A Click Chemistry Based Coordination Polymer Inside Small Heat Shock Protein

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Electronic Supplementary Information (ESI)

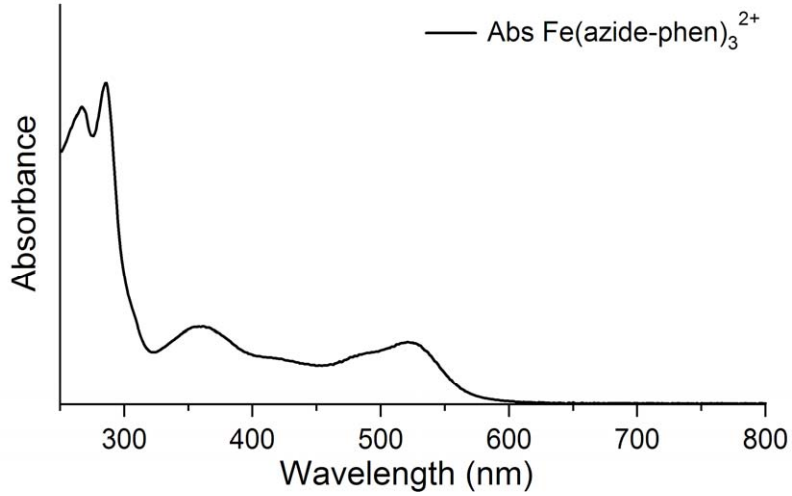
Experimental Section

Materials. All reagents were purchased from Aldrich Chemical Co. or Fisher Scientific and used without further purification. All water was purified through a Nanopure system to 18.2 MΩ resistivity before use. Tetrahydrofuran was distilled over sodium metal and benzophenone under nitrogen.

***N*-(3-azidopropyl)-1,10-phenanthrolin-5-amine (az-phen).** In a flask, under argon**,** 0.50 g 5,6-epoxy-5,6-dihydro-1,10-phenanthroline (0.50 g, 25 mmoles) was dissolved in 6 ml water and 3 ml THF.[1] To this solution 3-azidopropylamine (76.5 mmoles, 3-fold excess) was added dropwise.[2] This reaction was stirred at room temperature for 24 hrs followed by extraction 3 times with methylene chloride. The organic layer was dried over anhydrous sodium sulfate and the solvent was removed using a rotary evaporator. The crude compound was purified by column chromatography (silica gel, 9:1 CH_2Cl_2:TEA) to yield a dark yellow oil in near quantitative yield. The entire product from step 1 was added to a solution containing 0.7 g 95% NaH (12-fold excess) in 75 ml dry THF under Ar and refluxed for 4 hrs. The reaction was cooled to room temperature and 20 ml MeOH was slowly added dropwise to quench the reaction. The solvents were removed by rotary evaporation and the product was dissolved in 150 ml water followed by extraction 4 times with methylene chloride. The crude final product was purified by column chromatography (silica gel, 9:1 CH_2Cl_2:TEA) resulting in a yellow solid (0.24g, 35% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.06 (d, J = 4.5, 1H), 8.89 (d, J = 2.5, 1H), 8.33 (d, J = 8.5, 1H), 8.03 (d, J = 8.0, 1H), 7.56 (q, J = 4.5, 1H), 7.54 (q, J = 4.5, 1H), 6.63 (s, 1H), 4.91 (s, br, NH), 3.57 (t, J = 6.5, 2H), 3.47 (t, J = 6.5, 2H), 2.10 (m, J = 6.5, 2H). LC-MS: M + H 279.1388 (found), M + H 279.1358 (calculated).
Caution: Exercise extra caution when handling the reagents in this reaction scheme as azides can be an explosion hazard.

$Fe(az\text{-}phen)_3(PF_6)_2$. A fresh aqueous solution containing ammonium iron(II) sulfate hexahydrate (329 μmoles) was mixed in a 1:3 molar ratio with a solution of 5-aminopropylazide-1,10-phenanthroline (987 μmoles) instantly forming a dark red solution. The solution was allowed to stir at room temperature for 30 min followed by precipitation by adding a saturated aqueous solution of ammonium hexafluorophosphate dropwise to the mixture. The red solid was collected

through filtration and washed with ether and water. The dark red powder was recrystallized from dichloromethane and ether to yield the final product $Fe(az\text{-}phen)_3(PF_6)_2$ (0.25 g, 64% yield). ^1H NMR ($CDCl_3$, 500 MHz): δ 8.61 (t, J = 6.5, 1H), 8.20 (t, J = 7.5, 1H), 7.61 (td, J = 5.0, 20.5, 1H), 7.54 (m, J = 5.5, 1H), 7.35 (m, J = 3.5, 1H), 7.22 (td, J = 5.0, 24.0, 1H), 6.98 (d, J = 3.5, 1H), 5.86 (d, J = 3.0, 1H, NH), 3.54 (t, J = 6.5, 2H), 3.50 (d, J = 6.0, 2H), 2.06 (m, J = 6.5, 2H). LC-MS: M + H 890.3658 (found), M + H 890.3189 (calculated).



