Willem J.M. Mulder, Ph.D.
-Director Nanomedicine Laboratory at Mount Sinai
-Strategic appointment at the AMC Amsterdam

Particles and polymers:

Established particles and polymers

Educational Program WMIC 2012

September 5th, 2012, Dublin

Nanomedicine Laboratory at Mount Sinai

Overview

Part 1
- Self-assembly and cellular life
- Lipoproteins
- Amphiphiles and (phospholipids)
- Vesicles, disks, micelles

Part 2
- Liposomes
- Oil-in-water emulsions
- Nanocrystal core HDL
- Complex lipid/polymer hybrid nanoparticles

Complexity

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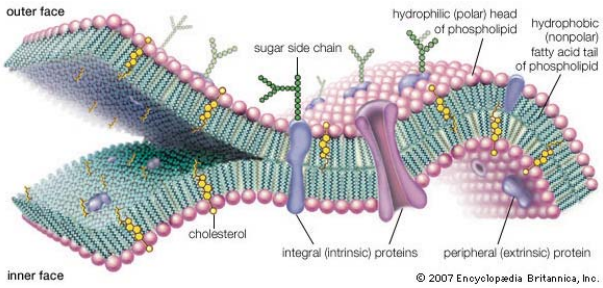
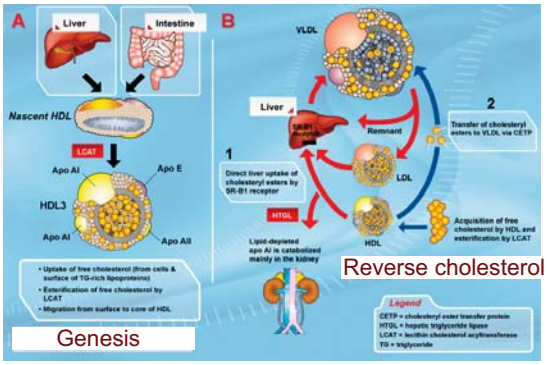
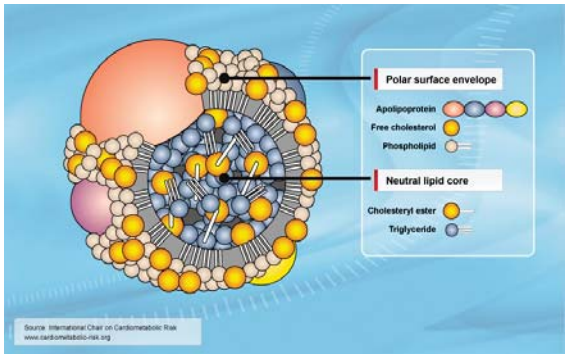
Self-assembly and (cellular) life



HDL: "good cholesterol"

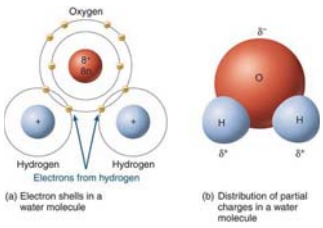


Architecture of lipoprotein

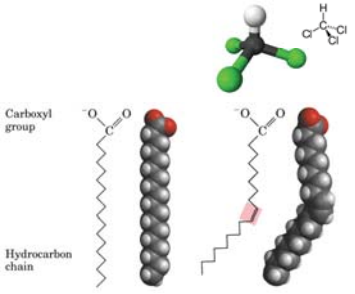


Polarity

http://www.bbc.co.uk/scotland/learning/bitesize/higher/chemistry/energy/bsp_rev2.shtml



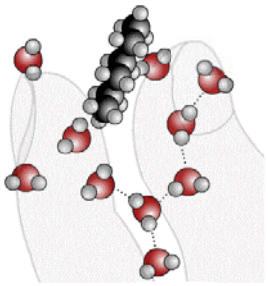
http://rst.gsfc.nasa.gov/Sect20/A12.html



http://www.biochem.arizona.edu/classes/bioc462/462a/NOTES/LIPIDS/Lipids.html

Oil in water

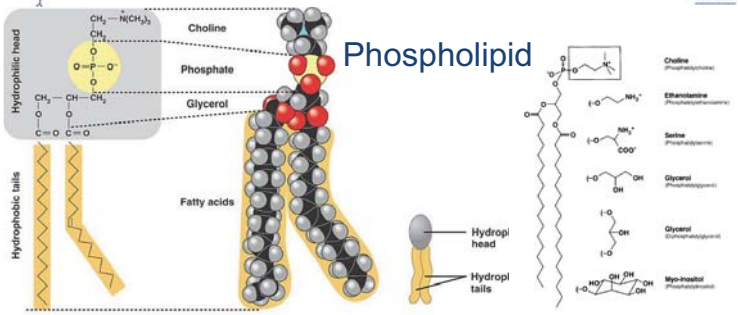
Hydrogen bonds



Attractive interaction of a hydrogen atom with an electronegative atom that comes from another molecule or chemical group

