SUPPORTING INFORMATION

Metal-Dependent Assembly of a Protein Nano-Cage

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Table S1

Protein sequence of the Tet8-M construct. *Orange*: maltose binding protein domain; *Green*: Spacer sequence 1; *Black*: trimeric esterase domain (TriEst); *Blue*: Spacer sequence 2; *Red*: metal-binding coiled coil domain

MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDT
GIKVTVEHPDKLEEKFPQVAATGDGPDIIFWA
HDRFGGYAQSGLLAEITPDKAFQDKLYPFTWD
AVRYNGKLIAYPIAVEALSLIYNKDLLPNPPK
TWEEIPALDKELKAKGKSALMFNLQEPYFTWP
LIAADGGYAFKYENGKYDIKDVGVDNAGAKAG
LTFLVDLIKNKHMNADTDYSIAEAAFNKGETA
MTINGPWAWSNIDTSKVNYGVTVLPTFKGQPS
KPFVGVLSAGINAASPNKELAKEFLENYLLTD
EGLEAVNKDKPLGAVALKSYEEELVKDPRIAA
TMENAQKGEIMPNIPQMSAFWYAVRTAVINAA
SGRQTVDEALKDAQTGGGGGGGGGGENLYFQGG
HMSYVTTKDGVQIFYKDWGPRDAPVIHFHHGW
PLSADDWDAQLLFFLAHGYRVVAHDRRGHGRS
SQVWDGHDMDHYADDVAAVVAHLGIQGAVHVG
HSTGGGEVVRYMARHPEDKVAKAVLIAAVPPL
MVQTPGNPGGLPKSVFDGFQAQVASNRAQFYR
DVPAGPFYGYNRPGVEASEGIIGNWWRQGMIG
SAKAHYDGIVAFSQTDFTEDLKGIQQPVLVMH
GDDDQIVPYENSGVLSAKLLPNGALKTYKGYP
HGMPTTHADVINADLLAFIRSGTGGGGGGIEKK
IEAIEKKIEAHEKKHEAIEKKIEAG

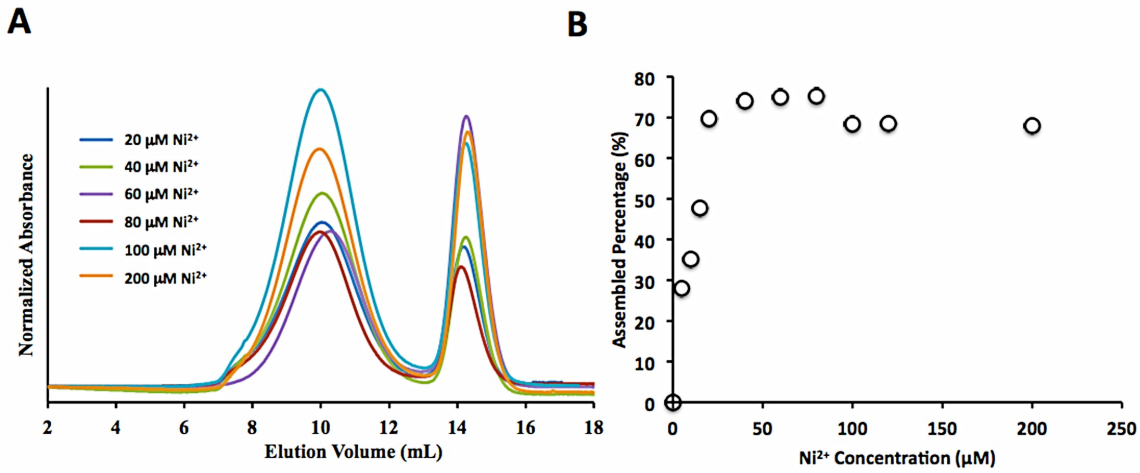

Figure S1.

Size exclusion column chromatography of Tet8-M in the presence of various concentrations of Ni^{2+}. **A:** 20 μM protein assembled and chromatographed in the presence of 20 μM, 40 μM, 60 μM, 80 μM, 100 μM and 200 μM Ni^{2+}. **B:** Percentage of assembled Tet8-M cages as a function of Ni^{2+} determined by integrating the peak areas of the SEC elution profiles.

