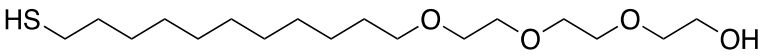
Electronic Supplementary Material (ESI) for Analyst.

Figure S1. Structures of thiols used for self-assembled monolayers (SAMs)

1: 3,6,9-Trioxa-19-mercapto-nonadecan-1-ol



2. 1-amino-3,6,9,12,15,18-hexaoxanonacosane-29-thiol

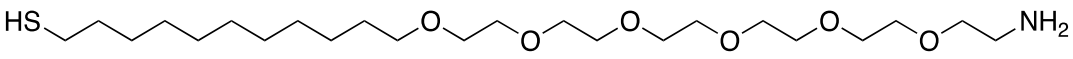


Figure S2. LPS Binding Profiles for SMAP-29 Mutants to a small panel of LPS Samples (expanded version of that shown in Figure 3).

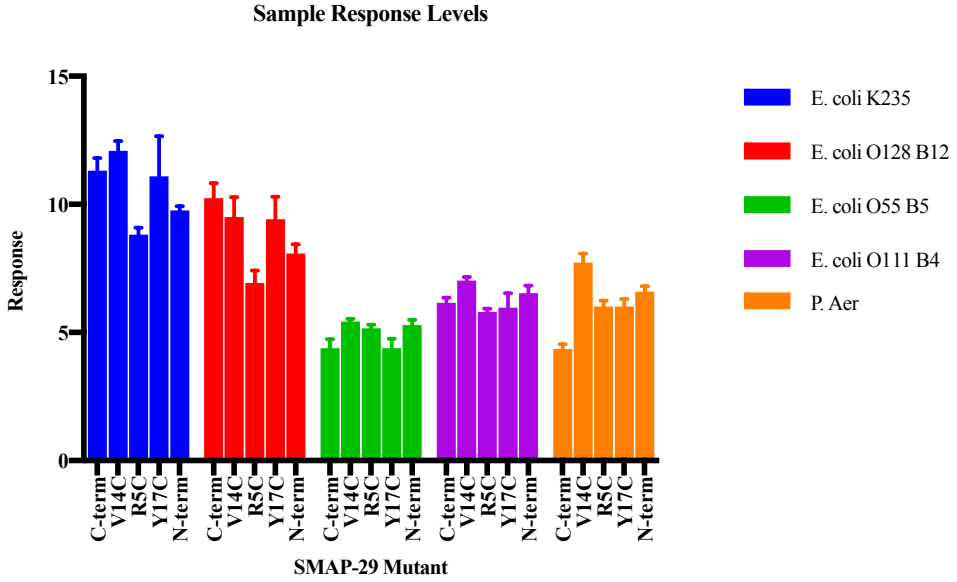


Figure S3. Full panel of SMAP-29 Binding to LPS Samples from Figure 4.

