Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Immuno-Targeting of *Staphylococcus aureus via* Surface Remodeling Complexes

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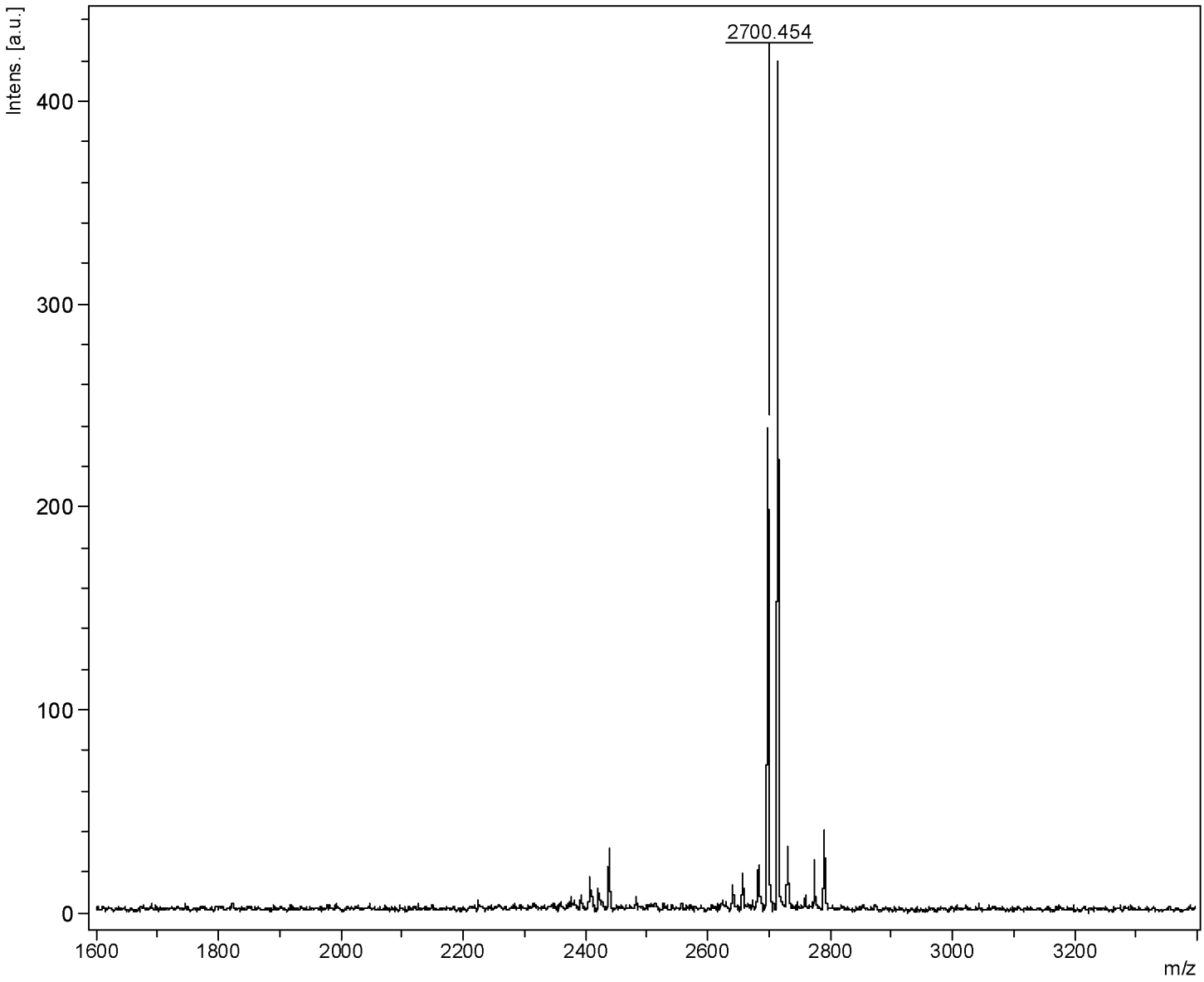

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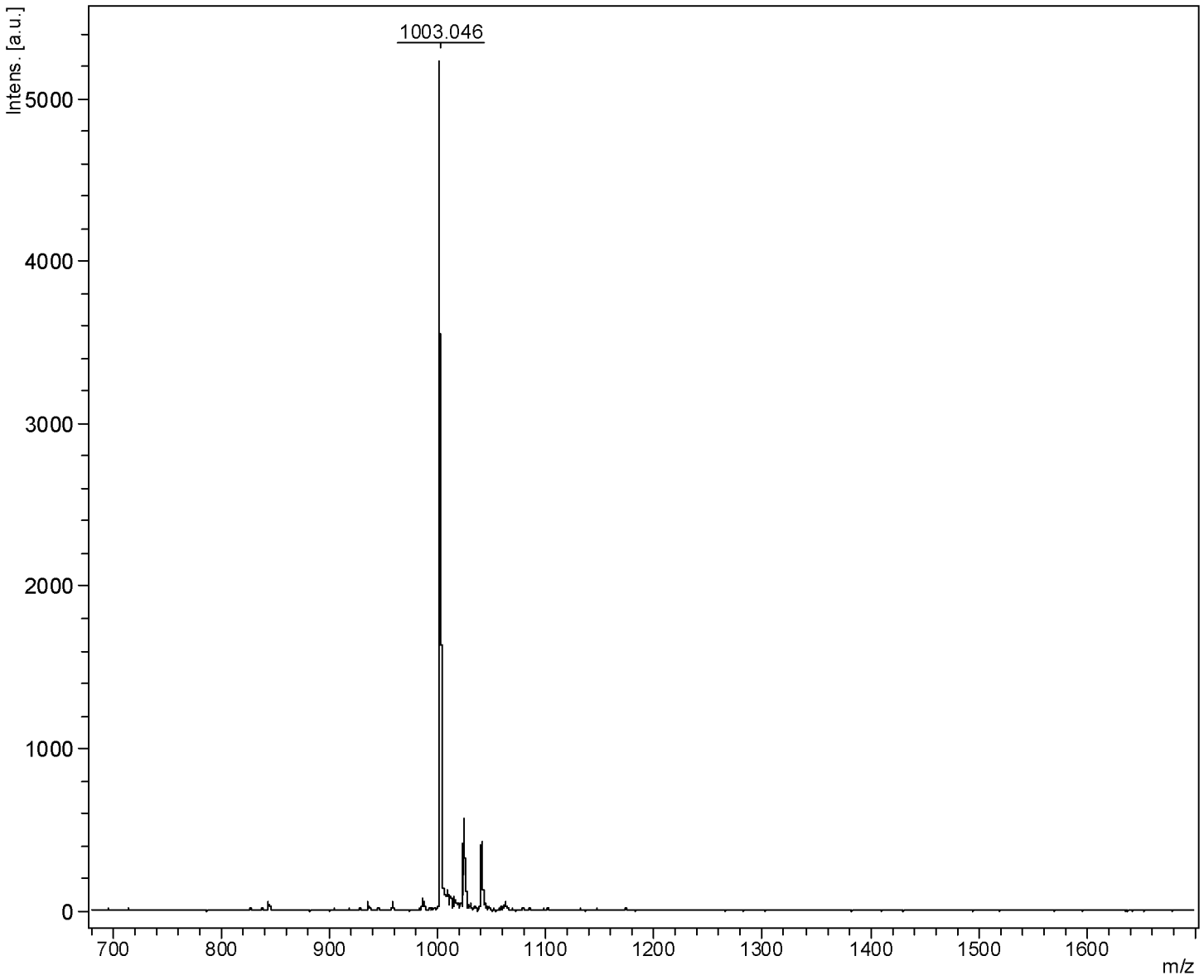

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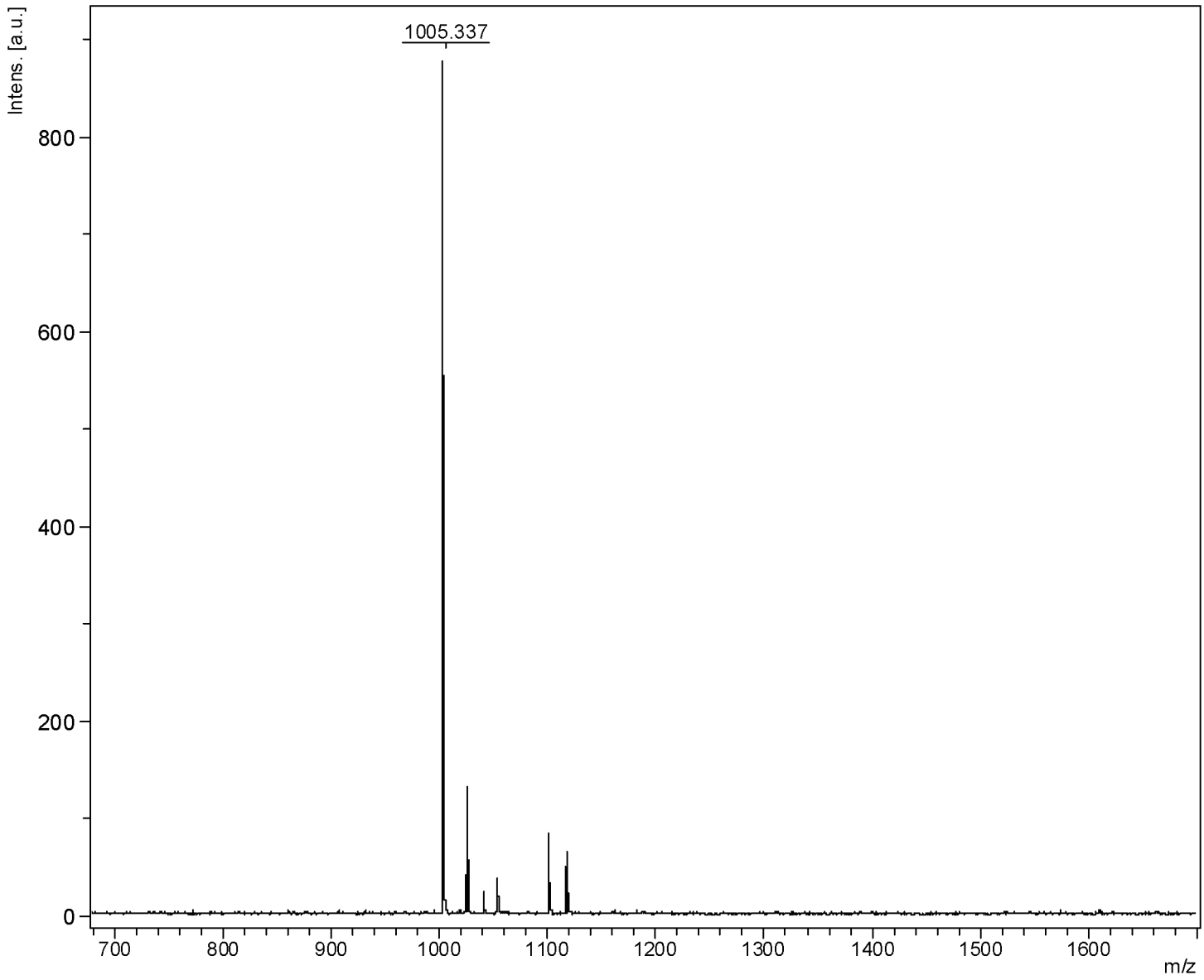

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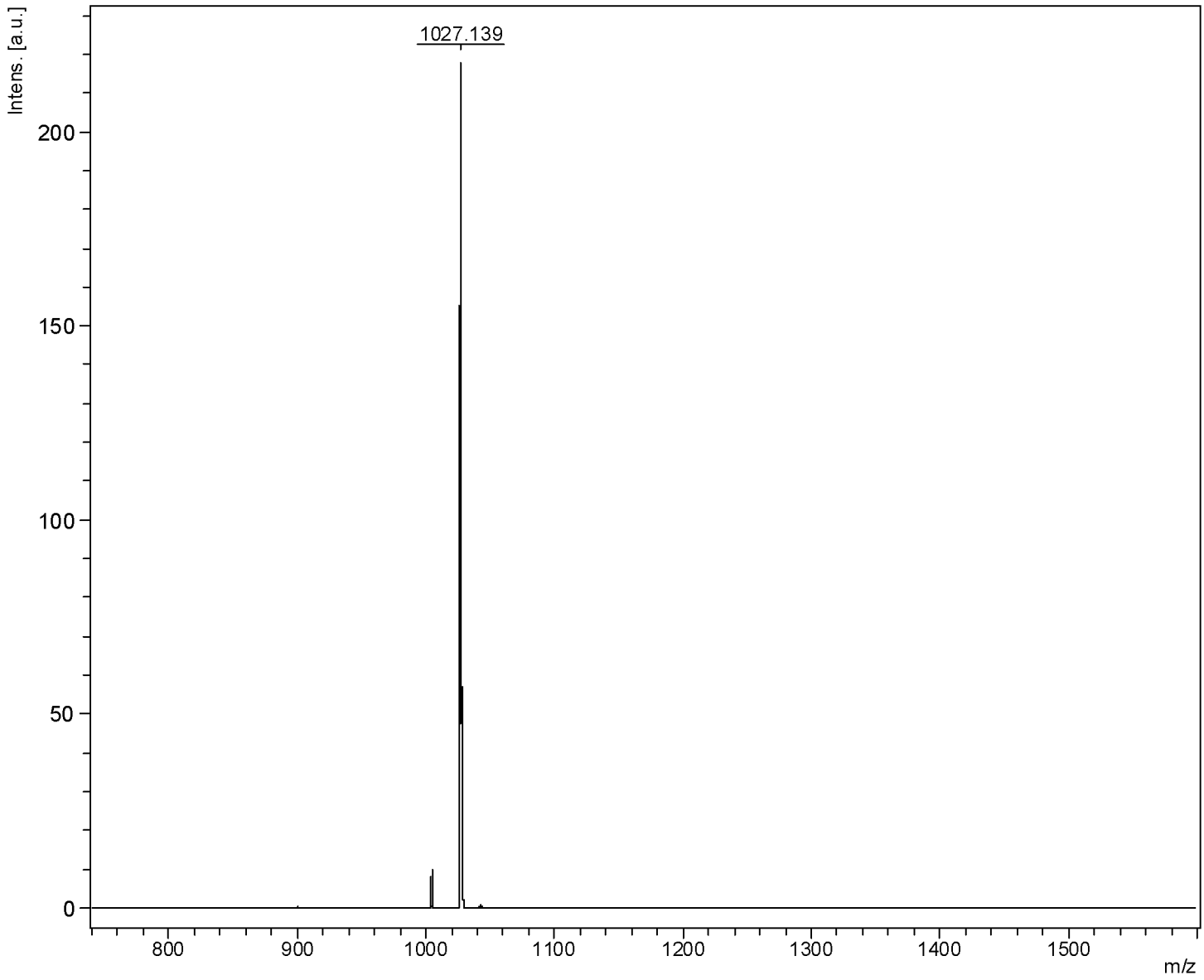

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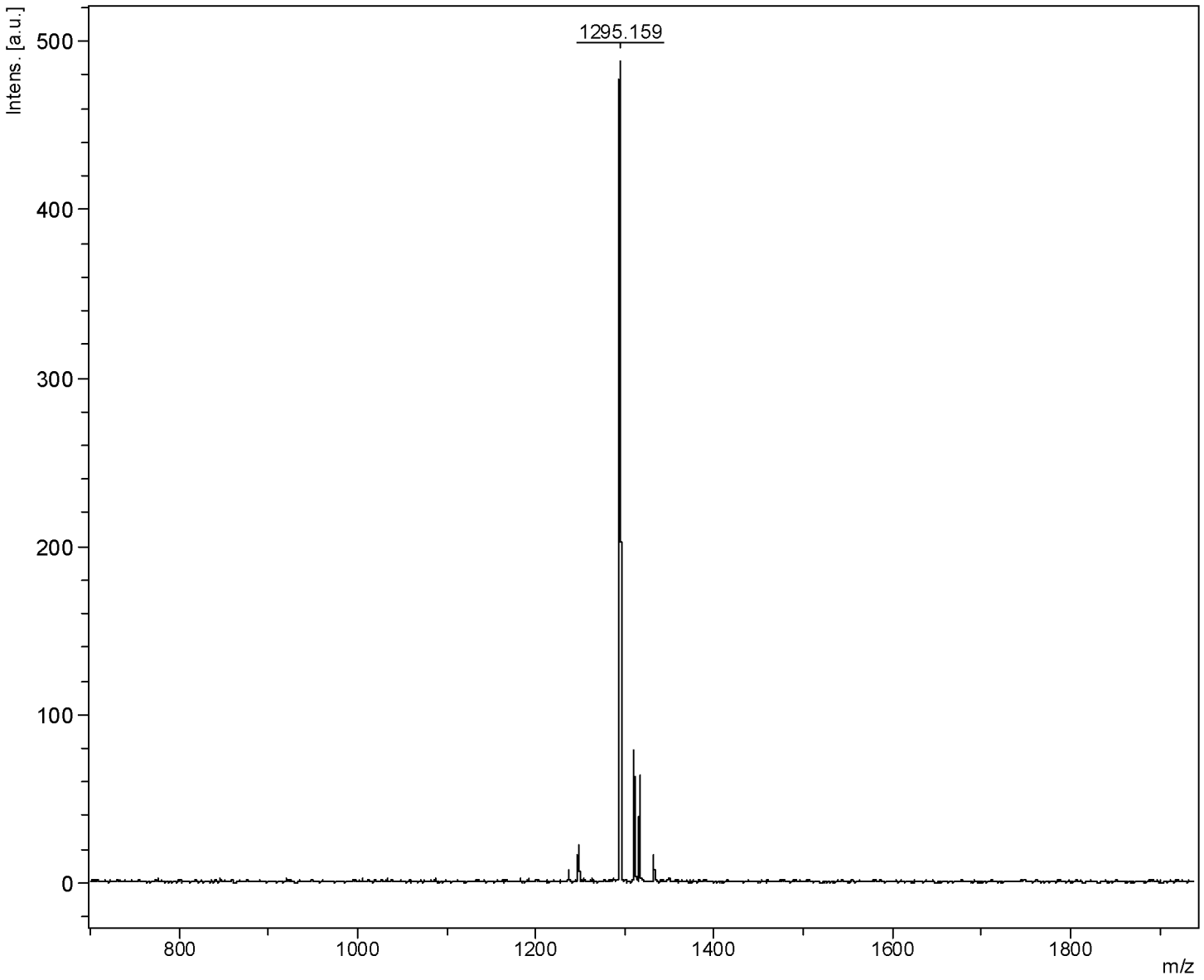

