Structure-based inhibitors of tau aggregation

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Supplementary Methods

Protein expression and purification

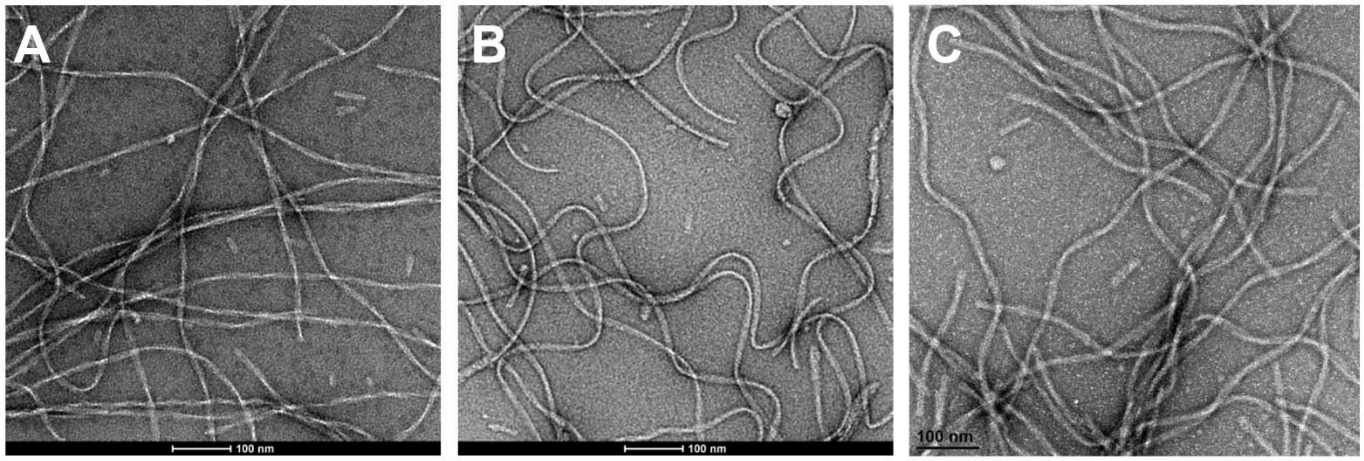
Human Tau wild-type K18 (residues 244-372), 2xIN (V308I/Y309N) and 2xVY (I278V/N279Y) were expressed in a pNG2 vector[33] in BL21-Gold E. coli cells grown in LB to an OD600 = 0.8. Cells were induced with 0.5 mM IPTG for 3 hours at 37 ºC and lysed by sonication in 20 mM MES buffer (pH 6.8) with 1 mM EDTA, 1 mM $MgCl_2$, 1 mM DTT and HALT protease inhibitor before addition of NaCl 500 mM final concentration. Lysate was boiled for 20 minutes and the clarified by centrifugation at 15,000 rpm for 15 minutes and dialyzed to 20 mM MES buffer (pH 6.8) with 50 mM NaCl and 5 mM DTT. Dialyzed lysate was purified on a 5 ml HighTrap SP ion exchange column and eluted over a gradient of NaCl from 50 to 550 mM. Proteins were polished on a HiLoad 16/600 Superdex 75 pg in 10 mM Tris (pH 7.6) with 100 mM NaCl and 1 mM DTT, and concentrated to ~20-60 mg/ml by ultrafiltration using a 3 kDa cutoff.

Human Tau40 (residues 1-441) was expressed in pET28b with a C-terminal His-tag in BL21-Gold E. coli cells grown in TB to an OD600 = 0.8. Cells were induced with 0.5 mM IPTG for 3 hours at 37 ºC and lysed by sonication in 50 mM Tris (pH 8.0) with 500 mM NaCl, 20 mM imidazole, 1 mM beta-mercaptoethanol, and HALT protease inhibitor. Cells were lysed by sonication, clarified by centrifugation at 15,000 rpm for 15 minutes, and passed over a 5 ml HisTrap affinity column. The column was washed with lysis buffer and eluted over a gradient of imidazole from 20 to 300 mM. Fractions containing purified Tau40 were dialyzed into 50 mM MES buffer (pH 6.0) with 50 mM NaCl and 1 mM beta-mercaptoethanol and purified by cation exchange as described for K18. Peak fractions were polished on a HiLoad 16/600 Superdex 200 pg in 1X PBS (pH 7.4), and concentrated to ~20-60 mg/ml by ultrafiltration using a 10 kDa cutoff.

