

**Computational Design and Elaboration of a *De Novo* Heterotetrameric  
 $\alpha$ -Helical Protein that Selectively Binds an Emissive Abiological  
(Porphinato)zinc Chromophore**

H. Christopher Fry,<sup>‡</sup> Andreas Lehmann,<sup>¶‡</sup> Jeffrey G. Saven,<sup>\*,\*\*</sup> William F.  
DeGrado,<sup>\*,†\*</sup> and Michael J. Therien<sup>§</sup>

<sup>‡</sup>*Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104-6323;* <sup>¶</sup>*Present address: Fox Chase Cancer Center, Philadelphia, PA 19111-2434;* <sup>†</sup>*Department of Biochemistry and Molecular Biophysics, Johnson Foundation, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6059;* <sup>§</sup>*Department of Chemistry, Duke University, Durham, NC 27708-0354*

**Contents:**

**Table S1.** Doming heights (the distance between the least-squares plane defined by the four coordinating N atoms of the porphyrin macrocycle and the position of the Zn ion) for 20 representative crystal structures from the Cambridge Structural Database (CSD).<sup>1</sup>

***Binding model***

**Figure S1.** UV/vis spectra (in a 1 mm quartz cell) of (DPP)Fe(III) (50  $\mu$ M) binding to 400  $\mu$ M of the parent homotetramer peptide (Black) and (DPP)Fe(III) (50  $\mu$ M) mixed with 400  $\mu$ M of the  $A_{His}$  peptide (red).

***Size Exclusion Chromatography***

**Figure S2.** Size exclusion chromatograms of  $A_{His}$  (red),  $B_{Thr}$  (blue),  $A_{His}:B_{Thr}$  (1:1 mixture, black), and  $(DPP)Zn:A_{His}:B_{Thr}$  (1:1:1 mixture, purple). Evaluated MWs:  $A_{His}$ , 3953.4;  $B_{Thr}$ , 4027.5;  $[A_{His}:B_{Thr}]_2$ , 15944,  $[(DPP)Zn:A_{His}:B_{Thr}]_2$ , 17309.8.

**Figure S3.** Normalized excitation (solid purple) and emission spectra (purple dots) of  $(DPP)Zn:A_{His}:B_{Thr}$  in 50 mM Pi, 150 mM NaCl, pH 7.5 buffer.

CSD ID (Refcode)	Dist. Centroid-Zn [Å]	Reference
AQUDOF	0.334	A.Tsuda, S.Sakamoto, K.Yamaguchi, T.Aida, <i>J.Am.Chem.Soc.</i> (2003), 125, 15722
BABBAI	0.287	Y.Diskin-Posner, G.K.Patra, I.Goldberg, <i>J.Chem.Soc.,Dalton Trans.</i> (2001), , 2775
BABBEM	0.278	Y.Diskin-Posner, G.K.Patra, I.Goldberg, <i>J.Chem.Soc.,Dalton Trans.</i> (2001), , 2775
BABBIQ	0.332	Y.Diskin-Posner, G.K.Patra, I.Goldberg, <i>J.Chem.Soc.,Dalton Trans.</i> (2001), , 2775
BABBOW	0.257	Y.Diskin-Posner, G.K.Patra, I.Goldberg, <i>J.Chem.Soc.,Dalton Trans.</i> (2001), , 2775
BADRII	0.344	T.E.O.Screen, I.M.Blake, L.H.Rees, W.Clegg, S.J.Borwick, H.L.Anderson, <i>J.Chem.Soc.,Perkin Trans.1</i> (2002), , 320
BADRUU	0.322	T.E.O.Screen, I.M.Blake, L.H.Rees, W.Clegg, S.J.Borwick, H.L.Anderson, <i>J.Chem.Soc.,Perkin Trans.1</i> (2002), , 320
BUZTEV	0.265	Y.Diskin-Posner, G.K.Patra, I.Goldberg, <i>J.Chem.Soc.,Dalton Trans.</i> (2001), , 2775
EACWAG	0.346	S.G.DiMagno, V.S.-Y.Lin, M.J.Therien, <i>J.Org.Chem.</i> (1993), 58, 5983
EBUQOH	0.284	Y.Diskin-Posner, I.Goldberg, <i>New J.Chem.(Nouv.J.Chim.)</i> (2001), 25, 899
FUPCEY	0.371	K.Hatano, K.Kawasaki, S.Munakata, Y.Iitaka, <i>Bull.Chem.Soc.Jpn.</i> (1987), 60, 1985
GETLAS	0.285	A.M.Shachter, E.B.Fleischer, R.C.Haltiwanger, <i>Chem.Commun.</i> (1988), , 960
HALTOD	0.269	M.P.Byrn, C.J.Curtis, Yu.Hsiou, S.I.Khan, P.A.Sawin, S.K.Tendick, A.Terzis, C.E.Strouse, <i>J.Am.Chem.Soc.</i> (1993), 115, 9480
IFOKAP	0.313	Y.Diskin-Posner, G.K.Patra, I.Goldberg, <i>Chem.Commun.</i> (2002), , 1420
OBUTUA	0.335	R.K.Kumar, Y.Diskin-Posner, I.Goldberg, <i>J.Inclusion Phenom.Macrocyclic Chem.</i> (2000), 37, 219
OBUVAI	0.334	R.K.Kumar, Y.Diskin-Posner, I.Goldberg, <i>J.Inclusion Phenom.Macrocyclic Chem.</i> (2000), 37, 219
QARSAD	0.294	A.D.Shukla, P.C.Dave, E.Suresh, A.Das, P.Dastidar, <i>J.Chem.Soc.,Dalton Trans.</i> (2000), , 4459
QARSIL	0.266	A.D.Shukla, P.C.Dave, E.Suresh, A.Das, P.Dastidar, <i>J.Chem.Soc.,Dalton Trans.</i> (2000), , 4459
TACKOY	0.273	Ting-Bin Tsao, Gene-Hsiang Lee, Chen-Yu Yeh, Shie-Ming Peng, <i>Dalton Trans.</i> (2003), , 1465
VAJYIP	0.263	A.L.Litvinov, D.V.Konarev, A.Yu.Kovalevsky, P.Coppens, R.N.Lyubovskaya, <i>CrystEngComm</i> (2003), 5, 137
<b>AVERAGE</b>	<b>0.303</b>	

