SUPPORTING INFORMATION

Structure-Function Analyses of a *Staphylococcus epidermidis* Autoinducing Peptide Reveals Motifs Critical for AgrC-type Receptor Modulation

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Contents.

- Additional experimental details
- MS and HPLC data for *S. epidermidis* AIPs and AIP analogs
- HPLC traces for *S. epidermidis* AIPs and AIP analogs
- *S. epidermidis* AgrC fluorescence dose response curves
- Tables of IC_{50} and EC_{50} values for active compounds with 95% confidence intervals
- Single-concentration screening data for AIPs using fluorescence reporter strains
- Crystal violet biofilm assay data

Additional experimental details.

Chemical reagents and instrumentation. Chemical reagents, resin, and solvents were purchased from commercial sources (Sigma-Aldrich and Chem-Impex International) and used as obtained, with the exception of anhydrous dichloromethane (CH_2Cl_2), which was kept dry using molecular sieves. Water (18 MΩ) was purified using a Barnstead Nanopure (Thermo Scientific).

Reverse-phase high performance liquid chromatography (RP-HPLC) was performed using a Shimadzu system equipped with a SLC-10Avp controller, a LC-10AT pump, a FCV-ALvp solvent mixer, and a SPC-10MAvp UV/Vis diode array detector. Preparative RP-HPLC work was performed on a semi-preparative Phenomenex Gemini C18 column (5 μm, 10 x 250 mm^2, 110 Å), and analytical RP-HPLC work was performed on an analytical Phenomenex Gemini C18 column (5 μm, 4.6 x 250 mm^2, 110 Å). The RP-HPLC conditions were as follows: flow rate = 5 mL min^{-1} for semi-preparative work and 1 mL min^{-1} for analytical work; mobile phase A = 18 MΩ water + 0.1% trifluoroacetic acid (TFA); mobile phase B = acetonitrile + 0.1% TFA. Linear peptides were purified using a linear gradient (80% → 50% A over 30 min). Cyclic peptides were purified using a linear gradient (80% → 65% A over 27 min). Peptide purity was determined using a linear gradient (90% → 5% A over 27 min) and integrating peaks detected at 220 nm.

A Micromass LCT ESI-TOF mass spectrometer (Waters) was used to obtain Exact Mass (EM) measurements for all peptides. The samples were sprayed with a sample cone voltage of 20 V.

Peptide synthesis. *S. epidermidis* AIPs and analogs were synthesized using our previously reported solid-phase synthesis and macrocyclization methods.[1] Briefly, linear peptide was synthesized on Boc-protected, amino acid pre-loaded 4-hydroxymethyl-phenylacetamidomethyl resin using standard Fmoc/tBu solid-phase synthesis protocols. The linear protected peptide was cleaved under anhydrous conditions using ethanethiol activated by dimethylaluminum chloride, and then treated with TFA to effect deprotection. The resulting linear peptide-thioester was purified by preparative RP-HPLC before being subjected macrocyclization. Upon macrocyclization completion, cyclic peptide was purified by preparative RP-HPLC, and its identity and purity were confirmed by exact mass (EM) measurement and analytical RP-HPLC, respectively. Purified cyclic peptide product was lyophilized prior to analysis in biological assays. See below for EM and HPLC characterization data for all of the peptides in this study. The native *S. aureus* AIPs I–IV and AIP-III D4A were previously prepared in our laboratory[1] and were not re-synthesized for the current study.

[1] Tal-Gan, Y., Stacy, D. M., Foegen, M. K., Koenig, D. W., and Blackwell, H. E. (2013) Highly Potent Inhibitors of Quorum Sensing in *Staphylococcus aureus* Revealed Through a Systematic Synthetic Study of the Group-III Autoinducing Peptide, *J. Am. Chem. Soc. 135*, 7869–7882.

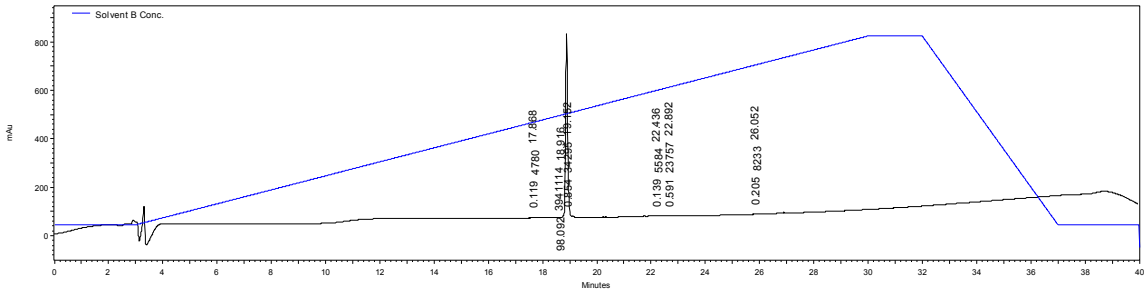
MS and HPLC data for *S. epidermidis* AIPs and AIP analogs.

Table S-1. MS and HPLC data for the native *S. epidermidis* AIPs and AIP analogs in this study. EM = exact mass. Rt = retention time.

Peptide name	Structure	Calc. EM	Obs. EM	Rt (min)	% Purity
AIP-I	D-S-V-(C-A-S-Y-F)	873.3448	873.3456	18.9	>98
AIP-II	N-A-S-K-Y-N-P-(C-S-N-Y-L)	678.3056 $[M+2H]^{2+}$	678.3058 $[M+2H]^{2+}$	17.6	>99
AIP-III	N-A-A-K-Y-N-P-(C-A-S-Y-L)	648.8053 $[M+2H]^{2+}$	648.8043 $[M+2H]^{2+}$	18.0	>99
AIP-I D1A	A-S-V-(C-A-S-Y-F)	829.3550	829.3538	17.9	>99
AIP-I S2A	D-A-V-(C-A-S-Y-F)	857.3499	857.3494	18.1	>97
AIP-I V3A	D-S-A-(C-A-S-Y-F)	845.3135	845.3126	18.3	>99
AIP-I S6A	D-S-V-(C-A-A-Y-F)	857.3499	857.3495	19.7	>97
AIP-I Y7A	D-S-V-(C-A-S-A-F)	781.3186	781.3186	16.5	>97
AIP-I F8A	D-S-V-(C-A-S-Y-A)	797.3135	797.3135	15.5	>99
AIP-I D-D1	dD-S-V-(C-A-S-Y-F)	873.3448	873.3442	22.5	>97
AIP-I D-S2	D-dS-V-(C-A-S-Y-F)	873.3448	873.3458	19.9	>96
AIP-I D-V3	D-S-dV-(C-A-S-Y-F)	873.3448	873.3448	21.6	>97
AIP-I D-C4	D-S-V-(dC-A-S-Y-F)	873.3448	873.3455	23.0	>96
AIP-I D-A5	D-S-V-(C-dA-S-Y-F)	873.3448	873.3452	23.2	>94
AIP-I D-S6	D-S-V-(C-A-dS-Y-F)	873.3448	873.3447	22.5	>94
AIP-I D-Y7	D-S-V-(C-A-S-dY-F)	873.3448	873.3447	22.6	>92
AIP-I D-F8	D-S-V-(C-A-S-Y-dF)	873.3448	873.3447	22.5	>98
AIP-I A5Y	D-S-V-(C-Y-S-Y-F)	965.3710	965.3712	19.5	>98
AIP-I A5S-1	D-S-V-(C-S-S-Y-F)	889.3397	889.3372	18.5	>98
AIP-I A5S-2	D-S-V-(C-S-S-Y-F)	889.3397	889.3372	18.6	>96
AIP-I V3AA5Y	D-S-A-(C-Y-S-Y-F)	937.3397	937.3399	17.9	>99
AIP-I V3AA5S	D-S-A-(C-S-S-Y-F)	861.3084	861.3065	16.8	>98
AIP-I D1AS6A	A-S-V-(C-A-A-Y-F)	813.3600	813.3604	18.1	>99
tAIP-I	Ac-(C-A-S-Y-F)	614.2280	614.2279	18.9	>99
AIP-I D1AS6AV3A	A-S-A-(C-A-A-Y-F)	785.3287	785.3284	17.6	>99
AIP-I D1AS6AV3AA5S	A-S-A-(C-S-A-Y-F)	801.3237	801.3239	17.1	>99
AIP-II 11aa	A-S-K-Y-N-P-(C-S-N-Y-L)	1241.5620	1241.562	17.7	>99
AIP-II 10aa	S-K-Y-N-P-(C-S-N-Y-L)	1170.5249	1170.5249	17.3	>99
AIP-II 9aa	K-Y-N-P-(C-S-N-Y-L)	1083.4928	1083.4962	16.5	>99
AIP-II 8aa	Y-N-P-(C-S-N-Y-L)	955.3979	955.4007	17.3	>99
AIP-II 7aa	N-P-(C-S-N-Y-L)	792.3346	792.3314	16.5	>99
tAIP-II	Ac-(C-S-N-Y-L)	623.2494	623.2491	17.7	>99

HPLC traces for *S. epidermidis* AIPs and AIP analogs.

AIP-I



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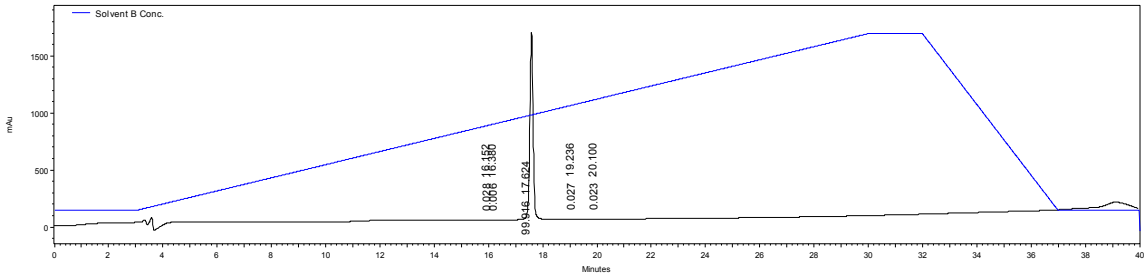

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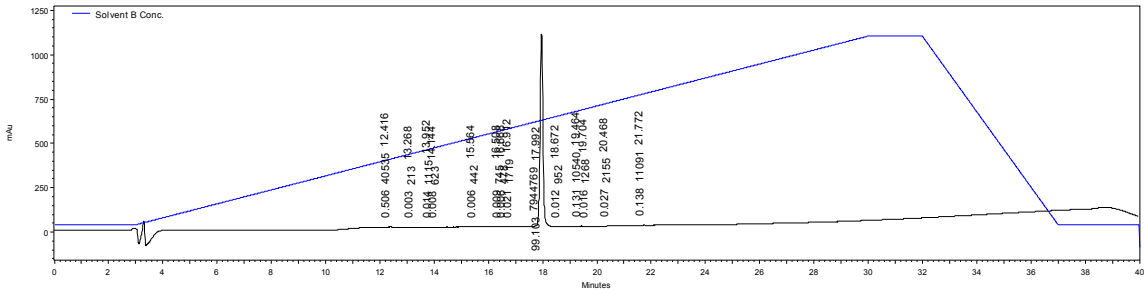