http://www.indiana.edu/~oso/animations/water&oil.html

Amphiphilic molecules

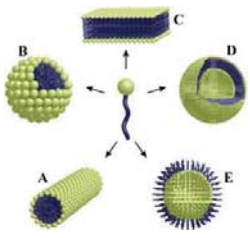


Phospholipid

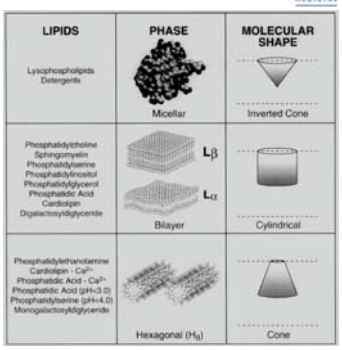
An amphiphile is a molecule with both a hydrophilic part and hydrophobic part

Lipid aggregates in water

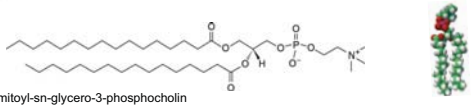
Phospholipids



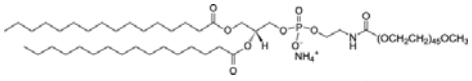
Biochemistry of Lipids, Lipoproteins and Membranes.
J.E. Vance & Dennis E. Vance



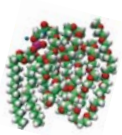
Lipid aggregates in water



DPPC
1,2-dipalmitoyl-sn-glycero-3-phosphocholin



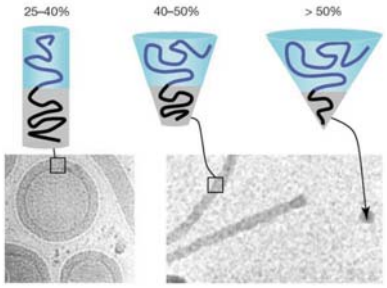
PEG-DSPE
1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]



Johnsson M.
Biophys J. 2003

Lipid aggregates in water

Hydrophilic fraction f dictates aggregate morphology



Discher & Ahmed.
Annu Rev Biomed Eng 2006

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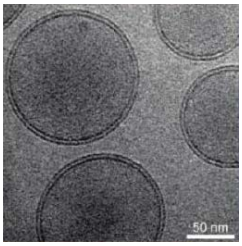
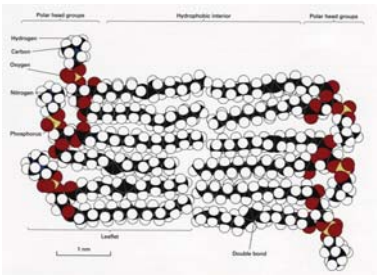
Phospholipid vesicles: Liposomes

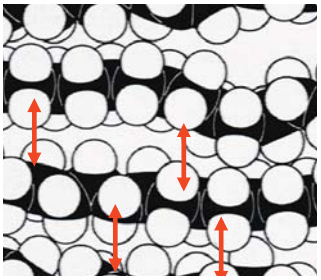


http://astrobiology.nasa.gov/team/images/2006/ciw/1829/1.jpg

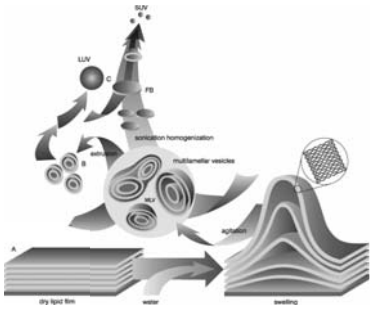
Van der Waals interactions

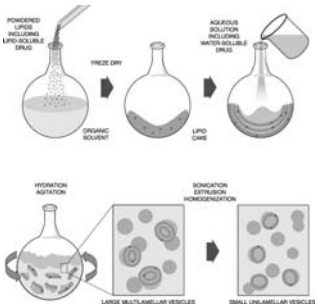
Van der Waals force is the sum of the attractive or repulsive forces between molecules





