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SUPPORTING INFORMATION

Characterization of Structural Elements in Native Autoinducing Peptides and Non-Native Analogues that Permit the Differential Modulation of AgrC-type Quorum Sensing Receptors in *Staphylococcus aureus*

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Contents.

- MS and HPLC data for the peptides in this study
- Biological assay data
- Tables of NMR chemical shift assignments, numbers of assigned ROEs, and RMSD values
- Additional images of calculated peptide structures

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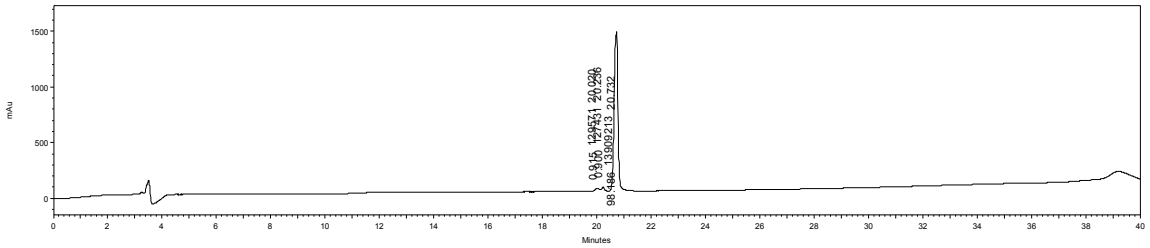
MS and HPLC data for the peptides in this study.

Table S-1. MS and HPLC data for AIPs and AIP analogs. EM = Exact Mass. See Experimental Section for methods.

Compound Name	Structure	Calc. EM	Obs. EM	% Purity
AIP-I D5N	Y-S-T-(C-N-F-I-M)	960.3954	960.3950	>98
AIP-I D5A	Y-S-T-(C-A-F-I-M)	917.3896	917.3886	>99
tAIP-I	Ac-(C-D-F-I-M)	652.2470	652.2462	>99
tAIP-II	Ac-(C-S-S-L-F)	580.2436	580.2434	>99
S. epi. AIP-I	D-S-V-(C-A-S-Y-F)	873.3448	873.3435	>99
S. lug. AIP-I	D-I-(C-N-A-Y-F)	827.3393	827.3402	>99
AIP-III D4N	I-N-(C-N-F-L-L)	818.4230	818.4253	>99

HPLC traces.

AIP-I D5N



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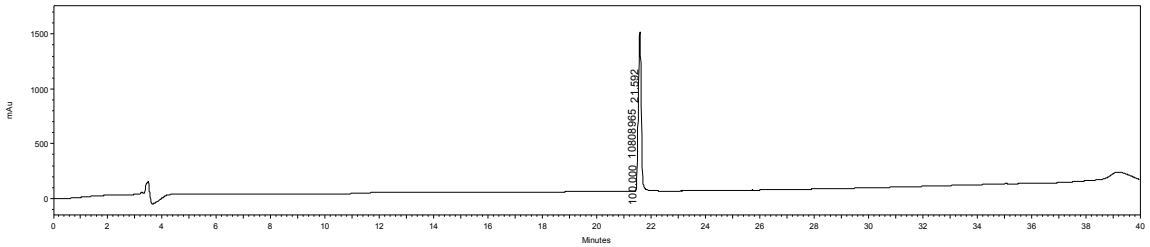
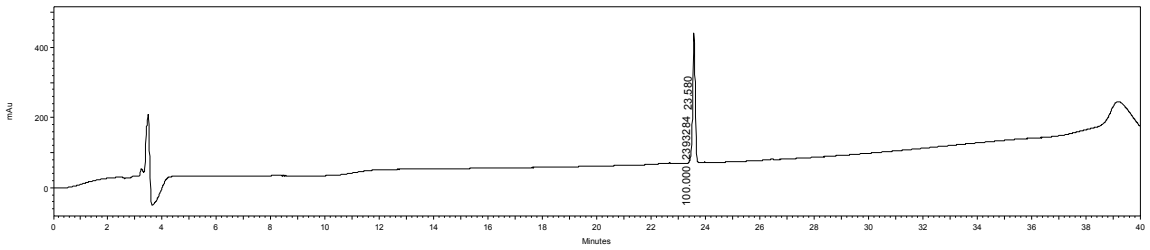


tAIP-I



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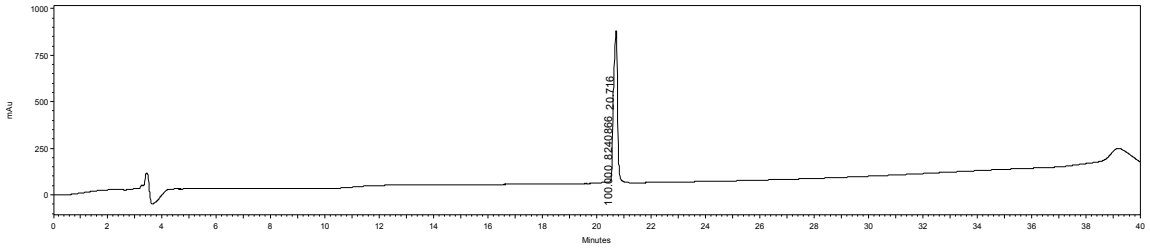
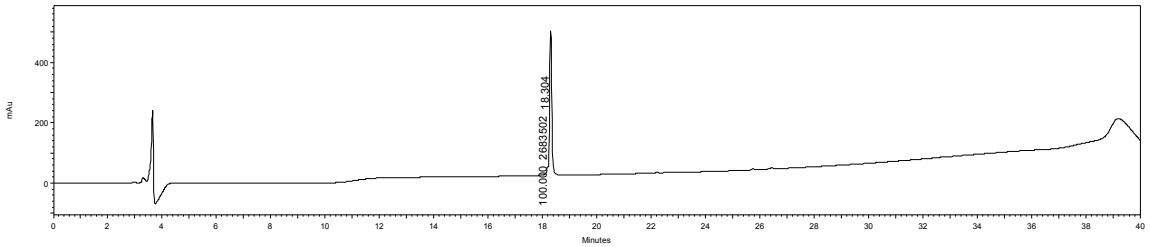


