# Supporting Information

# Antimicrobial Peptides Against Multidrug-Resistant *Pseudomonas aeruginosa* Biofilm from Cystic Fibrosis Patients

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**Table S1. *P. aeruginosa* CF isolates identification and resistance index**

| Isolate No. | Gentamicin | Tobramycin | Amikacin | Ofloxacin | Ciprofloxacin | Ceftazidime | Cefepime | Aztreonam | Piperacillin | Pipera/Tazobactam | Imipenem | Meropenem |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | R | R | R | R | R | R | R | R | R | R | R | R |
| 25 | R | R | S | R | R | R | R | R | R | R | R | R |
| 29 | R | R | R | R | R | R | R | R | I | I | R | R |
| 40 | R | R | R | R | R | R | R | R | R | R | R | R |
| 46 | R | S | R | R | R | R | R | R | R | R | R | S |
| 52 | R | R | R | R | R | R | R | R | R | R | S | S |
| 53 | R | R | R | R | R | R | R | R | R | R | R | R |
| 59 | R | S | S | R | R | S | S | S | S | S | S | S |
| 71 | R | S | S | R | R | R | R | R | R | R | R | R |
| 72 | R | R | R | R | R | R | R | R | R | R | R | R |
| 82 | R | R | S | S | S | R | R | R | R | R | R | R |
| 94 | R | R | R | R | R | R | R | R | R | R | R | R |
| 95 | R | R | R | R | R | R | R | R | R | R | R | R |
| 99 | S | S | S | S | S | S | S | S | S | S | S | S |
| 172 | R | R | R | R | R | R | R | R | R | R | R | R |
| 238 | R | R | R | R | R | R | R | R | R | R | R | R |
| 251 | R | R | R | R | R | R | R | R | R | R | R | R |
| 629 | S | R | S | S | S | R | R | R | R | S | R | R |
| 895 | R | R | R | R | R | R | R | R | R | R | R | R |
| 896 | R | S | S | S | S | S | S | S | S | S | S | S |
| 995 | R | R | R | R | R | R | R | S | S | R | R | R |

■ - Sensitive  ■ - Intermediate  ■ - Resistance

