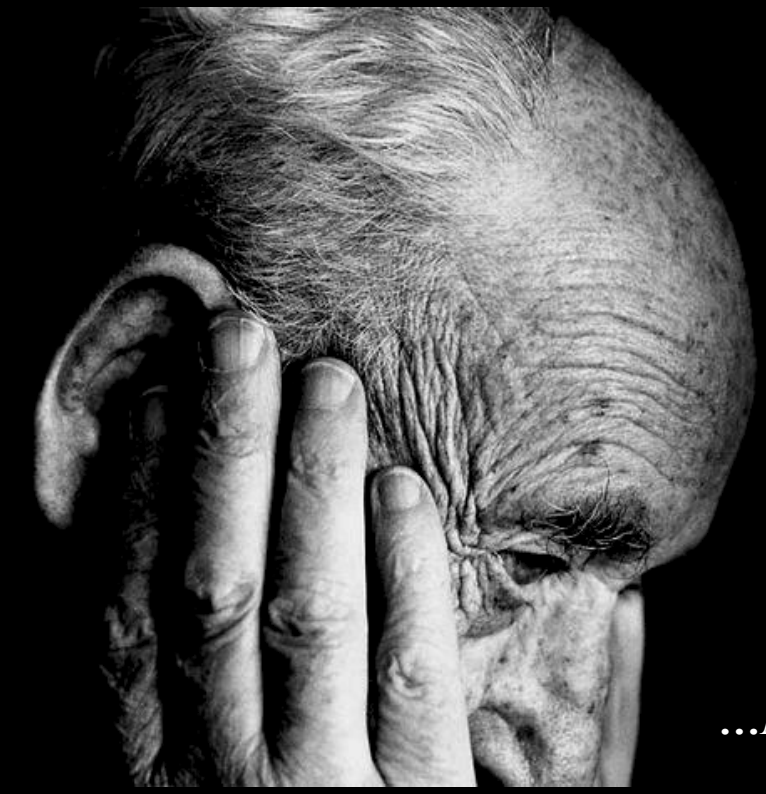
Nano Innovation 2016
Rome, 20-23 September
Conference & Exhibition

Antibody-conjugated PEGylated cerium oxide nanoparticles for specific targeting of Aβ aggregates modulate neuronal survival pathways

Roma, 22/09/2016

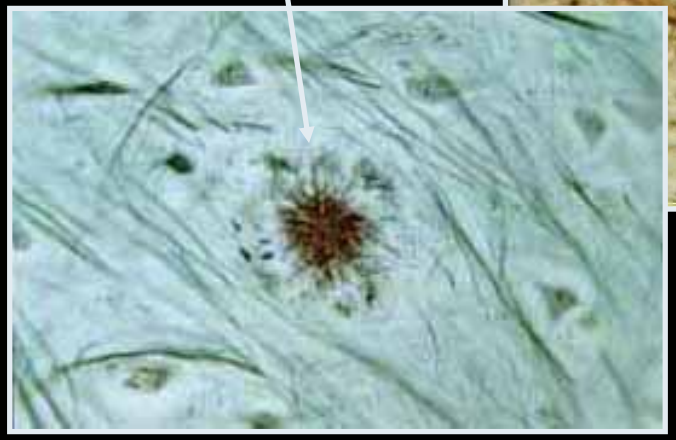
Alzheimer…
…the Search for New Treatments!



…No one knows what causes AD to begin, but we do know a lot about what happens in the brain once AD takes hold…

Alzheimer's Disease

β-amyloid plaques



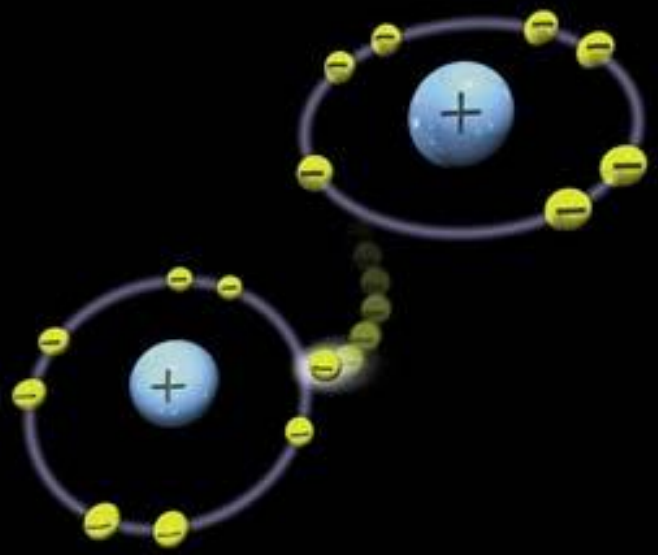
Neurofibrillary tangles

Oxidative stress

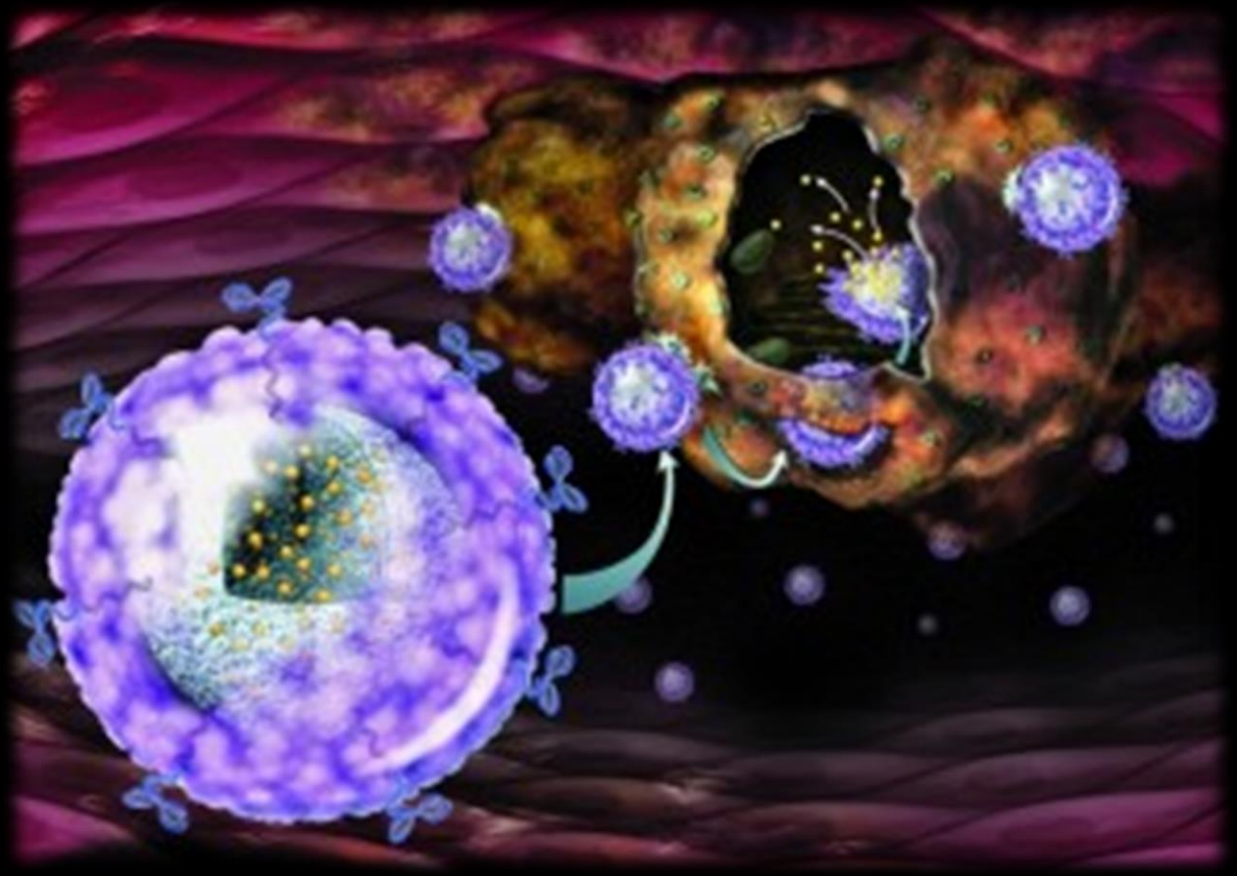
"An imbalance in pro-oxidants and antioxidants with associated disruption of redox circuitry and macromolecular damage"

Antioxidant

" A substance that, when present at a low concentration compared with that of an oxidizable substrate, inhibits oxidation of the substrate"

