Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Information

For

Tight Binding of Heme to *Staphylococcus aureus* IsdG and IsdI Precludes Design of a Competitive Inhibitor

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Supplemental Experimental

The UV/Vis absorption-detected heme titrations into IsdG and IsdI were analyzed in order to determine the dissociation constant (K_d) of the heme substrate. The UV/Vis absorption intensity of a solution consisting of: IsdG, IsdG–heme, and heme at 411 nm depends on equation 1:

$$A_{411} = \varepsilon_{IsdG-heme}[IsdG-heme] + \varepsilon_{heme}[heme] \quad (1)$$

where $\varepsilon_{IsdG-heme}$ and ε_{heme} are the extinction coefficients of these species at 411 nm. The K_d for heme binding to IsdG is defined by equation 2:

$$K_d = \frac{[IsdG][heme]}{[IsdG-heme]} \quad (2)$$

The total amount of IsdG ($IsdG_T$) is defined by equation 3:

$$[IsdG_T] = [IsdG] + [IsdG-heme] \quad (3)$$

which can be rearranged to give free IsdG in terms of $IsdG_T$ and IsdG–heme as defined by equation 4:

$$[IsdG] = [IsdG_T] - [IsdG-heme] \quad (4)$$

The total amount of heme ($heme_T$) is defined by equation 5:

$$[heme_T] = [heme] + [IsdG-heme] \quad (5)$$

which can be rearranged to give free heme in terms of $heme_T$ and IsdG–heme as defined by equation 6:

$$[heme] = [heme_T] - [IsdG - heme] \quad (6)$$

Substituting equations 4 and 6 into equation 2 gives the K_d for heme binding to IsdG in terms of the total concentration of IsdG and total concentration of heme (rather than concentration of free heme and free IsdG) as defined by equation 7:

$$K_d = \frac{([IsdG_T] - [IsdG - heme])([heme_T] - [IsdG - heme])}{[IsdG - heme]} \quad (7)$$

Rearrangement of equation 7 simplifies the K_d for heme binding to IsdG as given by equation 8:

$$K_d[IsdG - heme] = ([IsdG_T] - [IsdG - heme])([heme_T] - [IsdG - heme]) \quad (8)$$

Expansion of the binomial side of equation 8 gives equation 9:

$$K_d[IsdG - heme] = ([IsdG_T][heme_T]) - ([IsdG_T][IsdG - heme]) - ([heme_T][IsdG - heme]) + [IsdG - heme]^2 \quad (9)$$

Setting equation 9 equal to zero, rearranging the terms, and combining all [IsdG–heme] terms gives equation 10:

$$[IsdG - heme]^2 - ([IsdG_T] + [heme_T] + K_d)[IsdG - heme] + ([IsdG_T][heme_T]) = 0 \quad (10)$$

which is a quadratic equation of the form $ax^2 + bx + c = 0$, where:

$$a = 1 \quad (11a)$$

$$b = -([IsdG_T] + [heme_T] + K_d) \quad (11b)$$

$$c = ([IsdG_T][heme_T]) \quad (11c)$$

$$x = [IsdG - heme] \quad (11d)$$

Solving the quadratic equation for x yields equation 12:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \quad (12)$$

Substitution of 11a, 11b, and 11c into the solved quadratic equation gives equation 13, the quadratic equation in terms of [IsdG–heme]:

$$[IsdG - heme] = \frac{-(-([IsdG_T] + [heme_T] + K_d)) \pm \sqrt{(-([IsdG_T] + [heme_T] + K_d))^2 - 4([IsdG_T][heme_T])}}{2} \quad (13)$$

Simplification of the quadratic equation 13, and separating it into the two possible roots of x, $[IsdG - heme]$, gives equations 14a and 14b:

$$[IsdG - heme] = \frac{([IsdG_T] + [heme_T] + K_d) - \sqrt{([IsdG_T] + [heme_T] + K_d)^2 - 4([IsdG_T][heme_T])}}{2} \quad (14a)$$

$$[IsdG - heme] = \frac{([IsdG_T] + [heme_T] + K_d) + \sqrt{([IsdG_T] + [heme_T] + K_d)^2 - 4([IsdG_T][heme_T])}}{2} \quad (14b)$$

