



LUND
UNIVERSITY

Cellular mechanisms of A β antibody reduction of neuronal A β

GUNNAR K. GOURAS, NILS LINDBLOM AND GEHUA WEN

EXPERIMENTAL DEMENTIA RESEARCH UNIT, LUND UNIVERSITY, LUND, SWEDEN

Introduction

A β immunotherapy is the first approved disease-modifying therapy for Alzheimer's disease (AD). Reduction of plaques by microglia is widely viewed as the central mechanism of A β antibodies. However, decades of research indicate that more soluble forms of amyloid- β (A β) track better with AD symptoms than amyloid plaques. It is widely *believed* that soluble A β oligomers accumulate extracellularly, despite lack of evidence for this. In fact, levels of soluble A β 42 in normal brain tissue are markedly higher than extracellular levels, with this brain A β 42 rising exponentially prior to plaques while extracellular levels of A β 42 in CSF remain remarkably stable with aging until they drop prior to A β 42 symptoms. Familial AD mutations raise the ratio of A β 42/40 but like sporadic AD don't show increases in CSF A β 42 with aging.

In contrast to lack of evidence for pre-plaque increases in soluble extracellular A β 42, hundreds of papers have reported on the early accumulation and aggregation of A β 42 *inside* AD vulnerable neurons. A β 42 micro-aggregates inside neurons, particularly in dystrophic neurites, show ultrastructural subcellular pathology even prior to plaques.¹

Our group spearheaded, and over the years focused on, the pivotal role of intraneuronal A β 42/43 accumulation in initiating AD pathology. In a prior cellular study, we showed that A β antibodies are taken up by neurons, reduce intraneuronal A β and protect against AD-like synaptic alterations in AD transgenic mouse primary neurons (see Figure 1, below).² Recently, we extended this work to *in vivo* in Wen et al., *Alzheimer's & Dementia* (2026; see Figure 2, above right).³

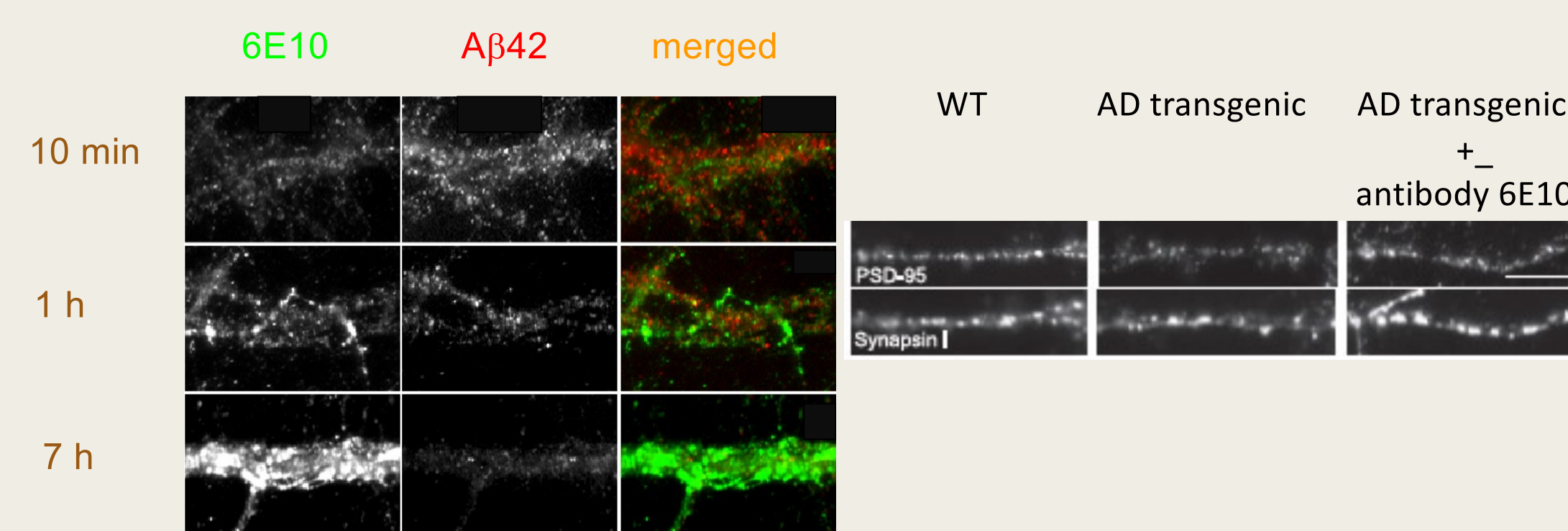


Figure 1. A β antibodies internalize, reduce A β and protect synapses

Left side: left panel, A β antibody (6E10) is internalized over time into neurites (dendritic segments); middle panel, concomitantly iA β 42 is reduced over time; and right panel, merged images of 6E10 and A β 42.

Right side: concomitantly, AD-like synaptic reductions of PSD-95 in AD transgenic neurons in culture are reversed upon antibody 6E10 treatment. Synapsin I is not altered in these AD neurons.