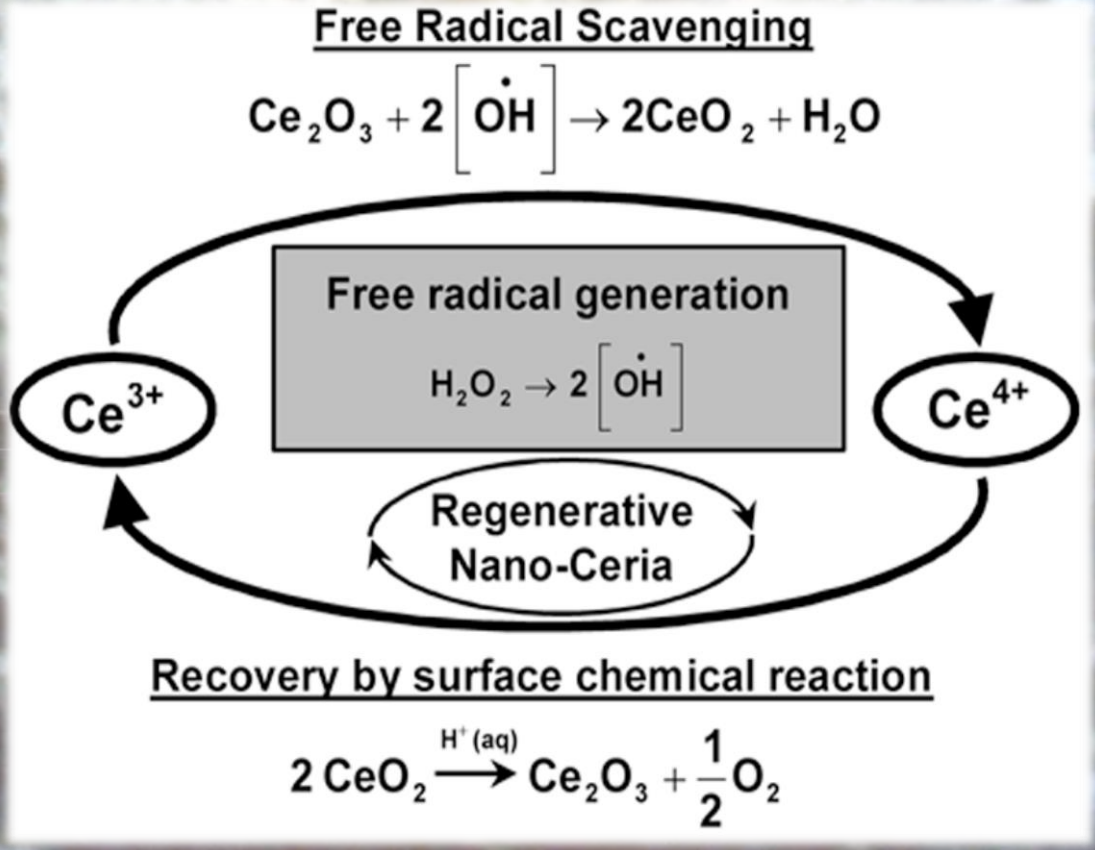


Free radicals are molecules that have at least one unpaired electron and then go around just taking electrons from other healthy molecules causing damage (oxidative stress).

