Supplementary Information

Biophysical Characterization of Cationic Antibacterial Oligothioetheramides

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Instrumentation

All NMRs were performed on a Bruker Avance III 500 MHz spectrometer equipped with a cryogenic probe. NMR spectra were analyzed in MestReNova (version 12.0.3-21384).

HPLC purification was carried out on a reverse-phase Agilent Eclipse XDB-C18 column (9.4 x 250 mm, 5 µM), by a 1100 Series Agilent HPLC system equipped with a UV diode array detector and a 1100 Infinity analytical scale fraction collector. Solvents for HPLC were water and acetonitrile with 0.1% trifluoroacetic acid (TFA) added.

LCMS characterization was performed on an Agilent 1100 LCMS system (single-quad G1956B) in positive ion mode using a Poroshell 120 EC-C18 column (3x100 mm, 2.7 µm) purchased from Agilent Technology, with absorbance recording at 210 nm and 254 nm. All samples were analyzed on a gradient of 5-100% acetonitrile in water with 0.1% acetic acid added.

Most tabulated data were processed with GraphPad Prism 7.01. Model fitting of SPR data was performed using MATLAB R2018b (9.5.0.944444).

Description of supplementary video files

Supplementary Video S1: 5 µM PDT-4Am disrupting SLB membrane to generate retracted aggregates.
Time-lapse fluorescence microscopy of 5 µM PDT-4Am placed onto an R18-labeled S. aureus mimic supported lipid bilayer (SLB). Time of oligomer exposure to membrane surface is indicated in black in the upper left corner in minutes:seconds. 100 µm scale bar provided.

Supplementary Video S2: 5 µM PDT-4G disrupting SLB membrane to generate longer aggregates.
Time-lapse fluorescence microscopy of 5 µM PDT-4G placed onto an R18-labeled S. aureus mimic supported lipid bilayer (SLB). Time of oligomer exposure to membrane surface is indicated in black in the upper left corner in minutes:seconds. 100 µm scale bar provided.
**Guanidine Monomer Synthesis**

Reaction 1: 2-(2-aminoethyl)-1,3-di-Boc-guanidine synthesis: 1 equivalent 1,3-Di-boc-2-methylisothiourea at a concentration of 250 mM in dichloromethane (DCM) was reacted with 2.5 eq ethylenediamine for 70 min at room temperature. The reaction mixture was washed 3x with water and 1x with brine. It was used immediately in the acylation reaction.

Reaction 2: Acylation reaction: Additional DCM was added to the resulting solution of Reaction 1 for a final concentration of 150 mM. 3 eq triethylamine were added and the mixture was stirred on ice for 10-15 min. 1.5 eq acryloyl chloride was dissolved in 25% the total volume of DCM to be used and added dropwise to the reaction mixture over 20 min. The reaction was stirred 1 hr on ice and 1 hr at room temperature, then quenched with water and extracted three times with DCM. All organic layers were combined, washed with brine, and dried with anhydrous sodium sulfate before solvent removal under reduced pressure.

Reaction 3: Alkylation reaction: 1 eq acylation product was dissolved in dry dimethylformamide (DMF) for a final reaction concentration of 200 mM. 4 eq sodium hydride was added and the mixture was stirred at room temperature for 10 minutes. 2.5 eq allyl bromide was dissolved in 25% the final volume DMF and added dropwise over 15 minutes. The mixture was stirred 1 hour at room temperature before the reaction was quenched with water. The product was extracted three times with diethyl ether and washed with brine. Solvent was removed under reduced pressure to yield a yellow oil which was purified using silica-column flash chromatography on a gradient of 0-100% ethyl acetate in hexanes. The desired guanidine monomer product eluted at 24% hexanes in ethyl acetate. Product was verified by \(^1\)H NMR and LCMS.

**Boc-Amine Monomer Synthesis**

Reaction 1: 1 equivalent Boc-bromoethylamine was dissolved in twice its volume of dichloromethane and added dropwise to a mixture of 6 eq potassium carbonate dissolved in 50 eq allylamine. After 3 hours, the mixture was filtered through celite to remove the potassium carbonate, and allylamine was removed under reduced pressure.

Reaction 2: The product of reaction 1 was dissolved in dichloromethane for a final reaction concentration of 200 µM, and stirred for 15 min at 0°C. Acryloyl chloride, diluted in five times its volume of dichloromethane, was added dropwise over 30 min. The mixture was stirred for one hour at 0°C and one hour at room temperature before being quenched with water. Product was extracted with dichloromethane and dried with anhydrous sodium sulfate. Solvent was removed under reduced pressure to yield a yellow oil. This was further purified using silica column flash chromatography on a gradient of 15-75% ethyl
acetate in hexanes. The desired product, the amine monomer, eluted at 55% ethyl acetate in hexanes and was verified using $^1$H NMR and LCMS.

