



Preliminary investigations on the synthesis, physicochemical and biological characterization of a new stilbene derivative grafted to USPIO dedicated to MRI diagnosis of Alzheimer disease

Abstract: 138

Congress: ESMRMB 2005

Type: Scientific

Topic: Contrast agents

Authors: S. Laurent¹, C. Burtea¹, L. Larbanoix¹, G. Toubeau¹, F. Van Leuven², L. Vander Elst¹, R.N. Muller¹; ¹Mons/BE, ²Leuven/BE

Keywords: MRI, Molecular Imaging

Any information contained in this pdf file is automatically generated from digital material submitted to e-Poster by third parties in the form of scientific presentations. References to any names, marks, products, or services of third parties or hypertext links to third-party sites or information are provided solely as a convenience to you and do not in any way constitute or imply ESMRMB's endorsement, sponsorship or recommendation of the third party, information, product, or service. ESMRMB is not responsible for the content of these pages and does not make any representations regarding the content or accuracy of material in this file.

As per copyright regulations, any unauthorised use of the material or parts thereof as well as commercial reproduction or multiple distribution by any traditional or electronically based reproduction/publication method is strictly prohibited.

You agree to defend, indemnify, and hold ESMRMB harmless from and against any and all claims, damages, costs, and expenses, including attorneys' fees, arising from or related to your use of these pages.

Please note: Links to movies, ppt slideshows and any other multimedia files are not available in the pdf version of presentations.

<http://www.esmrmb.org>

1. Purpose

Alzheimer disease (AD):

- neurodegenerative pathology and the principal cause of dementia in the elderly in the developed countries.

Pathological hallmarks of AD ([\[ESMRMB_STB_Fig1.JPG\] Figure 1](#)):

- substantial neuronal loss in the late clinical phases
- deposition of senile plaques (SPs) and neurofibrillary tangles (NFTs)

MRI:

- high spatial resolution
- better suited to assess SPs in AD, which could require a spatial resolution of less than 200 micrometers ([\[ESMRMB_STB_Fig2.jpg\] Figure 2](#)) [1]
- However, the image acquisition requires high magnetic fields (often higher than 7T) and long acquisition times (1 – 2 hours)

Stilbene derivatives:

- extensively explored for the detection of SPs by PET and SPECT, as well as by histochemistry

Purpose: This study reports preliminary investigations on the synthesis, physicochemical and biological characterization of a new stilbene derivative [4-amino-4(N,N-dimethylamino)stilbene] grafted to USPIO (USPIO-g-STB) dedicated to MRI-based diagnosis of AD. The stilbene vectorization of USPIO particles may help to the detection of SPs by MRI, which has the advantage of a better spatial and anatomical resolution as compared to the imaging techniques of nuclear medicine.

2. Methods and Materials

Synthesis and physico-chemical characterization of USPIO-g-STB

- Synthesis: 4-amino-4(N,N-dimethylamino)stilbene grafted on magnetic nanoparticles by reaction with the dextran coating of USPIO previously treated with epichlorhydrin ([\[ESMRMB_STB_Fig3.jpg\] Figure 3](#)).
- Iron concentration: determined by relaxometry at 20 MHz (Bruker Minispec, Bruker, Karlsruhe, Germany) and 37°C after mineralization in acidic conditions (0.6 ml HNO₃ and 0.3 ml H₂O₂) by microwaves (Milestone MSL-1200, Sorisole, Italy).
- The hydrodynamic size: measured by PCS and evaluated to 33 nm.
- Relaxometric characterization:
 - the proton NMRD profile was recorded between 0.01 MHz and 10 MHz
 - additional measurements at 20 and 60 MHz were respectively obtained on Minispec PC-20 and Mq 60 Series systems (Bruker, Karlsruhe, Germany)

In vivo MRI evaluation

Animal model of Alzheimer disease: eighteen months old double transgenic mice APP[V717I] x PS1-A246E [2]. The histology and MRI investigations previously performed [2, 3] demonstrated the presence of amyloid plaques (SPs) both in the cortex and in the thalamic areas (

[\[ESMRMB STB Fig2.jpg\] Figure 2](#)).

Contrast agent administration:

- i.v. injection of 80 micromol Fe/kg of USPIO-g-STB; USPIO was used as control.
- to open the blood-brain barrier, the mice were first injected with a solution of 25% mannitol i.v. The wild type (WT) control mice received the same treatment.