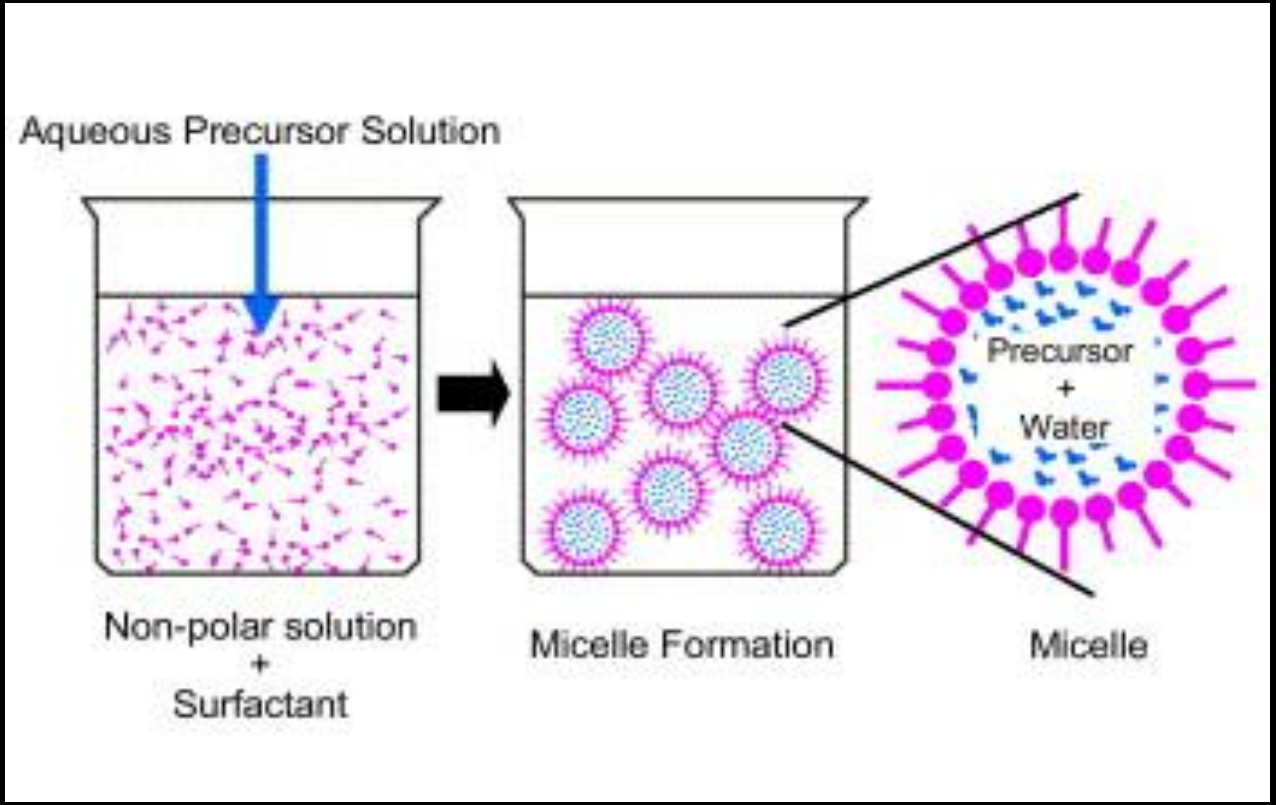


Specific targeting of nanoceria for Alzheimer therapy

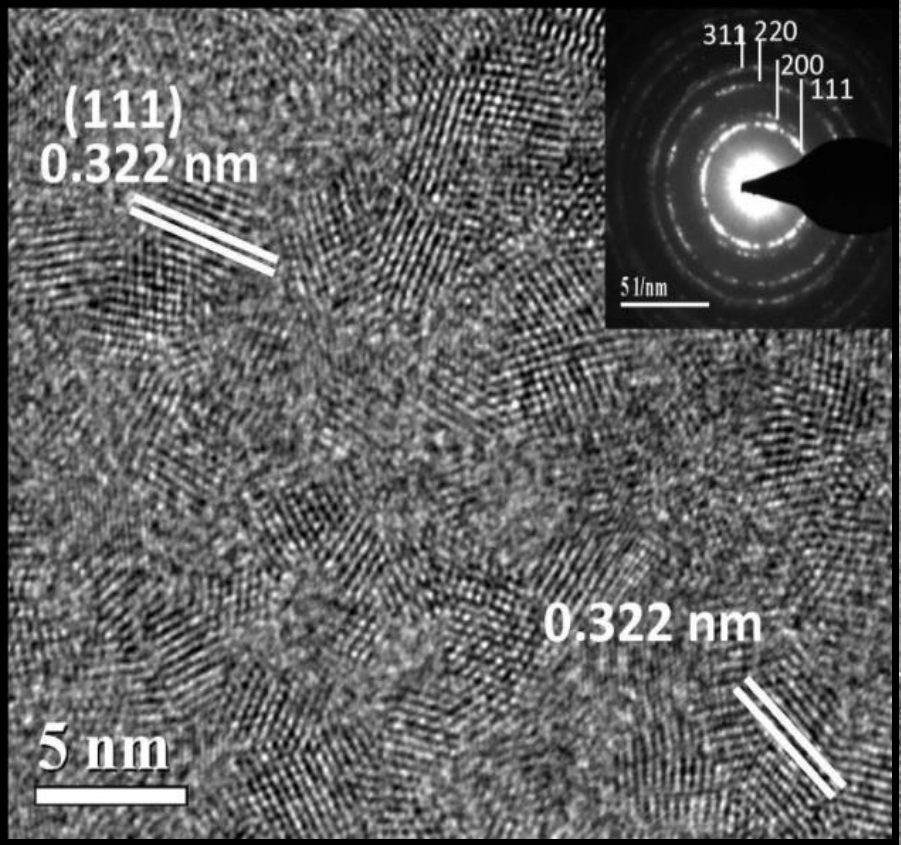
Cerium oxide

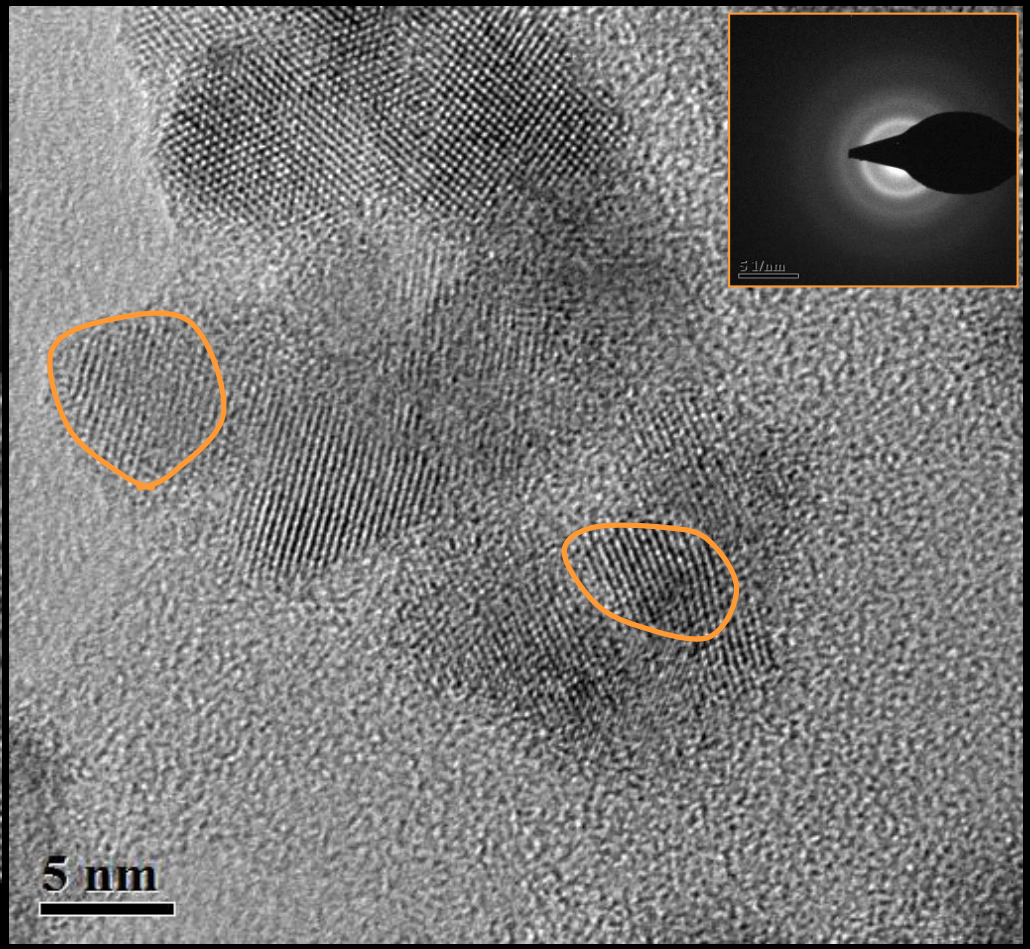


Synthesis and characterization of cerium oxide nanoparticles

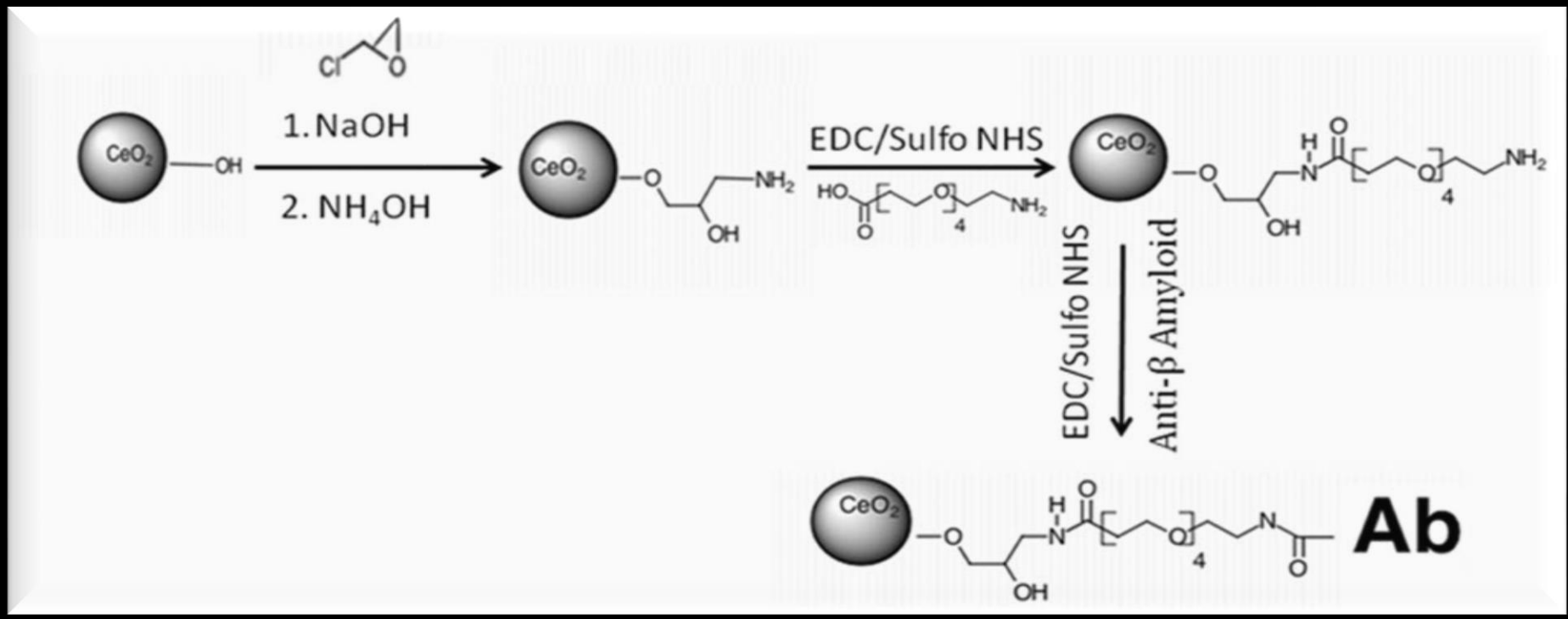


TEM image of microemulsion nanoparticles showing controlled particle size distribution (5 nm)





FUNCTIONALIZATION NP-EPICHLOROHYDRIN

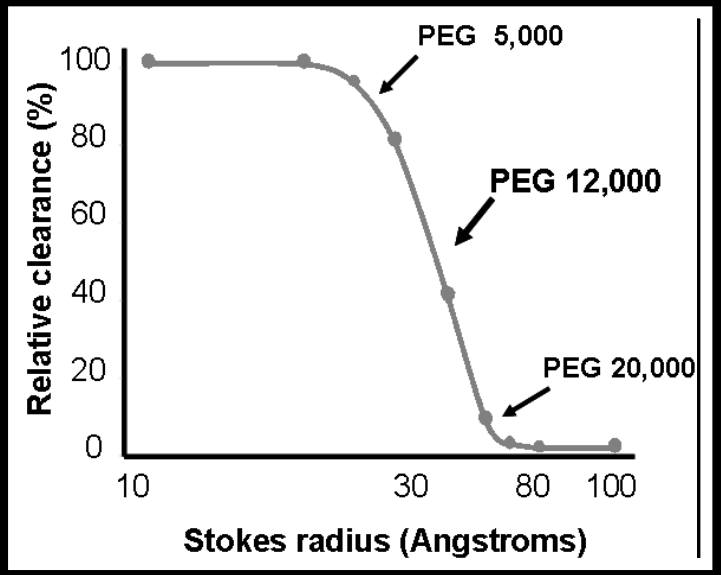


CONJUGATION NP- POLYETHYLENEGLYCOL

For its excellent physical, chemical and biological characteristics the PEG is the polymer of choice for the modification of therapeutic proteins:

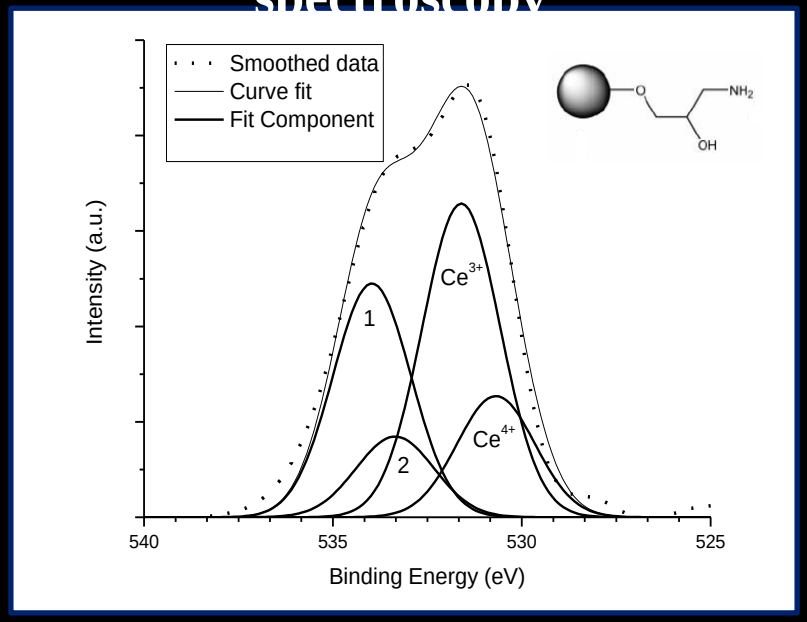
- neutral
- biocompatible
- not-biodegradable
- not-toxic
- not immunogenic
- soluble
- low cost
- FDA approval

Although it is not biodegradable, it can be rapidly eliminated via the renal and liver without undergoing structural changes. Both deletions renal and hepatic depend on the molecular weight of the polymer. PEG to below 20 kDa are freely filtered at the glomerulus, while polymers with higher molecular weights are removed from the circulation more slowly.

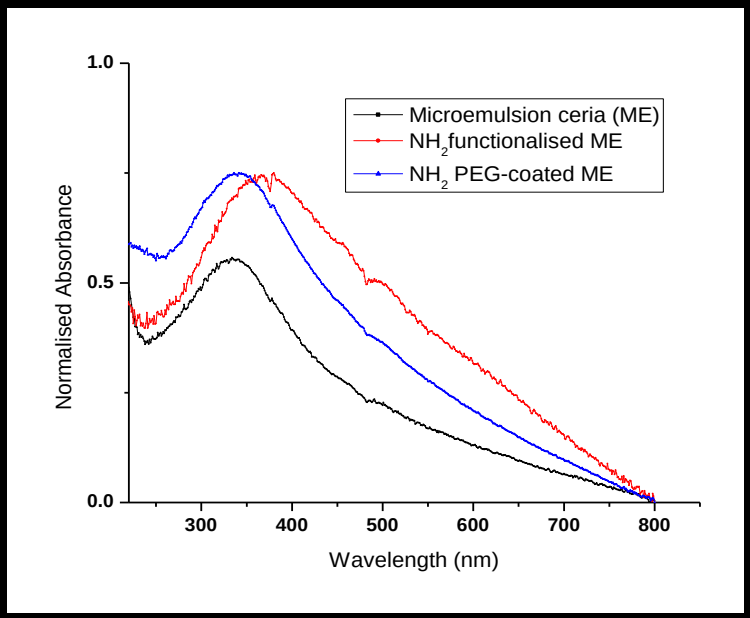


Renal *Clearance* of polyethylene glycol as a function of molecular weight .
[Yamaoka T. Tabata Y, Ikada Y. 1994].

