

Highly Potent Inhibitors of Quorum Sensing in *Staphylococcus aureus*

Revealed Through a Systematic Synthetic Study of the Group-III

Autoinducing Peptide

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Additional experimental details.

HPLC analyses. An analytical Phenomenex Gemini C18 column ($5 \mu\text{m}$, $4.6 \text{ mm} \times 250 \text{ mm}$, 110 \AA) was used for analytical RP-HPLC work. A semi-preparative Phenomenex Gemini C18 column ($5 \mu\text{m}$, $10 \text{ mm} \times 250 \text{ mm}$, 110 \AA) was used for preparative RP-HPLC work. Standard RP-HPLC conditions were as follows: flow rates = 1 mL min^{-1} for analytical separations and 5 mL min^{-1} for semi-preparative separations; mobile phase A = $18 \text{ M}\Omega$ water + 0.1% trifluoroacetic acid (TFA); mobile phase B = acetonitrile (ACN) + 0.1% TFA. Purities were determined by integration of peaks with UV detection at 220 nm. Peptide thioesters were purified using a linear gradient ($75\% \rightarrow 45\% \text{ A}$ over 30 min). Cyclic peptides were purified using a linear gradient ($70\% \rightarrow 55\% \text{ A}$ over 27 min). Overall sample purity was determined using a linear gradient ($90\% \rightarrow 5\% \text{ A}$ over 27 min).

Peptide synthesis. To deprotect the resin, a portion of resin (50 mg) was first swelled by suspension in CH_2Cl_2 (2 mL) for 30 min at rt and then drained. The resin was then treated with TFA (50% in CH_2Cl_2 , 2 mL, $2 \times 2 \text{ min}$, rt) and washed with dimethylformamide (DMF; $3 \times 2 \text{ mL}$). To couple each amino acid, Fmoc-protected amino acids (2 equiv. relative to resin), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU; 2 equiv.), and diisopropylethylamine (DIPEA; 2 equiv.) were dissolved in DMF (2 mL). The solution was allowed to pre-activate for 1 min prior to being added to the resin and agitated for 1 h at rt. After each coupling step, the resin was drained and washed with DMF ($2 \times 2 \text{ mL}$). To remove the Fmoc-protecting group after each coupling, the resin was treated with piperidine (2 mL of 20% in DMF, $2 \times 10 \text{ min}$) and washed with DMF ($3 \times 2 \text{ mL}$). To acetylate the amino terminus, acetic anhydride (10 equiv.) and DIPEA (7 equiv.) were dissolved in DMF (2 mL), and the solution was added to the resin and agitated for 15 min. The resin was then drained and washed with DMF ($2 \times 2 \text{ mL}$). Upon synthesis of a complete linear peptide sequence, the resin was washed with diethyl ether ($1 \times 2 \text{ mL}$) and dried under vacuum for 48 h.

Peptide cleavage protocol. The linear peptidyl-resin (70–90 mg) was placed in a dry, three-neck round bottom flask and suspended in anhydrous CH_2Cl_2 under argon at rt for 15 min. Me_2AlCl (20 equiv., 1 mL of 1M hexane solution) and anhydrous CH_2Cl_2 (3 mL) were stirred in a separate, dry round bottom flask under argon for 5 min at 0°C . Ethanethiol (EtSH) (60 equiv.) was added drop-wise at 0°C , and the solution was stirred for 15 min at 0°C . This solution was then added to the suspended resin and stirred under argon for 5 h at rt to effect cleavage of the linear peptide as a thioester. The cleavage product solution was transferred into a new round bottom flask containing a TFA solution (95% (aq), 3 mL) and the solvents were removed *in vacuo* to yield a yellow or orange oil. The resulting oil was subjected again to TFA solution (3 mL, 30 min) and filtered from the resin. The resin was washed with TFA solution (1 x 2 mL) to collect any additional peptide. A cooled solution of diethyl ether:hexane (1:1, 40 mL, 0°C) was added to the filtrate, and the peptide was allowed to precipitate overnight in a freezer at -20°C . The precipitated peptide solution was centrifuged and the supernatant removed to yield a white solid. This solid was dissolved in ACN (50% (aq)), lyophilized, redissolved in ACN (50% (aq)) and purified by semi-preparative RP-HPLC. Collected HPLC fractions were lyophilized to yield the linear peptide thioester as a white powder (25–50% isolated yields).

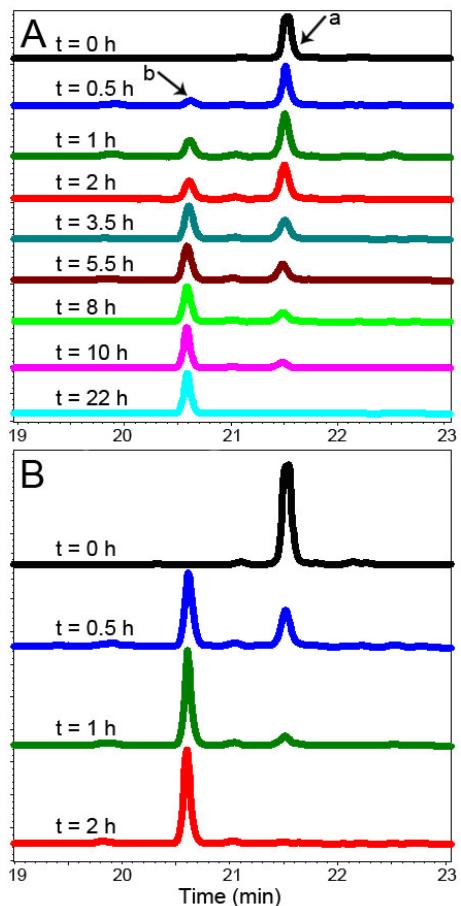


Figure S-1. Representative peptide macrocyclization reaction progression as monitored by HPLC (UV detection at 220 nm; see HPLC methods above). AIP-III I1A thioester was the substrate. A. Reaction temp. = rt. B. Reaction temp. = 50 °C. Peak a is the peptide thioester precursor, and peak b is the macrocyclic product. Buffer conditions: 60% 6M guanidinium chloride in 0.1M phosphate buffer, 40% ACN, pH = 6.8.

MS and HPLC data for AIP analogs.

Table S-1. MS and HPLC data for AIP analogs. Rt = retention time (minutes). EM = Exact mass. See main text for methods.

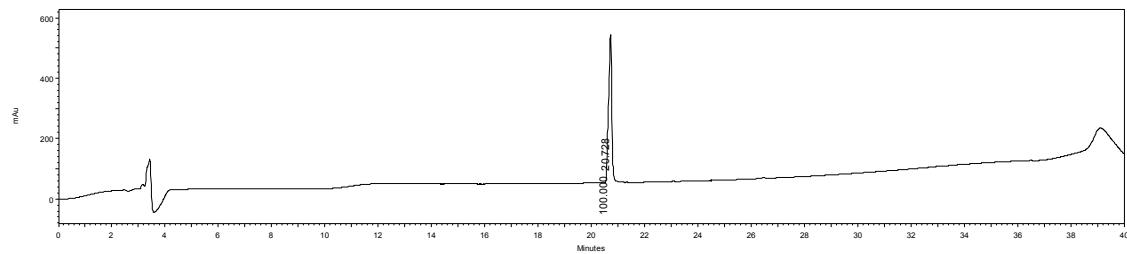
Compound Name	Structure	Thioester (-S-Et)							Cyclic peptide			
		Calc. MH ⁺	Meas. MH ⁺	Calc. MNa ⁺	Obs. MNa ⁺	Calc. MK ⁺	Obs. MK ⁺	Rt HPLC	Calc. EM	Obs. EM	Rt HPLC	% Purity
AIP-I	Y-S-T-(C-D-F-I-M)	1023.4	-	1045.4	1045.3	1061.4	1061.3	21.2	961.4*	961.4	20.7	>99
AIP-II	G-V-N-A-(C-S-S-L-F)	941.4	941.3	963.4	963.3	979.4	979.4	19.7	879.4*	879.5	18.5	>98
AIP-III	I-N-(C-D-F-L-L)	881.4	881.2	903.4	903.2	919.4	919.3	22.3	819.4*	819.2	21.5	>95
AIP-IV	Y-S-T-(C-Y-F-I-M)	1071.4	-	1093.4	1093.5	1109.4	1109.4	22.0	1009.4*	1009.2	21.4	>99
tAIP-I D2A	Ac-(C-A-F-I-M)	670.3	-	692.3	692.0	708.2	708.0	25.7	630.2391 (MNa ⁺)	630.2393	23.9	>99
AIP-III D-I1	DI-N-(C-D-F-L-L)	881.4	881.2	903.4	903.3	919.4	919.2	22.1	819.4069	819.4072	21.0	>98
AIP-III D-N2	I-DN-(C-D-F-L-L)	881.4	881.4	903.4	903.4	919.4	919.4	22.2	819.4069	819.4069	21.1	>98
AIP-III D-C3	I-N-(DC-D-F-L-L)	881.4	881.3	903.4	903.4	919.4	919.4	22.0	819.4069	819.4088	20.4	>99
AIP-III D-D4	I-N-(C-DD-F-L-L)	881.4	881.4	903.4	903.4	919.4	919.4	22.2	819.4069	819.4082	21.5	>99
AIP-III D-F5	I-N-(C-D-DF-L-L)	881.4	881.2	903.4	903.2	919.4	919.1	22.3	819.4069	819.4048	20.8	>99
AIP-III D-L6	I-N-(C-D-F-DL-L)	881.4	-	903.4	902.9	919.4	918.9	22.2	819.4069	819.4091	21.1	>99
AIP-III D-L7	I-N-(C-D-F-L-DL)	881.4	881.3	903.4	903.3	919.4	919.3	22.2	819.4069	819.4048	21.4	>99
AIP-III I1A	A-N-(C-D-F-L-L)	839.4	-	861.4	861.2	877.3	877.1	21.5	777.3600	777.3600	20.6	>99
AIP-III N2A	I-A-(C-D-F-L-L)	838.4	-	860.4	859.9	876.4	875.9	22.7	776.4012	776.4021	21.7	>95
AIP-III D4A	I-N-(C-A-F-L-L)	837.4	-	859.4	859.0	875.4	875.0	22.6	775.4172	775.4173	21.7	>99
AIP-III F5A	I-N-(C-D-A-L-L)	805.4	-	827.4	827.0	843.4	843.0	20.4	743.3757	743.3765	18.8	>98
AIP-III L6A	I-N-(C-D-F-A-L)	839.4	-	861.4	861.3	877.3	877.3	20.7	777.3600	777.3575	19.0	>99
AIP-III L7A	I-N-(C-D-F-L-A)	839.4	839.1	861.4	861.1	877.3	877.0	20.3	777.3600	777.3620	19.3	>99
AIP-III I1A/N2A	A-A-(C-D-F-L-L)	796.4	796.3	818.4	818.3	834.3	834.3	22.0	734.3542	734.3527	20.9	>99
AIP-III I1A/D4A	A-N-(C-A-F-L-L)	795.4	795.4	817.4	817.4	833.3	833.4	21.8	733.3702	733.3690	20.9	>99
AIP-III N2A/D4A	I-A-(C-A-F-L-L)	794.4	794.2	816.4	816.3	832.4	832.3	23.1	732.4113	732.4132	22.2	>99
AIP-III I1A/N2A/D4A	A-A-(C-A-F-L-L)	752.4	752.1	774.4	774.3	790.3	790.2	22.3	690.3643	690.3632	21.3	>99
tAIP-III	Ac-(C-D-F-L-L)	696.3	-	718.3	718.2	734.3	734.2	26.1	656.2725 (MNa ⁺)	656.2735	23.9	>98
tAIP-III D2A	Ac-(C-A-F-L-L)	652.3	-	674.3	674.0	690.3	689.9	27.3	612.2826 (MNa ⁺)	612.2813	24.9	>99
tAIP-III D2A/F3Y	Ac-(C-A-Y-L-L)	668.3	668.3	690.3	690.0	706.3	706.0	25.0	628.2776 (MNa ⁺)	628.2780	22.2	>99
tAIP-III D2A/F3W	Ac-(C-A-W-L-L)	691.3	-	713.3	713.1	729.3	729.1	27.1	651.2935 (MNa ⁺)	651.2928	24.5	>99

Compound Name	Structure	Thioester (-S-Et)							Cyclic peptide			
		Calc. <i>MH</i> ⁺	Meas. <i>MH</i> ⁺	Calc. <i>MNa</i> ⁺	Obs. <i>MNa</i> ⁺	Calc. <i>MK</i> ⁺	Obs. <i>MK</i> ⁺	Rt HPLC	Calc. <i>EM</i>	Obs. <i>EM</i>	Rt HPLC	% Purity
Ac-AIP-III	Ac-I-N-(C-D-F-L-L)	923.4	-	945.4	945.7	961.4	961.8	25.7	861.4175	861.4160	23.9	>99
G-AIP-III	G-I-N-(C-D-F-L-L)	938.4	-	960.4	960.3	976.4	976.3	22.5	876.4284	876.4292	21.2	>98
A-AIP-III	A-I-N-(C-D-F-L-L)	952.5	952.4	974.4	974.5	990.4	990.5	22.4	912.4260 (<i>MNa</i> ⁺)	912.4236	21.5	>99
Y-AIP-III	Y-I-N-(C-D-F-L-L)	1044.5	1044.3	1066.5	1066.4	1082.4	1082.3	22.9	982.4703	982.4717	21.6	>99

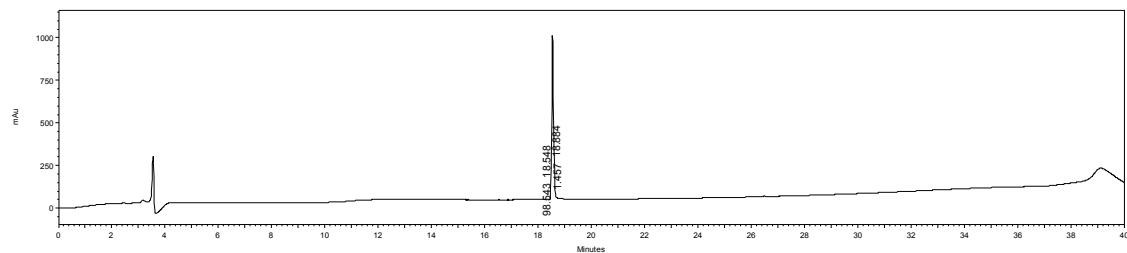
* Identified by MALDI-TOF MS.

HPLC traces for AIP analogs.

