Supplementary Information

Abbreviations of proteins used in the text

Abbreviation	Description
Cyt	Wild type cytochrome b_{562}, both apo- and holo- forms discussed
$(SH3)_2$	Tandem repeat of bovine PI3-SH3 domain, both monomeric and fibrillar forms discussed
$(SH3)_2Cyt$	Tandem repeat of bovine PI3-SH3 domain, fused to wild type cytochrome b_{562}. Apo-,holo- , monomeric and fibrillar forms are discussed.

Full sequence of designed construct

$SH3 - GGGGSGGGGS - SH3 - GSGGGG - Cytb_{562}$

G S M S A E G Y Q Y R A L Y D Y K K E R E E D I D L H L G D I L T V N K G S L V A L G F S D G Q E A K P E E I G W L N G Y N E T T G E R G D F P G T Y V E Y I G R K K I S P G G G G S G G G G S G S M S A E G Y Q Y R A L Y D Y K K E R E E D I D L H L G D I L T V N K G S L V A L G F S D G Q E A K P E E I G W L N G Y N E T T G E R G D F P G T Y V E Y I G R K K I S P G S G G G G A D L E D N M E T L N D N L K V I E K A D N A A Q V K D A L T K M R A A A L D A Q K A T P P K L E D K S P D S P E M K D F R H G F D I L V G Q I D D A L K L A N E G K V K E A Q A A A E Q L K T T R N A Y H Q K Y R

Protein Expression

The sequence cloned into a pGEX2-GST from Amersham Biosciences and transformed into. The GST fusion protein was expressed from this construct in E.coli strain HMS174 in Luria Bretani (LB) medium. After cell lysis, the soluble GST fusion was passed down a Glutathione-sepharose column, washed and cleaved on-column with thrombin. The SH3-cytochrome fusion protein released was passed down a mono-Q column with a KCl gradient of 0-200 mM KCl (to separate holo protein due to endogenous heme) on a Waters HPLC, achieving separation of apo and holo constructs typically in the ratio of 5:1. The pooled fractions of each of these species were passed down a gel filtration column in ammonium bicarbonate. Fractions were then lyophilised giving final yields of 3-7mg per litre of culture.

Fibril formation and purification

Lyophilised protein was resuspended in 3mM Tris-HCl and taken down to pH 2 with 1M HCl. Sodium acetate (5.6M) buffered at pH 3.6 is added and the mixture is held at room temperature for 15 minutes. The mixture is then taken back down to pH 2 and held at room temperature for 4-6 days. The mixture is purified by ultracentrifugation at 90,000rpm in a TLA 120 rotor 10S at 20^{o}C to remove remaining smaller aggregated states. The pelleted material is readily resuspended in 3mM HCl and its morphology confirmed by TEM. Total protein concentration is measured by UV-Vis using the extinction coffecient of 33,000 $M^{-1}cm^{-1}$. Yields of pelleted material vary between 70-90%, with balance of the protein present as monomer or small aggregates.

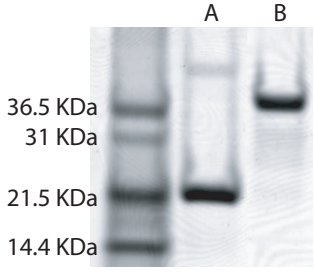

Figure 1: SDS PAGE experiment showing composition of fibrils. SH3 dimer fibrils (A), and cytochrome fibrils (B) are shown to be made of just one peptide species - no hydrolysis of starting peptide observed.

Heme Binding

The UV-Vis binding data was analysed as an equilibrium between two unassociated species combining to give a single bound species, with a 'hidden' population of the unbound species, according to the following scheme, and corresponding equilibrium constant.

$$\begin{gathered}(n)Heme(H) + Apo - Cyt(P) \rightleftharpoons (n)Holo - Cyt(B) + (n-1)Apo - Cyt \\ K = \frac{[B_{eq}]}{[P_{eq}][H_{eq}]}\end{gathered} \tag{1}$$

