Nanomedicine:
Colloidal systems for drug delivery into the CNS

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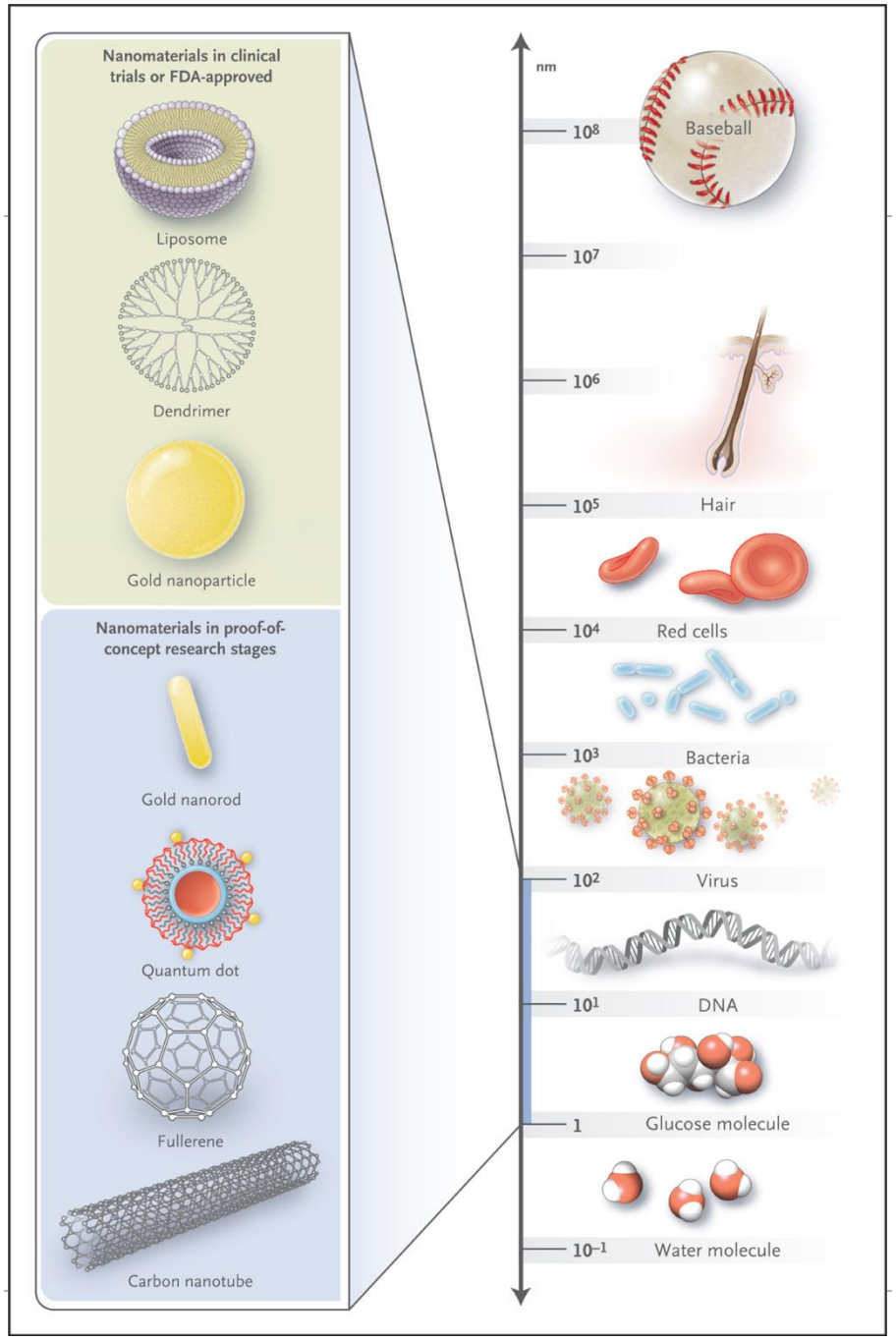
Karl Frontzek, Institute of Neuropathology

Nanotechnology in the Medical Sciences

Intentional design, characterization, production, and applications of materials, structures, devices, and systems by controlling their size and shape in the nanoscale range (1 to 100 nm)

- Terminology for nanomaterials. Publicly available specification 136. London: British Standards Institute, 2007.

> Nanovectors for drug delivery may be able to overcome prior drug efficiacy difficulties of mostly pharmokinetical or pharmacodynamical causes



Kim et al., N Engl J Med 2010

Table 1. **Examples of Nanomaterials in Clinical Use.***

Nanomaterial	Trade Name	Application	Target	Adverse Effects	Manufacturer	Current Status
Metallic						
Iron oxide	Feridex	MRI contrast	Liver	Back pain, vasodilatation	Bayer Schering	FDA approved
	Resovist	MRI contrast	Liver	None	Bayer Schering	FDA approved
	Combidex	MRI contrast	Lymph nodes	None	Advanced Magnetics	In phase 3 clinical trials
	NanoTherm	Cancer therapy	Various forms	Acute urinary retention	MagForce	In phase 3 clinical trials
Gold	Verigene	In vitro diagnostics	Genetic	Not applicable	Nanosphere	FDA approved
	Aurimmune	Cancer therapy	Various forms	Fever	CytImmune Sciences	In phase 2 clinical trials
Nanoshells	Auroshell	Cancer therapy	Head and neck	Under investigation	Nanospectra Biosciences	In phase 1 clinical trials
Semiconductor						
Quantum dot	Qdots, EviTags, semiconductor nanocrystals	Fluorescent contrast, in vitro diagnostics	Tumors, cells, tissues, and molecular sensing structures	Not applicable	Life Technologies, eBioscience, Nanoco, CrystalPlex, Cytodiagnostics	Research use only
Organic						
Protein	Abraxane	Cancer therapy	Breast	Cytopenia	Abraxis Bioscience	FDA approved
Liposome	Doxil/Caelyx	Cancer therapy	Various forms	Hand–foot syndrome, stomatitis	Ortho Biotech	FDA approved
Polymer	Oncaspar	Cancer therapy	Acute lymphoblastic leukemia	Urticaria, rash	Rhône-Poulenc Rorer	FDA approved
	CALAA-01	Cancer therapy	Various forms	Mild renal toxicity	Calando	In phase 2 clinical trials
Dendrimer	VivaGel	Microbicide	Cervicovaginal	Abdominal pain, dysuria	Starpharma	In phase 2 clinical trials
Micelle	Genexol-PM	Cancer therapy	Various forms	Peripheral sensory neuropathy, neutropenia	Samyang	For phase 4 clinical trials

* MRI denotes magnetic resonance imaging.

Surface design: size matters

- Up to 5 µm cellular internalization
- Below 400 nm preferrable for internalization into tumor cells (dependent on vascular pores - Beija, Trends in Biotechnology 2012)
- Particles between 10 and 100 nm won't be cleared by the reticulo-endothelial system (RES, that captures anything over 200 nm)
- Particles below 10 nm will be filtered through the glomerular capillary wall

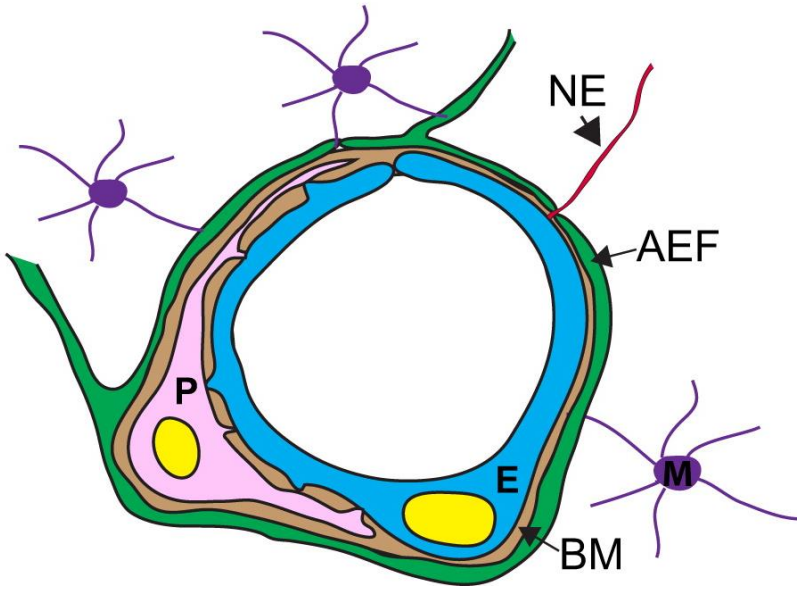
Surface design affects uptake

- Positively-charged NPs rather interact with negatively-charged components of the outer leaflet of the plasma membrane (e.g. proteoglycans)
  - Internalisation
- Negatively-charged NPs and Liposomes are more useful to target monocytes and macrophages

