



**The Abdus Salam
International Centre for Theoretical Physics**



2268-16

**Conference on Nanotechnology for Biological and Biomedical
Applications (Nano-Bio-Med)**

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Nanoparticles and the Blood Brain Barrier

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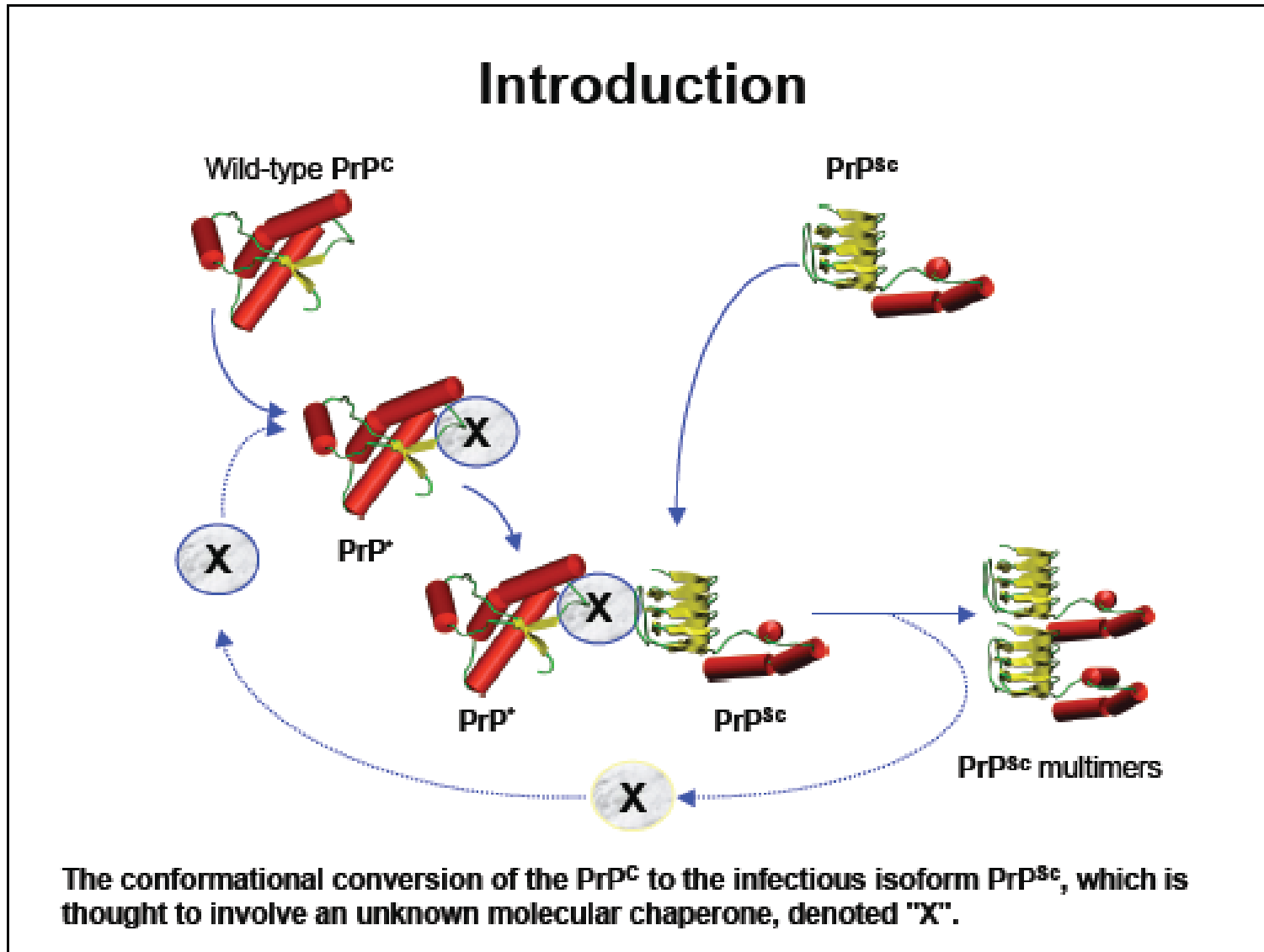
A fluorescence microscopy image showing a field of cells. The cells exhibit a mix of red and green fluorescence, with some cells showing both colors, suggesting the presence of different markers or components. The background is dark, making the fluorescent spots stand out.

Nanoparticles and the blood brain barrier

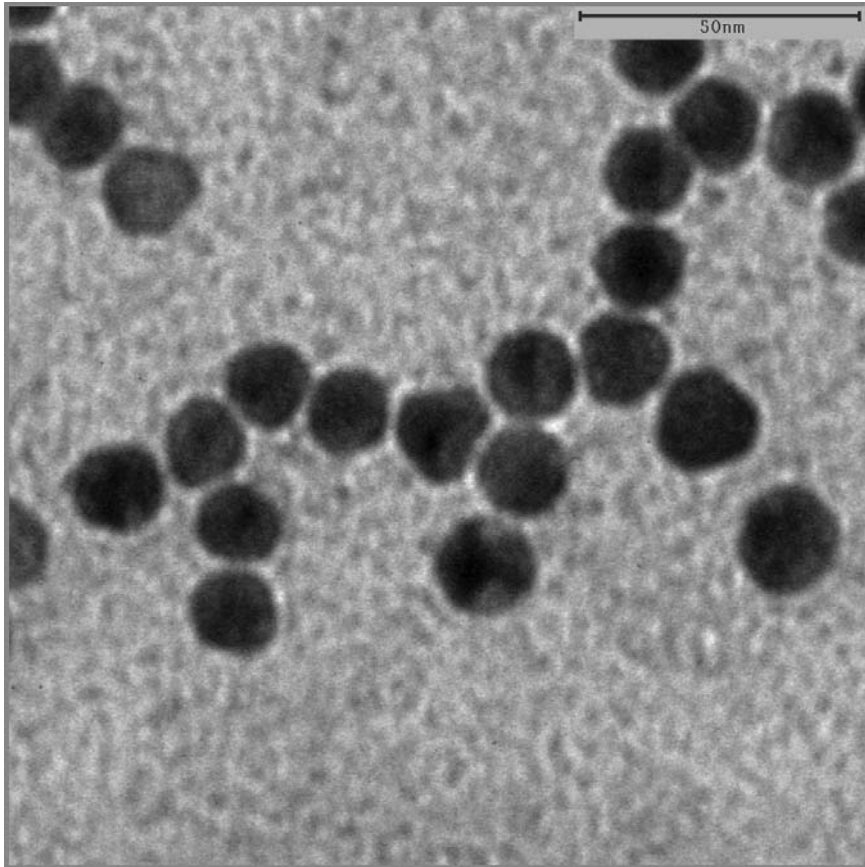
Silke Krol

Fondazione IRCCS Istituto Neurologico
"Carlo Besta", Milan, Italy

Prion diseases



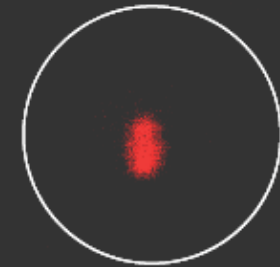
Theranostics



Nanogold:

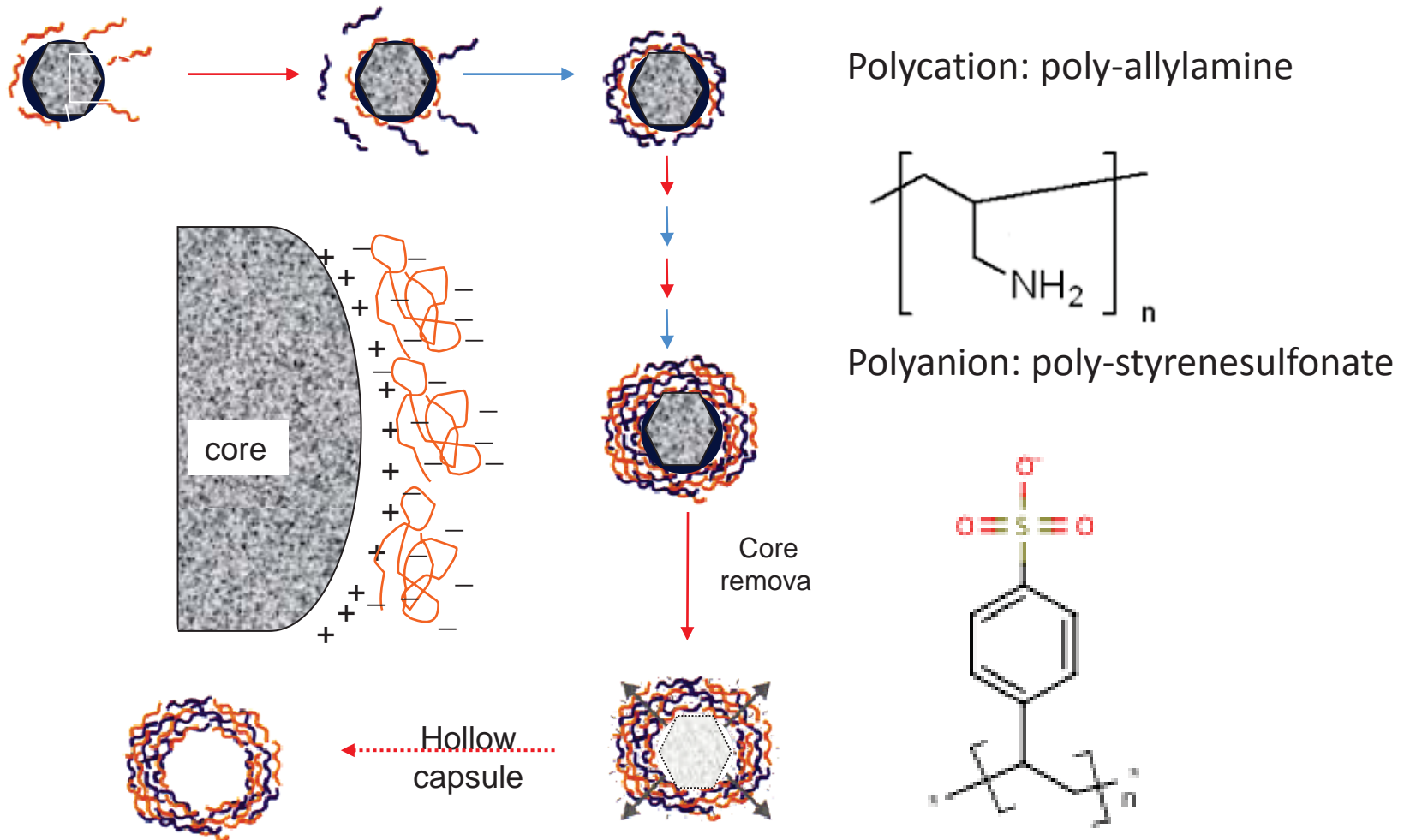
- diameter: 10 to 50 nm in diameter
 - monodispersed
 - Non-cytotoxic (particle diameter: >5 nm)
 - Chemically inert
-
- Visible in two-photon excitation (TPE) fluorescence, electron microscopy, x-ray

