Biomimetic Nanoformulations for Targeting Inflamed Vasculature

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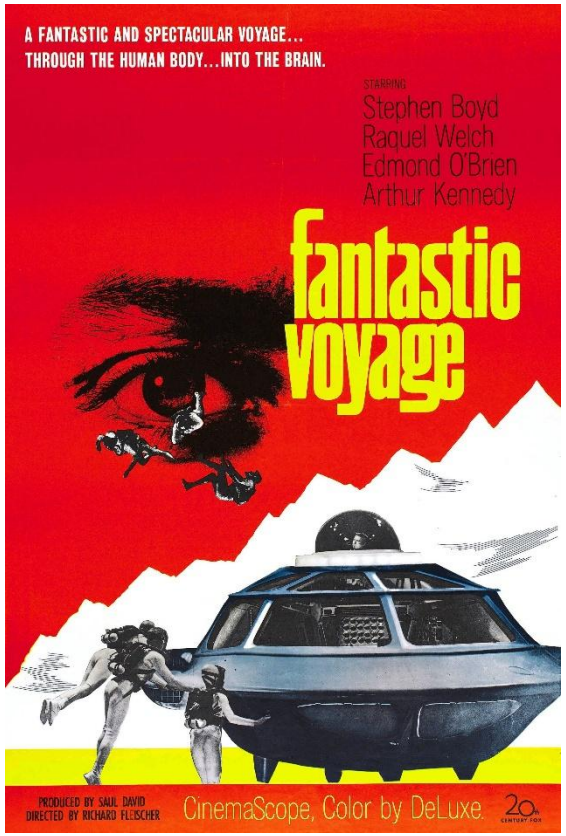
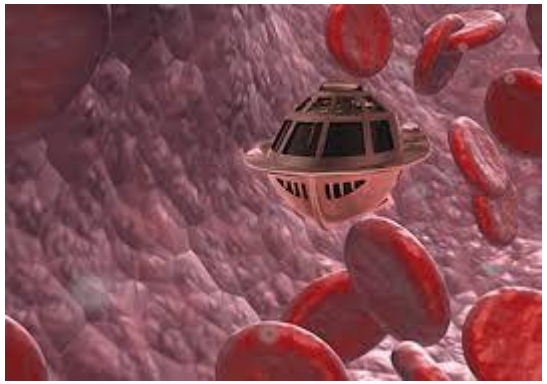
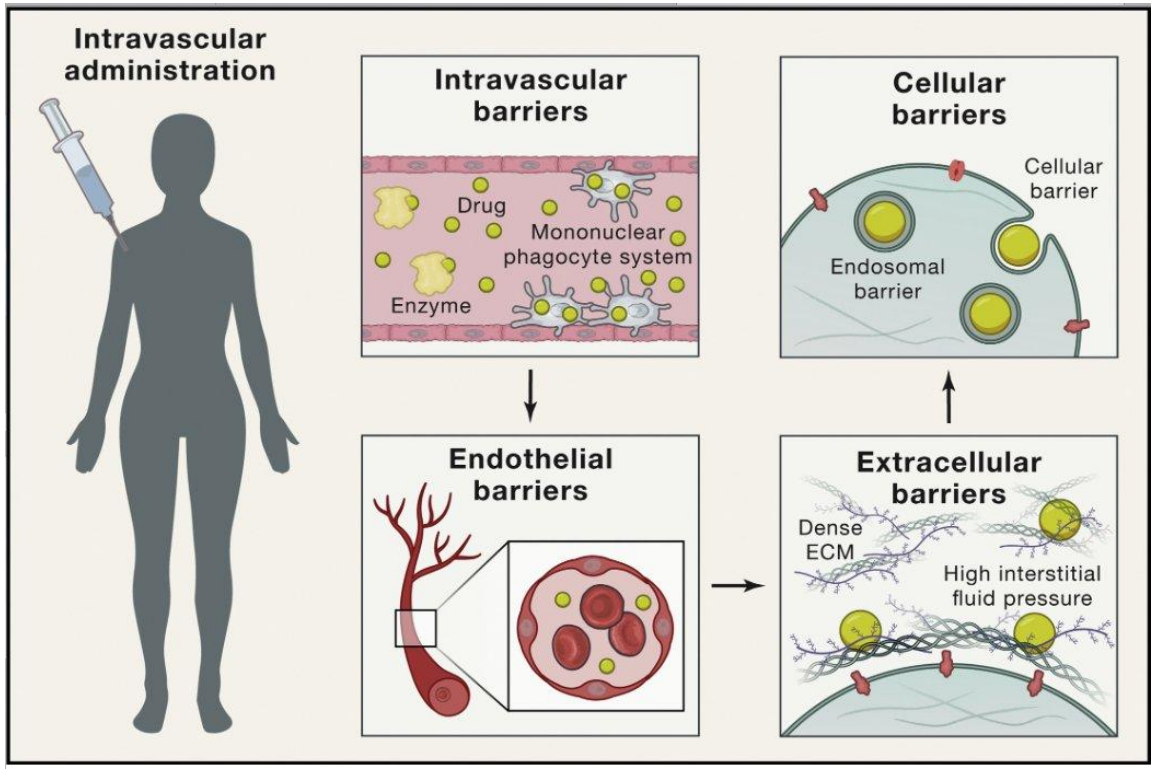
- Director Center for Musculoskeletal Regeneration
- Houston Methodist Research Institute

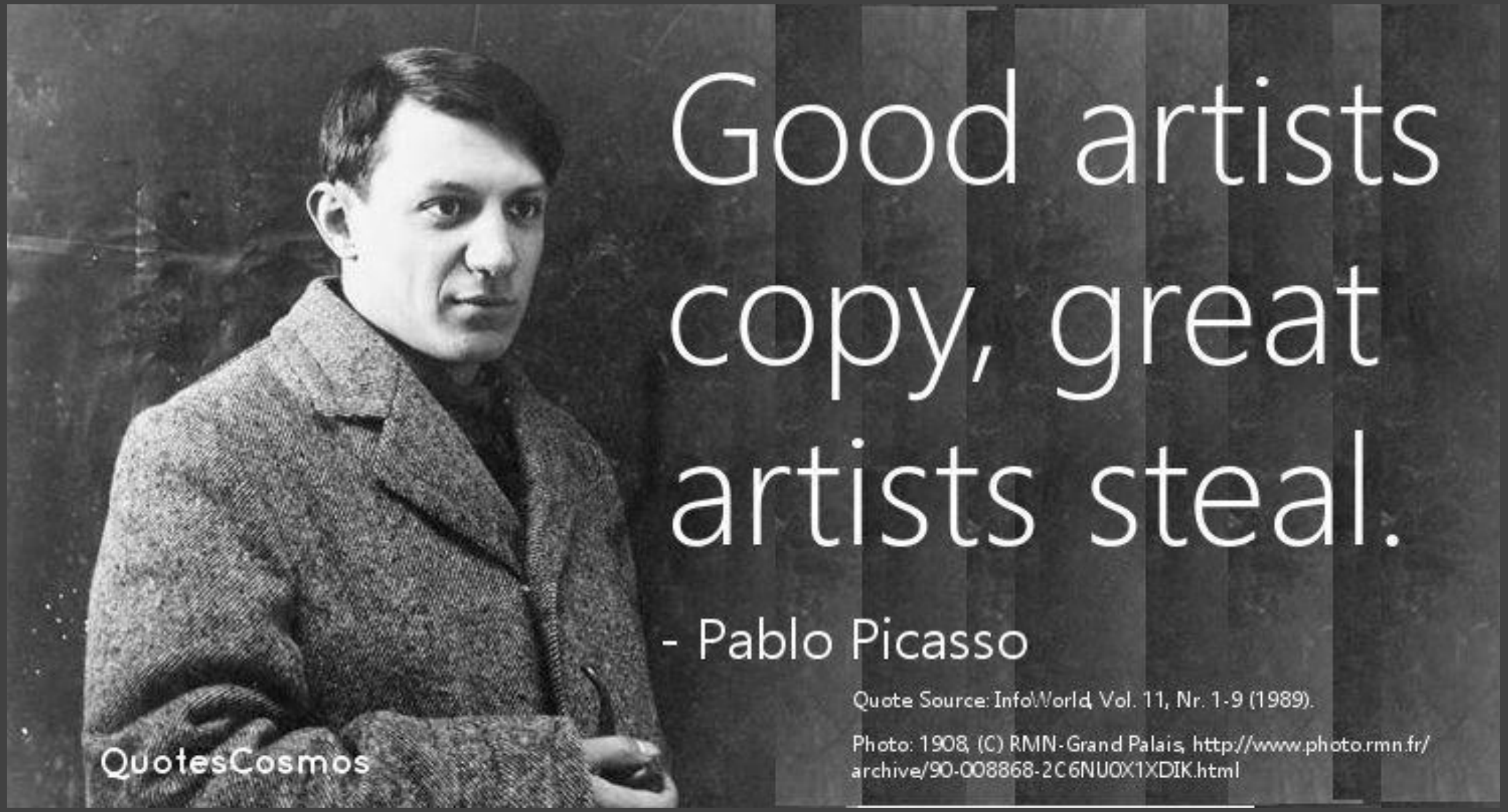
NANOMEDICINE – THE SEMI-UNTOLD STORY



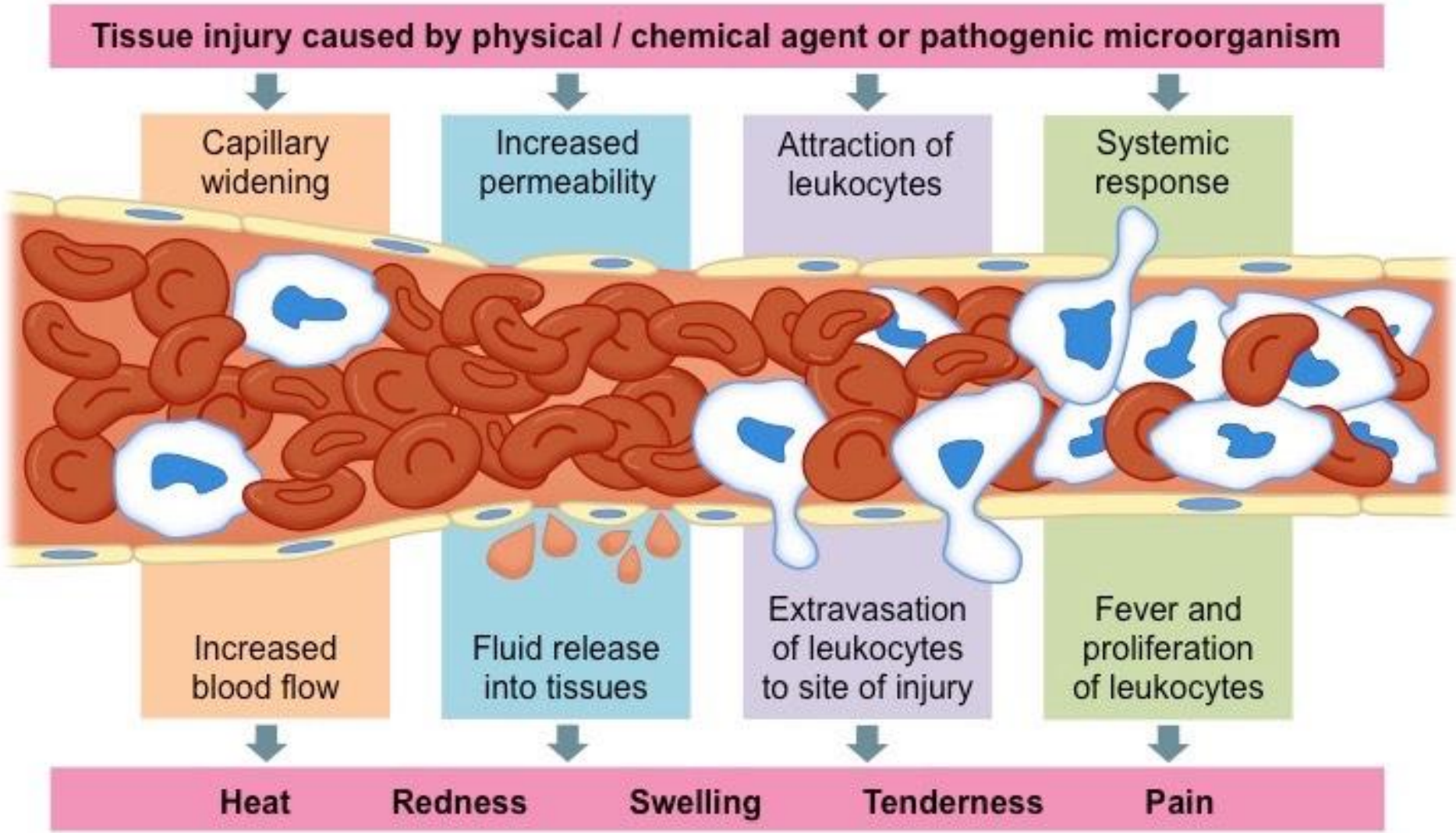




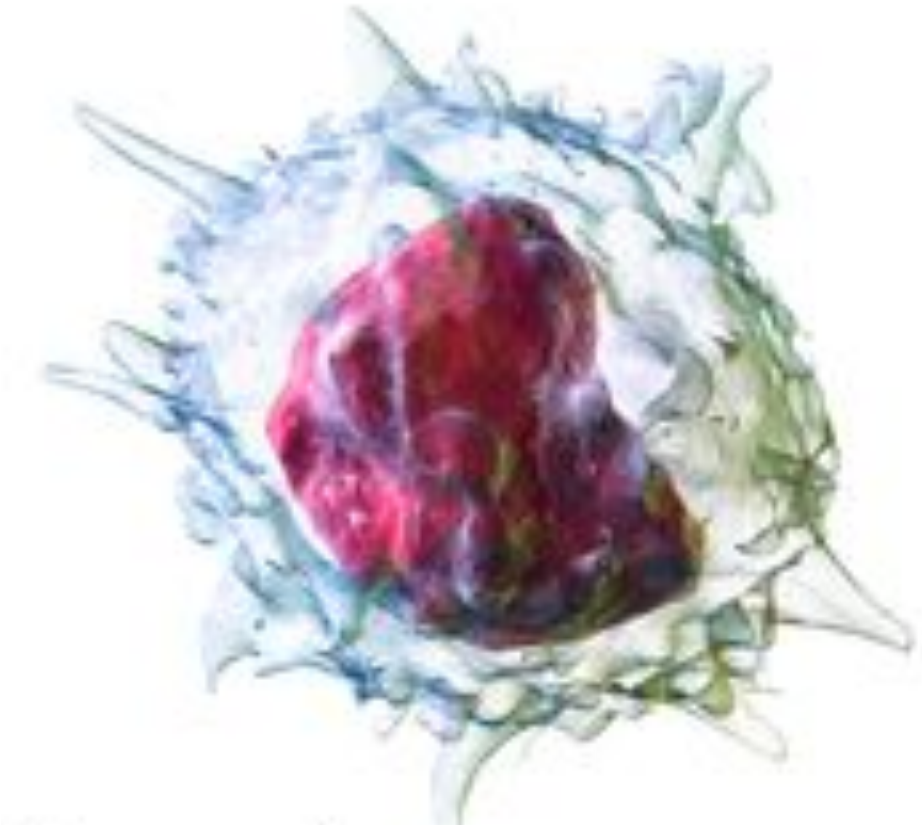
Zongmin Zhao, et al. Cell 2020 181,:1, 151-167


Good artists copy, great artists steal.
- Pablo Picasso
Quote Source: InfoWorld, Vol. 11, Nr. 1-9 (1989).
Photo: 1908, (C) RMN-Grand Palais, http://www.photo.rmn.fr/archive/90-008868-2C6NU0X1XDIK.html
QuotesCosmos

….LET’S STEAL FROM NATURE

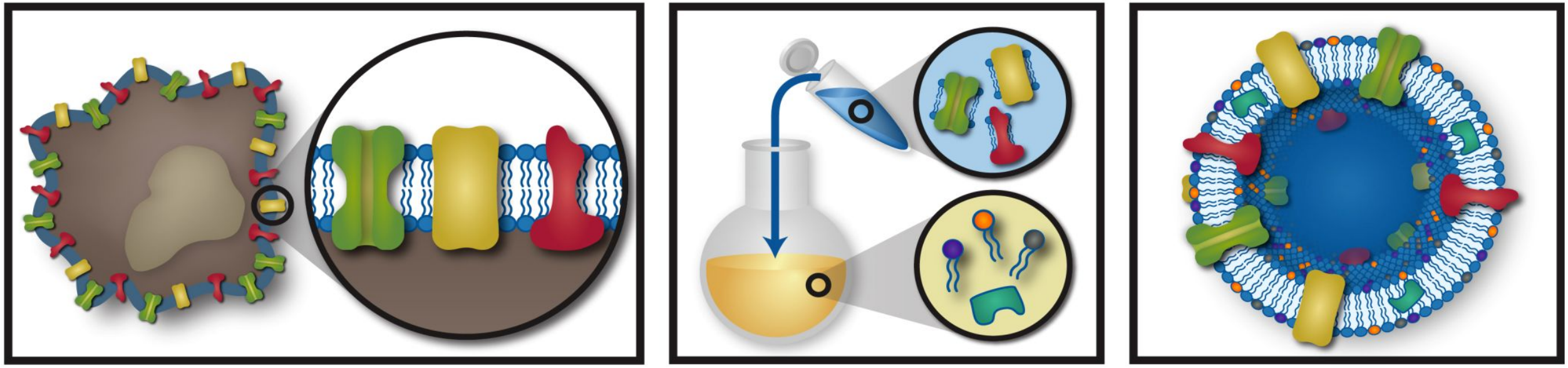


HIDING UNDER A MASK



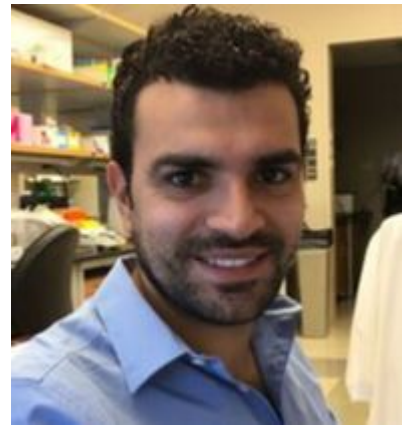
LEUKOSOMES

LEUKOSOMES ASSEMBLY: FROM A CELL TO A TARGETING NANOPARTICLE

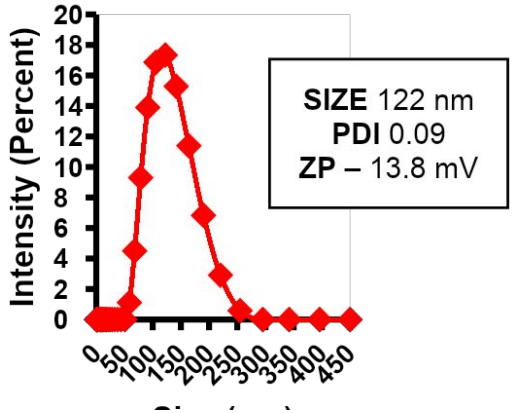


Roberto Molinaro, et al. Nature Materials 2016

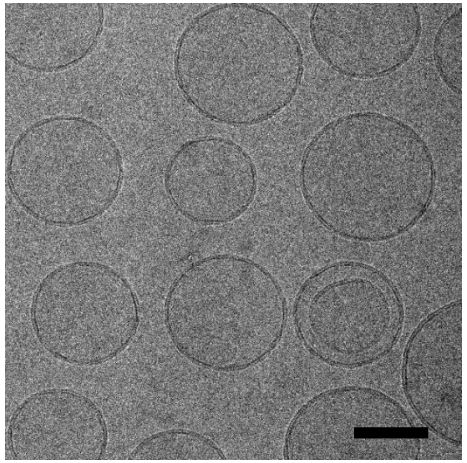
LEUKOSOMES



DLS



cryoEM analysis



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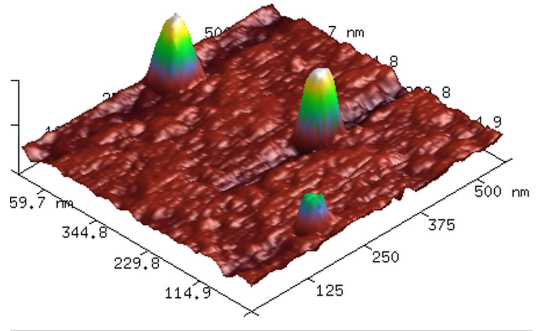

