An Entirely Solid Phase Peptide Synthesis-Based Strategy for Synthesis of Gelatinase Biosynthesis-Activating Pheromone (GBAP) Analogue Libraries: Investigating the Structure-Activity Relationships of the *Enterococcus faecalis* Quorum Sensing Signal

Dominic N. McBrayer, Brooke K. Gantman, Crissey D. Cameron, Yftah Tal-Gan*

Department of Chemistry, University of Nevada, Reno, 1664 North Virginia Street, Reno, NV 89557

Supporting Information.

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

Experimental Methods

General: Unless otherwise specified, all coupling reactions were manually conducted in 6 mL polypropylene reaction vessels containing porous frits at their base for ease of filtering and washing the resin between reaction steps. All reference to equivalents used are relative to the initial resin loading. All bioassays were conducted with *Enterococcus faecalis* reporter strain TX5274, expressing β-galactosidase as the reporter.

Chemical Reagents and Instrumentation: All chemical reagents and solvents were purchased from Sigma-Aldrich or Chem-Impex and used without further purification. Water (18 MΩ) was purified using a Millipore Analyzer Feed System. Solid-phase resin was purchased from Advanced ChemTech.

Reversed-phase high-performance liquid chromatography (RP-HPLC) was performed using a Shimadzu system equipped with a CBM-20A communications bus module, two LC-20AT pumps, an SIL-20A auto sampler, an SPD-20A UV/VIS detector, a CTO-20A column oven, and an FRC-10A fraction collector. All RP-HPLC solvents (18 MΩ water and HPLC-grade acetonitrile (ACN)) contained 0.1% trifluoroacetic acid (TFA). Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) data were obtained on a Bruker Microflex spectrometer equipped with a 60 Hz nitrogen laser and a reflectron. In positive ion mode, the acceleration voltage on Ion Source 1 was 19.01 kV. Exact mass (EM) data were obtained on an Agilent Technologies 6230 TOF LC/MS spectrometer. The samples were sprayed with a capillary voltage of 3500 V and the electrospray ionization (ESI) source parameters were as follows: gas temperature of 325 °C at a drying gas flow rate of 8 L/min at a pressure of 35 psi.

Initial Resin Deprotection and Loading Determination: Unless otherwise noted, peptide synthesis was conducted using Rink Amide MBHA (Knorr) resin with a loading capacity of 0.89 mmol/g. Peptide synthesis was conducted on either 50 mg (0.045 mmol) or 100 mg (0.09 mmol) resin scales. Resin loading was verified by measuring Fmoc removal during the initial deprotection of the resin. 2 mL of 20% piperidine in *N*,*N*-dimethylformamide (DMF) was added to the resin, which was subsequently shaken at 200 rpm for 8 min. The 2 mL solution was collected, and an aliquot (10-20 µL) was taken and diluted to 3 mL in DMF before the absorbance was read at 290 nm. This step was repeated after the resin was subjected to a second, identical deprotection step (for a total of 16 min of deprotection). The combined moles of removed Fmoc were determined using an extinction coefficient of 5253 $M^{-1}cm^{-1}$ and corrected for the dilution factor used.[1] Division by the resin mass verified the resin loading to be as stated by the manufacturer.

Fmoc Removal: Fmoc removal was accomplished with treatment of the resin by 2 mL of 20% piperidine in DMF and shaking at 200 rpm for 7 min. This process was then repeated once more (for a total of 14 min deprotection time). The resin was then washed with shaking three times with DMF for 1 min. The solution from the first 7 min reaction was collected, an aliquot taken, diluted to 3 mL in DMF, and the absorbance read at 290 nm after blanking with a solution of 20% piperidine that had been similarly diluted. This absorbance was compared with that of the first deprotection solution value from the original deprotection of the resin as a means of following the resin loading throughout the synthesis (Figures S-1 and S-2).

Resin Capping: After coupling the first amino acid to the resin, the resin was capped with acetic anhydride to ensure that any remaining unreacted primary amines were blocked before continuing synthesis. 10 equiv of acetic anhydride and 7 equiv of *N*,*N*-Diisopropylethylamine (DIPEA) in 2 mL DMF were added to the resin, which was then shaken at 200 rpm for 15 min. The resin was washed with shaking three times with DMF for 1 min prior to conducting additional synthetic steps.

HATU and DIPEA-Facilitated Coupling: Most amino acid coupling steps were accomplished by using (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) (HATU) as the coupling reagent and DIPEA as the base. 3 equiv of the desired amino acid was combined with 2.85 equiv of HATU before being dissolved in approximately 2 mL of 25% dichloromethane (DCM) in DMF. 2.85 equiv of DIPEA was then added, and the mixture briefly shaken, before addition to the resin and reaction for 1.5, 2, 4, or 16 h (see Tables S-3 and S-4). Immediately following coupling, the resin was washed with shaking three times with DMF for 1 min.

Selective Deprotection of the Serine OTrt Protecting Group: Before the lactone linkage reaction could be conducted, the OTrt protecting group on Ser3 was selectively removed. The resin was washed three times with DCM for 1 min, followed by treatment with a 2 mL solution of 1% TFA and 5% triisopropylsilane (TIPS) in DCM for 2 min. The treatment with the TFA solution was repeated an additional three times (for a total deprotection time of 8 min). Following deprotection, the resin was washed four times with DCM for 1 min, then four times with shaking with DMF for 1 min prior to beginning the next reaction step.

Verification of Deprotection of Serine OTrt Protecting Group: To aid in the determination of the reaction conditions for the selective deprotection of the serine OTrt group, we developed a calibration curve using Trt-*N*-Glutamine as a standard. The amino acid stock solutions were prepared in the deprotection solution, and a 500 µL aliquot was diluted to 3 mL with DMF before the absorbance at 271 nm was read (following blanking with a 500 µL of deprotection solution diluted to 3 mL). A calibration curve made through serial dilution of the Trt-*N*-Glutamine standard allowed the progression of resin-bound peptide deprotection to be approximated. Unknown samples were measured by conducting the same dilution (500 µL to 3 mL in DMF) and reading at 271 nm.

DIC with Catalytic DMAP-Facilitated Coupling: Formation of the lactone "branch" in the peptide was accomplished *via* reaction with a symmetrical anhydride formed from the preliminary reaction of the desired amino acid with *N*,*N*'-diisopropylcarbodiimide (DIC). 10 equiv of the desired amino acid was dissolved in DCM that had been dried over 3 Å molecular sieves. To this vigorously stirred solution was added 5 equiv of DIC dropwise at 0 °C. The flask was then sealed with continued stirring for 25 min. After the initial reaction was complete, additional dry DCM was added as necessary to dissolve any precipitate, and the solution was allowed to warm back to room temperature with additional stirring for 10 min. The DCM was then removed *via* rotary evaporation under reduced pressure and the resulting solid dissolved in 2 mL DMF that had been dried over 3 Å molecular sieves. The resulting solution was then added to the resin and the mixture shaken for 25-30 min at 200 rpm. At this point, 0.04 equiv of 4-Dimethylaminopyridine (DMAP) was added as an 11 µg/µL solution in DMF to the resin mixture. The resin mixture was shaken for an additional

3.5 h. The resin was then washed with shaking three times with DMF for 1 min before coupling the next amino acid.

Palladium Catalyzed Removal of OAlloc Protecting Group: The resin was washed three times in DCM for 1 min before being dried down and placed under argon. Approximately 5 mL of DCM dried over 3 Å molecular sieves in a 15 mL polypropylene centrifuge tube was sparged with argon for 3 min. 10 equiv of phenyl silane was then added and sparging continued for an additional 2-3 min. 0.5 equiv of tetrakis(triphenylphosphine)palladium(0) was added and sparging continued 4 min. The resulting solution was added to the resin and the reaction vessel was sealed with parafilm. The reaction vessel was then placed in a 50 mL polypropylene centrifuge tube wrapped in aluminum foil. The air in the centrifuge tube was displaced with argon before the centrifuge tube was sealed with parafilm. The 50 mL tube was then shaken at 200 rpm for 2 h. The resin was washed with shaking four times with 0.5% sodium diethyldithiocarbamate trihydrate in DMF for 2 min and four times with shaking with DMF for 1 min before proceeding onto the next reaction step.

Pyoxim and DIPEA-Facilitated On-Resin Peptide Cyclization: The final cyclization of the peptide was accomplished through reaction with pyoxim and DIPEA. 1.25 equiv of pyoxim was dissolved in 25% DCM in DMF. To this solution was added 2.5 equiv of DIPEA. The mixture was briefly shaken and then added to the resin. The resulting mixture was shaken at 200 rpm for 4 h. The solution was then drained, and a second, identical coupling reagent solution was added to the resin. The mixture was shaken at 200 rpm for 16 h. Completion of the cyclization was verified using the Kaiser test (see below). After the reaction was complete, the resin was washed with shaking three times with DMF for 1 min and three times with DCM for 1 min. The resin was then dried prior to final cleavage.

Kaiser Test: The Kaiser (ninhydrin) test for primary amines was conducted as previously described.[1] Briefly, a small amount of dried resin was placed in a small glass culture tube. To the dried resin was added 2 drops of 5% (w/v) ninhydrin in ethanol, 2 drops of 80% (w/v) phenol in ethanol, and 2 drops of 20 µM potassium cyanide (KCN) in pyridine. The tube was then heated for 6 min at 110-120 °C. Blue or purple coloration in the beads and/or solution is indicative of an incomplete reaction (presence of unreacted primary amines).

Final Cleavage and Precipitation: The resin was transferred from the original reaction vessel to a 15 mL polypropylene centrifuge tube. 3 mL of 2.5% water and 2.5% TIPS in TFA was then added and the tube was shaken at 200 rpm for 3 h. The resin was then filtered through a cotton plug in a polypropylene syringe and washed with a small amount of TFA. The filtrate was collected in a 50 mL polypropylene centrifuge tube. 45 mL of -20 °C 1:1 ether:hexane solution was then added as a precipitant and the sealed tube mixed by upending several times before being placed in a flammables-compatible freezer at -20 °C for 10 min. The 50 mL tube was centrifuged in a Beckman Coulter Allegra 6 centrifuge equipped with a GH3.8 rotor at 2990 rpm (approx. 2550 x g) for 5 min in order to pellet the crude peptide. The supernatant was decanted off and the pellet dissolved in approximately 5-10 mL of 1:1 ACN:water. The solution was frozen by placing the tube in an acetone/dry ice bath, and then lyophilized for at least 18 h.

Purification by HPLC: Crude peptide was purified using RP-HPLC. The crude pellet was dissolved in either 4 mL (0.045 mmol scale) or 8 mL (0.09 mmol scale) of 3-36% ACN (depending on solubility) in water and purified in 4 mL portions on a Phenomenex Kinetex 5

μm C18 semi-preparative column (10 mm × 250 mm, 110 Å). The crude purification was accomplished with a gradient from 20% ACN to 60% ACN over 40 min. These conditions were typically sufficient to purify the peptide to >= 80-90%. Fraction purity was determined through analysis on a Phenomenex Kinetex 5 μm analytical C18 column (4.6 mm × 250 mm, 110 Å). Typically, 90 μL of either a 2-fold-diluted fraction sample or a 50:50 mix of aliquots from two different HPLC fractions (to verify peak identity between fractions) was injected. The gradient used for analytical analysis was from 5% ACN to 95% ACN over 24 min. Fractions with similar purity were combined, frozen using an acetone/dry ice bath, and lyophilized for at least 18 h. Secondary purification to get the peptide to >= 95% purity was conducted on the same semi-preparative column mentioned above and generally used a gradient from 25% ACN to 35% ACN over 40 min. Before the final masses and yields of purified peptides were determined, they were dissolved in 30% acetic acid in up to 50% ACN in water to displace and permit removal of any residual TFA. The solution was then frozen in an acetone/dry ice bath, and lyophilized for at least 18 h before the yield was determined.