Au plasmon luminescence at
800 nm excitation
wavelength



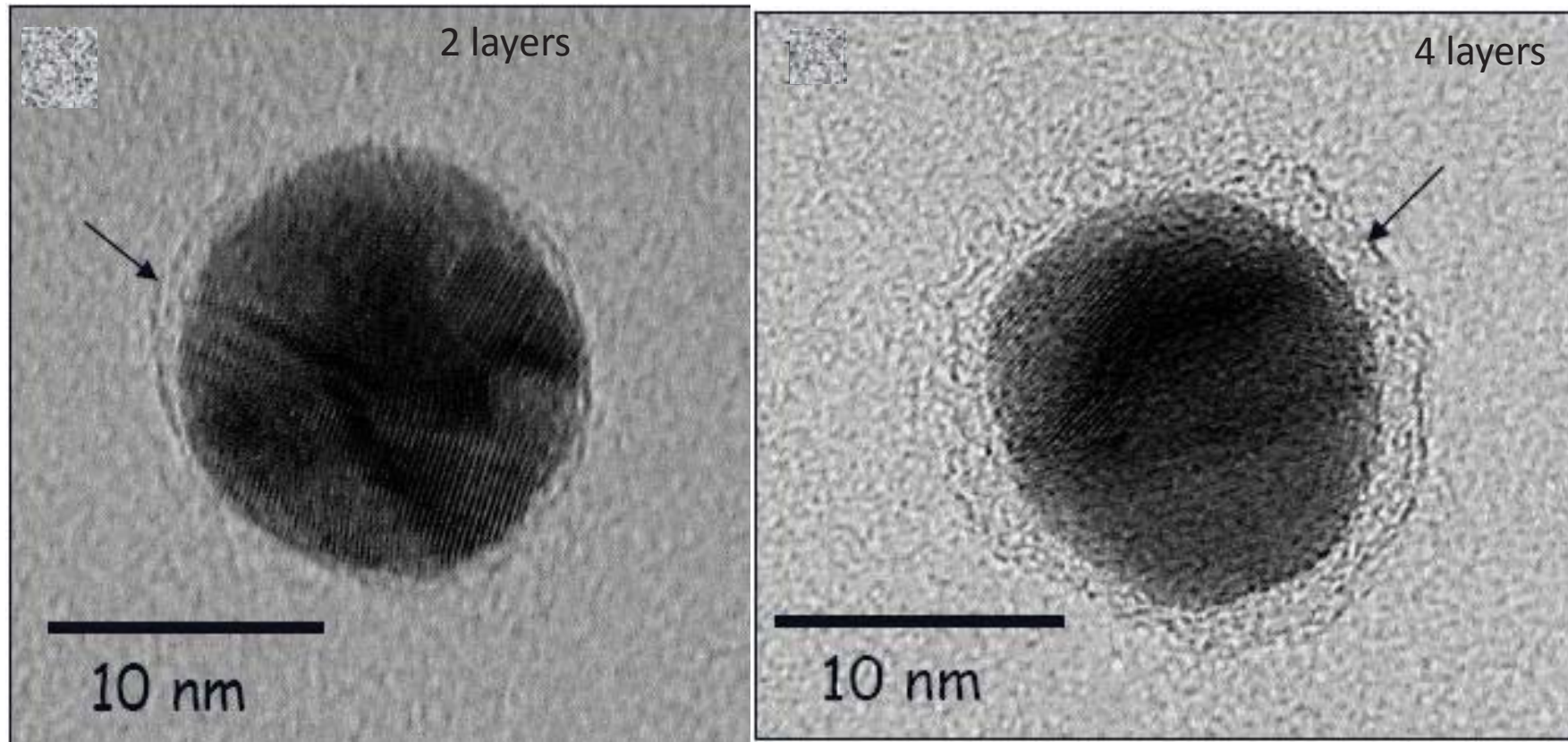
—
700 nm

Layer-by-Layer-technique



- 1) Decher, G., Science (1997), 277, 1232-1237
- 2) G.B. Sukhorukov, M. Brumen, E. Donath, H. Möhwald, J. Phys. Chem. (1999), 103, 6434

Nanoencapsulation with polyelectrolyte multilayers

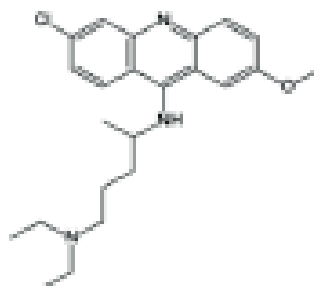


Chanana M., Gliozzi A., Diaspro A., Chodnevskaja I., Huelwel S., Moskalenko V., Ulrichs K., Galla H.-J., Krol S. (2005) *Nano Letters* 5(12), 2605-2612

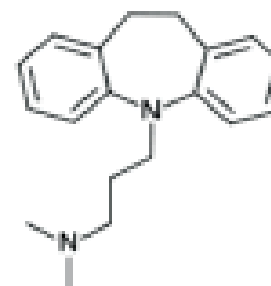
Comparison in efficacy with other drugs considered for treatment

Materials

- Cell lines:
 - Mouse hypothalamic GT1 and ScGT1
 - Mouse neuroblastoma N2a and ScN2a
- Small molecules:
 - Known antiprion compounds:



Quinacrine (Fluka)



Imipramine (Sigma)

PrP^{Sc} inhibition and cellular toxicity of quinacrine, imipramine and the nanoparticles in ScGT1 and ScN2a cells.

Compounds	PrP ^{Sc} inhibition ^(a)		% cell viability \pm SE ^(b)	
	ScGT1 (EC ₅₀ \pm SE, μ M)	ScN2a (EC ₅₀ \pm SE, μ M)	ScGT1	ScN2a
Quinacrine	0.4 \pm 0.1	0.3 \pm 0.1	100 \pm 4	100 \pm 2
Imipramime	6.2 \pm 0.4	5.5 \pm 0.5	100 \pm 7	100 \pm 5
Nanoparticles	ScGT1 (EC ₅₀ \pm SE, pM)	ScN2a (EC ₅₀ \pm SE, pM)	ScGT1	ScN2a
	Positive surface charge –PAH (NG-15nm)			
1A	8.3 \pm 0.5	8.4 \pm 0.6	100 \pm 6	100 \pm 3
2A	8.8 \pm 0.2	24.5 \pm 1.0	100 \pm 1	97 \pm 1

PCT/IB2009/054922

PCT/EP2009/056042

Tran et al. 2010 Nanoscale 2, 2724-2732

**PROBLEM IS THE TRANSLATION
FROM *IN VITRO* TO *IN VIVO***

If we are injecting NP intravenously to hit the target organ, nature is against us!

- 4.7-5 liter of blood
- 96,500 kilometers blood vessels in the human body (Vogel, Steven. Vital Circuits, pp. 15-16; World Book Encyclopedia, vol. 2, p. 424.).
- brain microvessels: surface area of 100 cm²/g brain tissue / surface of 130,000 cm² in a brain of an average male subject (1.3-1.4 kg for an adult).
- brain blood flow : 54 milliliters per 100 grams of brain weight per minute /740 milliliters of blood circulating in the brain every minute

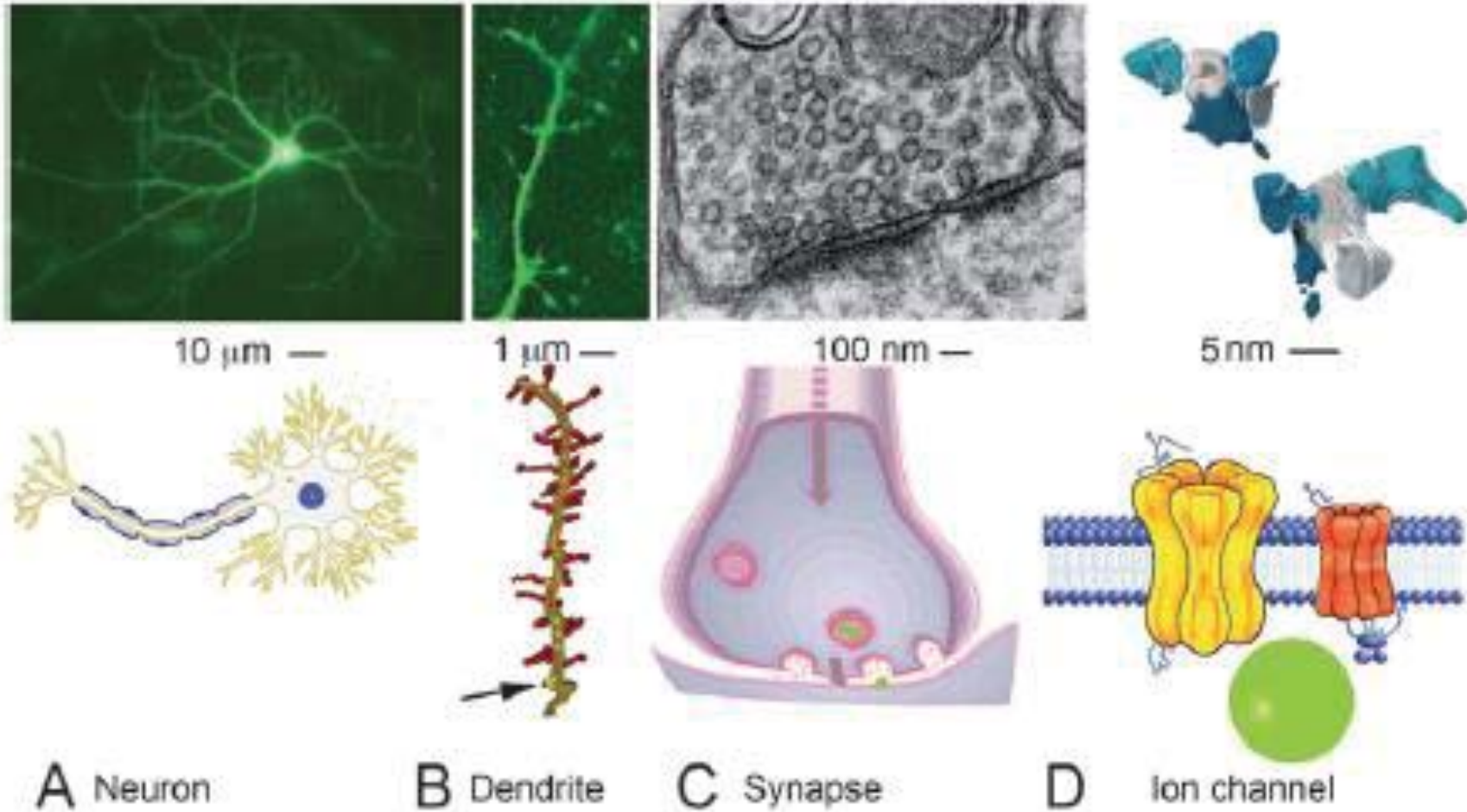
If our target are specific neurons it is
even worse!

