Supporting Information for *BIOCHEMISTRY* Paper:

Structural Studies of the Transmembrane C-Terminal Domain of the Amyloid Precursor Protein: Does APP Function as a Cholesterol Sensor?[†,‡]

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4 Supporting Figures and Captions

Supporting Figure 1. Comparison of the effects of *bona fide* cholesterol and cholesterol analogs on the TROSY spectra of C99. Depicted are Δδ values (in units of ppm) for C99 in the presence of 5 mol % cholesterol (blue), 4 mol % α-CHOLBIMALT (red), and 4 mol % β-CHOLBIMALT (yellow). The reported chemical shift perturbations represent a composite of the CHOLBIMALT-induced changes in the proton and nitrogen-15 shifts according to the equation given in the Methods. The effects of cholesterol and β-CHOLBIMALT exhibit considerable similarity throughout most regions of C99, suggesting that this derivative is an appropriate surrogate for cholesterol. In contrast, the structural perturbations induced by α-CHOLBIMALT diverge from those induced by cholesterol, suggesting that this particular derivative is not suitable for

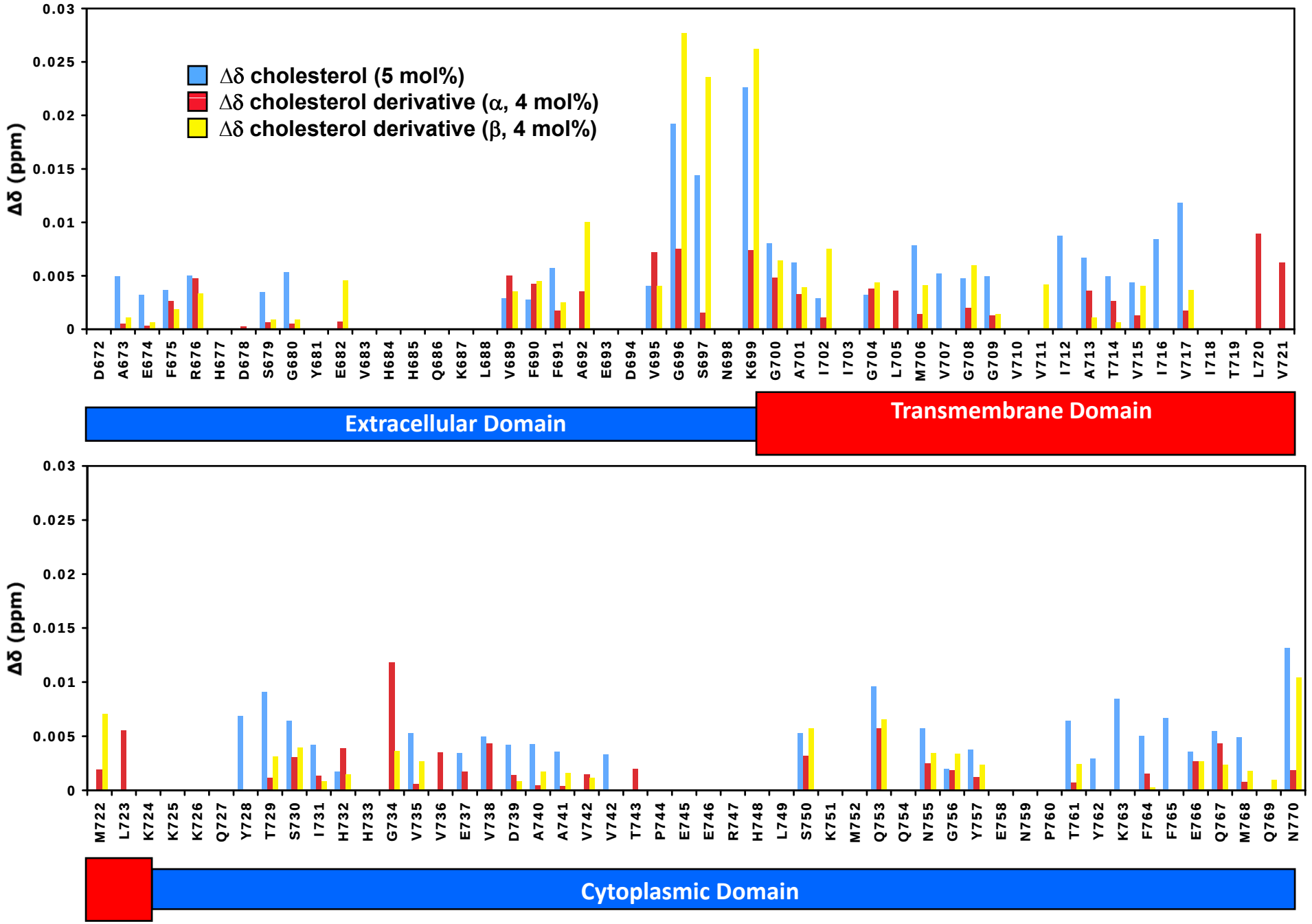
studying the impact of cholesterol on C99 structure. Points lacking data represent unresolvable peaks, unassigned residues, or resonances that disappeared during the course of the titration.

