

*Chemical & Biomolecular Eng'g.,
Cell & Molecular Biol. and Physics Graduate Groups*

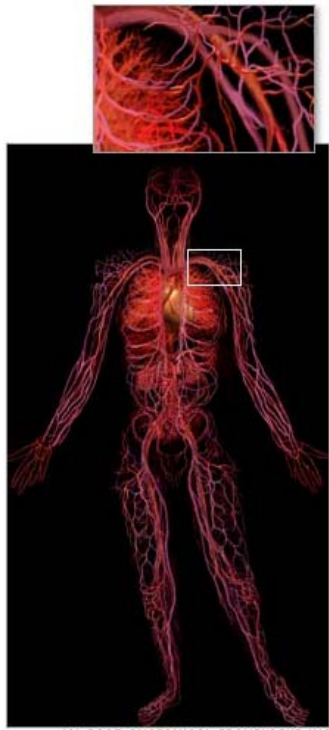
*Dennis Discher
NanoBioPolymers & Biophysical Eng. Lab
www.seas.upenn.edu/~discher*

***'NanoBiology' -
Natural or Engineered Particles of Diverse Shape
and their Interactions with Cells***

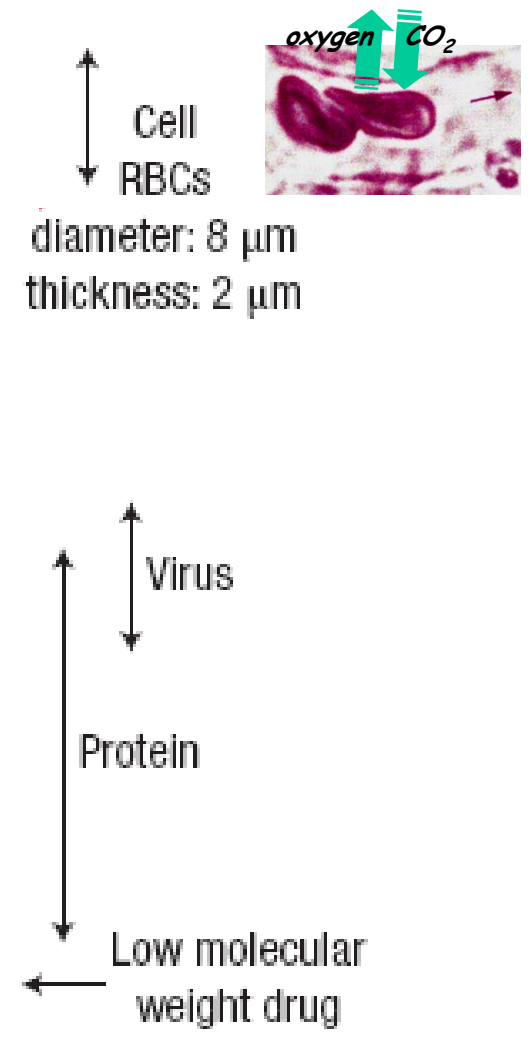
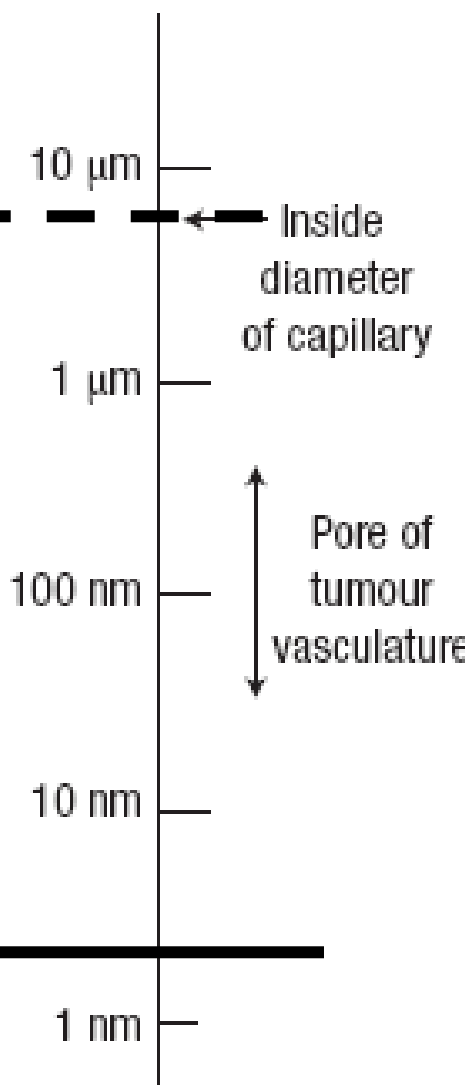
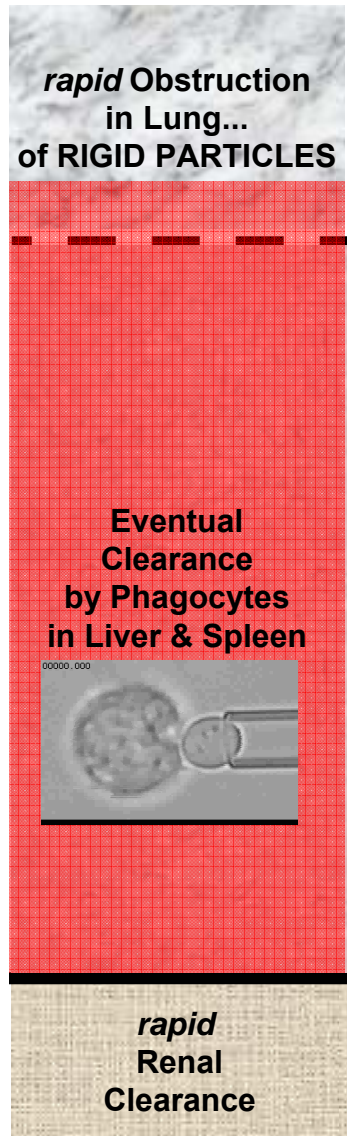


University of Pennsylvania

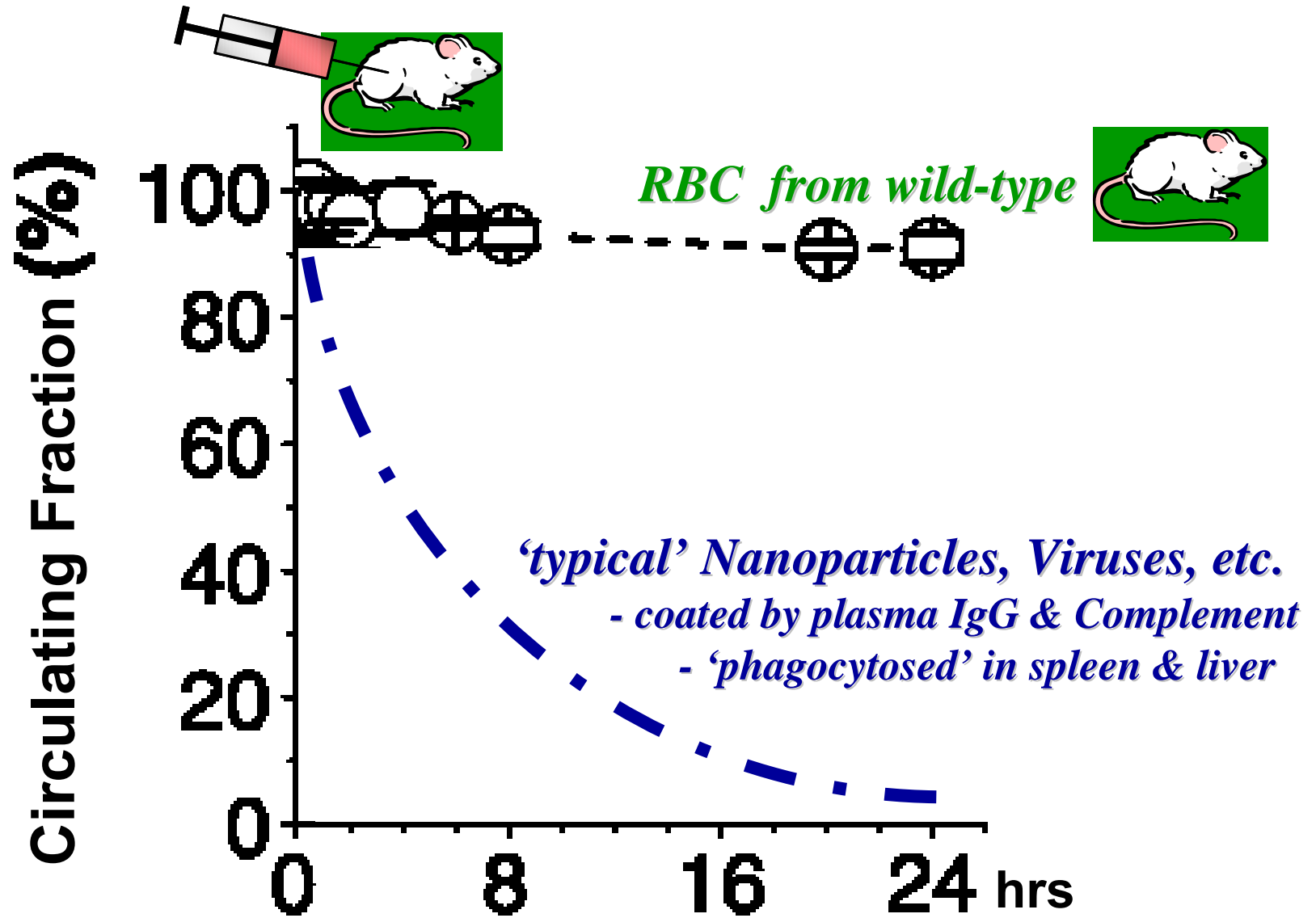
Length scales & Circulation within the Vasculature System