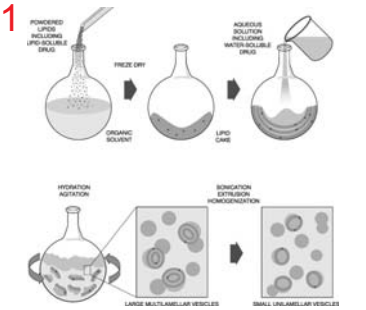
Liposome formation



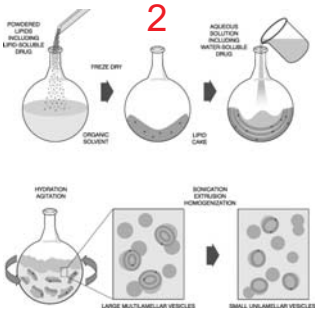


Dissolving lipids

1

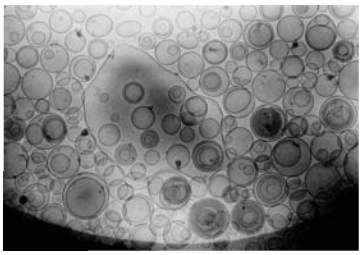


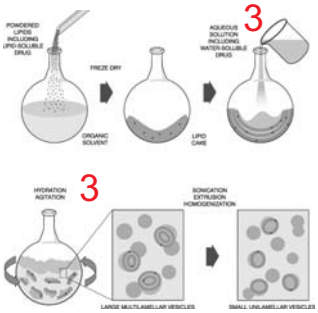
Lipid film formation



Lipid film hydration

Multilamellar vesicles (MLVs)

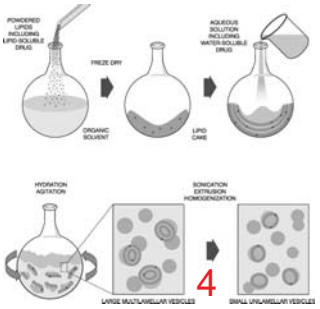




Sizing of MLVs

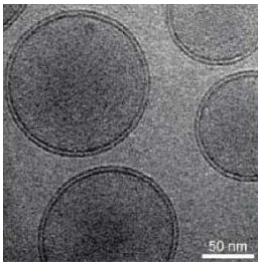
Sonication

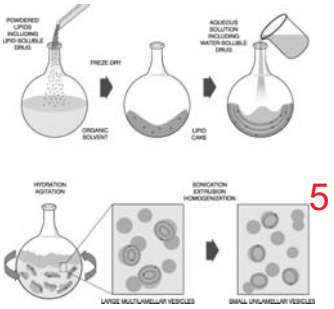
Extrusion



Liposomes: unilamellar vesicles

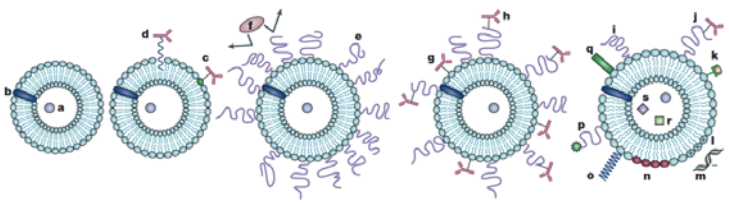
Unilamellar vesicles





Evolution of liposomes

Torchilin. Nat Rev Drug Discov. 2005



Features of liposomes

Torchilin. Nat Rev Drug Discov. 2005

Box 1 | **Liposomes**

Liposomes are spherical, self-closed structures formed by one or several concentric lipid bilayers with an aqueous phase inside and between the lipid bilayers. There are a number of different types of liposomal vesicle:

- Multilamellar vesicles: these range in size from 500 to 5,000 nm and consist of several concentric bilayers.
- Small unilamellar vesicles: around 100 nm in size and formed by a single bilayer.
- Large unilamellar vesicles: range in size from 200 to 800 nm.
- Long-circulating liposomes: liposomes modified in such a way (usually surface-grafted with certain polymers) that they can stay in the blood much longer (for hours) than non-modified liposomes.
- Immunoliposomes: liposomes carrying antibodies attached to their surfaces and able to accumulate in the area within the body where an attached antibody recognizes and binds its antigen.

Attractive biological properties of liposomes:

- Liposomes are biocompatible.
- Liposomes can entrap water-soluble (hydrophilic) pharmaceutical agents in their internal water compartment and water-insoluble (hydrophobic) pharmaceuticals into the membrane.
- Liposome-incorporated pharmaceuticals are protected from the inactivating effect of external conditions, yet do not cause undesirable side reactions.
- Liposomes provide a unique opportunity to deliver pharmaceuticals into cells or even inside individual cellular compartments.
- Size, charge and surface properties of liposomes can be easily changed simply by adding new ingredients to the lipid mixture before liposome preparation and/or by variation of preparation methods.