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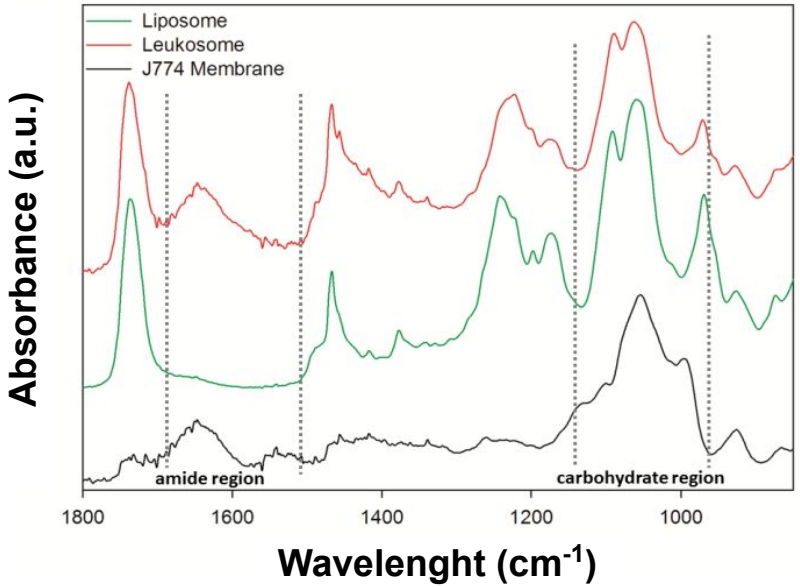

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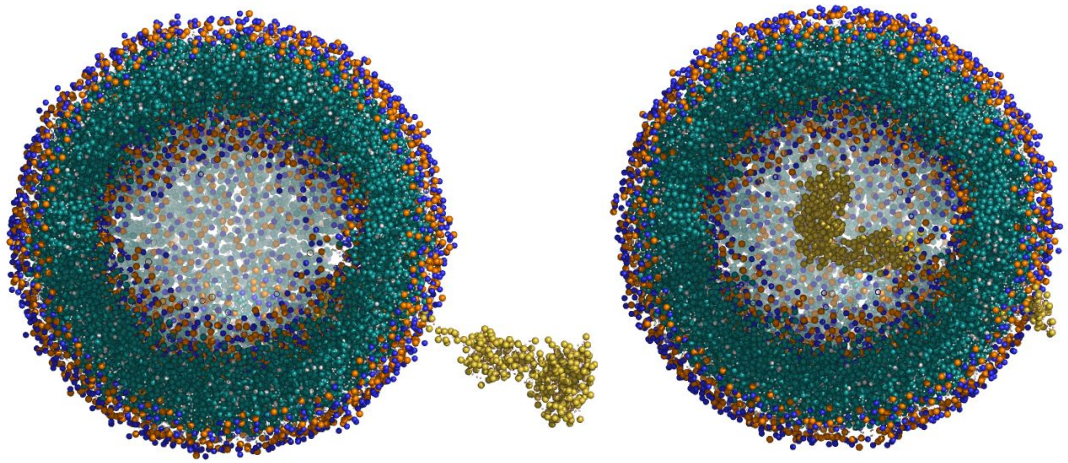
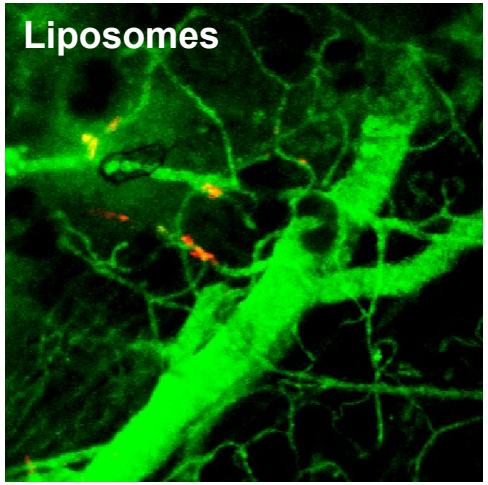
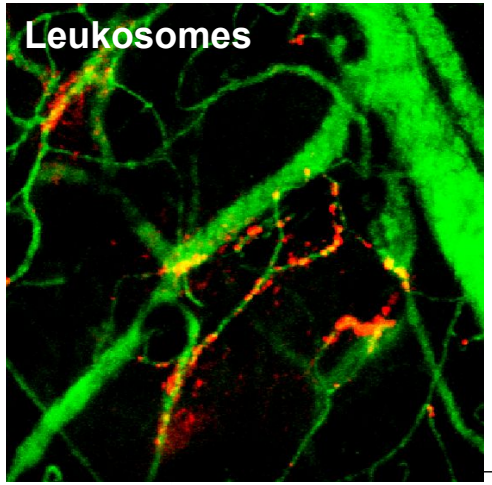
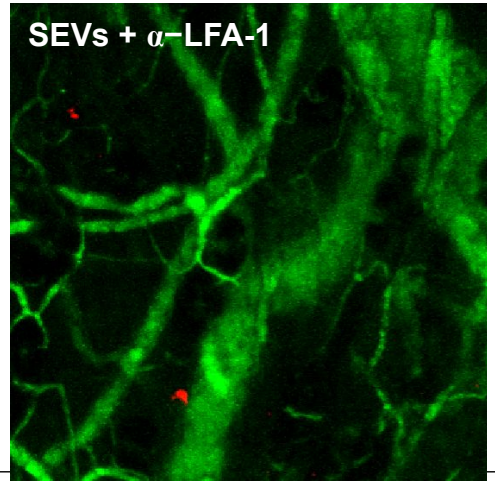


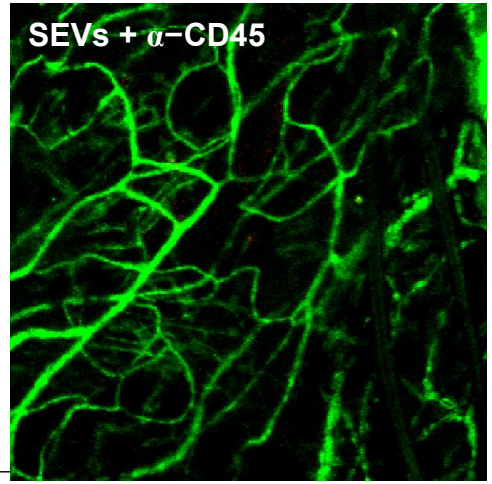
Roberto Molinaro, et al. Nature Materials 2016
Roberto Molinaro, et al. Advanced Materials 2018

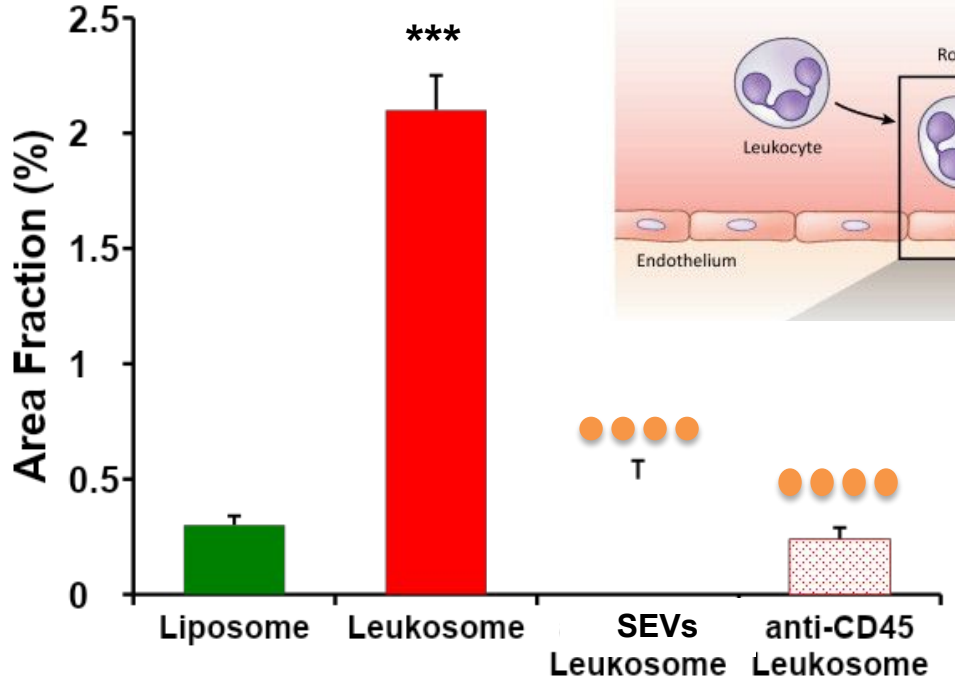
TARGETING MECHANISMS

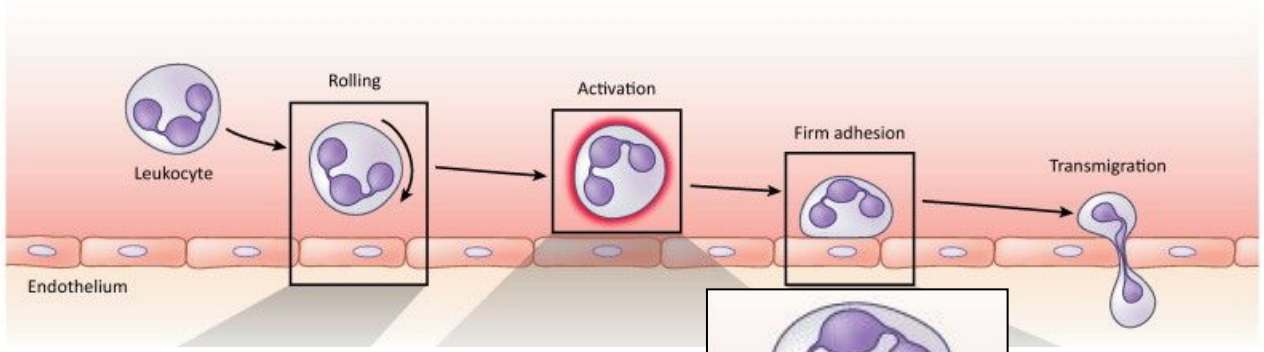


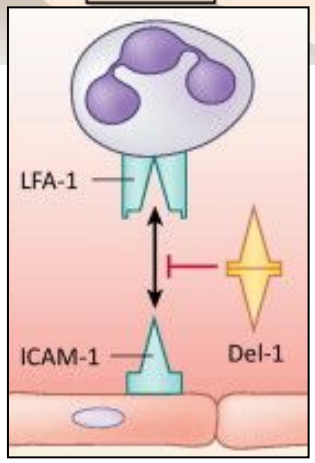


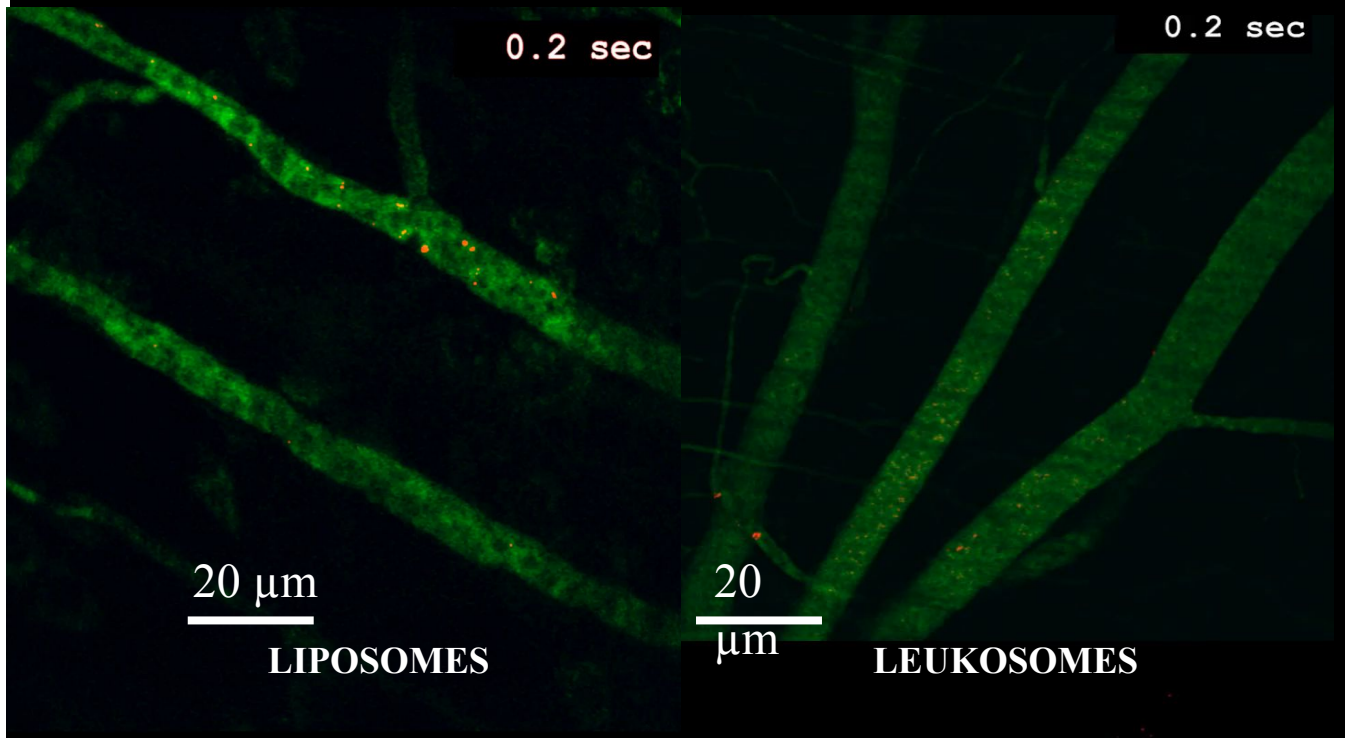








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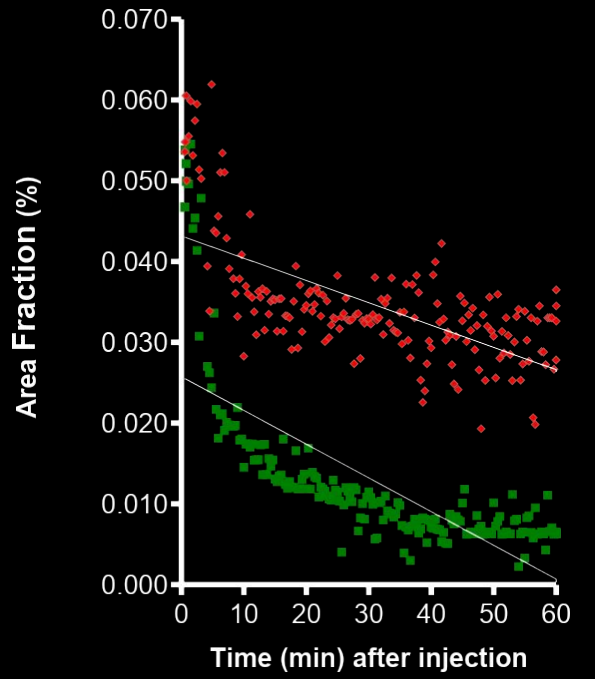
20 µm
LIPOSOMES
0.2 sec
20
µm
LEUKOSOMES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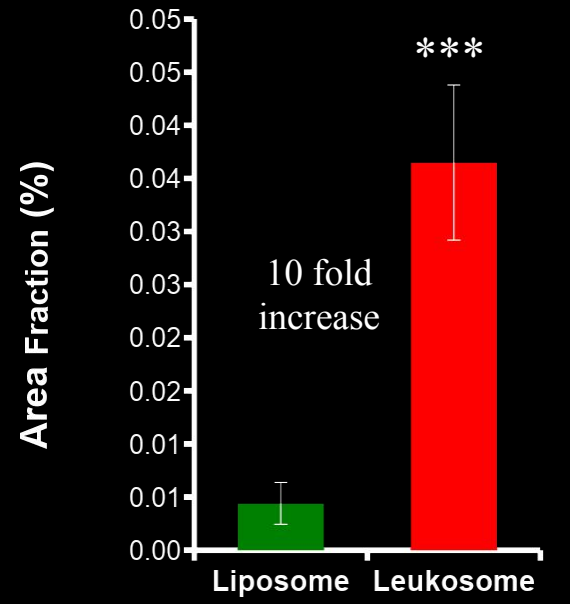
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
0
10
20
30
40
50
60
Time (min) after injection