MRI protocol:

- 4.7 T Bruker AVANCE-200 system, vertical bore, mini-imaging device
- SE T₂-weighted, TR/TE = 2000/15-60 ms, NA = 3, NE = 4, matrix 256x256, slice thickness = 2 mm, FOV = 4 cm, spatial resolution = 1.56 mm

3. Results

Relaxometric characterization: The NMRD data demonstrate that grafting did not significantly alter the particle size and relaxometric properties (). In vivo MRI evaluation: The molecule used to target the SPs in this experiment is a stilbene derivative, which is specific for the protein beta-sheets. As a consequence, this molecule can target not only the SPs, but also the NFTs [4]. Nevertheless, solely the SPs are accessible for targeting since they are located extracellularly. Mannitol was used to open the blood-brain barrier (BBB), although its integrity might already be compromised and the permeability probably enhanced in AD [5, 6] due to the lesions produced by SPs on different types of cerebral blood vessels ([\[ESMRMB STB Fig5.jpg\] Figure 5](#)) [7]. Two hours post-contrast, USPIO-g-STB produced a significant decrease of the signal intensity in the cortex and the thalamic areas in the brain of the transgenic mouse ([\[ESMRMB STB Fig6.jpg\] Figure 6](#)) [3], which is probably associated to the SPs localization. The negative contrast is more important in the brain of the AD mouse as compared to the WT control mouse (). It is also remarkable that the negative contrast in cortex is solely present in the brain of the AD mouse but not in the WT control. The effect produced by USPIO-g-STB is not explained by a simple diffusion of the contrast agent in the brain of the transgenic mouse, since amyloid deposits are known to restrict the diffusion within the interstitial space [8]. The spatial resolution is low (1.56 mm), which means that the precise epitope of the contrast agent interaction cannot be certainly identified. The micro-imaging seems to be indispensable for the SPs detection. On the other hand, the blow-up effect of USPIO-g-STB allows to diagnose the disease. Of course, it remains to be proven if this brain distribution is specific, and not common to other neurodegenerative pathologies. The patchy distribution of USPIO-g-STB in the brain of AD mouse argues for a rather specific accumulation at the level of SPs compared with USPIO, which seems to diffuse homogeneously in the nervous tissue ([\[ESMRMB STB Fig8.jpg\] Figure 8](#)).

4. Conclusion

The preliminary characterization of the new USPIO-grafted stilbene derivative emphasizes its potential as a tool for the non-invasive diagnosis of AD, which may help in the treatment and monitoring of this pathology.

5. References

1. Clifford RJ Jr et al, Magn Reson Med, 52, 2004, 1263 - 1271.
2. Dewachter I et al, J Neurosci, 20, 2000, 6452 - 6458.
3. Vanhoutte G et al, MRM, 53, 2005, 607 - 613.
4. Ono M et al, Nucl Med Biol, 30, 2003, 565 - 71.
5. Wisniewski HM et al, Ann N Y Acad Sci, 826, 1997, 161-172.