Liposome-cell interaction

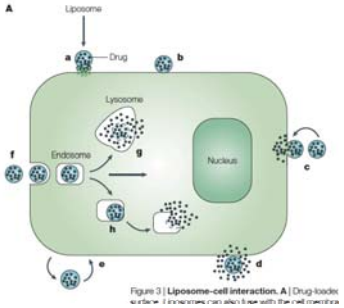


Figure 3 | **Liposome-cell interaction. A** | Drug-loaded

Torchilin. Nat Rev Drug Discov. 2005

Clinically approved liposomes

Torchilin. Nat Rev Drug Discov. 2005

Table 1 | **Liposomal drugs approved for clinical application or undergoing clinical evaluation***

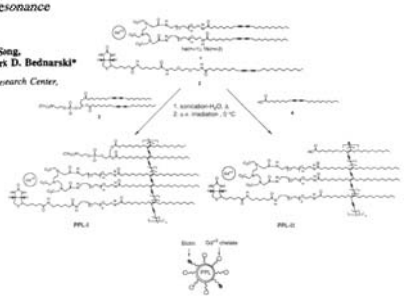
Active drug	Product name‡	Indications
Daunorubicin	DaunoXome	Kaposi's sarcoma
Doxorubicin	Myocet	Combinational therapy of recurrent breast cancer
Doxorubicin in PEG-liposomes	Doxil/Caelyx	Refractory Kaposi's sarcoma; ovarian cancer; recurrent breast cancer
Amphotericin B	AmBisome	Fungal infections
Cytarabine	DepoCyt	Lymphomatous meningitis
Vincristine	Onco TCS	Non-Hodgkin's lymphoma
Lurtotecan	NX211	Ovarian cancer
Nystatin	Nyotran	Topical antifungal agent
All-trans retinoic acid	Altragen	Acute promyelocytic leukaemia; non-Hodgkin's lymphoma; renal-cell carcinoma; Kaposi's sarcoma
Platinum compounds	Platar	Solid tumours
Annamycin		Doxorubicin-resistant tumours
E1A gene		Various tumours
DNA plasmid encoding HLA-B7 and α_2 microglobulin	Allovectin-7	Metastatic melanoma
Liposomes for various drugs and diagnostic agents (lipoMASC)		Broad applications

Liposomes and imaging

Paramagnetic Polymerized Liposomes: Synthesis, Characterization, and Applications for Magnetic Resonance Imaging

Richard W. Storrs, François D. Tropper, Henry Y. Li, Curtis K. Song, Jeremy K. Kuniyoshi, Dorothy A. Sipkins, King C. P. Li, and Mark D. Bednarski*

Contribution from the Stanford University School of Medicine, Lucas MRS Research Center, Stanford, California 94305



Liposomes and imaging

1: Li KC, **Bednarski MD**. Vascular-targeted molecular imaging using functionalized polymerized vesicles. J Magn Reson Imaging. 2002.

2: Sipkins DA, Cheresh DA, Kazemi MR, Nevin LM, **Bednarski MD**, Li KC. Detection of tumor angiogenesis in vivo by alphaVbeta3-targeted magnetic resonance imaging. Nat Med. 1998.

3: **Bednarski MD**, Lee JW, Callstrom MR, Li KC. In vivo target-specific delivery of macromolecular agents with MR-guided focused ultrasound. Radiology. 1997.

4: Storrs RW, Tropper FD, Li HY, Song CK, Sipkins DA, Kuniyoshi JK, **Bednarski MD**, Strauss HW, Li KC. Paramagnetic polymerized liposomes as new recirculating MR contrast agents. J Magn Reson Imaging. 1995.

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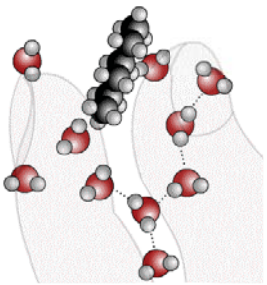
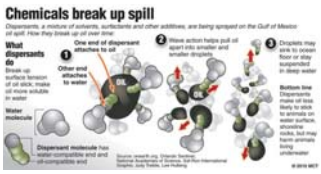
Hydrogen bonds

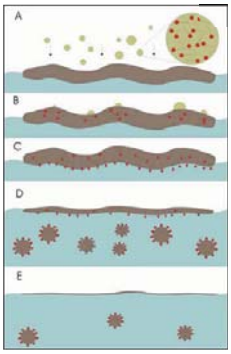


Attractive interaction of a hydrogen atom with an electronegative atom that comes from another molecule or chemical group

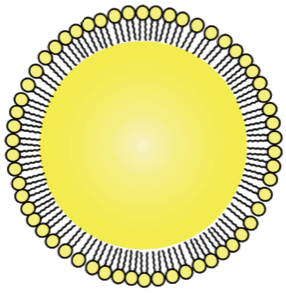
http://www.indiana.edu/~oso/animations/water&oil.html

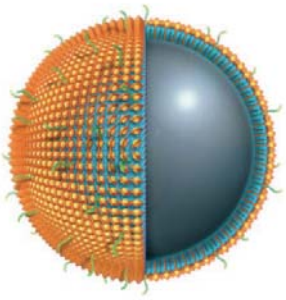
Oil spill





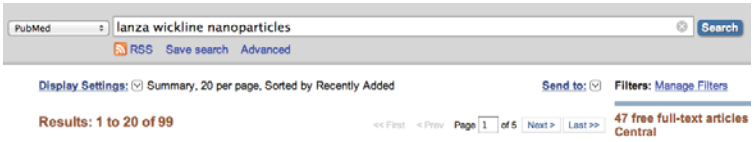
Oil-in-water emulsion



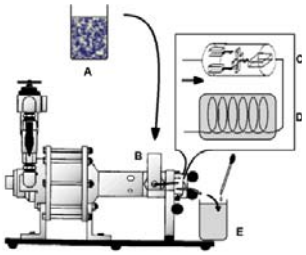


Chen J, Lanza GM, Wickline SA. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2010

