

Design and Characterization of Biomimetic Vesicles Deriving from Leukocyte Plasma Membrane

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FORTUNE 100 BEST COMPANIES TO WORK FOR

BEST HOSPITALS U.S.News & WORLD REPORT NATIONAL RANKED IN 13 SPECIALTIES 2012-13

2014 prc national excellence in healthcare AWARD WINNER

The Voice of Female Consumers AMERICA'S BEST HOSPITALS for Patient Experience 2012 WOMEN'S CHOICE AWARD

NanoInnovation 2016, Sep 20-23, Rome

Current therapeutic treatments

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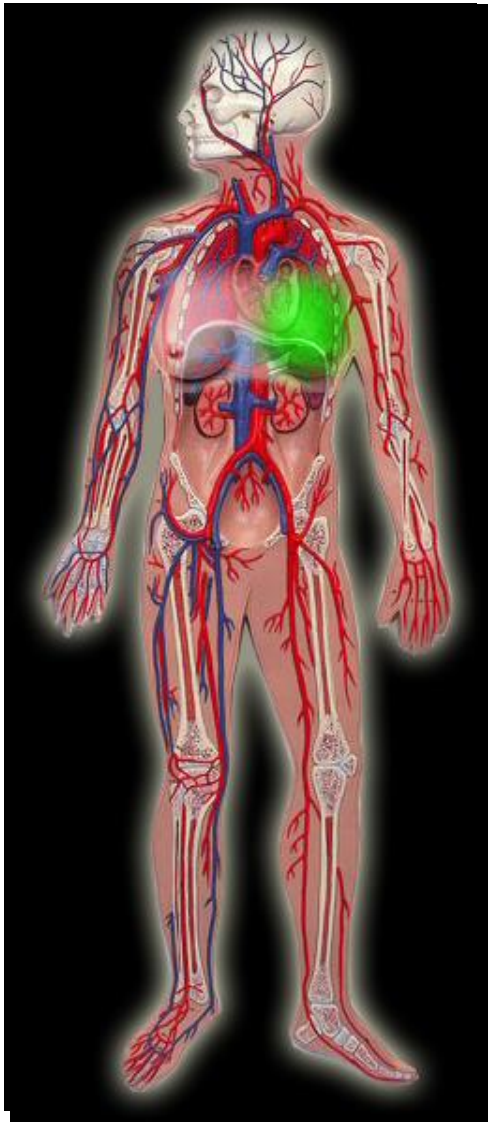
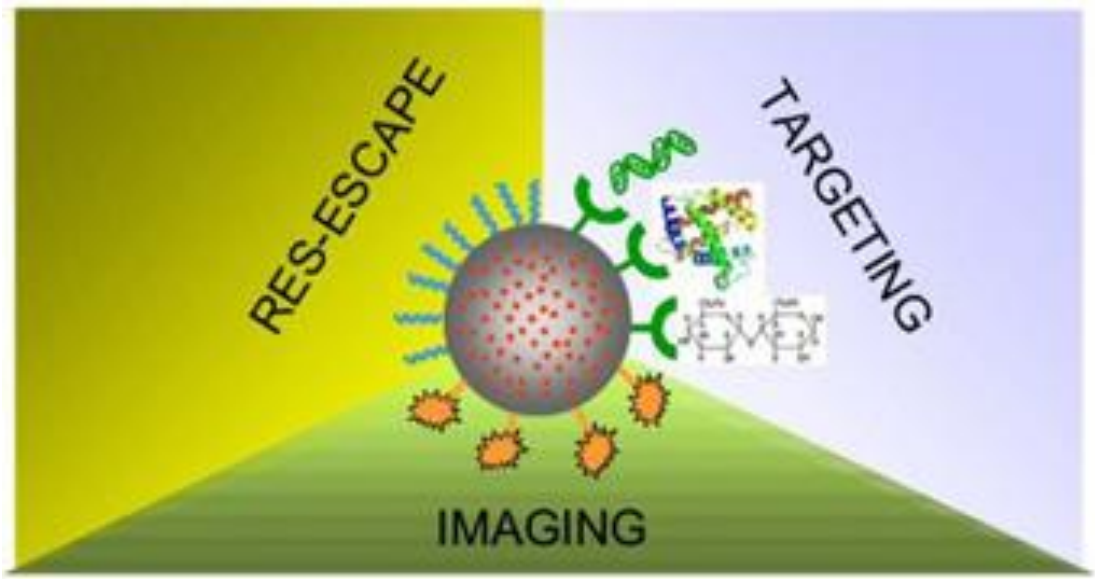


CONVENTIONAL THERAPY

- 1 in 100,000 drug molecules actually reaches target
- 1 in 10,000 of therapeutic antibodies reaches target

FUTURE NANOTHERAPY



Biological Barriers

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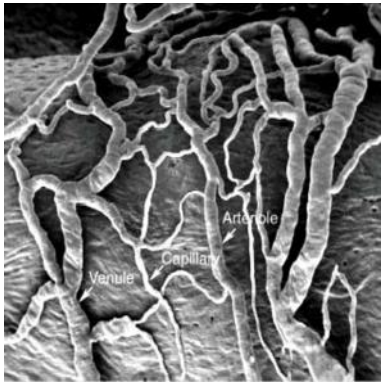
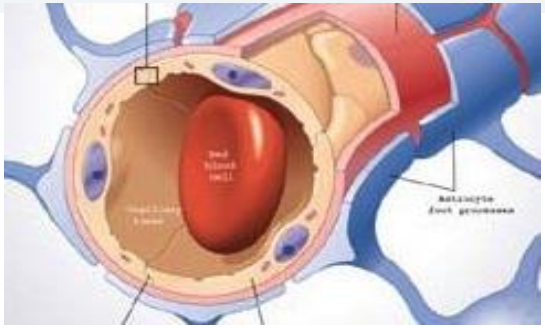
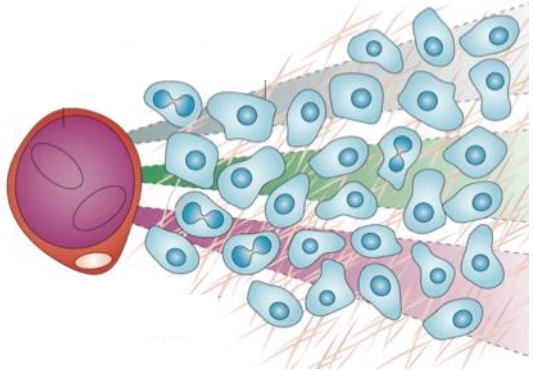
Normal vasculature

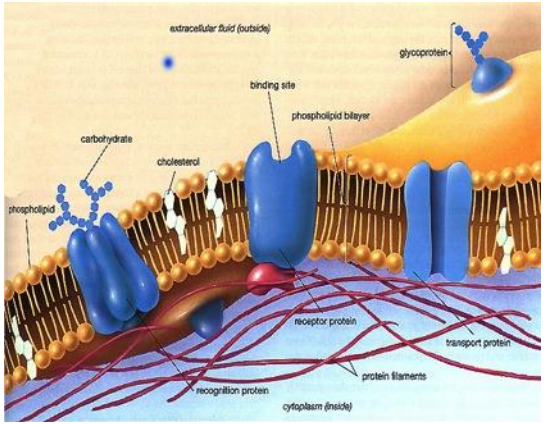


Tumor vasculature

- Hemo-Rheology
- Endothelial Barriers
- Extracellular Matrix
- Cell Membrane
- Ionic & Molecular Pumps
- Enzymatic Degradation
- Nuclear Membrane







Clearance of foreign particles from the MPS

How to overcome biological barriers?

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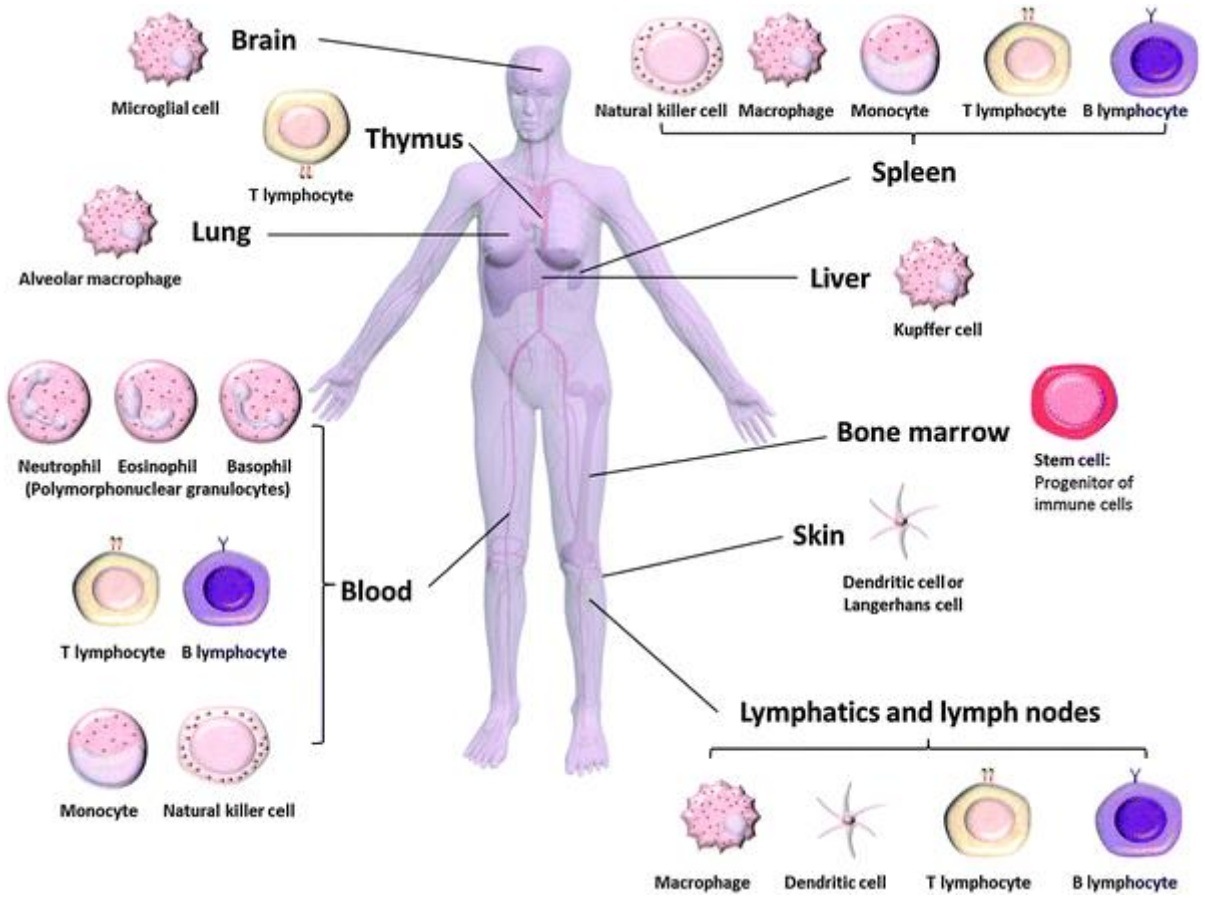

