 9.38 nM
95% CI: 8.44-10.4
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

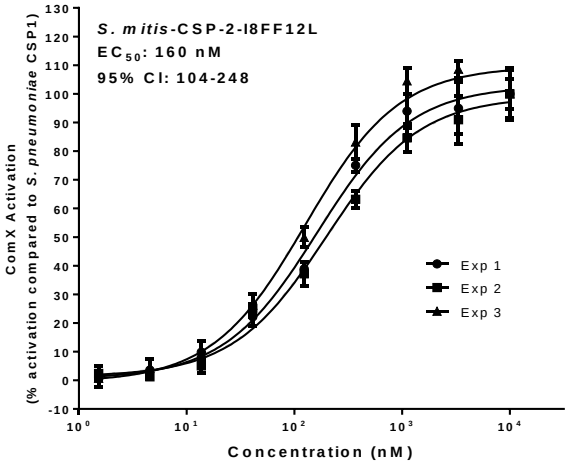

S. mitis-CSP-2-Q4LF12L
EC50: 68.7 nM
95% CI: 50.6-93.2
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

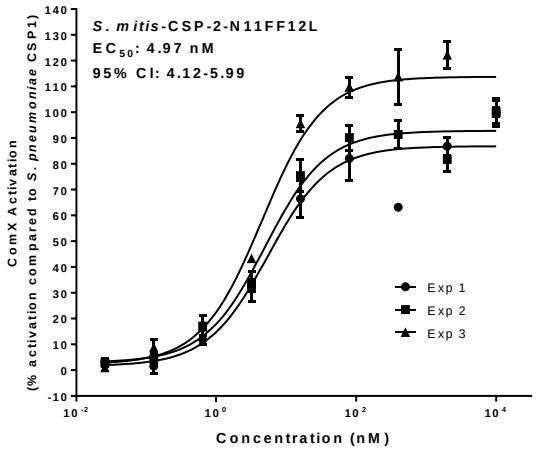

S. mitis-CSP-2-N7FF12L
EC50: 156 nM
95% CI: 84.9-287
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

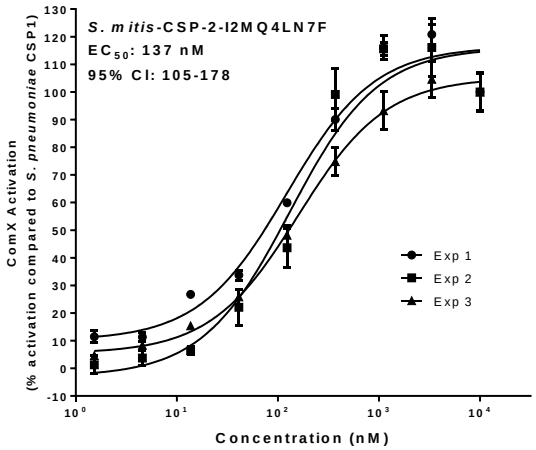

S. mitis-CSP-2-N7II8F
EC50: 87.2 nM
95% CI: 56.8-134
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

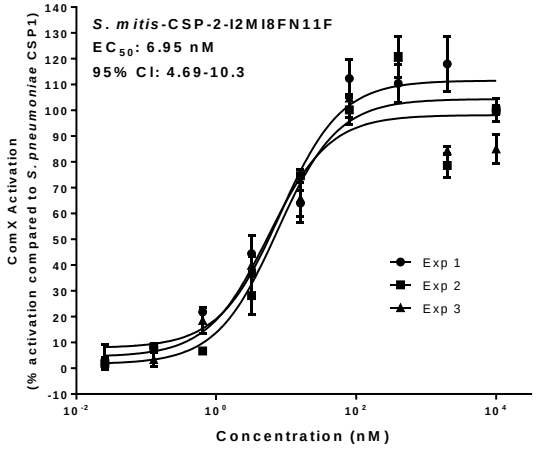

S. mitis-CSP-2-N7IF12L
EC50: 613 nM
95% CI: 506-743
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Legend

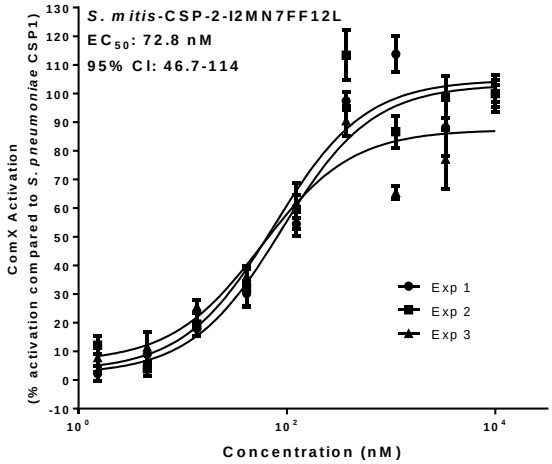

S. mitis-CSP-2-I8FN11F
EC50: 12.2 nM
95% CI: 5.73-26.0
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

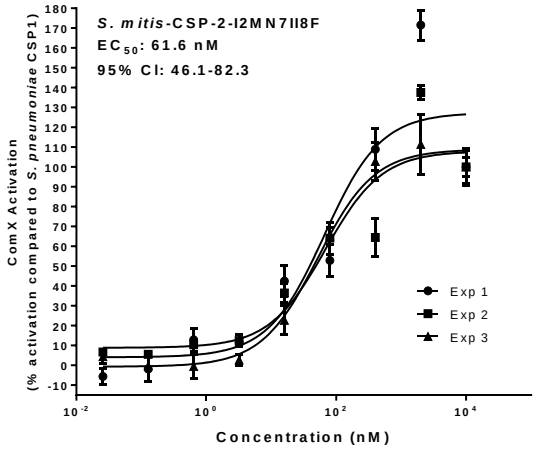

S. mitis-CSP-2-I8FF12L
EC50: 160 nM
95% CI: 104-248
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

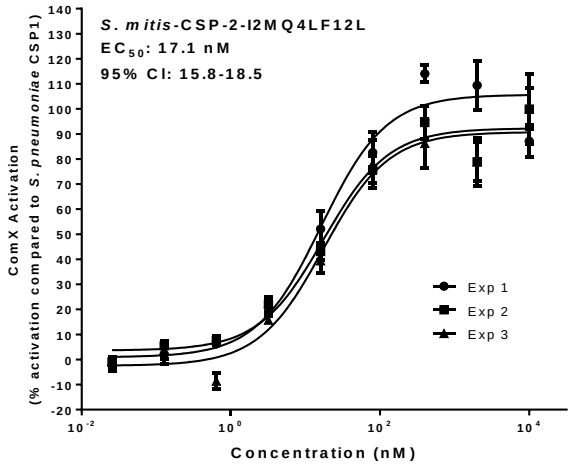

S. mitis-CSP-2-N11FF12L
EC50: 4.97 nM
95% CI: 4.12-5.99
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

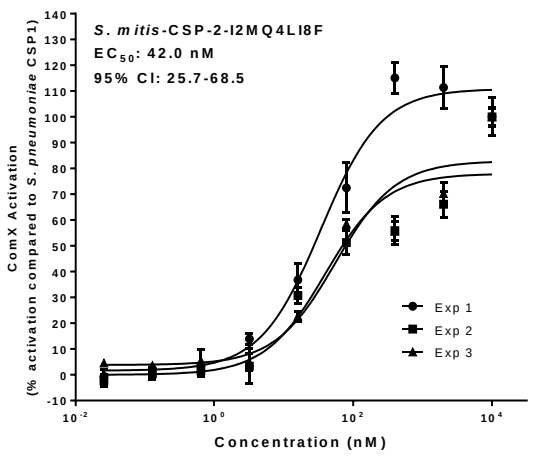

S. mitis-CSP-2-I2MQ4LN7F
EC50: 137 nM
95% CI: 105-178
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

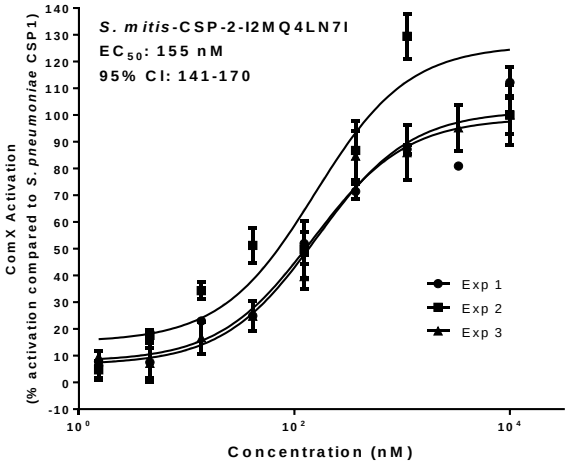

S. mitis-CSP-2-I2MI8FN11F