Equation 14a is the physically meaningful root of the quadratic equation, while the slope of the curve described by equation 14b is inconsistent with experiment. Thus, we will use equation 14a for the remainder of the analysis. Substitution of equation 6 into equation 1 relates the observed absorbance at 411 nm to total amount of heme in solution by equation 15:

$$A_{411} = \varepsilon_{IsdG-heme}[IsdG-heme] + \varepsilon_{heme}([heme_T] - [IsdG-heme]) \quad (15)$$

Expansion of equation 15 gives equation 16:

$$A_{411} = \varepsilon_{IsdG-heme}[IsdG-heme] + \varepsilon_{heme}[heme_T] - \varepsilon_{heme}[IsdG-heme] \quad (16)$$

As the extinction coefficients are constants, equation 16 can be simplified to equation 17:

$$A_{411} = (\varepsilon_{IsdG-heme} - \varepsilon_{heme})[IsdG-heme] + \varepsilon_{heme}[heme_T] \quad (17)$$

Isolation of the $IsdG-heme$ term gives equation 18:

$$[IsdG-heme] = \frac{A_{411} - \varepsilon_{heme}[heme_T]}{(\varepsilon_{IsdG-heme} - \varepsilon_{heme})} \quad (18)$$

Substitution of equation 18 into equation 14a gives equation 19:

$$\frac{A_{411} - \varepsilon_{heme}[heme_T]}{(\varepsilon_{IsdG-heme} - \varepsilon_{heme})} = \frac{([IsdG_T] + [heme_T] + K_d) - \sqrt{([IsdG_T] + [heme_T] + K_d)^2 - 4([IsdG_T][heme_T])}}{2} \quad (19)$$

Rearrangement of equation 19 to get an equation in terms of A_{411}, the observed variable in the titration, gives equation 20:

$$A_{411} = \frac{(([IsdG_T] + [heme_T] + K_d) - \sqrt{([IsdG_T] + [heme_T] + K_d)^2 - 4([IsdG_T][heme_T])}) * (\varepsilon_{IsdG-heme} - \varepsilon_{heme}[heme_T]}{2} \quad (20)$$

A similar equation was previously used to model heme binding to human heme oxygenase-2.[1, 2]

Equation 20 was defined within GraphPad Prism 6.0g where:

$P = [IsdG_T]$

$X = [heme_T]$

$K = K_d$

$Y = A_{411}$

$e = \varepsilon_{IsdG-heme}$

$f = \varepsilon_{heme}$

For UV/Vis absorption-detected heme titrations into IsdG and IsdI, Equation 20 was rewritten in GraphPad prism 6.0g as equation 21 in order to estimate the K_d where:

$a = 1$

$b = (P + X + K)$

$c = (P * X)$

$Y = (((b - sqrt((b * b) - 4 * a * c)) * (e - f))/(2 * a)) + (f * X)$ (21)

A plot of the observed absorbance at 411 (A_{411}) as a function of heme concentration $[heme_T]$ was fit to the user defined equation 21 in GraphPad Prism 6.0g. $\varepsilon_{IsdG-heme}$ and ε_{heme} (e and f) were constrained to their known values while total concentration of protein and K_d (P and K) were fit simultaneously.