Synthetic NP

- Size and surface potential
- Loading and release
- Shape
- Stability
- Surface chemistry

MPS organs

- Bone marrow
- Liver
- Spleen
- Lungs
- Gastrointestinal tract
- Lymphatic system

Interaction with biological barriers and cells

- Recognition from serum proteins - opsonization
- Cell uptake
- Rapid clearance

Elsabahy and Wooley Chem. Soc. Rev., 5552-5576, 42 2013

Biomimicry: BIOMIMETIC COATINGS to BEAT BIOLOGICAL BARRIERS

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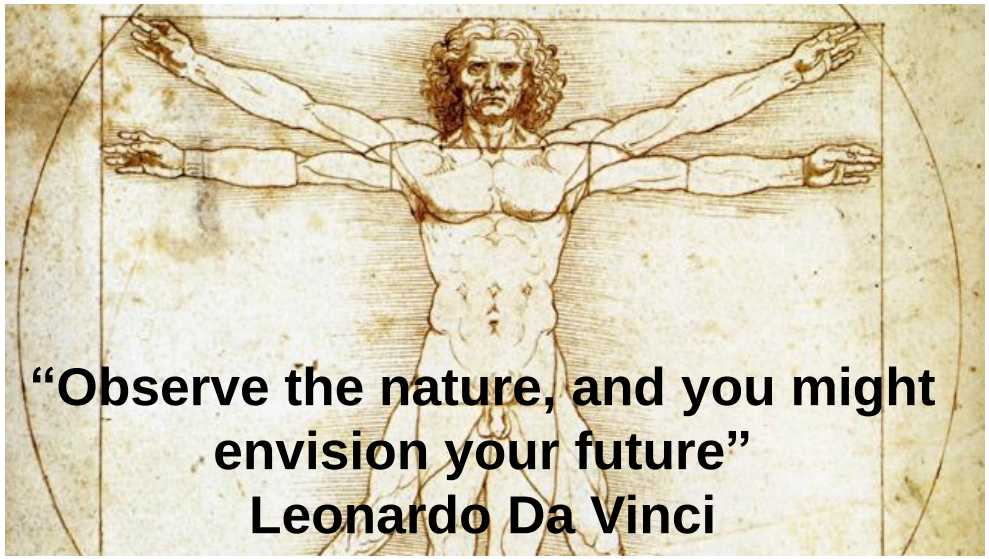


Mimicry is mostly a prey technique to look like a predator

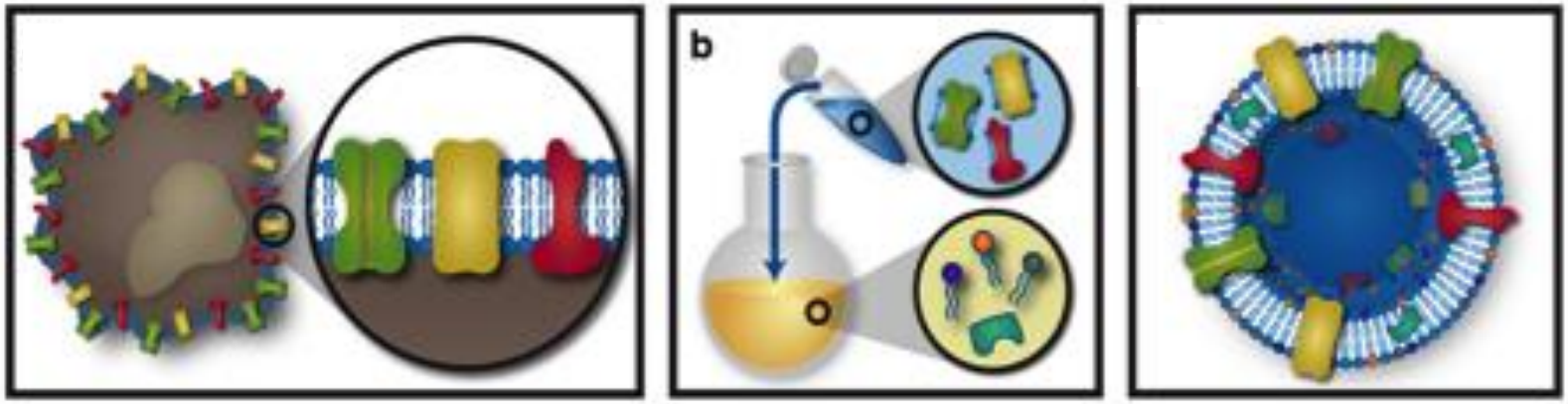

Eastern coral snake
(venomous)


Scarlet king snake
(nonvenomous)

Biomimetic approach

Leukosome

*The **Leukosome:** the membrane proteins of **leuko**cytes combined with synthetic phospholipids in order to obtain a lipo**some**-like nanovesicle.*



Physical and pharmaceutical characterization

- Size, shape, surface charge and polydispersity index
- Protein integration into lipid bilayer
- Protein composition of leukosome surface
- Key markers identification and orientation
- Loading and release properties

Molinaro et al., Nature Materials 2016.

Top down and bottom-up approaches

Background

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Two Different Approaches to Nanofabrication

- **Top ⇨ Down:**
  - Start with the bulk material and "cut away material" to make what you want

- **Bottom ⇨ Up:**
  - Building what you want by assembling it from building blocks (such as atoms and molecules).
  - Atom-by-atom, molecule-by-molecule, or cluster-by-cluster

Pros and cons

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Top-down methods

Cells

Cell-derived nanoparticles
Nano-ghosts

- Retain complexity of cellular surface
- Lack of control of physical parameters
- Poor encapsulation and retention of payload

Bottom-up methods

Macromolecules

Synthetic nanoparticles

- Physicochemical control over final formulation
- Inadequate to reproduce the complexity of cellular membrane on carrier surface

Leukosomes are liposome-like nanovesicles

DLS and cryoEM analysis

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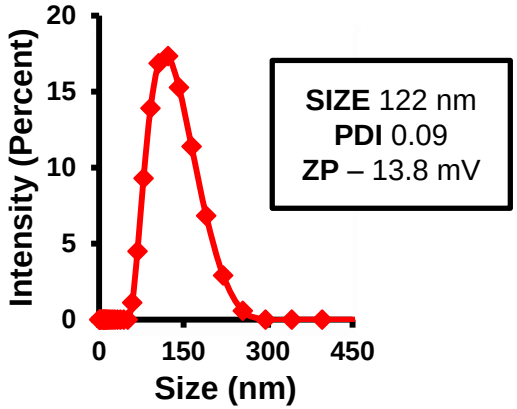
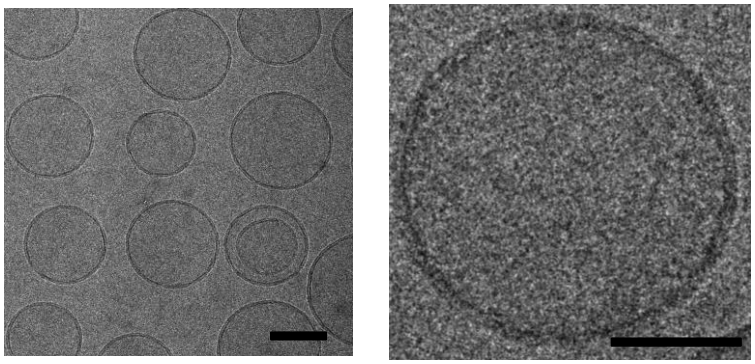
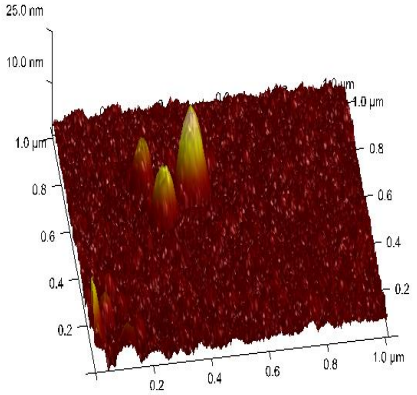
DLS