Blood vessels
Liposomes
Blood vessels
Leukosomes


Area Fraction (%)
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0.04
0.03
0.03
0.02
0.02
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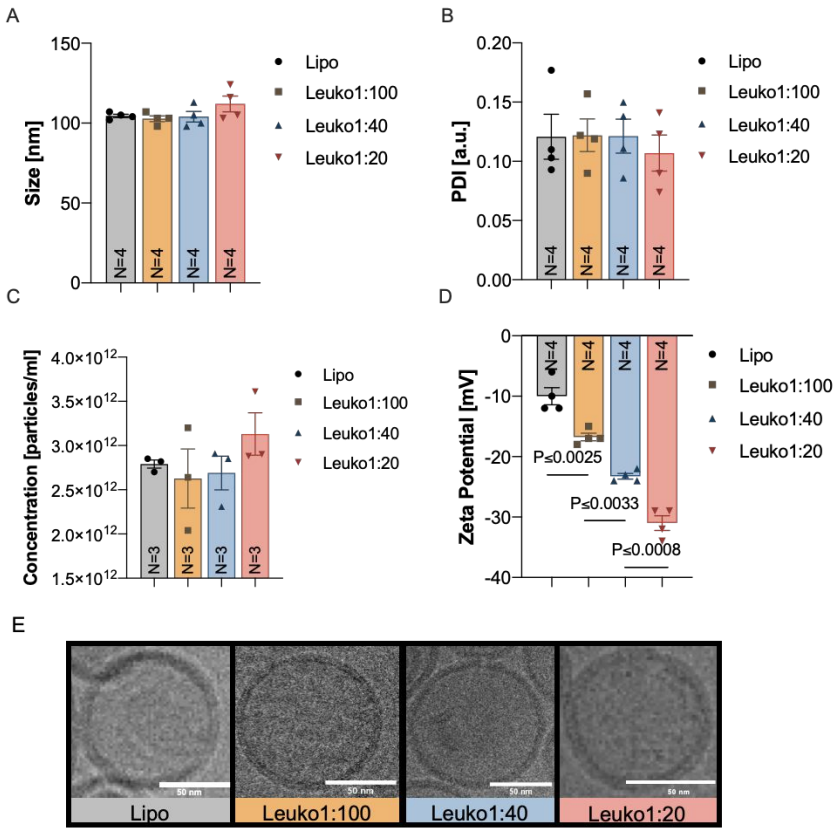
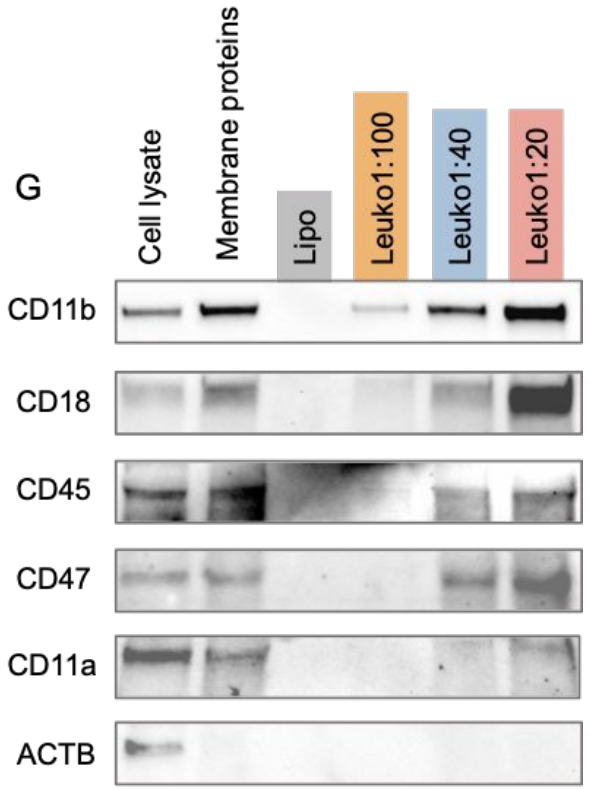
0.01
0.00
***
10 fold
increase
Liposome
Leukosome

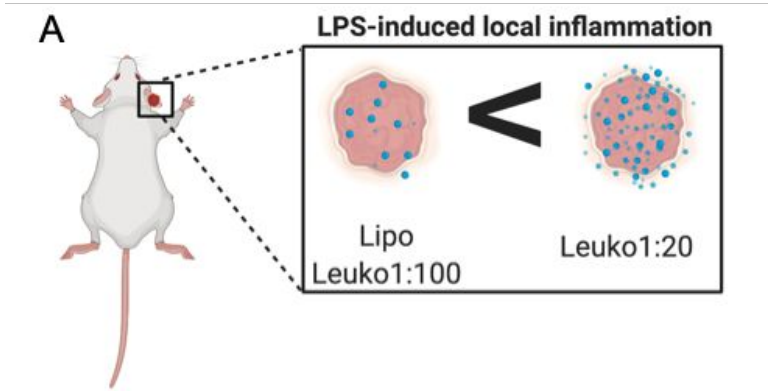
The **physiochemical** effect of lipids to proteins ratio on leukosomes

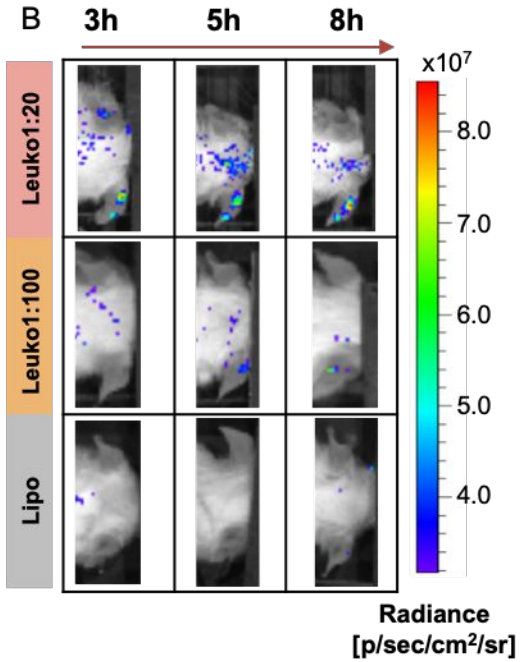


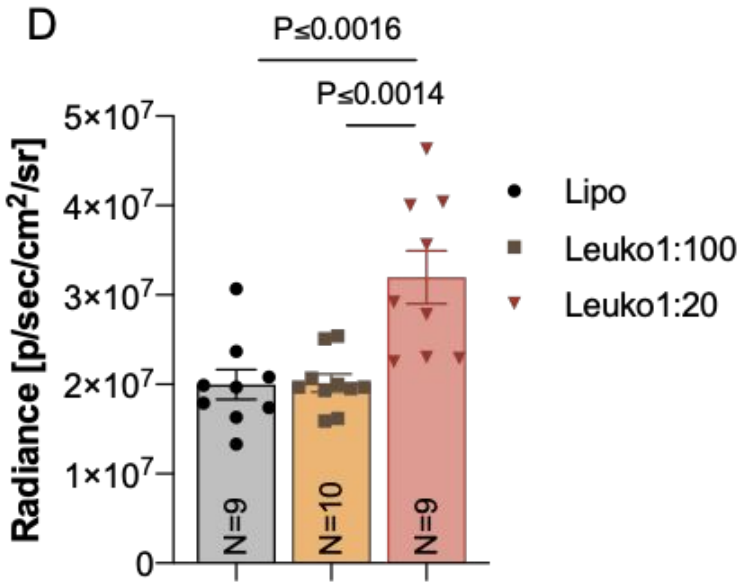


A. Zinger and M. Sushnitha et al. ACS NANO 2021

In vivo targeting of NP in **LPS induced local inflammation**







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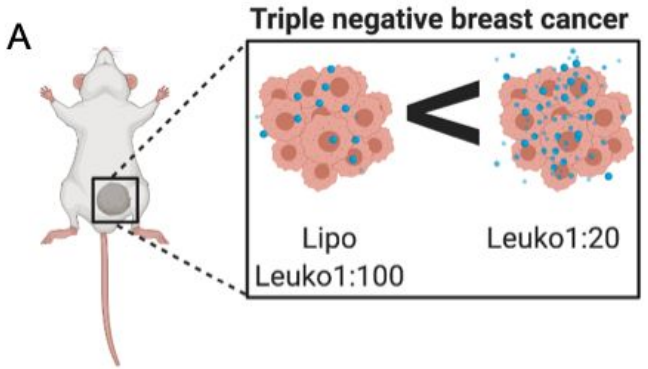
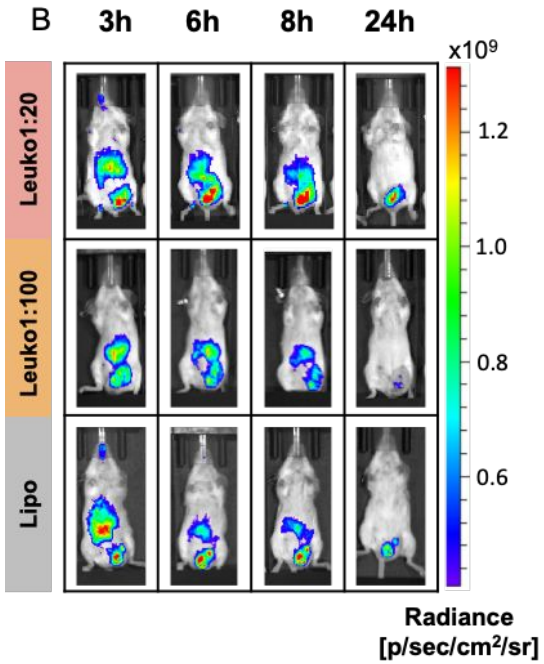
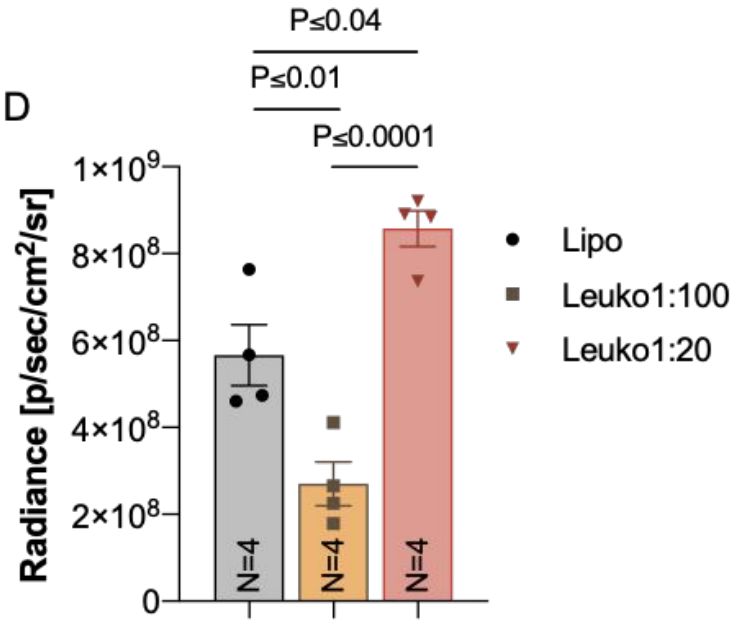
In vivo targeting of NP in **triple negative breast cancer**







A. Zinger and M. Sushnitha et al. ACS NANO 2021

Cancer

Pulmonary diseases

Cardiovascular diseases

Neurological diseases

INFLAMMATION

Alzheimer

Autoimmune diseases

Arthritis

Diabetes II

- Osteosarcoma (OS) arises from primitive mesenchymal bone forming cells.
- It is the **second highest cause of cancer-related death**.
- The **5-years survival** rate is still less than 70%.
- **Absence** of specific molecular targets.

F. Giordano, S. Lenna et al. submitted

ACS APPLIED BIO MATERIALS

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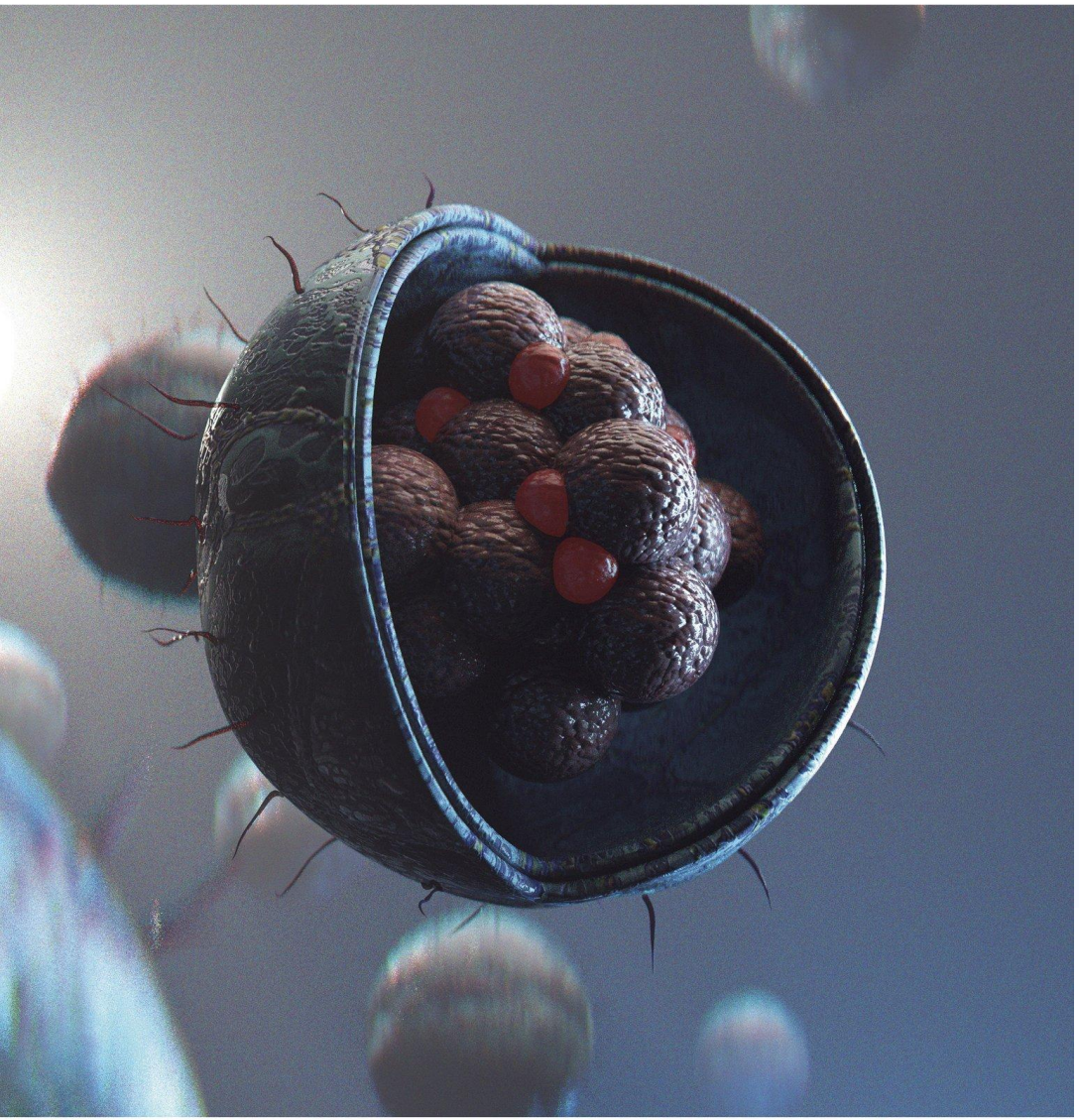

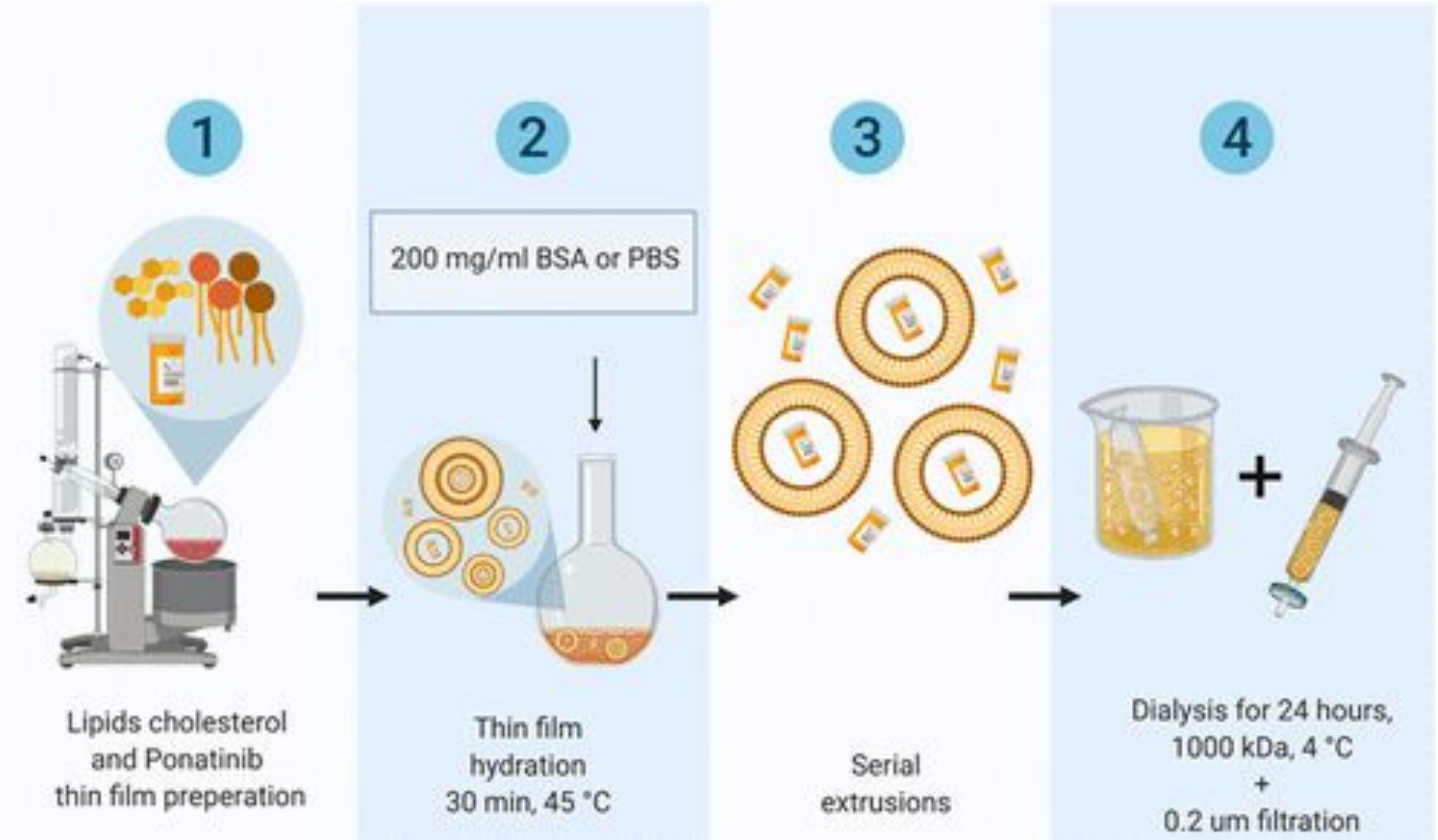

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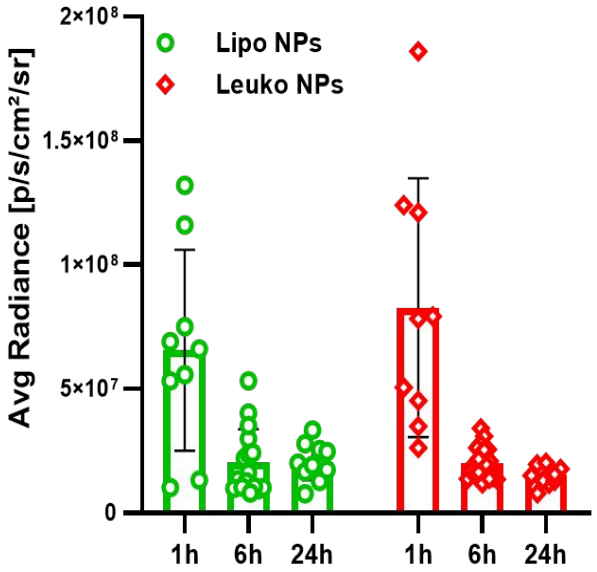
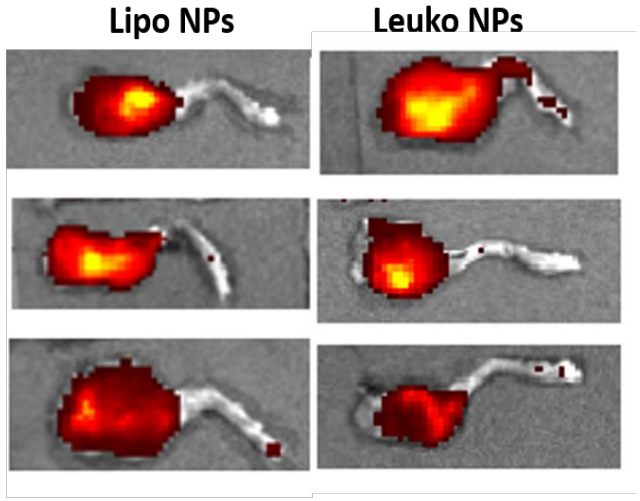
A. Zinger et al. 2020, ACS AppL Bio Mater