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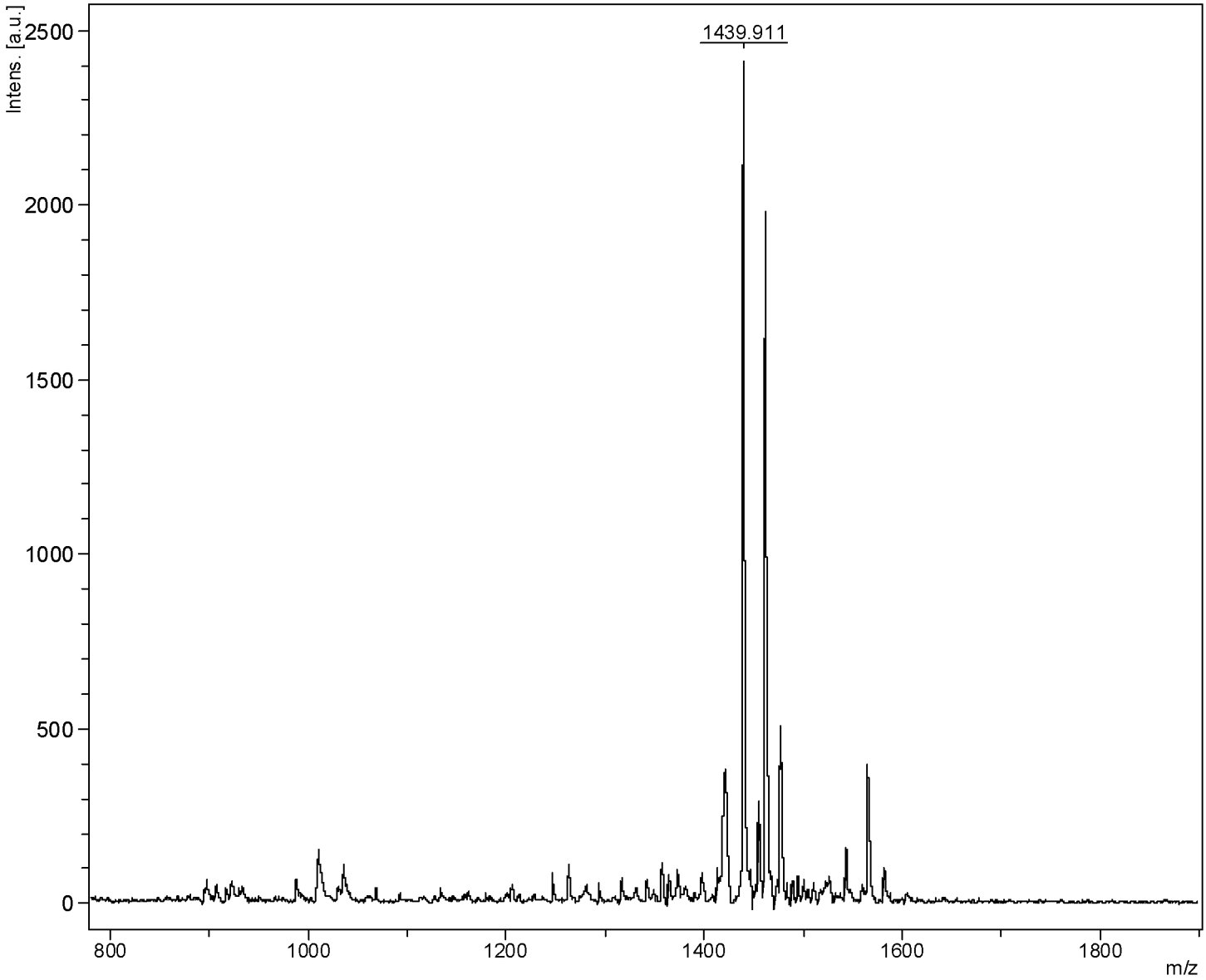

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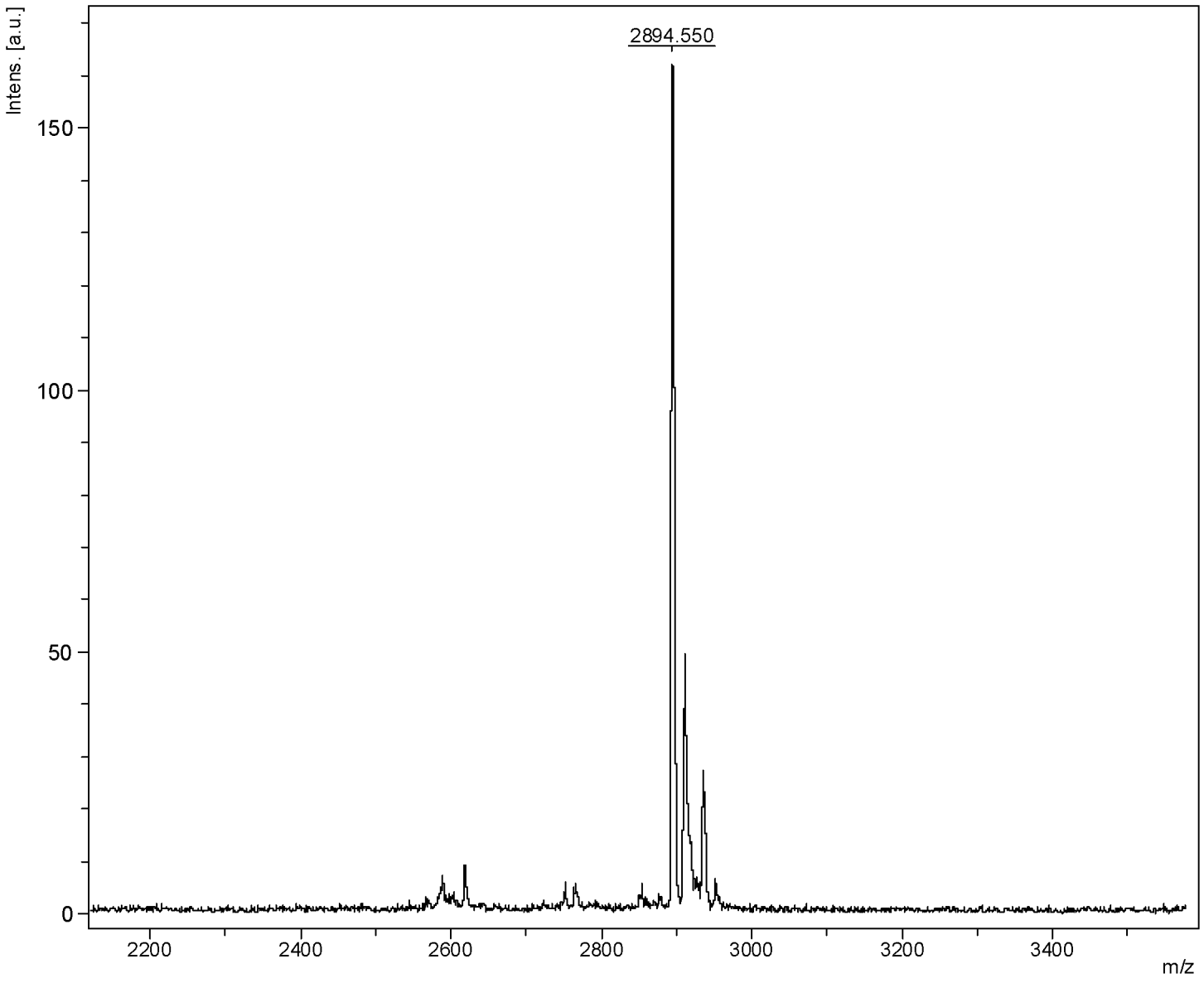

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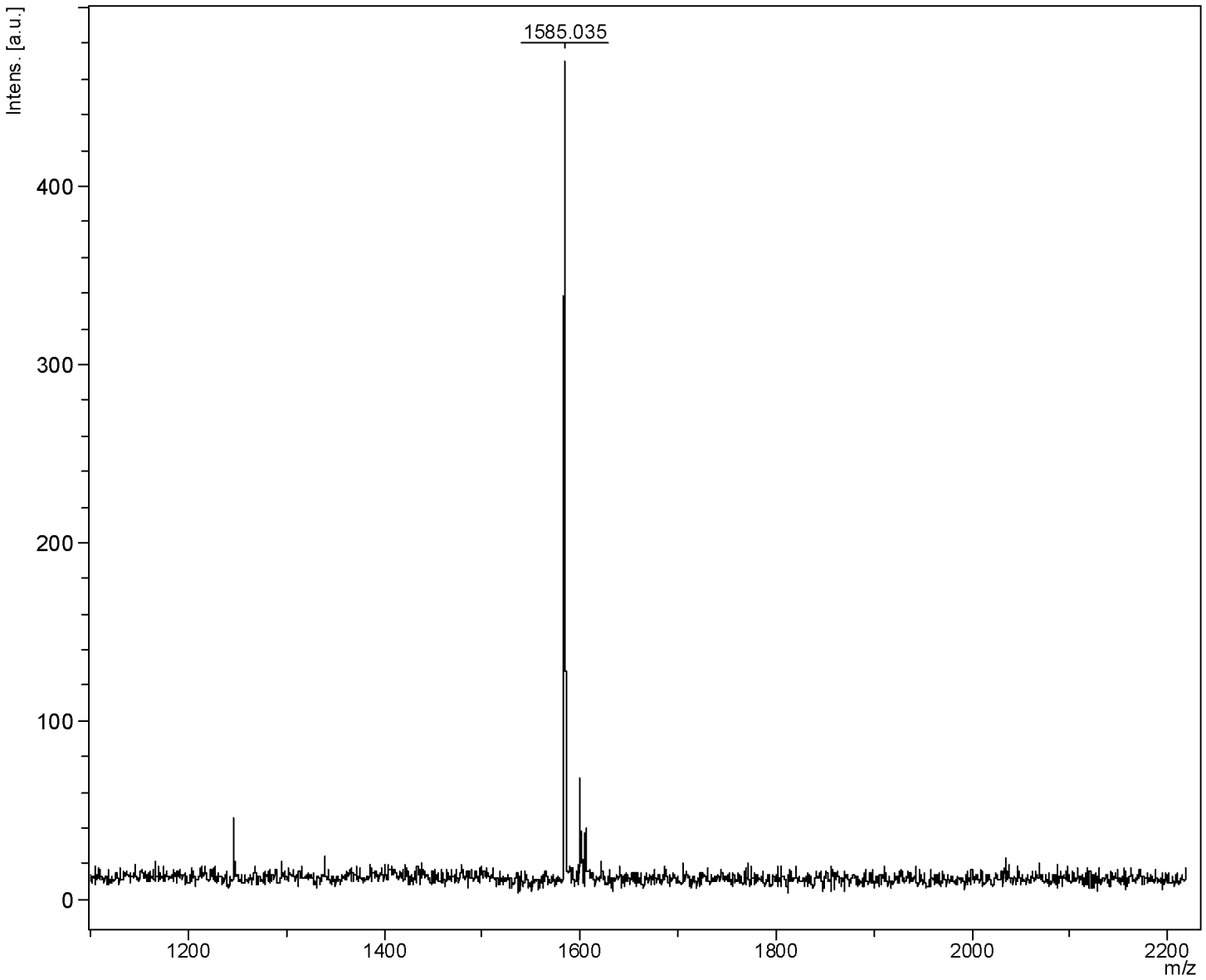

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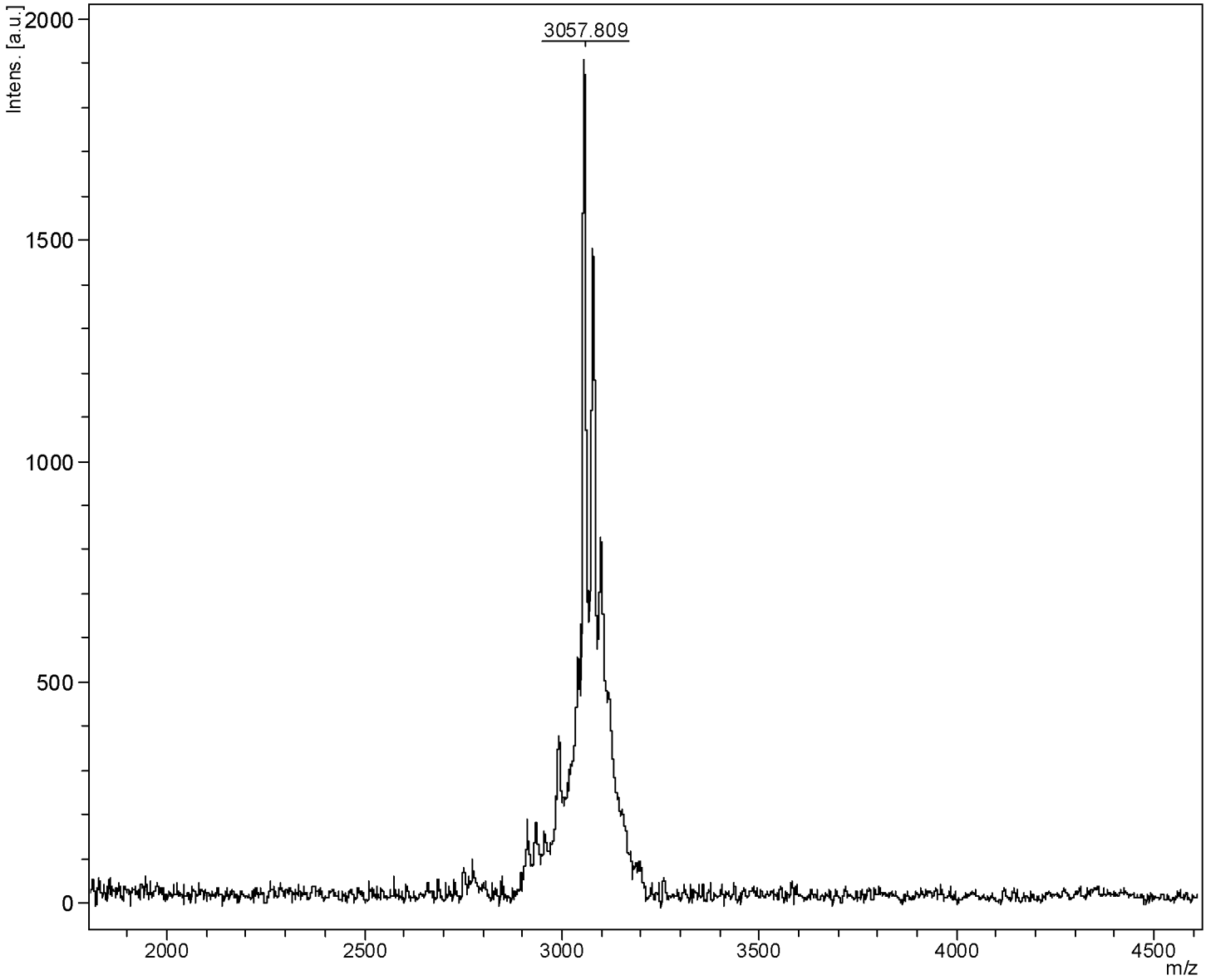

