Supporting Information for *Biochemistry* paper:

Purification and Characterization of the Human γ-Secretase Activating Protein[†]

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Supporting Table 1. Summary of Attempts to Refold Non-Immobilized GSAP

Experiment	Method	Outcome
Dialysis-Based Refolding	After solubilization of IB by SDS and 8M urea, sample was dialyzed against decreasing amounts of denaturant at pH 7.8.	GSAP precipitated out of solution during dialysis in 0M urea.
Dialysis-based refolding after purification in denaturant	IBs were solubilized in 8M urea overnight, followed by metal ion affinity chromatography-based purification of GSAP in the presence of 8M urea. After purification, the denatured protein was dialyzed against successively decreasing amounts of urea. This was performed in different buffer conditions at pH 5.5, 6.5 and 7.8.	Purified GSAP eluted cleanly from the nickel column and was soluble indefinitely in 8M urea. Protein began to precipitate during the 4M urea dialysis step, ending with a total loss of GSAP due to precipitation by the final 0M urea stage.
Rapid dilution refolding after purification	IB was solubilized in 8M urea overnight, then purified as usual in 8M urea. After purification, the urea-denatured protein was slowly added drop-wise into a large volume of rapidly stirring buffer containing 50 mM HEPES, 300 mM NaCl, and 400 mM L-Arginine, pH 7.8.	Protocol initially seemed to be effective; there was no visible precipitation in the large rapidly stirring volume. However, when sample concentration was initiated through both centrifugal and stirred cell concentration the protein catastrophically precipitated from solution.
Refolding after purification in detergent	After solubilization of IB by SDS and 8M urea, sample was bound to column and purified in 0.2% SDS. SDS was then dialyzed out of solution at pH 7.8	The protein precipitated out of solution as SDS was fully dialyzed out of the sample.

Supporting Table 2: Attempts to Refold GSAP on Column

Experiment	Method	Outcome
Refolding on the column by urea removal	After solubilization of IB by SDS and 8M urea, sample was bound to column and refolded with 15% glycerol and decreasing amounts denaturant on the column.	GSAP could not be eluted from the resin after all of the denaturant had been rinsed away.
Purification with DM	IB solubilized with Empigen, bound to the metal ion affinity resin, followed by equilibration and attempted elution with a solution containing the mild non-ionic detergent, β-decylmaltoside.	GSAP failed to elute from column with 250 mM imidazole and 0.5% DM at pH 7.8.
Purification without detergent	IB were solubilized with Empigen, followed by binding of GSAP to metal ion affinity resin and successive re-equilibration with DPC and DM solutions, with a detergent free solution, followed by attempted elution of the protein using a detergent-free buffer.	GSAP failed to elute from column upon attempted elution using 250 mM imidazole, pH 7.8.