cardiovasculaire conferentie
Waar vasculair Nederland elkaar ontmoet!

Mount Sinai
MOUNT SINAI SCHOOL OF MEDICINE

TMII
TRANSLATIONAL AND MOLECULAR IMAGING INSTITUTE

Willem J.M. Mulder, Ph.D.

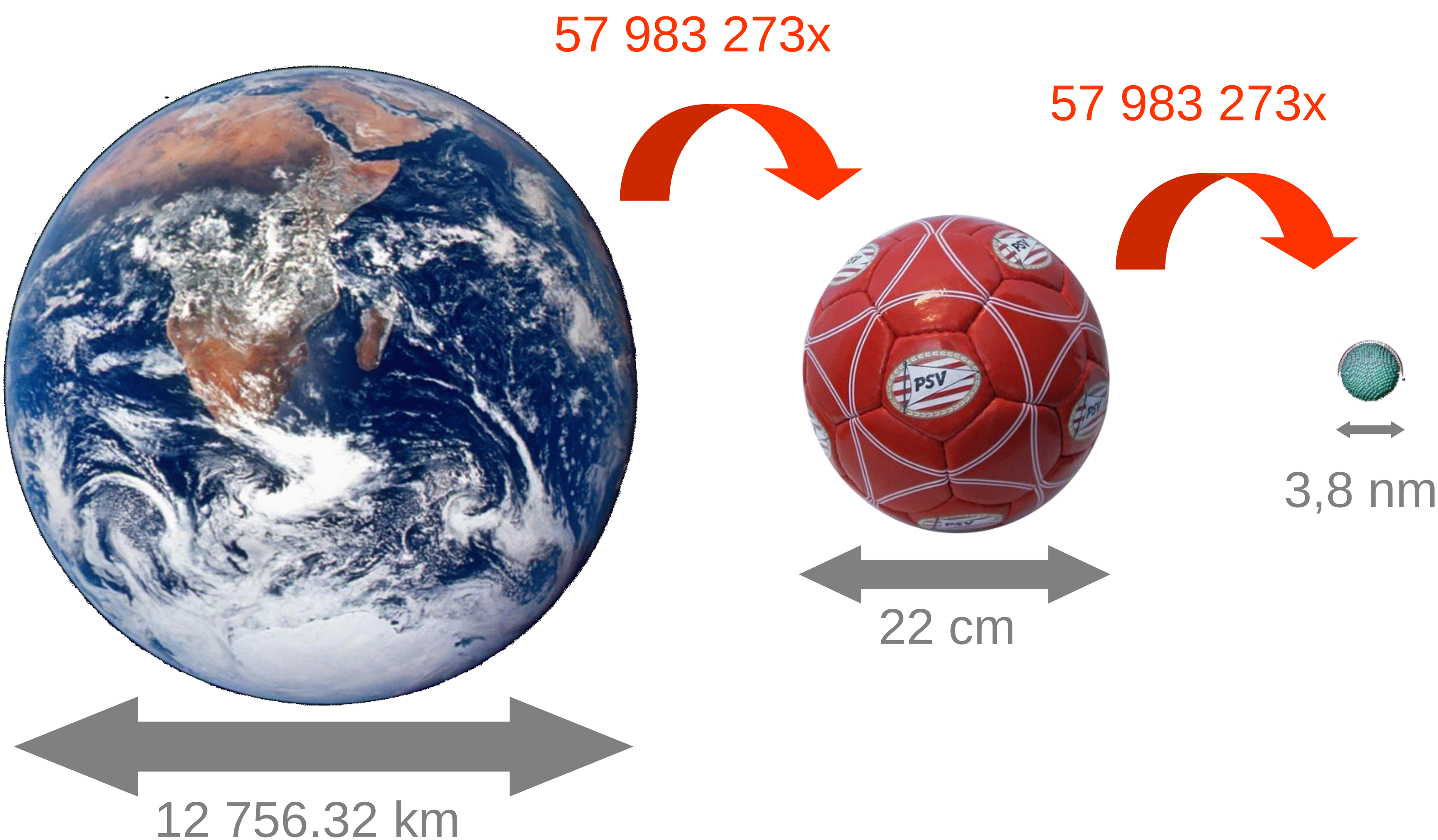
Assistant Professor of Radiology
Assistant Professor of Gene and Cell Medicine
Director Nanomedicine Laboratory

Nanomedical strategies for atherosclerosis

Noordwijkerhout, The Netherlands

17-18 March 2011

Nanometer (10^{-9} meter)



Nanotechnology!

Nano = 10^{-9} nanus=νανοζ=dwarf

Nanotechnology deals with structures sized between 1 nm to 100 nm in at least one dimension

What is special about nano?

-Fundamental properties of materials change

-Large surface to volume ratio

-Multifunctionality

Gold nanoparticles

Nanomedicine

- Wikipedia definition
  - Nanomedicine is the **medical application of nanotechnology**. Nanomedicine ranges from the medical applications of nanomaterials, to nanoelectronic biosensors, and even possible future applications of molecular nanotechnology. Current problems for nanomedicine involve understanding the issues related to toxicity and environmental impact of nanoscale materials.
- NIH definition
  - Applications of nanotechnology for treatment, diagnosis, monitoring, and control of biological systems.

Applications of nanomedicine

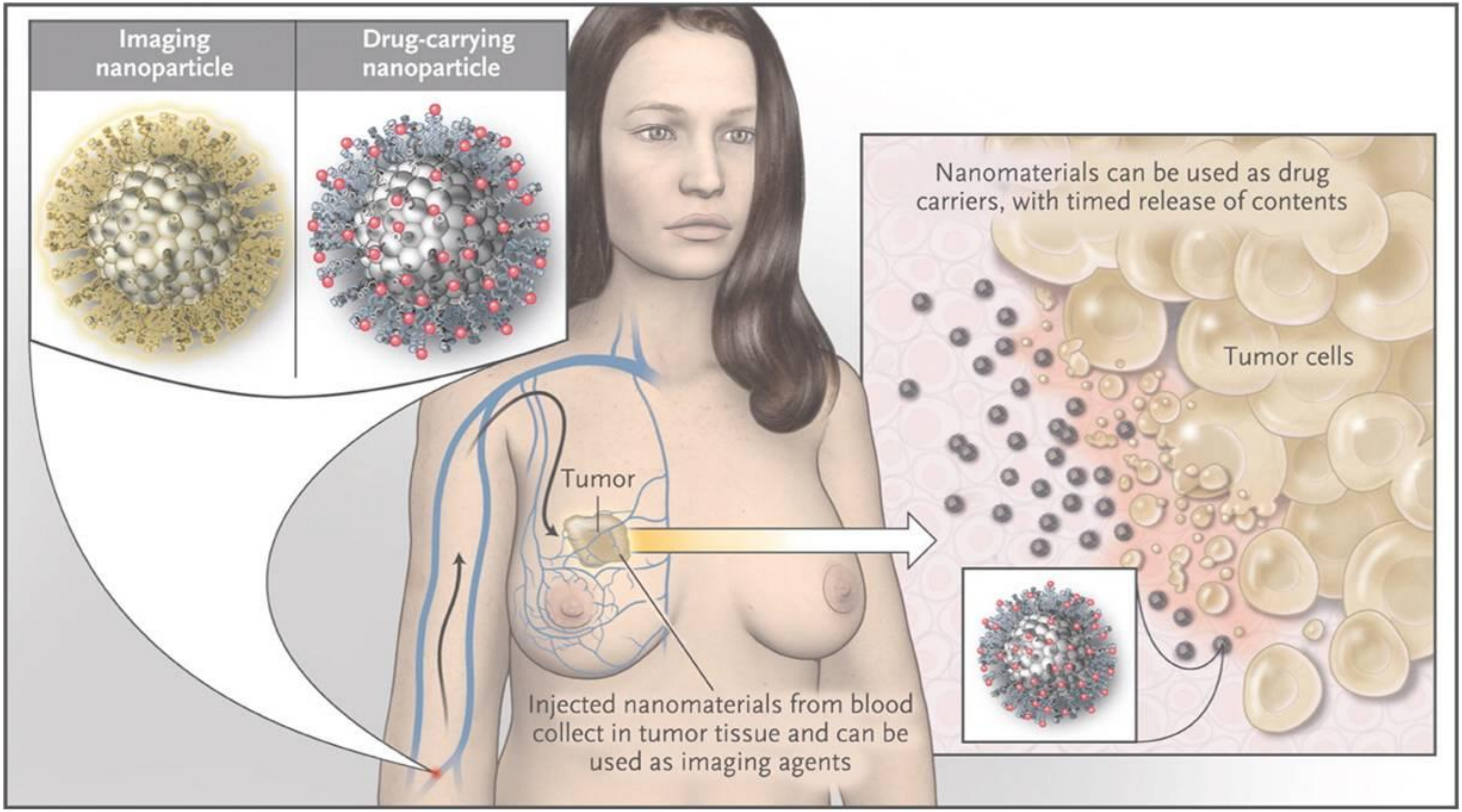
Drug delivery. Nanoscale particles/molecules developed to improve the bioavailability and pharmacokinetics of therapeutics. Examples are liposomes (and virosomes), polymer nanoparticles, nanosuspensions and polymer therapeutics. Drugs in which a protein is combined with a polymer nanoparticle or chemical nanostructure to improve its pharmacokinetic properties would be classified as nanomedicine-based drug delivery.

Drugs and therapy. Nanoscale particles/molecules used in the treatment of diseases that according to their structure have unique medical effects and as such differ from traditional small-molecule drugs. Examples include drugs based on fullerenes or dendrimers.

***In vivo* imaging.** Nanoparticle contrast agents, particularly for MRI and ultrasound, that provide improved contrast and favorable biodistribution. For example, superparamagnetic iron oxide nanoparticles for use as MRI contrast agents.

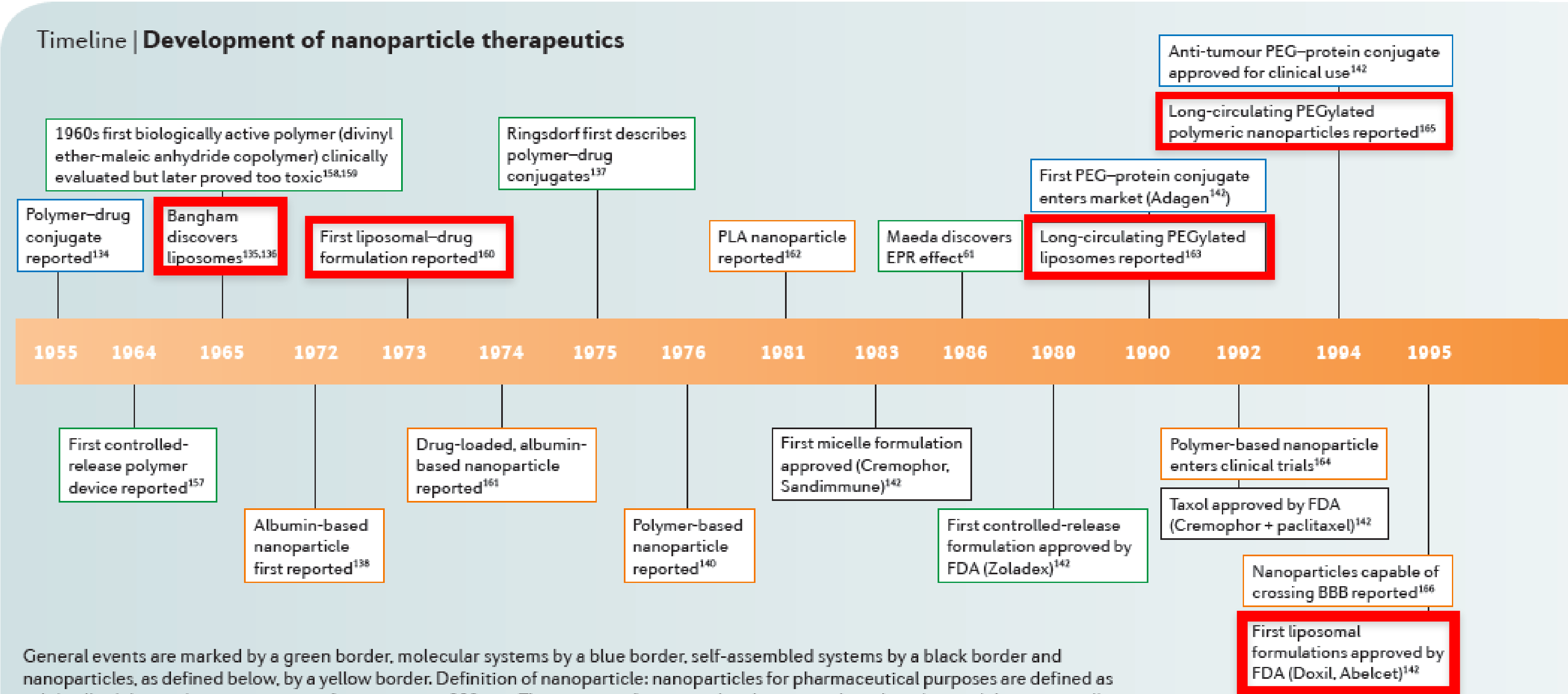
Wagner *et al*. Nat Biotechnol. 2006

Applications of nanomedicine



Kim *et al*. N Engl J Med. 2010

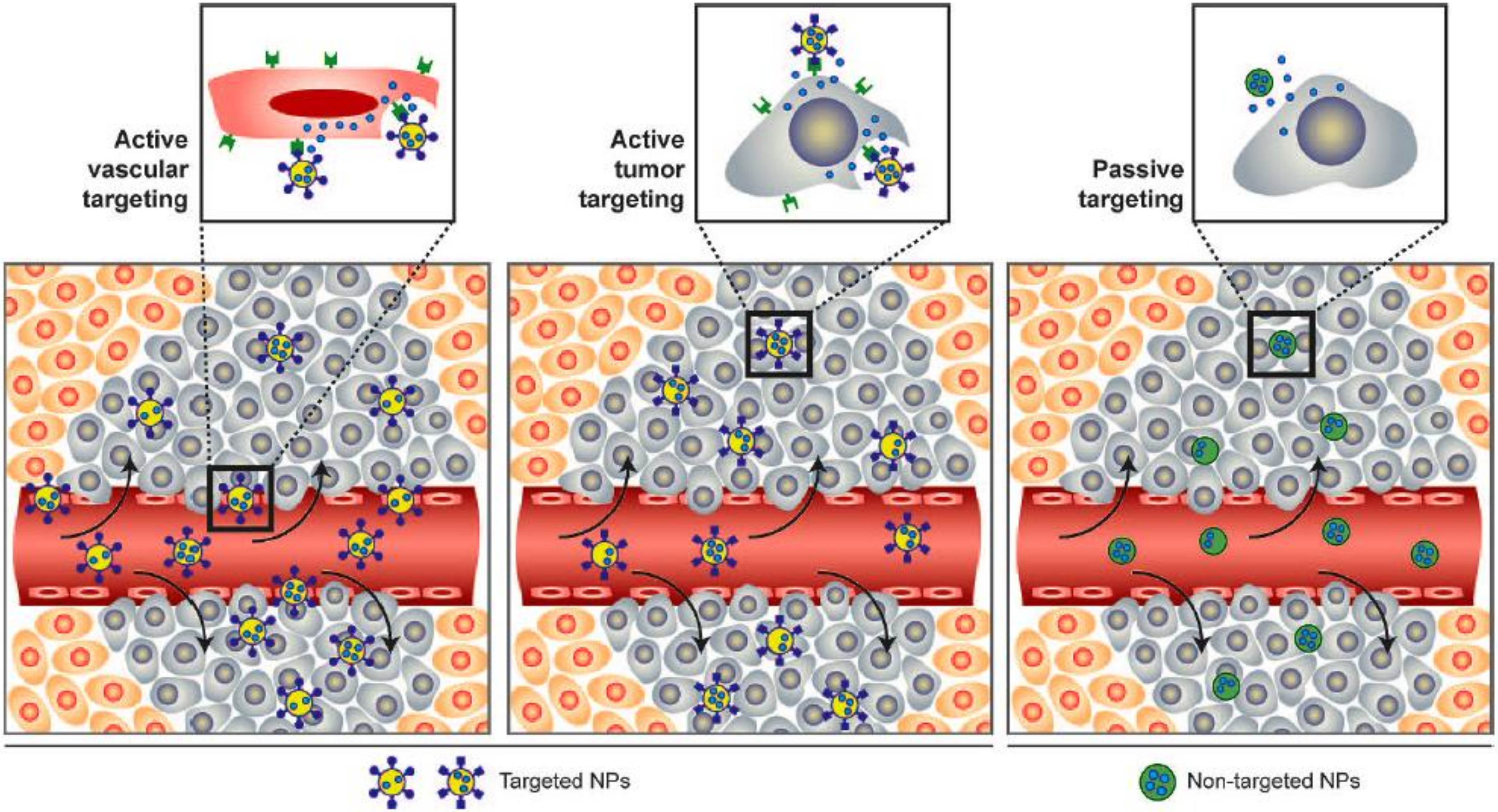
History of nanoparticle therapeutics



General events are marked by a green border, molecular systems by a blue border, self-assembled systems by a black border and nanoparticles, as defined below, by a yellow border. Definition of nanoparticle: nanoparticles for pharmaceutical purposes are defined as solid colloidal particles ranging in size from 1 nm to 1,000 nm. They consist of macromolecular materials and can be used therapeutically as drug carriers, in which the active principle (drug or biologically active material) is dissolved, entrapped or encapsulated, or to which the active principle is adsorbed or attached. Abraxane, paclitaxel protein-bound particles for injectable suspension (Abraxis/AstraZeneca); Adagen, PEG–adenosine deaminase (Enzon); BBB, blood–brain barrier; Copaxone, glatiramer acetate for injection (Teva Pharmaceuticals); Cremophor, polyoxyethylated castor oil (BASF); EPR, enhanced permeability and retention; FDA, US Food and Drug Administration; Gliadel, polifeprosan 20 with carmustine implant (Eisai); PEG, polyethylene glycol; PLA, polylactic acid; Sandimmune, cyclosporine injection (Novartis); Zoladex, goserelin acetate implant (AstraZeneca).