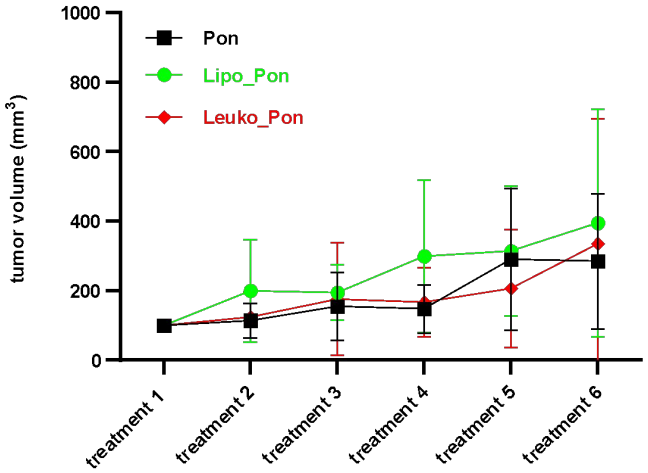
Primary Tumor



Left

Right + Tumor



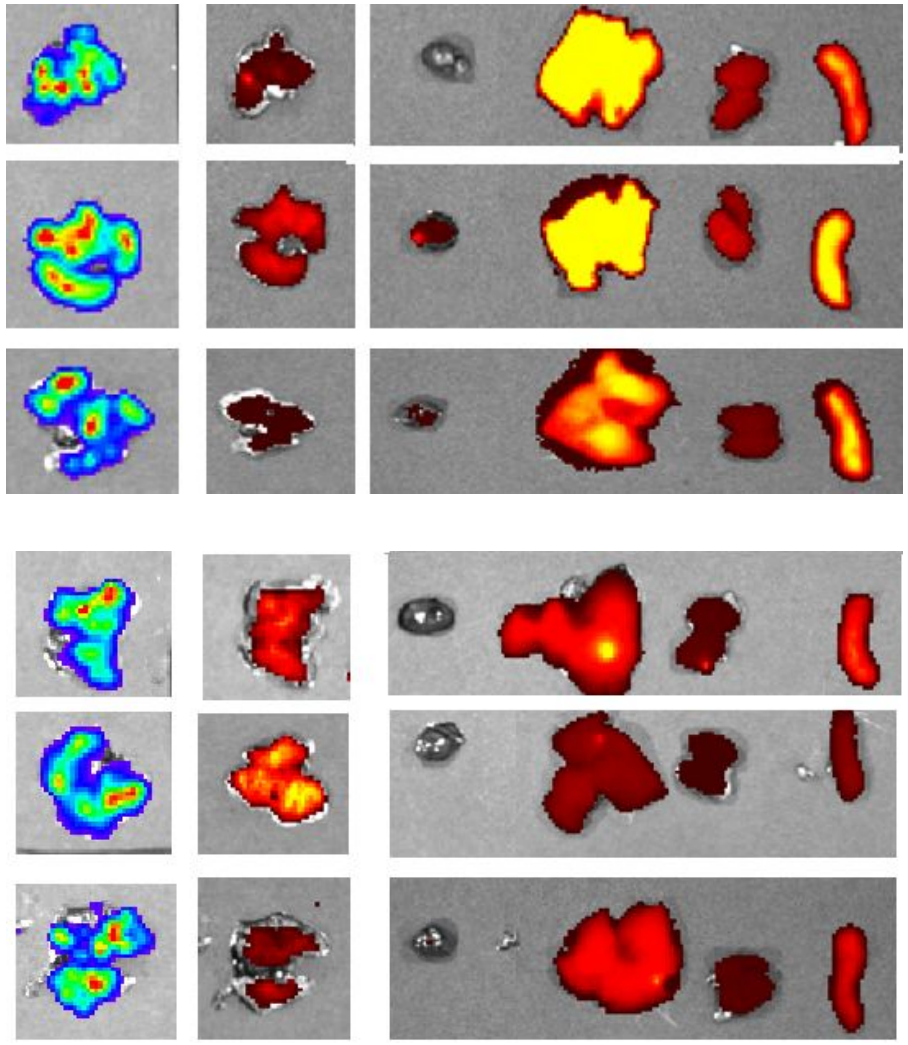


Lungs Mets

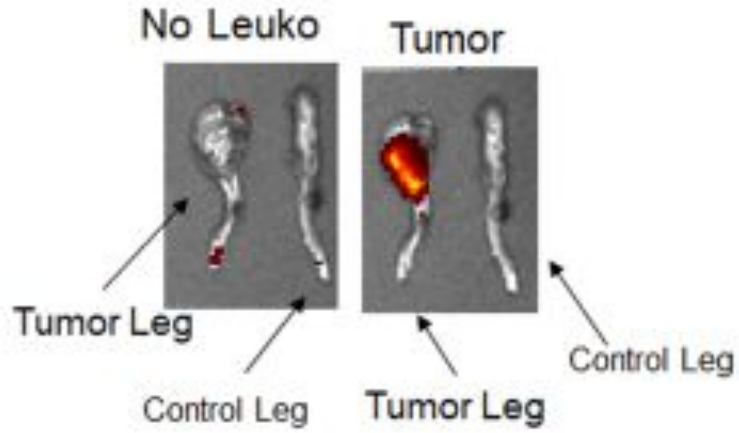
Lungs

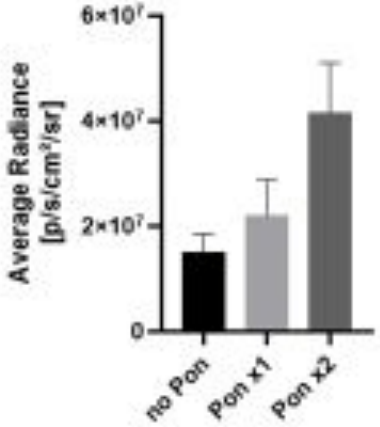
Lipo 3h

Leuko 3h



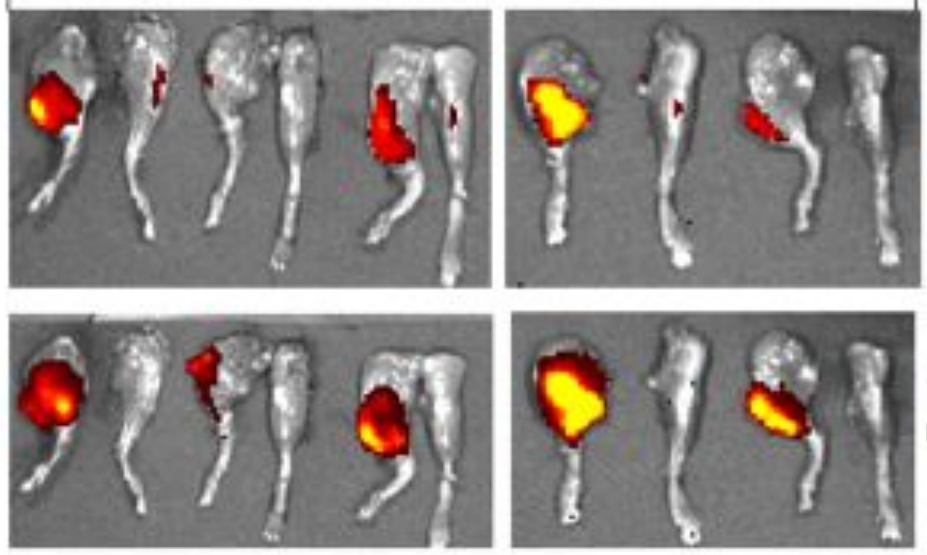
F. Giordano et al. submitted





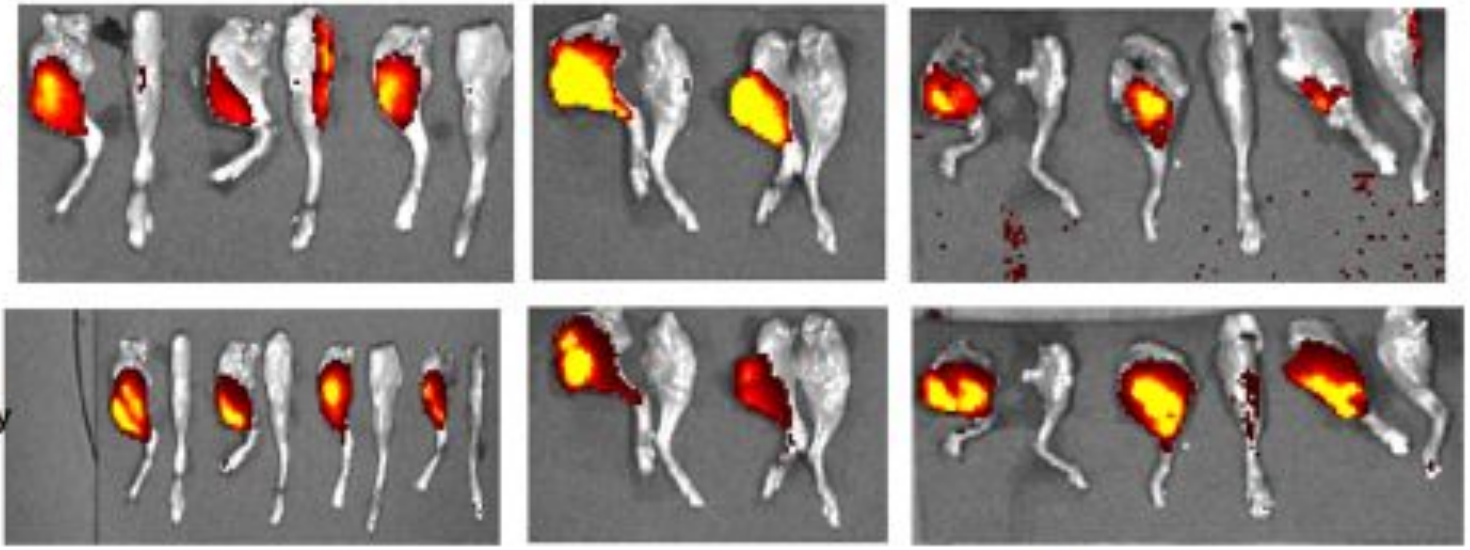
Control group

Ponatinib group



Leuko DiD NP 1h

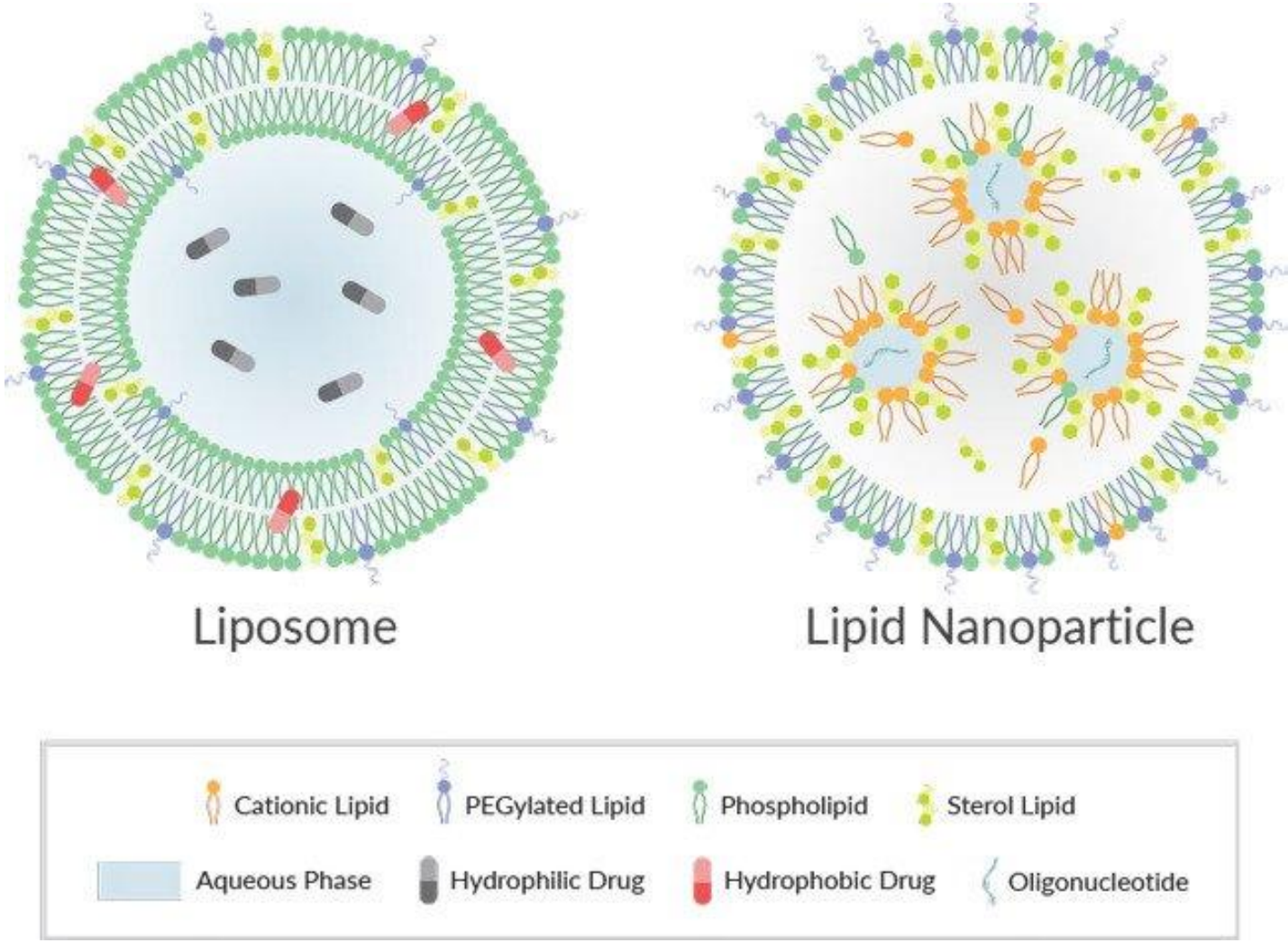
Tumor mCherry



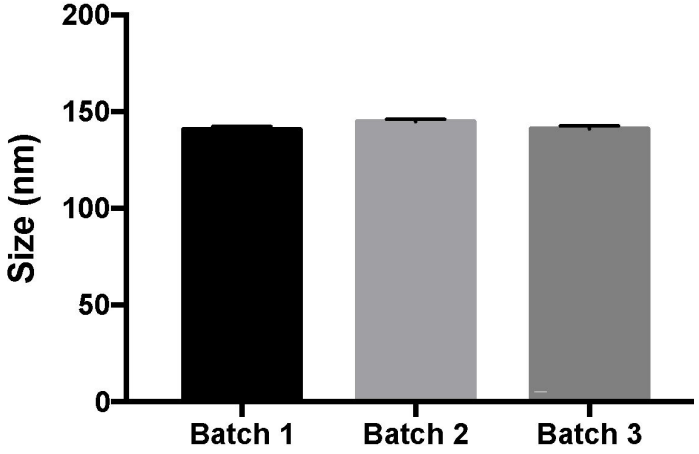
Mice analyzed by IVM

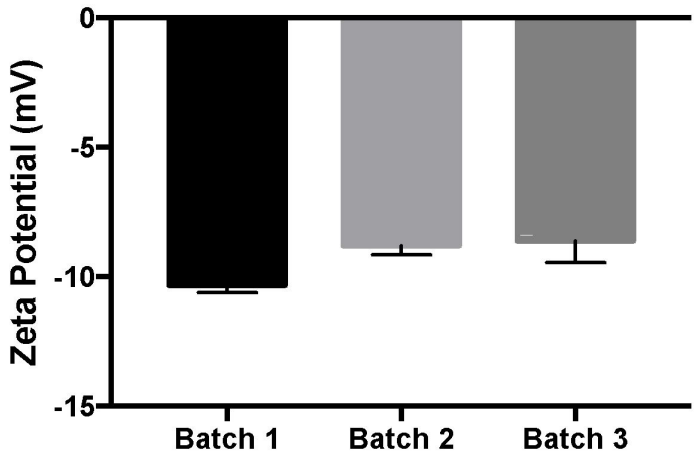
Mice analyzed By IVM

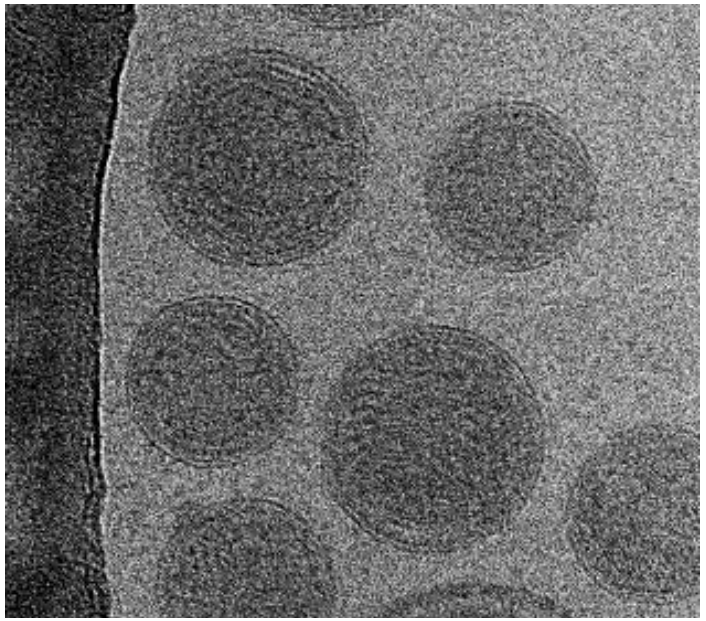
Leukosome to Leuko-LNP: is this even possible?