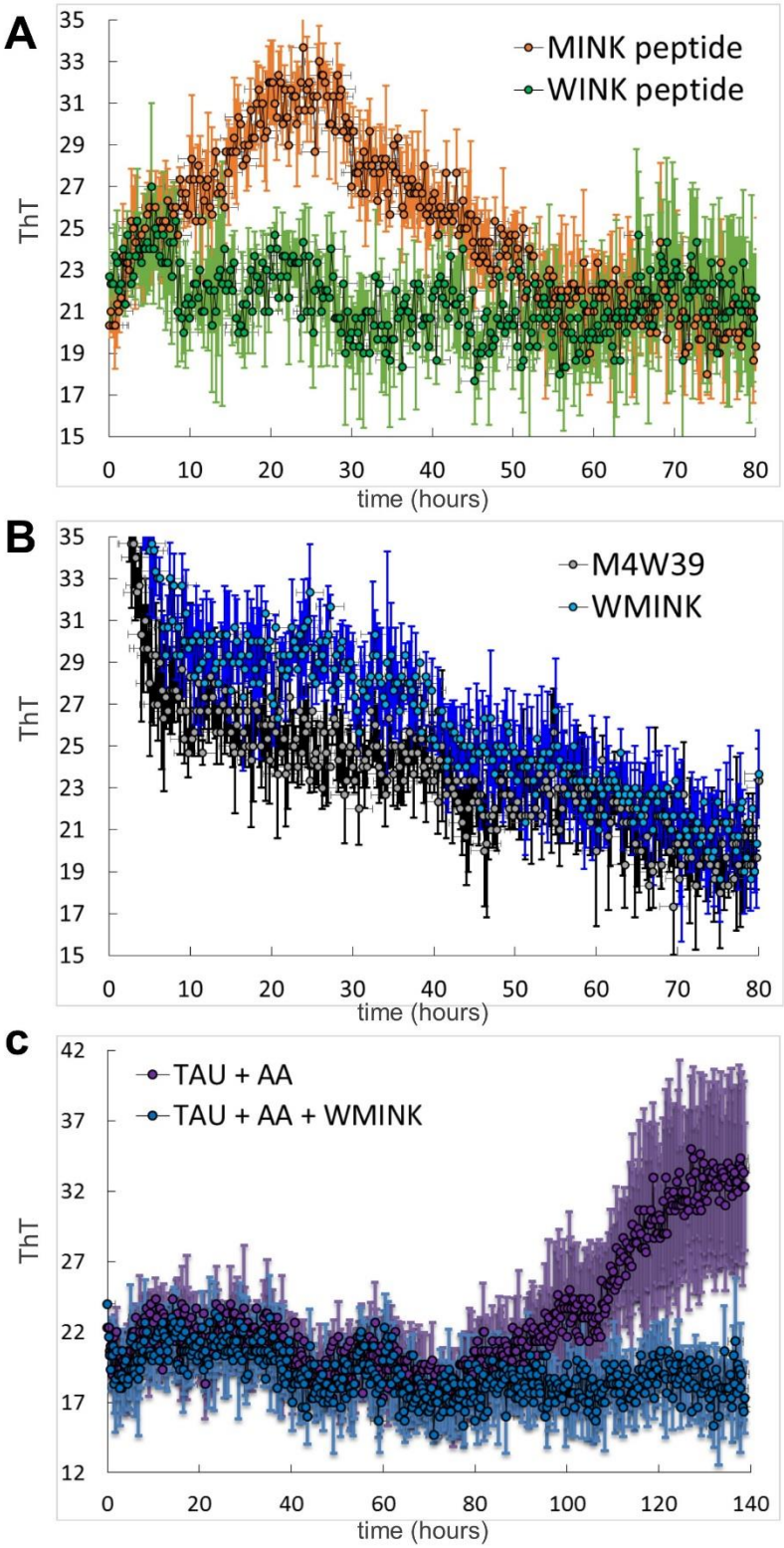
Tau biosensor cell line maintenance and seeding