number of neurons:

100.000.000.000

(Ndabahaliye, 2002)

Structures in the brain



REVIEW

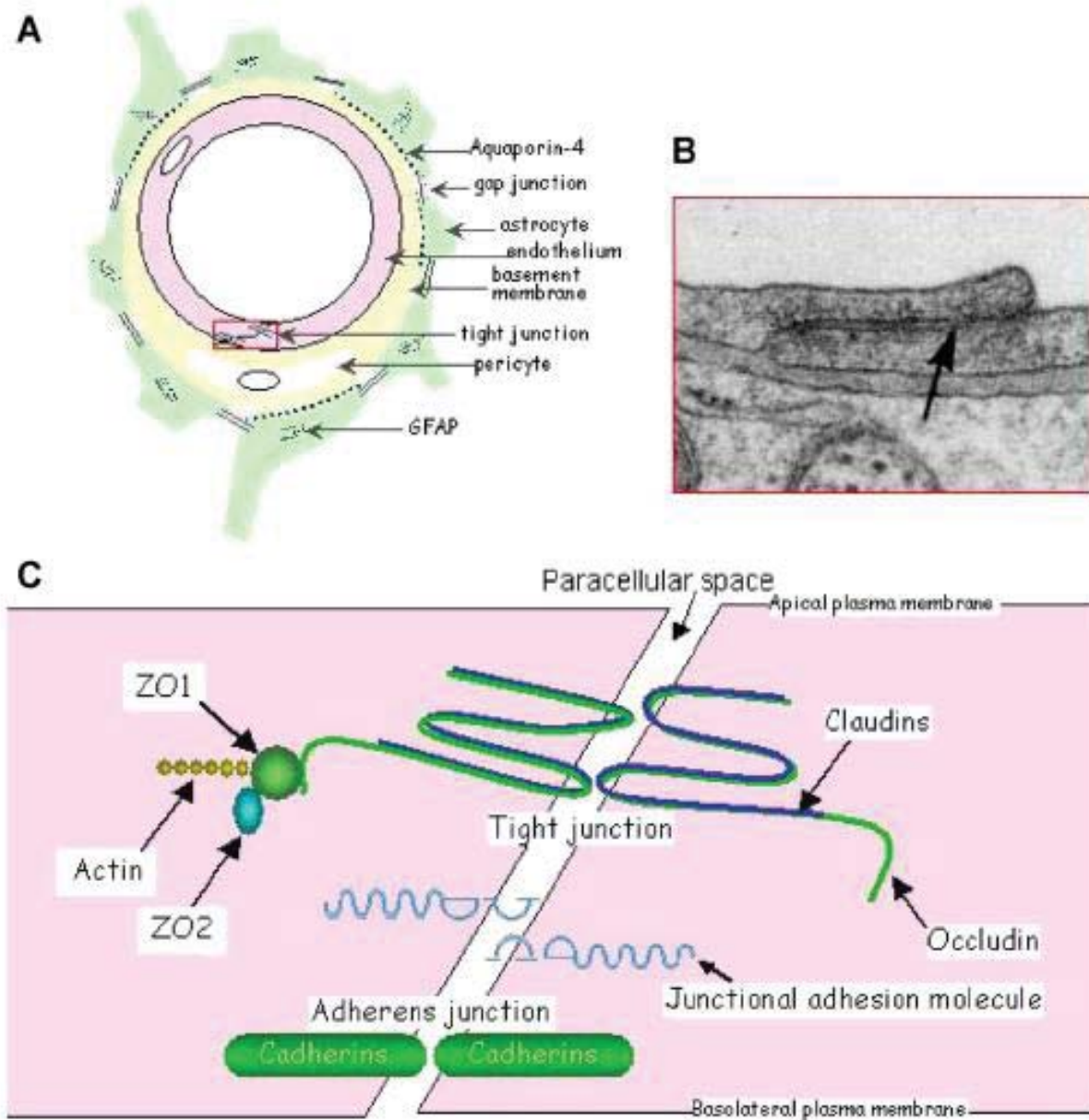
www.rsc.org/nanoscale | Nanoscale

Nanotechnology for *in vitro* neuroscience

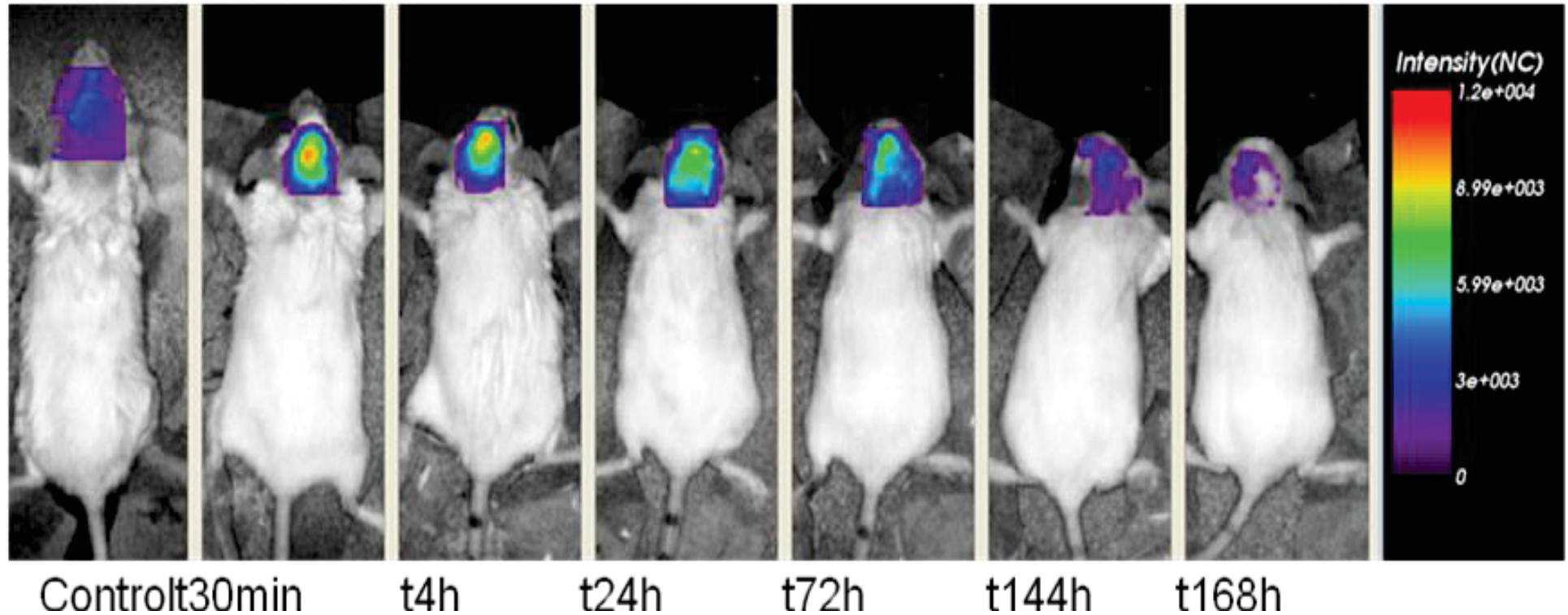
Daniel R. Cooper and Jay L. Nadeau*

Problem: The blood brain barrier

P. Ballabh et al. / Neurobiology of Disease 16 (2004) 1-13



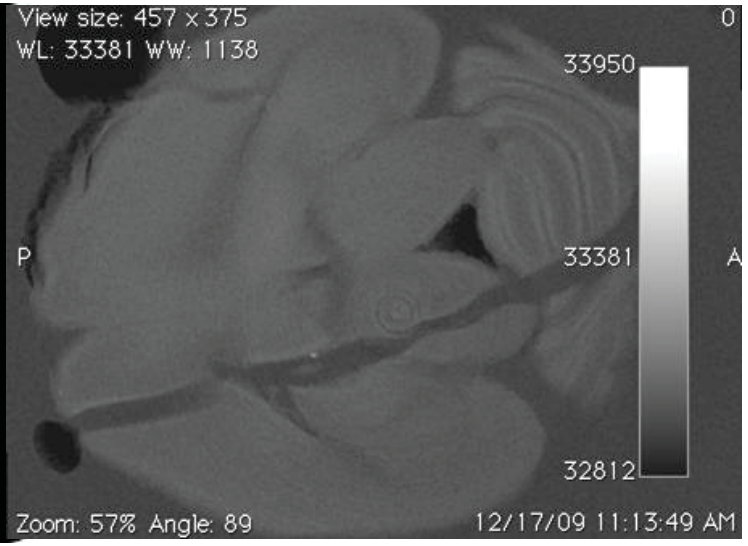
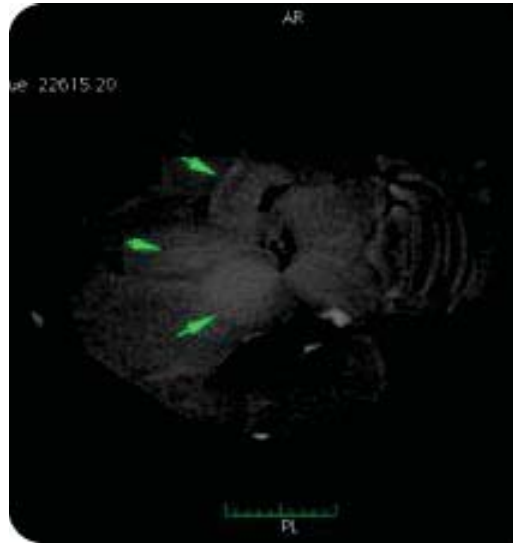
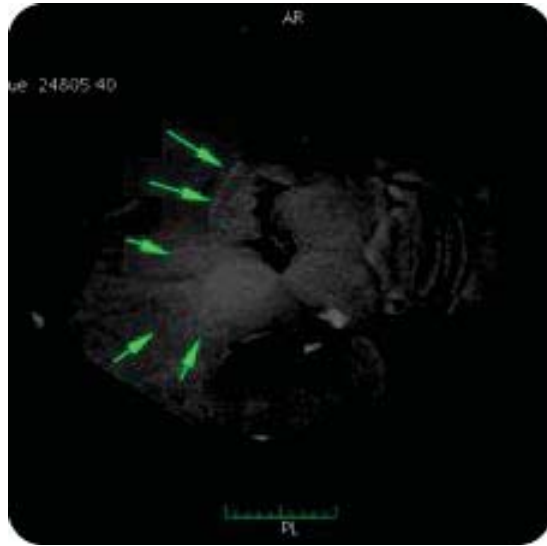
Near infrared time-domain Optical Imaging