S. epi. AIP-I



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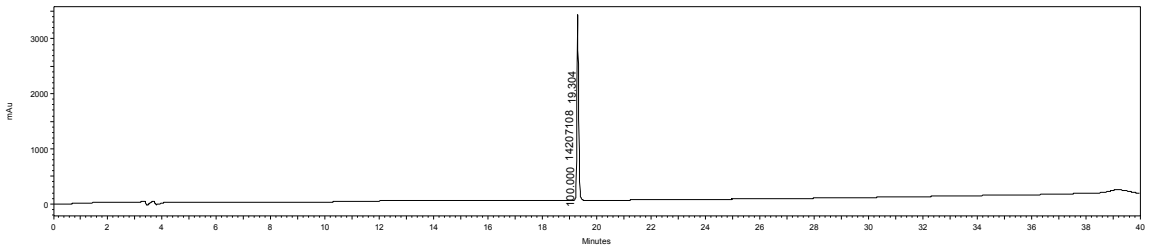
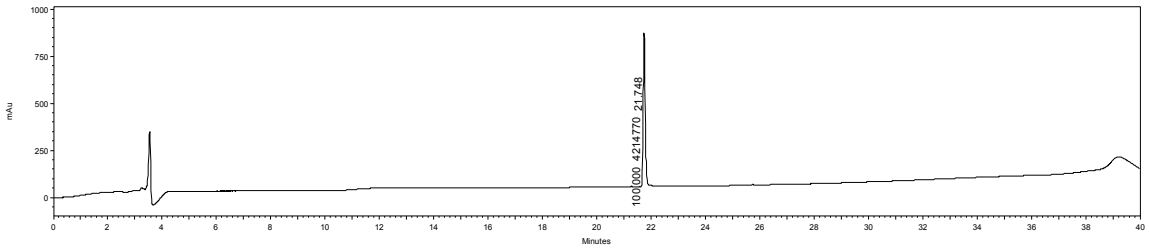


AIP-III D4N

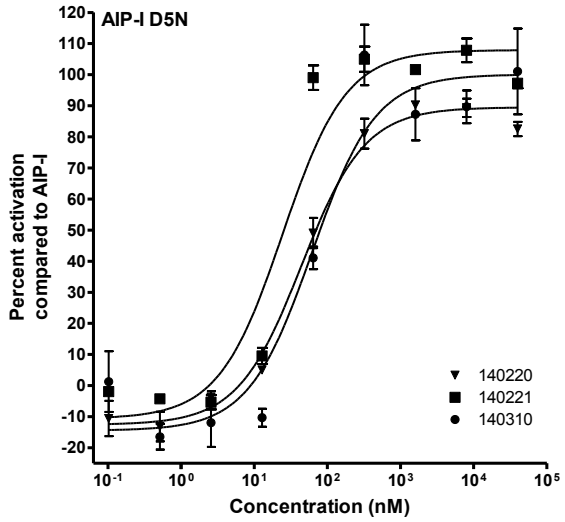


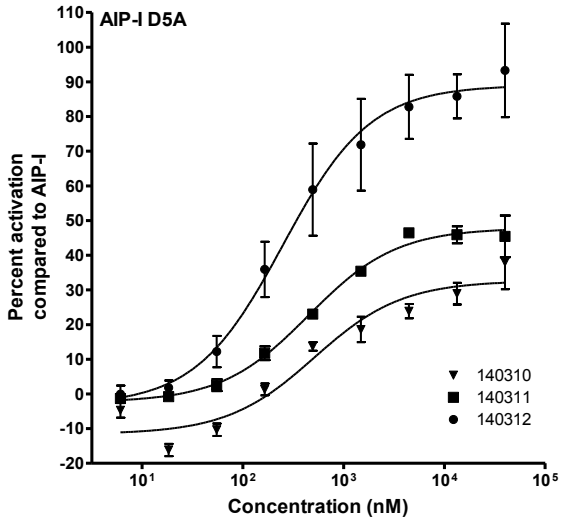
Biological assay data.

β-lactamase agonism dose response curves.

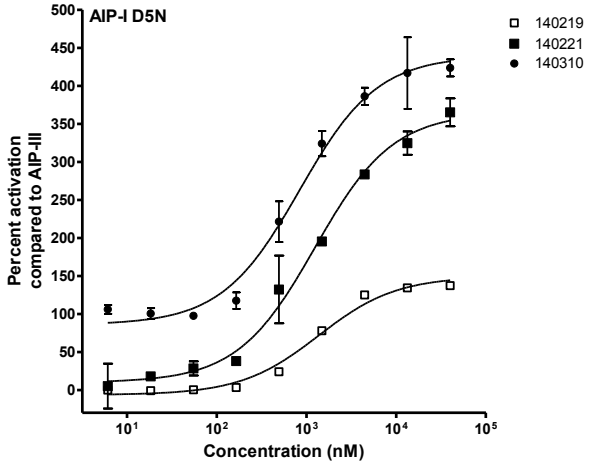
Peptides were screened over varying concentrations in the indicated *S. aureus* reporter strains for AgrC receptor agonism. Each dose response experiment was performed in triplicate on three separate occasions. Each plot below is labeled with the appropriate peptide name in bold at top left. Error bars indicate standard error of the mean of triplicate values. See Experimental Section for details of methods and strains. None of the peptides in this study could activate the *S. aureus* group-II or –IV reporter strains.

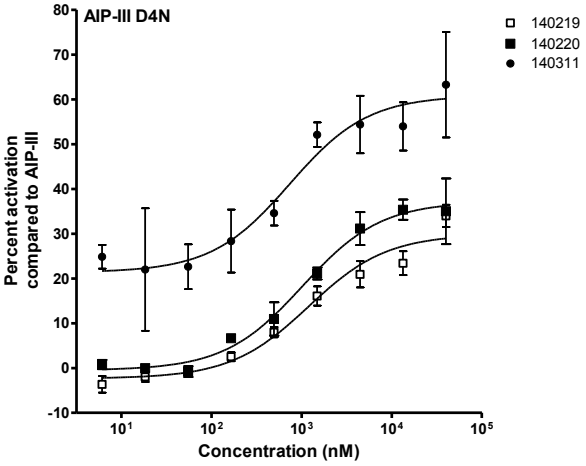
S. aureus RN9222 (group-I, AgrC-I reporter)