6. Van Dorpe J et al, Am J Pathol, 157, 2000, 1283 - 1298.

7. Mueggler T et al, Eur J Neurosci, 20, 2004, 811- 817.

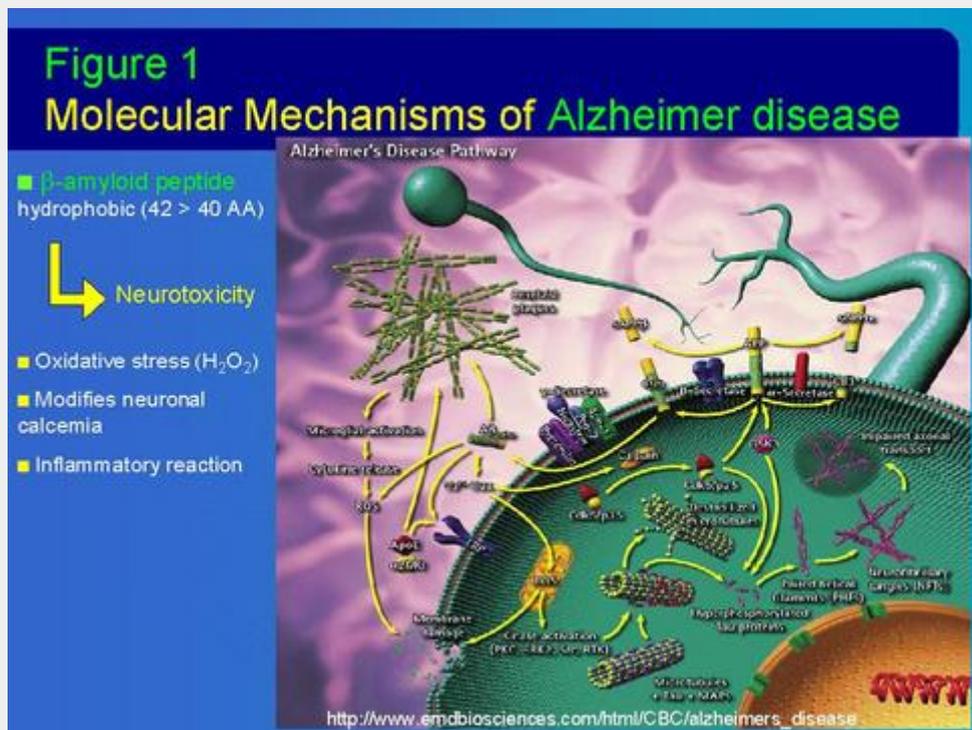
6. Personal Information

Acknowledgements

This work was financially supported by the ARC program of the French Community of Belgium (research contract no. 00-05/258).

7. Mediafiles

ESMRMB_STB_Fig1.JPG



ESMRMB_STB_Fig1.JPG

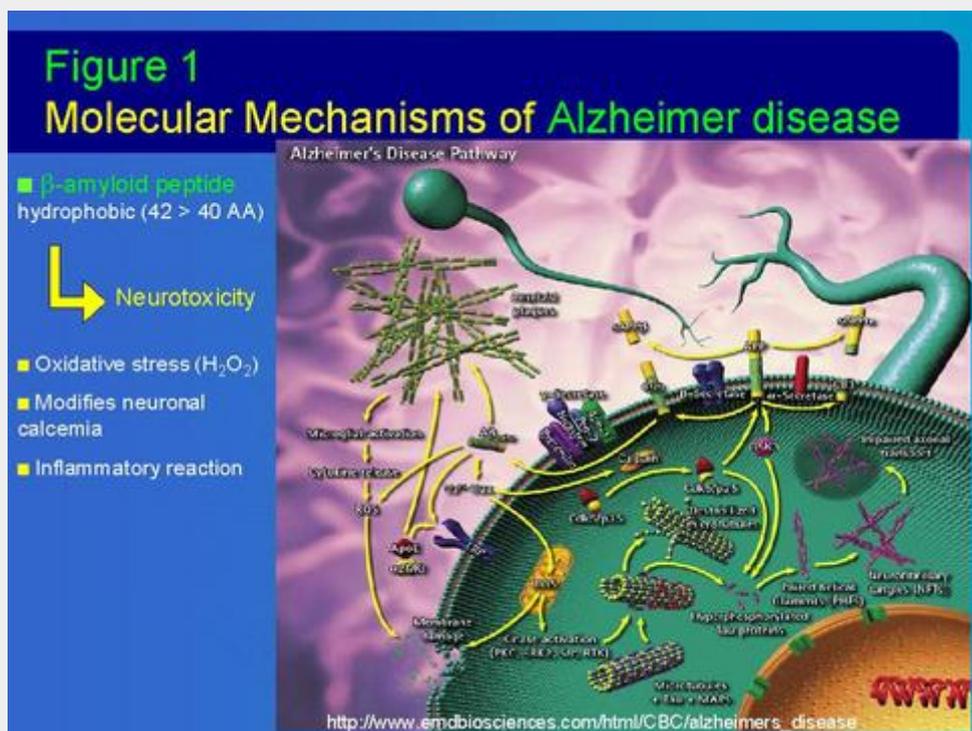
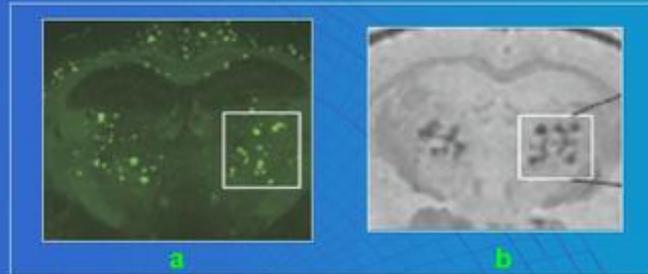


