

# CRYOEM STRUCTURE OF GAMMA SECRETASE: A KEY COMPONENT IN ALZHEIMER NEURODEGENERATIVE DISEASE

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## **BACKGROUND**

Gamma-secretase (g-sec) is a membrane protein complex. It is a 180kDa tetrameric protease, assembled from a stable inactive trimeric core, composed of the catalytic subunit presenilin, the substrate binding protein nicastrin and the accessory protein APh1; the incorporation of the fourth component PEN2 (presenilin enhancer 2) triggers the endoproteolysis of presenilin resulting in an active tetrameric g-sec. By cleaving into the transmembrane domain of membrane signaling precursors, g-sec is responsible for their proteolytic maturation; among these precursors the Amyloid Protein Precursor (APP) is particularly important for its biomedical implications: its cleavage by g-sec results, in fact, in the production of the amyloid beta-peptides, that are implicated in Alzheimer disease (AD).

## **AIMS**

The SCIENTIFIC AIM of this project was to carry out **structural studies of g-sec and to provide its 3D reconstruction by single particle Electron Microscopy (EM)**. This is relevant both in basic research to understand the so far uncharacterized mechanism of intramembrane proteolysis and in applied biomedical research for targeted drug design to interfere with the malfunctioning of g-sec in AD.

The LONG-TERM GOALS were to enlarge the background of Dr Renzi in structural studies: previous X-ray crystallography studies, suitable to determine the structure of small proteins at high-resolution, would be implemented by the **EM techniques**, suitable to study large complexes at low- to medium-resolution. Combination of the two techniques would allow to study large assemblies at high resolution. In addition a permanent **collaboration between EU and US** would be established.

## **RESULTS**

Regarding the LONG-TERM GOALS: Dr Renzi learnt to prepare **specimen** suitable for EM, to handle **electron microscopes** and to collect EM images; she learnt to reduce and process raw data, to carry out a **3d reconstruction** both with and without an initial model, using the **EM software** (EMAN, iMagic,

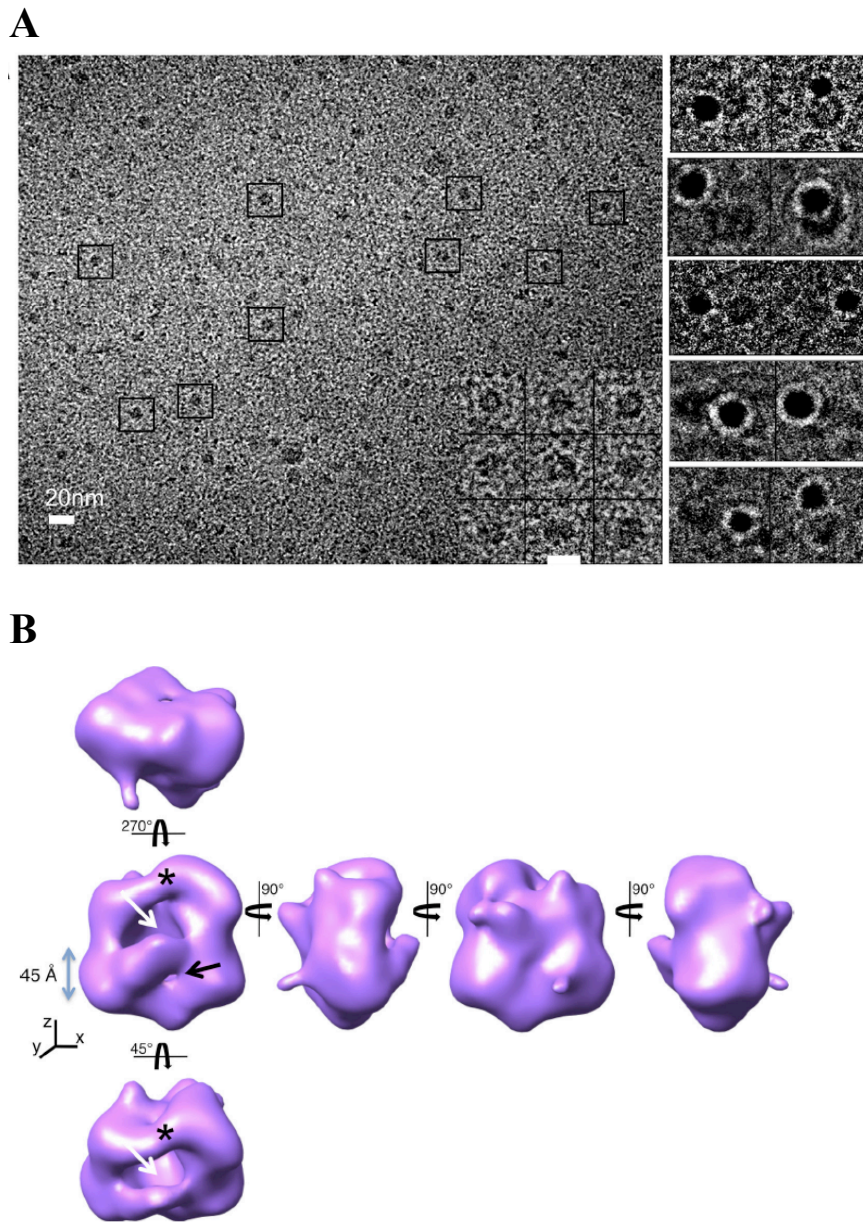
SPIDER, FReAlign, XMIPPS, eTomo, Chimera...). Dr Renzi established a stable **collaboration with the US** institution by applying for grants in common projects.

