The small molecule luteolin inhibits *N*-acetyl-α-galactosaminyltransferases and reduces mucin-type *O*-glycosylation of amyloid precursor protein

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Running title: Luteolin inhibits ppGalNAc-Ts

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Key words: *O*-glycosylation, ppGalNAc-T, glycosylation inhibitor, glycoprotein, glycosyltransferase, luteolin, amyloid precursor protein (APP), crystal structure

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Table S1. Data collection and refinement statistics. Values in parentheses refer to the highest resolution shell. Ramachandran plot statistics were determined with PROCHECK.

	ppGalNAc-T2 in complex with the luteolin and UDP/Mn^{+}
Space group	$P2_12_12_1$
Wavelength (Å)	0.97
Resolution (Å)	20-2.30 (2.42-2.30)
Cell dimensions a, b, c (Å) α, β, γ (°)	a = 116.98 b = 123.11 c = 248.15 90, 90, 90
Mn(I) half-set correlation CC (1/2)	0.997 (0.590)
Unique reflections	151903
Completeness	95.5 (99.5)
R_{pim}	0.050 (0.535)
I/σ(I)	10.3 (1.6)
Redundancy	6.7 (6.9)
R_{work} / R_{free}	0.213/0.251
RMSD from ideal geometry, bonds (Å)	0.013
RMSD from ideal geometry, angles (º)	1.710
<B> GalNAc-T2 ($Å^2$)	54.66
<B> UDP ($Å^2$)	49.23
<B> luteolin ($Å^2$)	103.02
<B> solvent ($Å^2$)	44.38
<B> ethylenglycol($Å^2$)	64.17
Ramachandran plot: Most favoured (%) Additionally allowed (%) Disallowed (%)	 96.50 3.03 0.48
PDB ID	5NDF