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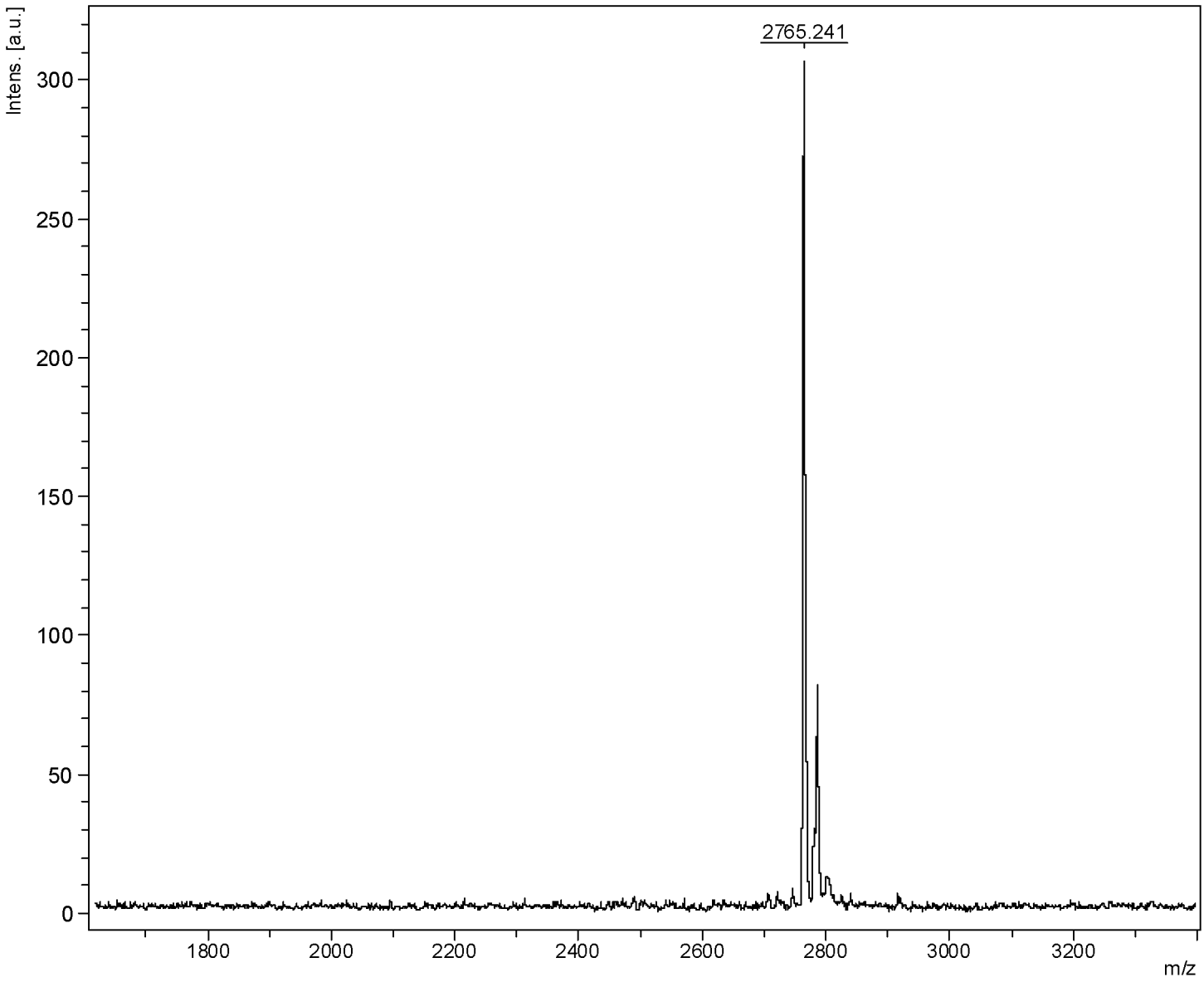

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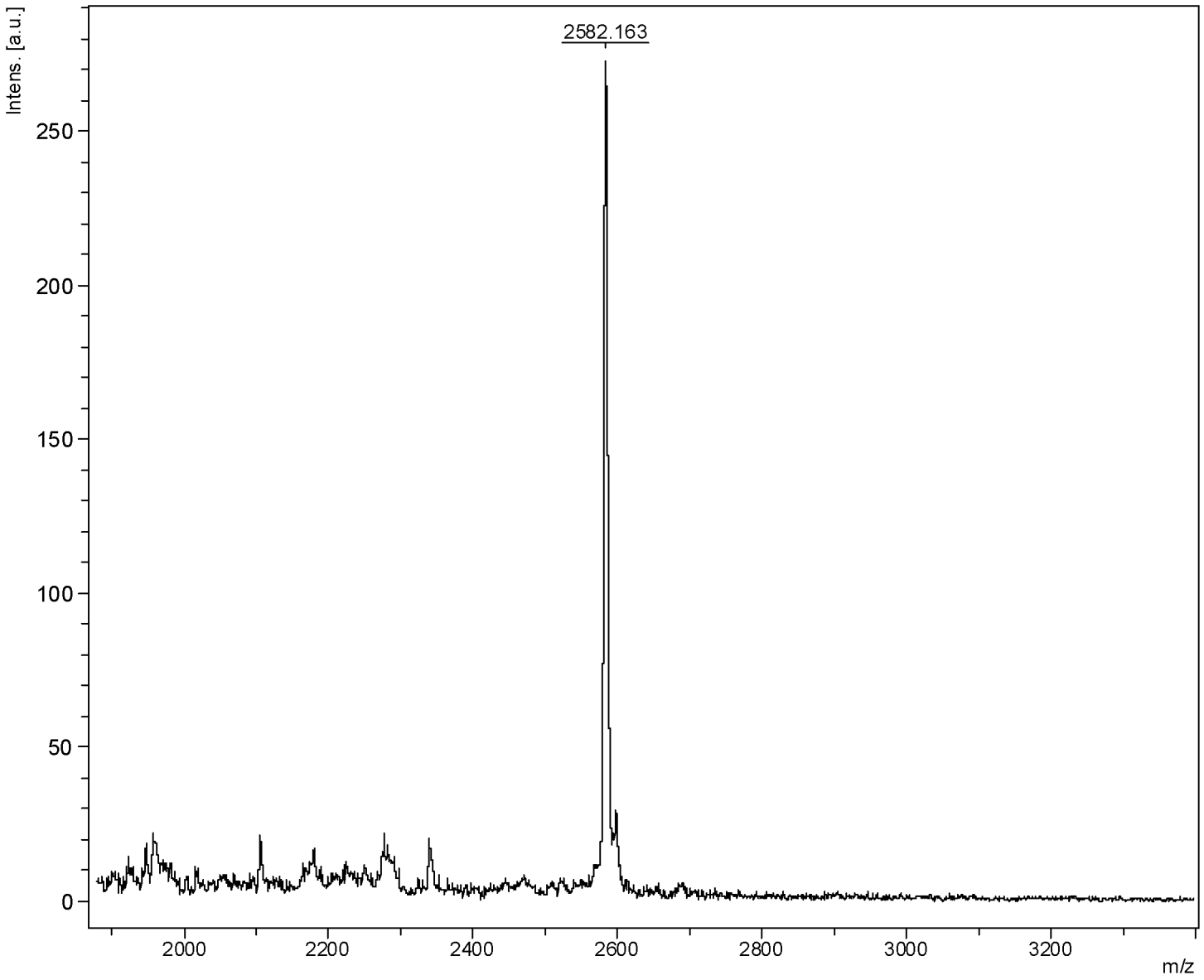

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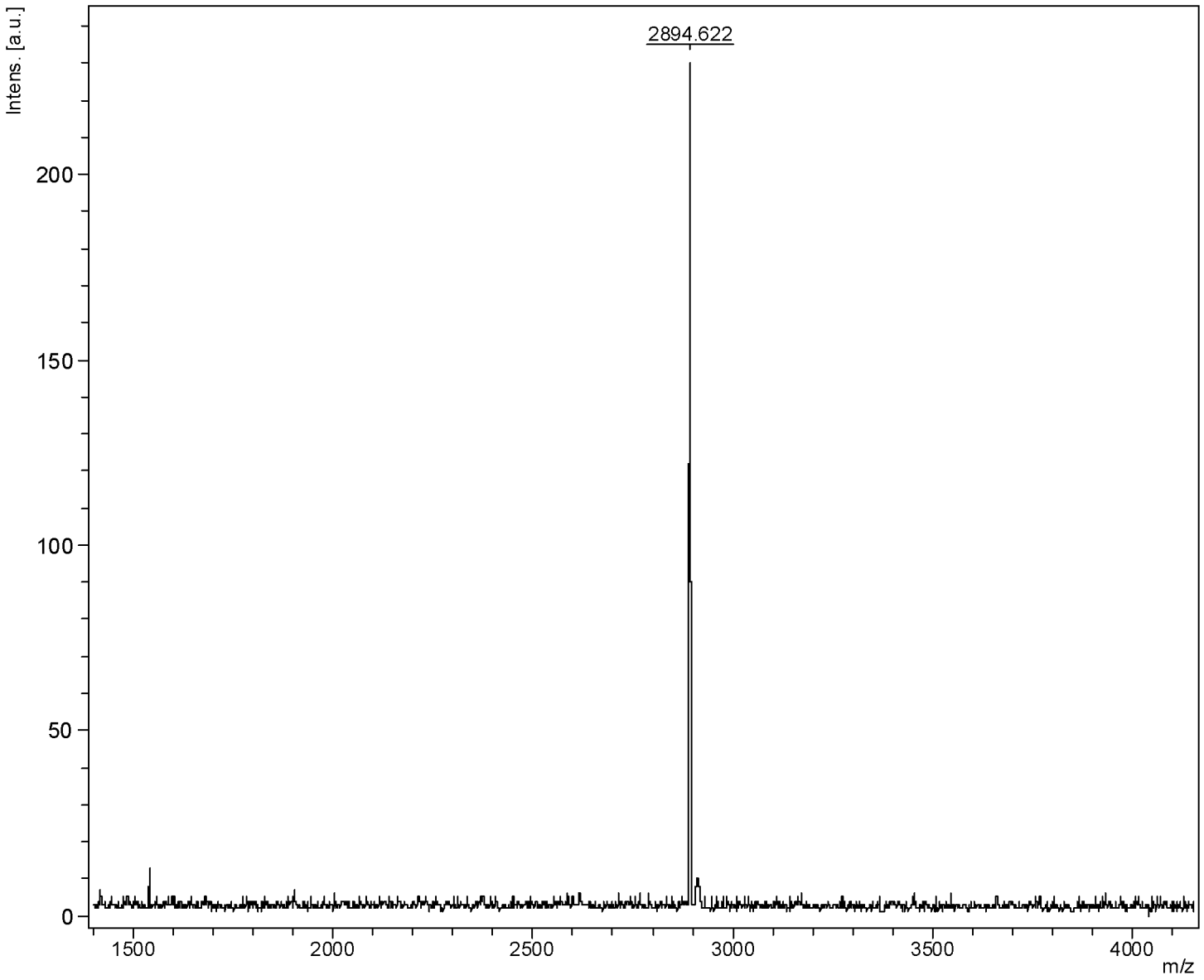

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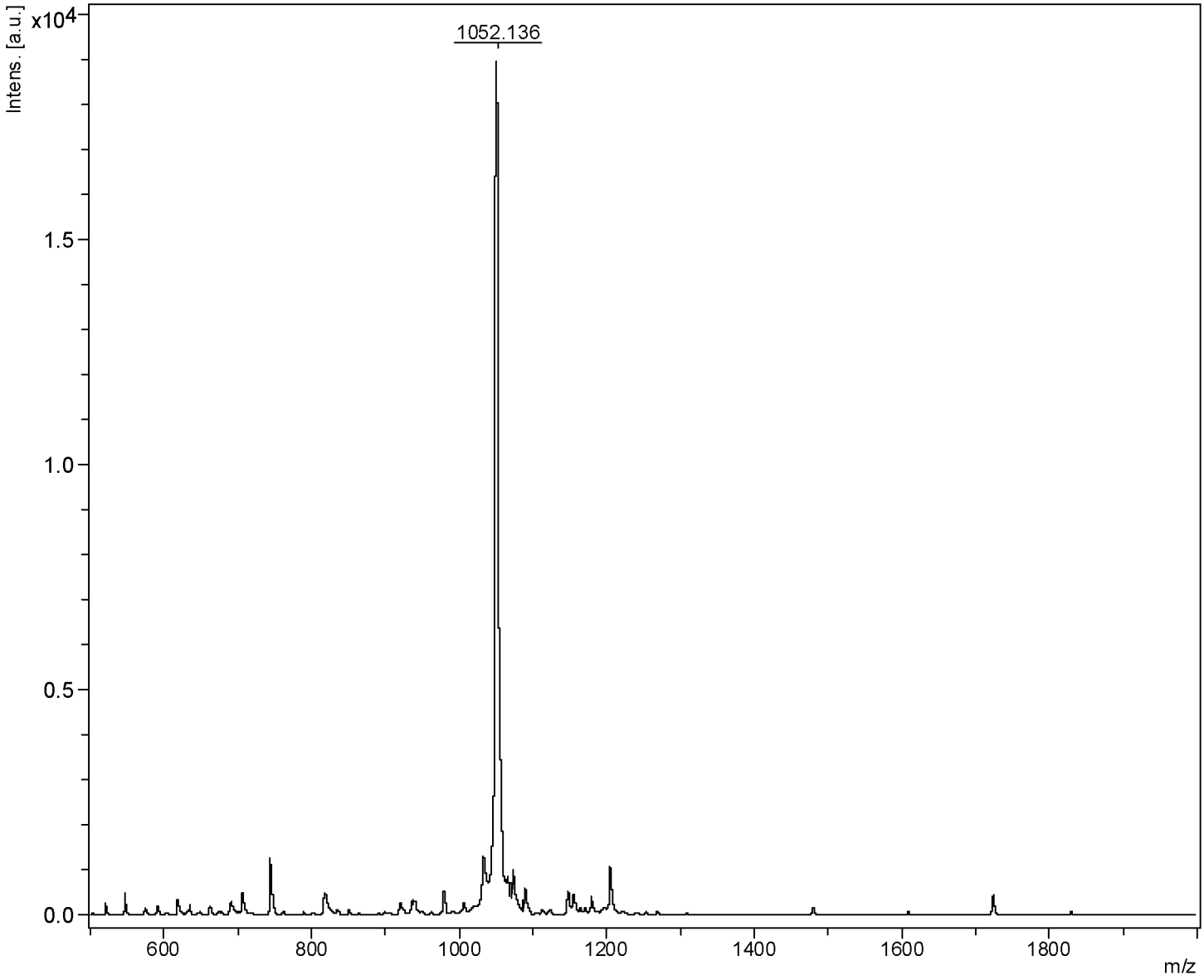

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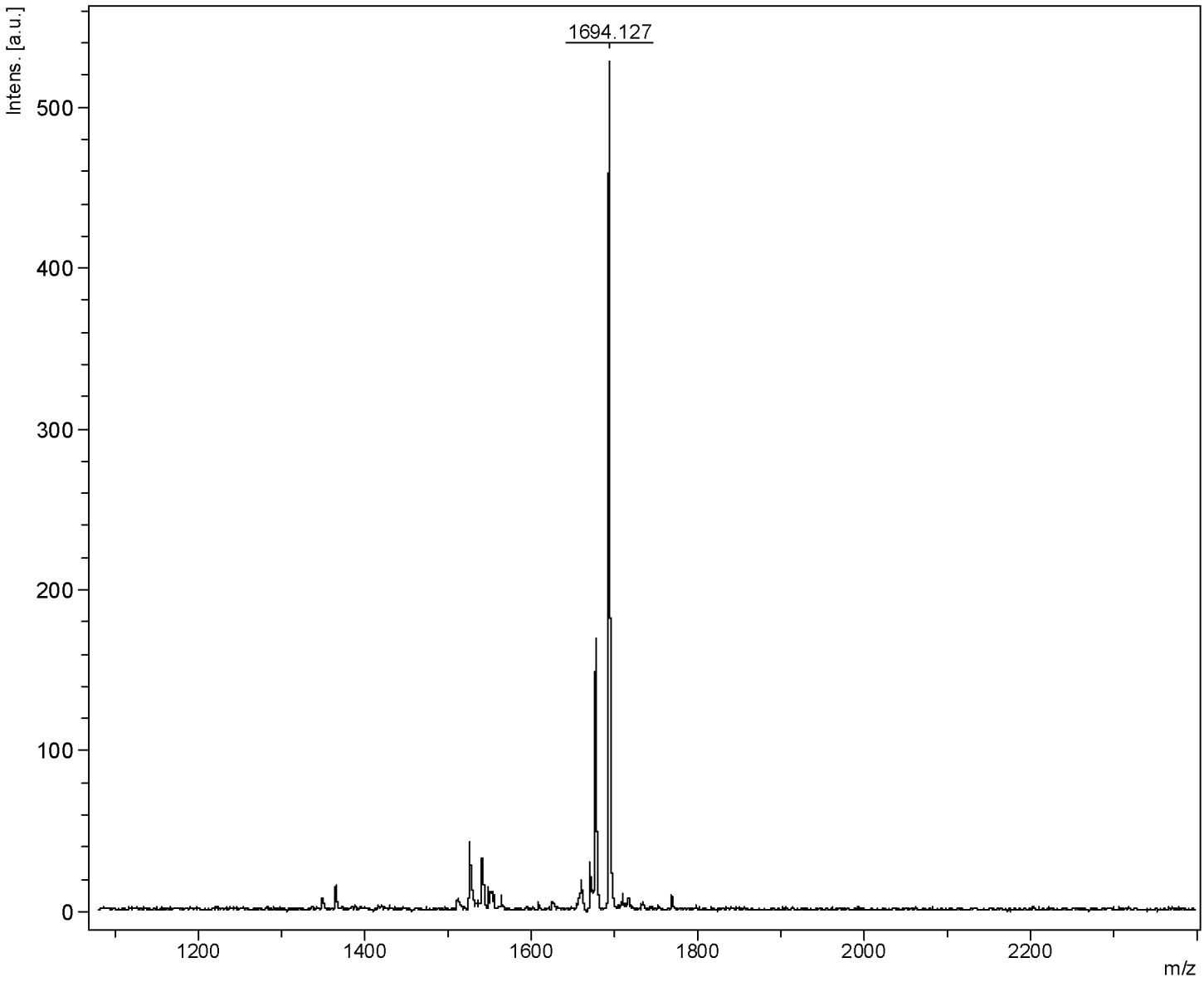