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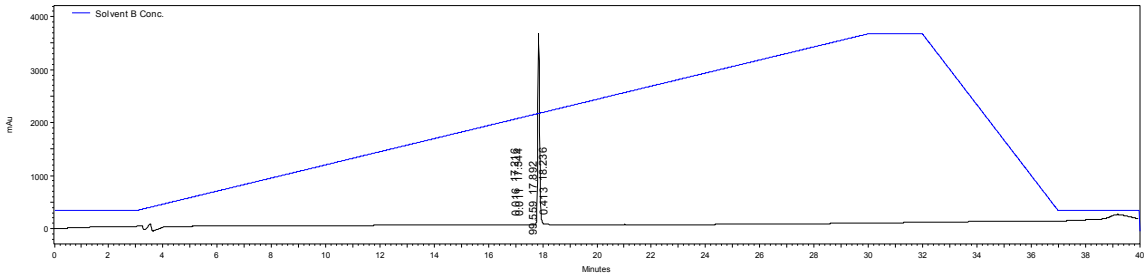

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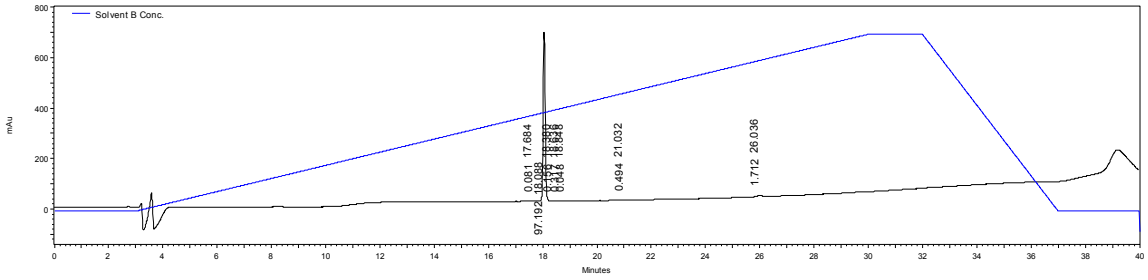

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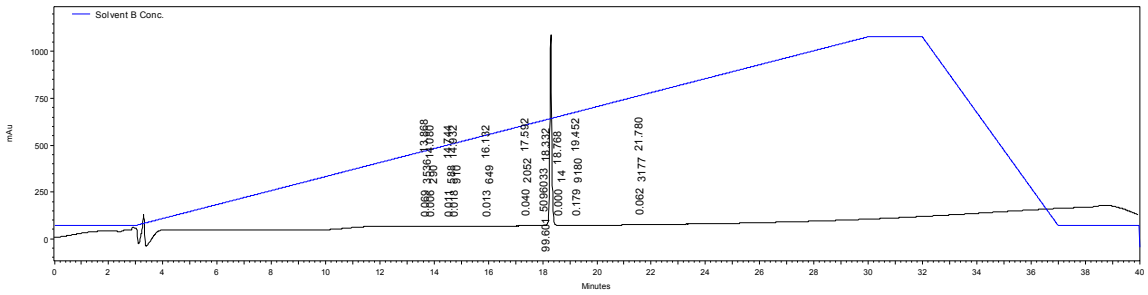

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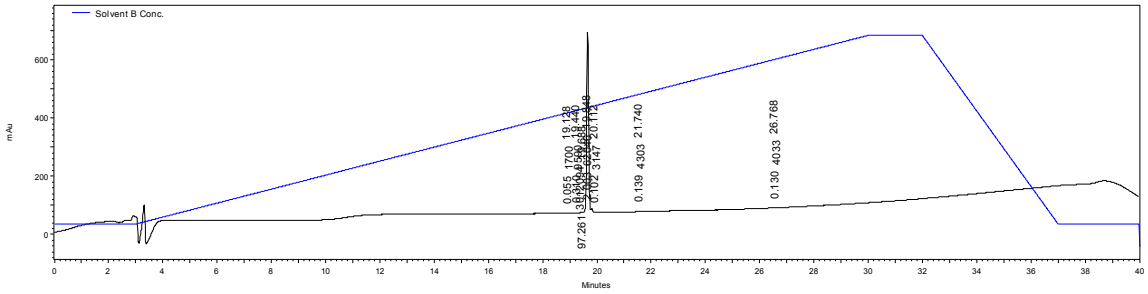

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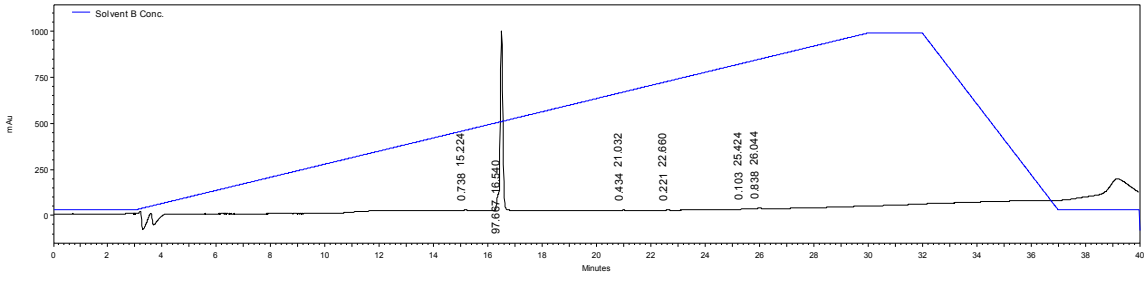

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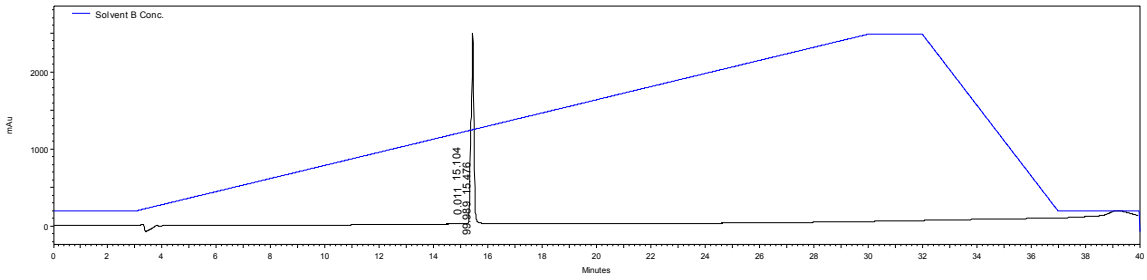

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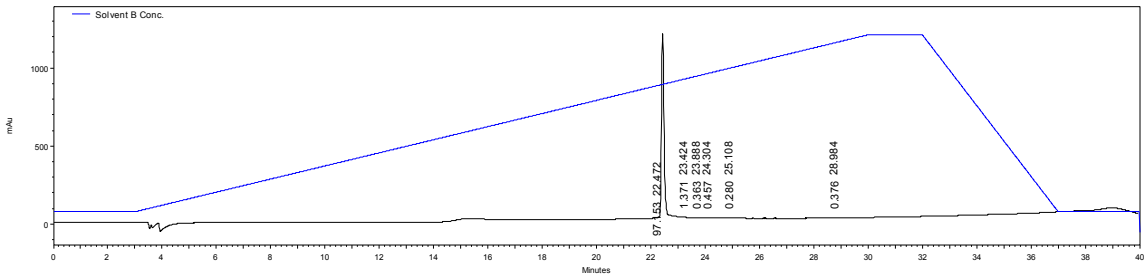

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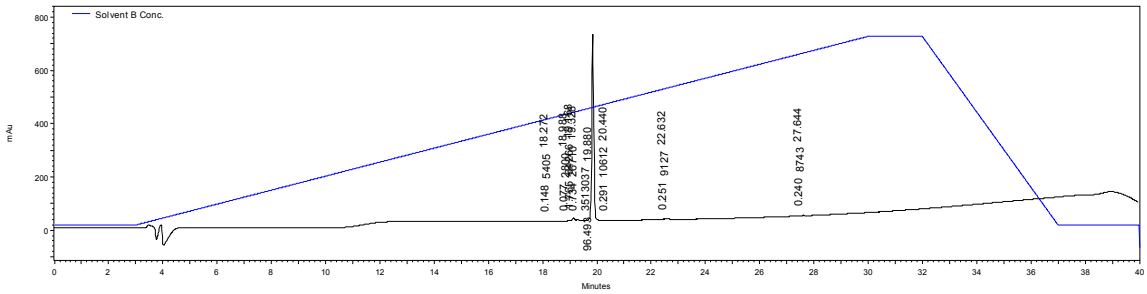

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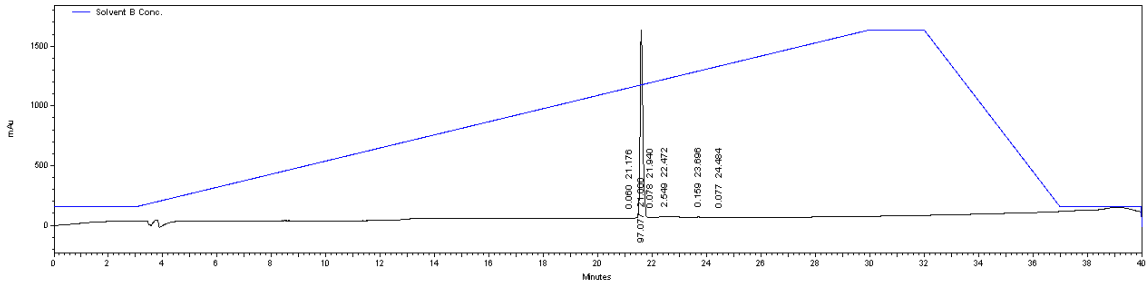

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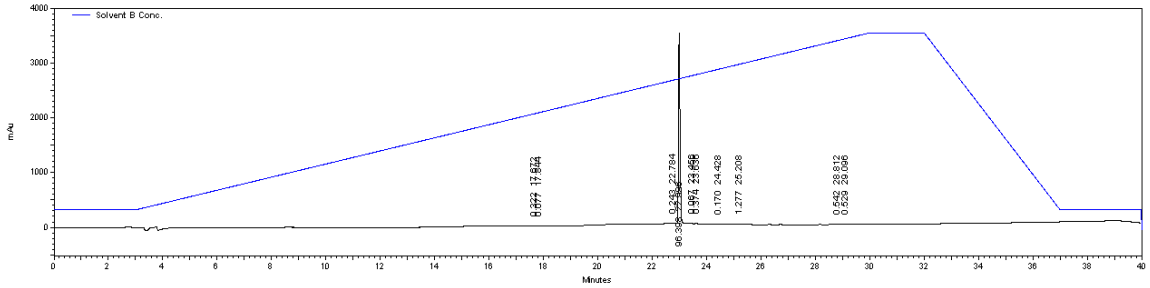