EC50: 6.95 nM
95% CI: 4.69-10.3
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

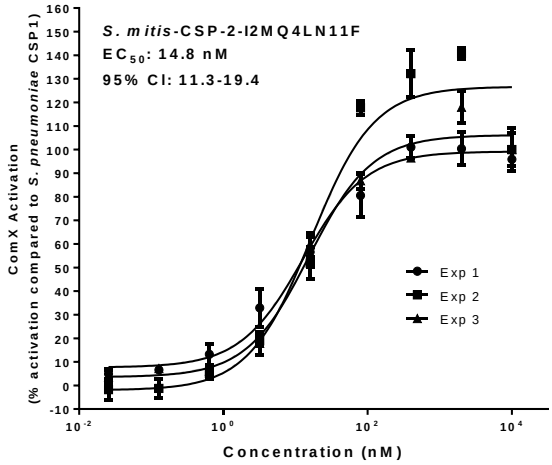

S. mitis-CSP-2-I2MN7FF12L
EC50: 72.8 nM
95% CI: 46.7-114
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

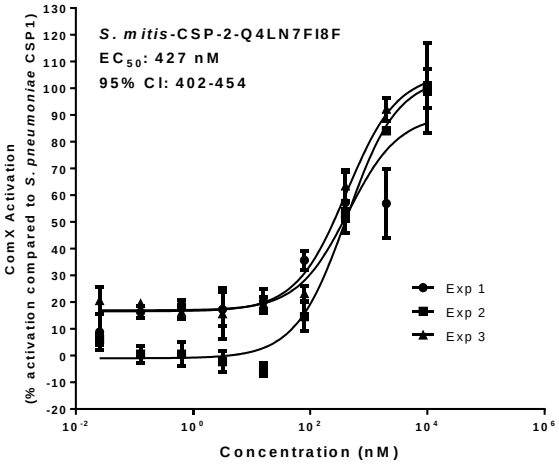

S. mitis-CSP-2-I2MN7II8F
EC50: 61.6 nM
95% CI: 46.1-82.3
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

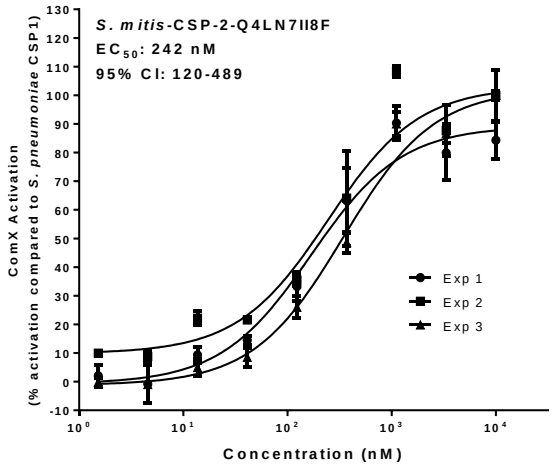

S. mitis-CSP-2-I2MQ4LF12L
EC50: 17.1 nM
95% CI: 15.8-18.5
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

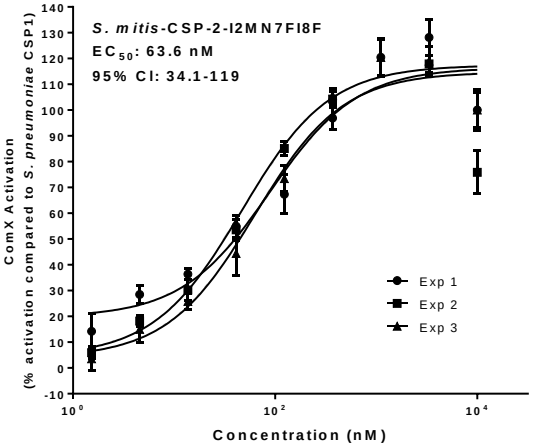

S. mitis-CSP-2-I2MQ4LI8F
EC50: 42.0 nM
95% CI: 25.7-68.5
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

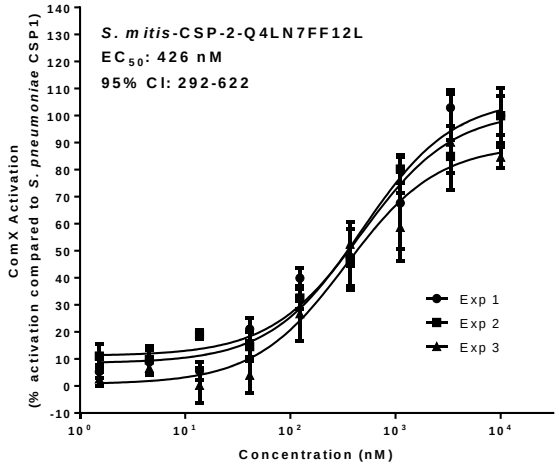

S. mitis-CSP-2-I2MQ4LN7I
EC50: 155 nM
95% CI: 141-170
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

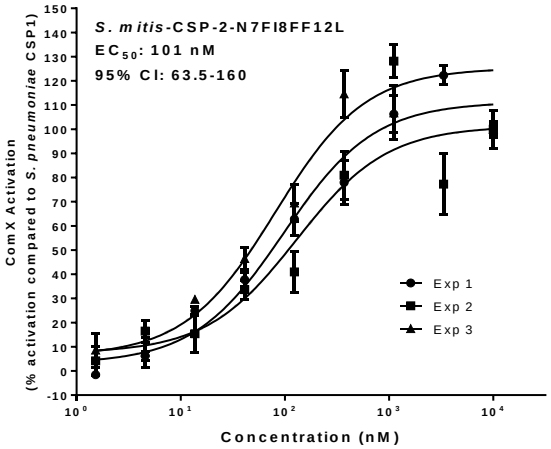

S. mitis-CSP-2-I2MQ4LN11F
EC50: 14.8 nM
95% CI: 11.3-19.4
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

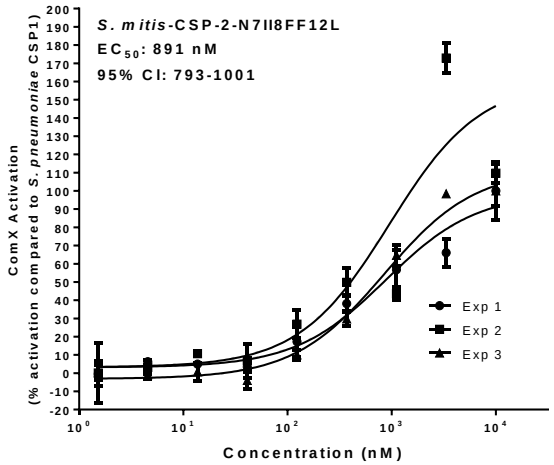

S. mitis-CSP-2-Q4LN7FI8F
EC50: 427 nM
95% CI: 402-454
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

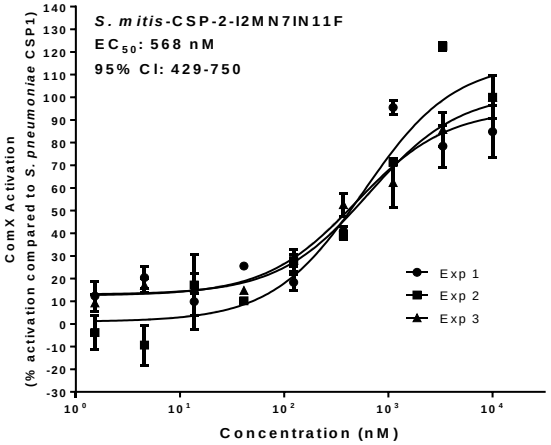

S. mitis-CSP-2-Q4LN7II8F
EC50: 242 nM
95% CI: 120-489
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3


S. mitis-CSP-2-I2MN7FI8F
EC50: 63.6 nM