Supporting Figure 2. NMR relaxation data for C99 in the presence of 16 mol% β-CHOLBIMALT. Sample and spectroscopic conditions are the same as described in the caption of Figure 5 except for the presence of the β-CHOBIMALT.

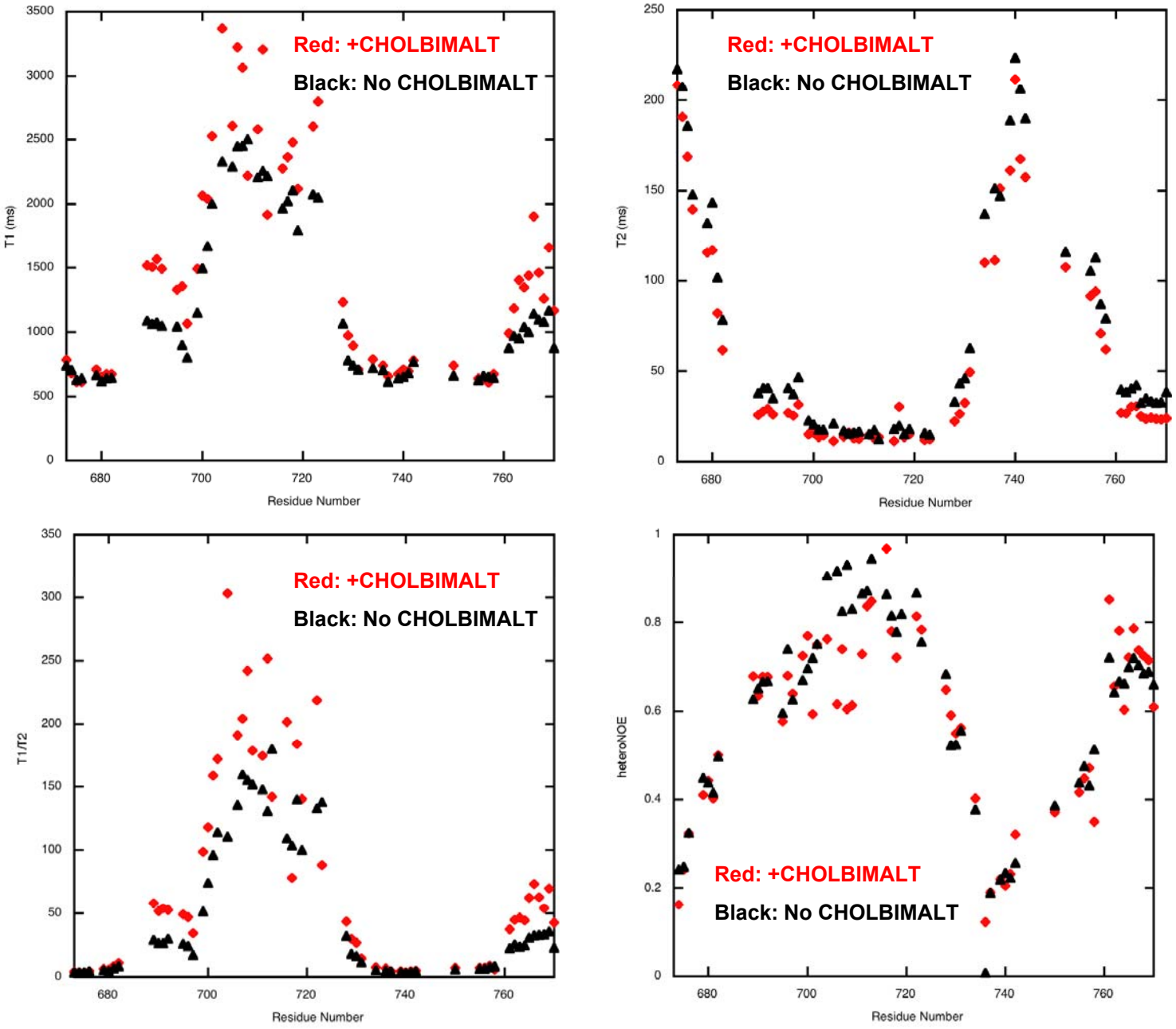
Supporting Figure 3. Comparison of paramagnet-induced site-specific reductions in 800 MHz 1H,15N-TROSY NMR peak intensities for C99 in the absence and presence of 16 mol% β-CHOLBIMALT. The data for the sample where no CHOLBIMALT was present is for the same sample to which solid β-CHOLBIMALT was then added. The sample conditions for the β-CHOLBIMALT-containing sample are similar to the samples described in the caption to Figure 8, except that the LMPG concentration was 9%.