The three constants in the equilibrium expression are linked to their initial (0) values by the following stoichiometries; $H_0 = H_{eq} + B_{eq}$ and $P_0 = P_{eq} + nB_{eq}$. The analysis uses the following parameters, $x = H_0/P_0$, $y = B_{eq}/P_0$ and $g = K.P_0$. Substituting these into the equilibrium expression, solving the quadratic for y and discarding the positive solution, we have;

$$\begin{aligned}y &= (1-y)(x-y)g \\ y &= \frac{1}{2n}\left(\left[nx + 1 + \frac{1}{g}\right] - \sqrt{(nx)^2 + 2nx\left(\frac{1}{g} - 1\right) + \left(\frac{1}{g} + 1\right)^2}\right)\end{aligned} \tag{2}$$

As free heme contributes marginally to the absorbance at 420nm, the effective measured absorbance at 420nm will be a linear sum of both bound and free heme, leading to the following expression.

$$\frac{A_{420}}{P_0} = \epsilon_{420}^{Holo}Y + \epsilon_{420}^{Heme}(X - Y) \frac{A_{420}}{\epsilon_{420}^{Holo}P_0} = \left(1 - \frac{\epsilon_{420}^{Heme}}{\epsilon_{420}^{Holo}}\right)Y + \frac{\epsilon_{420}^{Heme}}{\epsilon_{420}^{Holo}}X \tag{3}$$

The absorbance data at 420nm can be understood in terms of the equilibrium constant, the fraction of the population competent to bind (n) and the experimental extinction coefficients of the free heme and the holo-cytochrome. A least squares analysis performed in sigmaplot 8.0 with this scheme yield the parameters referred to in the text. A plot of the data in this form is shown in figure 2.

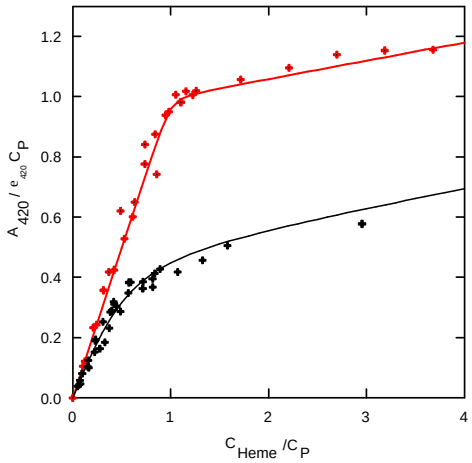

Figure 2: Hyperbolic plot of dataset

Job plot

$$\begin{aligned} A + mB &\rightleftharpoons AB_m \\ \beta &= \frac{AB_m}{(A)(B)^m} \\ [A_0] - [A_{eq}] &= [AB_m] \\ [B_0] - [B_{eq}] &= m[AB_m] \end{aligned} \tag{4}$$

Let $C = [A_0] + [B_0]$, and $x = \frac{B_0}{A_0 + B_0} = \frac{B_0}{c}$. y is defined as a quantity proportional to the concentration of $[AB_m]$, here the absorbance at 420nm after heme deconvolution as described above to give a y_{eff}. C is fixed either during experiment or during analysis by plotting $\frac{y}{C}$ against x and fitting to the following expression.

$$0 = \beta \left(c(1-x) - m y_{eff}\right) \left(cx - m y_{eff}\right)^m - y_{eff} \tag{5}$$

Taking the derivative if the logarithm shows that the x ordinate of maximum y can be related to the stoichiometric constant, m. Thus the apparent discontinuity in the plot gives a strong visual indication of the stoichiometry of binding.

NMR titration

NMR spectra acquired on a Bruker advance 500MHz with a cryoprobe. Results shown from three independent fibril sample preparations of concentration 40uM, 52uM and 63uM. Low volume 4mm NMR tubes were used to ease sample transference to 100μl cuvettes for UV-Vis measurements.

Concentration Estimate

Assuming fibrils are cylinders of radius r, and length l and have constant density, we can calculate the number of protein monomers N of molecular weight M_w expected in a given volume. Taking into account that only half of the total protein is competent to bind heme, the following relation describes the number of metalloporphyrins N_{Iron} expected per nm of fibril. The density of the fibrils is given by the inverse of the partial specific volume $\overline{v}$.

$$\begin{aligned} V = \pi r^2 L &= \frac{\overline{v} M_w N}{N_a} \\ N_{iron} &= \frac{2 N_a \pi r^2 \times 10^{-9}}{M_w \overline{\nu}} \end{aligned} \tag{6}$$