Molecular MRI of apoptosis in atherosclerotic plaque by using a peptide-vectorized paramagnetic imaging probe
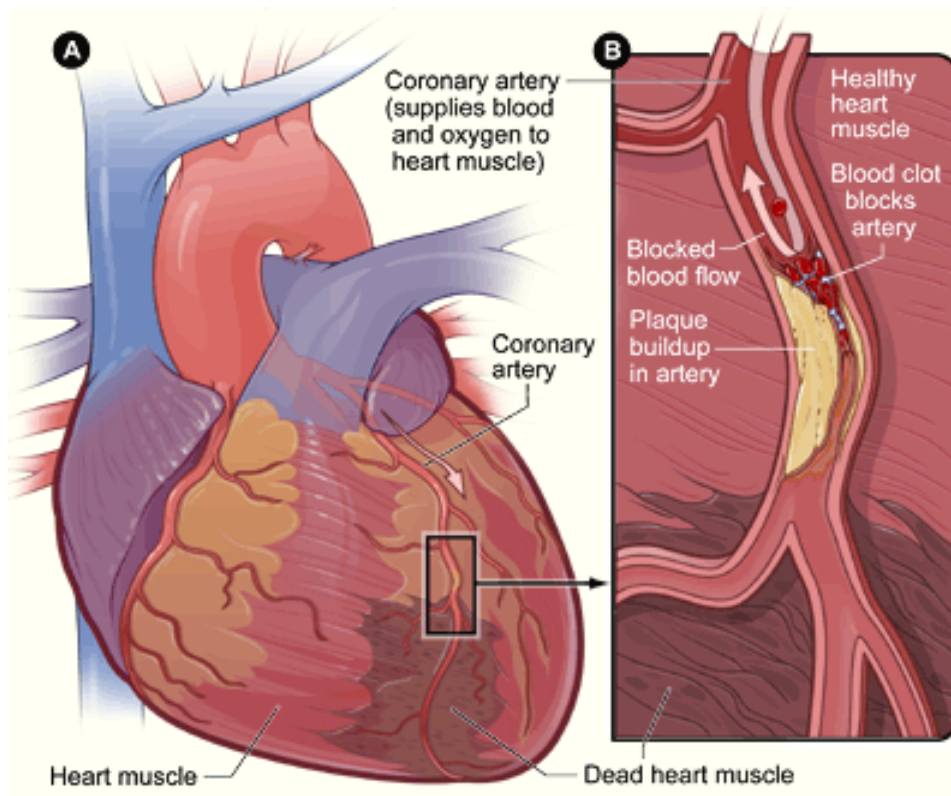
Carmen Burtea[1], Sophie Laurent[1], Eric Lancelot[2], Marc Port[2], Sébastien Ballet[2], Olivier Rousseaux[2], Luce Vander Elst[1], Claire Corot[2], Robert N. Muller[1]

[1]Department of General, Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, University of Mons-Hainaut, 24, Avenue du Champ de Mars, B-7000 Mons, Belgium; [2]Guerbet, Research Center, 16-24 rue Jean Chaptal, 93600 Aulnay-sous-Bois, France

http://www.umh.ac.be/~nmrlab/

Cardiovascular diseases: complications of atherosclerosis

A
- Coronary artery (supplies blood and oxygen to heart muscle)
- Coronary artery
- Heart muscle
- Dead heart muscle

B
- Healthy heart muscle
- Blood clot blocks artery
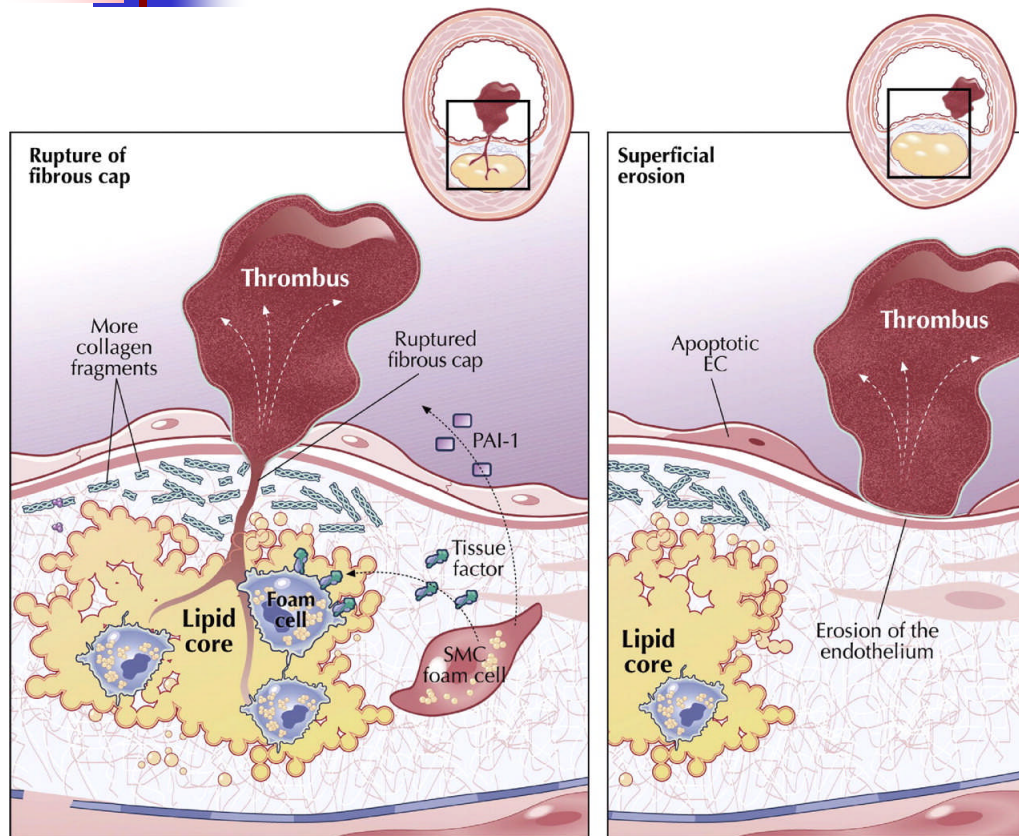- Blocked blood flow
- Plaque buildup in artery