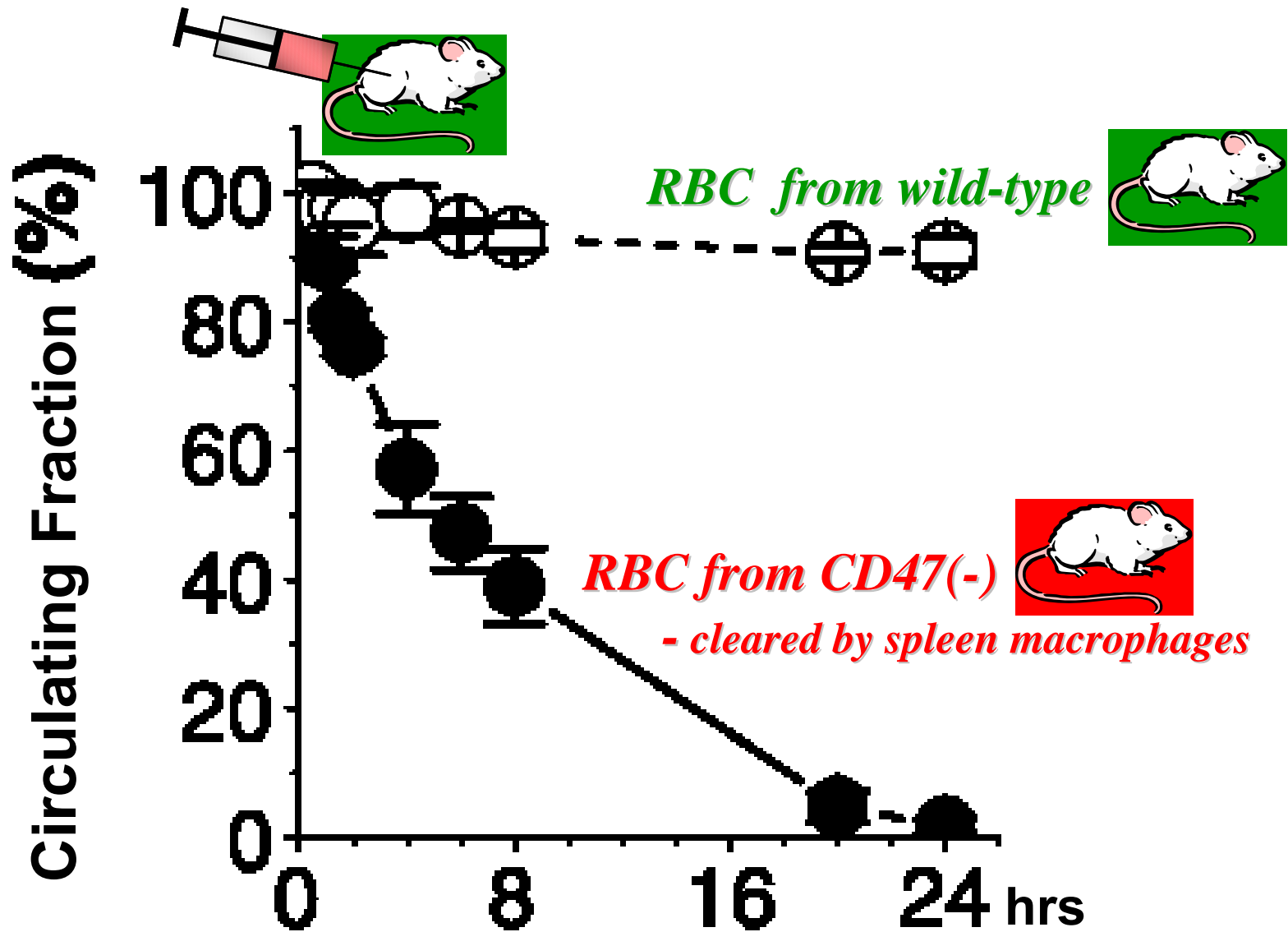
© 2006 ANATOMICAL TRAVELOGUE INC



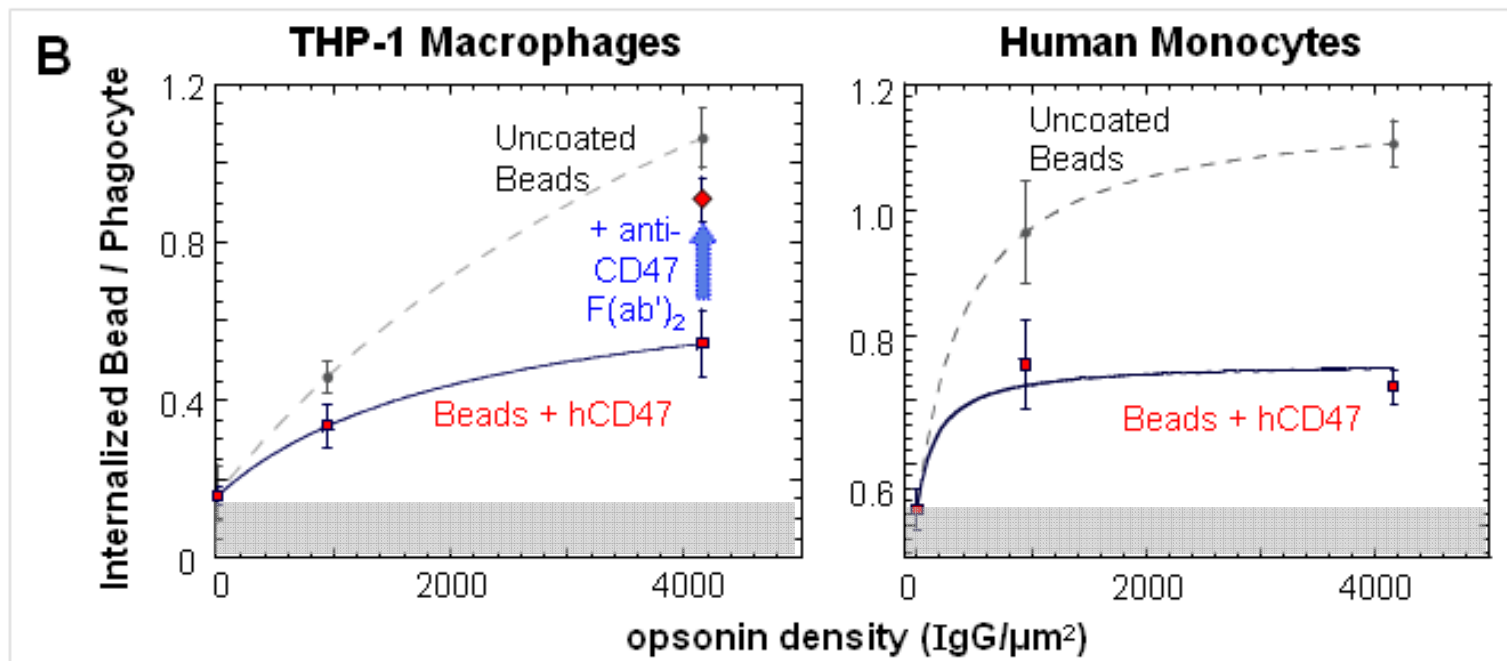
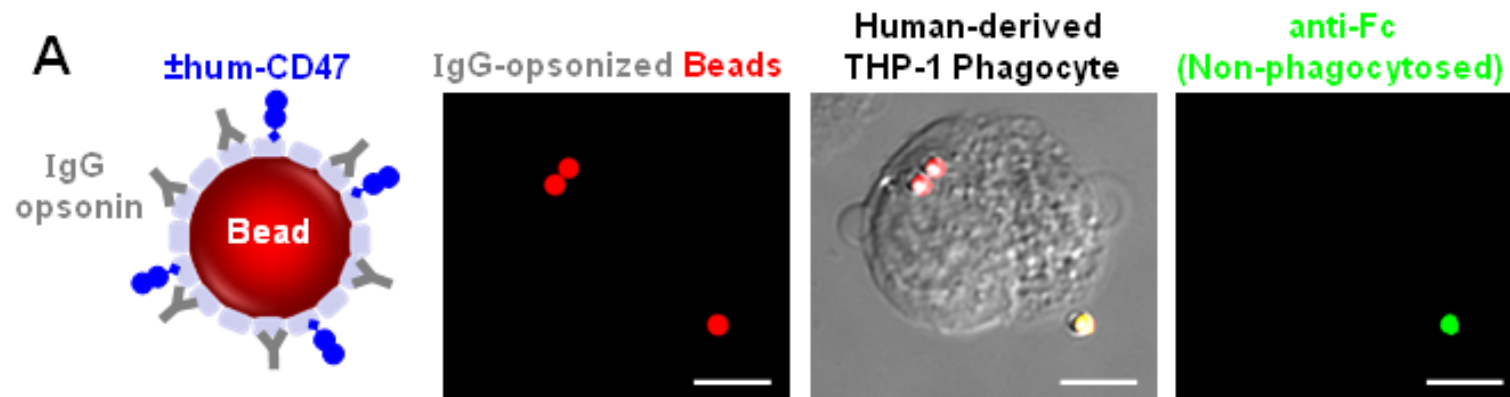
Red Blood Cells Circulate for month(s)



Membrane protein CD47 required for Circulation



human-CD47 on micron-size particles Inhibits phagocytosis

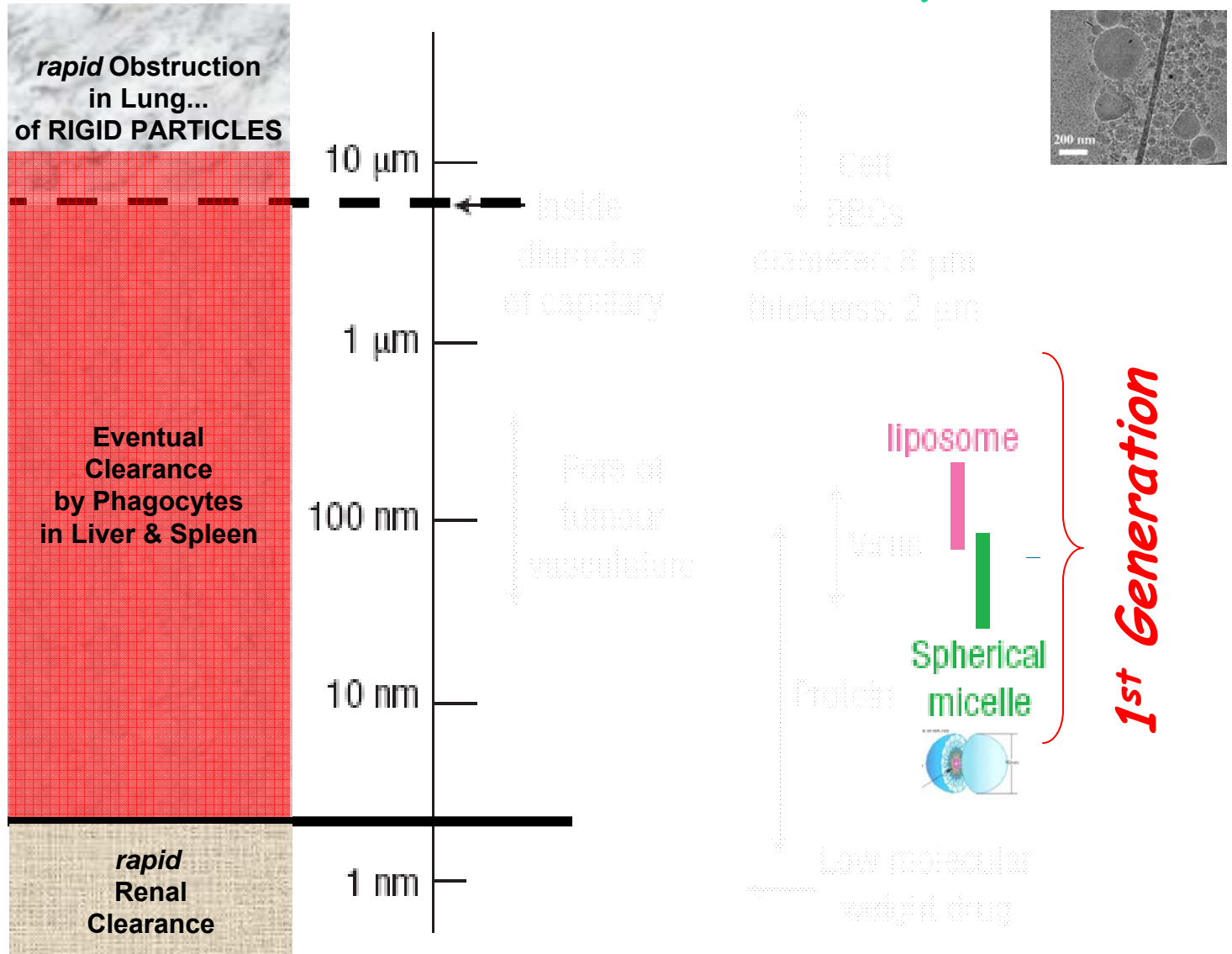


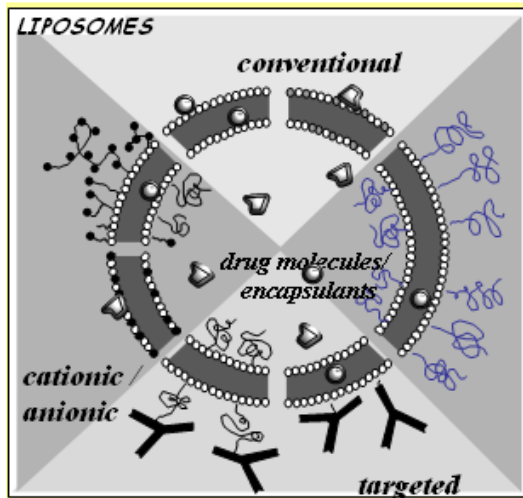
Nano

decades of work on Lipid-based Vesicles, Micelles, & Oily Emulsions



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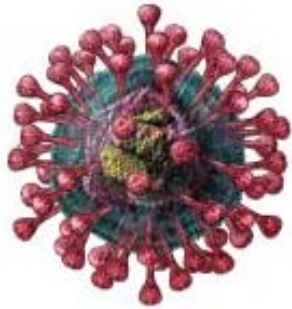


1st Generation Nano-Delivery Systems:

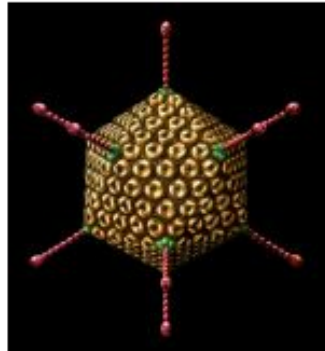
- + *Delivery of many Drugs is improved*
- + *Synthetic Polymers (eg. PEG) prolong circulation*
- + *Entry into cells can occur but...*

- *Drug release is uncontrolled*
- *Clearance from Circulation occurs within hours*
(versus months for Red Blood Cells)

Nature's Nano-Carriers



SARS virus



Adenovirus



*Self-assembled
Shell of Polypeptides
(protect & RELEASE)*

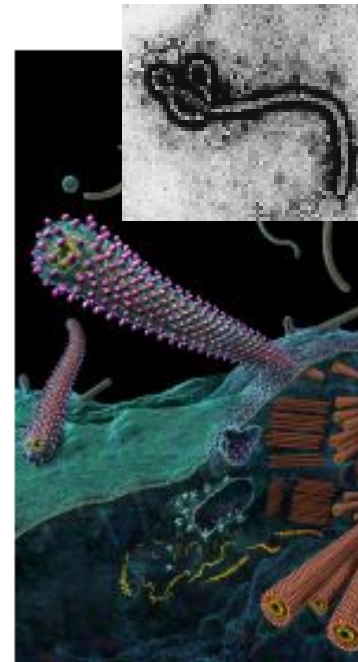
Targeting moiety

*Spherical Shells
& Filaments*

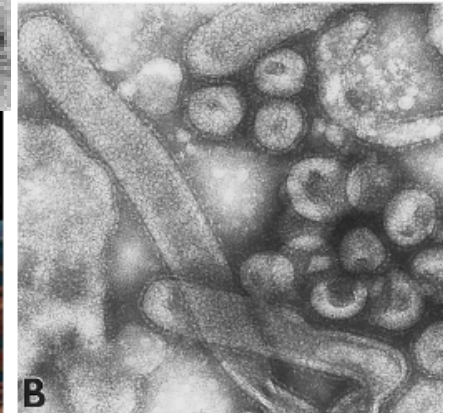


Filamentous Phages

M13, fd, TMV...