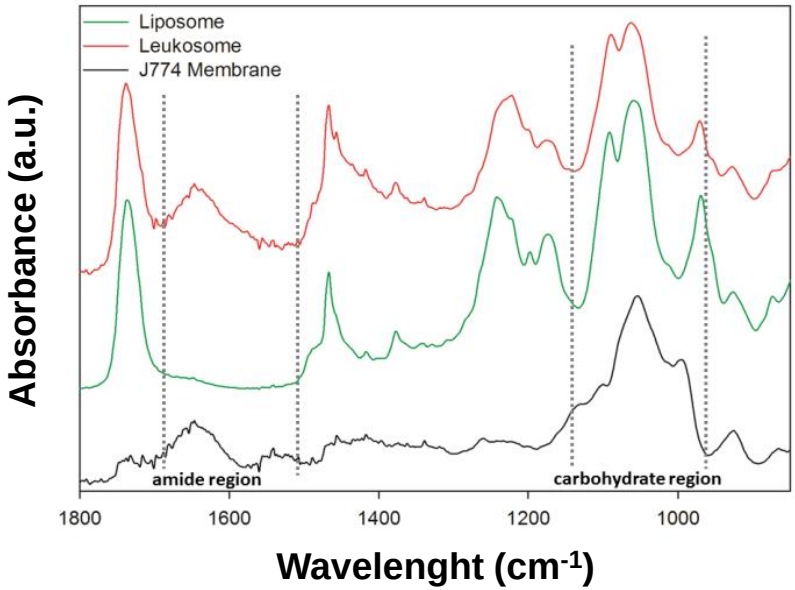
cryoEM analysis



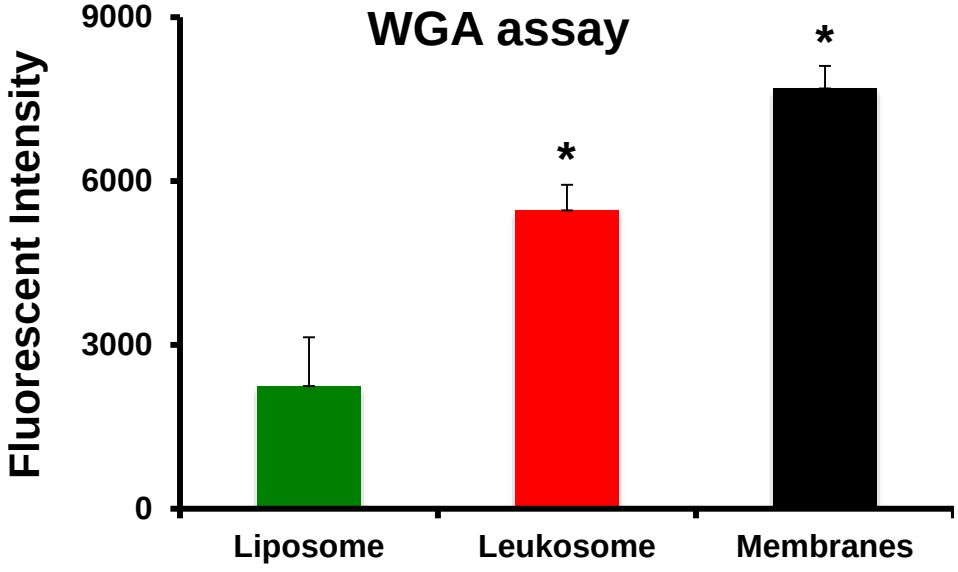
AFM



FTIR



WGA assay



Membrane proteins are integrated into lipid bilayer

DSC and deformability analysis

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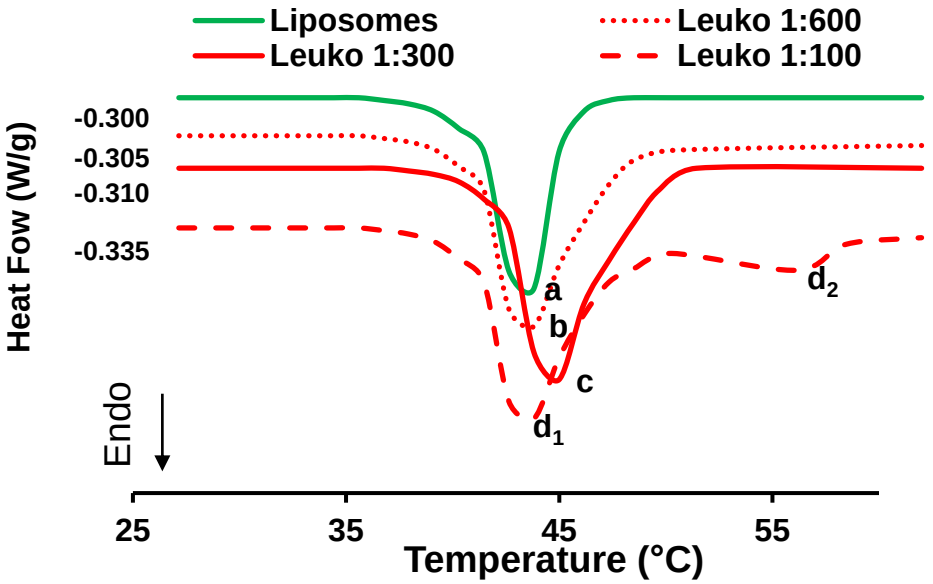
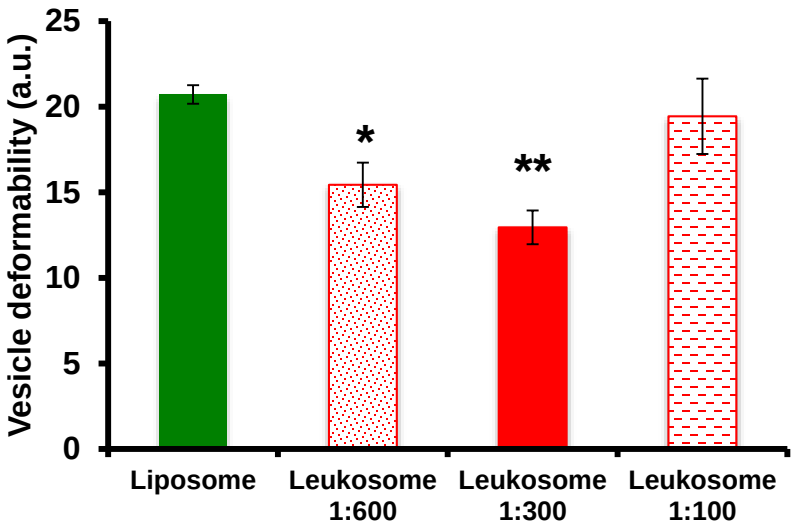
Differential Scanning Calorimetry

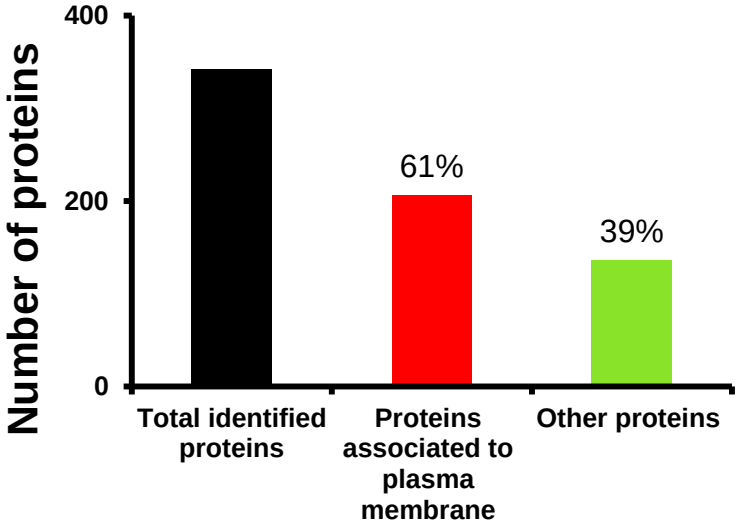


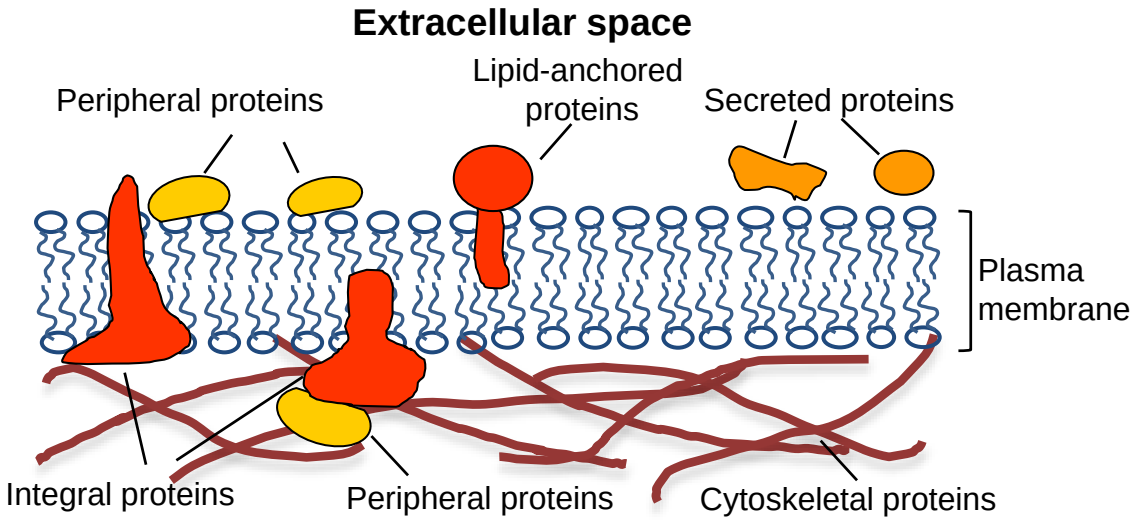
Extrusion assay

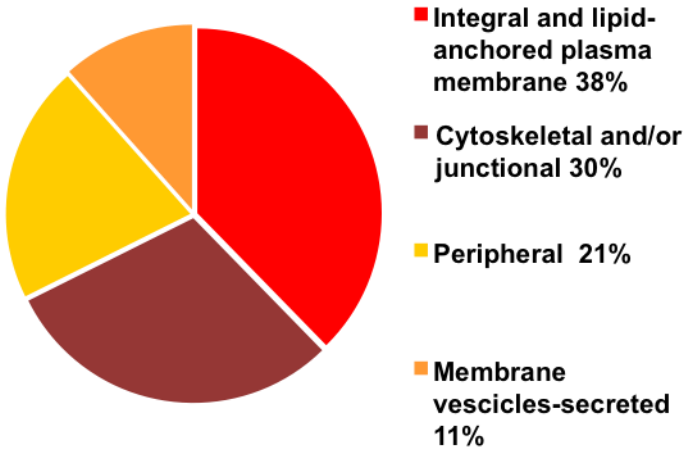


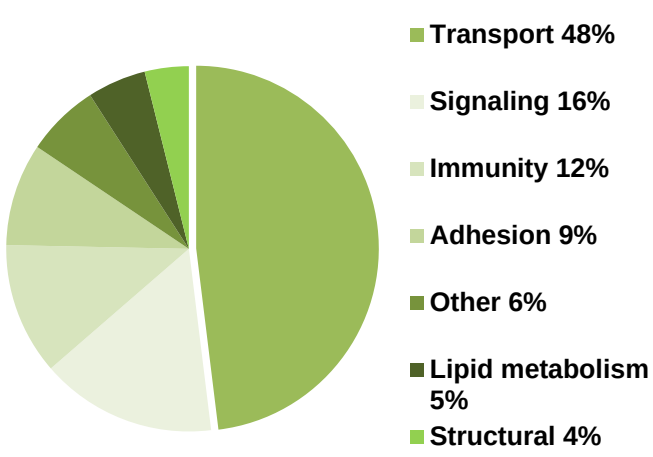
- Proteins are integrated into lipid bilayer
- 1:300 results the best compromise between vesicle stability and level of protein integration into leukosome bilayer
- Protein incorporation results in a reduced flexibility of leukosome membrane

