Supplementary Material

O-GlcNAc modification of tau directly inhibits its aggregation without perturbing the conformational properties of tau monomers.

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Table S1. *O*-GlcNAcylation of Ser400 does not markedly perturb the cis/trans X-Pro conformational equilibria of Tau353-408

	S396-P397 [a]	**S404-P405** [a]
cTau353-408	0.09 ± 0.001	0.11 ± 0.001
ogTau353-408	0.08 ± 0.004	0.08 ± 0.004

[a] *cis/trans* ratios determined from relative $^{1}H^{N}$-^{15}N peak intensities of amides closest to the indicated X-Pro bonds. The data were obtained from ^{15}N-HSQC spectra recorded with a 10 sec recycle delay to ensure complete relaxation. Errors were estimated from signal-to-noise ratios.

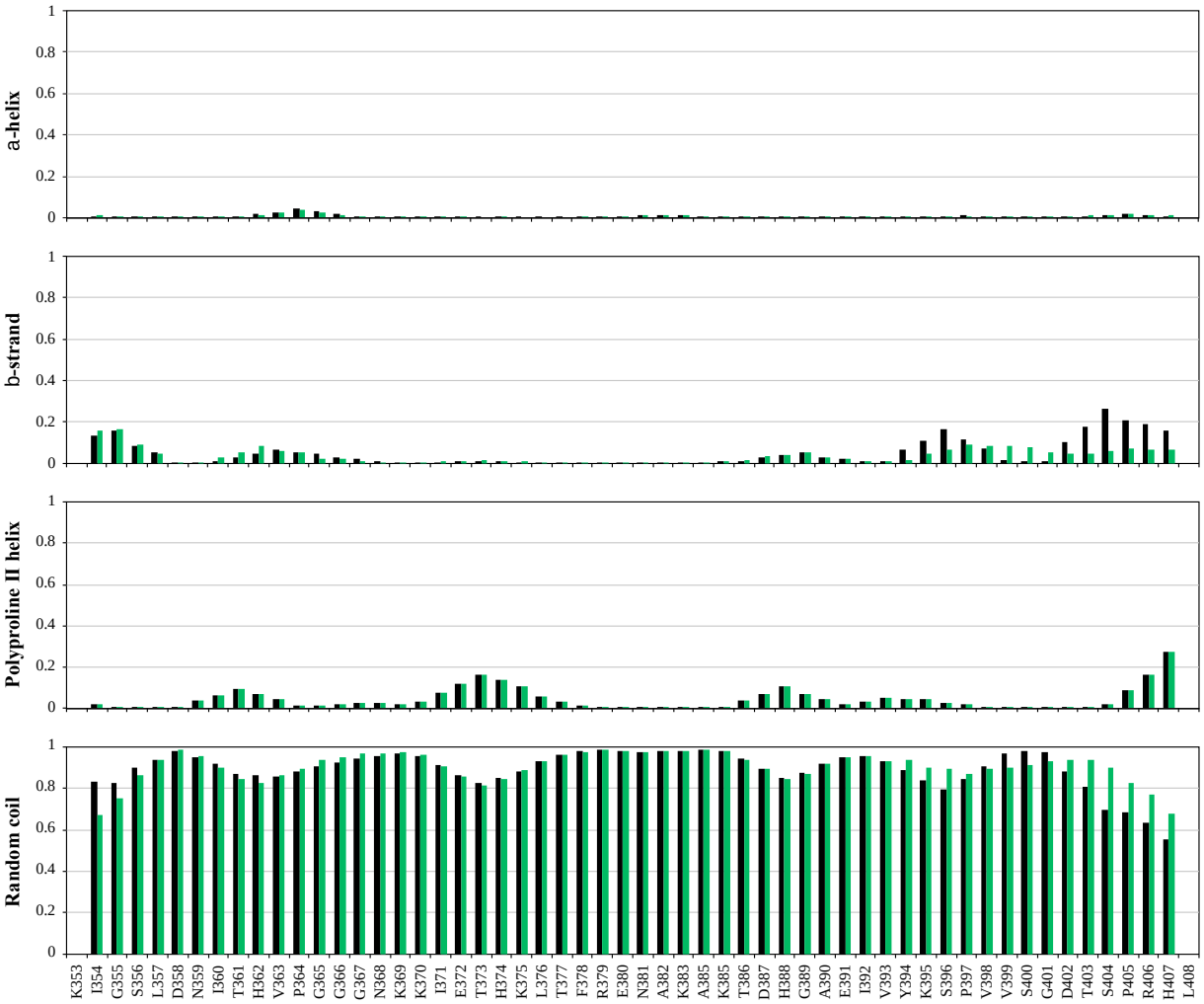

Figure S1. Both ogTau353-408 and cTau353-408 are intrinsically disordered and the *O*-GlcNAc-modification of Ser400 does not induce any persistence secondary structure. Secondary structure propensities for ogTau353-408 (green) and cTau353-408 (black) were calculated from main chain chemical shifts ($^{1}H^{N}$, $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, ^{13}CO, and ^{15}N) using the δ2D algorithm[49]. The small apparent increase in random coil propensities for the C-terminal residues of ogTau353-408 versus cTau353-408 is accompanied by a corresponding decrease in β-strand propensities. However, the δ2D algorithm was not calibrated with modified amino acids, and thus the origin and significance of these predictions is unclear as the terminal residues have very similar chemical shifts.

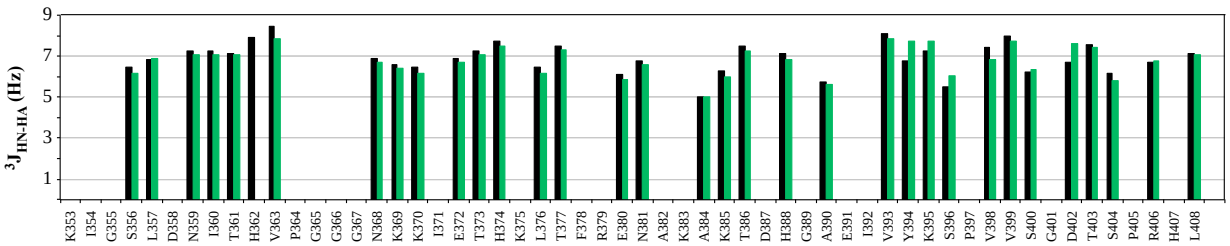

Figure S2. Both ogTau353-408 and cTau353-408 are intrinsically disordered and the *O*-GlcNAc-modification of Ser400 does not induce any persistence secondary structure. Plotted are $^3J_{HN-H\alpha}$ couplings for cTau353-408 (black) and ogTau353-408 (green). Note that the three-bond $^3J_{HN-H\alpha}$ coupling is dependent upon the ϕ dihedral angle. Values around 3-4 Hz are typical for α-helices, values around 9-10 Hz are typical for β-strands, and values around 7 Hz, as observed above, are typical for random coils. The data were measured from a HNHA spectrum[66]. Missing data correspond to glycine and proline residues as well as those residues with overlapping signals.