  (Wang et al., Small 2011)
- Non-charged (e.g. PEGylated) are more likely to avoid macrophage uptake

Delivery of pharmaceuticals into the CNS

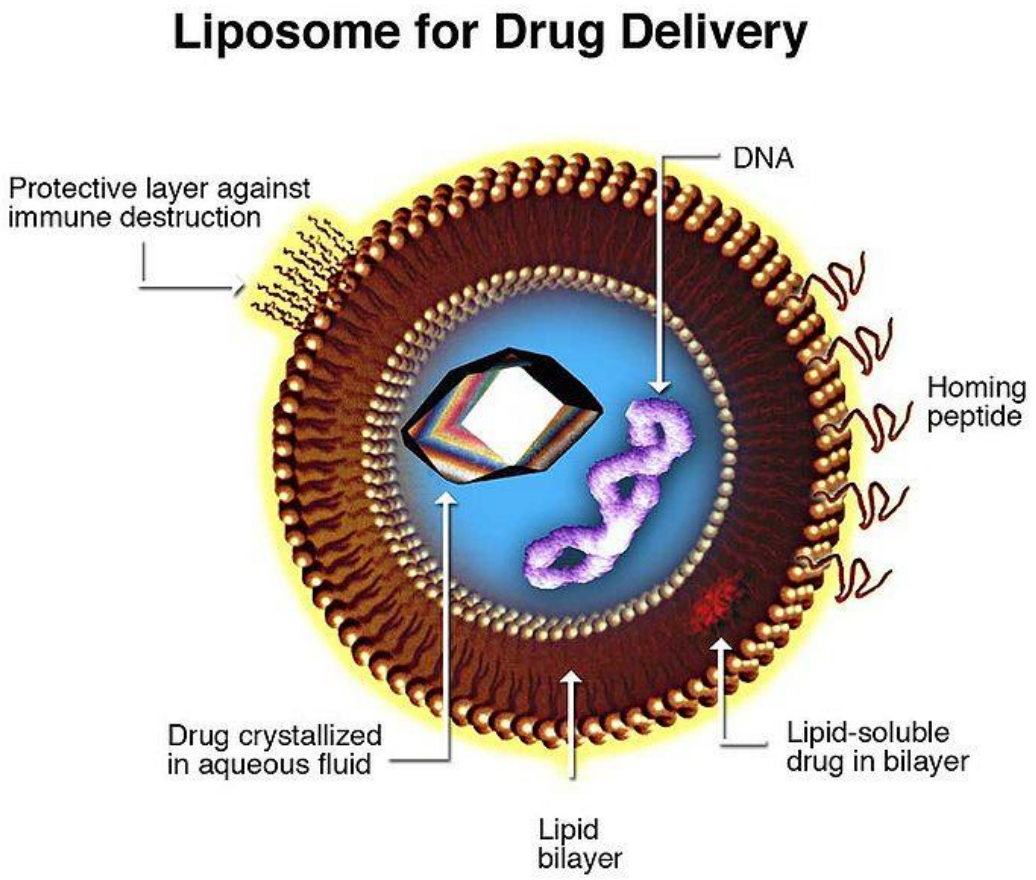
- Blood-brain-barrier endothelial cells limit paracellular flux of hydrophilic molecules and only nonionic, lipophilic and low molecular weight molecules may passively cross the BBB
- In addition, passive diffusion is limited through effective efflux pump systems (multiple organic anion carriers, esp. P-glycoprotein)



The «neurovascular unit», comprised of endothelial cells (E), pericytes (P), basal membrane (BM), nerve endings (NE) astrocytic endfeet (AEF) and resident microglia (M)
Armulik et al., Dev Cell 2011

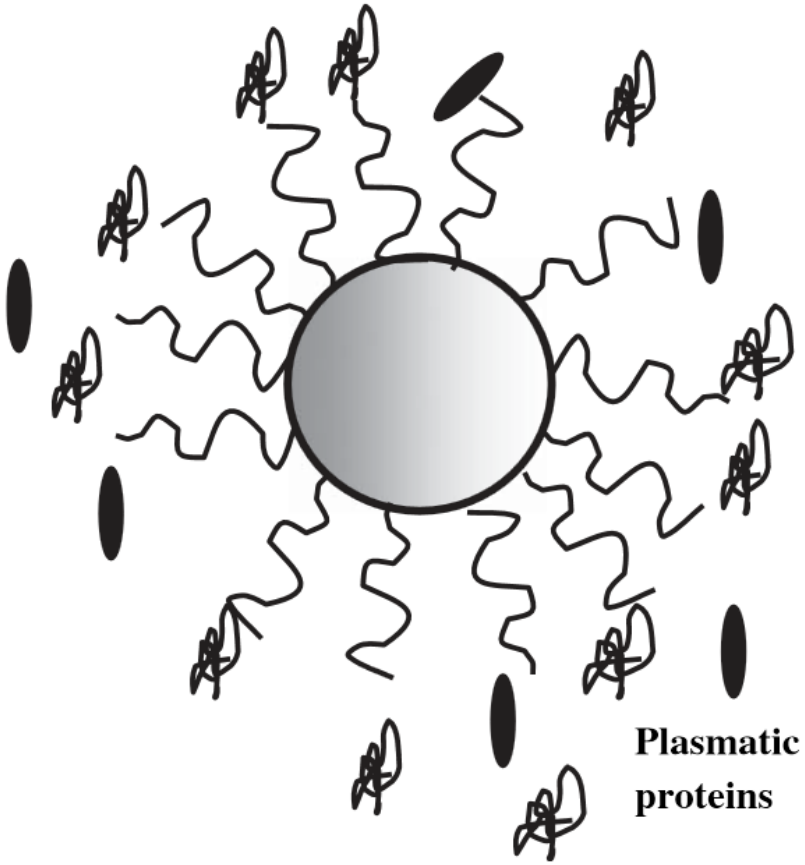
Several generations of nanocarriers in over 30 years

- Liposomes (artificial lipid bilayers in aqueous solution)