HEK293 cell lines stably expressing Tau 4R1N P301S-EYFP , referred to as “4R1N Tau biosensor cells” were engineered by Marc Diamond’s lab at UTSW[22] and used without further characterization or authentication. Cells were maintained in DMEM (Life Technologies, cat. 11965092) supplemented with 10% (vol/vol) FBS (Life Technologies, cat. A3160401), 1% penicillin/streptomycin (Life Technologies, cat. 15140122), and 1% Glutamax (Life Technologies, cat. 35050061) at 37 °C, 5% CO2 in a humidified incubator. As described above, seeding was performed with Tau40 fiber treated with inhibitor as indicated. Just before transfection with Lipofectamine 2000, fibers diluted in OptiMEM (Life Technologies) were sonicated in a cuphorn water bath for 3 minutes according to the manufacturer’s instructions. Three days after the addition of pre-capped Tau40 fibers, the number of seeded aggregates in 4R1N Tau biosensor cells were determined by imaging the entire well of a 96-well plate in triplicate using a Celigo Image Cytometer (Nexcelom) in the YFP channel. Aggregates were counted using ImageJ[34] by subtracting the background fluorescence from unseeded cells and then counting the number of peaks with fluorescence above background using the built-in Particle Analyzer. Example images before and after counting are shown in Supplementary Fig. 2-5. The number of aggregates was normalized to the confluence of each well, and dose-response curves were generated by calculating the average and standard deviations for each inhibitor concentration from triplicate measurements. Resulting does-response curves were fit by nonlinear regression in Graphpad Prism to determine IC50s. For high quality images, cells were photographed on a ZEISS Axio Observer D1 fluorescence microscope using the YFP fluorescence channel.