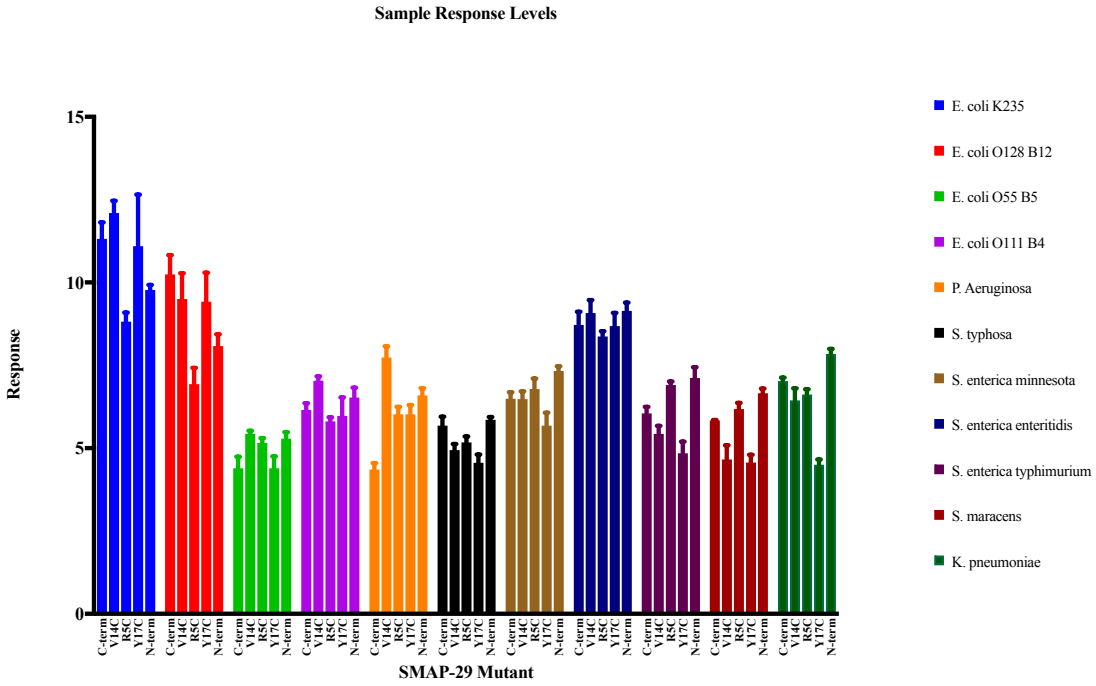


Figure S4. Heat map showing success rates of pairs of AMPs in identifying LPS samples.

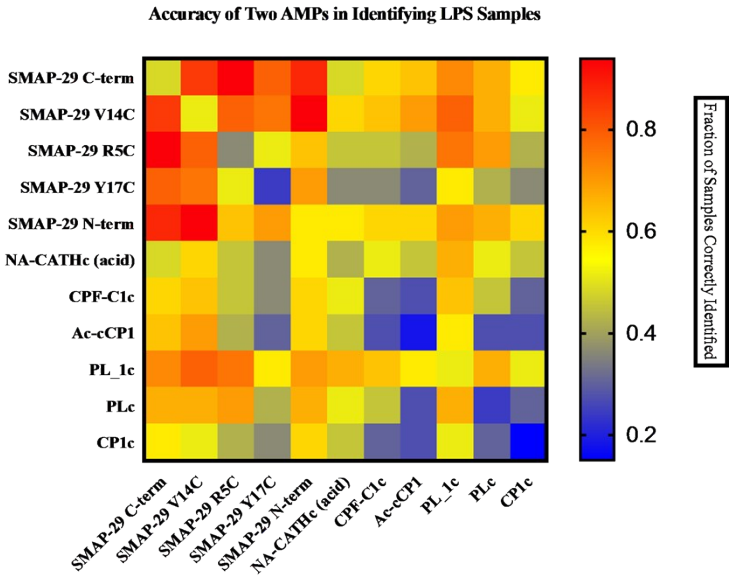
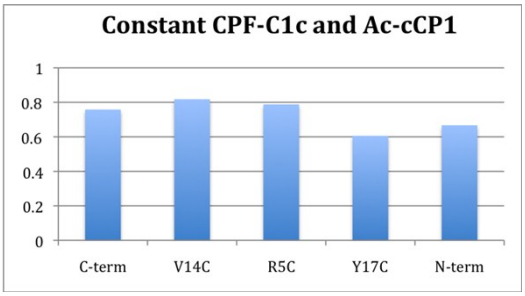


Figure S5. Comparison of Discrimination Accuracy of SMAP-29 Mutants. LDA functions were generated using one SMAP-29 mutant (labeled on each bar) and two other AMPs (shown in each title). The full panel of 11 LPS samples were used, and the fraction of samples correctly identified by the discrimination function (by jackknife analysis) is plotted.