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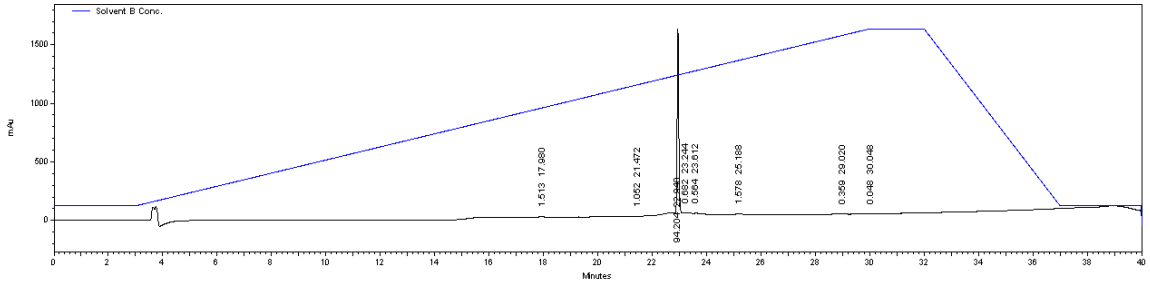

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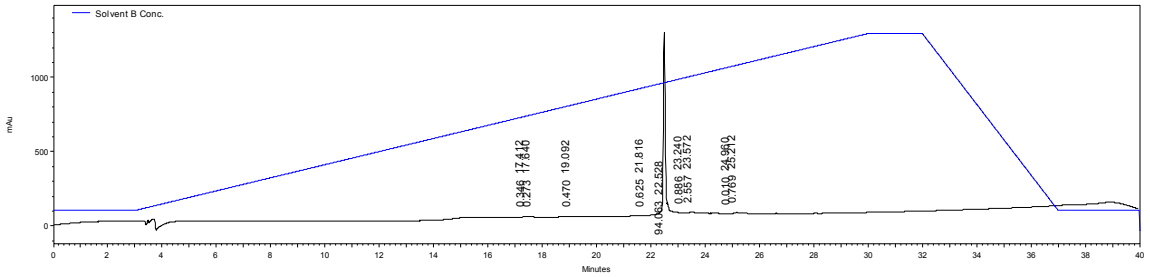

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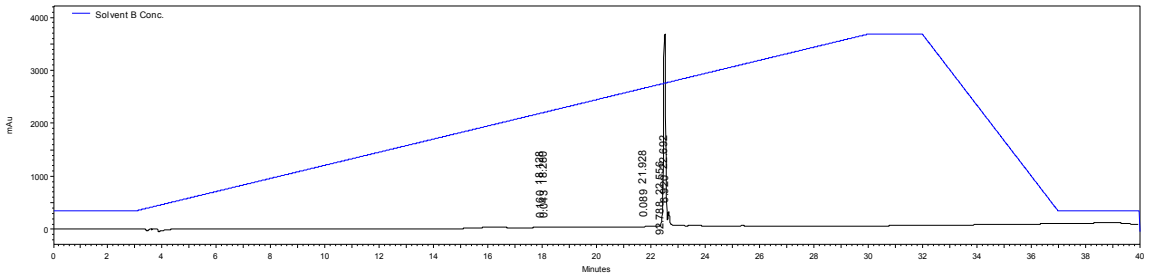

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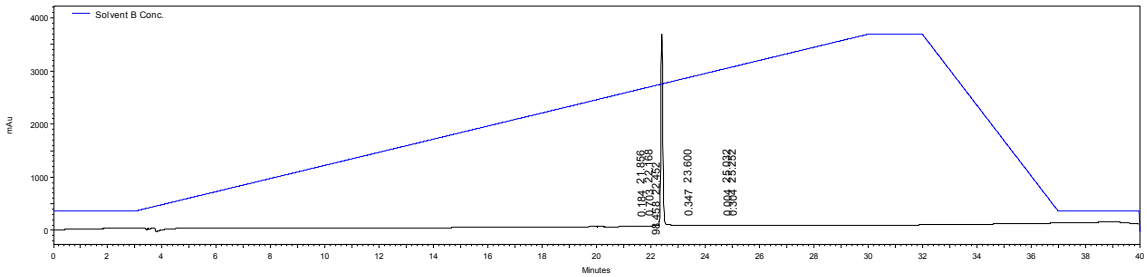

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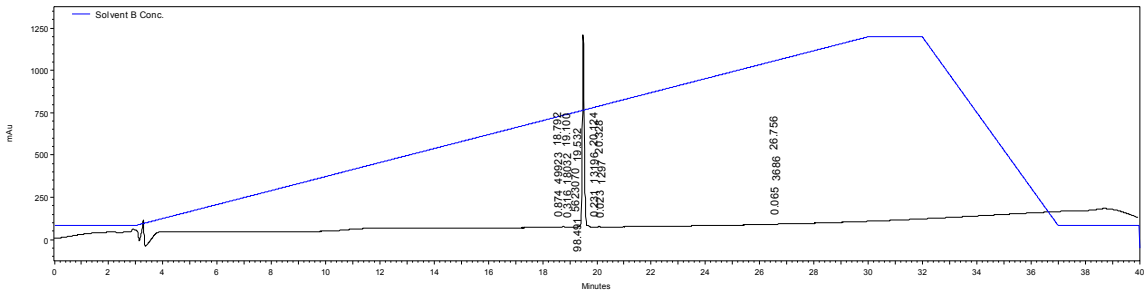

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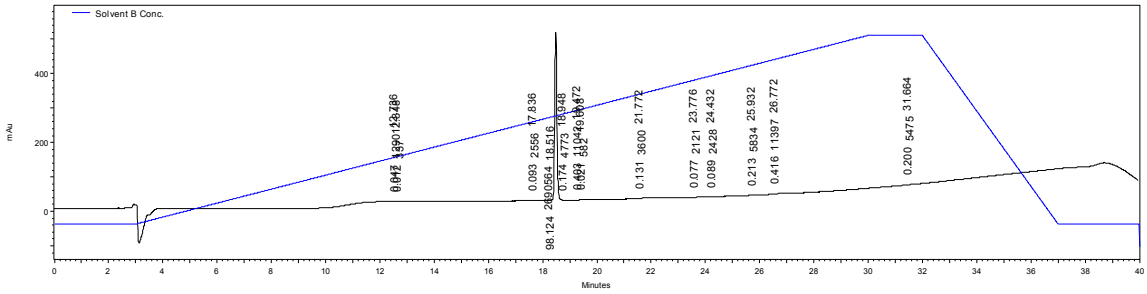

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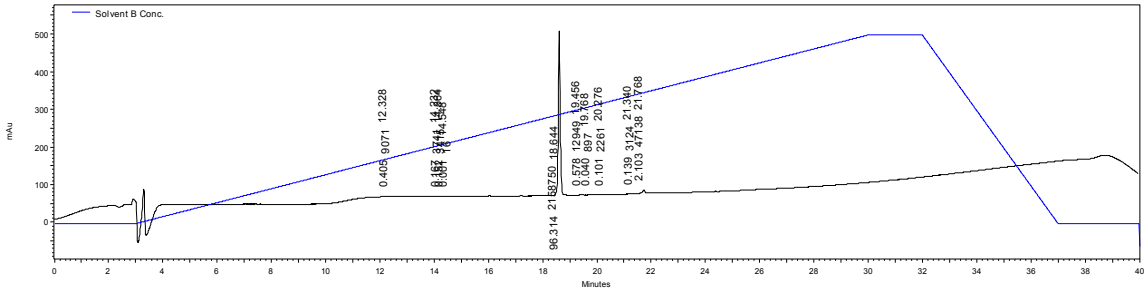

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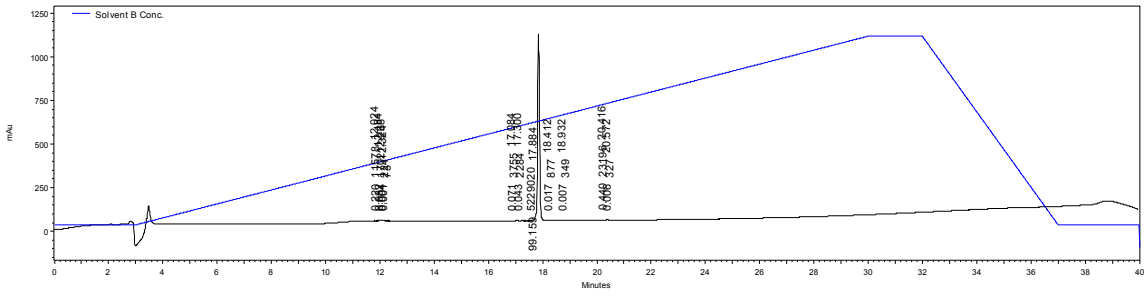

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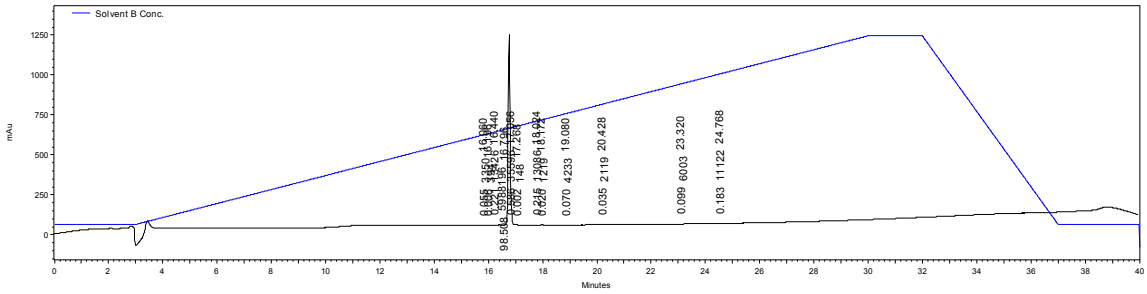

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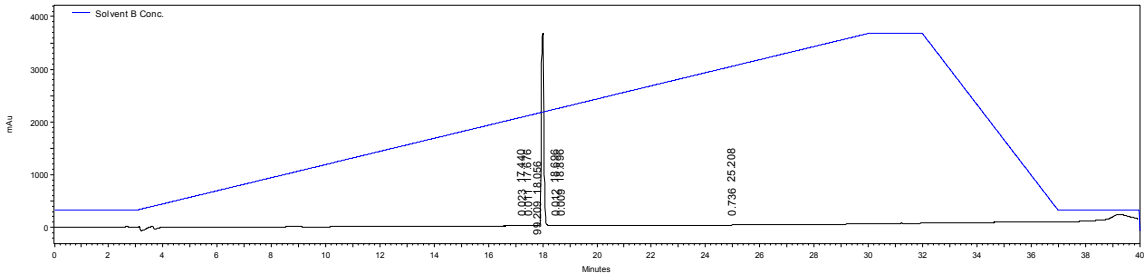