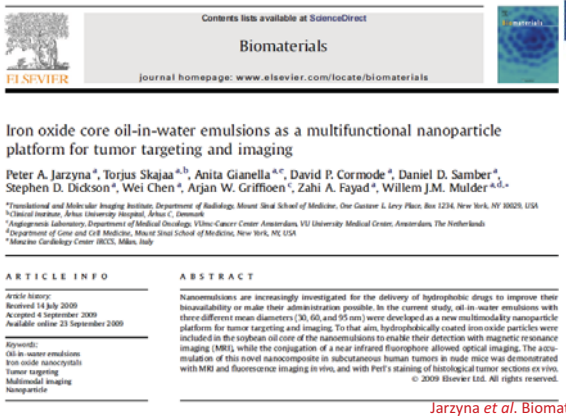
Oil-in-water emulsion



Microfluidics

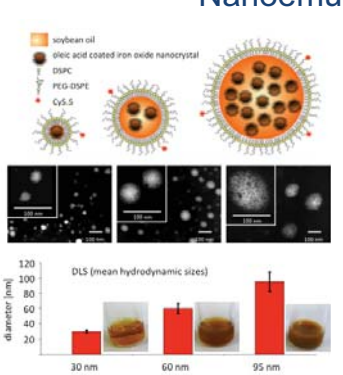


http://www.microfluidicscorp.com



Jarzyna *et al*. Biomaterials. 2009

Nanoemulsions



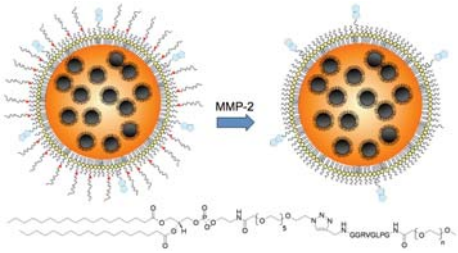
-Size control: 25 nm to 100 nm

-Biocompatible components

-Delivery of water insoluble materials

-High inclusion efficiency

Surface-switching



Anita Gianella

"Development of a multifunctional surface-switchable platform for combined imaging and therapy"

Poster session 3:
September 7, 2012 from 3:15 PM to 4:45 PM

Overview

Part 1

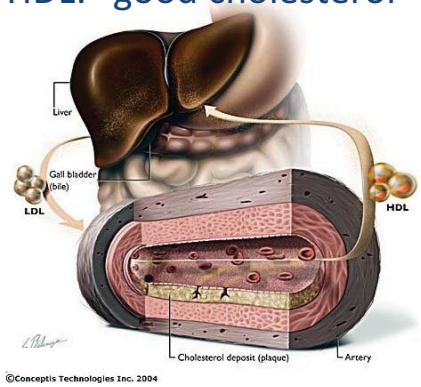
- Self-assembly and cellular life
- Lipoproteins
- Amphiphiles and (phospholipids)
- Vesicles, disks, micelles

Part 2

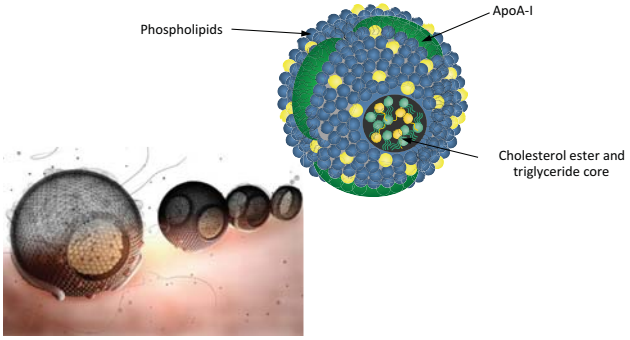
- Liposomes
- Oil-in-water emulsions
- Nanocrystal core HDL
- Complex lipid/polymer hybrid nanoparticles

Complexity

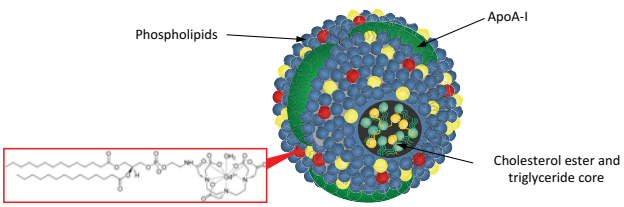
HDL: "good cholesterol"