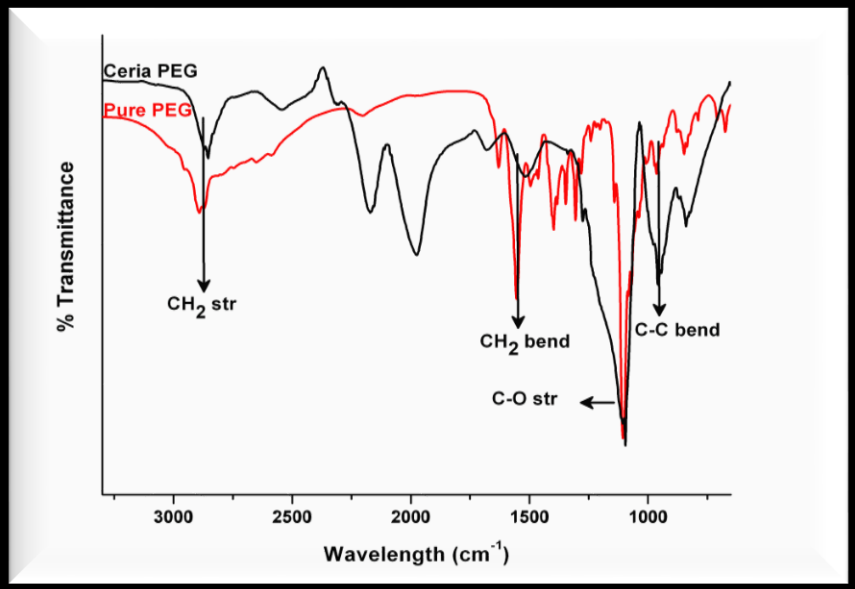
X-ray photoelectron spectroscopy



Ultraviolet–visible spectroscopy

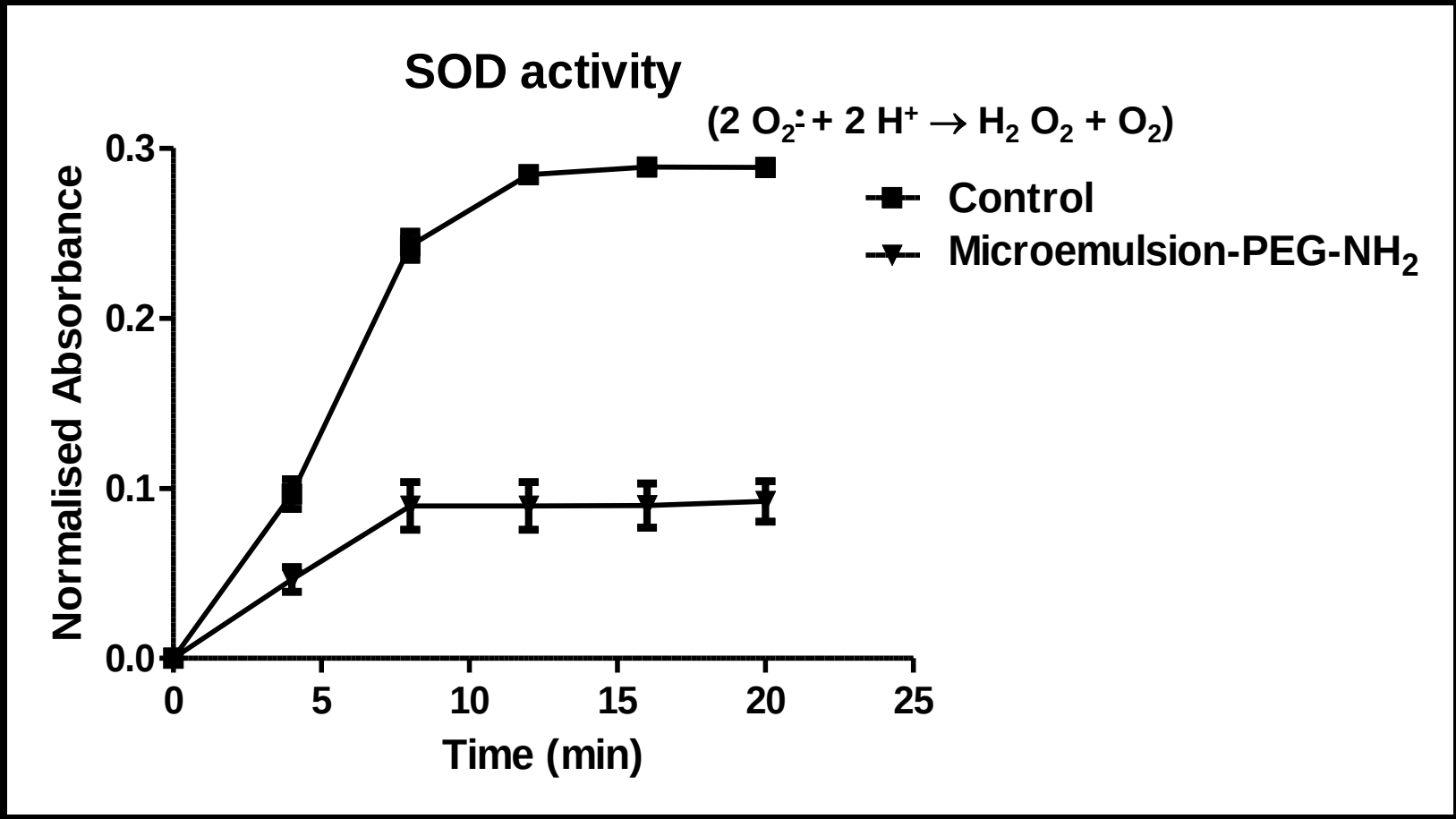


Fourier transform infrared spectroscopy

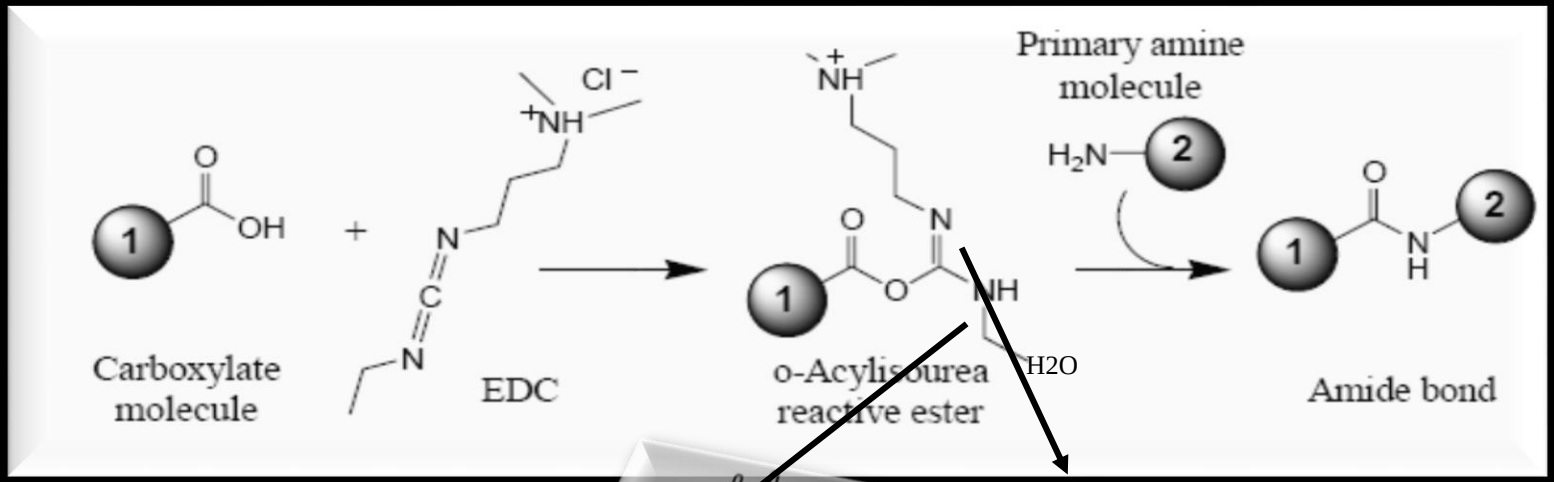


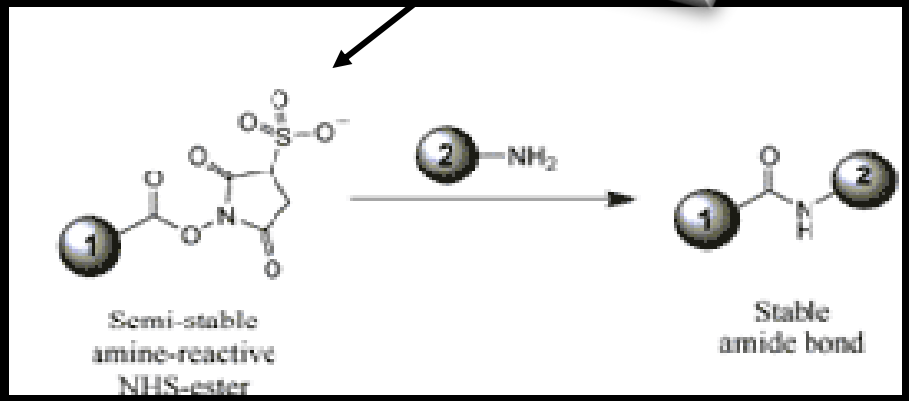
ZETA POTENTIAL:

Ceria micro-emulsion	**-32.8**	**-31.9**
Ceria micro-emulsion NH_2 (functionalised)	**-10.3**	**-10.7**
Ceria micro-emulsion NH_2-PEG-NH_2	**-37.7**	**-37.8**


SOD activity
(2 O2:- + 2 H+ → H2 O2 + O2)
Control
Microemulsion-PEG-NH2
Normalised Absorbance
0.3
0.2
0.1
0.0
0
5
10
15
20
25
Time (min)

CONJUGATION NP-PEG-Ab BY EDC (Ethyldiethylaminopropylcarbodiimide) & Sulfo-NHS (N-Hydroxysuccinimide)

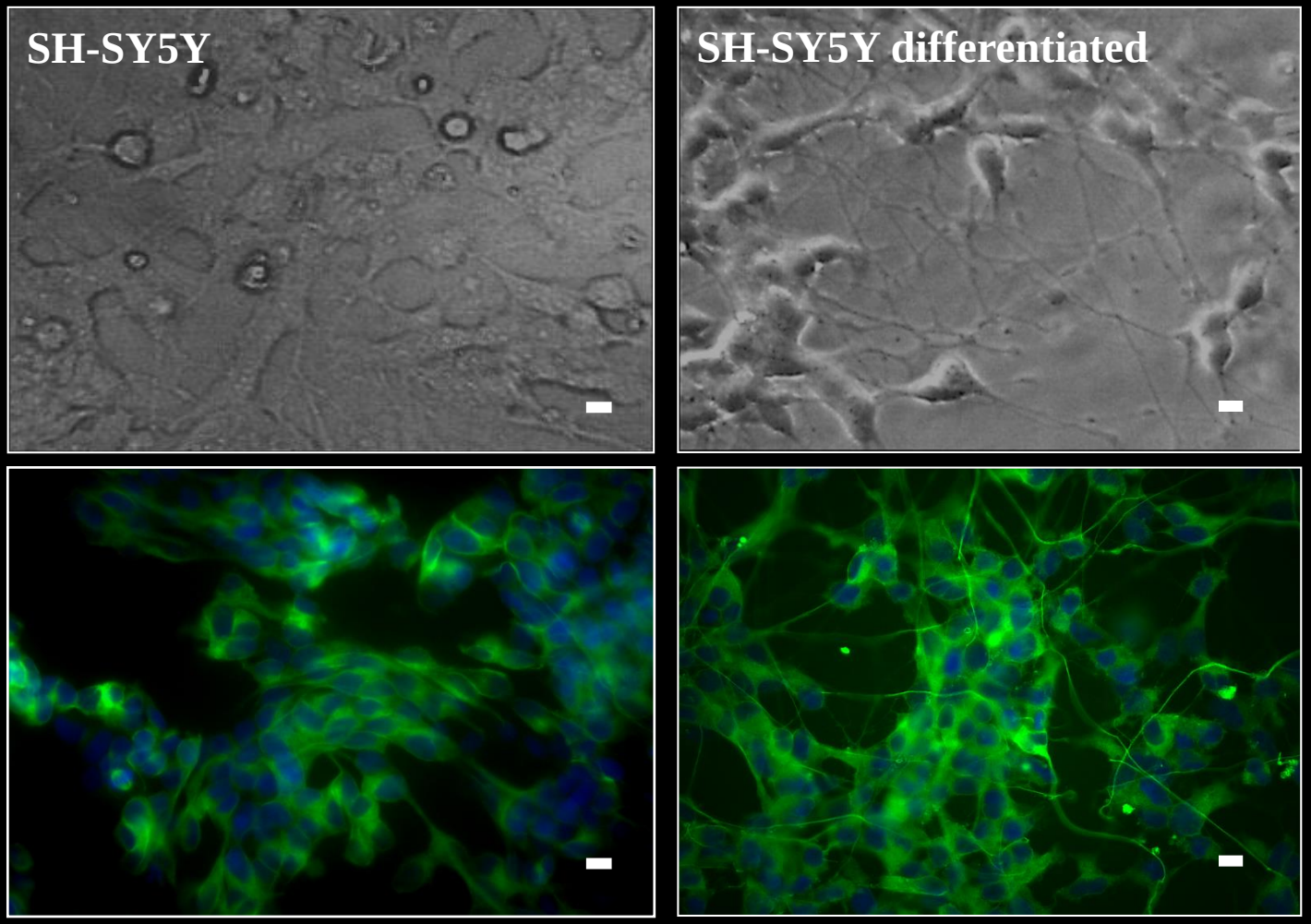




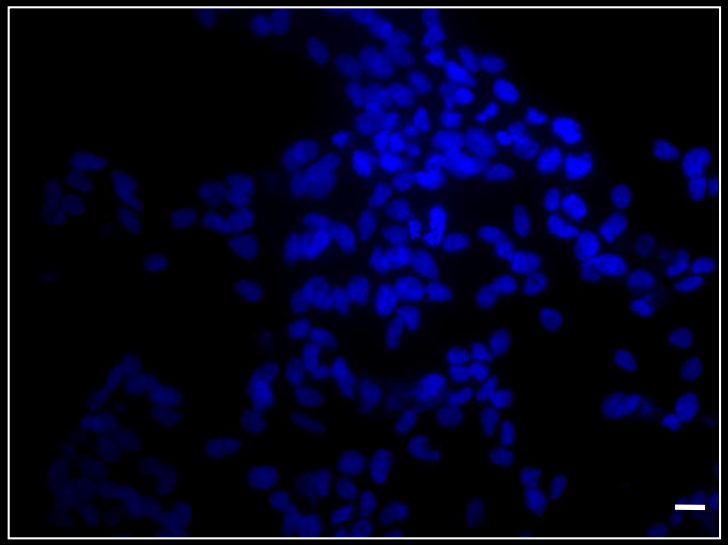
Samples	Ceria NH_2-Ab	Ceria-PEG-NH_2-Ab
Amount of Protein conjugated	140ug-protein/ml	549.01ug-protein/ml

➢ **Human in vitro model of Alzheimer's disease:**

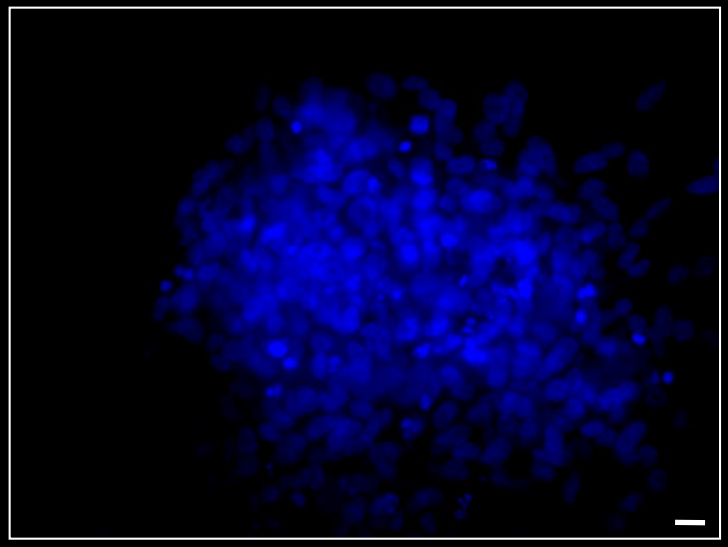
The SH-SY5Y neuroblastoma cells induced to neuronal differentiation are treated with an acute dose of Aß (fragment 25-35).

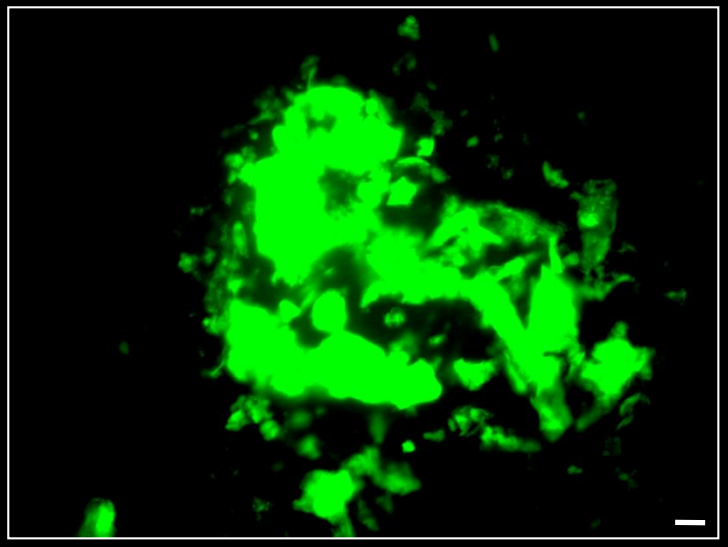


PRESENCE OF PLAQUES:



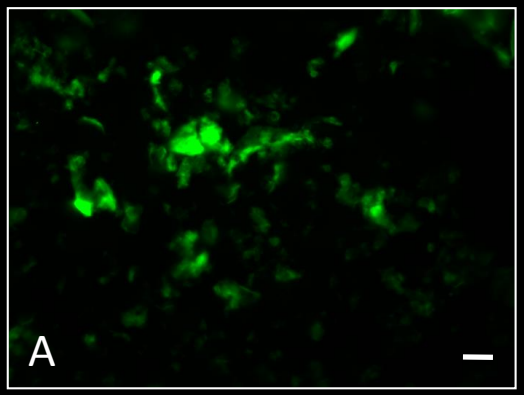
N2 differentiated cells

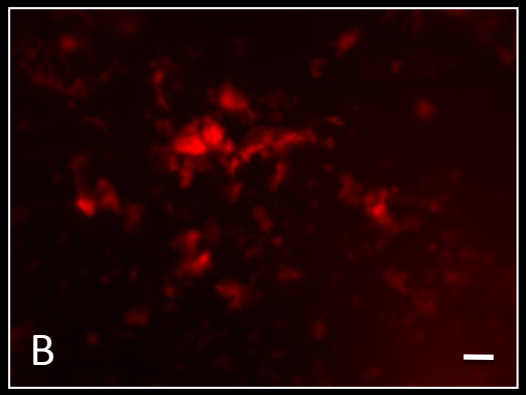


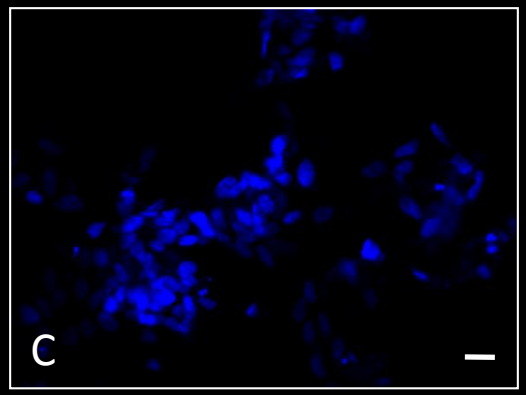


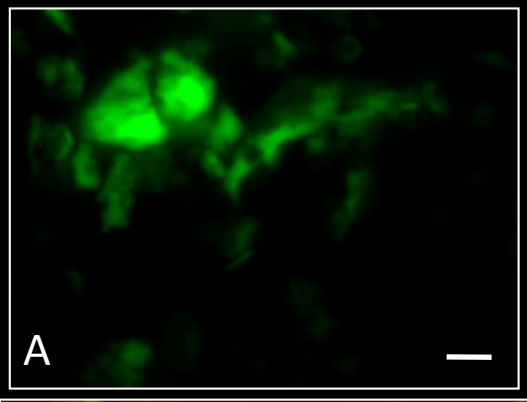
β-Amyloid treated cells

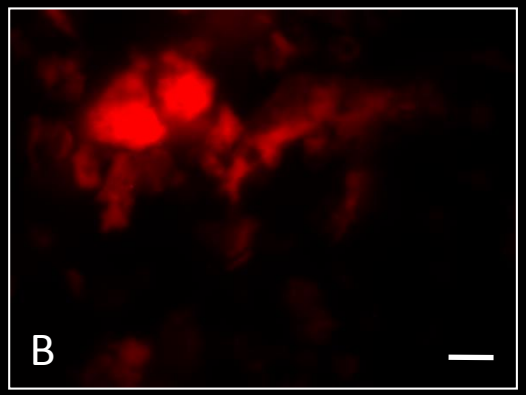
β-Amyloid + CeO_2 PEG_Ab

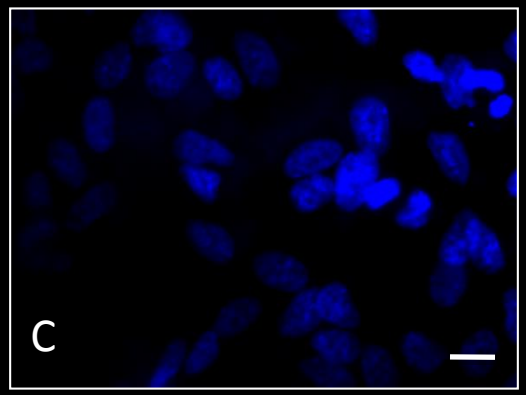


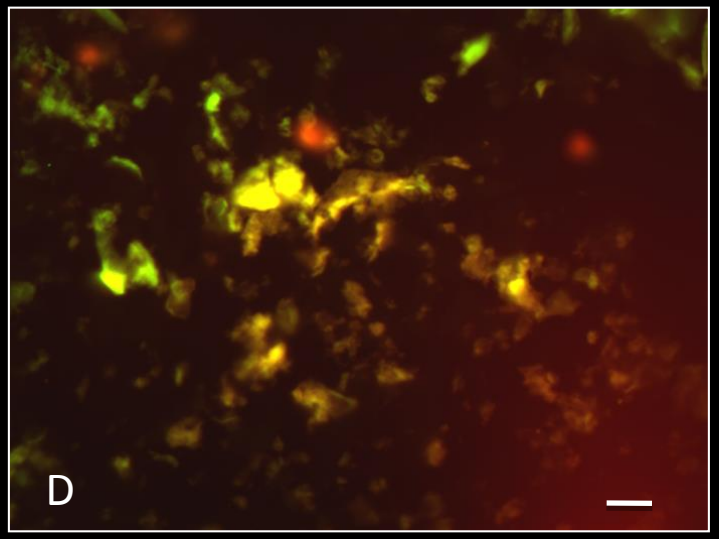


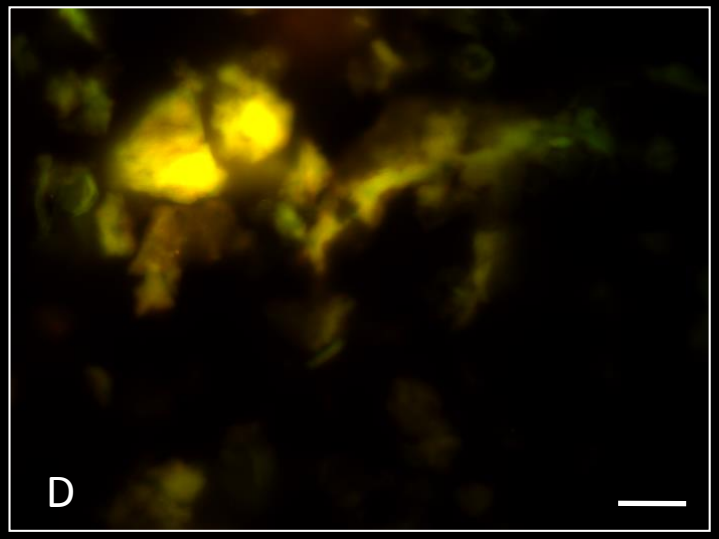






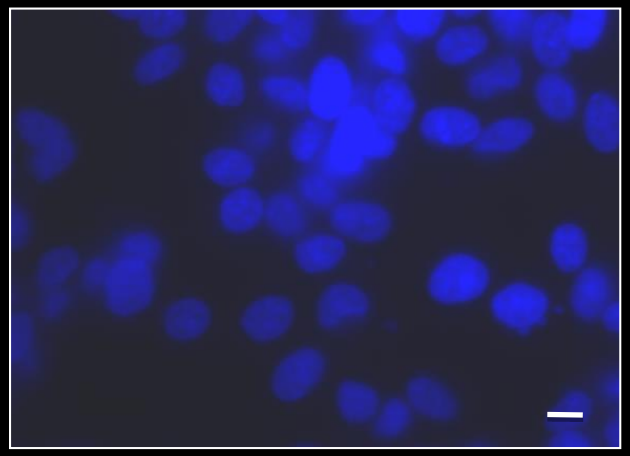




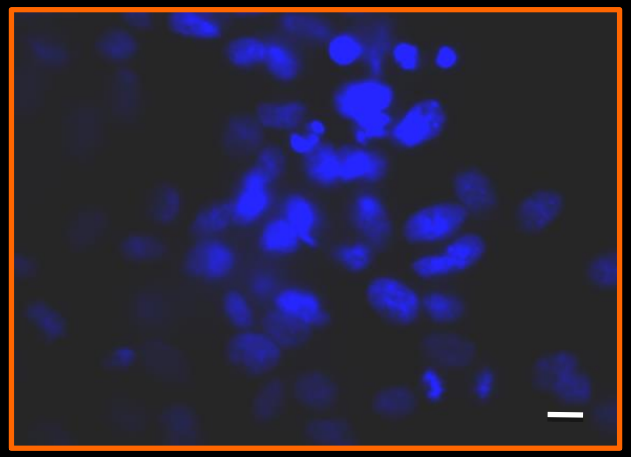


A: merge B and C (antibody in red and plaques in green):
B: localization of plaques;
C: localization of antibody;
D: nuclei stained with DAPI,
Bar. 17 μm.

