Evaluating the Ribosome Binding Activity of the Natural Product Antimicrobial Peptide Pdi1

TT Dean[†], A Ziyad[†], D Klepacki[†‡], N Nazquez-Laslop[†‡], A Mankin[†‡], TW Moore[†]

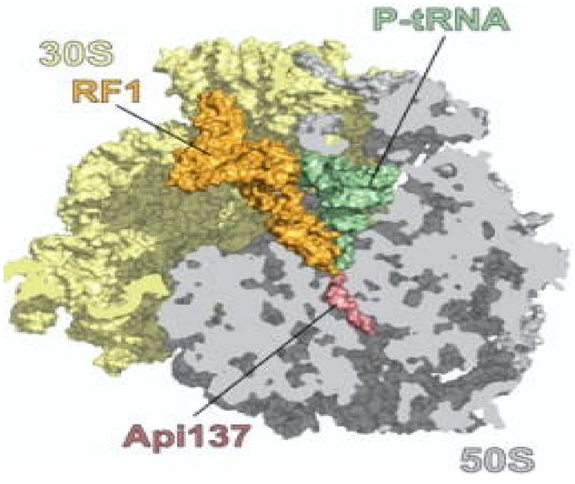
[†]Department of Pharmaceutical Sciences, College of Pharmacy, University of Illinois Chicago

[‡]Center for Biomolecular Sciences, University of Illinois Chicago

Background

- Apidaecins are natural product antimicrobial peptides found in a variety of insect species
- These peptides inhibit translation termination by sequestering the ribosomal release factor within the occluded nascent peptide exit tunnel (nPET). This activity results in the depletion of cellular release factor and abhorrent protein synthesis
- Honeybee-derived Api1b has been the subject of several medicinal chemistry campaigns which yielded the most potent and pharmacologically relevant derivative Api137
- Newly identified Apidaecin peptides, such as Pdi1 and Fva1, demonstrate potencies that rivals Api137

Figure 1: Molecular Model of Apidaecin Bound to Translation Termination Complex



Rationale

- Binding data and structure-activity relationships for Pdi1 are critical for the rational development of therapeutically relevant Pdi1 analogues
- Alanine scanning of Pdi1 can be used to explore the relationship between individual residues and ribosome binding affinity
- Biolayer interferometry (BLI) measures binding events in real-time and can link alanine substitutions to kinetics and affinity data
- N-terminal PEG_{24}-Biotin linkers were chosen to provide sufficient length to span the >50Å distance between the Apidaecin N-terminus and the mouth of the nPET
- Drop diffusion and liquid broth microdilution assays contribute antibacterial activity measurements to validate our BLI binding data

Table 1: Pdi1 Alanine Scan BLI Binding Data

Peptide	K_D(pM)	k_a(1/Ms)	k_{dis}(1/s)
Pdi1 Wild-Type	209	3.33e6	7.07e-4
Pdi1 (R14A)	Poor fit	Poor fit	Poor fit
Pdi1 (P15A)	405	1.62e6	6.34e-4
Pdi1 (P16A)	514	1.17e6	5.34e-4
Pdi1 (H17A)	677	1.02e6	6.82e-4
Pdi1 (P18A)	Poor fit	Poor fit	Poor fit
Pdi1 (R19A)	Poor fit	Poor fit	Poor fit
Pdi1 (L20A)	323	1.55e6	4.83e-4
Scrambled Pdi1	NBD[⸸]	NBD[⸸]	NBD[⸸]