Ebola virus



Influenza (H5N1)

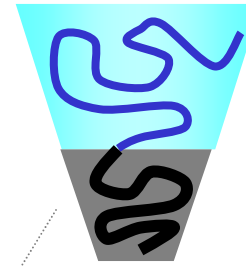
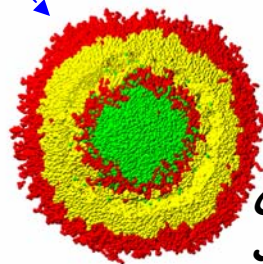
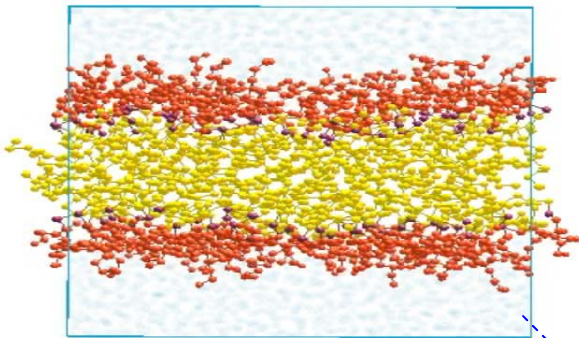
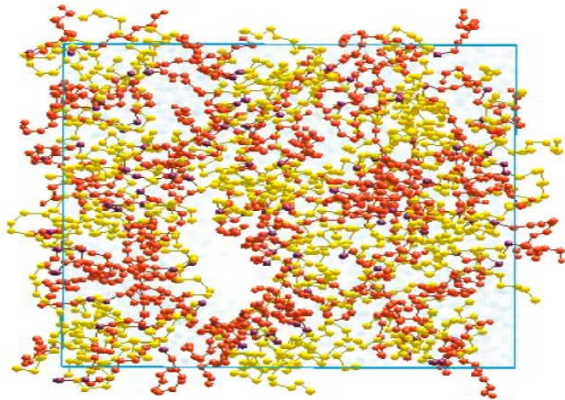
PEG-based Diblock Amphiphiles



44% PEG

108 diblock copolymers

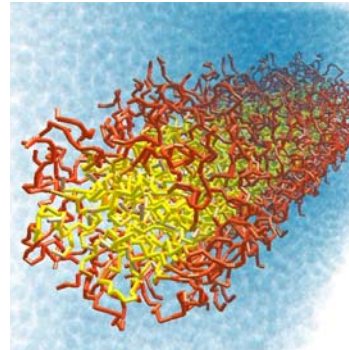
2200 waters



51% PEG

80 diblock copolymers

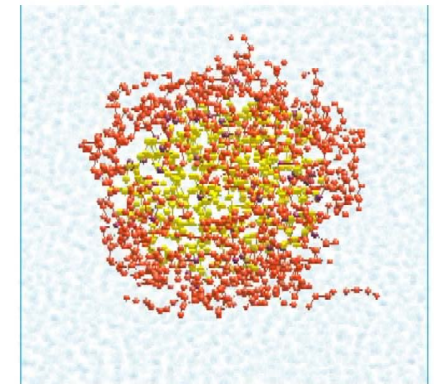
4000 waters



60% PEG

48 diblock copolymers

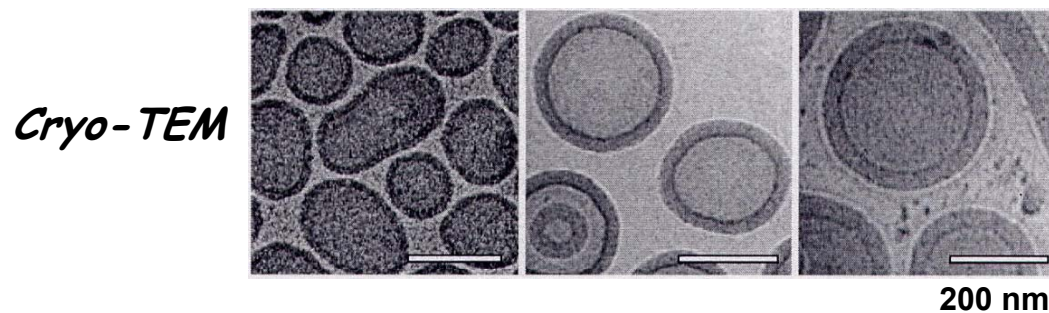
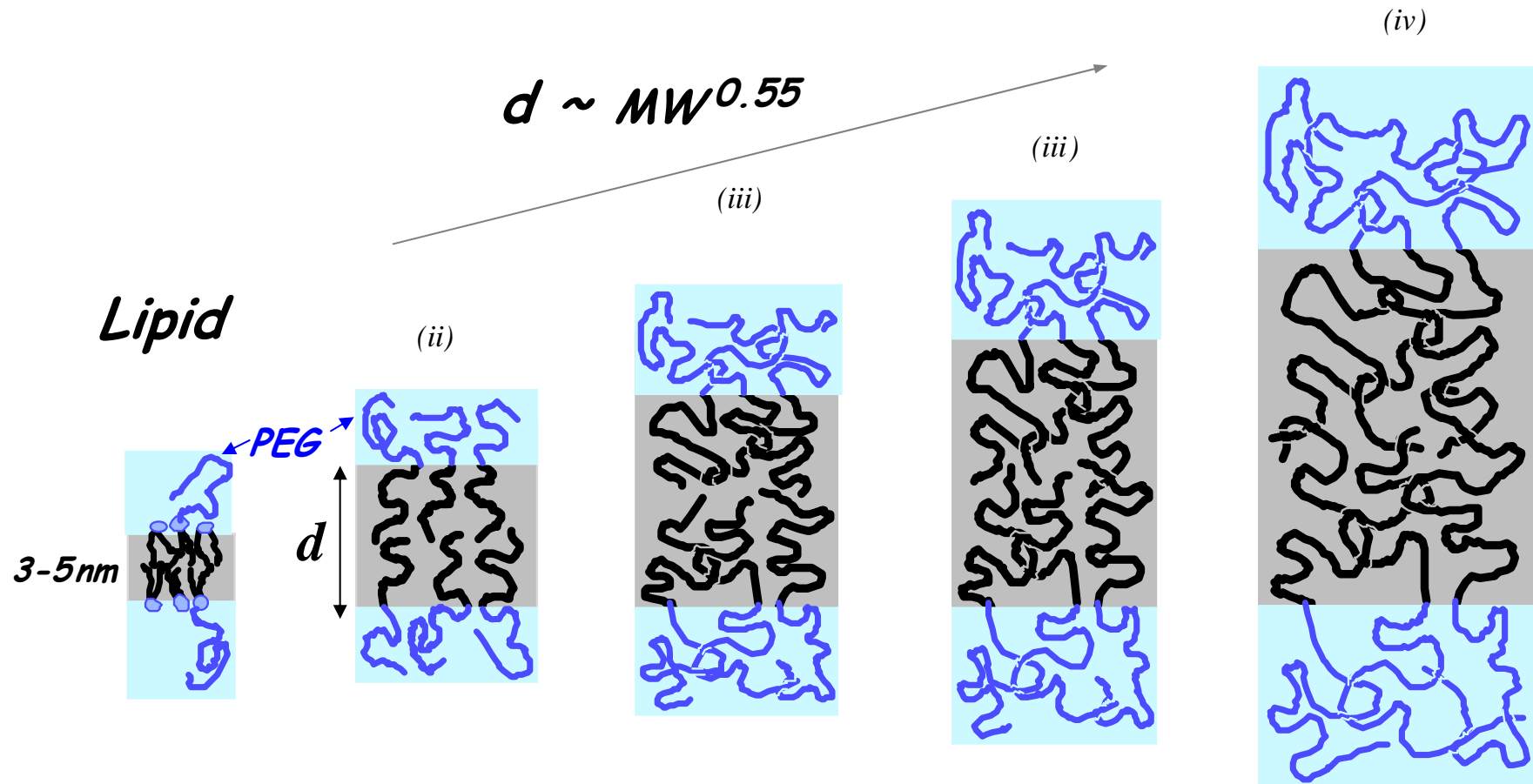
~6000 waters



