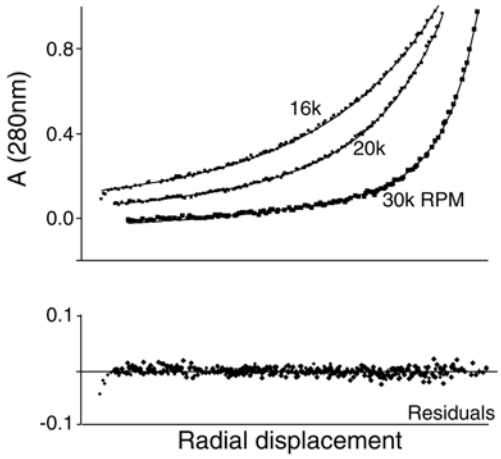
A regulatable switch mediates self-association in an immunoglobulin fold
Matthew F. Calabrese, Catherine M. Eakin, Jimin M. Wang, and Andrew D. Miranker



Supplementary Figure 1: Sedimentation equilibrium analytical ultracentrifugation of $H13F_{holo}$. Displayed in the upper panel is a portion of the global analysis derived from 30 μM $H13F_{holo}$. Data (points) and fit (solid-line) are overlaid. Lower panel shows a superposition of the residuals.

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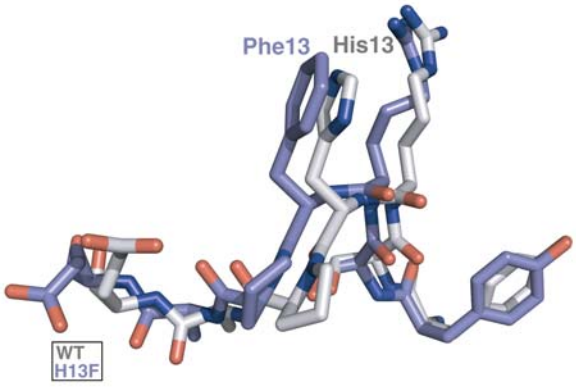

Supplementary Figure 2 Overlay of position 13 in WT and H13F. Stick representation of WT_{apo} (gray) and $H13F_{holo}$ (blue) in the region surrounding residue 13.

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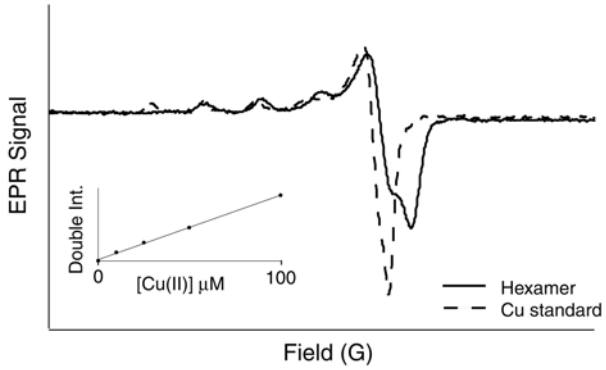

Supplementary Figure 3 Cu^{2+} binding stoichiometry by EPR. Shown as a solid line is a sample of Cu^{2+}-bound hexameric H13F (at 50 μM in monomeric units). Dashed line shows a 50 μM Cu^{2+} standard. Inset is a linear fit to a standard curve. The Cu^{2+} stoichiometry in these oligomers is 1.1 +/- 0.2. Error represents one standard deviation.

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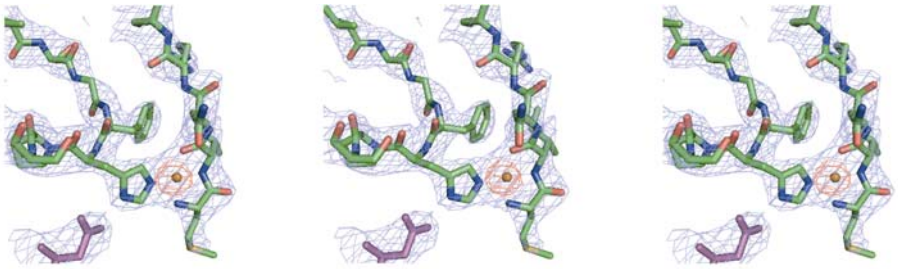

Supplementary Figure 4 Electron Density map of Cu^{2+} binding site. Cu^{2+} binding site and surrounding residues shown in wall eye (left pair) and cross-eye (right pair) stereo. The model is presented with an overlay of an annealed composite omit map contoured at 1.5 σ (blue). Also shown is an anomalous difference Fourier map contoured at 7 σ (red). The location of the Cu^{2+} ion is marked by an orange sphere. Shown in magenta is Asp34 from an adjacent chain.

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Chain	Atoms	Bond (Å)	Atoms	Angle (°)
A	Nterm-Cu	2.4	Nterm-Cu-His	113
	His-Cu	1.9	His—Cu-CO	97
	CO-Cu	2.5	CO-Cu-NH	77
	NH-Cu	2.2	NH-Cu-Nterm	72
B	Nterm-Cu	2.2	Nterm-Cu-His	99
	His-Cu	2.0	His—Cu-CO	111
	CO-Cu	1.9	CO-Cu-NH	75
	NH-Cu	2.4	NH-Cu-Nterm	74
C	Nterm-Cu	2.2	Nterm-Cu-His	95
	His-Cu	2.0	His—Cu-CO	114
	CO-Cu	2.7	CO-Cu-NH	74
	NH-Cu	2.2	NH-Cu-Nterm	75
D	Nterm-Cu	2.5	Nterm-Cu-His	107
	His-Cu	1.9	His—Cu-CO	99
	CO-Cu	2.0	CO-Cu-NH	78
	NH-Cu	2.3	NH-Cu-Nterm	73
E	Nterm-Cu	2.3	Nterm-Cu-His	110
	His-Cu	2.0	His—Cu-CO	83
	CO-Cu	2.0	CO-Cu-NH	88
	NH-Cu	2.0	NH-Cu-Nterm	79
F	Nterm-Cu	2.4	Nterm-Cu-His	103
	His-Cu	2.1	His—Cu-CO	111
	CO-Cu	2.7	CO-Cu-NH	72
	NH-Cu	2.3	NH-Cu-Nterm	72
Average	**Nterm-Cu**	**2.3±0.1**	**Nterm-Cu-His**	**104±7**
	His-Cu	**2.0±0.1**	**His—Cu-CO**	**103±12**
	CO-Cu	**2.3±0.4**	**CO-Cu-NH**	**77±6**
	NH-Cu	**2.2±0.1**	**NH-Cu-Nterm**	**74±3**

Supplementary Table 1 Bond lengths and angles for Cu^{2+} coordination in each chain of the hexamer. Nterm refers to the N-terminus, NH and CO refer to the backbone amide and carbonyl of Ile1, His refers to the epsilon-Nitrogen of the His31 imidazole ring. Errors on averages represent ± 1 standard deviation.