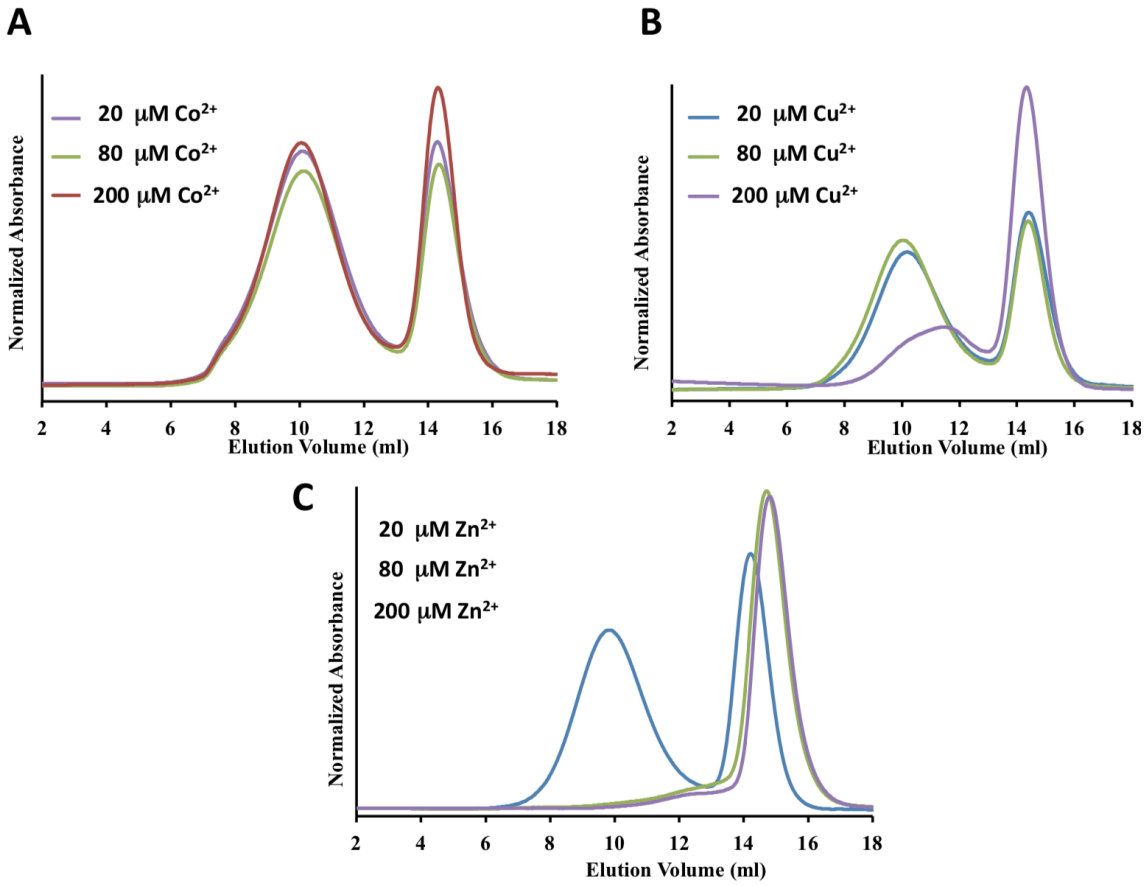

Figure S2

Assembly of Tet8-M in the presence of Co^{2+}, Cu^{2+} and Zn^{2+}. Size exclusion chromatographs of Tet8-M assembled and chromatographed in the presence of the indicated concentrations of metal ions: **A** Co^{2+}; **B** Cu^{2+}; **C** Zn^{2+}.

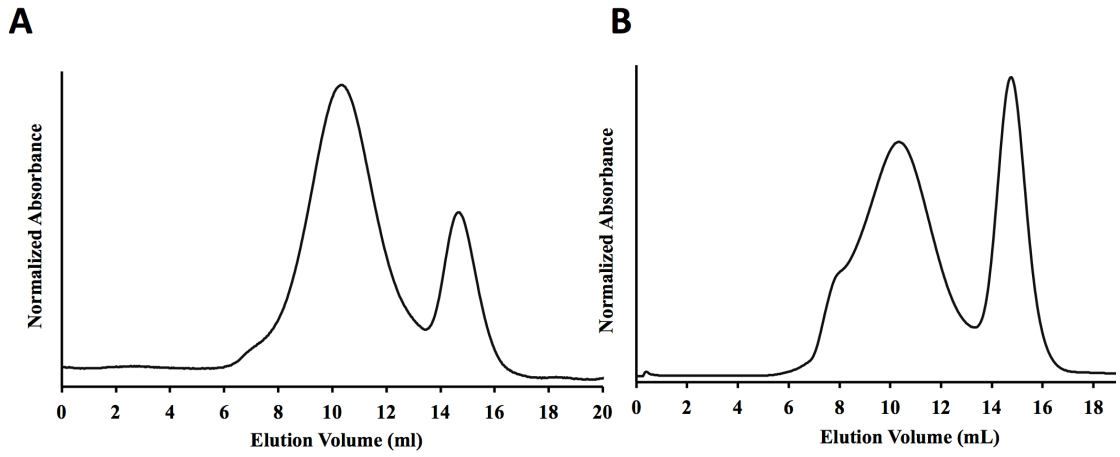

Figure S3.

Reversibility of cage assembly. Size exclusion chromatographs of Ni^{2+}-Tet8-M complexes. **A:** Ni^{2+}-Tet8-M complexes were dissociated to trimers by chelation of Ni^{2+} with EDTA; EDTA was removed and the protein re-equilibrated with Ni^{2+}. **B:** Ni^{2+}-Tet8-M complexes were dissociated to trimers by lowering the pH to 4.5; subsequently the re-pH was re-adjusted to pH 8.0. Some aggregated material is evident that elutes in the void volume at ~ 8 mL.