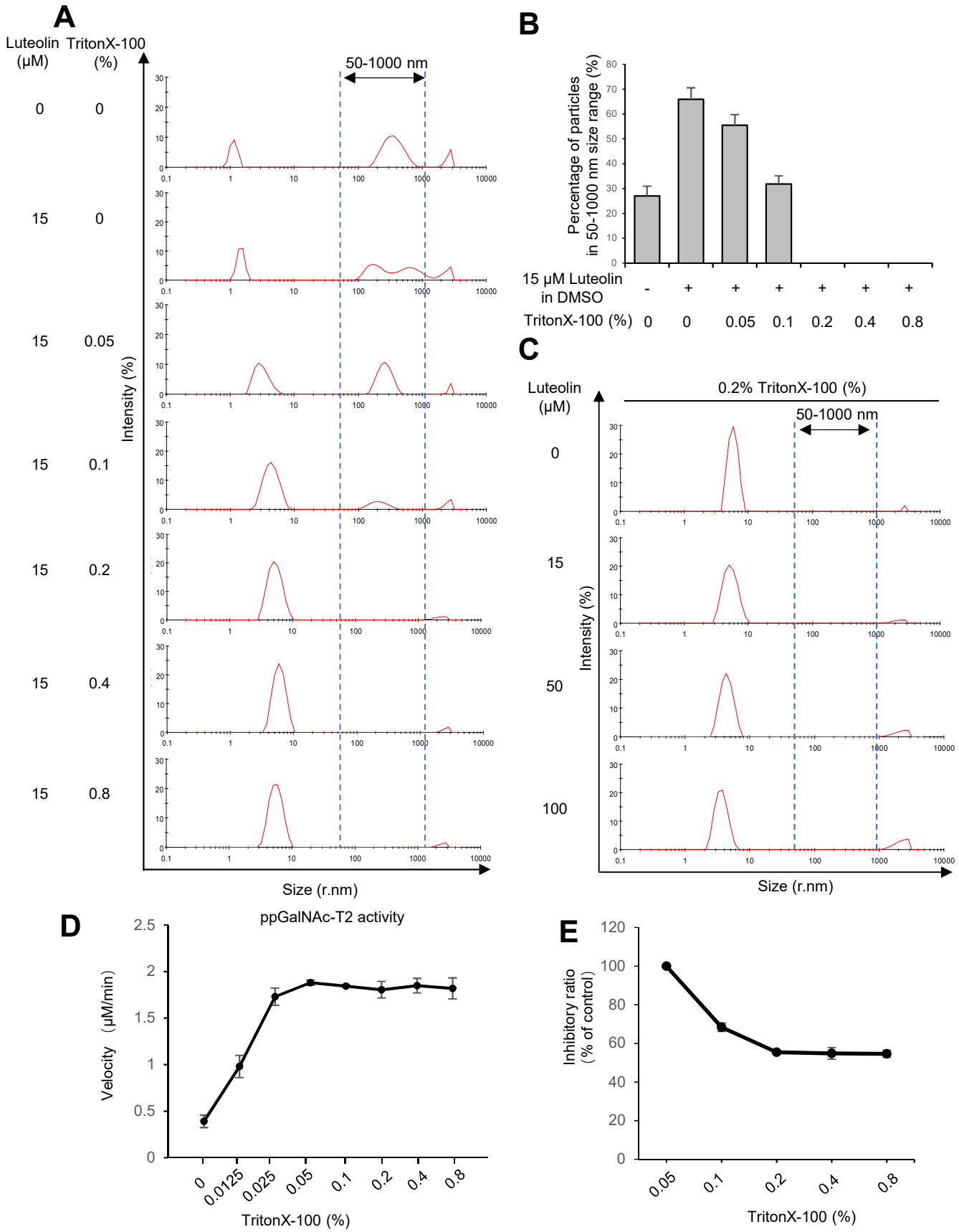

Fig. S1 Aggregation-based activity of luteolin with ppGalNAc-T2. (**A-C**) The particle spectrum change of luteolin in different concentration of TritonX-100 (v/v). The particles spectrum was measured by dynamic light scattering (DLS, Particle Size Analyzer, Z90s, Malvern Instruments Ltd., UK). (**D**) The change of ppGalNAc-T2 catalytic velocity with different concentrations of TritonX-100 (v/v). (**E**) The inhibitory potency of luteolin (15 mM) under the different concentrations of TritonX-100 (v/v). 10 mM EDTA was used as 100% inhibitor control. Data are shown as the mean ± SD (n=3). Two-tailed Student's t-test, **, P<0.01; ***, P<0.001; n.s., no significant difference.