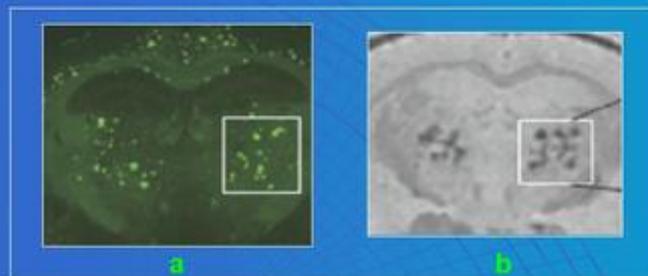
Figure 2 Micro-MRI of amyloid plaques in Alzheimer disease



G. Vanhoutte, I. Dewachter, P. Borghgraef, F. Van Leuven, A. Van der Linden, Magn Reson. Med. 53, 607-613, 2005 (reproduced with authors' permission)

Thioflavin-S-stained brain sections of APP[V717I] transgenic mouse showing occurrence of amyloid plaques in the cortex and the thalamic area (a) and the corresponding MR image (7T, T_2^* -weighted images, TA = 68 min, voxel resolution = $78 \times 156 \times 234 \mu\text{m}^3$) displaying hypointense brain inclusions exclusively in the thalamic region (b).

Figure 2 Micro-MRI of amyloid plaques in Alzheimer disease



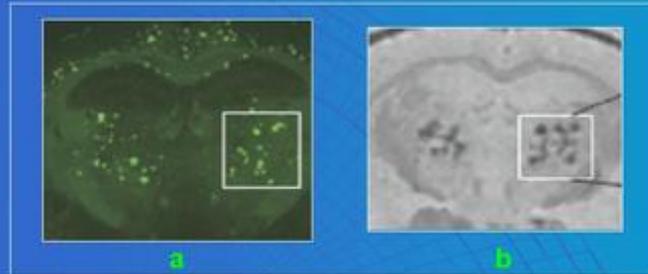
G. Vanhoutte, I. Dewachter, P. Borghgraef, F. Van Leuven, A. Van der Linden, Magn Reson. Med. 53, 607-613, 2005 (reproduced with authors' permission)

Thioflavin-S-stained brain sections of APP[V717I] transgenic mouse showing occurrence of amyloid plaques in the cortex and the thalamic area (a) and the corresponding MR image (7T, T_2^* -weighted images, TA = 68 min, voxel resolution = $78 \times 156 \times 234 \mu\text{m}^3$) displaying hypointense brain inclusions exclusively in the thalamic region (b).

ESMRMB_STB_Fig2.jpg

Figure 2

Micro-MRI of amyloid plaques in Alzheimer disease



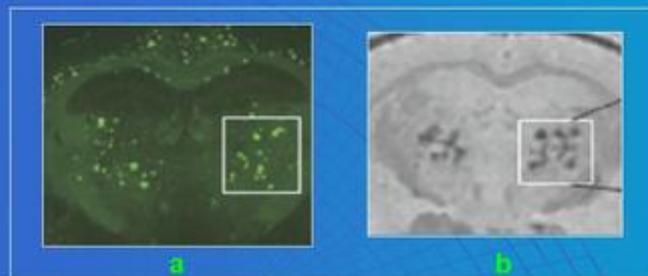
G. Vanhoutte, I. Dewachter, P. Borghgraef, F. Van Leuven, A. Van der Linden, Magn Reson. Med. 53, 607-613, 2005 (reproduced with authors' permission)

Thioflavin-S-stained brain sections of APP[V717I] transgenic mouse showing occurrence of amyloid plaques in the cortex and the thalamic area (a) and the corresponding MR image (7T, T_2^* -weighted images, TA = 68 min, voxel resolution = $78 \times 156 \times 234 \mu\text{m}^3$) displaying hypointense brain inclusions exclusively in the thalamic region (b).

ESMRMB_STB_Fig2.jpg

Figure 2

Micro-MRI of amyloid plaques in Alzheimer disease



G. Vanhoutte, I. Dewachter, P. Borghgraef, F. Van Leuven, A. Van der Linden, Magn Reson. Med. 53, 607-613, 2005 (reproduced with authors' permission)

Thioflavin-S-stained brain sections of APP[V717I] transgenic mouse showing occurrence of amyloid plaques in the cortex and the thalamic area (a) and the corresponding MR image (7T, T_2^* -weighted images, TA = 68 min, voxel resolution = $78 \times 156 \times 234 \mu\text{m}^3$) displaying hypointense brain inclusions exclusively in the thalamic region (b).