**Table S1:** Dome heights (distances between the four coordinating N atoms of the porphyrin macrocycle and the position of the Zn ion) for 20 crystal structures from the Cambridge Structural Database (CSD)<sup>1</sup>.

**Binding model**

The size-exclusion chromatography, analytical ultracentrifugation, and Job analysis are consistent with the apo form of the protein having an A<sub>2</sub>B<sub>2</sub> heterotetrameric structure. Therefore a simple model involving the binding of two cofactors to the preformed A(His)<sub>2</sub>B(Thr)<sub>2</sub> protein is applied to the titration experiments, where protein is added into cofactor.

The model makes use of laws of mass action for the binding of the first and second equivalents of the cofactor.

$$K_1 = [P][C]/[PC] \quad (1)$$

$$K_2 = [PC][C]/[PC_2] \quad (2)$$

Here [P] denotes the concentration of the uncomplexed tetrameric A<sub>2</sub>B<sub>2</sub> protein, [C] is the concentration of the unbound cofactor; [PC] and [PC<sub>2</sub>] are the concentrations of protein complexes bound to one and two equivalents of the cofactor respectively.

The conservation equations yield

$$[C]_t = [C] + [PC] + 2[PC_2] \quad (3)$$

$$[P]_t = [P] + [PC] + [PC_2] \quad (4)$$

The absorbance A at 420 nm is taken to be of the form

$$A = d_0 [C] + d_1 ([PC] + 2[PC_2]) \quad (5)$$

$$= d_1 [C]_t + (d_0 - d_1)[C]$$

$$A = c_0 + c_1 [C] \quad (6)$$

where d<sub>0</sub> and d<sub>1</sub> are the absorptivities of the free and bound cofactor. The constants c<sub>0</sub> and c<sub>1</sub> are independent of concentration.

The chemical equilibria, Eqs. (1) and (2), and conservation equations, Eqs. (3) and (4), lead to the following equation involving [C]:

$$l = ((al + 2abl^2)/(1 + al + abl^2)) y \quad (7)$$

where

$$l = [C]/[C]_t$$

$$y = [P]_t/[C]_t \quad (8)$$

$$a = [C]_t / K_1 \quad (9)$$

$$b = [C]_t / K_2 \quad (10)$$

Equations (6) and (10) are used to fit the titration data in Figure 3, where the cubic equation (10) for  $l$  is solved using a root finding algorithm. Here  $y$  is the ratio of the tetrameric protein concentration to the total cofactor concentration.

$$y = [A(His)_2B(Thr)_2]_t / [(DPP)Zn]_t = [A(His):B(Thr)]_t / 2[(DPP)Zn]_t$$

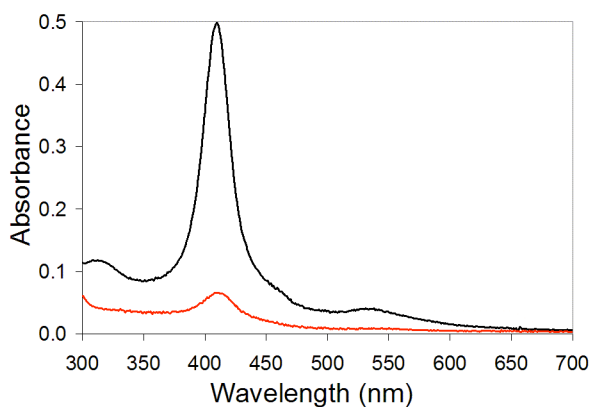
The data may be fit allowing  $c_0$ ,  $c_1$ ,  $a$ , and  $b$  as variable parameters. The absorbance  $A$  (Eq. 6) presented in Figure 3 may be fit using