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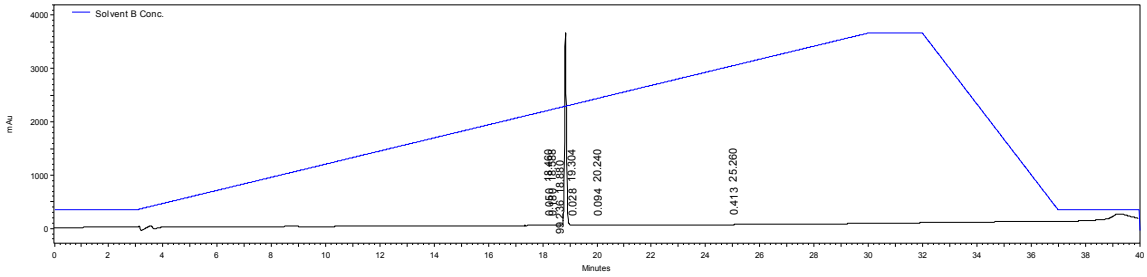

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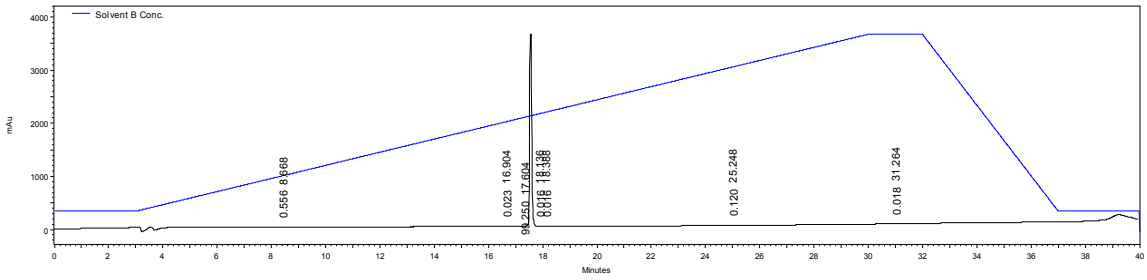

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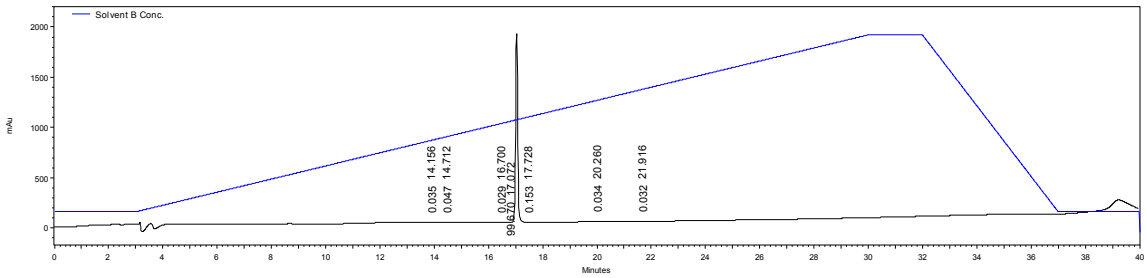
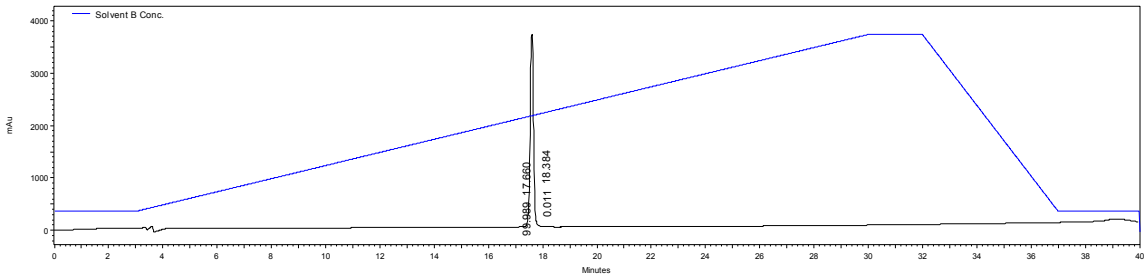


AIP-II 11aa



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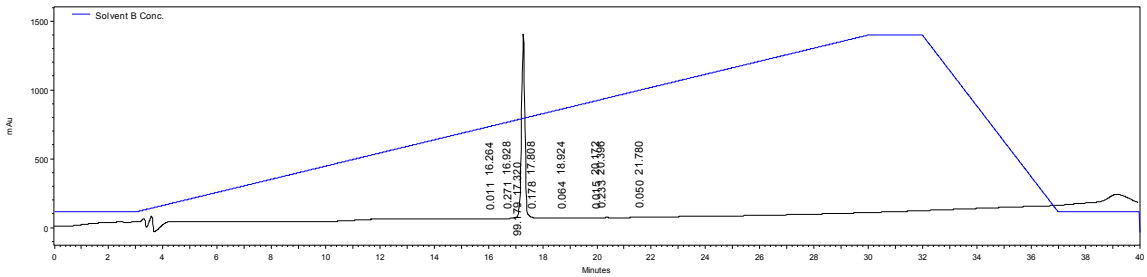

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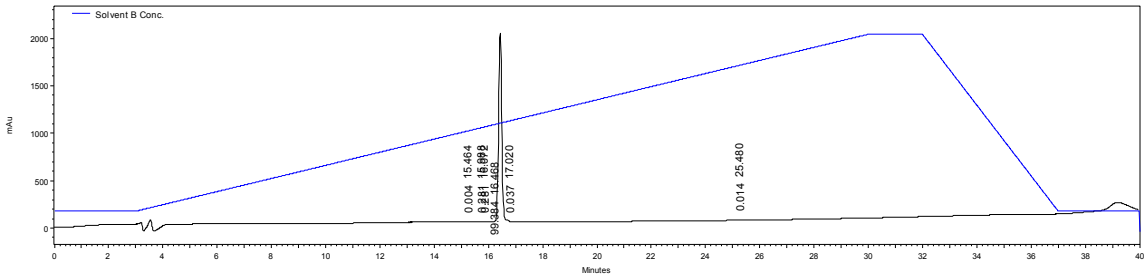

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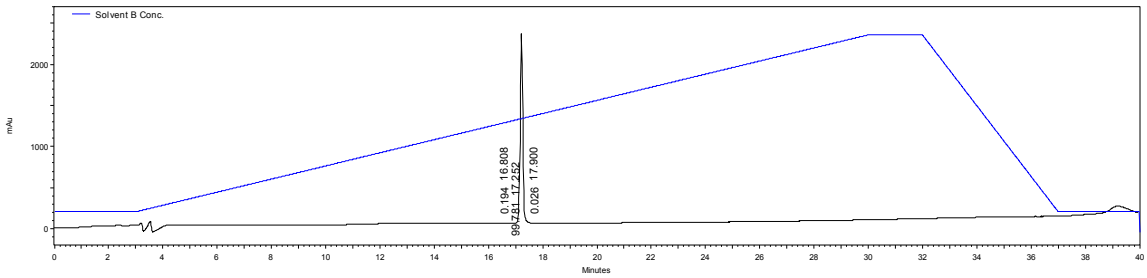

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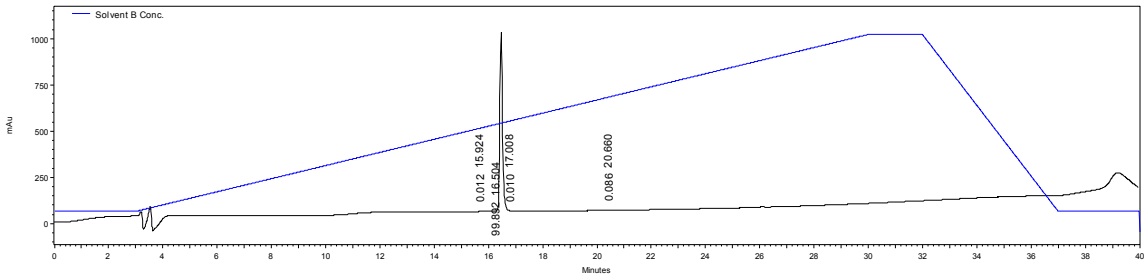