Supplemental Figures

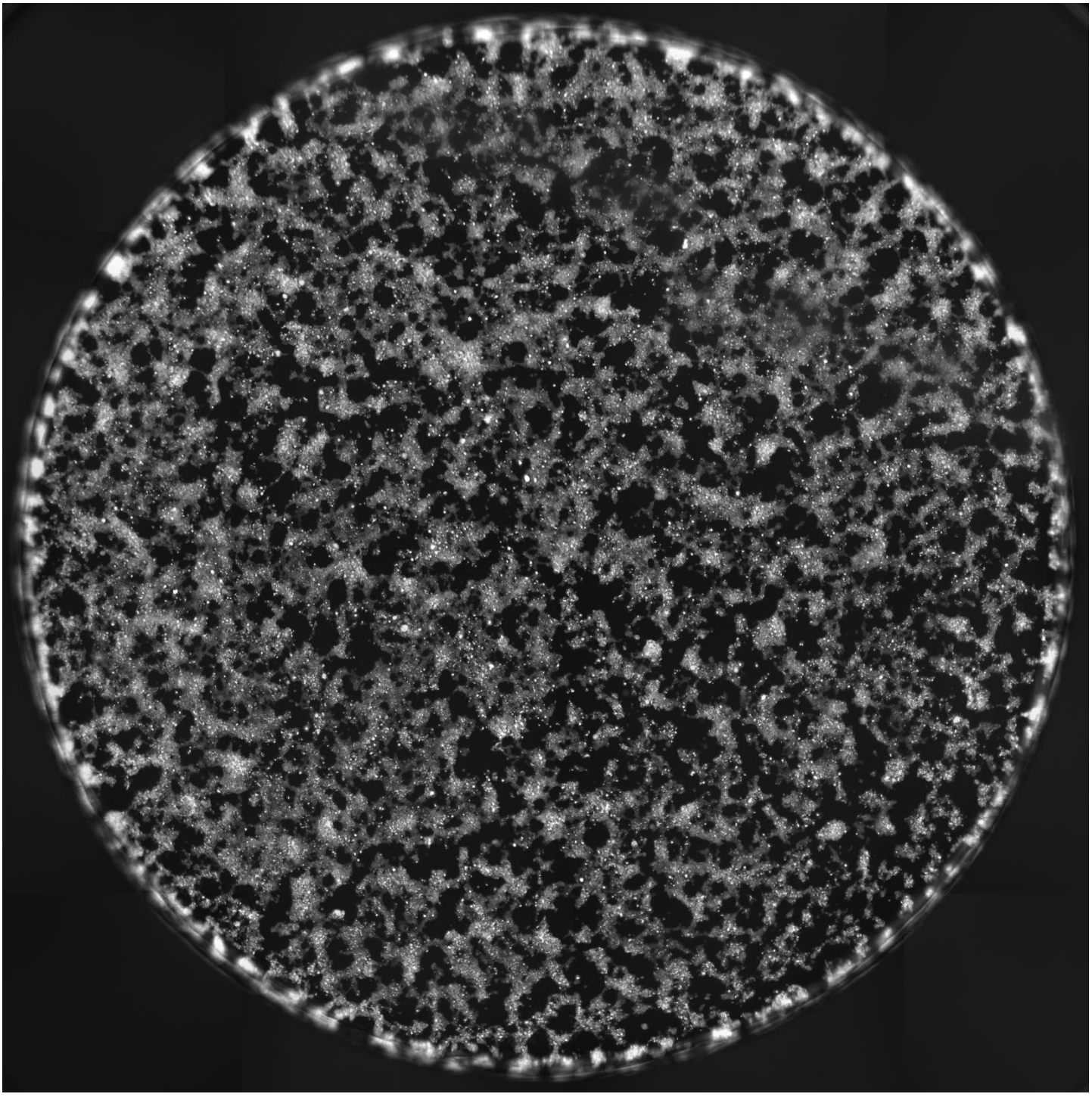


Supplemental Figure 1 Negative stain EM images of fibers from Tau (A) K18, (B) 2xIN, and (C) 2xVY collected on a FEI TF20 TEM. No differences in fibril morphology are apparent at the resolution of imaging shown.