http://www.nhlbi.nih.gov/health/dci/Diseases/Cad/CAD_All.html

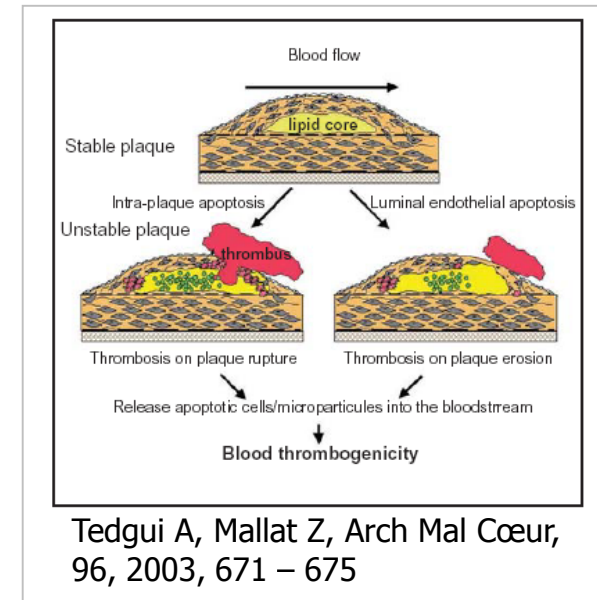- Important progress in the therapy and prevention of cardiovascular diseases

- Still, myocardial infarction and brain stroke ➔ the main causes of death in Occidental countries

# Vulnerable plaques, thrombosis and apoptosis

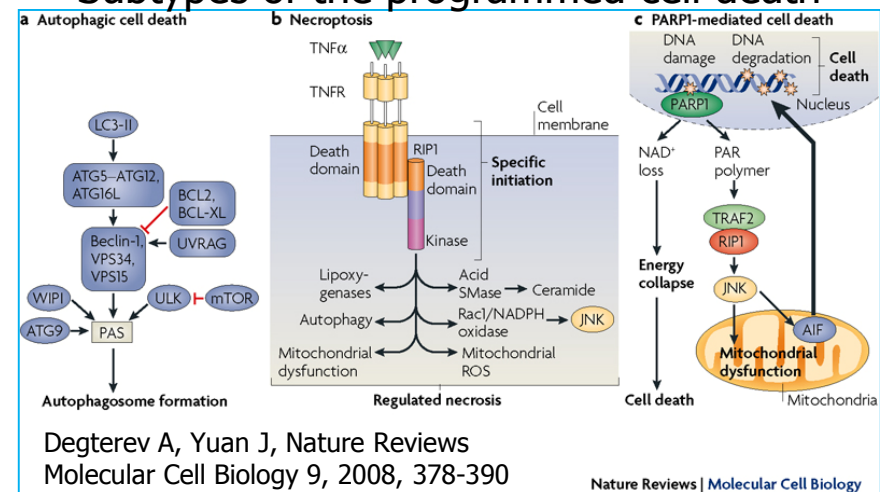Libby, P. et al. J Am Coll Cardiol, 2006, 48, A33-A46

Tedgui A, Mallat Z, Arch Mal Cœur, 96, 2003, 671 – 675

- Macrophages ➜ MMPs ➜ collagen degradation ➜ weakening of the fibrous plaque ➜ erosion and rupture of the fibrous plaque ➜ thrombus
- Apoptosis of SMCs, macrophages, lymphocytes T ➜ positive and negative effects