Constant CPF-C1c and Ac-cCP1
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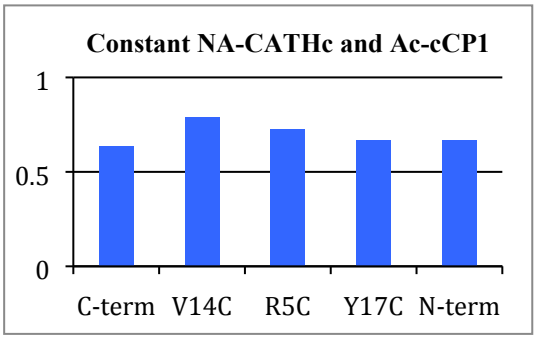
0.6
0.4
0.2
0
C-term
V14C
R5C
Y17C
N-term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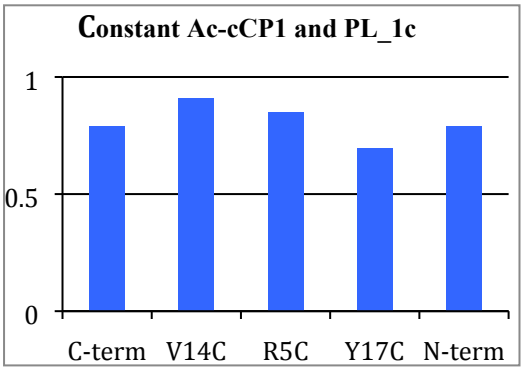
Y17C
N-term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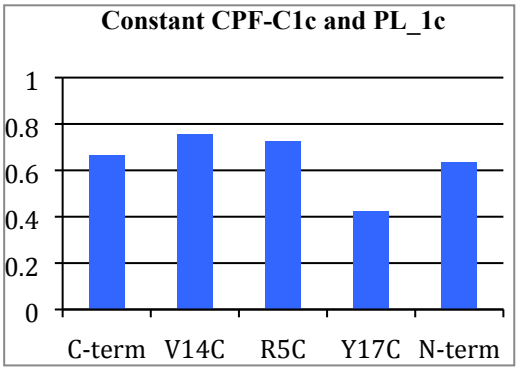
C-term
V14C
R5C
Y17C
N-term


Constant CPF-C1c and PL_1c
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0.8
0.6
0.4
0.2
0
C-term
V14C
R5C
Y17C
N-term

Figure S6. Comparison of Discrimination Accuracy of SMAP-29 Mutant Pairs. LDA functions were generated using two SMAP-29 mutants (labeled on the x- and y-axes) and PL_1c. The full panel of 11 LPS samples were used, and the fraction of samples correctly identified by the discrimination function (by jackknife analysis) is plotted.

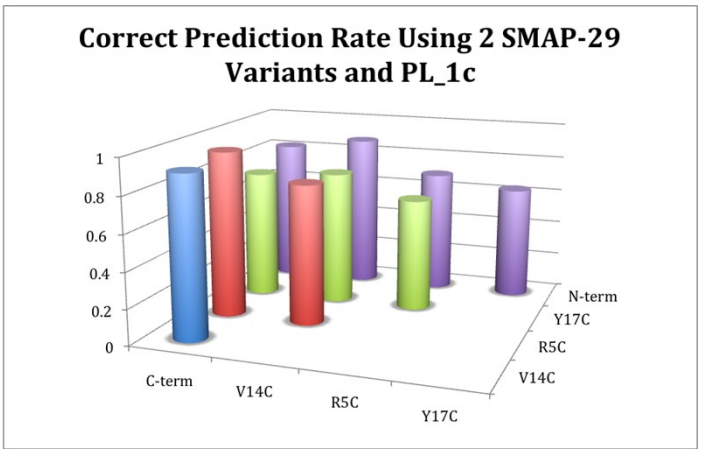


Figure S7. LDA canonical score plot for the training data set shown in Figure 8A.