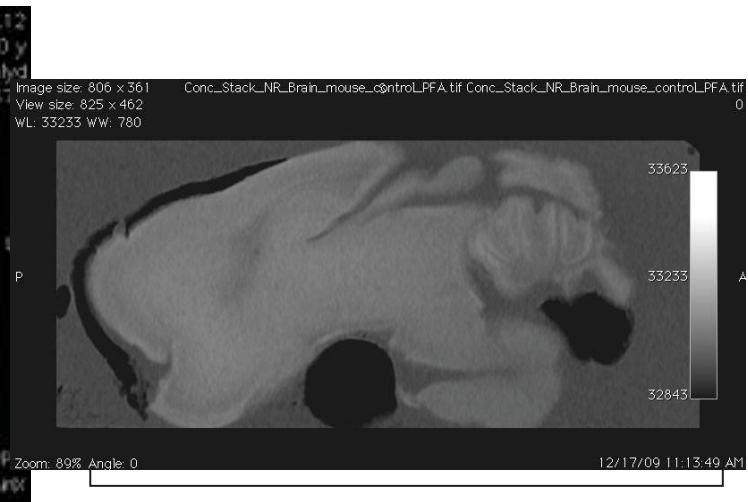
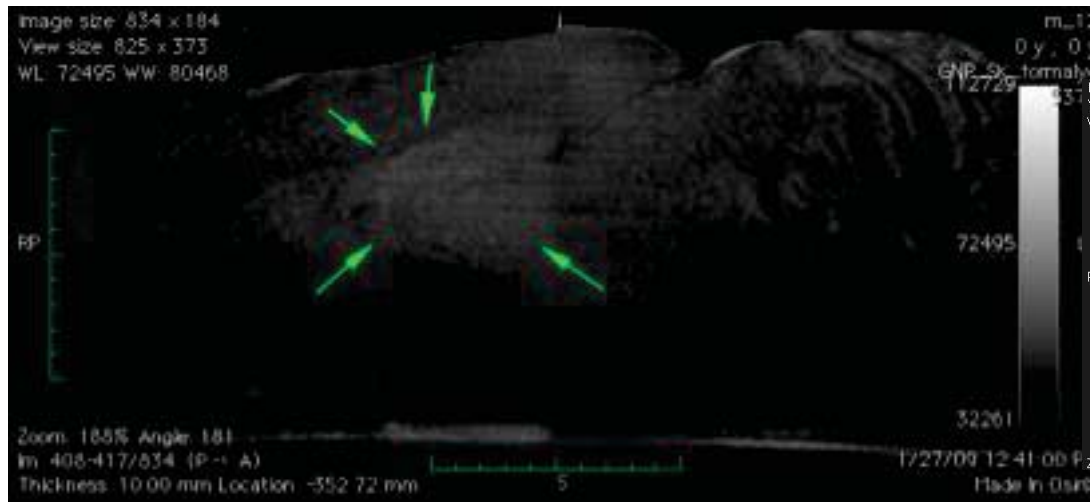
Collaboration with the CBM
optical imaging core facility