Regarding the SCIENTIFIC AIMS: Dr Renzi determined the **3d reconstruction** of a mature and catalytically active **tetrameric g-sec** using single-particle cryo-EM. After assessing the quality of the sample by site-specific gold labeling, she collected EM images (Fig.1a), reduced and analysed raw data to carry out a 3d reconstruction (Fig.1b). G-sec has a cup-like shape with a lateral belt of about 45Å in height that encloses a water-accessible internal chamber. Active site labeling with a gold-coupled transition state analog inhibitor suggested that the active site is inside this chamber. The structure thus suggests that the water dependent proteolysis can have place in the hydrophobic membrane, if intramembrane proteases isolate their active site in a hydrophilic environment in their matrix while inserted in the membrane, where to allocate the substrate.

Dr Renzi also collected EM images of the catalytically inactive **trimeric core g-sec** (Fig.3A) and carried out a 3d reconstruction: the aim was to compare the tetramer and the trimer and to understand how the addition of PEN2 would enhance the activity of the complex. **Comparison** (Fig.3B and C) suggests that the incorporation of PEN-2 might contribute to the maturation of the active site architecture in enlarging the internal cavity and the accessibility of the active site to the substrate. The location of the surface cavities, their position relative to the internal chamber and, possibly, to the membrane, suggest as well a possible pathway for the substrate to access the active site and the cleavage products to be released.

## FIGURES

**Fig.1**



**FIGURE 1. Single-particle electron microscopy of gamma-secretase.** *A*, representative CCD image of in vitreous ice tetrameric gamma-secretase (left) and gold labeling with antibodies against each subunit and with a substrate analog (right). *B*, **Three-dimensional reconstruction of  $\gamma$ -secretase.** Shown are views of the final three-dimensional volume of  $\gamma$ -secretase filtered at 18 Å and set to a threshold equivalent to a molecular mass of ~200 kDa. The *double-headed arrow* delimits the proposed location of the transmembrane region of the complex. The *asterisks* depict the proposed location of the ectodomain of NCT. The *arrows* mark a cavity (*white arrows*) in the extracellular region that extends to the transmembrane region ending in a lateral opening (*black arrows*)

**Fig.2**

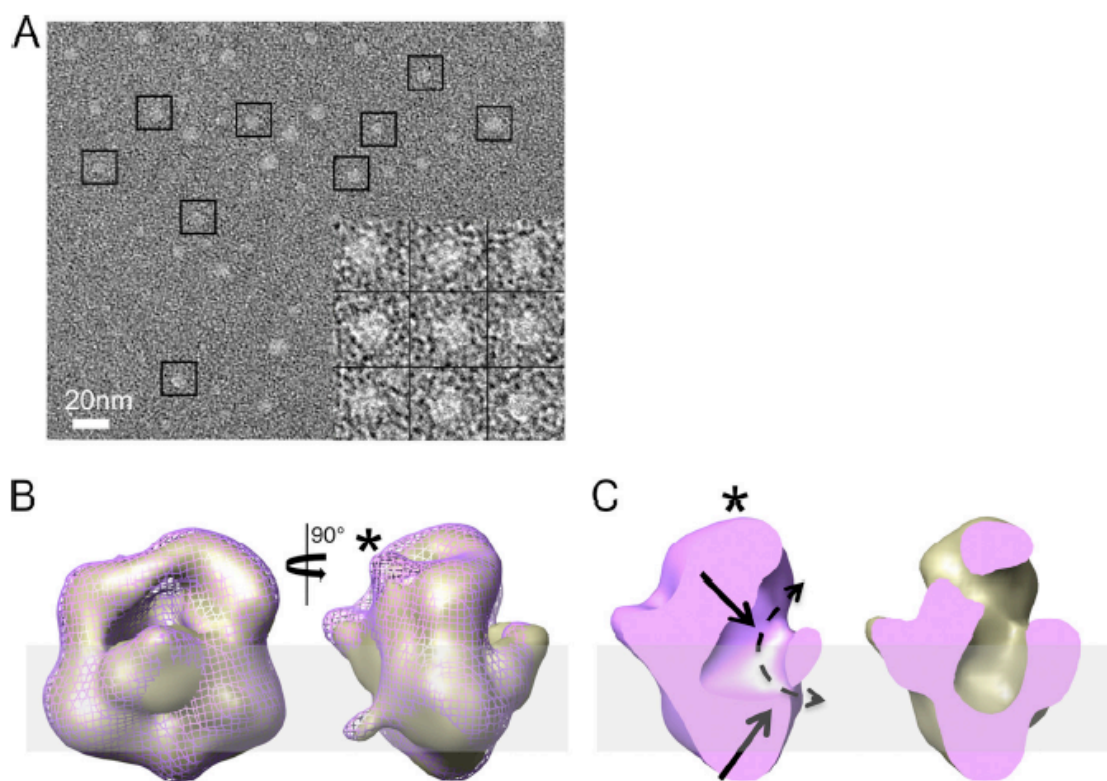


FIGURE 2. **Comparison of gamma-secretase with a trimeric pre-activation intermediate lacking PEN-2.** *A*, representative CCD image of the trimeric preactivation intermediate under negative stain. *B*, for comparison, the three-dimensional reconstruction of the pre-activation intermediate (*yellow*) was docked manually in the envelope corresponding to gamma-secretase (*magenta mesh*). An *asterisk* marks differences in density between the two reconstructions. *C*, cut-through views of gamma-secretase (*magenta*) and the pre-activation intermediate (*yellow*). The *black arrows* mark the widening of the mouth and the lateral opening. The *double-headed arrow* depicts the putative path of substrate/product release/entry. The proposed location of the lipid membrane is depicted as a *slab*.