The mechanisms of cell death
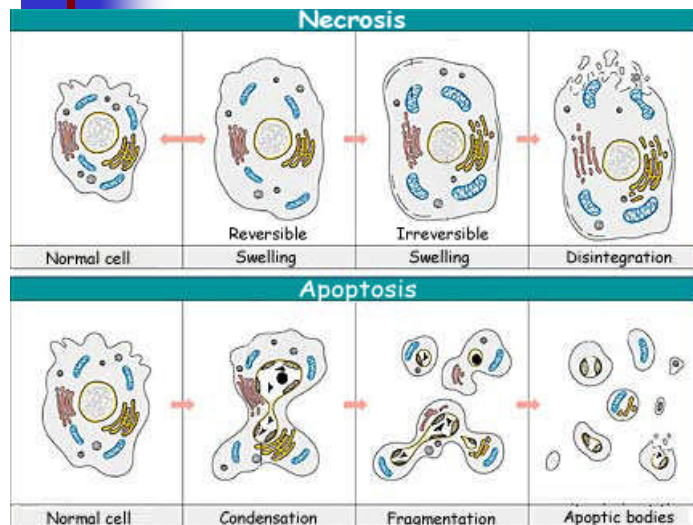
http://thebrain.mcgill.ca/flash/capsules/outil_bleu17.html

### Subtypes of the programmed cell death

Degterev A, Yuan J, Nature Reviews
Molecular Cell Biology 9, 2008, 378-390

Apoptosis	Autophagy	Necroptosis	PARPtosis
- Chromatin fragmentation - Membrane blebbing - Apoptotic bodies - Caspase dependent - Early PS exposure	- Expression of autophagy-related genes - Degradation of cell organelles - Accumulation of membrane-closed vesicles	- Cell disintegration - Independent of caspases - Late PS exposure	- Chromatin fragmentation independent of caspases -Energy collapse - Activation of PARP-1

AIM:

Search for peptide ligands that target apoptotic cells by a specific interaction with phosphatidylserine (PS)
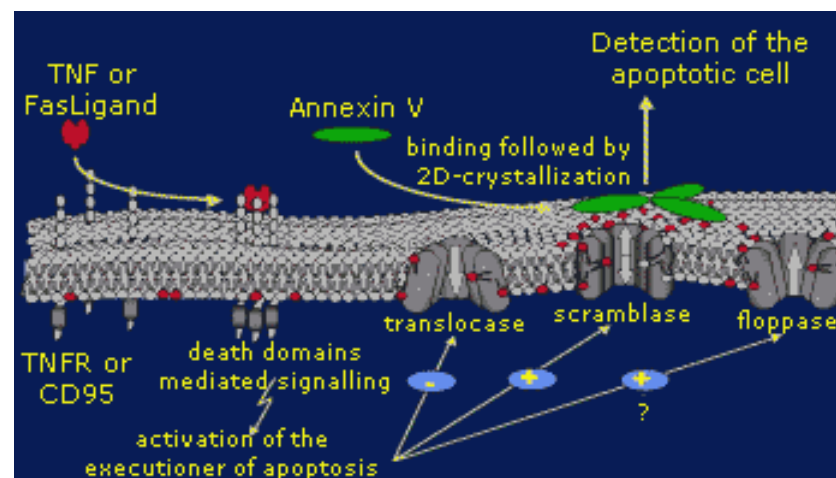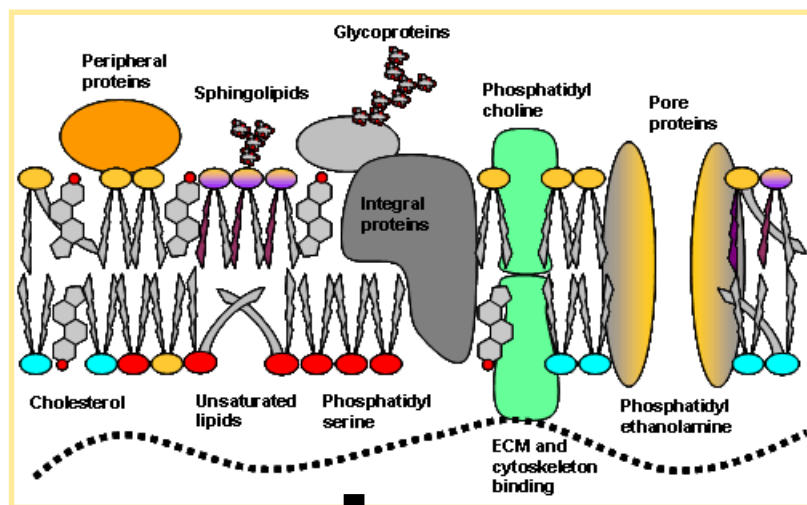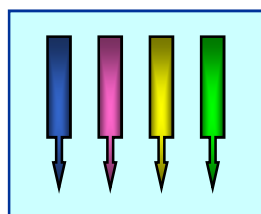