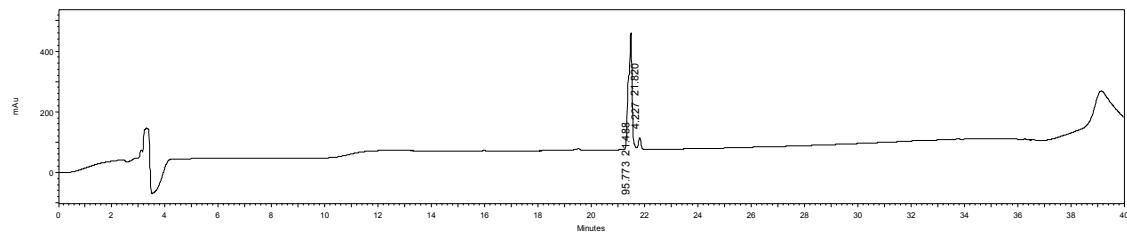
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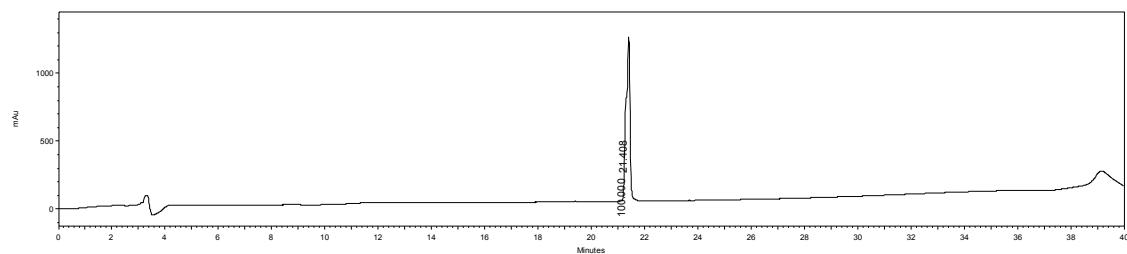
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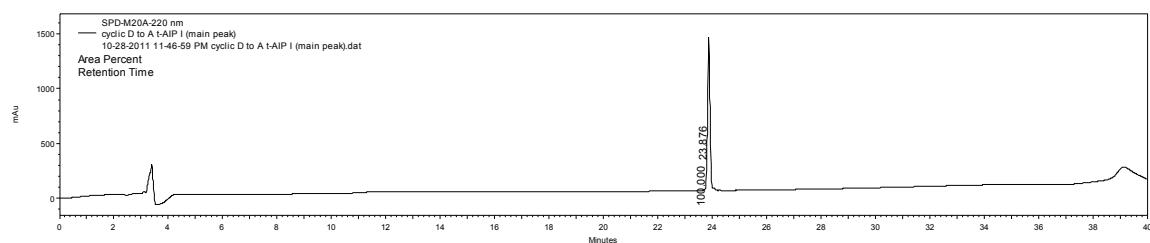
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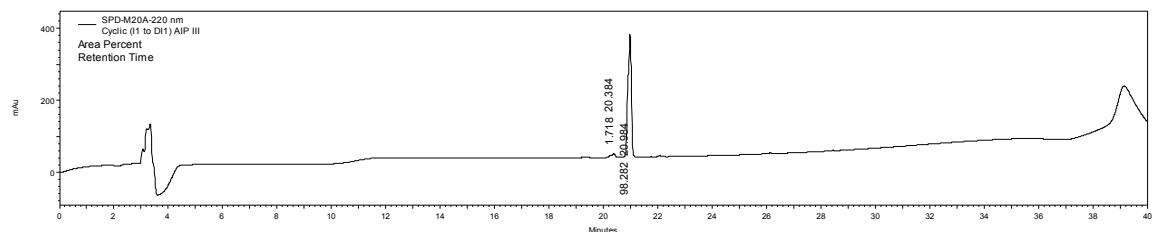
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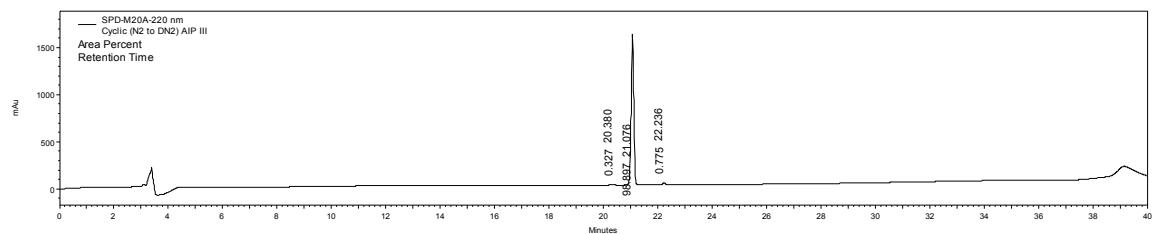
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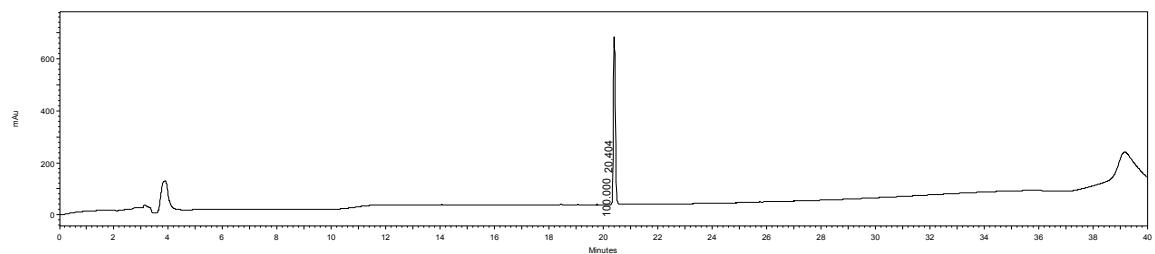
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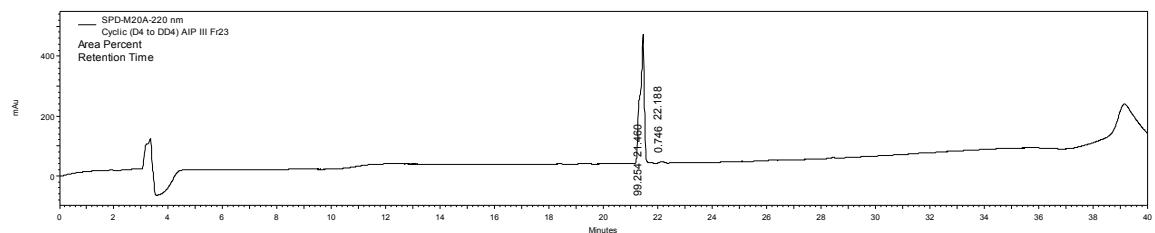
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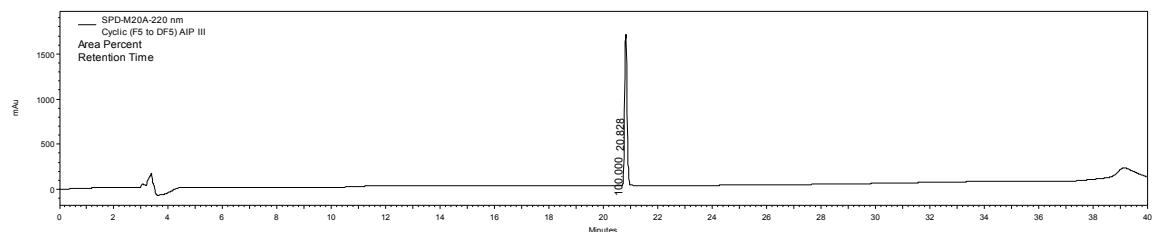
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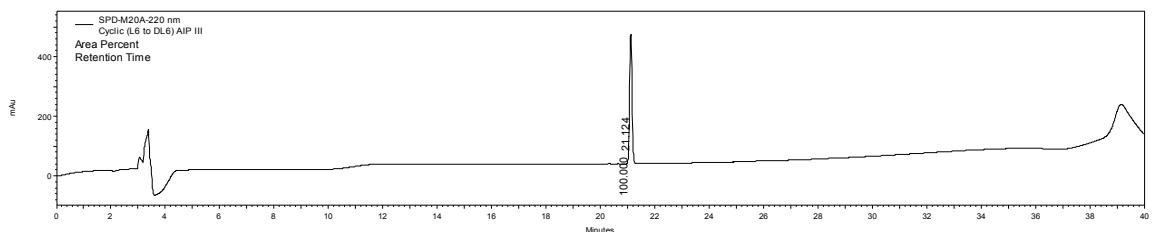
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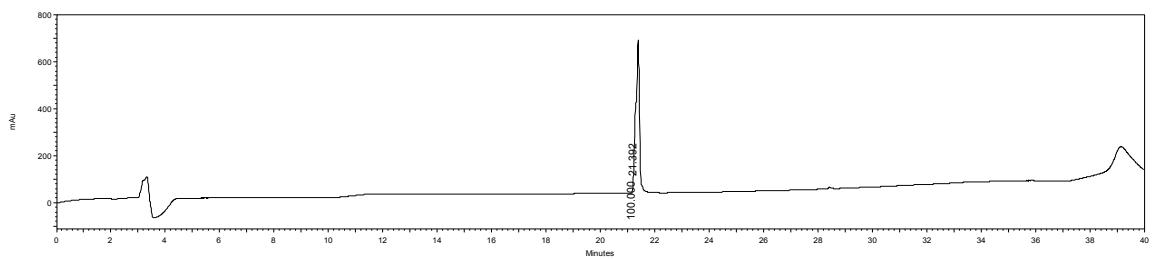
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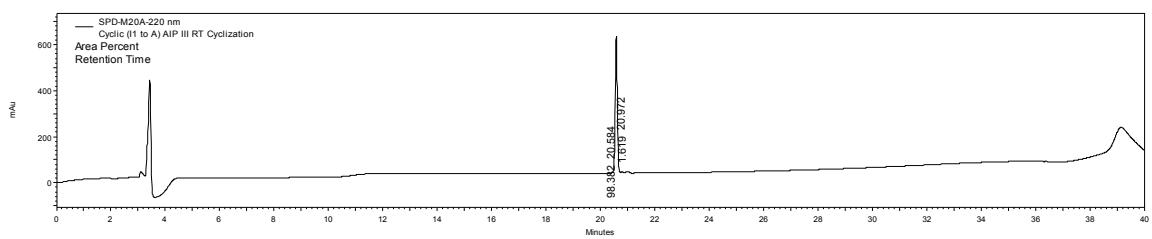
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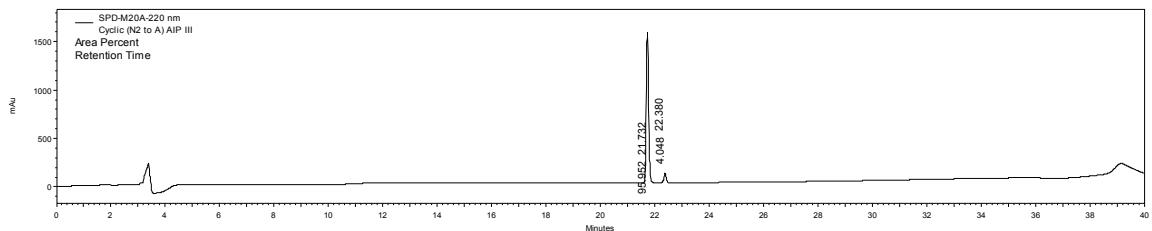
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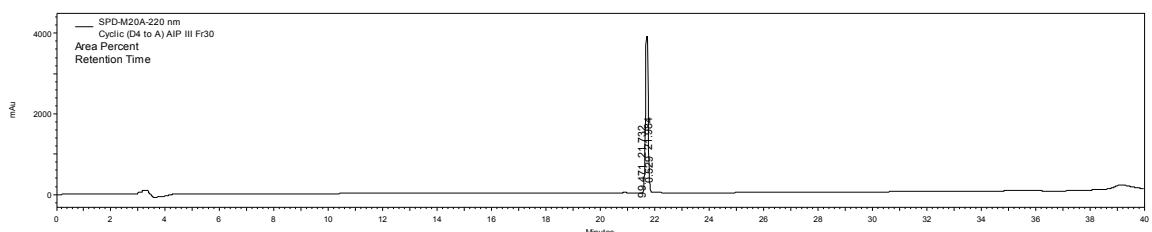
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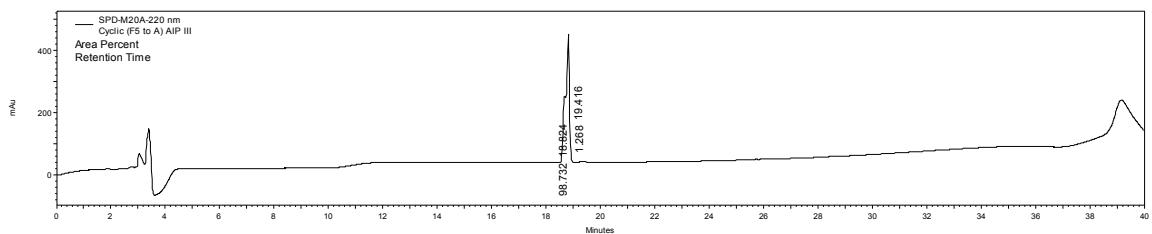
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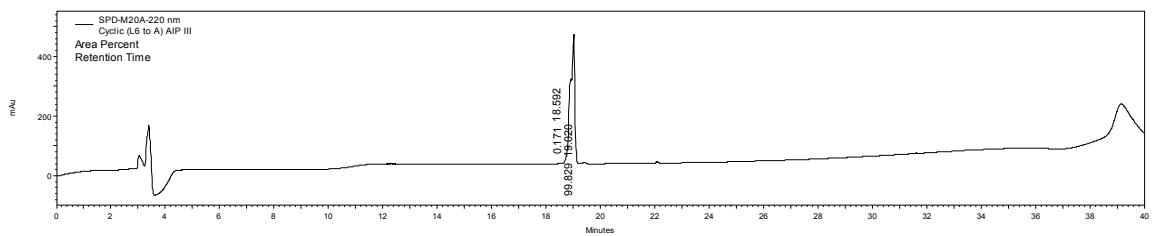
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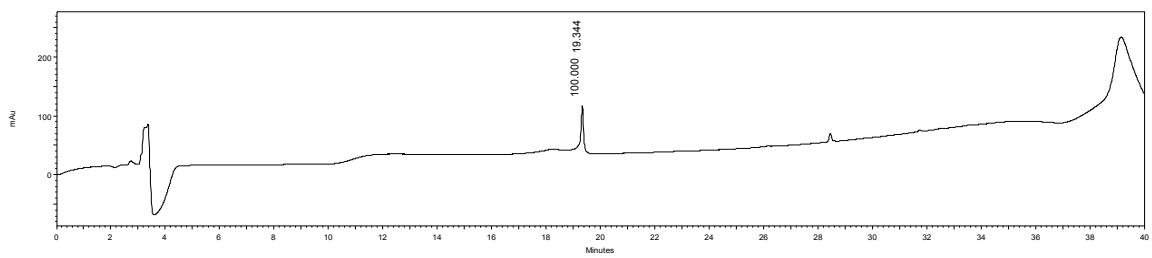
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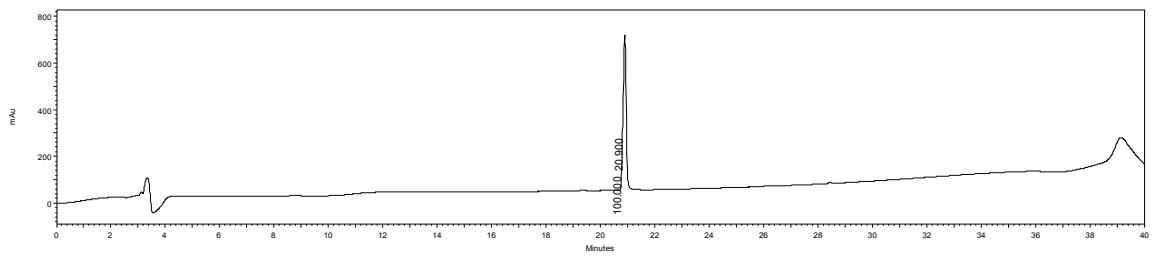
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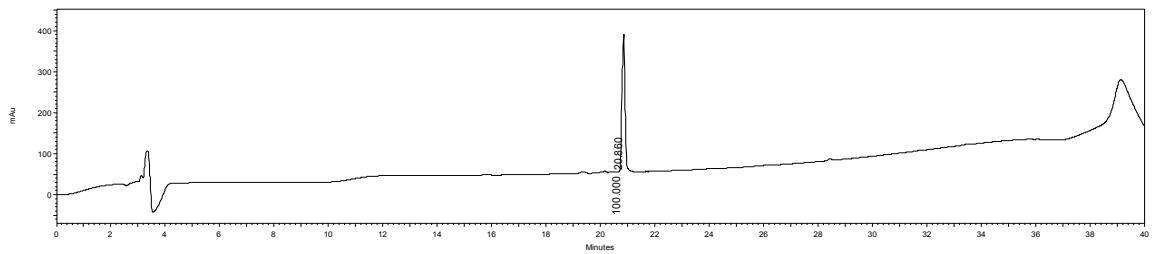
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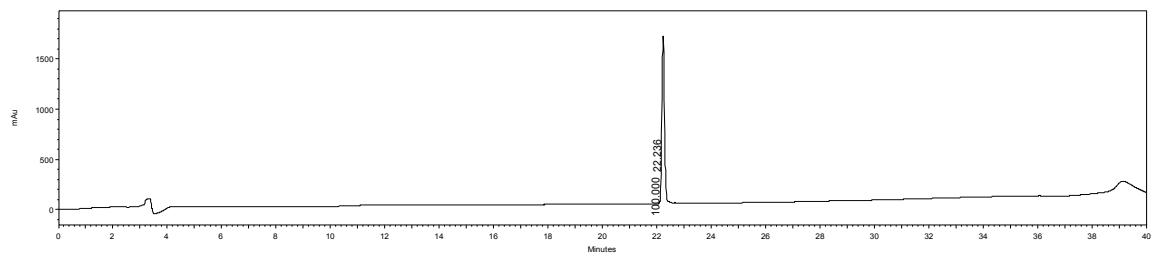
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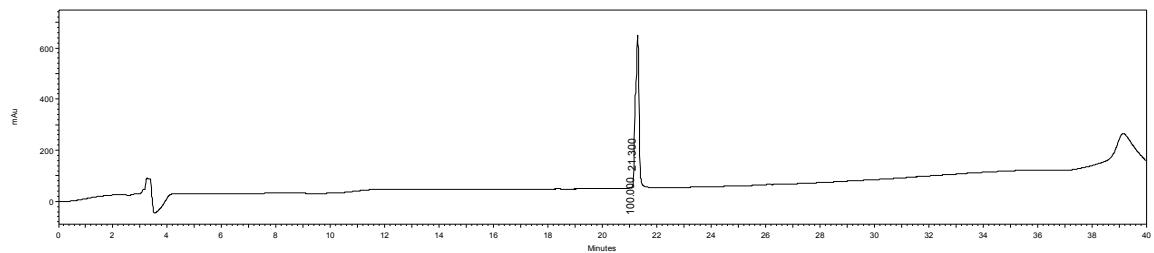
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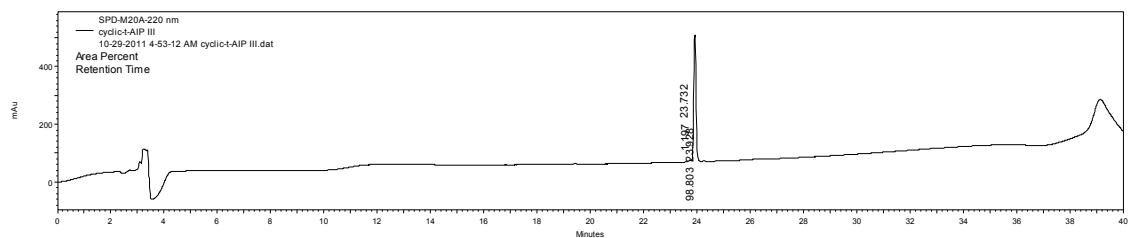
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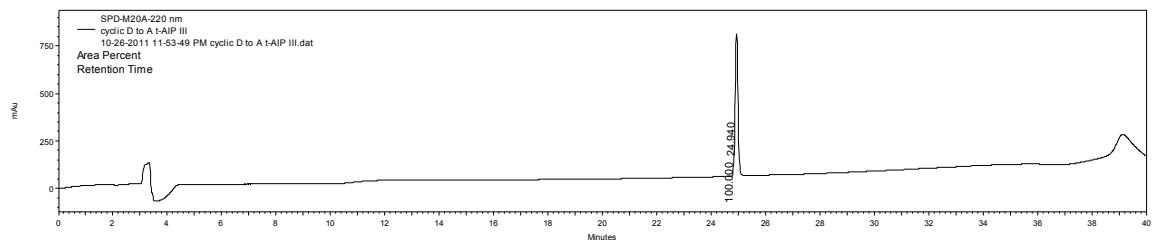
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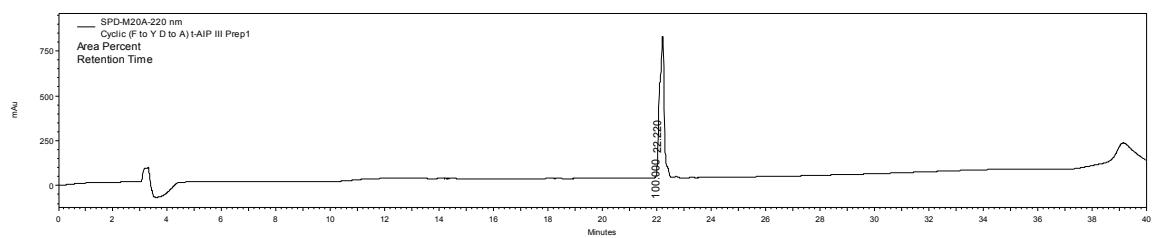
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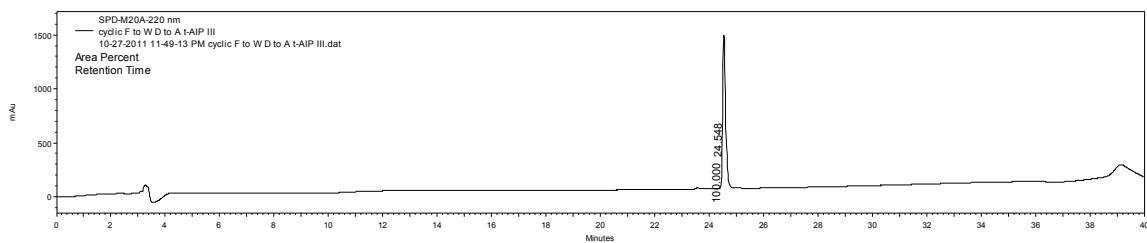
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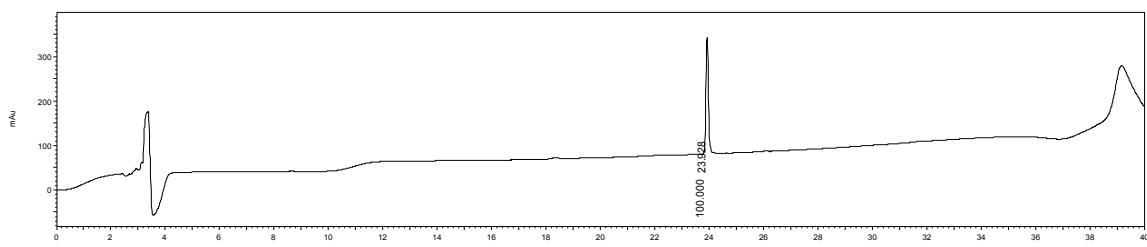
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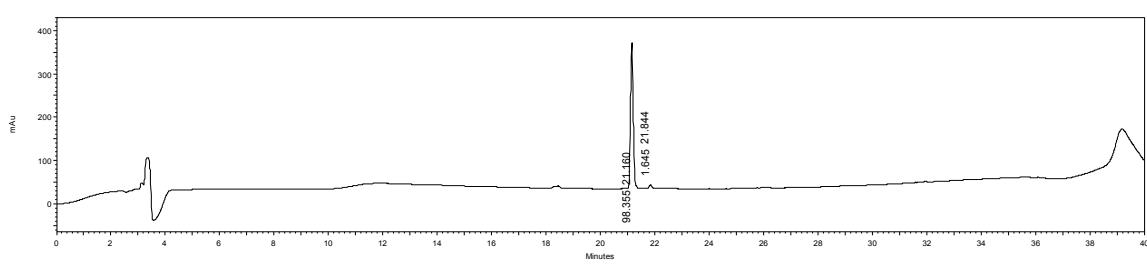
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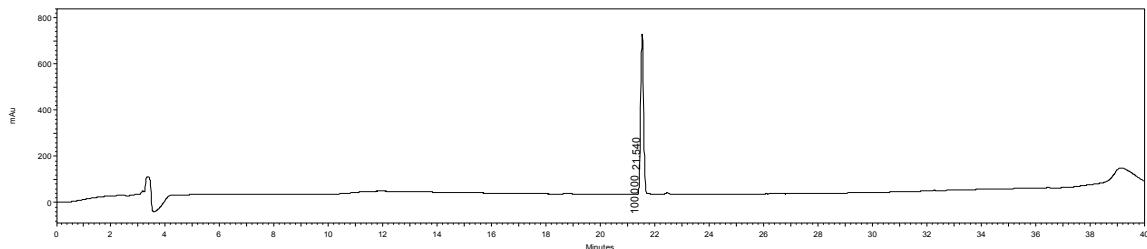
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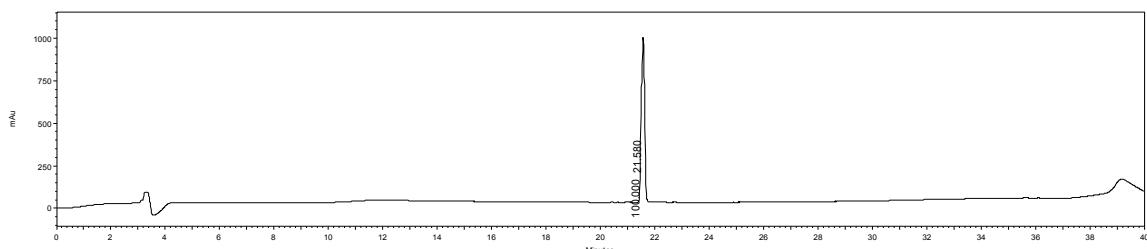
G-AIP-III