Proteomic Analysis

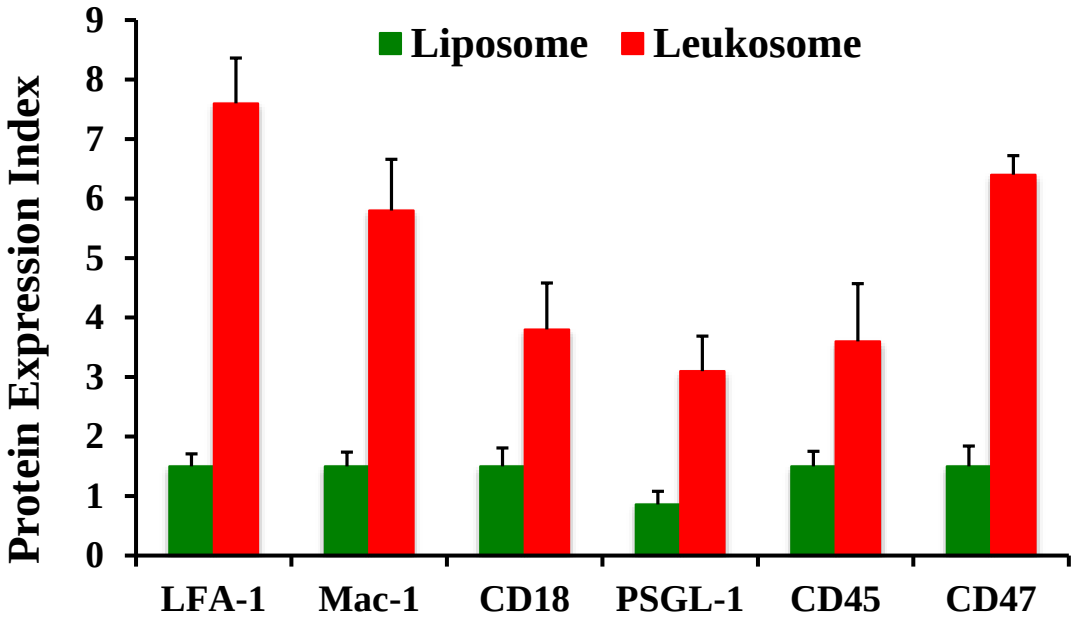


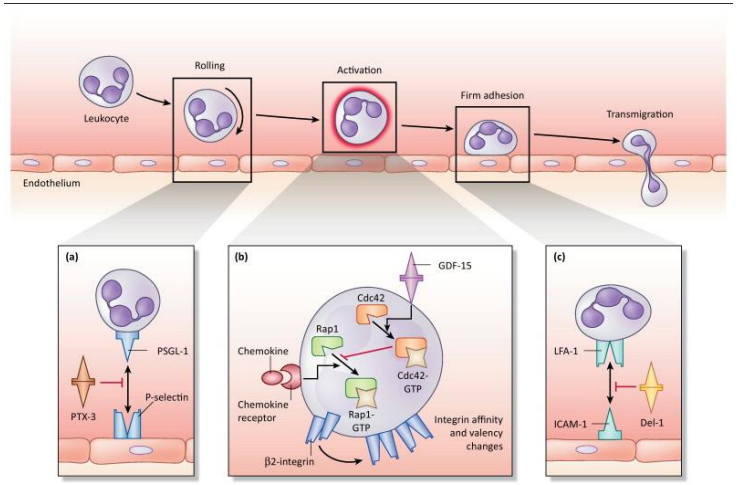






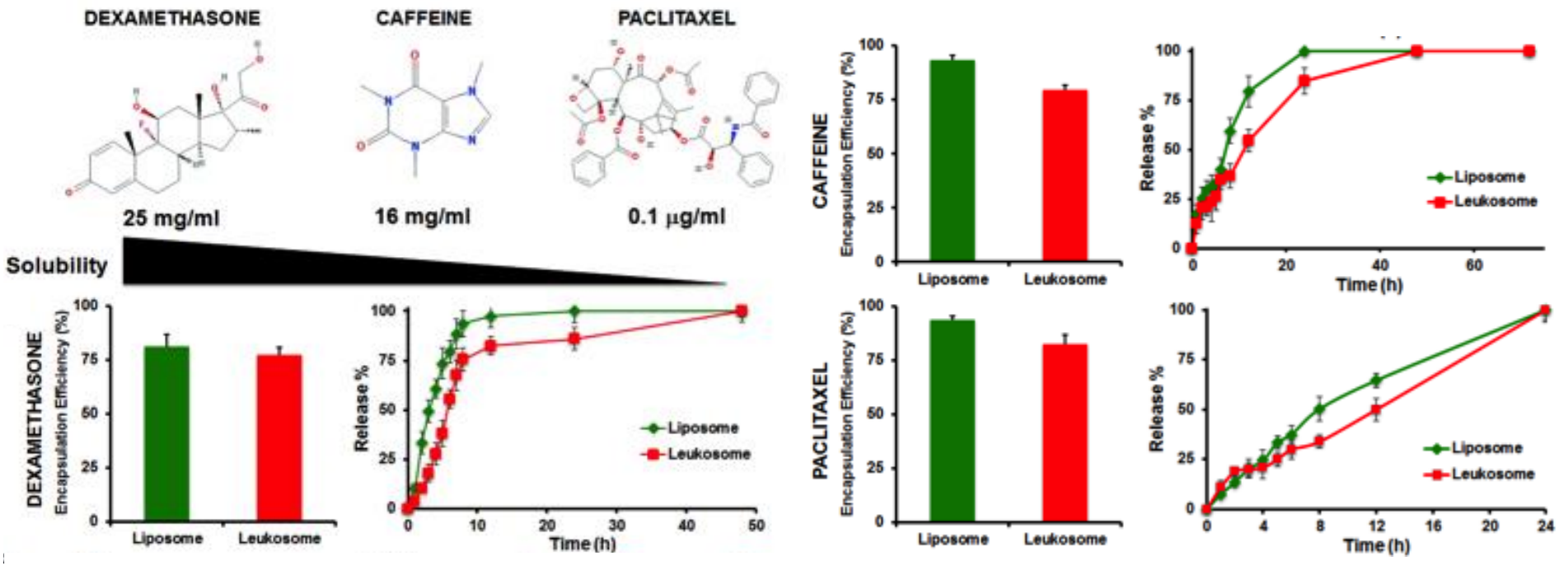
Leukocyte surface marker identification on leukosome surface





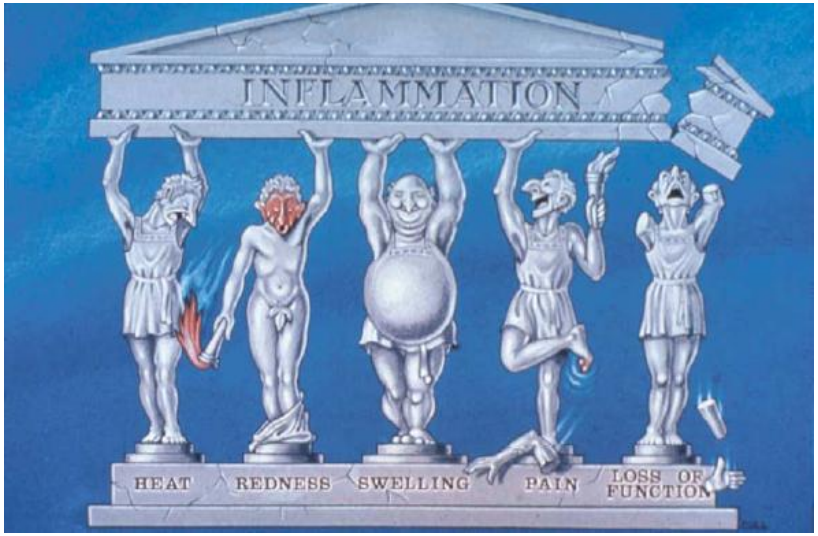
Theoretical calculations	LFA-1	MAC-1	PSGL-1	CD18	CD45	CD47	IgG
Number of copies	1.37×10^{14}	9.93×10^{13}	5.62×10^{13}	9.60×10^{13}	7.24×10^{13}	1.24×10^{14}	7.78×10^{11}
Number of copies/particle	≈ 10	≈ 7	≈ 4	≈ 7	≈ 5	≈ 9	≈ 0.1
Number of copies/surface area (mm^2)	**≈ 206**	**≈ 149**	**≈ 85**	**≈ 144**	**≈ 109**	**≈ 187**	**≈ 1**

Loading and release properties



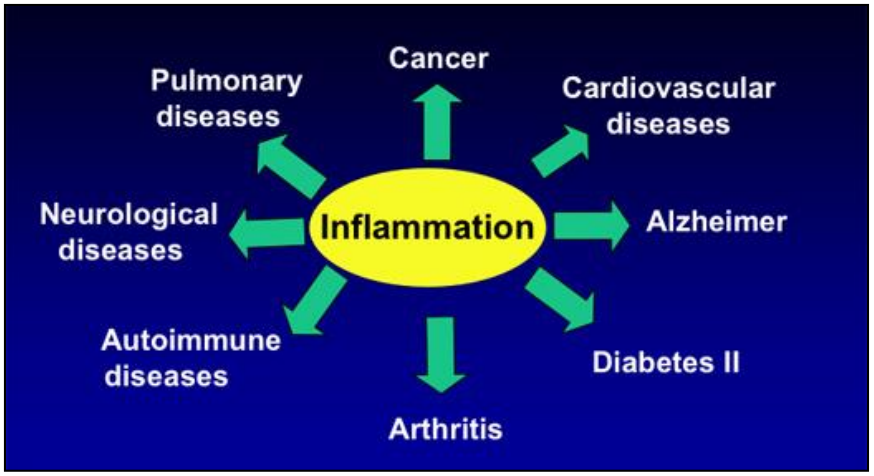
- Leukosomes showed high versatility for the encapsulation of chemically different drugs
- Leukosomes retain loading and release properties typical of liposomes
- Leukosomes showed delayed release kinetic