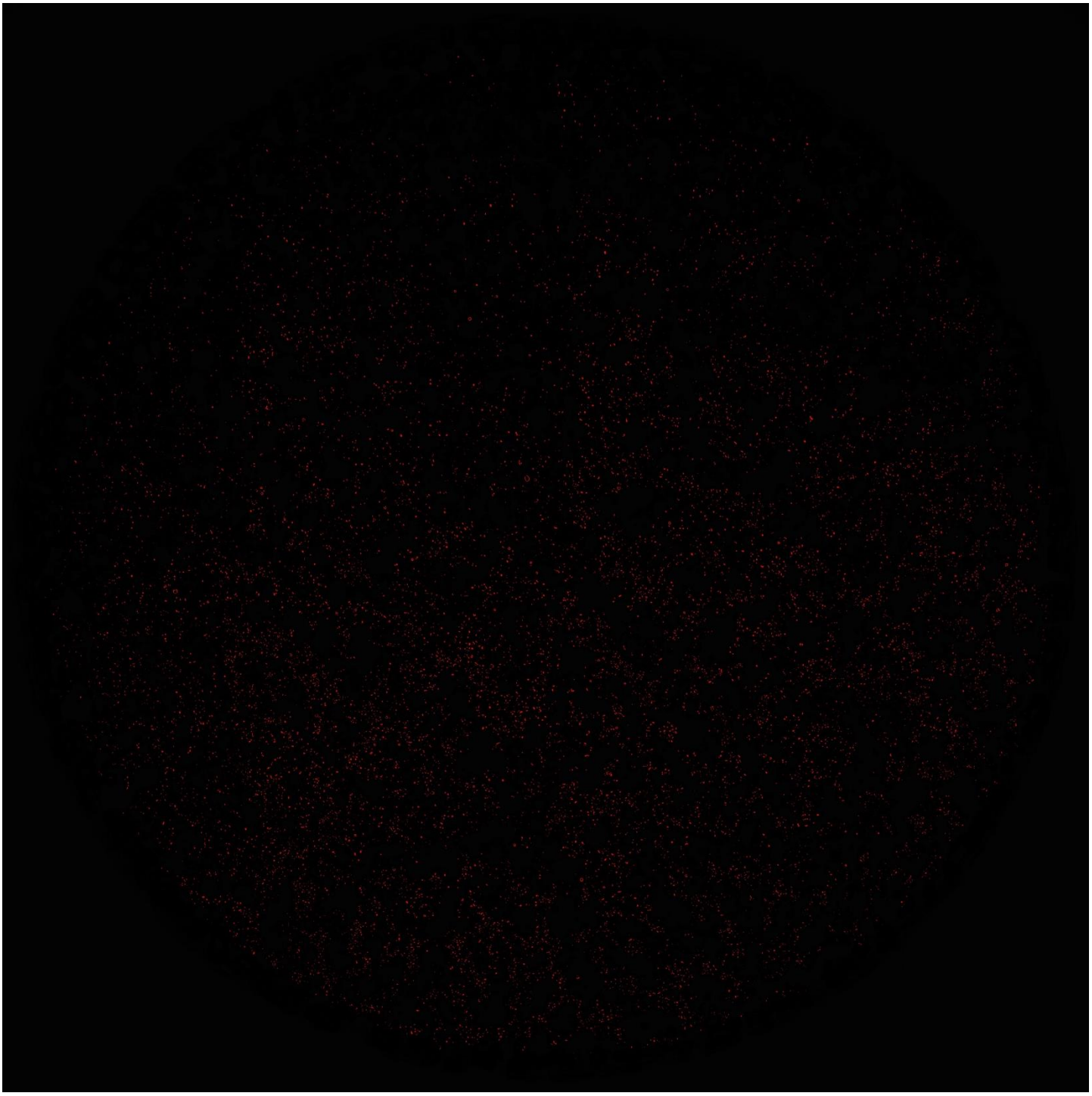


Supplemental Figure 2 (A) 25 µM MINK (orange) and WINK (green) peptides without Tau protein, in the presence of 0.5 mg/ml heparin shaking at 700 rpm 37 ºC. WINK shows no detectable ThT signal, and MINK shows ThT signal peaking at 24 hours suggesting the low level formation of amyloid-like aggregates. By comparison, we estimate the level of MINK aggregates produced to be nearly 20 times less than Tau40. (B) As in A, but for 25 µM the W-MINK (blue) and M4W39 peptides (black and grey). Neither W-MINK nor M4W39 show appreciable ThT aggregation by shaking with heat and heparin. (C) 12 µM Tau40 + 300 µM arachidonic