95% CI: 34.1-119
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

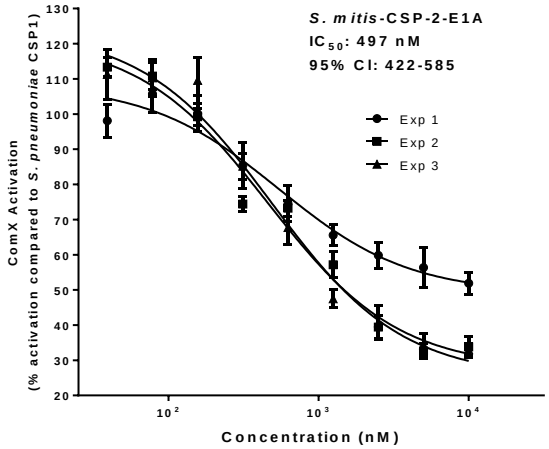


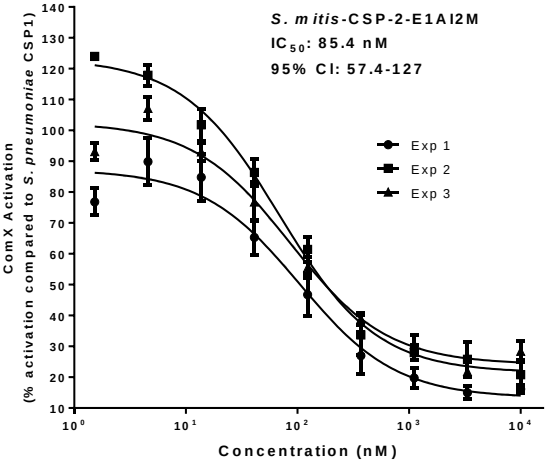


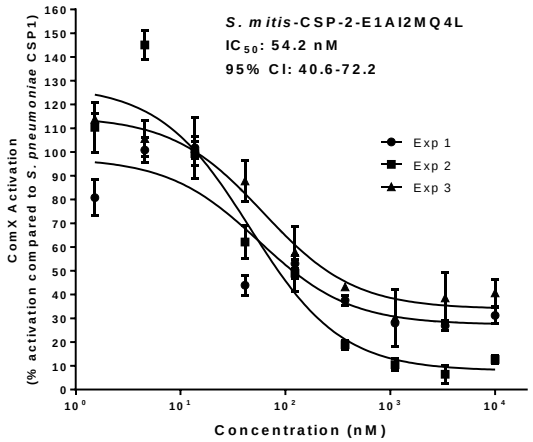


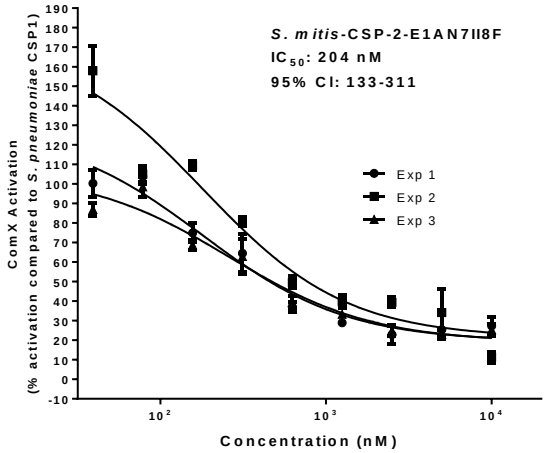


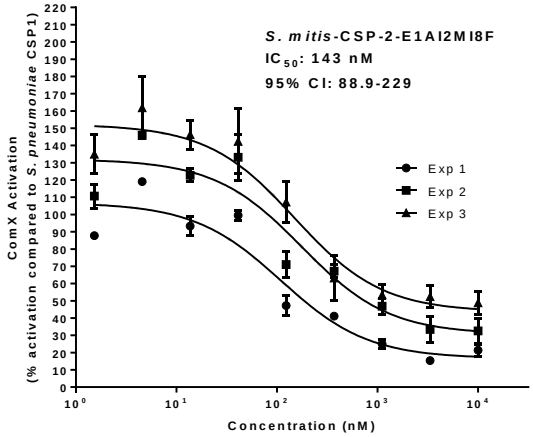
Inhibition dose response curves

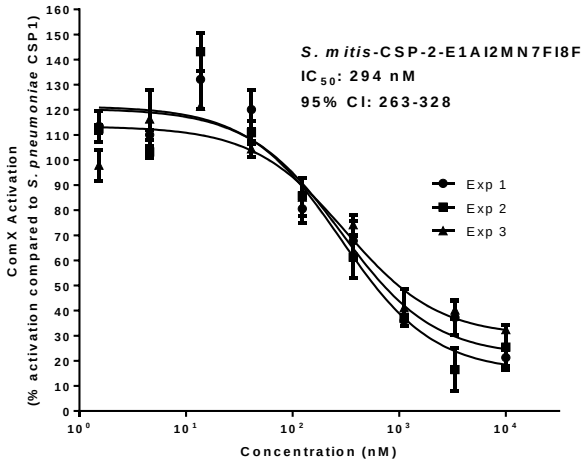


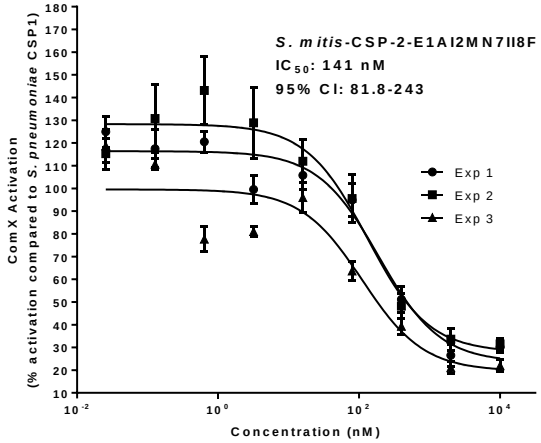


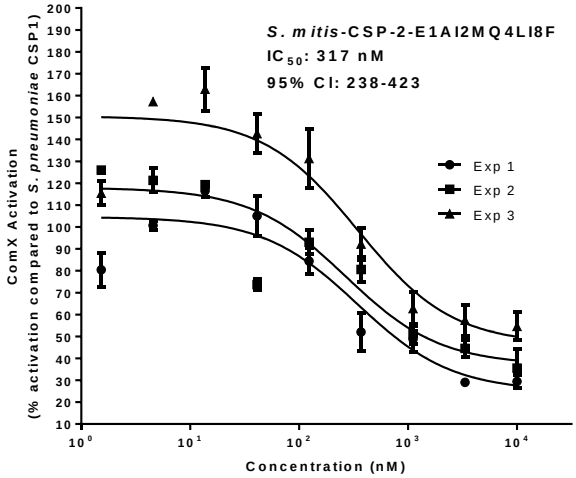

S. mitis-CSP-2-E1AI2MQ4L
IC50: 54.2 nM
95% CI: 40.6-72.2
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)


S. mitis-CSP-2-E1AN7II8F
IC50: 204 nM
95% CI: 133-311
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)


S. mitis-CSP-2-E1AI2MI8F
IC50: 143 nM
95% CI: 88.9-229
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)

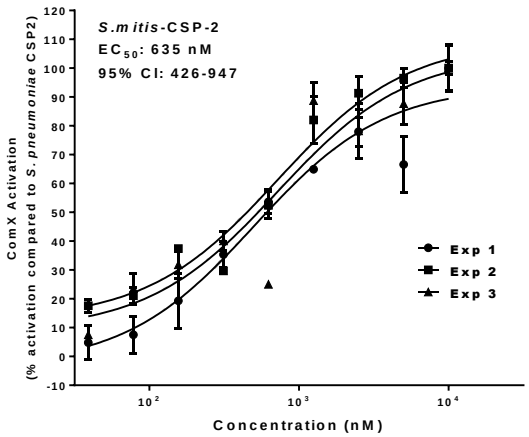

S. mitis-CSP-2-E1AI2MN7FI8F
IC50: 294 nM
95% CI: 263-328
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)

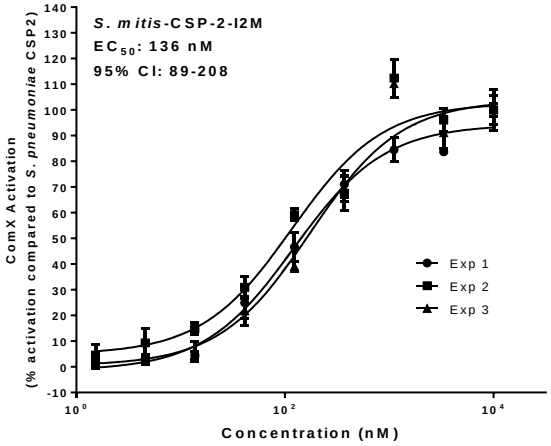

S. mitis-CSP-2-E1AI2MN7II8F
IC50: 141 nM
95% CI: 81.8-243
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)

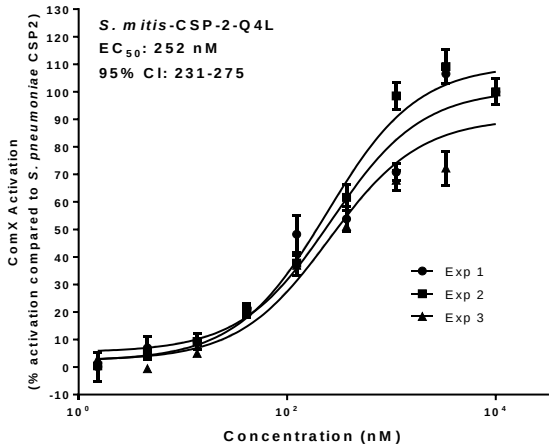

S. mitis-CSP-2-E1AI2MQ4LI8F
IC50: 317 nM
95% CI: 238-423
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to S. pneumoniae CSP1)
Concentration (nM)

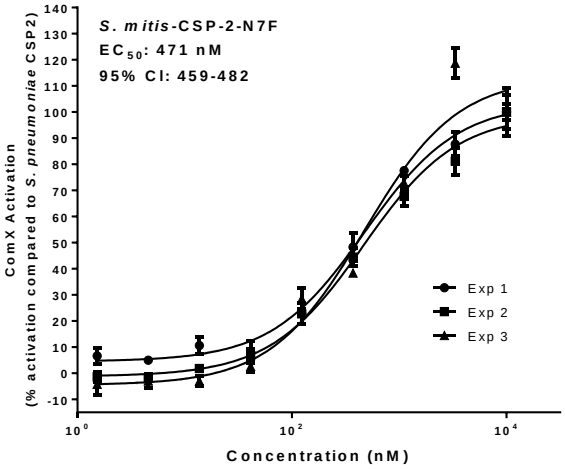
S. pneumoniae TIGR4 pcomX::lacZ (ComD2)