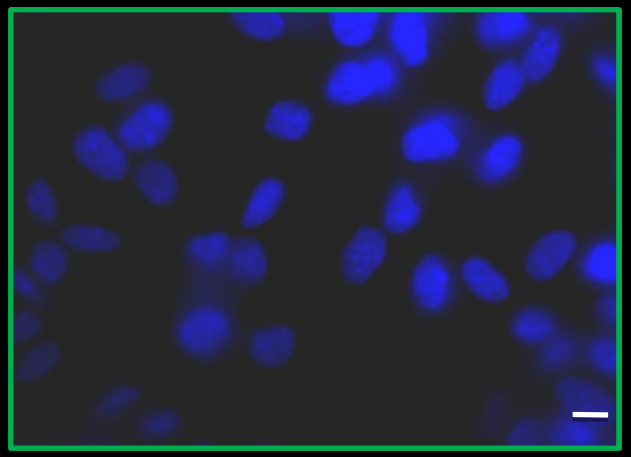
N2 differentiated cells

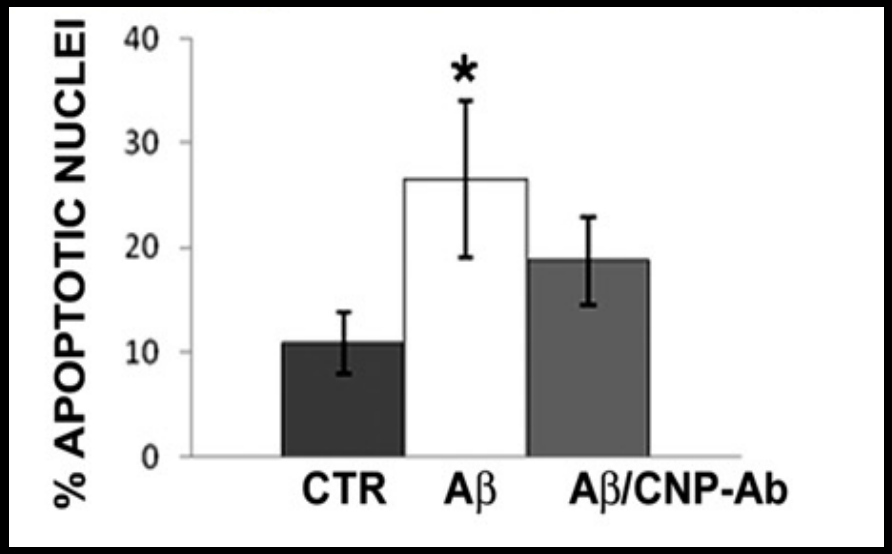


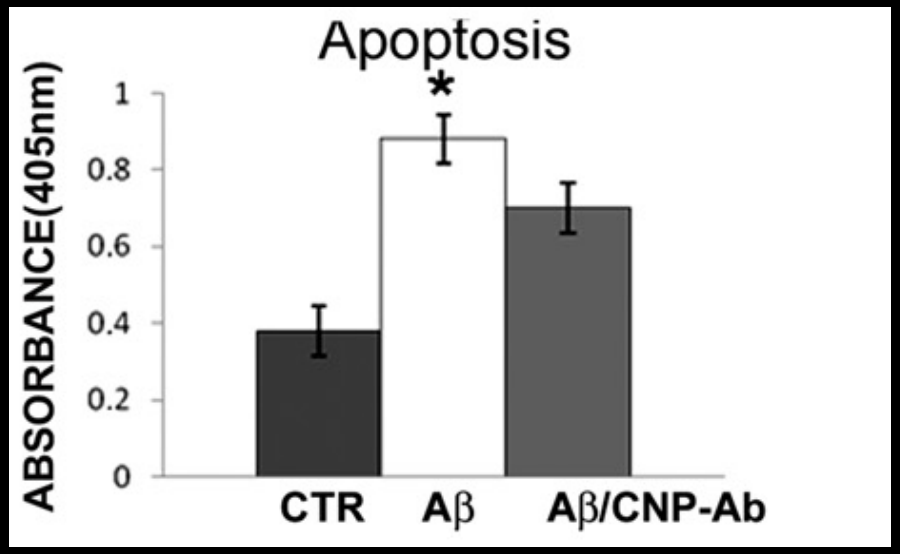
Amyloid beta

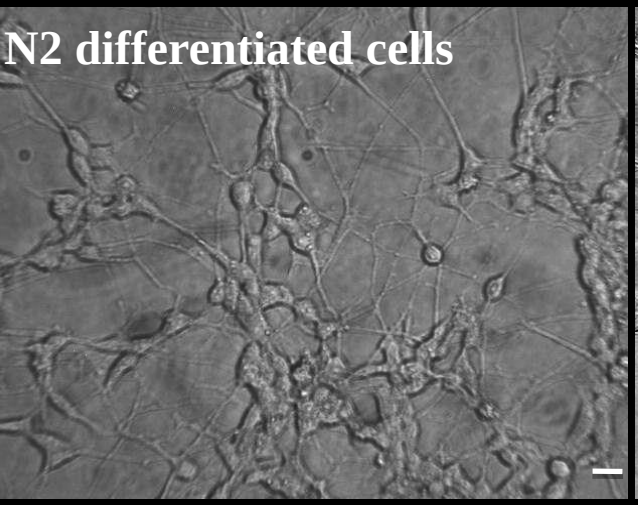


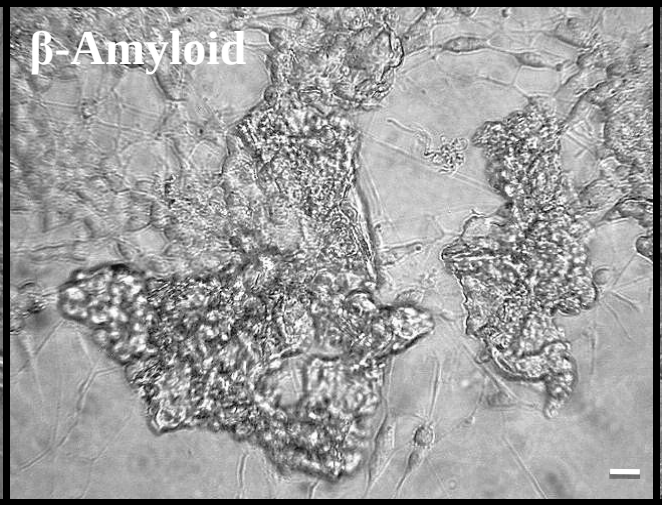
Aβ + CeO_2 PEG Ab

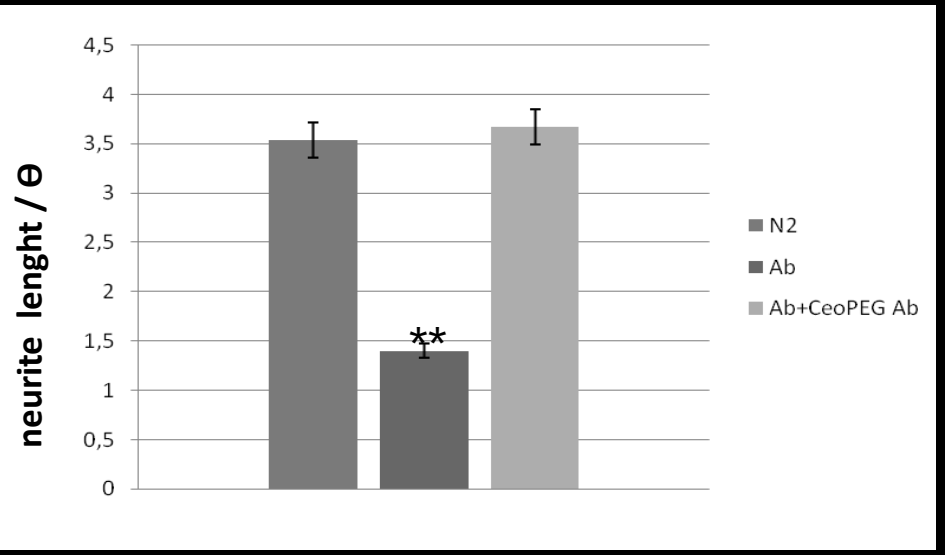


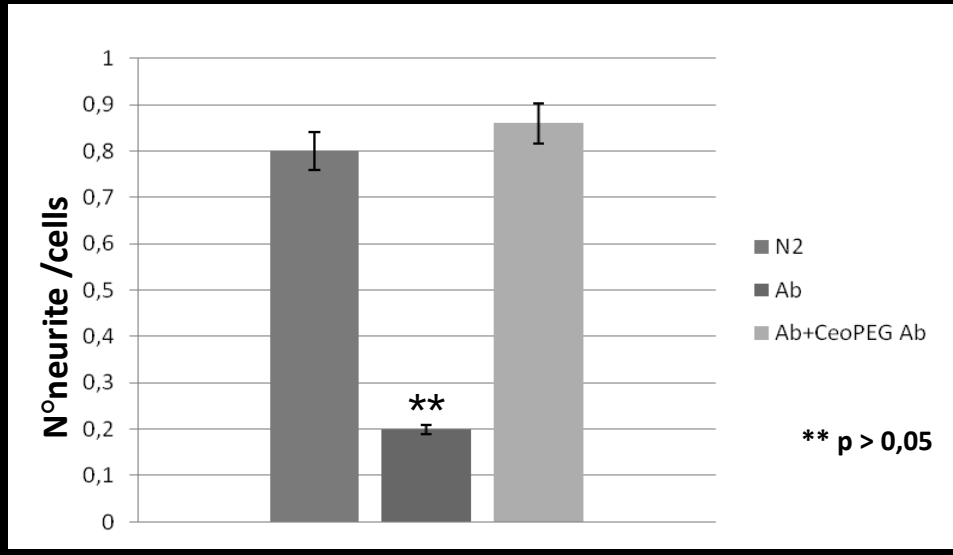












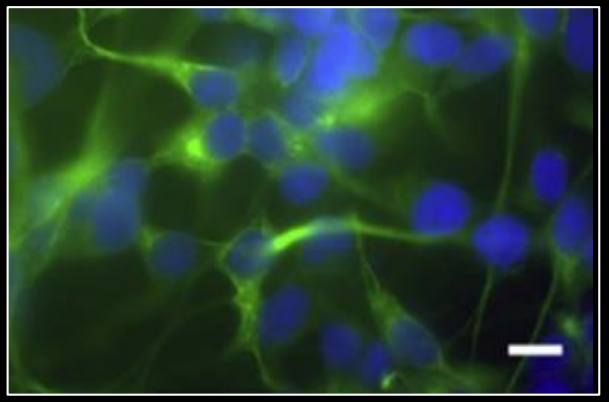
Quantification of the number and length of neurites in control cells and treated.

