Supplementary Information

Proteome reactivity profiling for the discrimination of pathogenic bacteria**

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Materials and general methods

Electrophile probes were purchased from Life Technology (1: 5-(Bromomethyl)Fluorescein; B1355, 2: MitoTracker Orange CMTMRos; M7510, 3: 5-(and-6)-Carboxyfluorescein, Succinimidyl Ester; C1311, 4: 5-(and-6)-Carboxytetramethylrhodamine, Succinimidyl Ester; C1171). The spectroscopy grade dimethyl sulfoxide (DMSO, 99.9% purity) was purchased from Sigma-Aldrich, and all electrophile probes were dissolved in DMSO stock solution (20 mM) and further diluted for desired concentration (final concentration of DMSO < 1%). De-ionized water was prepared using Milli-Q Direct (Milipore Inc.). All solutions were prepared with de-ionized water. Phosphate buffer solution (20 mM, pH 7.4) was purchased from Wellgene (ML008-01). For protein mixture model study, purified proteins were purchased from Sigma-Aldrich for Protease from *Bacillus amyloliquefaciens* (P1236) and Protease from *Bacillus polymyxa* (P6141), and Lipase from *Pseudomonas fluorescens* (62312). 11 bacteria were purchased from ATCC; *Staphylococcus aureus*: ATCC 65389, *Enterococcus faecalis*: ATCC 29212,: ATCC27508, *Escherichia coli*: ATCC 10536, *Bacillus subtillis*: ATCC 6633, *Bacillus cereus*: ATCC 27348, *Salmonella typhimurium*: ATCC 13311, *Acinetobacter calcoaceticus*: ATCC 15473, *Micrococcus luteus*: ATCC 9341, *Moraxella catanhalis*: ATCC 25240, *Serratia marcescens*: ATCC 27117.

Instruments and computer software

Quantitation of whole cell lysate was performed using NanoDrop™ Lite (ThermoScientific Inc.). Protein electrophoresis was performed using Mini-PROTEAN® system (Bio-Rad) and in-house made SDS gels. After electrophoresis, all fluorescence images of SDS gel were obtained using fluorescence scanner Typhoon 9400 (GE Healthcare) using 488 nm and 532 nm laser depending on fluorophores (compound **1**, **3** for 488 nm; compound **2**, **4** for 532 nm). Obtained images were analysed using ImageJ program. Reactivity profile was further analysed by Matlab (R2012a) for principal component analysis. Graphs were generated using OriginPro 8.6 software.

Proteome reactivity profile of bacterial proteins

Protein solution was prepared in 5 mg/ml final concentration of each protease from *Bacillus amyloliquefaciens*, protease from *Bacillus polymyxa*, and lipase from *Pseudomonas fluorescense* in PBS solution (20 mM, pH 7.2). Electrophile probes (1 μM) were added to the proteome mix, and incubated in room temperature for 10 min. To reaction mixture, 5x SDS loading buffer supplemented with β-mercaptoethanol was added and boiled at 98 °C for 2 min. Final 20 μl was loaded on 12 % SDS gel, and electrophoresis was performed. Reactivity profiles of protein mixture were obtained by measurement of fluorescent signal using Typhoon 9400 scanner.

Enzyme inhibitor competition assay

Enzyme solutions were prepared in 5 mg/ml final concentration of each protease from *Bacillus amyloliquefaciens*, protease from *Bacillus polymyxa*, and lipase from *Pseudomonas fluorescense* in PBS solution (20 mM, pH 7.2). Enzyme inhibitors, protease inhibitor cocktail (Sigma P2714) for proteases and olistat (Sigma O4139) for ligase, were pre-incubated for 10 min at room temperature followed by incubation with electrophile probes (1 μM) for 10 min at room temperature. To reaction mixture, 5x SDS loading buffer supplemented with β-mercaptoethanol was added and boiled at 98 °C for 2 min. Final 20 μl was loaded on 12 % SDS gel, and electrophoresis was performed. Reactivity profiles of protein mixture were obtained by measurement of fluorescent signal using Typhoon 9400 scanner.

Proteome reactivity profile of 11 bacteria lysate

Individual bacterium was cultured overnight and lysed using pulsed ultrasonication for 1 min. Membranes and bacterial DNAs were removed by centrifugation (18,000 g for 20 min at 4 °C). Soluble fraction of proteome was obtained from supernatants, and proteme concentration was quantified using Nanodrop™ Lite. To excess amount of proteome (1 mg/ml), electrophile probes (1 μM final concentration) were treated and incubated in room temperature for 30 min. To reaction mixture, 5x SDS loading buffer supplemented with β-mercaptoethanol was added and boiled at 98 °C for 2 min. Final 16 μg proteome was loaded on 12 % SDS gel, and electrophoresis was performed. Reactivity profiles of bacteria proteome were obtained by fluorescence scanning using Typhoon 9400. After obtaining fluorescence image, the SDS-gel was further stained by coomassie brilliant blue staining for abundance profile.

Fluorescence image analysis for discrimination

Original 64-bit fluorescence image was analysed using Transform:ImagetoResult module in *ImageJ* program[1] to obtain quantitative numeric values of fluorescence intensity. First, open the fluorescence image. Second, select the fluorescence reactivity profile area. Third, run "Transform::ImagetoResult" module. It will generate numeric

matrix consist of fluorescence intensity. Forth, digitalized intensity values were further analysed using princomp function in Matlab, and 1st and 2nd principal component values were visualized in 2D scatter plot.

Figure S1. Fluorescence SDS-gel image of reactivity profiling. Individual 4 chemical probes were incubated with bacterial proteins (Protease_*Bacillus amyloliquefaciens*, Protease_*Bacillus polymyxa*, Lipase_*Pseudomonas fluorescens)* for 10 min. After SDS-PAGE, fluorescently labeled proteins were visualized using Typhoon 9400 fluorescence scanner (left: excitation 488 nm laser, emission 526-SP filter; right: excitation 532 nm laser, emission 580SP30 filter).