Fluorescence-detected heme titrations into IsdG and IsdI were analyzed in order to measure the K_d of the heme substrate. The fluorescence intensity of substrate-free IsdG is defined as F_{max} while the fluorescence intensity of fully substrate-bound IsdG is defined as F_{min}. Quenching of the tryptophan fluorescence intensity via Förster resonance energy transfer is governed by the amount of substrate bound in the active site. Thus, the observed fluorescence intensity of a solution consisting of $IsdG$ and $IsdG-heme$ is given by the linear combination of the fractions of IsdG that are substrate-free and substrate-bound as defined by equation 22:

$$F = F_{max}\frac{[IsdG]}{[IsdG_T]} + F_{min}\frac{[IsdG-heme]}{[IsdG_T]} \quad (22)$$

Substitution of equation 4 into equation 22 gives the fluorescence intensity in terms of [$IsdG_T$] and [IsdG–heme] as defined by equation 23:

$$F = F_{max}\frac{([IsdG_T]-[IsdG-heme])}{[IsdG_T]} + F_{min}\frac{[IsdG-heme]}{[IsdG_T]} \quad (23)$$

Expansion and simplification of the equation 23 gives equation 24:

$$F = F_{max}(1-\frac{[IsdG-heme]}{[IsdG_T]}) + F_{min}\frac{[IsdG-heme]}{[IsdG_T]} \quad (24)$$

Multiplication of F_{max} through equation 24 gives equation 25:

$$F = F_{max} - F_{max}\frac{[IsdG - heme]}{[IsdG_T]} + F_{min}\frac{[IsdG - heme]}{[IsdG_T]} \quad (25)$$

Rearrangement of equation 25 gives equation 26:

$$(F_{min} - F_{max})\frac{[IsdG - heme]}{[IsdG_T]} = (F - F_{max}) \quad (26)$$

Rearrangement of equation 26 gives equation 27:

$$[IsdG - heme] = \frac{F - F_{max}}{F_{min} - F_{max}}[IsdG_T] \quad (27)$$

Equation 27 relates the concentration of IsdG–heme to the observed fluorescence intensity when F_{max}, F_{min}, and $[IsdG_T]$ are known. Substitution of equation 27 into equation 14a gives a quadratic equation with respect to fluorescence intensity, as defined by equation 28:

$$\frac{F - F_{max}}{F_{min} - F_{max}}[IsdG_T] = \frac{([IsdG_T] + [heme_T] + K_d) - \sqrt{([IsdG_T] + [heme_T] + K_d)^2 - 4([IsdG_T][heme_T])}}{2} \quad (28)$$

Rearrangement of equation 28 to get an equation in terms of observed fluorescence intensity (F) gives equation 29:

$$F = \frac{F_{min} - F_{max}}{[IsdG_T]} * \frac{([IsdG_T] + [heme_T] + K_d) - \sqrt{([IsdG_T] + [heme_T] + K_d)^2 - 4([IsdG_T][heme_T])}}{2} + F_{max} \quad (29)$$

A similar equation was previously used to model heme binding to fluorescently-labeled human heme oxygenase-1,[3] and the soluble domain of human heme oxygenase-2.[4]

Equation 29 was defined within GraphPad Prism 6.0g as equation 30 where:

$P = [IsdG_T]$

$X = [heme_T]$

$K = K_d$

$Y = F$

$d = F_{max}$

$m = F_{min}$

For the fluorescence-detected heme titration into IsdG and IsdI; Equation 29 was rewritten in GraphPad prism 6.0g as equation 30 in order to estimate the K_d where:

$a = 1$

$b = (P + X + K)$

$c = (P * X)$

$$Y = (((m - d)/P)((b - sqrt((b * b) - 4 * a * c))/(2 * a)) + d) \quad (30)$$

A plot of the observed fluorescence (F) as a function of total heme $[heme_T]$ titrated into the solution was fit to the user defined equation 30 in GraphPad Prism 6.0g. F_{max} and F_{min} were

constrained to the values observed in the experiment and the total amount of protein (P) was constrained to the value calculated based on the UV/Vis absorption-detected heme titrations.

All fits are the average of three independent trials plus or minus the standard deviation between the three trials.