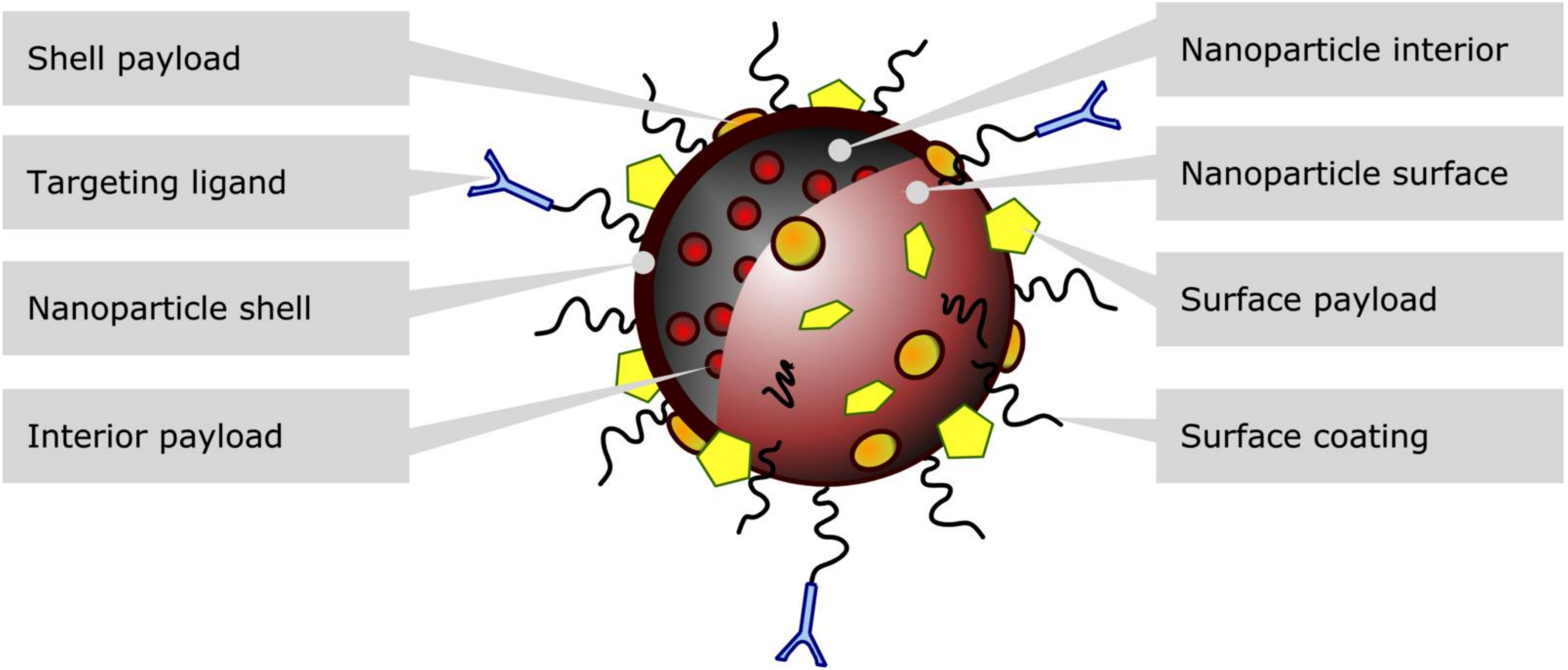
Petros & DeSimone. Nat Rev Drug Discov. 2010

Nanoparticle targeting



Farokhzad, Langer. ACS Nano. 2009

Nanoparticle schematic



Mulder *et al*. NMR Biomed. 2006
Mulder *et al*. Nanomed. 2007
Mulder *et al.* Nat Clin Pract Cardiovasc Med 2008
Cormode *et al*. Arterioscler Thromb Vasc Biol. 2009
Mulder *et al*. Acc Chem Res. 2009
Skajaa *et al*. Arterioscler Thromb Vasc Biol. 2009
Jarzyna *et al*. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2009

Making nanostructures

Distinguish between two strategies:

Top-down

Start with a homogeneous substrate and remove material until the desired nanostructures are formed

Bottom-up

Start with molecules or atoms and let them react until nanostructures are grown

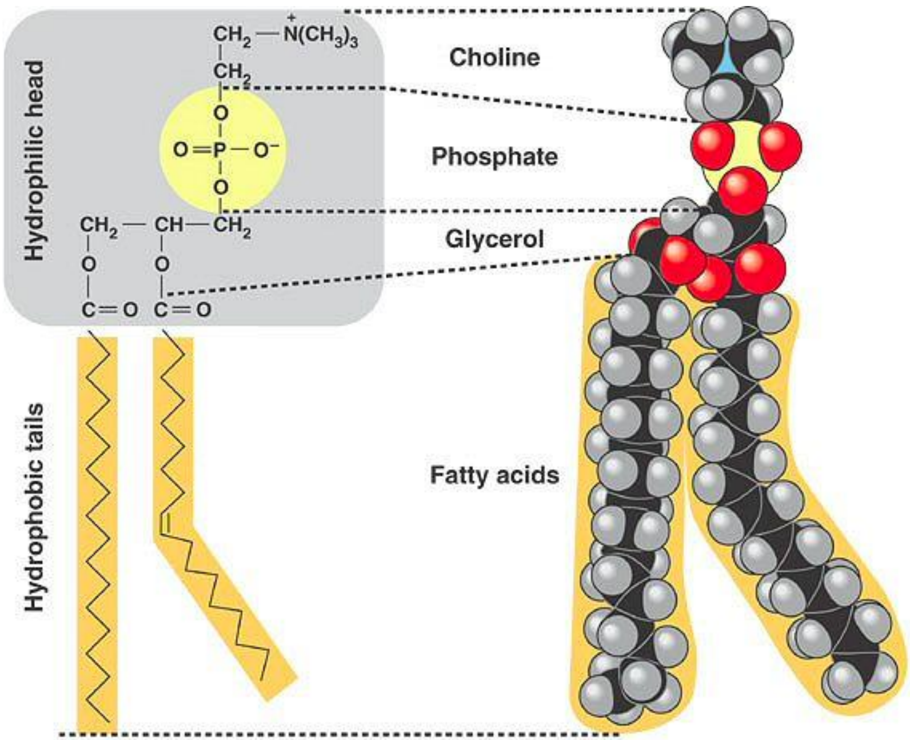
“Artificial structures"   “Self-assembly"

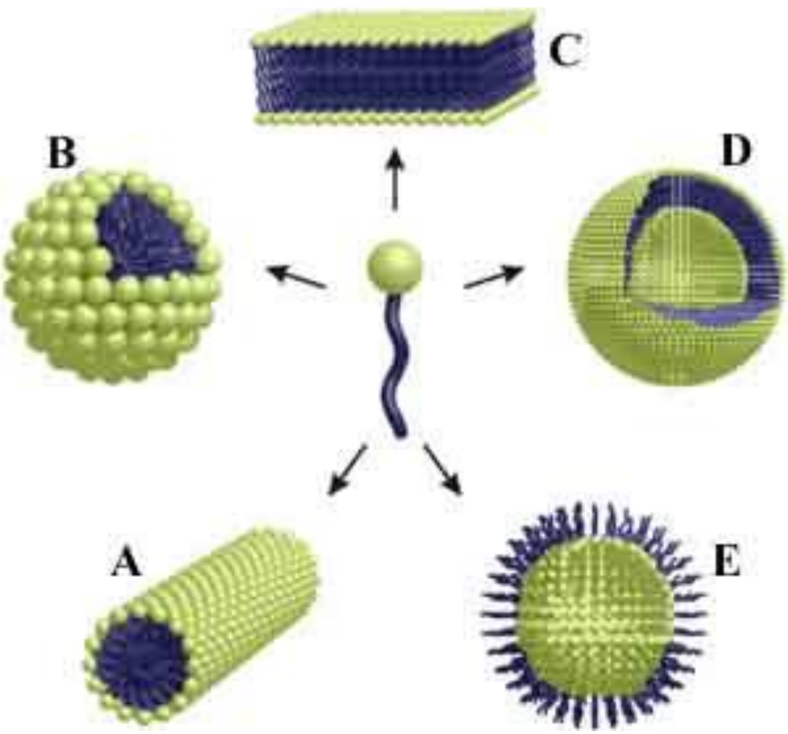
Self-assembly and amphiphiles

Amphiphile

From Wikipedia, the free encyclopedia

Amphiphile (from the Greek αμφις, amphis: both and φιλία, philia: love, friendship) is a term describing a chemical compound possessing both hydrophilic (*water-loving*) and lipophilic (*fat-loving*) properties. Such a compound is called *amphiphilic* or *amphipathic*. This forms the basis for a number of areas of research in chemistry and biochemistry, notably that of lipid polymorphism. Organic compounds containing hydrophilic groups at **both** ends of a prolate molecule are called bolaamphiphilic. Common amphiphilic substances are soaps and detergents.

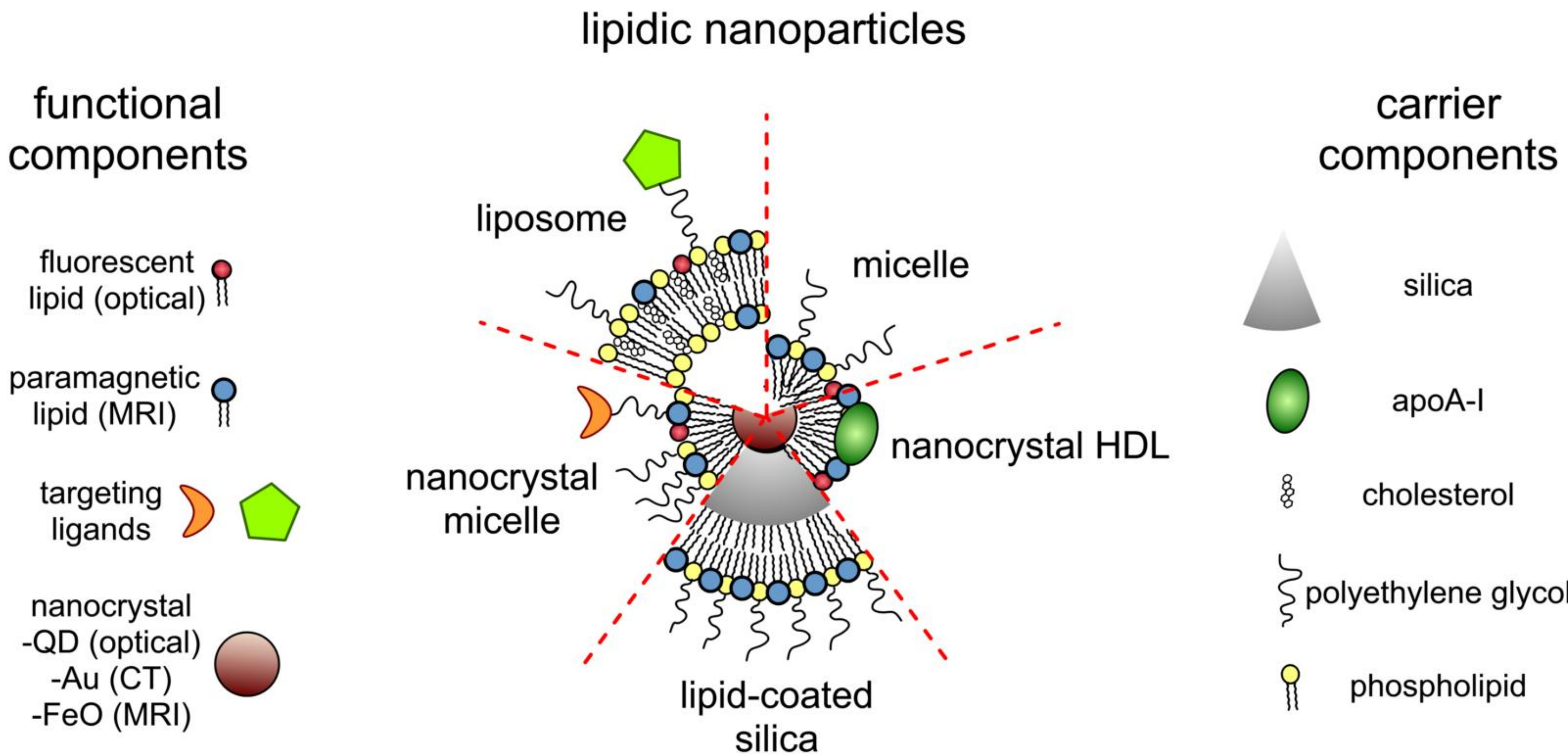




ACCOUNTS
of chemical research

Nanoparticulate Assemblies of Amphiphiles and Diagnostically Active Materials for Multimodality Imaging

CONSPECTUS



Mulder *et al*. Acc Chem Res. 2009

Outline

Classes of nanoparticles to be discussed

- Micellar nanoparticles
  - Collagen imaging in atherosclerosis and AAA
  - Apoptosis imaging in atherosclerosis
- High density lipoprotein nanoparticles
  - Nanocrystal HDL: HDL-cell biology ➔ In vivo imaging
  - HDL statin delivery as anti-atherosclerosis therapy
- Multimodal liposomes
  - Theranostics of atherosclerosis

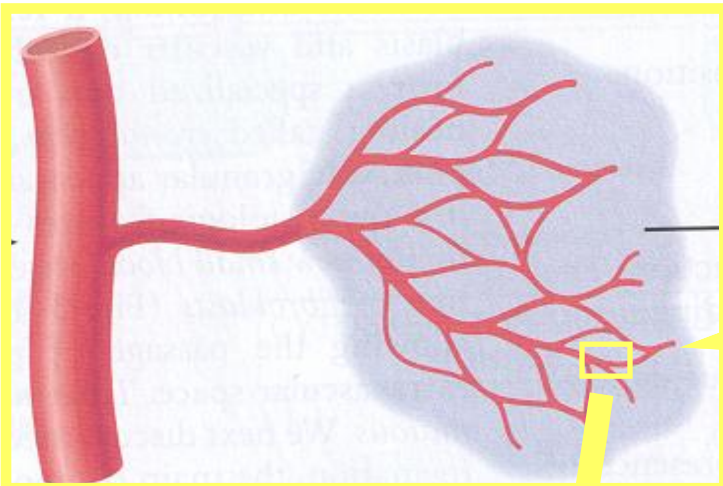
Molecular imaging

Theranostics

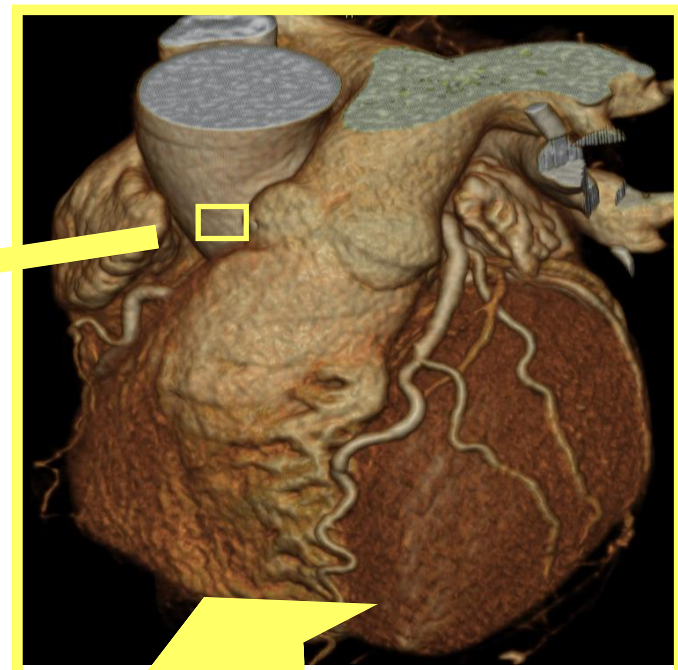
Multimodal imaging

Anatomical to molecular imaging

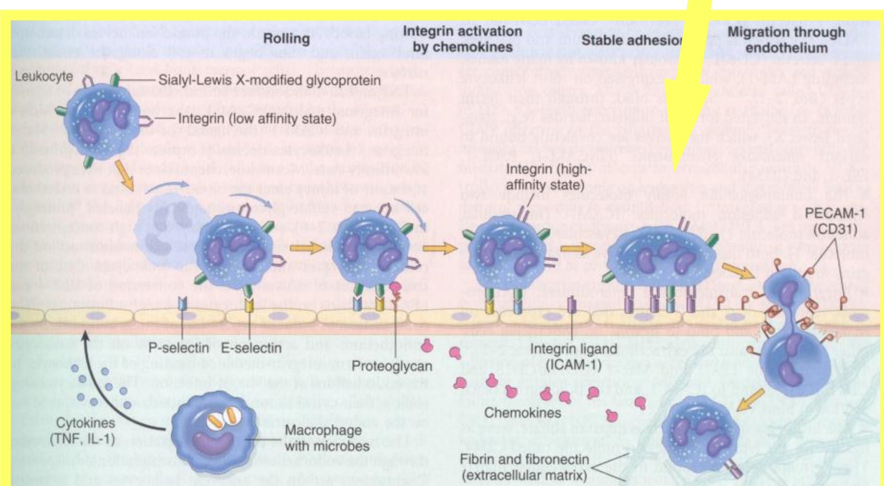
Tissue level (e.g. myocardial perfusion)



Anatomical level



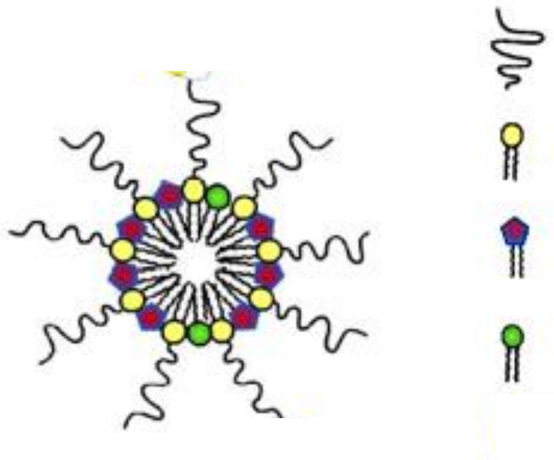
Cellular and molecular level



Nanoparticles!