Physicochemical Properties

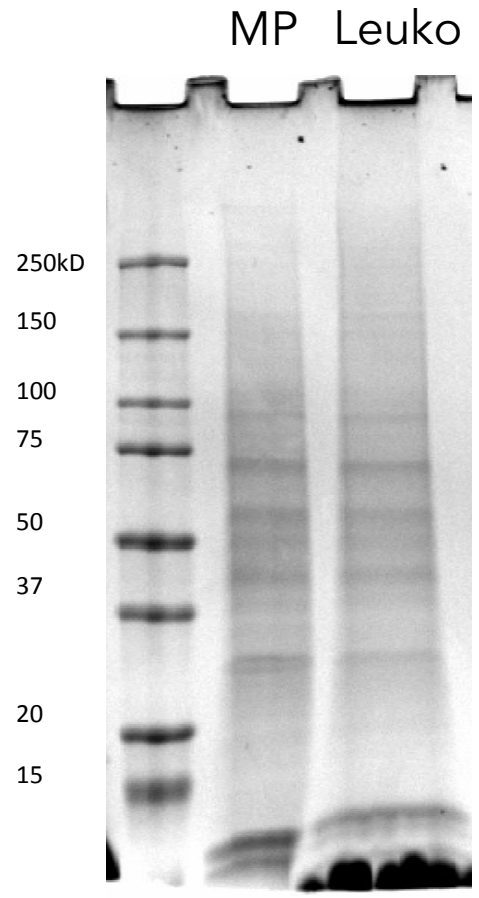






	siRNA amount loaded	Encapsulation Efficiency
Batch 1	74.2 ug/mL	49.3%
Batch 2	80.8 ug/mL	53.9%
Batch 3	86.1 ug/mL	57.4%

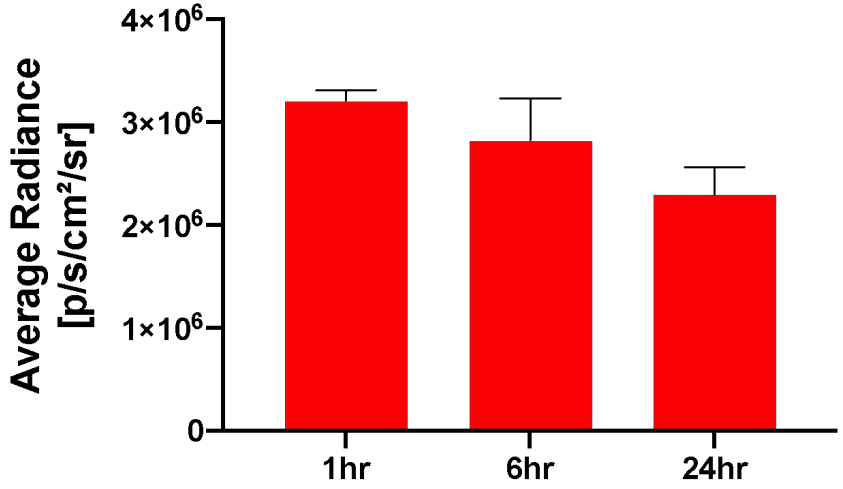
Proteins on particles



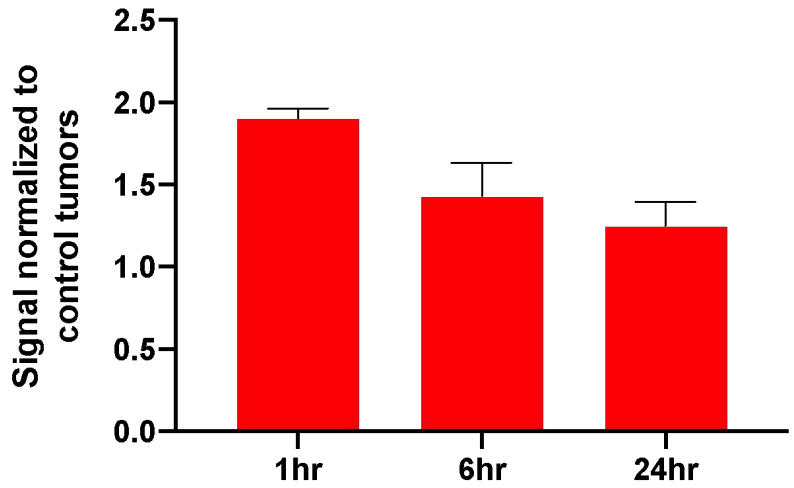
MP = extracted membrane protein
Leuko = Leukosomes