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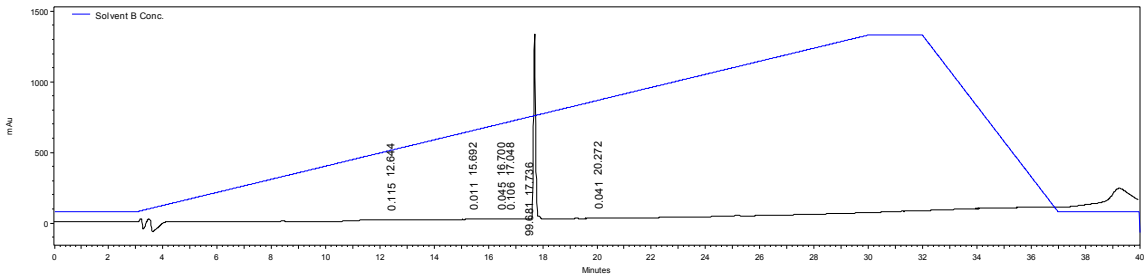
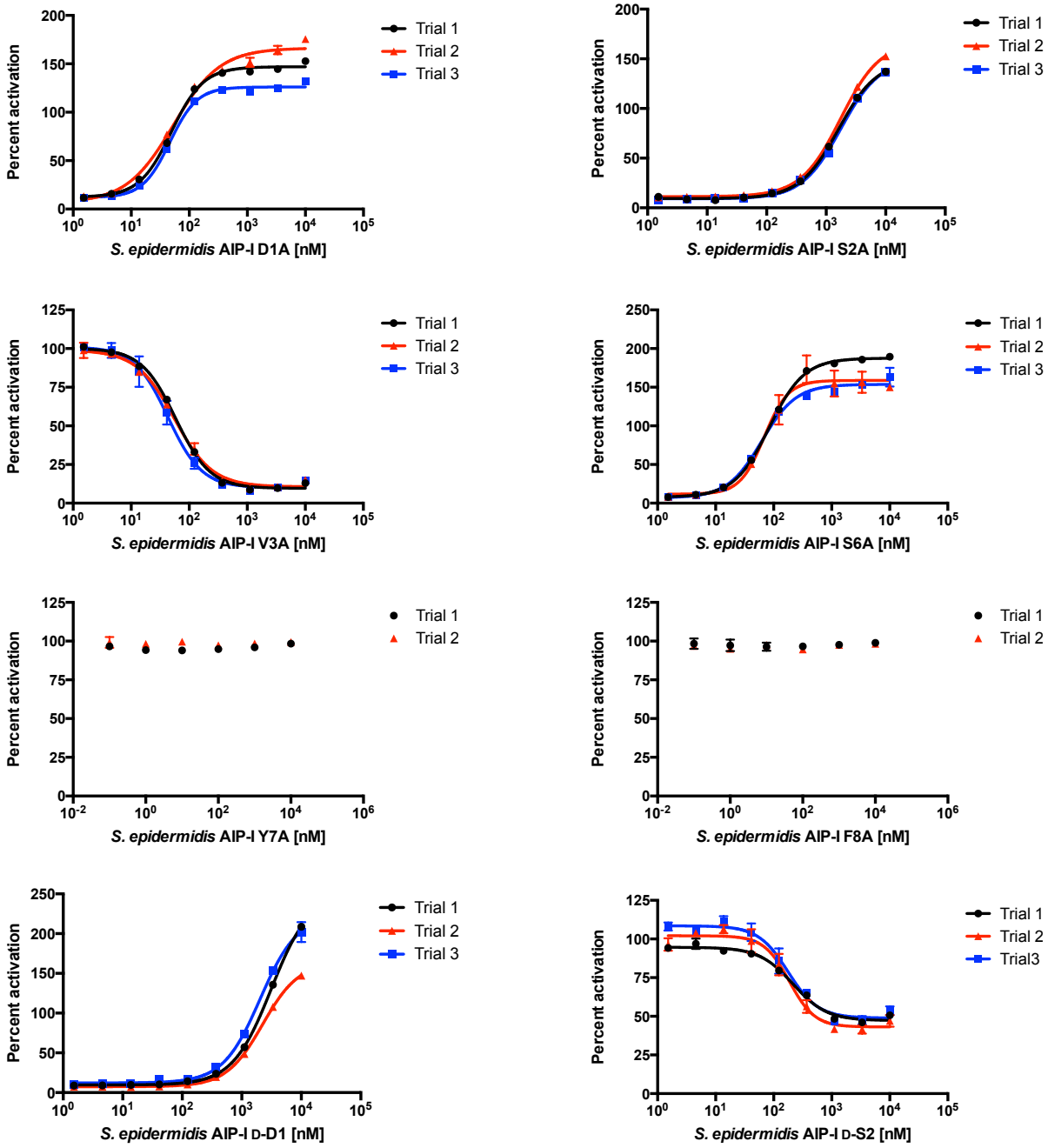
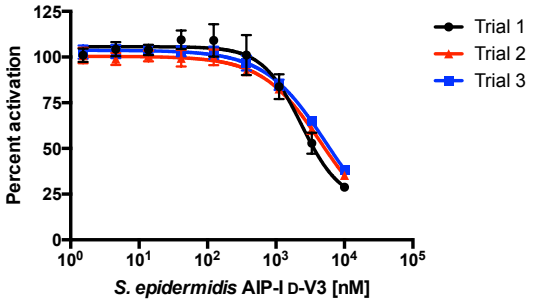


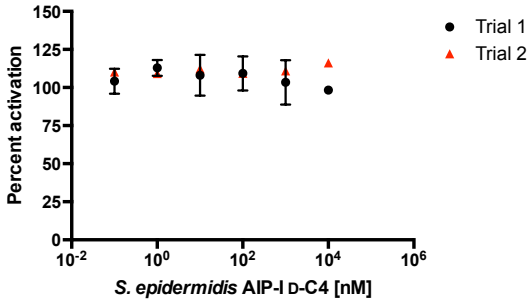
S. epidermidis **AgrC fluorescence dose response curves.**

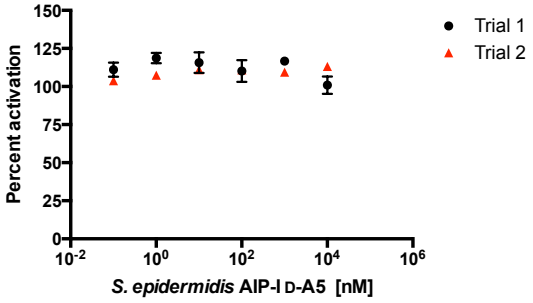
The analogs were screened over varying concentrations in the *S. epidermidis* fluorescence reporter strains for AgrC receptor agonism and antagonism (see main text for details of strains and protocols). For AgrC-I agonism dose response assays, 50 nM *S. epidermidis* AIP-II was introduced to all wells to block AgrC-I activation due to endogenously produced AIP-I. Curves for each of the three biological replicates (Trials 1–3) are shown; error bars represent standard deviation of triplicate values. Percent activation was normalized to a DMSO control.

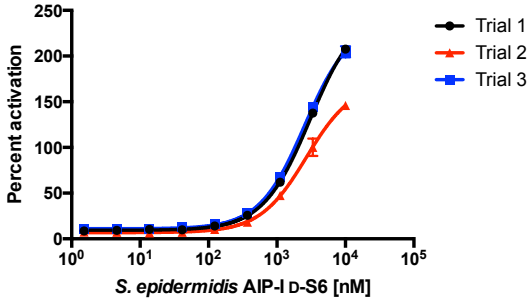
- *S. epidermidis* AH3408 (Group-I)

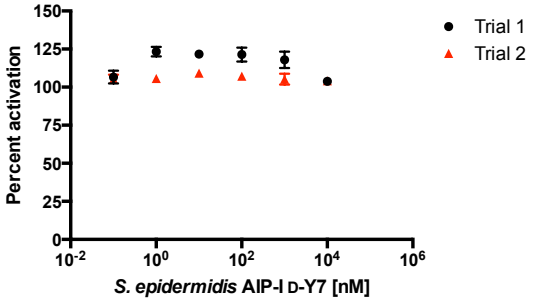


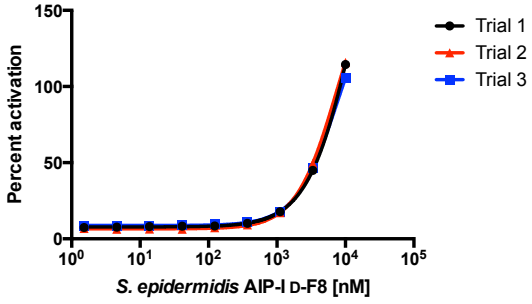

Percent activation
S. epidermidis AIP-I D-V3 [nM]
Trial 1
Trial 2
Trial 3

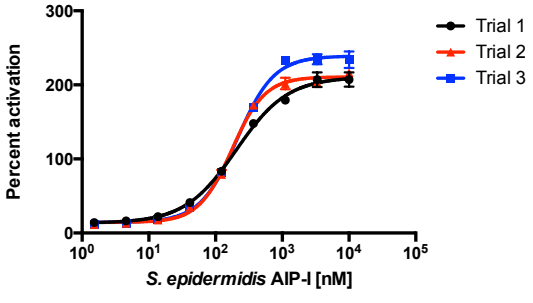

Percent activation
S. epidermidis AIP-I D-C4 [nM]
Trial 1
Trial 2

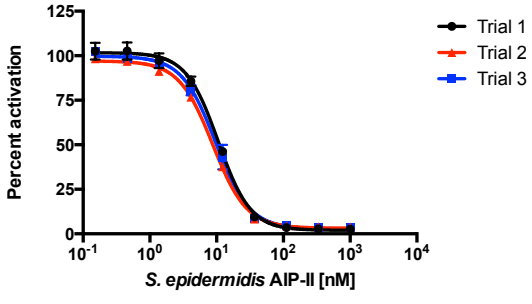

Percent activation
S. epidermidis AIP-I D-A5 [nM]
Trial 1
Trial 2

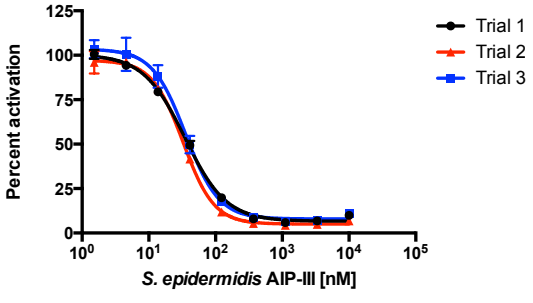

Percent activation
S. epidermidis AIP-I D-S6 [nM]
Trial 1
Trial 2
Trial 3

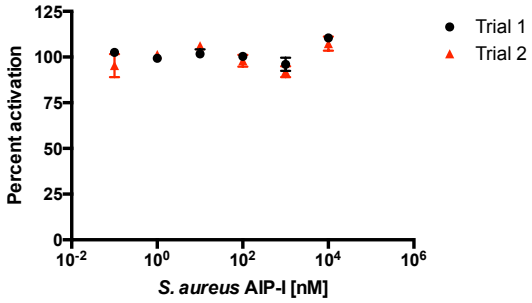

Percent activation
S. epidermidis AIP-I D-Y7 [nM]
Trial 1
Trial 2

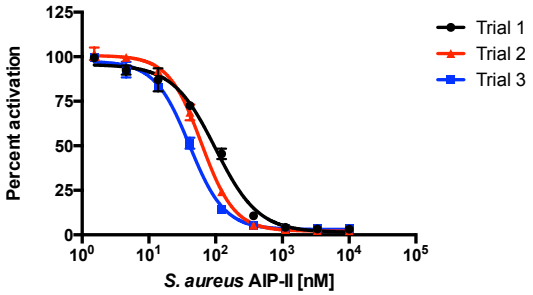

Percent activation
S. epidermidis AIP-I D-F8 [nM]
Trial 1
Trial 2
Trial 3

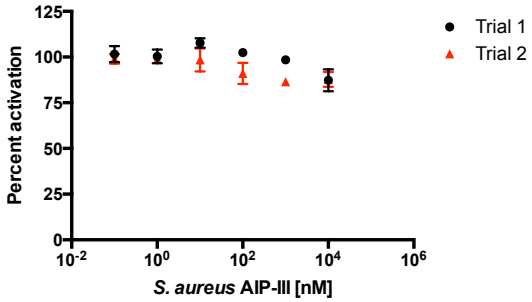

Percent activation
S. epidermidis AIP-I [nM]
Trial 1
Trial 2
Trial 3

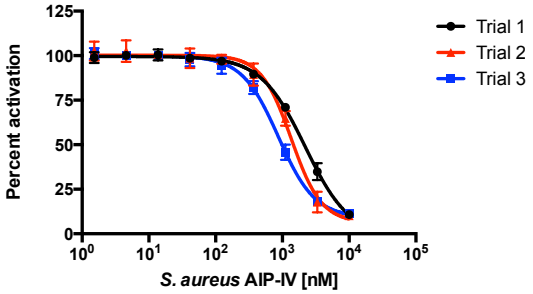

Percent activation
S. epidermidis AIP-II [nM]
Trial 1
Trial 2
Trial 3

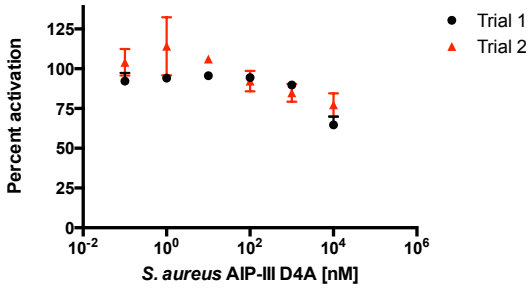

Percent activation
S. epidermidis AIP-III [nM]
Trial 1
Trial 2
Trial 3

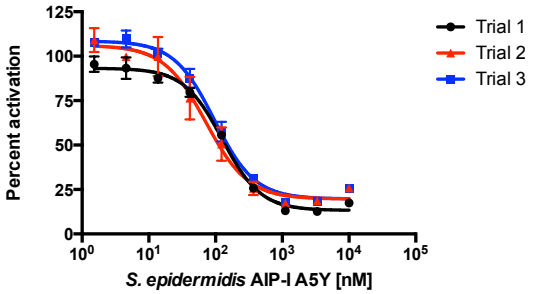

Percent activation
S. aureus AIP-I [nM]
Trial 1
Trial 2

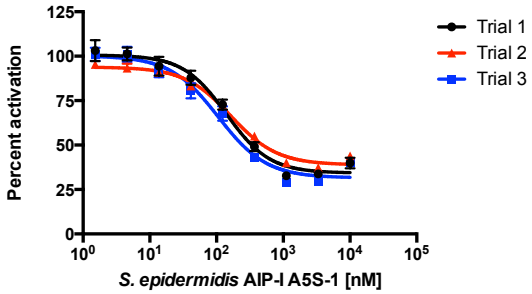

Percent activation
Trial 1
Trial 2
Trial 3
S. aureus AIP-II [nM]