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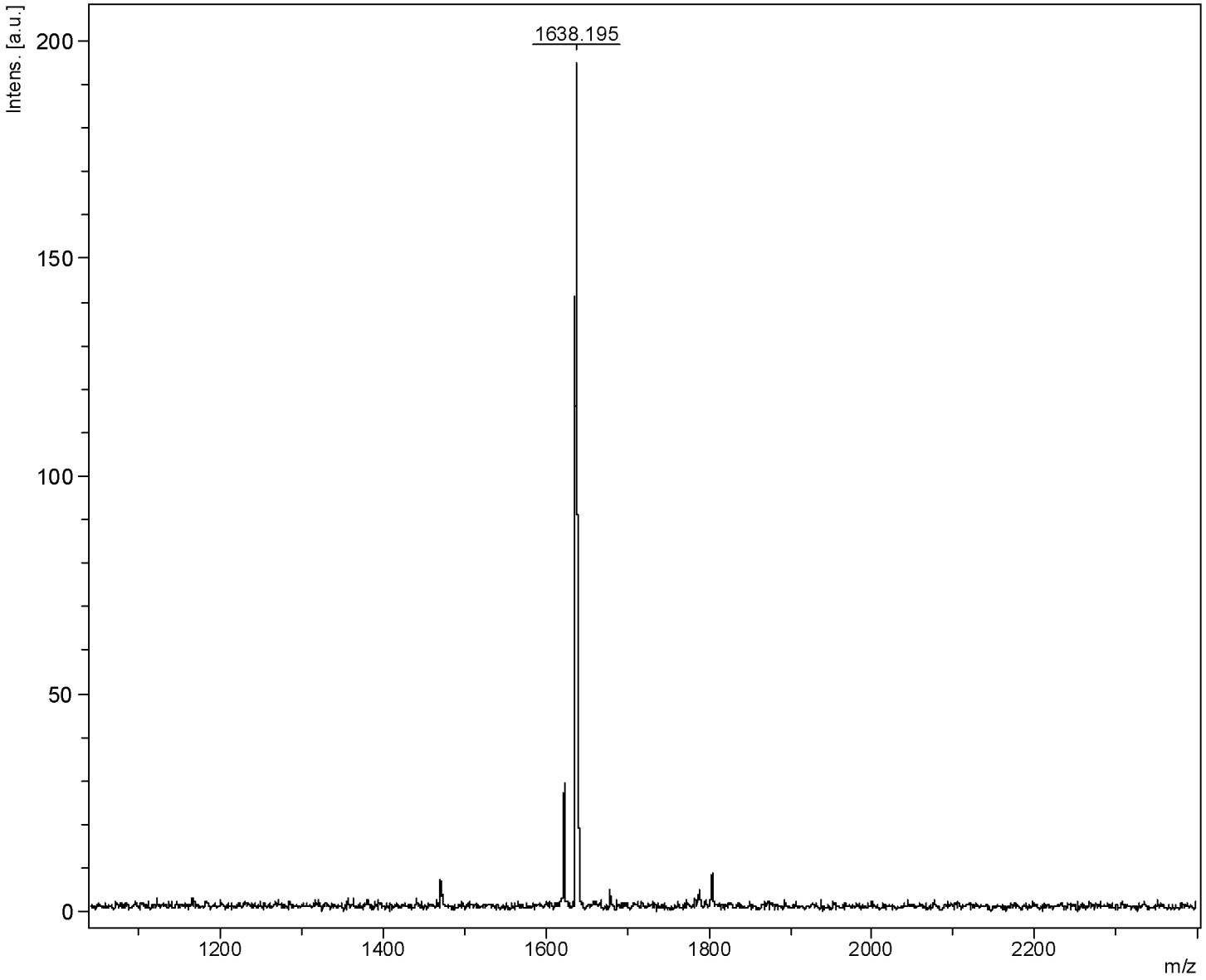

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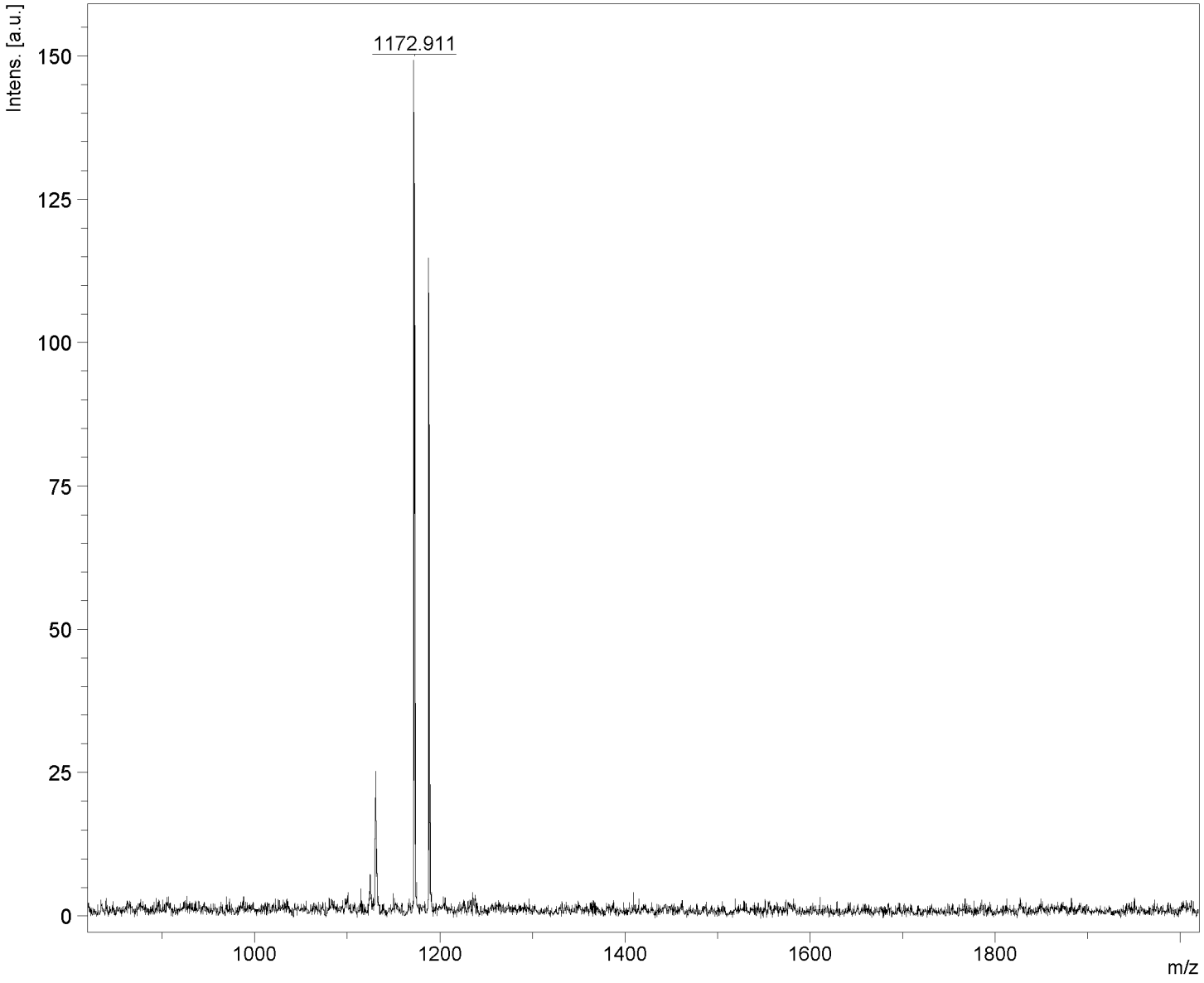

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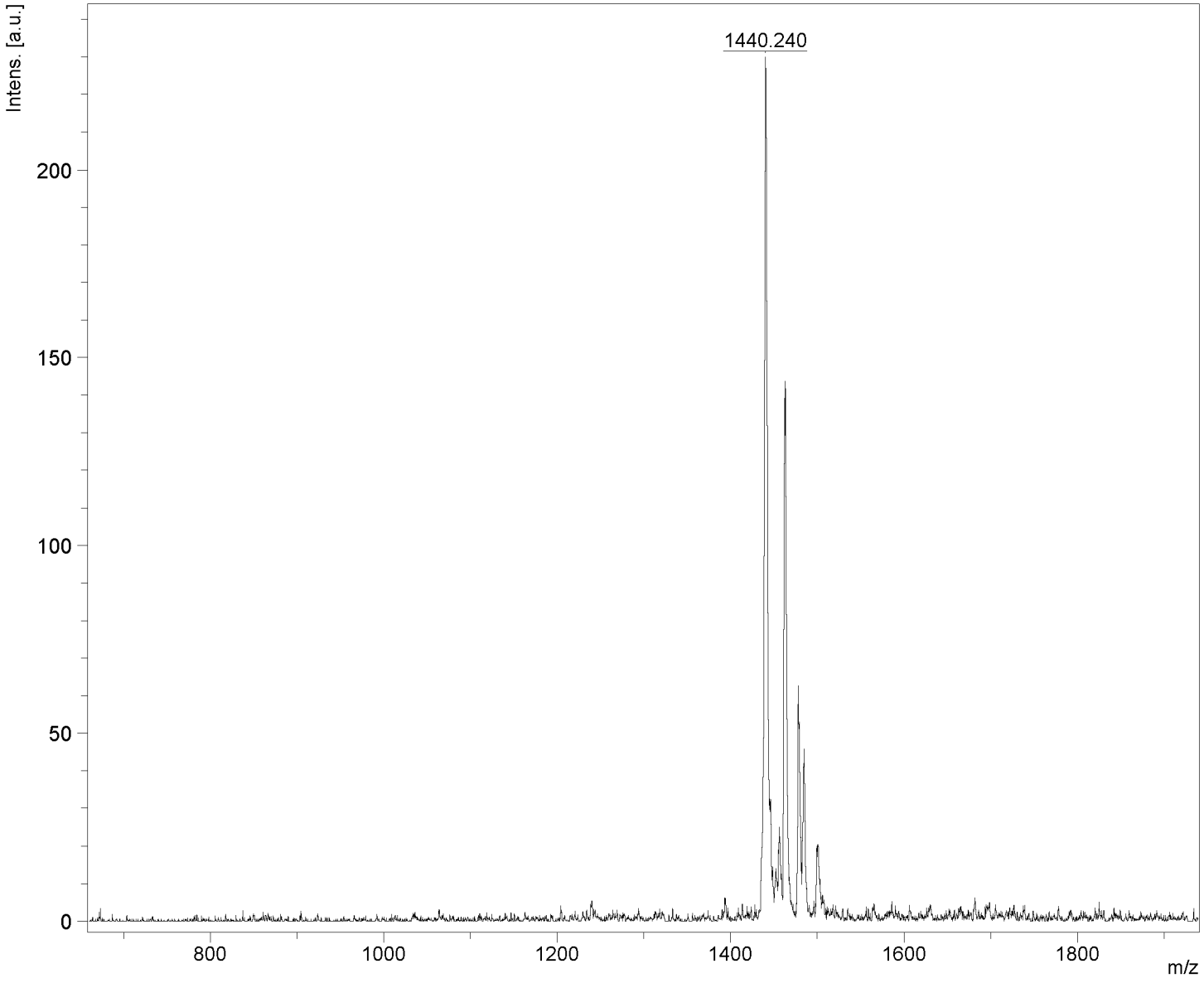

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Instrument Info

Supporting Information

Supporting Figures

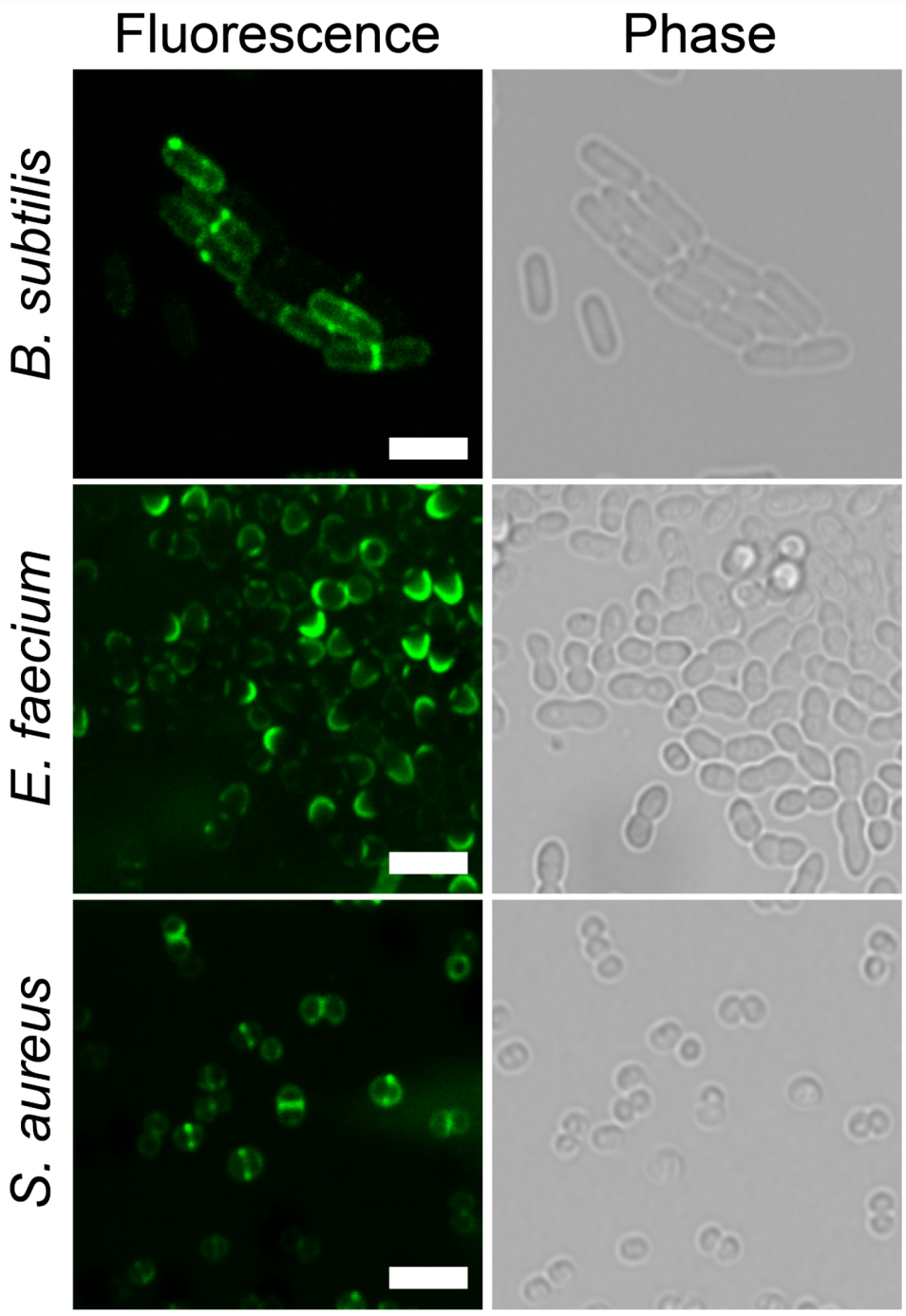


Figure S1. Confocal microscopy images of *B. subtilis*, *S. aureus*, and *E. faecium* treated with BODIPY® FL vancomycin (1 μg/mL) for 30 min, washed three times with 1X PBS, and analyzed. Scale bar = 2 μm.

