α1-FANGs—Protein Ligands Selective for the α-Bungarotoxin site of the α1-Nicotinic Acetylcholine Receptor

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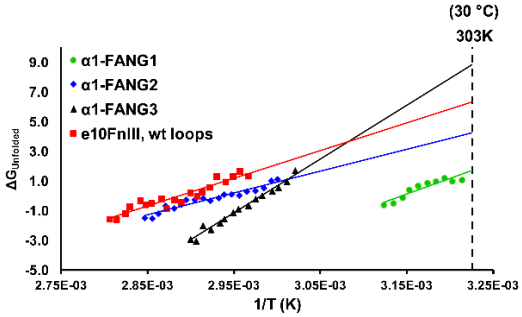
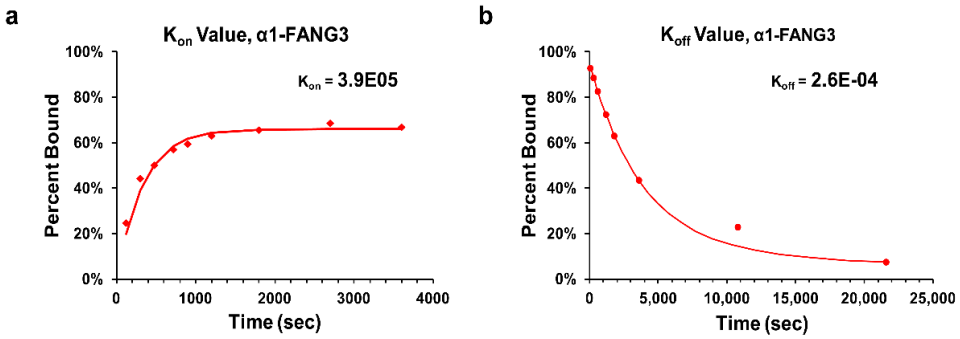


Supplemental Figure 1. Plot of $\Delta G_{unfolding}$ versus inverse temperature of α1-FANG1 and derivatives. Direct comparison of $\Delta G_{unfolding}$ with 1/T for α1-FANG ligands using the experimentally derived circular dichroism melting data shows the different ΔH contributions (slope) of each protein to overall stability. When extrapolated to 3.25E-03 (30°C) to compare with historical data, the high stability of α1-FANG3 versus parent clones is clear.



Supplemental Figure 2. Determination of α1-FANG3 K_{on} and K_{off} rates via radiolabeled binding assays. A) K_{on} of α1-FANG3 was determined by the pulldown of excess ^{35}S labeled α1-FANG3 against α211 immobilized on beads. K_{on} was fit using excel and determined to be 3.9E5. B) K_{off} of α1-FANG3 was determined by a direct competition of α-Btx with ^{35}S labeled α1-FANG3 bound to α211 immobilized on beads.