**OligoTEA Synthesis**

OligoTEA synthesis was performed by alternating thiol-ene and thiol-Michael addition reactions, starting with fluorous-allylamine.

*Fluorous solid-phase extraction (FSPE)*: Crude reaction product was loaded onto a column packed with 2g fluorous silica. The column was washed using a fluorophobic wash of 80:20 MeOH:water to remove any non-fluorous material. The desired fluorous product was eluted using pure MeOH.

*Fluorous allylamine synthesis*: 1 molar equivalent Fluorous BOC-ON was combined with 1.6 equivalents allylamine and 2.5 equivalents triethylamine in tetrahydrofuran and stirred overnight at room temperature. After removal of tetrahydrofuran under reduced pressure, the product was purified using FSPE and dried to yield fluorous-allylamine, which was used without further purification.

*Thiol-ene reaction*: 1 equivalent fluorous-olefin was dissolved in MeOH (150 µM first thiol-ene reaction, 100 µM each subsequent thiol-ene reaction). 5 molar equivalents of propanedithiol (PDT) and 10 mol% (of the dithiol) of 2,2-dimethoxy-2-phenylacetophenone (DMPA) were added and the solution was UV irradiated at 20 mW/cm$^2$ for 270 seconds. The mixture was purified using FSPE to yield the desired fluorous-thiol, which was used before removal of MeOH.

*Thiol-Michael addition reaction*: Fluorous-thiol in MeOH was combined with 2 equivalents of Boc-protected amine or guanidine monomer and 10% (of the monomer) dimethylphenylphosphine. MeOH was removed under reduced pressure, during which time the reaction went to completion. The product was purified using FSPE as described previously to yield the desired fluorous-olefin.

*Fluorous tag cleavage and Boc-deprotection*: Completed oligoTEA was dissolved at 20 µM in TFA for 1 hour, which was subsequently removed under reduced pressure to yield the desired deprotected oligoTEA.

*HPLC purification*: Deprotected oligoTEA was purified on a gradient of 0-100% acetonitrile with 0.1% TFA in water with 0.1% TFA. Compounds were verified using LCMS and $^1$H NMR and quantified using $^1$H NMR with integration against a known standard (acetonitrile or 1,4-dioxane).
Figure S1: $^1$H NMR of PDT-3G, taken in methanol D$_4$. Acetonitrile used for quantification.

Figure S2: $^1$H NMR of PDT-2GAm, taken in methanol D$_4$. Acetonitrile used for quantification.
Figure S3: $^1$H NMR of PDT-Am2G, taken in methanol D4. Acetonitrile used for quantification

Figure S4: $^1$H NMR of PDT-G2Am, taken in methanol D4. Acetonitrile used for quantification
Figure S5: $^1$H NMR of PDT-3Am, taken in methanol D4. Acetonitrile used for quantification.

Figure S6: $^1$H NMR of PDT-4G, taken in D$_2$O. 1,4-dioxane used for quantification.
Figure S7: $^1$H NMR of PDT-4Am, taken in D$_2$O. 1,4-dioxane used for quantification.

Figure S8: $^1$H NMR of BDT-4G, taken in D$_2$O. 1,4-dioxane used for quantification.
Figure S9: $^1$H NMR of BDT-4Am, taken in D$_2$O. 1,4-dioxane used for quantification.
Figure S10: LCMS of PDT-3G

Figure S11: LCMS of PDT-2GAm
Figure S12: LCMS of PDT-Am2G

Figure S13: LCMS of PDT-G2Am
Figure S14: LCMS of PDT-3Am

Figure S15: LCMS of PDT-4G
Figure S16: LCMS of PDT-4Am

Figure S17: LCMS of BDT-4G
Figure S18: LCMS of BDT-4Am
Ordinary differential equations from SPR kinetic model

\[
\text{AOT} + \text{Lipid} \xrightarrow{k_1} \xrightarrow{k_2} \text{OL} \xrightarrow{k_4} \xrightarrow{k_5} \text{OL}^* \xrightarrow{\text{Loss?}} \xrightarrow{\text{Loss?}}
\]

\[
\frac{d\text{OL}}{dt} = k_1[A\text{OT}][C_{\text{Lipid,total}} - \text{OL} - \text{OL}^* - \text{Loss(OL)} - \text{Loss(OL')} - k_2\text{OL} - k_3\text{OL} - k_4\text{OL} \quad (1)
\]

\[
\frac{d\text{OL}^*}{dt} = k_3\text{OL} - k_5\text{OL}^* \quad (2)
\]

\[
\frac{d\text{Loss(OL)}}{dt} = k_4\text{OL} \quad (3)
\]

\[
\frac{d\text{Loss(OL')}^*}{dt} = k_5\text{OL}^* \quad (4)
\]

\[
\frac{d\text{Signal}_{\text{SPR}}}{dt} = \frac{d\text{OL}}{dt} + \frac{d\text{OL}^*}{dt} \quad (5)
\]

Excerpt of Figure 4a of translated to mass action kinetics rates (Equations 1-5). SPR observes effective refractive index changes near the gold sensor surface, encoding both mass and structural changes. Thus, the SPR cannot observe material lost from the surface seen in Equation 3 and 4 and Equation 5 sums up the combined observations.

