Supporting Information for World Wide Web Edition:

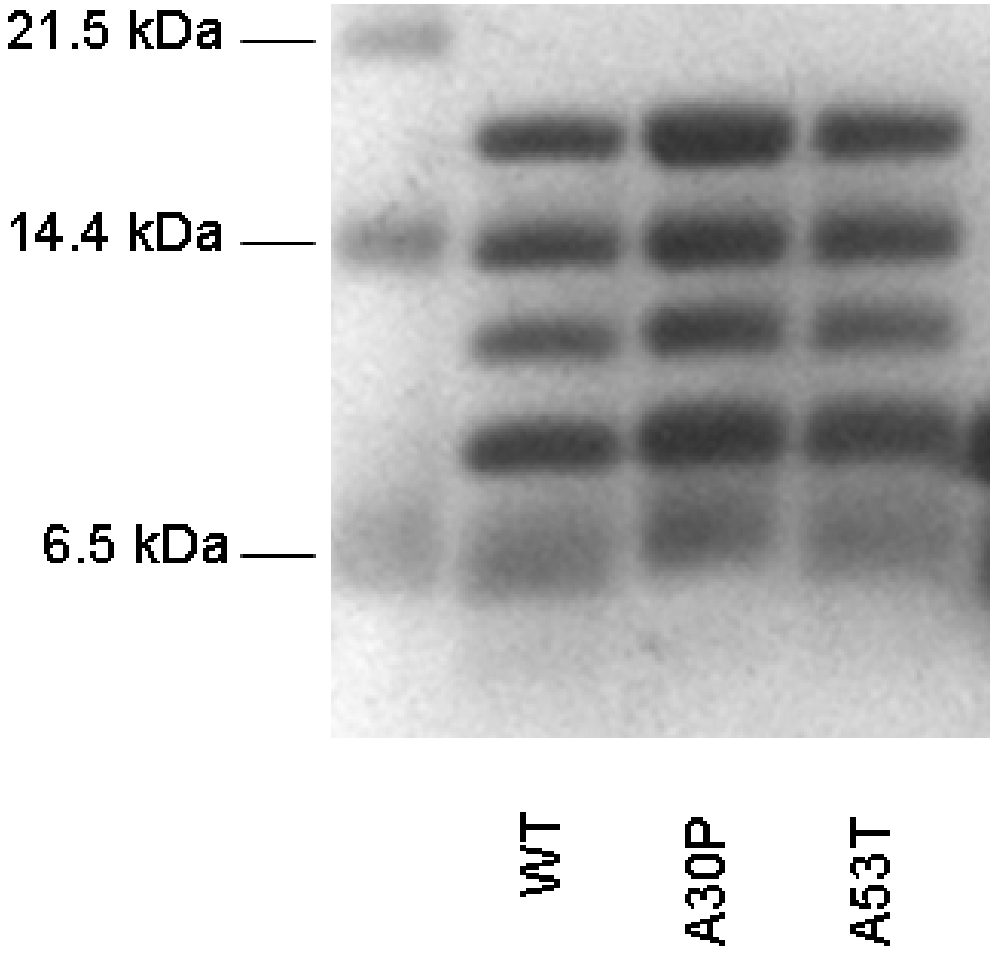

Figure S1: SDS-PAGE monitored proteolytic digestion of SDS micelle bound wild type, A30P and A53T αS after 45 minutes at a protease to protein mass ratio of 1:1000. A labeled molecular weight standard is included for reference.

Expected concentration dependence of free and bound protein concentrations:

For two interacting species, A and B, at equilibrium, the concentrations of free and bound species A (or if desired B) can be determined from the relationships describing equilibrium and the conservation of the total amount of A and of B.

1) $Kd = [A][B]/[AB]$

2) $[A] + [AB] =$ constant (i.e. total concentration of A)

3) $[B] + [AB] =$ constant (i.e. total concentration of B)

Using these relationships, the fraction of bound A can be plotted as a function of the total concentration of A for a given total concentration of B and equilibrium constant Kd. For tight binding, the bound fraction of A will remain very nearly 1 until the total concentrations of A and B are almost equal, after which the concentration of bound A will fall off in a non-linear fashion. For weaker binding, the bound fraction of A will start at $1/(1+Kd/[B])$, and will fall off immediately in a non-linear fashion. These two cases are illustrated in Figures S2 and S3 below.