A-AIP-III



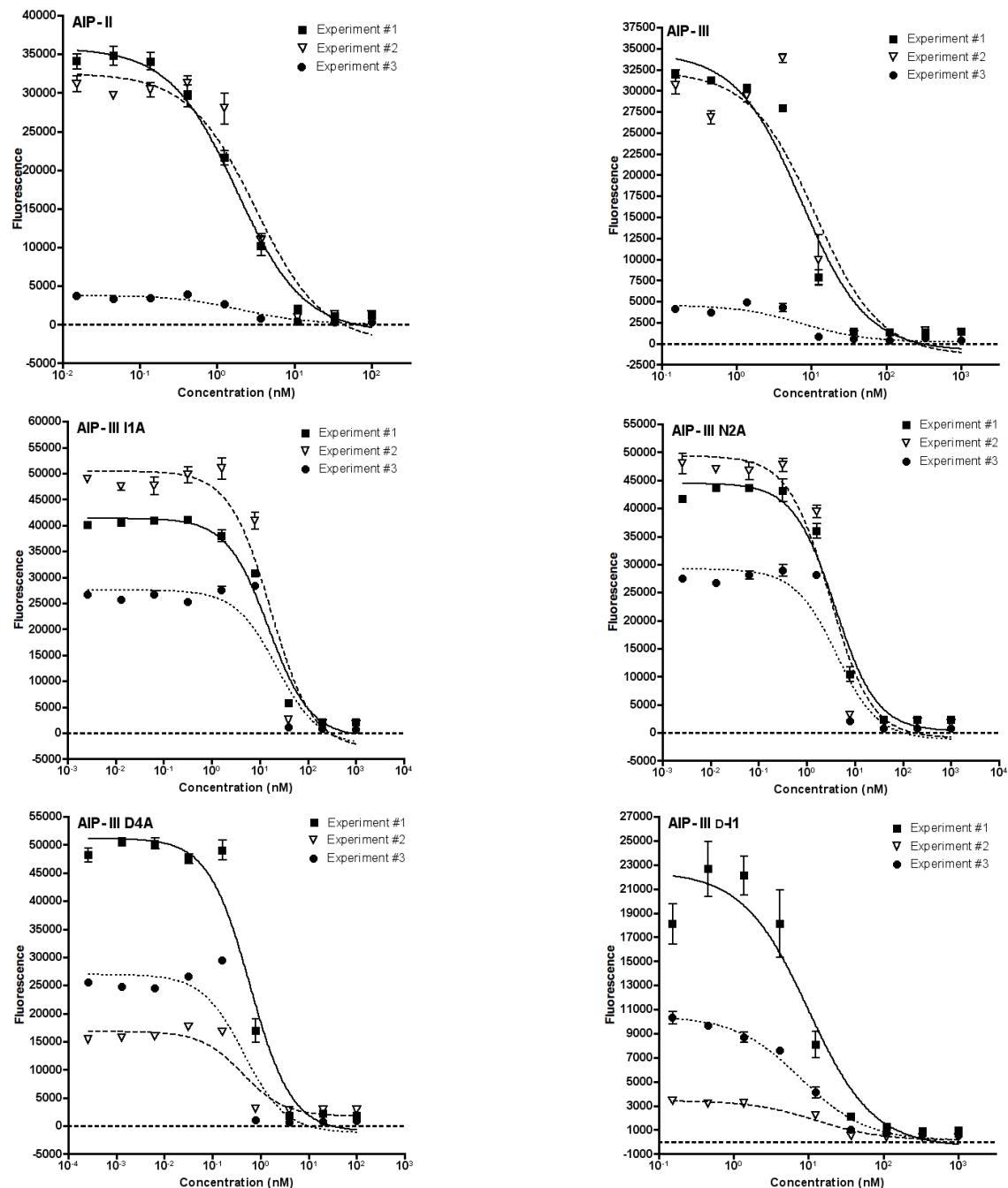
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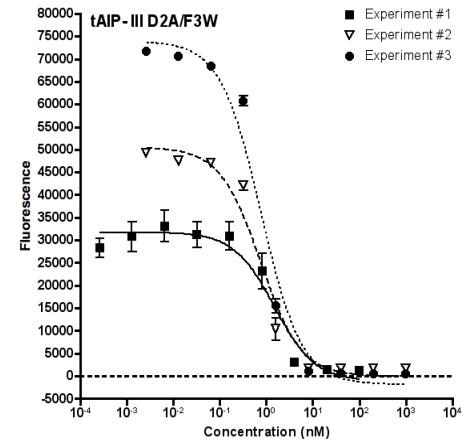
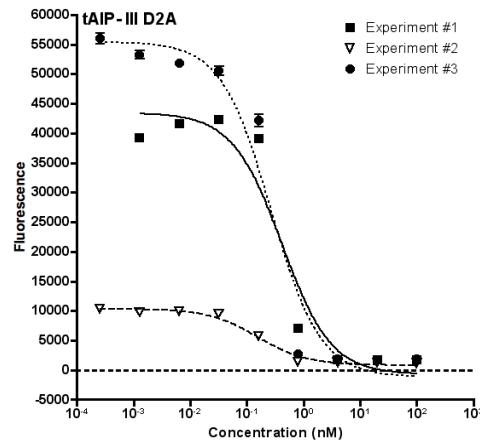
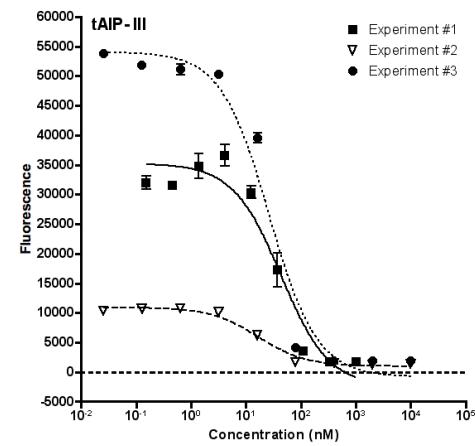
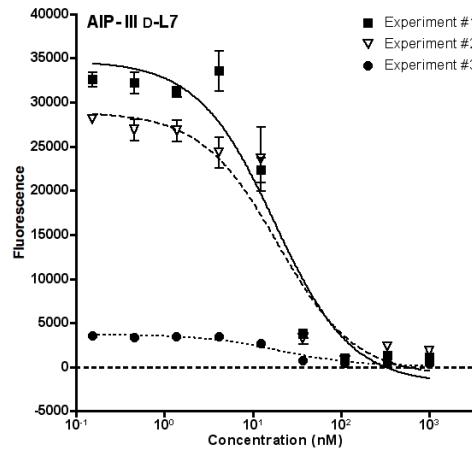
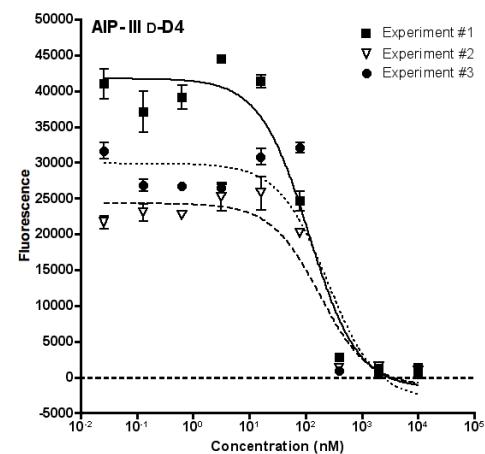
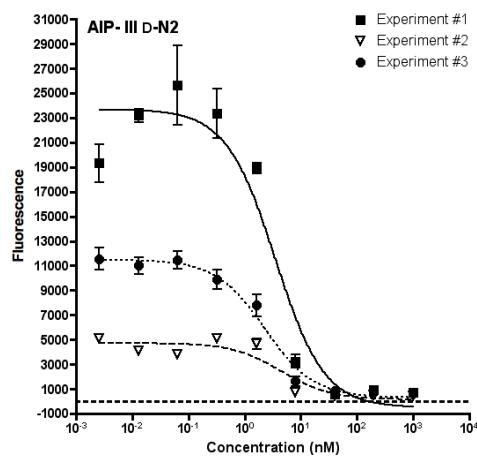


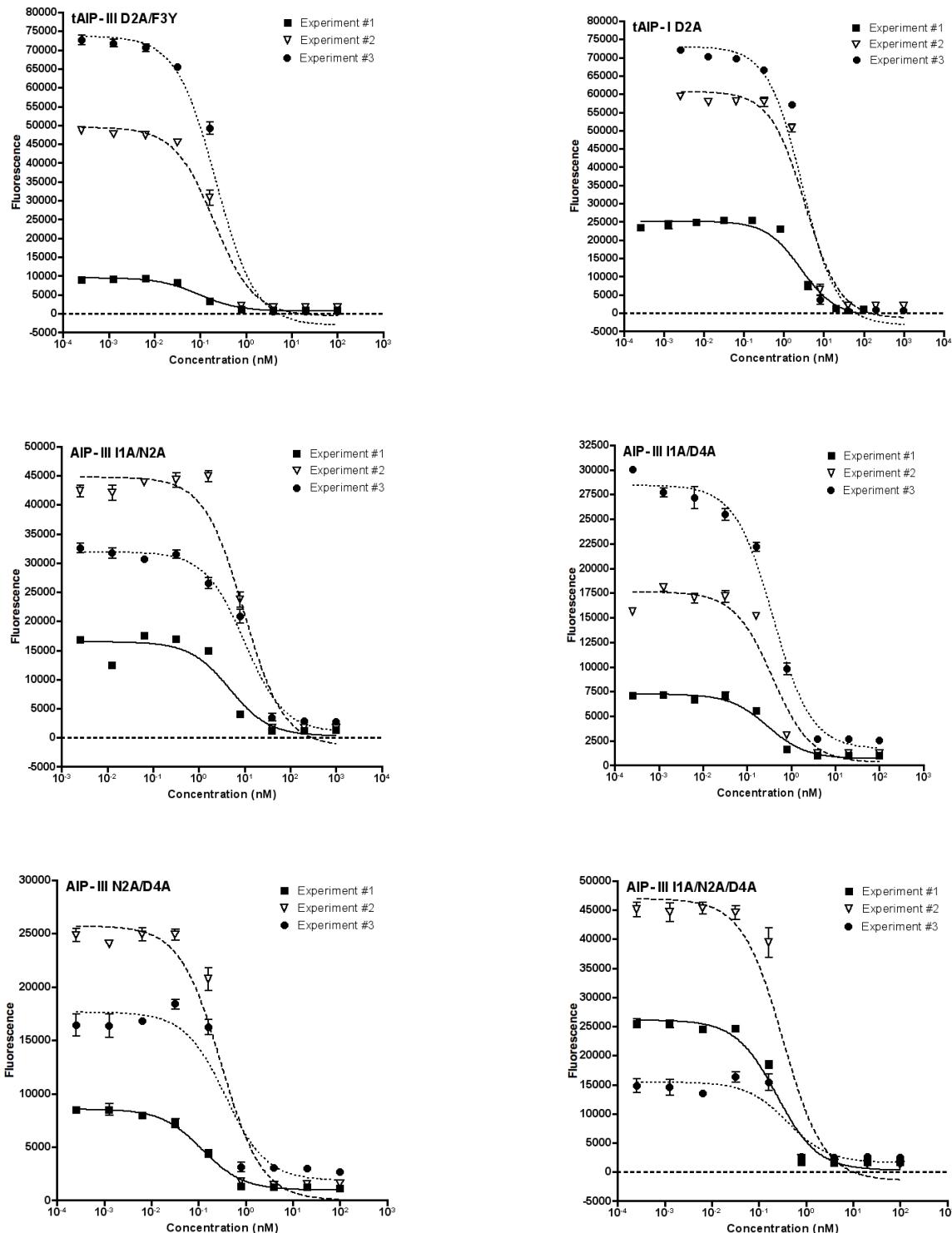
AgrC antagonism fluorescence dose response curves.

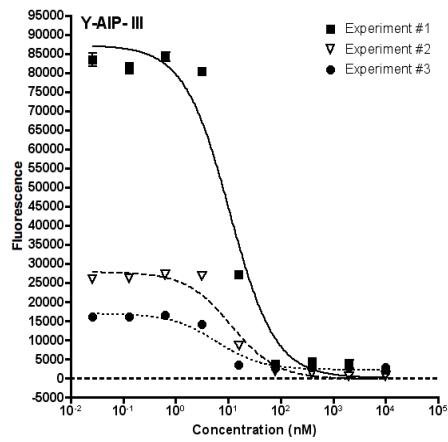
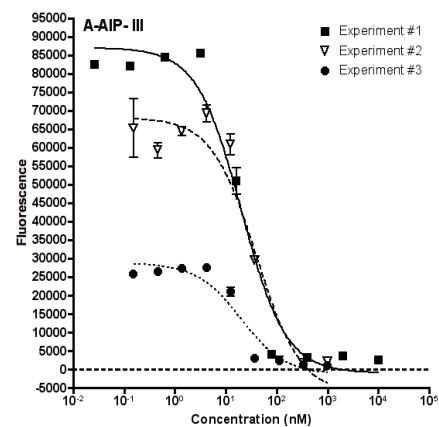
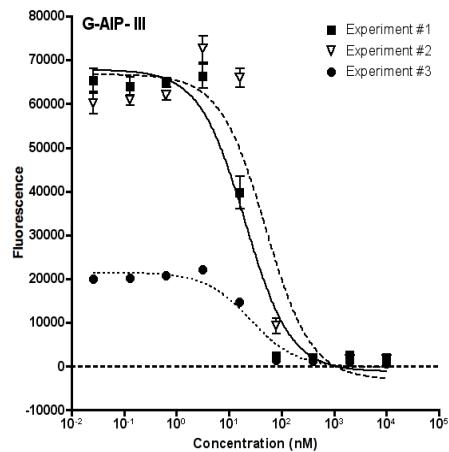
The most potent peptides were screened over varying concentrations in the four indicated *S. aureus* gfp-reporter strains (groups-I–IV). The peptide tested in each plot below is indicated in bold at top left. Each dose response experiment was performed in triplicate on three separate occasions (*i.e.*, experiments #1–3; shown for each peptide below). Error bars indicate standard error of the mean of triplicate values. See main text for details of methods and strains.

S. aureus AH1677 (group-I)

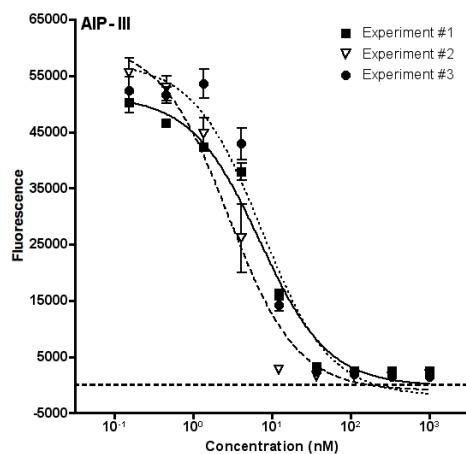
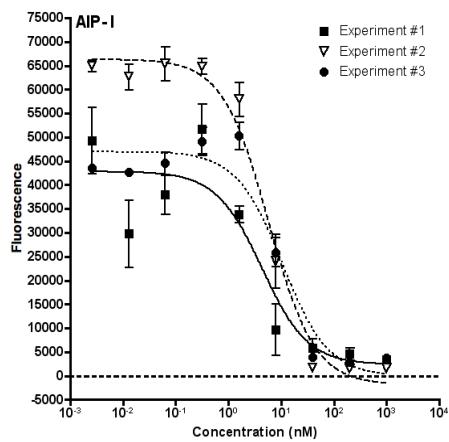


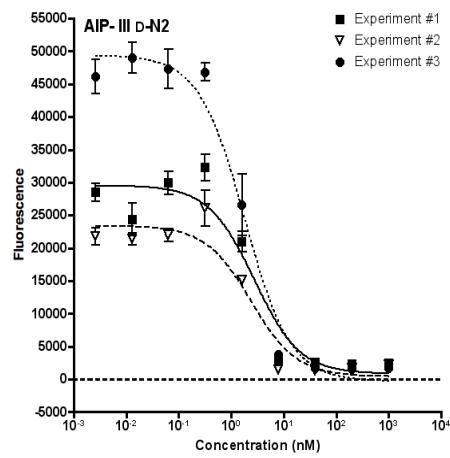
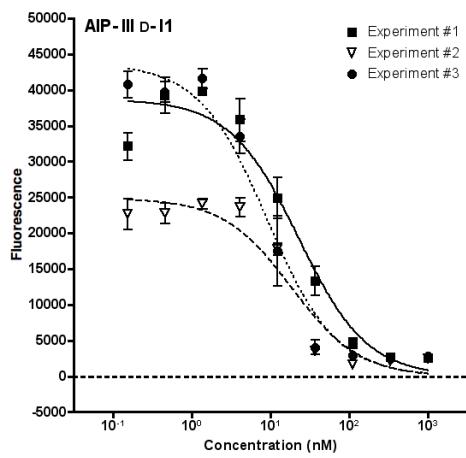
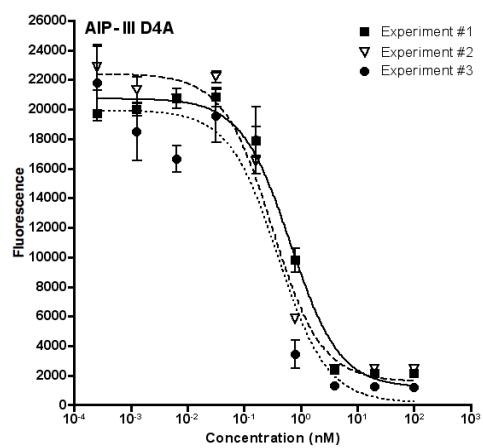
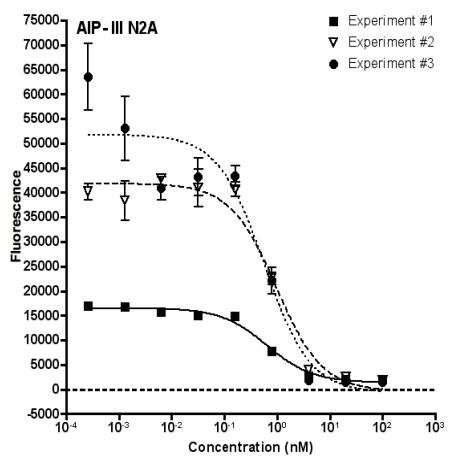
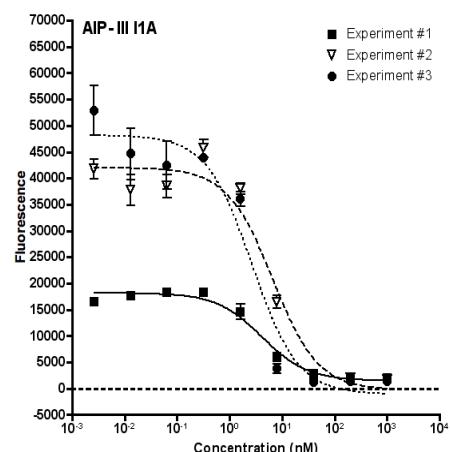
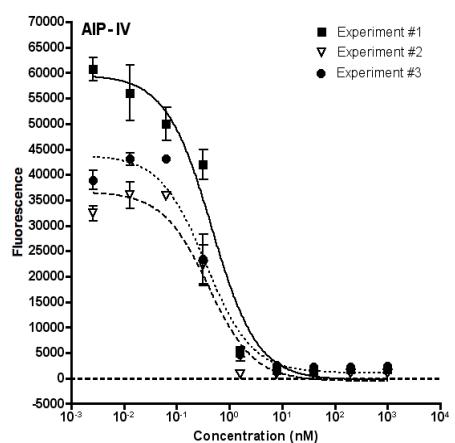


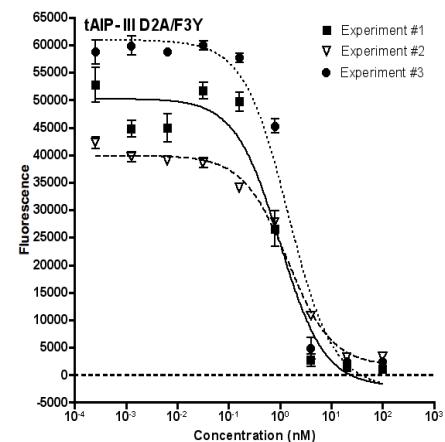
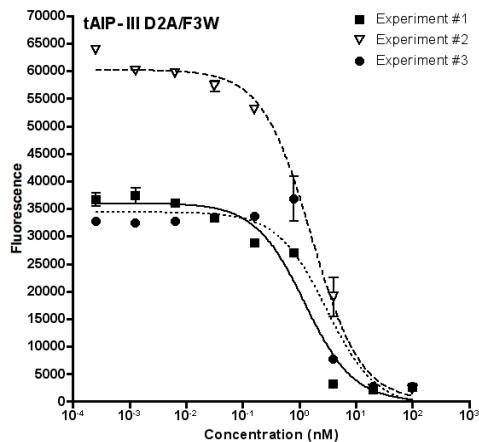
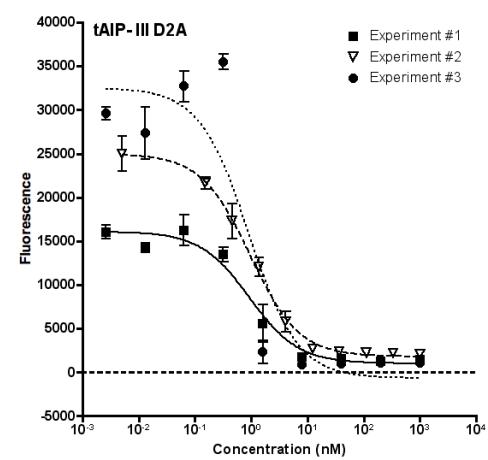
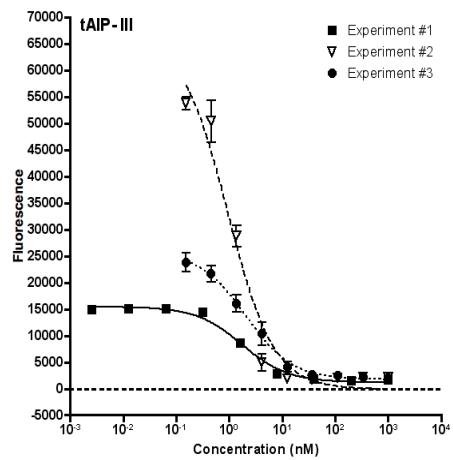
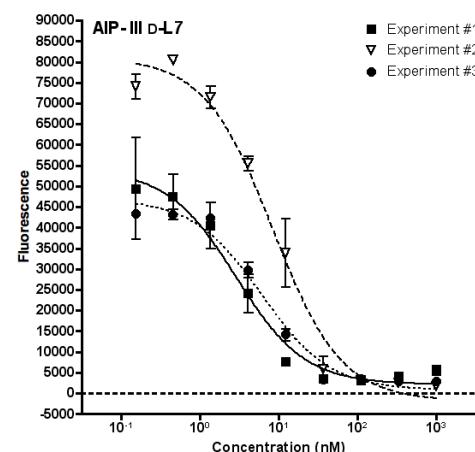
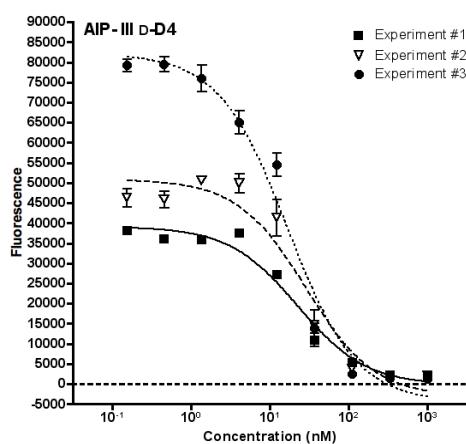