van Engeland M et al. Cytometry, 31, 1998, 1– 9
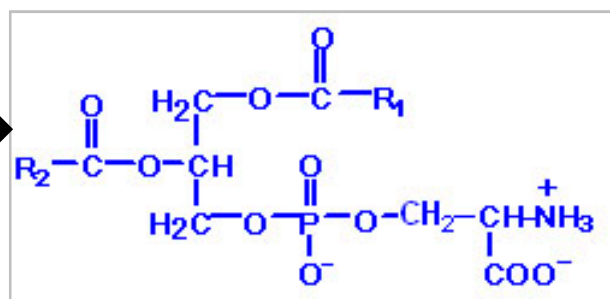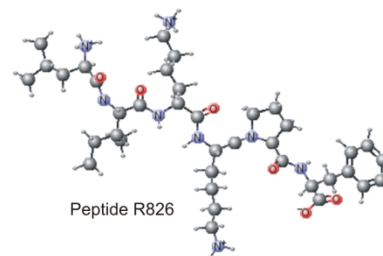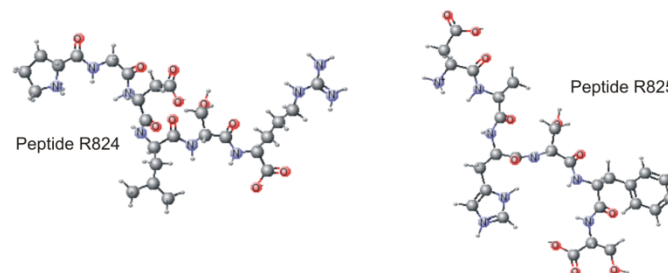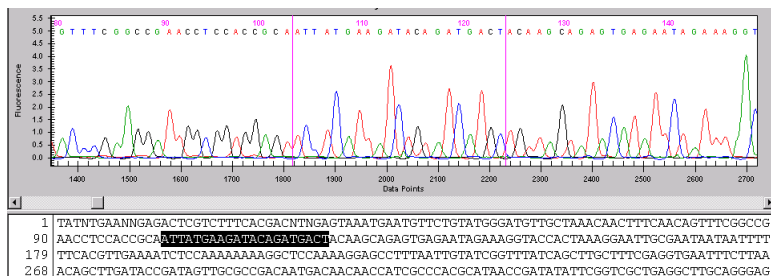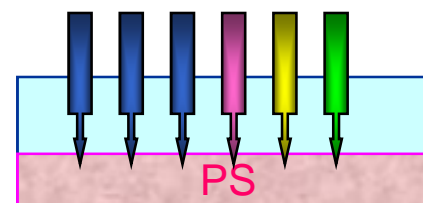

Phosphatidylserine

METHOD:
Phage display screening

3 rounds of panning

PS

Library of linear
hexapeptides

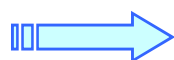Peptide R824

Peptide R825

Peptide R826
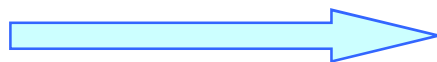
RESULTS:
Peptide sequence
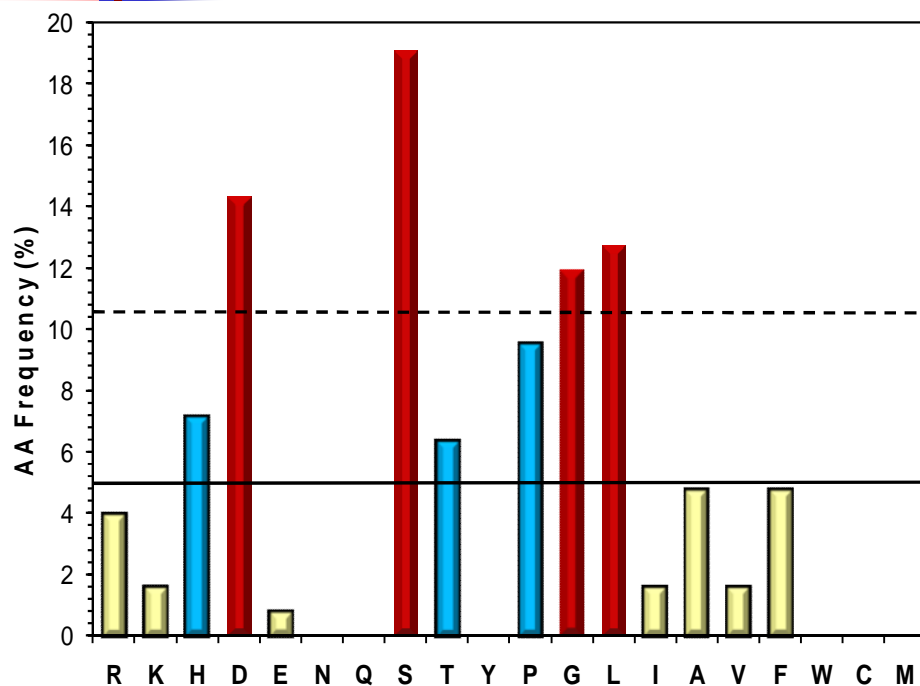
3 first positions (N-terminal): Asp, His