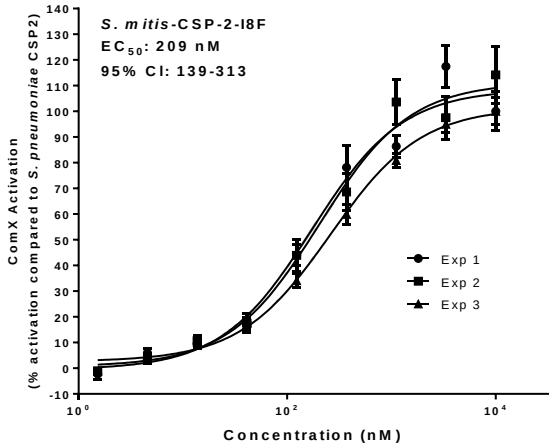
Activation dose response curves

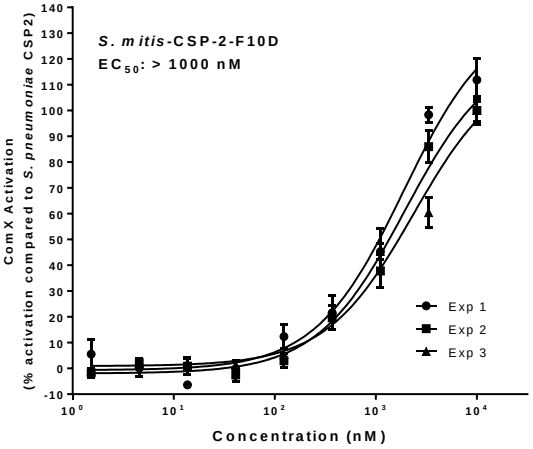


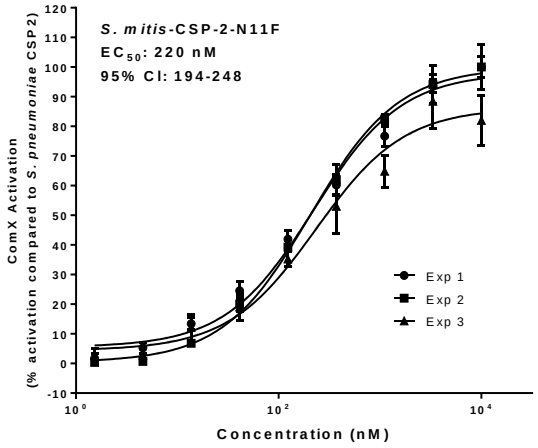


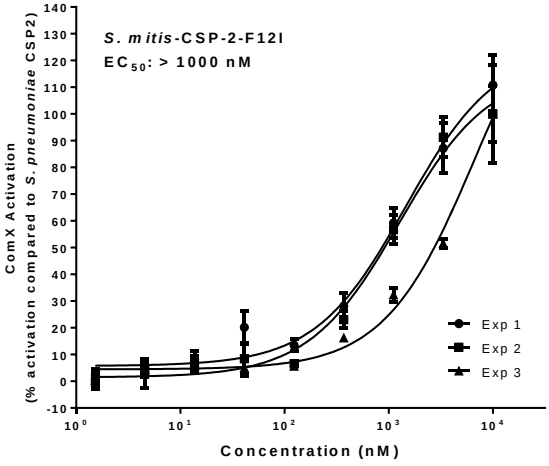


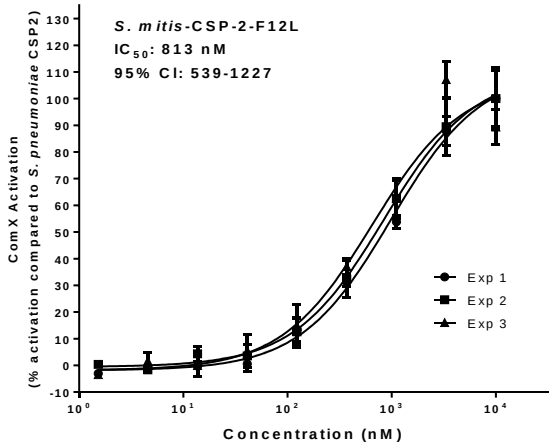


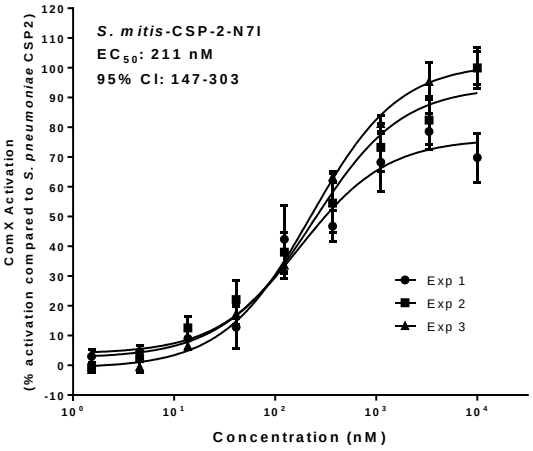


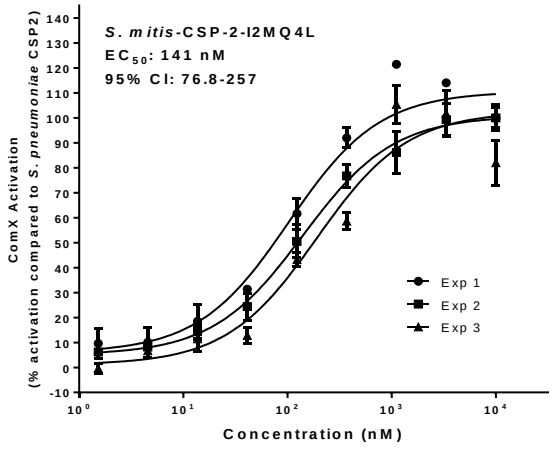


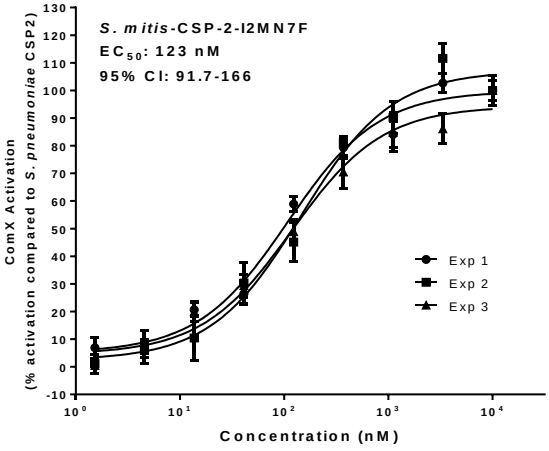

S. mitis-CSP-2-N11F
EC50: 220 nM
95% CI: 194-248
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

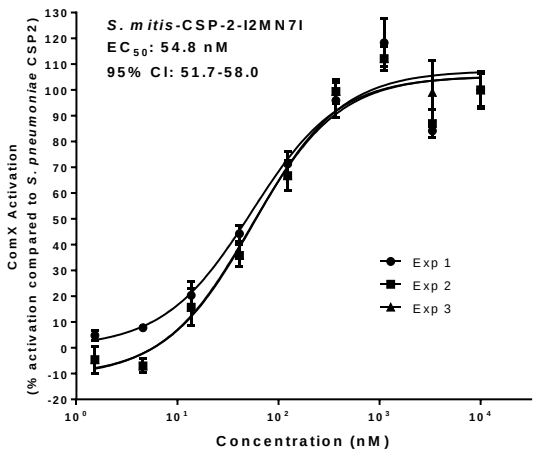

S. mitis-CSP-2-F12I
EC50: > 1000 nM
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

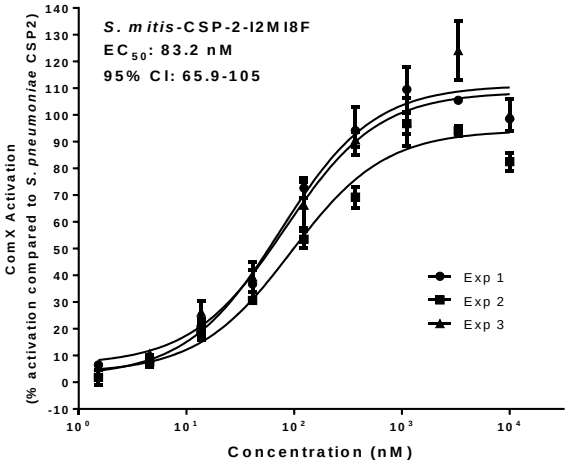

S. mitis-CSP-2-F12L
IC50: 813 nM
95% CI: 539-1227
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

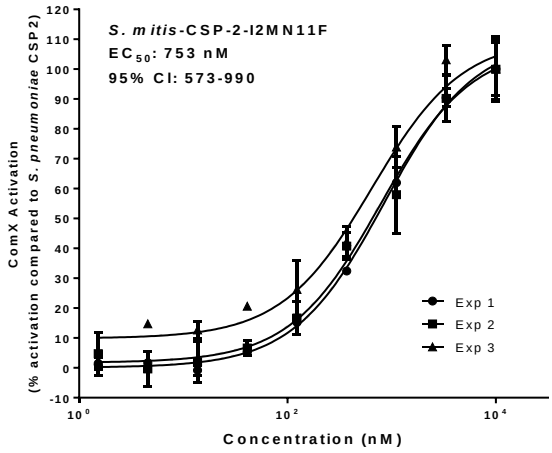

S. mitis-CSP-2-N7I
EC50: 211 nM
95% CI: 147-303
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

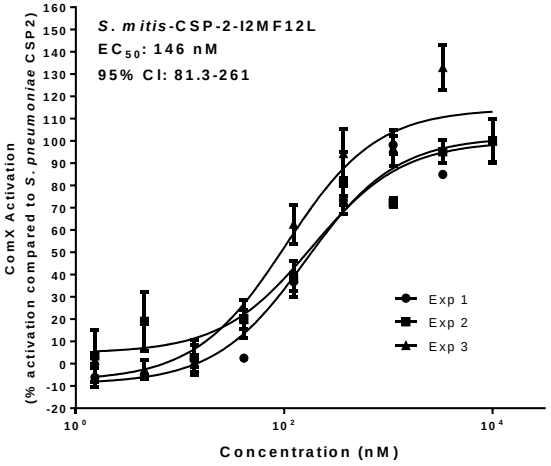

S. mitis-CSP-2-I2MQ4L
EC50: 141 nM
95% CI: 76.8-257
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

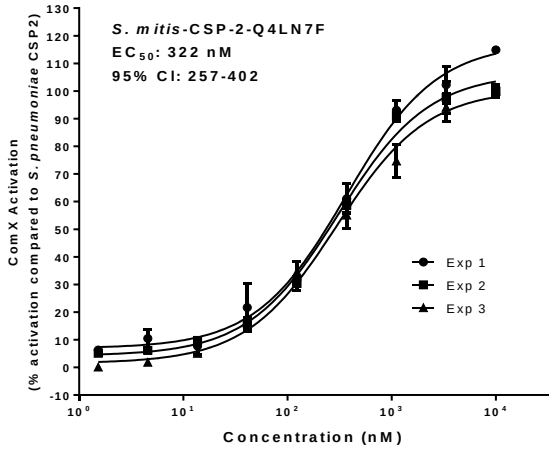

