## **Supporting Information**

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**Fig. S1.** Spectroscopic properties of ThT in the presence and absence of  $\beta 2m$ . These are 2D representations of excitation and emission spectra for  $\pm 100 \mu M \beta 2m$  and  $\pm 200 \mu M Cu^{2+}$ . Contour levels in all panels are drawn in decreasing intervals of  $10^c$ , where c = -0.1, -0.2, -0.3..., relative to the maximum intensity of the hexamer ( $+\beta 2m + Cu^{2+}$ ). All contours with c < -1 are drawn in gray. Samples additionally contained 100  $\mu M$  ThT, 200 mM potassium acetate and 25 mM MOPS at pH 7.4 and 25° C. 10 mM EDTA was added to all  $-Cu^{2+}$  samples.



**Fig. S2.** Visualization of ThT in aggregates. False-color fluorescence images collected using blue (440 nm) excitation. (*A*) Amyloid fibers derived from human islet amyloid polypeptide (identical to image in Fig. 1*B* so as to facilitate comparison with Fig. S2 *B–D*). (*B*) Avidin-crosslinked membrane Nanodiscs containing 5% biotinylated lipids. (*C*) Avidin-crosslinked aggregates of biotin-labeled bovine serum albumin. (*D*) A droplet of a nonamyloidogenic sequence variant of islet amyloid polypeptide from rat in the same buffer as *A*. The right half of each image has had contrast increased for clarity. Ratiometric measures use signal integrated across the areas indicated with a white box. Ratios were computed using the same areas on images collected using 340 nm excitation. These ratios are ~15, ~6, ~6, and ~5 for *A–D*, respectively. Note that background regions in Fig. 1*C–D* also yield a ratio of ~6, indicating that this represents the instrumental response for ratiometric-based assessments at these wavelengths.



**Fig. S3.** Alternate conformations of ThT bound to  $\beta 2m_{holo}$ . (A) The side-view of ThT (yellow) bound at the  $\beta 2m_{holo}$  intersheet interface (*Left*) and the head-on view (*Right*). (B) The second ThT (cyan) conformation at the  $\beta 2m_{holo}$  intersheet interface viewed from the side (*Left*) and looking down the  $\phi$  axis (*Right*). (C) An overlay of both ThT conformations at the  $\beta 2m_{holo}$  intersheet interface.



**Fig. 54.** The  $F_o - F_c$  electron difference density for ThT. (A) Difference density of ThT from  $\beta 2m_{holo}$ . Density around the benzothiazole ring is clearly apparent; however, clear density for the dimethylaminobenzene ring is not observed. (*B* and C) Difference density for the two ThT binding sites observed in  $\beta 2m_{apo}$ . Density for the entirety of two fluorophores can be seen for the first site (*B*) and the entirety of one fluorophore for the second site (*C*). For *A*–*C*, two panels are shown related by the indicated 90° rotation. In the right panel of *B* only the topmost ThT is shown for clarity. (*A*–*C*) All density is contoured at  $3\sigma$ .

	$\beta 2m_{holo}$	$\beta 2m_{apo}$
Cell Dimensions (Å)		
A	92.89	48.55
В	116.59	28.79
С	66.32	74.13
Space Group	P21212	P21
Resolution (Å)	50-2.7	50-1.6
R <sub>merge</sub>	12.7 (92.2)	8.2 (37.4)
1/ol	11.8 (1.7)	8.9 (1.6)
Completeness (%)	97.7 (87.4)	98.3 (88.3)
Redundancy	6.5 (4.9)	1.9 (1.6)
Refinement		
Resolution	50-2.7	50-1.6
$R_{\rm work}/R_{\rm free}$	23.9/25.6	18.6/22.1
rms deviations		
Bond lengths	0.013	0.014
Bond angles	1.4	1.6

Table S1. Data and refinement statistics for  $\beta 2m_{holo}$  and  $\beta 2m_{apo}$ 

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