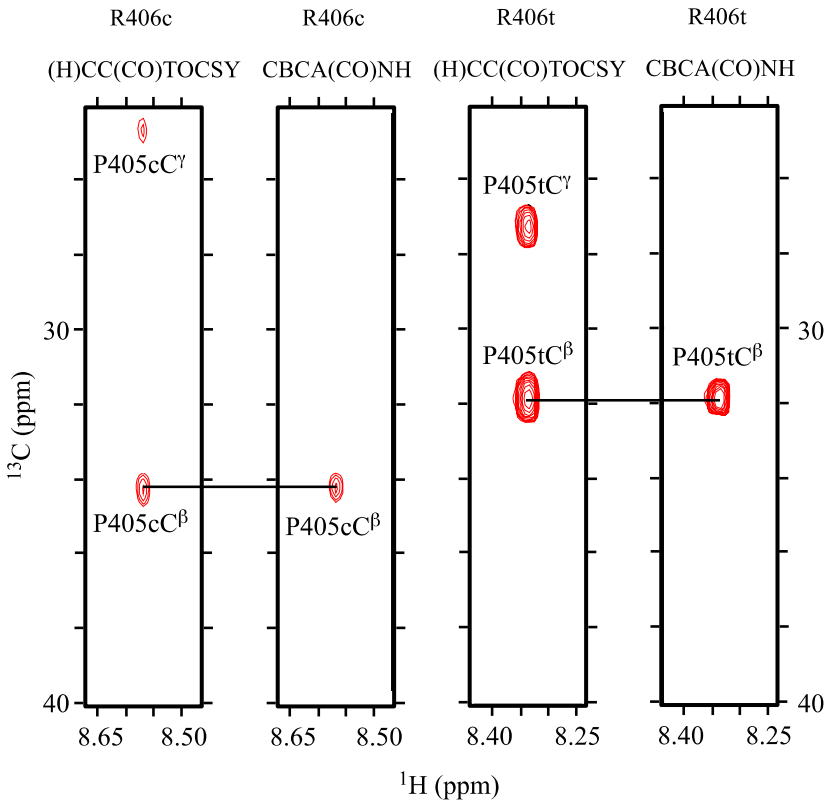

Figure S3. Signals arising from amides perturbed by nearby X-Pro groups in cis or trans conformations are assigned based on diagnostic $^{13}C^{\beta}$ versus $^{13}C^{\gamma}$ chemical shift differences. Shown are selected portions of strip plots taken at the ^{15}N chemical shifts of the major ("R406t") and minor ("R406c") amide peaks of Arg406 detected in the cTau353-408 ^{15}N-HSQC spectrum presented in Figure 6a. These plots show correlations to the $^{13}C^{\beta}$ and $^{13}C^{\gamma}$ ((H)CC(CO)TOCSY-NH) or $^{13}C^{\beta}$ only (CBCACONH) signals of Pro405. A $^{13}C^{\beta}$ versus $^{13}C^{\gamma}$ chemical shift difference of ~ 9 ppm indicates that the R406c peak corresponds to a polypeptide with Ser404-Pro404 in a cis conformation. In contrast, a chemical shift difference of ~ 5 ppm indicates the R406t peak corresponds to a polypeptide with Ser404-Pro404 in a trans conformation[50].