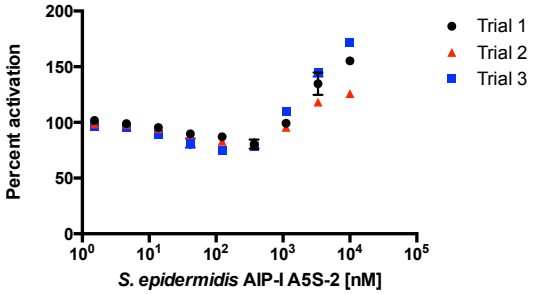

Percent activation
Trial 1
Trial 2
S. aureus AIP-III [nM]

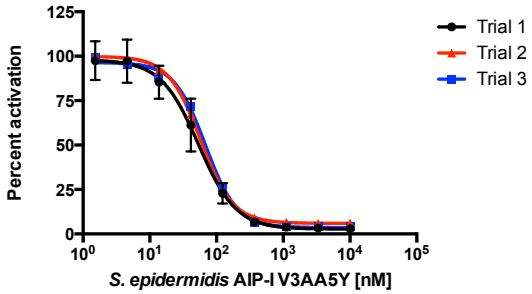

Percent activation
Trial 1
Trial 2
Trial 3
S. aureus AIP-IV [nM]

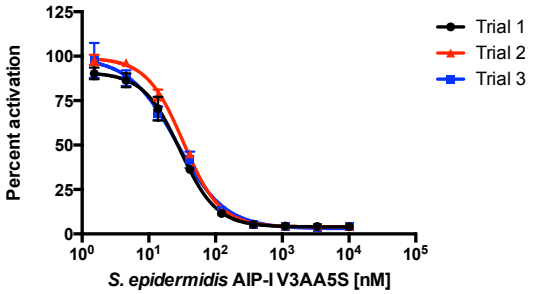

Percent activation
Trial 1
Trial 2
S. aureus AIP-III D4A [nM]

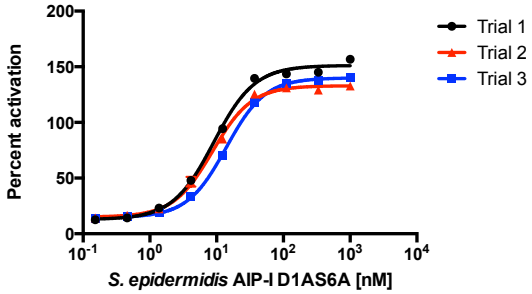

Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis AIP-I A5Y [nM]

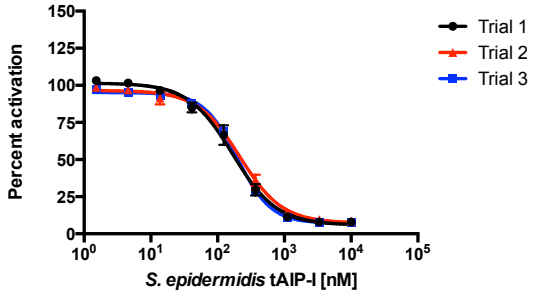

Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis AIP-I A5S-1 [nM]

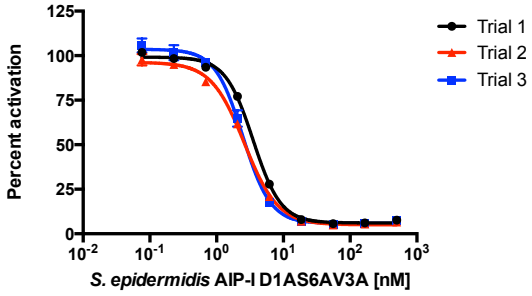

Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis AIP-I A5S-2 [nM]

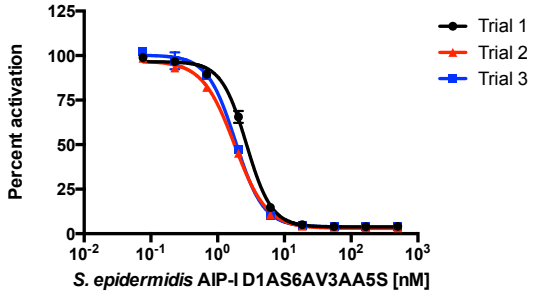

Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis AIP-I V3AA5Y [nM]

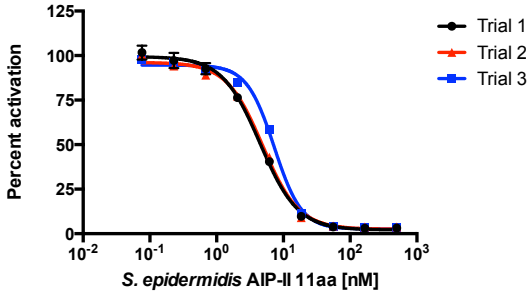

Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis AIP-I V3AA5S [nM]

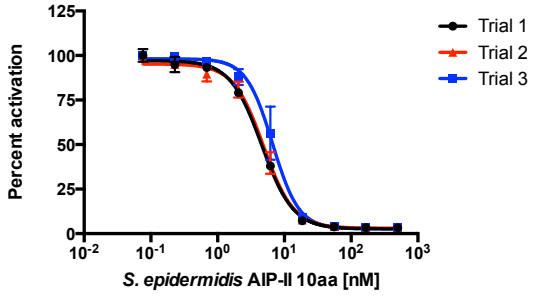

Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis AIP-I D1AS6A [nM]

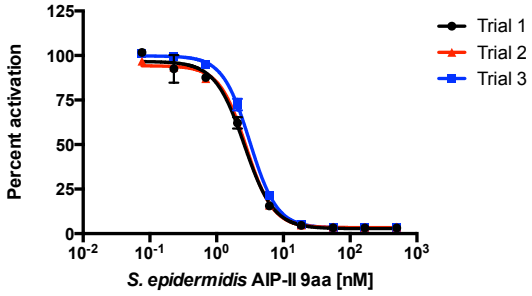

Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis tAIP-I [nM]

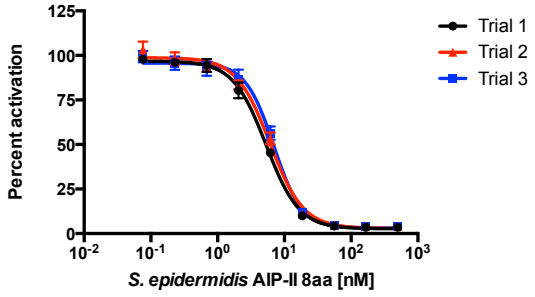

Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis AIP-I D1AS6AV3A [nM]

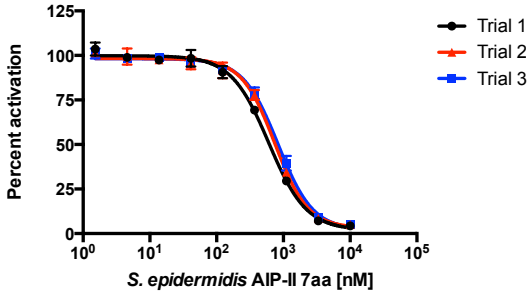

Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis AIP-I D1AS6AV3AA5S [nM]

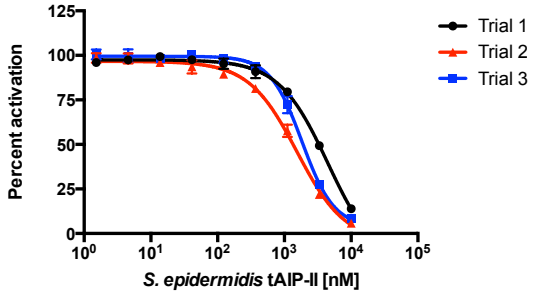

Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis AIP-II 11aa [nM]


Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis AIP-II 10aa [nM]

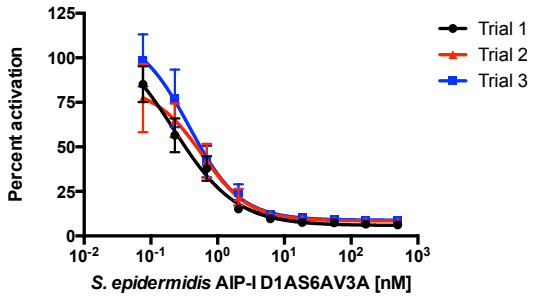

Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis AIP-II 9aa [nM]

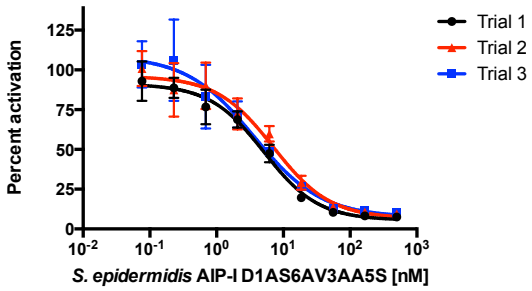

Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis AIP-II 8aa [nM]


Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis AIP-II 7aa [nM]

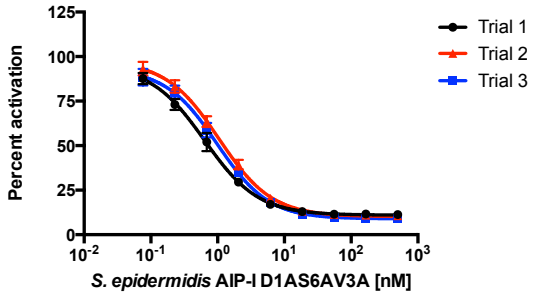

Percent activation
Trial 1
Trial 2
Trial 3
S. epidermidis tAIP-II [nM]

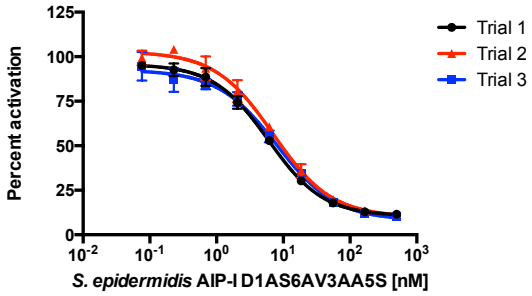
- *S. epidermidis* AH3567 (Group-II)





- *S. epidermidis* AH3408 (Group-III)





Tables of IC_{50} and EC_{50} values of active compounds with 95% confidence intervals.