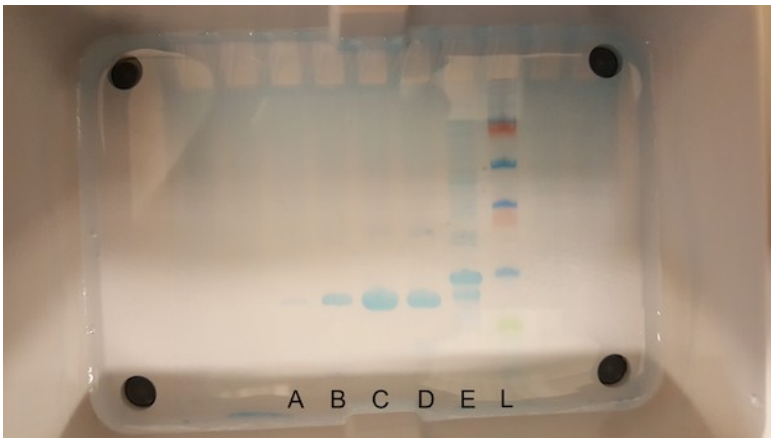

Figure S1. SDS-PAGE gel of WT IsdG Purification. The lanes represent: 1/100 dilution of purified WT IsdG (A), 1/10 dilution of purified WT IsdG (B), Pure WT IsdG (C), S219V TEV protease cleavage reaction (D), WT IsdG lysate (E), PageRuler Plus prestained protein ladder (Pierce) (L).

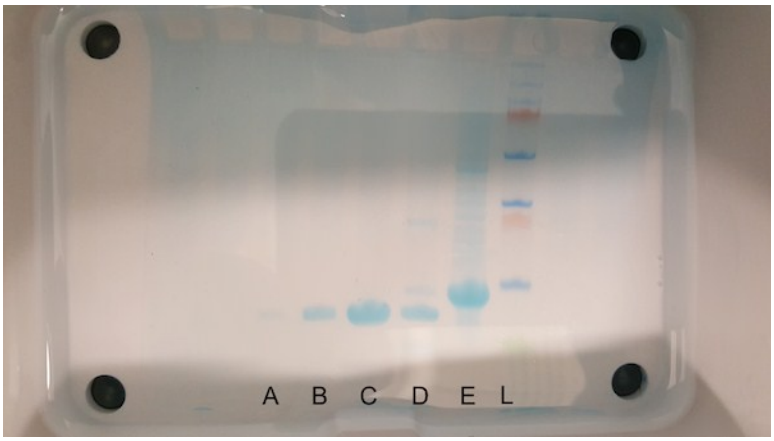

Figure S2. SDS-PAGE gel of WT IsdI Purification. The lanes represent: 1/100 dilution of purified WT IsdI (A), 1/10 dilution of purified WT IsdI (B), Pure WT IsdI (C), S219V TEV protease cleavage reaction (D), WT IsdI lysate (E), PageRuler Plus prestained protein ladder (Pierce) (L).