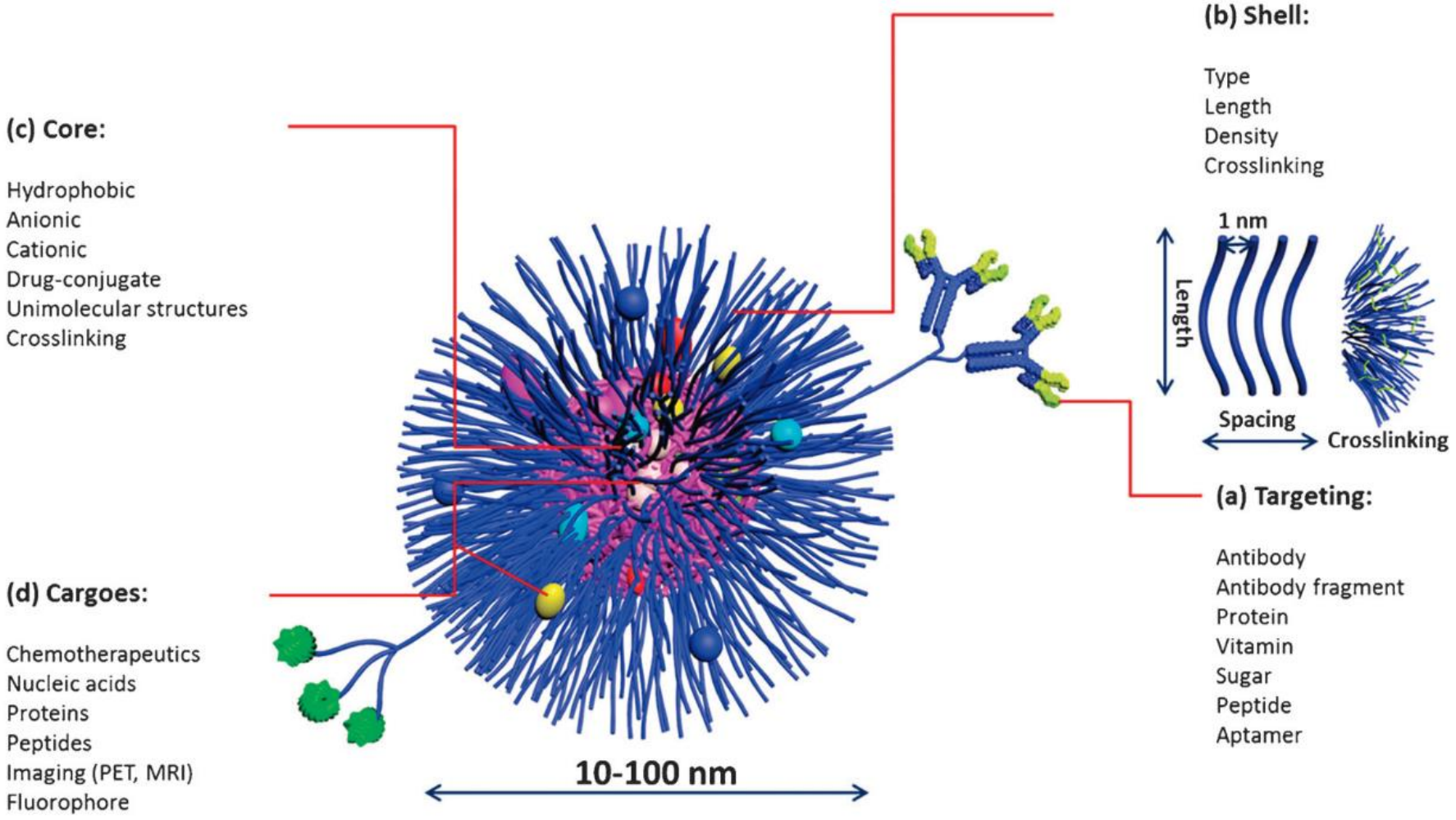


PEGylation of liposomes

P. Couvreur et al. / Progress in Solid State Chemistry 34 (2006) 231–235



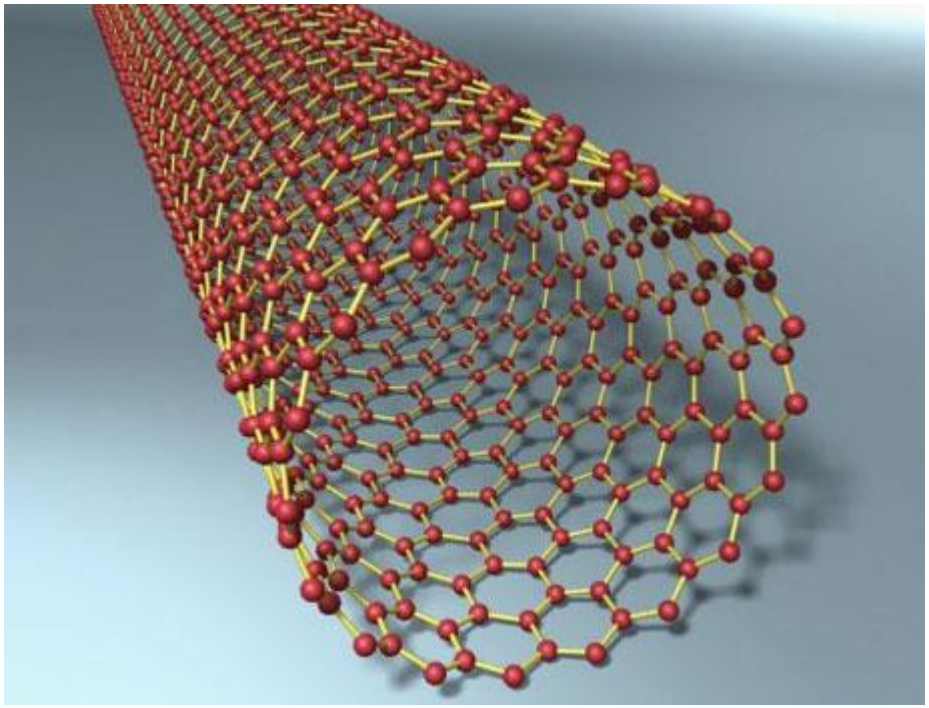
Polymeric nanoparticles (NPs)



Elsabahy and Wooley, Chem. Soc. Rev. 2012

«Hard» nanocarriers (i.e. metal based)

- Carbon nanotubes

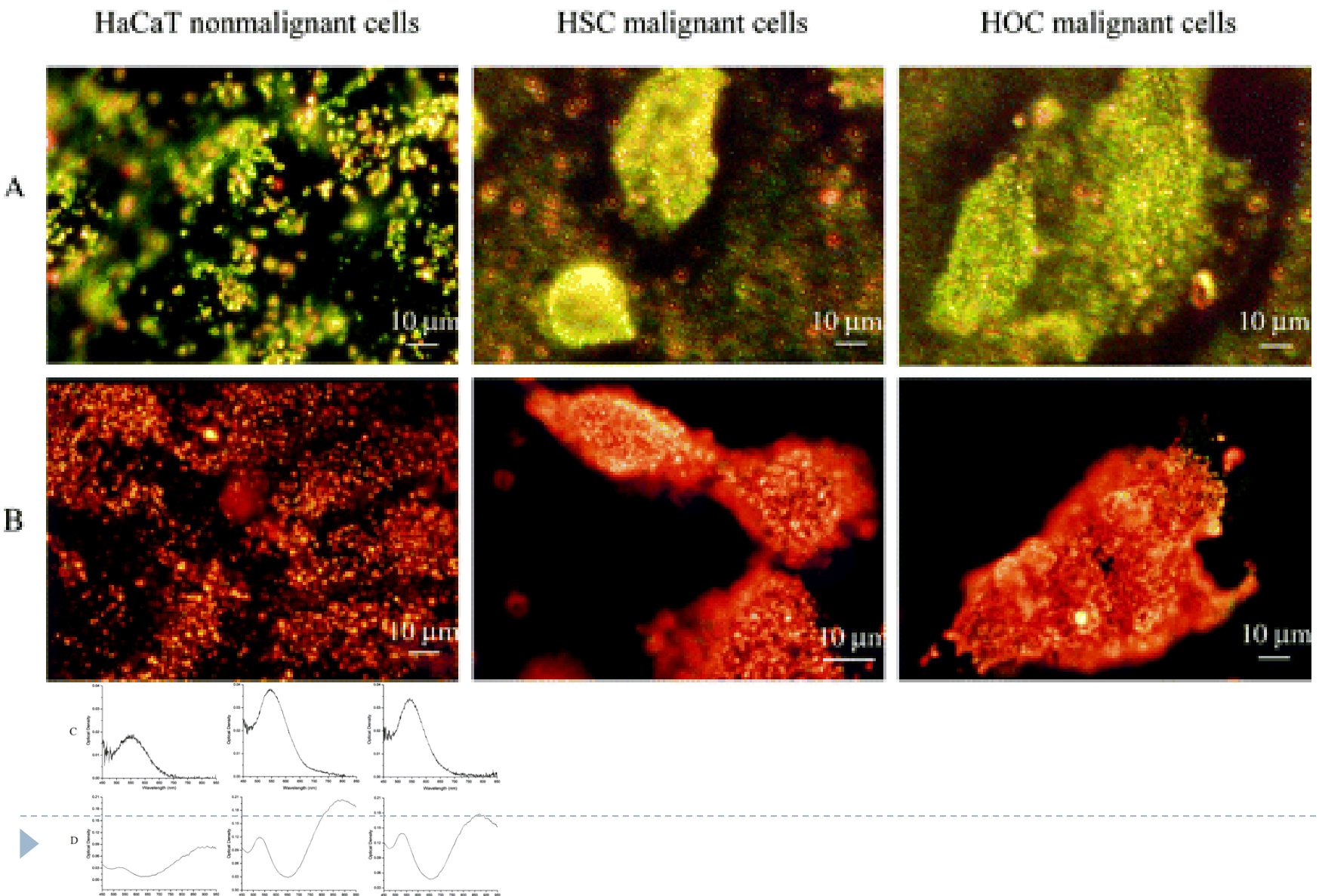


http://static.guim.co.uk/sys-images/Guardian/Pix/pictures/2008/05/20/nanotube.article.jpg