3 last positions (C-terminal): Ser, Thr

Ionic or hydrogen binding with the polar head of PS

No clones	Homology
5	**Low voltage-activated T-type calcium channel α-1 subunit**
1	Tyrosine protein kinase pp60-c-src Neuronal pp60c-src
7	Matrix metalloproteinase 14 preprotein Matrix metalloproteinase 1, 9, 14
3	**Transient rec. potential Ca^{2+} channel 6C** Acyl-coenzyme A oxydase 2
1	**Transient rec. potential Ca^{2+} channel 6C** **Fas antigen ligand**
1	**K$^+$ inwardly-rectifying channel** **K$^+$ large conductance pH-sensit. channel**
1	Protein Tyr phosphatase 2C Alanine:glyoxylate aminotransferase 2
1	**Apoptosis associated Tyr-kinase** **Ca^{2+} channel β-subunit**
1	**Transient receptor potential calcium channel 5 (TRPC5)** **Capacitative calcium entry channel 2**

Theoretical biochemical parameters of the three candidate peptides R824, R825 and R826, respectively, as estimated by using ExPASy Proteomics Server, Proteomics and sequence analysis tools; LogP was calculated by using the ACDLabs 12.0 software

Parameter	R824	R825	R826
Half-life	>20 h	1.1 h	5.5 h
Instability index	40.43	-5.82	13.72
pI	6.27	5.08	10.00
LogP	-2.33 ± 0.86	-1.79 ± 0.88	2.51 ± 0.86
GRAVY	-1.167	-0.617	0.283
Aliphatic index	65	16.67	130

Half-life: theoretically estimated in mammalian reticulocytes *in vitro*; Instability index: when smaller than 40, the protein (or peptide) is predicted as stable; pI = Isoelectric point; LogP = Partition coefficient; GRAVY = Grand average of hydropathicity (predicts the hydrophobicity); Aliphatic index = the relative volume occupied by aliphatic side chains.

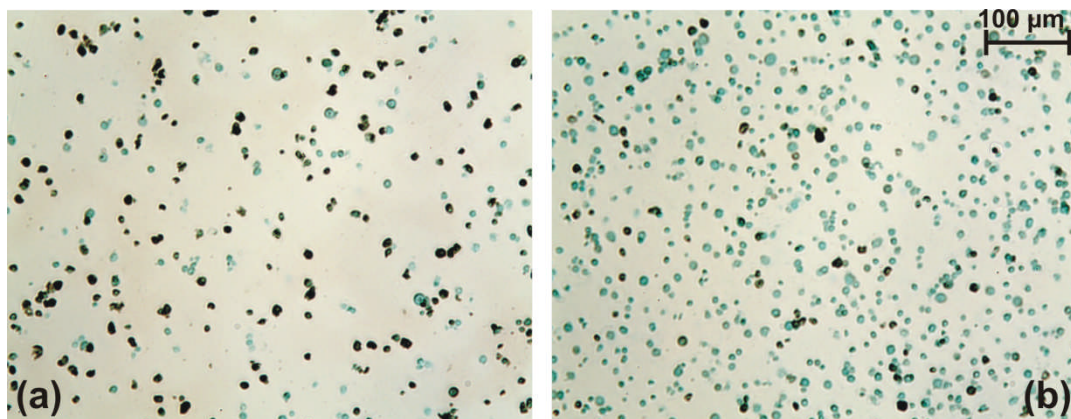# Specific affinity for PS of the selected peptides
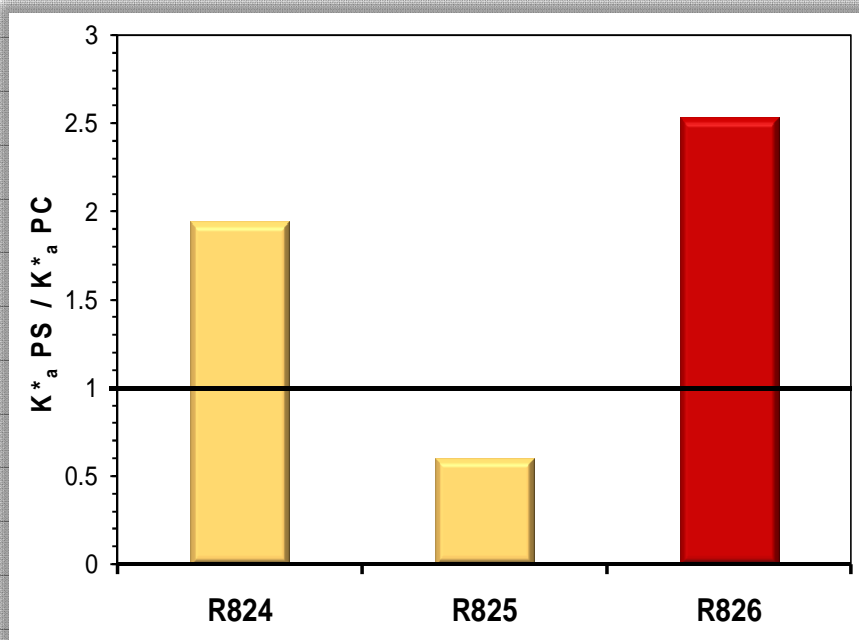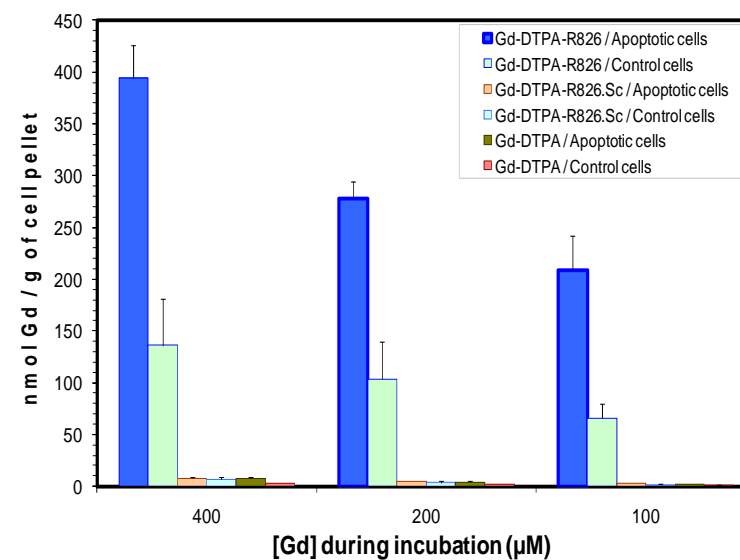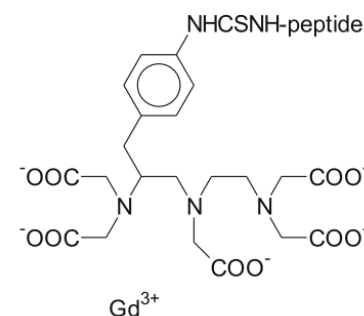

