Investigation of human neutrophil elastase inhibition by *Staphylococcus aureus* EapH1: The key role

played by Arginine 89

Timothy J. Herdendorf and Brian V. Geisbrecht*

Department of Biochemistry& Molecular Biophysics, Kansas State University, Manhattan, KS 66506

*To whom correspondence should be addressed: Brian V. Geisbrecht, tel : 785-532-3154, fax: 785-532-7278, Geisbrechtb@ksu.edu
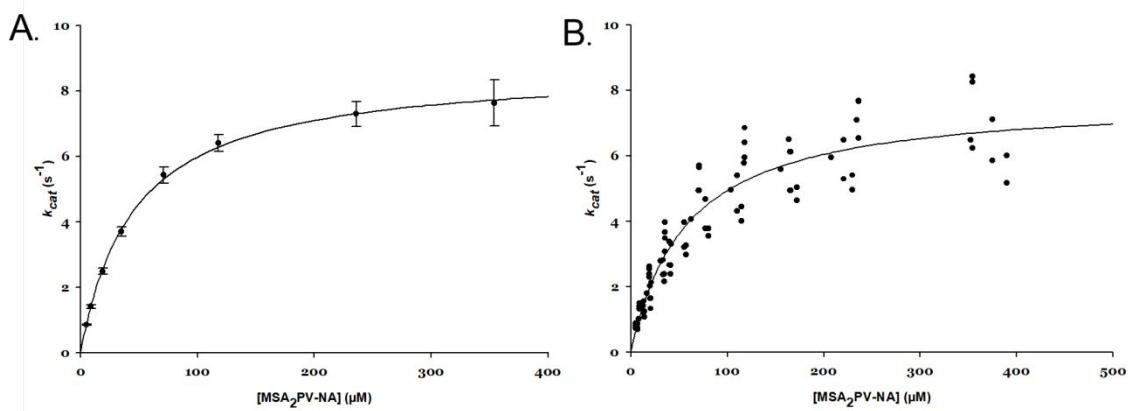
Figure S1. Steady-State kinetics of Human Neutrophil Elastase. (A) Cuvette-based assay, (B) Plate-based assay.

```
[task]
task = fit
data = progress
[mechanism]
E + S <==> ES : k1 k2
ES ---> E + P : k3
E + I <==> EI : k4 k5
[constants]   ; units: uM, min
k1 = 15 ?
k2 = 57 * k1
k3 = 500 ?
k4 = 15 ?
k5 = 0.01 ?
[concentrations]
[data]
directory ./dynafit_data/ST_data/EapH1/new_for_paper/180219
file Set1_1.txt  | conc E = 0.004 | conc S = 64 | conc I = 0
file Set1_2.txt  | conc E = 0.004 | conc S = 64 | conc I = 0.0025 ?
file Set1_3.txt  | conc E = 0.004 | conc S = 64 | conc I = 0.0051 ?
file Set1_4.txt  | conc E = 0.004 | conc S = 64 | conc I = 0.0068 ?
file Set1_5.txt  | conc E = 0.004 | conc S = 64 | conc I = 0.0085 ?
file Set2_1.txt  | conc E = 0.004 | conc S = 111 | conc I = 0
file Set2_2.txt  | conc E = 0.004 | conc S = 111 | conc I = 0.0025 ?
file Set2_3.txt  | conc E = 0.004 | conc S = 111 | conc I = 0.0051 ?
file Set2_4.txt  | conc E = 0.004 | conc S = 111 | conc I = 0.0068 ?
file Set2_5.txt  | conc E = 0.004 | conc S = 111 | conc I = 0.0085 ?
file Set3_1.txt  | conc E = 0.004 | conc S = 159 | conc I = 0
file Set3_2.txt  | conc E = 0.004 | conc S = 159 | conc I = 0.0025 ?
file Set3_3.txt  | conc E = 0.004 | conc S = 159 | conc I = 0.0051 ?
file Set3_4.txt  | conc E = 0.004 | conc S = 159 | conc I = 0.0068 ?
file Set3_5.txt  | conc E = 0.004 | conc S = 159 | conc I = 0.0085 ?
file Set4_1.txt  | conc E = 0.0081 | conc S = 64 | conc I = 0
file Set4_2.txt  | conc E = 0.0081 | conc S = 64 | conc I = 0.0042 ?
file Set4_3.txt  | conc E = 0.0081 | conc S = 64 | conc I = 0.0085 ?
file Set4_4.txt  | conc E = 0.0081 | conc S = 64 | conc I = 0.0127 ?
file Set4_5.txt  | conc E = 0.0081 | conc S = 64 | conc I = 0.0169 ?
file Set5_1.txt  | conc E = 0.0081 | conc S = 111 | conc I = 0
file Set5_2.txt  | conc E = 0.0081 | conc S = 111 | conc I = 0.0042 ?
file Set5_3.txt  | conc E = 0.0081 | conc S = 111 | conc I = 0.0085 ?
file Set5_4.txt  | conc E = 0.0081 | conc S = 111 | conc I = 0.0127 ?
file Set5_5.txt  | conc E = 0.0081 | conc S = 111 | conc I = 0.0169 ?
file Set6_1.txt  | conc E = 0.0081 | conc S = 159 | conc I = 0
file Set6_2.txt  | conc E = 0.0081 | conc S = 159 | conc I = 0.0042 ?
file Set6_3.txt  | conc E = 0.0081 | conc S = 159 | conc I = 0.0085 ?
file Set6_4.txt  | conc E = 0.0081 | conc S = 159 | conc I = 0.0127 ?
file Set6_5.txt  | conc E = 0.0081 | conc S = 159 | conc I = 0.0169 ?
file Set7_1.txt  | conc E = 0.0101 | conc S = 64 | conc I = 0
file Set7_2.txt  | conc E = 0.0101 | conc S = 64 | conc I = 0.0051 ?
file Set7_3.txt  | conc E = 0.0101 | conc S = 64 | conc I = 0.0102 ?
file Set7_4.txt  | conc E = 0.0101 | conc S = 64 | conc I = 0.0152 ?
file Set7_5.txt  | conc E = 0.0101 | conc S = 64 | conc I = 0.0203 ?
file Set8_1.txt  | conc E = 0.0101 | conc S = 111 | conc I = 0
file Set8_2.txt  | conc E = 0.0101 | conc S = 111 | conc I = 0.0051 ?
file Set8_3.txt  | conc E = 0.0101 | conc S = 111 | conc I = 0.0102 ?
file Set8_4.txt  | conc E = 0.0101 | conc S = 111 | conc I = 0.0152 ?
file Set8_5.txt  | conc E = 0.0101 | conc S = 111 | conc I = 0.0203 ?
file Set9_1.txt  | conc E = 0.0101 | conc S = 159 | conc I = 0
file Set9_2.txt  | conc E = 0.0101 | conc S = 159 | conc I = 0.0051 ?
file Set9_3.txt  | conc E = 0.0101 | conc S = 159 | conc I = 0.0102 ?
file Set9_4.txt  | conc E = 0.0101 | conc S = 159 | conc I = 0.0152 ?
file Set9_5.txt  | conc E = 0.0101 | conc S = 159 | conc I = 0.0203 ?
file PC1_180219.txt  | conc E = 0.004 | conc S = 4 | conc I = 0
file PC2_180219.txt  | conc E = 0.004 | conc S = 6 | conc I = 0
file PC3_180219.txt  | conc E = 0.004 | conc S = 7.9 | conc I = 0
file PC4_180219.txt  | conc E = 0.004 | conc S = 9.9 | conc I = 0
file PC5_180219.txt  | conc E = 0.004 | conc S = 11.9 | conc I = 0
file PC6_180219.txt  | conc E = 0.004 | conc S = 13.9 | conc I = 0
file PC7_180219.txt  | conc E = 0.0081 | conc S = 7.9 | conc I = 0
file PC8_180219.txt  | conc E = 0.0081 | conc S = 15.9 | conc I = 0
file PC9_180219.txt  | conc E = 0.0081 | conc S = 23.8 | conc I = 0
file PC10_180219.txt  | conc E = 0.0081 | conc S = 31.8 | conc I = 0
file PC11_180219.txt  | conc E = 0.0081 | conc S = 39.7 | conc I = 0
file PC12_180219.txt | conc E = 0.0081 | conc S = 59.6 | conc I = 0
[responses]   ; p-nitroanalide ext. coef. uM-1 cm-1
 P = 0.0123
[output]
directory ./KSU/Eap_data/Eap_Kinetics/Dynafit_Results/EapH1/WT/
180219/Set1_9_floatI_Progresscurves
```