Collagen-targeted paramagnetic micellar nanoparticle

Paramagnetic micelle



PEG

Phospholipid

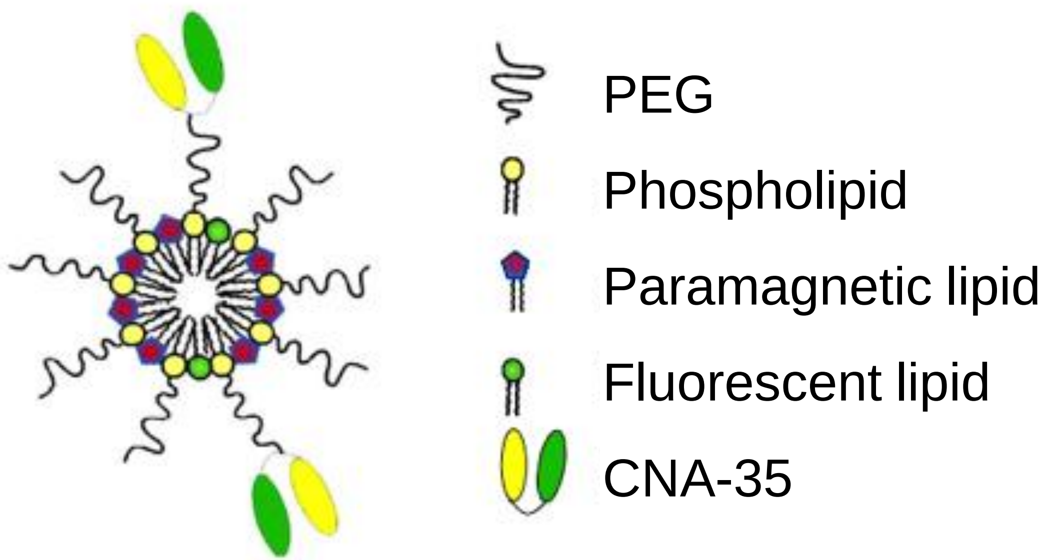
Paramagnetic lipid

Fluorescent lipid

~ 70 lipids / micelle
~ 25 nm diameter
1-2 CNA35 / micelle
$K_{d\_CNA35} = 0.5\ \mu M$

Collagen-targeted paramagnetic micellar nanoparticle

Paramagnetic micelle conjugated with CNA-35,
a 35 kDa collagen-specific adhesion protein

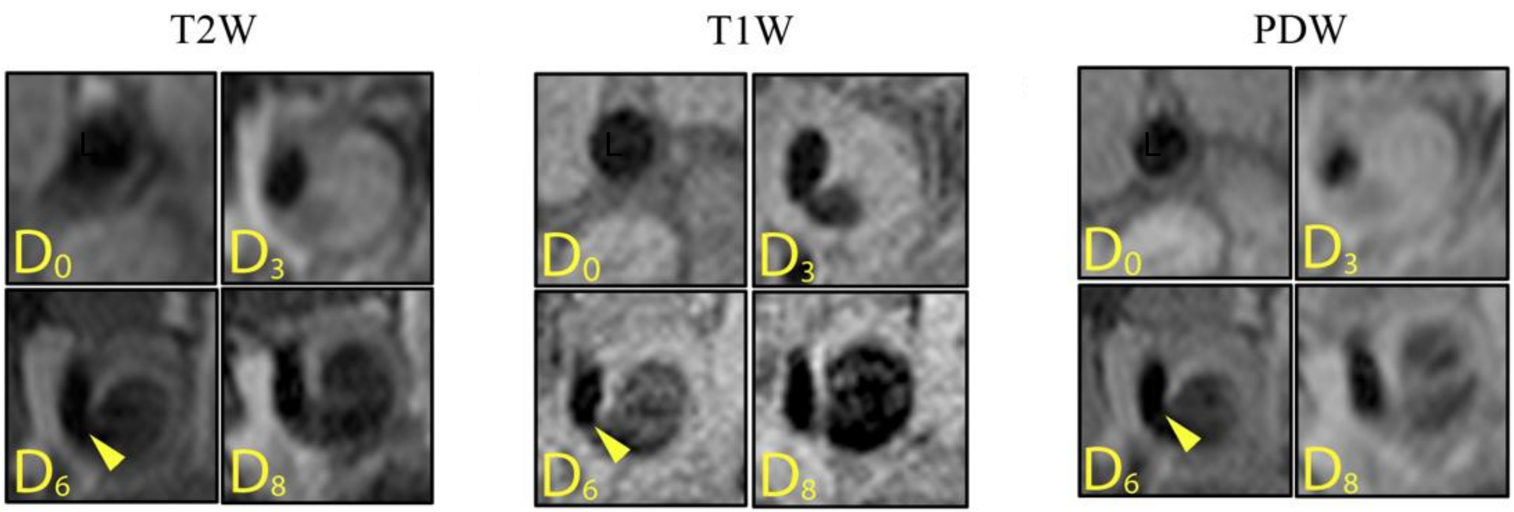


CNA35

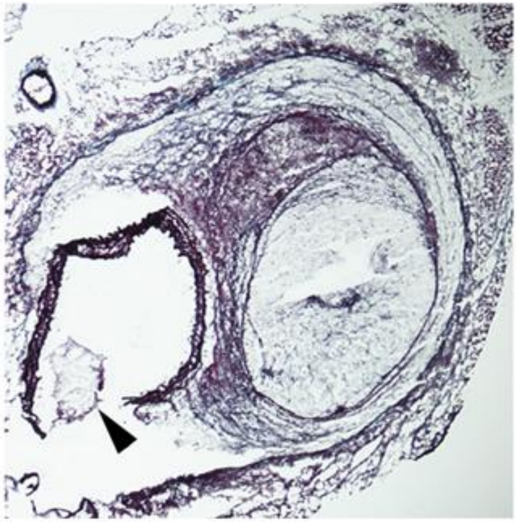
binds type I
but also II/III/IV

~ 70 lipids / micelle
~ 25 nm diameter
1-2 CNA35 / micelle
$K_{d\_CNA35} = 0.5\ \mu M$

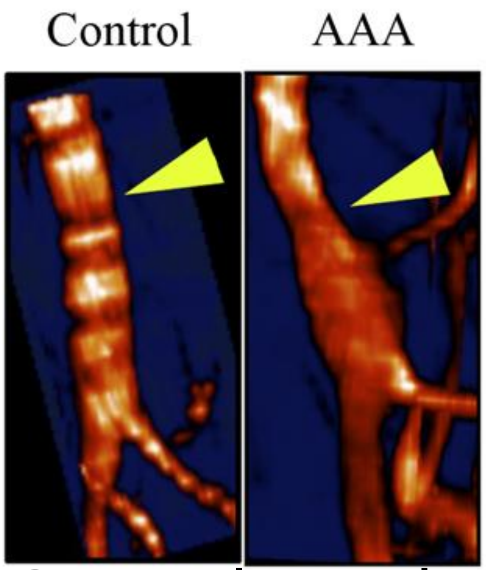
MRI of abdominal aortic aneurysm (AAA)



Histology - CME

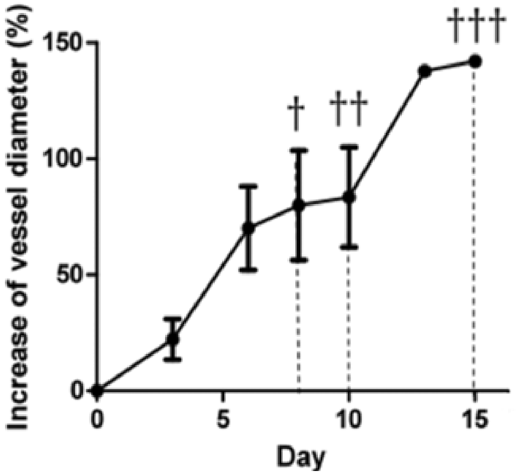


Collagen; AAA

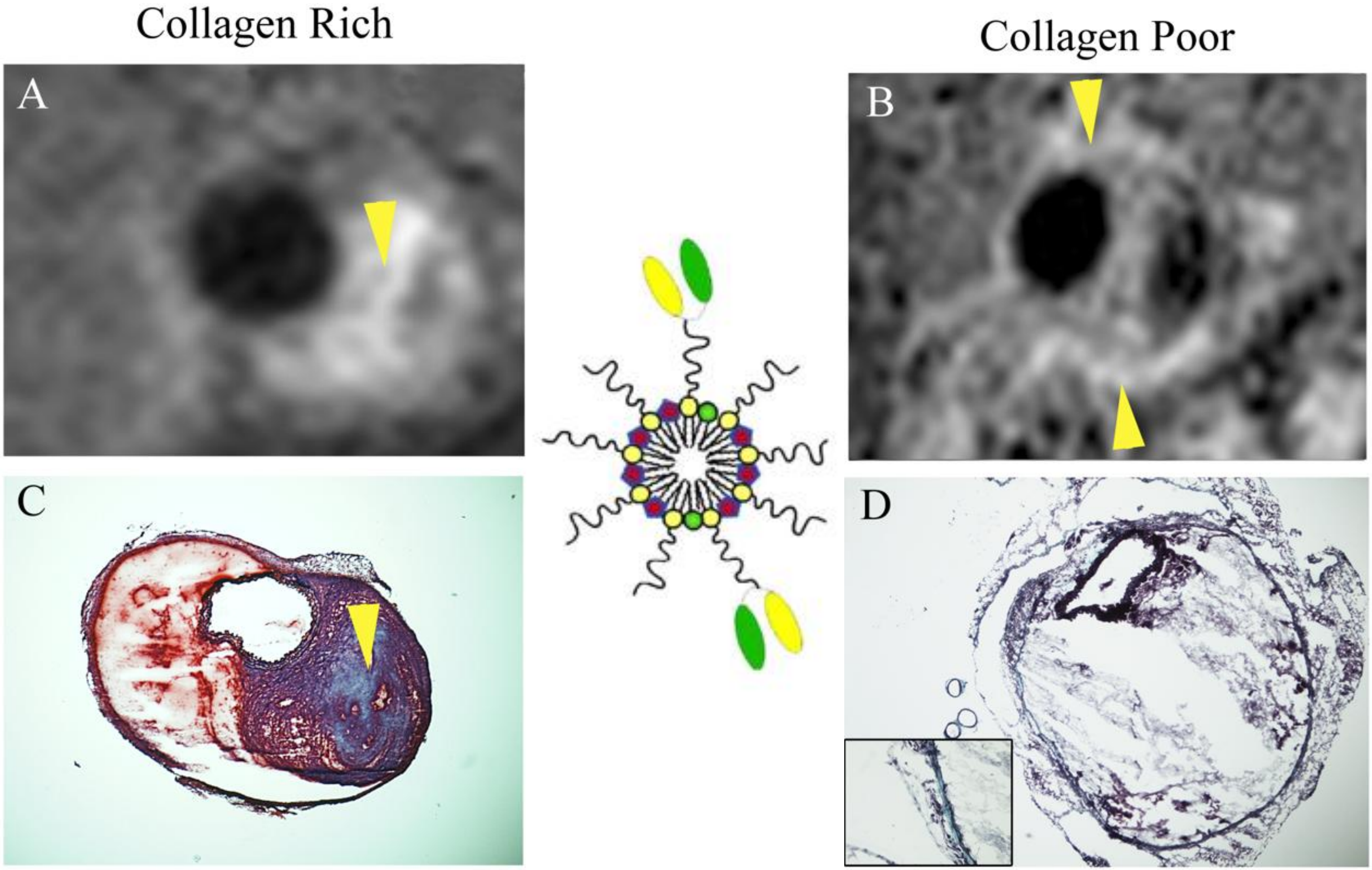


TOF angiography

Aortic diameter



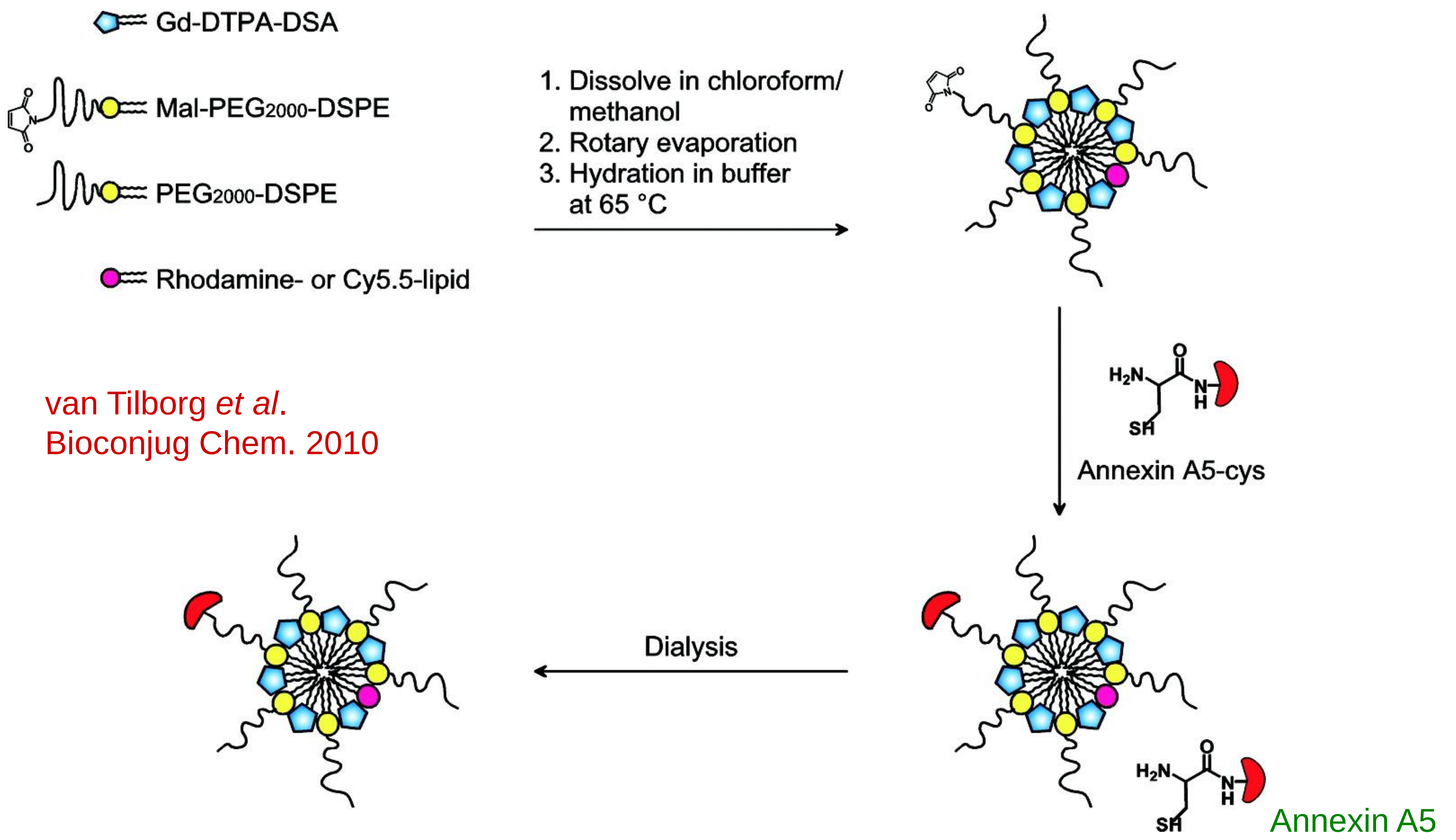
Molecular MRI of collagen



Collagen; AAA

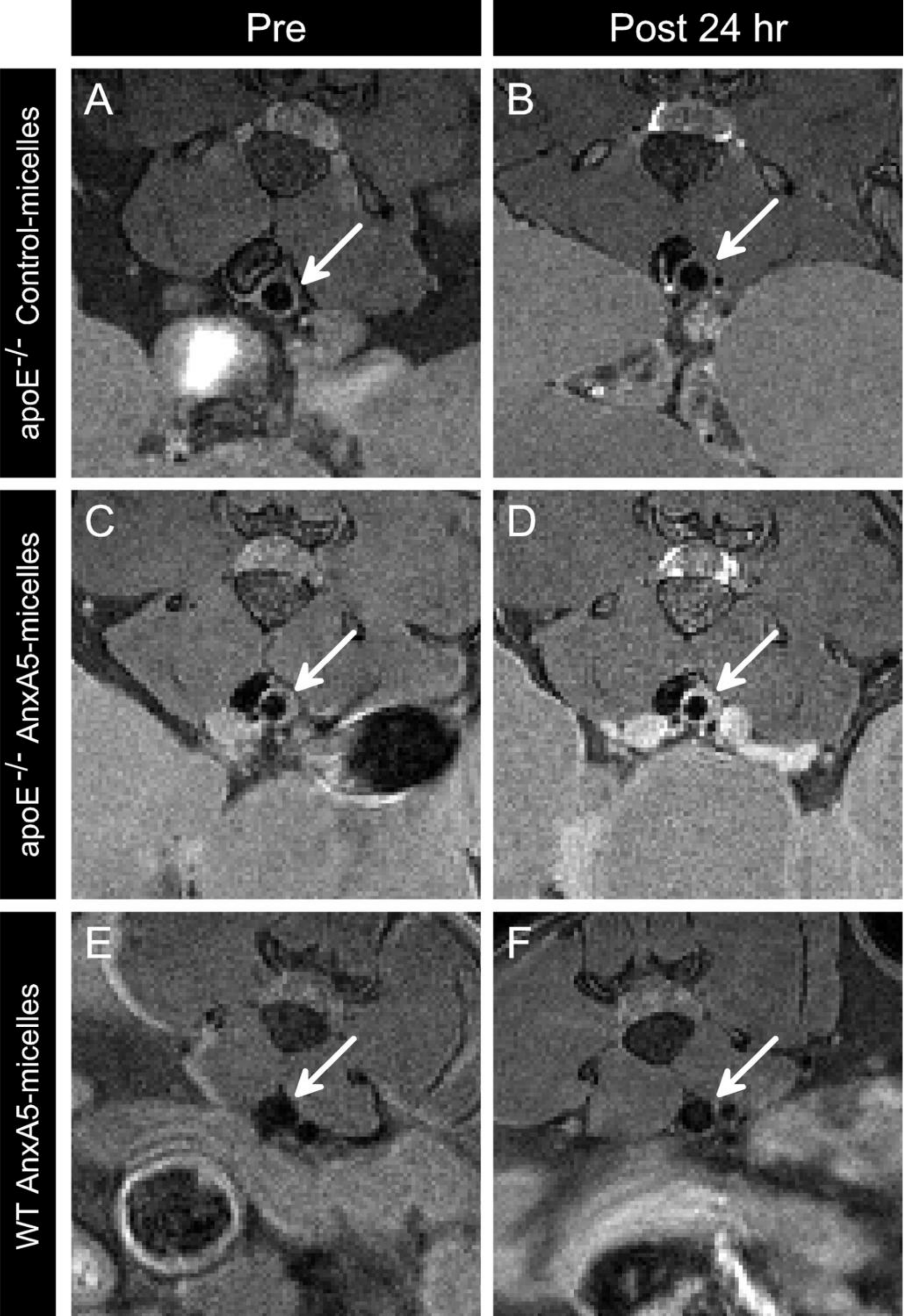
Annexin A5-Functionalized Bimodal Nanoparticles for MRI and Fluorescence Imaging of Atherosclerotic Plaques

Geralda A. F. van Tilborg,*[,†,‡] Esad Vucic,[§] Gustav J. Strijkers,[†] David P. Cormode,[§] Venkatesh Mani,[§] Torjus Skajaa,[§] Chris P. M. Reutelingsperger,[||] Zahi A. Fayad,[§] Willem J. M. Mulder,[§] and Klaas Nicolay[†]