Identification and susceptibility were conduct by BD Phoenix™ automated identification and susceptibility testing system

**Table S2: Susceptibility test parameters of the Phoenix system**

| Drug class | Drug name | Drug range | MIC (S) | MIC (I) | MIC (R) |
|---|---|---|---|---|---|
| Aminoglycoside | Gentamicin | 0.25-16 | ≤4 | 8 | ≥16 |
| Aminoglycoside | Tobramycin | 0.12-16 | ≤4 | 8 | ≥16 |
| Aminoglycoside | Amikacin | 0.5-64 | ≤16 | 32 | ≥64 |
| 5-Fluoroquinolone | Ofloxacin | 0.25-8 | ≤2 | 4 | ≥8 |
| 5-Fluoroquinolone | Ciprofloxacin | 0.25-4 | ≤1 | 2 | ≥4 |
| Cephem | Ceftazidime | 0.5-64 | ≤8 | 16 | ≥32 |
| Cephem | Cefepime | 0.5-64 | ≤8 | 16 | ≥32 |
| Monobactam | Aztreonam | 0.5-64 | ≤8 | 16 | ≥32 |
| B-Lactam Pen | Piperacillin | 0.5-128 | ≤16 | 32-64 | ≥128 |
| B-Lac/B-Lac. Inh | Pipera/ Tazobactam | 0.5/4-128/4 | ≤13 | 32-64 | ≥128 |