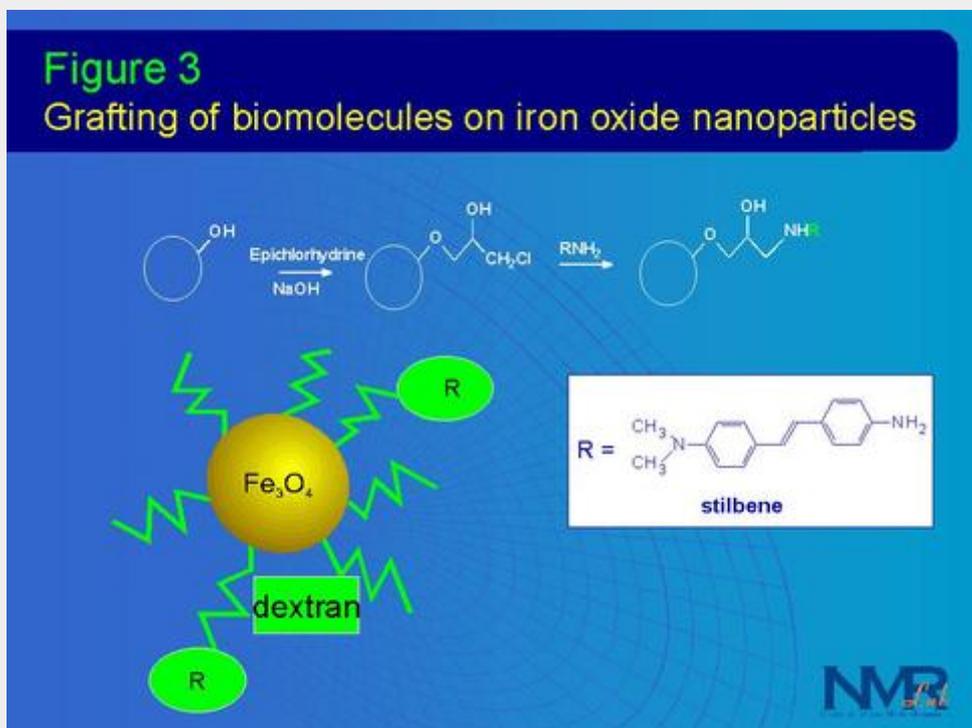
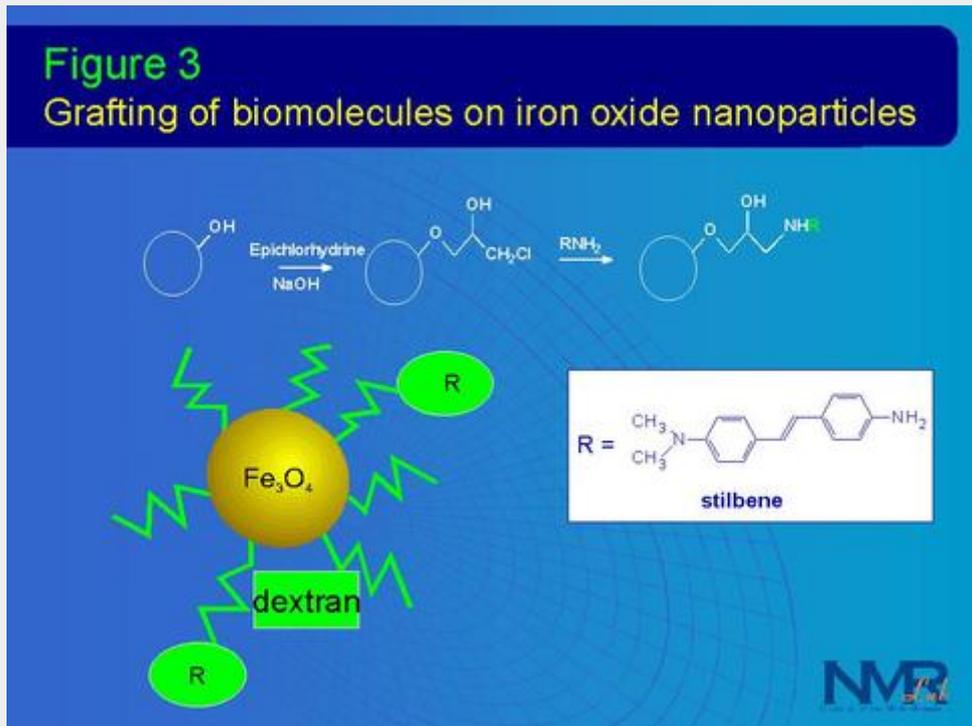
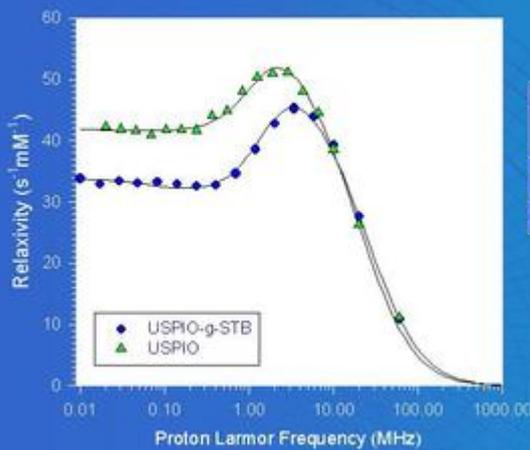