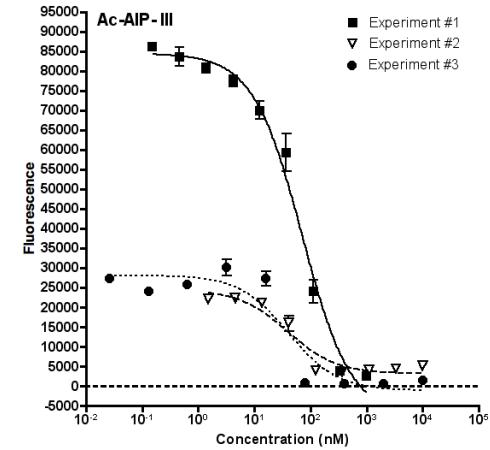
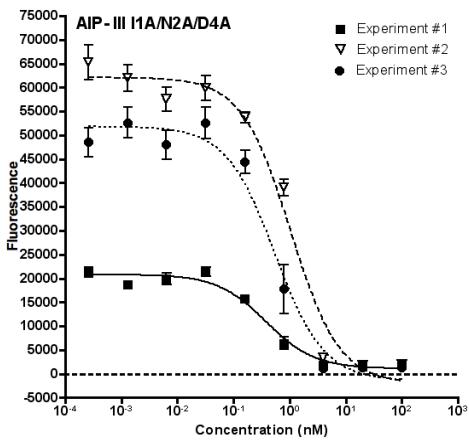
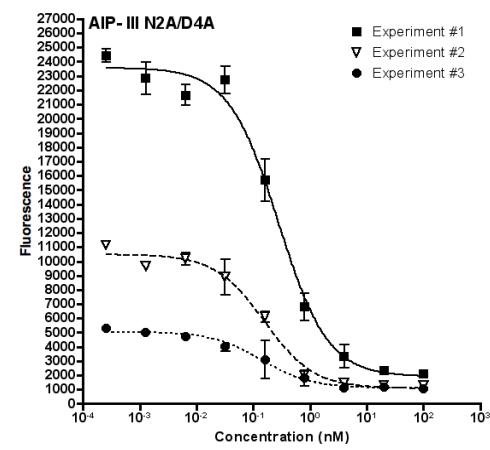
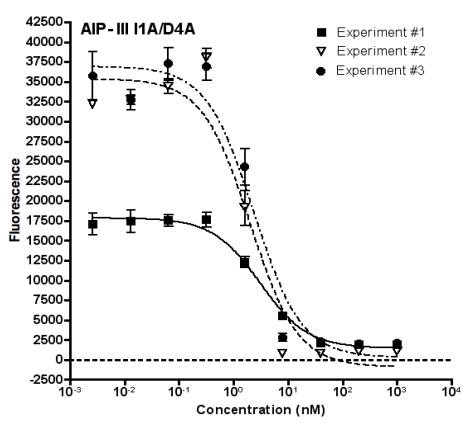
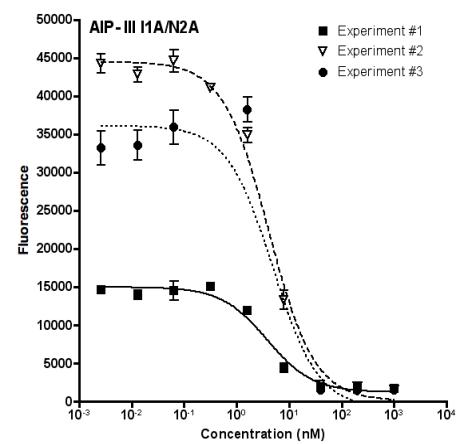
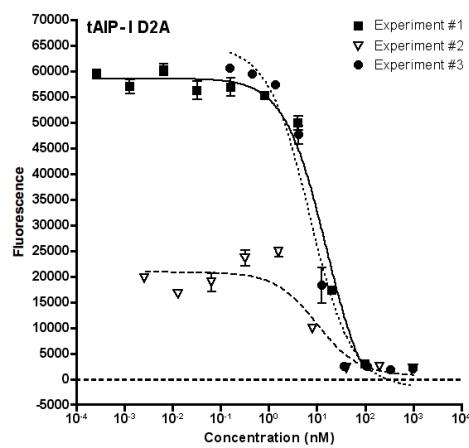


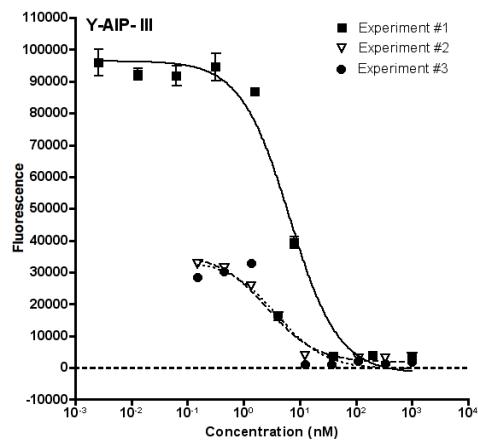
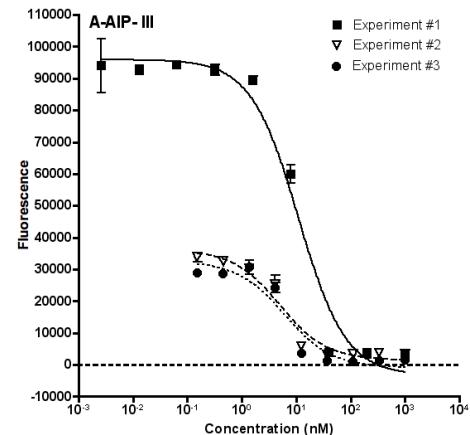
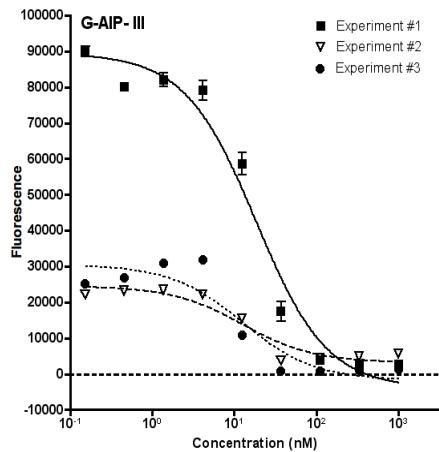
S. aureus AH430 (group-II)



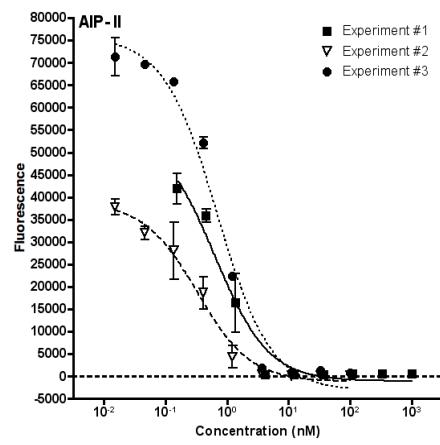
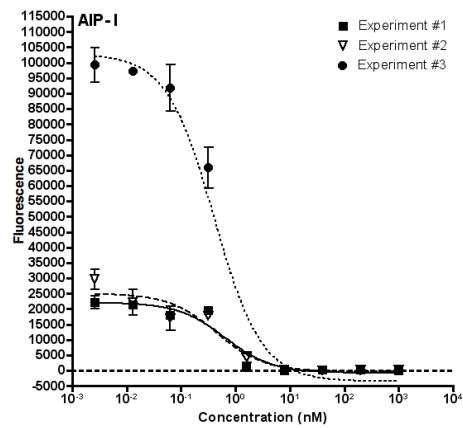


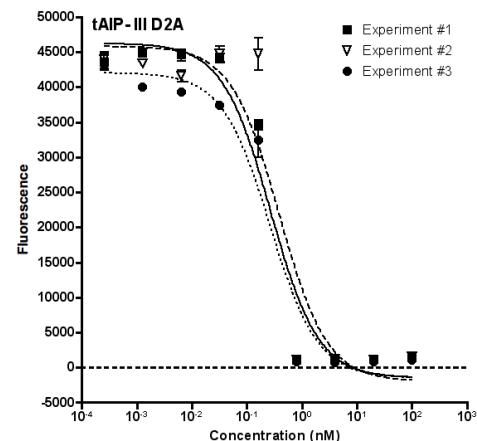
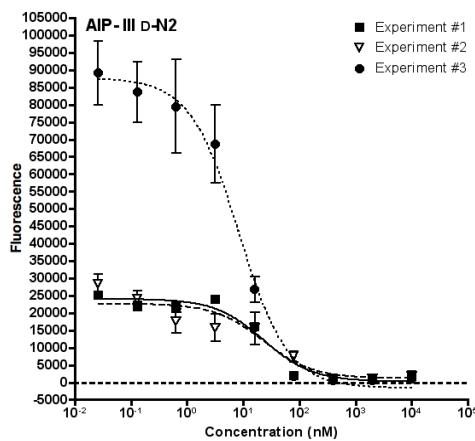
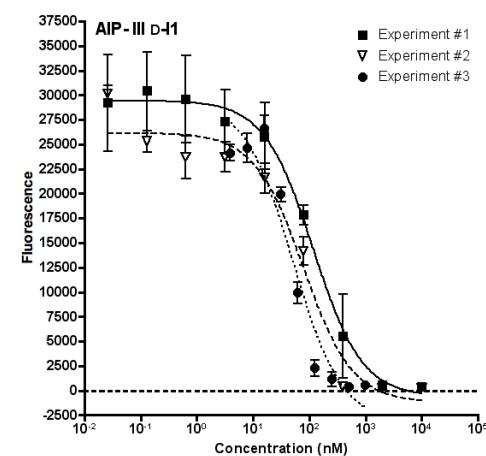
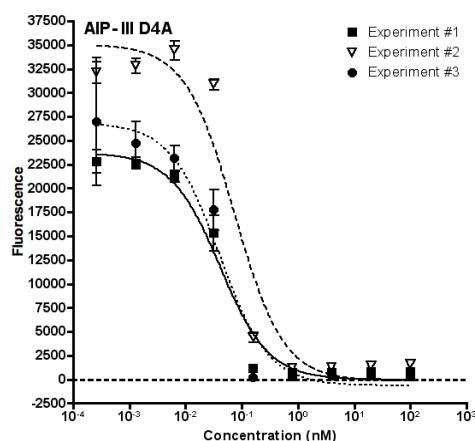
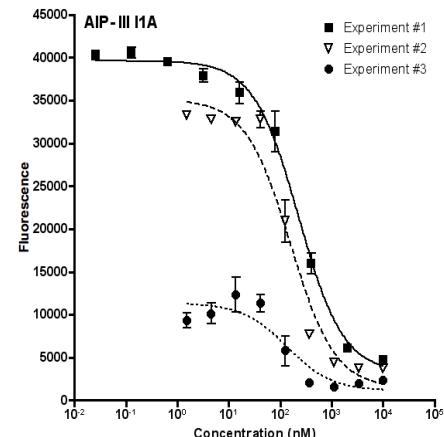
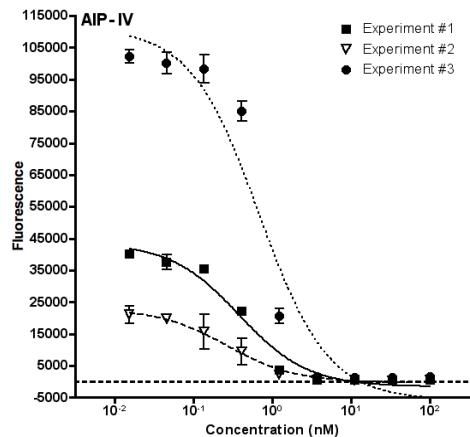


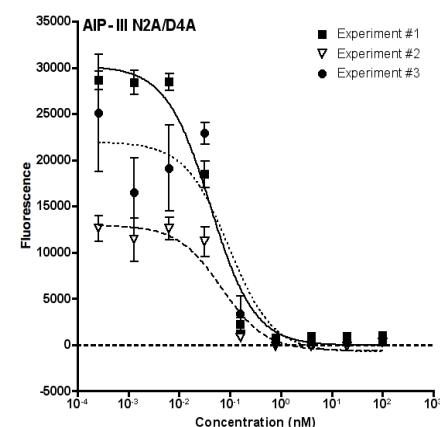
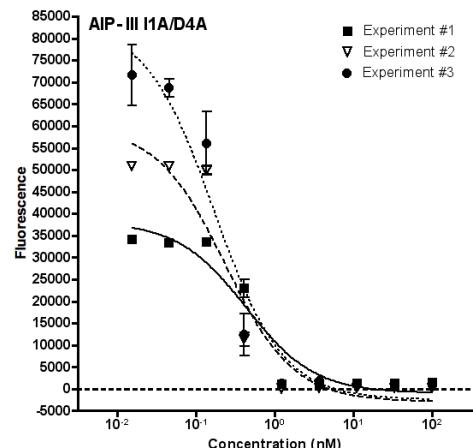
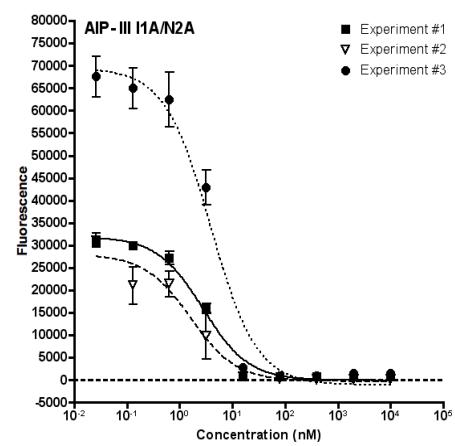
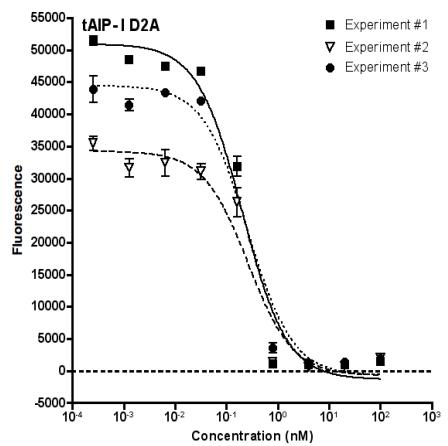
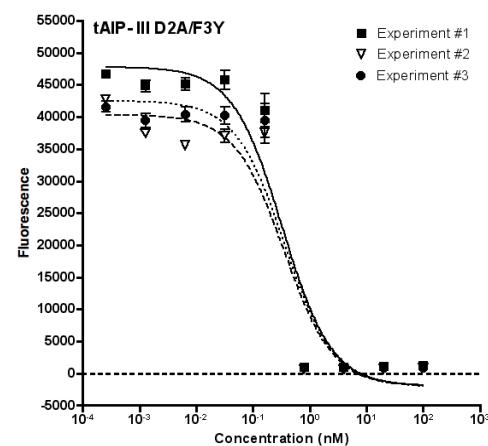
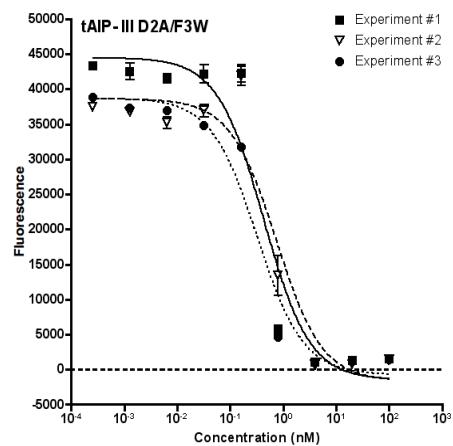


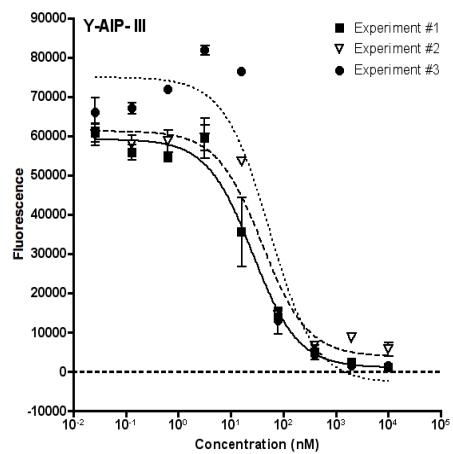
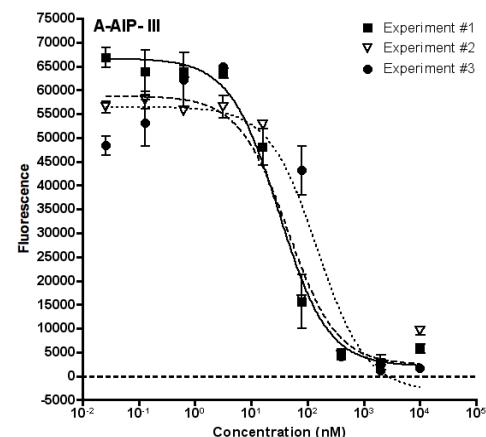
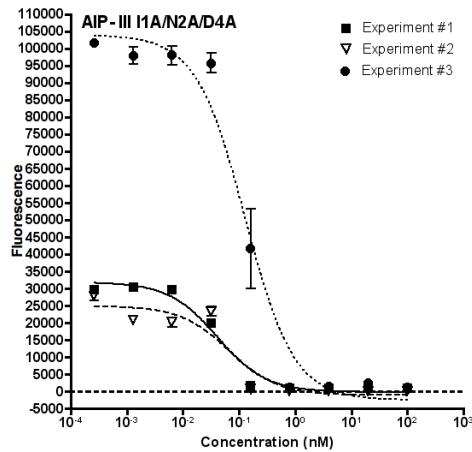


S. aureus AH1747 (group-III)

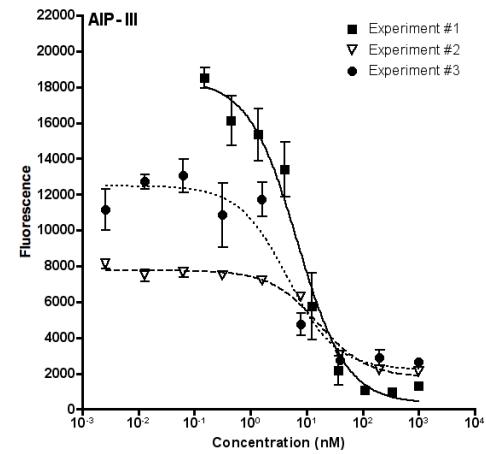
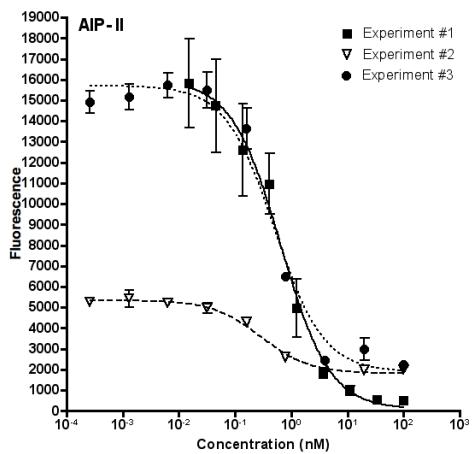