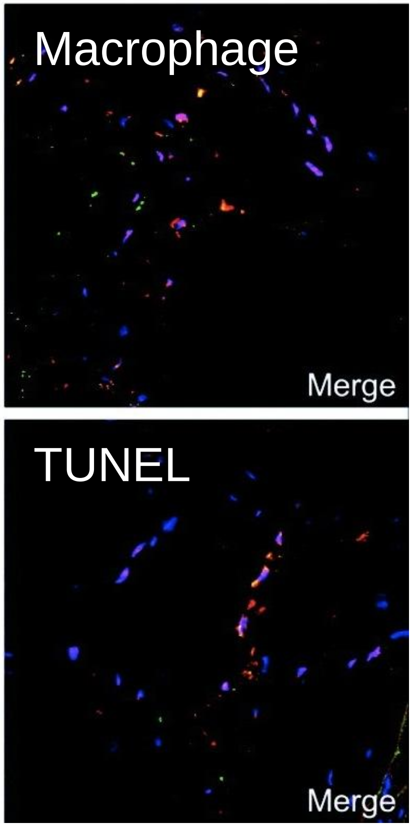


van Tilborg *et al*.
Bioconjug Chem. 2010

MRI signal enhancement

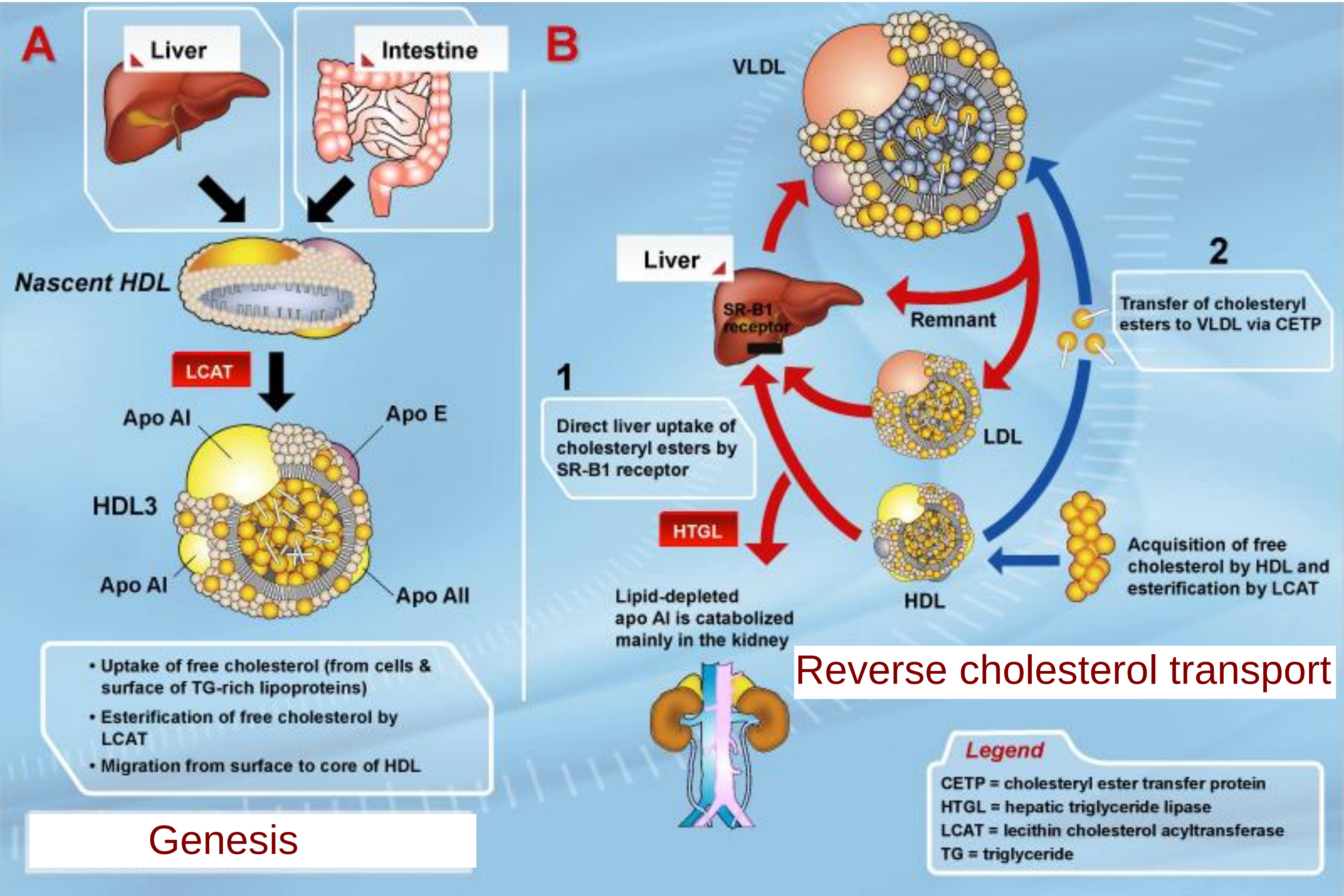


Immunofluorescence

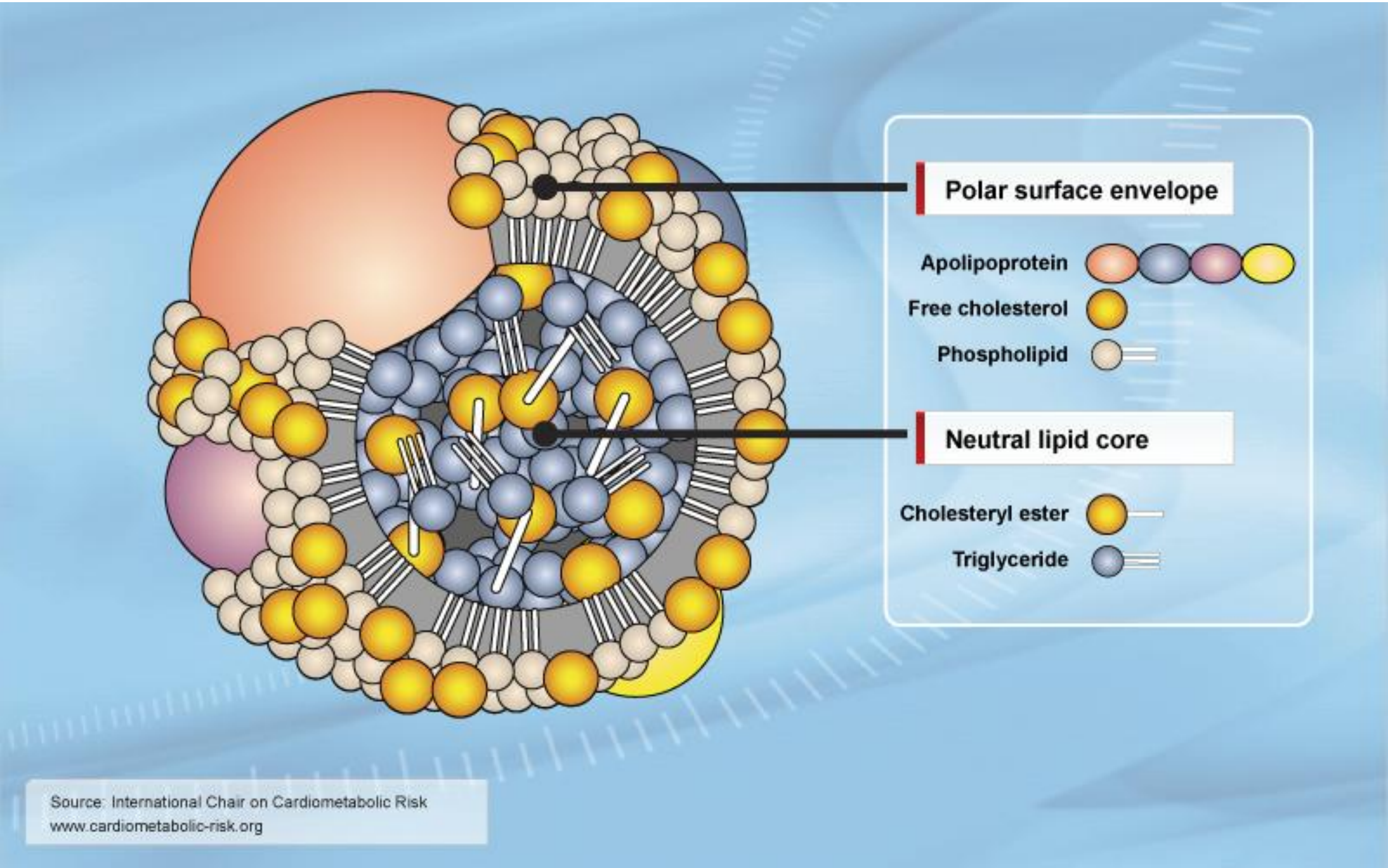


Annexin A5

HDL: “good cholesterol”



Architecture of HDL



How to use HDL to create multimodal agents

Highest resemblance

- Isolate HDL from blood and (post) insert labels
- Isolate HDL from blood, disassemble, add labels and reconstitute
- Isolate apoA-I and reconstitute from lipids and labels
- Redirect HDL-like particle to new target by the conjugation of targeting moieties
- Synthesize peptides derived from apolipoproteins
- Synthesize a lipidic particle of which the overall design resembles HDL

Lowest resemblance

NANO LETTERS

2008
Vol. 8, No. 11
3715-3723

Nanocrystal Core High-Density Lipoproteins: A Multimodality Contrast Agent Platform

David P. Cormode,[†] Torjus Skajaa,[†,‡] Matti M. van Schooneveld,[§] Rolf Koole,[§] Peter Jarzyna,[†] Mark E. Lobatto,[†] Claudia Calcagno,[†] Alessandra Barazza,[†,§] Ronald E. Gordon,[⊥] Pat Zanzonico,[#] Edward A. Fisher,[||] Zahi A. Fayad,[*,†] and Willem J. M. Mulder[*,†]

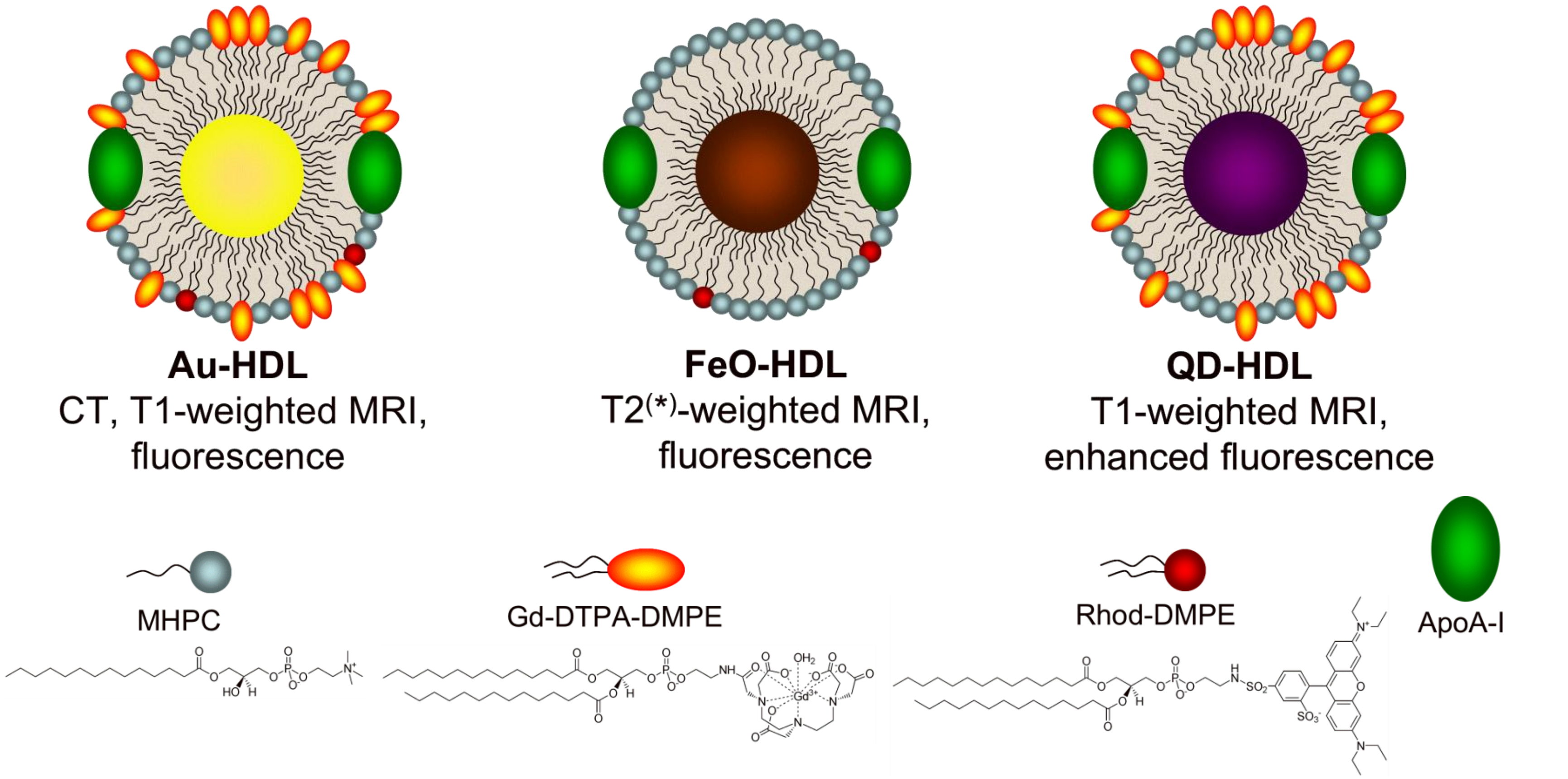
Translational and Molecular Imaging Institute, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1234, New York, New York 10029, Faculty of Health Sciences, Århus University, Vennelyst Boulevard 9, 8000 Århus C, Denmark, Condensed Matter and Interfaces, Debye Institute, Utrecht University, Princetonplein 5, 3584 CC Utrecht, The Netherlands, Department of Medicine (Cardiology), Marc and Ruti Bell Vascular Biology and Disease Program and the NYU Center for the Prevention of Cardiovascular Disease, New York University School of Medicine, New York University, Smilow 8 522 First Avenue, New York, New York 10016, Department of Pathology, Mount Sinai Hospital, One Gustave L. Levy Place, New York, New York 10029, Departments of Medical Physics and Radiology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021

Received July 3, 2008; Revised Manuscript Received September 30, 2008

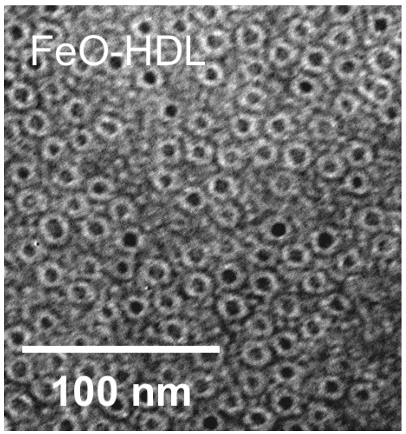
Cormode *et al*. Nano Lett. 2008

Multimodal inorganic core HDL

Nanocrystals with hydrophobic coatings replaced the triglyceride and cholesterol ester core of native HDL



Particle characterization
Physical properties



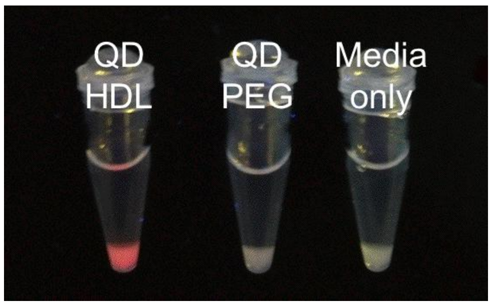
Property (units)	Au-HDL	FeO-HDL	QD-HDL
r1 ($mM^{-1}s^{-1}$)	13.1 ± 0.3	8.9 ± 0.3	11.7 ± 0.4
r2 ($mM^{-1}s^{-1}$)	16.8 ± 0.5	94.2 ± 6.8	14.8 ± 0.5
Number of apoA-I molecules per particle	3.0	3.9	3.1
Core diameter (nm)	5.6 ± 1.0	6.3 ± 1.0	6.5 ± .08
Diameter: TEM (nm)	9.7 ± 1.4	11.9 ± 1.2	12.9 ± 1.4

→ Diameters confirmed by DLS and gel electrophoresis. Native HDL 7-13 nm

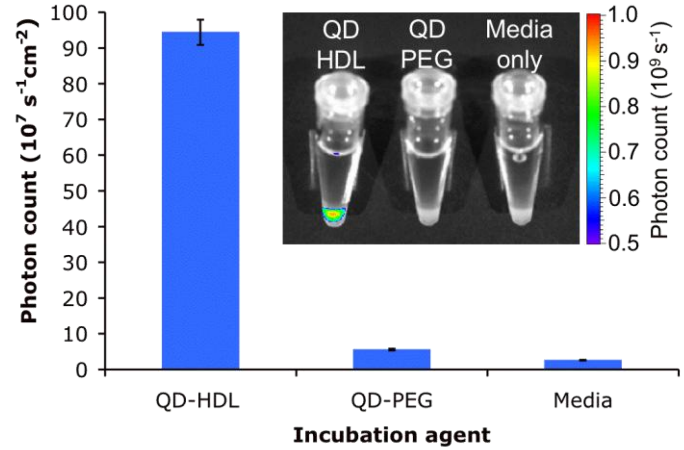
QD-HDL: Macrophage cell incubations

2 hours incubation time
0.02 mM Gd dose

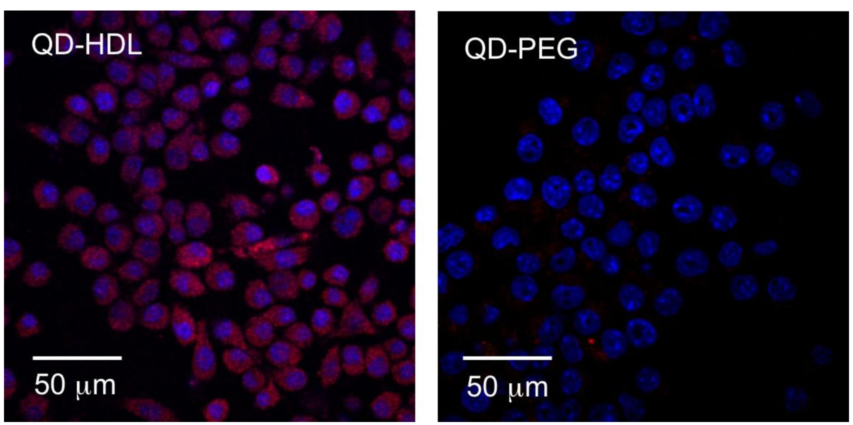
Photo under UV illumination



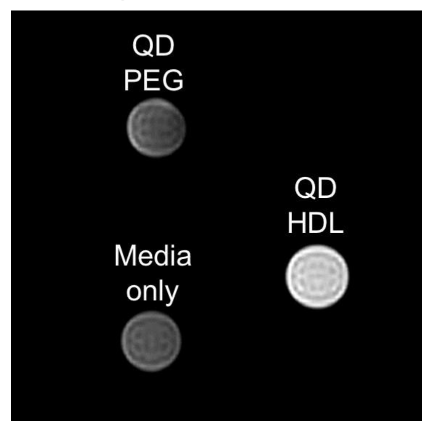
Fluorescence imaging



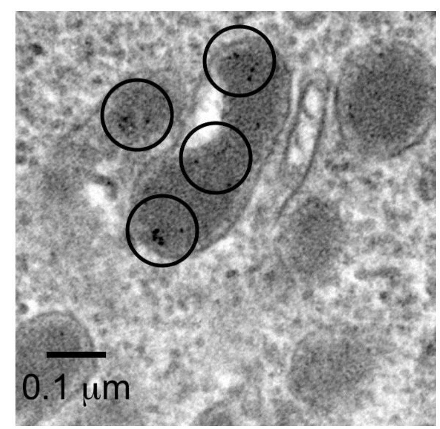
Confocal microscopy



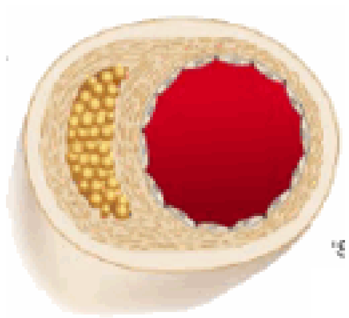
T1-weighted MRI (9.4T)