Leuko-siRNA: In vivo targeting - TNBC

Leukosomes in 4T1 tumors
Raw signal value



Leukosomes in 4T1 tumors
Raw signal value /control signal



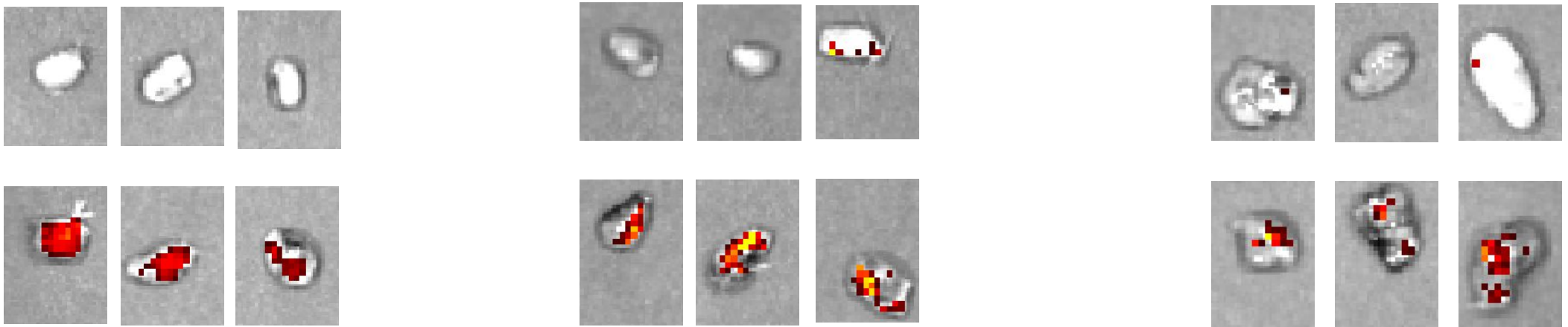
1 hour

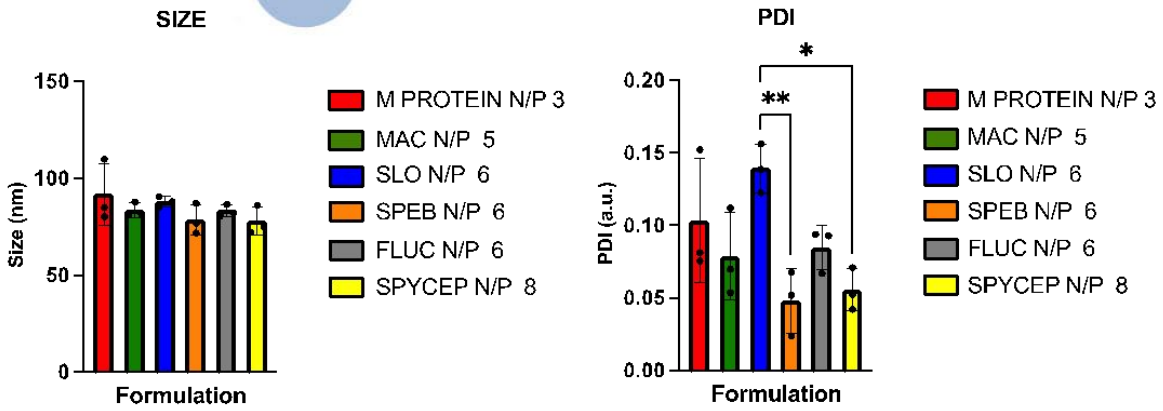
6 hours

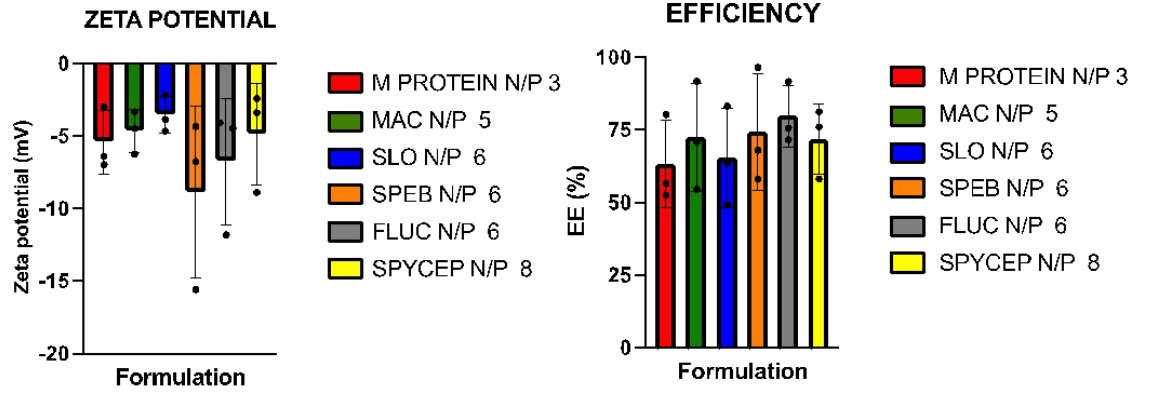
24 hours

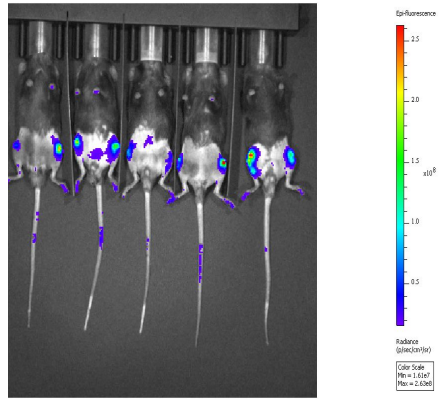
Control

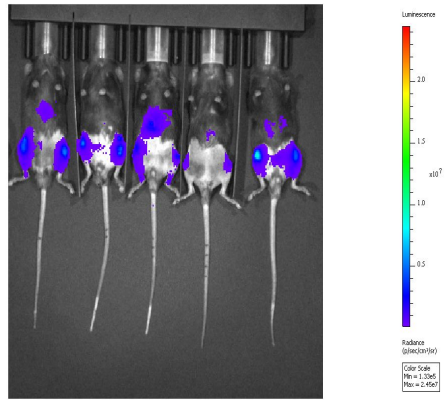
Leuko





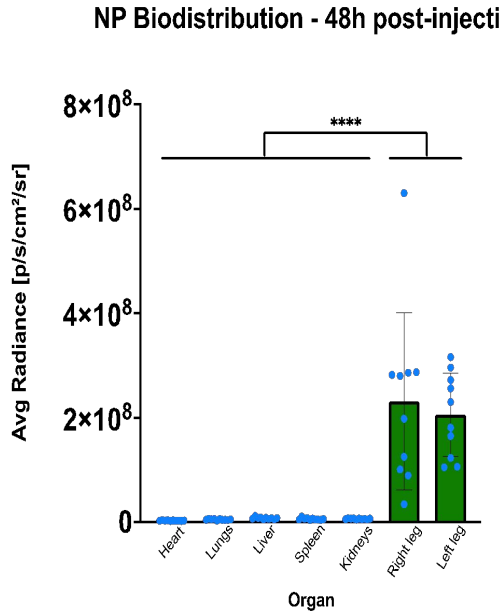


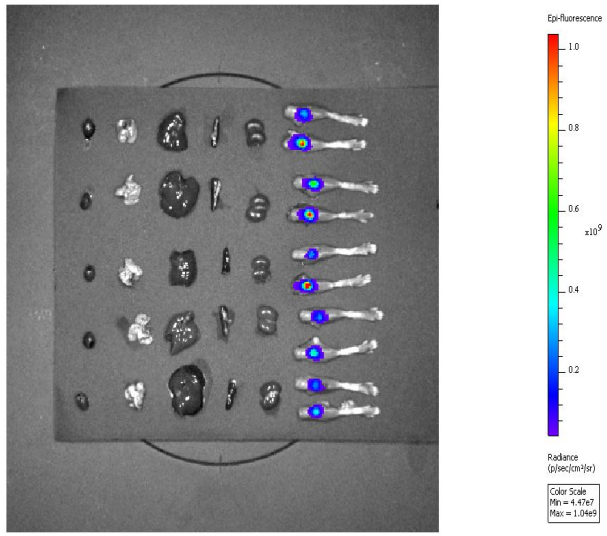




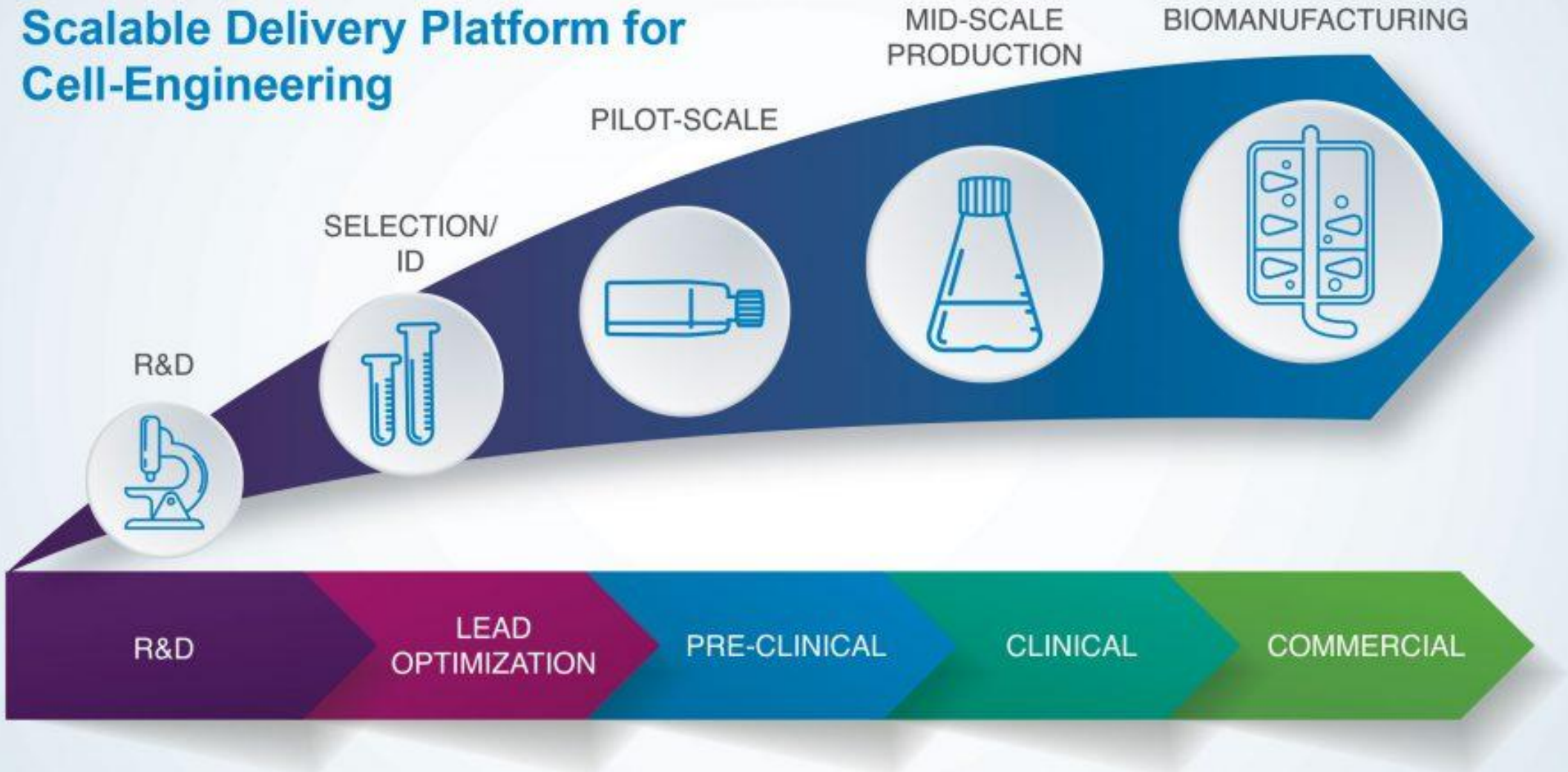
NPs Fluorescence

mRNA- Bioluminescence



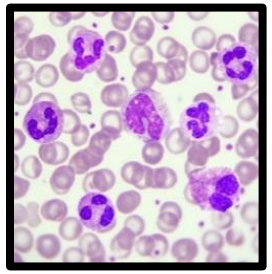


Scalable Delivery Platform for Cell-Engineering



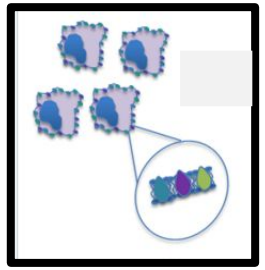
Challenges: SCALABILITY & TRANSLATION

A



Leukocytes cells

B

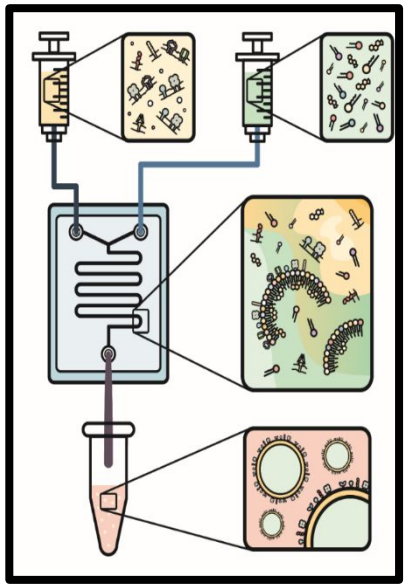


Membrane protein extraction

C

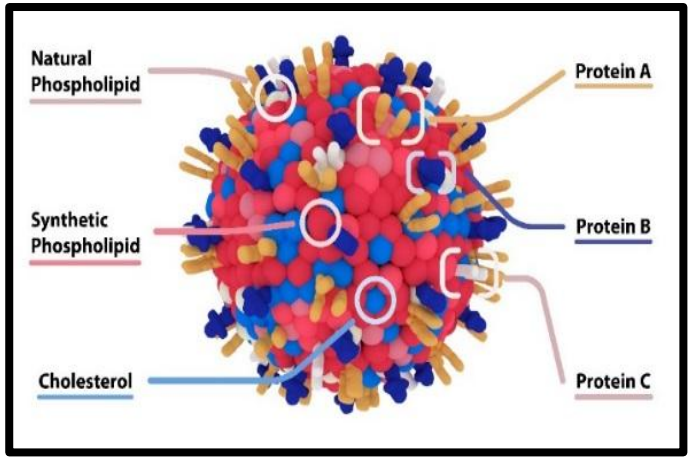
Cells Membrane proteins

Lipids formulation



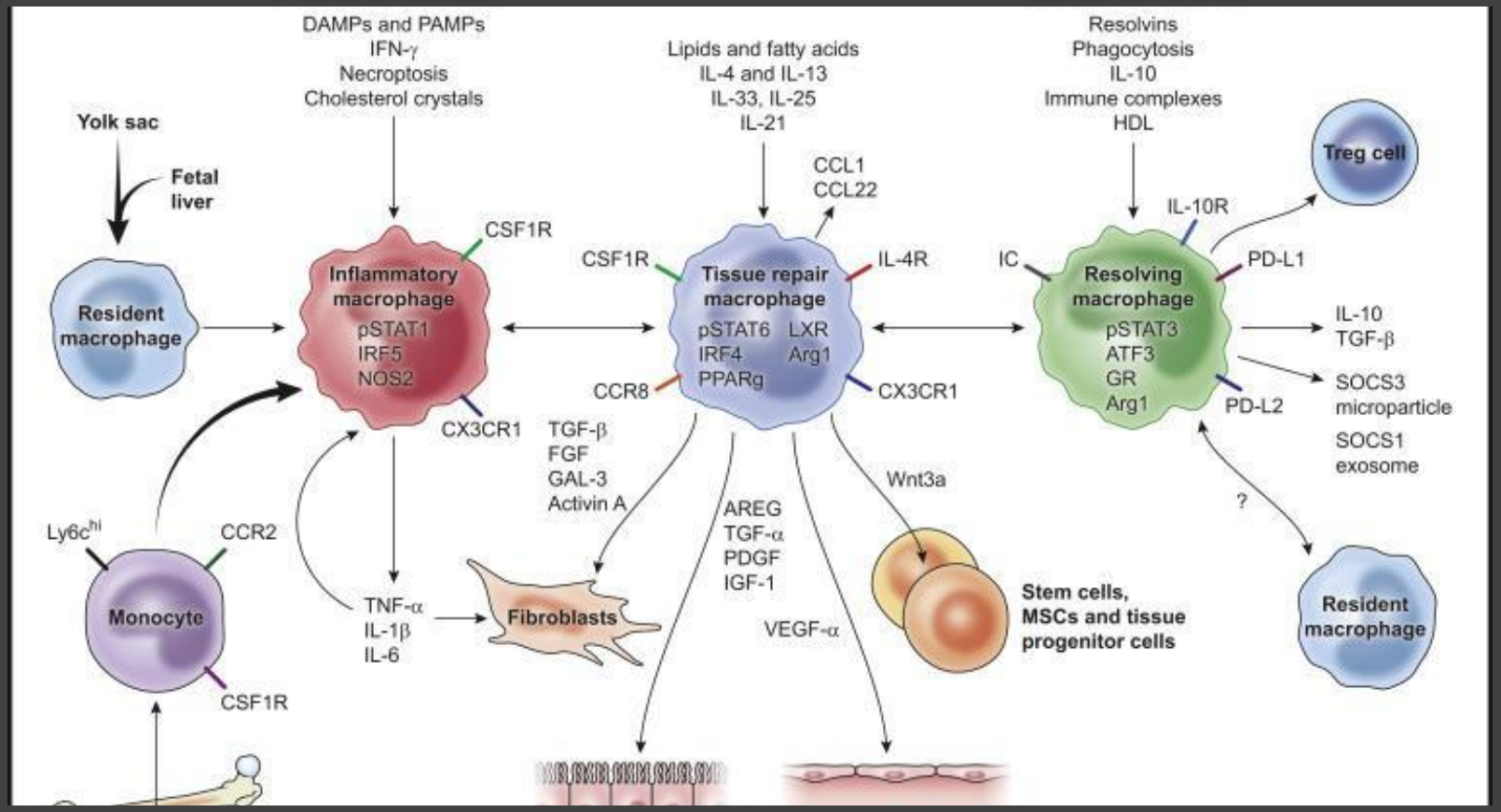
Synthesis

D



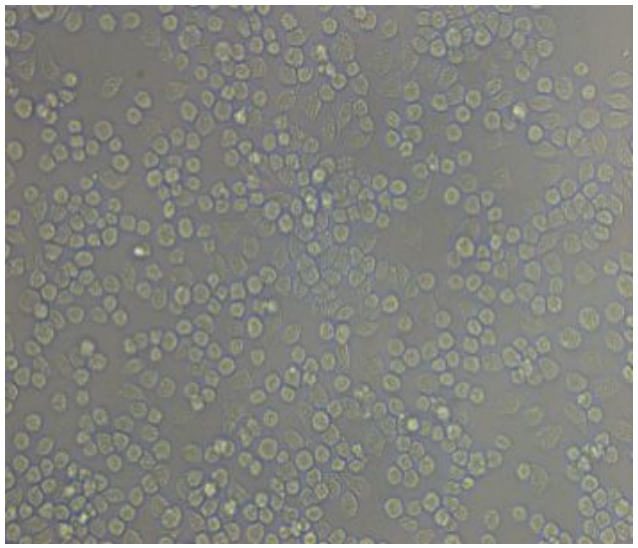
Providing medicine

Cells

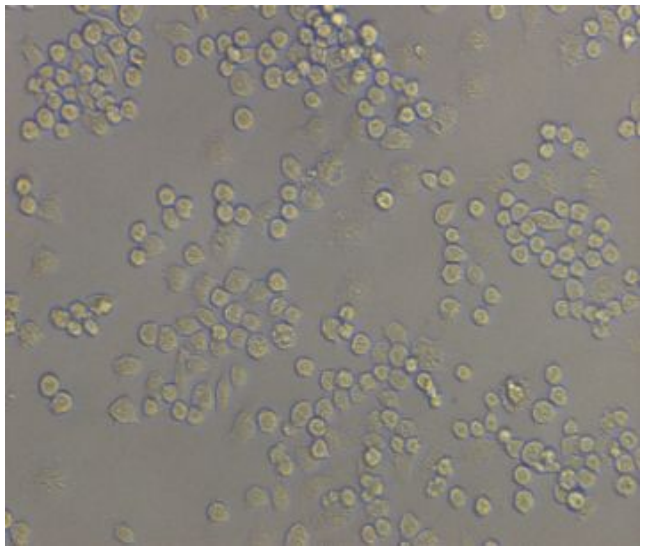

DAMPs and PAMPs
IFN-γ
Necroptosis
Cholesterol crystals
Lipids and fatty acids
IL-4 and IL-13
IL-33, IL-25
IL-21
Resolvins
Phagocytosis
IL-10
Immune complexes
HDL
Yolk sac
Fetal liver
CCL1
CCL22
Treg cell
IL-10R
CSF1R
CSF1R
IL-4R
IC
PD-L1
Resident macrophage
Inflammatory macrophage
pSTAT1
IRF5
NOS2
Tissue repair macrophage
pSTAT6
IRF4
PPARg
LXR
Arg1
Resolving macrophage
pSTAT3
ATF3
GR
Arg1
IL-10
TGF-β
SOCS3
microparticle
SOCS1
exosome
CX3CR1
CCR8
CX3CR1
PD-L2
TGF-β
FGF
GAL-3
Activin A
Wnt3a
?
Ly6c^hi
CCR2
AREG
TGF-α
PDGF
IGF-1
Monocyte
TNF-α
IL-1β
IL-6
Fibroblasts
VEGF-α
Stem cells,
MSCs and tissue
progenitor cells
Resident macrophage
CSF1R

WHEATON
RP

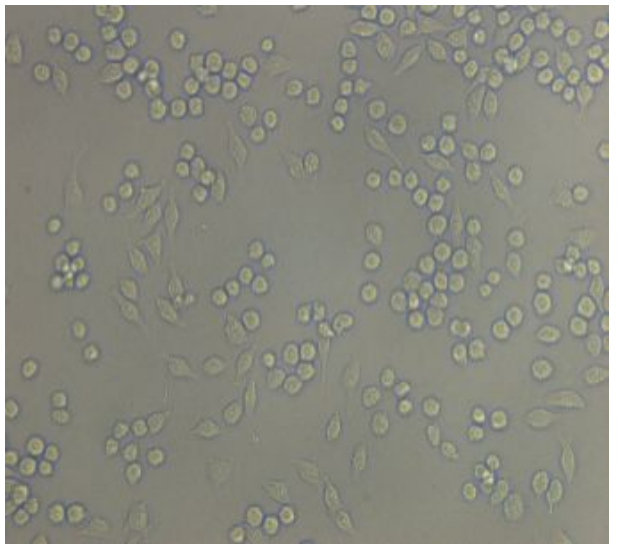
NT

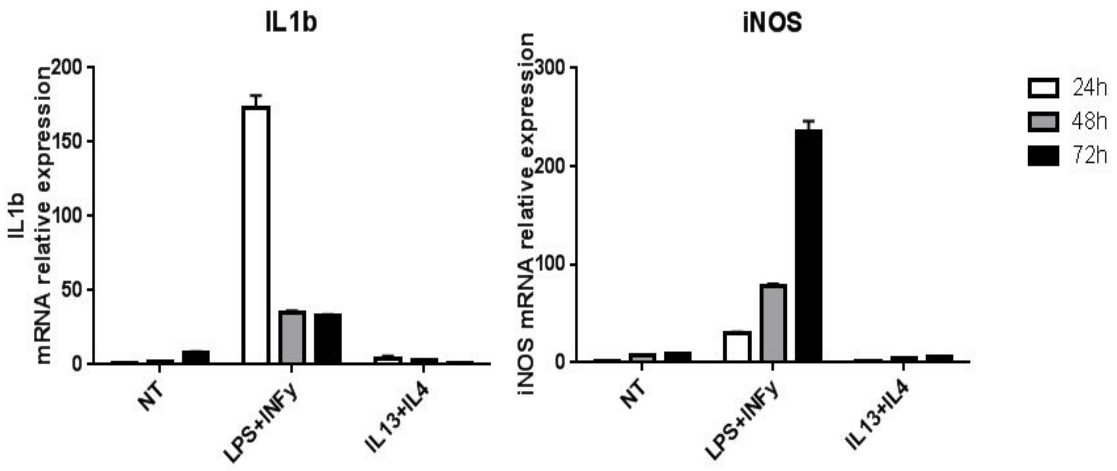


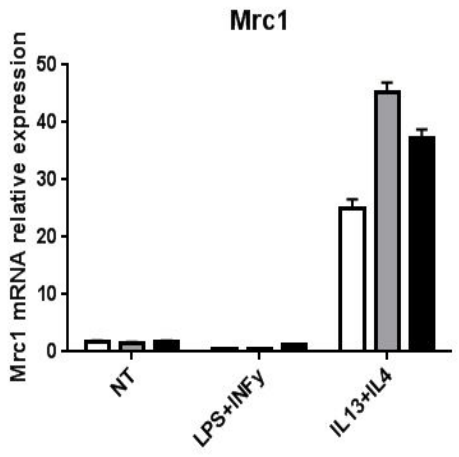
LPS+INFy

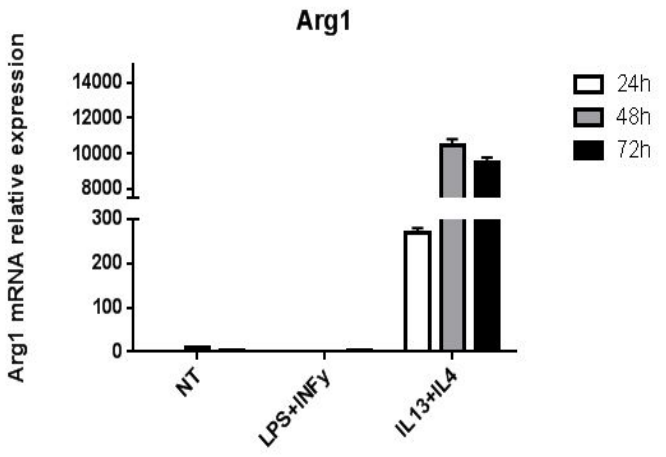


IL13+IL4









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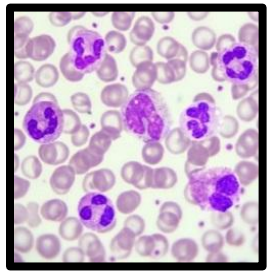
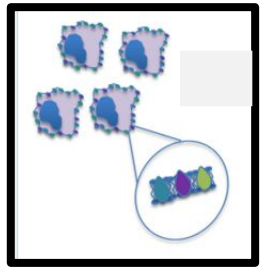


