

NANOTECHNOLOGIES ET INTERACTION AVEC LES TISSUS

Aspects pharmaco-toxicologiques et
biopharmaceutiques

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Professeur au Collège de France

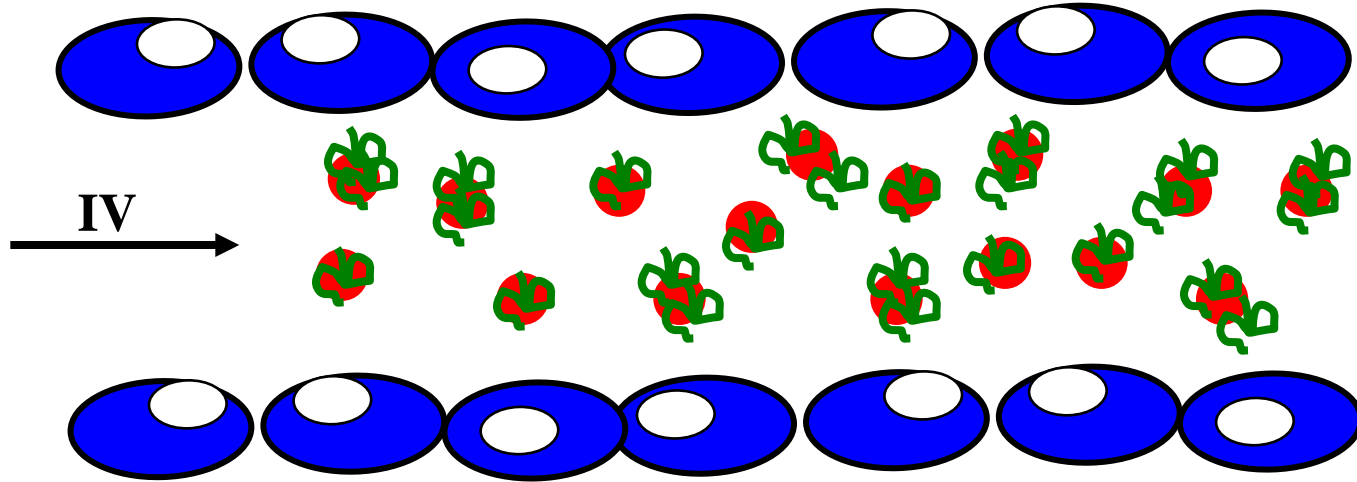
Chaire d'Innovation Technologique

2009-2010

BIODISTRIBUTION APRES ADMINISTRATION INTRAVEINEUSE

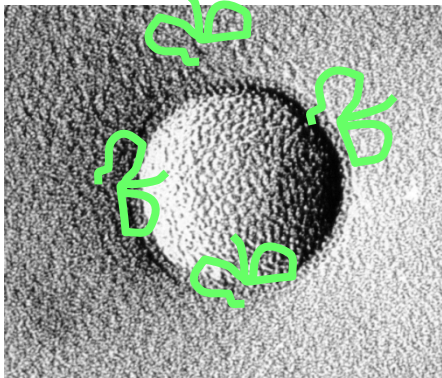
Opsonisation et capture hépato-
splénique

OPSONISATION

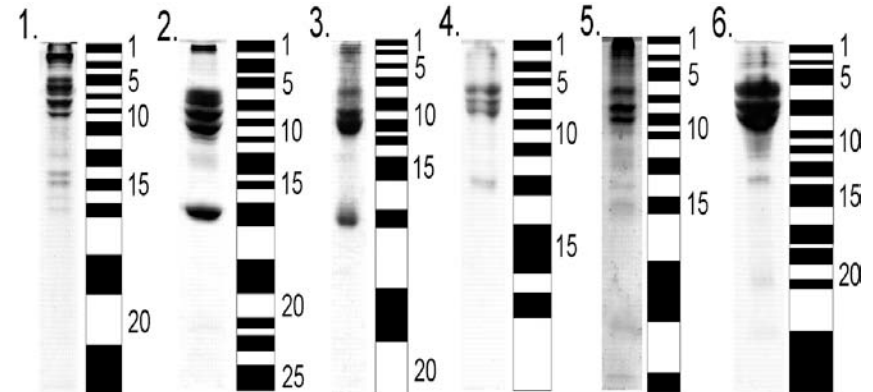
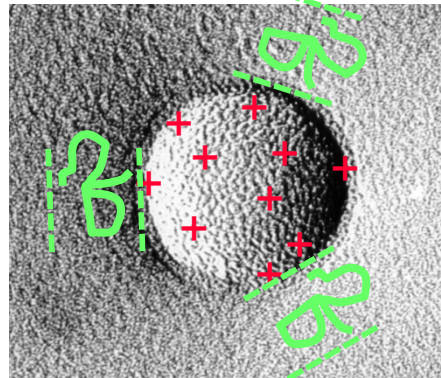