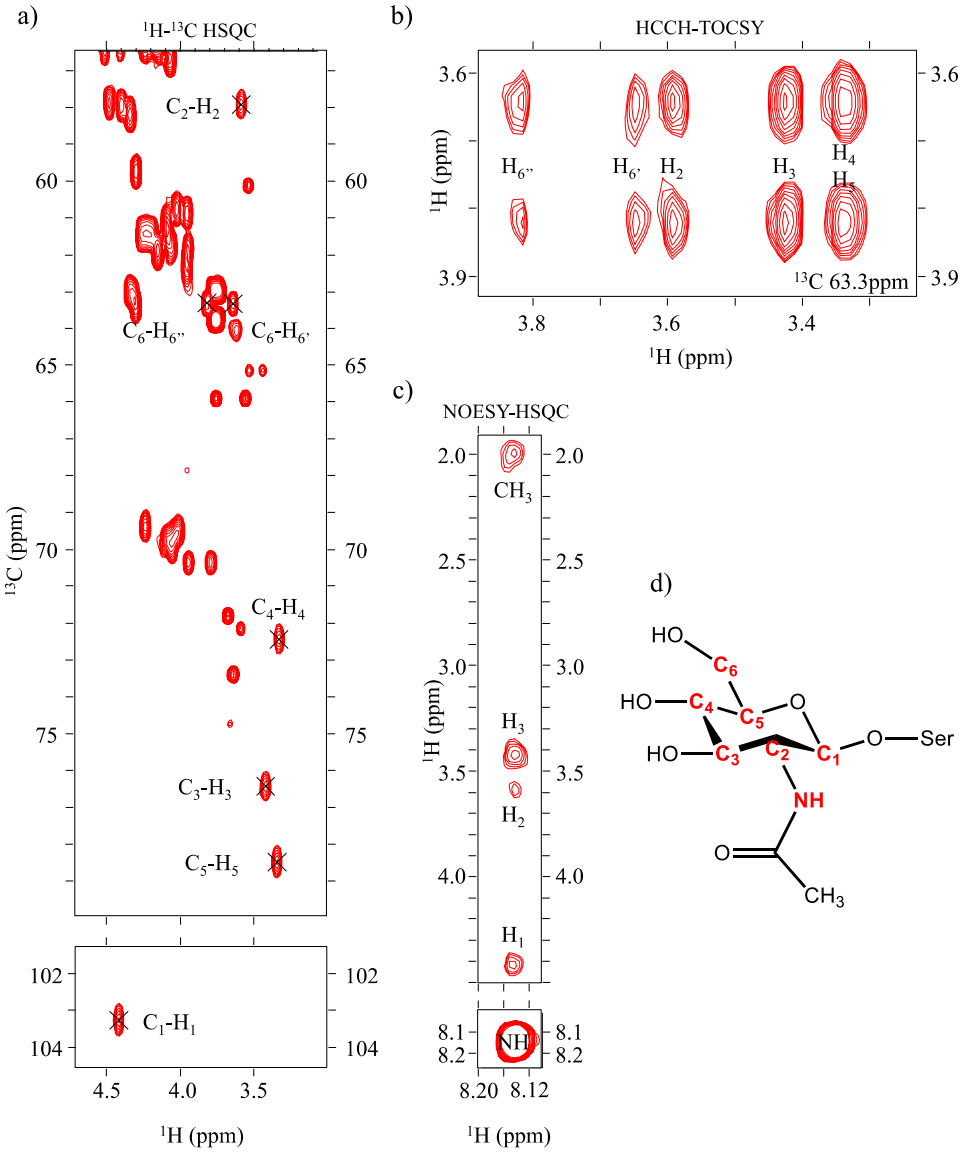

Figure S4: Assignment of *O*-GlcNAc NMR signals. (**a**) ^{13}C-HSQC with $^{13}C_1$ through $^{13}C_6$ assigned. The acetyl methyl signal at 24.8 ppm and 1.9 ppm is not shown. Although many additional peaks are seen in this spectrum, the (**b**) HCCH-TOCSY helped discern them from those of the tau polypeptide due to the distinct ^{13}C chemical shifts of the *O*-GlcNAc residue. These assignments are consistent with those reported for a smaller tau fragment (residues 392-411) without and with *O*-GlcNAc-modification of Ser400[35]. (**c**) The HNH-NOESY-HSQC (τ_{mix}= 200 ms) showing only intra-sugar NOE interactions to the amide. (**d**) Numbering scheme for *O*-GlcNAc.

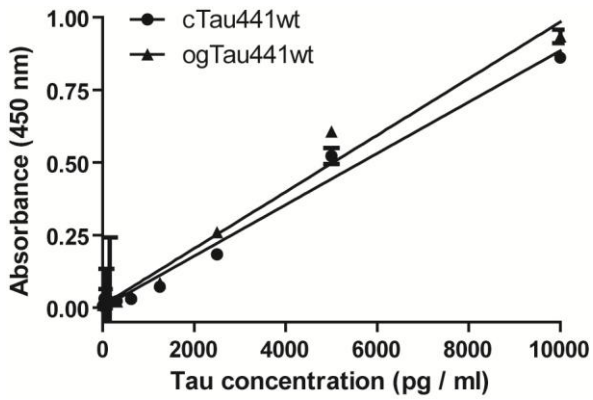

Figure S5. Total tau ELISA of the cTau441wt and the ogTau441wt samples. The total tau ELISA was performed with the $cTau441_{wt}$ and the $ogTau441_{wt}$ samples and shows that a nearly identical response in signal was generated by these samples. This result supports precision of the concentrations determined using the DC assay and indicates the aggregation assays contained equivalent concentrations of $cTau441_{wt}$ and $ogTau441_{wt}$. Analysis of covariance (ANCOVA) indicates that the slopes of the linear regressions shown above are not statistically different ($P = 0.13$).