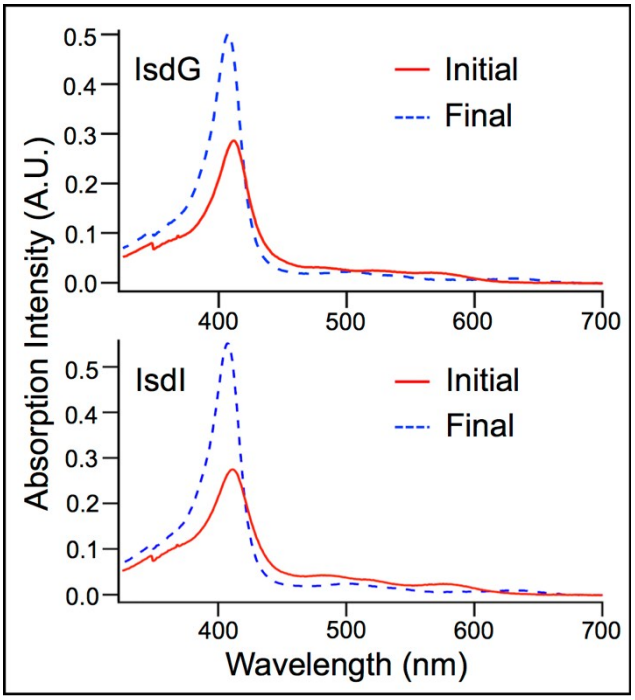

Figure S3. UV/Vis absorption spectra of 3 µM IsdG–heme prior to (solid red line) and 30 min after (dashed blue line) the addition of 30 µM apomyoglobin in 50 mM Tris pH 7.4, 150 mM NaCl (top). UV/Vis absorption spectra of 3 µM IsdI–heme prior to (solid red line) and 30 min after (dashed blue line) the addition of 30 µM apomyoglobin in 50 mM Tris pH 7.4, 150 mM NaCl (bottom). Heme transfer from IsdG or IsdI to apomyoglobin is complete after 30 min.

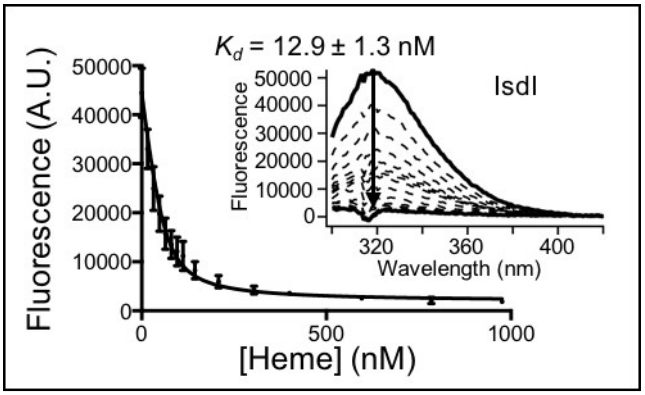

Figure S4. Fluorescence-detected titration of heme into 60 nM IsdI in 50 mM Tris pH 7.4, 150 mM NaCl. The emission spectrum for 285 nm excitation is shown in the inset. A fit of the emission intensity to equation 2 (main text) yielded a K_d of 12.9 ± 1.3 nM.

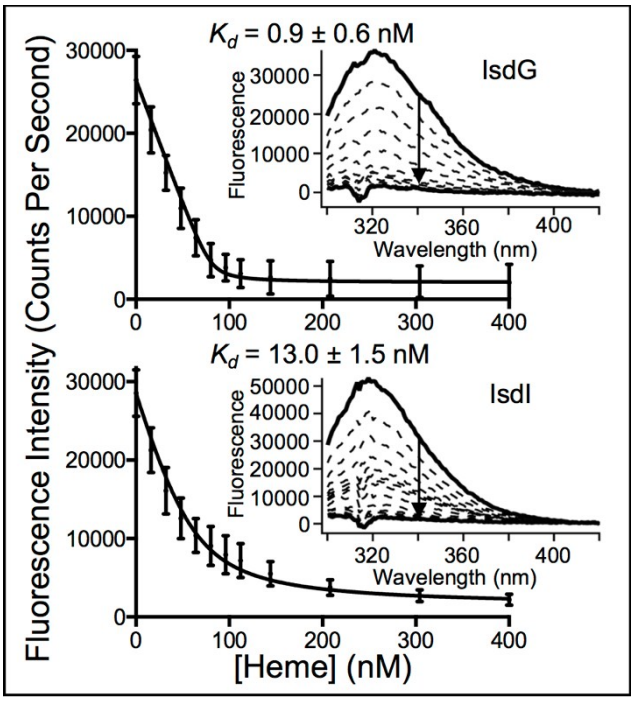

Figure S5. Fluorescence-detected titration of heme into 80 nM IsdG (top) and 60 nM IsdI (bottom) in 50 mM Tris pH 7.4, 150 mM NaCl. The emission spectra for 285 nm excitation are shown in the insets. Fits of the emission intensity at 340 nm to equation 2 (main text) yielded K_d values of 0.9 ± 0.6 nM for IsdG and 13.0 ± 1.5 nM for IsdI.

References

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