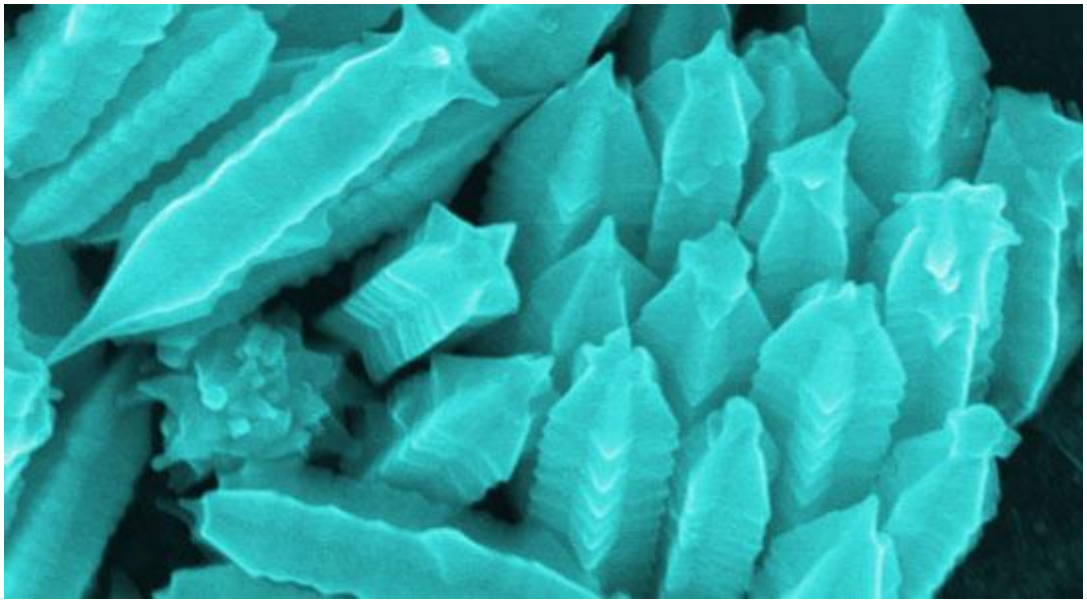
Near-infrared gold nanorods for photothermal cancer therapy (Huang et al., JACS 2006)

- Gold nanorods with an aspect ratio of 3.9 and a diameter of 35 nm

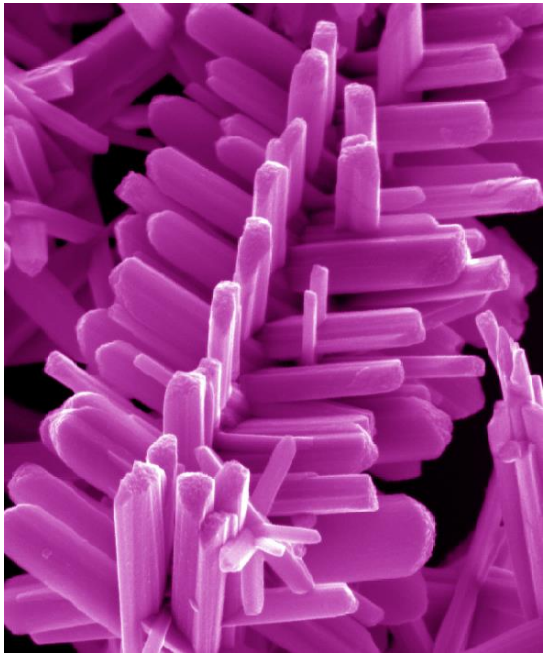
Uptake of gold nanorods in malignant and non-malignant cells



Emission of NIR spectra through design of noble metallic nanoparticles

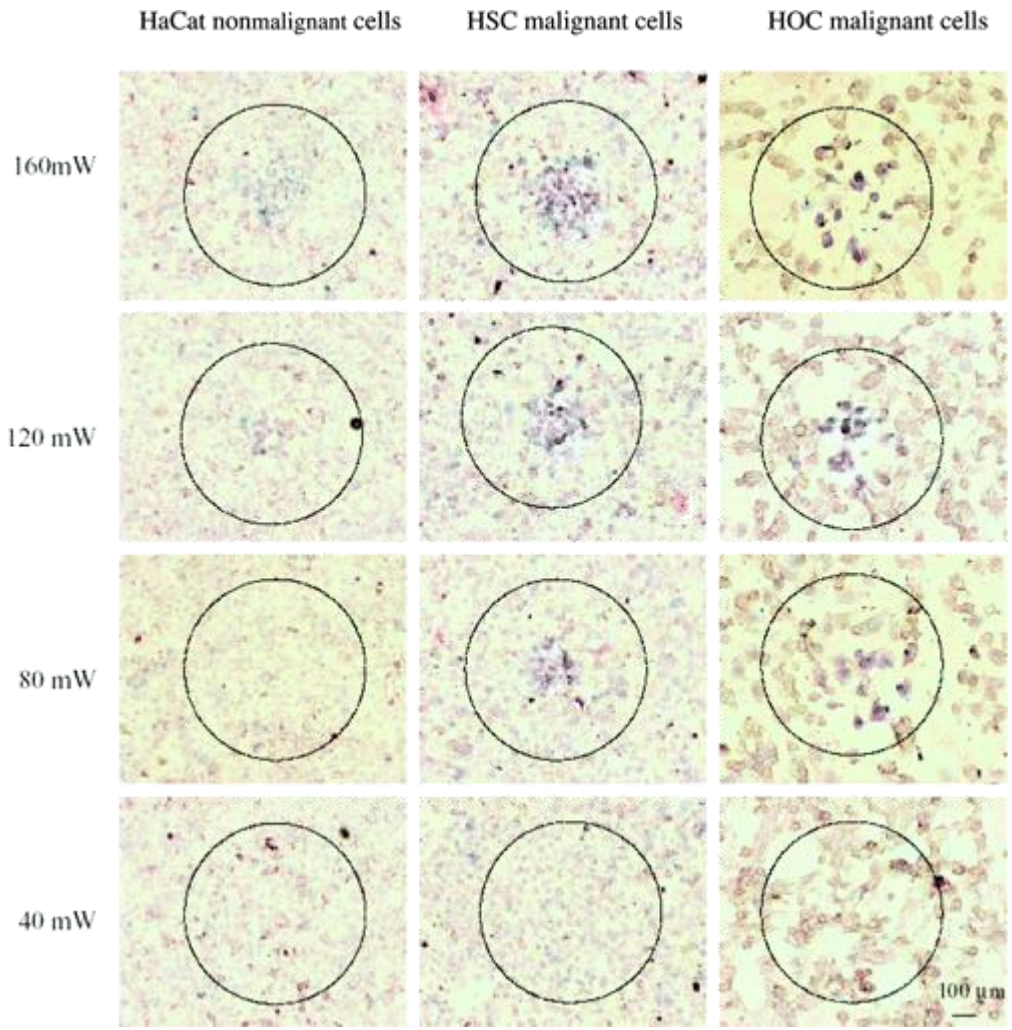


Zubarev Lab/Rice University



http://approach.rpi.edu/2009/05/05/1-%C3%97-103-words-branched-nanorods/

Selective photothermal therapy of cancer cells with anti-EGFR/Au nanorods



1 Dendrimer-Based Postnatal Therapy for Neuroinflammation and Cerebral Palsy in a Rabbit Model

Kannan et al., Science Translational Medicine 2012

Scientific question

- Cerebral palsy (CP) is a disease that affects children with a prevalence of 3.3/1000