*Srinivas, Discher, & Klein.
Nature Materials (2004)*

*Ortiz et al,
J.Phys.Chem 2005*

Polymersomes: Thickness-controlled polymer vesicles



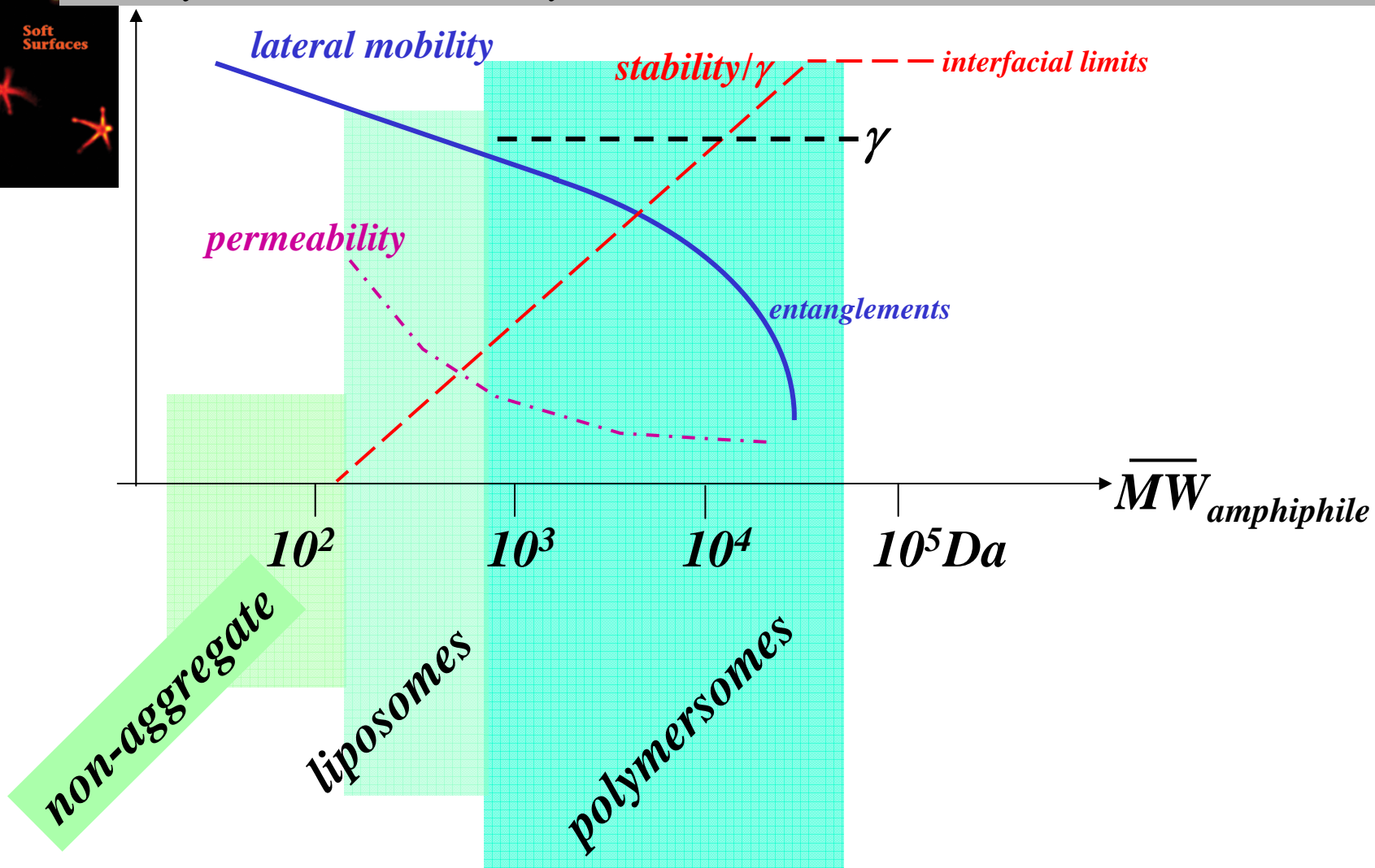
Polymer Vesicles

Dennis E. Discher^{1*} and Adi Eisenberg²



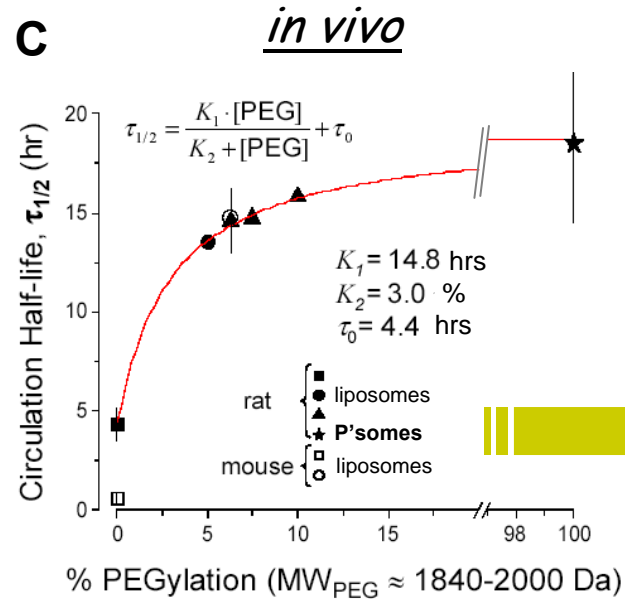
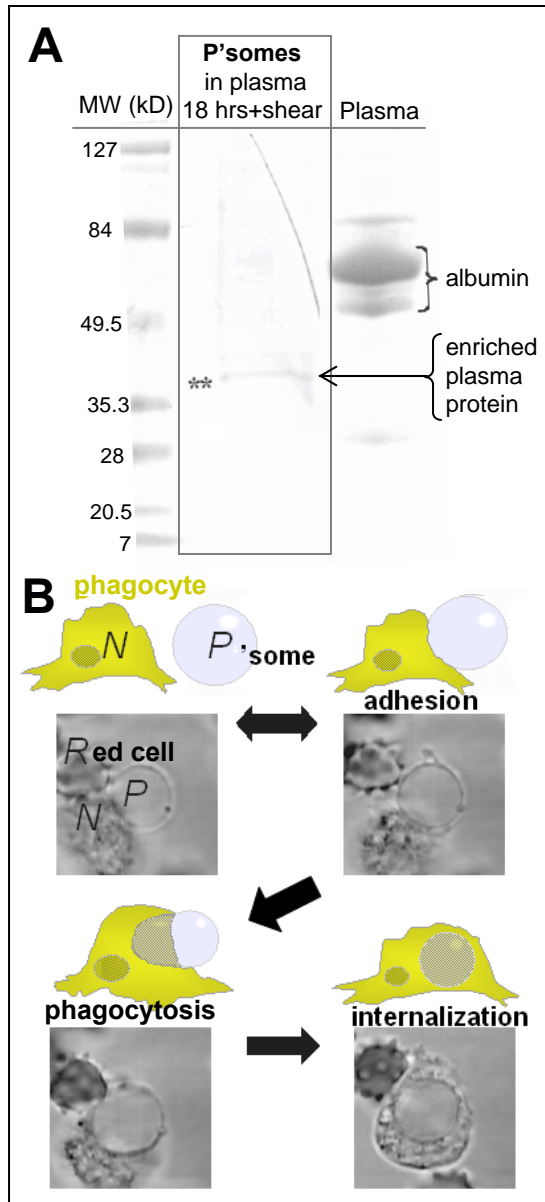
2002

Properties Summary: Transition from Interface to Bulk

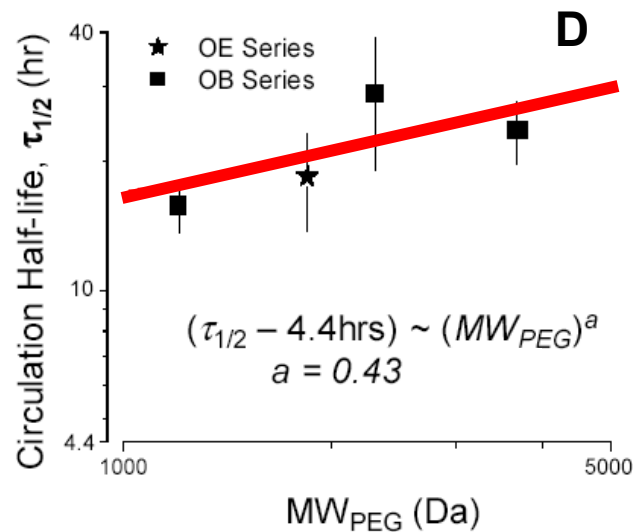


Fully PEGylated Vesicles are Super-Stealthy but still Opsonized and then Cleared

in vitro

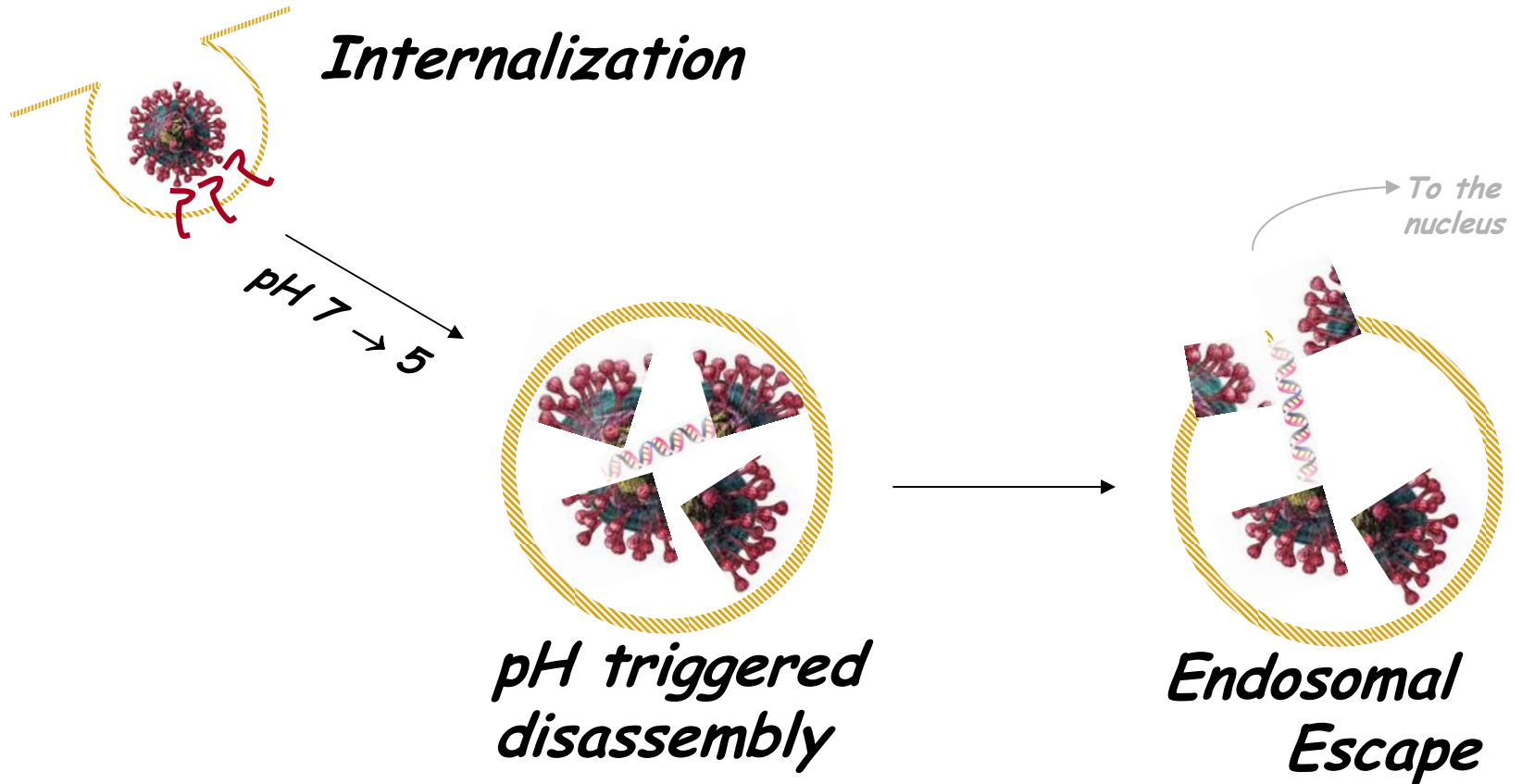
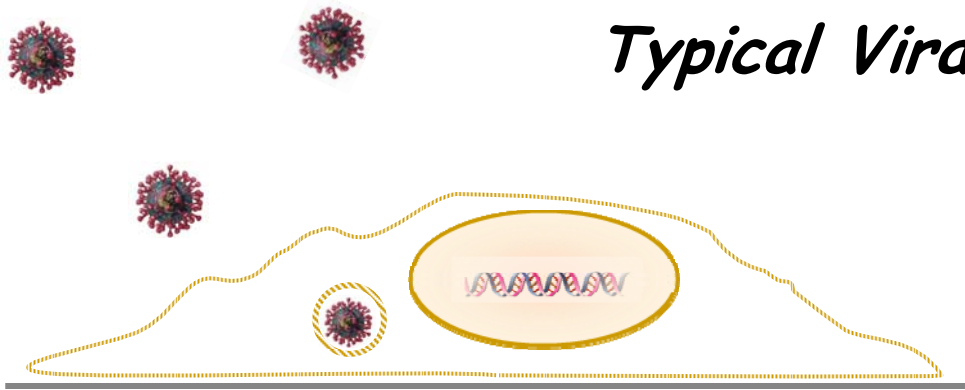


Cleared by Mono Phagocytic System of Liver & Spleen



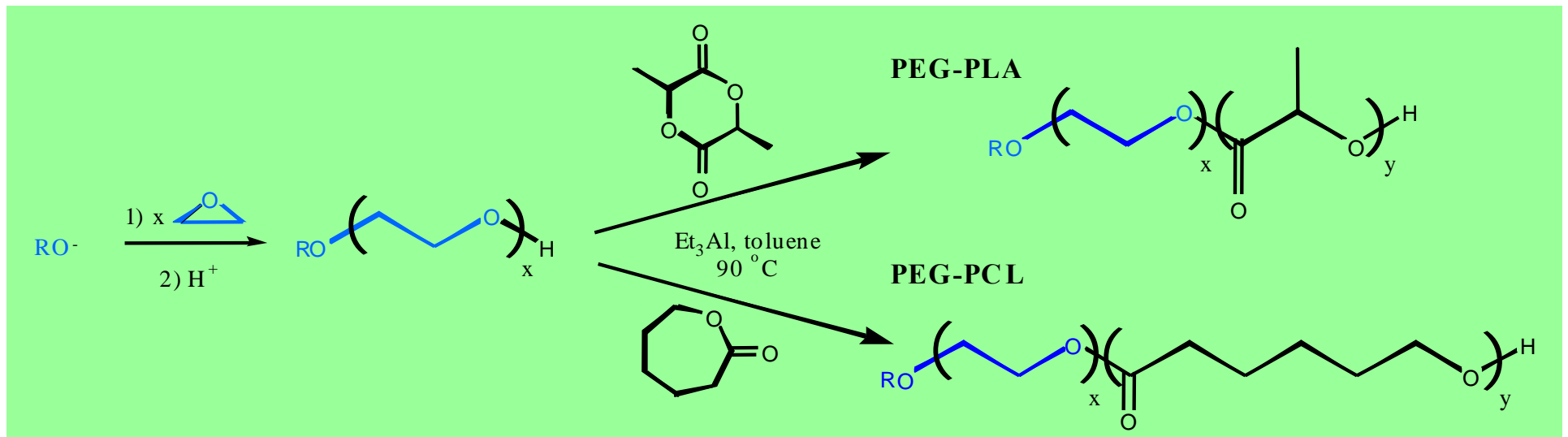
Photos et al.
J Controlled Release (2002)