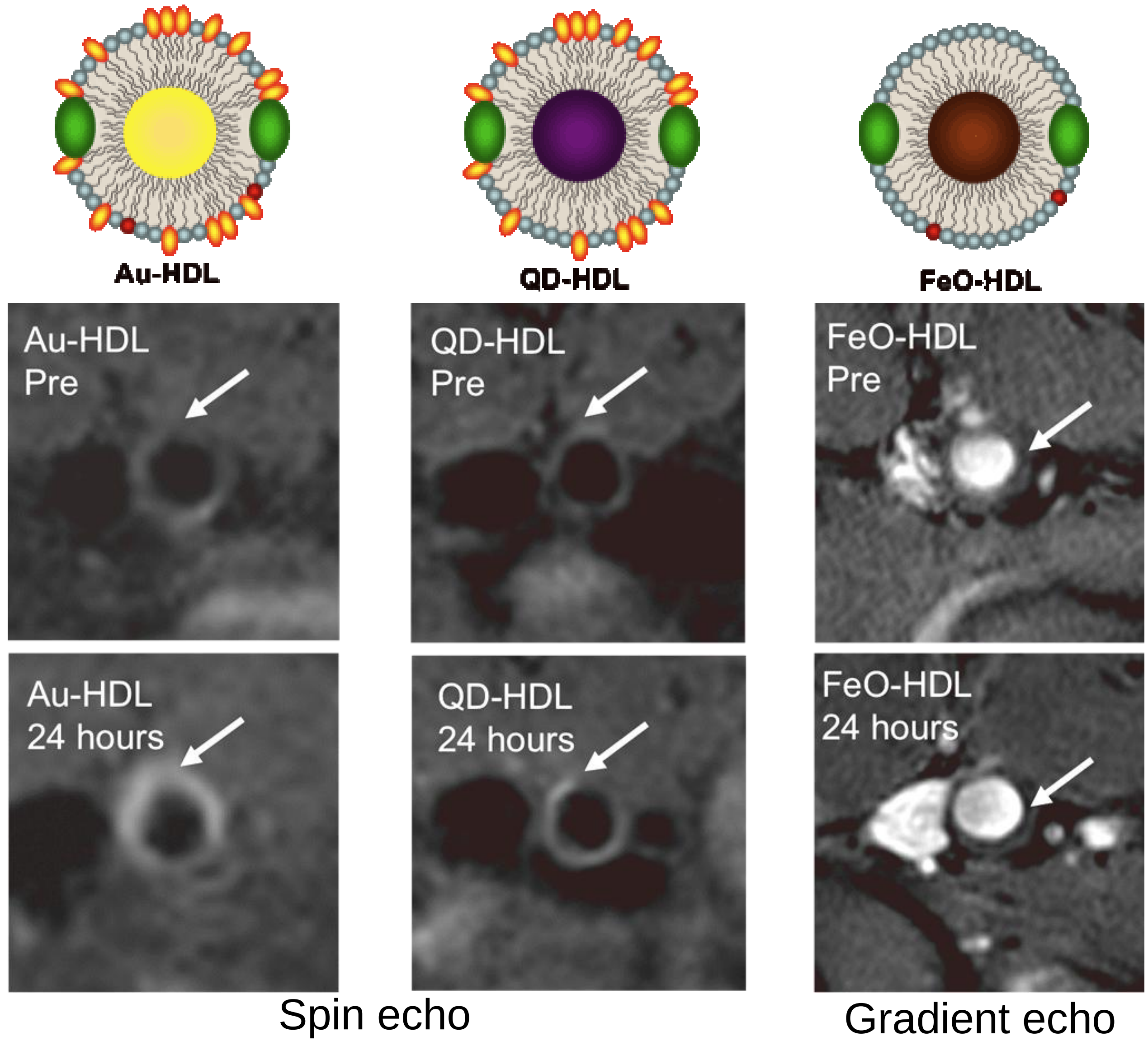


TEM



In vivo MRI studies

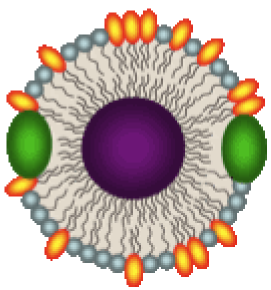




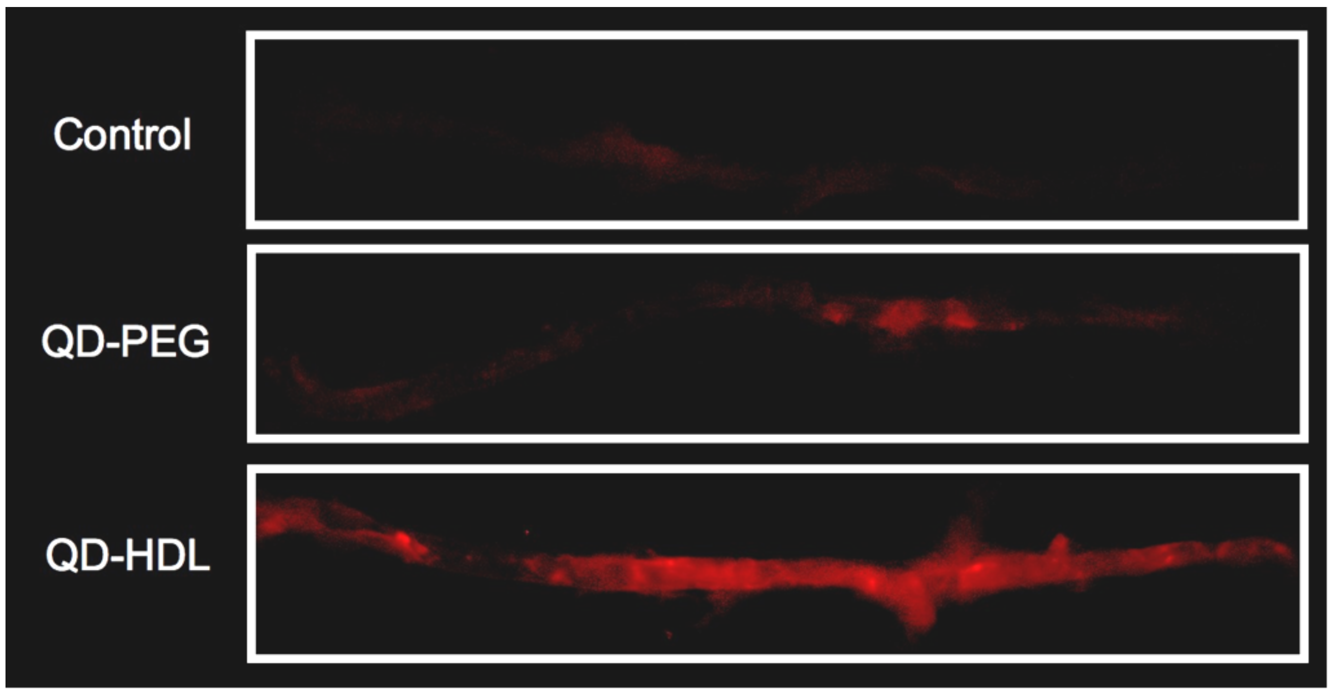
Nanocrystal HDL

Ex vivo fluorescence imaging

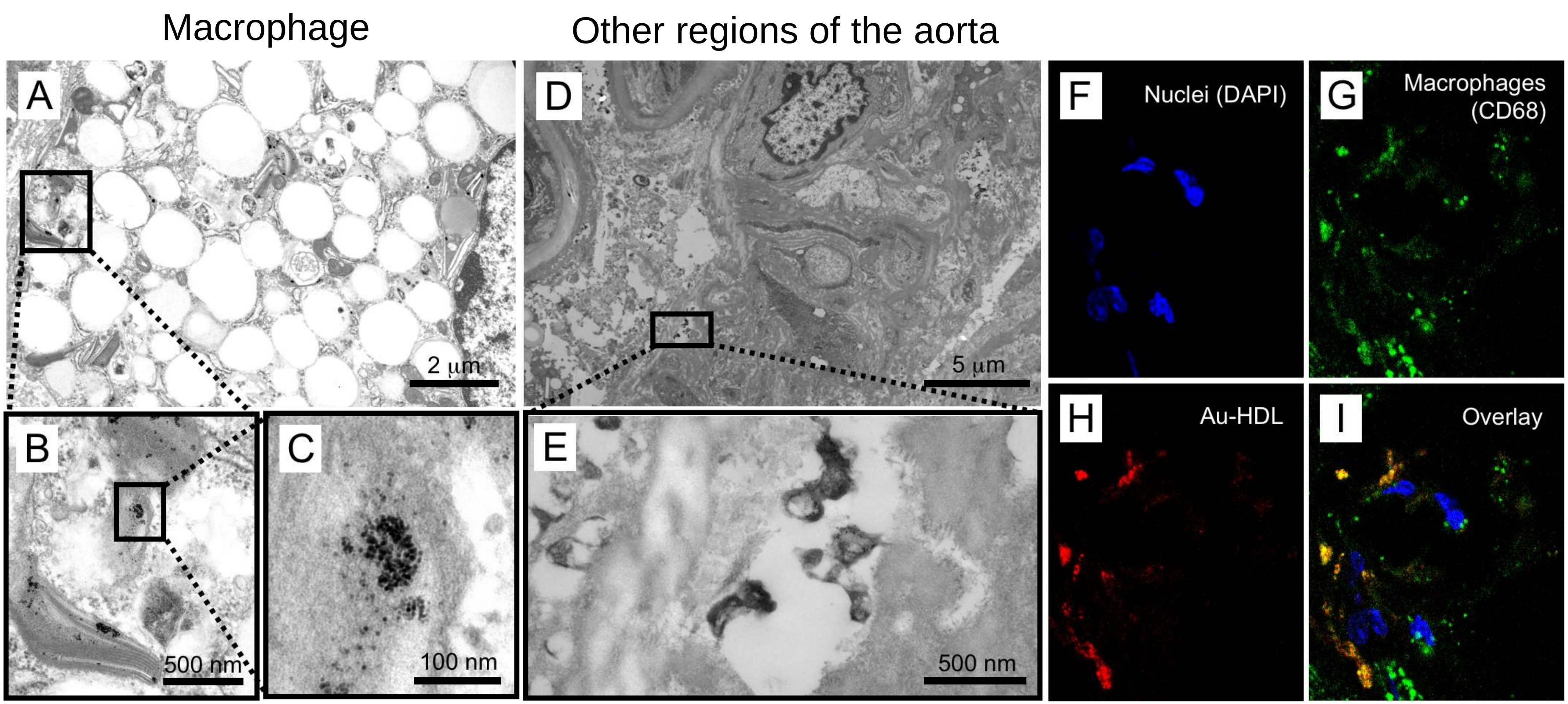
Excised aortas imaged using Maestro fluorescence imaging



QD-HDL
T1-weighted MRI,
enhanced fluorescence

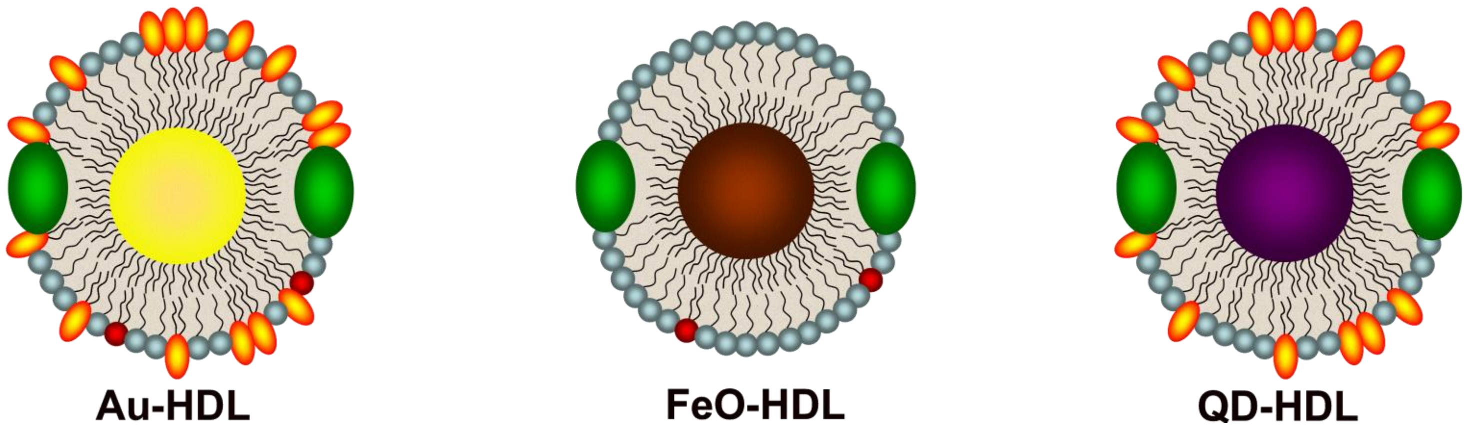


Microscopy of the aorta



Nanocrystal HDL were found almost exclusively in the macrophages

Inorganic core HDL - new directions



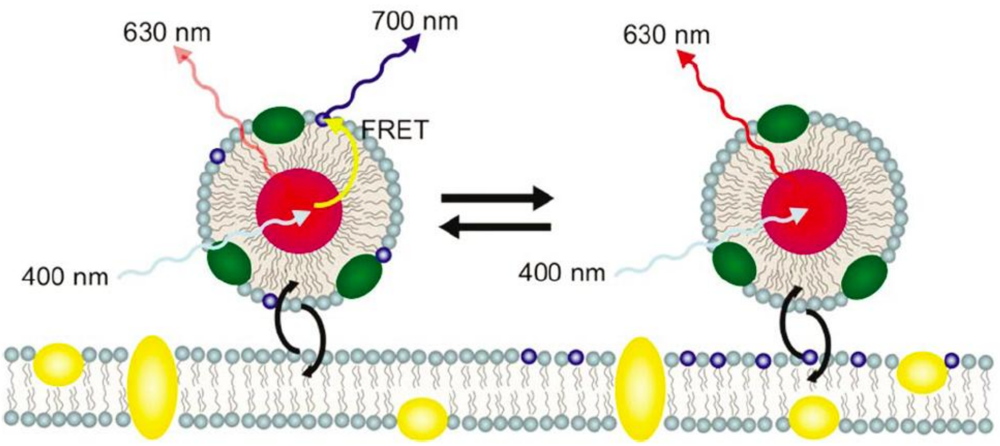
Multi-color CT

An HDL mimick?