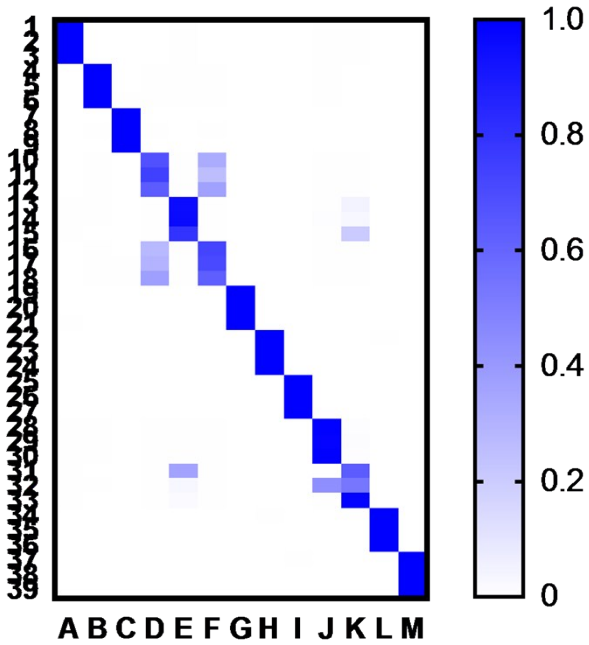


Figure S8. Success rate for LPS identification for the test data set shown in Figure 8B.

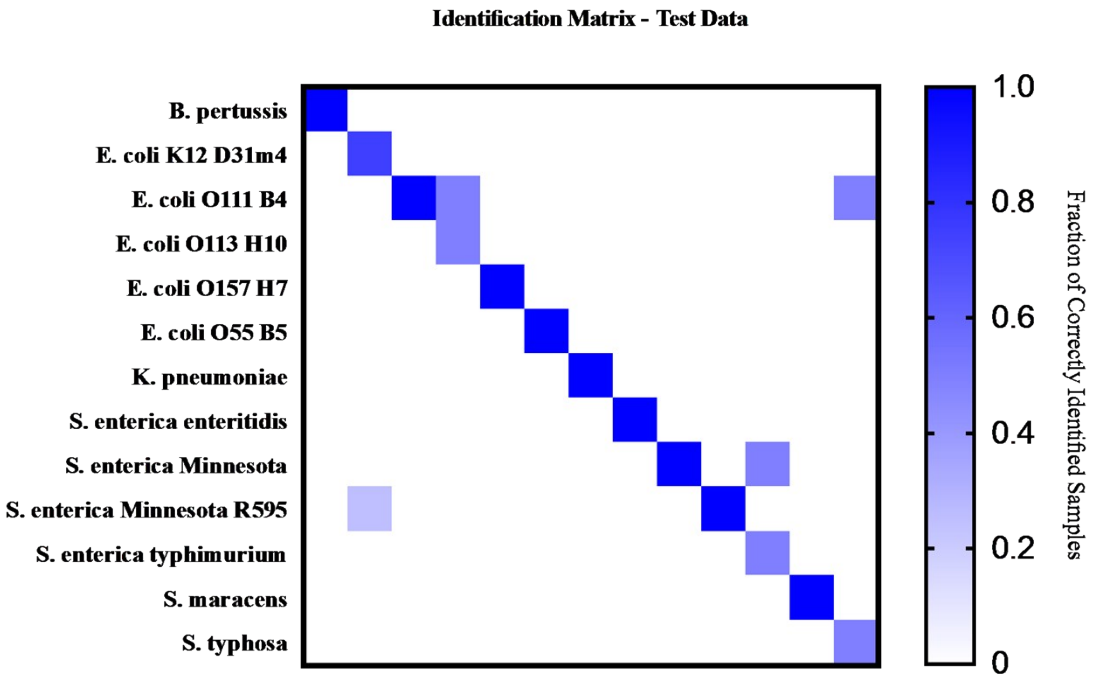


Figure S9. Plot of LDA function 1 versus function 2.