For αS the interaction with SDS micelles is strong, and the behavior we observe is consistent with Figure S2: all of the protein is in the micelle bound state over a range of protein concentrations, and we only observe proteolysis of the bound state. In contrast, the interaction of αS with lipid vesicles is weaker, and we observe behavior more consistent with Figure S3. We see complete or nearly complete binding only at low protein concentrations, with a decrease of the bound protein fraction as protein concentration increases (CD data, not shown), and we consistently observe that

proteolysis occurs via the free state. According to this analysis, the exact fractions of bound and unbound αS will be highly sensitive to both total αS concentration and to total lipid concentration. Since both and protein and lipid concentration determinations are subject to significant errors, our quantitative comparisons of the populations of lipid free αS must be considered estimates. Nevertheless, qualitatively, our results consistently show that the affinity of αS for different lipid compositions decreases in the order POPA, POPS/POPC or POPA/POPC, and POPC, and that the A30P mutant displays significantly lower affinity for POPA/POPC vesicles than the wild type or A53T mutant proteins.

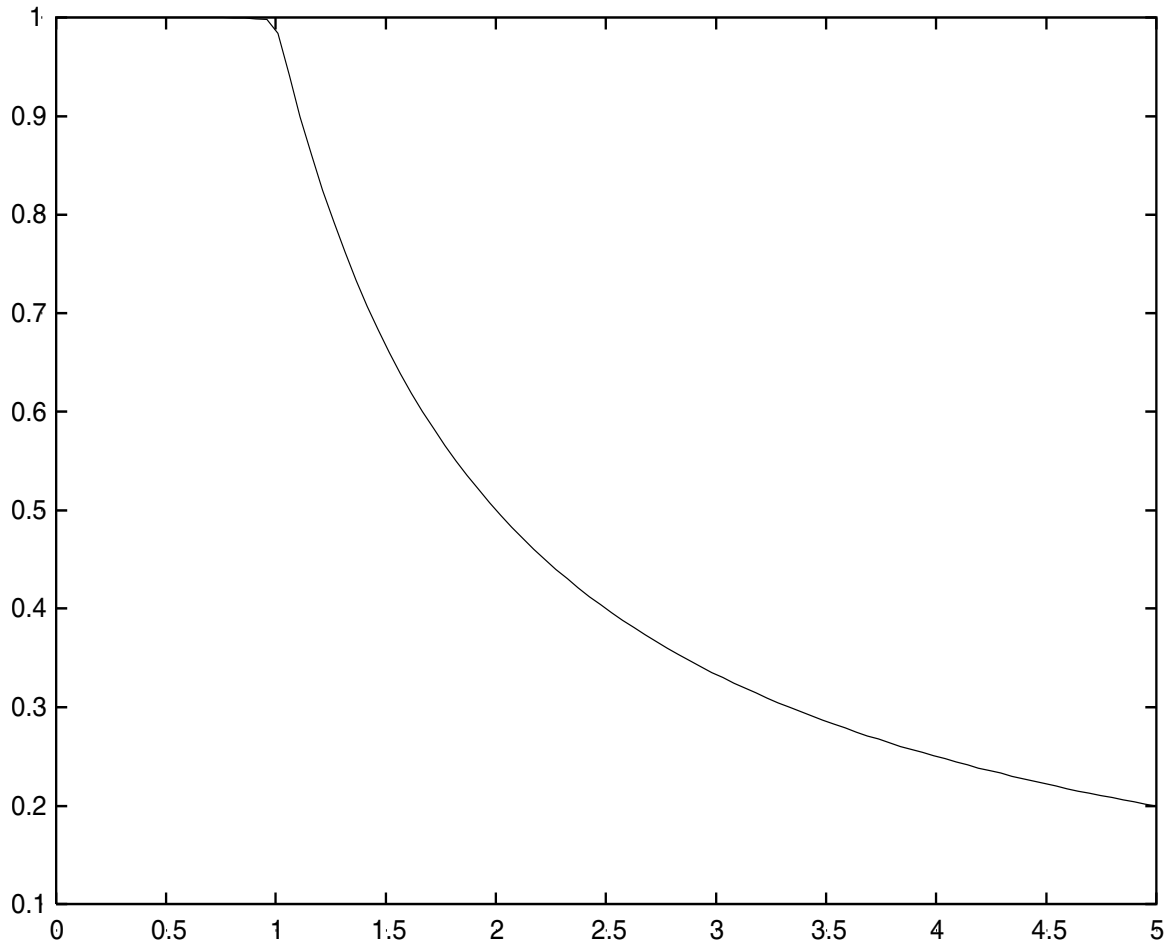

Figure S2: Bound fraction of A as a function of total A concentration using Kd = 0.0001 and total B concentration = 1 in arbitrary units.