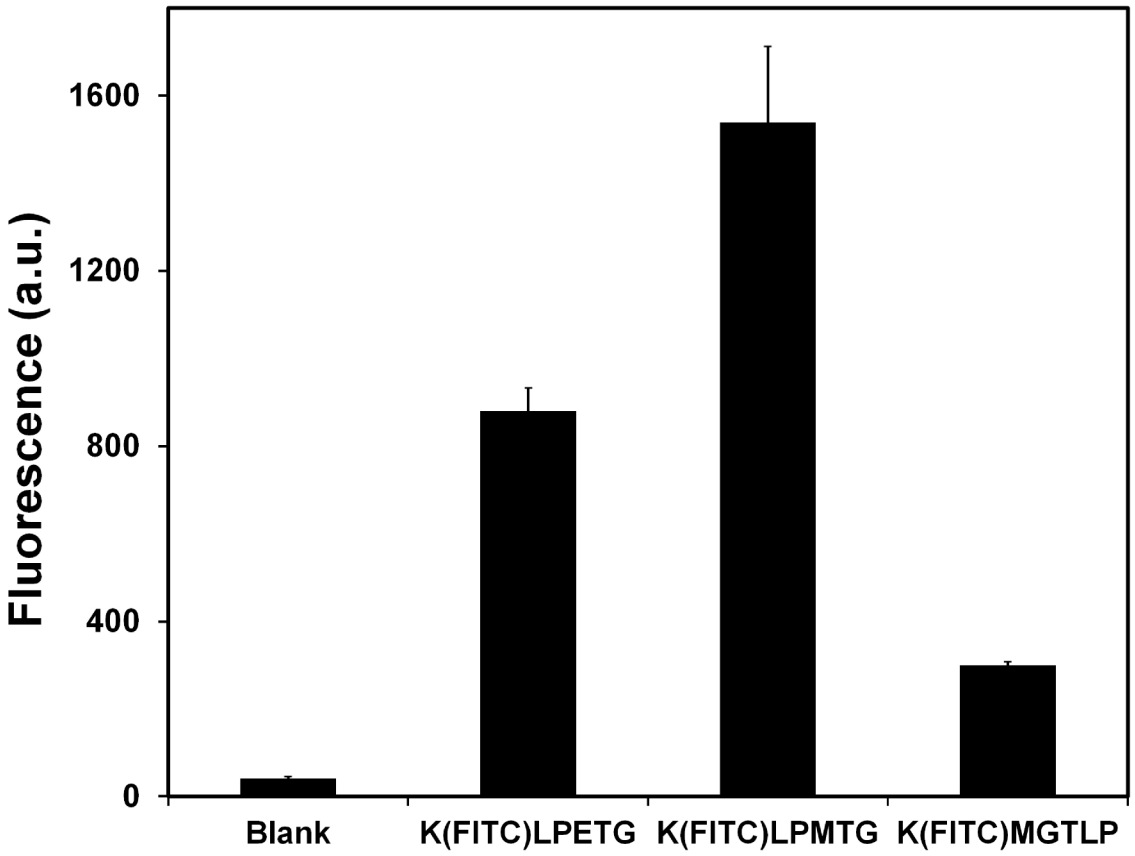


Figure S2. *S. aureus* Sc01 were grown in presence of FITC-labeled SrtA recognition peptides or a scrambled control at indicated concentrations overnight at 37 °C with shaking at 250 rpm in LB media. After 16 h, the bacteria were harvested, washed three times in 1X PBS, fixated with 2% formaldehyde solution and analyzed via flow cytometry. All data are represented as mean +SD (n = 3).

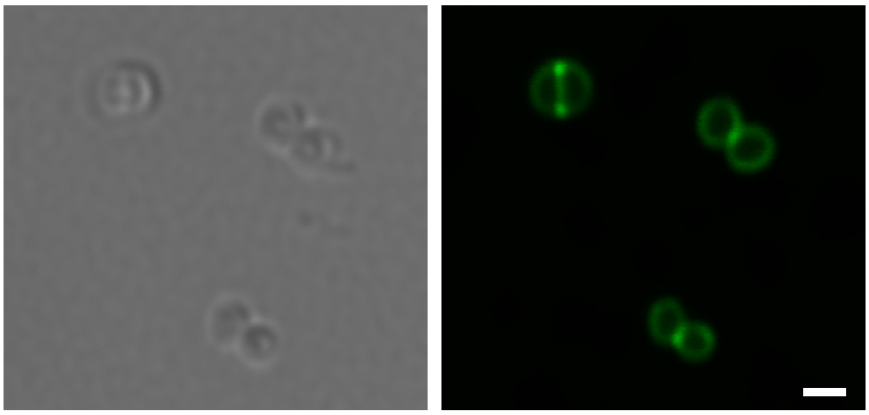


Figure S3. DIC and fluorescent confocal microscopy image of log phase *S. aureus* Sc01 treated with 5 μM **Sort1** for 15 min at 37 °C in LB media. Cells were washed and imaged. Scale bar represents 1 μm.

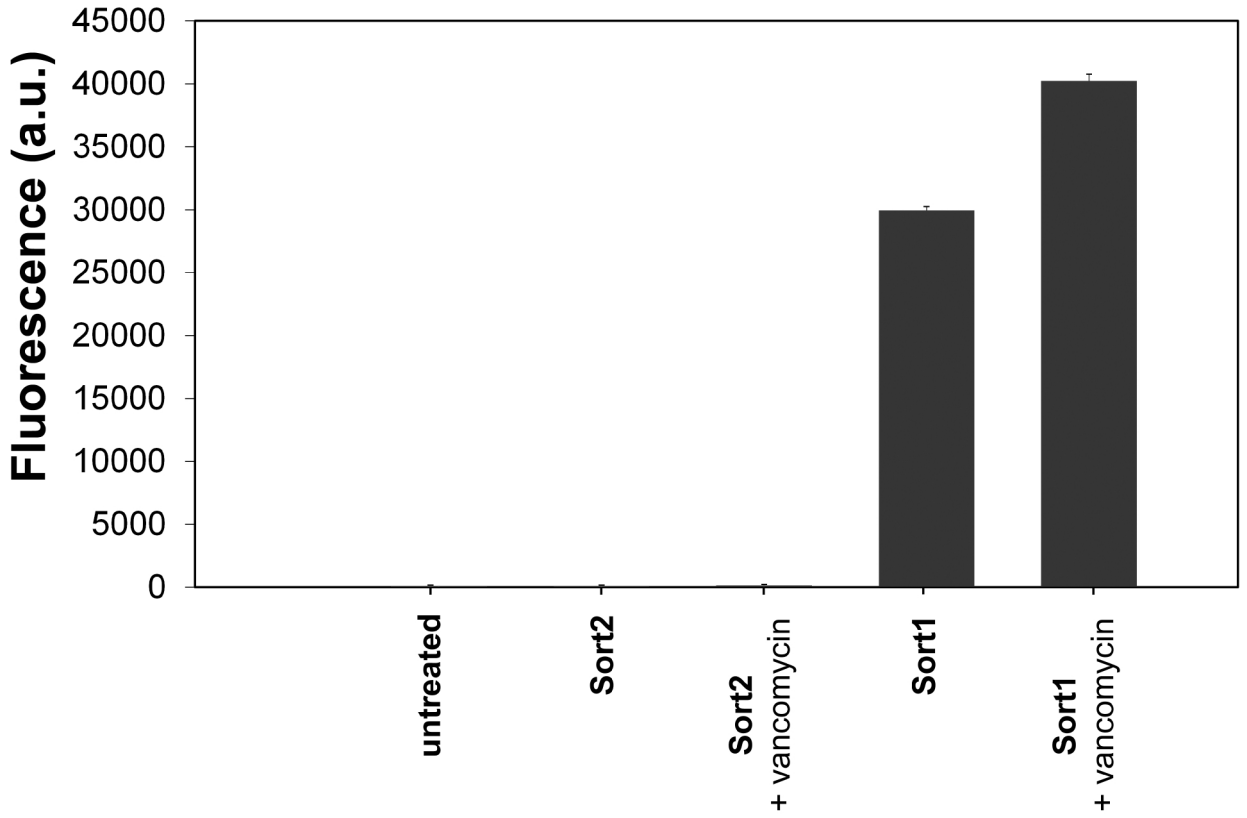


Figure S4. An overnight culture of *S. aureus* Sc01 was diluted to an OD = 0.4 in LB medium supplemented with 5 μM of **Sort1** with or without 5 μM vancomycin or 5 μM **Sort2** with or without 5μM vancomycin for 4 h at 37 °C with shaking at 250 rpm. Bacteria were then harvested, washed three times in 1X PBS, fixated with 2% formaldehyde solution and analyzed via flow cytometry. All data are represented as mean +SD (n = 3).

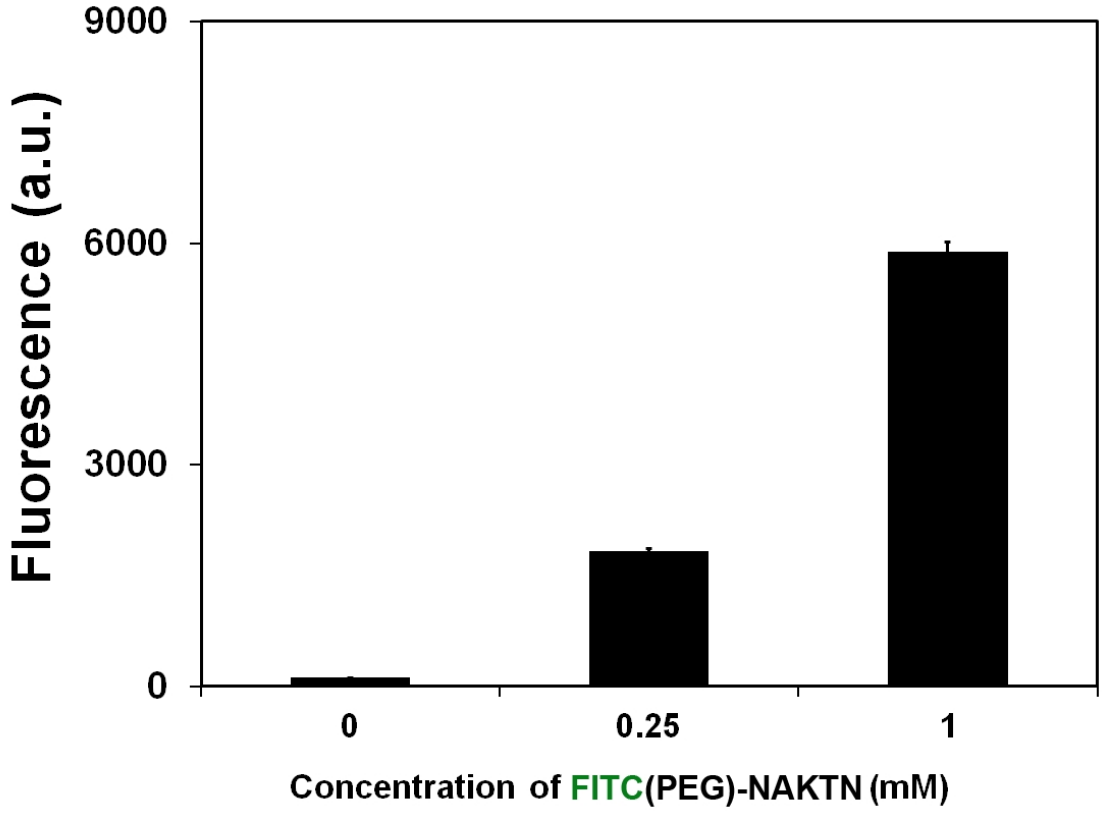


Figure S5. *Listeria monocytogenes* were grown in presence of FITC-labeled SrtB recognition peptide at indicated concentrations overnight at 37 °C with shaking at 250 rpm in BHI media. After 16 h, the bacteria were harvested, washed three times in 1X PBS, fixated with 2% formaldehyde solution and analyzed via flow cytometry. All data are represented as mean +SD (n = 3).

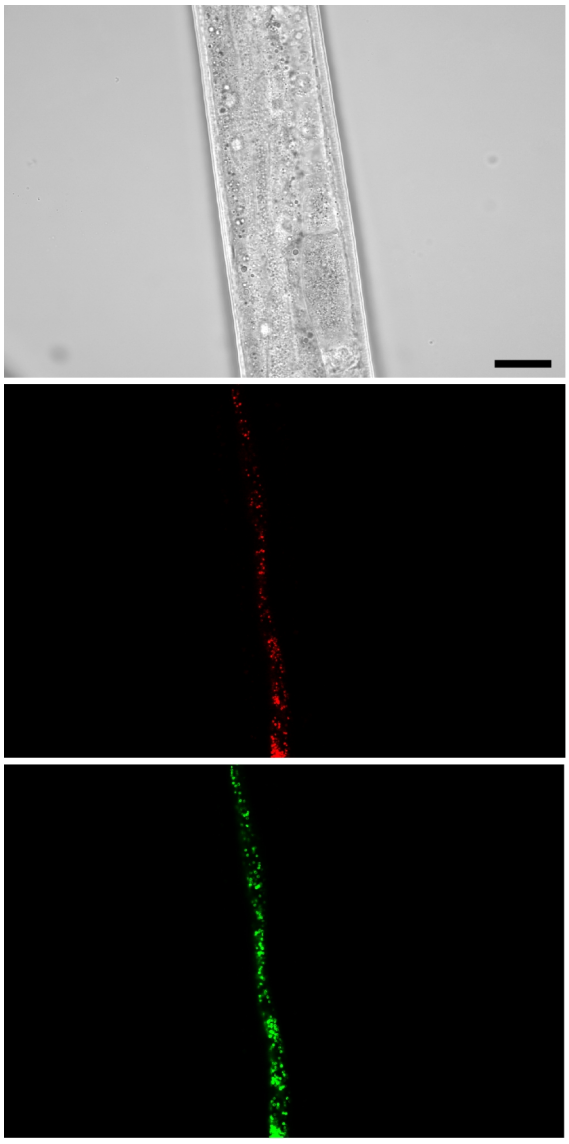


Figure S6. *C. elegans* infected with *S. aureus* (expressing mCherry) were treated with **Sort1** (50 μM), washed, anesthetized, mounted on a bed of agarose, and imaged using confocal microscopy. Scale bar presents 20 μm. All data has been represented from three separate experiments.

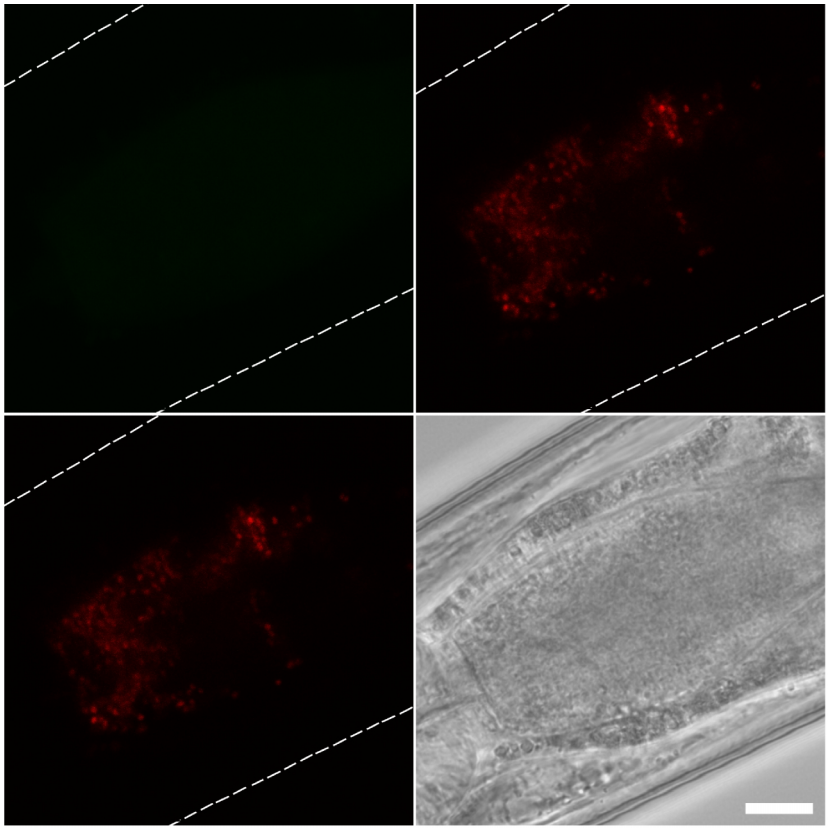


Figure S7. *C. elegans* infected with *S. aureus* (expressing mCherry) were treated with **Sort2** (50 μM), washed, anesthetized, mounted on a bed of agarose, and imaged using confocal microscopy. Scale bar presents 20 μm. All data has been represented from three separate experiments.