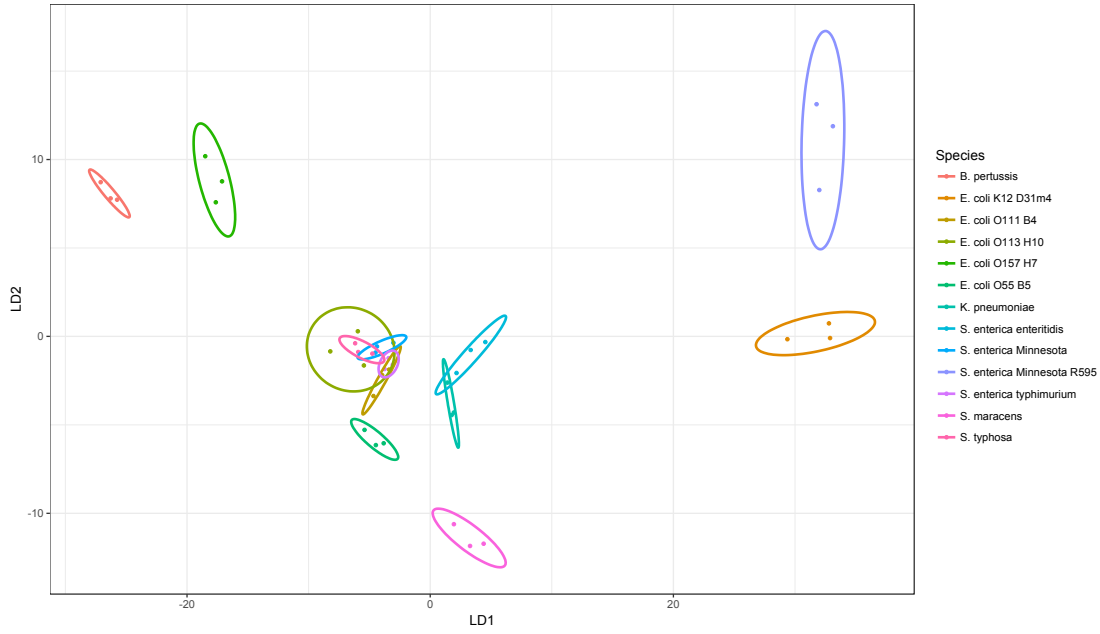


Figure S10. Plot of LDA function 1 versus function 3.