Figure 4
Relaxometric characterization of USPIO-g-STB



Contrast agent	r_2/r_1	
	20 MHz	60 MHz
USPIO-g-STB	3.15	7.07
USPIO	3.30	5.70

Hydrodynamic size:
measured by PCS = 33 nm

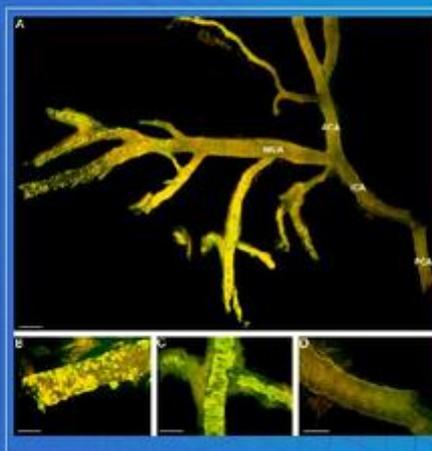


Figure 5
Prominent cerebral amyloid angiopathy in transgenic mice overexpressing the London mutant of human APP in neurons

J. Van Dorpe, L. Smeijers, I. Dewachter, D. Nuyens, K. Spittaels, C. Van den Haute, M. Mercken, D. Moechars, I. Laenen, C. Kuiperi, K. Bruynseels, I. Tesseur, R. Loos, H. Vanderstichele, F. Checler, R. Sciot, F. Van Leuven

Am. J. Pathol. 157: 1283-1298 2000

(Reproduced with authors' permission)



Thioflavine-S staining of the arterial circle of Willis from a 24-month-old APP^{LD} mouse. **A:** The main branches of the arteries at the base of the brain are almost completely free of amyloid, whereas the smaller branches show prominent amyloid deposition. **B:** High magnification of a branch of the middle cerebral artery showing focal amyloid deposits. **C:** Branch of middle cerebral artery with a pattern of fluorescence in concentric rings. **D:** No amyloid deposition in the internal carotid artery. ICA, internal carotid artery; MCA, middle cerebral artery; ACA, anterior cerebral artery; and PCA, posterior communicating artery. Scale bars, 200 μ m (**A**), 80 μ m (**B**), 100 μ m (**C**), and 90 μ m (**D**).



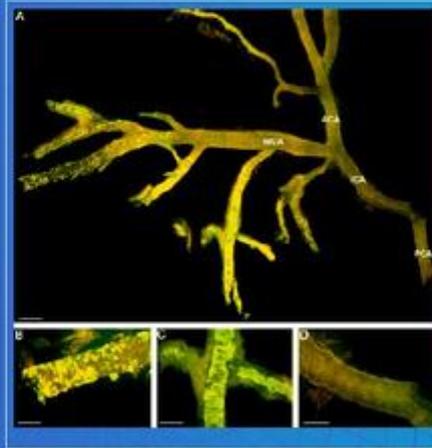
Figure 5

Prominent cerebral amyloid angiopathy in transgenic mice overexpressing the London mutant of human APP in neurons

