

In the Presenilin General Hypothesis a progressive enzymatic inhibition process is presented, in which a single active site (belonging to the gamma secretase monomeric complex) optionally receives the same substrate from two different sites or channels. When only two products were formed, it is assumed to be A β 40 and A β 42 because 2 channels gave rise to 2 products.

For this purpose an adaptation of the Michaelis-Menten equation were used with the following considerations and/or simplifications:

1. The highest processing speed of the enzyme will be the same, with the substrate coming from any channel (V_m).
2. At steady state, the processing of the substrate through each channel, will take place at a speed that is a fraction of this speed. Hypothetically, it is easier for the substrate to enter through *A β 40_forming* channel.
 - a. $vm40 = Vm40/Vm$, fraction of the speed at which the enzyme cleaves what comes from the *A β 40_forming* channel.
 - b. $vm42 = Vm42/Vm$, fraction of the speed at which the enzyme cleaves what comes from the *A β 42_forming* channel.

$$1\# \quad vm40 + vm42 = 1.$$

For demonstration purposes it seems easier to set the following sequence of discrete values of Michaelis and inhibition constants

$$km42 = 0.1 > km40 = 0.01 > ki42 = 0.001 > ki40 = 0.0001$$

(arbitrary units),

for a substrate concentration:

$$s = 1 \text{ (arbitrary units).}$$

Lets the concentration of inhibitor being splitted, by an amount di from *A β 40_forming* channel to *A β 42_forming* channel, as inhibitor concentration i increases.

Michaelis-Menten equation is proposed as expressed below:

2#

$$vm40 = \frac{1}{s + km40 \left(1 + \frac{i - di}{ki40} \right)}$$

3#

$$vm42 = \frac{1}{s + km42 \left(1 + \frac{di}{ki42} \right)}$$

Replacing expression 1 # ($vm40 + vm42 = 1$) with variables values ($km42 = 0.1 > km40 = 0.01 > ki42 = 0.001 > ki40 = 0.0001$ and $s = 1$), we have:

4#

$$\frac{1}{1 + 0.01 \left(1 + \frac{i - di}{0.0001} \right)} + \frac{1}{1 + 0.1 \left(1 + \frac{di}{0.001} \right)} = 1$$

Solving i in terms of di we have:

5#

$$i = \frac{10000000 * di^2 + 9000 * di + 999}{10000 * (1000 * di + 1)}$$

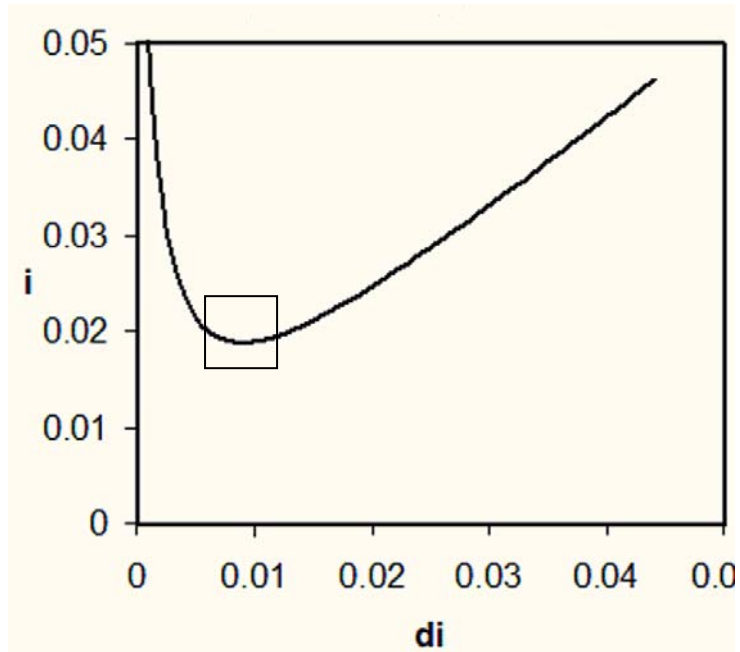


Figure 1. $i(di)$

Solving di in terms of i and we get two solutions, of which real solution is:

6#

$$di = \frac{\sqrt{(100000000 * i^2 + 220000 * i - 39879)} + 10000 * i - 9}{20000}$$