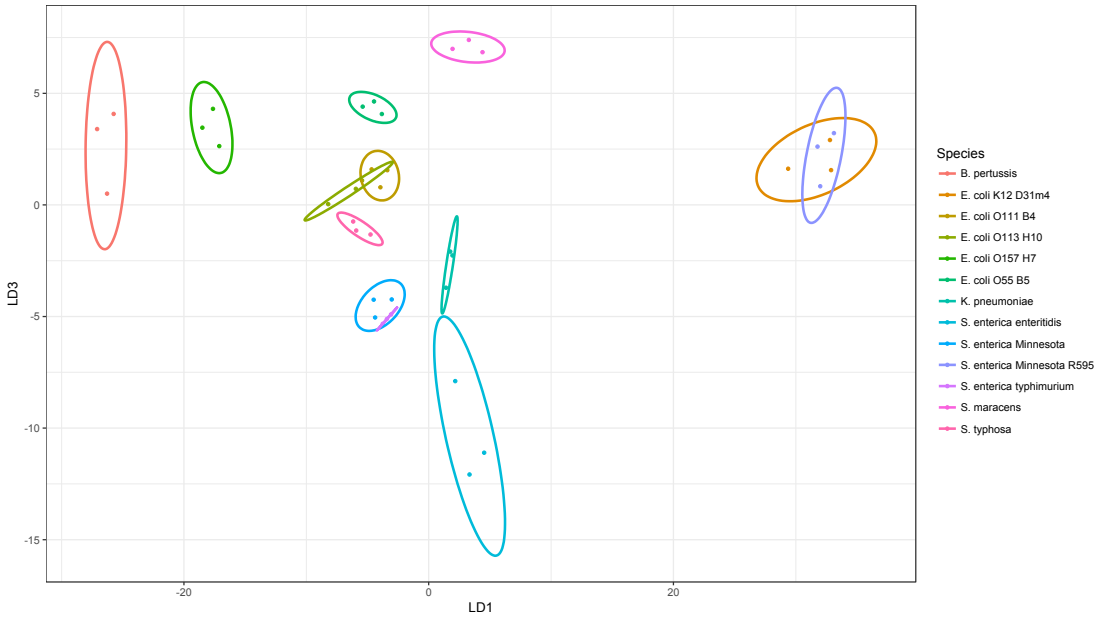
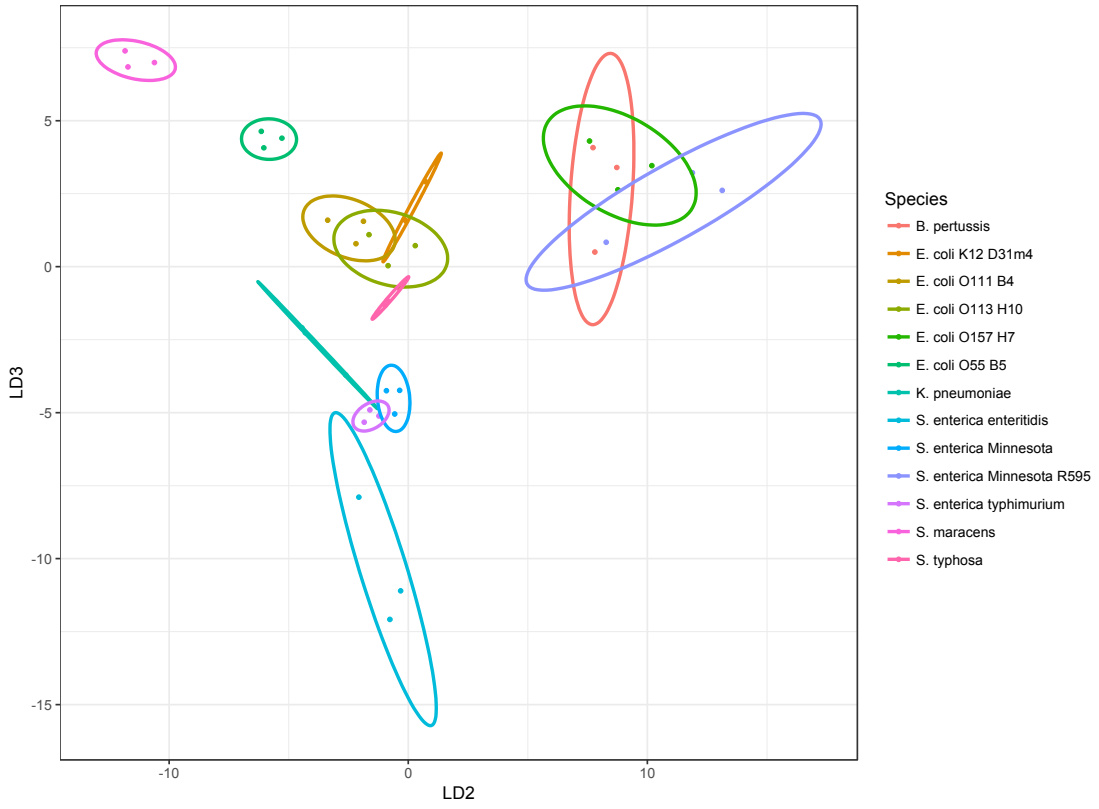


Figure S11. Plot of LDA function 2 versus function 3.



Materials and Methods Additional Details
Antimicrobial Peptides:

LPS Samples:
Antimicrobial peptides were purchased from New England Peptide (Gardner, MA) and were of minimum 85% purity. Thiols for self-assembled monolayers (**1:** 3,6,9-Trioxa-19-mercapto-nonadecan-1-ol and **2:** 1-amino-3,6,9,12,15,18-hexaoxanonacosane-29-thiol, structures shown in Figure S1) were purchased from ProChimia Surfaces (Sopot, Poland). Lipopolysaccharide samples from the following bacterial strains were obtained from Sigma-Aldrich:

Escherichia coli K235
Escherichia coli O111:B4
Escherichia coli O55:B5
Escherichia coli O128:B12
Pseudomonas aeruginosa
Serracia marcens
Klebsiella pneumonia
Salmonella typhosa
Salmonella enterica Minnesota
Salmonella enterica typhimurium

Lipopolysaccharide samples from the following bacterial strains were obtained from List Biological Laboratories:

Escherichia coli J5
Escherichia coli O157:H7
Escherichia coli O55: B5
Escherichia coli O111:B4
Escherichia coli K12, D31m4
Salmonella enterica Minnesota R595
Salmonella enterica typhimurium

All LPS stock solutions were sonicated for at least 20 minutes prior to use in order to disrupt any aggregates.