Table S-2. IC_{50} values, EC_{50} values, and 95% confidence ranges for the active alanine and D-amino acid scan analogs of AIP-I in AgrC-I determined using fluorescence reporter strains.[a] Shaded cells represent control peptides.

Peptide name	Sequence	IC_{50} (nM)	EC_{50} (nM)[b]
AIP-I D1A	A-S-V-(C-A-S-Y-F)	-	49.3 (43.3 – 56.2)
AIP-I S2A	D-A-V-(C-A-S-Y-F)	-	>1000
AIP-I V3A	D-S-A-(C-A-S-Y-F)	51.9 (37.9 – 71.0)	-
AiP-I S6A	D-S-V-(C-A-A-Y-F)	-	71.0 (52.8 – 95.4)
AIP-I D-D1	DD-S-V-(C-A-S-Y-F)	-	>1000
AIP-I D-S2	D-DS-V-(C-A-S-Y-F)	192[c] (159 – 233)	
AIP-I D-V3	D-S-DV-(C-A-S-Y-F)	>1000	-
AIP-I D-S6	D-S-V-(C-A-DS-Y-F)	-	>1000
AIP-I D-F8	D-S-V-(C-A-S-Y-DF)	-	>1000
AIP-I	D-S-V-(C-A-S-Y-F)	-	196 (162 – 238)
AIP-II	N-A-S-K-Y-N-P-(C-S-N-Y-L)	9.64 (7.99 – 11.6)	-
AIP-III	N-A-A-K-Y-N-P-(C-A-S-Y-L)	34.3 (31.4 - 37.4)	-
S. aureus AIP-II	G-V-N-A-(C-S-S-L-F)	62.9 (26.1 – 151)	-
S. aureus AIP-IV	Y-S-T-(C-Y-F-I-M)	>1000	-

[a] See main text for details of the reporter strains and assay procedures. See above for MS and HPLC characterization data and dose response curves. All IC_{50} and EC_{50} values are obtained from three biological replicates. [b] Activation dose response curves were performed in the presence of 50 nM *S. epidermidis* AIP-II to block AgrC-I activation caused by endogenously produced AIP-I. [c] Maximum inhibition did not exceed 60%.

Table S-3. IC_{50} values, EC_{50} values, and 95% confidence ranges for the second-generation *S. epidermidis* AIP analogs in AgrC-I determined using fluorescence reporter strains.[a]

Peptide name	Sequence	IC_{50} (nM)	EC_{50} (nM)[b]
AIP-I A5Y	D-S-V-(C-Y-S-Y-F)	95.4 (54.9 – 166)	-
AIP-I A5S	D-S-V-(C-S-S-Y-F)	135[c, d] (87.6 – 207)	
AIP-I V3AA5Y	D-S-A-(C-Y-S-Y-F)	59.6 (46.8 – 75.8)	-
AIP-I V3AA5S	D-S-A-(C-S-S-Y-F)	29.4 (23.6 – 36.6)	-
AIP-I D1AS6A	A-S-V-(C-A-A-Y-F)	-	10.3 (6.18 – 17.2)
tAIP-I	Ac-(C-A-S-Y-F)	192 (150 – 245)	-
AIP-I D1AS6AV3A	A-S-A-(C-A-A-Y-F)	2.84 (1.95 – 4.11)	-
AIP-I D1AS6AV3AA5S	A-S-A-(C-S-A-Y-F)	2.08 (1.32 – 3.26)	-
AIP-II 11aa	A-S-K-Y-N-P-(C-S-N-Y-L)	5.43 (3.26 – 9.03)	-
AIP-II 10aa	S-K-Y-N-P-(C-S-N-Y-L)	5.29 (3.56 – 7.87)	-
AIP-II 9aa	K-Y-N-P-(C-S-N-Y-L)	2.83 (2.34 – 3.42)	-
AIP-II 8aa	Y-N-P-(C-S-N-Y-L)	6.08 (4.70 – 7.86)	-
AIP-II 7aa	N-P-(C-S-N-Y-L)	721 (525 – 991)	-
tAIP-II	Ac-(C-S-N-Y-L)	>1000	-

[a] See main text for details of the reporter strains and assay procedures. See above for MS and HPLC characterization data and dose response curves. All IC_{50} and EC_{50} values are obtained from three biological replicates. [b] Activation dose response curves were performed in the presence of 50 nM *S. epidermidis* AIP-II to block AgrC-I activation caused by endogenously produced AIP-I. [c] Maximum inhibition did not exceed 75%. [d] Two different AIP-I A5S analogs with the same molecular weight (AIP-I A5S-1 and AIP-I A5S-2) were isolated from the synthesis that showed different activities; IC_{50} value listed is derived from the antagonism dose response curves for A5S-1 (see main text).

Table S-4. IC_{50} values and 95% confidence ranges for the potent pan-group *S. epidermidis agr* inhibitors against AgrC-II and AgrC-III determined using fluorescence reporter strains.[a]

Peptide Name	**Sequence**	**AgrC-II IC_{50} (nM)**	**AgrC-III IC_{50} (nM)**
AIP-I D1AS6AV3A	A-S-A-(C-A-A-Y-F)	0.36 (0.14 – 0.96)	0.87 (0.51 – 1.47)
AIP-I D1AS6AV3AA5S	A-S-A-(C-S-A-Y-F)	5.10 (2.64 – 9.84)	6.98 (5.23 – 9.32)

[a] See main text for details of the reporter strains and assay procedures. See above for MS and HPLC characterization data and dose response curves. All IC_{50} values are obtained from three biological replicates.

Single-concentration screening data for AIPs using fluorescence reporter strains.

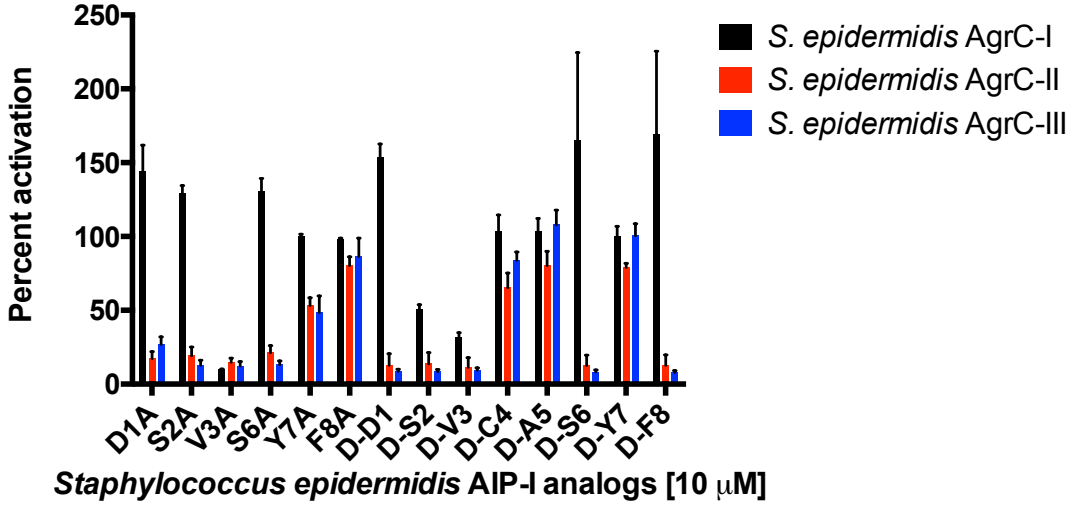


Figure S-1. Effects of alanine and D-amino acid analogs of *S. epidermidis* AIP-I on *S. epidermidis* AgrCs I–III at 10 μM as determined using fluorescence reporter strains. Error bars represent the standard deviation of measurements from three biological replicates. See main text for details of the reporter strains and assay procedures.

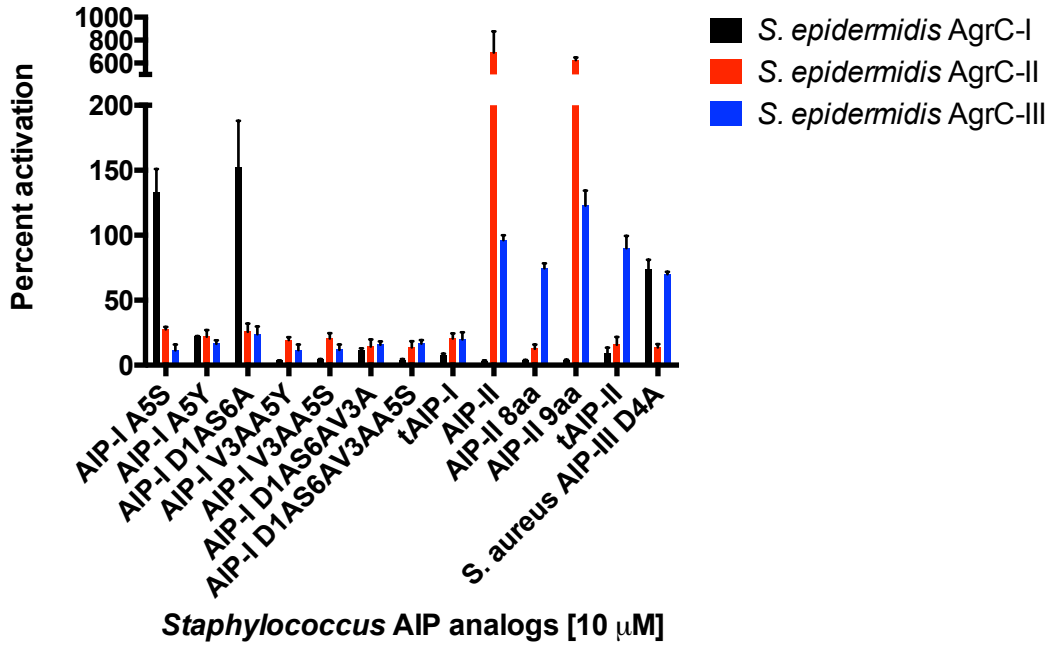


Figure S-2. Effects of the second-generation *S. epidermidis* AIP analogs and *S. aureus* AIP-III D4A on *S. epidermidis* AgrCs I–III at 10 μM as determined using fluorescence reporter strains. Error bars represent the standard deviation of measurements from three biological replicates. See main text for details of the reporter strains and assay procedures.