Use of MATLAB to model and fit SPR data

Fitting of the kinetic rates to the SPR sensorogram data was completed using lsqcurvefit. The function incorporated two separate nested ODEs for the association and dissociation phases, where \(C_{\text{oligoTEA}}\) was the designated concentration and 0uM, respectively. The ODEs were numerically solved by ode15s with RelTol and AbsTol as 1e-8. Convergence of lsqcurvefit was seen typically in less than 1k function iterations to a resnorm less than 5e5 to capture the behavior of the curve. Kinetic rate parameters \(k_1, k_2, k_3, k_4, \) and \(k_5\) were not bounded, while \(C_{\text{Lipid,total}}\) was kept constant (3000 ± <0.01%). \(C_{\text{Lipid,total}}\) should be dependent on the physical number of binding spots of the lipid surface, only changing based on SUV lipid composition, not the oligomer concentration (only the MRSA lipid composition was fit). \(C_{\text{Lipid,total}}\) must be greater than the highest RU value in the experimental data. For example, \(C_{\text{Lipid,total}}\) must be at least ~2100 RU worth of lipid in order to fit the BDT-4G from Figure S19. \(C_{\text{Lipid,total}}\) scales the curve response, with little change to the curve shape. Since the kinetic rates significantly affect curve shape, \(C_{\text{Lipid,total}}\) was held constant to allow comparison of kinetic rates.
S19: All SPR data for all oligoTEAs tested, at all concentrations tested.
S20: Model Fits of SPR of PDT-3G
S21: Model Fits of SPR of PDT-3Am
S22: Model Fits of SPR of PDT-4G
S23: Model Fits of SPR of PDT-4Am
S24: Model Fits of SPR of BDT-4G
S25: Model Fits of SPR of BDT-4Am
Table S1: All parameter from model fit of SPR data. $K_1$ (equilibrium constant) = $k_1/k_2$

| Conc, uM | PDT3G | PDT3Am | PDT4G | PDT4Am | BDT4G | BDT4Am |
|----------|-------|--------|-------|--------|-------|--------|
| K1       |       |        |       |        |       |        |
| 1        | 71550 | 1737699| 1844168| 671792 | 460794| 2000540|
| 2.5      | 261146 | 107363 | 2983064| 242892 | 1296666| 261159 |
| 5        | 63942  | 49461 | 112503 | 79981 | 146159 | 108327 |
| 10       | 30004  | 27562 | 58920  | 42318 | 100048 | 55907  |
| 25       | 16001  | 13076 | 31376  | 20837 | 56918  | 28333  |
| k3       |       |        |       |        |       |        |
| 1        | 0.00789 | 0.00464 | 0.00726 | 0.00609 | 0.00662 | 0.00665 |
| 2.5      | 0.00607 | 0.00378 | 0.00737 | 0.00475 | 0.00671 | 0.00570 |
| 5        | 0.00518 | 0.00371 | 0.00523 | 0.00486 | 0.00577 | 0.00564 |
| 10       | 0.00483 | 0.00339 | 0.00489 | 0.00477 | 0.00454 | 0.00574 |
| 25       | 0.00490 | 0.00331 | 0.00374 | 0.00415 | 0.00344 | 0.00460 |
| k4       |       |        |       |        |       |        |
| 1        | 0.0823 | 0.0303 | 0.0214 | 0.0300 | 0.0311 | 0.0299 |
| 2.5      | 0.0285 | 0.0205 | 0.0210 | 0.0172 | 0.0169 | 0.0201 |
| 5        | 0.0207 | 0.0147 | 0.0100 | 0.0122 | 0.0074 | 0.0105 |
| 10       | 0.0133 | 0.0113 | 0.0066 | 0.0090 | 0.0043 | 0.0078 |
| 25       | 0.0085 | 0.0081 | 0.0033 | 0.0056 | 0.0020 | 0.0044 |
| k5       |       |        |       |        |       |        |
| 1        | 0.001069 | 0.000596 | 0.000196 | 0.000250 | 0.000101 | 0.000336 |
| 2.5      | 0.000770 | 0.000767 | 0.000443 | 0.000235 | 0.000275 | 0.000235 |
| 5        | 0.000784 | 0.000875 | 0.000400 | 0.000396 | 0.000381 | 0.000321 |
| 10       | 0.000726 | 0.000706 | 0.000533 | 0.000484 | 0.000413 | 0.000465 |
| 25       | 0.000388 | 0.000557 | 0.000499 | 0.000426 | 0.000427 | 0.000420 |
S26: All parameters from model fits of SPR Data. K1 is the equilibrium constant (k1/k2)

S27: All populations from model fits of SPR Data.