Leukocytes cells

B

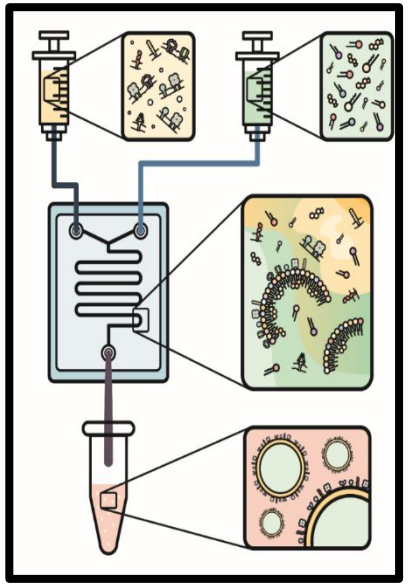


Membrane protein extraction

C

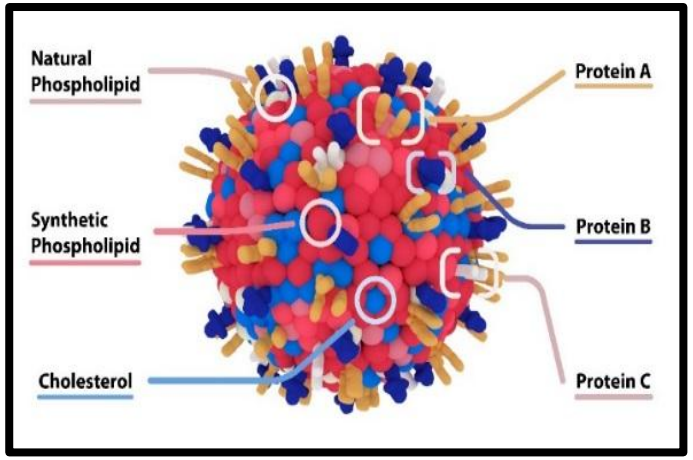
Cells Membrane proteins

Lipids formulation



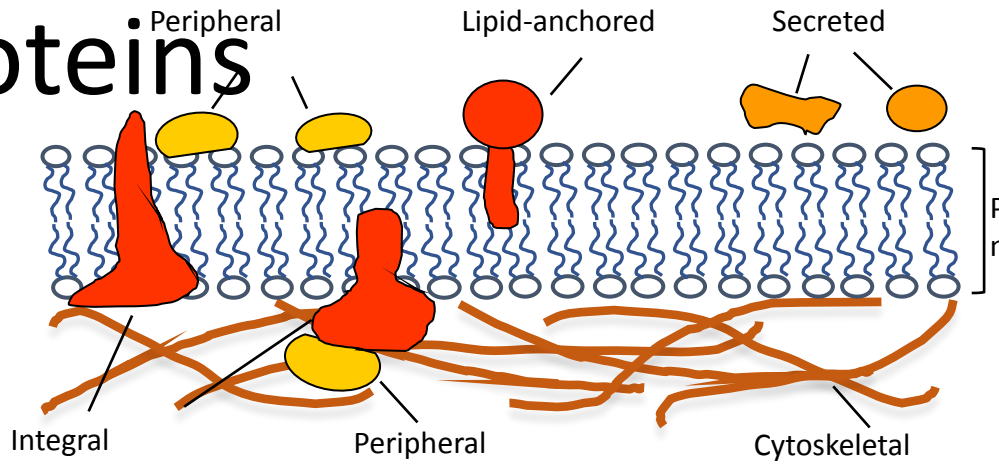
Synthesis

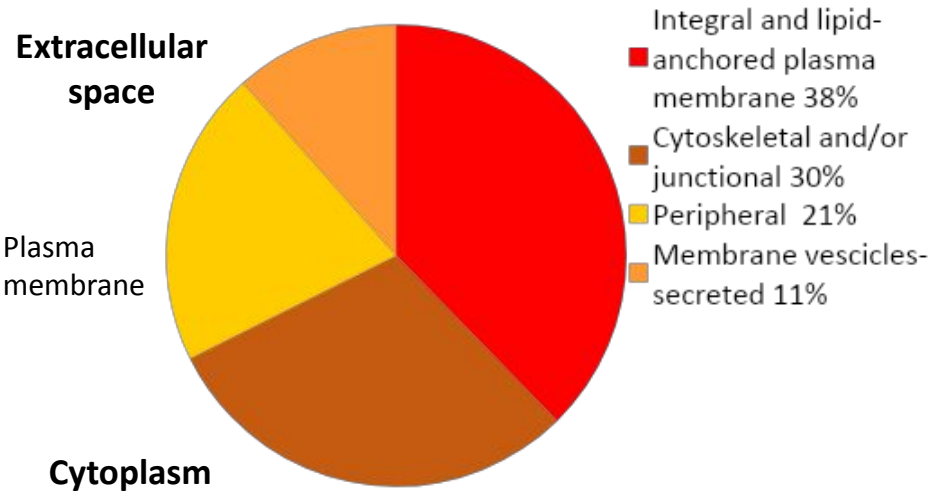
D

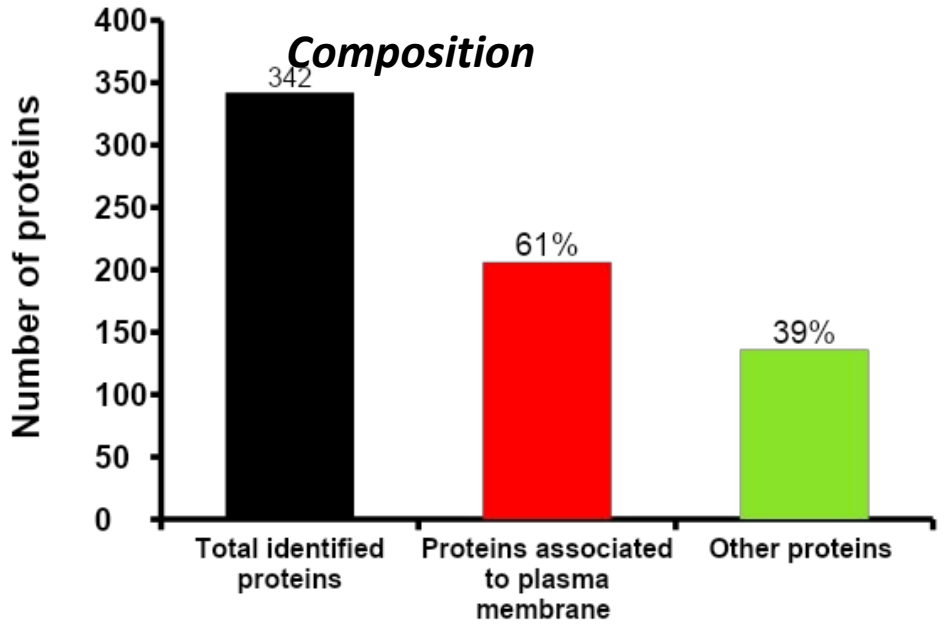


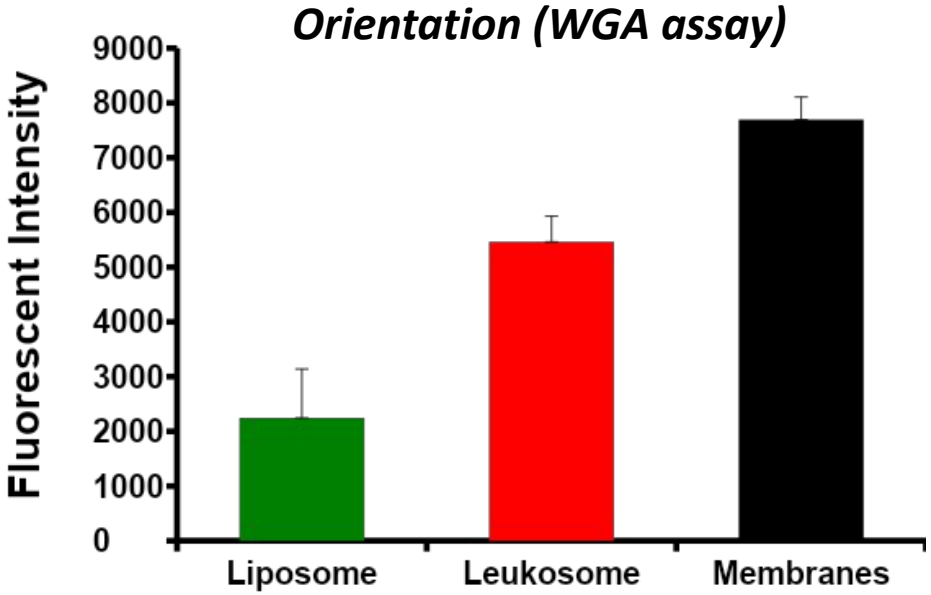
Providing medicine

Membrane Proteins



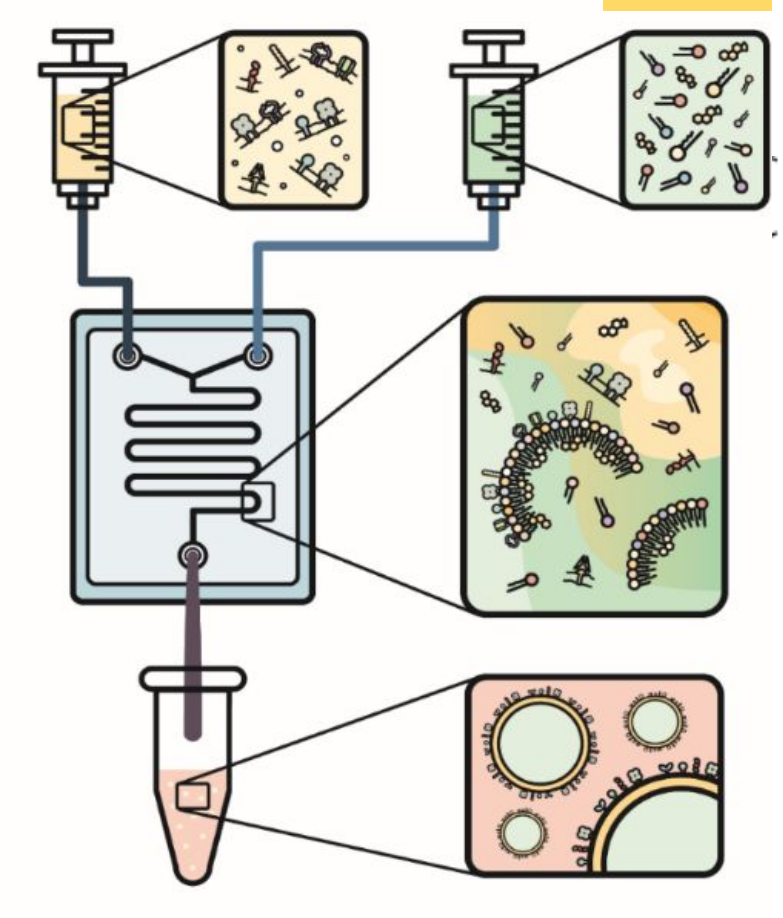




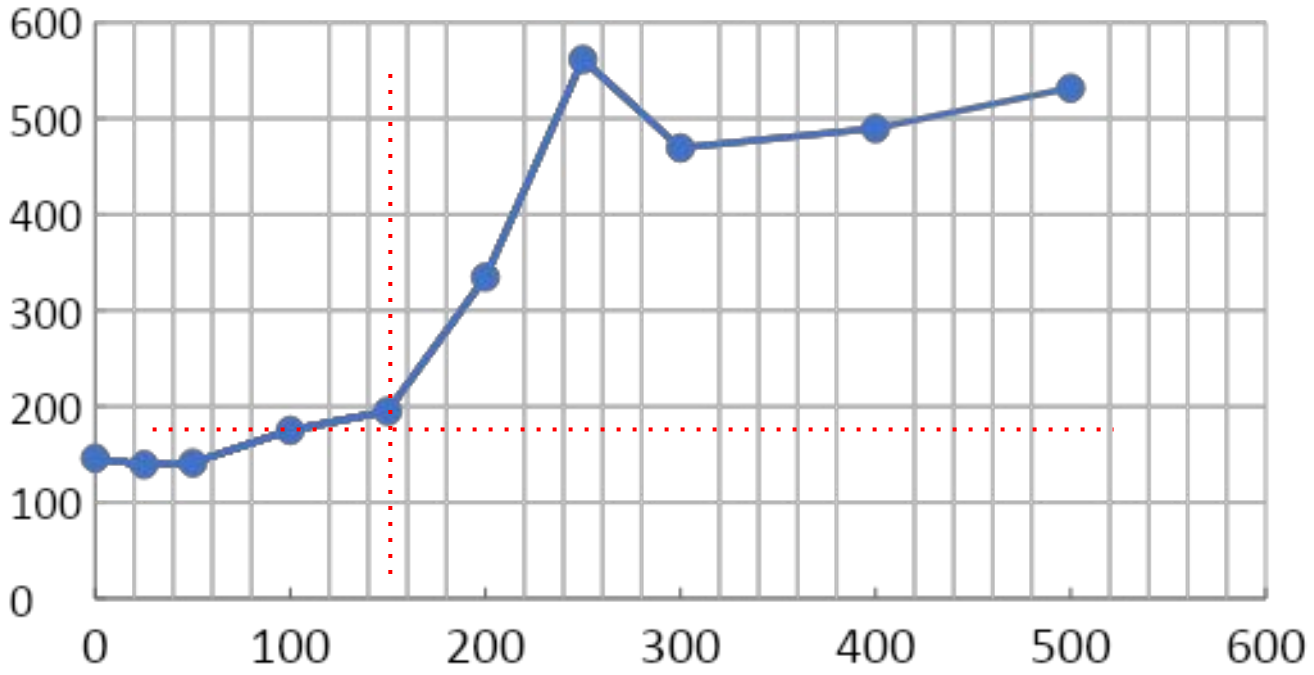


Roberto Molinaro, et al. Nature Materials 2016

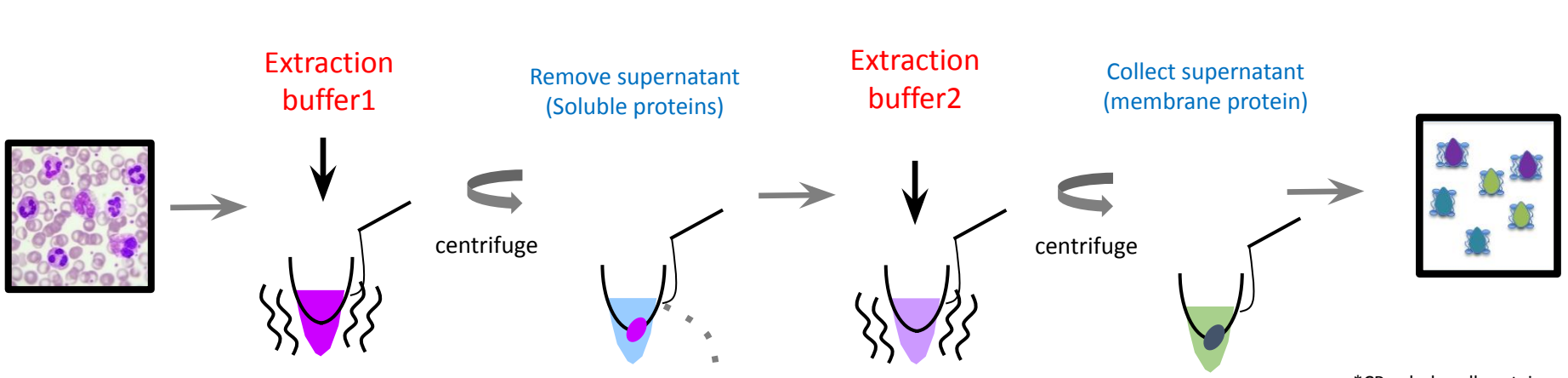
Water with Extraction Buffer 2

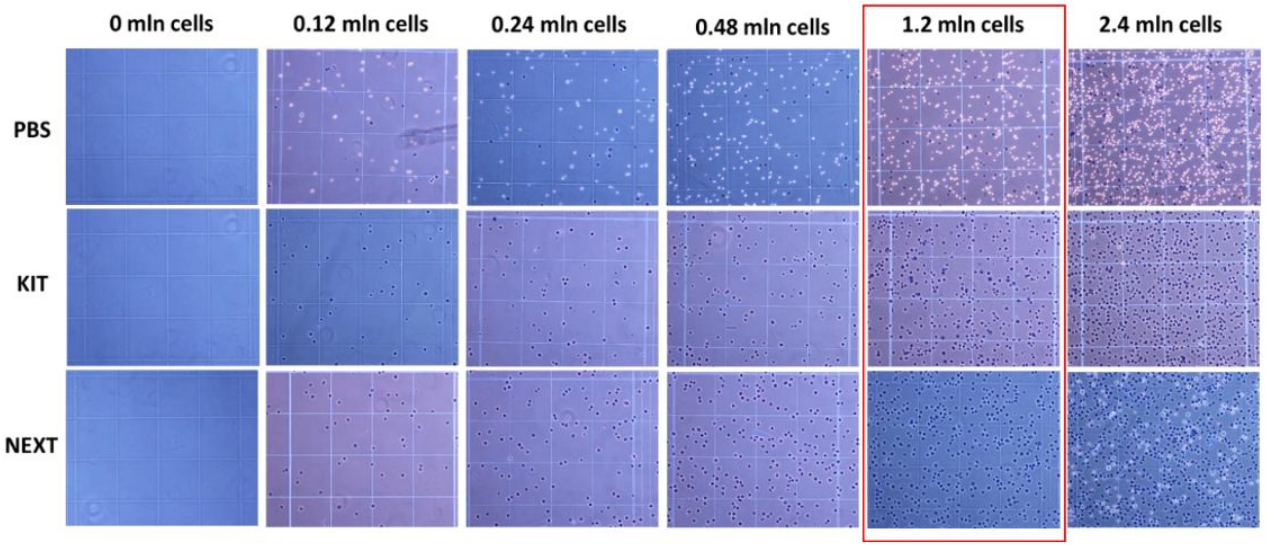


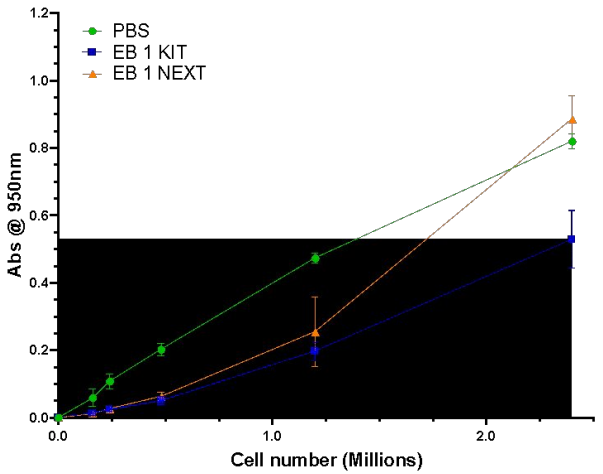
Liposome (with Extraction buffer2)

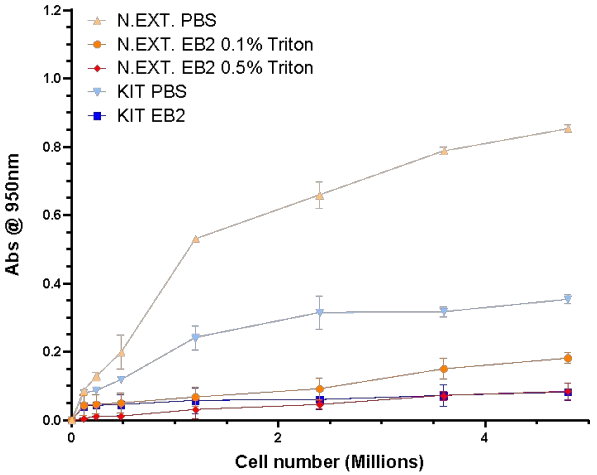


Extraction buffer2 volume in 1mL of formulation
(XX uL/Total 667uL)