J. Van Dorpe, L. Smeijers, I. Dewachter, D. Nuyens, K. Spittaels, C. Van den Haute, M. Mercken, D. Moechars, I. Laenen, C. Kuiperi, K. Bruynseels, I. Tesseur, R. Loos, H. Vanderstichele, F. Checler, R. Sciot, F. Van Leuven

Am. J. Pathol. 157: 1283-1298 2000

(Reproduced with authors' permission)

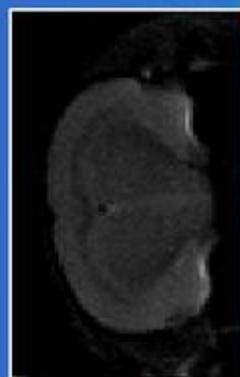


Thioflavine-S staining of the arterial circle of Willis from a 24-month-old APP(LD) mouse. **A:** The main branches of the arteries at the base of the brain are almost completely free of amyloid, whereas the smaller branches show prominent amyloid deposition. **B:** High magnification of a branch of the middle cerebral artery showing focal amyloid deposits. **C:** Branch of middle cerebral artery with a pattern of fluorescence in concentric rings. **D:** No amyloid deposition in the internal carotid artery. ICA, internal carotid artery; MCA, middle cerebral artery; ACA, anterior cerebral artery; and PCA, posterior communicating artery. Scale bars, 200 μ m (A), 80 μ m (B), 100 μ m (C), and 90 μ m (D).



Figure 6. Molecular targeting of SP in a transgenic mouse with USPIO-g-STB
4.7 T Bruker Avance-200, SE T₂ (TR/TE = 2000/45 ms, NA = 3, NE = 4, matrix 256x256, slice thickness = 2 mm, FOV = 4 cm, spatial resolution = 1.56 mm)