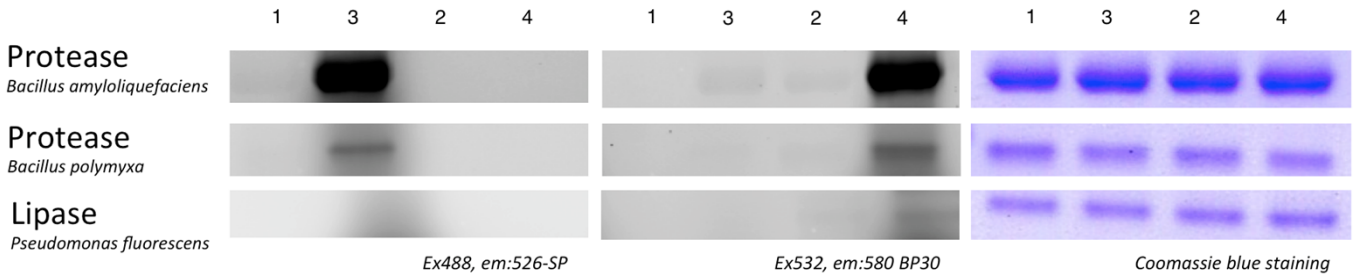


Figure S2. Fluorescence reactivity profile of chemical probes.

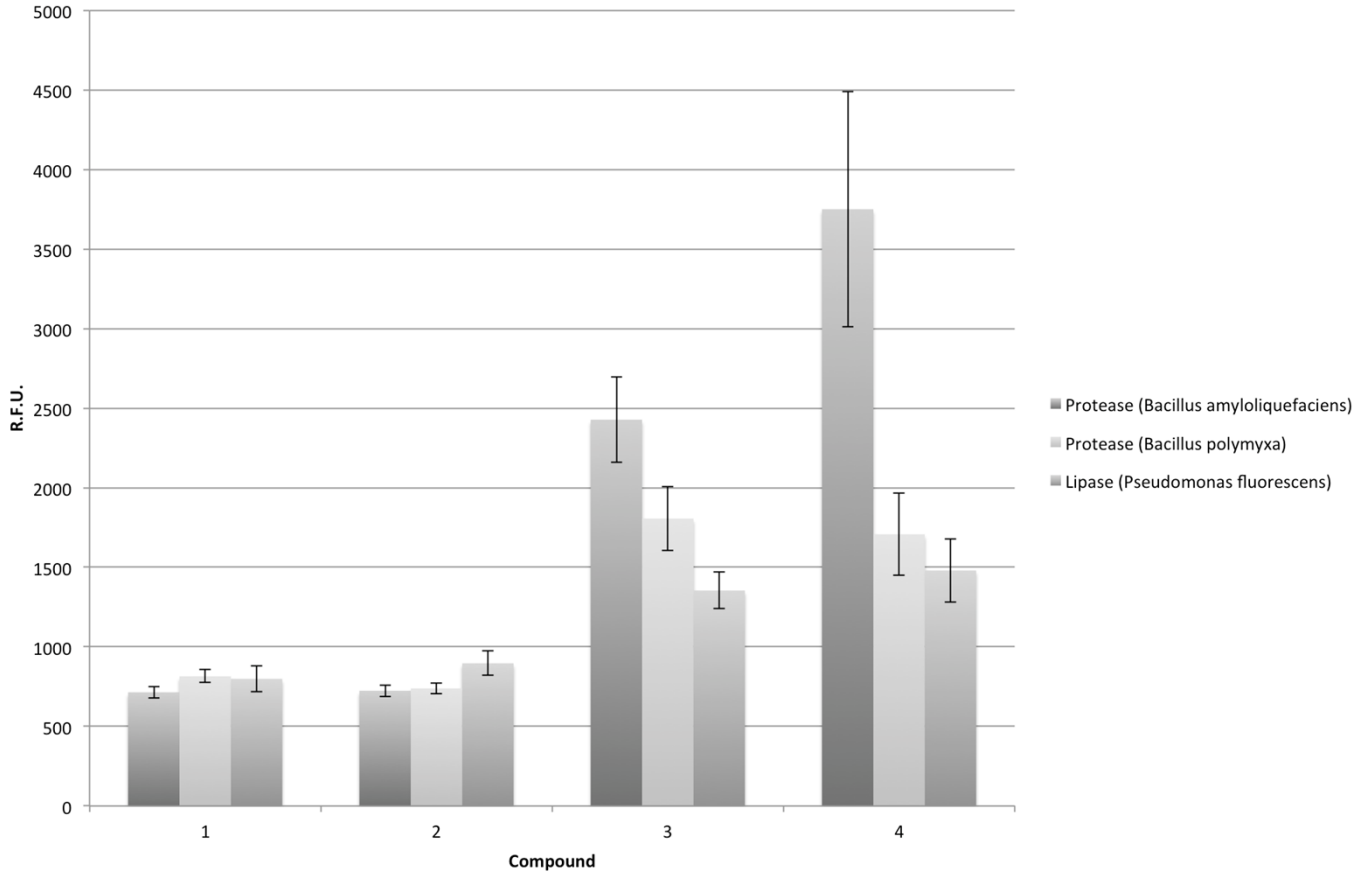


Figure S3. Competition assay between protease inhibitor (PI) and compounds. Left: SDS-gel images of protease loading control and fluorescence reactivity pattern. Right: Quantitative values of fluorescence intensity of SDS-gel image were plotted in bar graph.