Sup. Fig.1 Uv-vis trace of $Fe(az\text{-}phen)_3^{2+}$ in water.

sHsp G41C Cage Purification and Characterization. *Methanococcus jannaschii* small Hsp G41C was purified from an *E. coli* heterologous expression system as previously described.[3] One liter cultures of *E. coli* (BL21 [DE3] B strain) containing pET-30a(+) MjHsp16.5 plasmid were grown overnight in LB medium with kanamycin added (37°C, 220 rpm). Cells were harvested by centrifugation at 3700*g* for 15 min and resuspended in 30 mL of 100 mM HEPES, 50 mM NaCl, pH 8.0. Lysozyme, DNase, and RNase were added to the final concentrations of 50, 60, and 100 μg/mL, respectively. The sample was incubated for 30 min at room temperature, French pressed (American Laboratory Press Co., Silver Springs, MD), and sonicated on ice (Branson Sonifier 250, Danbury, CT, power 4, duty cycle 50%, 3 × 5 min with 3 min intervals). Bacterial cell debris was removed via centrifugation for 20 min at 12 000*g*. The supernatant was heated for 15 min at 65 °C, thereby denaturing many *E. coli* proteins. The supernatant was centrifuged for 20 min at 12 000*g* and purified by gel filtration chromatography (Superose-6, Bio-Rad Duoflow, Hercules, CA).

Coordination Polymer Synthesis Inside Hsp G41C.

Hsp G41C-alkyne Conjugate (G0.5). Hsp G41C (30 mg, 2.0 mg/ml, 1.8 μmoles subunit) in phosphate buffer (100mM phosphate, 5 mM EDTA, 100mM NaCl, pH 7.6) was reacted at room temperature for 3 hrs with N-propargylbromoacetamide (100mM, 11 μmoles, dissolved in DMF) at a 6-fold excess relative to subunit protein. The reaction was quenched with an equimolar (11 μmoles) amount of dithiothreitol followed by dialysis (100mM HEPES, 50 mM NaCl, pH 7.5)

with near quantitative yield as measured by absorbance at 280 nm using the published extinction coefficient (9322 M^{-1} cm^{-1}) for Hsp.[4]

Hsp G41C-alkyne-iron Conjugate (G1.0). G1.0 and following were completed according to a published procedure.[5] In brief, Hsp G41C-alkyne (20 mg, 2.0 mg/ml, 1.2 μmoles subunit) in 100mM HEPES, 50 mM NaCl, pH 7.5 was added to a 35 ml solution (25 ml water, 10 ml buffer) containing Fe(azide-phen)$_3^{2+}$ (4 μmoles). To this solution a mixture of $CuSO_4$ (0.17 μmoles) and tris(hydroxypropyl)triazolylmethyl-amine (THPTA) (4.25 μmoles), 100 mM aminoguanidine in water (34 μmoles), and freshly made 100 mM sodium ascorbate (34 μmoles) were added sequentially with thorough mixing between each additional reagent. This solution was allowed to mix at room temperature for 1 hr followed by dialysis and size exclusion chromatography on a Superose 6 column (Amersham Bioscience).

Hsp G41C-alkyne-iron-alkyne Conjugate (G1.5). Hsp G41C-alkyne (14 mg, 2.0 mg/ml, 0.85 μmoles subunit) in 100mM HEPES, 50 mM NaCl, pH 7.5 was added to a 40 ml solution (30 ml water, 10 ml buffer) containing tripropargylamine (5 μmoles). To this solution a mixture of CuSO4 (0.225 μmoles) and THPTA (5.5 μmoles), 100mM aminoguanidine in water (44 μmoles), and freshly made 100mM sodium ascorbate (44 μmoles) were added sequentially with thorough mixing between each additional reagent. This solution was allowed to mix at room temperature for 1 hr followed by dialysis and size exclusion chromatography on a Superose 6 column (Amersham Bioscience).

Hsp G41C-alkyne-iron-alkyne-iron Conjugate (G2.0). Hsp G41C-alkyne (6 mg, 2.0 mg/ml, 0.36 μmoles subunit) in 100mM HEPES, 50 mM NaCl, pH 7.5 was added to a 40 ml solution (30 ml water, 10 ml buffer) containing Fe(azide-phen)$_3^{2+}$ (5 μmoles). To this solution a mixture of CuSO4 (0.225 μmoles) and THPTA (5.5 μmoles), 100mM aminoguanidine in water (44 μmoles), and freshly made 100mM sodium ascorbate (44 μmoles) were added sequentially with thorough mixing between each additional reagent. This solution was allowed to mix at room temperature for 1 hr followed by dialysis and size exclusion chromatography on a Superose 6 column (Amersham Bioscience).