APP/PS1 mouse



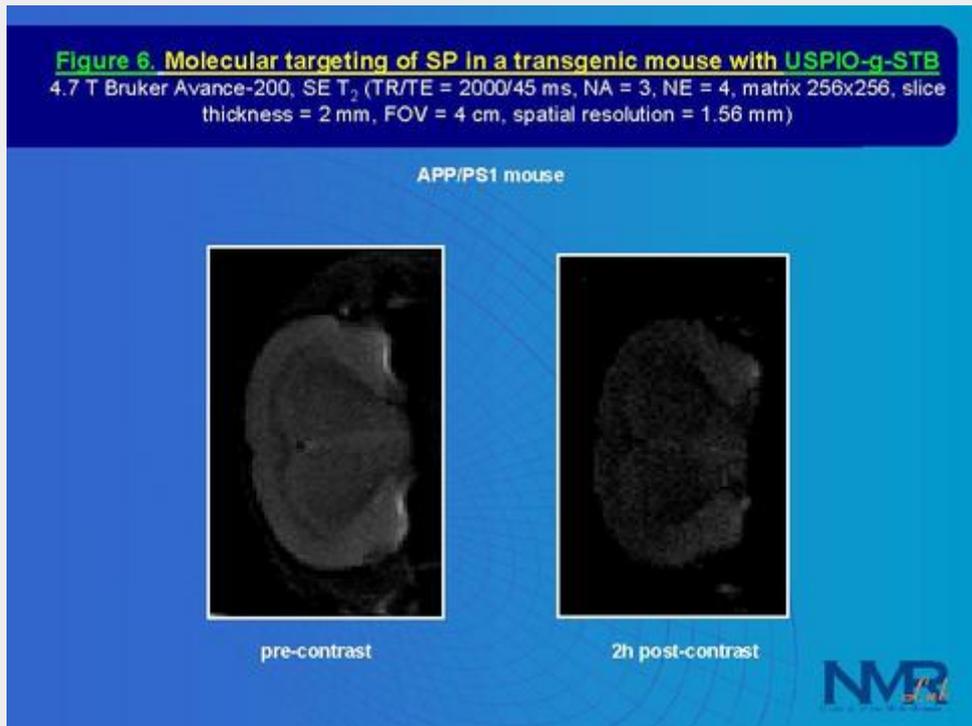
pre-contrast



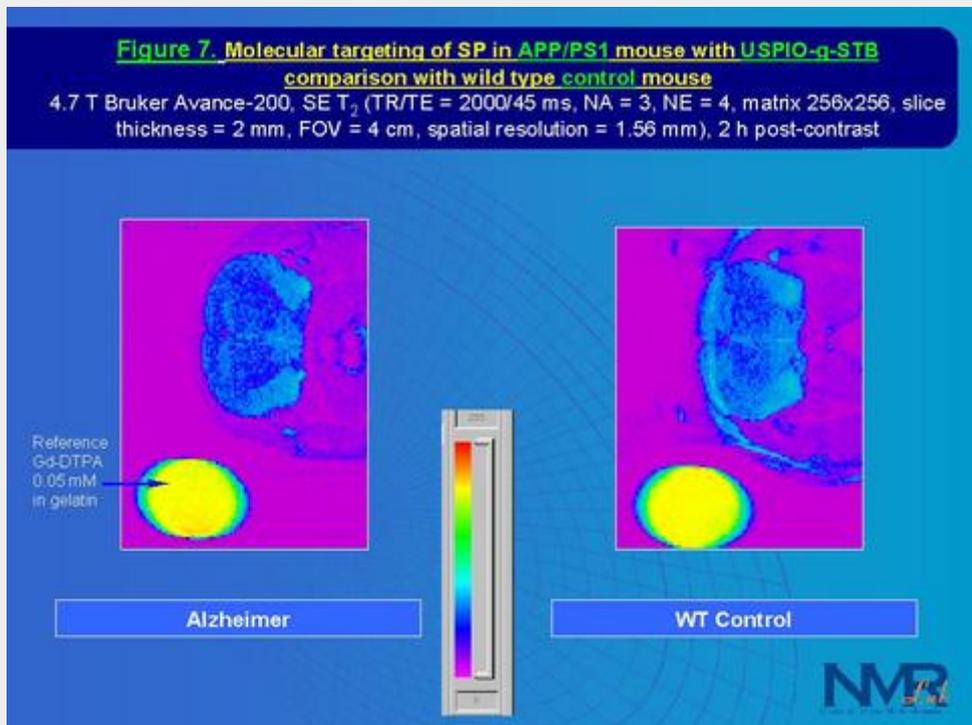
2h post-contrast



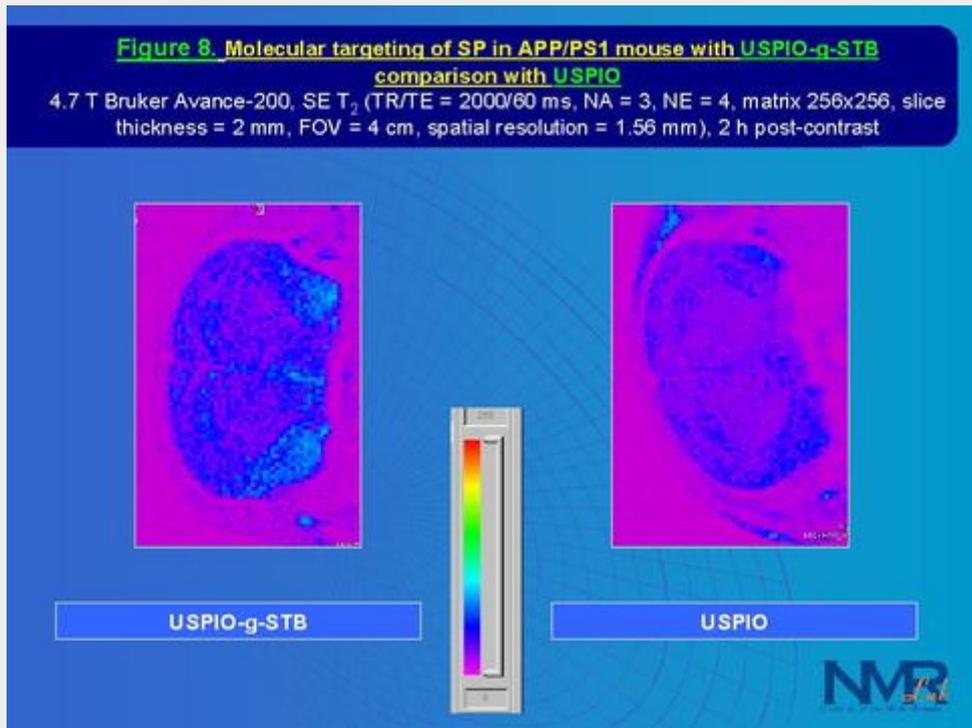
ESMRMB_STB_Fig6.jpg



ESMRMB_STB_Fig7.jpg



ESMRMB_STB_Fig8.jpg



ESMRMB_STB_Fig8.jpg