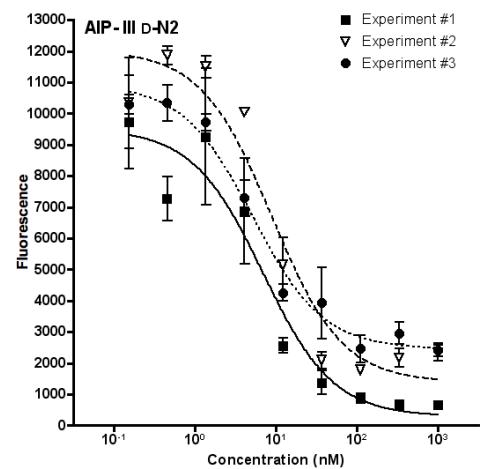
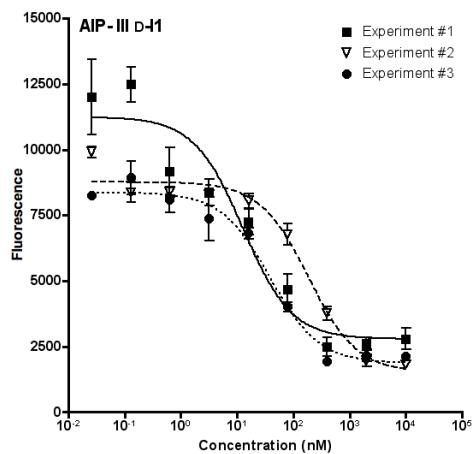
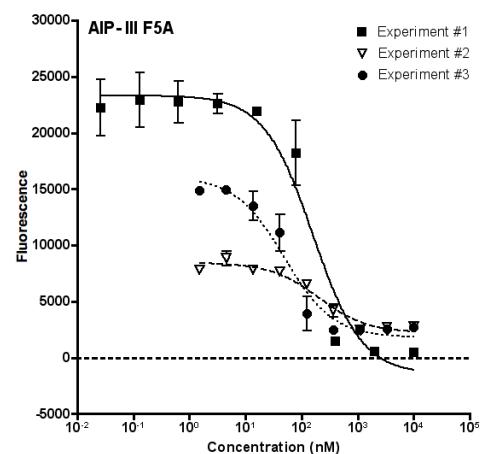
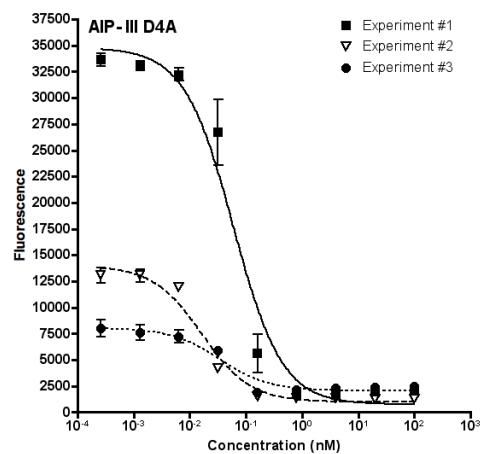
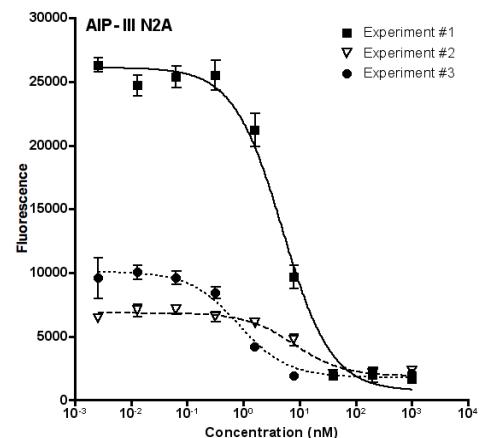
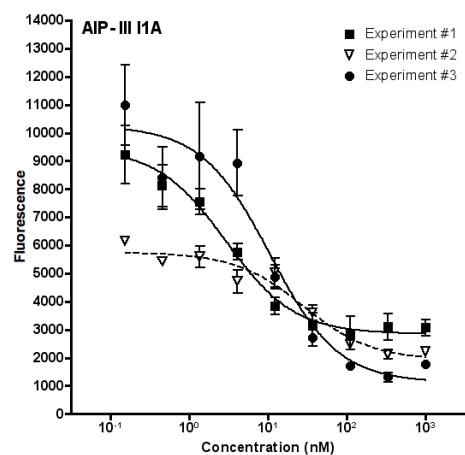


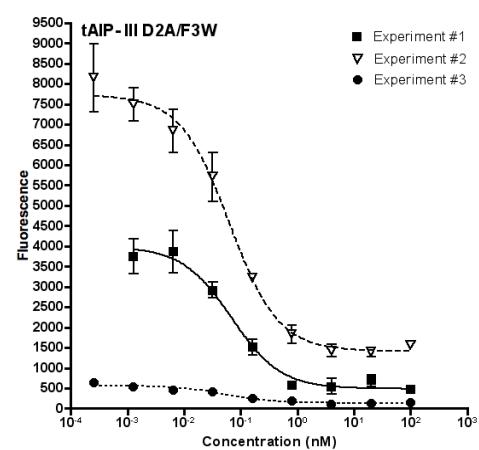
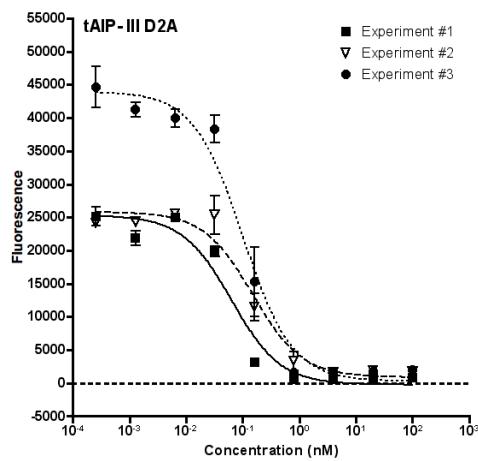
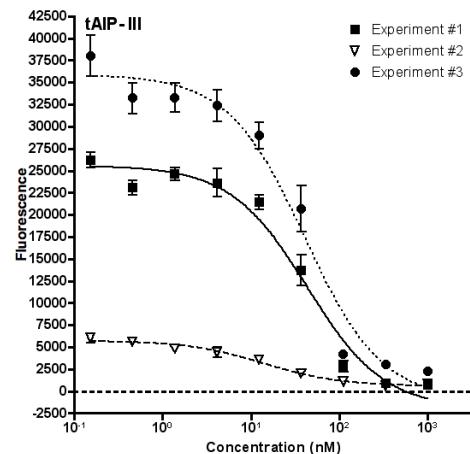
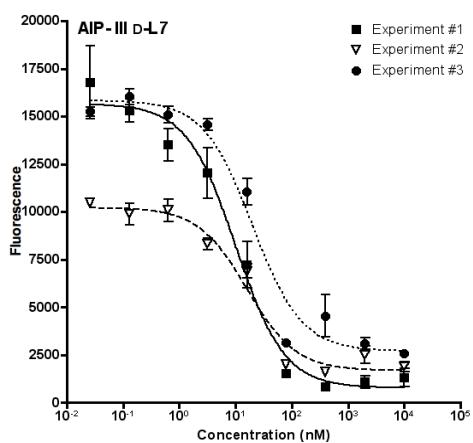
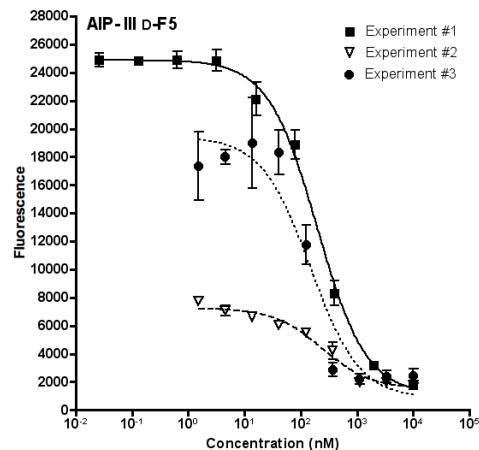
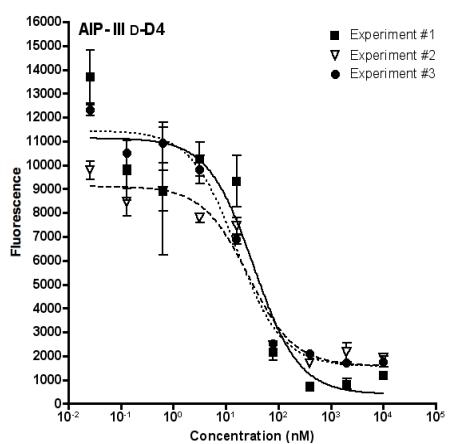


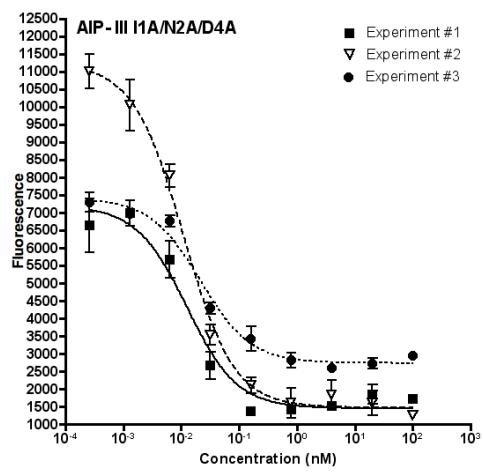
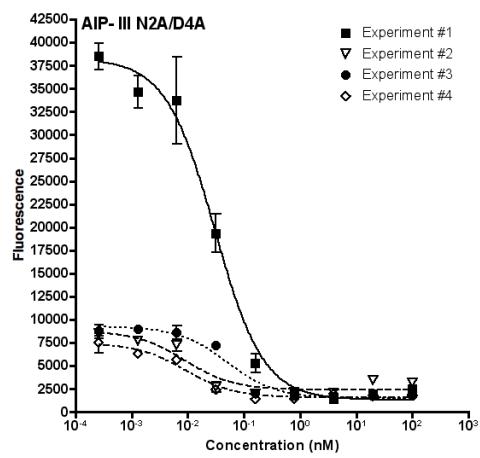
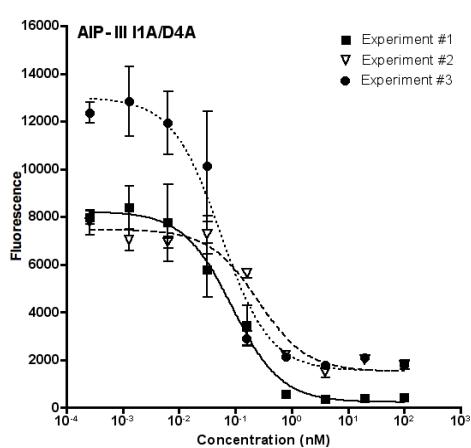
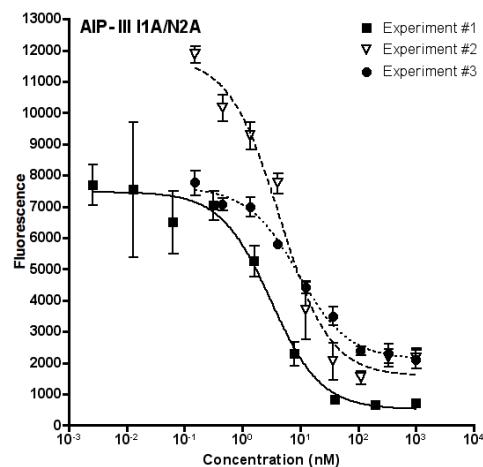
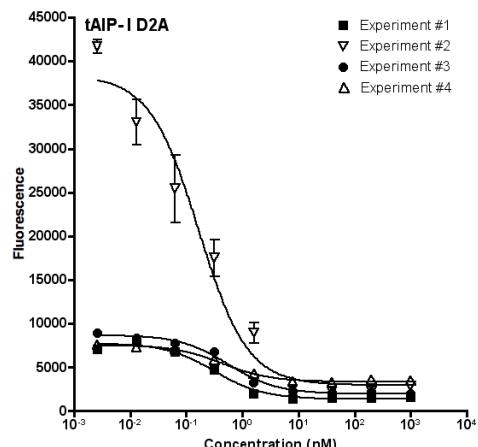
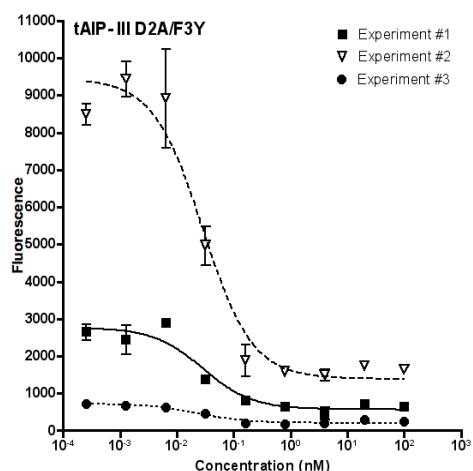


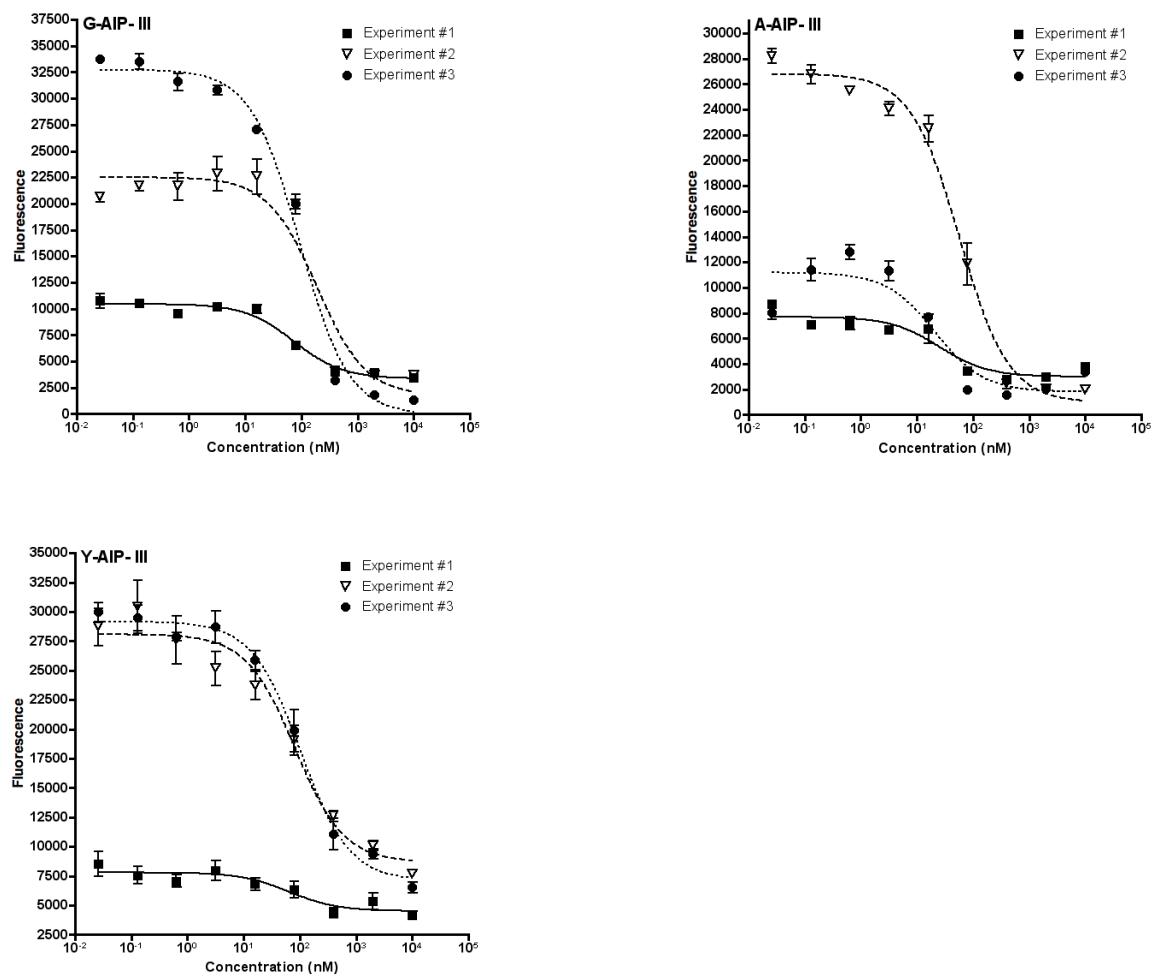
S. aureus AH1872 (group-IV)











AgrC competition fluorescence dose response curves.

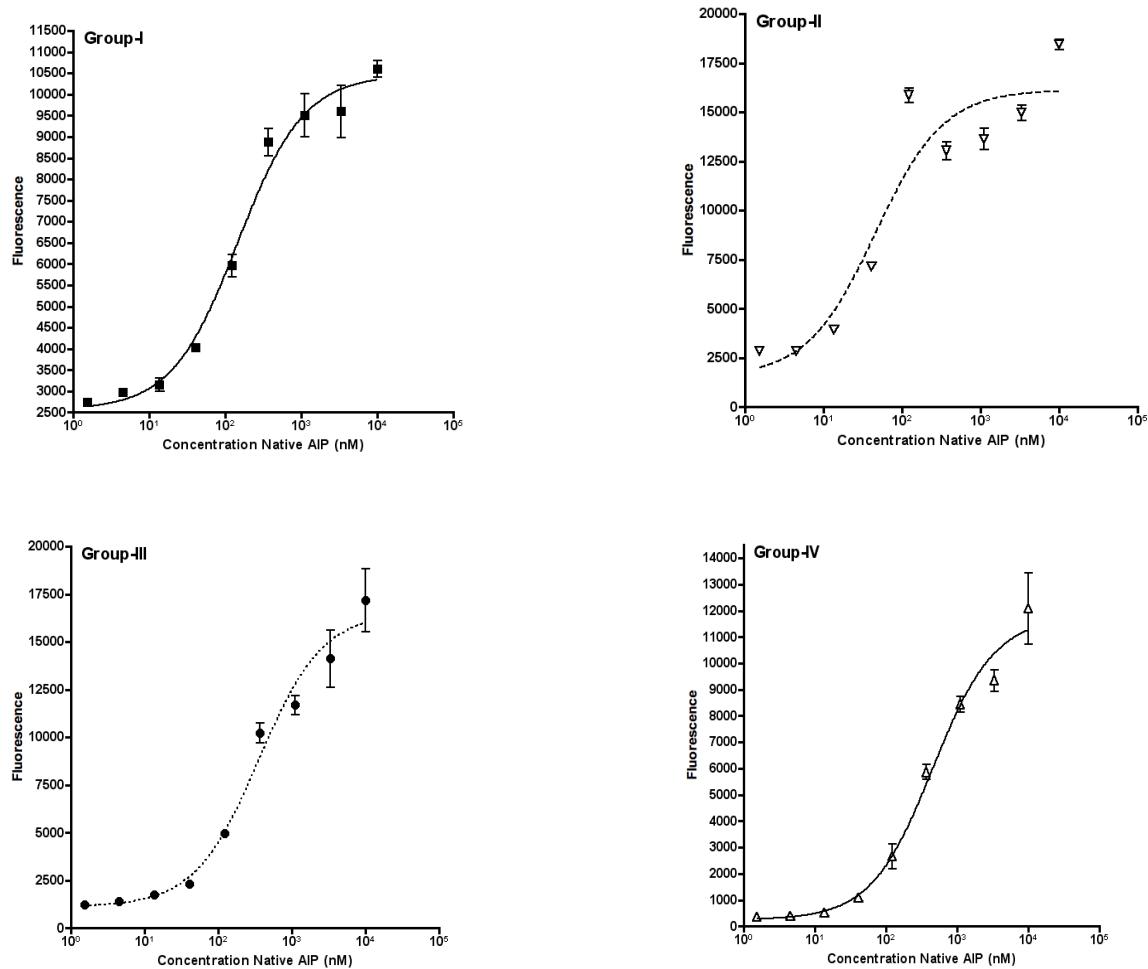


Figure S-2. Competition dose response agonism curves for the native AIPs (I–IV) in the presence of AIP-III D4A at 2 nM (group-I and group-II) or 0.3 nM (group-III and group-IV) in the group-I–IV fluorescence reporter strains. Error bars indicate standard error of the mean of triplicate values. See main text for details of methods and strains.

Hemolysis assay conditions.

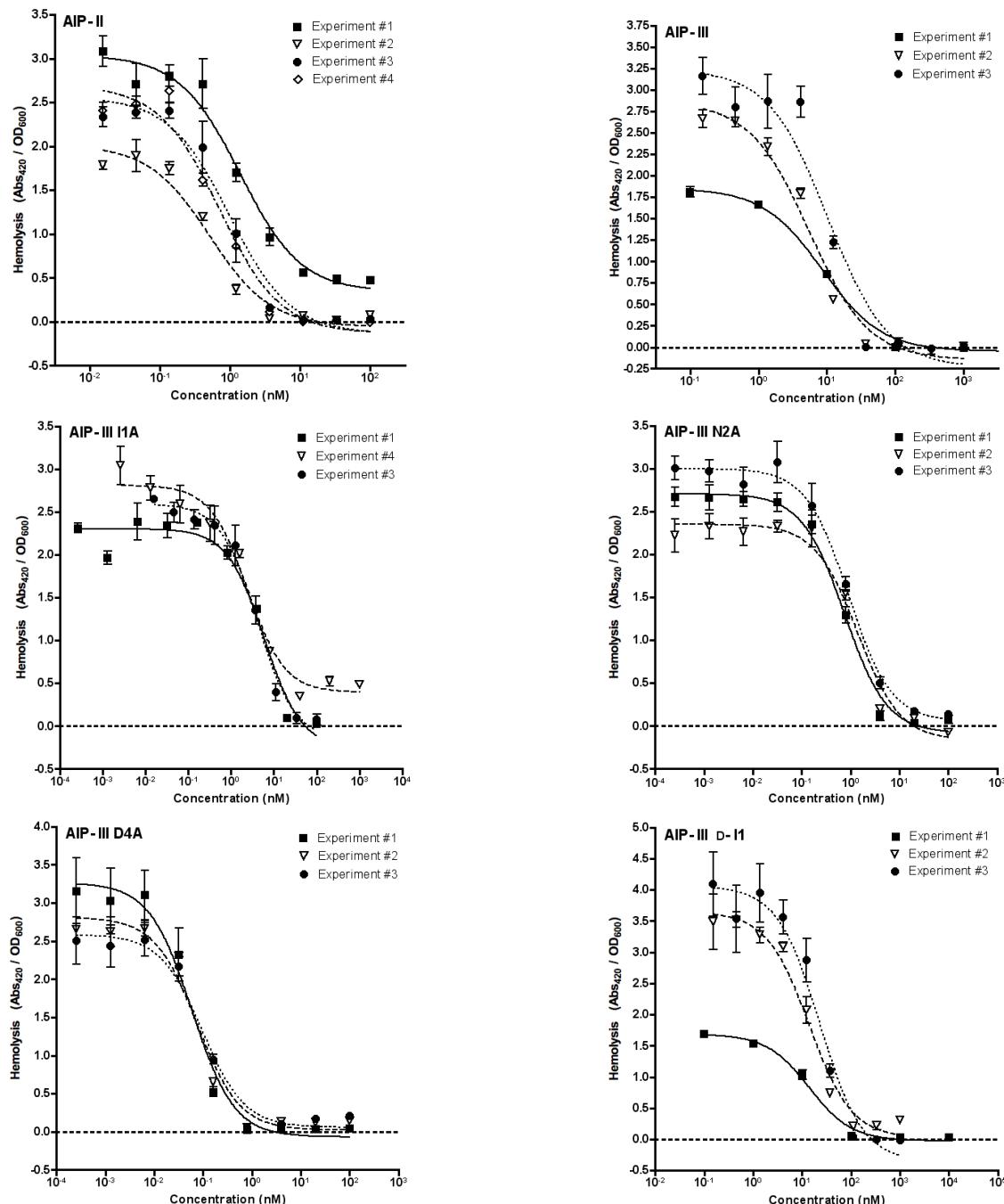
Table S-2. Conditions for hemolysis assay. TSB = tryptic soy broth. See main text for details of assay protocol and strains.