| Carbapenem | Imipenem | 1-16 | ≤14 | 4 | ≥8 |
| Carbapenem | Meropenem | 0.25-16 | ≤15 | 4 | ≥8 |
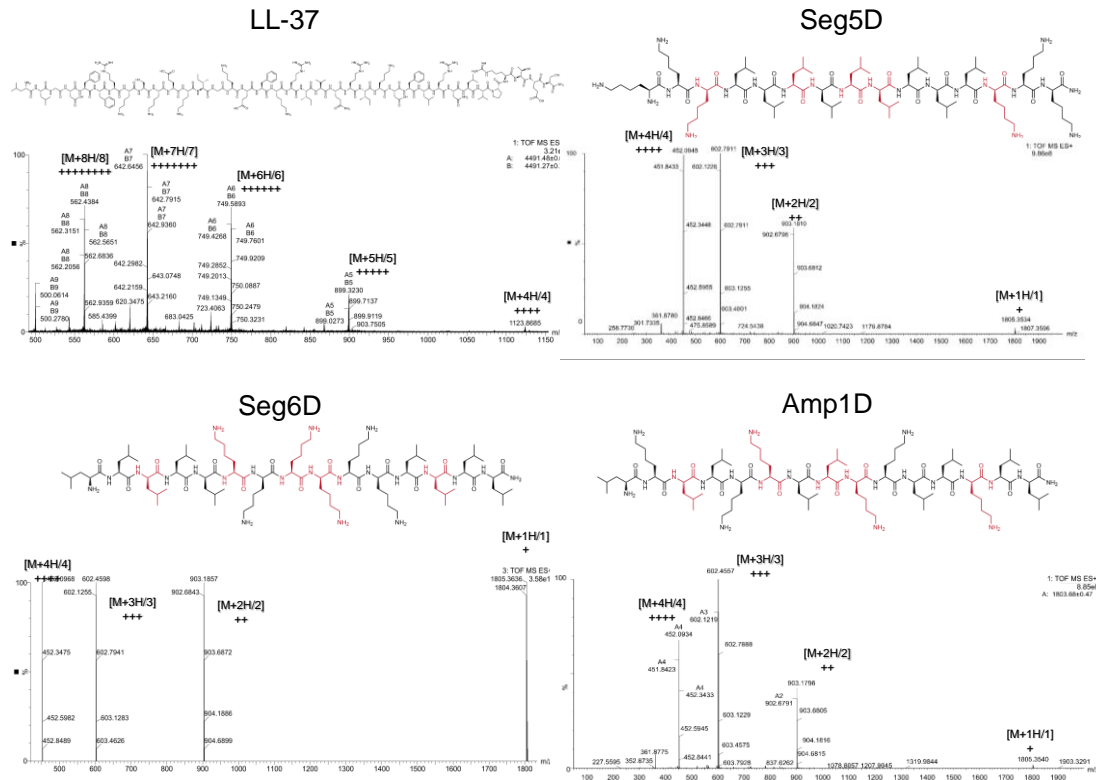
**Figure S1: Mass spectrum of peptides**
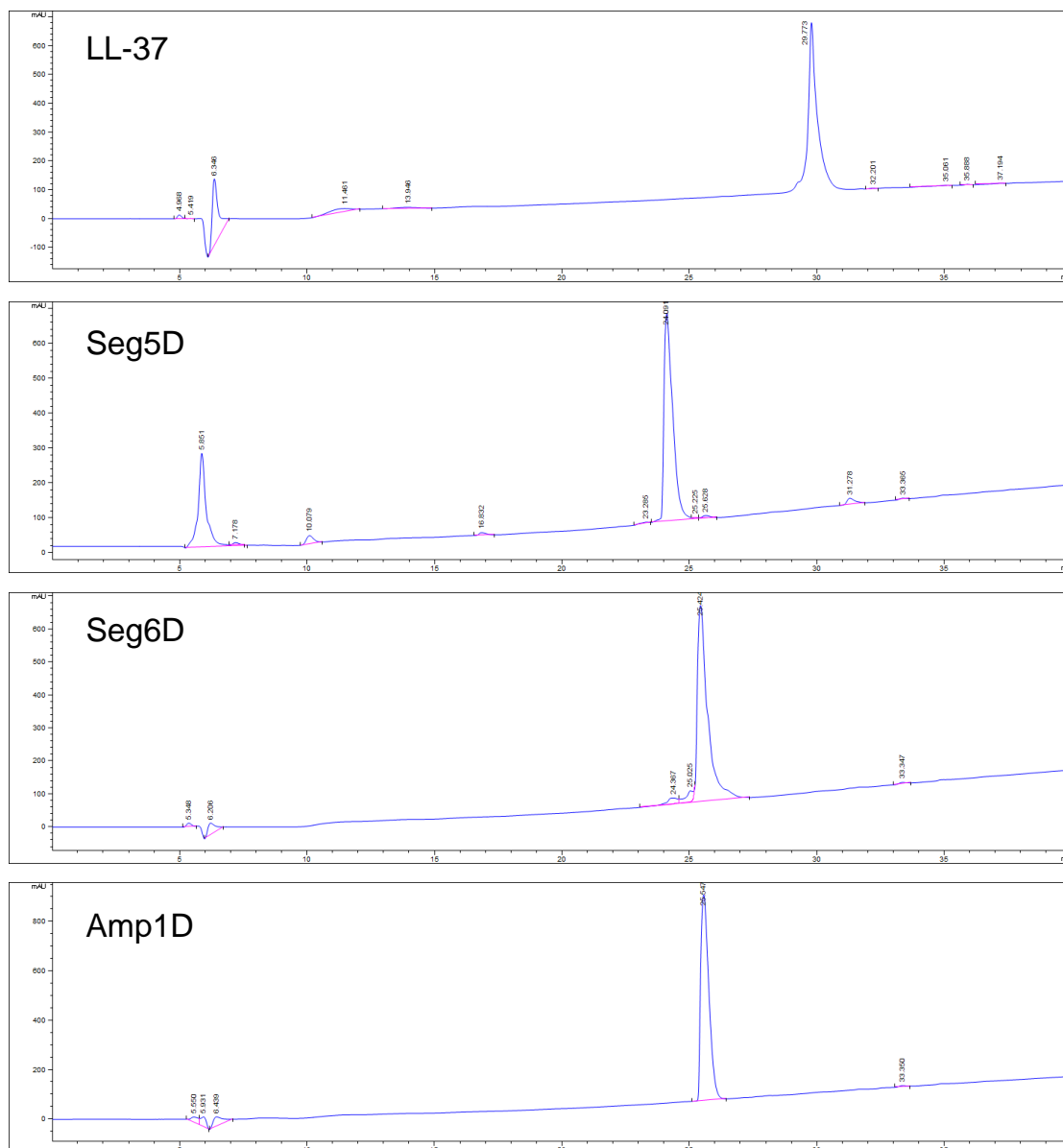
**Figure S2: Analytical HPLC chromatograms of peptides**
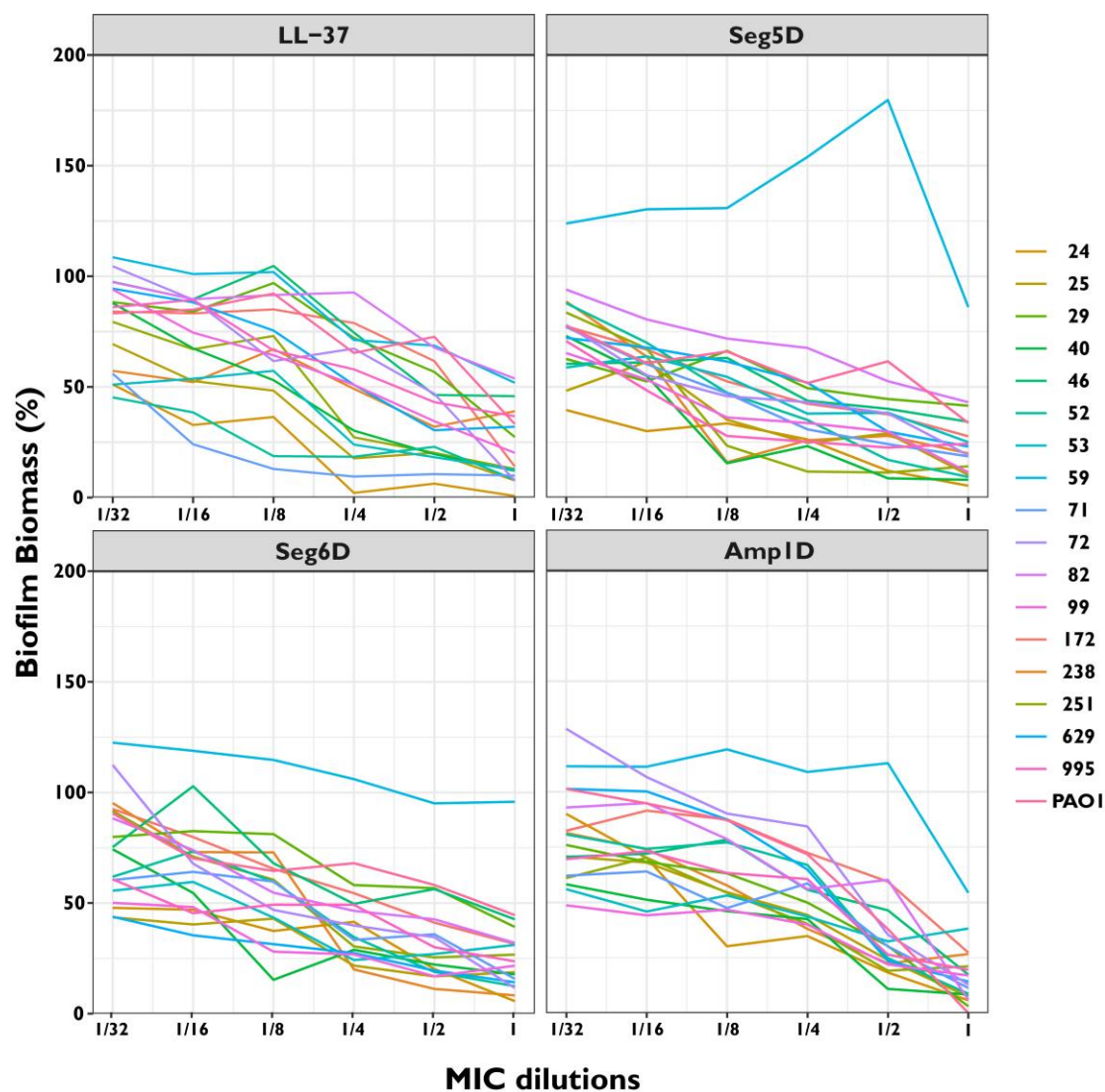
**Figure S3.** Inhibition of clinical CF patient isolates *P. aeruginosa* biofilm formations at sub-inhibitory concentration of AMPs. *P. aeruginosa* bacteria were incubated for 24 h in the presence of AMPs (at MIC dilutions). Surface-associated biofilm after treatment was examined using 0.1% CV staining followed by absorbance measurements at 590 nm. Results are reported relative to untreated biofilm. Each graph represents an isolated sample (shows in the left bottom of each graph). Background measurement with no added bacteria were preformed as blank.