Hsp G41C-alkyne-iron-alkyne-iron-alkyne Conjugate (G2.5). Hsp G41C-alkyne (2 mg, 2.0 mg/ml, 0.12 μmoles subunit) in 100mM HEPES, 50 mM NaCl, pH 7.5 was added to a 15 ml solution (30 ml water, 10 ml buffer) containing tripropargylamine (3.3 μmoles). To this solution a mixture of CuSO4 (0.15 μmoles) and THPTA (3.7 μmoles), 100mM aminoguanidine in water (30 μmoles), and freshly made 100mM sodium ascorbate (30 μmoles) were added sequentially with thorough mixing between each additional reagent. This solution was allowed to mix at room temperature for 1 hr followed by dialysis and size exclusion chromatography on a Superose-6 column (Amersham Bioscience).

Hsp G41C-alkyne-iron-alkyne-iron-alkyne-iron Conjugate (G3.0). Hsp G41C-alkyne (0.5 mg, 2.0 mg/ml, 0.03 μmoles subunit) in 100mM HEPES, 50 mM NaCl, pH 7.5 was added to a 14 ml solution (10 ml water, 4 ml buffer) containing Fe(azide-phen)$_3^{2+}$ (1.8 μmoles). To this solution a mixture of CuSO4 (0.08 μmoles) and THPTA (1.8 μmoles), 100mM aminoguanidine in water (15 μmoles), and freshly made 100mM sodium ascorbate (15 μmoles) were added sequentially with thorough mixing between each additional reagent. This solution was allowed to

mix at room temperature for 1 hr followed by dialysis and size exclusion chromatography on a Superose-6 column (Amersham Bioscience).

Hsp G41C coordination polymer composite analysis.
Hsp G41C and each coordination polymer generation were characterized by SEC (Superose 6, Bio-Rad Duoflow), DLS (Brookhaven 90Plus, Brookhaven, NY), TEM (Leo 912 AB), SDS-PAGE, and mass spectrometry (NanoAcquity/Q-Tof Premier; Waters, Milford, MA). The protein concentration was determined by absorbance at 280 nm using the published extinction coefficient (9322 M^{-1} cm^{-1}).[4] The assembled protein cages at each generation including the native Hsp G41C were imaged by transmission electron microscopy by negatively staining the sample with 2% uranyl acetate on formvar carbon coated grids.

Each Hsp G41C click generation was prepared for an acid urea gel in loading buffer (5% acetic acid, 6 M urea, and 30% glycerol) and heated at 60^{o}C for 6 min. The gel was prepared according to general acid urea gel protocols[6] with 12% acrylamide and 40% urea and run at 150V followed by coomassie blue staining and destaining.

Mass Spectrometry. MS analyses were performed on a ESI-Q-TOF mass spectrometer (Q-TOF Premier, Waters). Hsp G41C and each generation (0.1-2 μL, 1.0 mg/mL) were injected onto a BioBasic SEC-300 (Thermo Scientific) column and eluted with 40% IPA, 0.1% formic acid. Deconvoluted spectra were generated with the software MaxEnt1 provided by Waters. The organic ligand and iron coordination complex were analyzed using C18 column (218TP5115, Vydac, Deerfield, IL) and eluted with a water-acetonitrile linear gradient (eluent A, 0.1% formic acid in water; eluent B, 0.1% formic acid in acetonitrile).

References

1. M. Riklin, D. Tran, X. H. Bu, L. E. Laverman and P. C. Ford, *Dalton Trans.*, 2001, 1813-1819.
2. M. J. Abedin, L. Liepold, P. Suci, M. Young and T. Douglas, *J. Am. Chem. Soc.*, 2009, **131**, 4346-4354.
3. A. Miura, T. Hikono, T. Matsumura, H. Yano, T. Hatayama, Y. Uraoka, T. Fuyuki, S. Yoshii and I. Yamashita, *Jpn. J. Appl. Phys.,* Part 2, 2006, **45**, L1-L3.
4. K. K. Kim, H. Yokota, S. Santoso, D. Lerner, R. Kim and S.-H. Kim, *J. Struct. Biol.,* 1998, **121**, 76-80.
5. V. Hong, S. I. Presolski, C. Ma and M. G. Finn, *Angew. Chem., Int. Ed.*, 2009, in press.
6. J. H. Waite and C. V. Benedict, *Methods Enzymol.*, 1984, **107**, 397-413.