Clinical applications of leukosomes: The Role of Inflammation



- *Defense reaction caused by tissue damage or injury*
- *Calor, rubor, tumor, dolor, and functio laesa.*

- *Hemodynamic changes, increased permeability, and leukocyte exudation*



Leukosome targeting properties

Animal models

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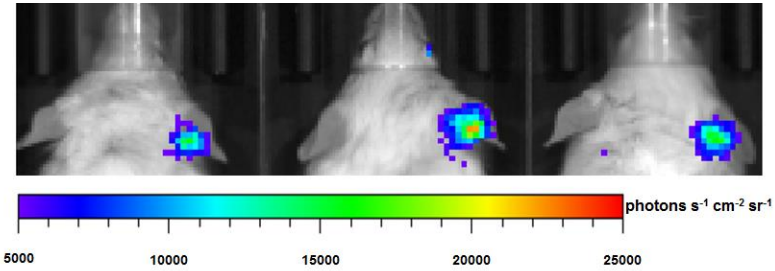
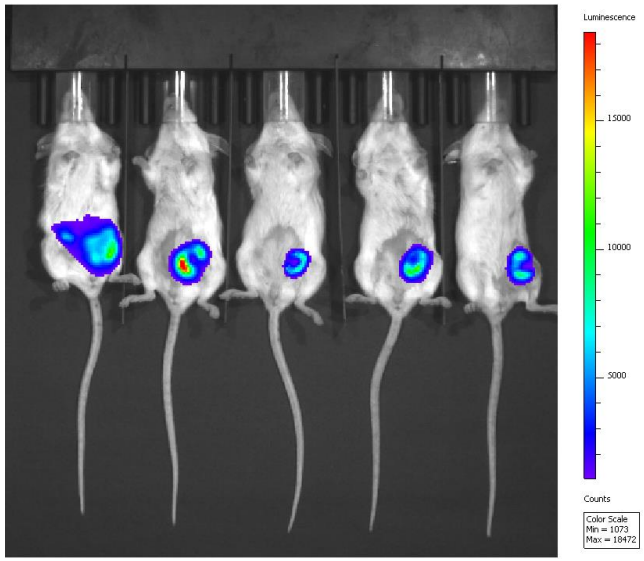
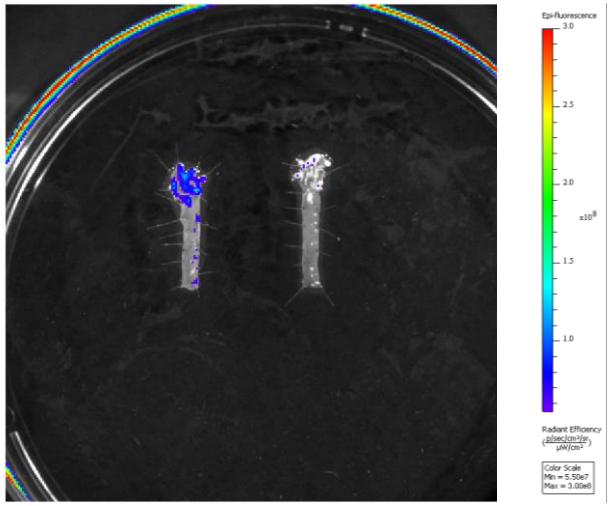
- Localized inflammation



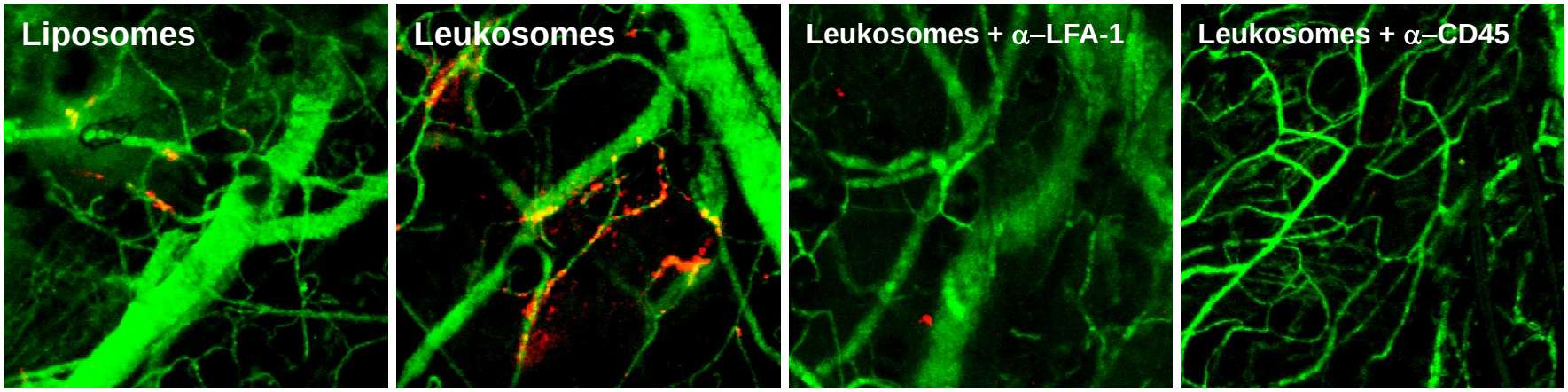


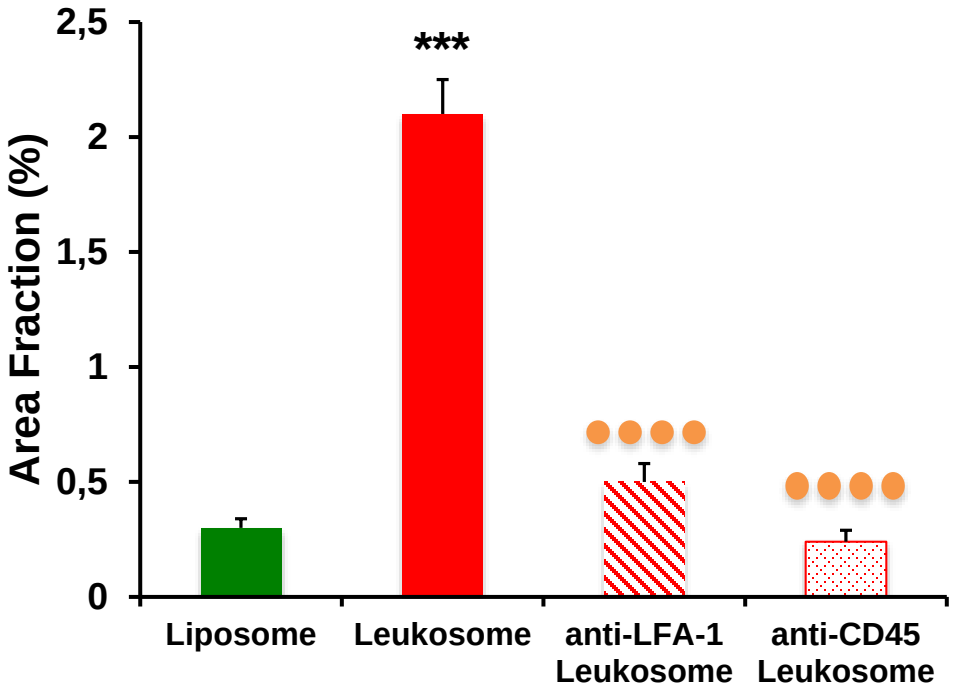
- Cancer

- Atherosclerotic plaques



In vivo targeting properties of leukosomes





- *In vivo* model of localized inflammation: subcutaneous injection of lipopolysaccharide (LPS) into the ears of mice.
- Compared to control liposomes, leukosomes showed an increased accumulation in the inflamed ear