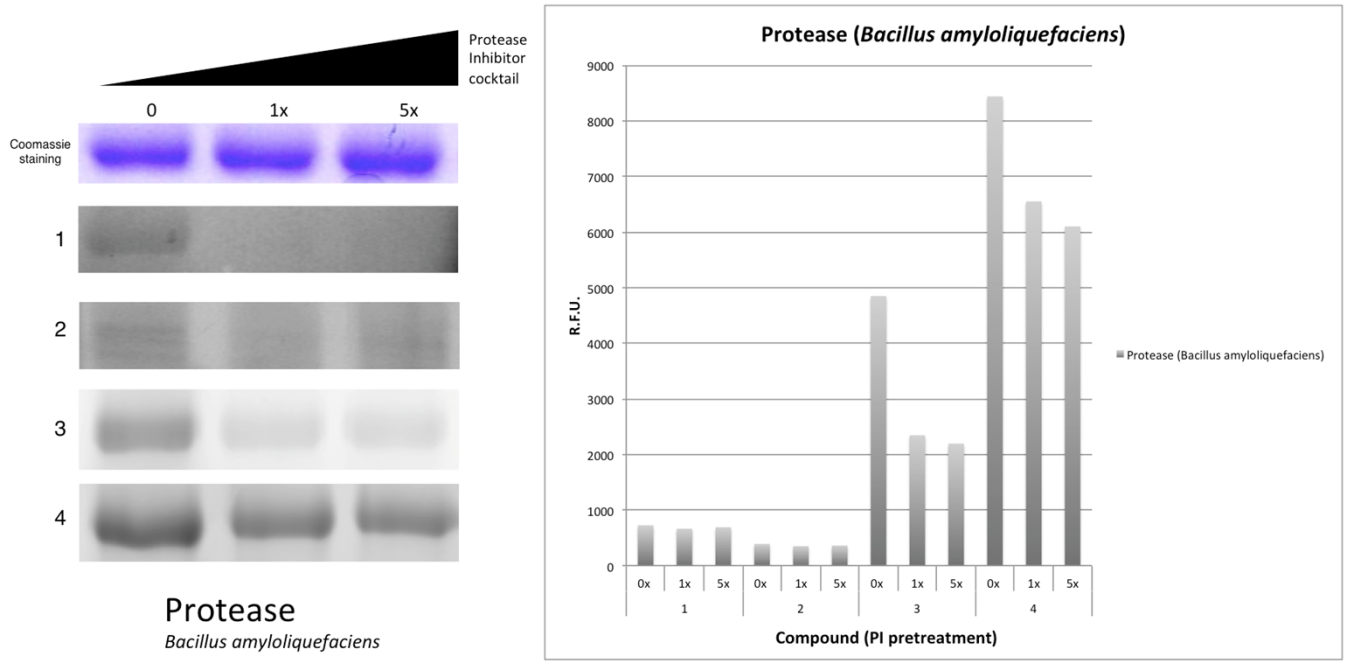


Figure S4. Competition assay between lipase inhibitor, Orlistat[2], and compound **2**. Left: SDS-gel image of lipase loading control and fluorescence reactivity pattern. Right: Quantitative values of fluorescence intensity of gel image were presented in bar-graph format.

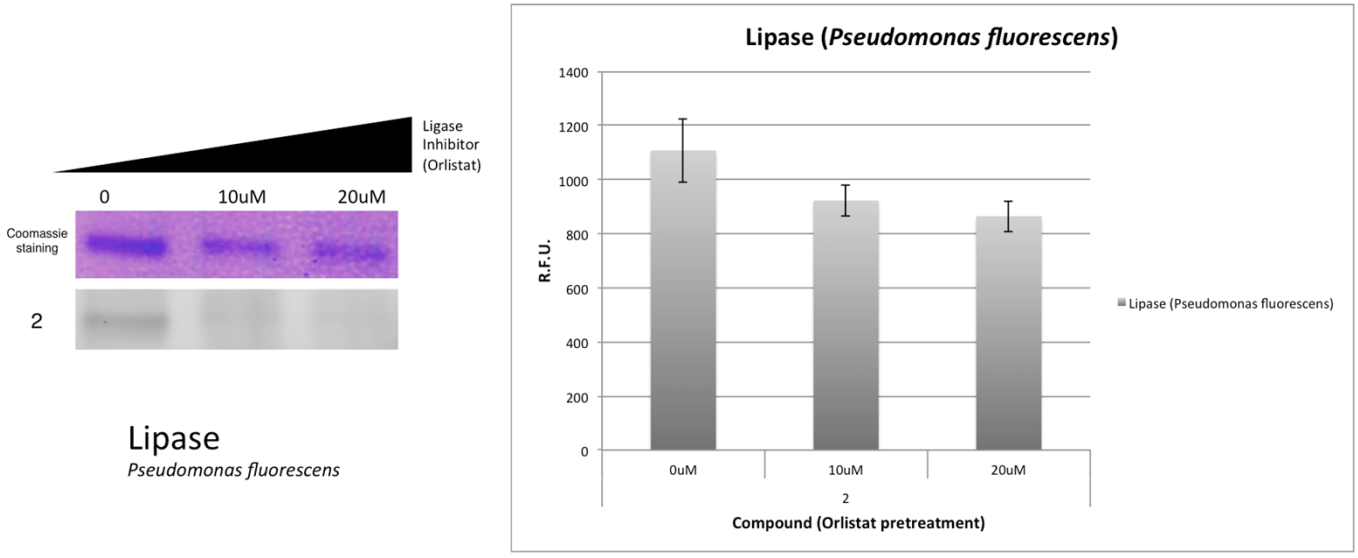


Figure S5. Comparison between protein abundance and reactivity (a), and reactivity profile with PI cocktail (b). Using whole proteome mixture of *Enterobacter cloacae,* abundant proteins were visualized by Coomassie brilliant blue staining, and reactivity profile was generated using compound **1** and **2**. In (b), protease inhibitor cocktails were pre-incubated with bacteria lysate for 10 min and chemical probes were incubated with 5 min. Asterisk marked bands were competed upon PI treatment.