Typical Viral Entry & Release



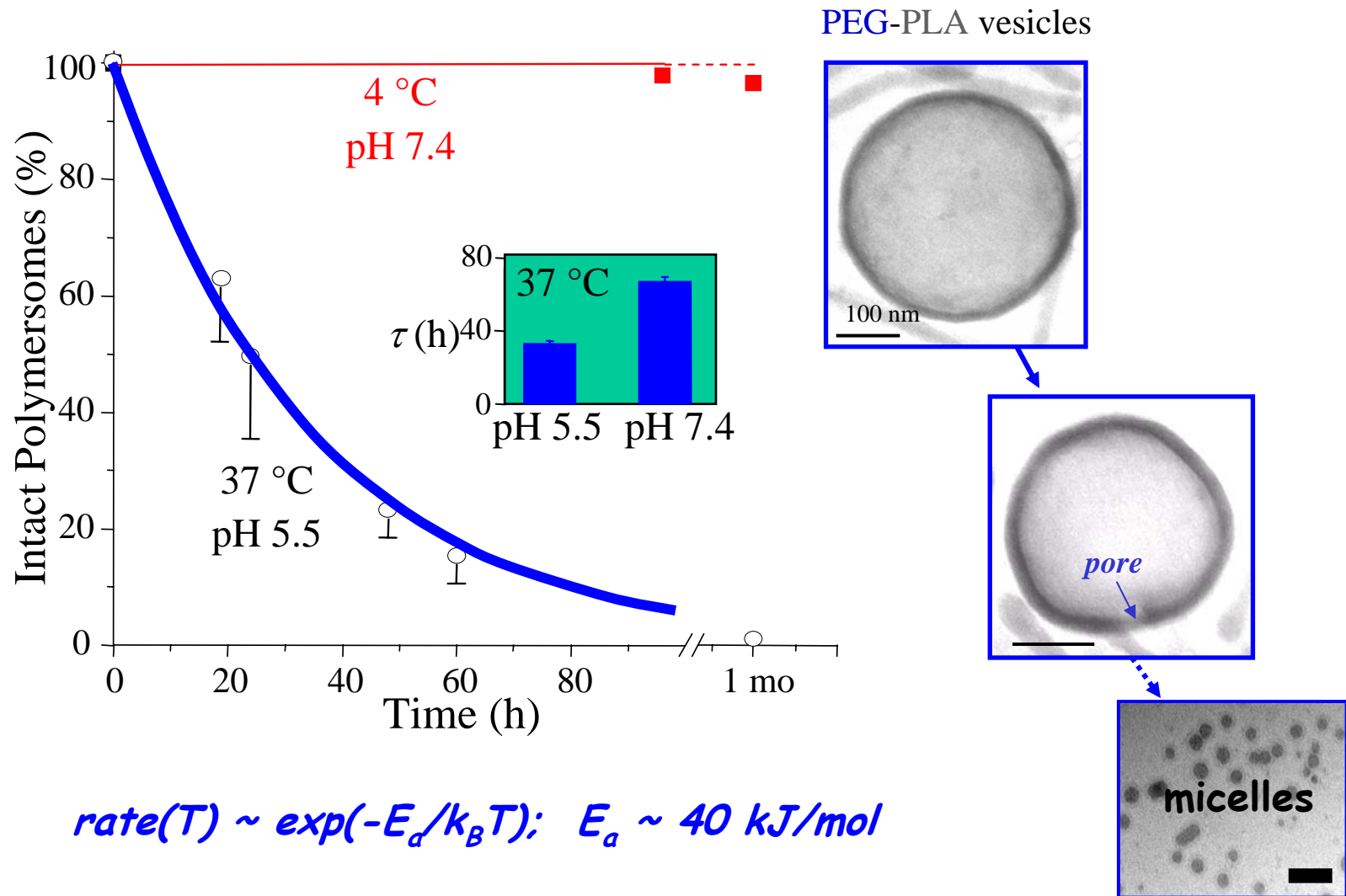
Controlled Release Polymer Vesicles

Hydrolysable Polyesters

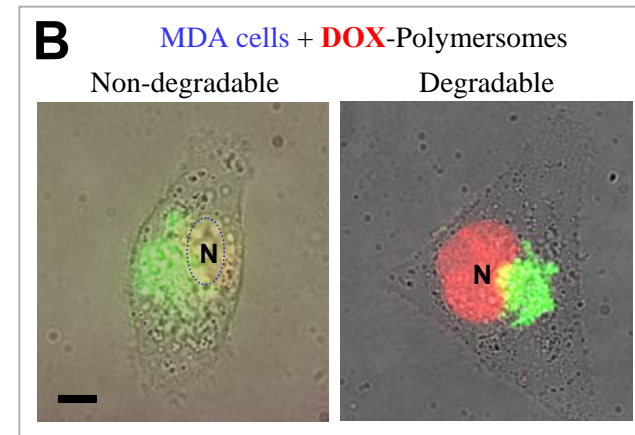
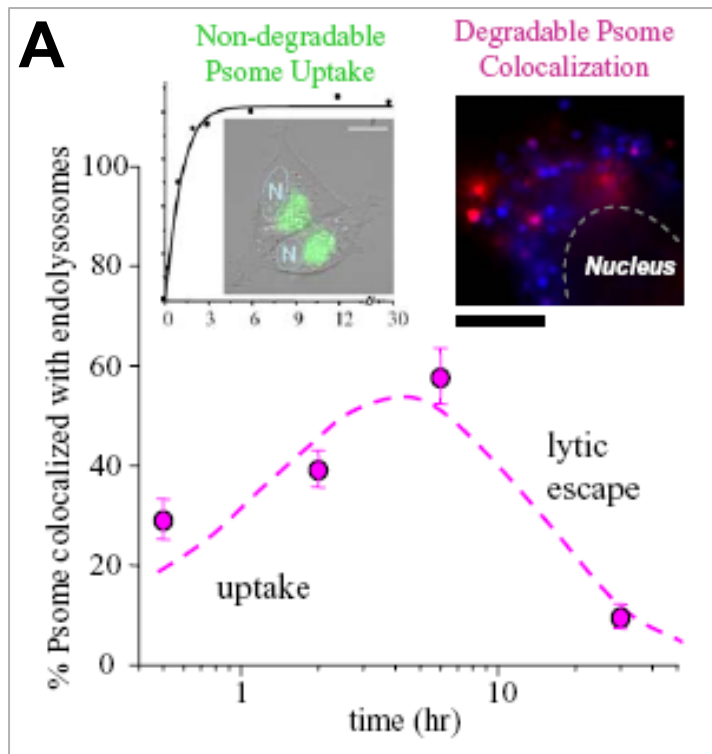


- *others have shown PEG-PLA makes sphere micelles (mid-90's)*
- *all blocks FDA approved*