AD transgenic is Tg2576 mice; bar: 10 μ m.

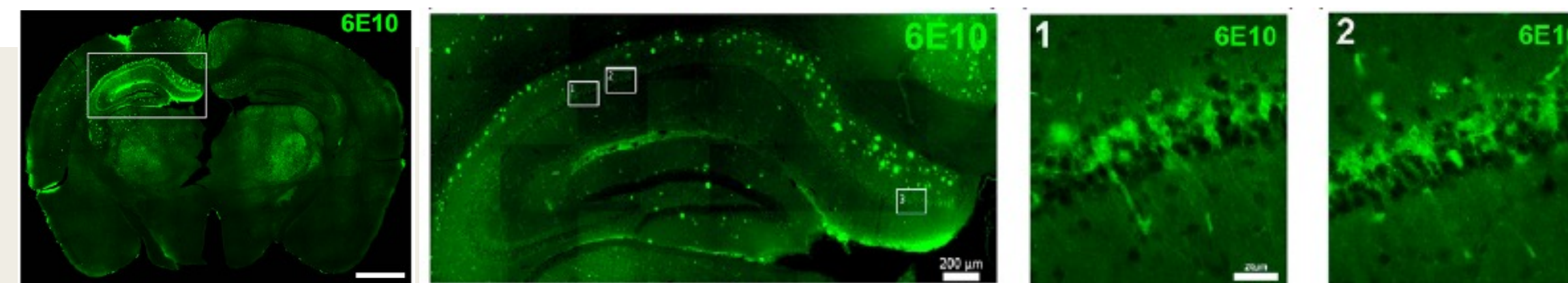


Figure 2. A β antibody is internalized into neurons in the brain

In Wen et al., 2026,³ we used intracerebral, cisterna magna and intraperitoneal injections of fluorescently conjugated or unconjugated N-terminal A β antibody 6E10 in three different AD mouse models, including the 5xFAD transgenic and two APP knockin mice: APP^{NL-F} and APP^{NL-G-F}. Shown here is unilateral injection of 5 μ L of Alexa Fluor-conjugated 6E10 antibody into the left hippocampus of 4-month-old 5xFAD mouse.

Left panel: note most fluorescence (green for 6E10) is in left hippocampus: scale bar = 1 mm. Next panel is higher power view where one can see plaque labeling, but also in insets 1 and 2 to right within CA1 pyramidal neurons; scale bar = 200 μ m.