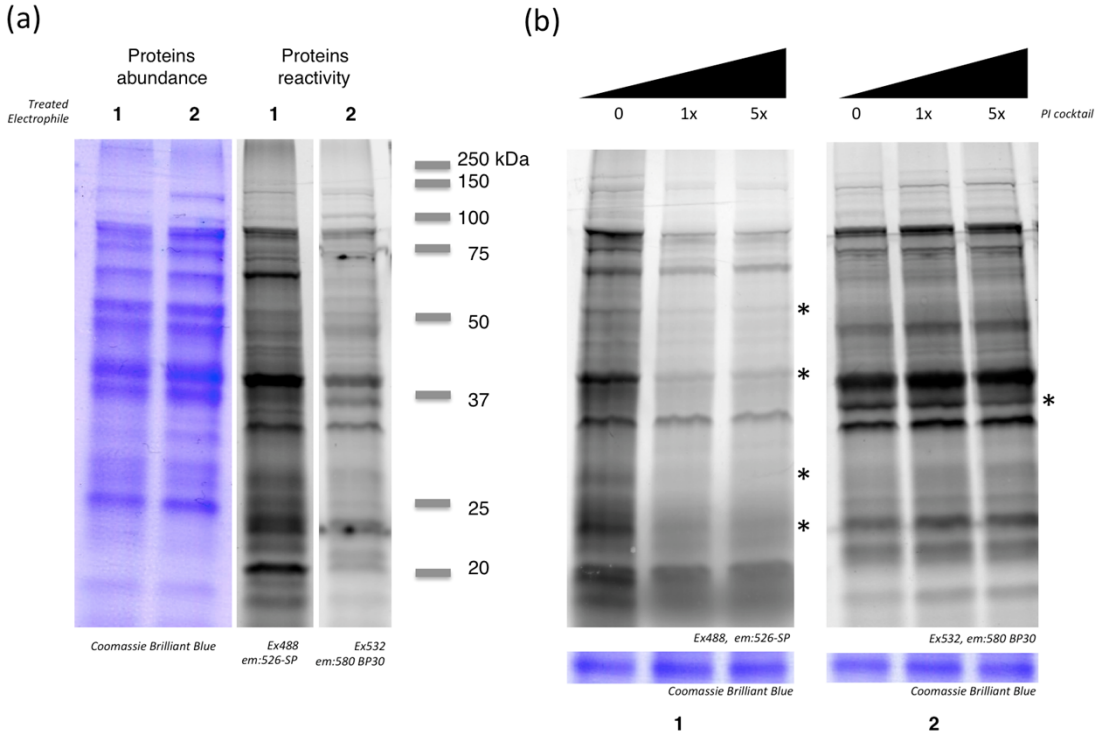


Figure S6. Bacterial proteome reactivity profile of compound **2**.

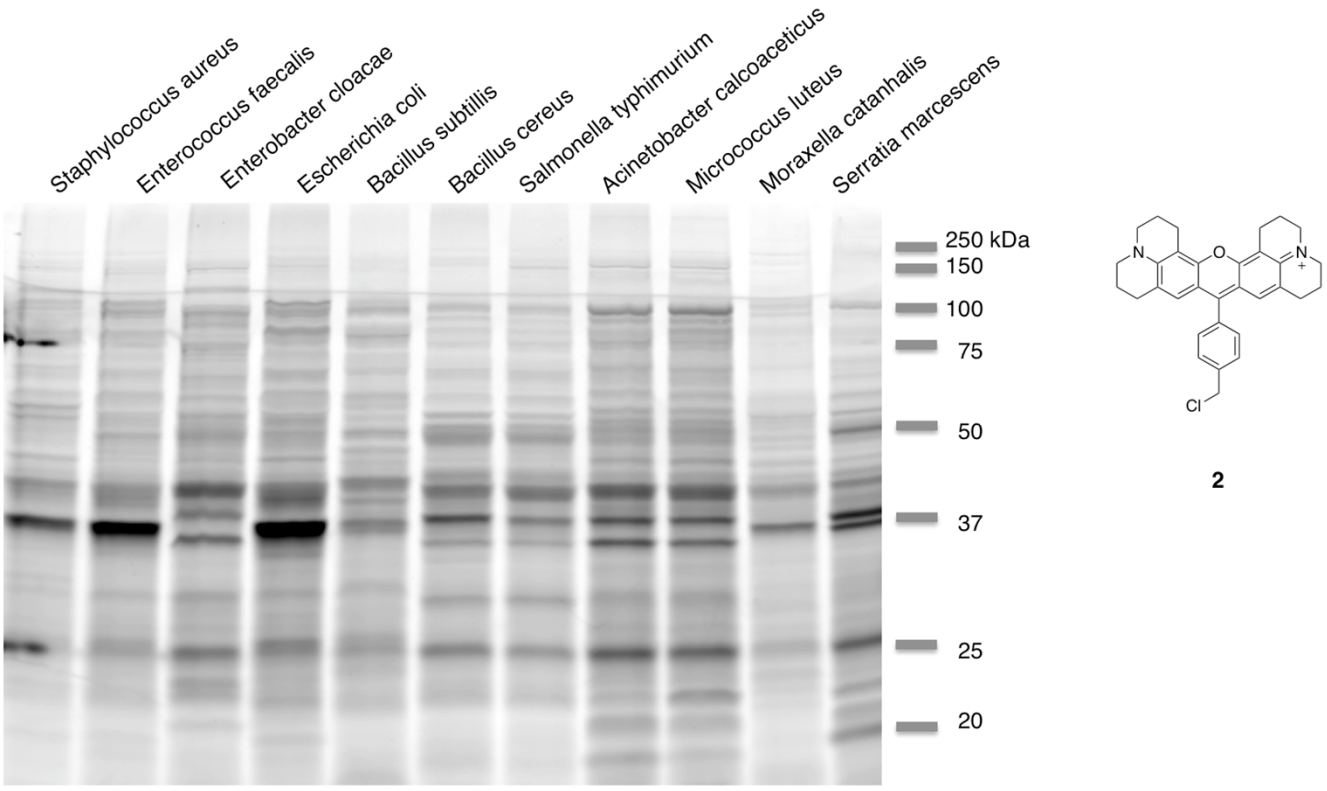


Figure S7. Bacterial proteome reactivity profile of compound **3**.