Self-Portating Polymersomes for Controlled Release

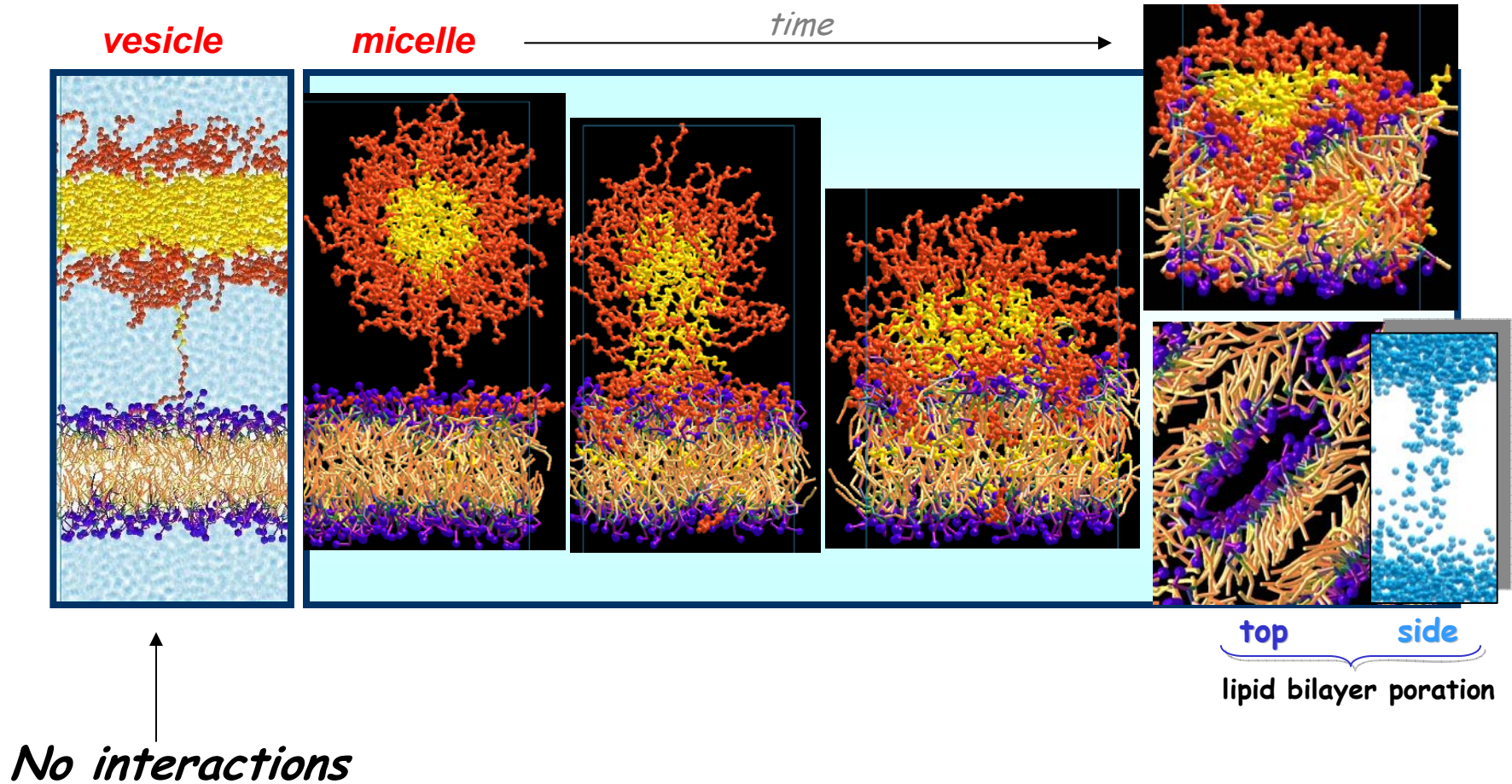


Endolysosomal poration of degradable polymersomes



- **Passive uptake via fluid phase endocytosis**

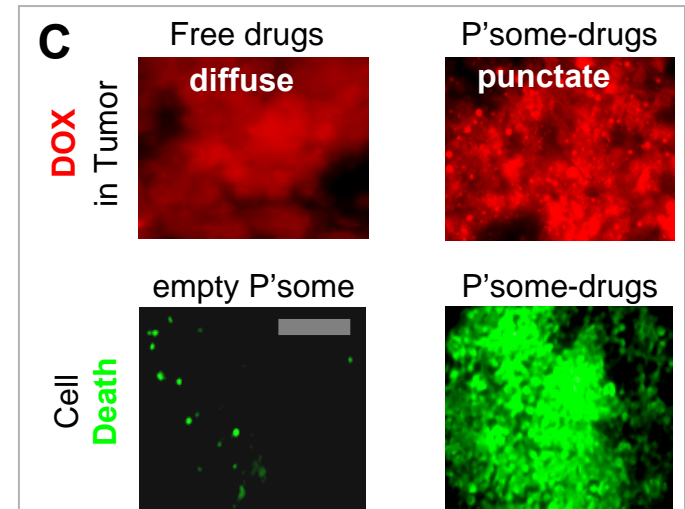
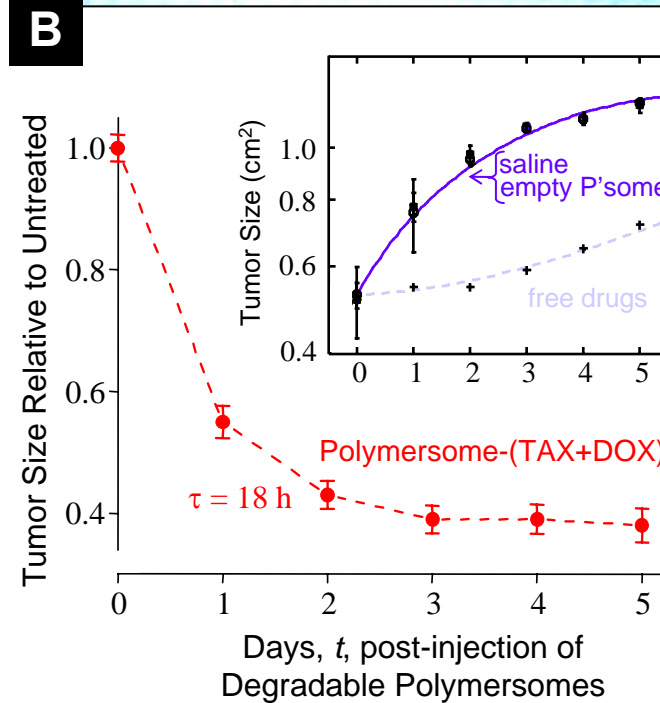
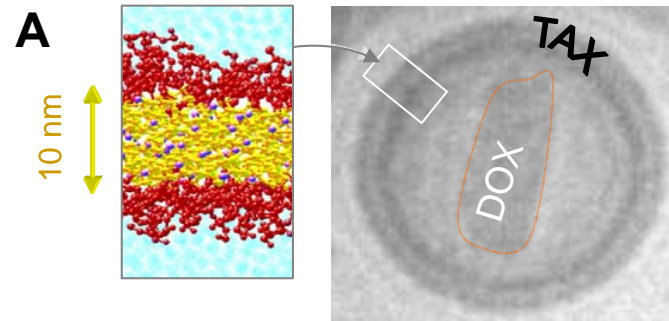
Endolysosome permeation with Micelles



Anticancer: Dual-drug delivery to Tumors with Degradable Polymersomes

Ahmed et al., *Molecular Pharm.* 2006

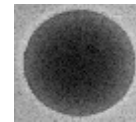
with
Tamara Minko,
Rutgers U.





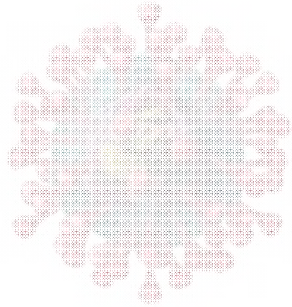
Encapsulants

- *hydrophobic drugs in thick membrane (Taxol)*
- *hydrophilic drugs (anti-cancer Doxorubicin)*
- *Antisense & RNAi Oligonucleotides*
- *peptides (antimicrobials ...)*
- *proteins (globins, catalase)*

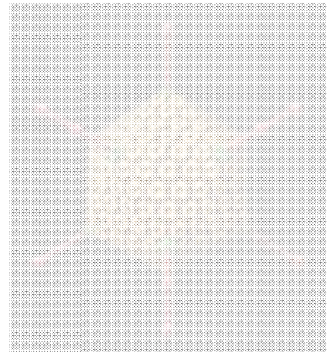


globin

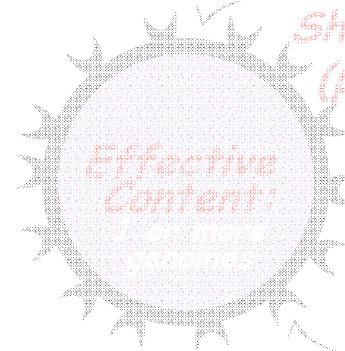
Nature's Nano-Carriers



SARS virus



Adenovirus



*Self-assembled
Shell of Polypeptides
(protect & RELEASE)*

*Effective
Content:*

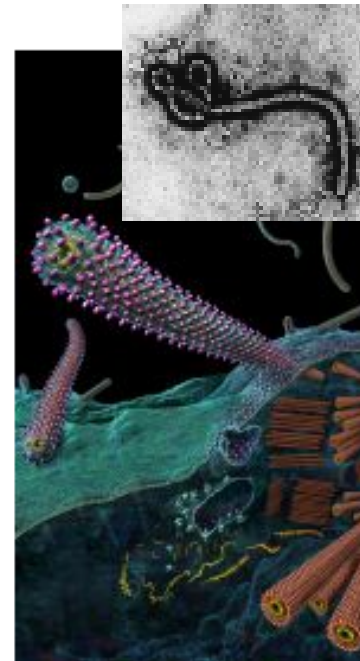
*Targeting
moiety*

*Spherical Shells
& Filaments*

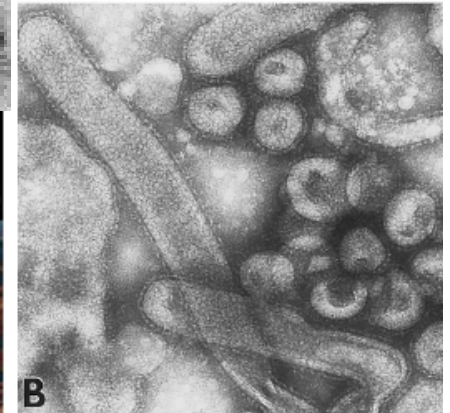


Filamentous Phages

M13, fd, TMV...



Ebola virus



Influenza (H5N1)

Ebola Virion:

polydisperse in length, nano-diameter, flexible

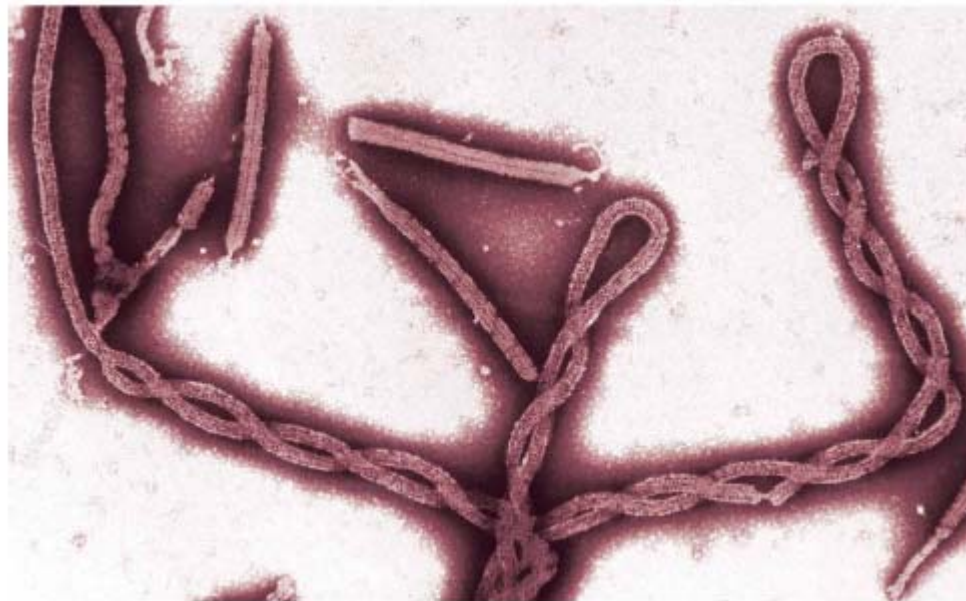


Figure 1 Transmission electron micrographs of negatively stained hemorrhagic fever virus particles.

_____ Ebola virus (bottom). Filovirus particles are mostly filamentous and vary in length up to 14,000 nm with a uniform diameter of 80 nm. Mean unit length is about 1,000 nm. Other forms of filovirus particles include U-shaped, '6'-shaped or circular configurations; branching of filamentous particles can also occur.

Influenza virus in the form of long filaments up to several micrometers in length has been observed as the predominant morphology in early passages of virus isolates from infected individuals and in allantoic fluid preparations of virus

In contrast, laboratory-adapted influenza viruses used in cell culture experiments exist in a predominantly spherical morphology,

Deadly Avian influenza virus - H5N1



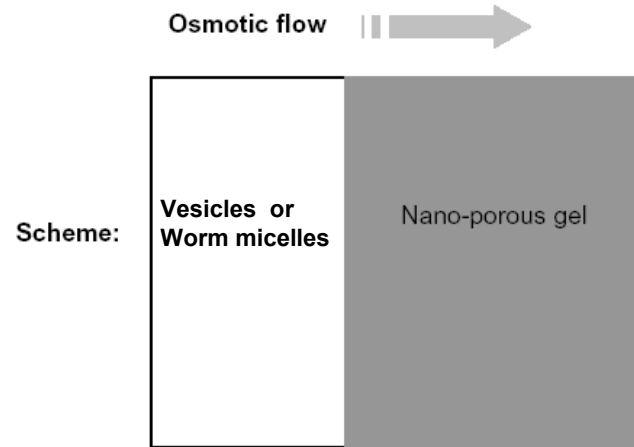
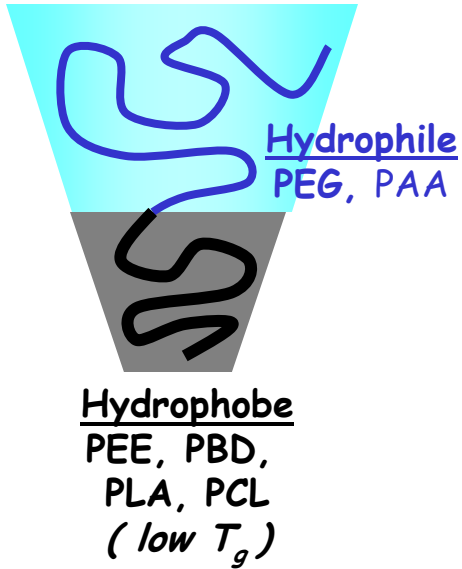
*30-70% infections
are fatal to humans*

Changeable beast. Because the H5N1 virus is constantly changing, diagnostics and vaccines must be updated regularly to remain effective.

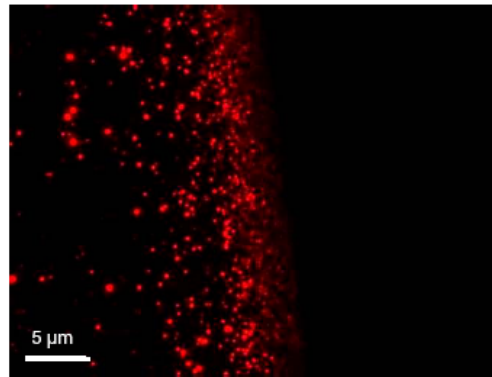
Science, July 2005

Inspiration??!!