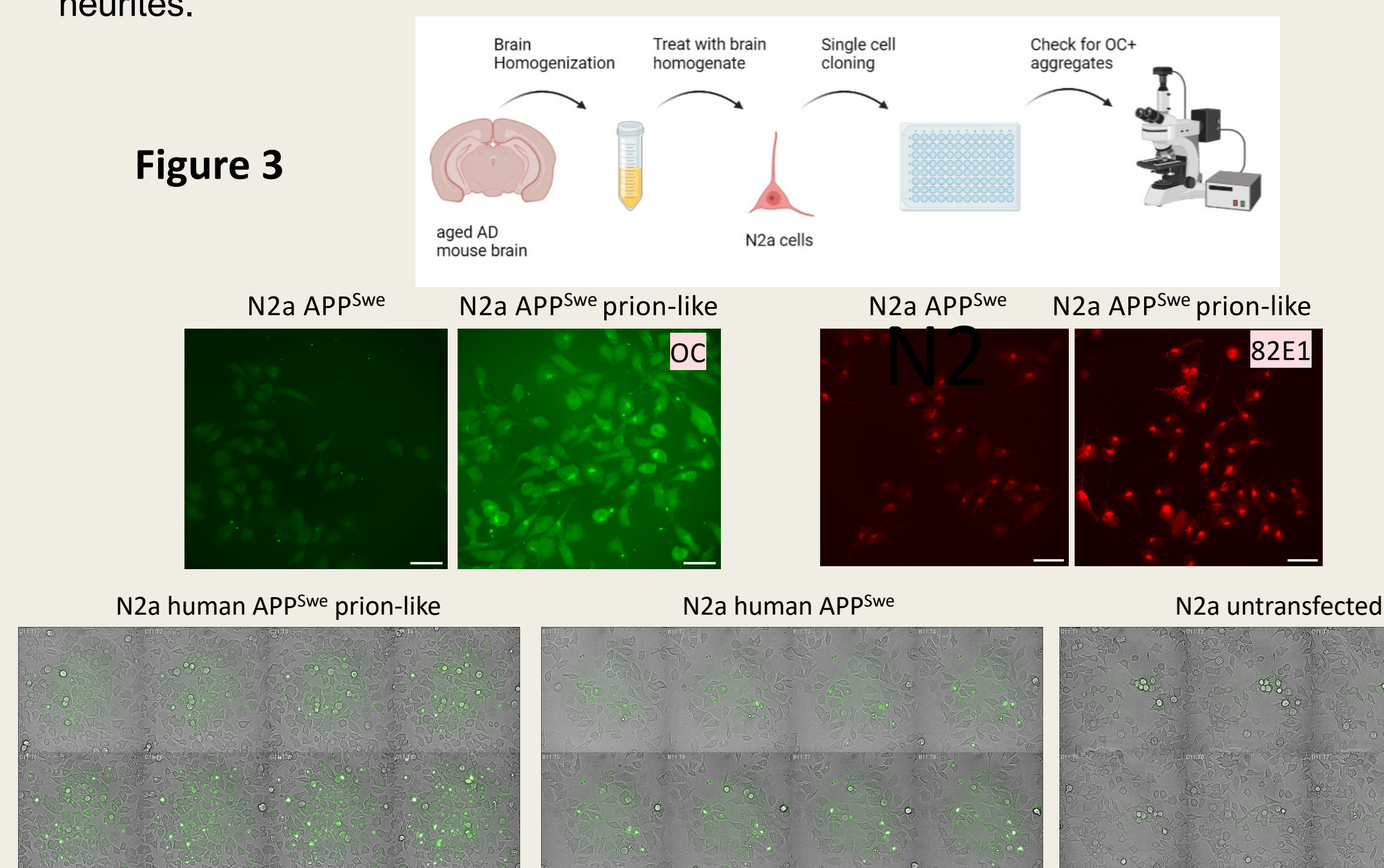
Methods

We use cell and AD mouse models (both transgenic and knock-in) to delineate the mechanisms of A β immunotherapy for AD at the subcellular level. We use antibodies directed at different epitopes and tagged and untagged A β antibodies, different neuronal AD cell models (N2a, and primary mouse and human iPSCs neurons), and different routes of *in vivo* injections (intracerebral, cisterna magna and intraperitoneal). N2a cells with antibody OC+ aggregates ("N2a APP^{Swe} prion-like") are described in Olsson et al., 2018.⁴ Analyses include biochemical methods (dot blot, Western blot) and immunofluorescence and live cell imaging. Functional properties of organelles are assayed using fluorogenic dyes.

Results

Our unpublished work shows that N-terminal domain A β antibody 6E10 is internalized in a time-dependent manner into LAMP1+ vesicles (not shown) where it colocalizes with A β aggregates (antibody OC positive). A β antibody uptake requires human APP/A β and appears dependent on clathrin-mediated endocytosis. A β antibodies colocalize with and reduce intracellular A β aggregates. Antibodies enter the endocytic pathway where A β peptides are both generated and along with APP CTFs initially accumulate in AD, particularly in dystrophic neurites.

Figure 3



(See Figure 3 legend at right column at top)

Figure 3 . A β antibody 6E10 is internalized in cells with human A β /APP (see images of figure at bottom of middle panel)

Top: schema for generation of N2a cells with prion-like aggregates.⁴

Middle panel: No labeling of human APP^{Swe} N2a cells with antibody OC (green) for A β 42 fibrillar oligomers but positive labeling of N2a APP^{Swe} prion-like cells. These prion-like cells also label with the N-terminus A β antibody 82E1.

Bottom panel: Human specific A β antibody 6E10 (Alexa fluor-488 conjugated) is readily internalized into N2a human APP^{Swe} prion-like cells (left) but also into N2a human APP^{Swe} cells (middle) but not N2a untransfected cells (right) which only contain endogenous mouse APP. Live cell imaging every 30 minutes over 4 hours.

Conclusions

- Cumulative evidence indicates that with aging A β 42 oligomers accumulate within rather than outside of neurons in brain with AD
- A β antibodies can target this pool of intraneuronal A β 42 (iA β 42) *in vitro* and *in vivo* in brain
- We hypothesize that plaques sequester valuable A β antibodies that are then prevented from reducing this critical pool of neuronal iA β 42
- Ongoing cellular work suggests that A β antibody appears to promote iA β 42 degradation in lysosomes

References

- Takahashi RH, Almeida CG, Kearney PF, Yu F, Lin MT, Milner TA, Gouras GK. Oligomerization of Alzheimer's beta-amyloid within processes and synapses of cultured neurons and brain. *J Neurosci*. 2004; 24(14):3592-9.
- Tampellini D, Magrané J, Takahashi RH, Li F, Lin MT, Almeida CG, Gouras GK. Internalized antibodies to the Abeta domain of APP reduce neuronal Abeta and protect against synaptic alterations. *J Biol Chem*. 2007; 282(26):18895-906.
- Wen G, Lindblom N, Zhan X, Garcia-Ryde M, Deierborg T, Kober-Flatmoen A, Gouras GK. A β antibodies target not only amyloid plaques but also distinct brain cells and vessels. *Alzheimer's & Dementia*. 2026; 22(1):e7112
- Olsson TT, Klementieva O, Gouras GK. Prion-like seeding and nucleation of intracellular amyloid- β . *Neurobiol Dis*. 2018 ;113:1-10.

Contact Email: gunnar.gouras@med.lu.se

Acknowledgements

Research funding: Swedish Research Council, Alzheimerfonden, Hjärnfonden and Kockska Stiftelsen

Infrastructure support: MultiPark (Multidisciplinary Research Environment in Parkinson's and Alzheimer's diseases)