$$a = 2 \times 10^{-1}, \quad b = 3 \times 10^2$$

A total cofactor concentration of  $[C]_t = 2.5 \mu M$  yields

$$K_1 = 10 \mu M$$

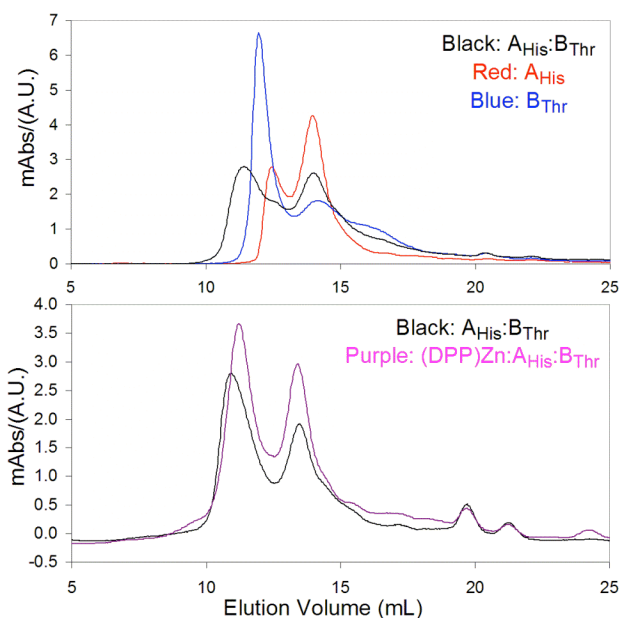
$$K_2 = 8 \times 10^{-3} \mu M$$



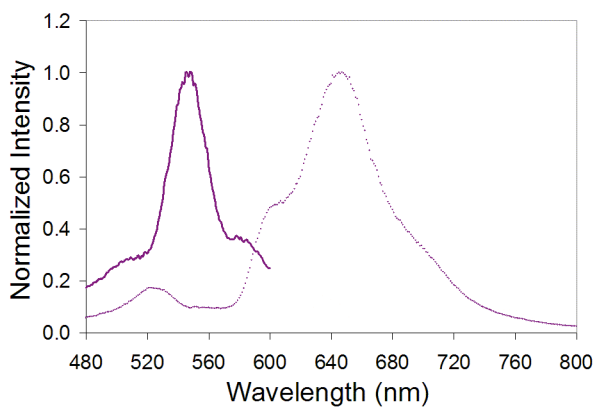
**Figure S1.** UV/vis spectra (in a 1 mm quartz cell) of (DPP)Fe(III) (50  $\mu M$ ) binding to 400  $\mu M$  of the parent homotetramer peptide ( $PA_{Tet}$ , Black) and (DPP)Fe(III) (50  $\mu M$ ) mixed with 400  $\mu M$  of the  $A_{His}$  peptide (red).

## Size Exclusion Chromatography:

Size exclusion chromatography was utilized to determine bundle formation of the individual components,  $A_{His}$  and  $B_{Thr}$ , as well as for the corresponding apo and holo states,  $A_{His}B_{Thr}$  and  $(DPP)Zn:A_{His}B_{Thr}$  (Figure 6).  $A_{His}$  elutes as two peaks at 12.5 mL and 14.0 mL corresponding to molecular weights of 11 and 5 kD respectively. Therefore,  $A_{His}$  appears to exist in a monomer-trimer equilibrium in solution.  $B_{Thr}$  also elutes as two peaks at 12.0 mL and 14.2 mL corresponding to molecular weights of 14 and 5 kD respectively, suggestive of trimer or tetramer formation. The mixture  $A_{His}B_{Thr}$  also elutes as two peaks at 11.5 mL and 14.0 mL corresponding to molecular weights of 17 and 5 kD respectively, which is consistent with a tetramer-monomer equilibrium. The apo and holo states are nearly identical in elution profile and the collected fractions did not exhibit any features in the visible spectrum. These data possibly suggest that the cofactor interacts with the chromatography matrix.



**Figure S2.** Size exclusion chromatograms of  $A_{His}$  (red),  $B_{Thr}$  (blue),  $A_{His}B_{Thr}$  (1:1 mixture, black), and  $(DPP)Zn:A_{His}B_{Thr}$  (1:1:1 mixture, purple). Evaluated MWs:  $A_{His}$ , 3953.4;  $B_{Thr}$ , 4027.5;  $[A_{His}B_{Thr}]_2$ , 15944,  $[(DPP)Zn:A_{His}B_{Thr}]_2$ , 17309.8.



**Figure S2.** Normalized excitation (solid purple) and emission spectra (purple dots) of (DPP)Zn:  $A_{His}$ : $B_{Thr}$  in 50 mM Pi, 150 mM NaCl, pH 7.5 buffer.

1. Allen, F. H., *Acta Crystallographica Section B-Structural Science* "The Cambridge Structural Database: a quarter of a million crystal structures and rising" **2002**, 58, 380-388.