

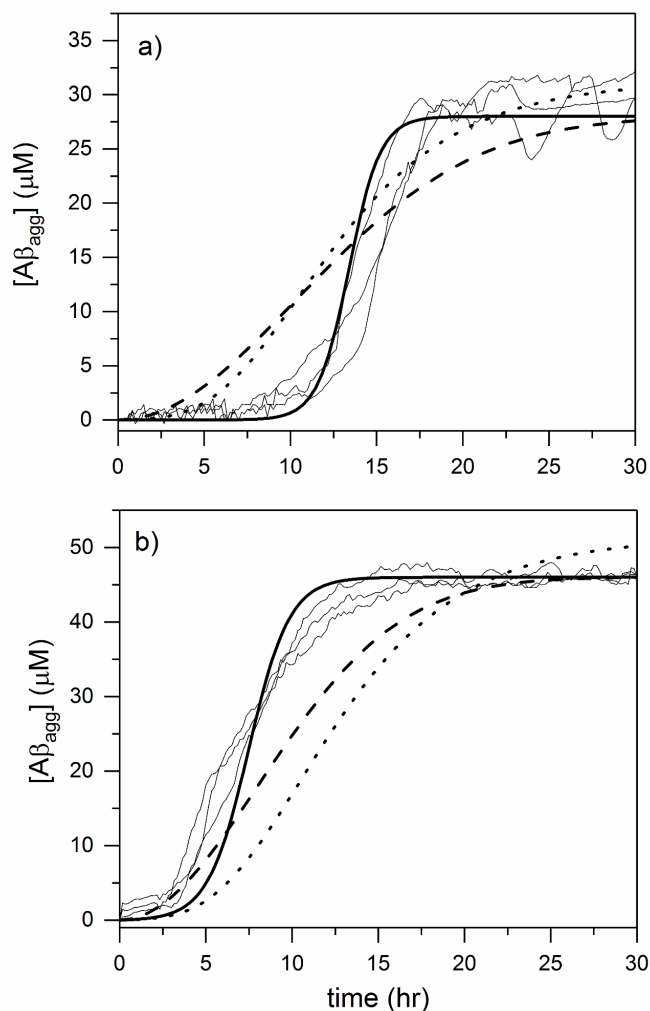
Supplemental Data

Inhibition of Aggregation or of Degradation: Cystatin C's Competing Effects on β -Amyloid

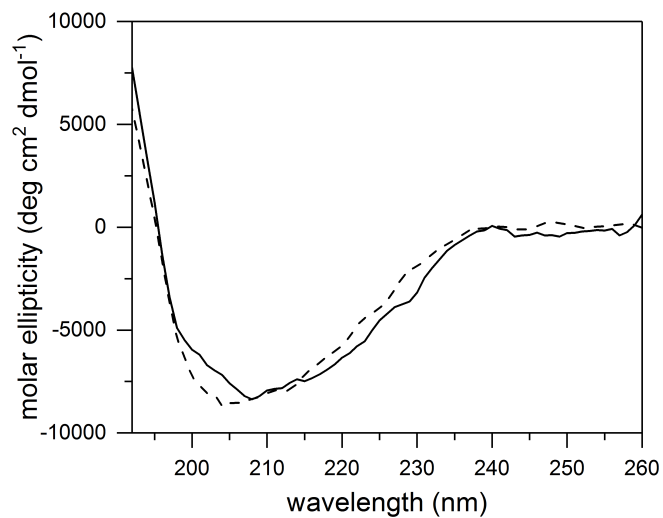
Tyler J. Perlenfein, Regina M. Murphy

This section contains the following supplementary data:

- Supplemental Figure S1: Fitting of $A\beta_{40}$ aggregation data to multiple aggregation models to determine best fit
- Supplemental Figure S2: CD spectra of wt and W106G CysC
- Supplemental Figure S3: CatB inhibitory activity of wt and W106G CysC
- Supplemental Figure S4: CatB degradation of CysC by dot blot
- Justification for neglecting CatB bound to $A\beta$ in material balance
- Derivation of $t_{50,agg}$ and $t_{50,deg}$