X-ray of the distribution of gold in the brain

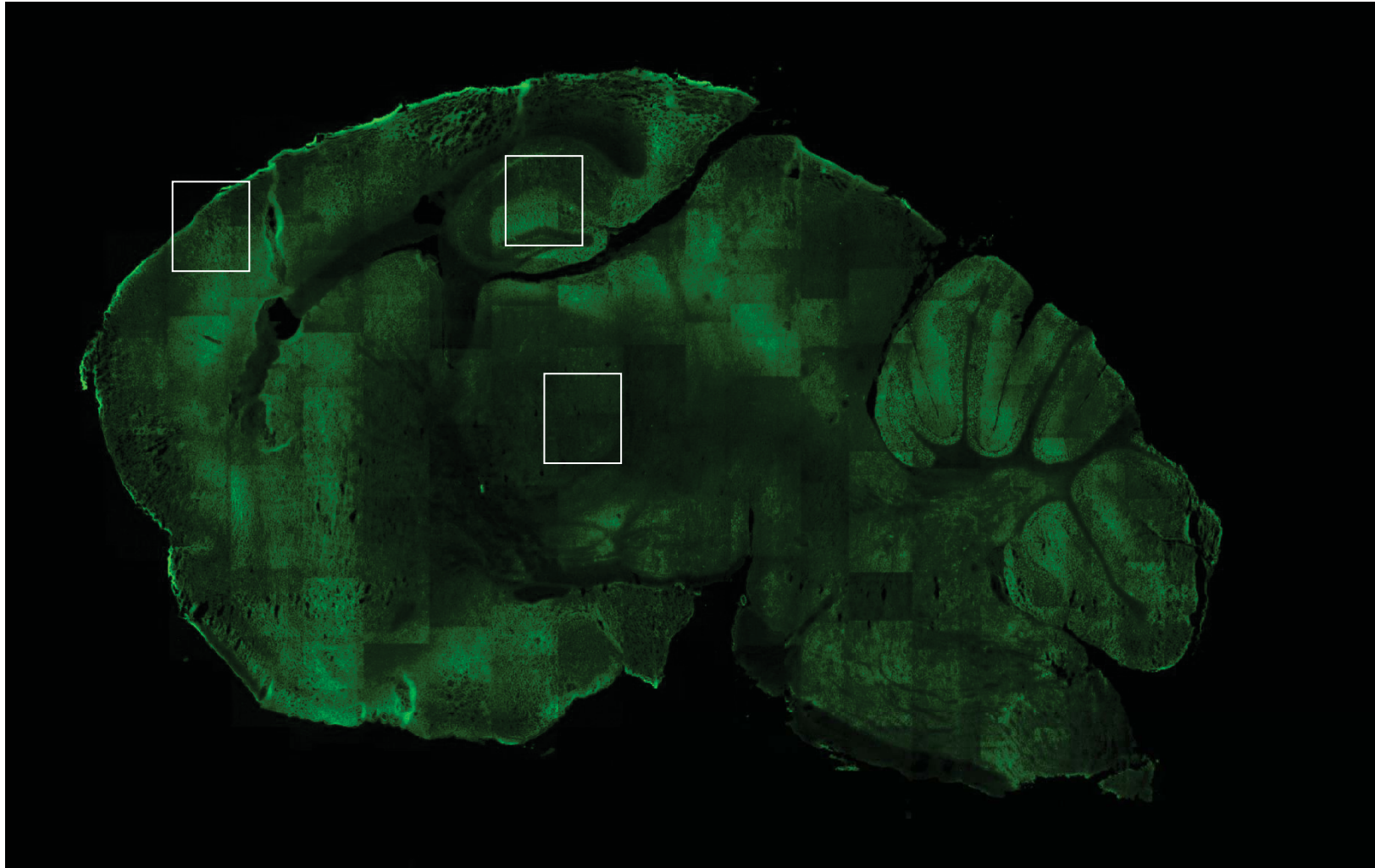
transverse plane



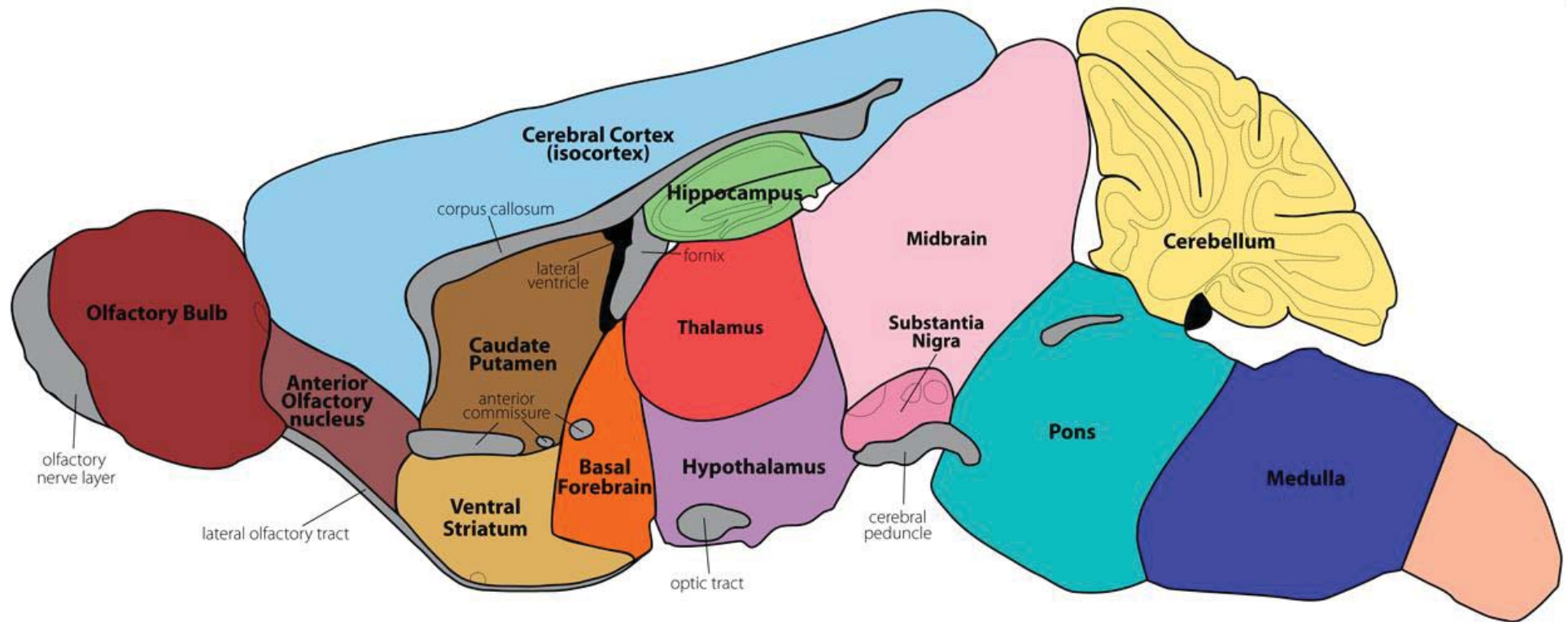
sagittal plane



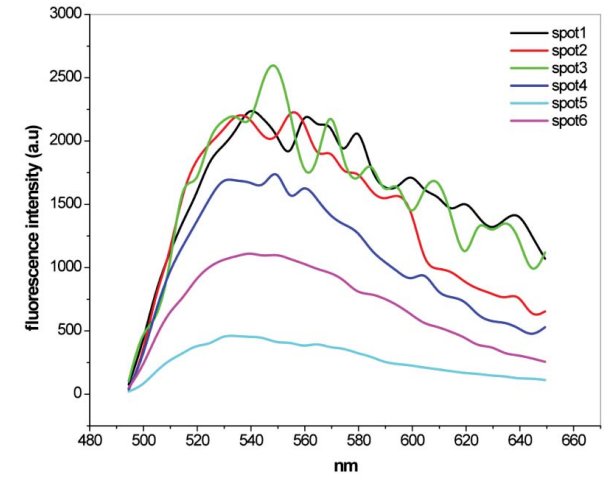
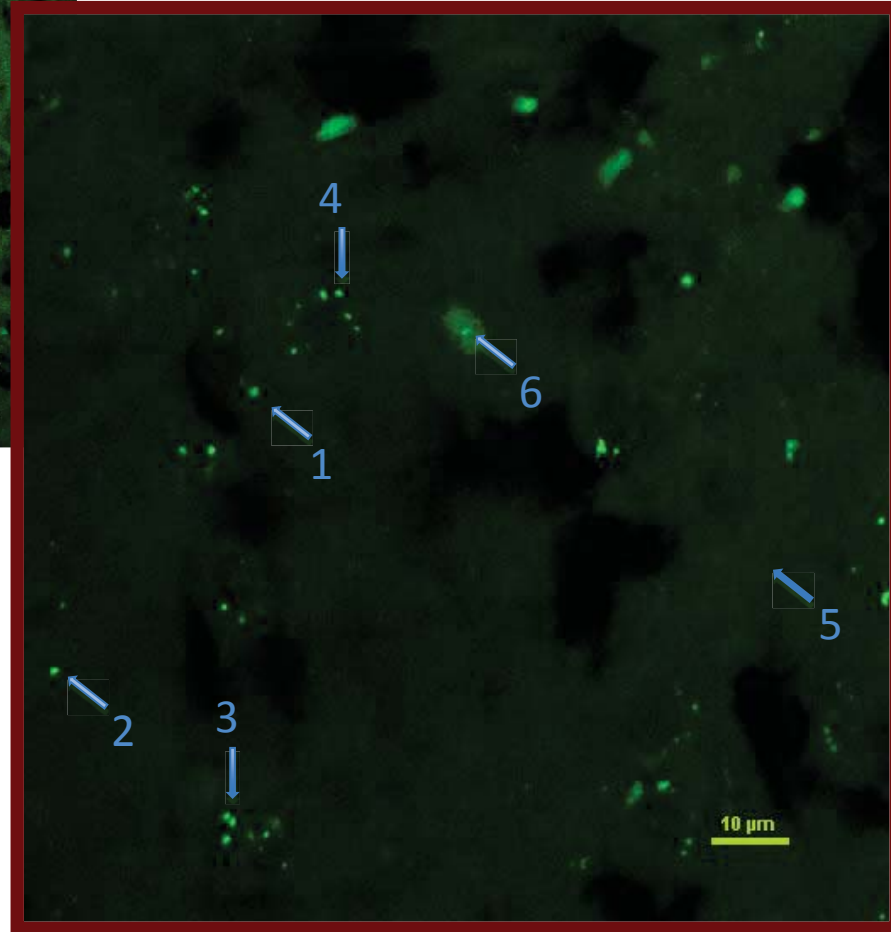
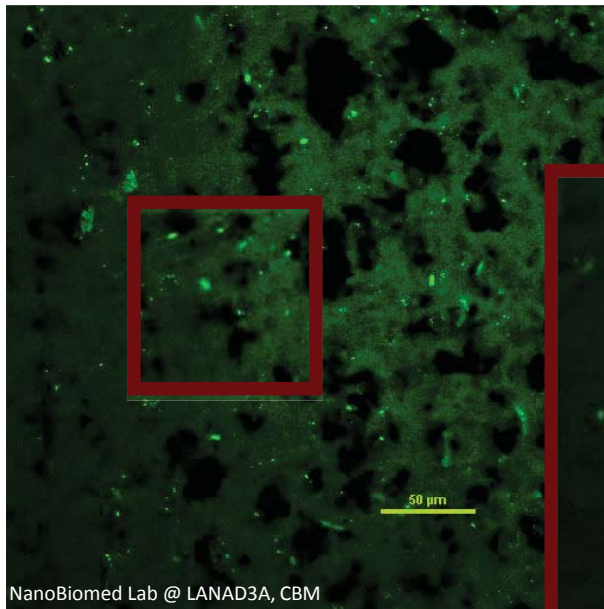
Confocal-laser-scanning microscopy (CLSM)



The mouse brain



Cortex – confocal microscopy

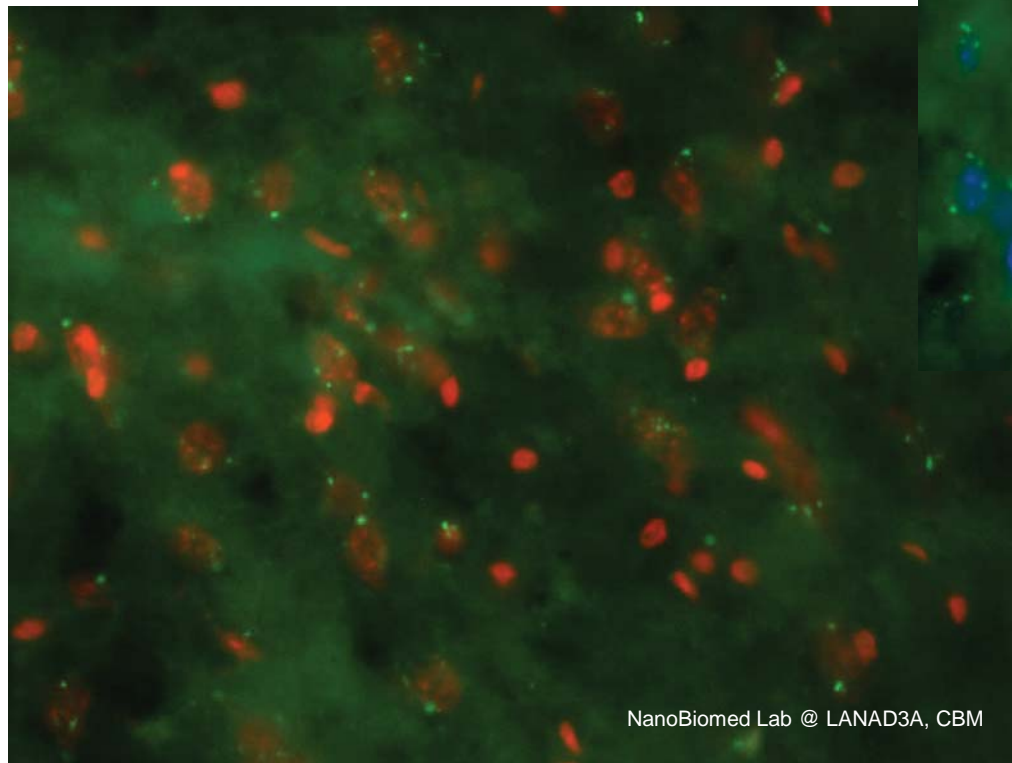
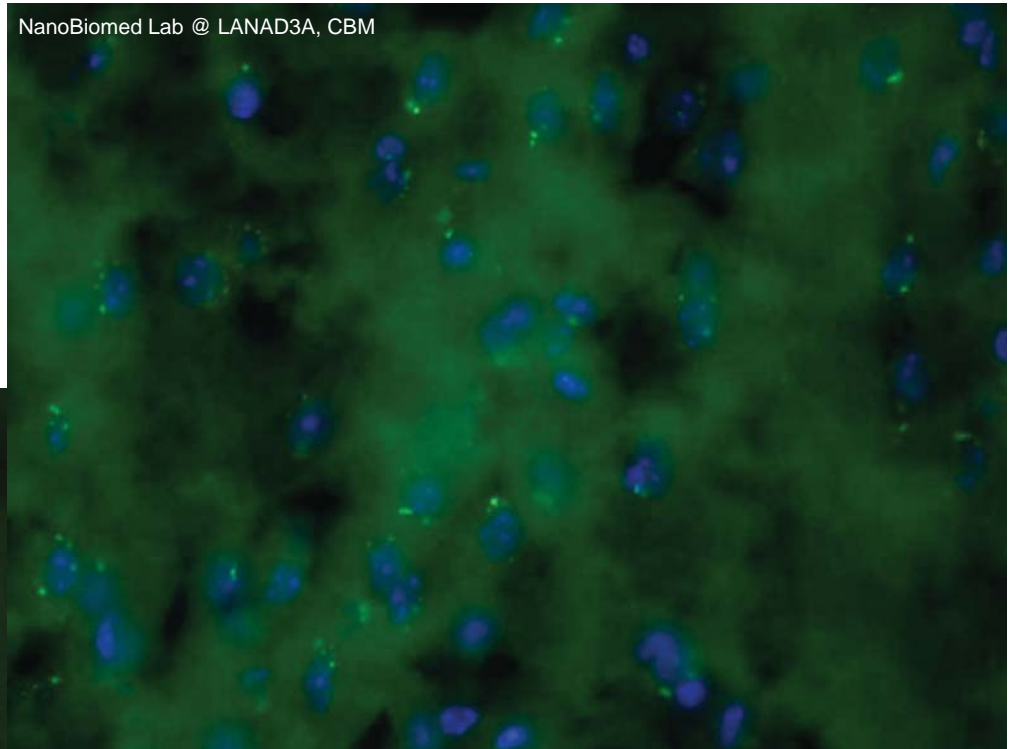