OPSONINES
Fragment Fc des IgG,
Fibronectine,
éléments du complément

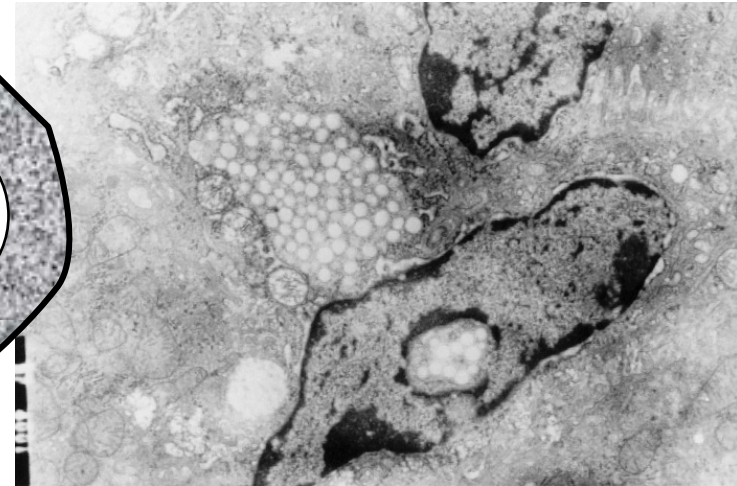
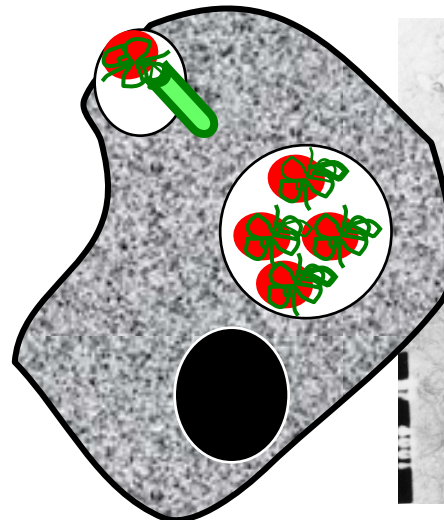
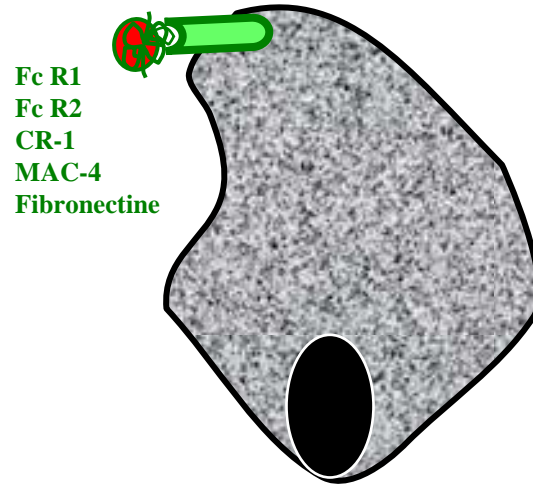
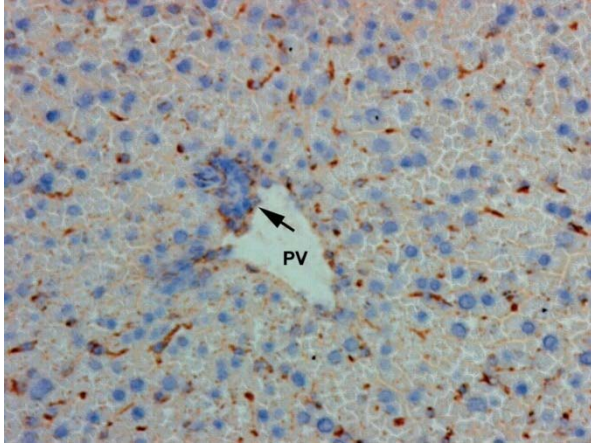
Interactions hydrophobes