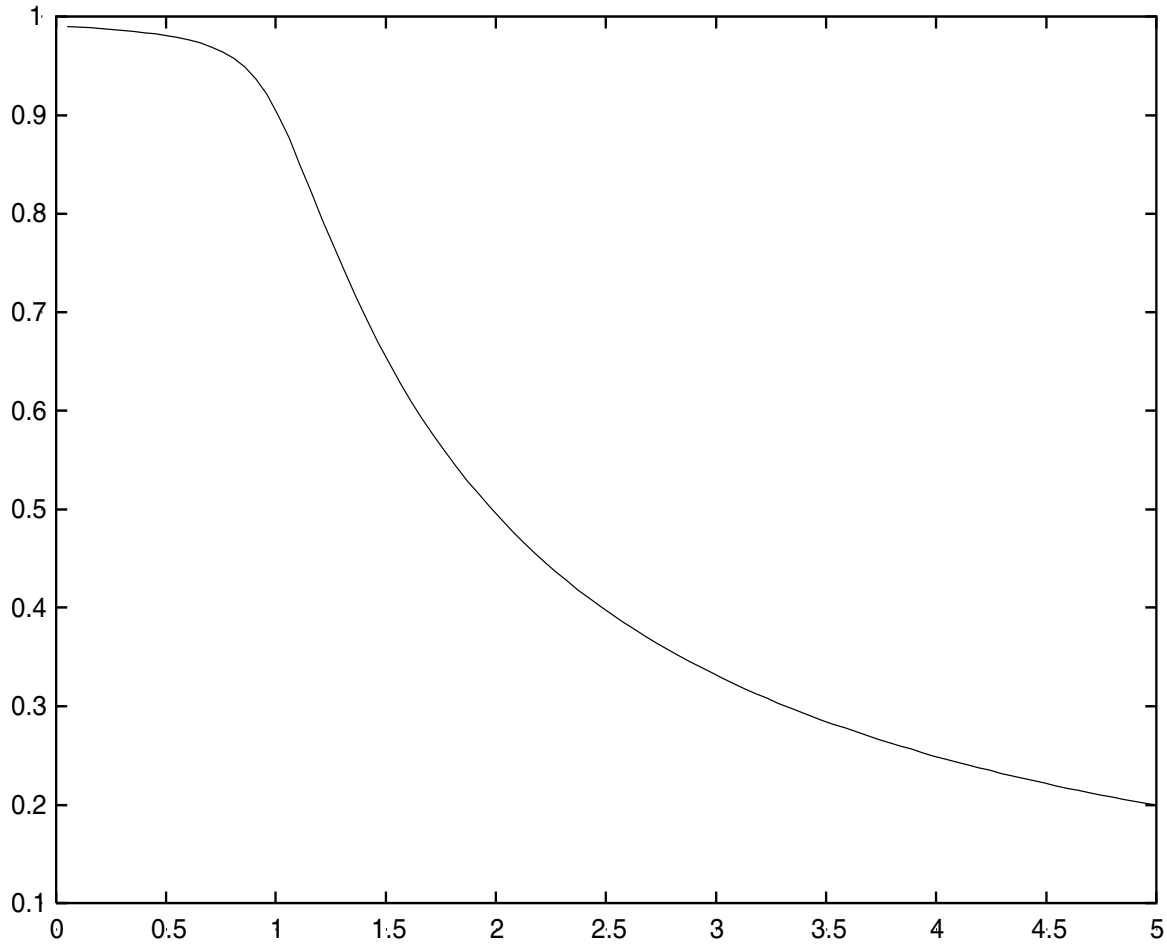

Figure S3: Bound fraction of A as a function of total A concentration, using Kd = 0.01 and total B concentration = 1 in arbitrary units.