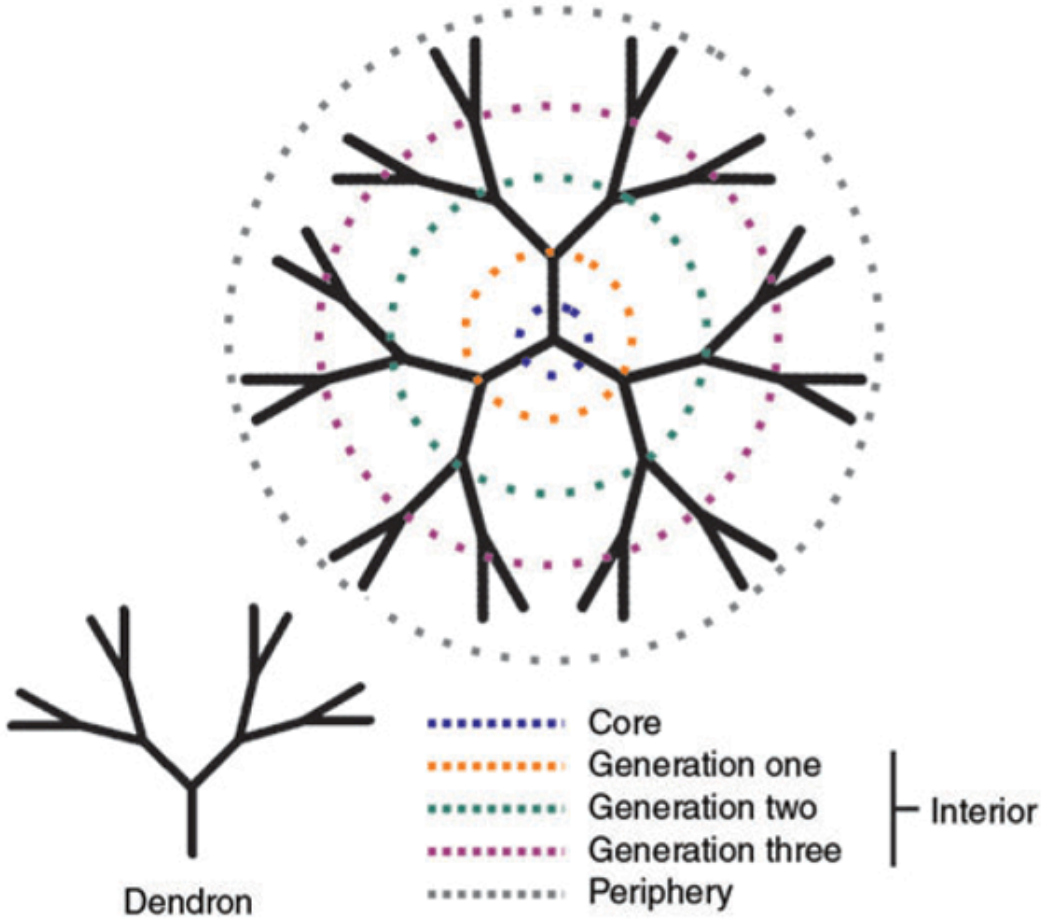
  Kirby et al., Res. Dev. Disabil. 32, 462–469 (2011)

- Periventricular leukomalacia is a pathophysiological mechanism proposed for development of CP
- Microglial activation is often observed next to CNS inflammation, while the neuroprotective abilities of astroglia are compromised in severe inflammation

- Does delivery of the antioxidans and anti-inflammatory agent N-acetyl-L-cysteine (NAC) via nanocarriers ameliorate inflammation and eventually disease progression?

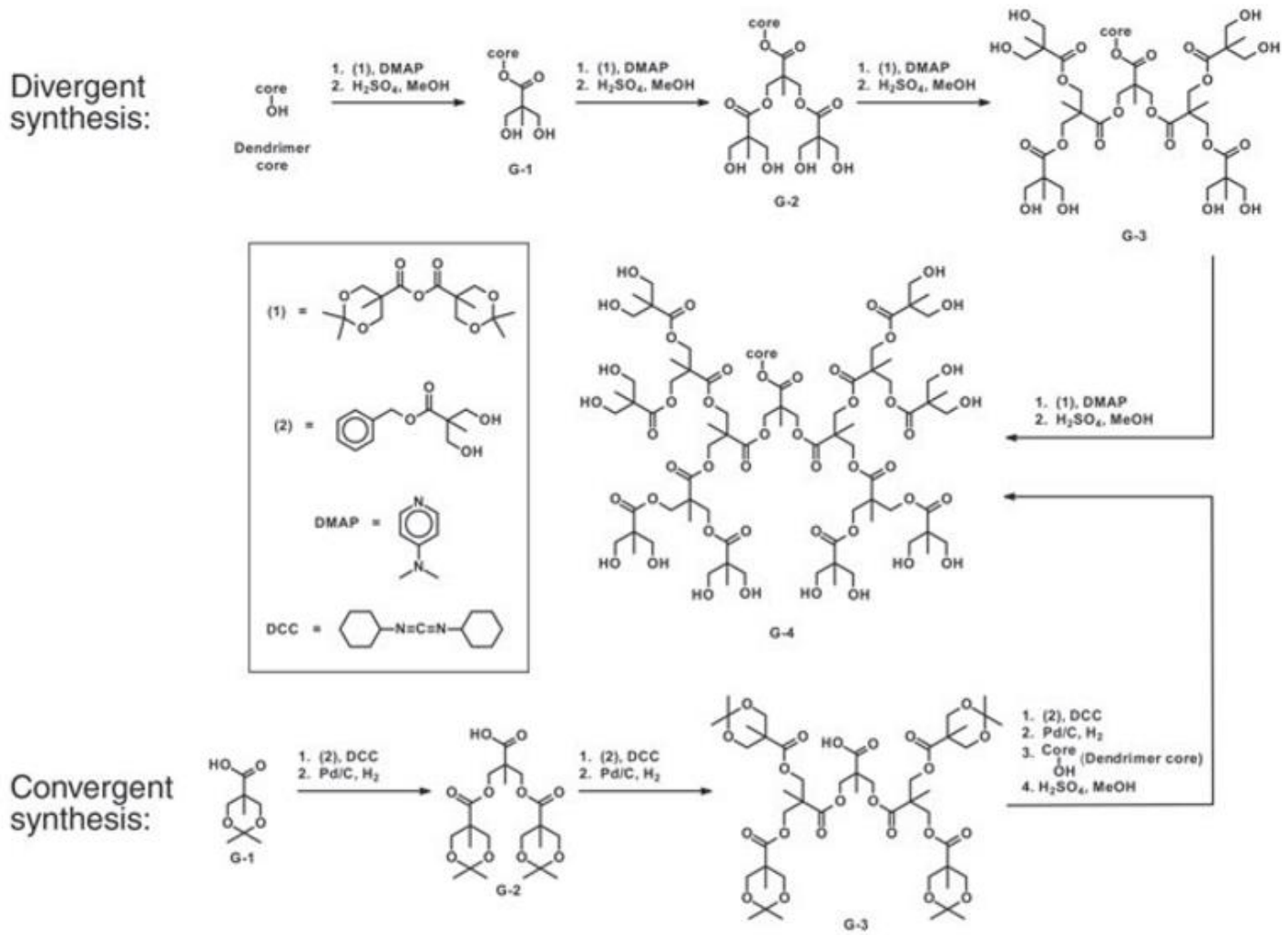
Dendrimers

- Polymeric molecules
- Multiple perfectly branched polymers radiating from a central core (>> dendrons)