Peptide Verification with Mass Spectrometry: During purification, peaks were verified to contain the desired peptide by MALDI-TOF MS. Samples were prepared using α-Cyano-4-hydroxycinammic acid as matrix and aliquots taken directly from the preparative HPLC fractions. Final verification of the peptides was conducted by obtaining their exact masses with a high resolution ESI-TOF MS (Tables S-1 and S-2). 1-8 μM stock solutions were prepared in either water or 50% ACN in water. The instrument was calibrated before each run and an internal reference mass standard was used.

Development of Bioassay Conditions: Peptide stocks for use in bioassays were prepared by dissolving the stock peptide in up to 50% ACN in water and transferring an aliquot to another vial. The contents of both vials were then frozen and the vials lyophilized for at least 18 h. The aliquot was then dissolved in DMSO in order to produce a 2 mM master stock solution, which was frozen at -80 °C and only thawed long enough to produce diluted working stocks in DMSO as necessary for a given bioassay.

Several growth times, incubation times, and kanamycin concentrations were investigated to identify the optimal conditions with which to conduct peptide activity assays. *E. faecalis* bacteria were grown on Mueller-Hinton Broth 2 (MHB-2) plates without antibiotics overnight at 37 °C. An isolated colony was then transferred to 5 mL of brain heart infusion (BHI) broth containing kanamycin (initially ranging from 250 μg/mL up to 2 mg/mL) and shaken overnight at 37 °C in ambient atmosphere. These overnight cultures were then diluted 50-fold into BHI broth containing 250 μg/mL to 2 mg/mL kanamycin. The cells were then grown with shaking at 37 °C for 0-2 h before 198 μL aliquots were placed in the wells of a 96-well plate containing either 2 μL DMSO or 2 μL synthetic GBAP dissolved in DMSO (the final GBAP concentration was 10 μM) and incubated at 37 °C for either 2 or 4 h. After this incubation time had elapsed, the absorbance at 600 nm (A600) was read. The wells were then treated with 20 μL 1% Triton X-100 in water for 30 min at 37 °C to lyse the bacteria cells. After lysis, 100 μL of the lysate was transferred to a new well containing 100 μL substrate buffer containing 0.8 mg/mL ortho-Nitrophenyl-β-galactoside (ONPG) and 2.7 μL/mL β-mercaptoethanol in Z-buffer (100 mM sodium phosphate buffer, 10 mM KCl, and 1 mM $MgSO_4$, pH 7.0). The substrate reaction was run for 30 min at 37 °C before being quenched with 20 μL of 1 M sodium carbonate in water. The absorbance of quenched

reaction wells was read at 420 nm (A420) and 550 nm (A550), allowing for the calculation of the activity in Miller units (Equation 1).

Equation 1: $Miller\ Unit = 1000 * \frac{A420 - c * A550}{t * v * A600}$

Where *c* is a species-specific correction factor (1.6 for *E. faecalis*), *t* is the substrate reaction time in minutes, and *v* is the volume of lysed cell culture used in the enzyme reaction in milliliters.[2] The equation allows for correction of background absorbance due to initial cell density, cell lysate turbidity, and enzyme reaction time.

EC_{50} Experiments: After experiments to determine the optimal conditions (Figure S-4), a bioassay protocol was developed to maximize the signal-to-noise ratio when determining peptide potency at activating the fsr quorum sensing circuit. The bacterial cultures were prepared as discussed above except that 500 µg/mL of kanamycin was used for all antibiotic treated cultures. The diluted culture was shaken for 1 h (the 1 h timing was found to be the most important determinant of a reproducibly maximized signal-to-noise ratio) at 37 °C. If the A600 value was much above 0.2 either at the time of the 50-fold dilution or after the 1 h initial growth period, drops in the maximum response signal were observed. After the 1 h initial growth period, 198 µL of the culture was added in triplicate to wells of a 96-well plate. The experimental peptide concentrations were varied along a 9-point, 5-fold serial dilution starting with 10 µM and ending with 0.052 nM. Each well was treated with 2 µL DMSO solution of experimental peptide, GBAP positive control, or DMSO negative control. The 96-well plate was then incubated at 37 °C for 2 h. The cell lysis, enzyme reaction, and signal reading steps were then conducted as discussed above. After Miller unit determination, the average value for the DMSO negative control was subtracted and the results normalized to the average value of the GBAP positive control. The EC_{50} value (Table S-5) was then determined through fitting using nonlinear regression with GraphPad Prism using Equation 2. In cases where the initial sigmoidal curve gave few baseline points, the 9-point dilution series was adjusted by starting at 1 µM and diluting 5-fold to a final concentration of 0.0052 nM. In cases where the initial sigmoidal curve gave few maximum signal points, the 9-point dilution series was adjusted by conducting a 3-fold dilution series starting at 10 µM and ending at 1.52 nM. If the maximum concentration (10 µM) activity of a peptide was less than or equal to 50% relative to the GBAP positive control, that peptide was tested as a potential competitive inhibitor.

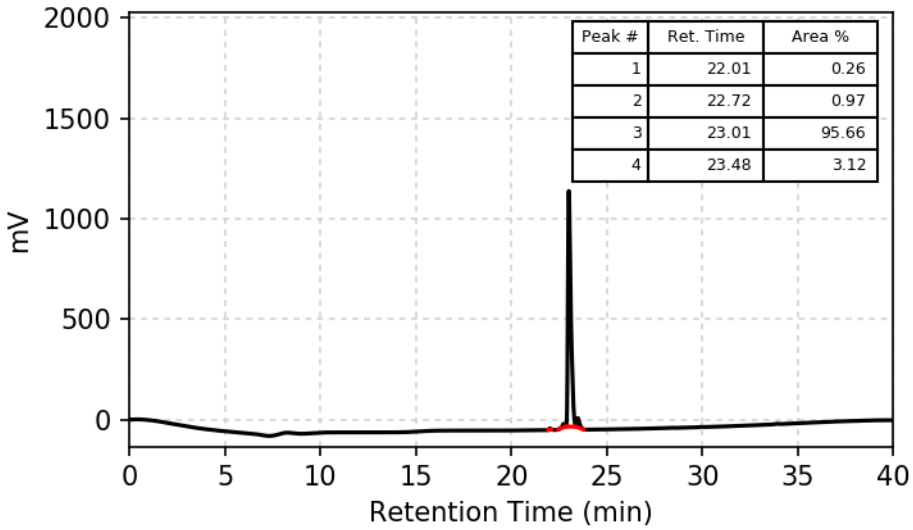
Equation 2: $y = min + \frac{\max - min}{1 + 10^{\log(EC50 - x)}}$

Where *min* is the average minimum signal and *max* is the average maximum signal.

IC_{50} Experiments: A 9-point dilution series of the experimental peptide was prepared similarly to those made for the EC_{50} determination. However, each sample also included 50 nM of GBAP as a competitor standard. This concentration of GBAP was determined to reproducibly give 90-95% of the GBAP maximum signal. After Miller unit determination, the DMSO negative control was subtracted and the results were normalized to the signal of 50 nM GBAP without the presence of any potential inhibitor. As none of the peptides tested showed sufficiently high dose-response to justify conducting nonlinear regression, the signal at the maximum experimental peptide concentration was compared with that of the 50 nM GBAP control to determine if the peptide exhibited weak inhibitory activity (Figure S-5).

HPLC Traces for GBAP Analogues

GBAP



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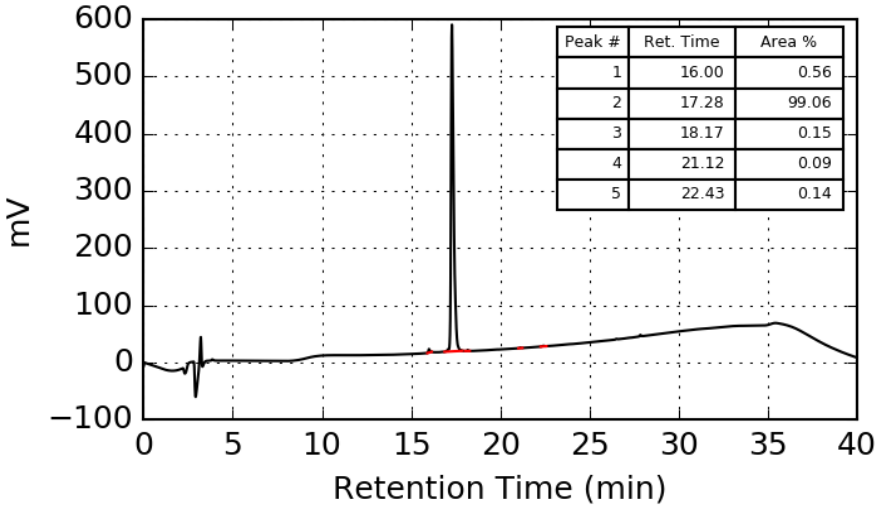

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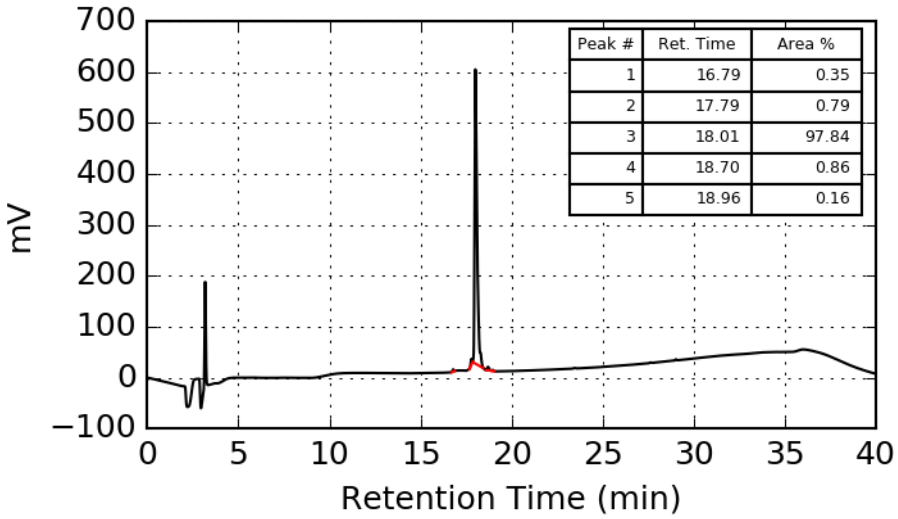