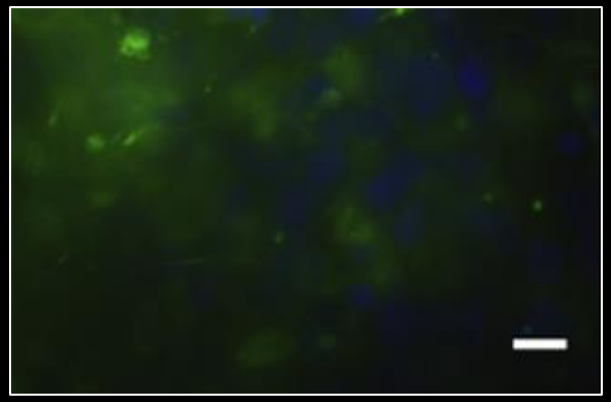
NF-H200

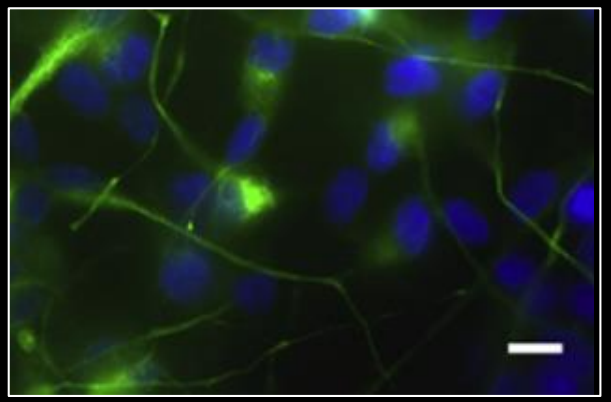
Control N2 differentiated cells

Amyloid beta treated cells

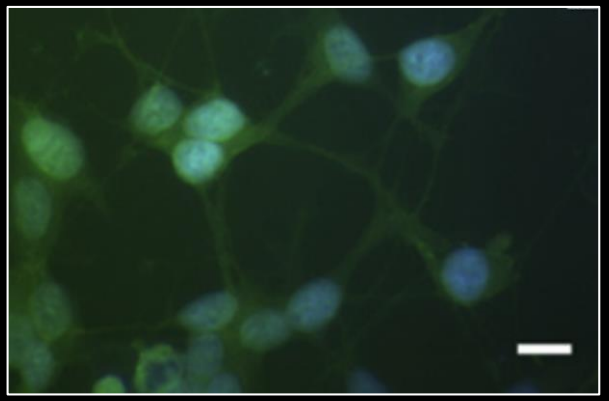
Amyloid beta treated cells + CeO2 PEG Ab

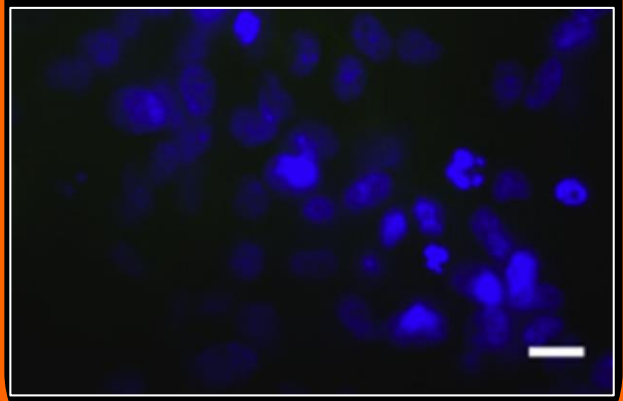


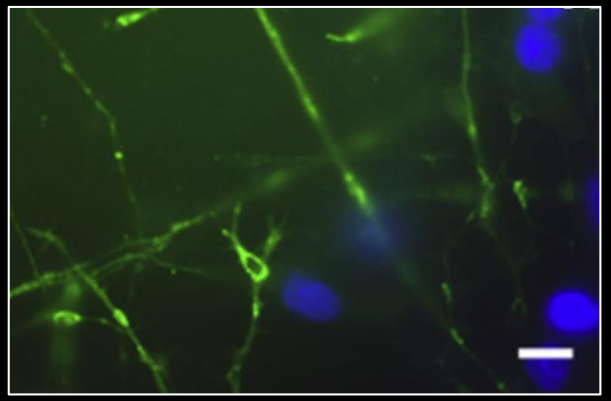




GAP-43

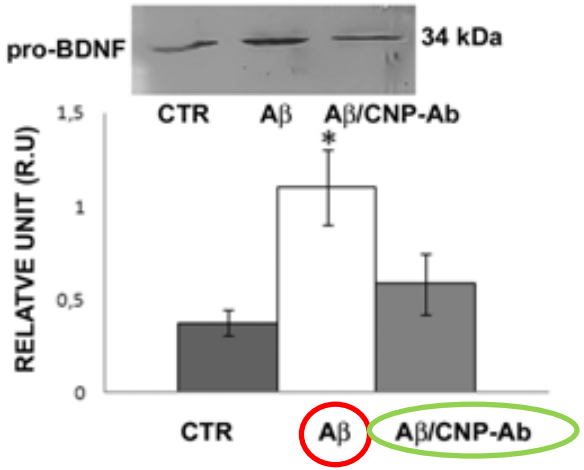


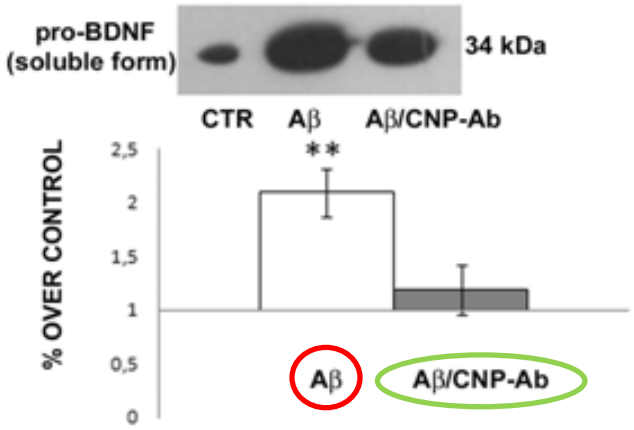




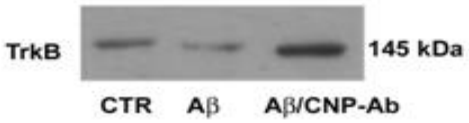
*p≤0.05
**p≤0.005
***p≤0.0005

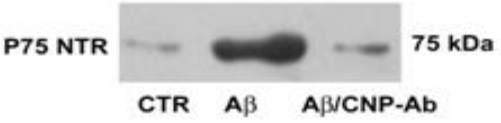
pro-BDNF

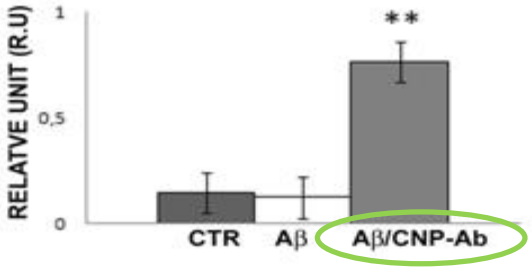




TrKB







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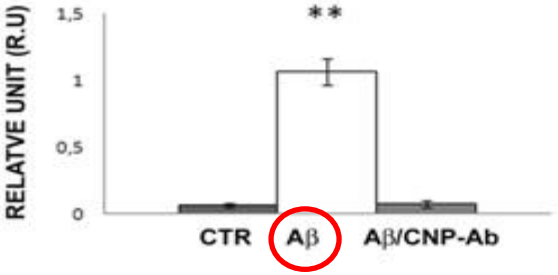
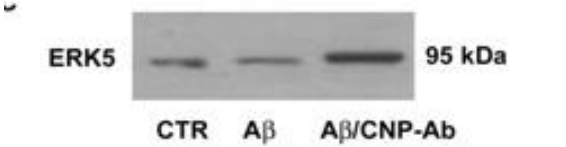
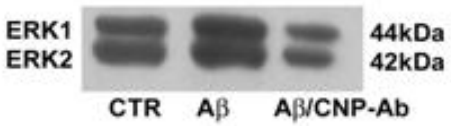
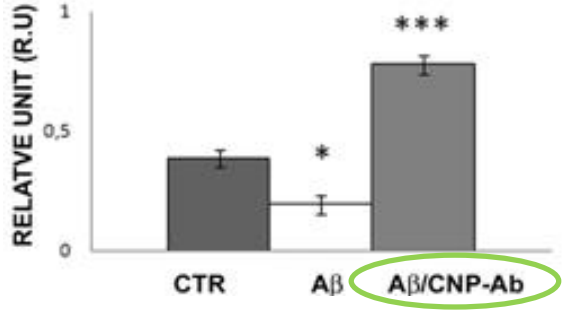
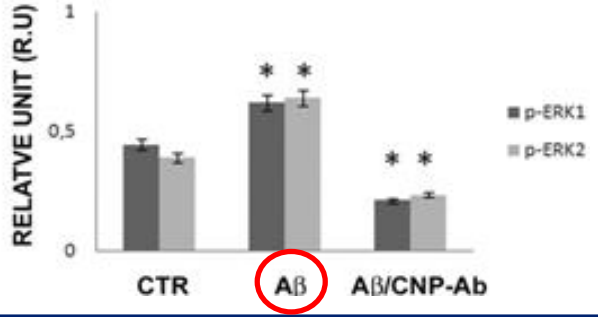






pERK5





pERK1,2

61. (WO2012036786) NANOPARTICLES OF CERIUM OXIDE TARGETED TO AN AMYLOID-BETA ANTIGEN OF ALZHEIMER'S DISEASE

Latest bibliographic data on file with the International Bureau ⇨ Submit observation PermaLink

Pub. No.: WO/2012/036786 **International Application No.:** PCT/US2011/044329
Publication Date: 22.03.2012 **International Filing Date:** 18.07.2011
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Title **(EN)** NANOPARTICLES OF CERIUM OXIDE TARGETED TO AN AMYLOID-BETA ANTIGEN OF ALZHEIMER'S DISEASE
(FR) NANOPARTICULES D'OXYDE DE CÉRIUM CIBLÉES POUR UN ANTIGÈNE BÊTA-AMYLOÏDE DE LA MALADIE D'ALZHEIMER

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THANKS FOR THE ATTENTION

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