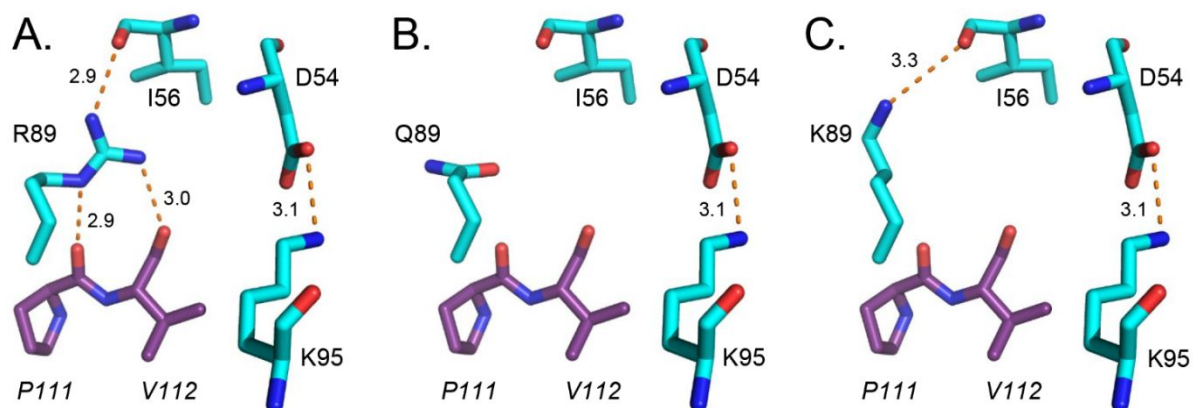**Figure S2. Representative Dynafit Script for Progress Curve Analysis.**

Figure S3. Homology Models of Selected R89 Mutants in EapH1 Bound to hNE. (A) Interactions observed in the wild-type EapH1/hNE crystal, as drawn from PDB entry 4NZL.[1] (B) Energy minimized homology model of R89Q EapH1/hNE, (C) Energy minimized homology model of R89K EapH1/hNE. EapH1 residues (normal font), hNE residues (italicized). EapH1 residues (carbon in cyan) are labeled with normal font, while hNE residues (carbon in purple) are labeled with italicized front. Potential polar interactions are depicted as orange dashes with associated distances given in Angstroms (Å).