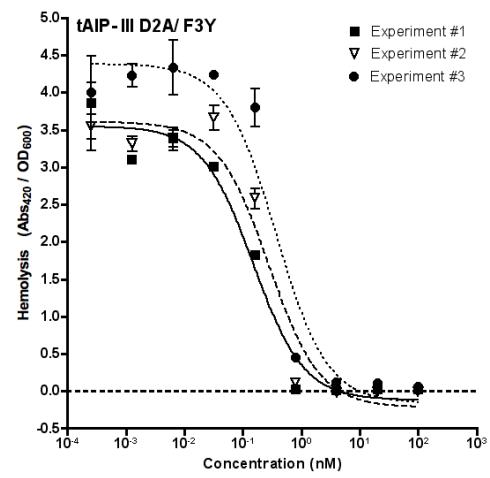
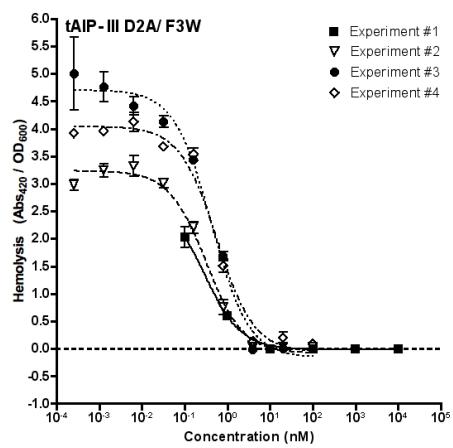
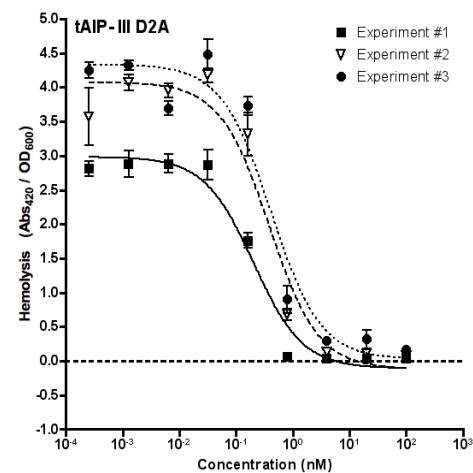
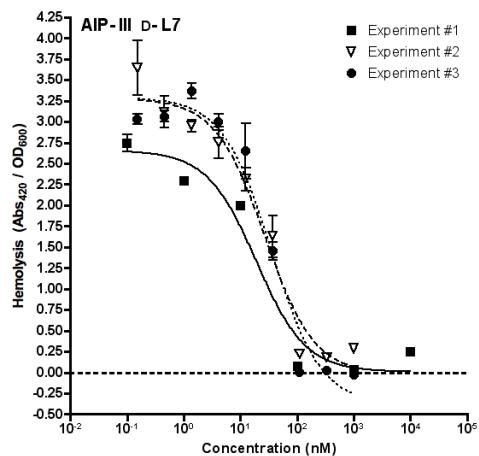
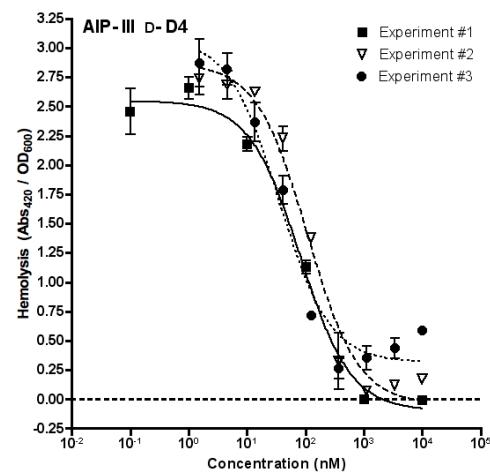
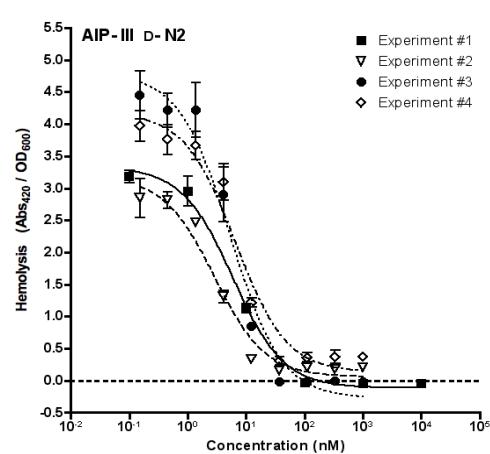
<i>S. aureus</i> strain	Dilution of culture with TSB	Bacteria + AIPs incubation time	Culture + red blood cells incubation time
RN6390B (group-I)	1:100	8 h	15 min
RN9623 (group-II)	1:25	8 h	15 min
MN8 (group-III)	1:10	6 h	25 min
RN4850 (group-IV)	1:10	6 h	15 min

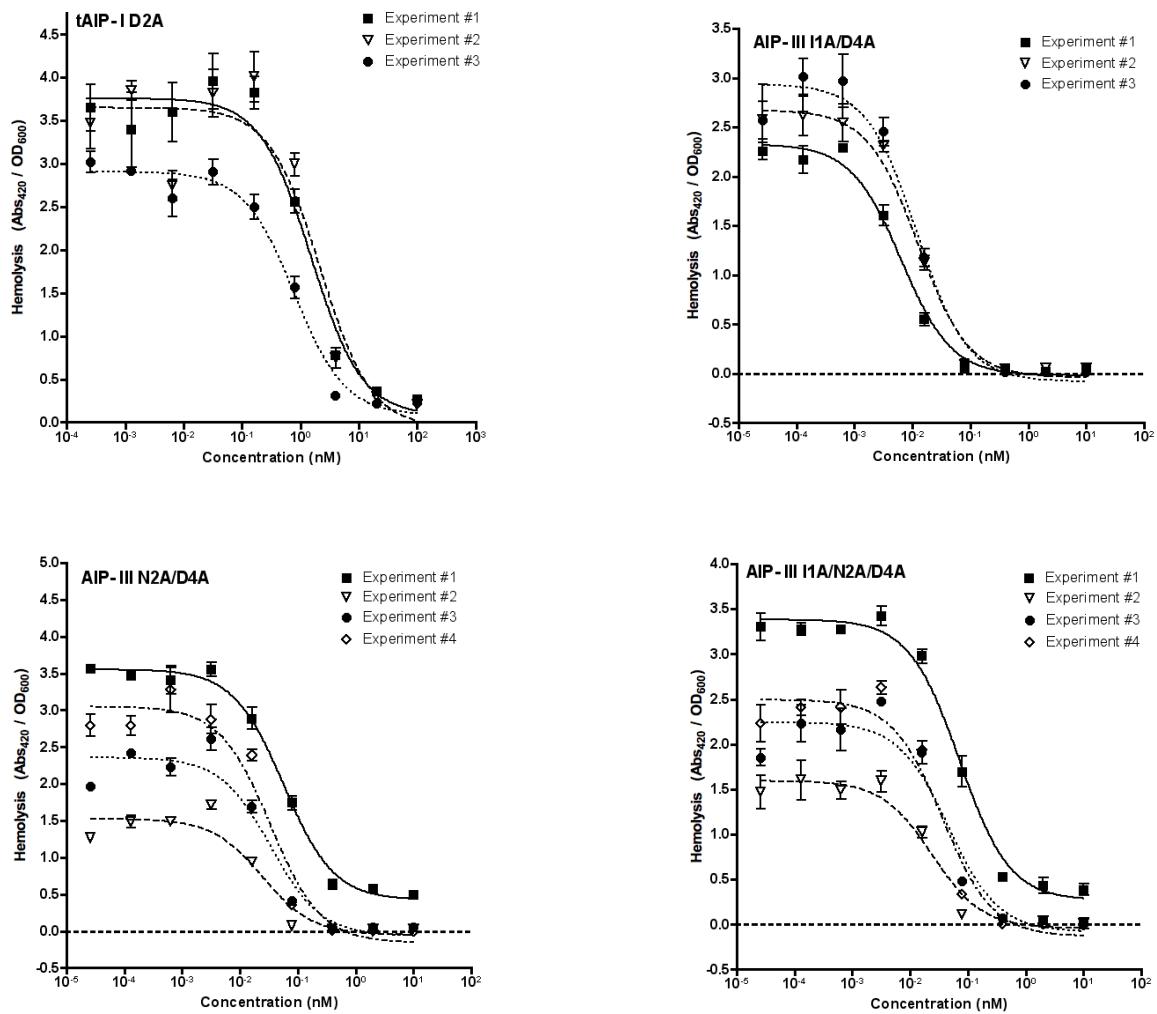
Hemolysis inhibition dose response curves.

The most potent peptides were screened over varying concentrations for their ability to inhibit the hemolysis of rabbit blood cells by wt group-I–IV *S. aureus*. The peptide tested in each plot below is indicated in bold at top left. Each dose response experiment was performed in triplicate on three separate occasions (*i.e.*, experiments #1–3; shown for each peptide below). Error bars indicate standard error of the mean of triplicate values. See main text for details of methods and strains.

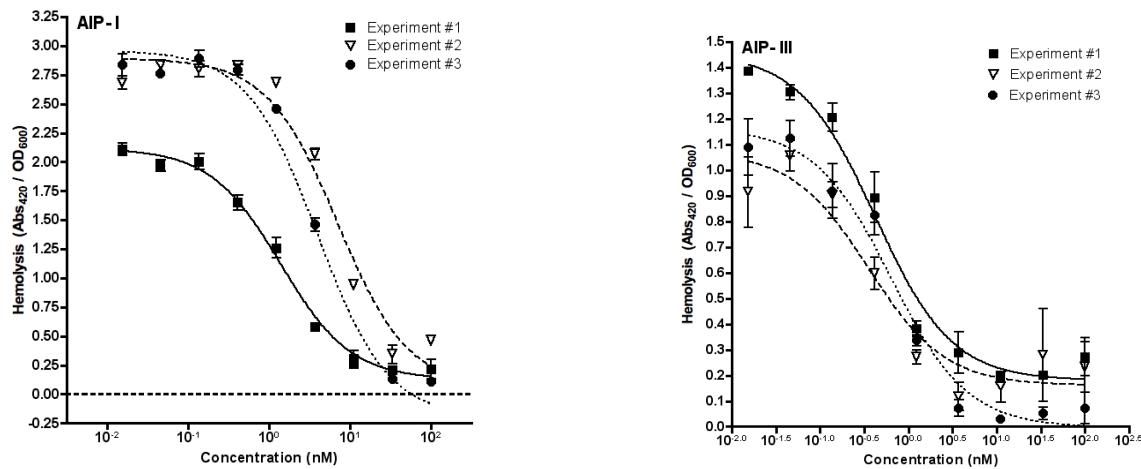
S. aureus RN6390B (group-I)

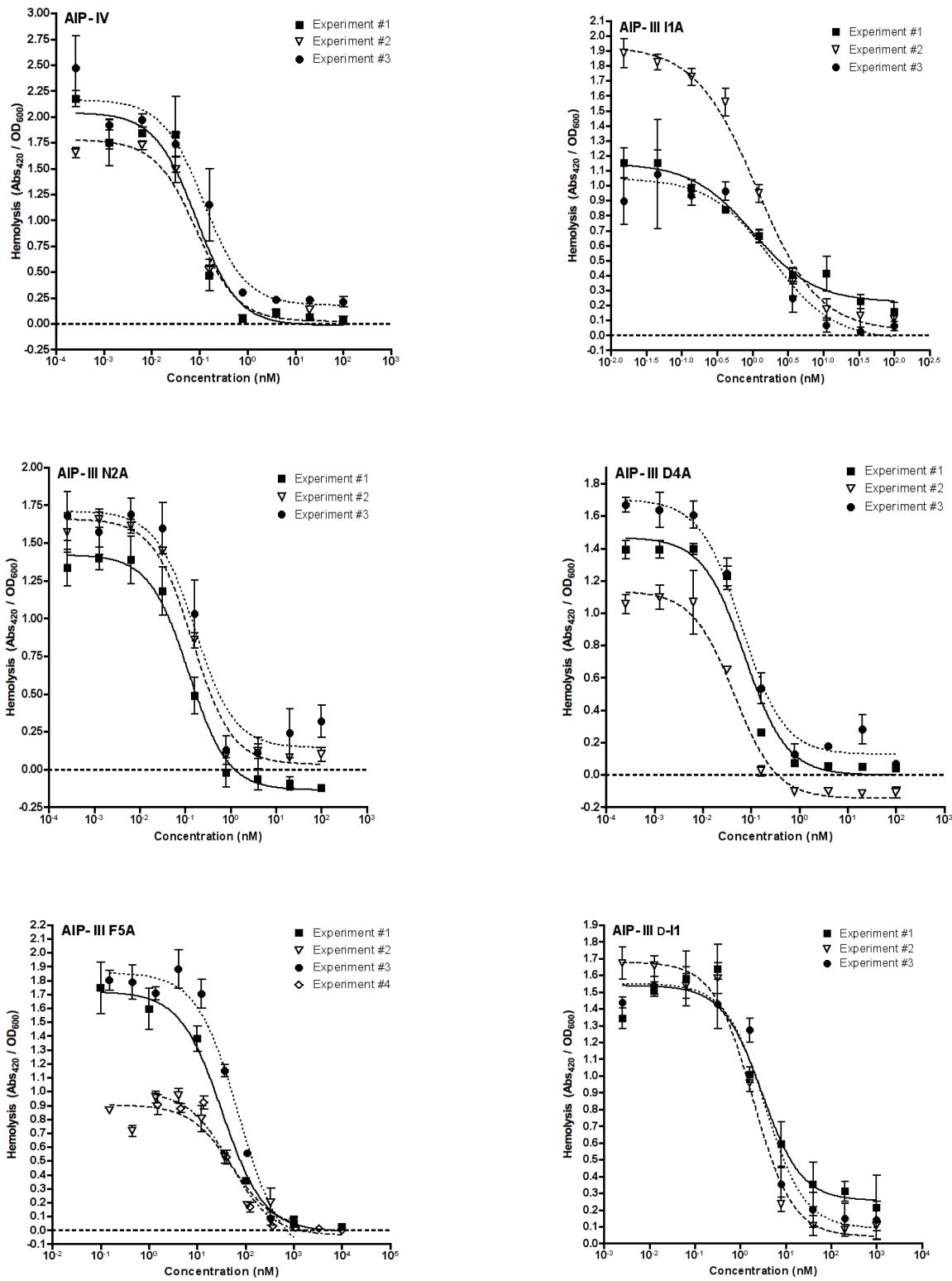


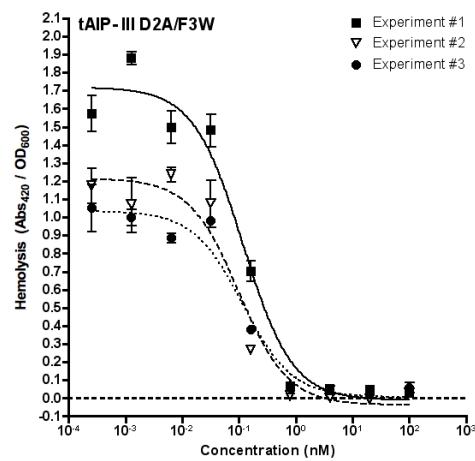
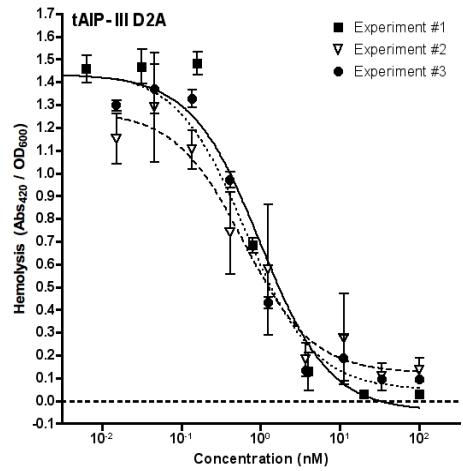
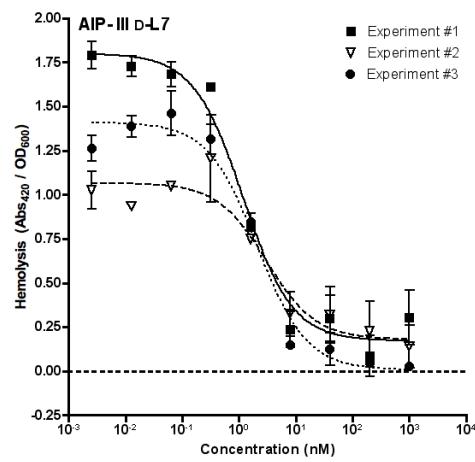
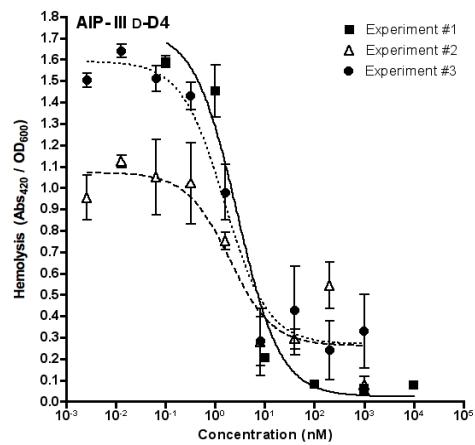
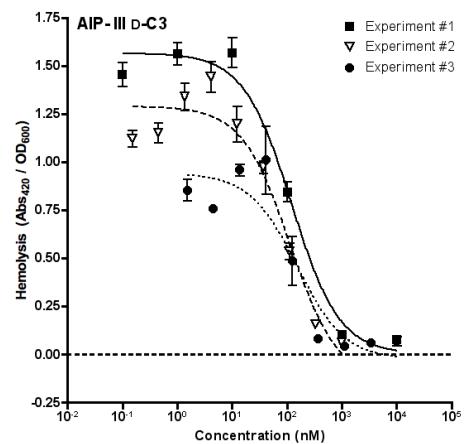
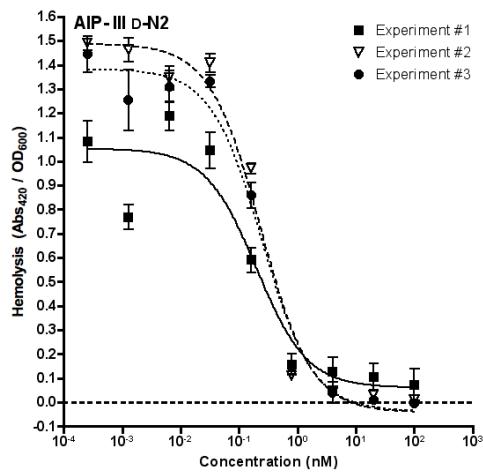