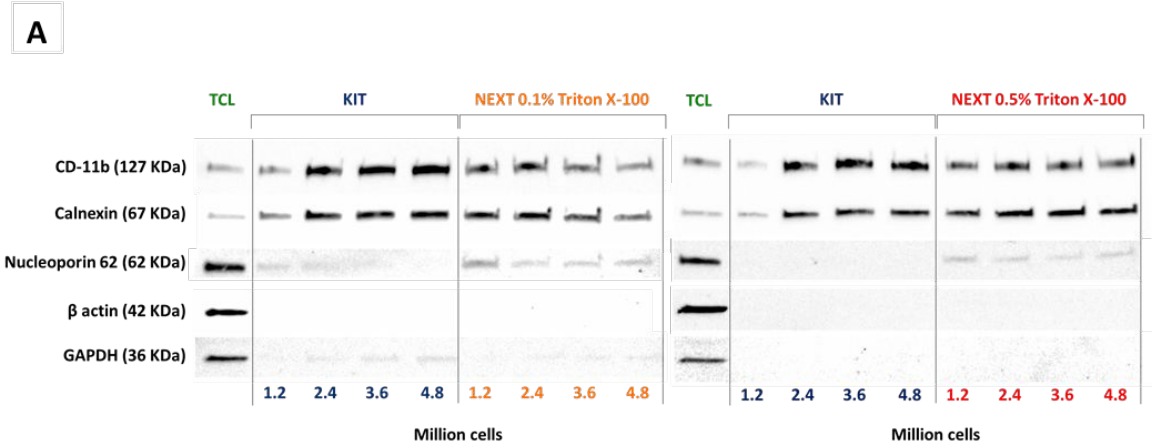


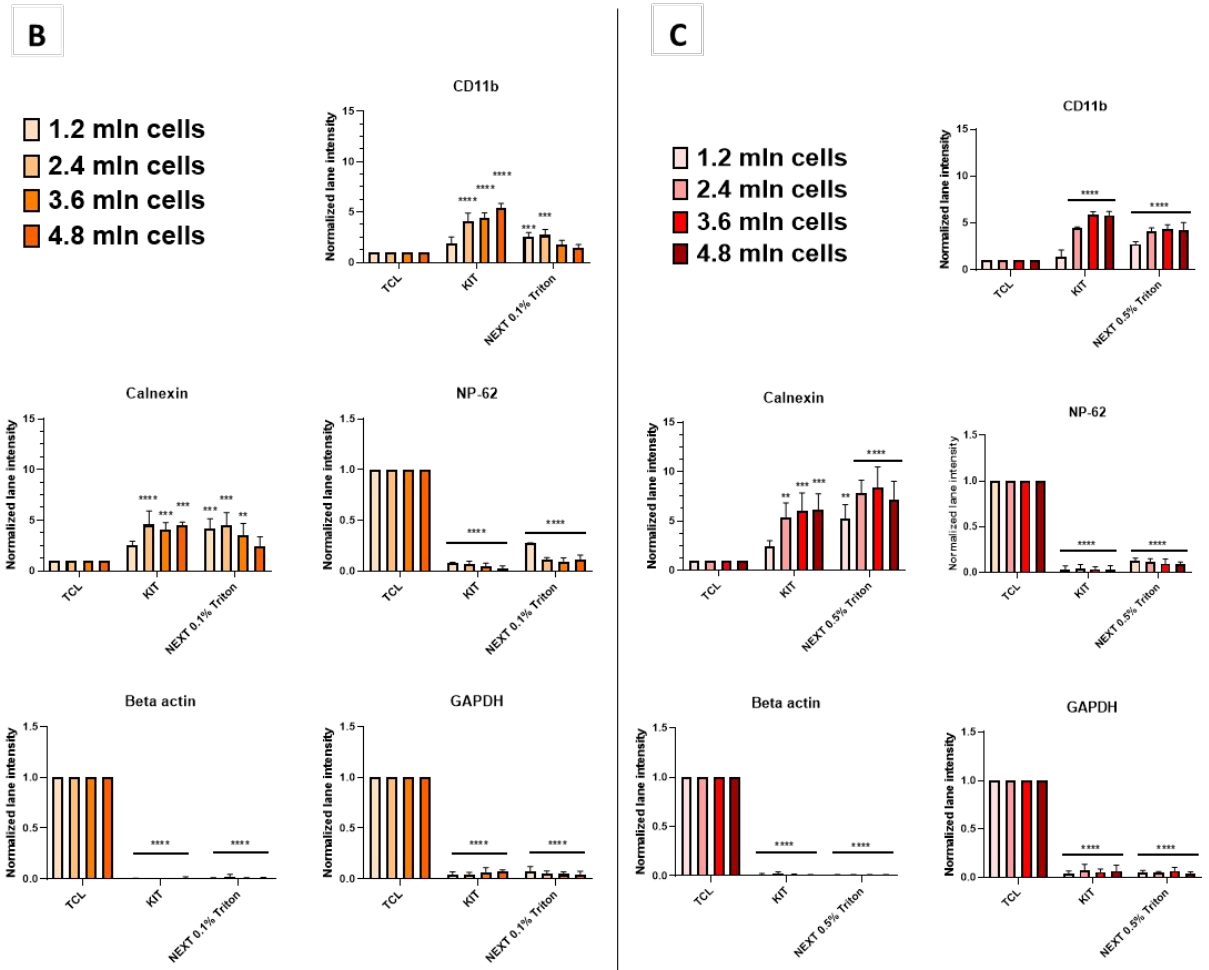


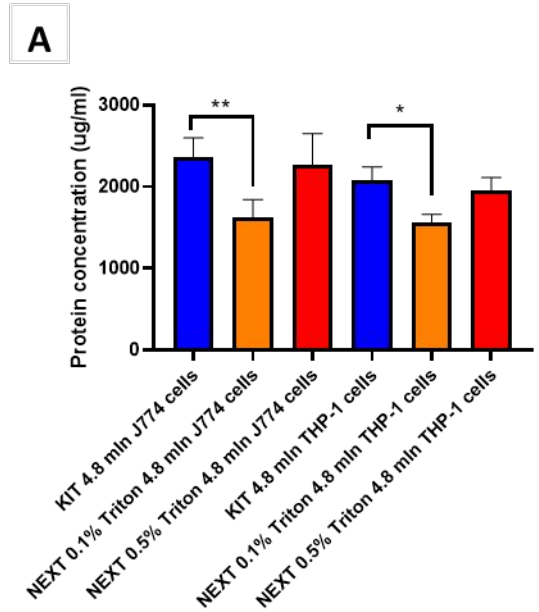


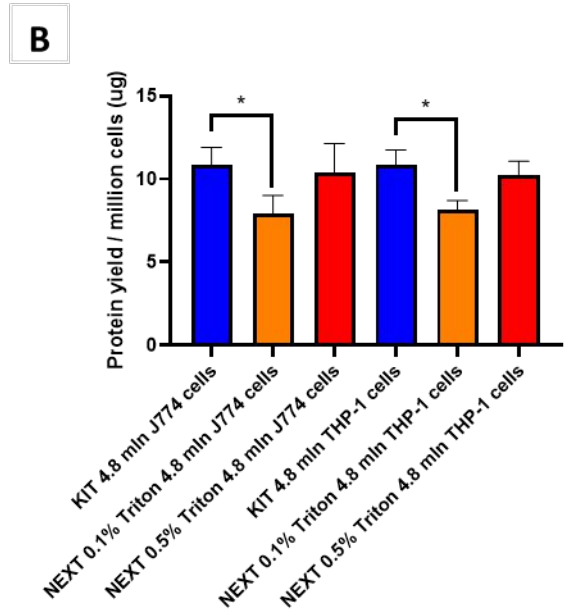


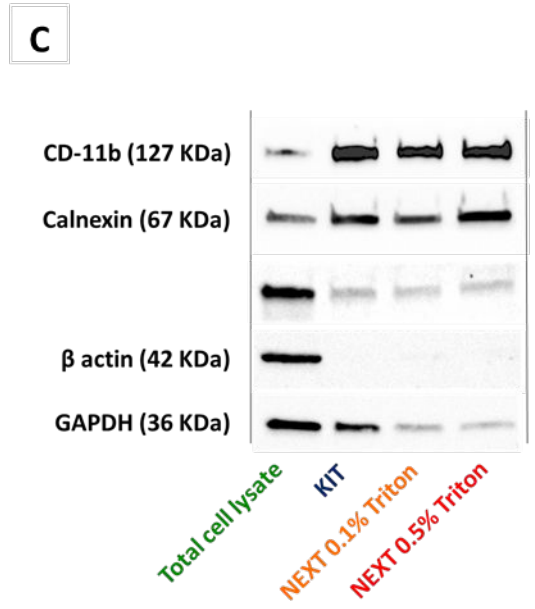
R. Rampado et al. Journal of Pharmaceutical and Biomedical Analysis 2022

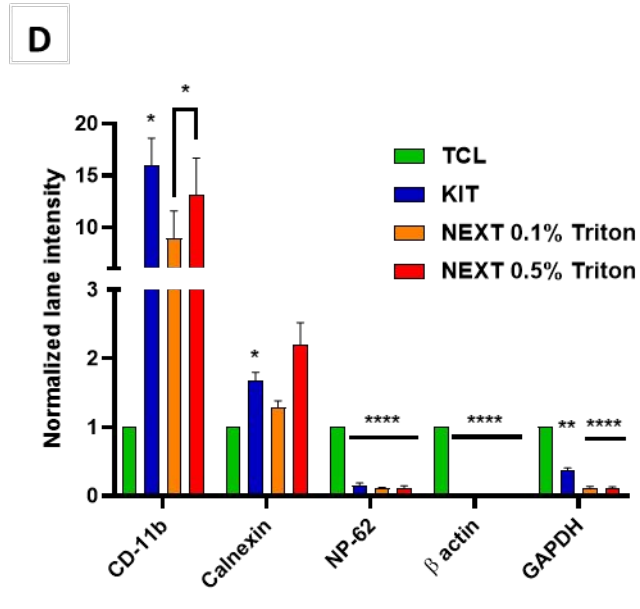












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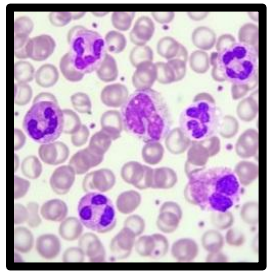
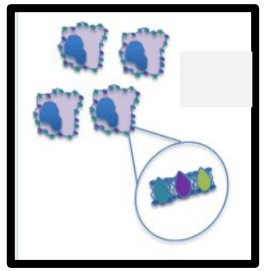


Leukocytes cells

B



Membrane protein extraction

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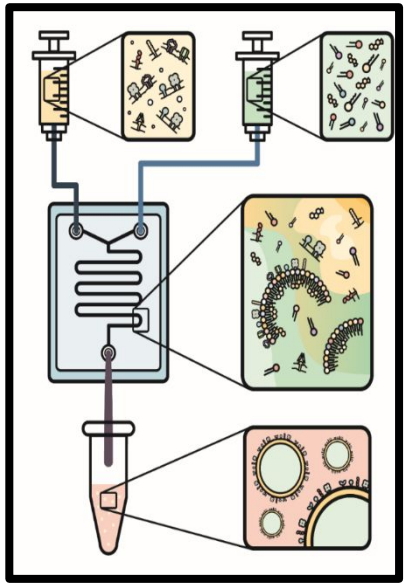
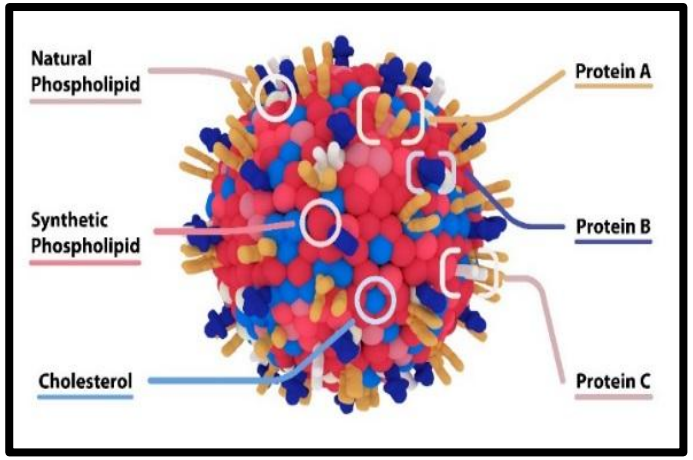
Lipids formulation



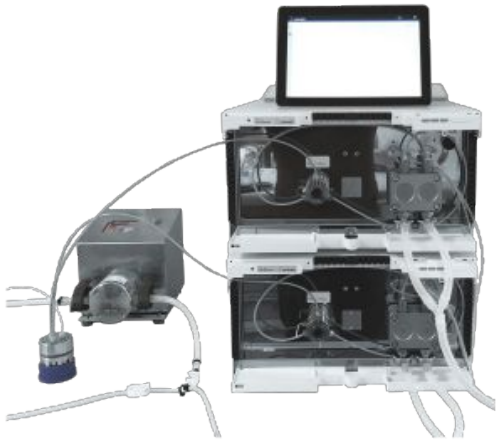
Synthesis

D



Providing medicine

Develop Medicines Faster with NxGen

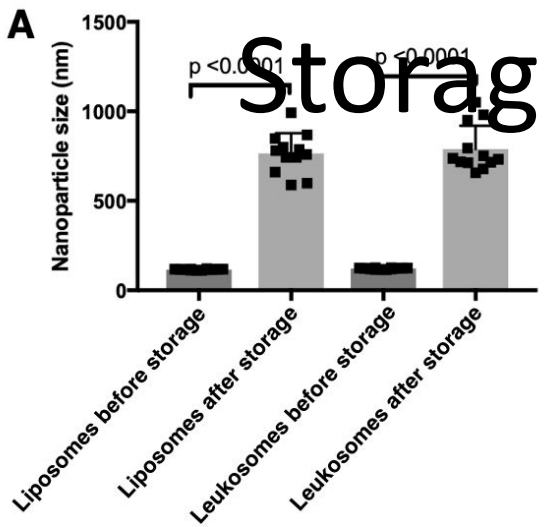


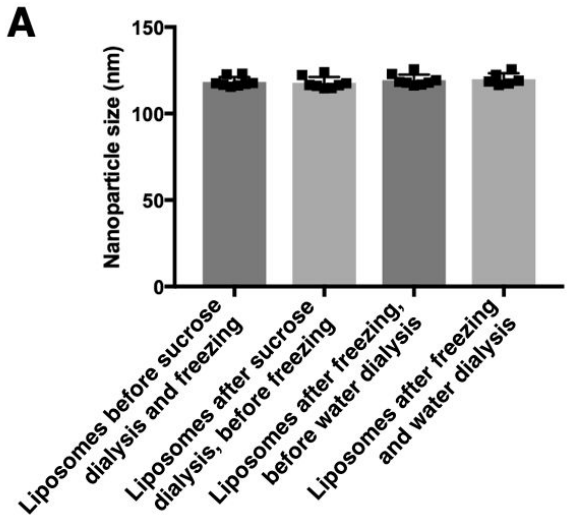
4 month acceleration

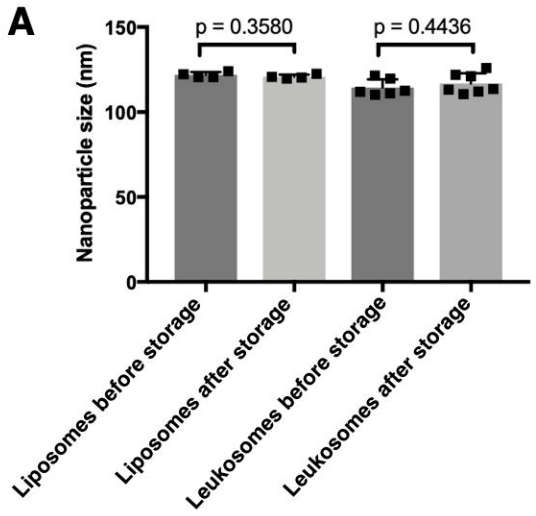
8 month acceleration

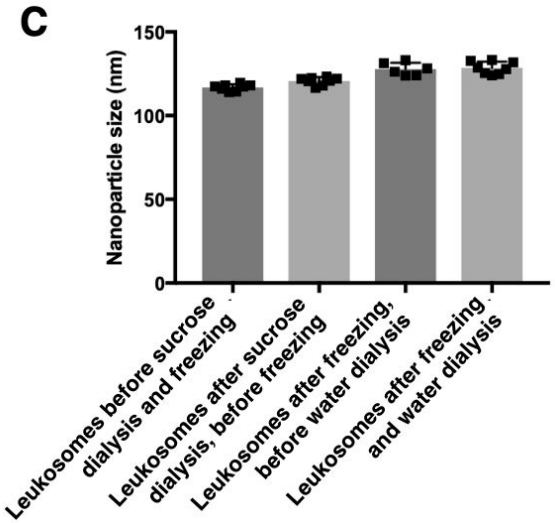
Discovery → Early preclinical → Late preclinical → Clinical development

Stability and Storage

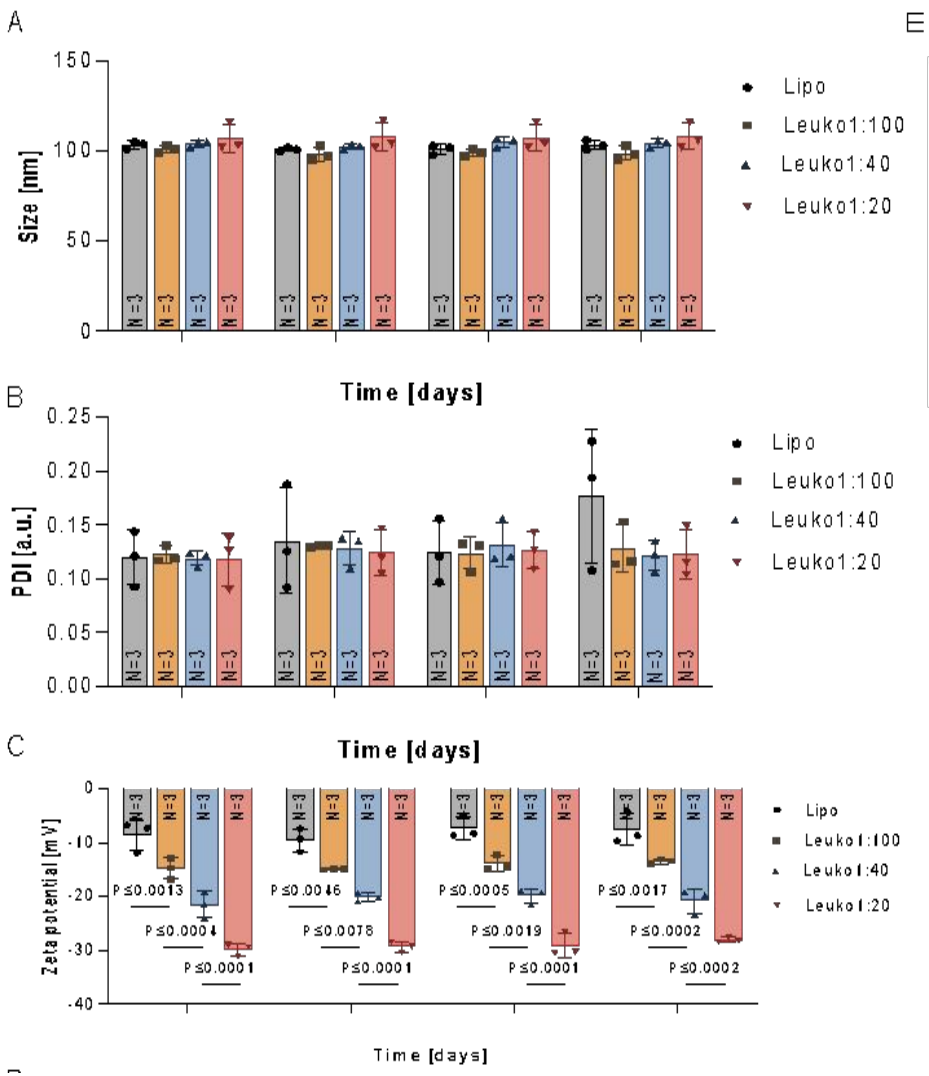


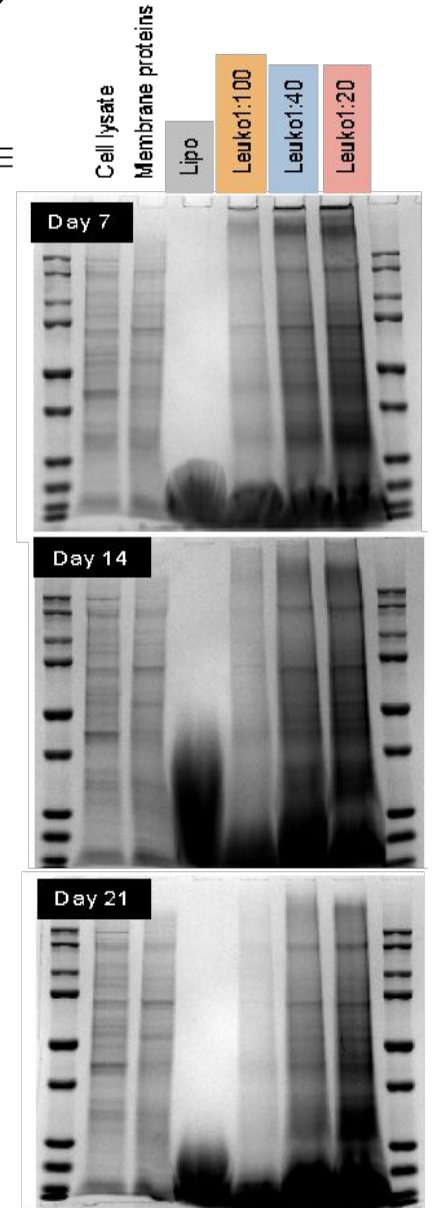






Stability- 21 days





A. Zinger and M. Sushnitha et al. ACS NANO under revision

Take Home Message

- Leukosome is a platform that can be tuned for the specific application.
- The potential of this platform for inflammatory based diseases.
- Depending on the payload the re-adjustment can be far to be easy and requires a secondary engineering
- Our preliminary data proved that there are hopes on the potential to translate this platform into the clinical practice

- If we want to reach the bedside we need to know HOW!
- What are the limitations?
- What is the next step?
- What is the final application?
- Where can I find funding for it?

ACKNOWLEDGMENTS

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Dr. Roberto Molinaro
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Dr. Bradley Weiner - HMH
Dr. John Cooke - HMRI

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