

SUPPORTING INFORMATION

Role of E270 in pH- and metal-sensitivities of firefly luciferases

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$$I_{max} = QY \cdot k_{cat} \cdot [Et]$$

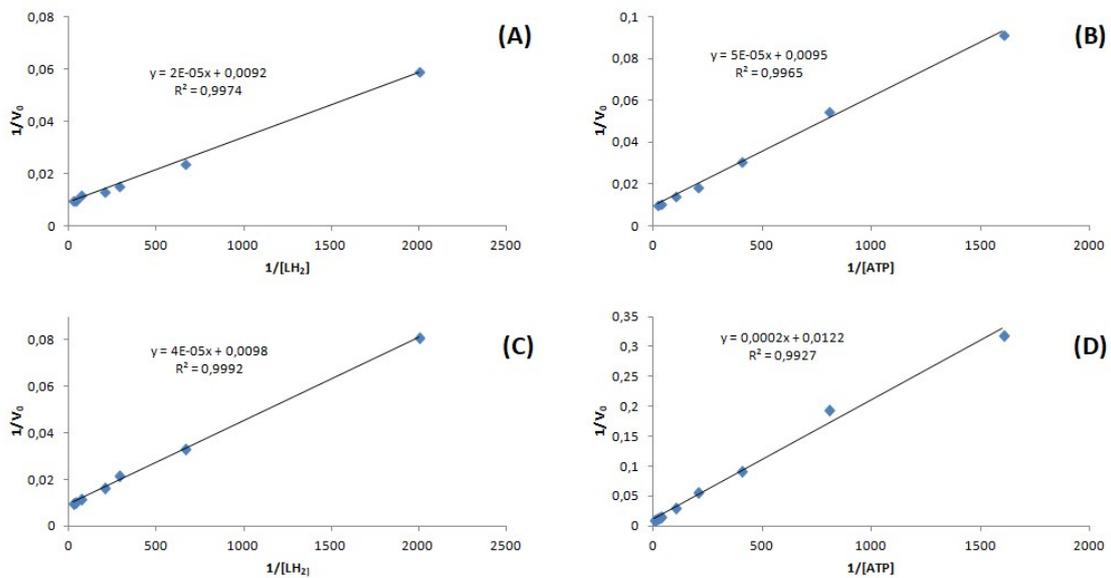
$$I_0 = QY \cdot k_{cat} \cdot [ES]$$

$$I_{max} \sim V_{max}$$

$$I_0 = V_0$$

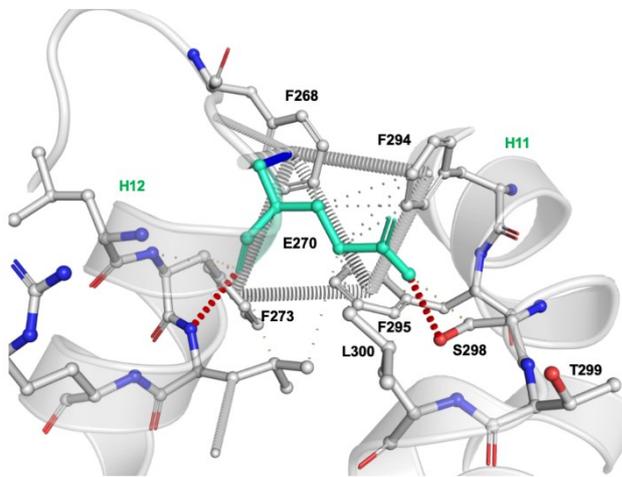
Equation 1. Relationships between luminescence intensity and Velocity: (I_{max})

maximum intensity; (I_0) Initial intensity; (QY) Quantum yield; (k_{cat}) catalytic constant; ($[Et]$) total enzyme concentration; ($[ES]$) enzyme-substrate complex concentration; (V_{max}) maximum velocity and (V_0) initial velocity.

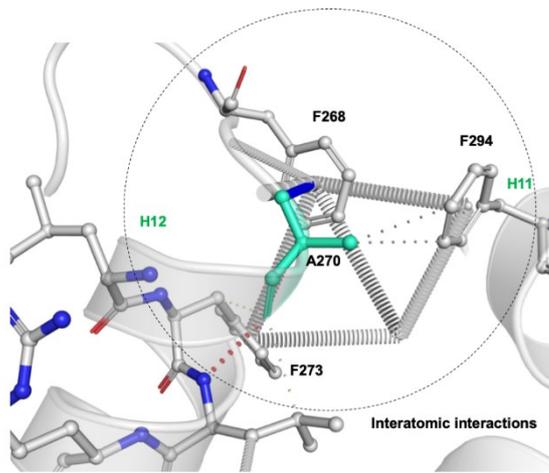


Supplementary Figure 1. Lineweaver-Burk Plots for determination of K_M values for *Amydetes vivianii* luciferase mutants: Amy-E270A: (A) D-luciferin and (B) ATP; Amy-E270G: (C) D-luciferin and (D) ATP. The V_0 values were normalized against V_{max} , and averages for each point reported.

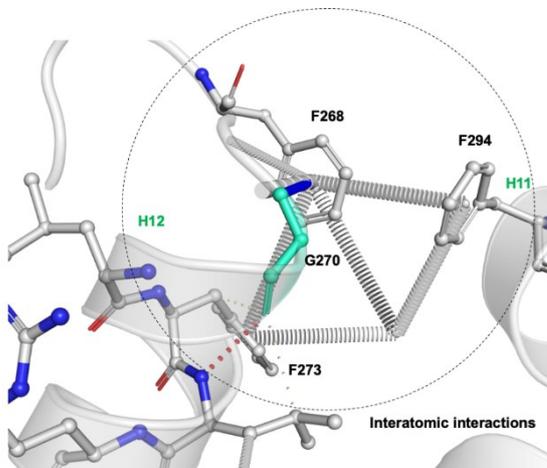
A



B



C



Supplementary Figure 2. The effects of mutations on folding, stability, and flexibility predicted by DynaMut. **A)** wild type, **B)** mutated E270A and **C)** mutated E270G luciferase from *Amydetes vivianii*. The interatomic interactions of wild-type and mutant proteins showed that bond interactions were affected as a result of mutation. Bond Type: red (Hydrogen bonds), orange (weak hydrogen bonds) and green (Hydrophobic contacts).