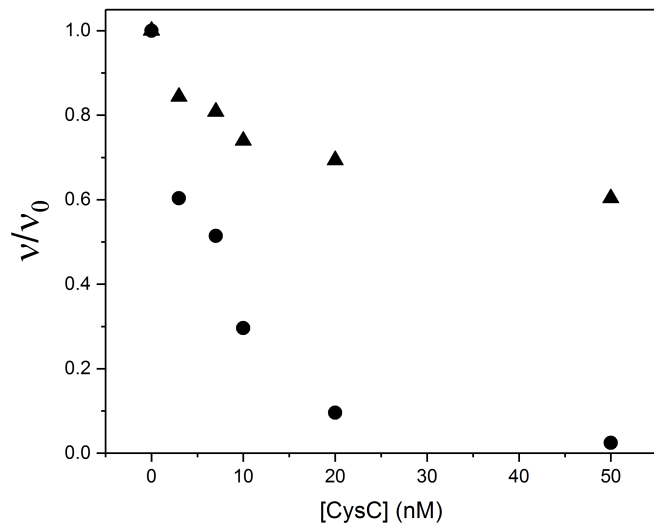
Supplemental Figure S1. Aggregation model choice by fit to $A\beta_{40}$ aggregation data. ThT fluorescence data were converted to equivalent aggregated $A\beta_{40}$ concentration by the empirically-determined quadratic equation (see main text). Autocatalytic model (solid line), monomer partitioning model (dashed line), and the Gompertz equation (dotted line) were globally fit to aggregation data including initial $[A\beta_{40}]_0 = 28$ and $46 \mu\text{M}$ to determine model parameters. Data (thin lines) are shown compared to model fits (thick lines) for (a) $[A\beta_{40}]_0 = 28$ and (b) $[A\beta_{40}]_0 = 46 \mu\text{M}$.



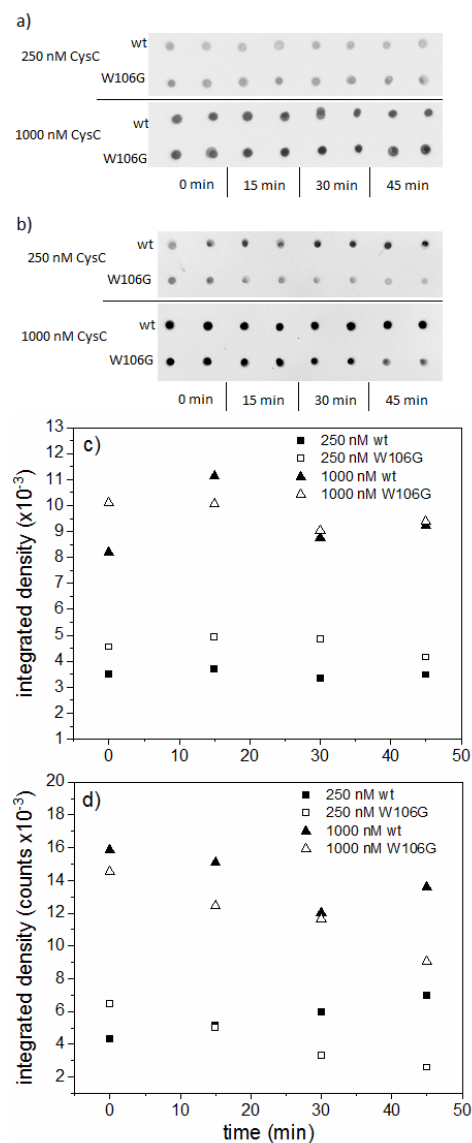
Supplemental Figure S2. CD spectra of wt CysC (solid line) and W106G (dashed line). Protein samples (20 μ M in PBSA-E) were dialyzed extensively into 10 mM phosphate, 50 mM NaF, pH 7.4.

Measurements of CD spectra were made on an Aviv model 420 and buffer background was subtracted.

Data shown is the average of three scans.