In vivo anti-inflammatory activity of leukosomes

Localized ear inflammation model

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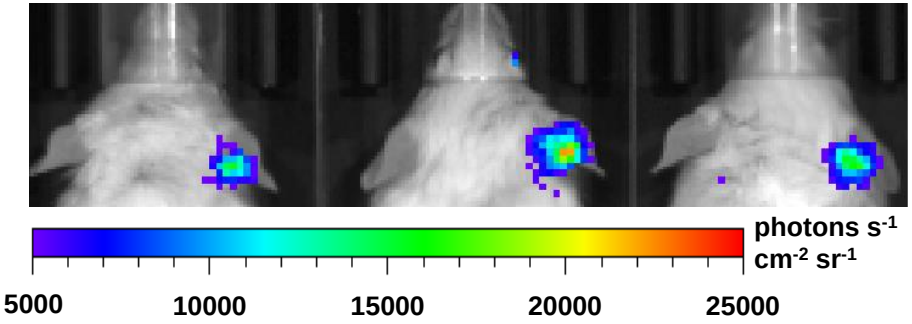
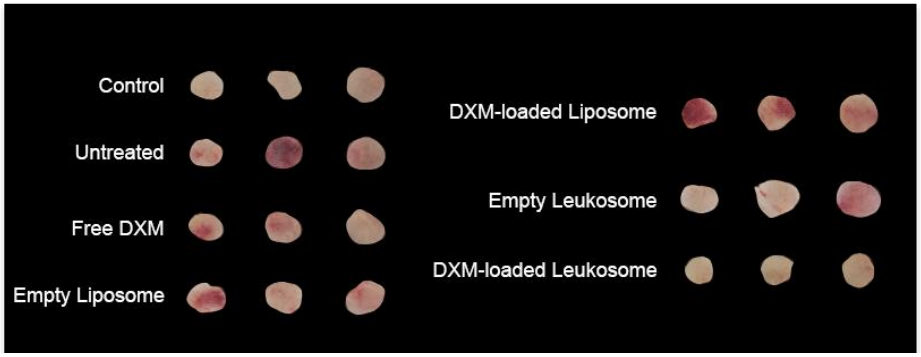
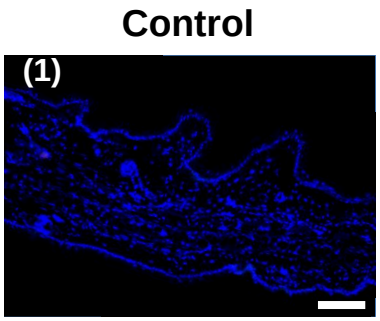
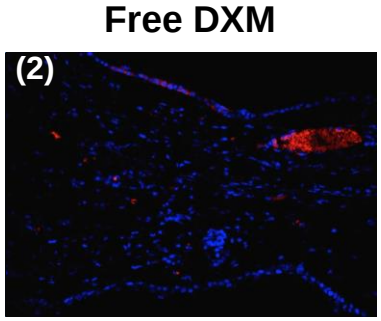
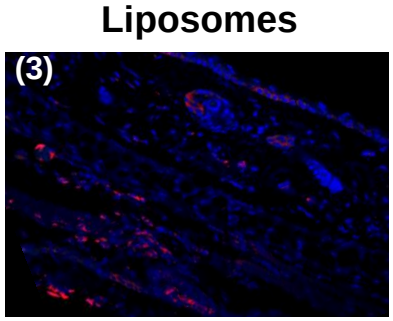
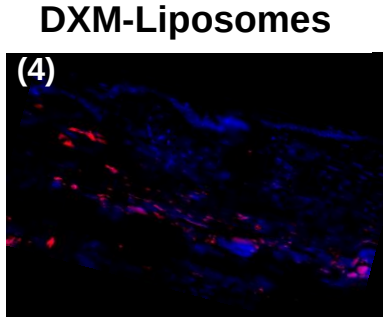


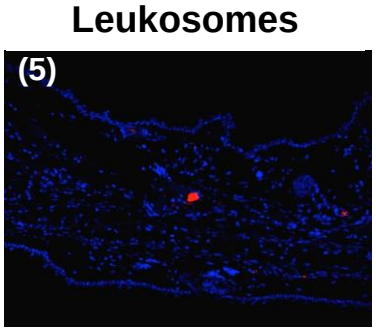


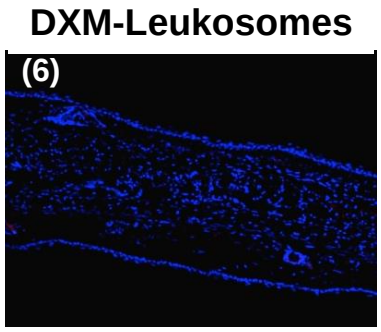


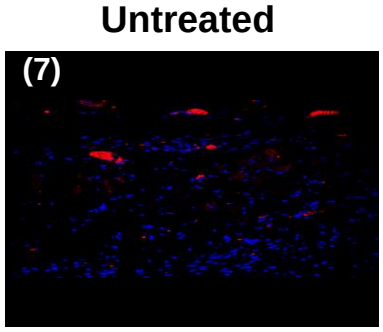


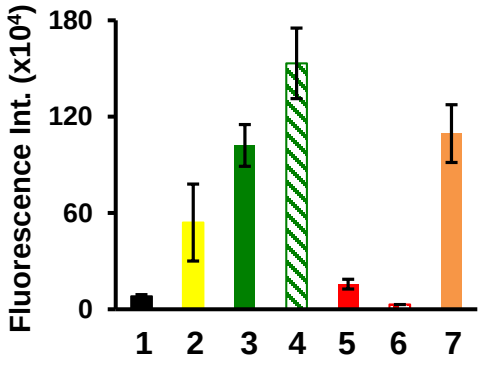






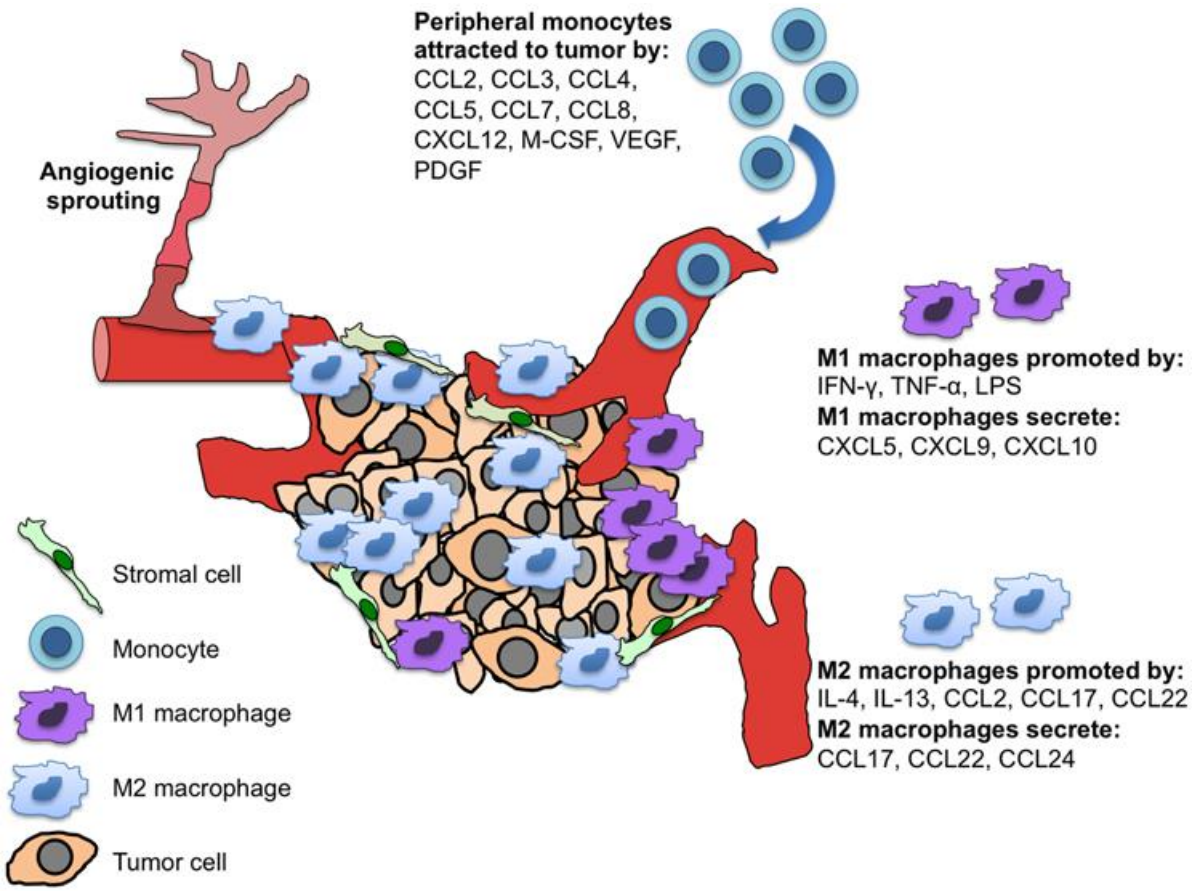






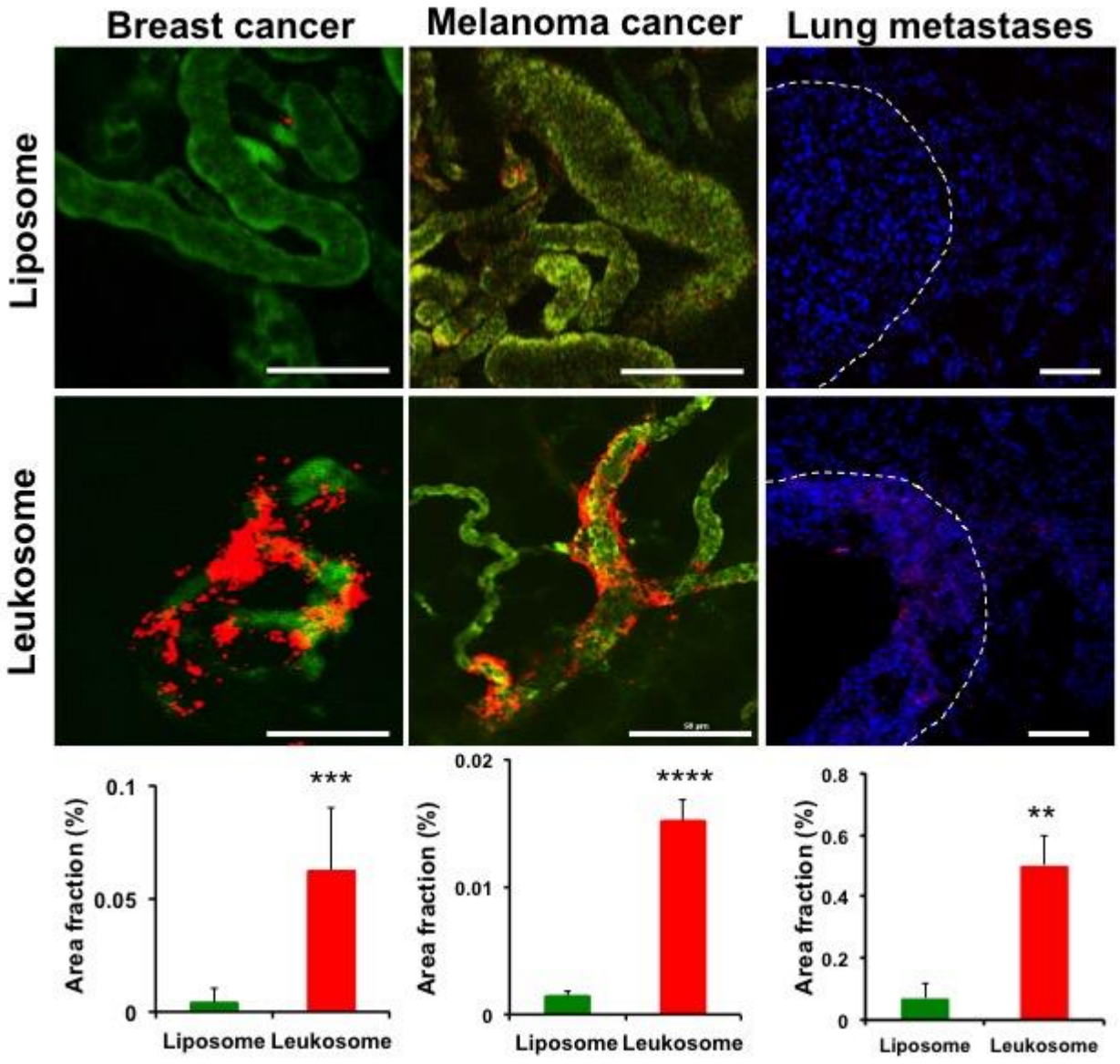
Molinaro et al., Nature Materials 2016.