The ratio K_a for PS/K_a for PC
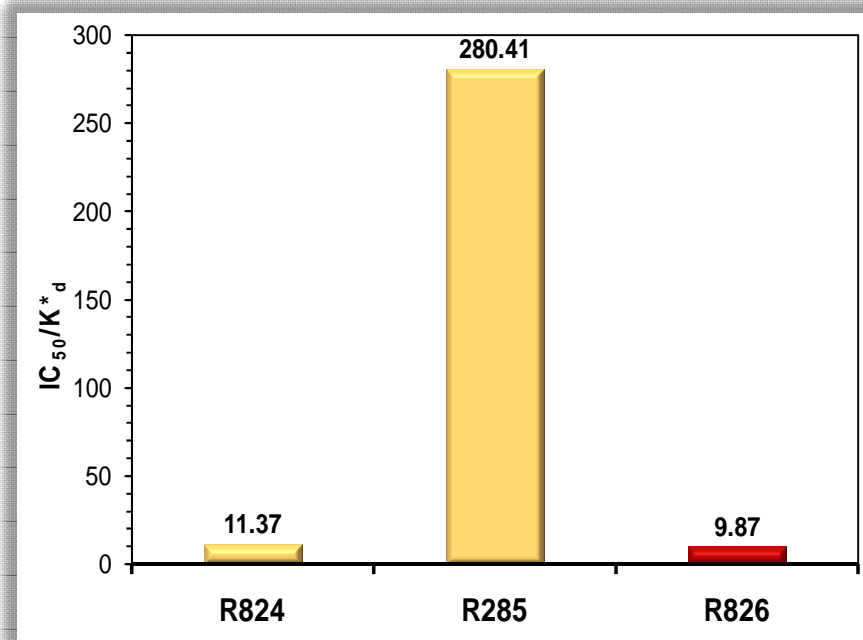
Ratio IC_{50}/K_d of PS-specific peptides. The IC_{50} of R824, R825, and R826 was determined in competition with Annexin V
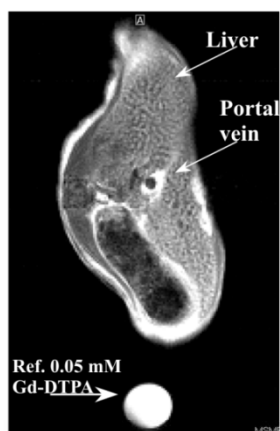
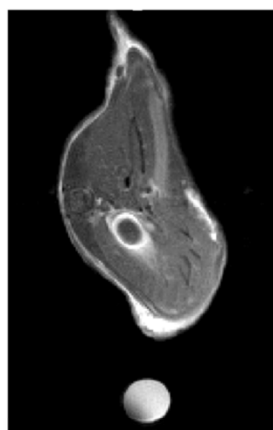# Binding of Gd-DTPA-g-R826 to apoptotic Jurkat cells as compared to various controls