S. aureus RN9532 (group-III, AgrC-III reporter)

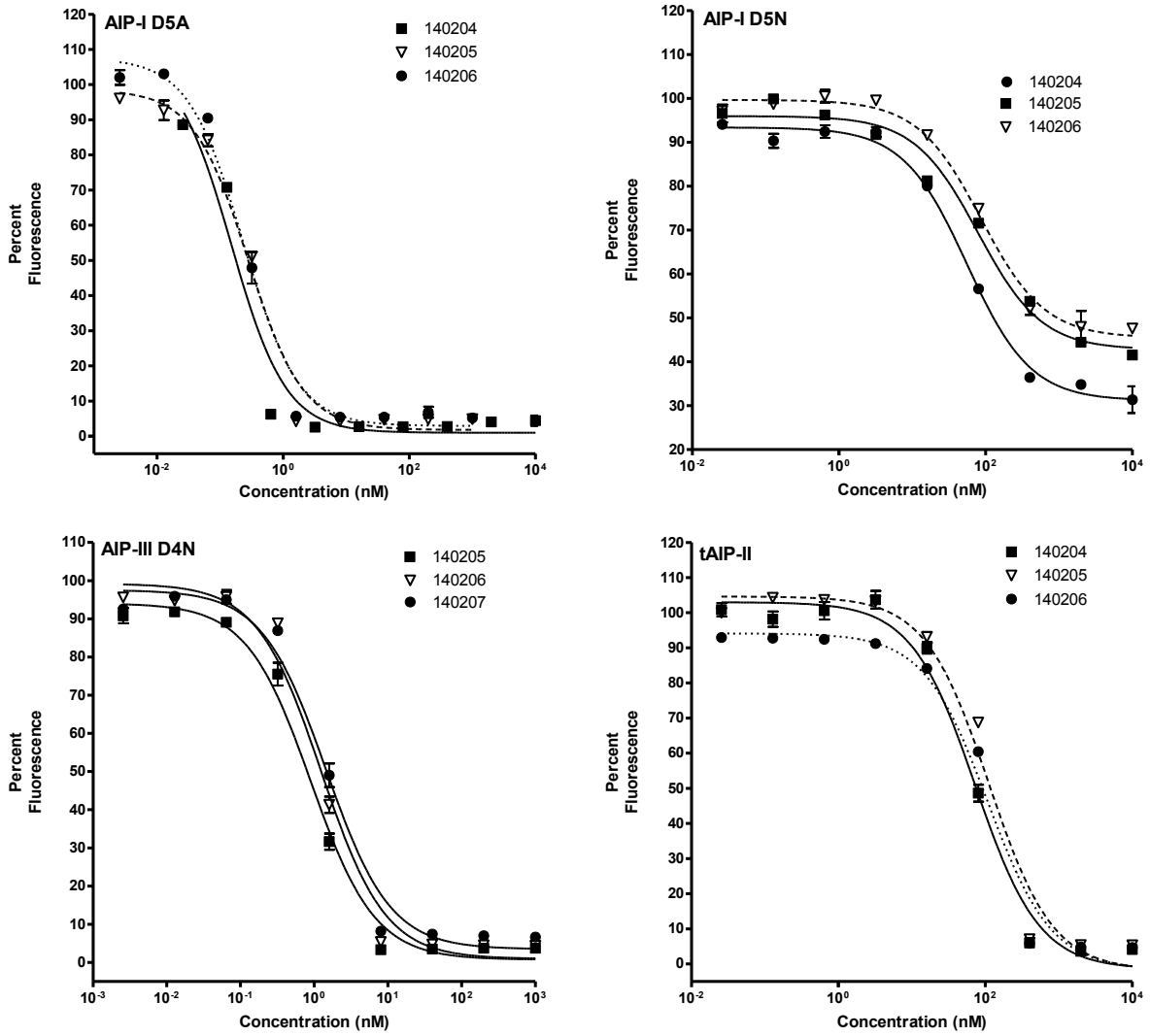


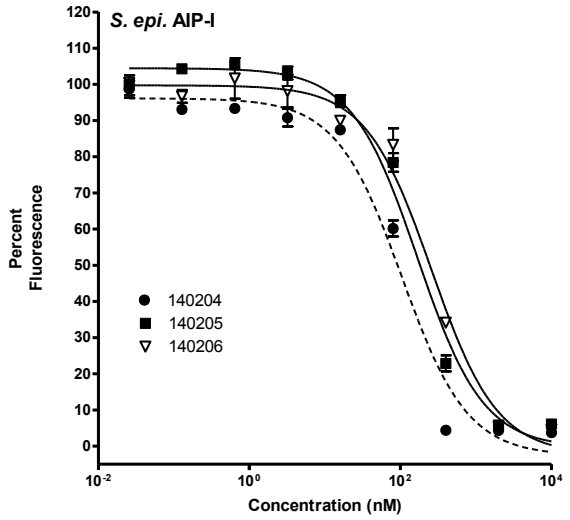


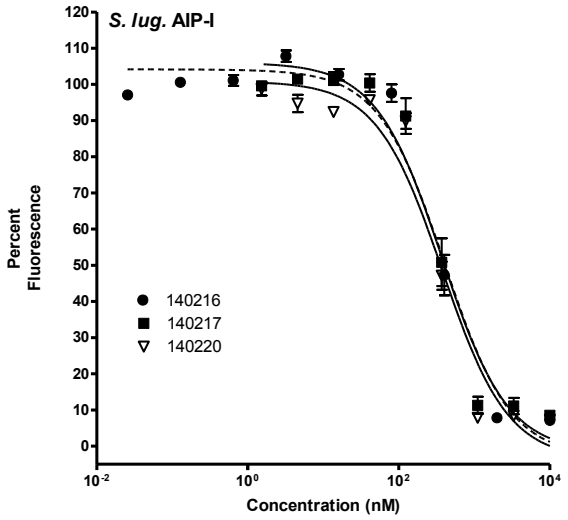
Fluorescence antagonism dose response curves.

Peptides were screened over varying concentrations in the four indicated *S. aureus* reporter strains for AgrC receptor antagonism. Each dose response experiment was performed in triplicate on three separate occasions. Each plot below is labeled with the appropriate peptide name in bold at top left. Error bars indicate standard error of the mean of triplicate values. See Experimental Section for details of methods and strains.

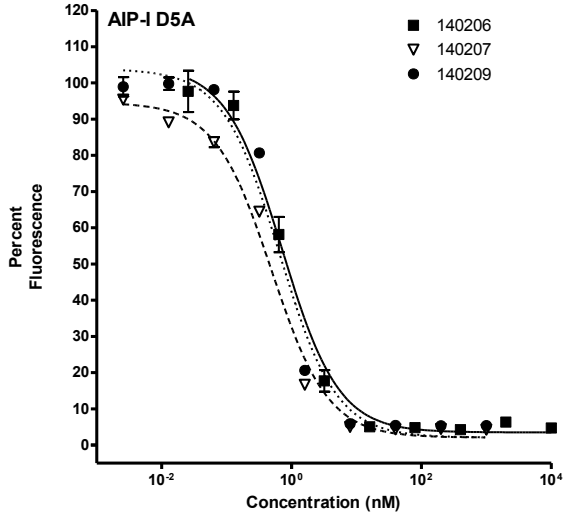
S. aureus AH1677 (group-I, AgrC-I reporter)