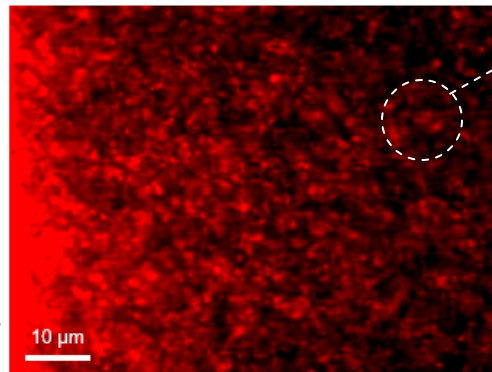
$f_{EO} \approx 0.5 \rightarrow$ *Stable, Flexible, Nano-Permeating Cylinders*



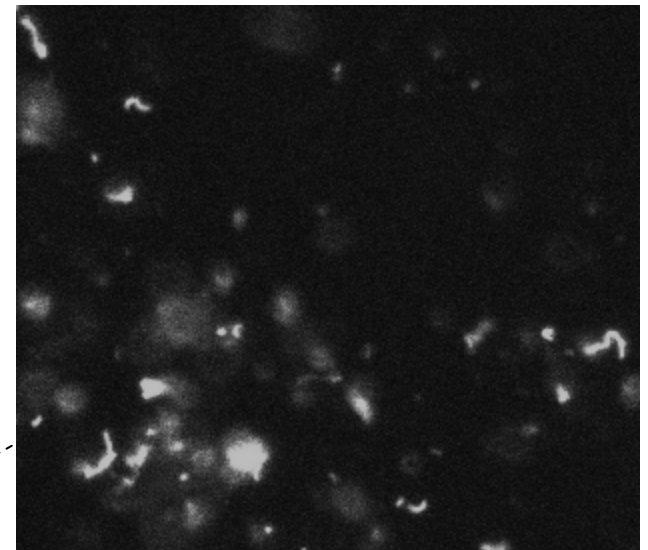
100 nm vesicles



Worm micelles



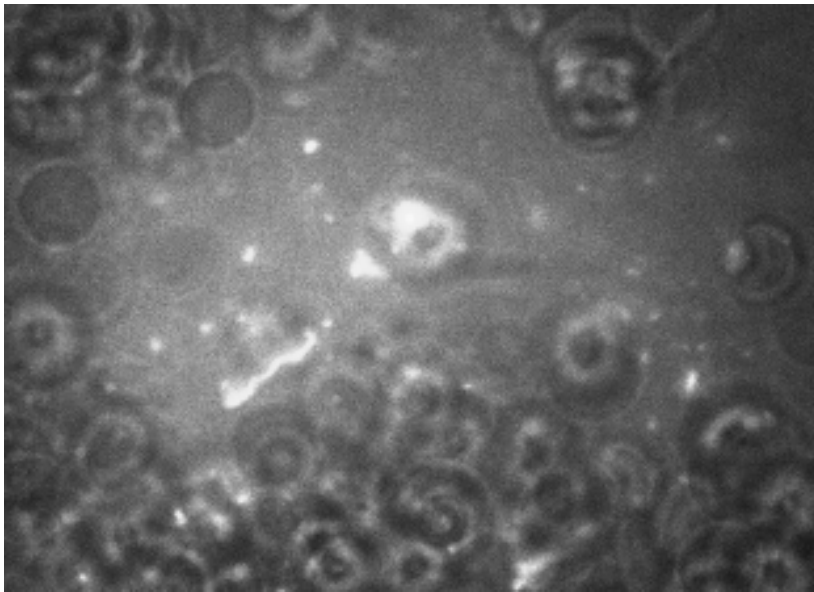
Pulled by the flow



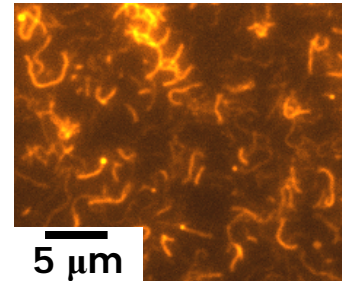
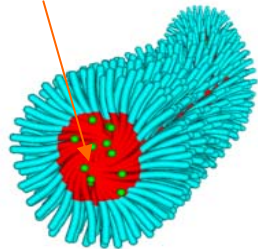
*Looks like
DNA electrophoresis*

*Geng Nature Nanotech 2007
Kim Nanotech 2005*

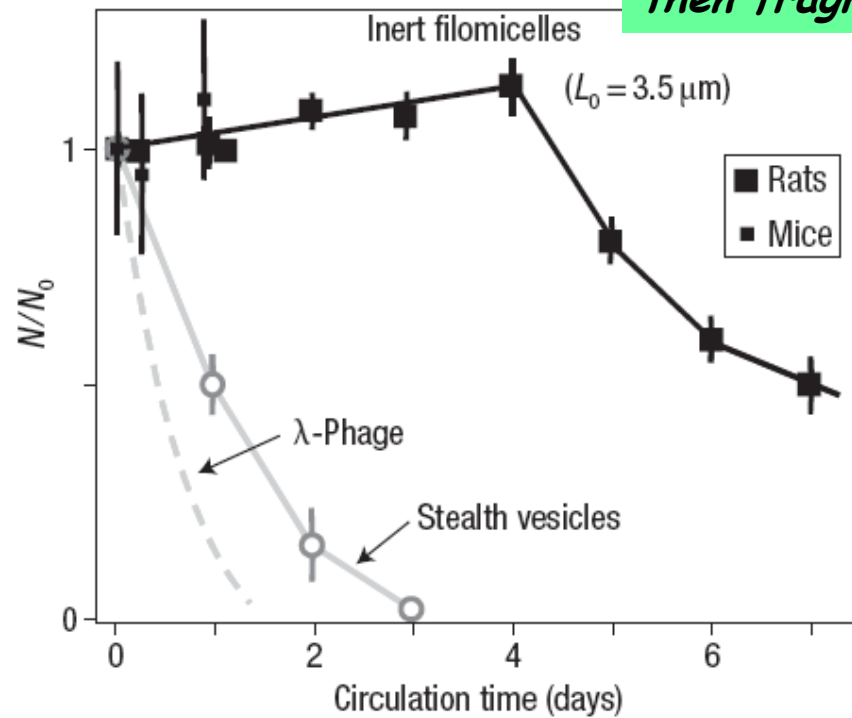
Flow among cells and extremely long Circulation



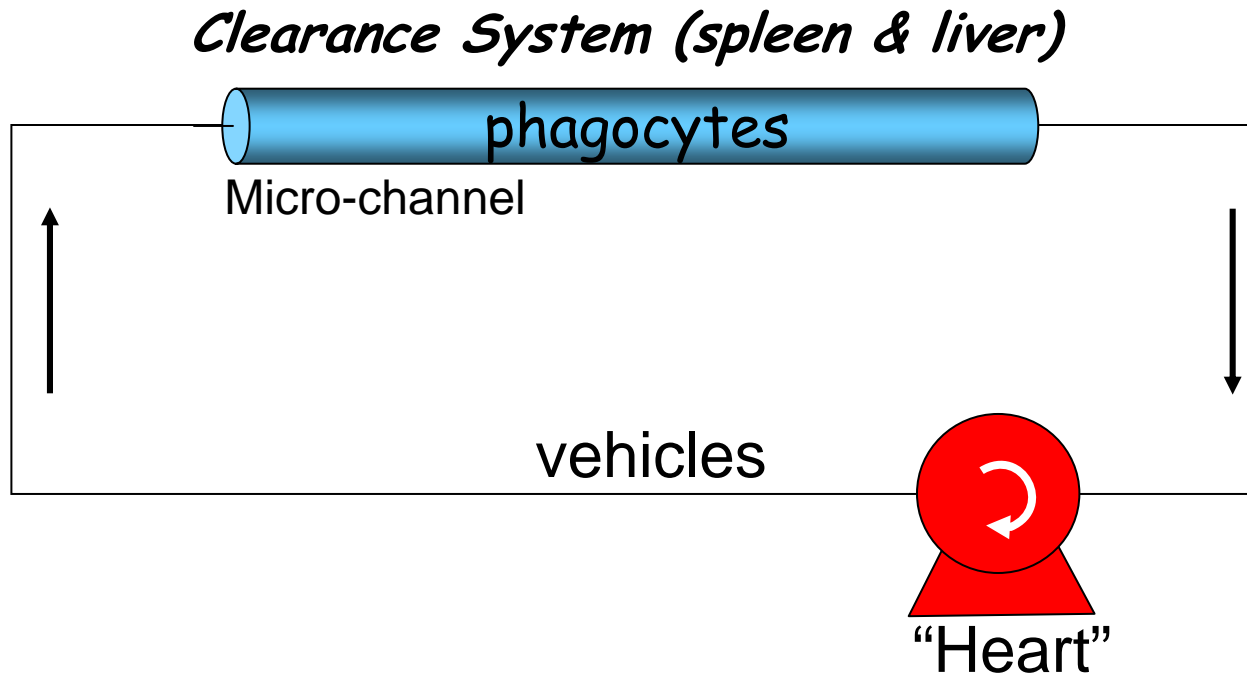
PKH26 dye



10 μm worms circulate longest, then fragment



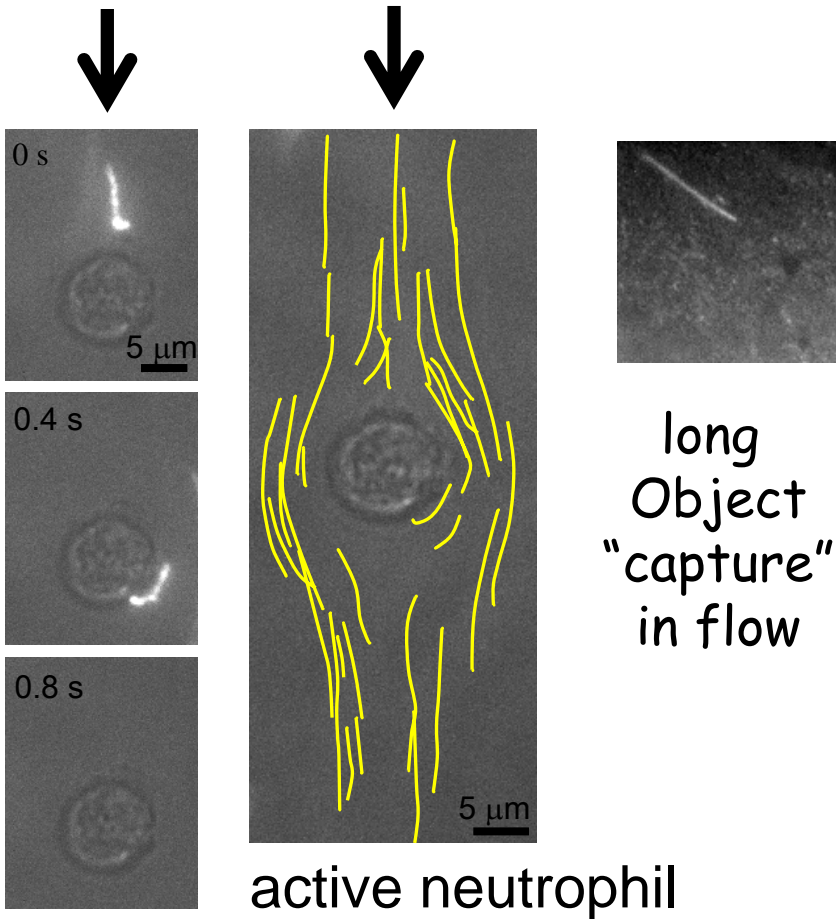
a Model Circulatory System



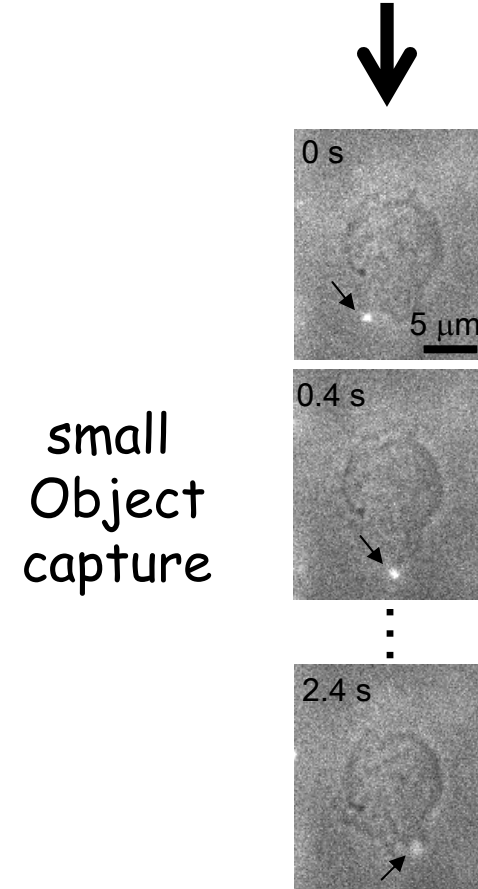
Length-dependent clearance *in vitro*

37° C

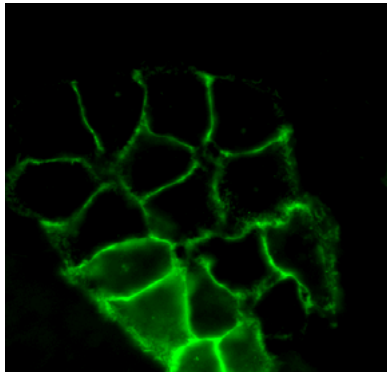
Flow (*Weissenberg#* > 1)