Apoptotic cells were stained (brown) in camptothecin treated **(a)** and control **(b)** samples with biotinylated Annexin V.
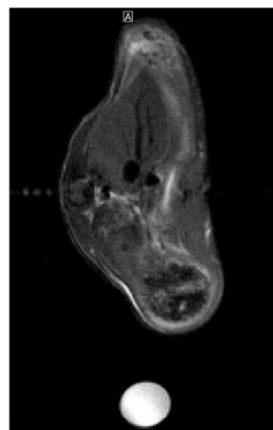
MRI (4.7T Bruker imaging system, T_1-weighted MSME, TR/TE = 307.4/14.7 ms) of PS in mouse liver 30 min post contrast and immunohistochemistry of apoptotic cells
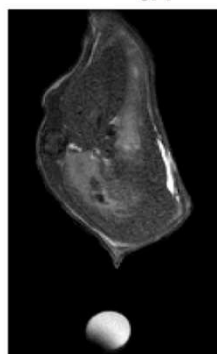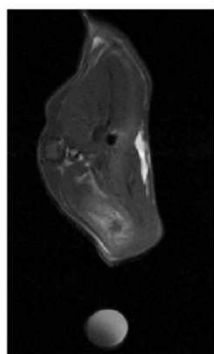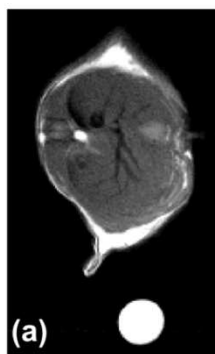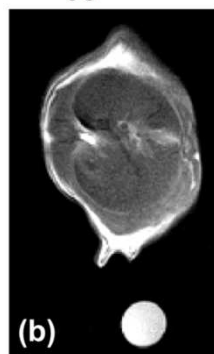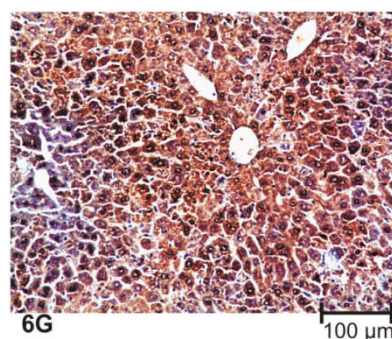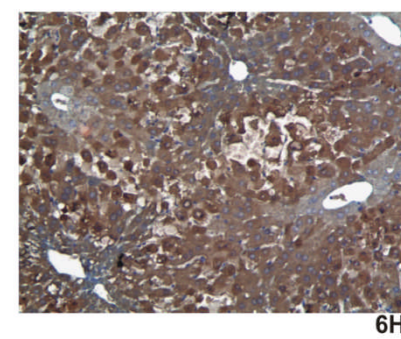

Apoptotic liver imaged with Gd-DTPA-g-R826 in the absence **(6A)** and in the presence of competitor R826 **(6B)** is compared to healthy liver **(6C)**. Apoptotic **(6D)** and healthy **(6E)** liver imaged with Gd-DTPA. **(6F)** Apoptotic liver in pre-contrast **(a)** and post injection of Gd-DTPA-g-R826.Sc **(b)**. Apoptotic cells immunostained (brown) with AnnV–Bt **(6G)** and anti-caspase-3 antibody **(6H)**.

MR images (4.7T Bruker imaging system, RARE sequence, TR/TE = 1048.5/4 ms, spatial resolution = 90 µm) of abdominal aorta in ApoE$^{-/-}$ mice
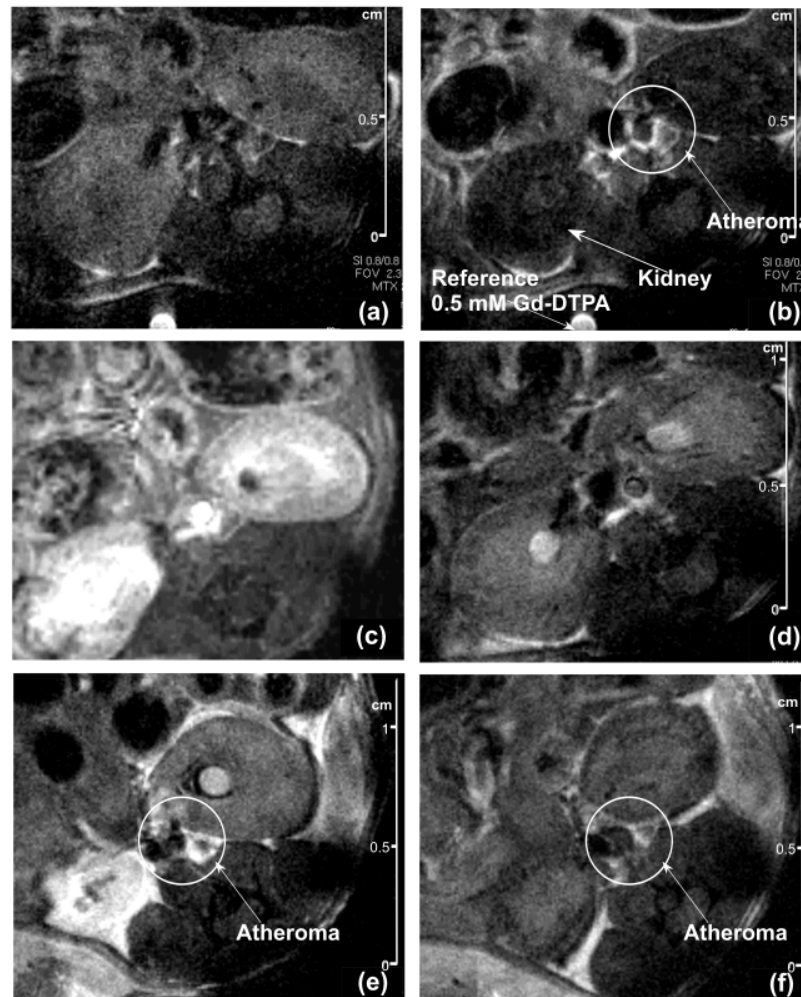