FRET imaging

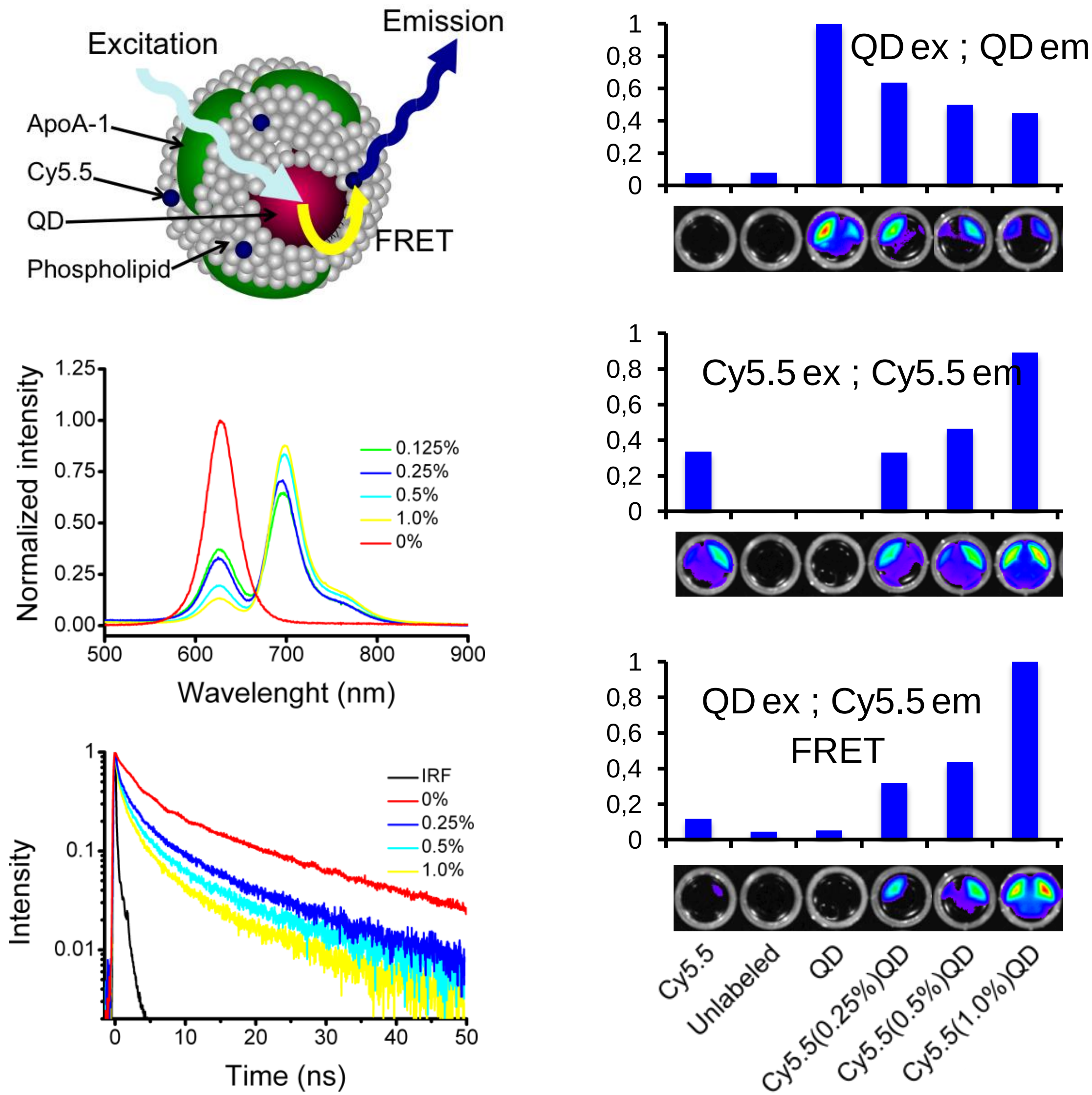
Quantum Dot and Cy5.5 Labeled Nanoparticles to Investigate Lipoprotein Biointeractions via Förster Resonance Energy Transfer

Torjus Skajaa,[†,§,◆] Yiming Zhao,[‖,◆] Dave J. van den Heuvel,[⊥] Hans C. Gerritsen,[⊥] David P. Cormode,[†] Rolf Koole,[‖] Matti M. van Schooneveld,[¶] Jan Andries Post,[#] Edward A. Fisher,[▽] Zahi A. Fayad,[†] Celso de Mello Donega,[‖] Andries Meijerink,[‖] and Willem J. M. Mulder*[,†,‡]



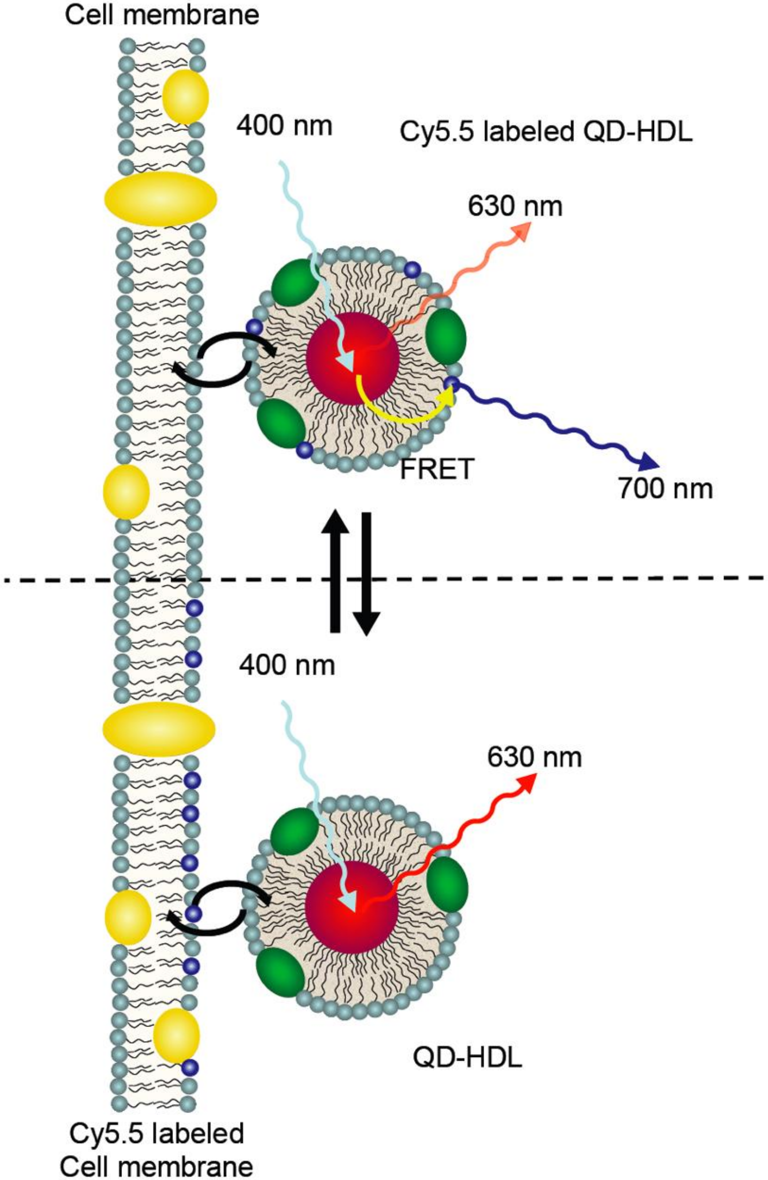
Skajaa *et al*. Nano Lett. 2010

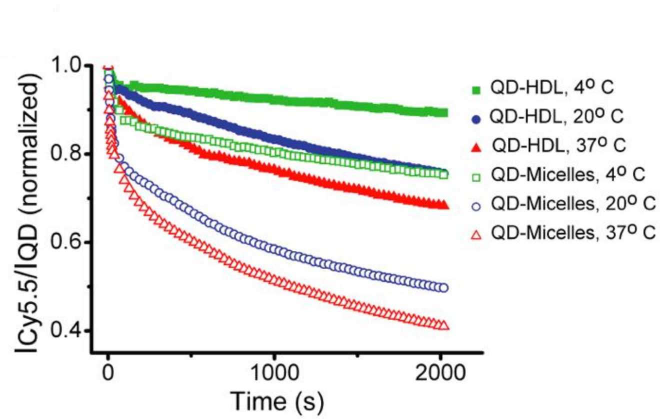
Förster resonance energy transfer

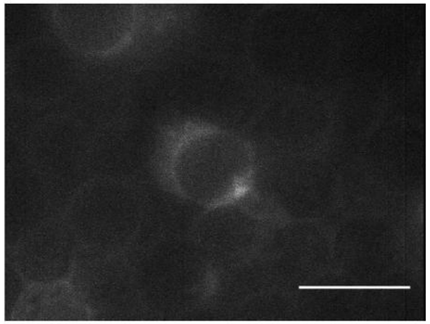


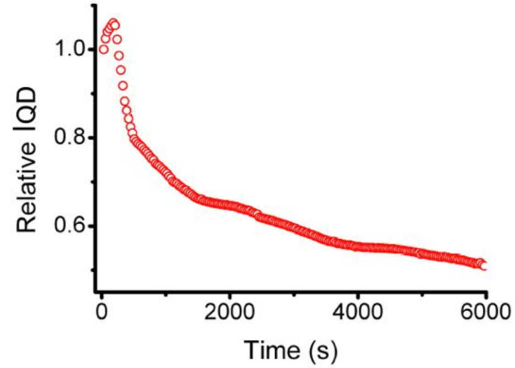
FRET

Interaction with cells



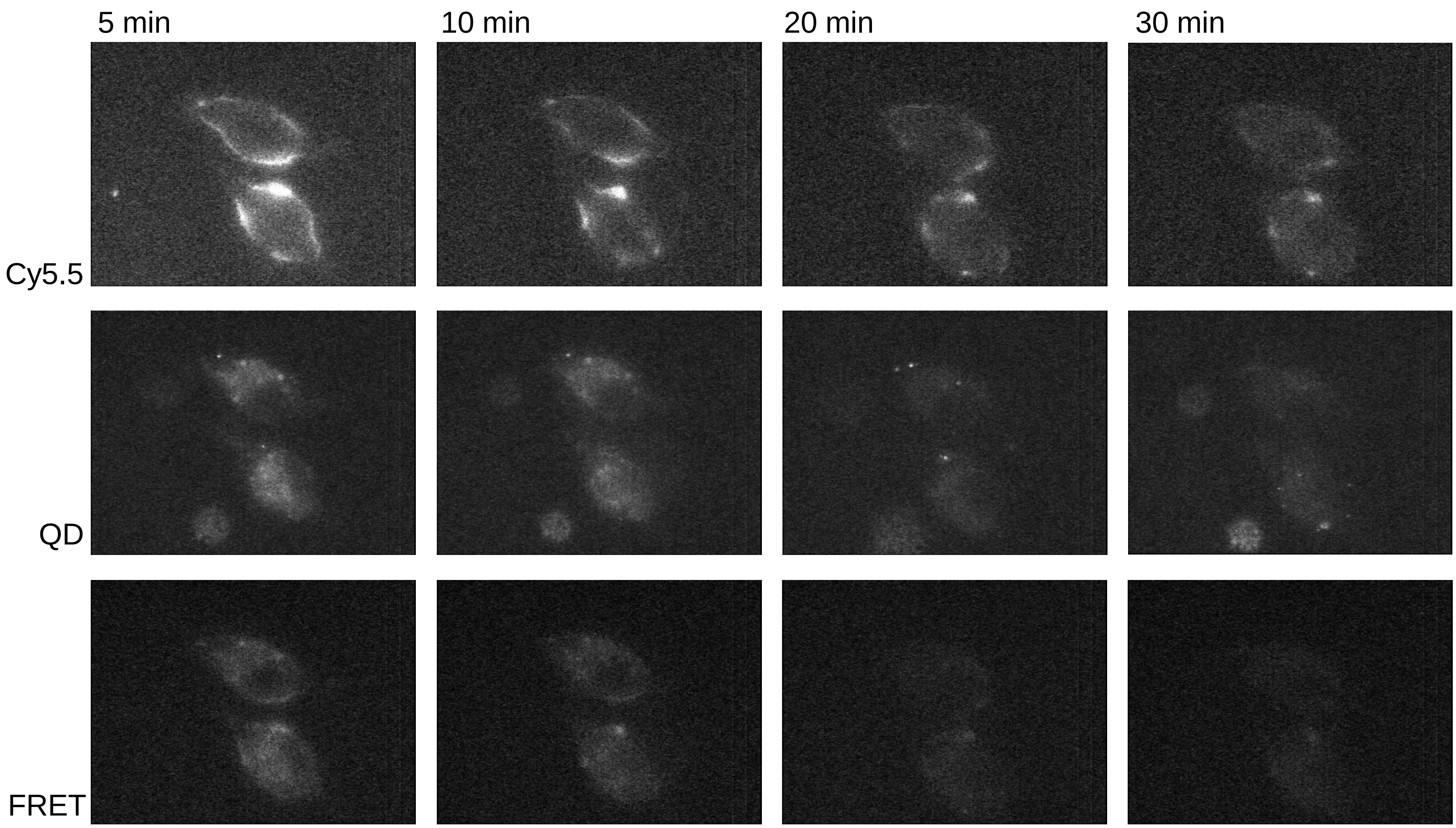






FRET

FRET in live cell imaging



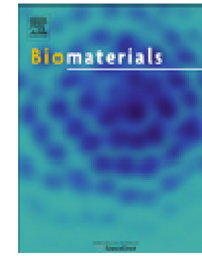
Is FeO-HDL like HDL?

Biomaterials 32 (2011) 206–213

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials



The biological properties of iron oxide core high-density lipoprotein in experimental atherosclerosis

Torjus Skajaa[a,b,1], David P. Cormode[a,1], Peter A. Jarzyna[a,1], Amanda Delshad[a,1], Courtney Blachford[c,2], Alessandra Barazza[c,2], Edward A. Fisher[c,2], Ronald E. Gordon[d], Zahi A. Fayad[a,*], Willem J.M. Mulder[a,e,*]

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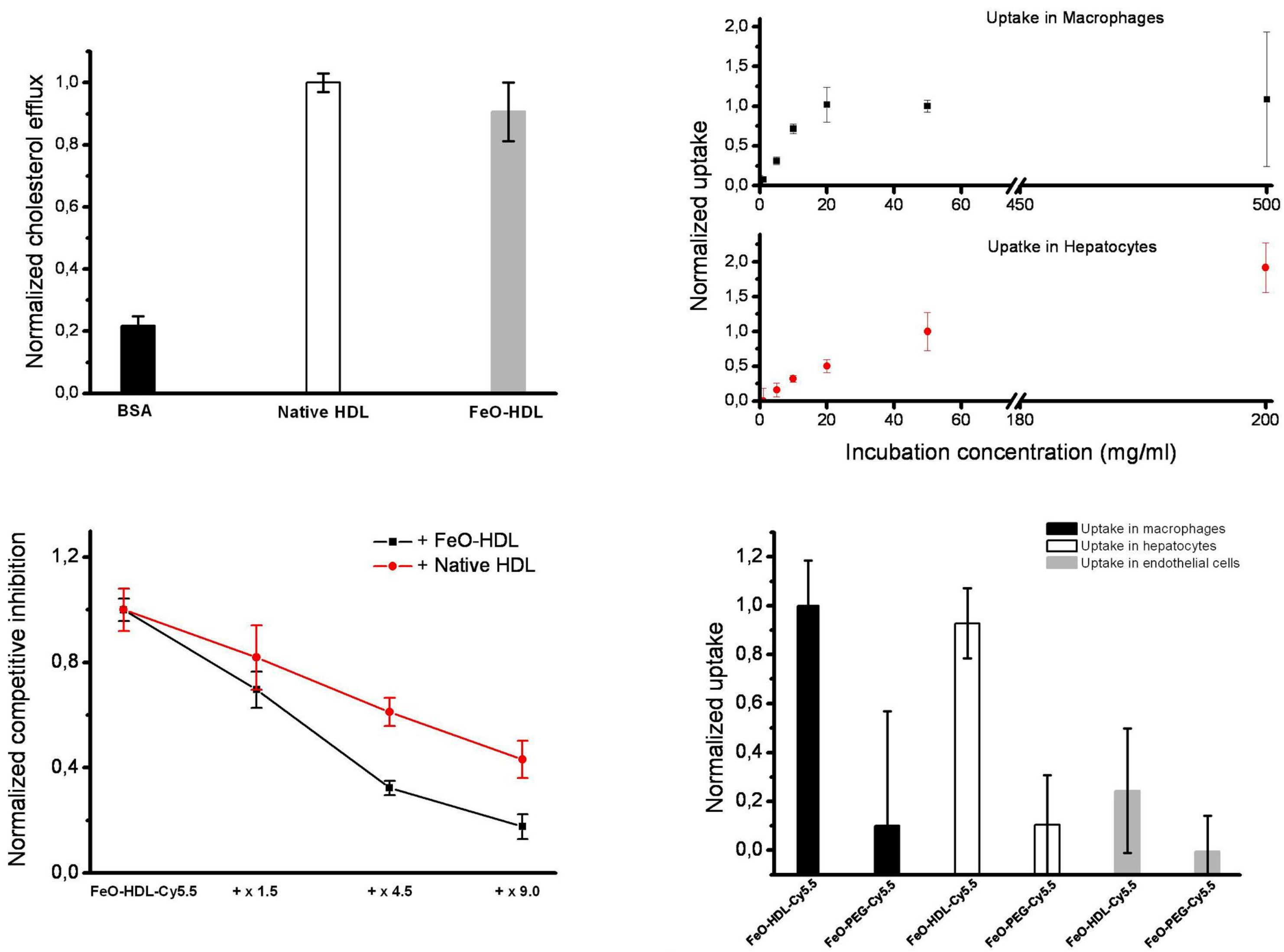
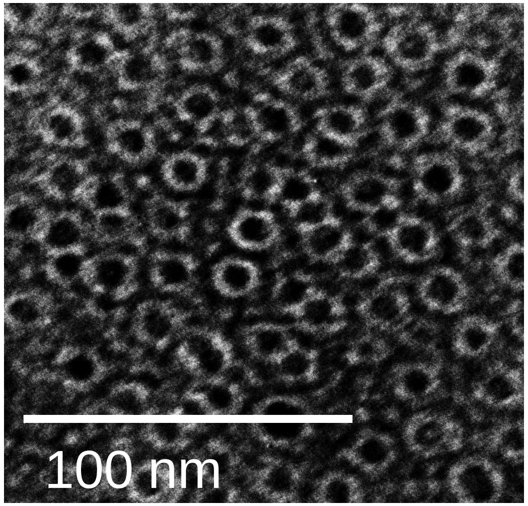
[b] *Clinical Institute and Dept. of Cardiology, Aarhus University Hospital (Skejby), Brendstrupgårdsvej 100, 8200 Århus N, Denmark*
[c] *Departments of Medicine (Cardiology) and Cell Biology, NYU School of Medicine, NY, 522 First Avenue, Smilow 8, New York, NY 10016, USA*
[d] *Department of Pathology, Mount Sinai Hospital, One Gustave L. Levy Place, New York, NY 10029, USA*
[e] *Department of Gene and Cell Medicine, Mount Sinai School of Medicine, One Gustave, L. Levy Place, Box 1234, New York, NY 10029, USA*