[⸸]No Binding Detected

Figure 3: Average BLI Response at 1nM Ribosome

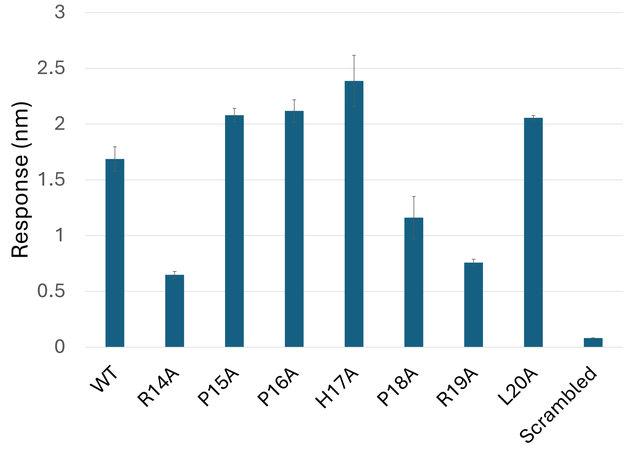


Figure 2: Representative BLI Sensogram for Wild-Type Pdi1

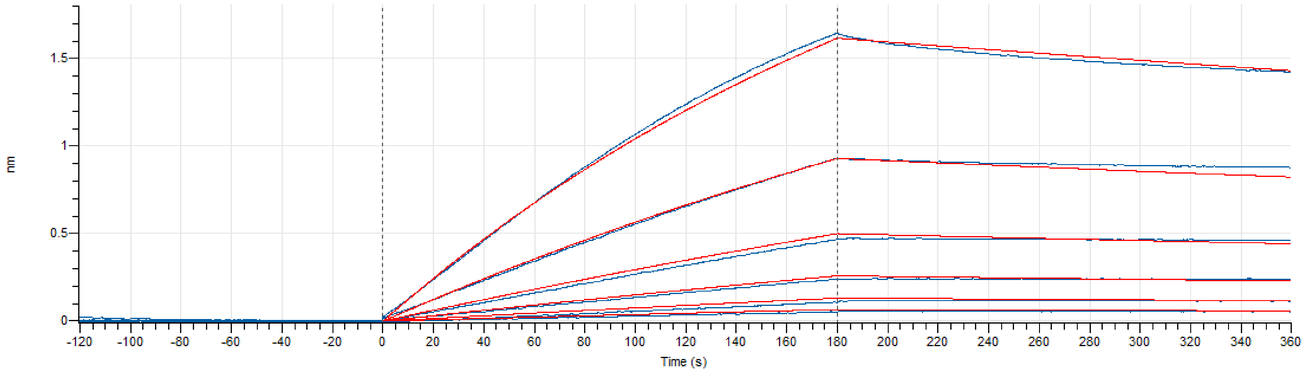


Figure 4: Drop Diffusion Test of Low Response/Affinity Pdi1 Peptides

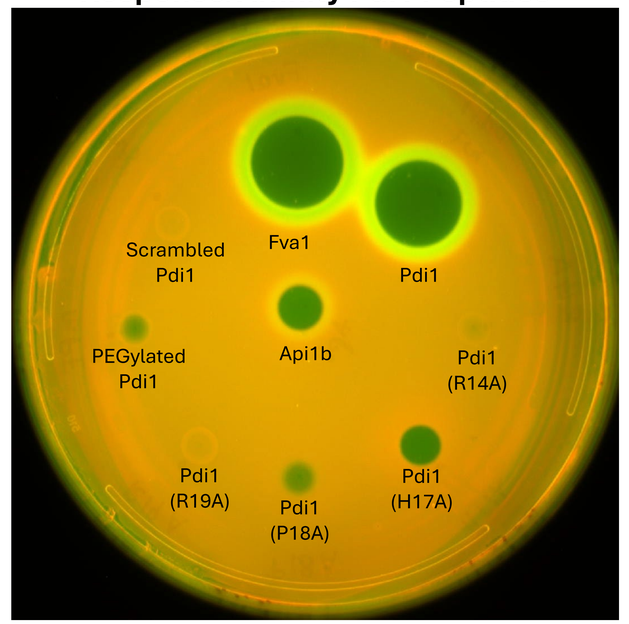


Table 2: Minimum Inhibitory Concentration Data for Pdi1 Peptides

Peptide	Minimum Inhibitory Concentration (μM)
Api1b	0.35
Api137	≤ 0.075
Pdi1	0.075
Pdi1 (R14A)	3.1
Pdi1 (H17A)	0.75
Pdi1 (P18A)	0.35
Pdi1 (R19A)	>100
PEGylated Pdi1	25
Scrambled Pdi1	100

Future Directions

- Fluorescence anisotropy experiments will be used as an orthogonal assay to determine IC_{50} values
- Circular dichroism spectroscopy will measure the impact of alanine substitutions on Pdi1 secondary structure