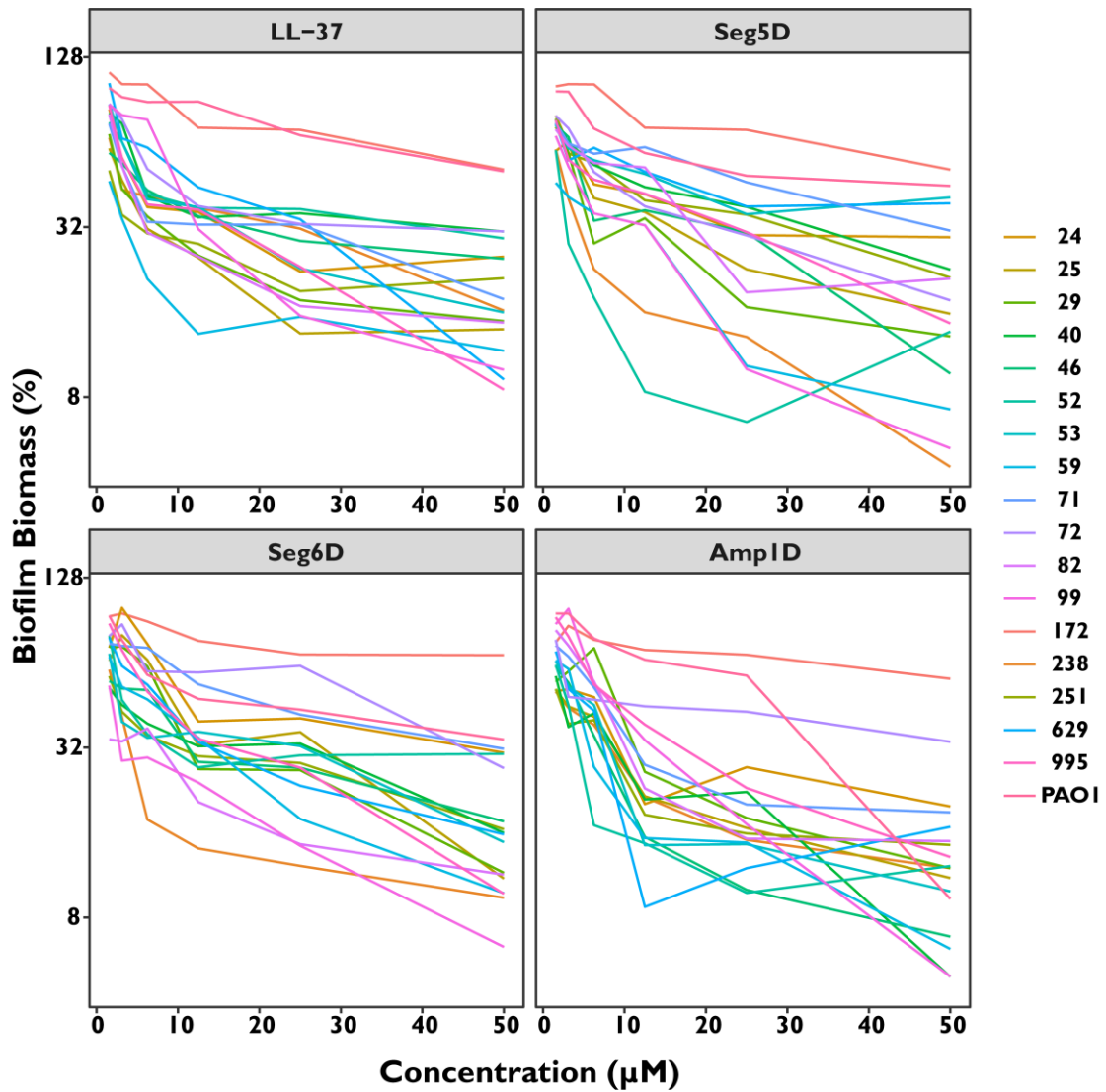
**Figure S4.** D,L-$K_6L_9$ peptides and LL-37 degrade established clinical isolates CF patient *P. aeruginosa* biofilms. *P. aeruginosa bacteria* were allowed to grew for 24-h and treated for 1-hour with peptides in a serial dilution concentrations. Surface-associated biofilm after treatment, examined using 0.1% CV staining followed by absorbance measurements at 590 nm. Results are reported relative to untreated biofilm. Each graph represent isolated sample (shows in the left bottom of each graph). Background measurement with no added bacteria were preformed as blank