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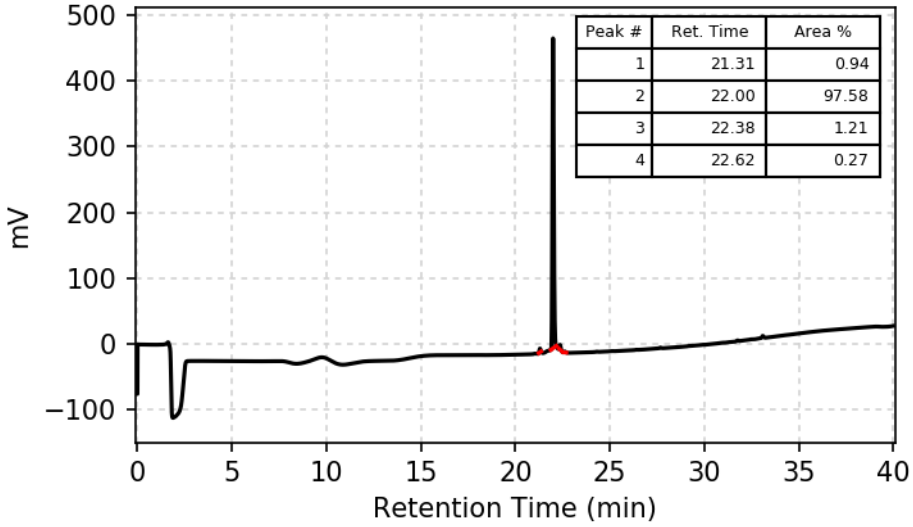

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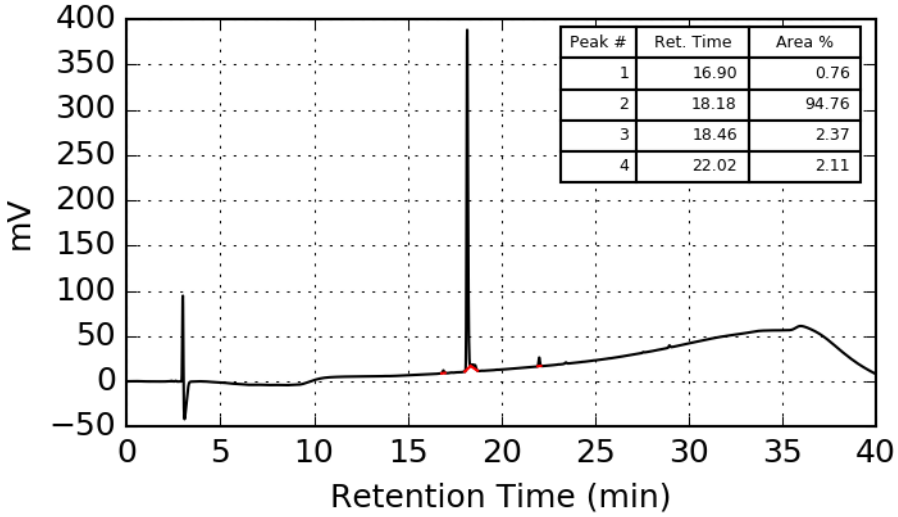

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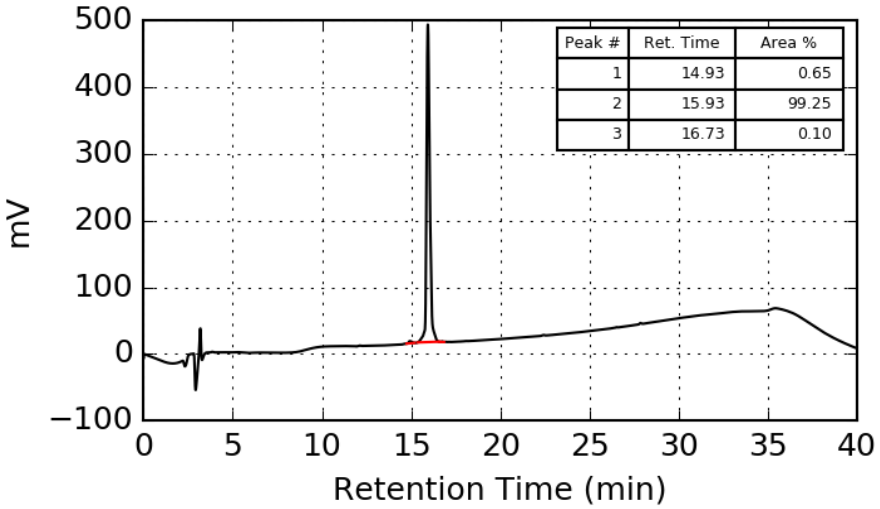

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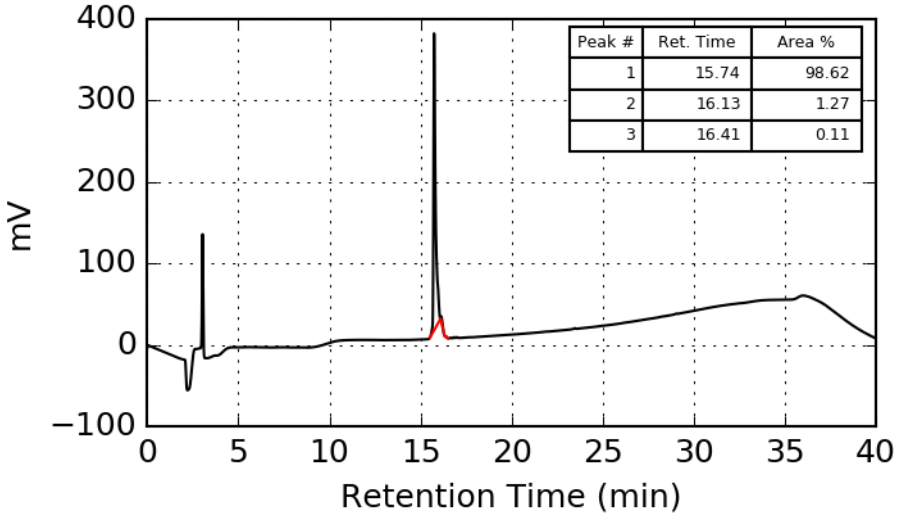

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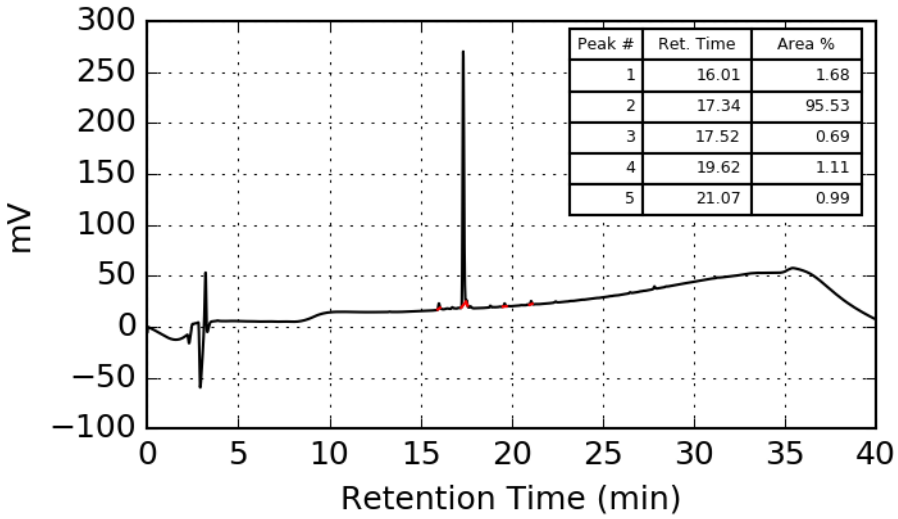

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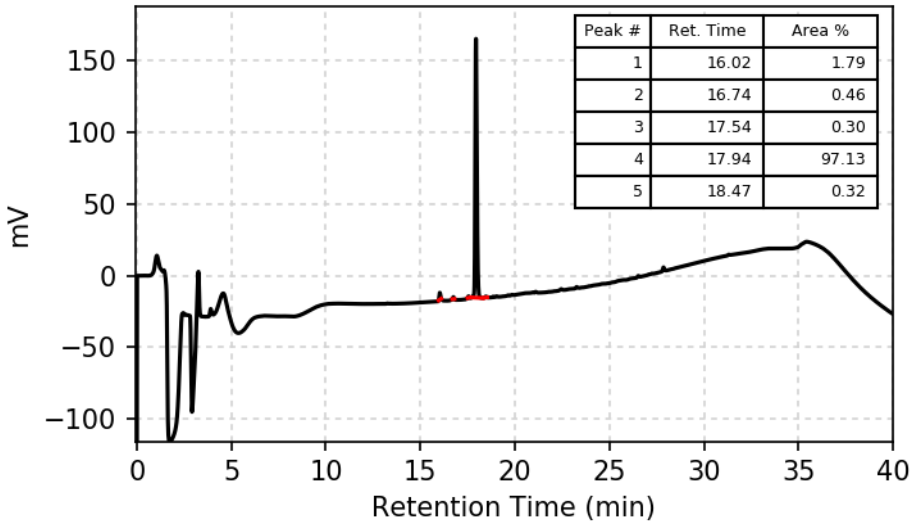

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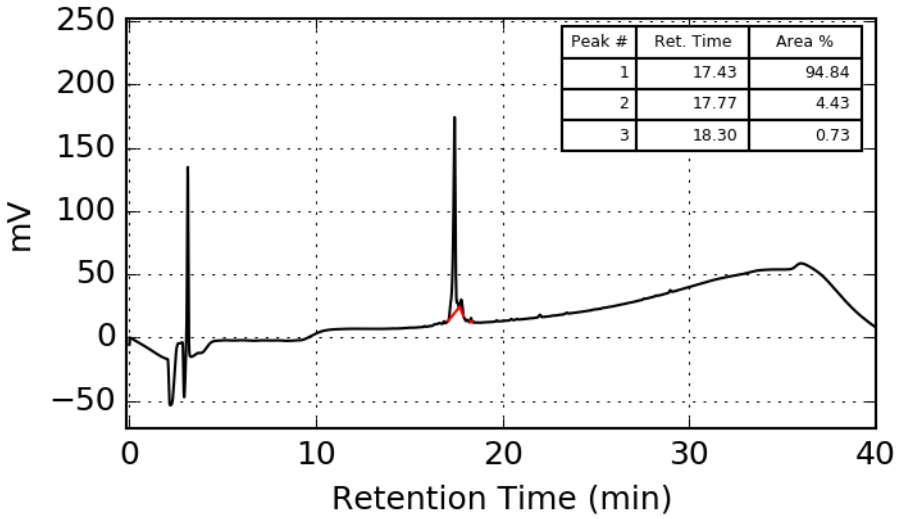

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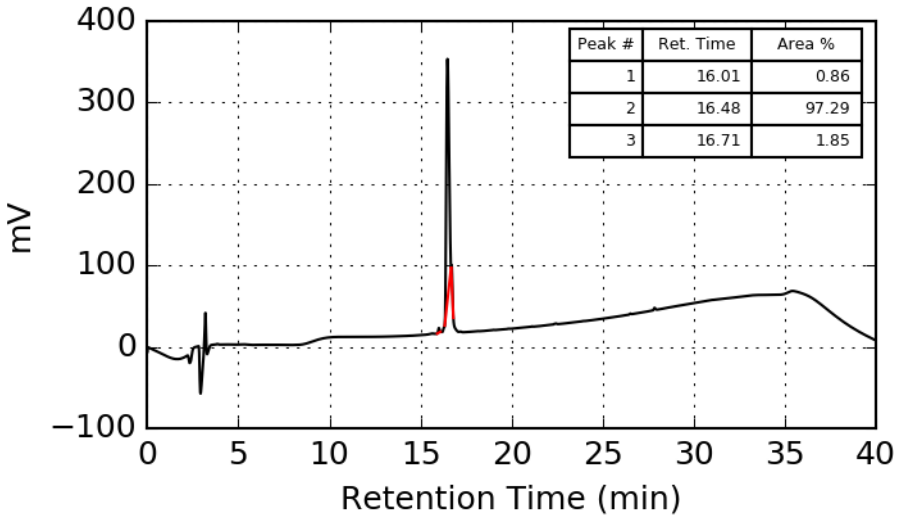