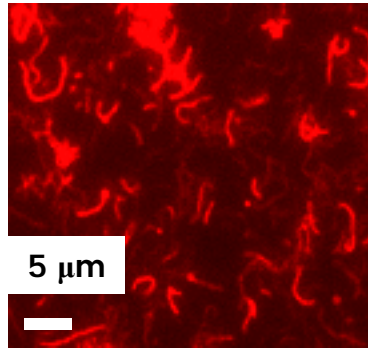
Flow



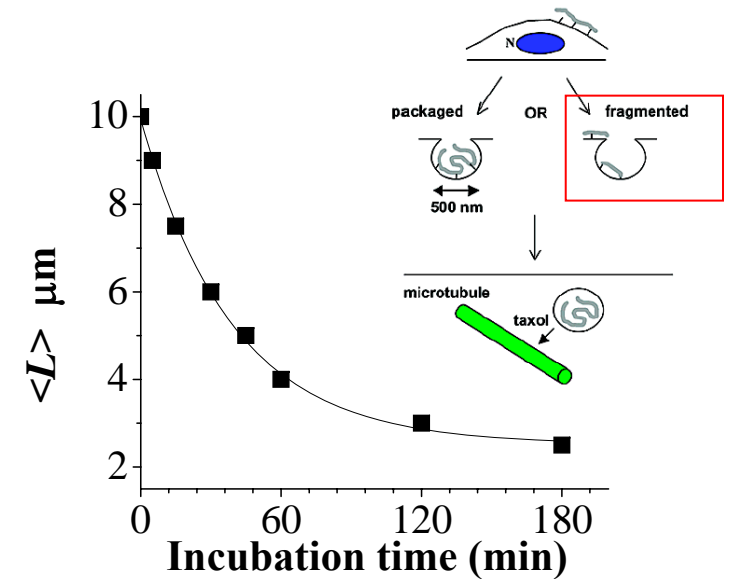
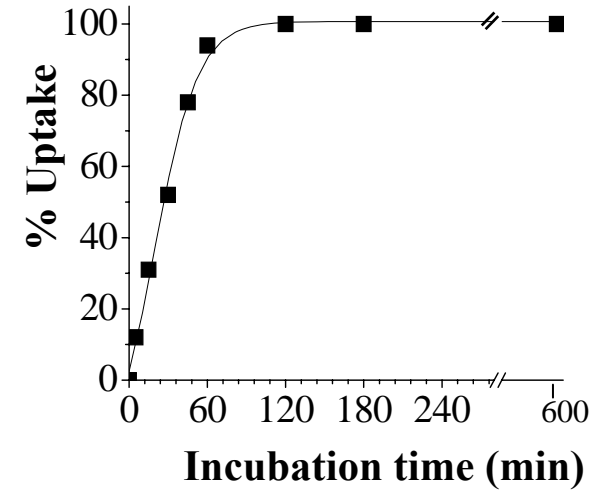
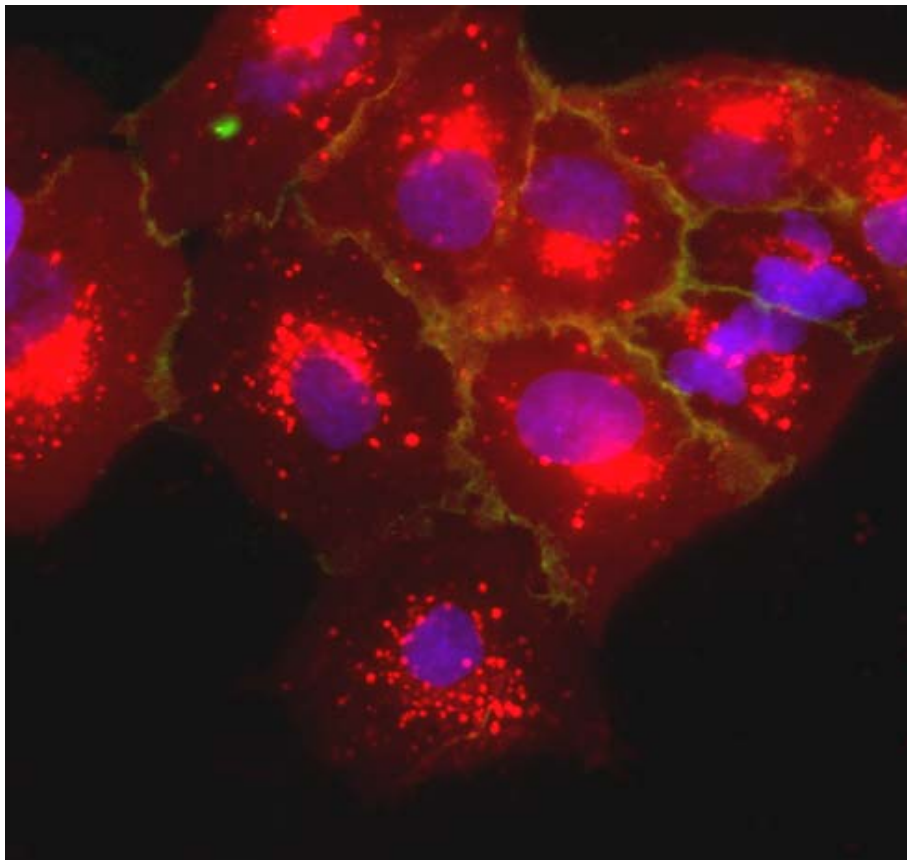
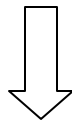
Entry into Cells despite their Length



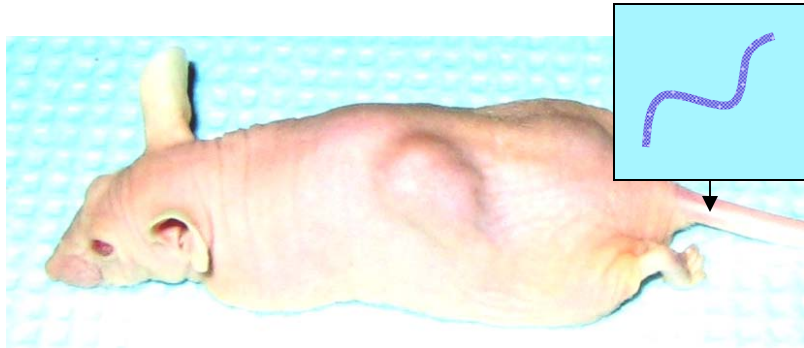
Human Lung Cancer Cell A549



Worm Micelle



Shrinking tumors with Taxol-loaded Filomicelles

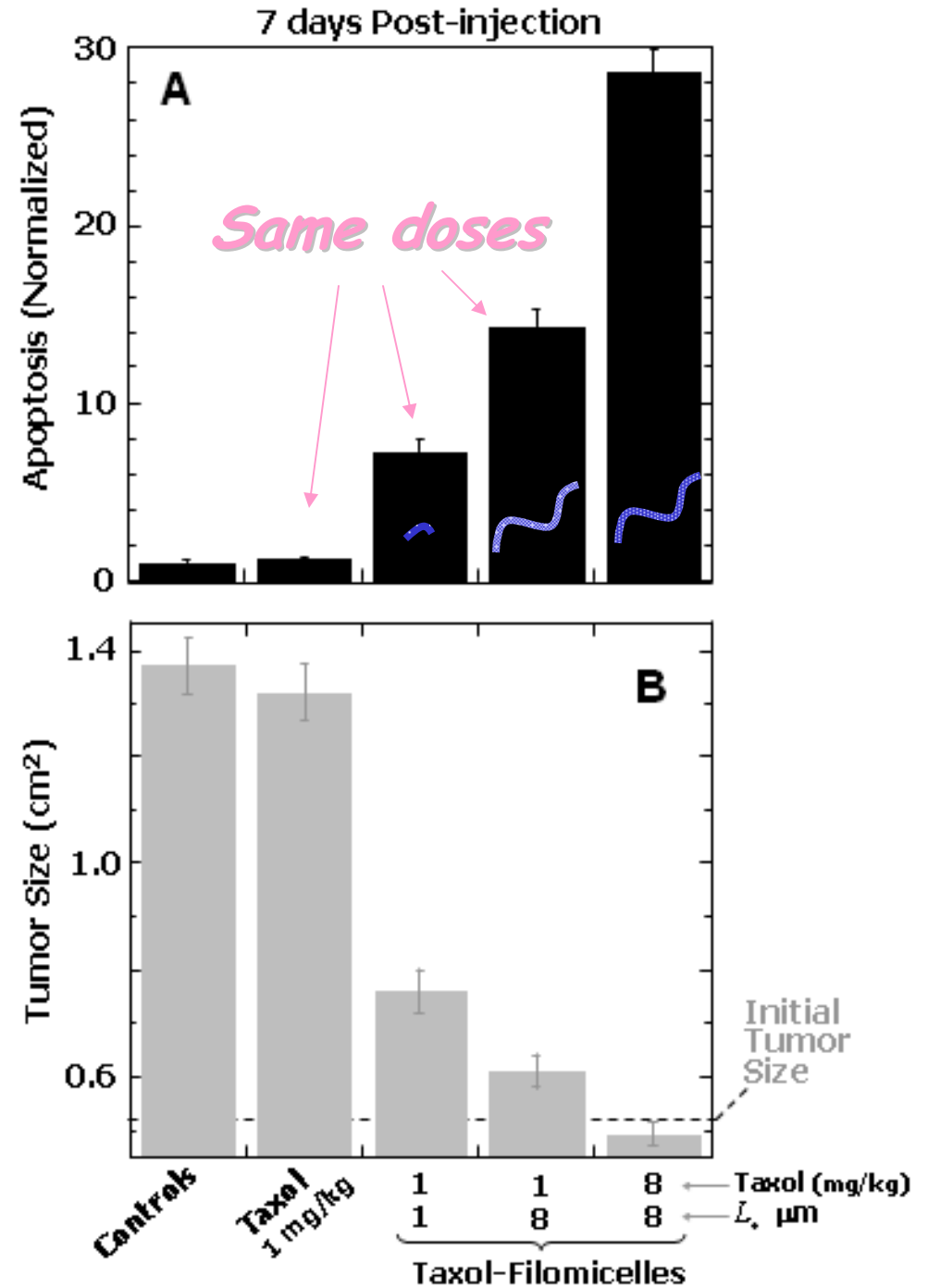


Human lung cancer A549 Xenograft

SHAPE MATTERS!

Effectiveness increases with Length of Filomicelles

Nature Nanotechnology 2007



Polymer Vesicles & Cylinders (amphiphilic tuned copolymers)

≈ idealized 'viral capsids' to test circulation,

cell-polymer interactions,

shape effects in delivery

→ more effective nano-therapeutics



Cell & Molecular Biophysics

David Pajerowski, BE grad Fellow
 Richard Tsai, BE grad. Fellow
 Andre Brown, Physics grad. Fellow
 Chistine Carag, CBE grad. Fellow
 Brian Chase, BE grad. Fellow
 Dr. Adam Engler, Asst. Prof. UCSD
 Dr. Kris Dahl, Asst. Prof. Carnegie Mellon
 Dr. Shamik Sen, UC Berk. Post-Doc. Fellow
 Dr. Shyam Subramanian, Merck
 Dr. Nishant Bhasin, Amgen
 Dr. Vanessa Ortiz, U.Wisc. Post-doc.
 Dr. Richard Law, Amgen
 Dr. Maureen Sheehan, Selectix
 Dr. Manu Tewari, Res. Assoc.
 Dr. Xiulian Du, Res. Assoc.
 Dr. Florian Rehfeldt, Physics Post-doc
 Dr. Colin Johnson, U.Wisc. Post-doc.

Sandy Harper, Wistar Institute
 Prof. D.W. Speicher, Wistar Institute
 Prof. Kevin Healey
 Prof. H.L. Sweeney, Physiology

Polymer vesicles & Filomicelles

David Christian, CBE grad. fellow
 Takamasa Harada, CBE grad. fellow
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 Dr. Fariyal Ahmed, Harvard Post-doc. Fellow
 Dr. Paul Dalhaimer, Yale Post-doc. Fellow
 Dr. Yan Geng, Asst. Prof. Univ. Georgia
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Dr. Goundla Srinivas, IBM-Almaden
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NSF-MRSEC, NSF-PECASE, Whitaker Fdn, NIH-R01,
 NIH-R21, Musc. Dystrophy Assocn, NanoTech Inst.,
 Am. Heart Assocn, IME-NIH training grant, IGERT, GAANN