Cortex – epifluorescence

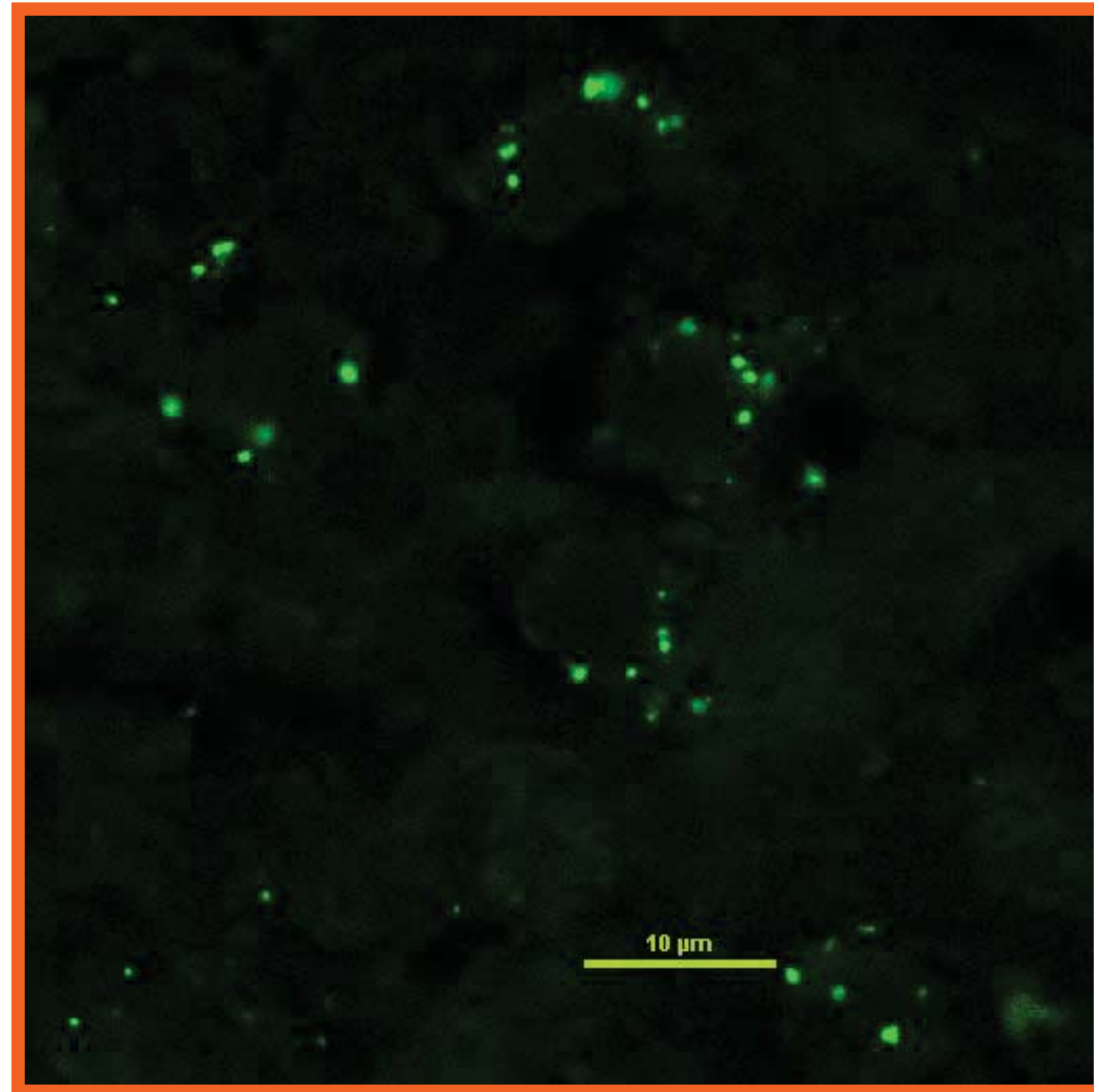
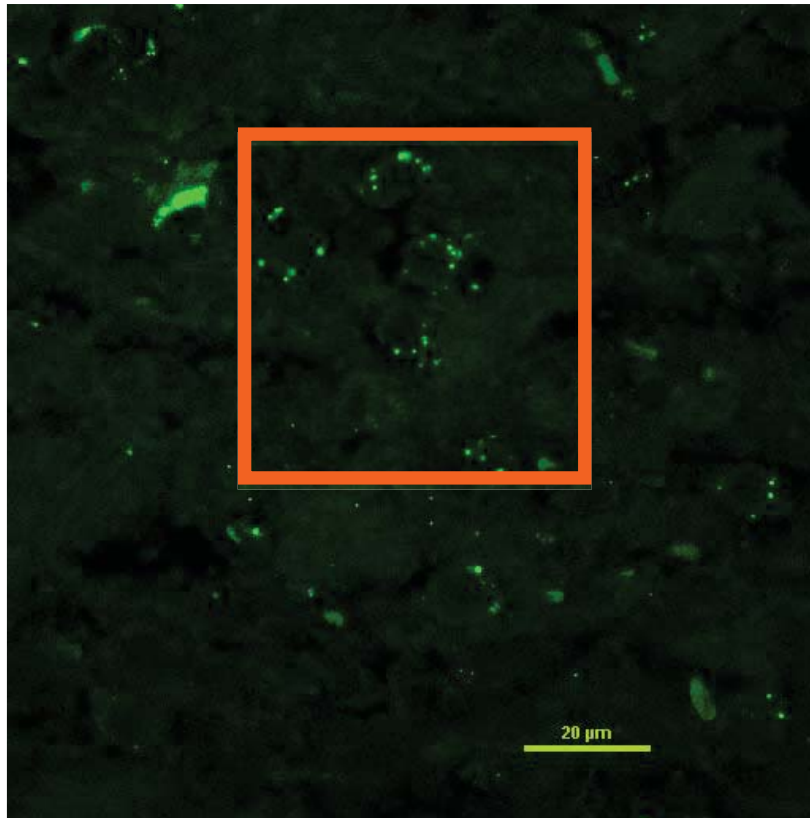
Nissl staining (red) – cell body of both, neurons and glia.

DAPI staining (blue) –stains nuclei specifically.

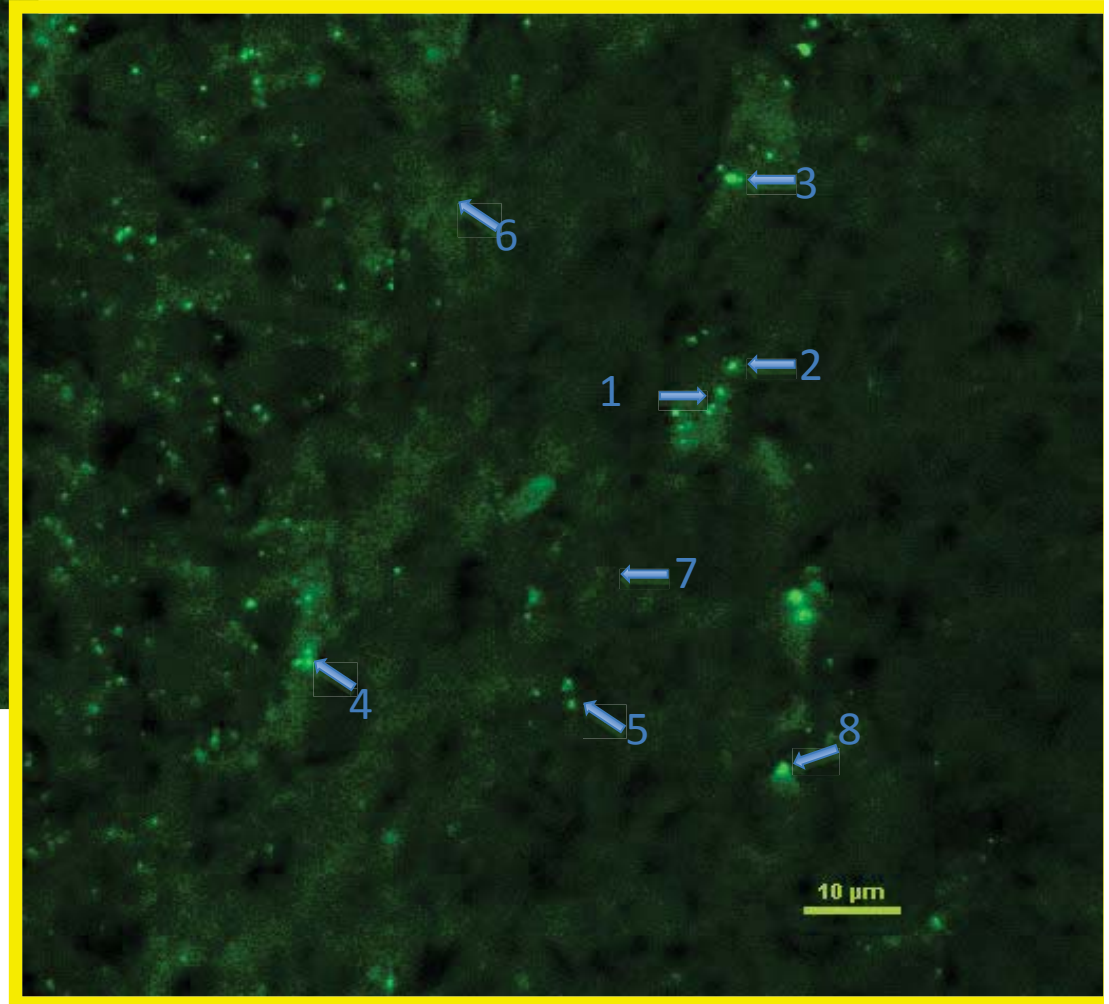
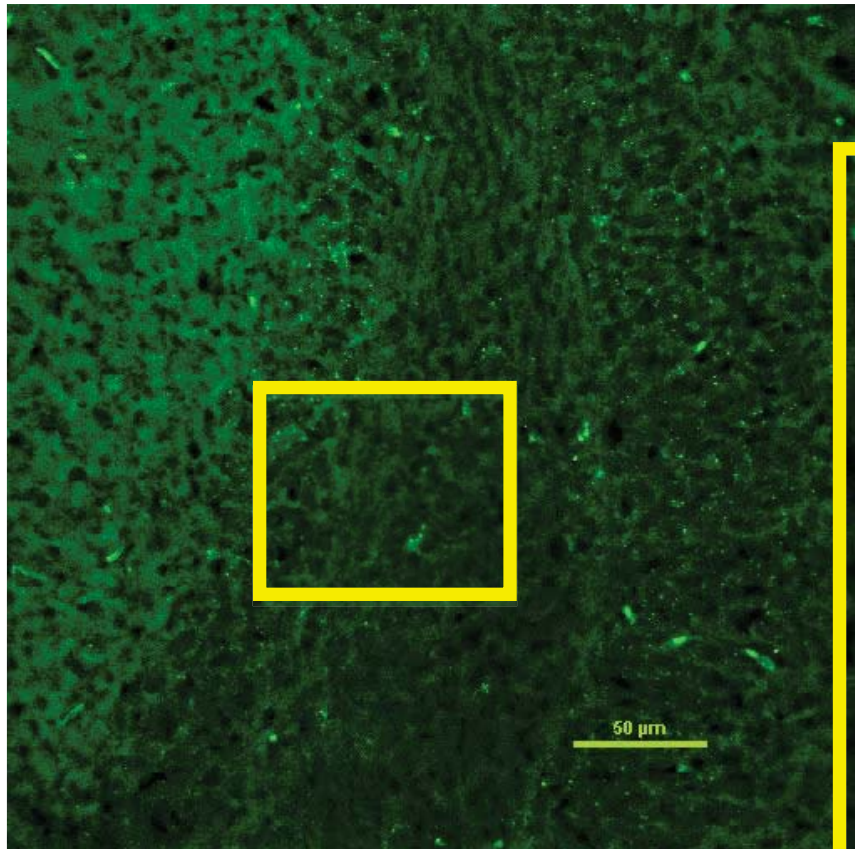
Nanoparticles labeled with FITC (green)