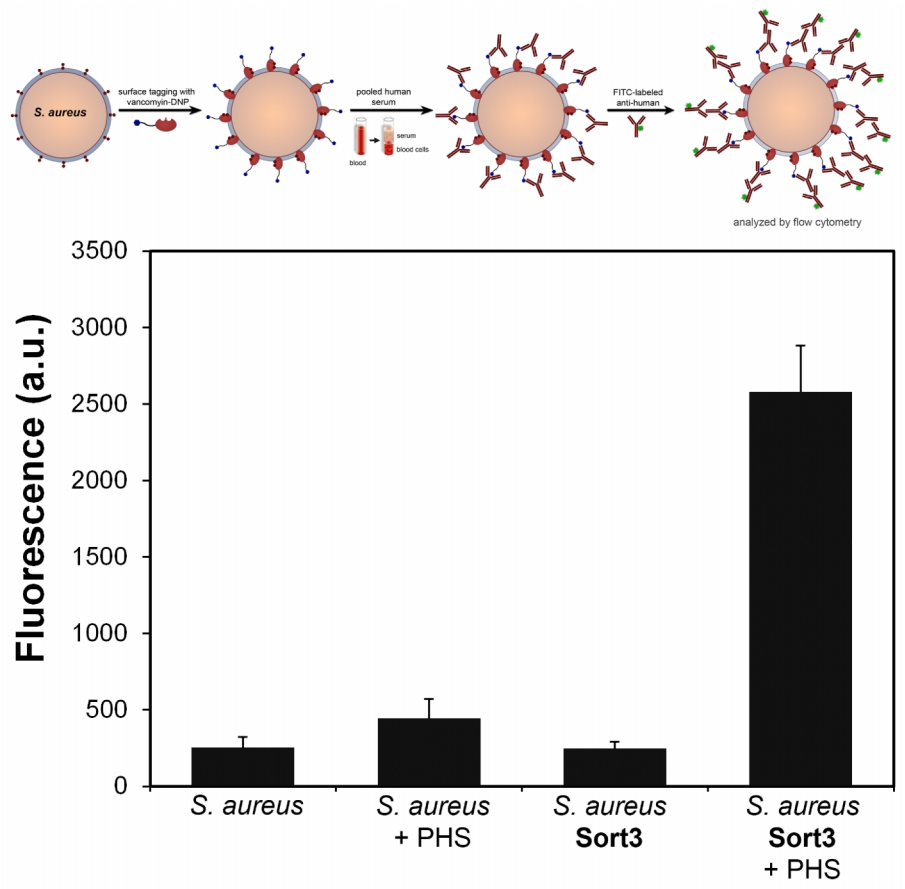


Figure S8. *Top:* Schematic diagram describing the steps used to detect anti-DNP recruitment directly from pool human serum (PHS). *Bottom: S. aureus* cells were incubated with or without 5 μM of **Sort3** overnight at 37 °C. Cells were subsequently incubated with either 15% pooled human serum or PBS, followed by the incubation with FITC-labeled anti-human IgG antibodies and analyzed using flow cytometry. Data are represented as mean + SD (n =3).

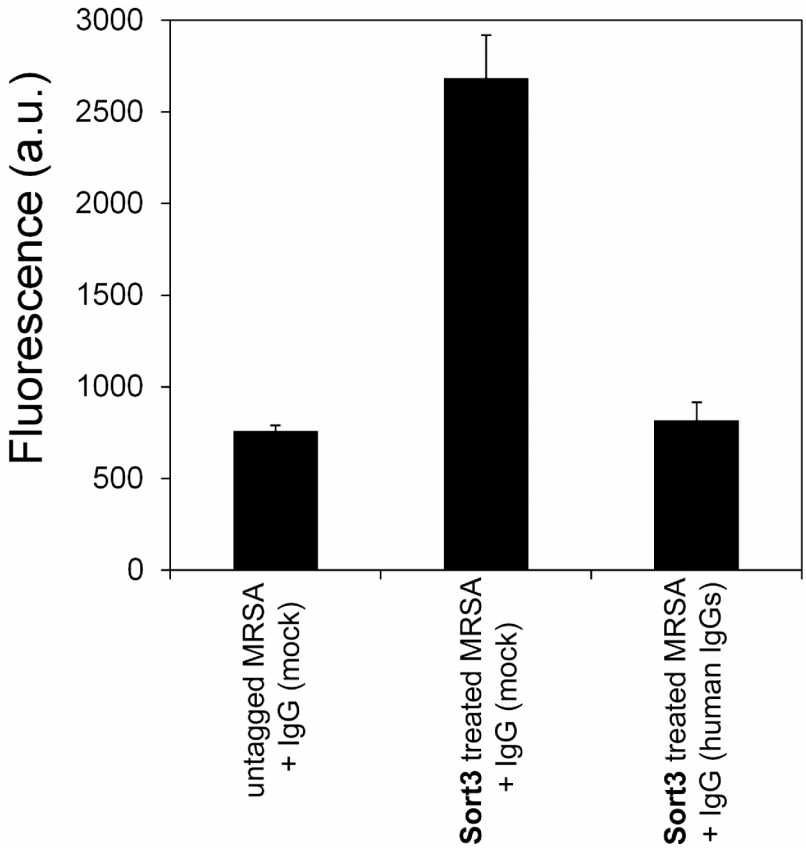


Figure S9. *S. aureus* Sc01 were grown at 37 °C overnight in LB broth, supplemented with 0.4 µg mL-1 tunicamycin and **Sort3** (5 µM) with shaking at 250 rpm. The bacteria were harvested, washed, and subsequently incubated with either polyclonal antibody or pooled human IgG antibodies for 20 min at 4 °C. Cells were washed and then incubated with FITC-conjugated rabbit anti-dinitrophenyl IgG for 30 min at 4 °C. Cells were washed 1X PBS, fixated in 2 % formaldehyde and analyzed by flow cytometry. Fluorescence data are represented as mean +SD (n = 3).

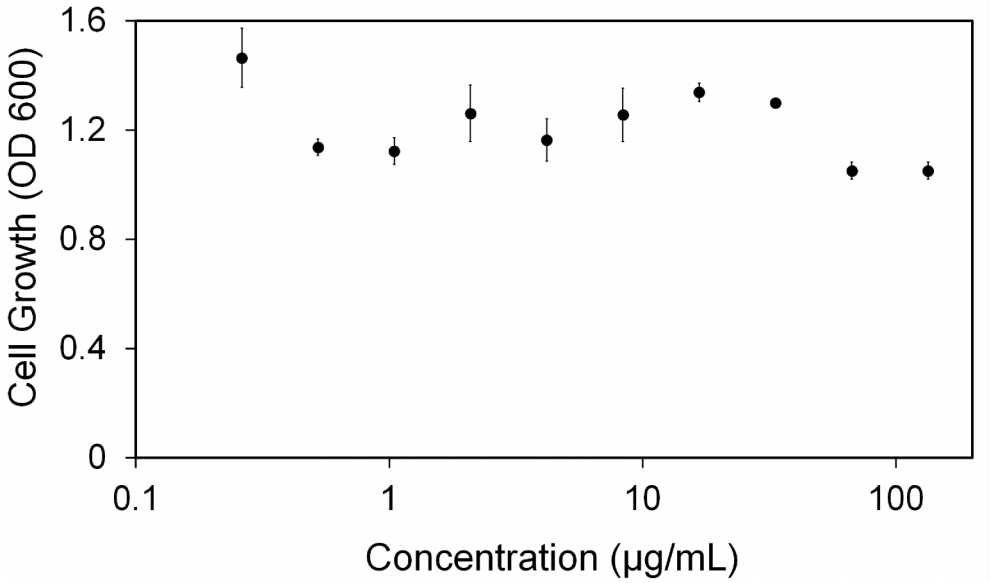


Figure S10. **Sort3** toxicity towards *S. aureus* Sc01. *S. aureus* Sc01 cells were incubated with varying concentrations of construct for 18 h at 37 °C. Bacterial viability was evaluated by measuring the absorbance at 600 nm. Data are represented as mean ± SD (n = 3).

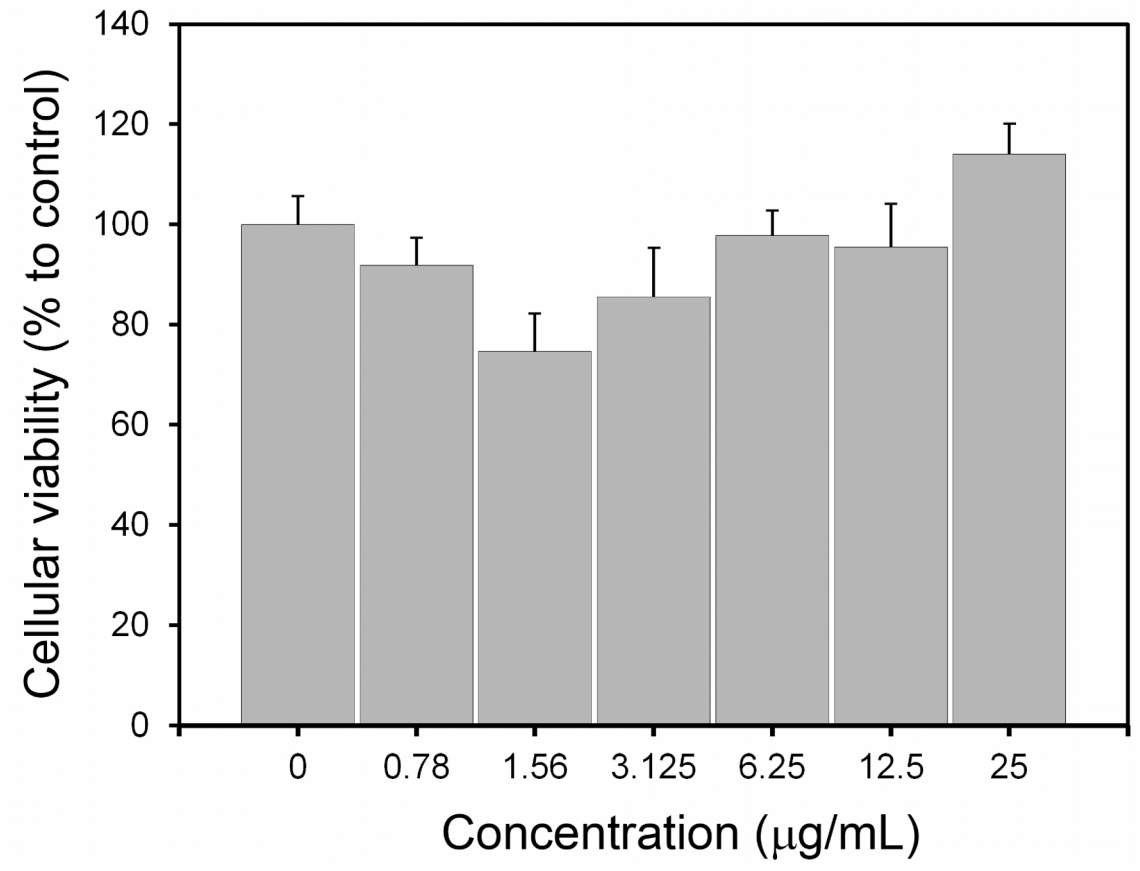


Figure S11. HEK293 cells were incubated for 24 hours in the absence or the presence of increasing concentrations of **Sort3**. Cellular viability was evaluated with MTT by measuring absorbance at 580 nm. All data are represented as mean + SD (n = 3).

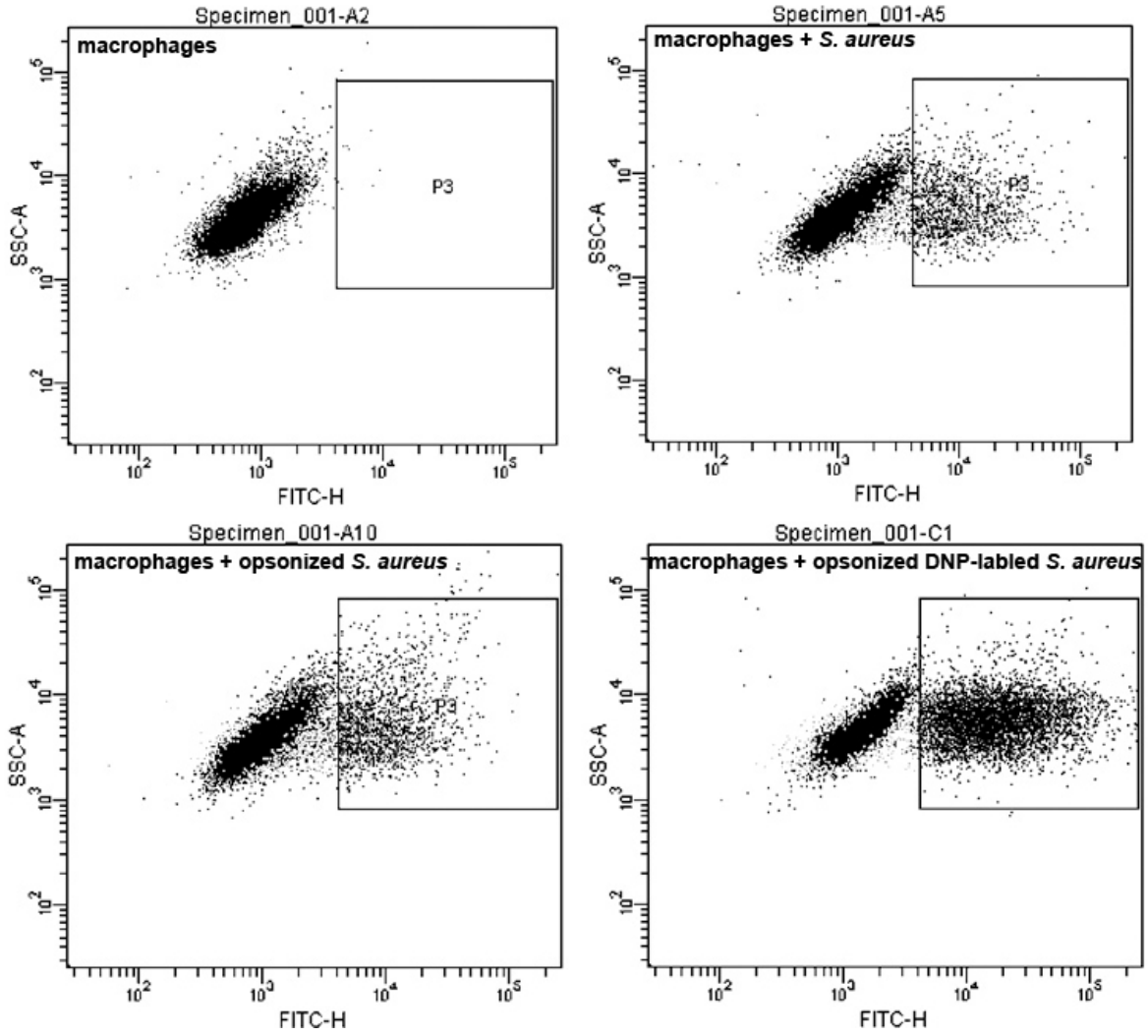


Figure S12. Plots generated from flow cytometry experiments measuring the **Sort3**-mediated antibody-dependent phagocytosis of calcein-AM labeled *S. aureus* by J774A.1 macrophages. Upper left plot represents J774A.1 macrophages alone and lower right plot represents J774A.1 macrophages incubated with opsonized **Sort3**-lableled *S. aureus*. The right rectangular gates in all plots represent cell events indicative of phagocytosis.

Materials and Methods

Materials. All peptide related reagents and protected amino acids were purchased from Chem-Impex. Fluorescein 5-isothiocyanate and 5, 6-carboxyfluorescein were purchased from Chem-Impex. Amino-PEG16-24-acid, DNP-PEG2-12-acid, and Fmoc-N-amido-PEG12-acid compounds were purchased from Broadpharm. Antibody reagents were purchased from Vector Laboratories. Purified Human IgG, Normal Serum was purchased from Bethyl Laboratories. Dimethyl sulfoxide (DMSO) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Thermo Fisher Scientific Inc. Fetal Bovine Serum (FBS) was purchased from Corning. Penicillin-Streptomycin was purchased from Sigma-Aldrich. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from EMD Millipore. All other organic chemical reagents were purchased from Fisher Scientific or Sigma Aldrich and used without further purification.

Bacteria Cell Culture. Bacterial cells were cultured in specific media in an aerobic environment shaking at 250 rpm at 37 °C. *S. aureus* (Sc01), *S. aureus* (Newman), *S. aureus* HG001(AH2183) + pAH9 (PsarA_mCherry, ermR), *S. epidermidis* (NRS101), *B. subtilis* (NCIB 3610), *E. coli* (MG1655) were all grown in Luria Bertani (LB) medium. *Enterococcus faecium* (ATCC BAA-2127) was grown in Tryptic Soy Broth (TSB) and *L. monocytogenes* (10403S) was grown in Brain Heart Infusion (BHI) medium.

Mammalian Cell Culture. Human embryonic kidney 293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 100 U/mL penicillin, and 0.1 mg/mL streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C.

SrtA Mediated Fluorescent Labeling. Bacteria were grown in presence of FITC-labeled SrtA recognition peptides or FITC-labeled vancomycin-conjugated SrtA recognition peptides (5 μM) overnight at 37 °C with shaking at 250 rpm in appropriate media. After 16 h, the bacteria were harvested, washed three times in 1X phosphate buffered saline (PBS), fixated with 2% formaldehyde solution and analyzed via flow cytometry on a BDFacs Canto II flow cytometer (BD Biosciences, San Jose, CA) equipped with a 488 nm argon laser and a 530 bandpass filter (FL1). A minimum of 10,000 events were counted for each data point. The data were analyzed using the FACSDiva version 6.1.1 software. For confocal microscopy, a Nikon Eclipse Ti-E was used with 488nm-excitation and 505-550 band pass emission filter for FITC.