Targeting of inflamed tumor-associated vasculature



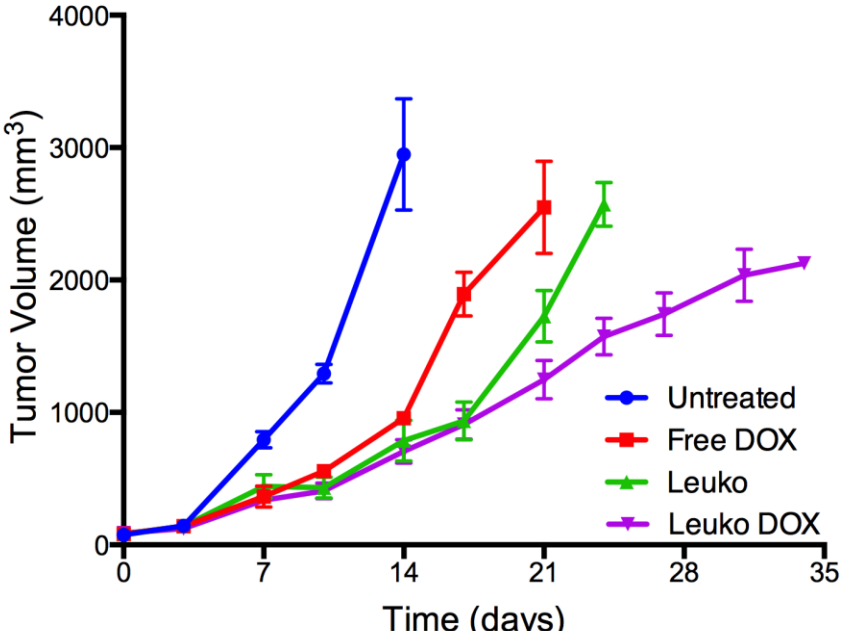
- Inflammatory cells are recruited to tumor tissues and extravasate to these areas via tethering to P-selectin expressed on activated endothelial cells and platelets (Egami et al., 2006).
- Neutrophil infiltration has been reported in various cancer entities and neutrophils are particularly abundant in the invasive front of the tumor (Stockmann et al. 2014).

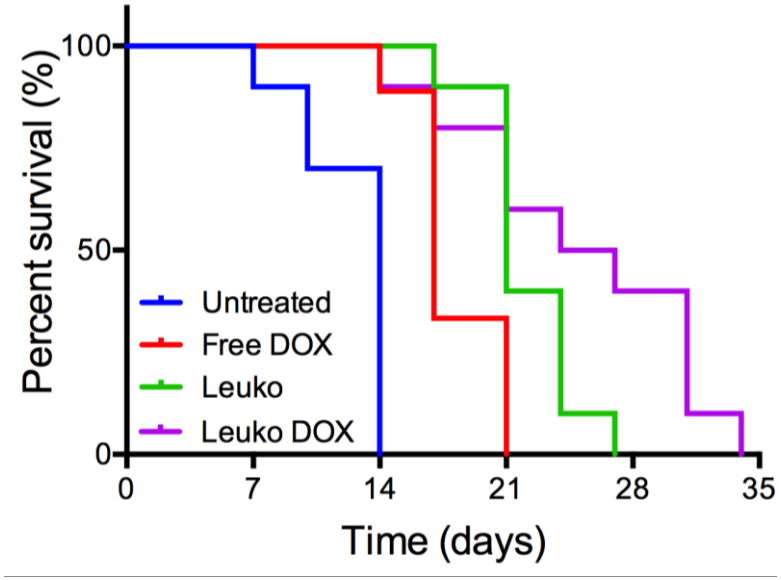
Leukosomes TARGET and ACCUMULATE around tumor-associated inflamed endothelia

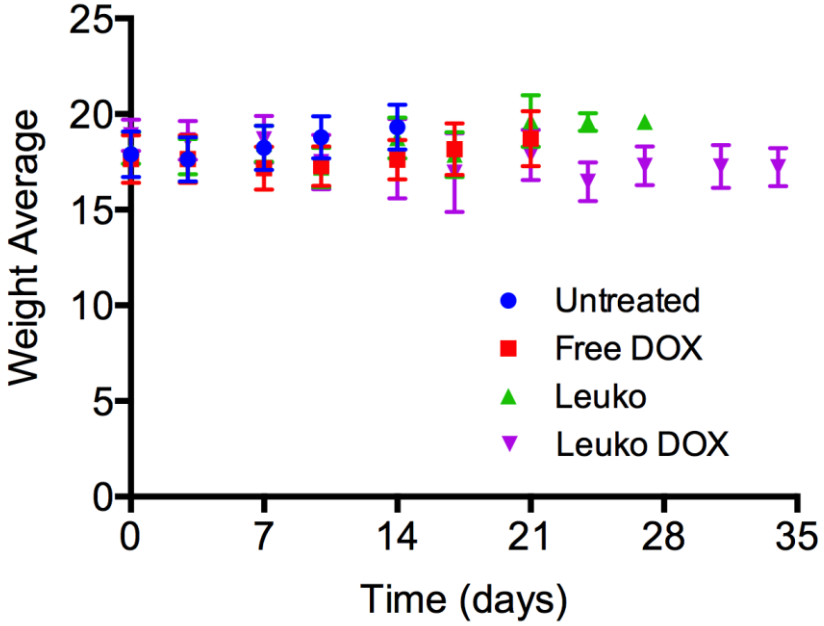


Paper in preparation

Melanoma model







Paper in preparation

The leukocyte's role in the athero-thrombotic disease

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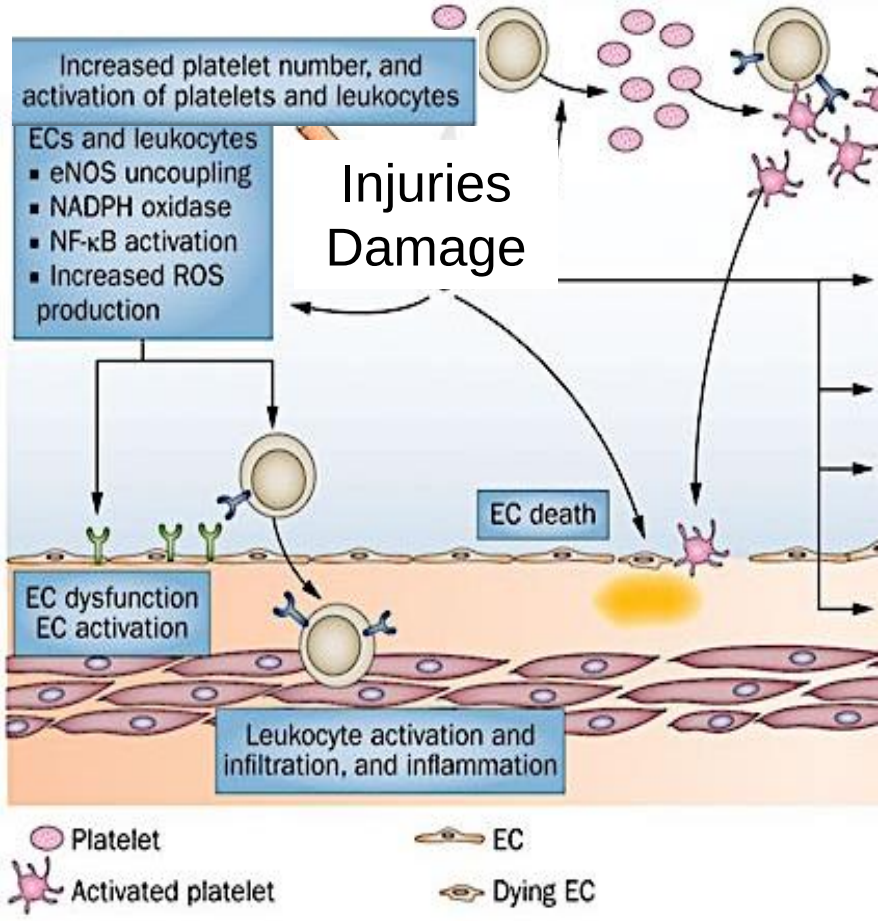
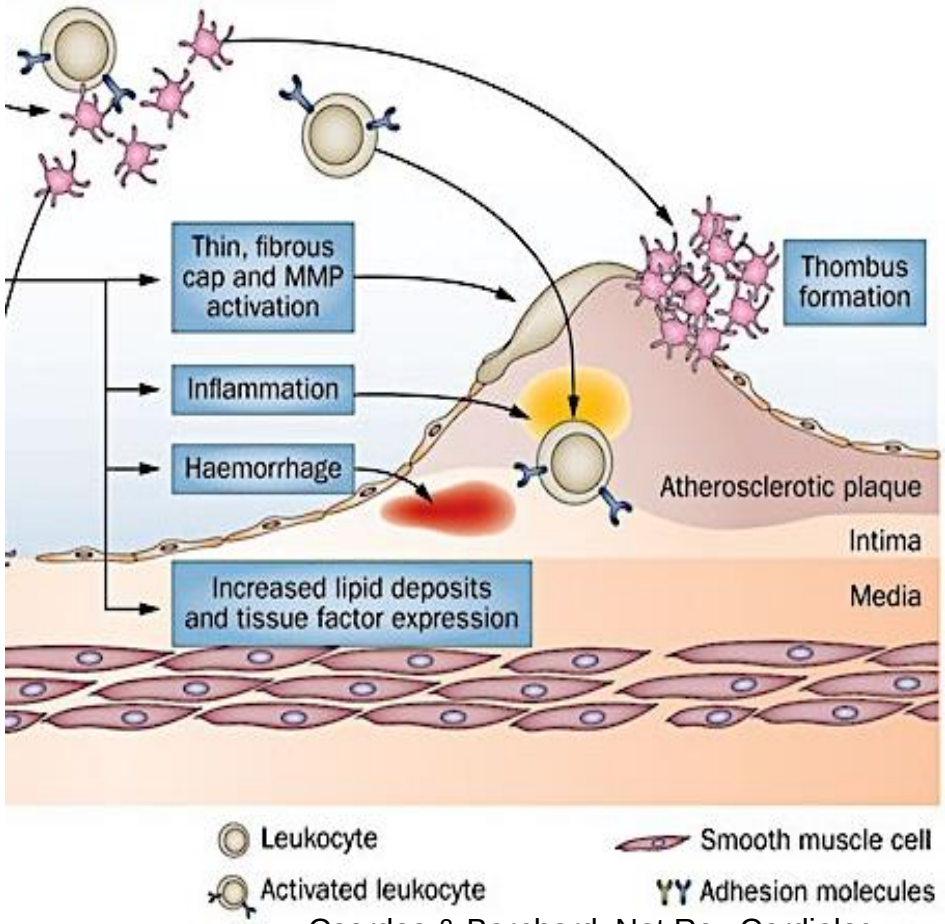
Two sides of the same coin