Lee et al., Nat Biotech 2005

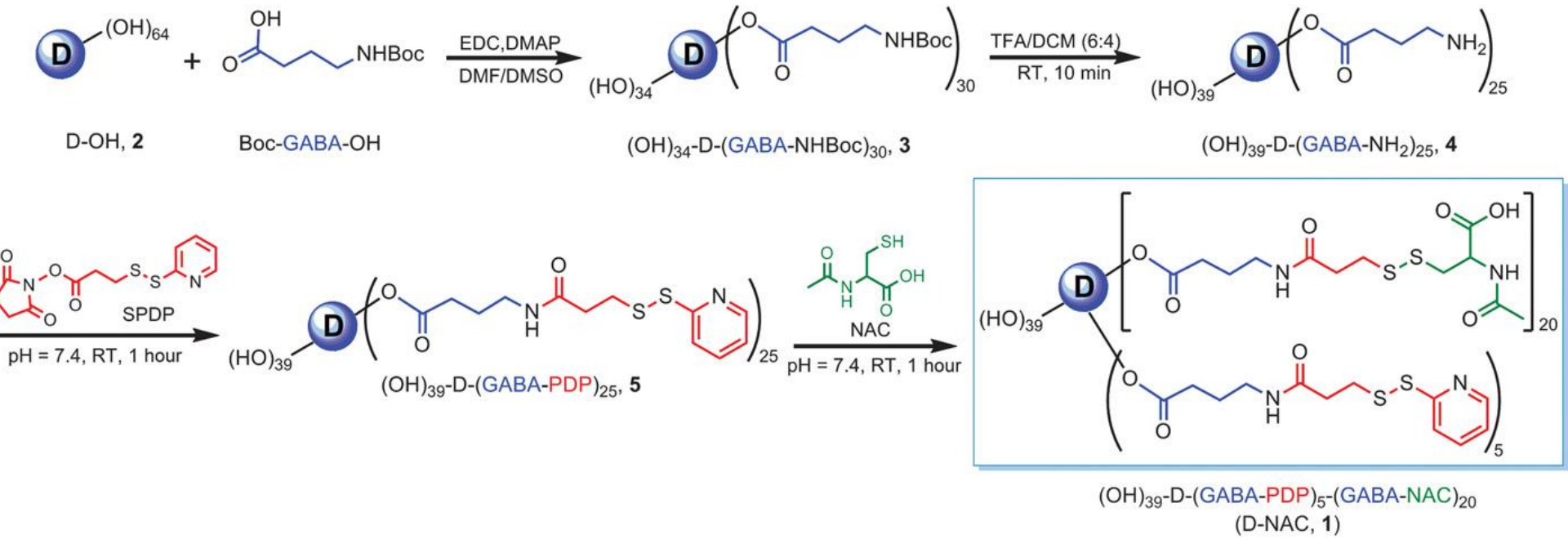
Dendrimer synthesis



Lee et al., Nat Biotech 2005

Production of dendrimer-NAC conjugate

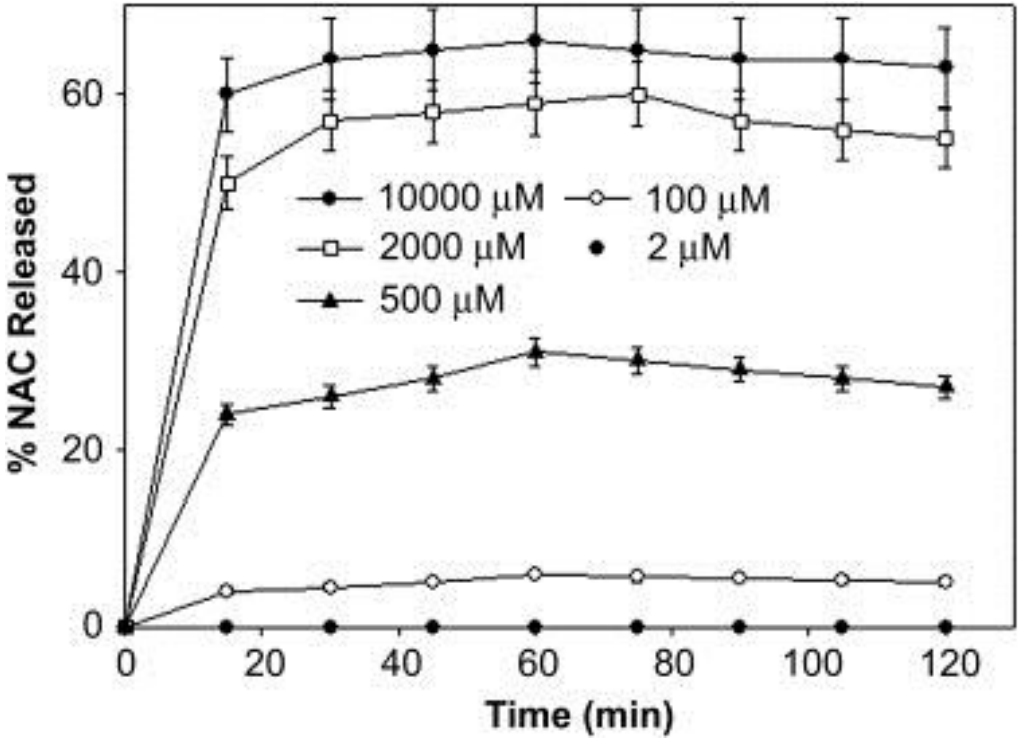
- … in 5 steps



Hydroxyl-terminated polyamidoamine (PAMAM) dendrimer with around 19% of drug payload

>> nontoxic, nonimmunogenic, cleared intact through the kidneys

Measuring in-vitro NAC release through exposure to different concentrations of glutathion



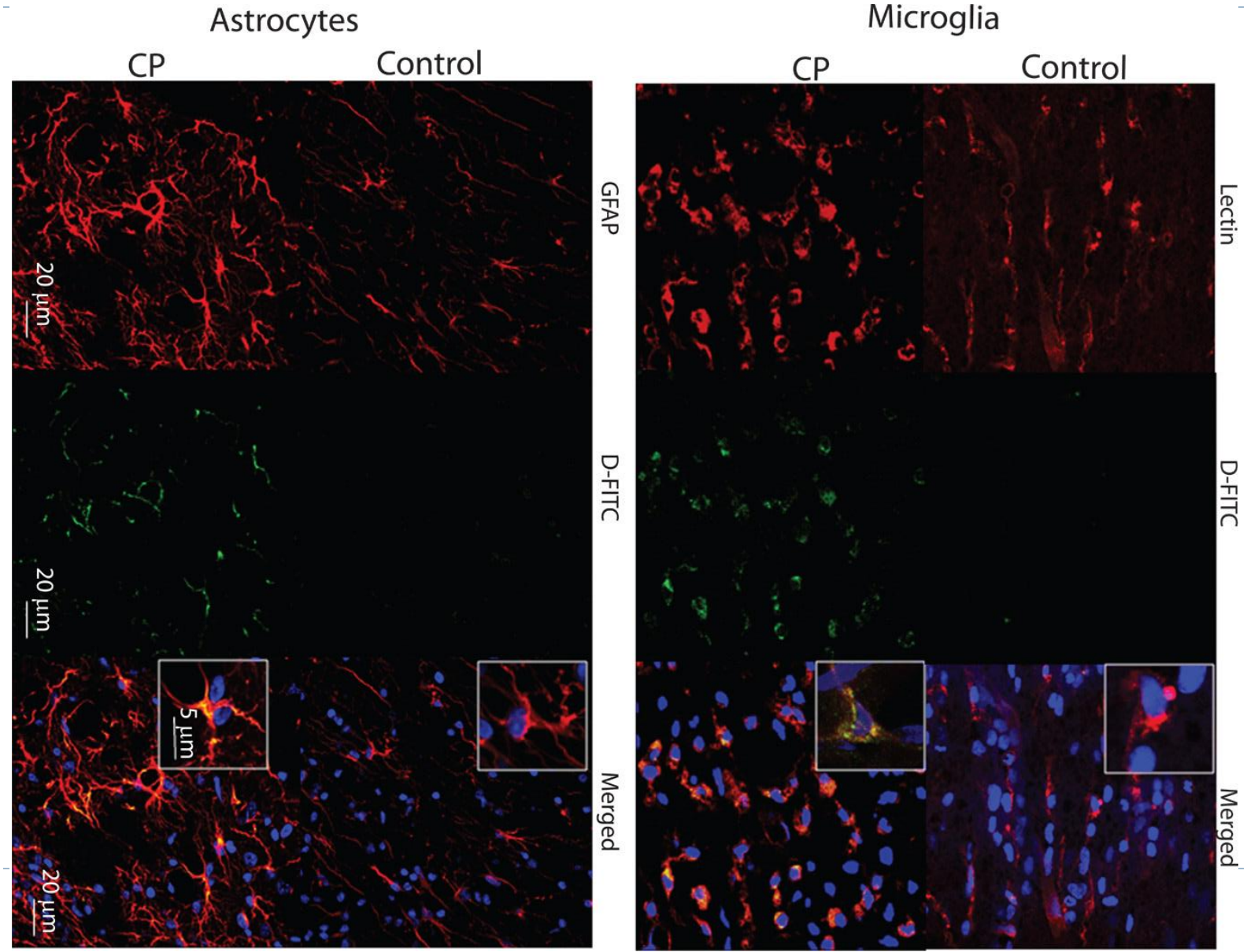
Kurtoglu et al., Biomaterials 2009

- NAC release was effective at 2 and 10 mM (concentration of GSH in astrocytes 4-25 mM, microglia around 20mM), but not at 2 µm (extracellular and plasma levels)