Architecture of HDL

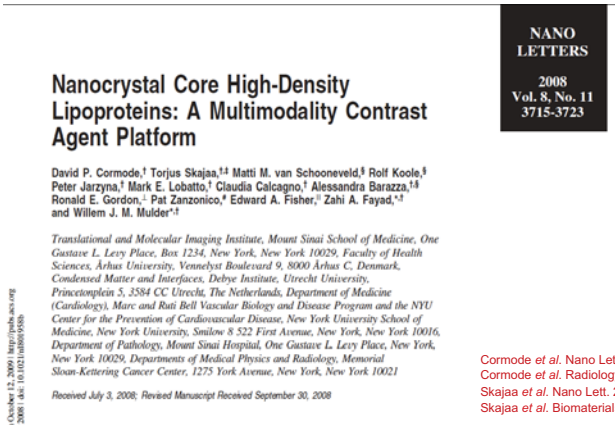


Labeling of HDL



- Endogenous nanoparticle, biodegradable, sized ~7-13 nm
- Has been adapted and used as an MRI contrast agent by including Gd-chelates

Frias *et al.* J. Am. Chem. Soc. 2004
JC Frias *et al.* Nano Lett. 2006
DP Cormode *et al.* Small 2008
W Chen *et al.* Contrast Med. Mol. Im. 2008
DP Cormode *et al.* Bioconjugate Chem. 2009
KC Briley-Saebo *et al.* J. Phys. Chem. B, 2009
T Skajaa *et al.* ATVB. 2010



NANO LETTERS
2008
Vol. 8, No. 11
3715-3723

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

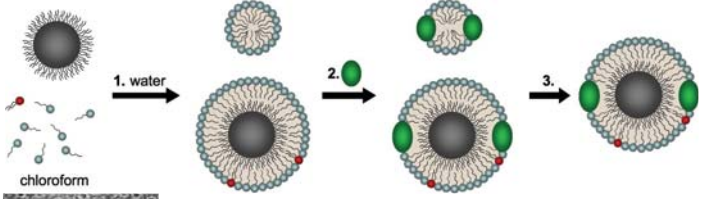
David P. Cormode,† Torjus Skajaa,†,‡ Matti M. van Schooneveld,§ Rolf Koole,§ Peter Jarzyna,† Mark E. Lobatto,† Claudia Calcagno,† Alessandra Barazza,†,§ Ronald E. Gordon,⊥ Pat Zanzonico,# Edward A. Fisher,∇ Zahi A. Fayad,*,† and Willem J. M. Mulder*,†

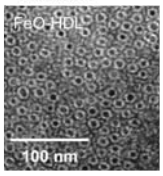
Translational and Molecular Imaging Institute, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1234, New York, New York 10029, Faculty of Health Sciences, Århus University, Vennelyst Boulevard 9, 8000 Århus C, Denmark, Condensed Matter and Interfaces, Debye Institute, Utrecht University, 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
Cormode *et al.* Radiology. 2010.
Skajaa *et al.* Nano Lett. 2010
Skajaa *et al.* Biomaterials. 2011