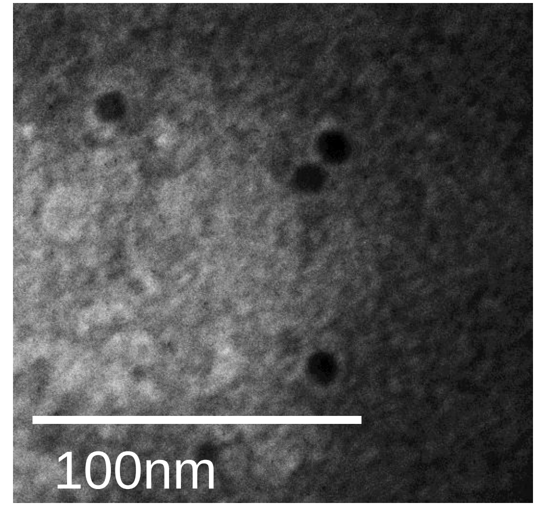
Skajaa *et al*. Biomaterials. 2011

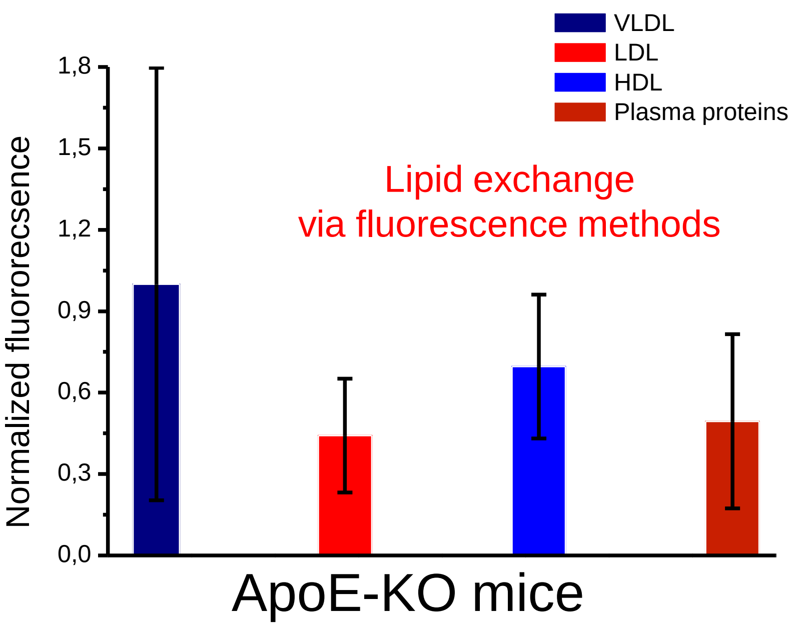
FeO-HDL interactions with macrophages

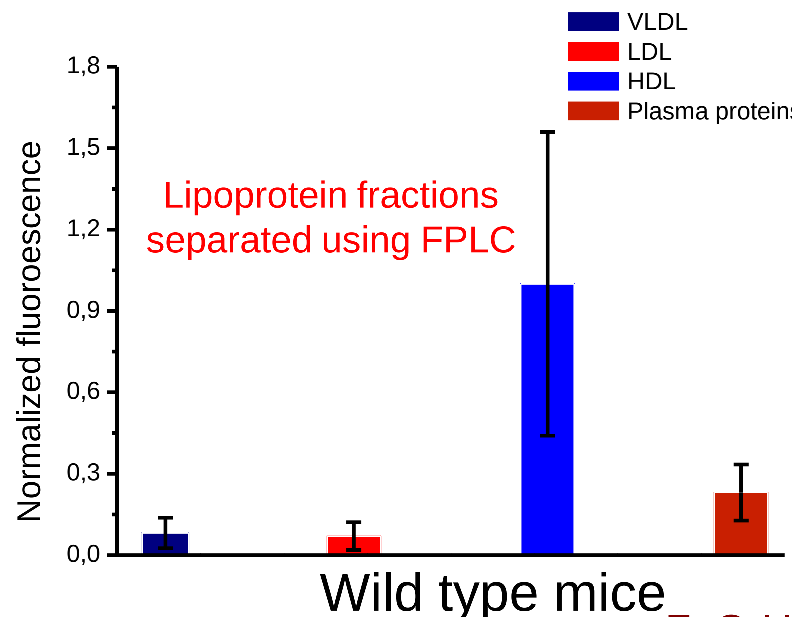


In vivo lipid transfer from FeO-HDL to circulating lipoproteins









FeO-HDL

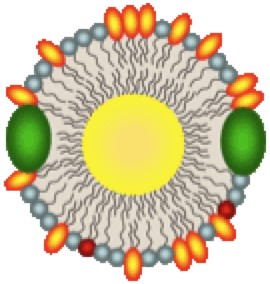
Atherosclerotic Plaque Composition: Analysis with Multicolor CT and Targeted Gold Nanoparticles[1]

Radiology

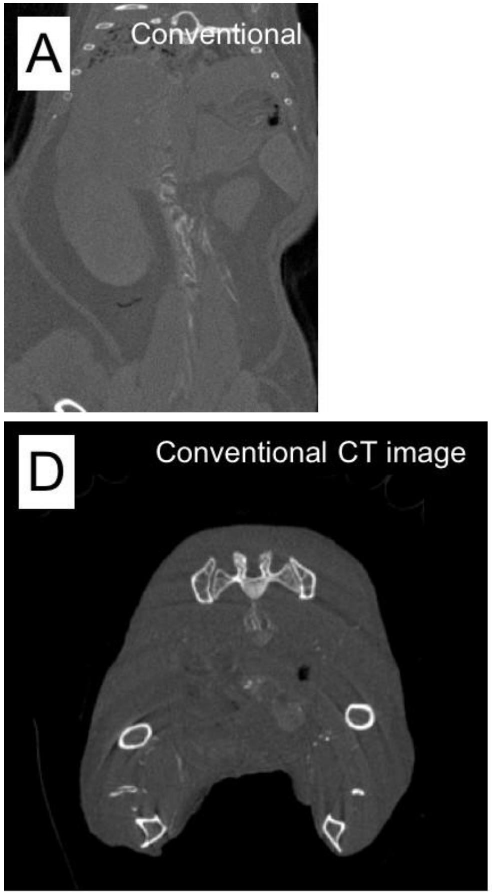
Cormode *et al*. Radiology. 2010.
In collaboration with Philips Hamburg