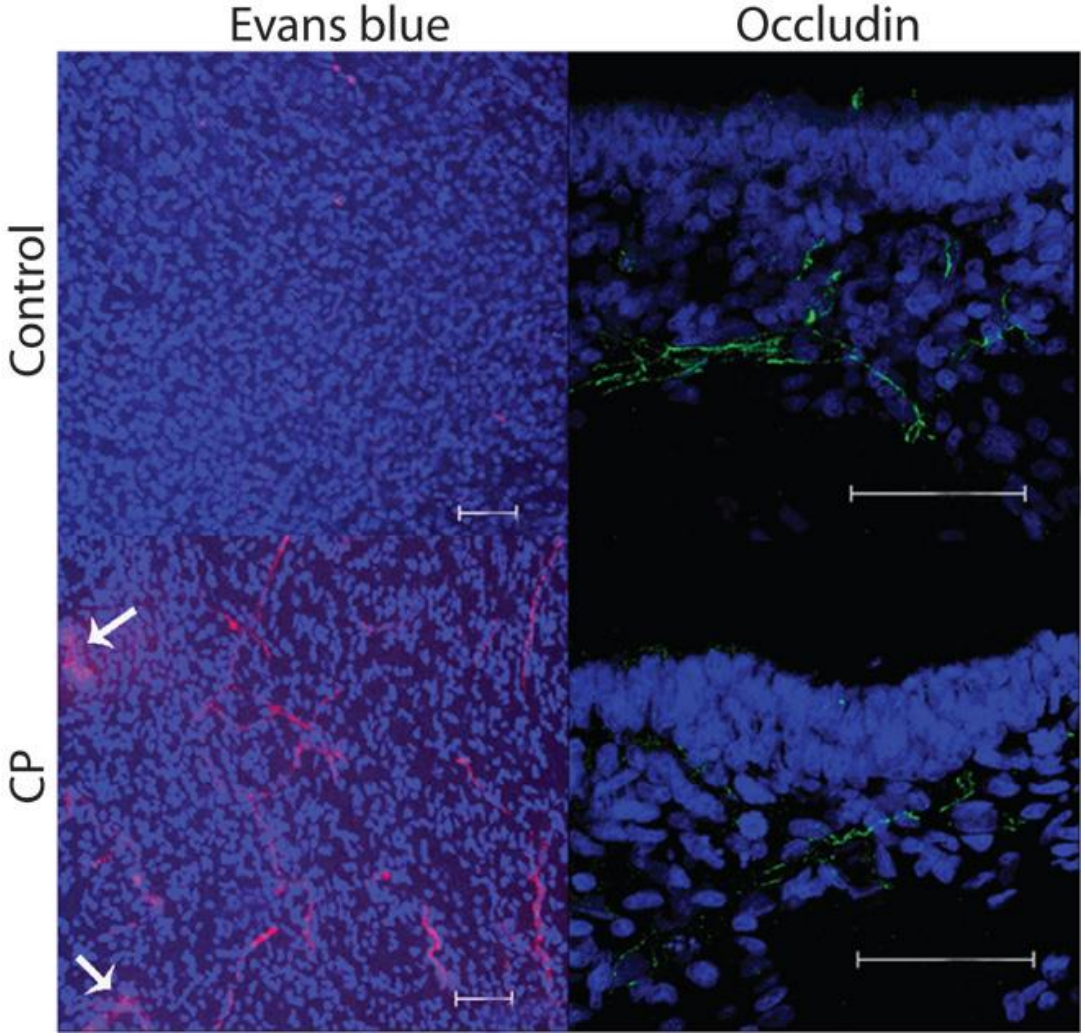
Rabbit model of cerebral palsy

- Pregnant rabbits were laparotomized, and 1 ml of E. coli endotoxine was injected along the length of the uterus
  - Uniform microglial activation in the periventricular region with phenotype of cerebral palsy

In vivo accumulation of FITC-labeled D-NAC in microglia and astrocytes (single i.v. injection 6h postnatally)



Breakdown of blood-brain-barrier

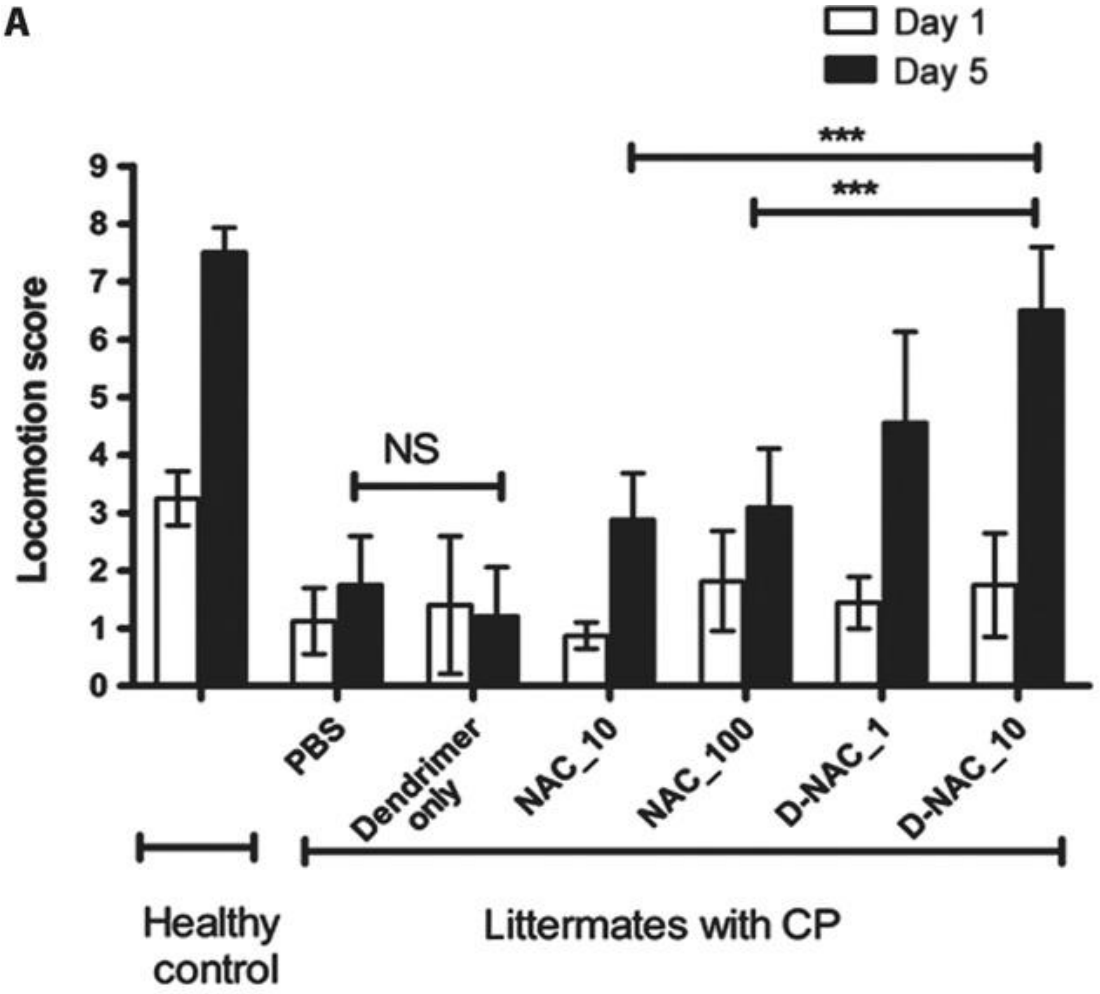


CP phenotype

- Inability to take steps
- decreased coordination
- impaired balance
- hypertonia of the hindlimbs

Videos S1 (healthy controls) and S2 (PBS-treated CP mice)