Supplemental Figure S3. CatB inhibitory activity of wt CysC (●) and W106G (▲). CysC and activated CatB were mixed to concentrations of 40 nM CatB and 0-100 nM CysC and incubated for 10 minutes at 37°C. Fluorogenic substrate Z-FR-AMC (200 μ M) was added to each sample to final concentrations of 20 nM CatB, 100 μ M Z-FR-AMC, and 0-50 nM CysC. Sample fluorescence was continuously monitored in a BioTek FL_x-800 plate reader for up to ten minutes. Initial rate of fluorescence increase, v , was determined for each sample and normalized to initial rate without CysC present, v_0 . Each sample shown is the average of two independent samples.



Supplemental Figure S4. CatB degradation of CysC. (a) wt and W106G CysC were incubated with 200 nM CatB for up to one hour at 37°C in pH 7.4 PBS. Samples were taken every 15 minutes and dotted onto a membrane. Membranes were probed for intact CysC using a C-terminal-specific CysC antibody (Cyst13, Novus). (b) Degradation experiments were repeated in pH 6.0 MES buffer. Integrated dot intensities (average of two dots) are shown for pH 7.4 (c) and pH 6.0 (d).

Justification for neglecting CatB bound to A β in material balance

Total CatB is distributed between free, bound to CysC, and bound to A β . This is expressed by the material balance equation:

$$[CatB]_0 = [CatB] + [CatB \cdot CysC] + [CatB \cdot A\beta]$$

We assumed that $[CatB] \gg [CatB \cdot A\beta]$ and that the last term in the material balance equation can be neglected. This assumption is justified from our experimental data as well as data in the literature. For Michaelis-Menten kinetics:

$$\frac{d[P]}{dt} = k_{cat}[ES] = \frac{k_{cat}[E]_{tot}[S]}{K_M + [S]}$$

where

$$K_M = \frac{[E][S]}{[E \cdot S]}$$

and

$$[E]_{tot} = [E] + [E \cdot S] = [E] \left(1 + \frac{[S]}{K_M} \right)$$

If $K_M \gg [S]$, then

$$\frac{d[P]}{dt} \cong \frac{k_{cat}[E]_{tot}[S]}{K_M}$$

and the rate of reaction is linear in substrate concentration. Furthermore,

$$[E]_{tot} \cong [E] \text{ or } [E] \gg [E \cdot S]$$

From Figure 5b, the reaction rate is linear in $[A\beta]$ so we conclude that $K_M \gg 1 \mu\text{M}$ and $[CatB] \gg [CatB \cdot A\beta]$.

Some experiments (e.g., Figure 7) were done at higher substrate concentrations (10 and 28 μM), and the assumption was still made that $K_M \gg [S]$ and $[CatB] \gg [CatB \cdot A\beta]$. Literature values for K_M of catB at

pH 7 are consistent with this assumption. For example, Moin et al. (1992) reports K_M of 600-900 μM for catB degradation of dipeptides, while Hasnain et al. (1993) reports K_M of 120-1000 μM for Arg-Phe or Phe-Phe dipeptides. While mass spectrometry analysis of catB degradation of A β has been described elsewhere (Mueller-Steiner et al., 2006), we are not aware of any reports of K_M for A β .

References:

Moin, K., Day, N.A., Sameni, M., Hasnain, S., Hiramata, T., and Sloane, B.F. (1992) Human tumor cathepsin B. Comparison with normal liver cathepsin B. *Biochem. J.* **285**, 427-434.

Hasnain, S., Hiramata, T., Huber, C. P., Mason, P., and Mort, J. S. (1993) Characterization of cathepsin B specificity by site-directed mutagenesis. *J. Biol. Chem.* **268**, 235-240.

Mueller-Steiner, S., Zhou, Y., Arai, H., Roberson, E. D., Sun, B., Chen, J., Wang, X., Yu, G., Esposito, L., Mucke, L., and Gan, L. (2006) Anti-amyloidogenic and neuroprotective functions of cathepsin B: implications for Alzheimer's disease. *Neuron*. **51**, 703–714

Derivation of $t_{50,deg}$ and $t_{50,agg}$

The autocatalytic model used to describe aggregation kinetics has an analytical solution (Eq. 2).