Supporting Figure 4. α-Helical wheel diagrams of the ten C-terminal residues of C99 alone (left) or of the final ten residues of C99 plus the first eight residues of the C-terminal purification tag (right). These diagrams show that the final ten residues of C99 map very well as an amphipathic helix, whereas the first eight residues of the tag do not. These plots were generated at an online server: “Helical Wheel Viewer” at the “Interactive Biochemistry” web site:
http://cti.itc.virginia.edu/~cmg/Demo/scriptFrame.html
(E. K. O'Neil and C. M. Grisham at the University of Virginia in Charlottesville, Virginia).

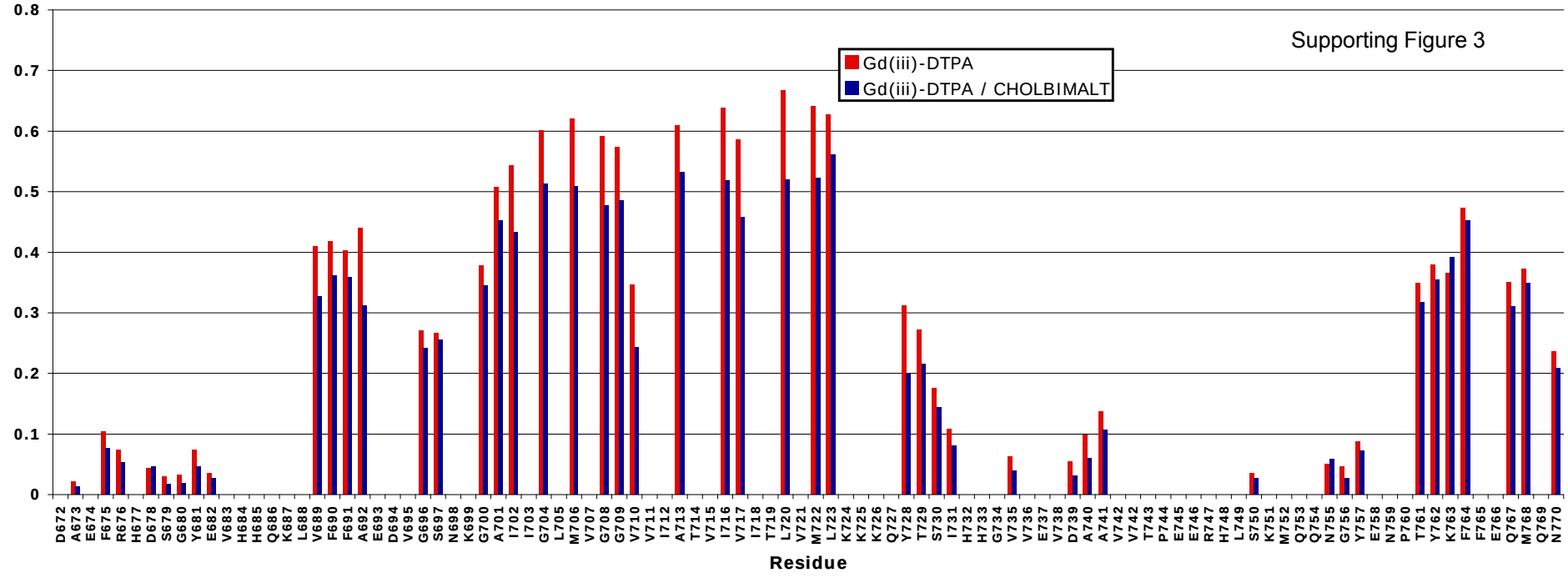
Supporting Figure 1

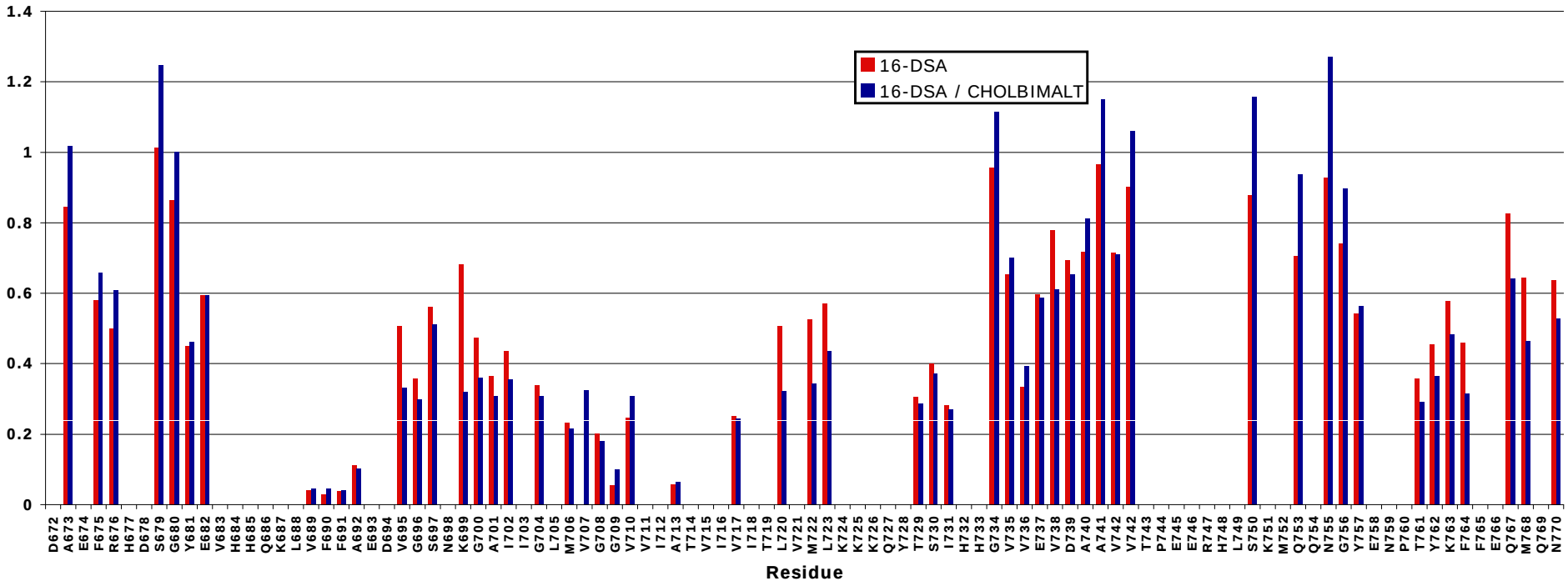