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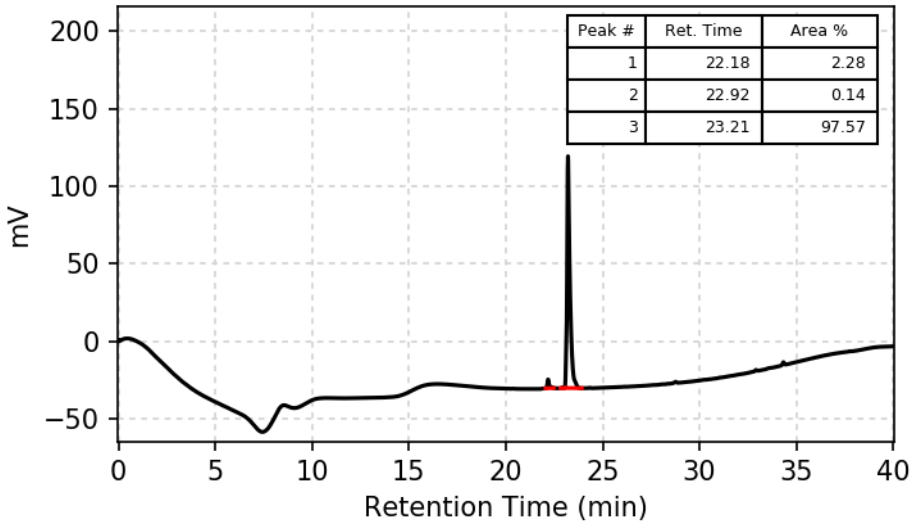

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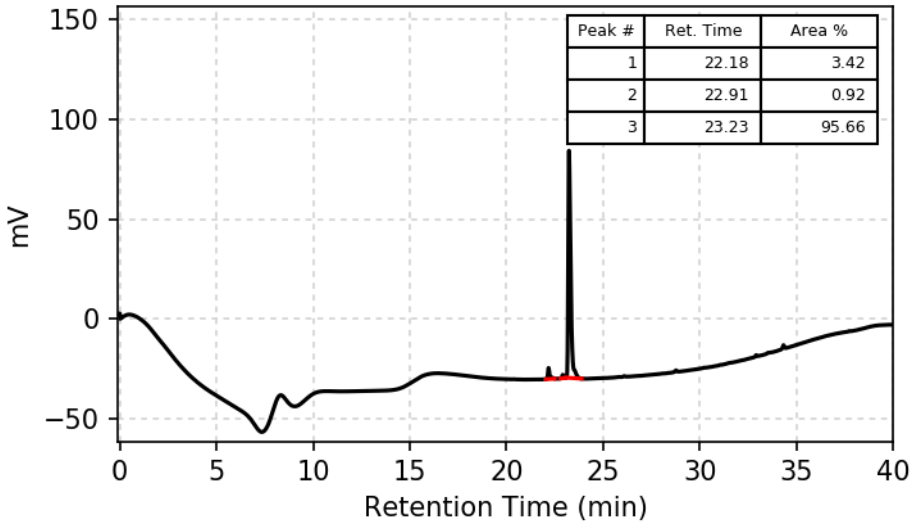

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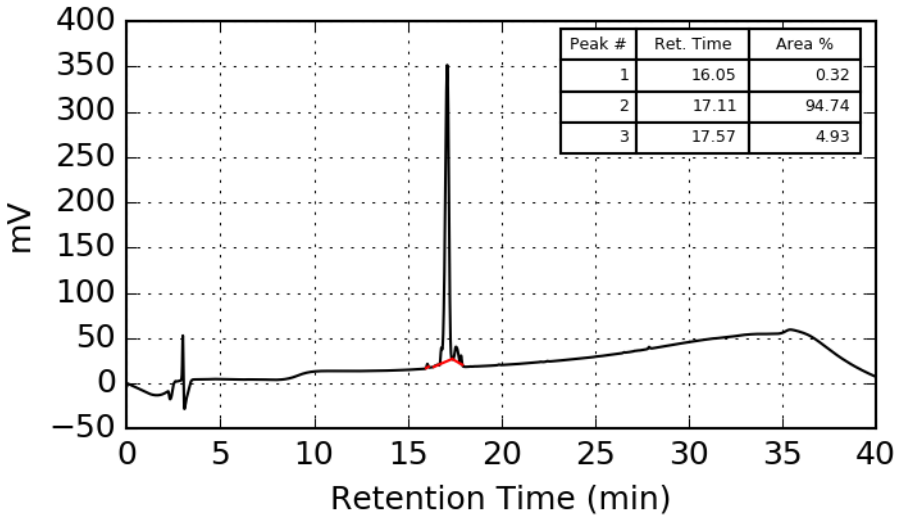

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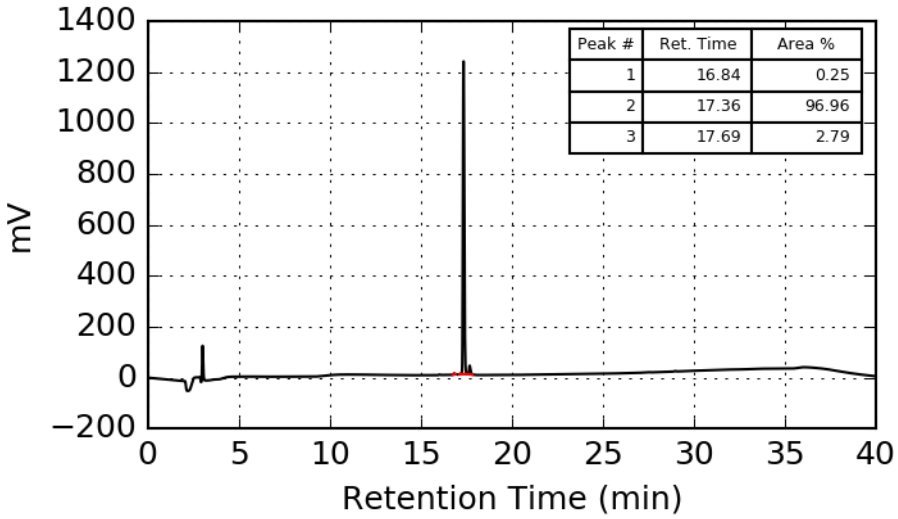

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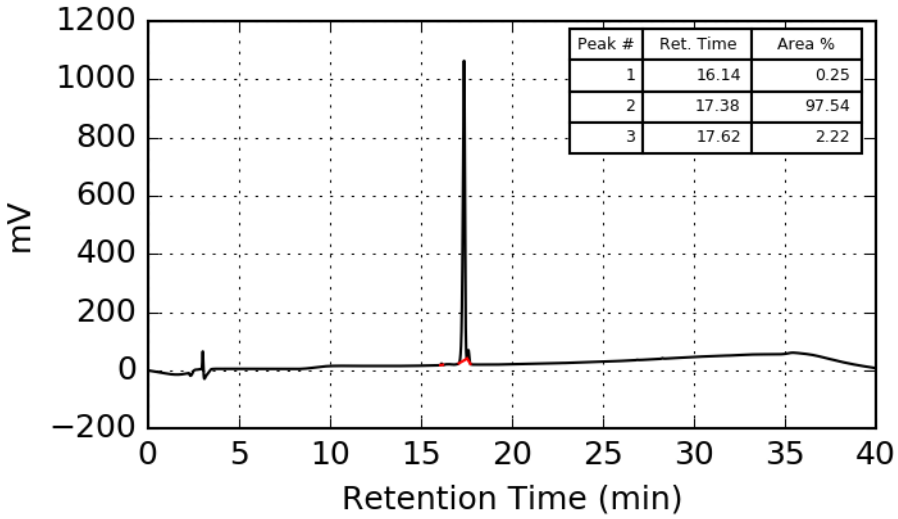

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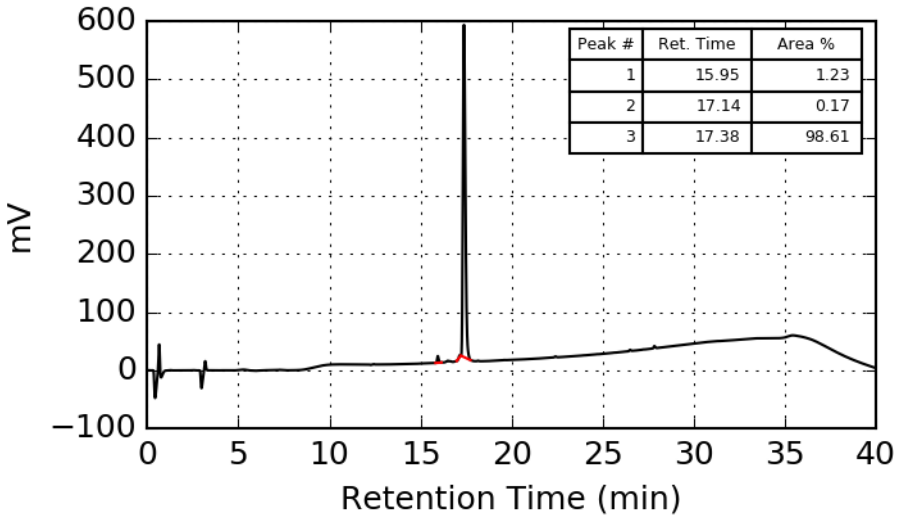

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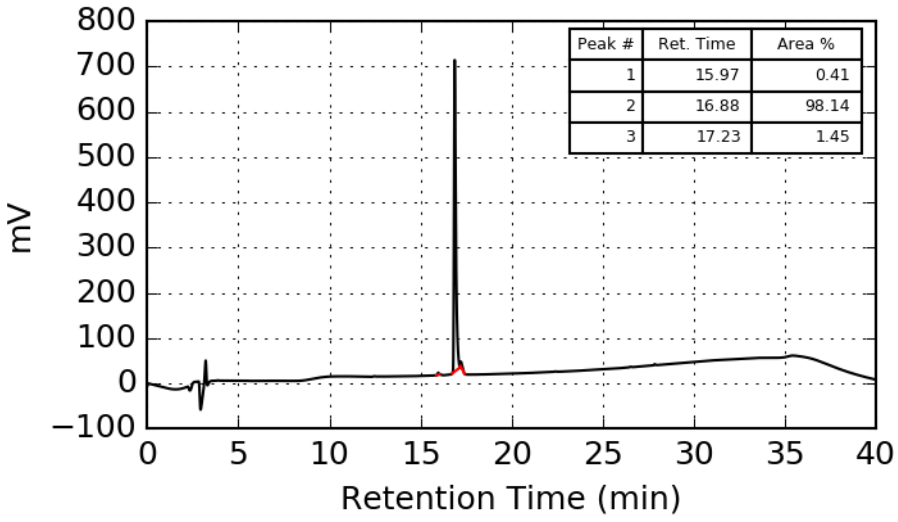