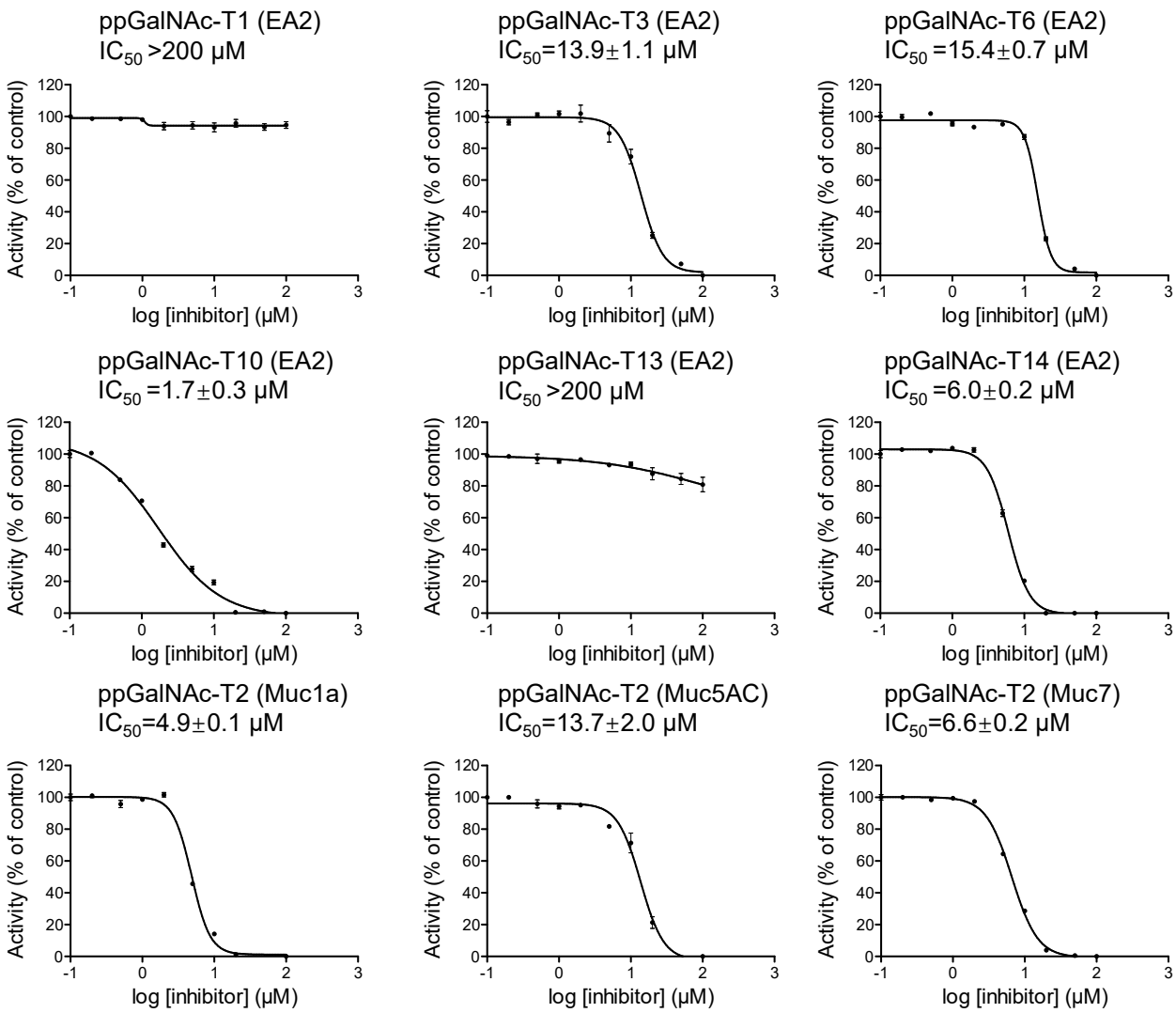

Fig. S2 Dose-dependent inhibition of ppGalNAc-Ts by luteolin in the presence of EA2 substrate and inhibition of ppGalNAc-T2 by luteolin in the presence of various substrates. The IC_{50} values of luteolin for ppGalNAc-Ts were determined under the standard conditions of the HPLC-based assay with EA2, Muc1a, Muc5AC or Muc7 as the substrate (Experimental Procedures). The data are expressed as percentages of the control (DMSO, 100%) and shown as the mean ± SD (n=3).