S. mitis-CSP-2-I2MN7F
EC50: 123 nM
95% CI: 91.7-166
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

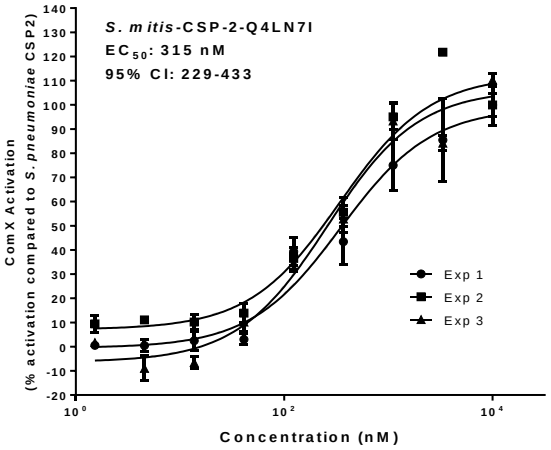

S. mitis-CSP-2-I2MN7I
EC50: 54.8 nM
95% CI: 51.7-58.0
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

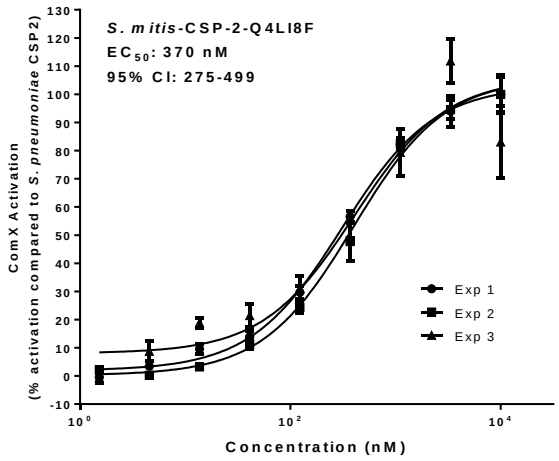

S. mitis-CSP-2-I2MI8F
EC50: 83.2 nM
95% CI: 65.9-105
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

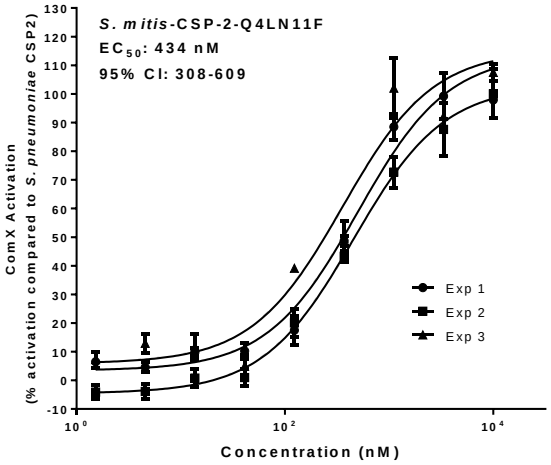

S. mitis-CSP-2-I2MN11F
EC50: 753 nM
95% CI: 573-990
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

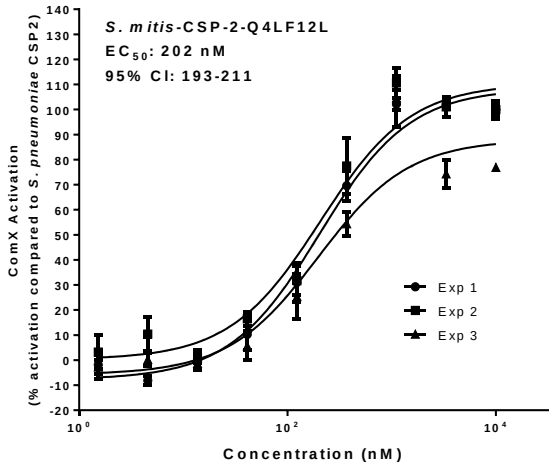

S. mitis-CSP-2-I2MF12L
EC50: 146 nM
95% CI: 81.3-261
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

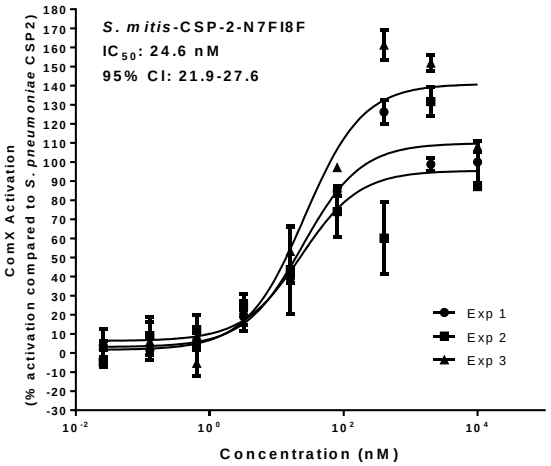

S. mitis-CSP-2-Q4LN7F
EC50: 322 nM
95% CI: 257-402
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

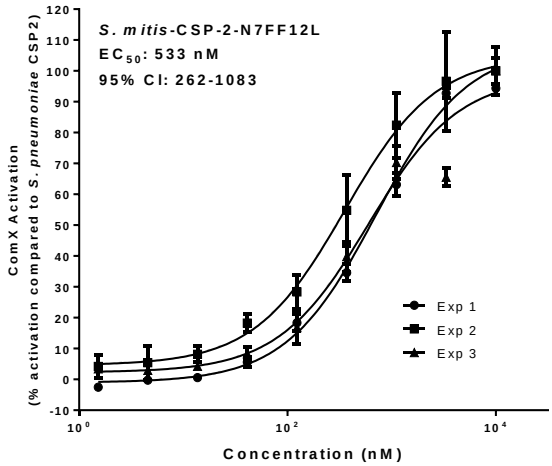

S. mitis-CSP-2-Q4LN7I
EC50: 315 nM
95% CI: 229-433
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

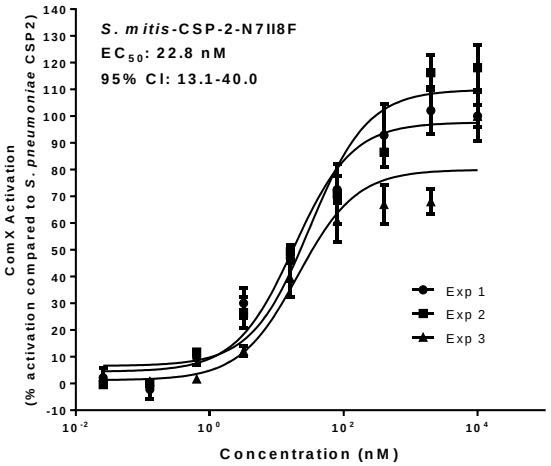

S. mitis-CSP-2-Q4LI8F
EC50: 370 nM
95% CI: 275-499