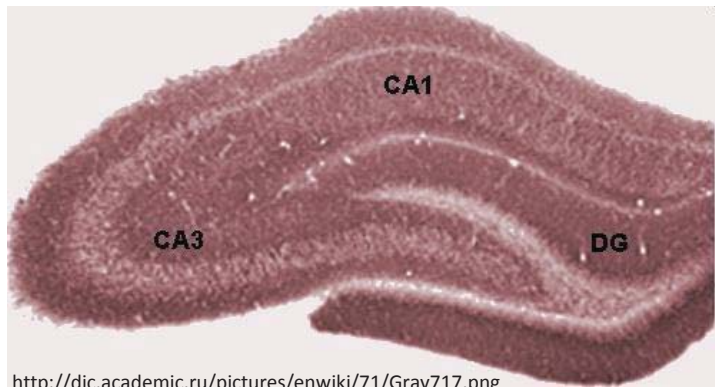
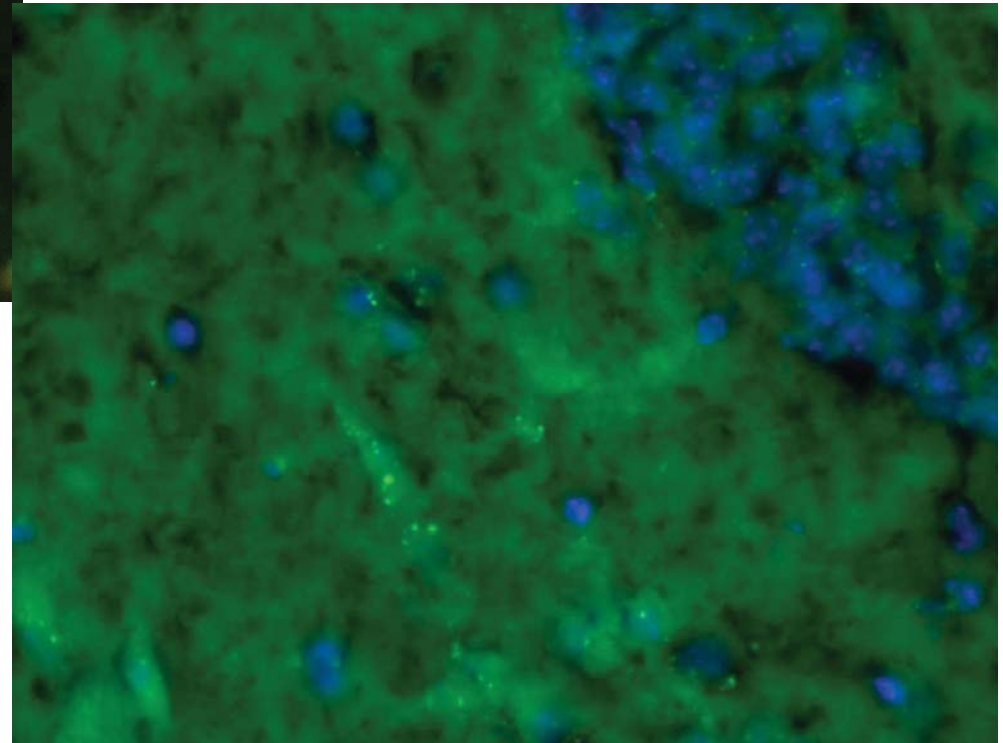
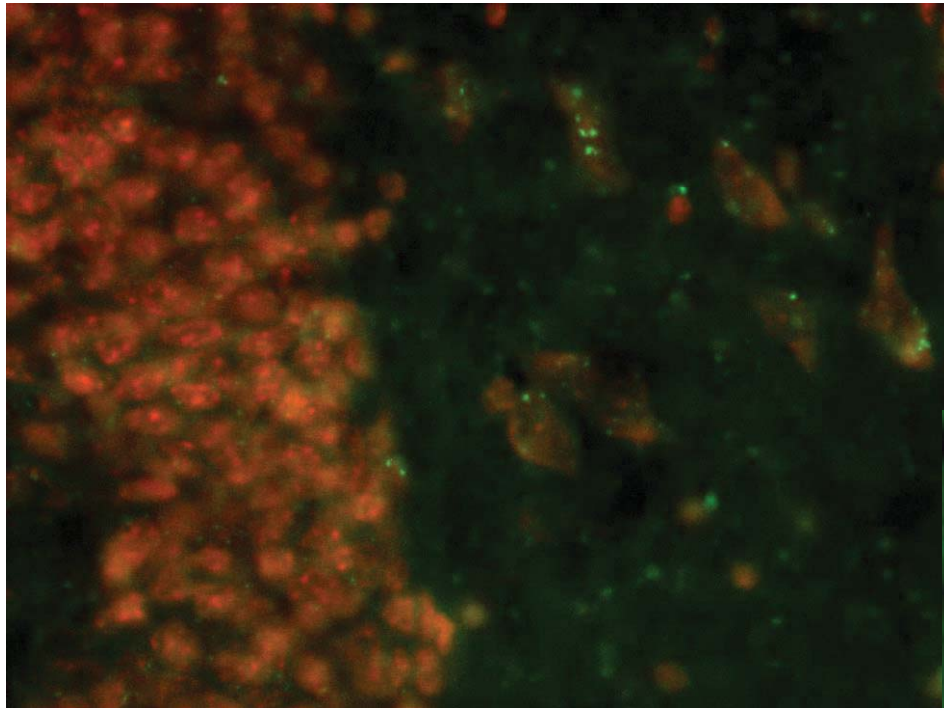
Thalamus – confocal microscopy



Hippocampus



Hippocampus – Dentate Gyrus

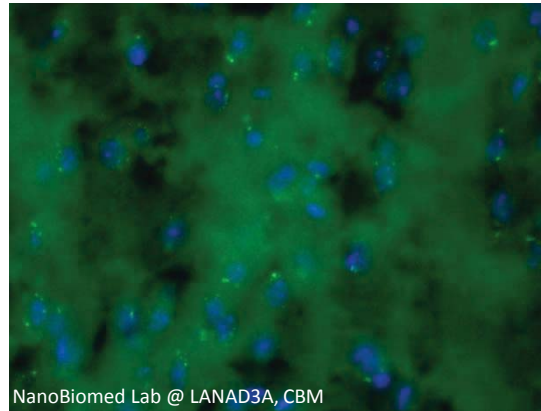
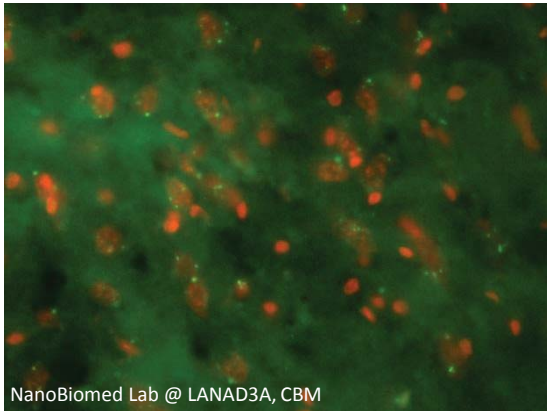


<http://dic.academic.ru/pictures/enwiki/71/Gray717.png>

Sousa et al. 2010 *Nanoscale*, 2, 2826-2834

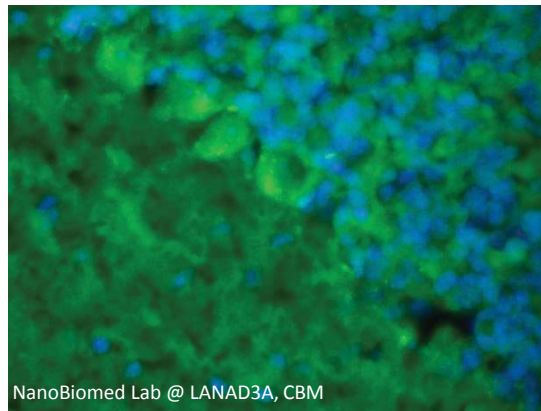
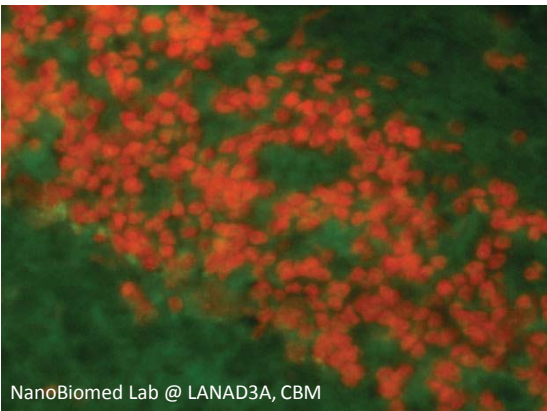
Some more images