Incorporation of nanocrystals into HDL

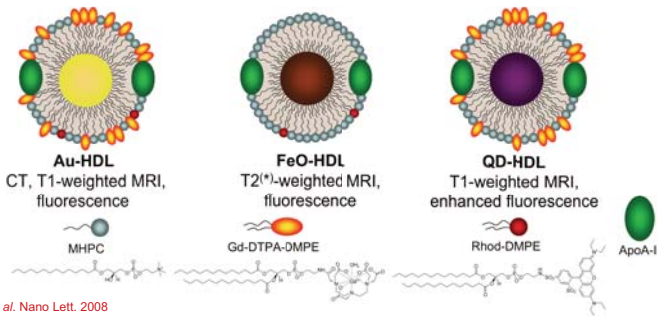




1. Addition of lipids and particles to hot water
2. Incubation with apoA-I
3. Separation via centrifugation in 30% KBr

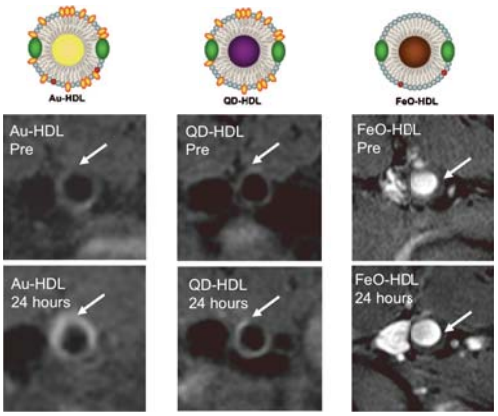
Multimodal nanocrystal core HDL

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

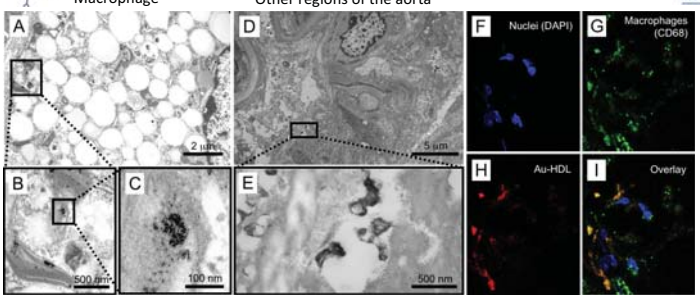


Cormode *et al.* Nano Lett. 2008

In vivo MRI studies

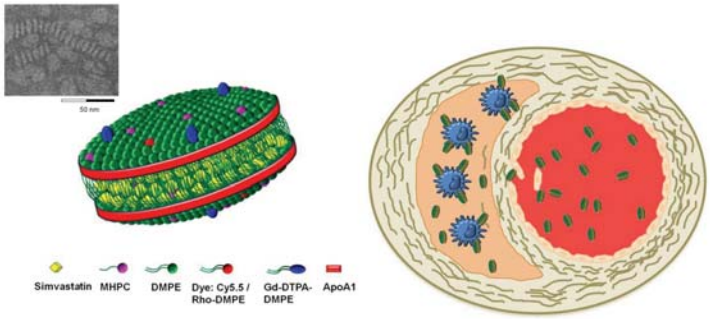


Microscopy of the aorta



Nanocrystal HDL were found almost exclusively in the macrophages

Statin loaded rHDL nanoparticles

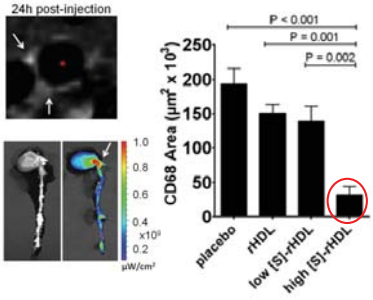


High dose treatment

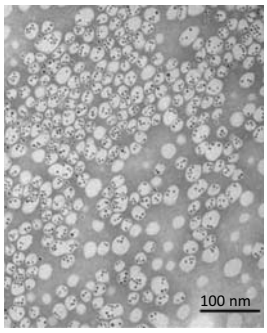
Jun Tang

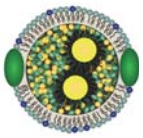
"Theranostic-based evaluation of simvastatin loaded high-density lipoprotein nanoparticles in atherosclerotic mice: mechanism of action of reduced macrophage inflammation"

Scientific Session 22: Preclinical In Vivo/Translational & Clinical – Cardiology
September 8, 2012 from 10:30 AM to 12:00 PM



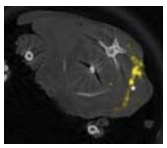
Au-LDL synthesis





David Cormode

"Gold Labeled Low Density Lipoprotein Nanoparticles: Imaging Lipoprotein Biointeractions with CT, TEM and Fluorescence "



Scientific Session 29: CT/US - Technology Methodology – Probes
September 8, 2012 from 1:00 PM to 2:30 PM

Overview

Part 1

- Self-assembly and cellular life
- Lipoproteins
- Amphiphiles and (phospholipids)
- Vesicles, disks, micelles

Part 2

- Liposomes
- Oil-in-water emulsions
- Nanocrystal core HDL
- Complex lipid/polymer hybrid nanoparticles

Complexity

Mass Production and Size Control of Lipid–Polymer Hybrid Nanoparticles through Controlled Microvortices

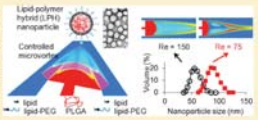
YongTae Kim,[†] Bomy Lee Chung,[†,‡] Mingming Ma,[†] Willem J. M. Mulder,[∥] Zahi A. Fayad,[∥] Omid C. Farokhzad,[⊥] and Robert Langer[*,†,‡,§]

[†]David H. Koch Institute for Integrative Cancer Research, [‡]Department of Chemical Engineering, and [§]Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States
[∥]Translational and Molecular Imaging Institute, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1234, New York, 10029, United States
[⊥]Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, United States

Supporting Information

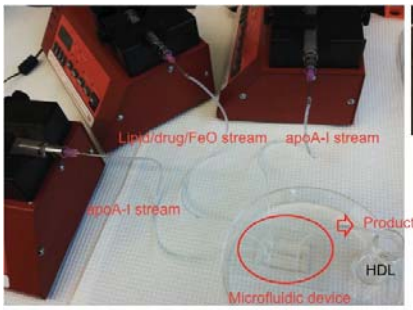
ABSTRACT: Lipid–polymer hybrid (LPH) nanoparticles can deliver a wide range of therapeutic compounds in a controlled manner. LPH nanoparticle syntheses using microfluidics improve the mixing process but are restricted by a low throughput. In this study, we present a pattern-tunable microvortex platform that allows mass production and size control of LPH nanoparticles with superior reproducibility and homogeneity. We demonstrate that by varying flow rates (i.e., Reynolds number (30–150)) we can control the nanoparticle size (30–170 nm) with high productivity (~3 g/hour) and low polydispersity (~0.1). Our approach may contribute to efficient development and optimization of a wide range of multicomponent nanoparticles for medical imaging and drug delivery.