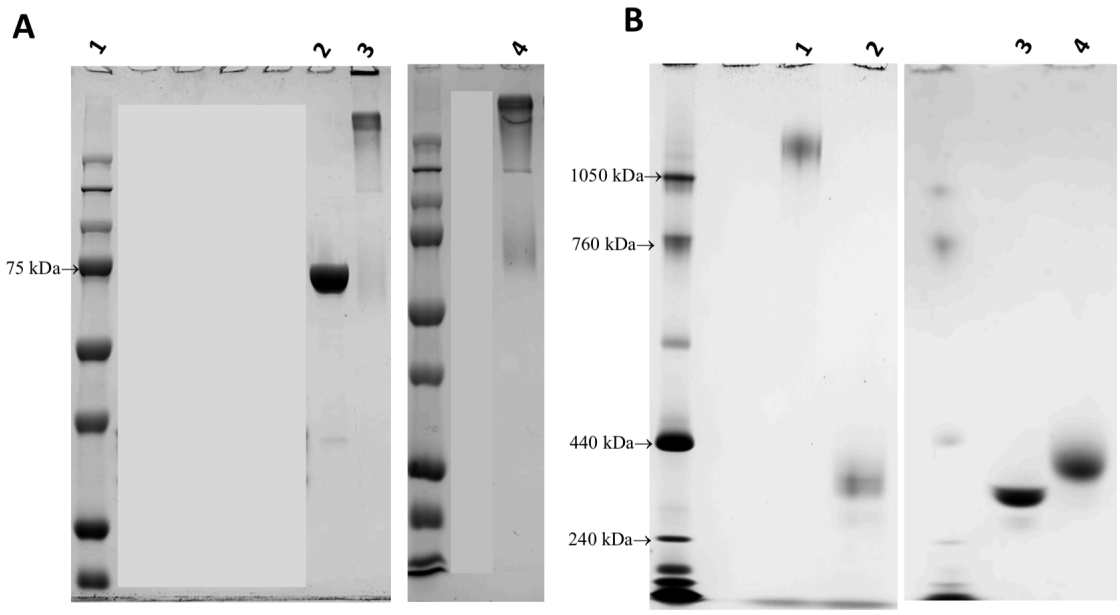

Figure S4.

Original images of gels used in Fig. 5 of the main text showing molecular weight markers for each gel. **A:** SDS-PAGE of Tet8-M: *lane 1* molecular weight markers; *lane 2* Tet8-M, non-crosslinked and in the absence of Ni^{2+}; *lane 3* Tet8-M crosslinked in the presence of Ni^{2+}; *lane 4* Tet8-M crosslinked in the absence of Ni^{2+}. **B:** Native PAGE of Tet8-M: *lane 1* Tet8-M assembled in the presence of Ni^{2+} and crosslinked; *lane 2* Tet8-M assembled in the presence of Ni^{2+} without crosslinking; *lane 3* Tet8-M, non-crosslinked and in the absence of Ni^{2+}; *lane 4* Tet8-M crosslinked in the absence of Ni^{2+}. (Boxes on gel in panel A cover unrelated samples)

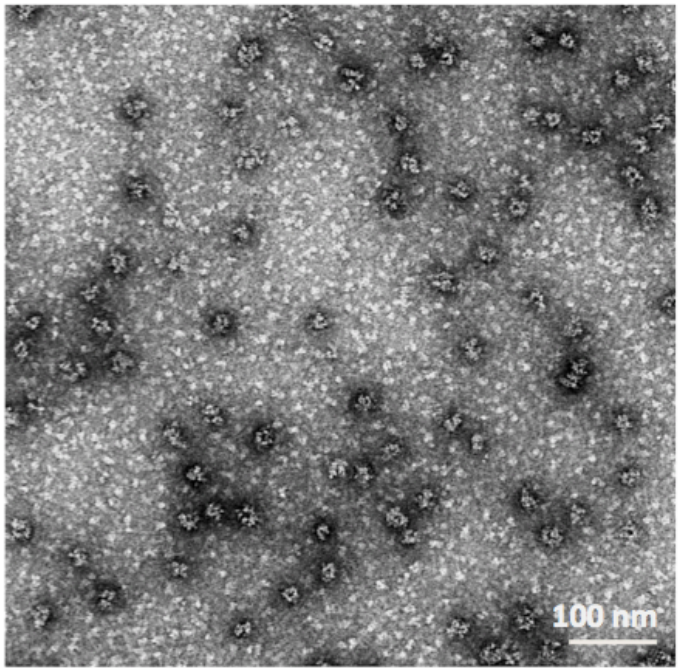

Figure S5.

Negative stain TEM image of un-crosslinked Ni^{2+}-Tet8-M complex. Some protein assemblies have dissociated into the trimeric form on the Cu grid.