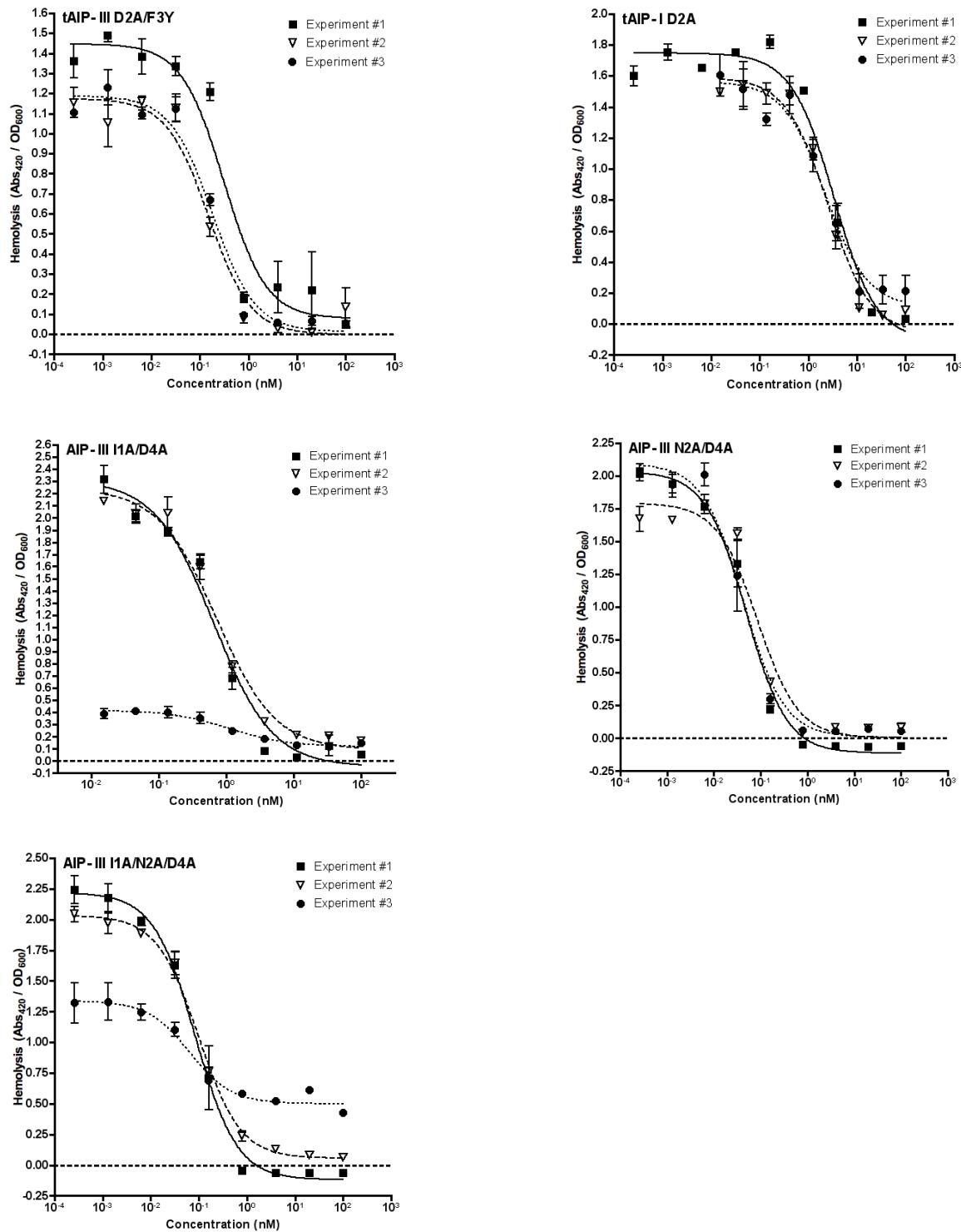


S. aureus RN6923 (group-II)

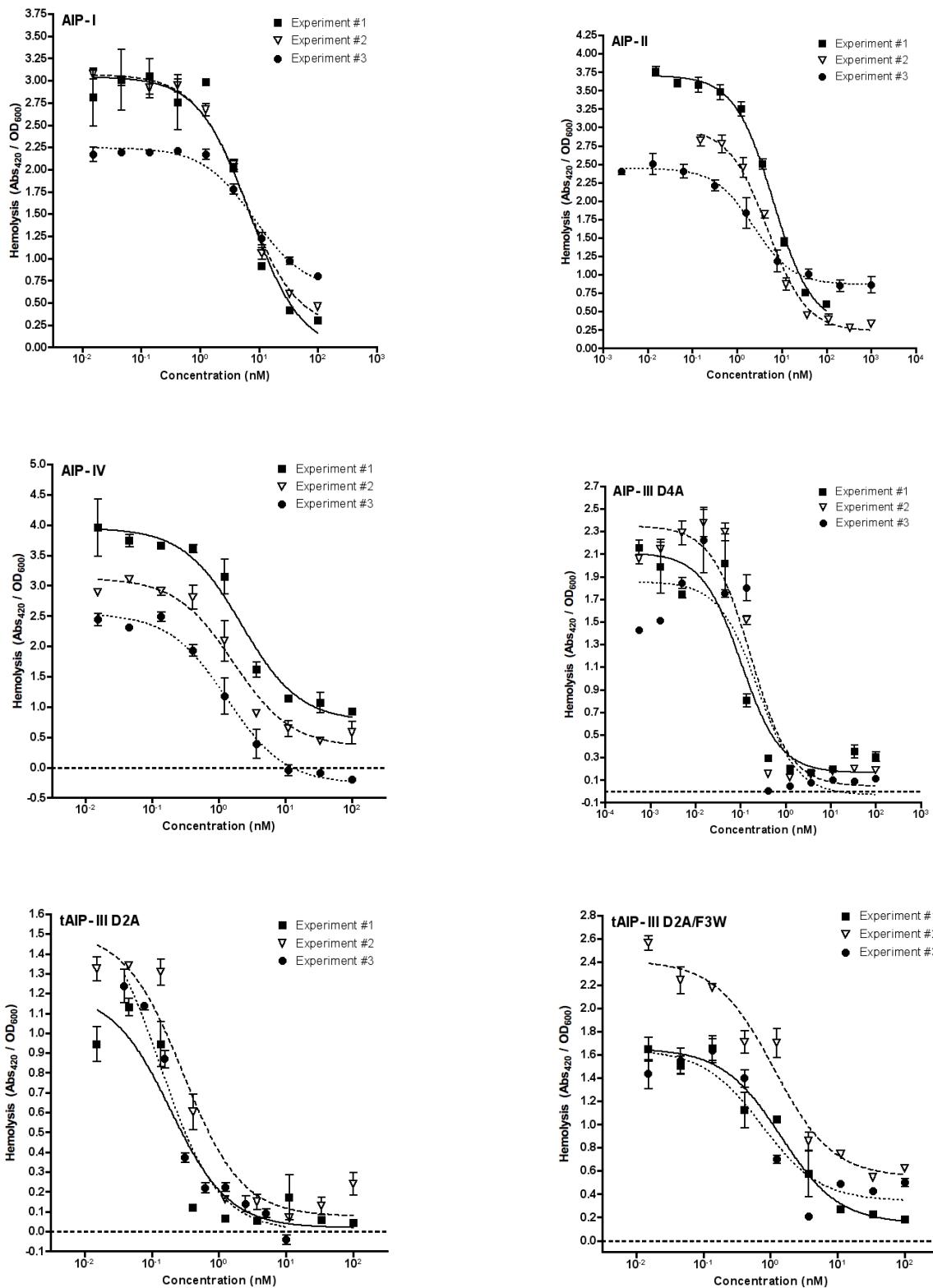


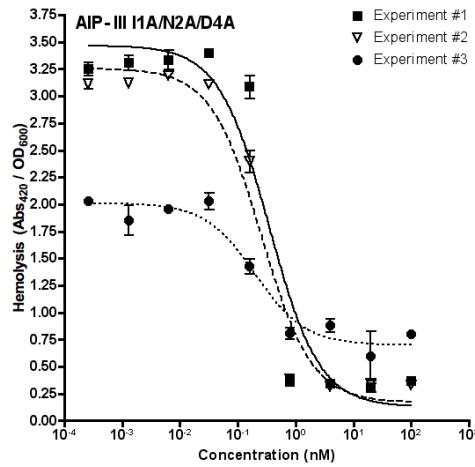
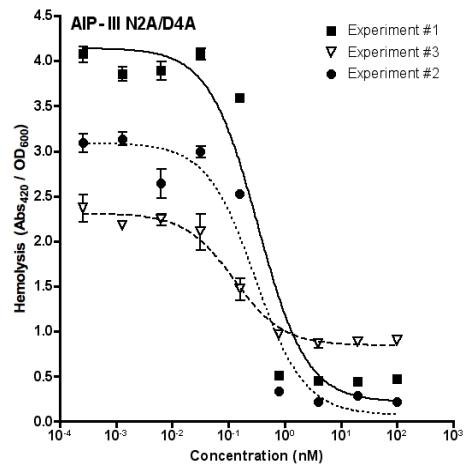
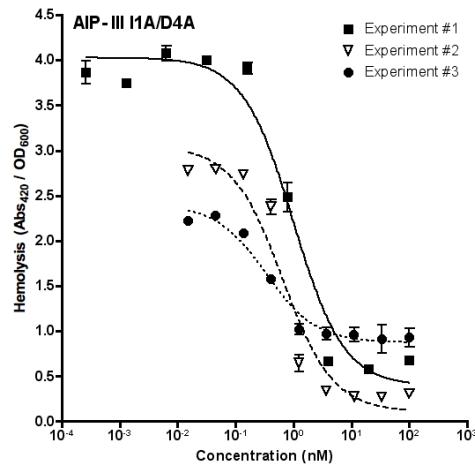
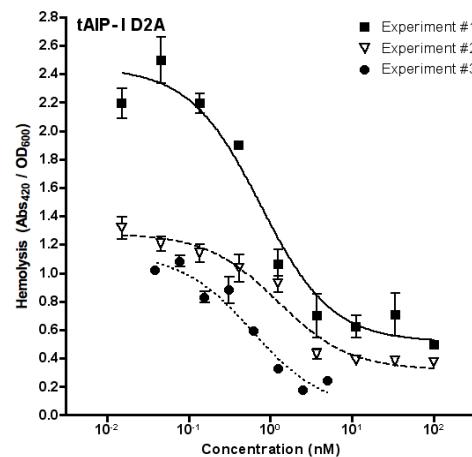
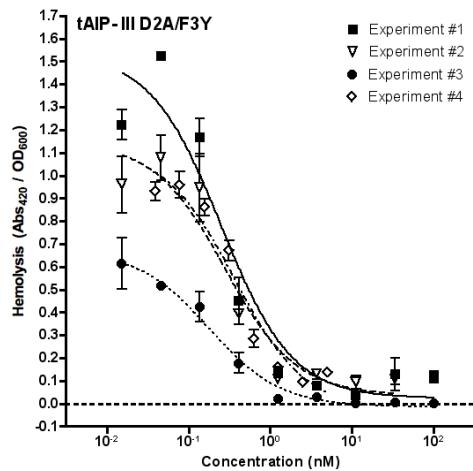




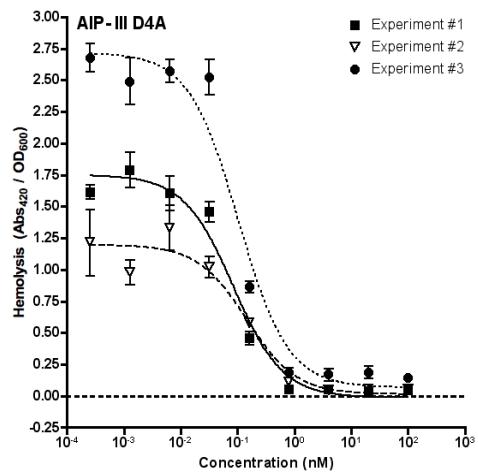
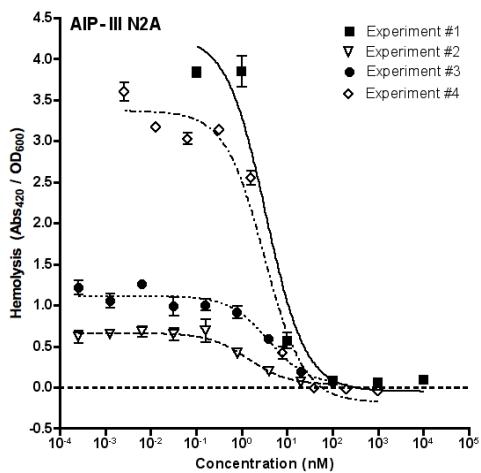
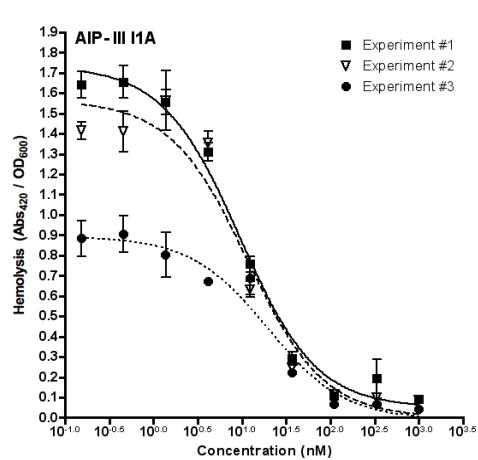
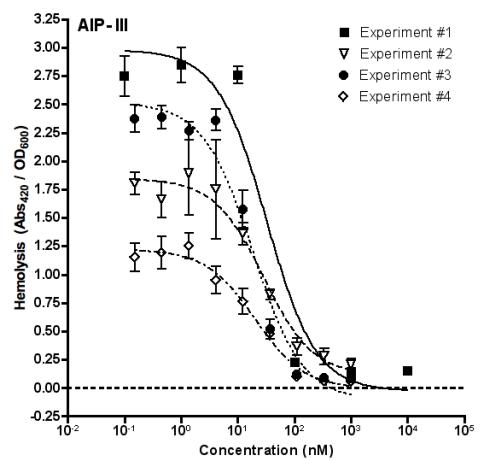
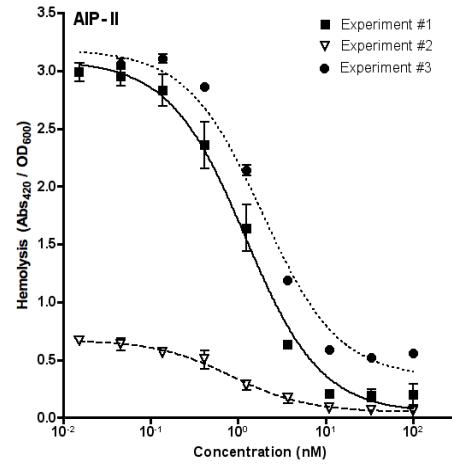
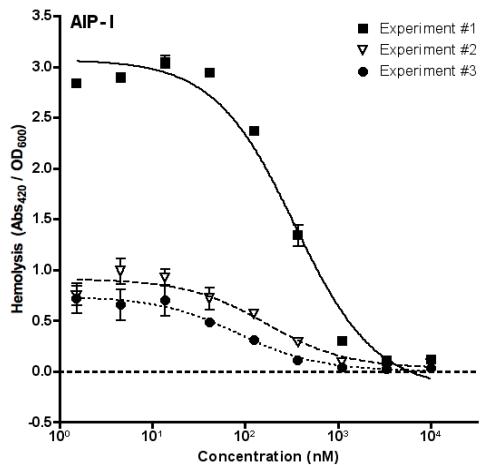


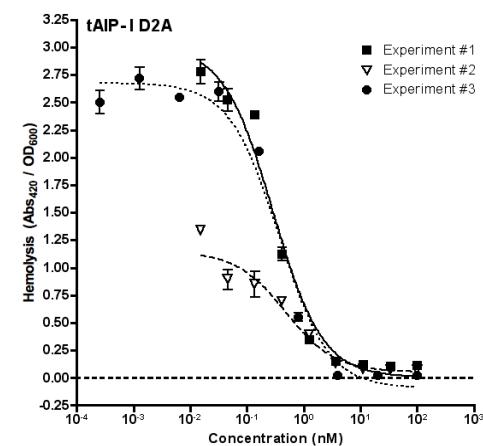
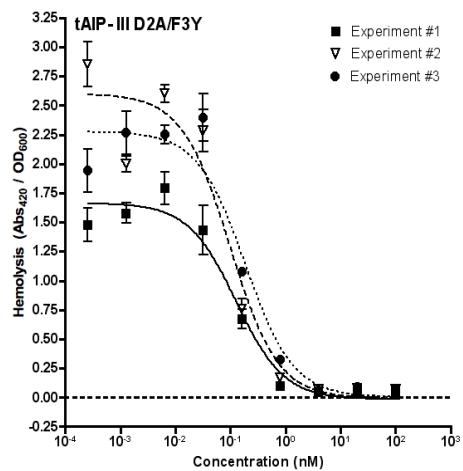
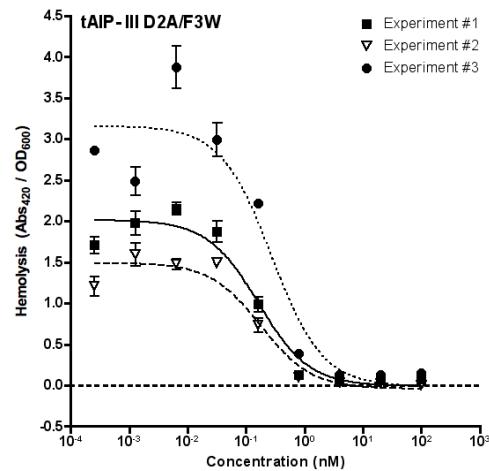
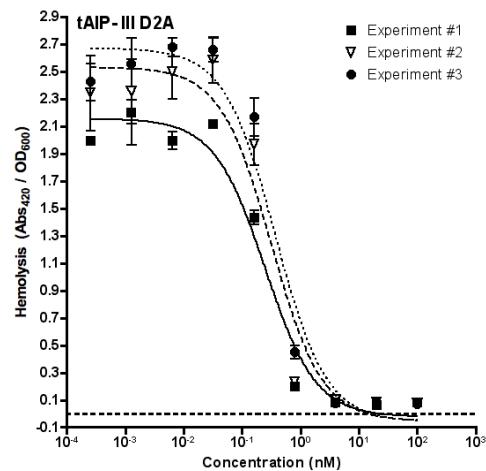
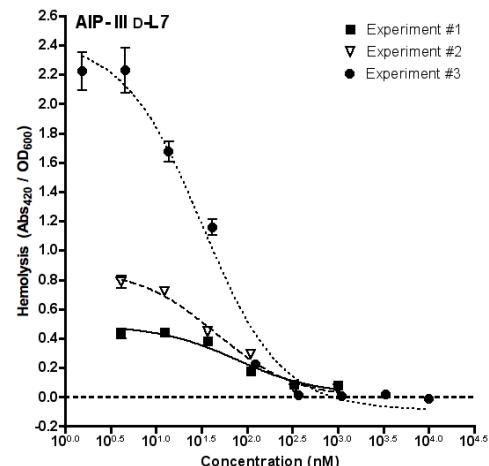
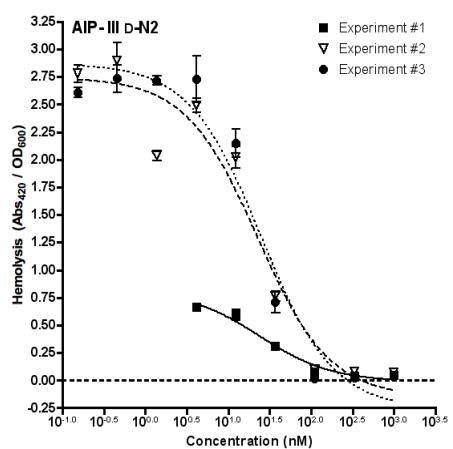
S. aureus MN8 (group-III)

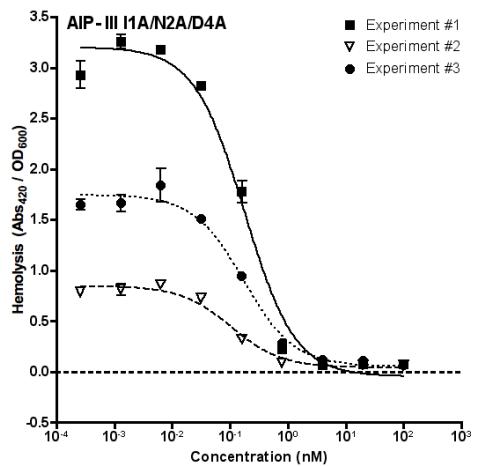
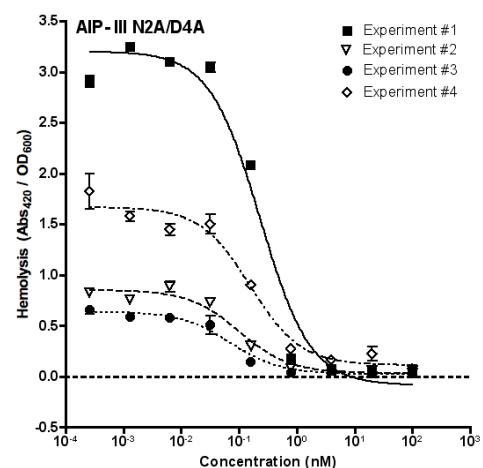
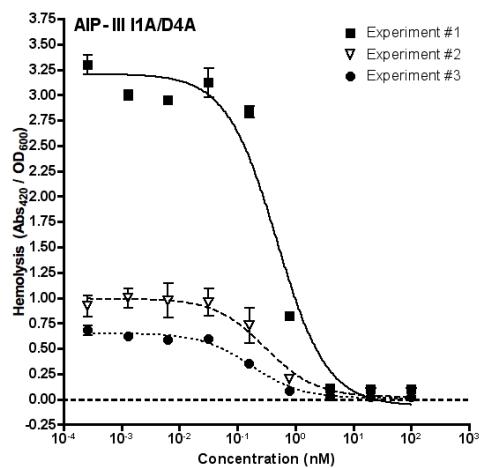




S. aureus RN4850 (group-IV)







Complete tables of IC₅₀ values for fluorescence and hemolysis assays.