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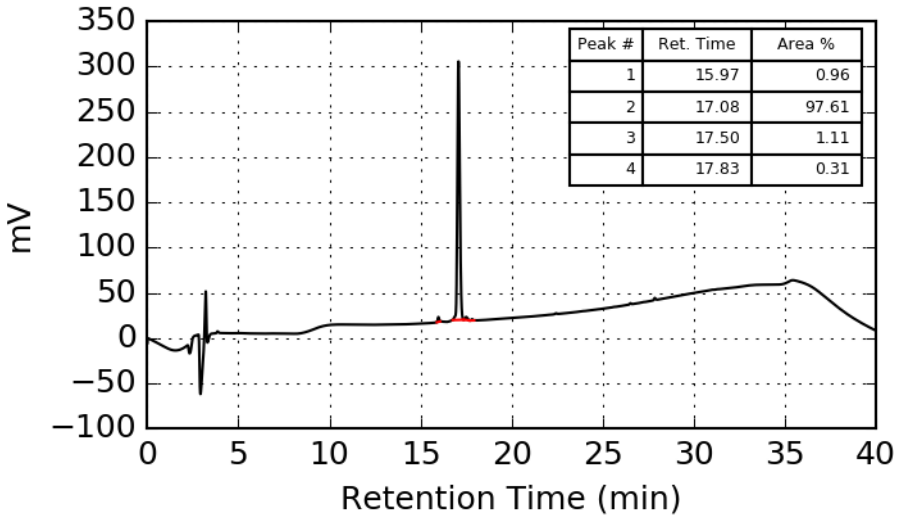

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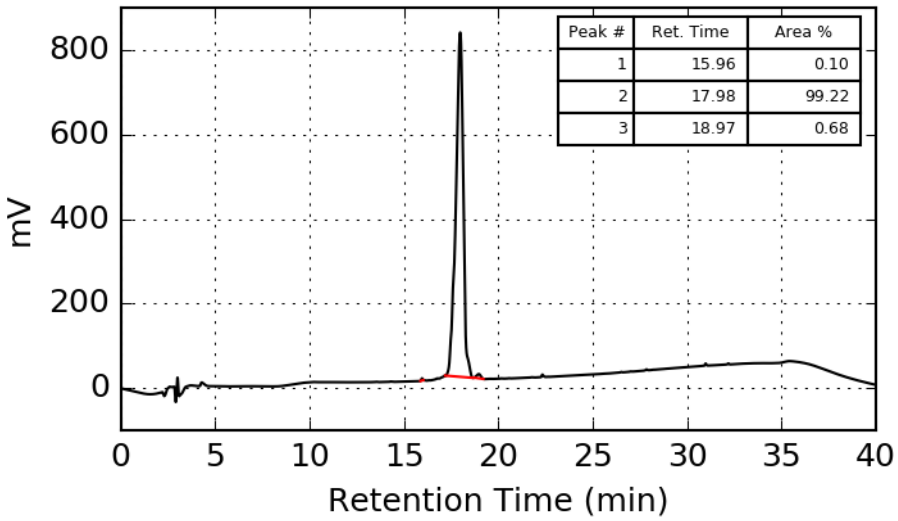

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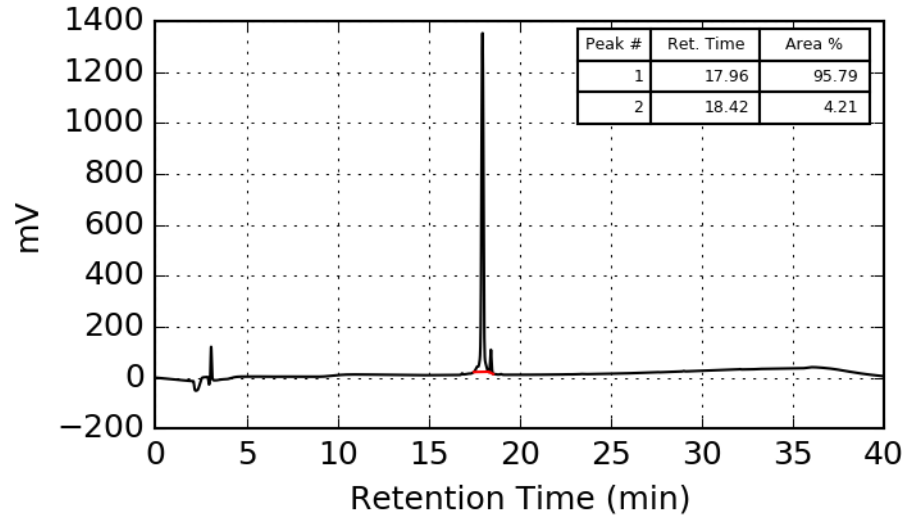

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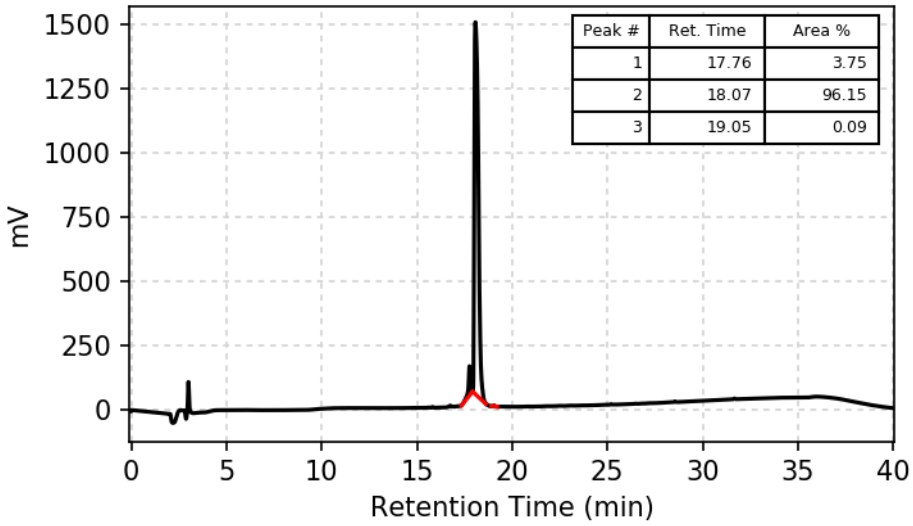

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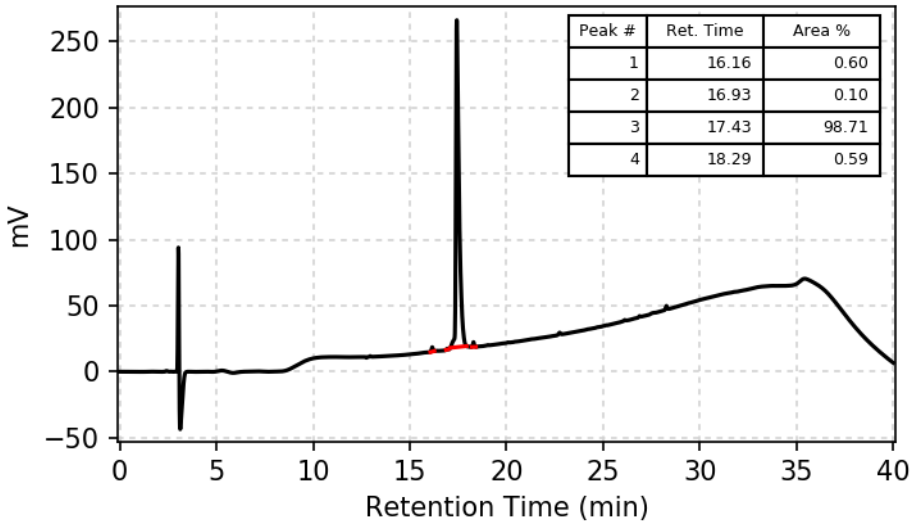

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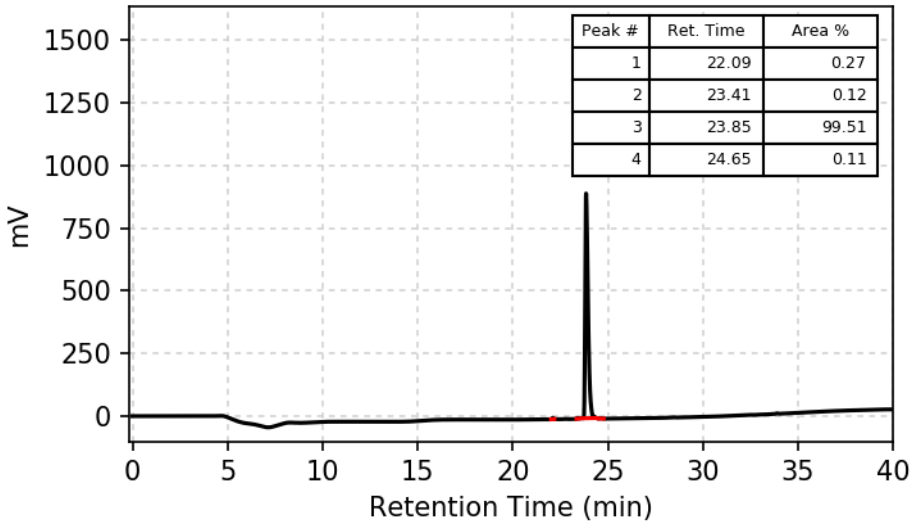
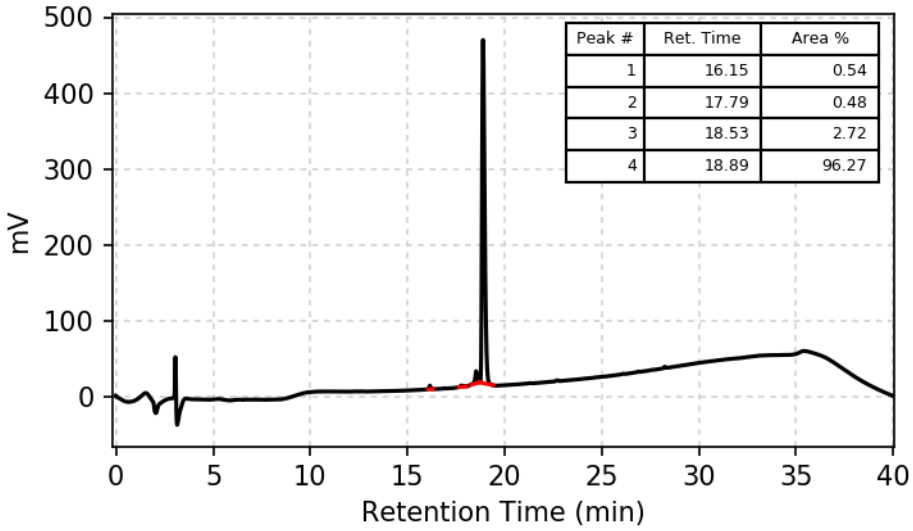
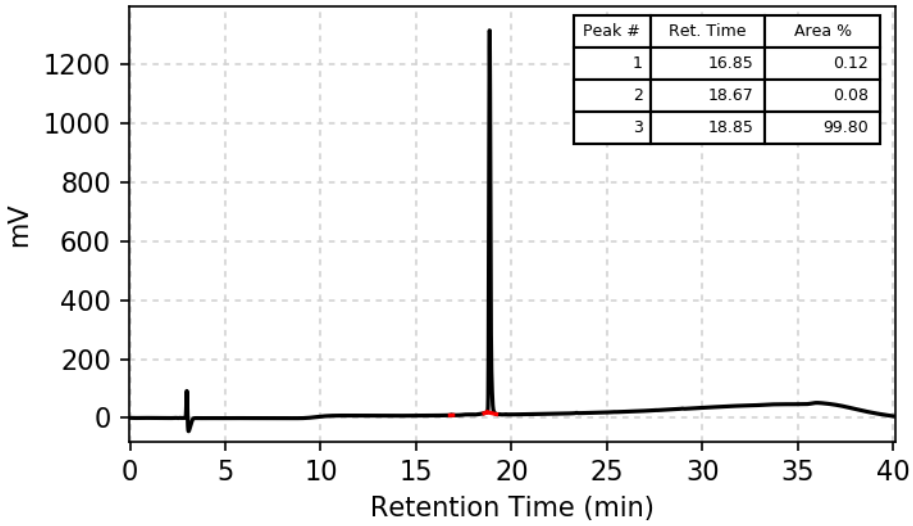


Ac-GBAP-DesQ1N2



GBAP-DesQ1N2 lactam



MS and HPLC Data for GBAP Analogues

Table S-1. HRMS and HPLC data for GBAP alanine & D-amino acid scanning library analogues.