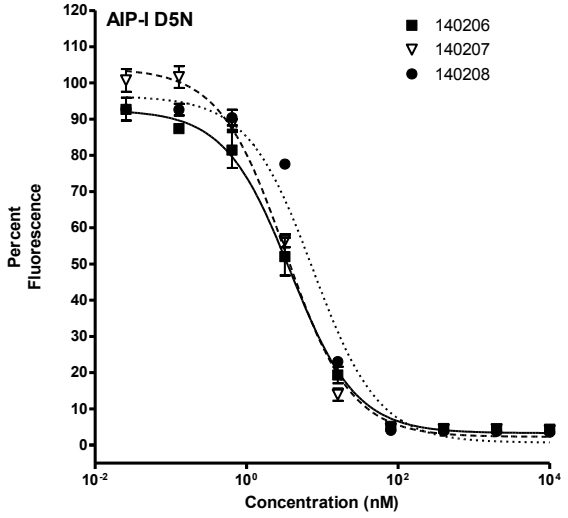


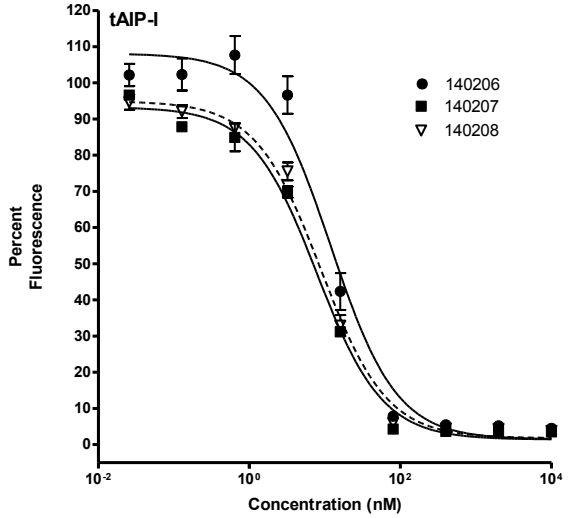


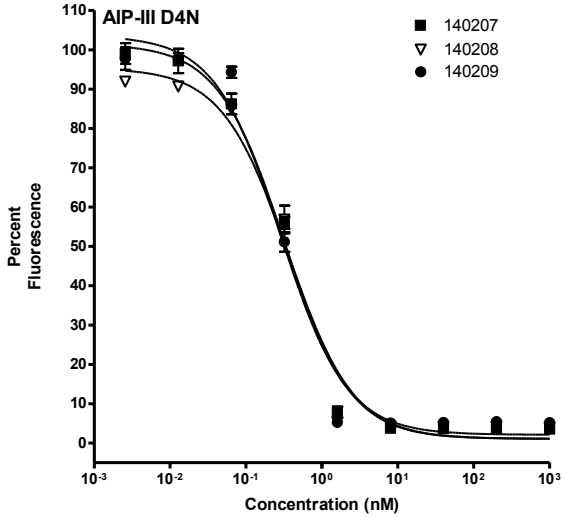


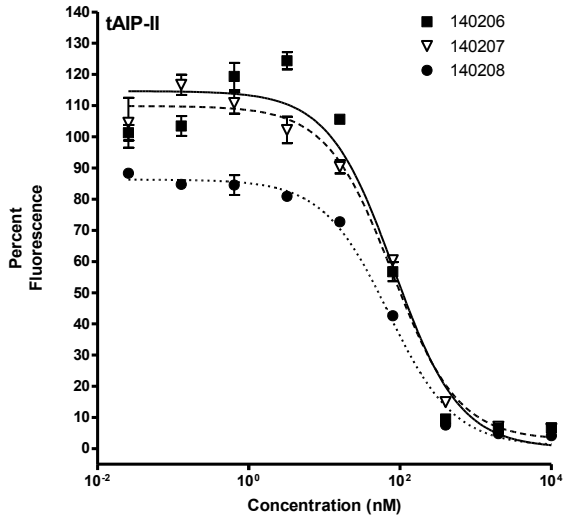
S. aureus AH430 (group-II, AgrC-II reporter)