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

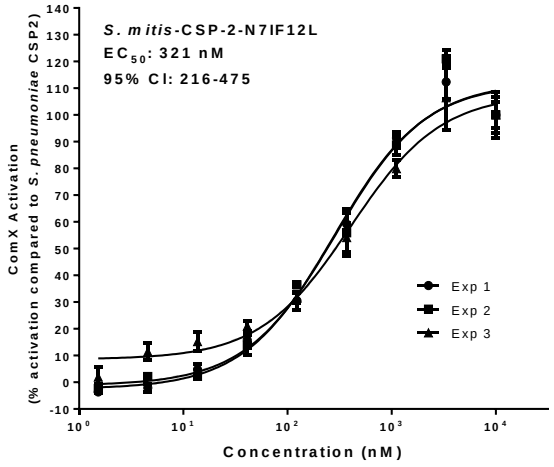

S. mitis-CSP-2-Q4LN11F
EC50: 434 nM
95% CI: 308-609
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

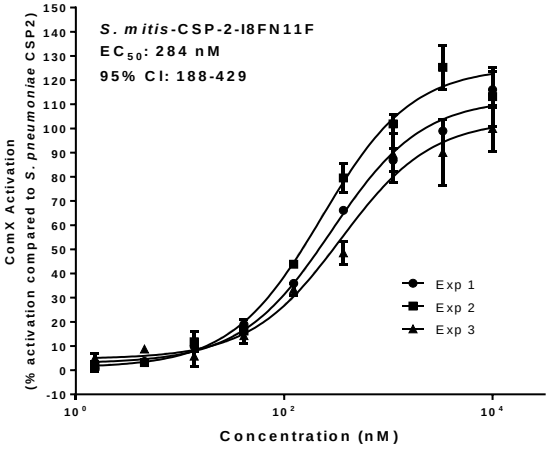

S. mitis-CSP-2-Q4LF12L
EC50: 202 nM
95% CI: 193-211
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

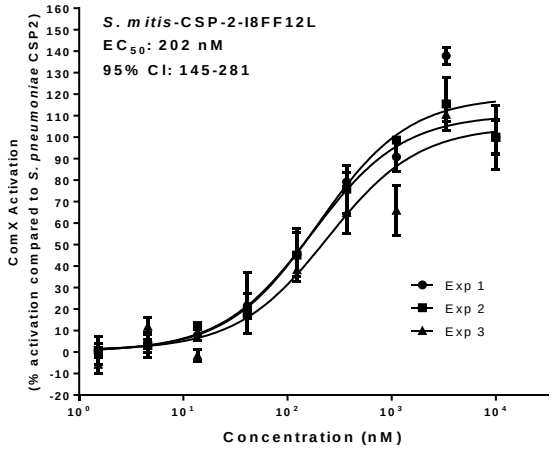

S. mitis-CSP-2-N7FI8F
IC50: 24.6 nM
95% CI: 21.9-27.6
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

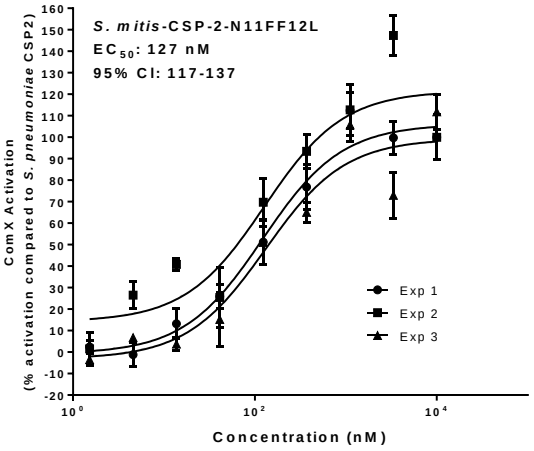

S. mitis-CSP-2-N7FF12L
EC50: 533 nM
95% CI: 262-1083
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

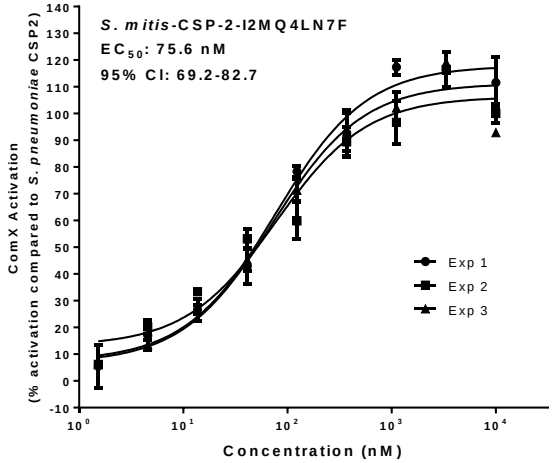

S. mitis-CSP-2-N7II8F
EC50: 22.8 nM
95% CI: 13.1-40.0
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

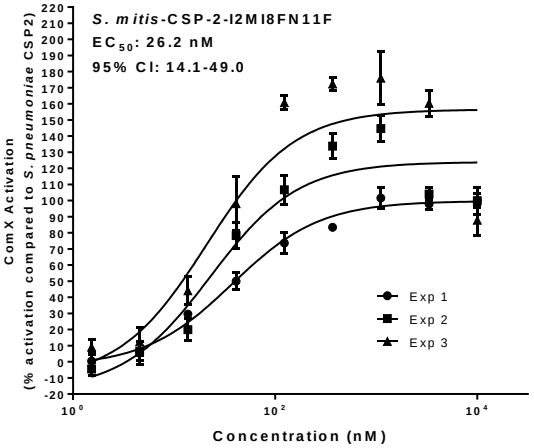

S. mitis-CSP-2-N7IF12L
EC50: 321 nM
95% CI: 216-475
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

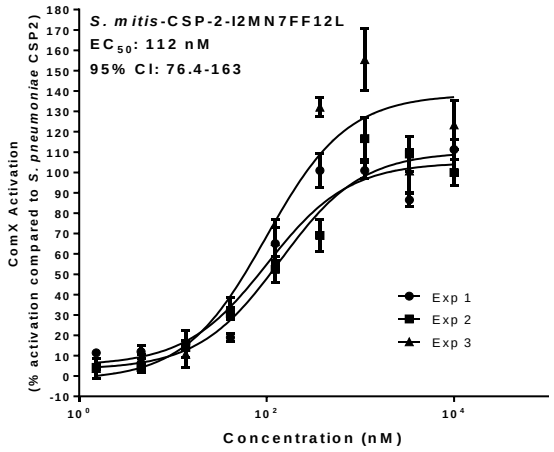

S. mitis-CSP-2-I8FN11F
EC50: 284 nM
95% CI: 188-429
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

