**Supplementary Data**

**Tuning site-specific dynamics to drive allosteric activation in a pneumococcal zinc uptake regulator**

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This file contains **Supplementary Tables S1-S2**

**Table S1.** Zinc binding affinities of wild-type AdcR and selected AdcR mutants characterized here.

|  |  |  |
| --- | --- | --- |
|  | ZnII binding to site 2 in the homodimer | |
| AdcR | *K*Zn, 3  (x109 M-1) | *K*Zn, 4  (x109 M-1) |
| wild-type | ≥1 | 0.0205 ±0.0013 |
| V34A | 0.0022±0.0017 | (9.4±8.2) 10-5 |
| L81V | ≥1 | 0.025±0.0027 |
| L57M | ≥1 | 0.0169±0.001 |
| L57V | ≥1 | 0.119±0.018 |
| I16A | ≥1 | 0.00479±0.0005 |
| V142A | 0.00085±0.00018 | <10-5 |
| L17A | ≥1 | .00158±0031 |
| I27A | ≥1 | .00349±.005 |

aConditions: 10 mM Hepes, pH 7.2, 0.4 M NaCl, 1 mM TCEP (chelexed), 15 M Mf2, 25.0 ºC titrated with ZnCl2 solutions. Experiments were conducted 3 times for each AdcR variant. Errors of the binding constant parameters were estimated from global fits.

b*K*Zn,1 and *K*Zn,2 were fixed to a value of 1 x1012 M-1. c *K*Zn,MF2 = (4.9 ± 0.6) x106 M-1 under these solution conditions.

**Table S2.** Differential Scanning Fluorimetry with SYPRO Orange

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | | Apo | |  | | ZnII |
| AdcR | pH 7.0a  Tm (oC) | | pH 5.5b  Tm (oC) | | pH 7.0  Tm (oC) | |
| wild-type | 47±1 | | 45±1 | | 69±1 | |
| I16A | 45±1 | | \_ | | \_ | |
| L17A | 52±1 | | \_ | | 71±1 | |
| I27A | 49±1 | | \_ | | 66±1 | |
| V34A | 46±1 | | 36±1 | | 70±1 | |
| L57V | 46±1 | | \_ | | 66±1 | |
| L57M | 46±1 | | 45±1 | | 67±1 | |
| L61V | 37±1 | | \_ | | \_ | |
| V63A | 41±1 | | \_ | | 64±1 | |
| L81V | 51±1 | | \_ | | \_ | |
| V142A | 49±1 | | \_ | | 67±1 | |

aConditions: 10 mM Hepes, pH 7.0, 0.23 M NaCl, 1 mM TCEP (chelexed), with 4 M protein, 5x SYPRO orange, 10 µM EDTA (For apo AdcRs) or 2 protomer mol•equivalents of ZnCl2 (for ZnII2 AdcR) added to these reactions. b25mM MES, pH 5.5, 50 mM NaCl, 1 mM TCEP (chelexed); all other conditions the same.