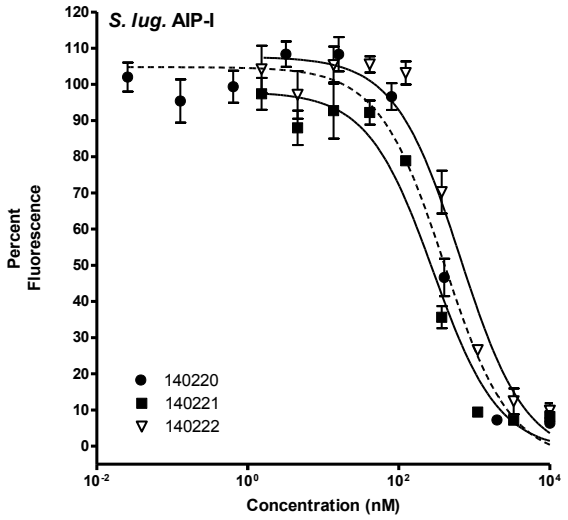




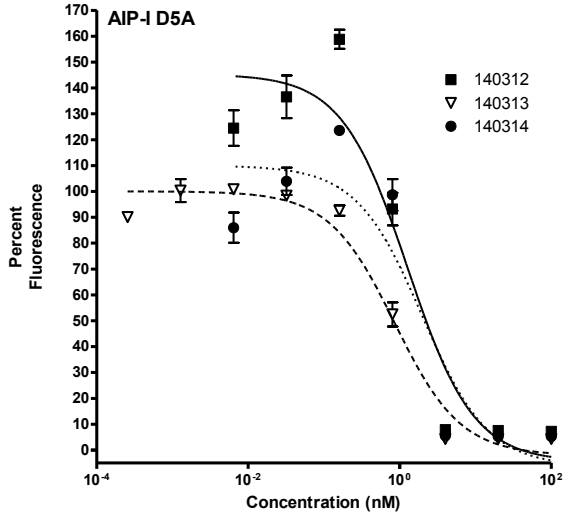


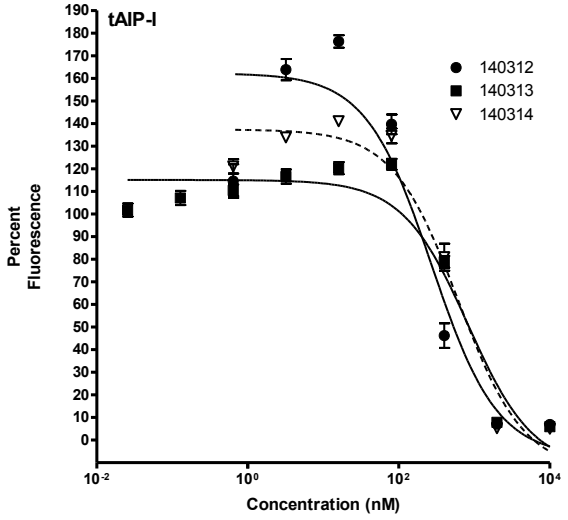


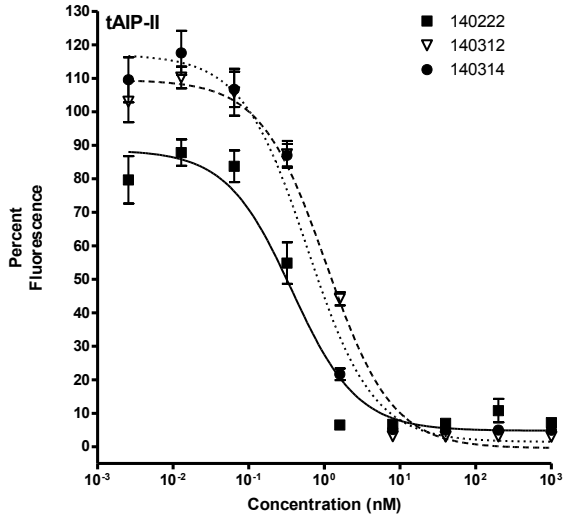


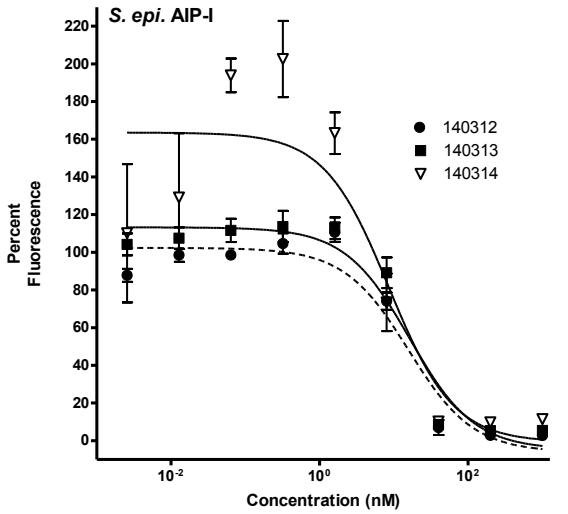


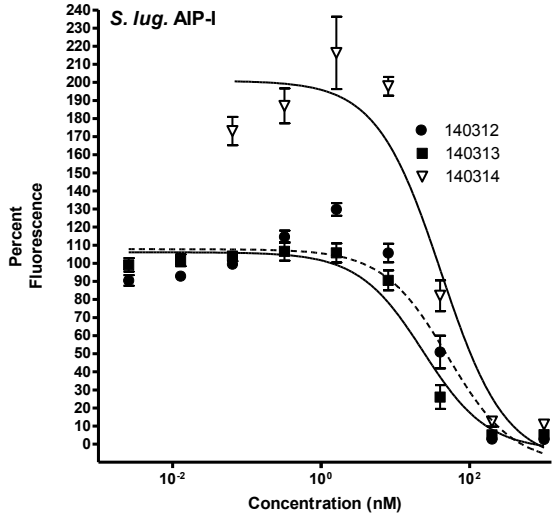
S. aureus AH1747 (group-III, AgrC-III reporter)



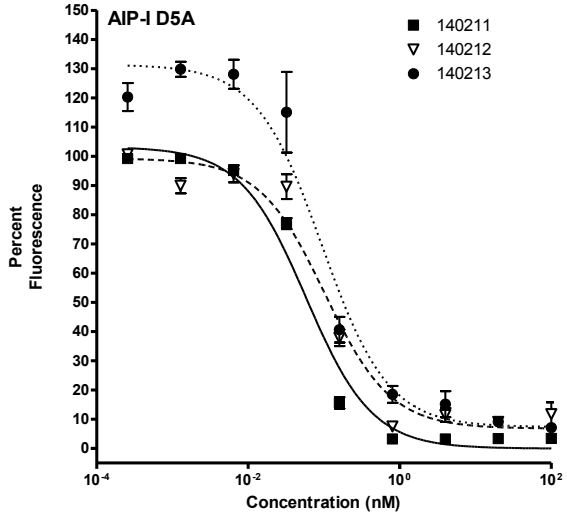


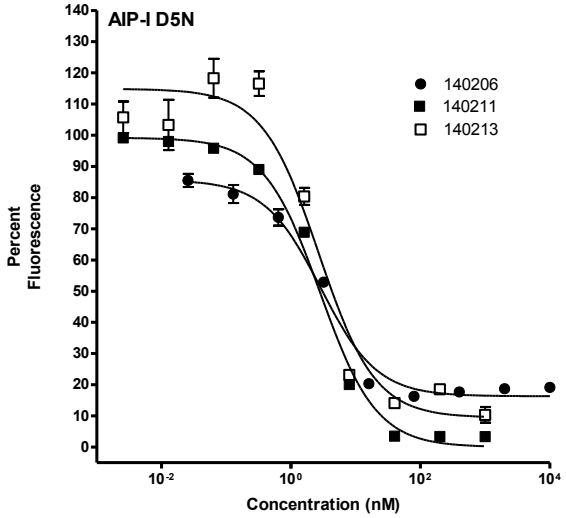


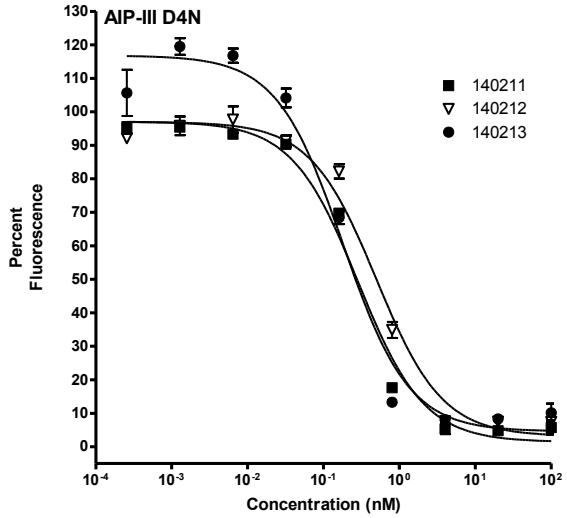




S. aureus AH1872 (group-IV, AgrC-IV reporter)







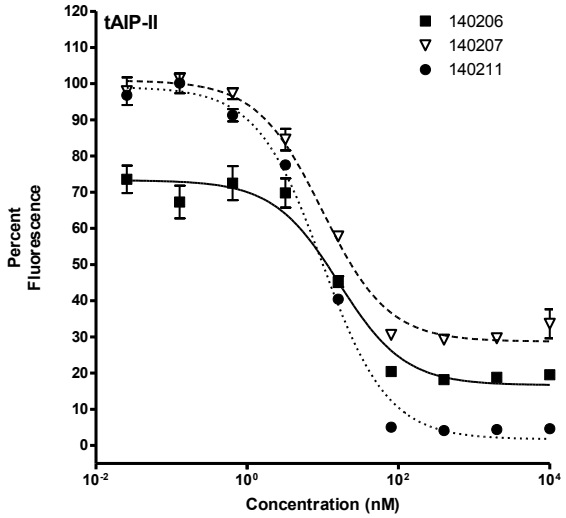


Table S-2. EC_{50} and IC_{50} values, and 95% confidence ranges of the peptides evaluated in this study in AgrC I–IV determined using *S. aureus* fluorescence and β-lactamase reporter strains.[a] EC_{50} values for native *S. aureus* AIPs I–III provided for comparison.