Inflamed Endothelia

Atherosclerotic plaque





Csordas & Bernhard. Nat Rev Cardiology.

Targeting atherosclerotic plaque

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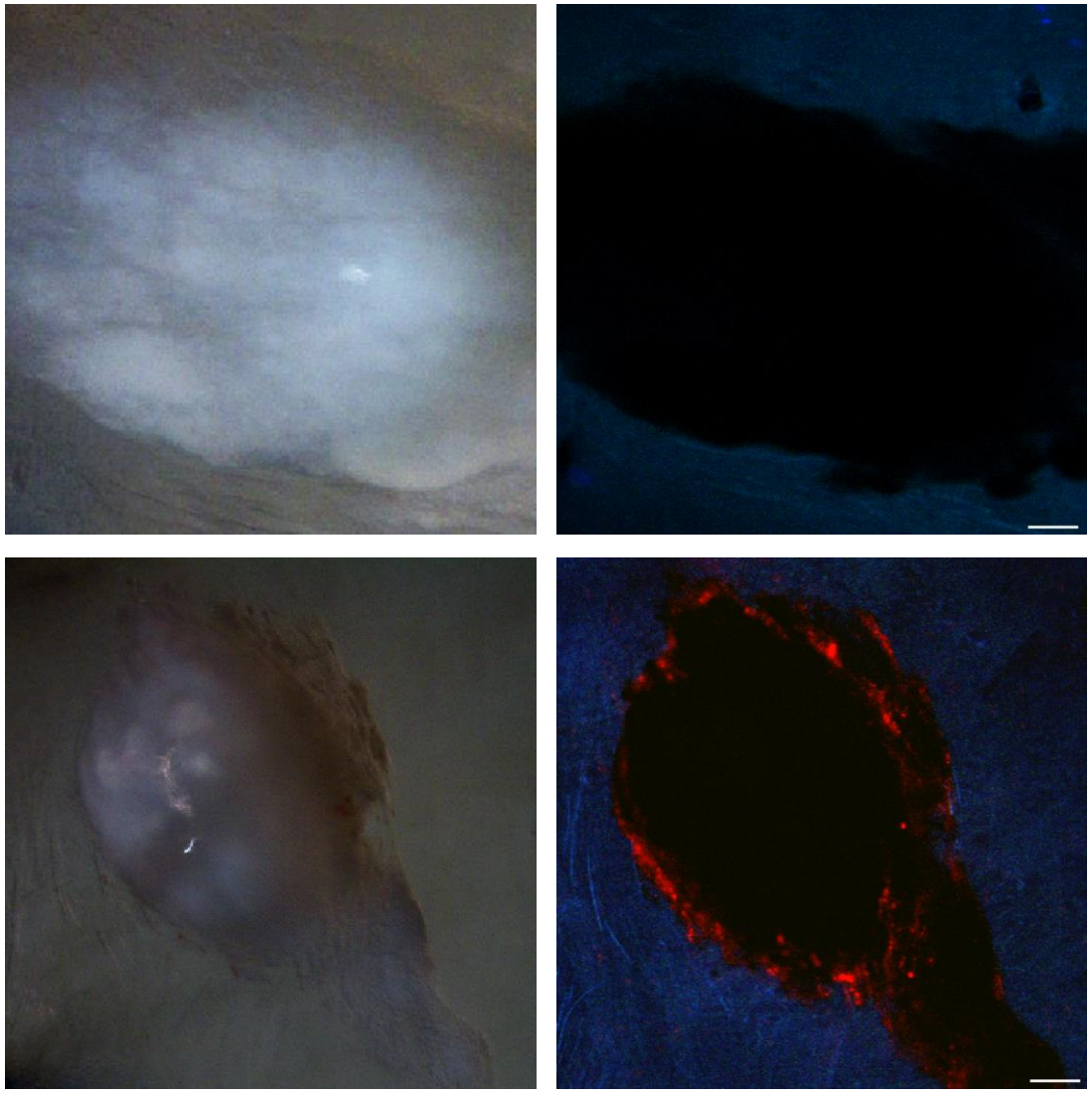
Liposome

Leukosome



- Atherosclerosis model
  - ApoE$^{-/-}$ obese mice
- Aorta imaged 1h after administration
- Leukosomes exhibited significant plaque targeting

Paper in preparation

Conclusions

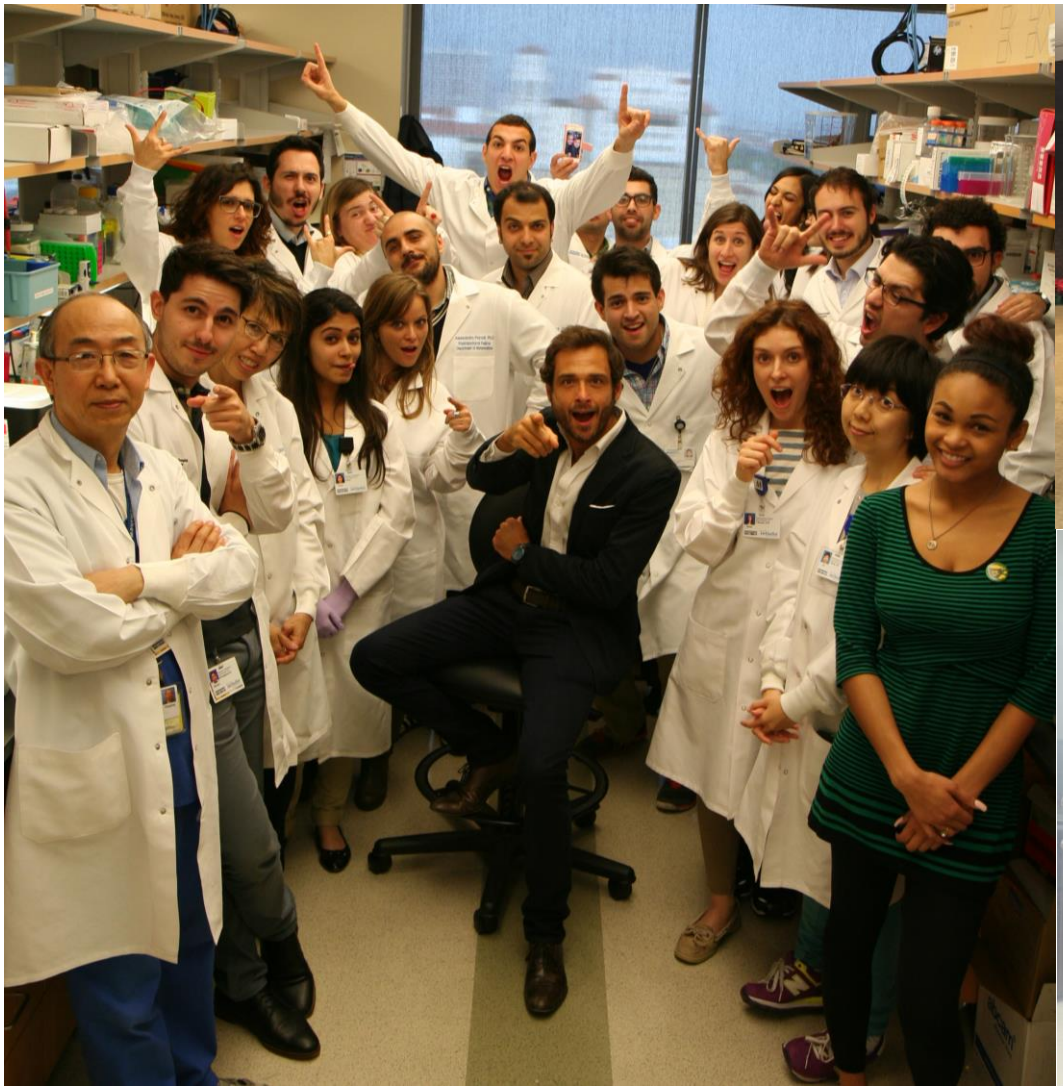
1. To the best of our knowledge, for the first time such a complex material as the plasma membrane is formulated into a lipid nanovesicle using an approach that combines both top-down and bottom-up methods.

2. Our biomimetic approach permitted the transfer of leukocyte membrane proteins to leukosome surface in their active post-transcriptional status.

3. The Leukosome retained loading capabilities similar to current liposomal formulations but delayed the release of the drug.

1. Leukosomes preferentially adhere to inflamed vasculature *in vivo* in several models that share the same inflammatory background.

The Dream Team



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HOUSTON Methodist LEADING MEDICINE

…If you don't want to get caught

blend in with your environment!