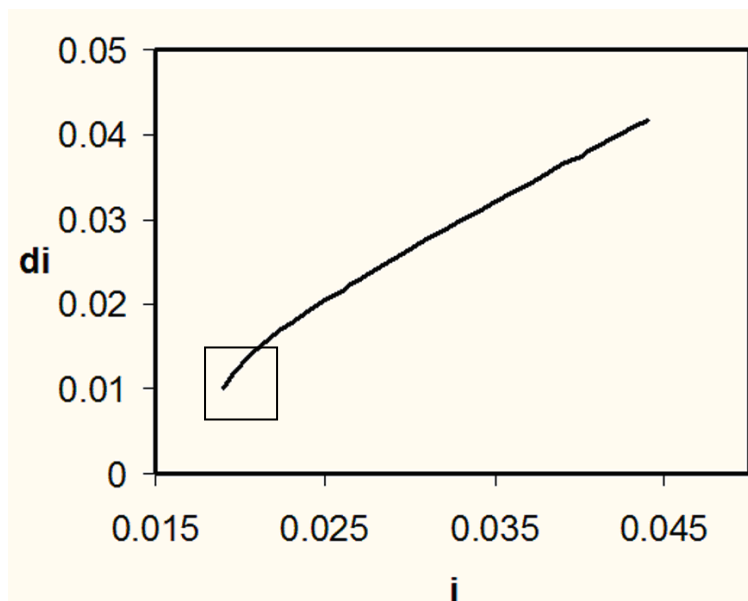
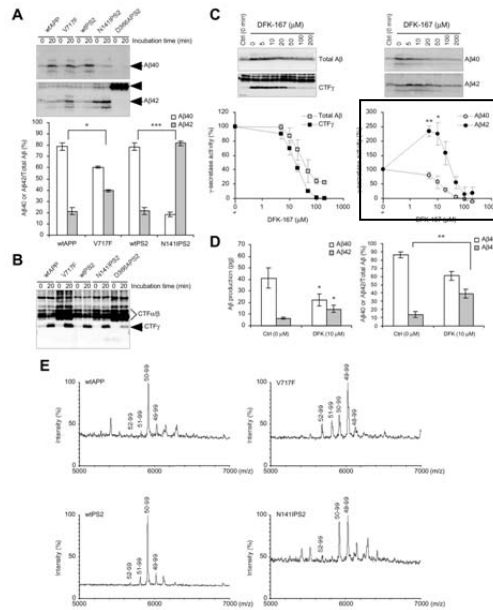


Figure 2. $di(i)$

We can see in this figure (figure 2, $di(i)$) a discontinuity point ($di = 0.01$) that corresponds with the minimum at the above figure (figure 1, $i(di)$) and is interpreted as the inhibitor concentration i which begins to inhibit $A\beta_{42}$ -forming channel (6#, $di(i)$ become a real solution).

At this point we consider some papers using a sensitive, membrane-based assay system, expressing *C99*, the substrate of gamma-secretase and inhibitors that inhibit the production of $A\beta_{40}$ in a dose-dependent fashion, while enhancing $A\beta_{42}$ production at low concentrations and inhibiting it at high concentrations [1, 2].

Production of $A\beta$ and CTF γ in membranes from various cell lines.



Sato T et al. J. Biol. Chem. 2003;278:24294-24301

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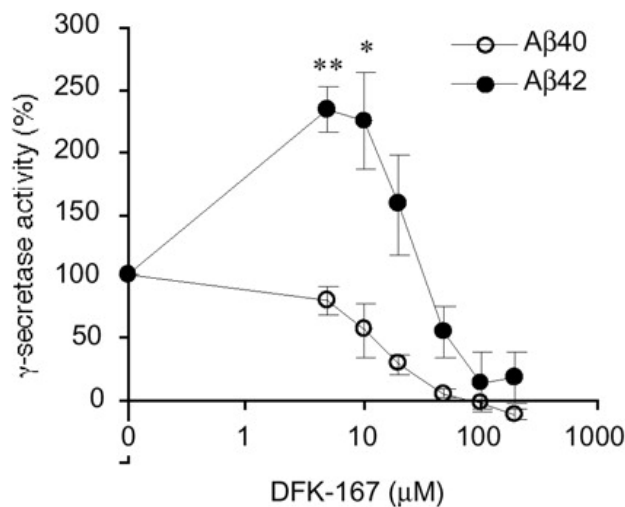


Figure 3. reference [2], figure 1C, right above 1D

When figure 5 $i(di)$ is compared with the percentage of $A\beta_{42}$ versus *DFK-167* inhibitor concentration, figure 3, it seems to be opposed to this one. It represents the exchange of

an inhibitor amount, di , between the two channels, from the $A\beta_{42}$ _forming channel standpoint. Figure 5 shows that as di increases “ i decreases” (not real solutions for di zone) and therefore increases the availability of $A\beta_{42}$ _forming channel; till a minimum (maximum production of $A\beta_{42}$) where $A\beta_{42}$ _forming channel begins to be loaded with inhibitor (concomitantly, starts $A\beta_{42}$ value fall down). Thus the inverse function, $di(i)$, only takes real and positive solutions when $A\beta_{42}$ _forming channel begins to be loaded with inhibitor.

The solutions of the equations are consistent with the model (in which a single monomer active site optionally receives the same substrate from two different sites or channels). It is qualitatively demonstrated, through competitive inhibition model, the inhibitor is exchanged between the two sites: from $A\beta_{40}$ _forming channel to $A\beta_{42}$ _forming channel.

Using the same mathematical strategy, regardless of the part that refers to the inhibitor in the model and introducing s/ds for the APP-substrate:

7#

$$\frac{1}{0.01+(s-ds)} + \frac{1}{0.1+ds} = 1$$

it can be shown that $A\beta_{42}$ _forming channel begins to be occupied from a threshold APP value. This means that the gamma-secretase complex will function like a high pass filter in the $Abeta_{42}$ formation. Both the alpha and beta secretase eliminate spurious APP, which comes from non-contiguous cells. The remnant APP finally reaches the gamma-secretase complex which initially should be converted to $Abeta_{40}$.

As the APP concentration increases, the complex would stop functioning when a reversible APP dimer (proposed by Sato et al. [3]) is formed inside the complex, with individual APP molecules coming from different channels. This blockage will impair gamma-secretase processing of Notch and many other substrates of the neurogenesis signal ...

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