Cortex



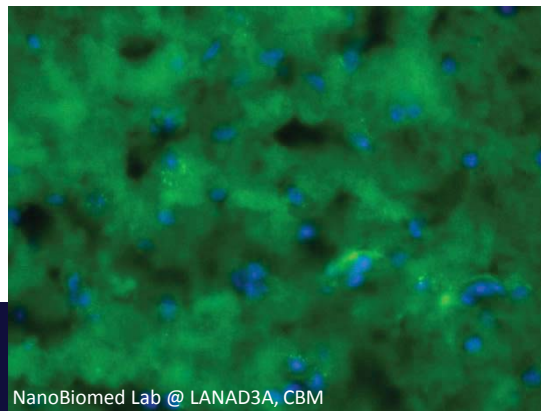
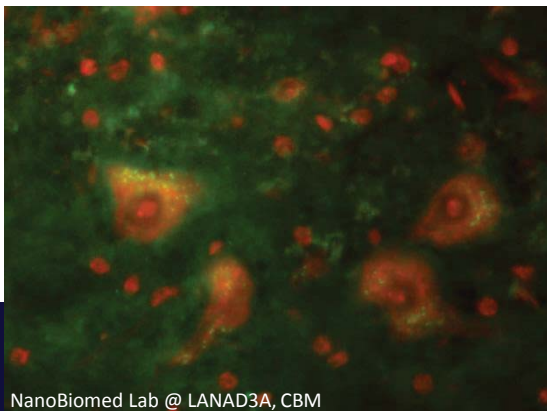
- Nanoparticles were observed in the cortex cells

Cerebellum



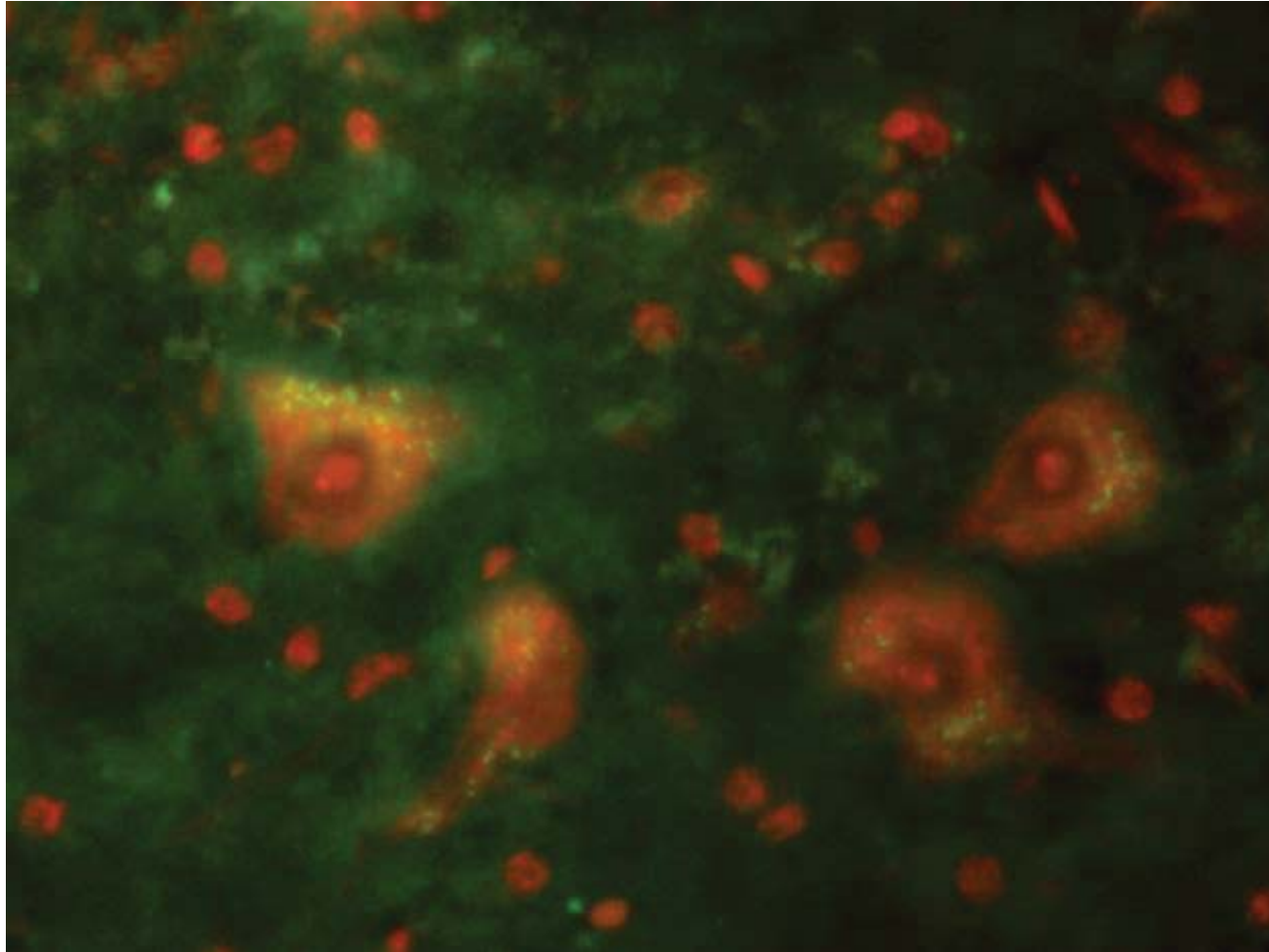
- they were not visualize in the cerebellum cells
- however it was observed that they accumulation inside of the Purkinje cells –GABAergic neurons located in the cerebellar cortex

Medulla



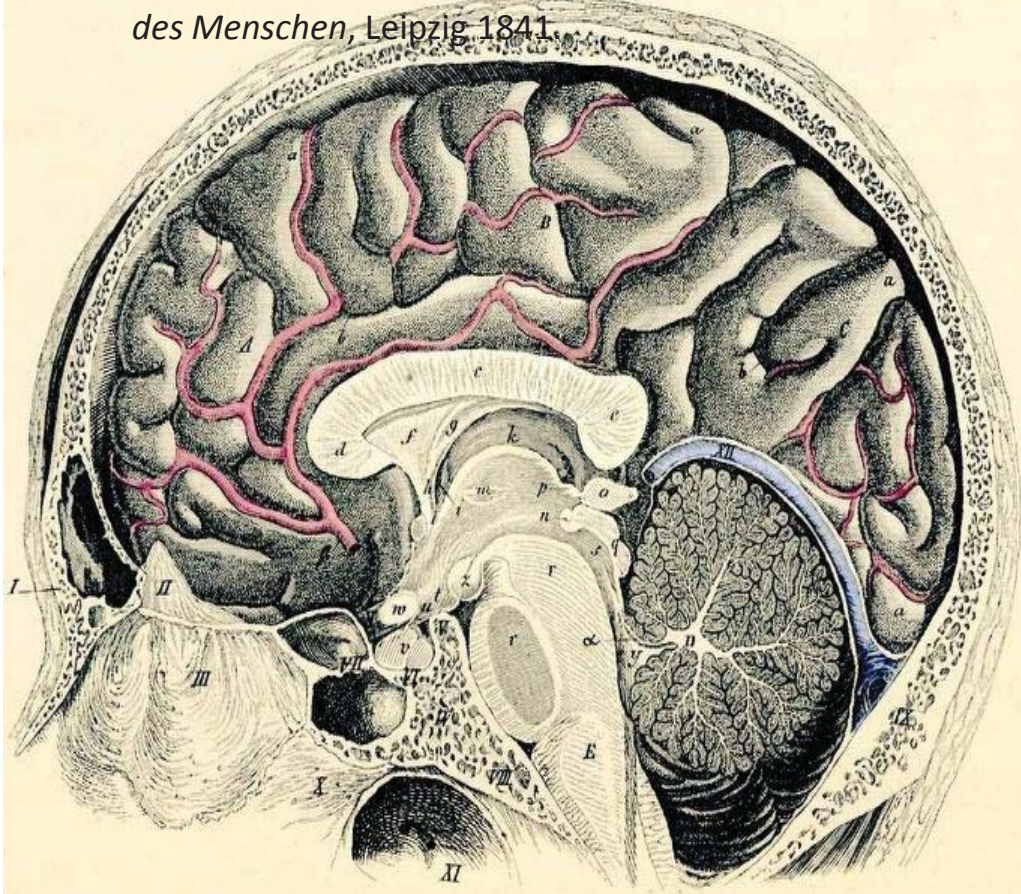
- These images were acquired in the medulla towards the spinal cord and it was clear observed that they are inside of the cells from medulla.

Some more images



Location of protein aggregates responsible for neurodegenerative disease

Source: Professor Dr. Carl Ernest Bock, *Handbuch der Anatomie des Menschen*, Leipzig 1841.



- Creutzfeldt-Jakob Disease (**CJD**), the cerebral cortex (prion protein aggregates) - memory and mental acuity are affected
- Alzheimer, manifests in the cerebral cortex (β -amyloid aggregates) – memory and mental acuity are affected
- Parkinson, manifests first in the substantia nigra (midbrain) (α -synuclein aggregates) - affect body movement

Acknowledgement

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TEM/EDS

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Marco Moeller

Fauzia Jabeen

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Martin Schaeffler

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and everlasting support.**

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Prion disease

(in vitro)

Giuseppe Legname

Diane Latawiec

Hoang Ngoc Ai Tran

Blood brain barrier

(TEER)

Hans-Joachim Galla

Sabine Huewel

SERS

Valter Sergo

Alois Bonifaccio

Prion disease animal models

Fabrizio Tagliavini

Fabio Moda

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