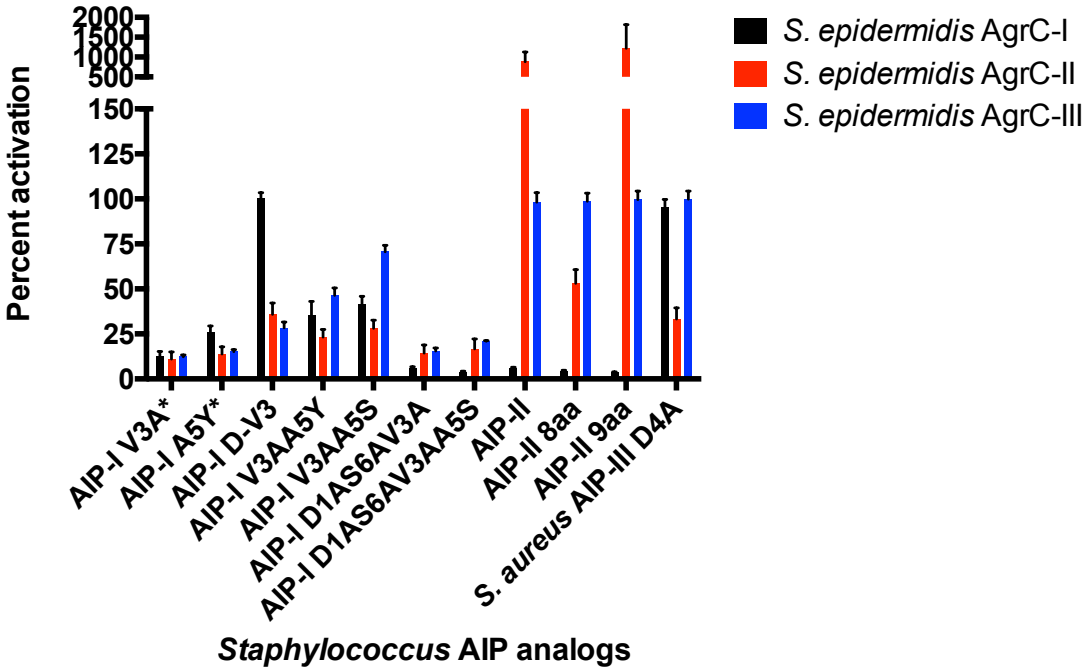


Figure S-3. Effects of selected *S. epidermidis* AIP analogs and *S. aureus* AIP-III D4A on *S. epidermidis* AgrCs I–III as determined using fluorescence reporter strains. Compounds were tested at 100 nM unless otherwise noted. Error bars represent the standard deviation of measurements from three biological replicates. * Compound tested at 1 μM. See main text for details of the reporter strains and assay procedures.

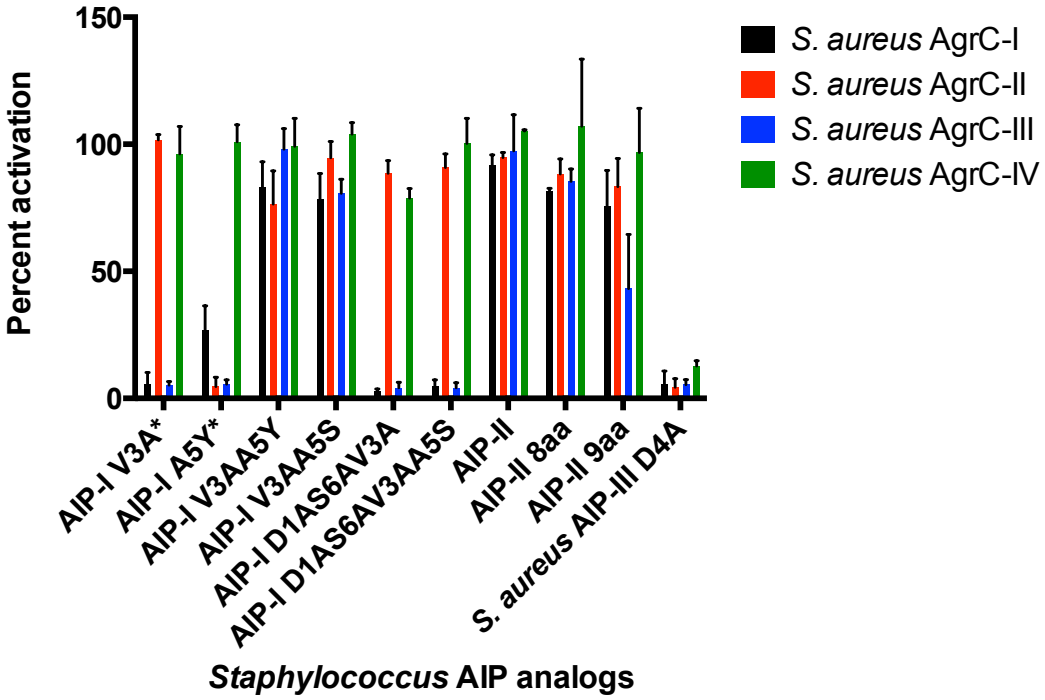


Figure S-4. Effects of selected *S. epidermidis* AIP analogs and *S. aureus* AIP-III D4A on *S. aureus* AgrCs I–IV as determined using fluorescence reporter strains. Compounds were tested at 100 nM unless otherwise noted. Error bars represent the standard deviation of measurements from three biological replicates. * Compound was tested at 1 μM. See main text for details of the reporter strains and assay procedures.

Crystal violet biofilm assay data.

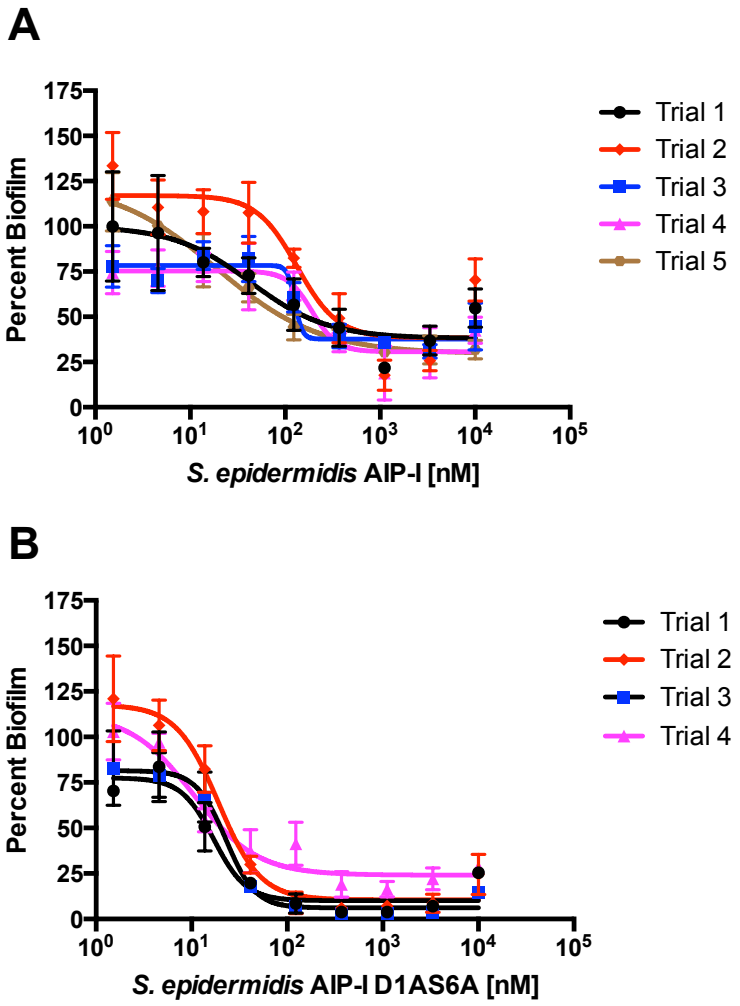


Figure S-5. *S. epidermidis* biofilm growth inhibition dose response curves for (A) AIP-I and (B) AIP-I D1AS6A. Peptides were screened over varying concentrations in (wild-type) *S. epidermidis* Group-I RP62A after 24 h in at least four separate experiments. Biofilm growth was measured by staining with crystal violet (CV). Error bars represent the standard deviation of triplicate values from each experiment. Percent of biofilm formation was normalized to a DMSO control. See main text for details of the strain and assay procedure.

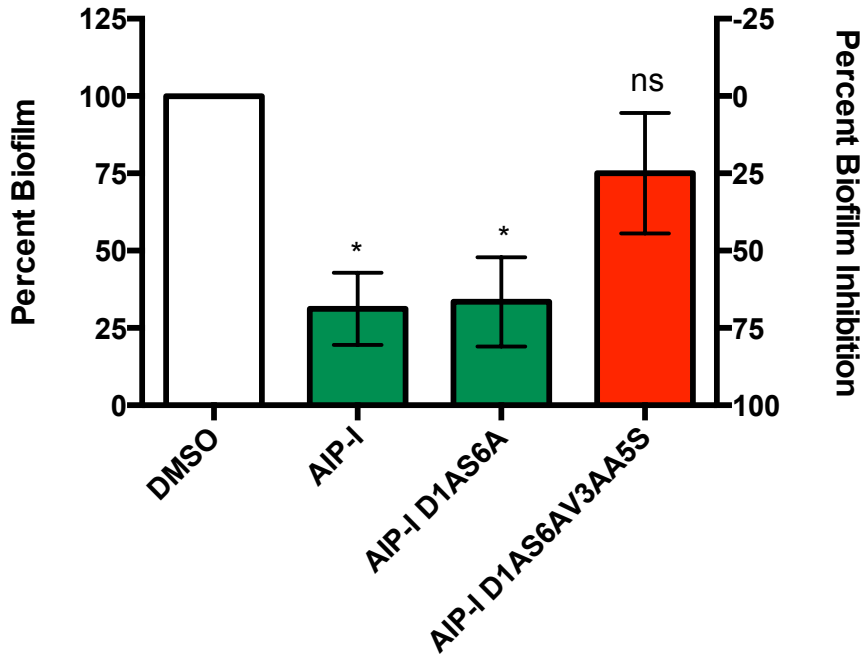


Figure S-6. Single-concentration *S. epidermidis* biofilm growth inhibition assay data. Selected peptides were tested for effects on biofilm formation in (wild-type) *S. epidermidis* Group-I RP62A at 10 μM after 24 h. Green: AgrC-I agonist; red: AgrC-I antagonist. Biofilm growth was measured by staining with crystal violet (CV). Data shown represent the average and the standard deviation of three biological replicates. P-values were calculated using an unpaired *t*-test compared to a no-peptide DMSO control. * = $P < 0.05$; ns = not significant. See main text for details of the strain and assay procedure.