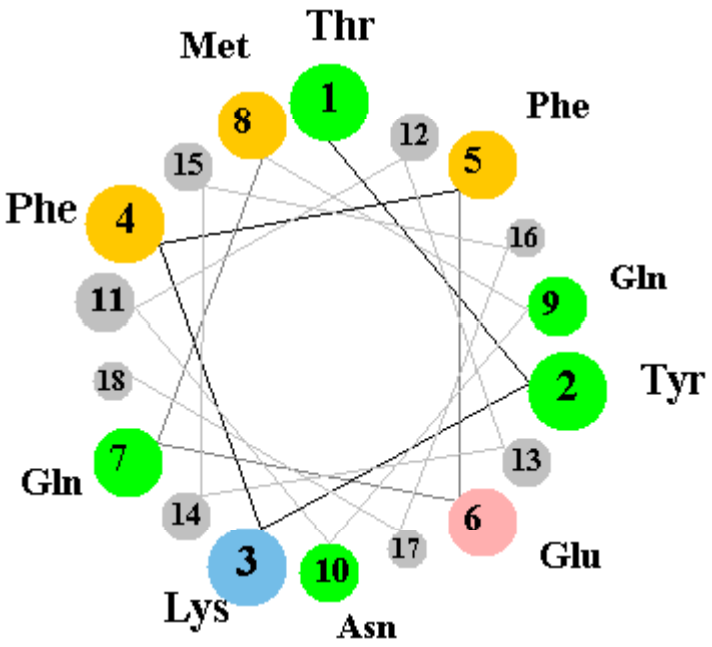
Supporting Figure 2



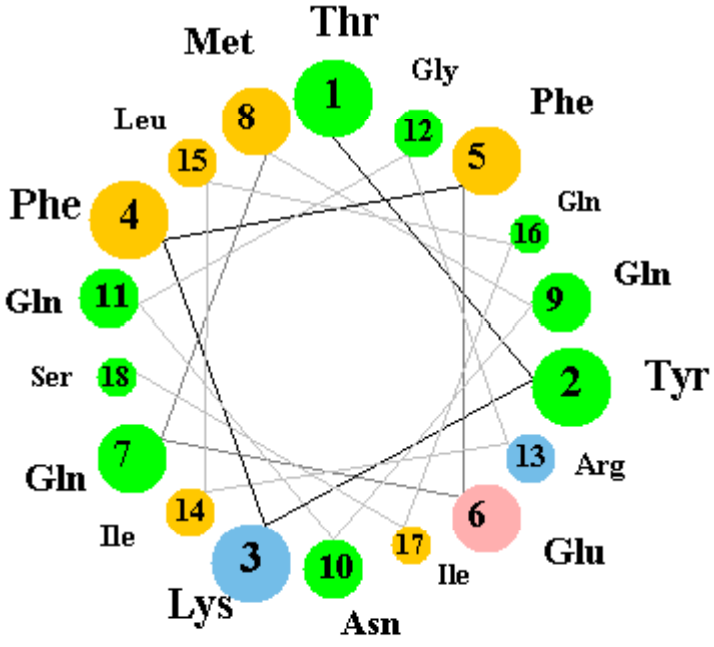
Supporting Figure 3







Wheel for sites 761-770 of APP Thr1 in the wheel is Thr761, Asn10 in the wheel is Asn770, and so forth.



Wheel for sites 761-770 of APP plus the first 8 amino acids of the C-terminal purification linker/tag. Again Thr1 in the wheel is Thr761.