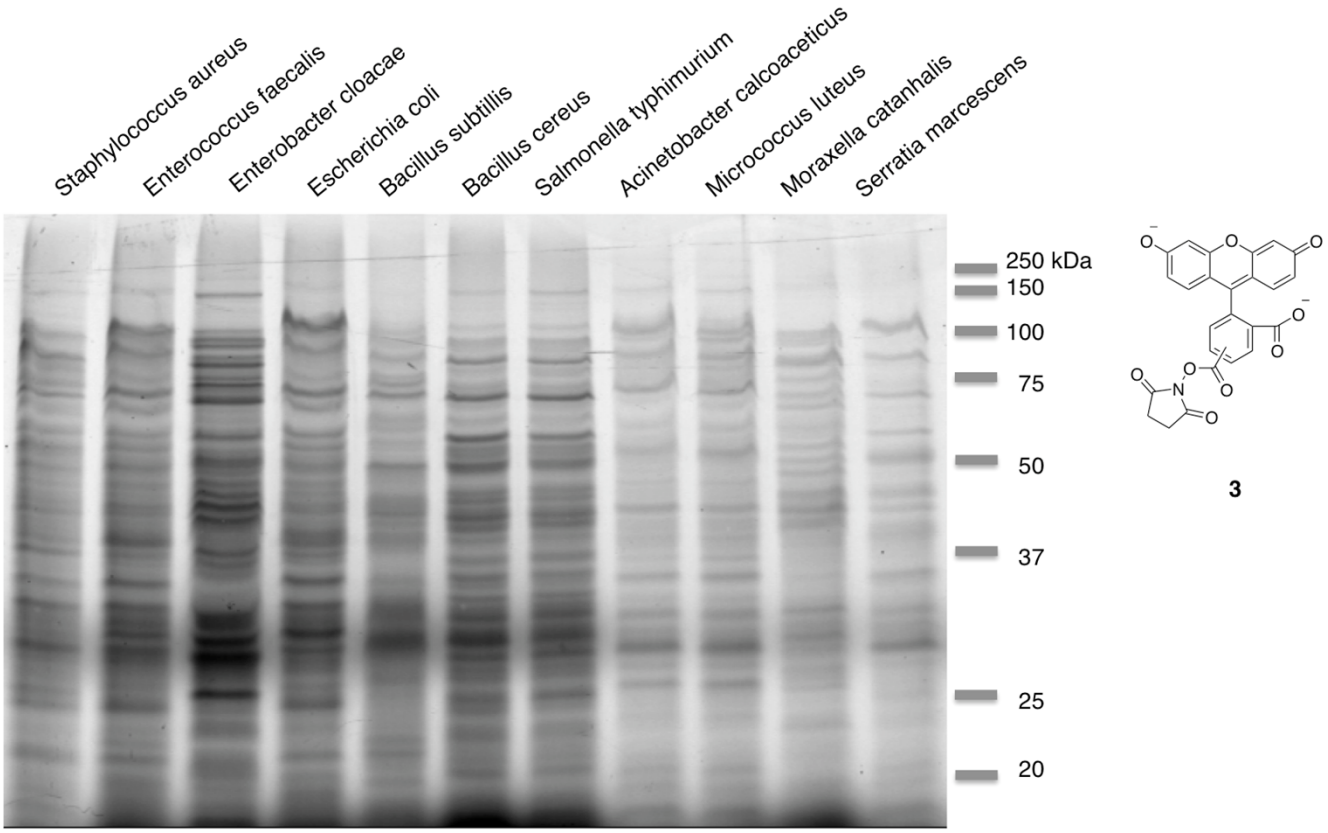


Figure S8. Bacterial proteome reactivity profile of compound **4**.