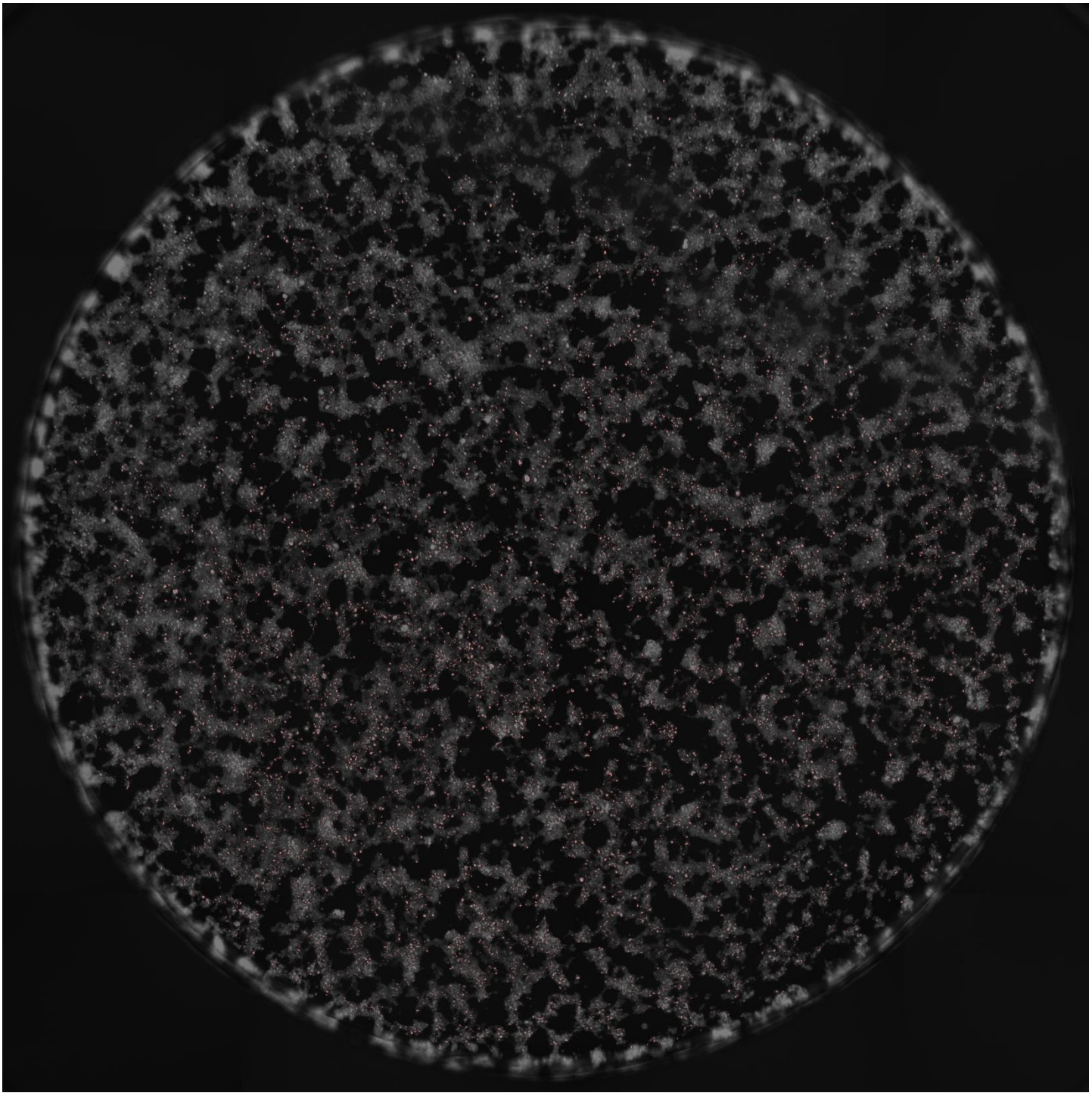
shaking at 700 rpm 37 ºC in the absence (purple) or presence (blue) of 24 µM W-MINK. Error bars show the standard deviation of triplicate ThT measurements



Supplemental Figure 3 Unedited image of a representative well containing seeded 4R1N Tau biosensor cells imaged using the Celigo Image Cytometer (Nexcelom) in the YFP channel.



Supplemental Figure 4 Same image as in Supplemental Figure 3 following background subtraction to eliminate unseeded cells and particle analysis. The red peaks are Tau aggregates included in the well count.



Supplemental Figure 5 Overlay of images in Supplemental Figures 3 and 4. The original image from Supplemental Figures 3 is shown in white, and seeded Tau aggregates appear as white puncta. Red puncta are counted aggregates from the processed image shown in Supplemental Figure 4. The red peaks align well with the white puncta, confirming the accuracy of the aggregate counting protocol.

Supplemental Table 1

X-ray Data collection and atomic refinement statistics.

Structure	KVQIINKKLD	VQIINK
Data collection		
Number of merged datasets	12	3
Space group	$P2_1$	$P2_12_12$
Cell dimensions		
a, *b*, *c* (Å)	27.1, 4.8, 32.4	20.4, 43.2, 4.8
α, β, γ (°)	90.0, 100.5, 90.0	90.0, 90.0, 90.0
Resolution (Å)	18.8-1.5 (1.68-1.50) *	10.2-1.25 (1.40-1.25) *
R_{merge} (%)	25.0	23.9
$I / \sigma(I)$	5.2 (2.8)	3.58 (1.59)
Completeness (%)	84.8 (82.3)	86.8 (71.6)
Redundancy	9.1	4.4
Refinement		
Resolution (Å)	18.8-1.5	10.18-1.25
No. reflections	2,203	1,226
R_{work} / R_{free}	19.0 / 21.2	21.9 / 26.6
No. atoms		
Protein	197	107
Ligand/ion	0	0
Water	2	0
Overall *B*-factors (Å^2)	16.9	15.3
R.m.s. deviations		
Bond lengths (Å)	0.01	0.01
Bond angles (°)	1.5	1.4

* Values in parentheses refer to outermost shell of data