Antibody Binding Assay in *S. aureus* (wood strain). *S. aureus* (wood strain) bacteria were grown at 37 °C overnight in LB broth, supplemented with 0.4 μg mL-1 tunicamycin and either Sort 3 or Sort4 at designated concentrations with shaking at 250 rpm. The bacteria were harvested and washed three times with 1X PBS. Approximately 2×10^6 colony forming units (CFU) were then incubated in 100 μL of PBS containing 10% (v/v) FBS and 0.02 μg / mL of FITC-conjugated rabbit anti-dinitrophenyl IgG. All experiments were protected from light and incubated at 37 °C for 1 hour. Samples were then washed with 1X PBS and fixated in 2% formaldehyde and analyzed by flow cytometry.

Fluorescence data are expressed as mean arbitrary fluorescence units and were gated to include all healthy bacteria.

Antibody Binding Assay in *S. aureus.* *S. aureus* Sc01 bacteria were grown at 37 °C overnight in LB broth, supplemented with 0.4 µg mL-1 tunicamycin and Sort4 (5 µM) with shaking at 250 rpm. The bacteria were harvested and washed three times with 1X phosphate buffer saline (PBS). Either polyclonal antibody (0.02 µg / mL) or pooled human IgG antibodies (0.02 µg / mL) was added to bacteria for 20 min at 4 °C. Cells were washed and roughly 2 × 106 colony forming units (CFU) were then incubated in 100 µL of PBS containing 10% (v/v) FBS and 0.02 µg / mL of FITC-conjugated rabbit anti-dinitrophenyl IgG. All experiments were protected from light and incubated at 4 °C for 30 min. Cells were washed 1X PBS, fixated in 2 % formaldehyde and analyzed by flow cytometry. Fluorescence data are represented as mean +SD (n = 3) and gated to include all healthy bacteria.

***S. aureus* Opsonization with Pooled Human Serum.** *S. aureus* (wood strain) bacteria were grown at 37 °C overnight in LB broth, supplemented with 0.4 µg mL-1 tunicamycin and either Sort3 or Sort4 (5 µM) with shaking at 250 rpm. The bacteria were harvested and washed three times with 1X PBS. Pooled human serum was diluted to 25% in PBS and incubated with bentonite for 20 min at 37 °C. The serum supernatant was obtained and diluted to 15% in PBS solution with 10 % FBS and incubated with the bacteria for 20 min on ice. The opsonized bacteria were then washed with PBS and 2 × 10^6 colony forming units (CFU) were incubated with Anti-Human IgG-FITC diluted 1:1000 in PBS containing 10% FBS at 4 °C for 30 min protected from light. Cells were washed 1X PBS and fixated in 2 % formaldehyde. Samples were then analyzed by flow cytometry as previously stated.

***S. aureus* Sc01 Viability Assay.** Toxicity of Sort3 in *S. aureus* Sc01 was determined by broth microdilution. Experiments were performed with Cation-adjusted Mueller-Hinton Broth (CaMHB) in 96-well polypropylene microtiter plates. Wells were inoculated with 200 µL of bacterial suspension prepared in CaMHB (containing ~10^6 colony forming units (CFU) / mL) and 200 µL of CaMHB containing increasing concentrations of the conjugates (0 to 100 µg/mL). Bacterial viability was evaluated by measuring the absorbance at 600 nm following an 18 h incubation at 37 °C. Data are represented as mean ± SD (n = 3).

Mammalian Cell Viability Assay. HEK293 cells were seeded in 96-well plates at a density of 6, 000 cells/well and incubated overnight. Prior to treatment, constructs were dissolved in DMSO to obtain desired aliquoted stock solutions. Appropriate volumes of these stock solutions were added to DMEM media so the final concentration of DMSO was equal to 1%. After removal of cell media, 200 µL of treatment solutions were added to each well and incubated at 37 °C for 72 h. After treatment, the media was removed and the cells were washed with 100 µL of complete DMEM. Next, 100 µL of complete DMEM was added to each well. Cell viability was determined using the colorimetric 3-(4,5-dimethylthiazol-2yl)-2,5 diphenyltetrazolium bromide (MTT) assay, in which 10 µL

of a 5 mg/mL MTT stock solution was added to the treated cells and incubated for 2 h at 37 °C. The resulting formazan crystals were solubilized in 200 µL of DMSO. Absorbance was measured at 580 nm using an Infinite 200 PRO microplate reader (Tecan). Cell viability was calculated against control cells treated with complete medium.

***S. aureus* Sc01 Labeling in live *C. elegans*.** *N2 Caenorhabditis elegans* were maintained by standard protocol[1] using nematode growth agar with bacterial lawns of E.coli OP50 (source) on a 60mm x 15mm cell culture dish. For bacterial labeling assays, *C. elegans* were grown to contain primarily L4 larval stage nematodes by incubation at 25 °C for ~48-52 h. On the day of experiments, *C. elegans* were washed off the plates with M9 buffer, and washed three times with M9 buffer. For washing steps, the *C. elegans* were pelleted at 1000g, resuspended in 450 µL of M9 buffer containing 10% LB broth and transferred to a sterile 24 multi-well plate. For infection, *Staphylococcus aureus* Sc01 or *Staphylococcus aureus* HG001(AH2183) + pAH9 (PsarA_mCherry, ermR) of an overnight growth was harvested at 6000g and washed three times with original culture volume of M9 buffer. The bacteria were resuspended in original culture volume in M9 buffer containing 10% LB broth and 50 µL of the bacterial cells were added to the 450 µL suspension of *C. elegans*. The *C. elegans* were incubated at 25 °C for 4 h, harvested at 1000g and washed three times with M9 buffer to remove excess bacteria in the extracellular space. The *C. elegans* were then resuspended in 500 µL of M9 buffer containing 10% LB broth and 50 µM Sort2. *C. elegans* were incubated for an additional 30 min at 25 °C. *C. elegans* were harvested at 1000g, and washed three times with M9 buffer, and put into a final suspension of 10 mM sodium azide in M9 buffer and analyzed by confocal microscopy.

Phagocytosis of Opsonized *S. aureus* (wood strain). *S. aureus* were inoculated (1:100) in LB broth supplemented with 5 µM Sort3 and allowed to grow overnight at 37 °C with shaking. After washing with PBS, the cells were resuspended in PBS containing 10 µM calcein-AM and incubated at 37 °C for 30 min. The bacteria were then washed three times with PBS and incubated with 20 µg/mL of rabbit antidinitrophenyl IgG-fraction KLH (2 mg mL−1, Life Technologies, catalog no. A6430) and 10% (v/v) heat inactivated FBS in PBS to opsonize the bacteria. J774A.1 cells were cultured at 37 °C (in the presence of 5% CO2) in DMEM medium supplemented with 50 µg/mL streptomycin, 50 IU/mL penicillin, 2 mM L-glutamine, and 10% heat inactivated FBS. On the day of the experiments, J774 cells were washed twice with Hank's Balanced Salt Solution (HBSS) by centrifuging 5 min at 250g at 4 °C. The washed J774A.1 cells were then mixed with opsonized *S. aureus* in a ratio of 1:3 and incubated in HBSS containing 5% FBS. The cell mixtures were then rotated at 37 °C for 20 min to induce phagocytosis, washed three times with cold HBSS, and fixed for 30 min in PBS with 2% formaldehyde. Following fixation, the cells were resuspended in 0.3 mL PBS and analyzed by flow cytometry. Cells were analyzed using a BDFacs Canto II flow cytometer (BD Biosciences, San Jose, CA) equipped with a 488 nm argon laser and a

530/30 band-pass filter (FL1). The fluorescence data are expressed as mean arbitrary fluorescence units and were gated to include all J774A.1 cells.

Compound Synthesis and Characterization

Synthesis of FITC-conjugated anti-DNP antibody. Anti-DNP rabbit (1 mg/mL) was suspended in 1 mL cold solution of 0.05 M boric acid, 0.2 M NaCl at a pH = 9.2 in a 30 KDa molecular weight cut-off centrifuge tube. Antibody was spun at 5,000g for 10 min at 4 °C (4X) to complete the wash process. A solution of 5 mg/mL of fluorescein 5-isothiocyanate (40 µL) was added to the washed antibody solution (1mL). Solution rotated for 2 h protected from light. After reaction time, solution was washed in with 10 mM phosphate, 0.15 M NaCl, 0.08% NaN3 at pH = 7.8, as before. Concentration was determined by absorbance at 280 nm and 480 nm via Shimadzu Biotech BioSpec-nano spectrophotometer.

Synthesis of N1-(2, 4-dinitrophenyl)propane-1,3-diamine. 1, 3-diaminopropane (2.475 mmol, 180 mg, 204 µL) was solubilized in 10 mL dry dichloromethane (DCM) and diisopropylethylamine (DIEA) (4.125 mmol, 532 mg, 717 µL). Solution was added to a 15 mL synthetic vessel charged with 750 mg 2-Chlorotrityl resin (1.1 mmol / g) and agitated for 0.5 h at room temperature. The resin was washed with dimethylformamide (DMF), DCM, methanol, DCM, and DMF (3 x 5 ml each). Reaction was monitored by Ninhydrin test for amines. 2, 4-dinitrofluorobenzene (8.25 mmol, 1535 mg, 2 mL) was added to 10 mL DMF and 2 mL DIEA. Solution was added to resin and agitated for 2 h at room temperature protected from light. Resin was washed as previously mentioned and cleavage occurred in a trifluoroacetic acid (TFA) cocktail solution (50% TFA, 50% CH2Cl2, 10 mL) for 1 h. The solvent was removed from the product under reduced pressure. The purity of the compound was verified by TLC analysis (10 % methanol: 98 % DCM: 2 % DIEA). MS (ESI) [$M+H^+$]: 240.23 (calculated) 241.20 (found).

Synthesis of Vancomycin-DNP (carboxy). Vancomycin hydrochloride (500 mg, 0.337 mmol, 1 eq) was dissolved in DMSO / DMF (1:1) in a 25 mL RBF equipped with stir bar. O-(6-Chlorobenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HCTU) (125 mg, 0.302 mmol, 0.9 eq) and DIEA (2 eq.) were added to the solution at 0 °C. N1-(2, 4-dinitrophenyl)propane-1,3-diamine (1.5 eq) was added to the solution. The reaction was stirred at 0 °C for 2 h. Cold ether was added and precipitate was purified via reverse-phase high-performance liquid chromatography (RP-HPLC) (Phenomenex Luna prep 10 µ 250 × 21.20 mm C8; flow rate 10 mL/min; phase A: water, 0.01% TFA; phase B: acetonitrile, 0.01% TFA; gradient 60 min from 95:5 A/ B to 0:100 A/B). The yellow fractions eluted at 44% B were pooled, concentrated, and lyophilized to afford a yellow solid. Due to complexity, the compound identity was confirmed by matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry and analytical RP-HPLC using a Phenomenex C18 column monitored at 230 nm. MS (MALDI-TOF) [M+Na+]: 1694.439 (calculated) 1694.127 (found).

Synthesis of Vancomycin-DNP (amino). Vancomycin hydrochloride (100 mg, 0.337 mmol, 1 eq) was dissolved in DMSO / DMF (1:1) in a 25 mL RBF equipped with stir bar. DIEA (2 eq.) was added to the solution at 0 °C. 2, 4-dinitrophenyl (1.5 eq) was added to the solution. The reaction was stirred at 0 °C for 2 h. Cold ether was added and precipitate was purified via reverse-phase high-performance liquid chromatography (RP-HPLC) (Phenomenex Luna prep 10 µ 250 × 21.20 mm C8; flow rate 10 mL/min; phase A: water, 0.1% TFA; phase B: acetonitrile, 0.1% TFA; gradient 60 min from 95:5 A/ B to 0:100 A/B). The yellow fractions eluted at 44% B were pooled, concentrated, and lyophilized to afford a yellow solid. Due to complexity, the compound identity was confirmed by matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry and analytical RP-HPLC using a Phenomenex C18 column monitored at 230 nm. MS (MALDI-TOF) [$M+Na^+$]: 1638.329 (calculated) 1638.195 (found).

Synthesis of FITC-Conjugated Sortase Recognition Peptides. A 25 ml synthetic vessel was charged with 564 g (0.41 mmol) of Rink Amide resin. The resin was initially deprotected in a solution of 6 M piperazine/100 mM HOBt in DMF (7 ml). The flask was agitated for 30 minutes and the deprotection solution was drained. The resin was washed with DMF, CH2Cl2, MeOH, CH2Cl2, and DMF (3 x 5 ml each). Initial loading of Fmoc-Gly (example for Sortase A peptide) (4 eq., 0.91 mmol), HCTU (3.9 eq., 0.88 mmol), and DIEA (8 eq., 1.80 mmol) in DMF (10 mL) was performed. The vessel was agitated for 2 h at room temperature. The resin was then washed with DMF, CH2Cl2, MeOH, CH2Cl2 and DMF (3 x 5 ml each). The Fmoc-protecting group was removed with a solution of 6 M piperazine/100 mM HOBt in DMF (7 ml). The flask was agitated for 25 minute and the deprotection solution was drained. The resin was washed and the addition of the second amino acid was performed by adding Fmoc-protected amino acid (4 eq., 0.91 mmol), HCTU (3.9 eq., 0.88 mmol), and DIEA (8 eq., 1.80 mmol) in DMF (10 mL) and agitating for 2 hours. Peptide synthesis was continued for remaining Fmoc-protected amino acids, including N^{α}-Fmoc-N^{ε}-methyltrityl-L-Lysine. The Nε-methyltrityl protecting group of the lysine was then deprotected by adding 10 mL of a TFA cocktail solution (1% TFA, 2% TIPS in CH2Cl2) to the resin and agitating for 10 minutes protected from light. The solution was drained and this procedure was repeated four additional times. For the FITC modification addition, the resin was deprotected and 5(6)-carboxyfluorescein (2 eq., 0.46 mmol), HCTU (1.9 eq., 0.44 mmol), and DIEA (4 eq., 0.93 mmol) in DMF (10 mL) was added to the resin. The flask was agitated in the dark overnight. The resin was drained and subsequently washed with DMF, CH2Cl2, MeOH, CH2Cl2 and DMF (3 x 5 ml each). For larger peptides, Fmoc-8-amino-3, 6-dioxaoctanoic acid (3 eq., 0.36 mmol), HCTU (0.36 eq., 0.36 mmol), and DIEA (6 eq., 0.72 mmol) in DMF was added to the side chain of deprotected lysine. After 2 h of agitation, the solution was then drained and washed as previously stated. Depending on the length of the PEG linker, additional PEG groups were added before cleaving the peptide from the resin. The cleavage of the peptide from resin was carried out by addition of trifluoroacetic acid (TFA) cocktail solution (95% TFA, 2.5% TIPS, 2.5% H2O, 20 ml). The mixture was agitated for 1 hour at room temperature. The resulting solution

was concentrated via compressed air to remove the TFA. The residue was triturated in cold diethyl ether and the precipitate was collected by centrifugation and dissolved in H2O. Compounds were then purified using RP-HPLC with a Phenomenex C8 prep column and an eluent consisting of solvent A (H2O / 0.1% TFA) and solvent B (MeOH / 0.1% TFA) using a 60 minute gradient transitioning from 5% B to 100% B at a flow rate of 10 ml min-1. The purity of the peptides was verified by analytical reverse phase HPLC using a Phenomenex C18 column with an eluent consisting of solvent A (CH3CN/0.1% TFA) and solvent B (H2O/0.1% TFA) with a 30 minute gradient consisting of 5 to 100 % B, and a flow rate of 1 ml min-1 and monitored at 230 nm. Purified peptides were subsequently characterized using MALDI-TOF MS.

Synthesis of DNP-Conjugated Sortase Recognition Peptide. Rink Amide resin was initially deprotected as previously stated and peptide built to specified sequence (-Fmoc$(PEG)_2$-K(Mtt)LPMTG). For the DNP modification step, after deprotection, the resin was treated with 2, 4-dinitrofluorobenzene (10 eq. 2.5 mmol) and DIEA (15 eq., 3.75 mmol) in DMF, and the flask was agitated in the dark for 1 hour at room temperature. The resin was drained and subsequently washed with DMF, CH2Cl2, and MeOH (3 x 5 mL each). The Nε-methyltrityl protecting group of the lysine was then deprotected by adding 10 mL of a TFA cocktail solution (1% TFA, 2% TIPS in CH2Cl2) to the resin and agitating for 10 minutes protected from light. The solution was drained and this procedure was repeated four additional times. The solution was then drained and washed as previously stated. Upon MTT deprotection, Fmoc-8-amino-3, 6-dioxaoctanoic acid (3 eq., 0.36 mmol), HCTU (0.36 eq., 0.36 mmol), and DIEA (6 eq., 0.72 mmol) in DMF was added to the resin. After 2 h of agitation, the resin was deprotected and washed as previously stated. To remove the peptide from resin, TFA cocktail solution (95%, TFA, 2.5% TIPS, and 2.5 % H2O) was added to the resin for 1 h and then concentrated to remove the TFA. The residue was triturated in cold diethyl ether and the precipitate was collected by centrifugation. Peptides were purified using RP-HPLC using a Phenomenex C8 prep column with an eluent consisting of solvent A (H2O / 0.1% TFA) and solvent B (MeOH / 0.1% TFA) using a 60 minute gradient transitioning from 5% B to 100% B at a flow rate of 10 ml min-1. The purity of the peptides was verified by analytical RP-HPLC using a Phenomenex C4 column with an eluent consisting of solvent A (H2O /0.01% TFA) and solvent B (CH3CN / 0.01% TFA) with a 30 minute gradient transitioning from 5% B to 100% B at a flow rate of 3 ml min-1 and monitored at 230 nm and molecular weight was confirmed using MALDI-TOF MS.