Peptide name	Activation (EC_{50} nM)[b]				Inhibition (IC_{50} nM)[b]			
	AgrC-I	AgrC-II	AgrC-III	AgrC-IV	AgrC-I	AgrC-II	AgrC-III	AgrC-IV
AIP-I D5N	40.4 (16.1-102)	-	1120[c] (675-1870)	-	72.4[d] (45.8-115)	4.53 (1.90-10.8)	-	2.80 (2.60-3.01)
AIP-I D5A	386[e] (181-822)	-	-	-	0.214 (0.115-0.398)	0.613 (0.418-0.898)	1.30 (0.573-2.94)	0.0827 (0.0451-0.152)
tAIP-I D2A	-	-	-	-	3.06 (2.16-4.35)	10.1 (5.82-17.7)	0.260 (0.193-0.351)	0.353 (0.235-0.530)
tAIP-I	-	-	-	-	>1000	9.42 (6.12-14.5)	516 (167-1590)	>1000
tAIP-II	-	-	-	-	95.9 (60.5-152)	77.5 (65.5-91.7)	0.616 (0.220-1.73)	11.7 (6.65-20.5)
***S. epidermidis* AIP-I**	-	-	-	-	166 (68.7-402)	>1000	13.0 (6.41-26.5)	>1000
***S. lugdunensis* AIP-I**	-	-	-	-	384 (353-418)	419 (187-941)	36.6 (16.8-79.5)	>1000
AIP-III D4N	-	-	967[e] (594-1570)	-	1.17 (0.729-1.89)	0.319 (0.257-0.398)	-	0.294 (0.108-0.799)
AIP-I	3.21[f] (1.21-8.57)	-	-	-				
AIP-II	-	40.9[f] (30.3-55.3)	-	-				
AIP-III	-	-	406[f] (281-586)	-				

[a] See Experimental Section for details of reporter strains and methods. See above for plots of agonism and antagonism dose response curves. All assays performed in triplicate. [b] EC_{50} and IC_{50} values determined by testing AIPs over a range of concentrations (200 fM – 40 μM). 95% confidence ranges indicated in parentheses below each value. [c] Agonism dose response exceeded 100% activation compared to the native ligand. [d] Antagonism dose response did not reach 100% inhibition. [e] Agonism dose response did not reach 100% activation compared to the native ligand. [f] Data reproduced from Ref. 11 in main text.

Tables of NMR chemical shift assignments, numbers of assigned ROEs, and RMSD values.

Table S-3. Chemical shift assignments for AIP-I D5N.

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Tyr1		4.12	3.06, 2.97	Hδ 7.03; Hε 6.73
Ser2	8.29	4.44	3.71	
Thr3	7.93	4.18	4.10	Hγ 1.07
Cys4	8.17	4.36	3.22, 2.89	
Asn5	8.29	4.46	2.49, 2.45	Hδ 7.26, 6.63
Phe6	8.03	4.42	3.12	Hδ 7.20; Hε 7.28
Ile7	7.69	3.91	1.88	Hγ 1.09, 0.82; Hδ 0.69
Met8	8.53	4.46	2.21, 1.97	Hγ 2.53, 2.42

Table S-4. Chemical shift assignments for AIP-I D5A.

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Tyr1		4.12	3.06, 2.97	Hδ 7.03; Hε 6.73
Ser2	8.28	4.44	3.71	
Thr3	7.94	4.19	4.13	Hγ 1.08
Cys4	8.12	4.35	3.22, 2.93	
Ala5	8.19	4.05	1.13	
Phe6	7.89	4.46	3.08	Hδ 7.19; Hε 7.28
Ile7	7.67	3.88	1.93	Hγ 1.10, 0.85; Hδ 0.72
Met8	8.58	4.50	2.21, 1.96	Hγ 2.54, 2.42

Table S-5. Chemical shift assignments for tAIP-I.

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Ac0		1.87		
Cys1	8.10	4.35	3.22, 2.89	
Asp2	8.26	4.46	2.57	
Phe3	7.97	4.44	3.11	Hδ 7.20; Hε 7.29
Ile4	7.69	3.90	1.89	Hγ 1.10, 0.84; Hδ 0.70
Met5	8.54	4.49	2.23, 1.99	Hγ 2.55, 2.43

Table S-6. Chemical shift assignments for tAIP-I D2A.

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Ac0		1.87		
Cys1	8.08	4.35	3.21, 2.92	
Ala2	8.16	4.07	1.14	
Phe3	7.87	4.48	3.08	Hδ 7.20; Hε 7.28
Ile4	7.70	3.85	1.94	Hγ 1.09, 0.83, 0.72; Hδ 0.69
Met5	8.58	4.52	2.22, 1.97	Hγ 2.55, 2.43

Table S-7. Chemical shift assignments for tAIP-II.

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Ac0		1.90		
Cys1	8.20	4.42	3.28, 2.90	
Ser2	8.25	4.33	3.78, 3.73	
Ser3	8.00	4.20	3.84	
Leu4	7.81	3.99	1.17, 1.01	Hγ 1.24; Hδ 0.71, 0.65
Phe5	8.21	4.79	3.39, 2.75	Hδ 7.17; Hε 7.25

Table S-8. Chemical shift assignments for *S. epidermidis* AIP-I.

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Asp1		4.22	2.83	
Ser2	8.44	4.42	3.74	
Val3	7.85	4.02	1.97	Hγ 0.81
Cys4	8.21	4.38	3.25, 2.89	
Ala5	8.21	4.19	1.27	
Ser6	8.03	4.01	3.74, 3.63	
Tyr7	7.87	4.16	2.63, 2.39	Hδ 6.80
Phe8	8.28	4.75	3.35, 2.80	Hδ 7.15

Table S-9. Chemical shift assignments for *S. lugdunensis* AIP-I.

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Asp1		4.22	2.89, 2.77	
Ile2	8.27	4.06	1.72	Hγ 1.36, 1.06, 0.77
Cys3	8.23	4.42	3.26, 2.85	
Asn4	8.24	4.55	2.58	Hδ 7.35
Ala5	8.21	3.89	1.24	
Tyr6	7.46	4.18	2.68, 2.52	Hδ 6.83
Phe7	8.20	4.74	3.39, 2.84	Hδ 7.19

Table S-10. Chemical shift assignments for AIP-III D4N.