At $t_{50,agg}$, half the A β is aggregated and in the absence of any degradation:

$$0.5 = \frac{(k_1 + k_2[A\beta]_0) \exp[-(k_1 + k_2[A\beta]_0)t_{50,agg}]}{k_2[A\beta]_0 \exp[-(k_1 + k_2[A\beta]_0)t_{50,agg}] + k_1}$$

Algebraic manipulation produces Eq. 12, the half-time for aggregation in the absence of any CatB or CysC:

$$t_{50,agg} = \frac{\ln\left(\frac{2k_1 + k_2[A\beta]_0}{k_1}\right)}{k_1 + k_2[A\beta]_0}$$

Degradation of A β by CatB in the absence of any aggregation is modeled as

$$\frac{d[A\beta]}{dt} = -k_{deg}[A\beta][CatB]$$

Making the approximation that $[CatB] \sim \text{constant}$, this equation can be integrated from $t = 0$ to $t = t_{50,deg}$

$$\ln(0.5) = -k_{deg}[CatB]t_{50,deg}$$

or

$$t_{50,deg} \approx \frac{\ln(2)}{k_{deg}[CatB]}$$

(Eq. S1)

Free (active) CatB concentration depends on CysC concentration (Eq. 3 in main text):

$$[CatB] = [CatB]_0 - \frac{[CysC][CatB]}{K_I}$$

(Eq. S2)

Re-arranging yields

$$[CatB] = [CatB]_0 \frac{K_I}{K_I + [CysC]}$$

(Eq. S3)

Combining Eq. S1 and S3 gives:

$$t_{50,deg} \approx \frac{\ln(2)(K_I + [CysC])}{k_{deg}K_I[CatB]_0}$$

(Eq. S4)

Now we need to solve for $[CysC]$, which depends on CatB and A β concentrations (Eq. 4 in main text):

$$[CysC] = [CysC]_0 - \frac{[CysC][A\beta]}{K_D} - \frac{[CysC][CatB]}{K_I}$$

(Eq. S5)

and considering only the A β bound to CysC (neglecting the loss of A β by degradation or aggregation)

$$[A\beta] = [A\beta]_0 - \frac{[CysC][A\beta]}{K_D}$$

(Eq. S6)

Eqs S2, S5 and S6 can be combined to yield a cubic equation:

$$\begin{aligned} & [CysC]^3 + [CysC]^2[K_D + K_I + [CatB]_0 + [A\beta]_0 - [CysC]_0] \\ & + [CysC][K_DK_I + K_I[A\beta]_0 + K_D[CatB]_0 - K_D[CysC]_0 - K_I[CysC]_0] - K_DK_I[CysC]_0 \\ & = 0 \end{aligned}$$

(Eq. S7)

Eq. S7 can be solved for $[CysC]$ using the trigonometric method for cubic equations (or an implicit equation solver), if a more accurate estimate of $[CysC]$ is desired in Eq. S4.

However, to simplify further, we return to Eq. S5. The second term on the rhs is CysC bound to A β , and the third term on the rhs is CysC bound to CatB. We note that $K_D \gg K_I$, so if $[A\beta] \sim [CatB]$, which is physiologically reasonable, then $\frac{[CysC][A\beta]}{K_D} \ll \frac{[CysC][CatB]}{K_I}$. We also note that the binding affinity for CysC and CatB (K_I) is strong, and that typically $[CysC] > [CatB]$, so the maximum amount of CysC that could be bound to CatB is $[CatB]_0$. With these approximations, Eq S5 simplifies to

$$[CysC] \approx [CysC]_0 - [CatB]_0 \tag{Eq. S8}$$

Plugging Eq S8 into Eq. S4 yields

$$t_{50,deg} \approx \frac{\ln(2)(K_I + [CysC]_0 - [CatB]_0)}{k_{deg}K_I[CatB]_0}$$

which is Eq. 13 in the main text.