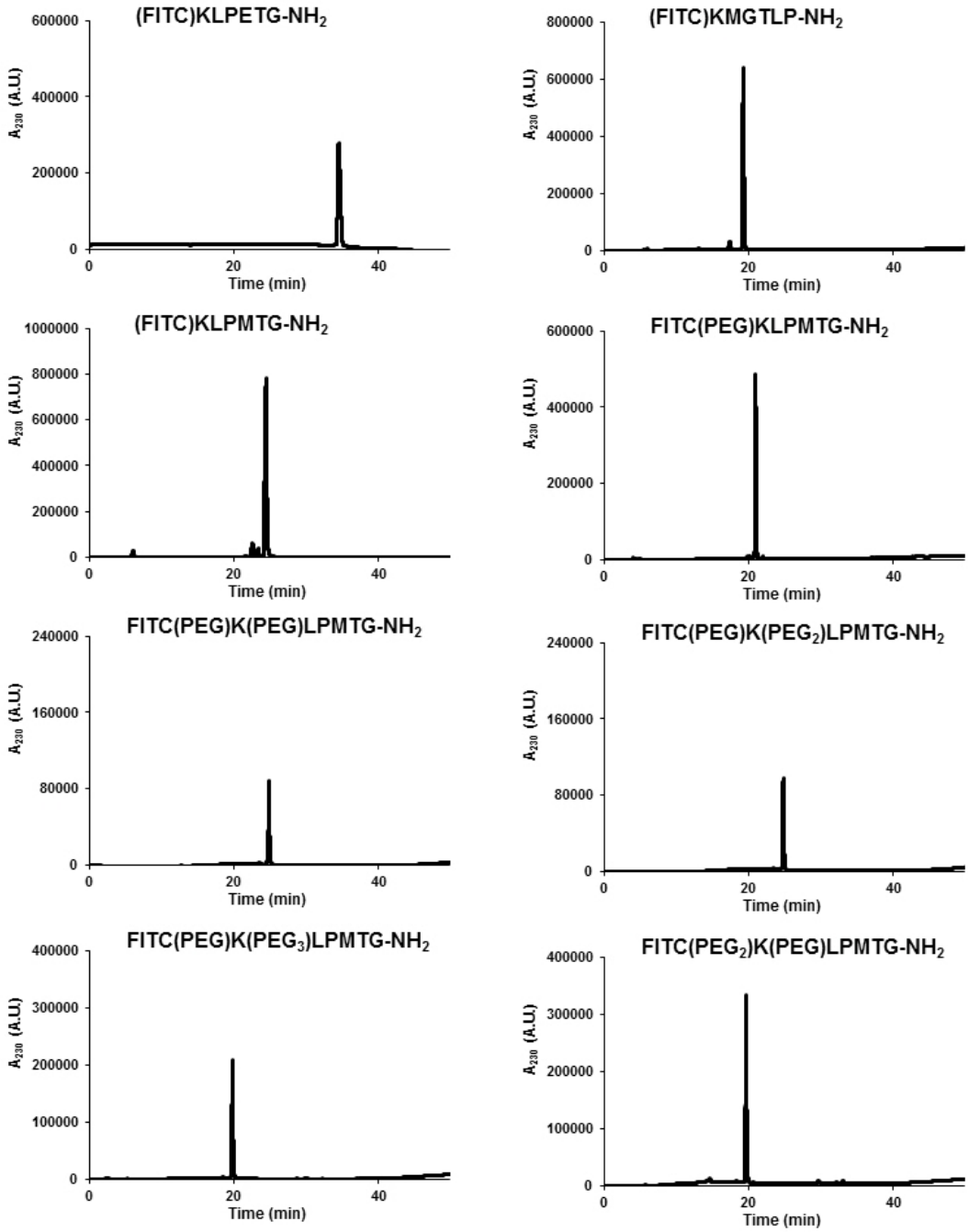
Synthesis of Vancomycin-conjugated SrtA Recognition Peptides. Vancomycin (20 mg, 0.0138 mmol) was dissolved in 1 mL 50/50 DMSO: DMF. Solution was cooled on ice and HCTU (5.13mg, 0.0124 mmol) and DIEA (7.2μL, 0.0414 mmol) were added. SrtA recognition peptide, FITC(PEGx)K(PEGy)LPMTG or DNP(PEG_2)K(PEG)LPMTG (1.5 eq.), was added to the mixture. Reaction continued on ice for 2 h with stirring. After time, mixture was filtered and injected into RP-HPLC for purification. Peptides were purified using RP-HPLC using a Phenomenex C8 prep column with an eluent consisting of solvent A (H2O / 0.001% TFA) and solvent B (MeOH / 0.001% TFA) using a 60 minute gradient transitioning from 5% B to 100% B at a flow rate of 10 ml min-1. The purity of the peptides was verified by analytical RP-HPLC using a Phenomenex C4 column with an eluent consisting of solvent A (H2O /0.001% TFA) and solvent B (CH3CN / 0.001% TFA) with a 30 minute gradient transitioning from 5% B to 100% B at a flow rate of 3 ml min-1 and monitored at 230 nm and molecular weight was confirmed using MALDI-TOF MS.

Molecular Weights and Purities of FITC-Conjugated SrtA Recognition Peptides

Compound	Calculated $[M + H^+]$	Found $[M + H^+]$	Purity
FITC-KLPETG	1003.43	1003.046	>95%
FITC-KLPMTG	1005.43	1005.337	>95%
FITC(PEG)K(PEG)LPMTG	1295.58	1295.159	>95%
FITC(PEG)K(PEG_2)LPMTG	1440.65	1439.911	>95%
FITC(PEG)K(PEG_3)LPMTG	1585.73	1585.035	>95%
FITC(PEG_2)K(PEG)LPMTG	1440.65	1440.24	>95%

Compound	Calculated $[M + Na^+]$	Found $[M + Na^+]$	Purity
FITC-KMGTLP	1027.42	1027.139	>95%
FITC(PEG)KLPMTG	1172.5	1172.911	>95%

Analytical RP-HPLC Profiles of FITC- Conjugated SrtA Recognition Peptides. The specified derivatives were analyzed on a Phenomenex C4 column by reverse phase HPLC with an eluent consisting of solvent A (H2O /0.1% TFA) and solvent B (CH3CN /0.1% TFA) with a 30 minute gradient consisting of 5 to 100 % B, a flow rate of 3 mL/min, and monitoring at 230 nm.



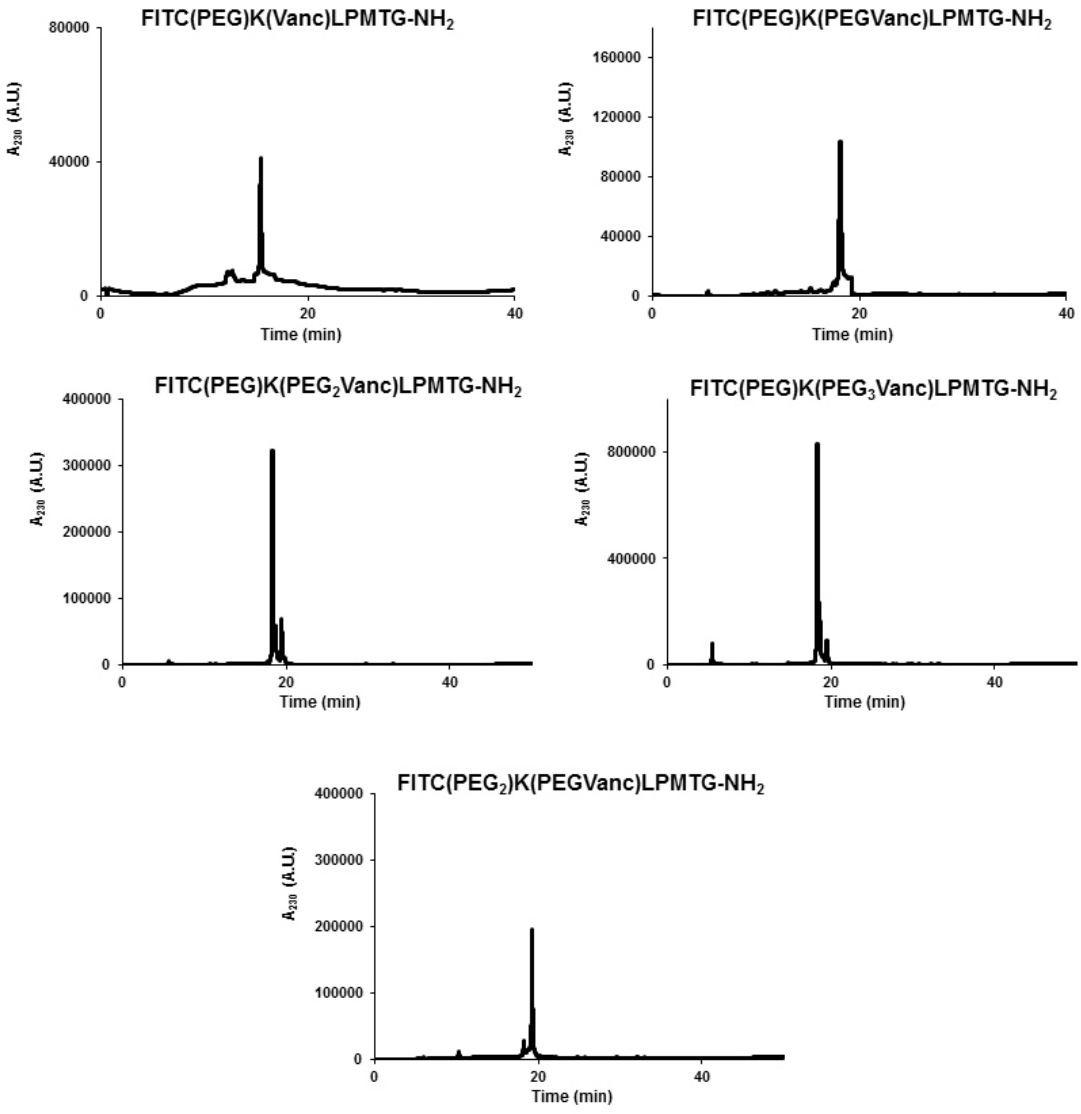
Molecular Weights and Purities of Synthesis of Vancomycin-Conjugated SrtA Recognition Peptides

Compound	Calculated $[M + H^+]$	Found $[M + H^+]$	Purity
FITC(PEG)-K(Vanc)LPMTG	2582.54	2582.163	>95%

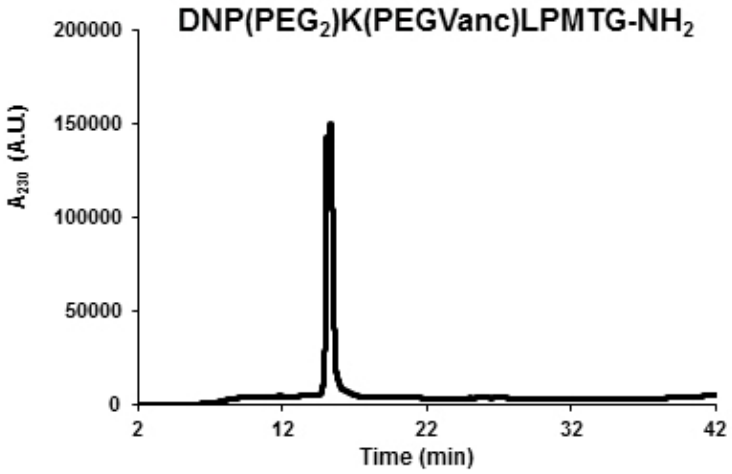
Compound	Calculated $[M + K^+]$	Found $[M + K^+]$	Purity
FITC(PEG)-K(PEGVanc)LPMTG	2765.78	2765.241	>95%
FITC(PEG)-K(PEG_3Vanc)LPMTG	3057.01	3057.809	>95%

Compound	Calculated $[M + Na^+]$	Found $[M + Na^+]$	Purity
FITC(PEG)-K(PEG_2Vanc)LPMTG	2894.84	2894.55	>95%
FITC(PEG_2)K(PEGVanc)LPMTG	2894.84	2894.622	>95%
DNP(PEG_2)K(PEGVanc)LPMTG	2700.62	2700.454	>95%

Analytical RP-HPLC Profiles of FITC(PEG$_x$)K(PEG$_y$Vanc)LPMTG Peptides. The specified derivatives were analyzed on a Phenomenex C4 column by reverse phase HPLC with an eluent consisting of solvent A (H2O /0.001% TFA) and solvent B (CH3CN /0.001% TFA) with a 30 minute gradient consisting of 5 to 100 % B, a flow rate of 3 mL/min, and monitoring at 230 nm.



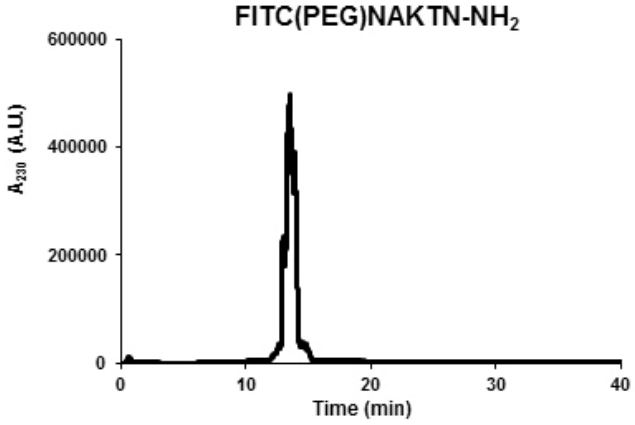
Analytical RP-HPLC Profile of DNP(PEG$_2$)K(PEGVanc)LPMTG. The specified derivative was analyzed on a Phenomenex C18 column by reverse phase HPLC with an eluent consisting of solvent A (H2O /0.001% TFA) and solvent B (CH3CN /0.001% TFA) with a 30 minute gradient consisting of 5 to 100 % B, a flow rate of 1 mL/min, and monitoring at 230 nm.



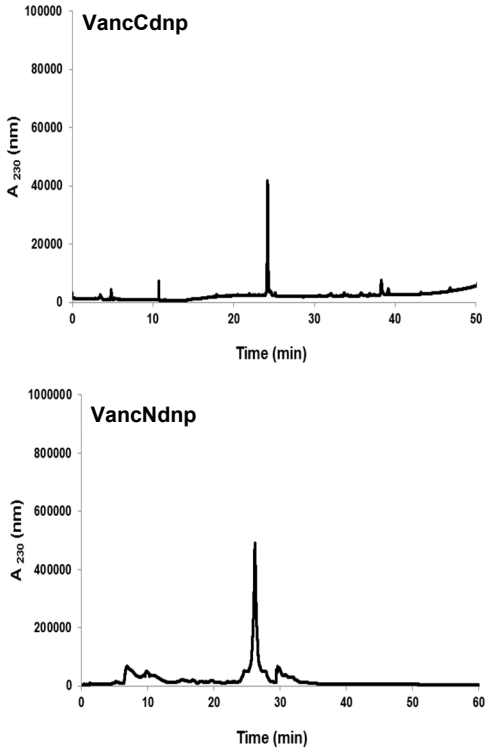
Molecular Weights and Purities of FITC-Conjugated SrtB Recognition Peptide

Compound	Calculated $[M + H^+]$	Found $[M + H^+]$	Purity
FITC(PEG)-NAKTN	**1052.06**	**1052.136**	**>95%**

Analytical RP-HPLC Profile of FITC(PEG)NAKTN Sortase B Peptide. The specified peptide was analyzed on a Phenomenex C18 column by reverse phase HPLC with an eluent consisting of solvent A (H2O /0.1% TFA) and solvent B (CH3CN /0.1% TFA) with a 30 minute gradient consisting of 5 to 100 % B, a flow rate of 1 mL/min, and monitoring at 230 nm.



Analytical RP-HPLC Profiles of Vancomycin-DNP constructs. The specified derivatives were analyzed on a Phenomenex C18 column by reverse phase HPLC with an eluent consisting of solvent A (H2O /0.01% TFA) and solvent B (CH3CN /0.01% TFA) with a 30 minute gradient consisting of 5 to 100 % B, a flow rate of 1 mL/min, and monitoring at 230 nm.



Supporting References

1. Lewis, J. A., and Fleming, J. T., Caenorhabditis elegans: Modern Biological Analysis of an Organism, eds. Epstein, H. F. & Shakes, D. C. (Academic, San Diego). **1995,** *48*, 3-29.