Axial slices of abdominal aorta are shown in pre-contrast **(a)** and ~30 min post Gd-DTPA-g-R826 **(b)**. They are compared to a TOF image **(c)** and to an image obtained post Gd-DTPA **(d)**. The comparison between Gd-DTPA-g-R826 **(e)** and Gd-DTPA-g-R826.Sc **(f)** is shown 60 min post-contrast. Images compared in **(a)**–**(d)** and those in **(e)**–**(f)** are located at the same level of abdominal aorta.

Immunostaining of apoptotic cells in atherosclerotic aorta of ApoE$^{-/-}$ mice
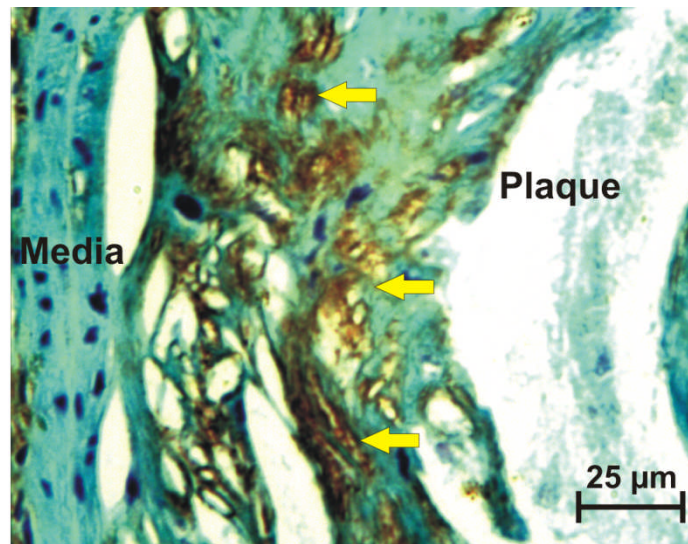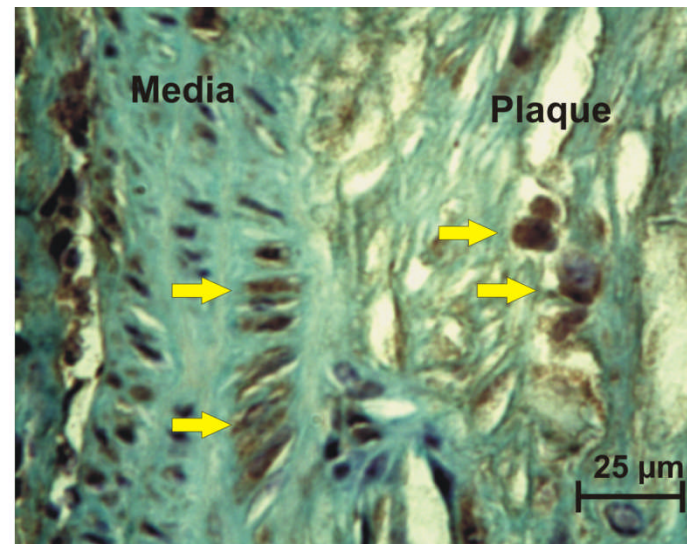
Apoptotic cells were immunostained (brown) with biotinylated Annexin V **(A)** and with anti-caspase-3 antibody **(B).**

Conclusions

- Peptide R826 ➔ the most important PS-specific peptide

  - diagnosis of atherosclerotic disease and of other apoptosis-associated pathologies, such as cancer, ischemia, chronic inflammation, autoimmune disorders, transplant rejection, neurodegenerative disorders, and diabetes mellitus

  - cardiovascular diseases: apoptosis associated with loss of cardiomyocytes subsequent to myocardial infarction, atherosclerotic plaque instability, congestive heart failure and allograft rejection of the transplanted heart