Compound Name	Calc. EM $[M+H]^+$	Obs. EM $[M+H]^+$	Purity (%)	Compound Name	Calc. EM $[M+H]^+$	Obs. EM $[M+H]^+$	Purity (%)
GBAP	1303.58882	1303.58470	> 96				
GBAP-Q1A	1246.56735	1246.56550	> 99	GBAP-q1	1303.58882	1303.58690	> 98
GBAP-N2A	1260.58300	1260.58159	> 98	GBAP-n2	1303.58882	1303.58753	> 96
GBAP-P4A	1277.57317	1277.57219	> 97	GBAP-s3	1303.58882	1303.58763	> 95
GBAP-N5A	1260.58300	1260.57913	> 95	GBAP-p4	1303.58882	1303.58680	> 97
GBAP-I6A	1261.54187	1261.54060	> 99	GBAP-n5	1303.58882	1303.58715	> 98
GBAP-F7A	1227.55752	1227.55689	> 99	GBAP-i6	1303.58882	1303.58686	> 99
GBAP-G8A	1317.60447	1317.60312	> 96	GBAP-f7	1303.58882	1303.58737	> 98
GBAP-Q9A	1246.56735	1246.56592	> 97	GBAP-q9	1303.58882	1303.58663	> 98
GBAP-W10A	1188.54662	1188.54477	> 95	GBAP-w10	1303.58882	1303.58718	> 99
GBAP-M11A	1243.58545	1243.58324	> 97	GBAP-m11	1303.58882	1303.58714	> 96

EM = Exact Mass. See above for methods

Table S-2. HRMS and HPLC data for GBAP tail modification library analogues.

Compound Name	Calc. EM $[M+Na]^+$	Obs. EM $[M+Na]^+$	Purity (%)
Ac-GBAP	1367.58188	1367.58151	> 96
GBAP-DesQ1	1197.51273	1197.51163	> 99
Ac-GBAP-DesQ1	1239.5233	1239.51893	> 99
Ac-GBAP-DesQ1N2	1125.48037	1125.47841	> 96
GBAP-DesQ1N2 lactam	1083.46981	1083.46946	> 99

EM = Exact Mass. See above for methods.

Synthesis Optimization Data

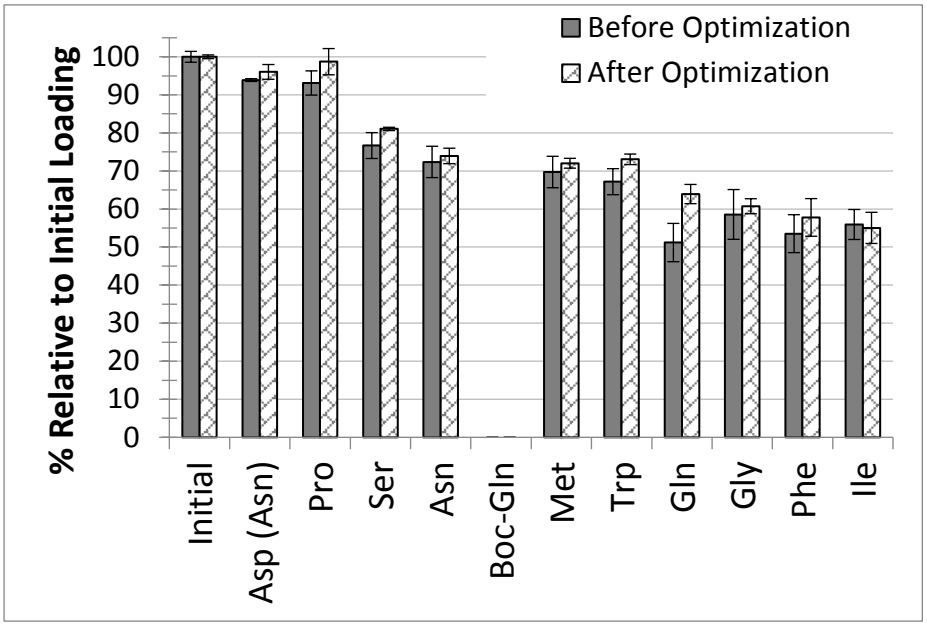


Figure S-1 Results of monitoring Fmoc removal by measuring the absorbance at 290 nm for the synthetic pathway beginning from Asn5. Quantification of Fmoc removal was used to direct synthesis optimization. Values are normalized to the initial apparent loading of the resin. Steps that saw large drops in apparent resin loading were targeted for optimization.

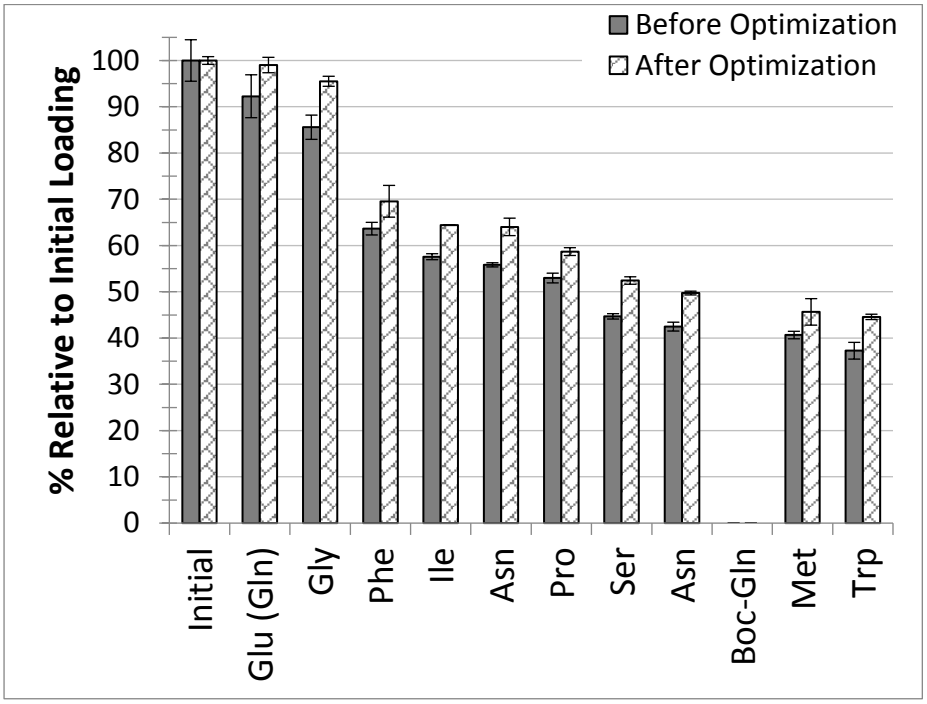


Figure S-2 Results of monitoring Fmoc removal by measuring the absorbance at 290 nm for the synthetic pathway beginning from Gln9. Quantification of Fmoc removal was used to direct synthesis optimization. Values are normalized to the initial apparent loading of the resin. Steps that saw large drops in apparent resin loading were targeted for optimization.

***Table S-3*:** Aspartic Acid Pathway Optimized Reaction Conditions

Step/Amino Acid	Equiv Added	Coupling	Time (h)
Fmoc-L-Asp-alpha-Alloc	3	HATU & DIPEA	16
Capping	10	Acetic Anhydride & DIPEA	0.25
Fmoc-L-Pro-OH	3	HATU & DIPEA	2
Fmoc-L-Ser(Trt)-OH	3	2X HATU & DIPEA 50 °C	4, 16
Fmoc-L-Asn(Trt)-OH	3	HATU & DIPEA	2
Boc-L-Gln(Xan)-OH	3	HATU & DIPEA	2
Fmoc-L-Met-OH	5 [a]	DIC (0.04 equiv DMAP)	4
Fmoc-L-Trp(Boc)-OH	3	HATU & DIPEA	2
Fmoc-L-Gln-OH	3	2X HATU & DIPEA 50 °C	4, 16
Fmoc-L-Gly-OH	3	HATU & DIPEA	2
Fmoc-L-Phe-OH	3	HATU & DIPEA	2
Fmoc-L-Ile-OH	3	HATU & DIPEA	2
Cyclization	1.25	2X Pyoxim & DIPEA	4, 16

Table S-4: Glutamic Acid Pathway Optimized Reaction Conditions

Step/Amino Acid	Equiv Added	Coupling	Time (h)
Fmoc-L-Glu-alpha-Alloc	3	HATU & DIPEA	16
Capping	10	Acetic Anhydride & DIPEA	0.25
Fmoc-L-Gly-OH	3	HATU & DIPEA	1.5
Fmoc-L-Phe-OH	3	2X HATU & DIPEA 50 °C	4, 16
Fmoc-L-Ile-OH	3	HATU & DIPEA	2
Fmoc-L-Asn(Trt)-OH	3	HATU & DIPEA	2
Fmoc-L-Pro-OH	3	HATU & DIPEA	2
Fmoc-L-Ser(Trt)-OH	3	2X HATU & DIPEA 50 °C	4, 16
Fmoc-L-Asn(Trt)-OH	3	HATU & DIPEA	2
Boc-L-Gln(Xan)-OH	3	HATU & DIPEA	2
Fmoc-L-Met-OH	5 [a]	DIC (0.04 equiv DMAP)	4
Fmoc-L-Trp(Boc)-OH	3	HATU & DIPEA	2
Cyclization	1.25	2X Pyoxim & DIPEA	4, 16

[a] 5 equiv of anhydride (10 equiv of amino acid)

	Mid-way Optimization		After Optimization	
	Yield	Purity	Yield	Purity
Initial	5.4%	93%	6.7%	97%
Secondary	2.4%	97%	NA	NA

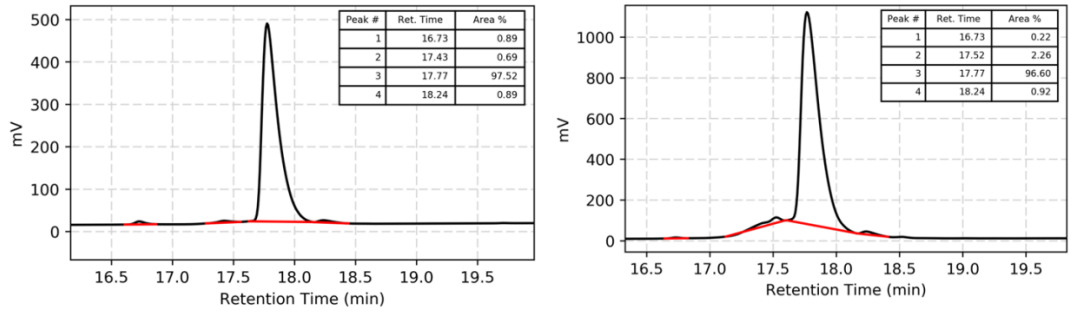


Figure S-3 Mid-way-optimization conditions are compared with after-optimization conditions both using the same purification protocol. The percent yield relative to initial resin loading and the purity are shown for each purification step. HPLC traces shown are for the final (bioassay-ready) product mid-way through (left) and after (right) optimization.