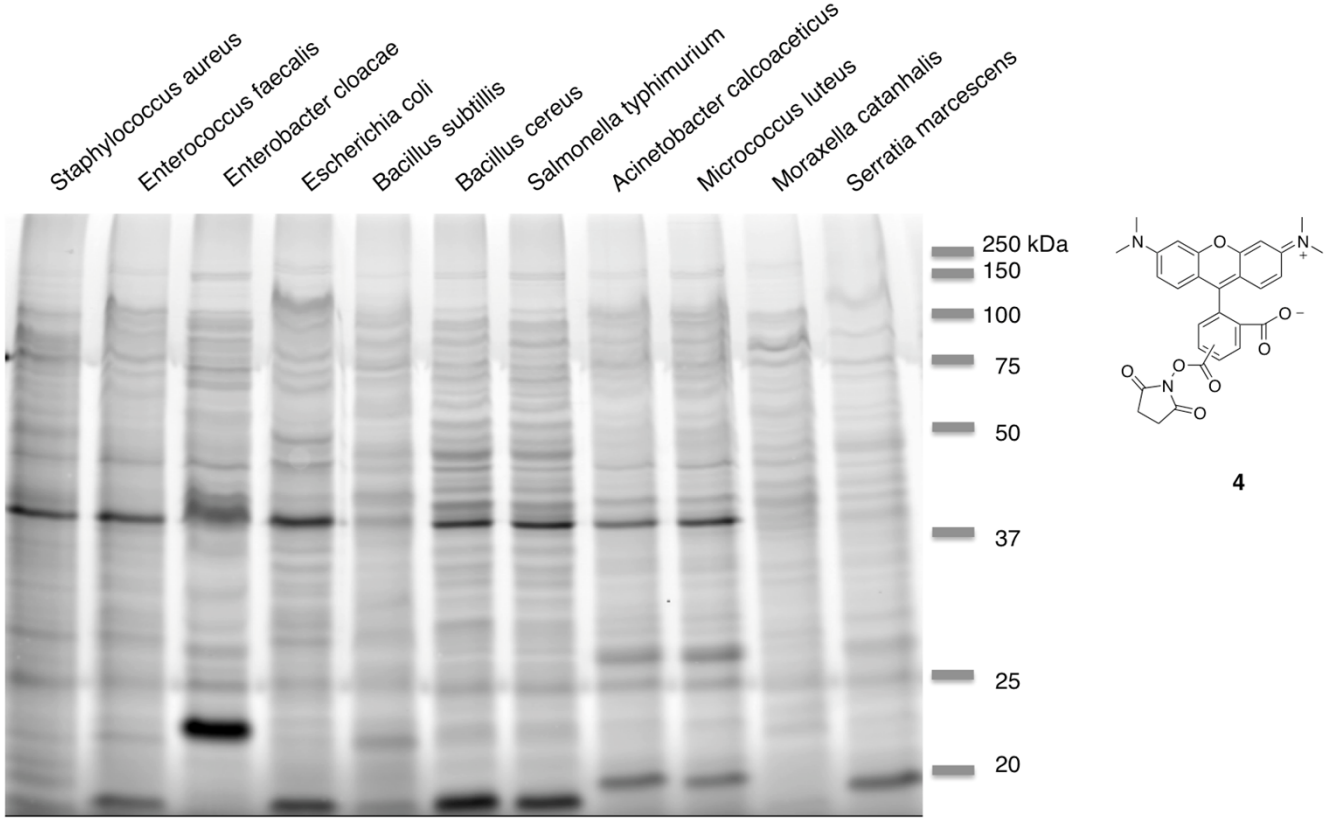
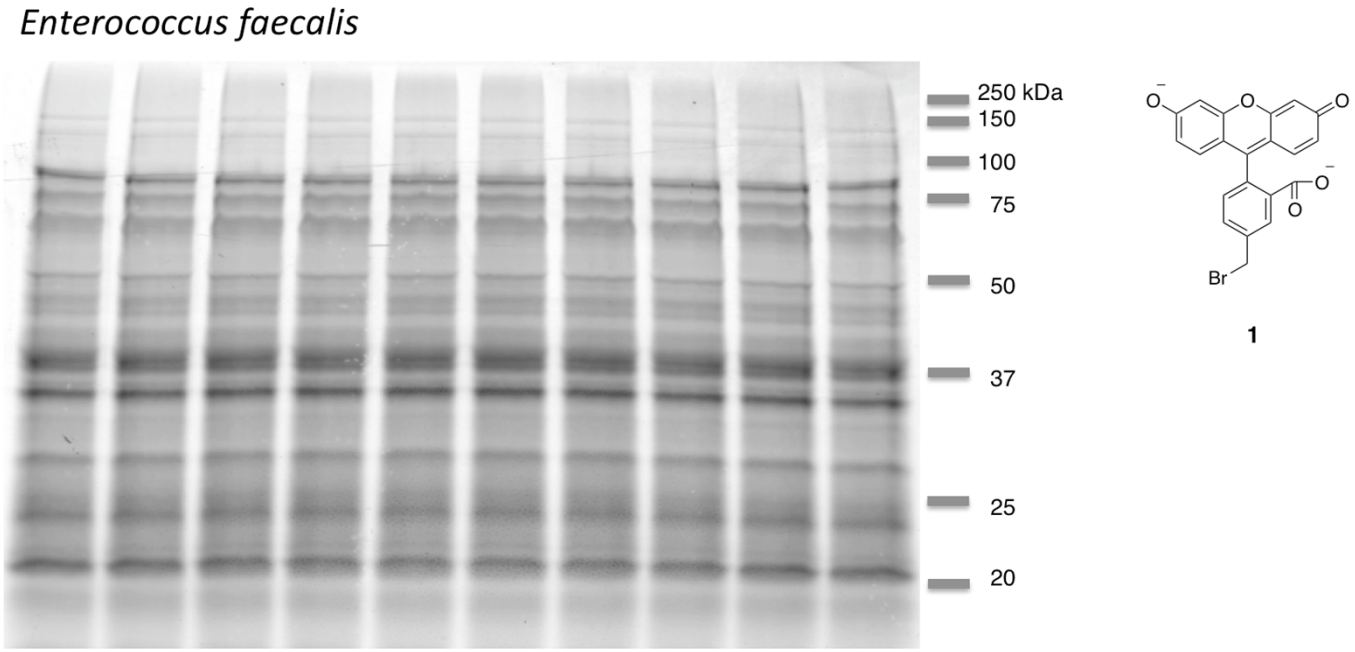
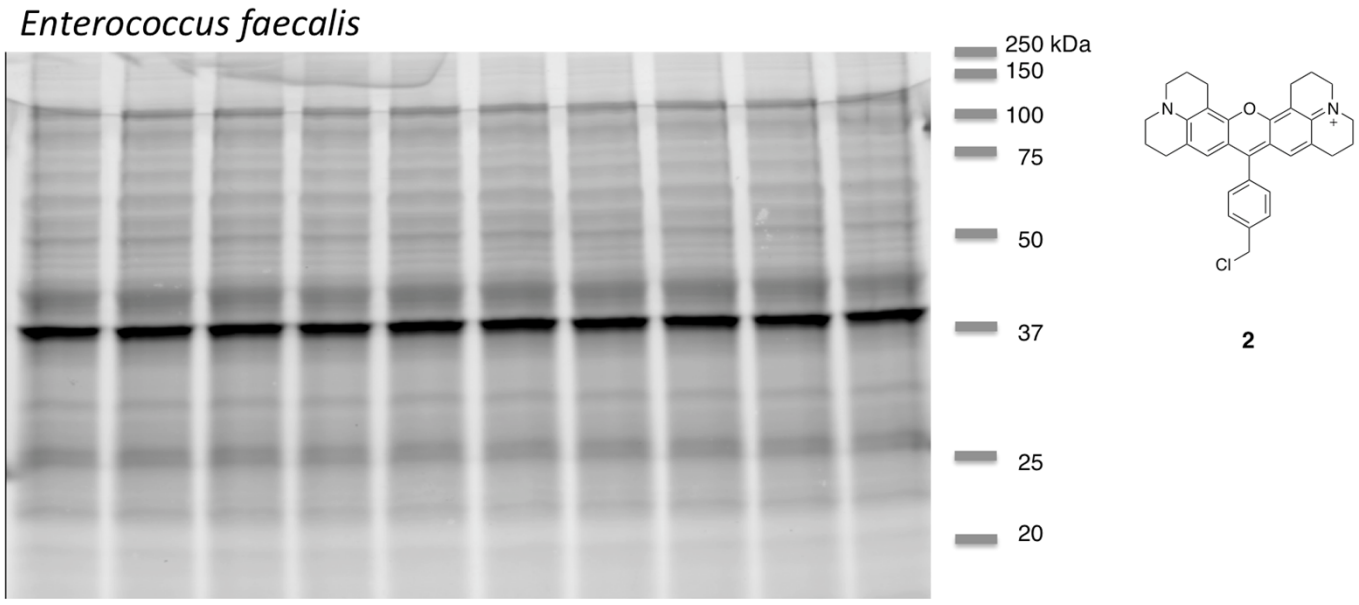