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Ile1		3.74	1.84	Hγ 1.39, 1.11, 0.89; Hδ 0.83
Asn2	8.47	4.61	2.66, 2.57	Hδ 7.37, 6.70
Cys3	8.17	4.36	3.20, 2.78	
Asn4	8.21	4.50	2.50, 2.40	Hδ 7.25, 6.64
Phe5	8.19	4.31	3.12	Hδ 7.18; Hε 7.28; Hζ 7.21
Leu6	7.86	4.12	1.65, 1.50	Hγ 1.14; Hδ 0.76, 0.68
Leu7	8.14	4.49	1.71, 1.66	Hγ 1.56; Hδ 0.88, 0.77

Table S-11. Number of assigned ROEs and RMSD values for each peptide.

Peptide name	**Total ROEs**	**Intra-residue ROEs**	**Inter-residue ROEs**			**Backbone RMSD (Å)**[a]	**Heavy atom RMSD (Å)**[a]
			i - i+1	*i - i+2*	other		
AIP-I[b]						0.13	0.58
AIP-I D5N	95	58	31	3	3	0.54	0.98
AIP-I D5A	86	50	31	5	0	0.78	1.79
tAIP-I	56	40	16	0	0	0.24	0.85
tAIP-I D2A	61	42	19	0	0	0.20	0.64
tAIP-II	70	41	22	3	4	0.05	0.42
S. epidermidis AIP-I	54	37	16	0	1	0.87	1.35
S. lugdunensis AIP-I	60	41	15	4	0	0.42	1.14
AIP-III D4N	115	76	37	1	1	0.61	1.16

[a] RMSD values calculated for the 10-structure ensemble of each peptide. [b] Control compound. Data reproduced from Ref. 10 in the main text.

Additional images of calculated peptide structures.

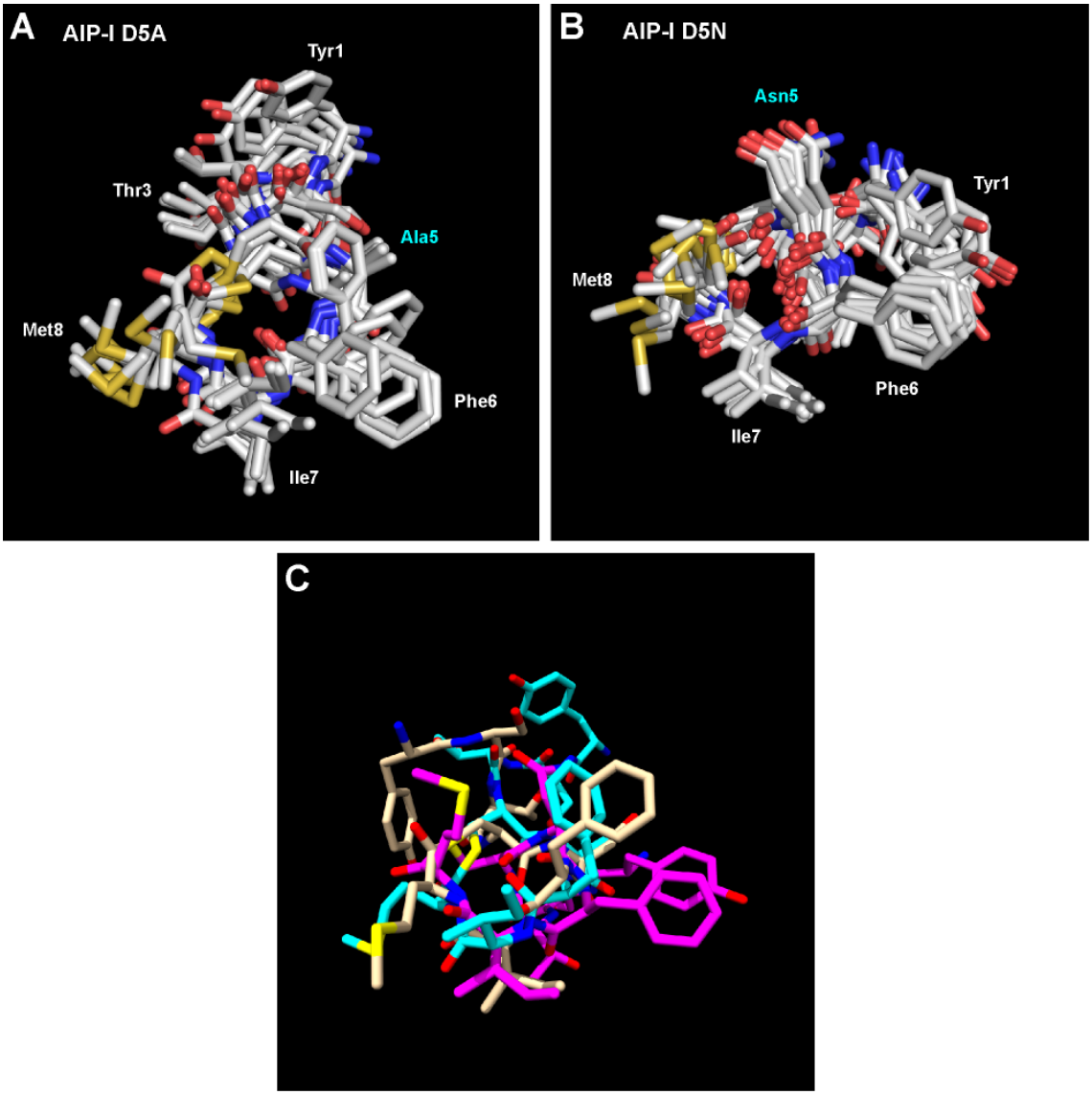


Figure S-1. Images of the heavy atom 10-structure ensembles for A) AIP-I D5A and B) AIP-I D5N. For all structures, carbon is silver, oxygen is red, nitrogen is blue, and sulfur is yellow. Residue labeled in cyan is the altered residue in each analog. C) Overlay of the heavy atom lowest energy structures for AIP-I (tan), AIP-I D5A (cyan) and AIP-I D5N (magenta).