Bioassay Condition Data

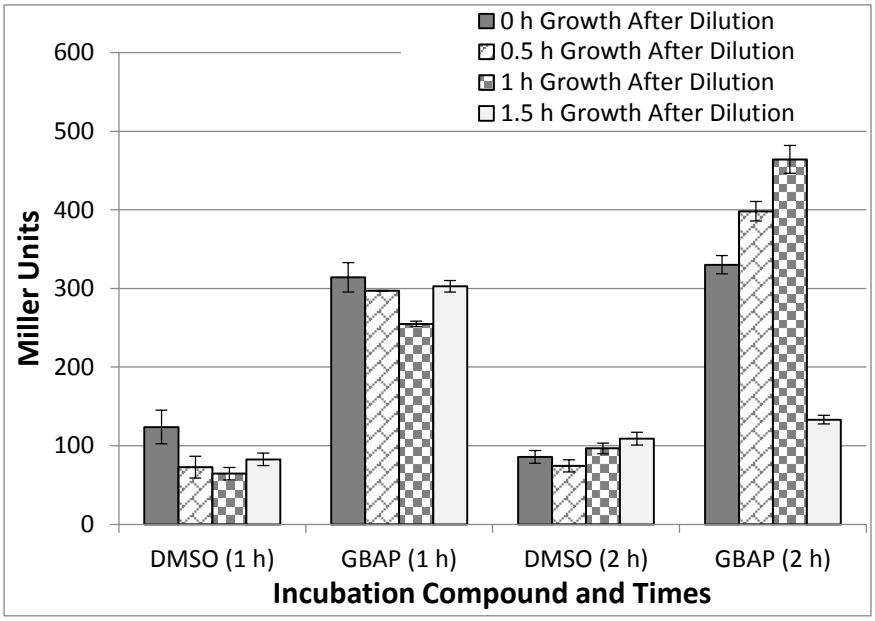


Figure S-4 Bioassay optimization for wild-type reporter strain TX5274 identified highest signal-to-noise conditions to be 1 h growth after dilution and peptide incubation time of 2 h when conducting a 30 min β-galactosidase reaction.

Bioassay Results

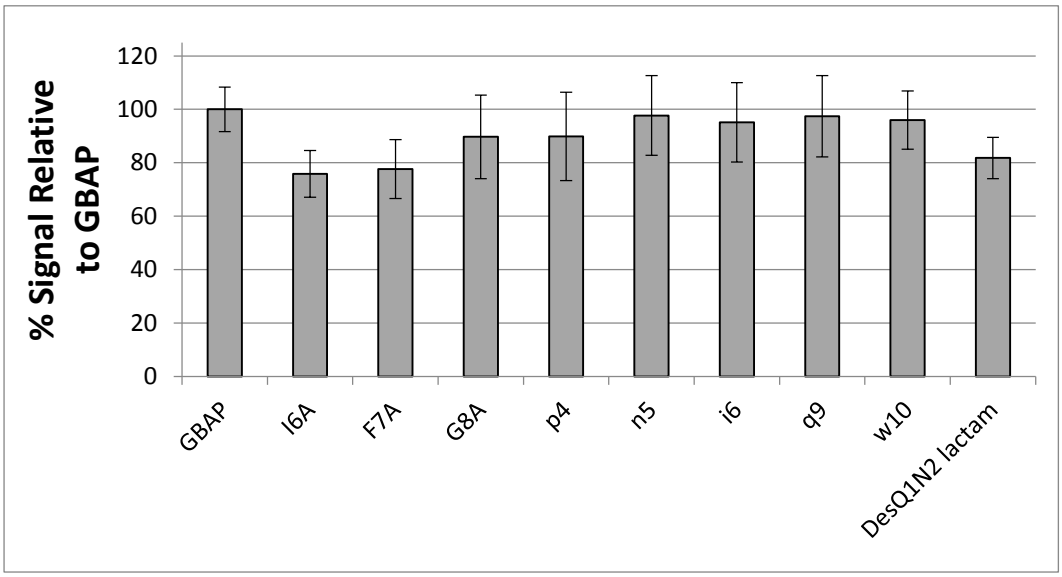


Figure S-5 Results of competitive inhibitor evaluation of select library analogues. The signal obtained with 50 nM GBAP is compared to 50 nM GBAP treated with 10 μM of tested peptide.

Table S-5. EC_{50} Data for all peptide analogues with confidence intervals

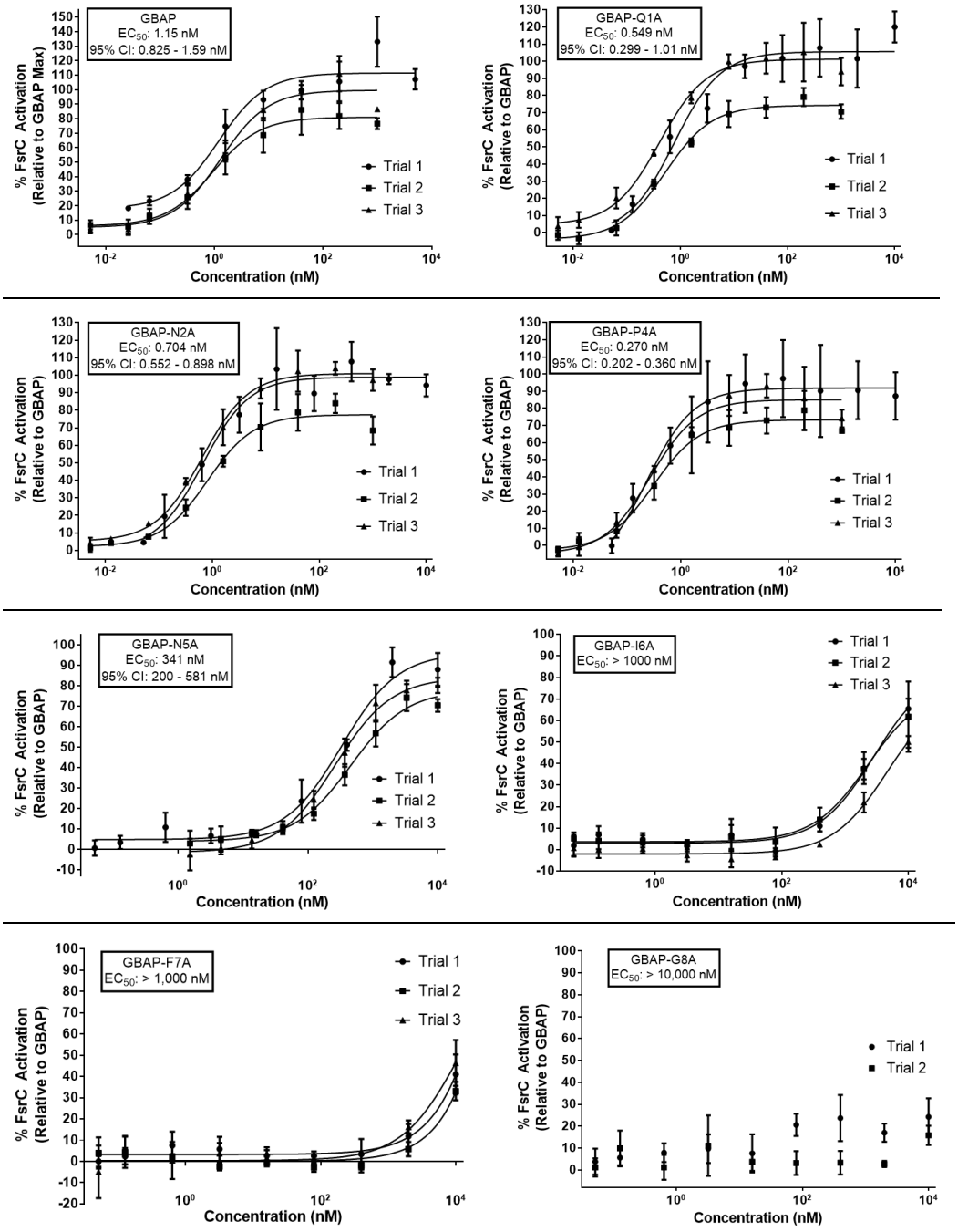
Peptide Name	Sequence	EC_{50} [95% CI] (nM)
GBAP	QN(SPNIFGQWM)	1.15 [0.825 – 1.59]
GBAP-Q1A	**A**N(SPNIFGQWM)	0.549 [0.299 – 1.01]
GBAP-N2A	Q**A**(SPNIFGQWM)	0.704 [0.522 – 0.898]
GBAP-P4A	QN(S**A**NIFGQWM)	0.270 [0.202 – 0.360]
GBAP-N5A	QN(SP**A**IFGQWM)	341 [200 – 581]
GBAP-I6A	QN(SPN**A**FGQWM)	> 1,000
GBAP-F7A	QN(SPNI**A**GQWM)	> 1,000
GBAP-G8A	QN(SPNIF**A**QWM)	> 10,000
GBAP-Q9A	QN(SPNIFG**A**WM)	312 [222 – 437]
GBAP-W10A	QN(SPNIFGQ**A**M)	246 [117 – 520]
GBAP-M11A	QN(SPNIFGQW**A**)	164 [93.8 – 288]
GBAP-q1	**q**N(SPNIFGQWM)	0.211 [0.0798– 0.599]
GBAP-n2	Q**n**(SPNIFGQWM)	0.304 [0.168 – 0.552]
GBAP-s3	QN(**s**PNIFGQWM)	45.1 [17.0 – 120]
GBAP-p4	QN(S**p**NIFGQWM)	> 1,000
GBAP-n5	QN(SP**n**IFGQWM)	> 1,000
GBAP-i6	QN(SPN**i**FGQWM)	> 1,000
GBAP-f7	QN(SPNI**f**GQWM)	4.97 [3.12 – 7.77]
GBAP-q9	QN(SPNIFG**q**WM)	> 1,000
GBAP-w10	QN(SPNIFGQ**w**M)	> 10,000
GBAP-m11	QN(SPNIFGQW**m**)	231 [129 – 414]
Ac-GBAP	**Ac**-QN(SPNIFGQWM)	1.50 [1.31 – 1.73]
GBAP-DesQ1	N(SPNIFGQWM)	1.26 [1.15 – 1.39]
Ac-GBAP-DesQ1	**Ac**-N(SPNIFGQWM)	1.44 [0.855 – 2.44]
GBAP-DesQ1N2 lactam	(SPNIFGQWM)	> 10,000
Ac-GBAP-DesQ1N2	**Ac**-(SPNIFGQWM)	1.01 [0.496 – 2.06]

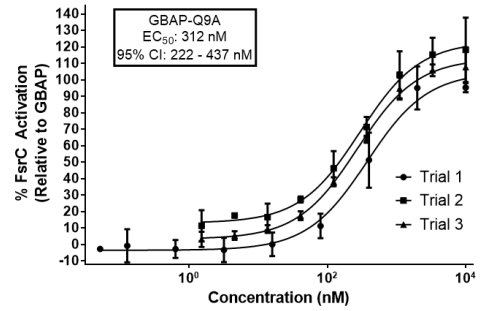
95% CI = 95% Confidence Interval

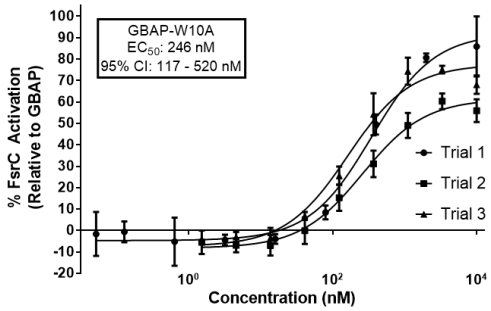
Dose Response Curves

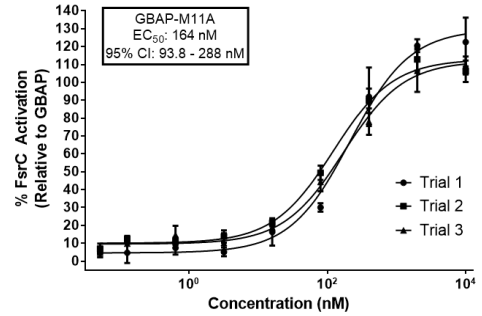
GBAP analogues were tested to determine their EC_{50} over varying concentrations. Each dose response experiment was performed in triplicate on three separate occasions* (i.e., experiments (Trial) #1-3; shown for each peptide below). Error bars indicate standard error of the mean of triplicate values. In each plot, the peptide, as well as its EC_{50} value (in nM) and 95% confidence interval (95% CI) values (in nM), when calculable, are indicated at top left. *Only two trials were conducted for GBAP G8A.