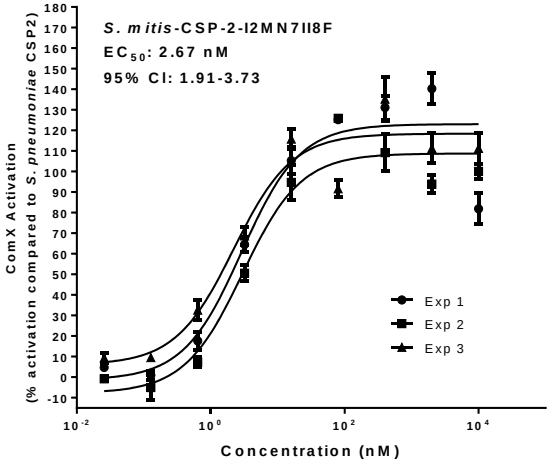

S. mitis-CSP-2-I8FF12L
EC50: 202 nM
95% CI: 145-281
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

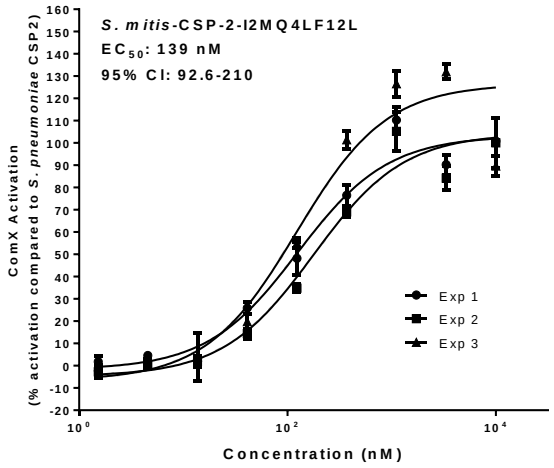

S. mitis-CSP-2-N11FF12L
EC50: 127 nM
95% CI: 117-137
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

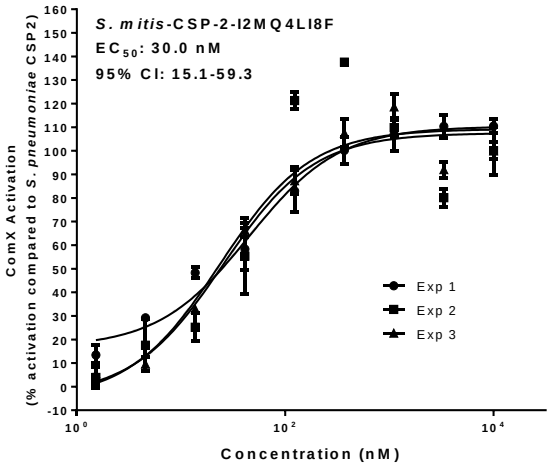

S. mitis-CSP-2-I2MQ4LN7F
EC50: 75.6 nM
95% CI: 69.2-82.7
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

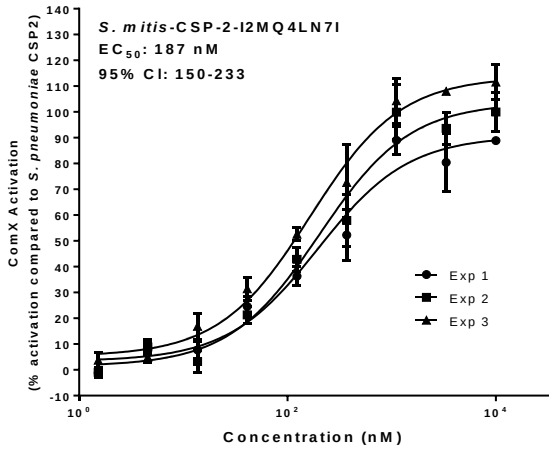

S. mitis-CSP-2-I2MI8FN11F
EC50: 26.2 nM
95% CI: 14.1-49.0
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

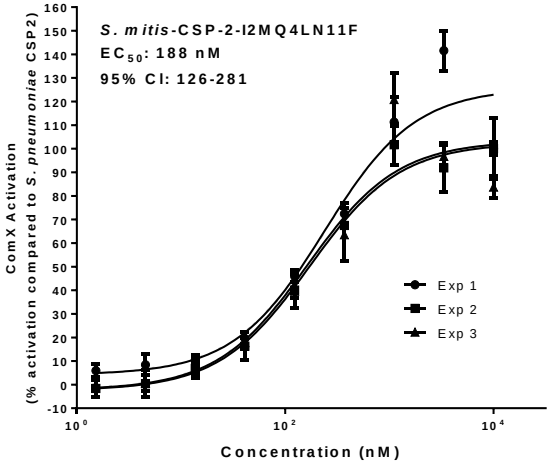

S. mitis-CSP-2-I2MN7FF12L
EC50: 112 nM
95% CI: 76.4-163
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

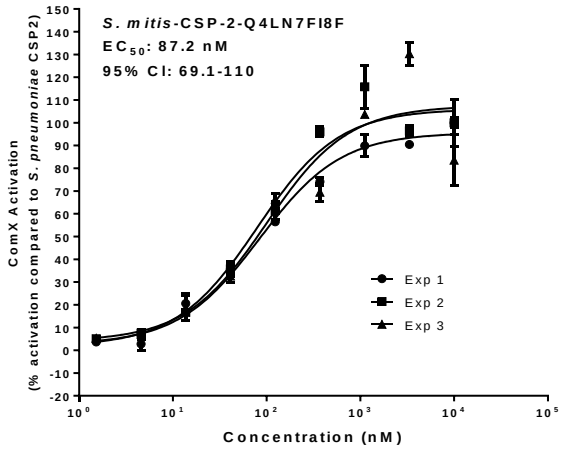

S. mitis-CSP-2-I2MN7II8F
EC50: 2.67 nM
95% CI: 1.91-3.73
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

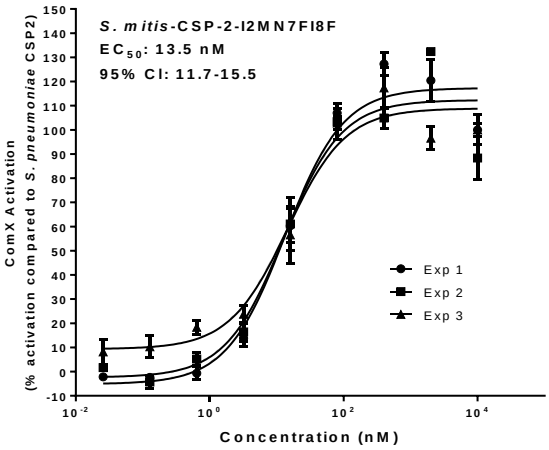

S. mitis-CSP-2-I2MQ4LF12L
EC50: 139 nM
95% CI: 92.6-210
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

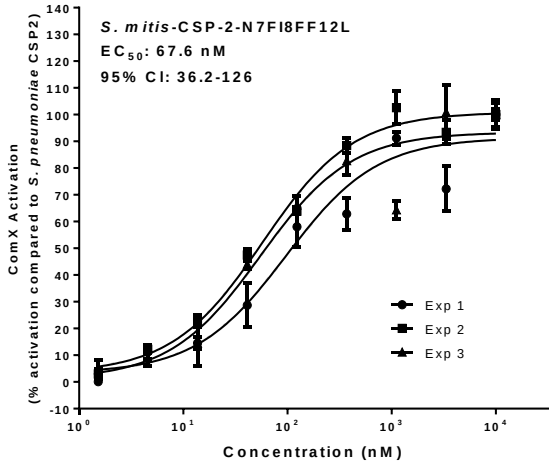