Surface Preparation.
Immediately prior to use, the gold surfaces were cleaned with 5 minutes sonication in methanol, drying with a stream of ultra-high purity nitrogen, then 15 minutes of ozonolysis in a UV-ozone generator (Novascan PSD Series), followed by rinsing with deionized water and drying with UHP nitrogen. Self-assembled monolayers (SAMs) of poly(ethylene glycol)-containing alkanethiols on the gold sutfaces to confer resistance to nonspecific adsorption of biomolecules. Thiols **1** and **2** (with functionalizable amine terminus) were used in a 1:1 to 4:1 ratio. SAMs were formed by layering ~ 300 μL of a 0.2 mM total thiol solution in 70% ethanol / 30% water for one hour.

Biacore:
After gold surface cleaning and SAM formation, the surface was attached to a blank BiaCore chip cassette and docked in the BiaCore T100 instrument. A "Normalize and Prime" protocol was immediately performed. The SAM was functionalized by flowing freshly dissolved 30 mM N-γ-maleimidobutyrl-oxysulfosuccinimide ester (sulfo-GMBS, Thermo Fisher) in phosphate buffer pH 8.5. After a short switch to running buffer (phosphate buffer, pH 7.4, 0.1% Tween 20), a solution of the antimicrobial peptide of interest (5 μM in phosphate buffer at pH 6.5 with 5 mM TCEP) was flowed over the appropriate flow channel, which was then capped with 50 mM mercaptoethanol. The reference channel had the same procedure, omitting only the AMP. This resulted in ~1000 RU immobilized peptide.

SPR Imager:
After washing with water and ethanol, and drying with UHP N_2, a thiol-reactive N-γ-maleimidobutyrl-oxysulfosuccinimide ester (sulfo-GMBS, Thermo Fisher) linker was attached by immersion in a 30 mM solution in a pH 8.5 phosphate buffer for 30 minutes. Antimicrobial peptide solutions (~ 5 μM in phosphate buffer at pH 6.5) were incubated with 5 mM of the reducing agent tris(2-carboxylethyl)phosphine hydrochloride (TCEP) for a minimum of 30 minutes and were then "printed" onto the functionalized gold surface using a continuous flow microspotter (Wasatch Microfluidics, Salt Lake City, UT) with two separate 30-minute flow cycles. A 50 mM solution of mercaptoethanol was flowed over the chip for 5 minutes to cap any unfunctionalized sites.

LPS Binding SPR Experiments. SPR experiments were performed on either a Biacore T100 instrument or SPRimagerII (GWC Technologies, Madison, WI). One peptide was immobilized per channel or spot, respectively, while one out of every four channels or spots was functionalized only with mercaptoethanol to serve as a reference channel.

Biacore T100:

Each analysis began with five "blank" injections of buffer, but included the regeneration step of flowing an aqueous solution of 50 mM NaOH containing 30% acetonitrile. These blank injections removed adsorbed or other loosely attached peptide. A minimum of three injections were performed for each LPS sample, all at 10 μg/mL (~ 0.5 - 1.0 μM), along with 5-10 injections of a reference LPS sample in order to correct for the gradual loss of peptide over time. The injection order was randomized by the Biacore control software.

SPRimagerII:

As on the Biacore, each analysis began with five "blank" injections of buffer, but included the regeneration step of flowing an aqueous solution of 50 mM NaOH containing 30% acetonitrile. These blank injections removed adsorbed or other loosely attached peptide. A minimum of one injection was performed for each LPS sample, providing a minimum of three measures for each pair of LPS and AMP.

Data Analysis. Loss of peptide activity over time was corrected by exponential or linear functions as described previously. A sample correction is shown in Figure S2. Time-corrected, reference-subtracted LPS binding was analyzed using R (R Foundation for Statistical Computing, Vienna, Austria). Linear discriminant analysis (LDA) was used to classify the LPS samples based on binding to the panel of AMPs. LDA generates classification functions that maximize inter-group variance while minimizing intra-group variance. Canonical scores for the top two classification functions are plotted to visualize the data clustering. The reported success rates for subgroups of peptides shown below are generated using the leave-one-out jackknife method, in which classification functions are generated without one data point, and the functions are used to classify that one data point.