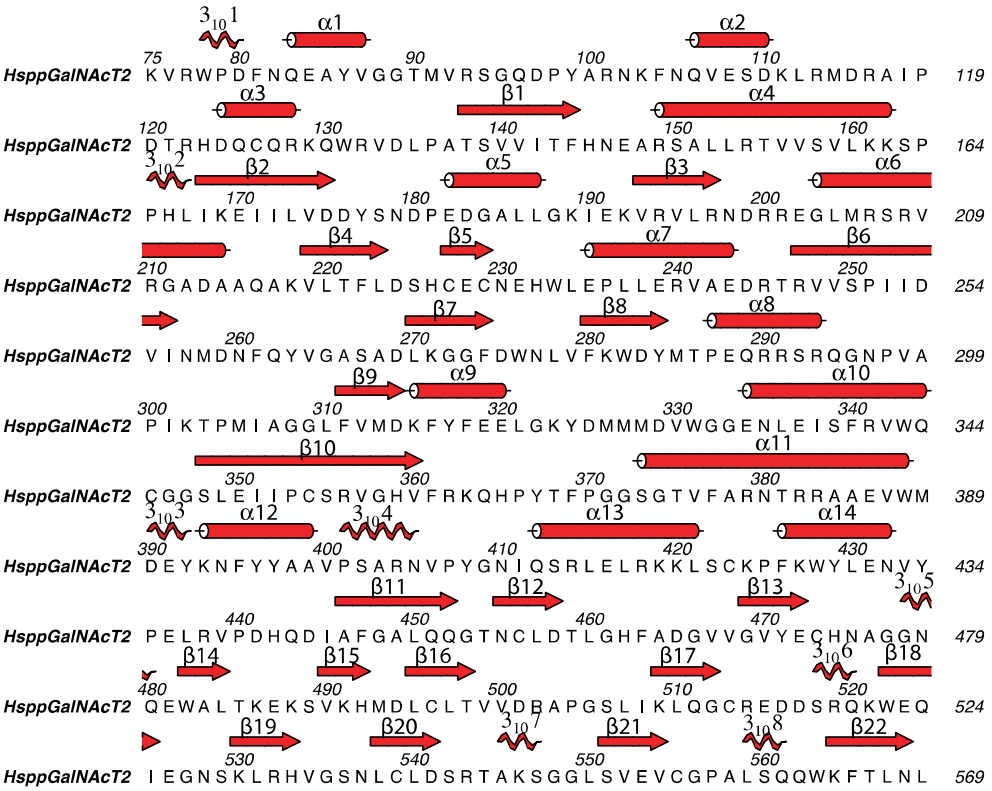

Fig. S3 Sequence of ppGalNAc-T2. Secondary structure elements from the ppGalNAc-T2 structure are shown, with a-helices, 3_{10}-helices and b-strands in red.

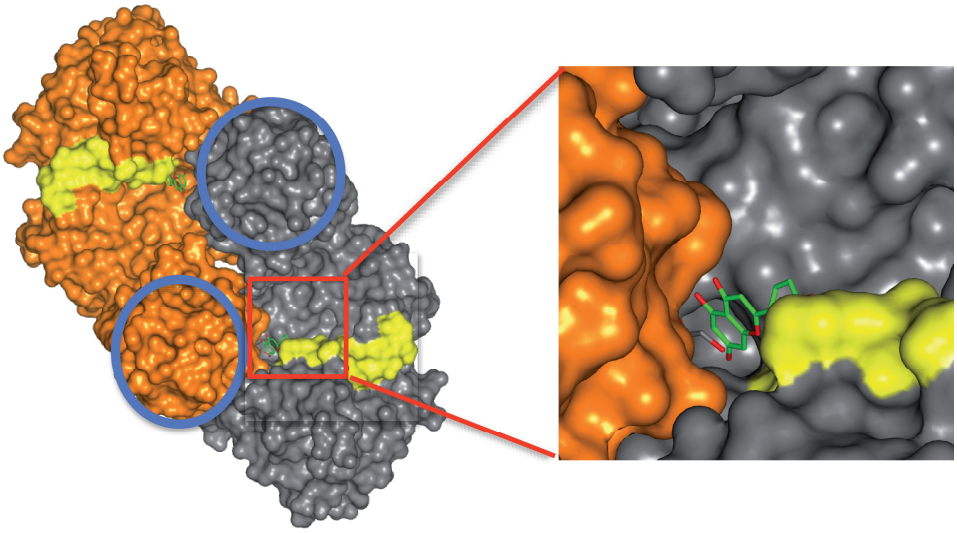

Fig.S4 Surface representation of one of the dimers present in the asymmetric unit. The surface of one of the monomers is depicted in grey whereas the other one is in orange. The flexible loop is shown in yellow in both ppGalNAc-T2 molecules. The two circles in blue and the red square point out the lectin domain position of both molecules, and the position of luteolin in one of the monomers, respectively.