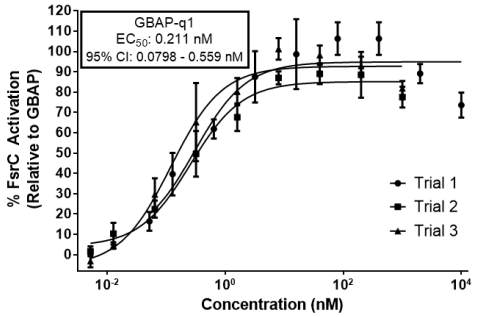
Activation Dose Response Curves

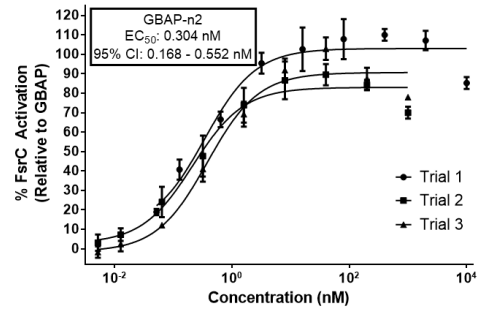


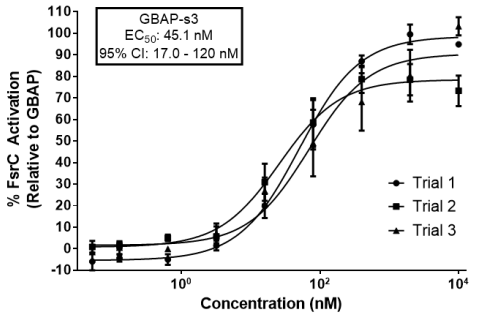

GBAP-Q9A
EC50: 312 nM
95% CI: 222 - 437 nM
% FsrC Activation (Relative to GBAP)
Concentration (nM)
Trial 1
Trial 2
Trial 3

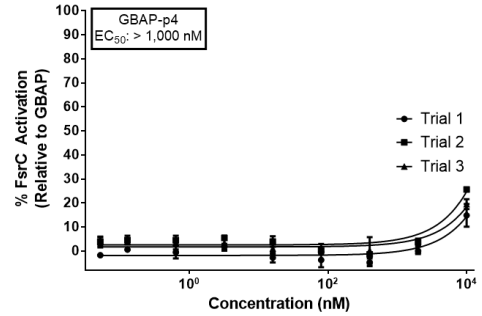

GBAP-W10A
EC50: 246 nM
95% CI: 117 - 520 nM
% FsrC Activation (Relative to GBAP)
Concentration (nM)
Trial 1
Trial 2
Trial 3

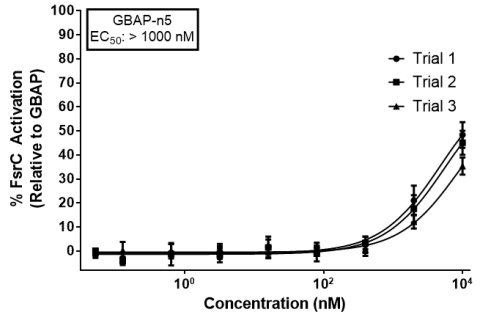

GBAP-M11A
EC50: 164 nM
95% CI: 93.8 - 288 nM
% FsrC Activation (Relative to GBAP)
Concentration (nM)
Trial 1
Trial 2
Trial 3

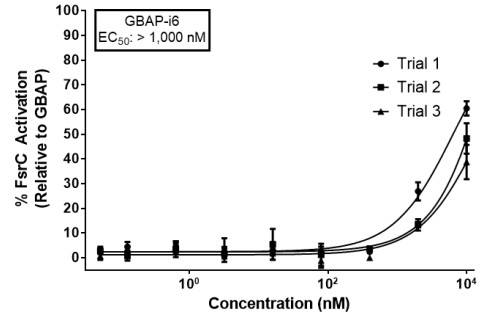

GBAP-q1
EC50: 0.211 nM
95% CI: 0.0798 - 0.559 nM
% FsrC Activation (Relative to GBAP)
Concentration (nM)
Trial 1
Trial 2
Trial 3

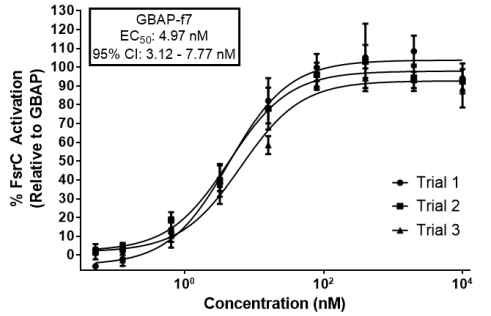

GBAP-n2
EC50: 0.304 nM
95% CI: 0.168 - 0.552 nM
% FsrC Activation (Relative to GBAP)
Concentration (nM)
Trial 1
Trial 2
Trial 3

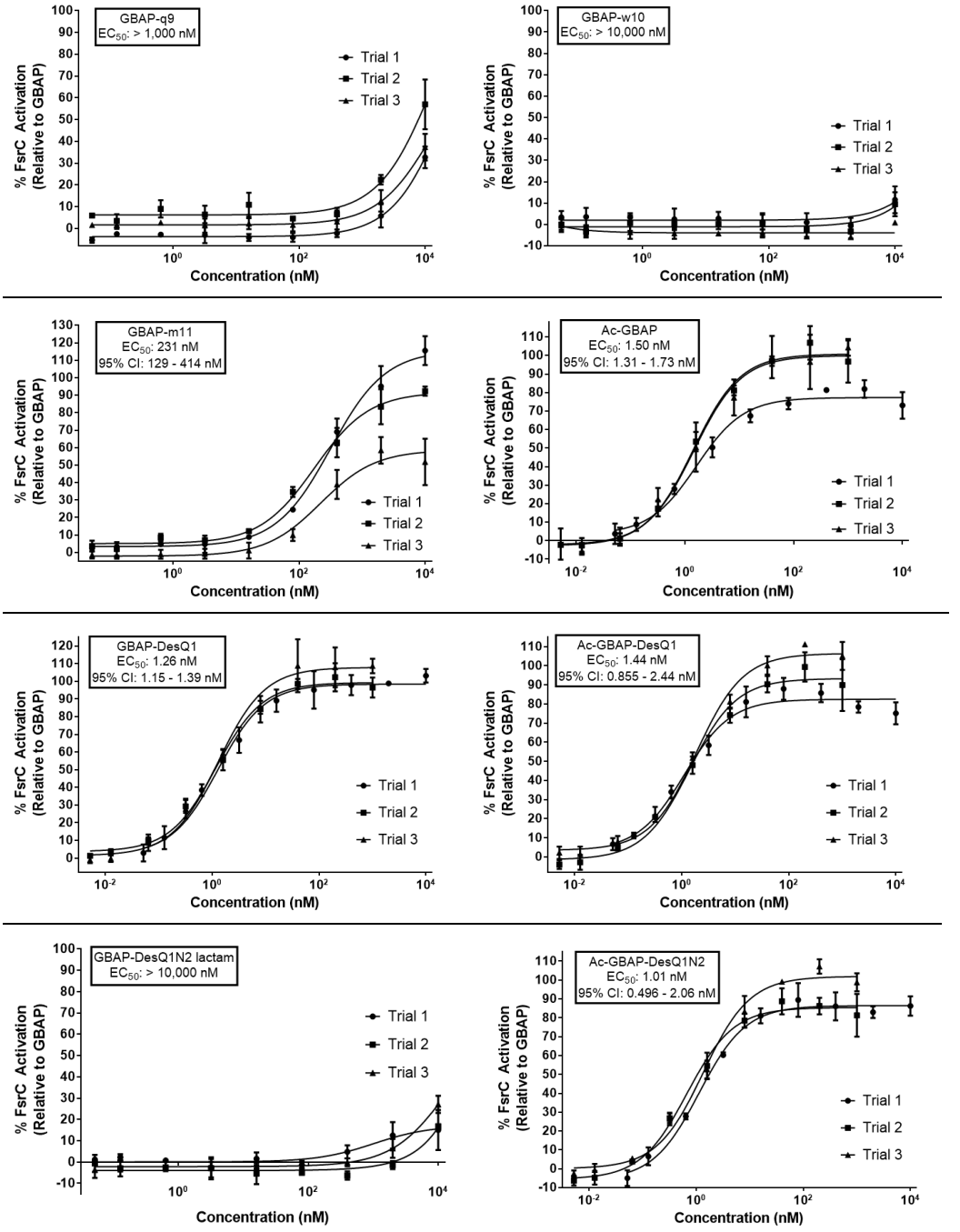

GBAP-s3
EC50: 45.1 nM
95% CI: 17.0 - 120 nM
% FsrC Activation (Relative to GBAP)
Concentration (nM)
Trial 1
Trial 2
Trial 3


GBAP-p4
EC50: > 1,000 nM
% FsrC Activation (Relative to GBAP)
Concentration (nM)
Trial 1
Trial 2
Trial 3


GBAP-n5
EC50: > 1000 nM
% FsrC Activation (Relative to GBAP)
Concentration (nM)
Trial 1
Trial 2
Trial 3


GBAP-i6
EC50: > 1,000 nM
% FsrC Activation (Relative to GBAP)
Concentration (nM)
Trial 1
Trial 2
Trial 3


GBAP-f7
EC50: 4.97 nM
95% CI: 3.12 - 7.77 nM
% FsrC Activation (Relative to GBAP)
Concentration (nM)
Trial 1
Trial 2
Trial 3



References

(1) *Fmoc solid phase peptide synthesis: a practical approach*; Chan, W. C., White, P. D., Eds.; Oxford University Press: New York, 2000.

(2) Qin, X.; Singh, K. V.; Weinstock, G. M.; Murray, B. E. *J. Bacteriol.* **2001,** *183*, 3372.