S. mitis-CSP-2-I2MQ4LI8F
EC50: 30.0 nM
95% CI: 15.1-59.3
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3


S. mitis-CSP-2-I2MQ4LN7I
EC50: 187 nM
95% CI: 150-233
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

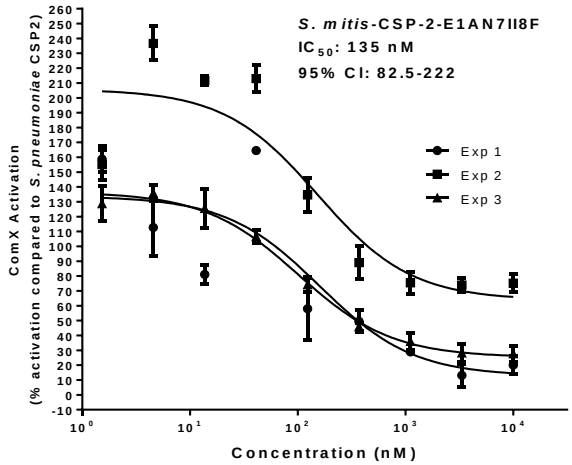

S. mitis-CSP-2-I2MQ4LN11F
EC50: 188 nM
95% CI: 126-281
ComX Activation
(% activation compared to S. pneumoniae CSP2)
Concentration (nM)
Exp 1
Exp 2
Exp 3

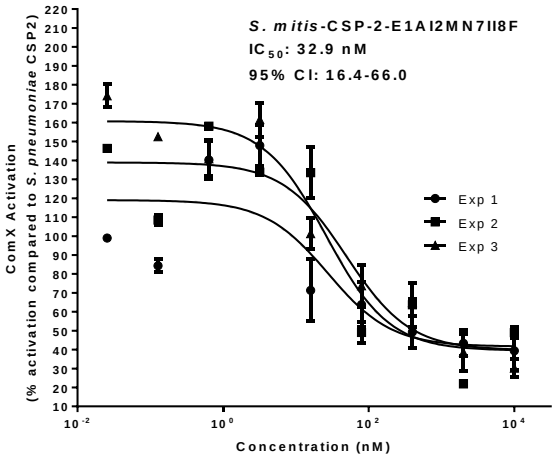


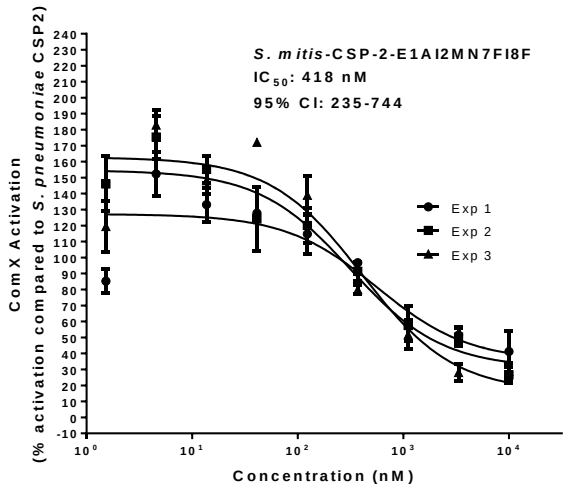




Inhibition dose response curves







References

1. Zhu, L.; Lau, G. W., Inhibition of competence development, horizontal gene transfer and virulence in Streptococcus pneumoniae by a modified competence stimulating peptide. *PLoS Pathog* **2011,** *7* (9), e1002241.
2. Yang, Y.; Koirala, B.; Sanchez, L. A.; Phillips, N. R.; Hamry, S. R.; Tal-Gan, Y., Structure–Activity Relationships of the Competence Stimulating Peptides (CSPs) in Streptococcus pneumoniae Reveal Motifs Critical for Intra-group and Cross-group ComD Receptor Activation. *ACS Chem Biol* **2017,** *12* (4), 1141-1151.