Au-HDL

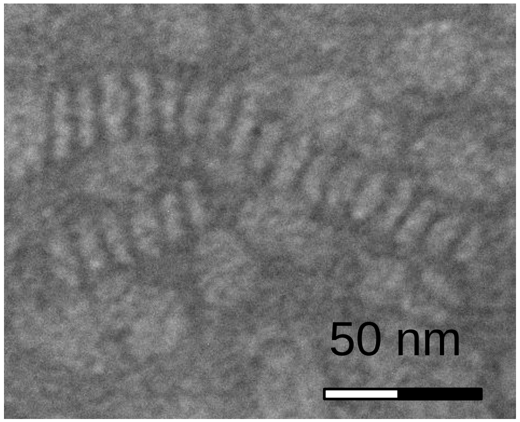
Multicolor molecular CT imaging

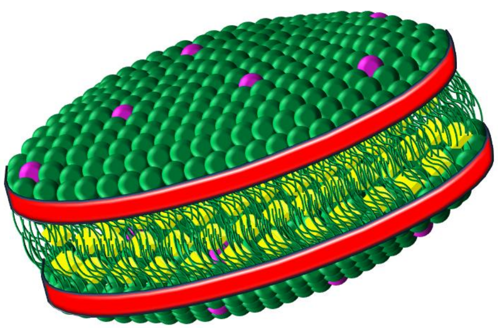


Au-HDL
CT, T1-weighted MRI, fluorescence

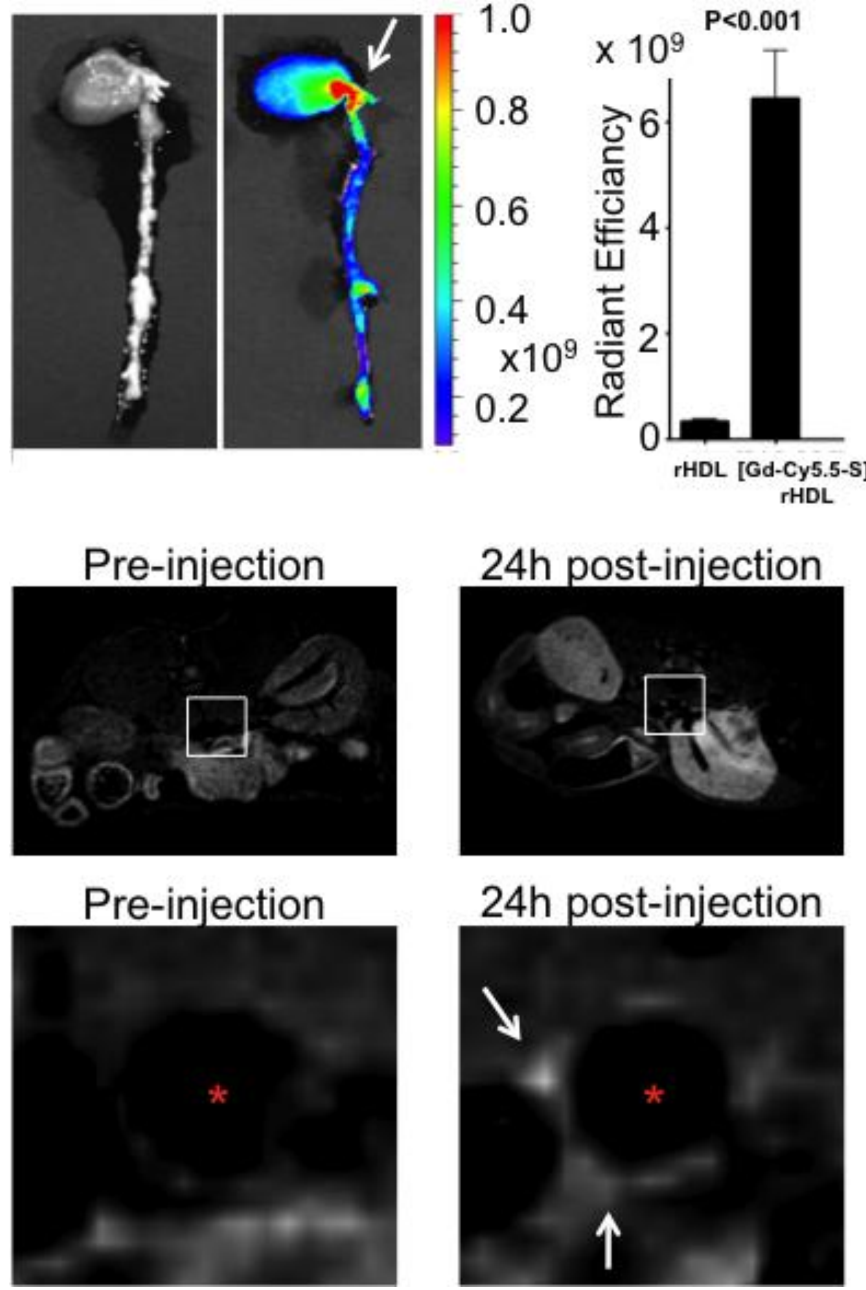


Statin loaded HDL



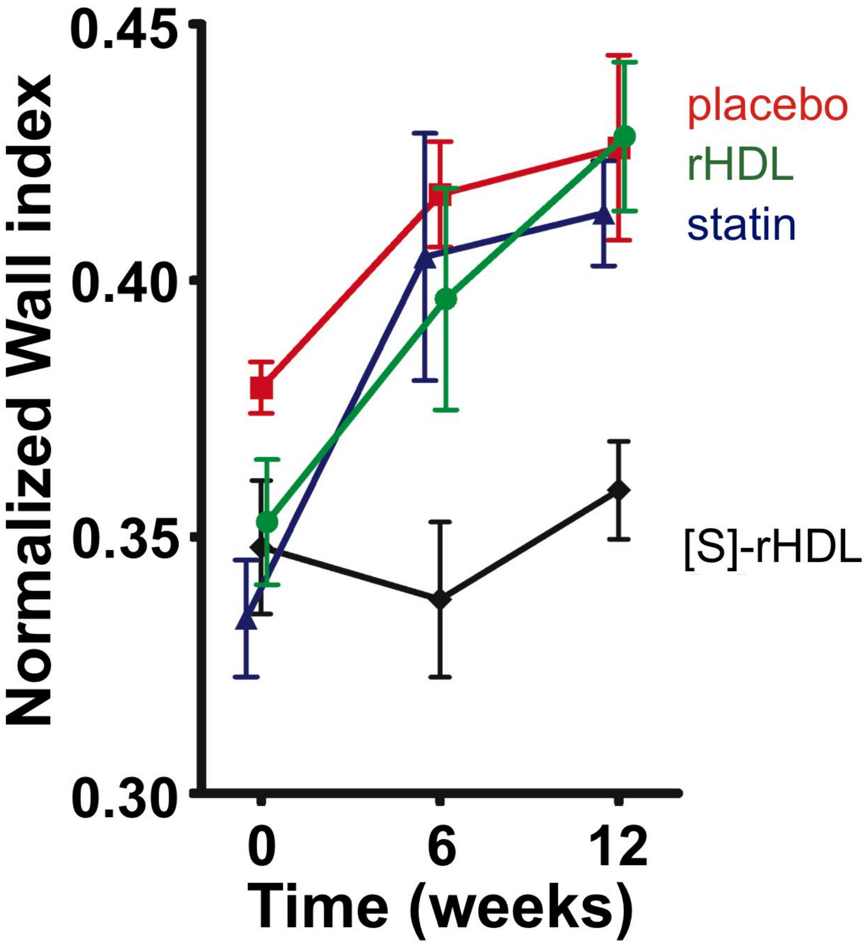


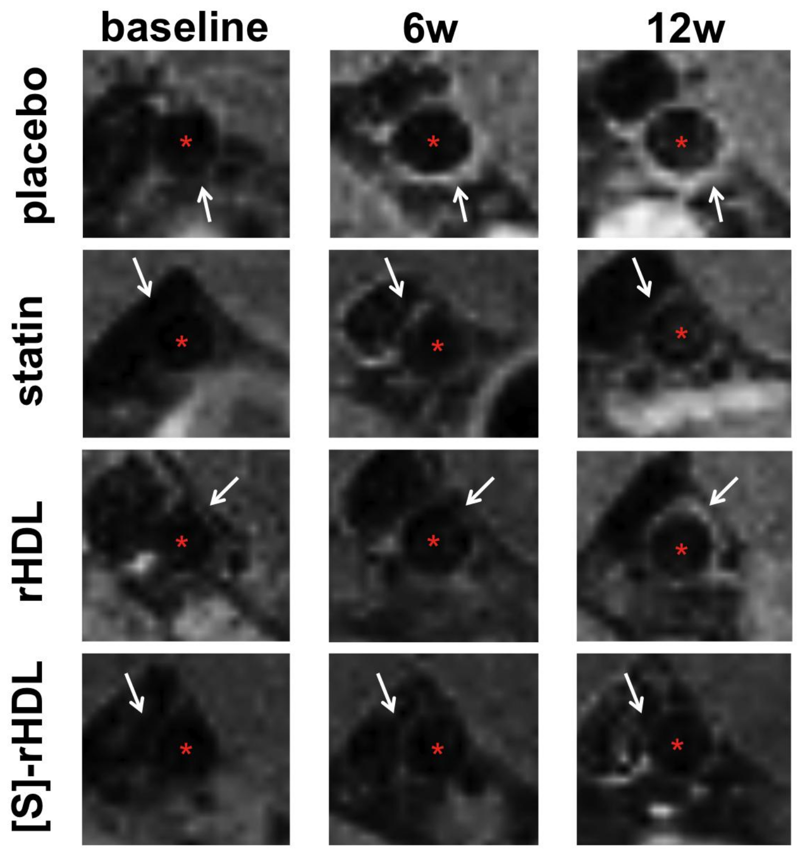
[S]-rHDL



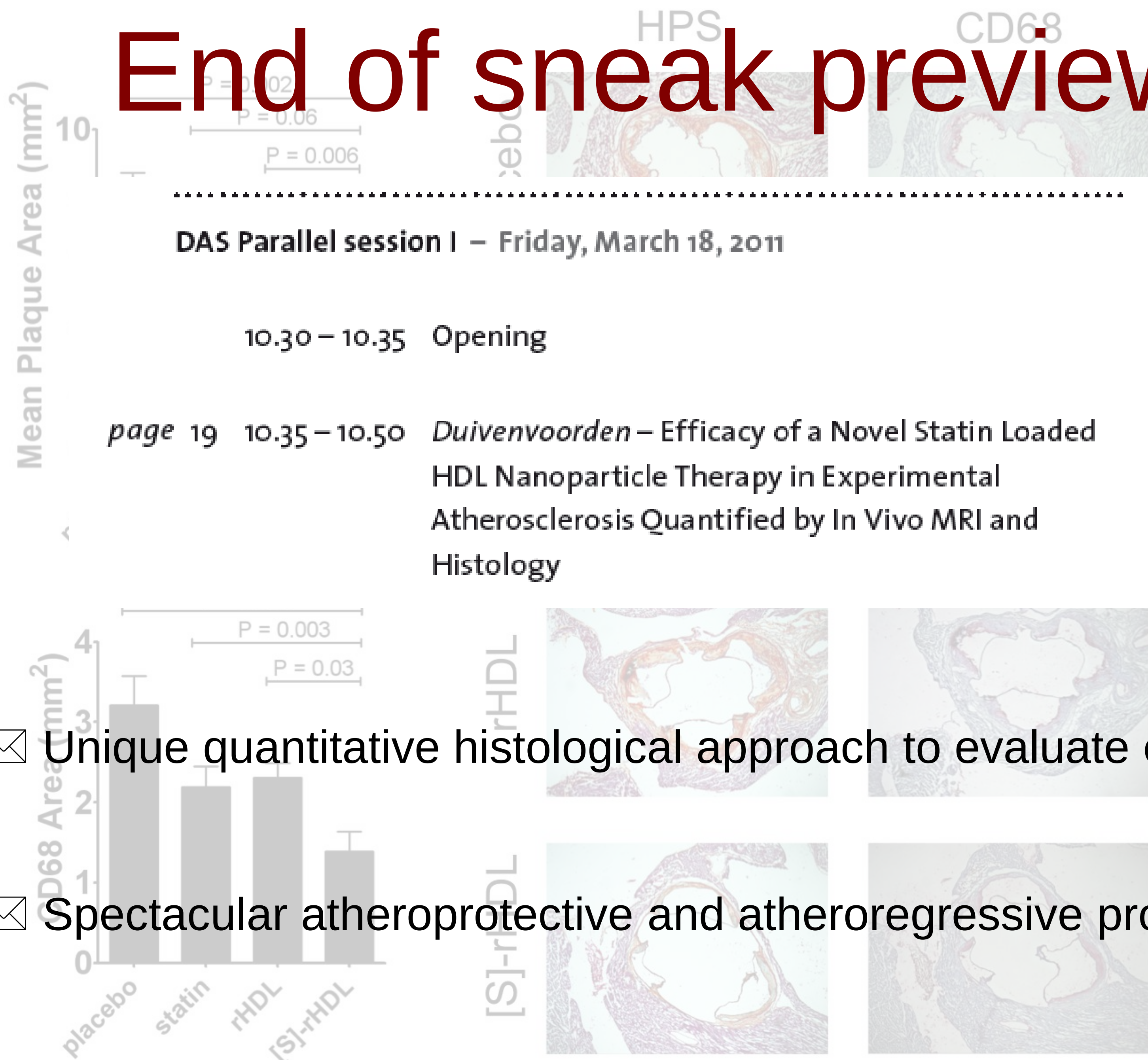
HDL drug delivery

MRI to assess therapeutic efficacy





End of sneak preview



DAS Parallel session I – Friday, March 18, 2011

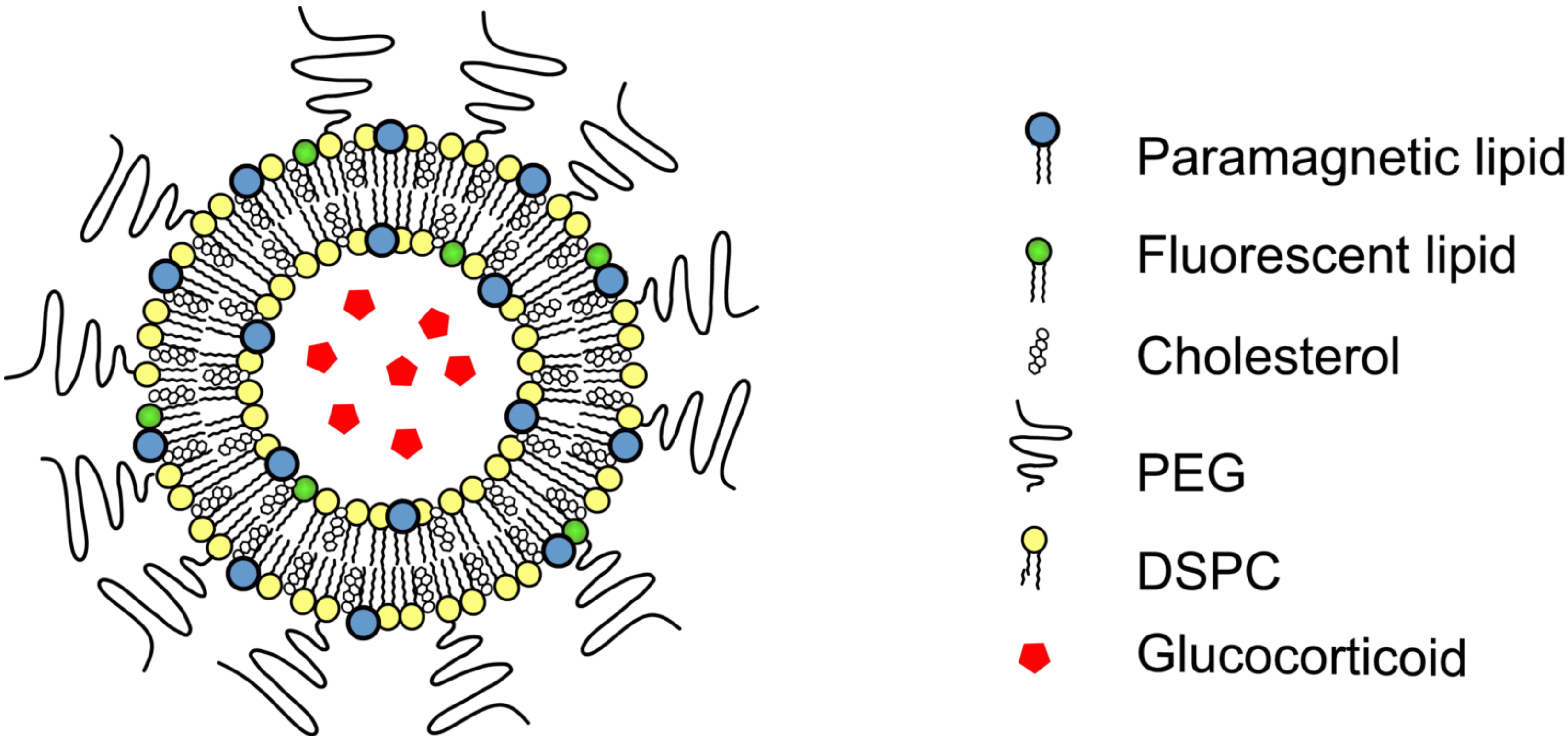
10.30 – 10.35 Opening

page 19 10.35 – 10.50 *Duivenvoorden* – Efficacy of a Novel Statin Loaded HDL Nanoparticle Therapy in Experimental Atherosclerosis Quantified by In Vivo MRI and Histology

- ⌧ Unique quantitative histological approach to evaluate efficacy
- ⌧ Spectacular atheroprotective and atheroregressive properties

Multimodal liposomes

Diagnostics and therapeutics

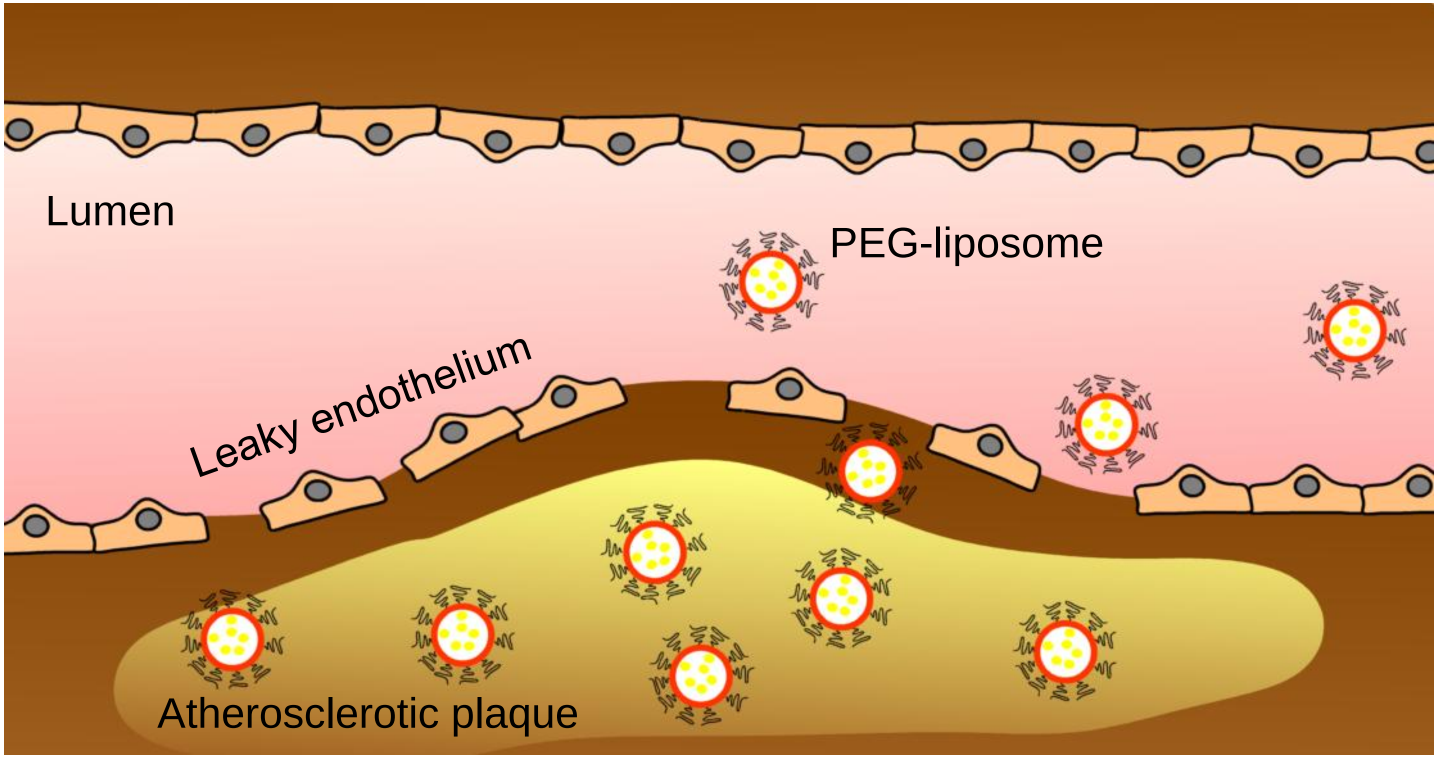


Mulder, Lobatto, Fayad *et al*. MSSM

Accumulation of PEG-liposomes in plaques

Vasa vasorum, neovascularization and EPR effect

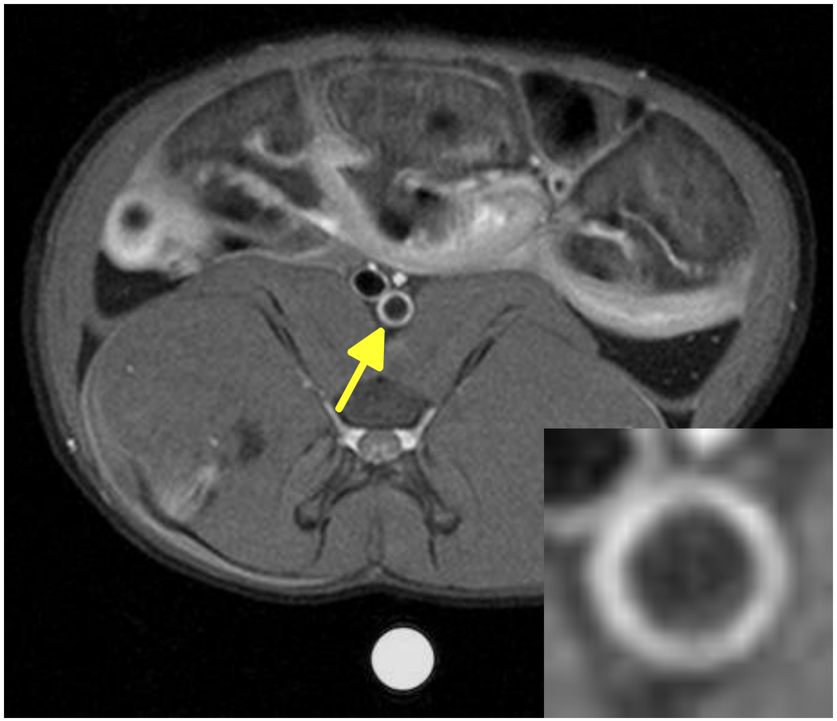
Enhanced permeability and retention effect



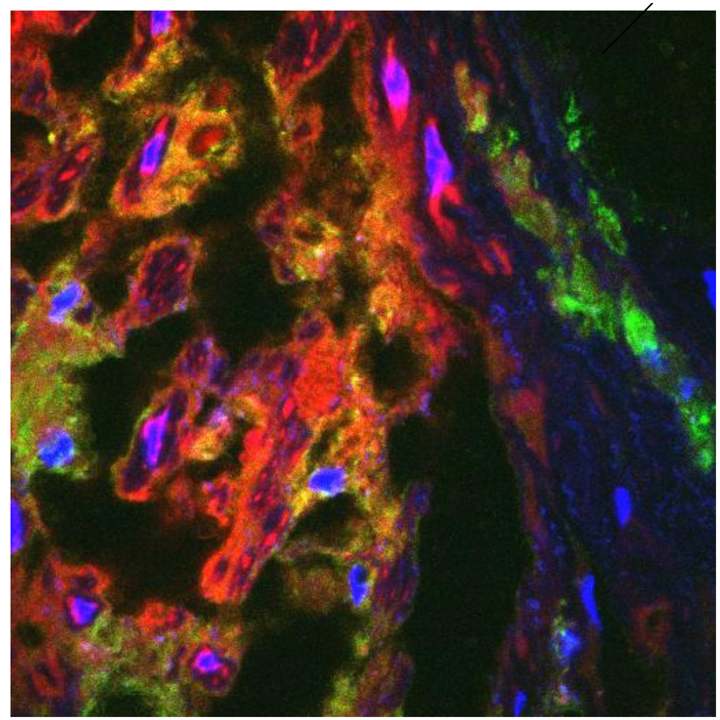
Langheinrich *et al*. Arterioscler Thromb Vasc Biol. 2006

Accumulation of liposomes in aortic plaques

MRI



Confocal microscopy



Liposomes
Macrophages
Nuclei

Quantitative analysis: 130 μg liposomes per mg aortic tissue

FDG-PET/CT imaging of plaque

Theory

High risk plaques

Active macrophages

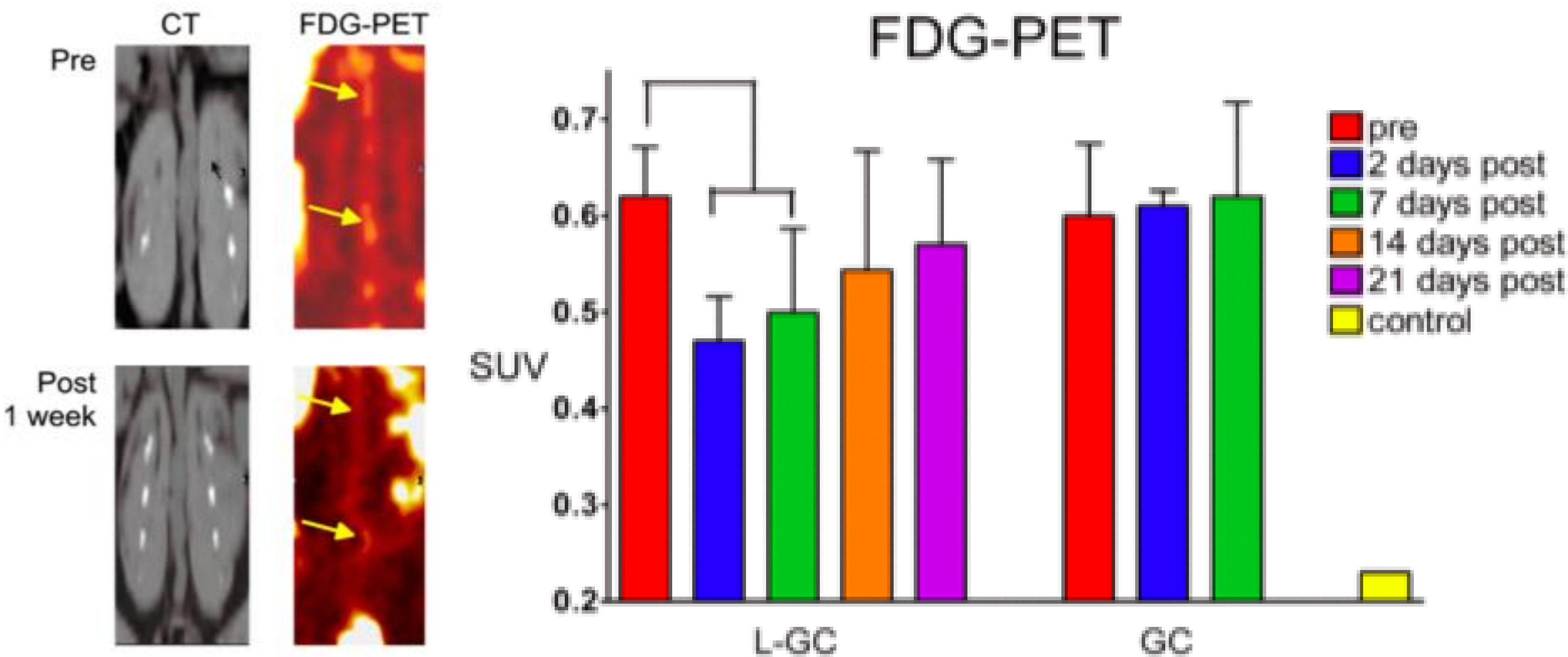
High metabolic activity

High glucose uptake

High FDG uptake

- Imaging of glucose transport in vivo with PET and ^{18}F-labeled 6-fluoro-6-deoxy-D-glucose (FDG), a tracer that is transported but not phosphorylated
- FDG (glucose analog) FDA approved

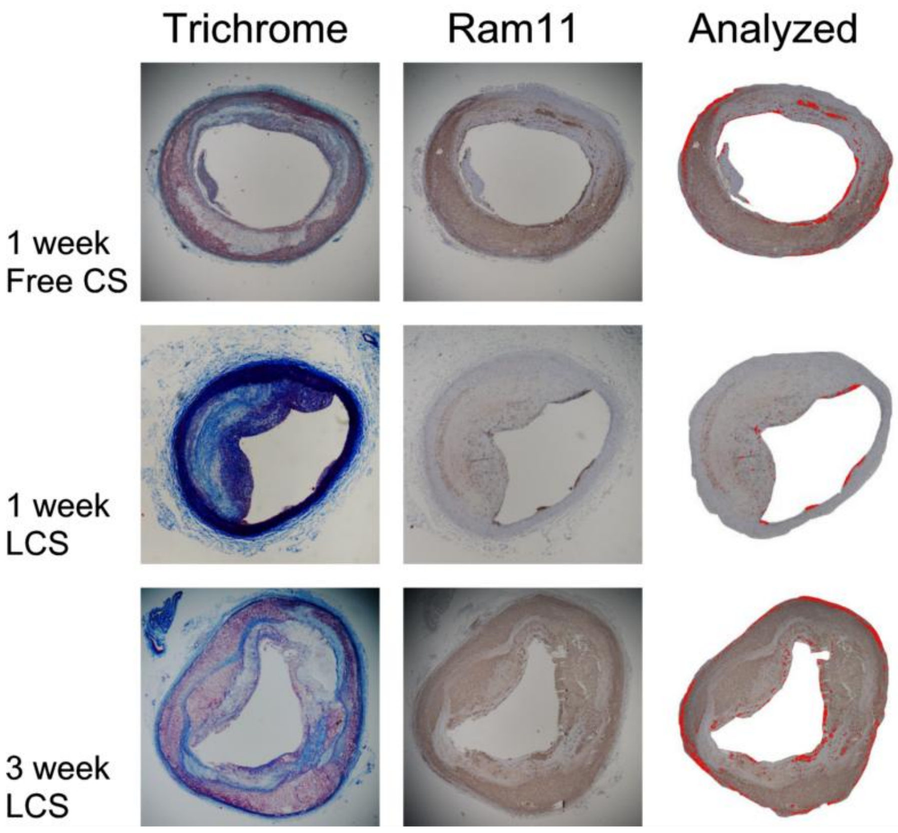
Liposomes selectively reduce inflammation



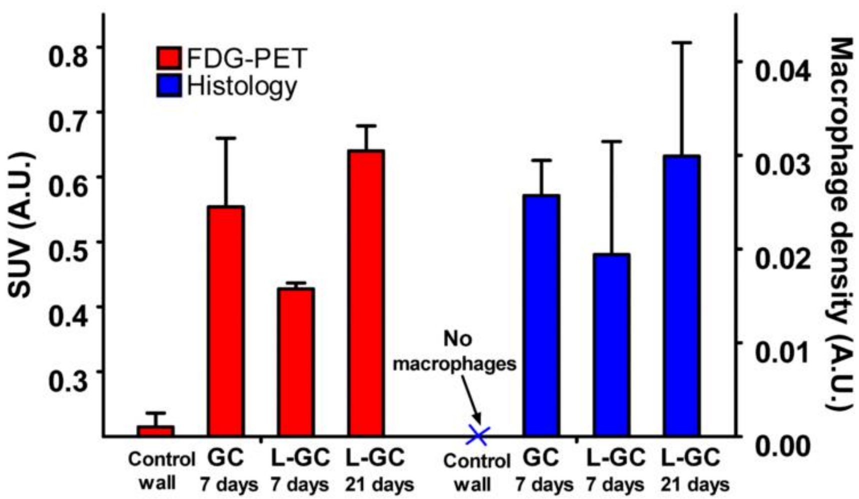
- Inflammation decreased significantly at 2 and 7 days after liposome injection
- Unprecedented therapeutic efficacy of a single treatment

Results confirmed with histology

Histology



FDG-PET imaging



Liposomes