Figure S9. Representative SDS-gel images of reproducibility of reactivity profiles; (a: compound **1**, b: compound **2**) *Enterococcus faecalis* and (c: compound **1**, d: compound **2**) *Enterobacter cloacae*.

(a)

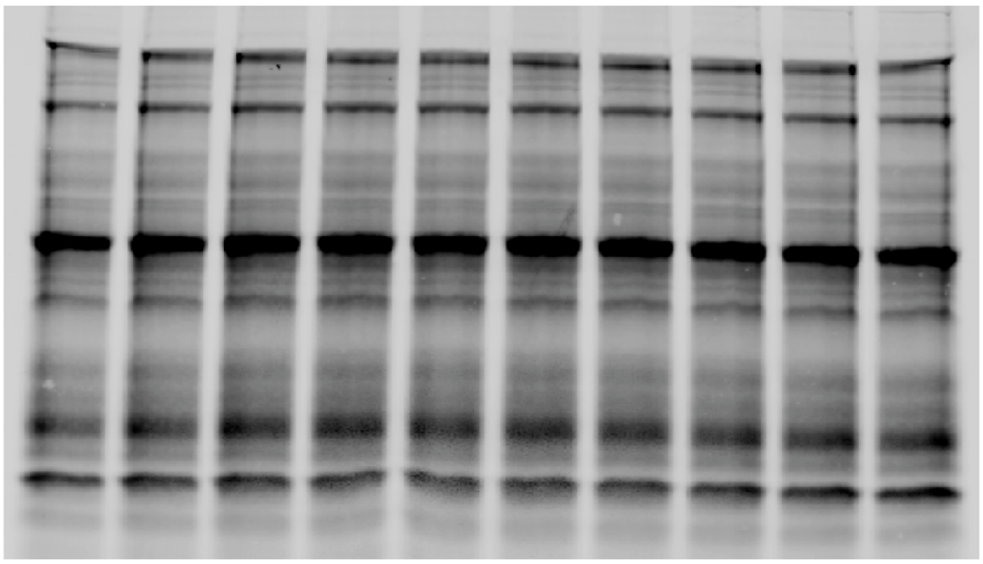


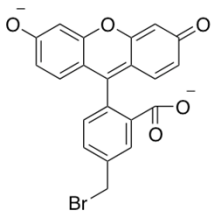
(b)



(c)

Enterobacter cloacae





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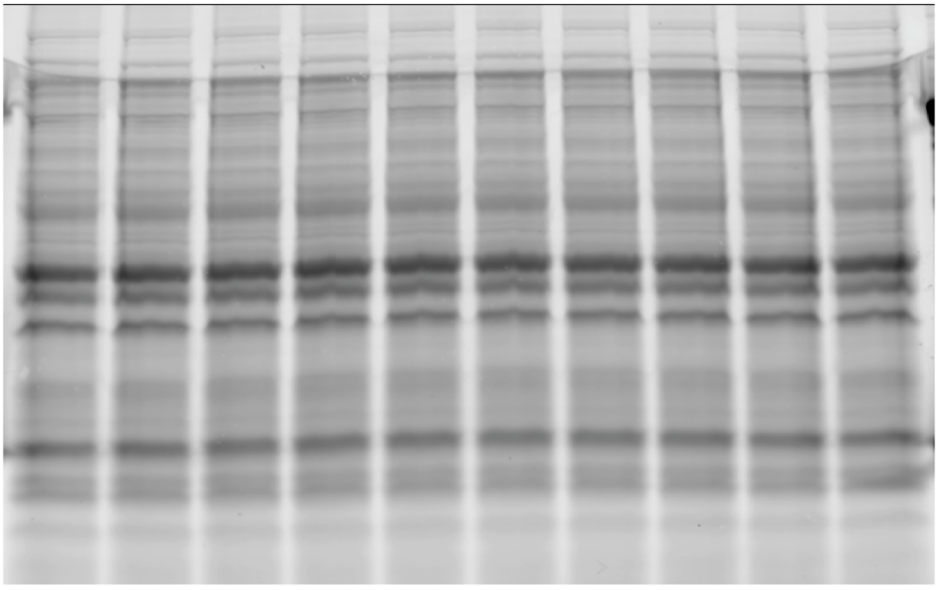
(d)