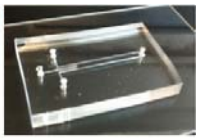
KEYWORDS: Nanoparticle, microvortex, synthesis, control, polymer, microfluidics

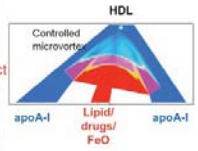


Kim *et al*. Nano Lett. 2012

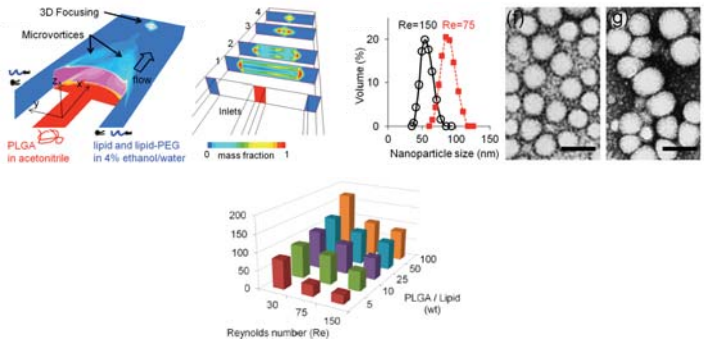
Microfluidics setup





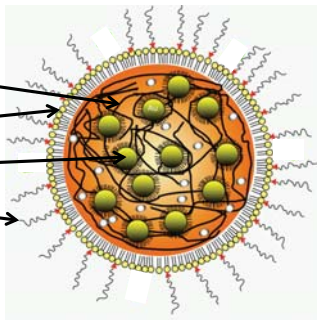


Microvortices

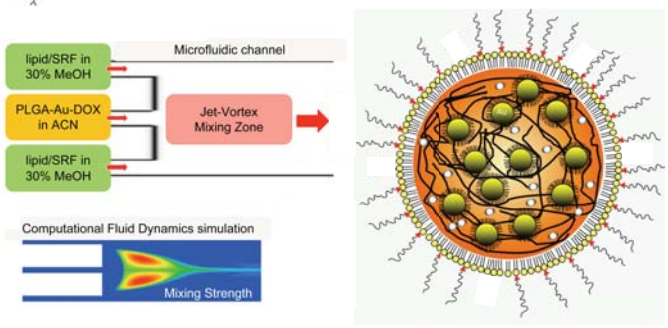


Lipid/polymer hybrid nanoparticle

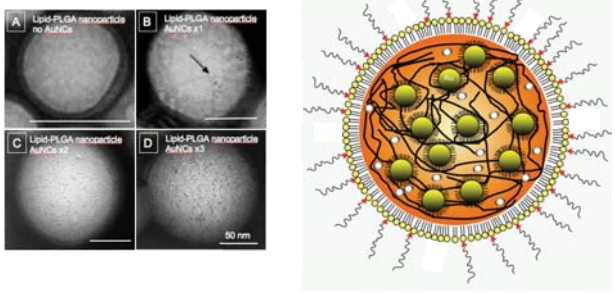
- Polymer (PLGA)
- Carrier lipids
- Au nanocrystals
- PEG-lipids



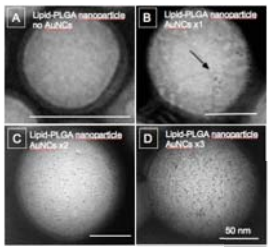
Lipid/polymer hybrid nanoparticle



Lipid/polymer hybrid nanoparticle



Lipid/polymer hybrid nanoparticle



Aneta Mieszawska

"Theranostic lipid-polymer nanoparticles for image guided delivery of dual modality cancer therapy"

Scientific session 6:
Chemistry and Probes-Multimodal,
September 6 from 1 pm to 2:30 pm.

Funding Nanomedicine Lab

Program of Excellence in Nanotechnology (PEN) Award: $16.5 Million

Translational Nanomedical Therapies for Cardiac and Vascular Diseases

NanoCarD: Nanomedicines for Cardiovascular Disease

Mount Sinai School of Medicine

Massachusetts Institute of Technology

New York University

Columbia University

Brigham and Women's Hospital

R01 EB009638 (Z.A.F.)

R21 CA159075 (A.R.)

R01 CA155432 (W.J.M.M.)

ECHO.06.B.047 (W.J.M.M.)

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