Amelioration of clinical phenotype through administration NAC and D-NAC



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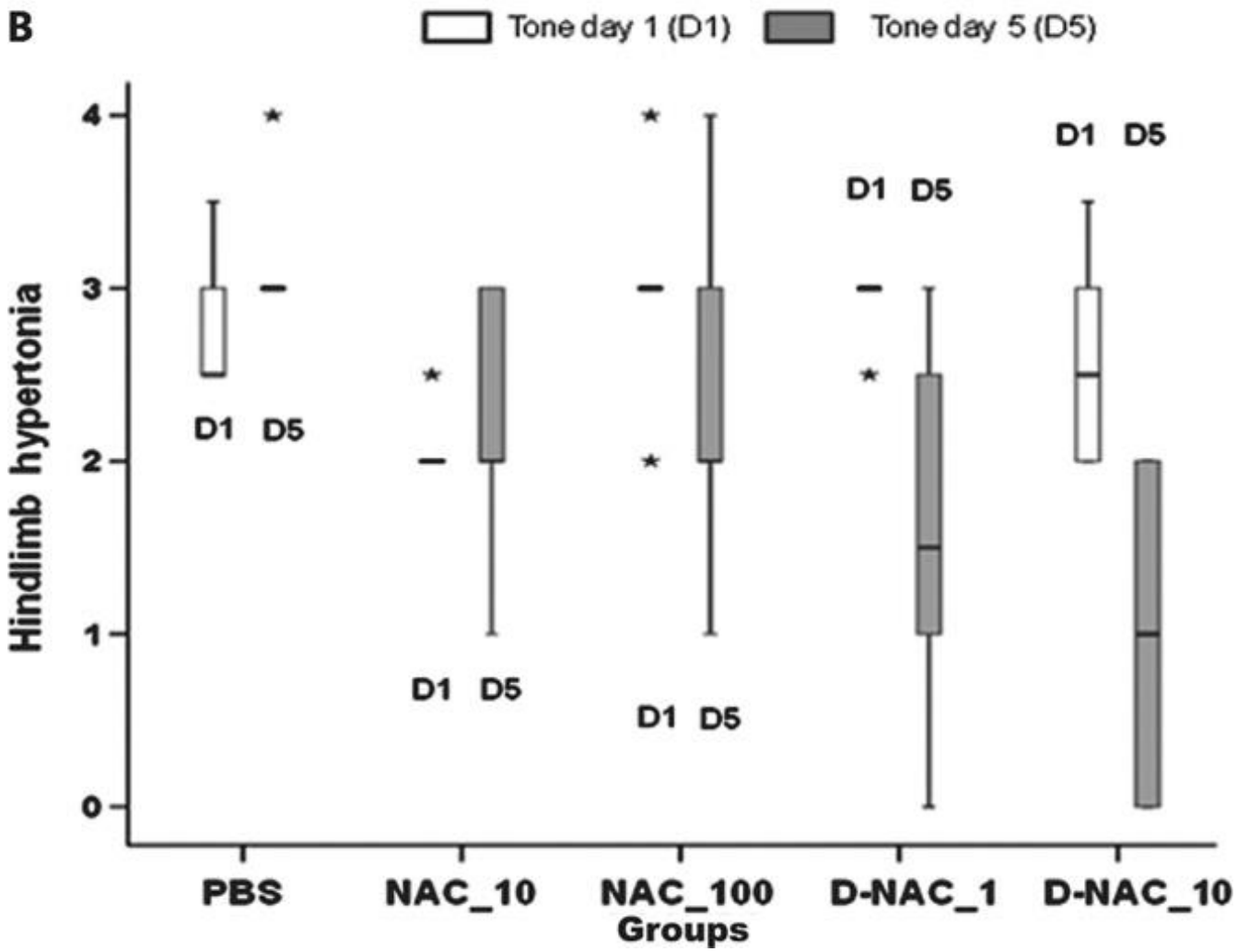
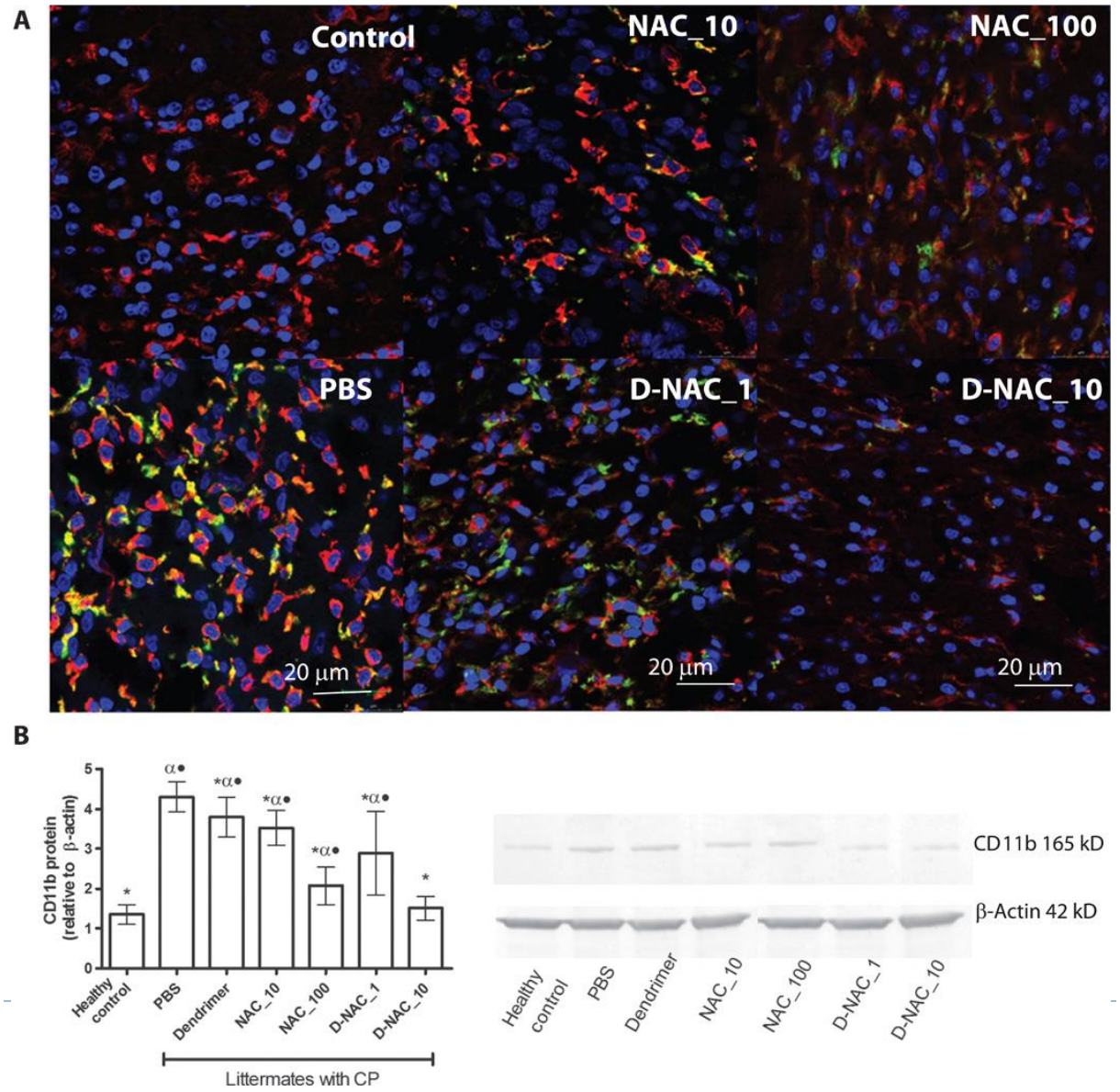


Amelioration of CP phenotype through NAC-dendrimer conjugate

- Videos of NAC-treated (S5) and D-NAC-treated (S3) CP mice

Suppression of proinflammatory microglia through D-NAC administration



Not shown

- Kit weight gain and survival
- D-NAC suppresses markers of oxidative injury and inflammation in the brains of CP kits
- D-NAC therapy suppresses proinflammatory microglia
- D-NAC therapy improves myelination and attenuates neuronal injury

Conclusions of the study

- Specific treatment effect in targeted cells through GSH-dependent activation
- Amelioration of clinical disease course through dendrimer-NAC conjugate
  - no significant treatment success through NAC alone
- Dendrimers pass the BBB in diseased conditions
  - possible translation into other human diseases (AD, stroke etc..)