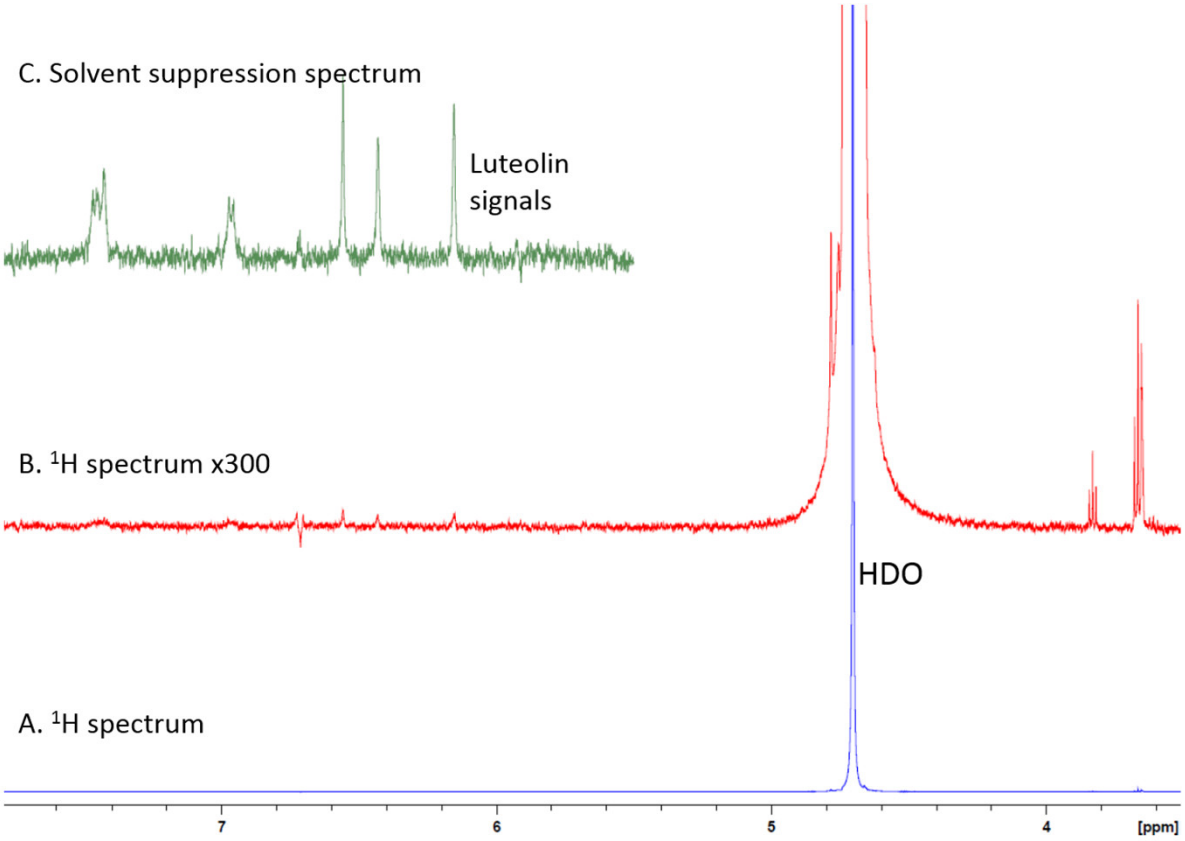

Fig.S5 Stacked ^{1}H NMR spectra of luteolin in deuterated buffer: A. Normal-scale spectrum. HDO is used as standard for chemical shift (4.70 ppm), B. Spectrum with increased intensity (300-fold), where luteolin signals arise, C. Aromatic region of the solvent suppression spectrum.