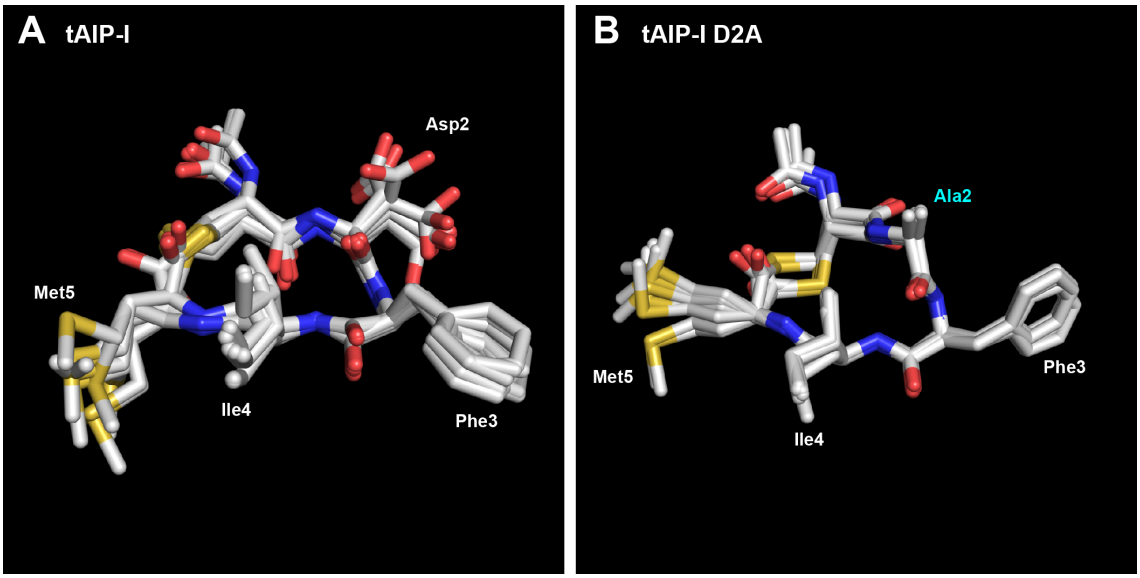


Figure S-2. Images of the heavy atom 10-structure ensembles for A) tAIP-I and B) tAIP-I D2A. For all structures, carbon is silver, oxygen is red, nitrogen is blue, and sulfur is yellow. Residue labeled in cyan is the altered residue.

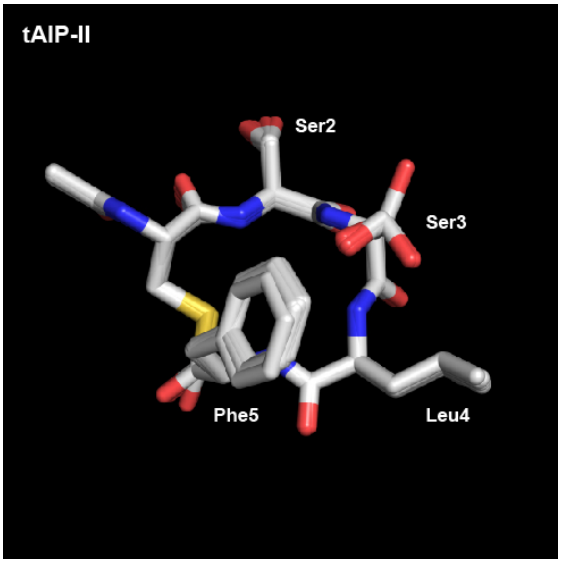


Figure S-3. Image of the heavy atom 10-structure ensemble of tAIP-II. Carbon is silver, oxygen is red, nitrogen is blue, and sulfur is yellow.

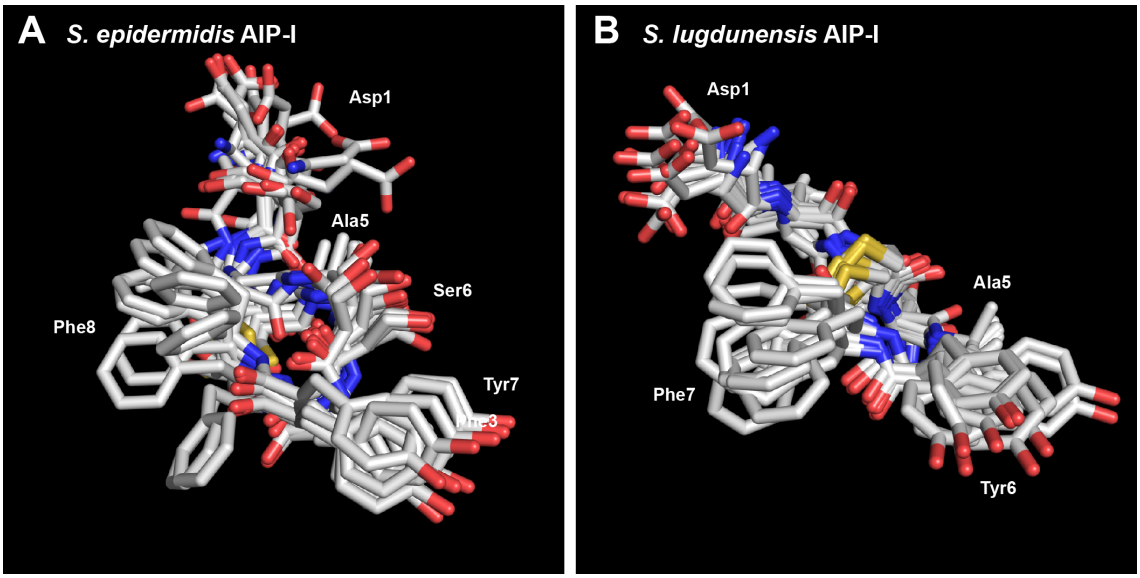


Figure S-4. Images of the heavy atom 10-structure ensembles for A) *S. epidermidis* AIP-I and B) *S. lugdunensis* AIP-I. For all structures, carbon is silver, oxygen is red, nitrogen is blue, and sulfur is yellow.

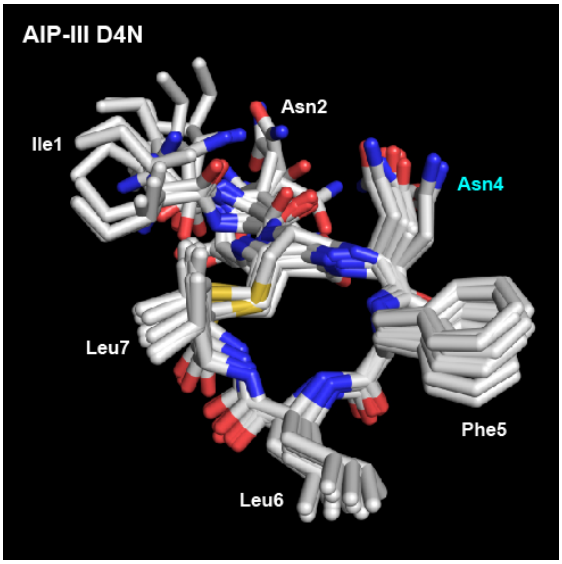


Figure S-5. Image of the heavy atom 10-structure ensemble of AIP-III D4N. Carbon is silver, oxygen is red, nitrogen is blue, and sulfur is yellow. Residue labeled in cyan is the altered residue.