Enterobacter cloacae



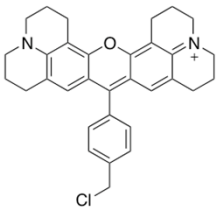


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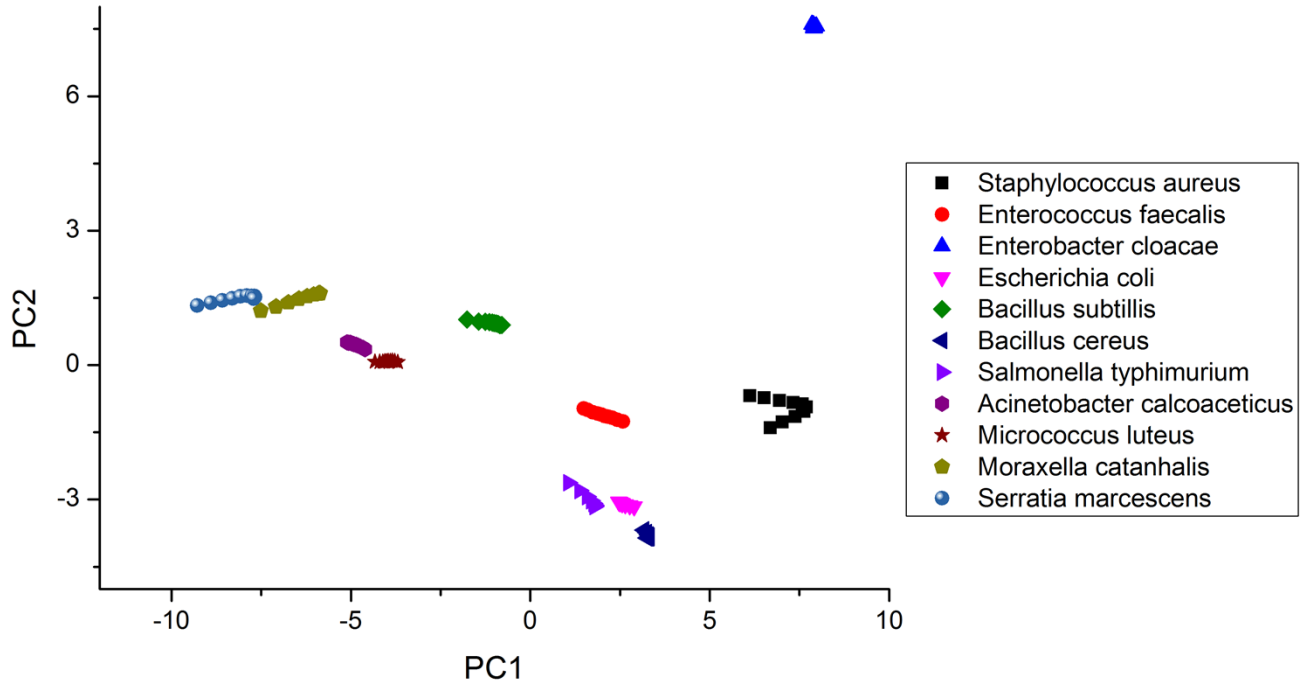
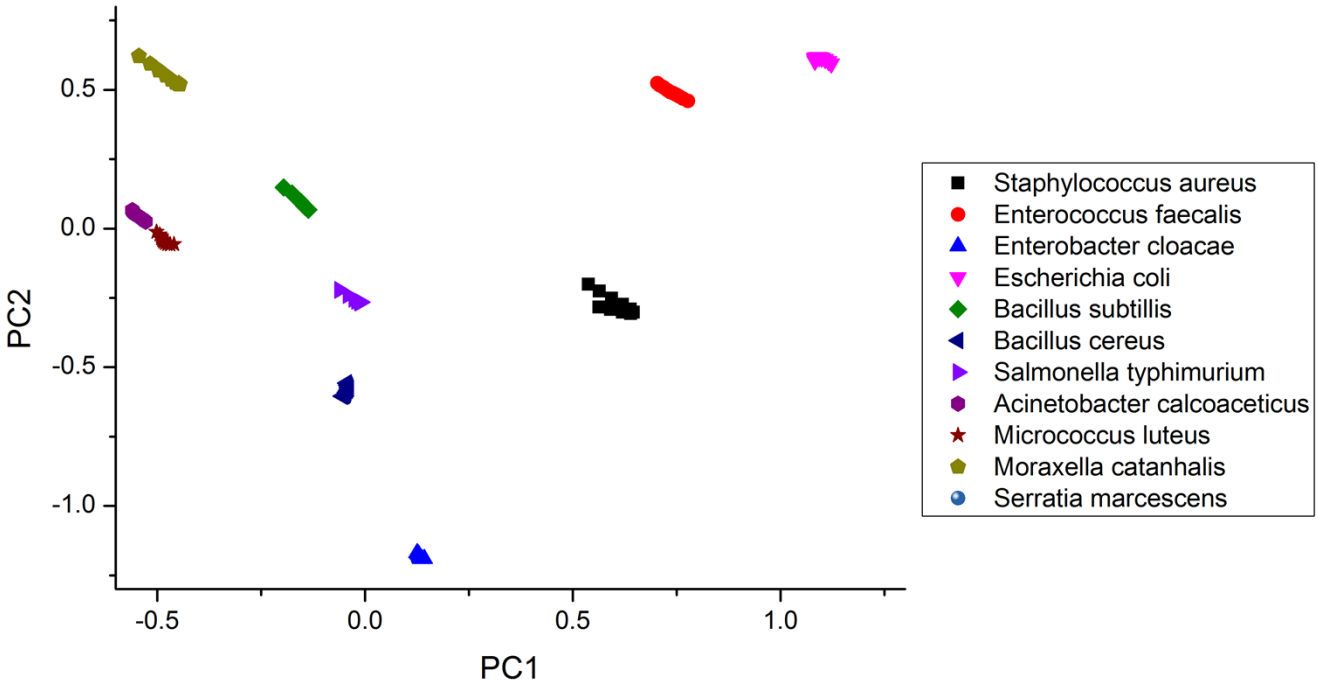
Figure S10. PCA plots for series of probe combinations

(a) NHS probes (compound **3**, **4**)



(b) All probes (compound **1**, **2**, **3**, **4**)



Reference

[1] C. A. Schneider, W. S. Rasband, K. W. Eliceiri, *Nat. Methods* **2012**, *9*, 671-675.

[2] J. O. Hill, J. Hauptman, J. W. Anderson, K. Fujioka, P. M. O'Neil, D. K. Smith, J. H. Zavoral, L. J. Aronne, *Am. J. Clin. Nutr.* **1999**, *69*, 1108-1116.