Table S-3. IC₅₀ values of the alanine and D-amino acid scan analogs of AIP-III against AgrC I–IV determined using *S. aureus* fluorescence reporter strains.^a

Peptide name	Sequence	AgrC-I IC ₅₀ (nM) ^b	AgrC-II IC ₅₀ (nM) ^b	AgrC-III IC ₅₀ (nM) ^b	AgrC-IV IC ₅₀ (nM) ^b
AIP-III D-I1	DI-N-(C-D-F-L-L)	8.42 (5.50-12.9)	16.4 (5.71-47.3)	78.3 (33.5-183)	77.7 (27.1-223)
AIP-III D-N2	I-DN-(C-D-F-L-L)	2.15 (1.90-2.43)	2.45 (1.60-3.75)	17.8 (5.08-62.6)	6.23 (2.41-16.1)
AIP-III D-C3	I-N-(DC-D-F-L-L)	>200	>200	>200	>200
AIP-III D-D4	I-N-(C-DD-F-L-L)	138 (53.2-359)	24.5 (11.2-53.5)	>200	29.2 (22.7-37.7)
AIP-III D-F5	I-N-(C-D-DF-L-L)	>200	>200 ^{c, d}	>200	174 (140-216)
AIP-III D-L6	I-N-(C-D-F-DL-L)	>200	- ^e	>200	>200
AIP-III D-L7	I-N-(C-D-F-L-DL)	12.0 (5.30-27.3)	5.36 (2.33-12.3)	- ^e	10.5 (5.92-18.7)
AIP-III I1A	A-N-(C-D-F-L-L)	17.9 (13.0-24.7)	4.26 (1.95-9.31)	194 (125-302)	7.85 (4.59-13.4)
AIP-III N2A	I-A-(C-D-F-L-L)	3.60 (2.74-4.74)	0.732 (0.456-1.17)	- ^{c, d}	3.53 (1.35-9.23)
AIP-III D4A	I-N-(C-A-F-L-L)	0.485 (0.289-0.813)	0.429 (0.208-0.886)	0.0506 (0.0227-0.113)	0.0349 (0.0123-0.0990)
AIP-III F5A	I-N-(C-D-A-L-L)	>200	>200 ^c	>200	118 (42.0-333)
AIP-III L6A	I-N-(C-D-F-A-L)	>200	>200	>200	>200
AIP-III L7A	I-N-(C-D-F-L-A)	>200	>200	>200	>200
AIP-I^f	Y-S-T-(C-D-F-I-M)	- ^e	8.00 (3.66-17.5)	0.522 (0.309-0.883)	- ^{c, d}
AIP-II^f	G-V-N-A-(C-S-S-L-F)	1.62 (0.927-2.82)	- ^e	0.532 (0.238-1.19)	0.396 (0.206-0.761)
AIP-III^f	I-N-(C-D-F-L-L)	5.05 (2.46-10.4)	5.63 (1.89-16.7)	- ^e	8.53 (4.15-17.5)
AIP-IV^f	Y-S-T-(C-Y-F-I-M)	- ^{c, d}	0.373 (0.218-0.638)	0.460 (0.213-0.994)	- ^e
tAIP-I D2A^f	Ac-(C-A-F-I-M)	3.06 (2.16-4.35)	10.1 (5.82-17.7)	0.260 (0.193-0.351)	0.353 (0.235-0.530)
Cyclo(Tyr-Pro)^f	(Y-P)	- ^g	- ^g	- ^g	- ^g
Cyclo(Phe-Pro)^f	(F-P)	- ^g	- ^g	- ^g	- ^g

^a See Experimental Section in main text for details of reporter strains and methods. See above for plots of antagonism dose response curves. All assays performed in triplicate. Shading in table included to enhance readability only. ^b IC₅₀ values determined by testing AIPs over a range of concentrations (200 fM – 100 μM). 95% confidence ranges are shown. ^c Dose response curve did not reach 100% inhibition over the concentrations tested. ^d Inhibition dose response curve upturned at higher concentrations, potentially indicative of partial agonism (see main text). ^e Dose response curve revealed agonism and no antagonism. ^f Control compound. ^g No activity at any concentration tested up to at least 100 μM.

Table S-4. IC₅₀ values of the alanine and D-amino acid scan analogs of AIP-III for inhibition of hemolysis by group-I–IV *S. aureus* strains.^a

Peptide name	Sequence	group-I IC ₅₀ (nM) ^b	group-II IC ₅₀ (nM) ^b	group-III IC ₅₀ (nM) ^b	group-IV IC ₅₀ (nM) ^b
AIP-III D-I1	DI-N-(C-D-F-L-L)	18.4 (10.4-32.7)	2.67 (1.56-4.56)	>200 ^c	>200
AIP-III D-N2	I-DN-(C-D-F-L-L)	5.31 (2.61-10.8)	0.222 (0.185-0.267)	- ^{c, d}	23.1 (20.3-26.4)
AIP-III D-C3	I-N-(DC-D-F-L-L)	>200	122 (84.9-175)	>200	>200
AIP-III D-D4	I-N-(C-DD-F-L-L)	77.0 (28.3-209)	1.15 (1.11-1.19)	>200	>200 ^d
AIP-III D-F5	I-N-(C-D-DF-L-L)	>200	>200	>200	>200
AIP-III D-L6	I-N-(C-D-F-DL-L)	>200	>200	>200	>200
AIP-III D-L7	I-N-(C-D-F-L-DL)	29.3 (15.0-57.2)	1.96 (0.786-4.91)	- ^e	47.8 (19.8-115)
AIP-III I1A	A-N-(C-D-F-L-L)	4.61 (2.68-7.92)	1.29 (0.691-2.41)	- ^f	12.5 (5.77-27.2)
AIP-III N2A	I-A-(C-D-F-L-L)	1.02 (0.591-1.77)	0.137 (0.102-0.183)	- ^{c, d}	2.64 (1.05-6.64)
AIP-III D4A	I-N-(C-A-F-L-L)	0.0820 (0.0472-0.142)	0.0596 (0.0379-0.0935)	0.163 (0.0689-0.388)	0.106 (0.0657-0.173)
AIP-III F5A	I-N-(C-D-A-L-L)	>200	44.7 (26.8-74.7)	>200	>200
AIP-III L6A	I-N-(C-D-F-A-L)	>200	>200	>200	>200
AIP-III L7A	I-N-(C-D-F-L-A)	>200	>200	>200	>200
AIP-I^g	Y-S-T-(C-D-F-I-M)	--	3.34 (0.676-16.5)	6.12 (5.20-7.21)	189 (84.0-424)
AIP-II^g	G-V-N-A-(C-S-S-L-F)	0.890 (0.369-2.15)	--	3.59 (1.22-10.6)	1.19 (0.549-2.60)
AIP-III^g	I-N-(C-D-F-L-L)	8.07 (4.34-15.0)	0.456 (0.271-0.769)	--	23.8 (13.7-41.3)
AIP-IV^g	Y-S-T-(C-Y-F-I-M)	- ^{c, d}	0.0897 (0.0782-0.103)	1.49 (0.704-3.14)	--
tAIP-I D2A^g	Ac-(C-A-F-I-M)	1.45 (0.600-3.51)	2.50 (1.85-3.38)	0.853 (0.373-1.95)	0.361 (0.214-0.607)

^a See Experimental Section in main text for details of strains and methods. See above for plots of antagonism dose response curves. All assays performed in triplicate. Shading in table included to enhance readability only. -- Not tested. ^b IC₅₀ values determined by testing AIPs over a range of concentrations (200 fM – 10 μM). 95% confidence ranges are shown. ^c Dose response curve did not reach 100% inhibition over the concentrations tested. ^d Inhibition dose response curve upturned at higher concentrations. ^e Dose response curve revealed agonism and no antagonism. ^f No activity at any concentration tested up to at least 10 μM. ^g Control compound.

Table S-5. IC₅₀ values of the second-generation AIP-III analogs against AgrC I-IV determined using *S. aureus* fluorescence reporter strains.^a

Peptide name	Sequence	AgrC-I IC ₅₀ (nM) ^b	AgrC-II IC ₅₀ (nM) ^b	AgrC-III IC ₅₀ (nM) ^b	AgrC-IV IC ₅₀ (nM) ^b
AIP-III I1A/N2A	A-A-(C-D-F-L-L)	7.40 (3.52-15.6)	4.38 (3.34-5.75)	2.60 (1.17-5.80)	5.41 (2.24-13.0)
AIP-III I1A/D4A	A-N-(C-A-F-L-L)	0.328 (0.296-0.364)	2.35 (1.35-4.07)	0.280 (0.0968-0.811)	0.101 (0.0333-0.309)
AIP-III N2A/D4A	I-A-(C-A-F-L-L)	0.331 (0.285-0.384)	0.204 (0.104-0.401)	0.0657 (0.0280-0.154)	0.0221 (0.00707-0.0693)
AIP-III I1A/N2A/D4A	A-A-(C-A-F-L-L)	0.304 (0.184-0.500)	0.604 (0.193-1.89)	0.0734 (0.0239-0.226)	0.0161 (0.00840-0.0307)
tAIP-III	Ac-(C-D-F-L-L)	26.7 (11.5-61.7)	1.53 (0.673-3.49)	>200	25.5 (7.31-88.7)
tAIP-III D2A	Ac-(C-A-F-L-L)	0.257 (0.102-0.646)	0.900 (0.842-0.963)	0.329 (0.215-0.502)	0.0957 (0.0375-0.245)
tAIP-III D2A/F3Y	Ac-(C-A-Y-L-L)	0.279 (0.0950-0.819)	1.15 (0.750-1.77)	0.387 (0.345-0.435)	0.0306 (0.0205-0.0456)
tAIP-III D2A/F3W	Ac-(C-A-W-L-L)	0.909 (0.431-1.92)	1.90 (0.746-4.84)	0.509 (0.224-1.16)	0.0363 (0.0112-0.117)
Ac-AIP-III	Ac-I-N-(C-D-F-L-L)	>200 (21.7-90.4)	44.3	>200	>200
G-AIP-III	G-I-N-(C-D-F-L-L)	29.9 (11.2-79.9)	13.7 (7.86-23.8)	>200 ^c	104 (36.4-296)
A-AIP-III	A-I-N-(C-D-F-L-L)	26.1 (10.3-66.0)	6.40 (2.46-16.7)	27.5 ^d (8.97-84.4)	28.5 ^d (9.42-86.4)
Y-AIP-III	Y-I-N-(C-D-F-L-L)	8.92 (4.56-17.4)	3.75 (1.34-10.5)	39.2 (20.1-76.4)	78.2 ^c (47.8-128)
AIP-III D4A^e	I-N-(C-A-F-L-L)	0.485 (0.289-0.813)	0.429 (0.208-0.886)	0.0506 (0.0227-0.113)	0.0349 (0.0123-0.0990)
tAIP-I D2A^e	Ac-(C-A-F-I-M)	3.06 (2.16-4.35)	10.1 (5.82-17.7)	0.260 (0.193-0.351)	0.353 (0.235-0.530)

^a See Experimental Section in main text for details of reporter strains and methods. See above for plots of antagonism dose response curves. All assays performed in triplicate. Shading in table included to enhance readability only. ^b IC₅₀ values determined by testing AIPs over a range of concentrations (200 fM – 10 μM). 95% confidence ranges are shown. ^c Dose response curve did not reach 100% inhibition over the concentrations tested. ^d Inhibition dose response curve upturned at higher concentrations, potentially indicative of partial agonism (see main text). ^e Data included for comparison.

Table S-6. IC₅₀ values of selected second-generation AIP-III analogs for inhibition of hemolysis by group-I-IV *S. aureus* strains.^a

Peptide name	Sequence	group-I IC ₅₀ (nM) ^b	group-II IC ₅₀ (nM) ^b	group-III IC ₅₀ (nM) ^b	group-IV IC ₅₀ (nM) ^b
AIP-III I1A/D4A	A-N-(C-A-F-L-L)	0.0103 (0.00504-0.0212)	0.793 (0.487-1.29)	0.551 (0.194-1.56)	0.284 (0.110-0.730)
AIP-III N2A/D4A	I-A-(C-A-F-L-L)	0.0362 (0.0171-0.0767)	0.0661 (0.0293-0.149)	0.216 (0.0767-0.609)	0.122 (0.0449-0.333)
AIP-III I1A/N2A/D4A	A-A-(C-A-F-L-L)	0.0411 (0.0160-0.106)	0.0606 (0.0284-0.129)	0.243 (0.143-0.415)	0.140 (0.0801-0.244)
tAIP-III D2A	Ac-(C-A-F-L-L)	0.332 (0.147-0.750)	0.711 (0.444-1.14)	0.197 (0.0925-0.419)	0.306 (0.213-0.440)
tAIP-III D2A/F3Y	Ac-(C-A-Y-L-L)	0.279 (0.123-0.633)	0.204 (0.0959-0.432)	0.265 (0.154-0.456)	0.134 (0.0763-0.235)
tAIP-III D2A/F3W	Ac-(C-A-W-L-L)	0.468 (0.192-1.14)	0.126 (0.0863-0.183)	1.08 (0.601-1.93)	0.194 (0.113-0.331)

^a See Experimental Section in main text for details of strains and methods. See above for plots of antagonism dose response curves. All assays performed in triplicate. Shading in table included to enhance readability only. ^b IC₅₀ values determined by testing AIPs over a range of concentrations (200 fM – 10 μM). 95% confidence ranges are shown.

IC₅₀ value comparisons between fluorescence and hemolysis assays (groups I–IV).

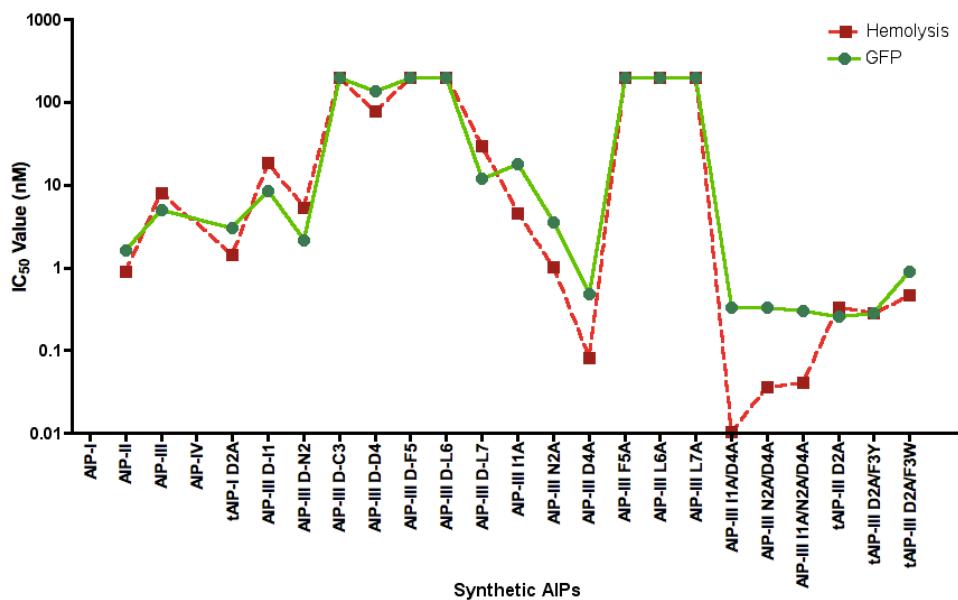


Figure S-3. IC₅₀ values for each AIP as determined by fluorescence (green, solid line) and hemolysis (red, dashed line) assays for AgrC-I/group-I *S. aureus*.

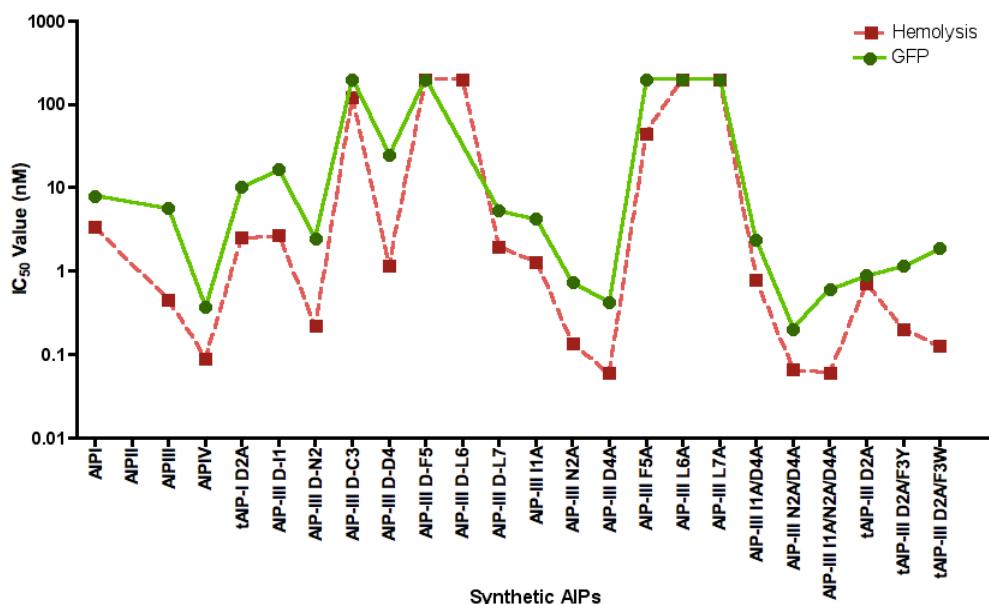


Figure S-4. IC₅₀ values for each AIP as determined by fluorescence (green, solid line) and hemolysis (red, dashed line) assays for AgrC-II/group-II *S. aureus*.

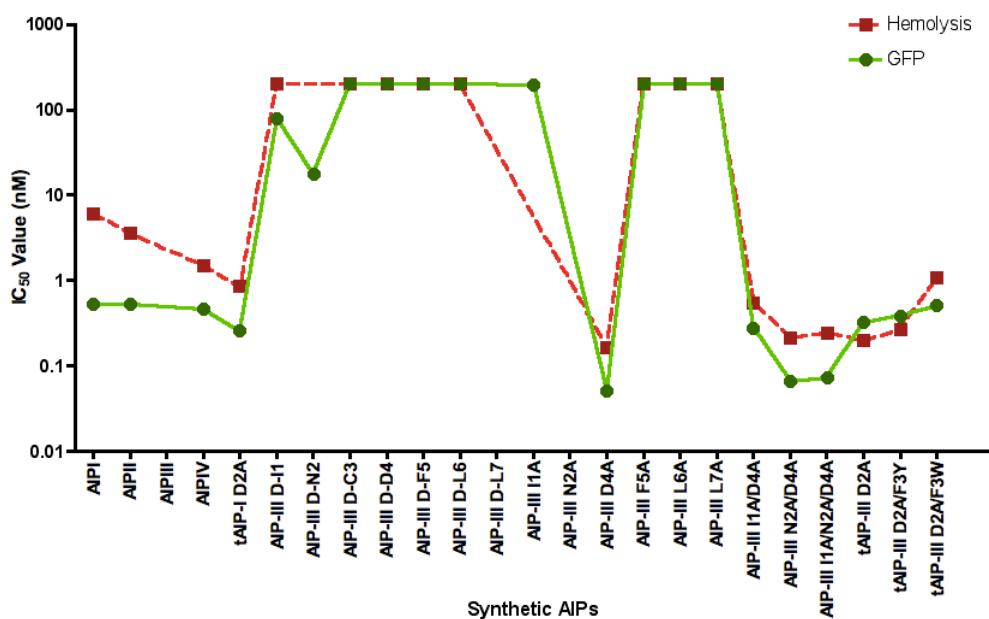


Figure S-5. IC₅₀ values for each AIP as determined by fluorescence (green, solid line) and hemolysis (red, dashed line) assays for AgrC-III/group-III *S. aureus*.

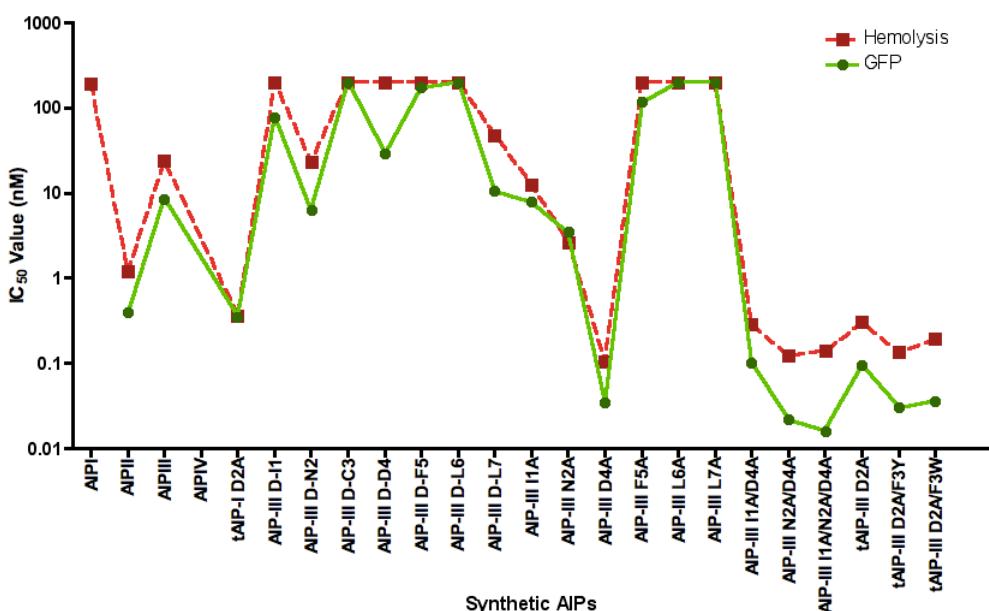


Figure S-6. IC₅₀ values for each AIP as determined by fluorescence (green, solid line) and hemolysis (red, dashed line) assays for AgrC-IV/group-IV *S. aureus*.