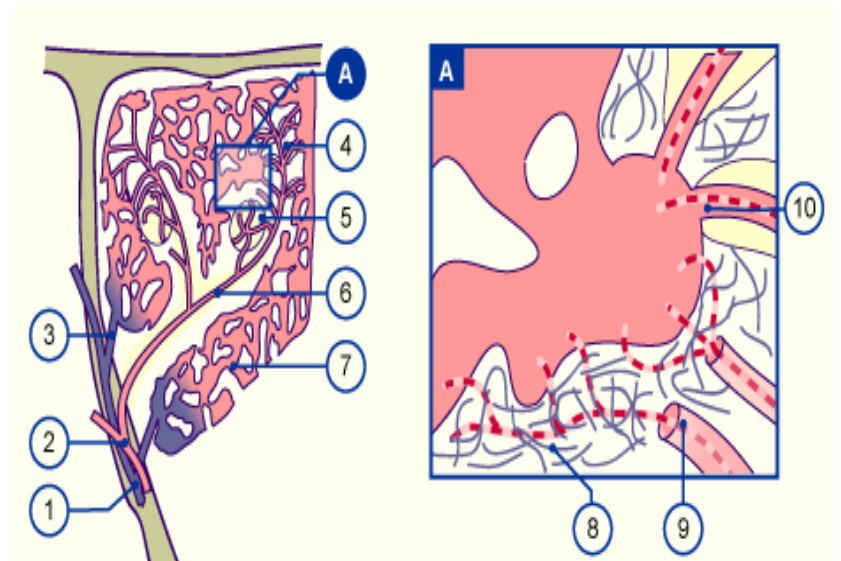
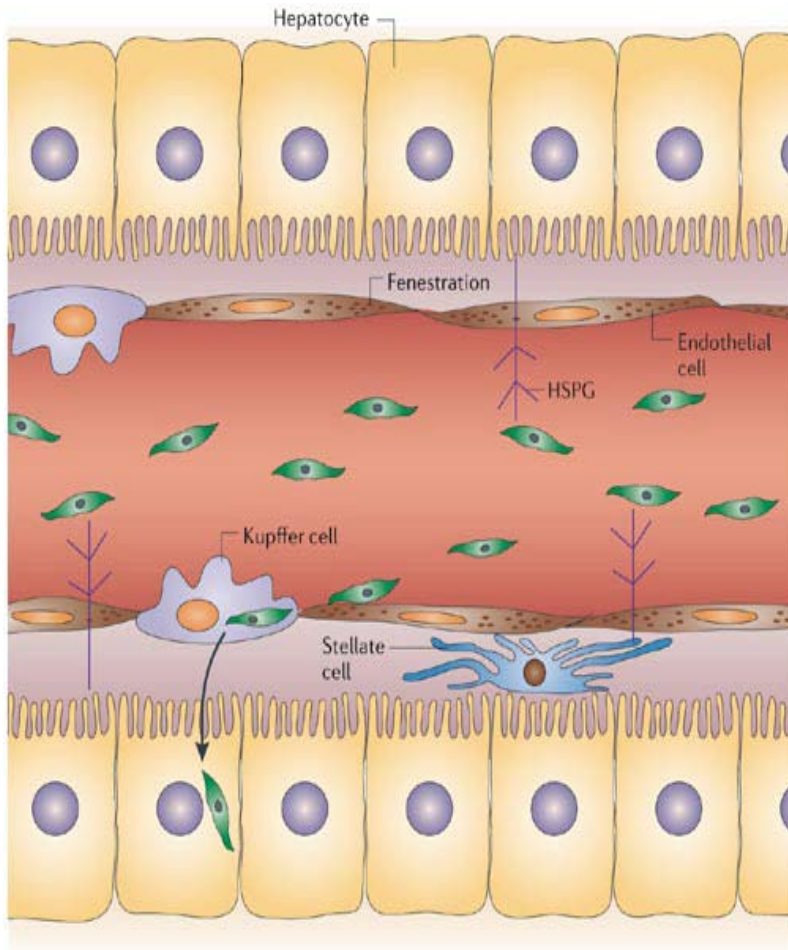
Interactions ioniques



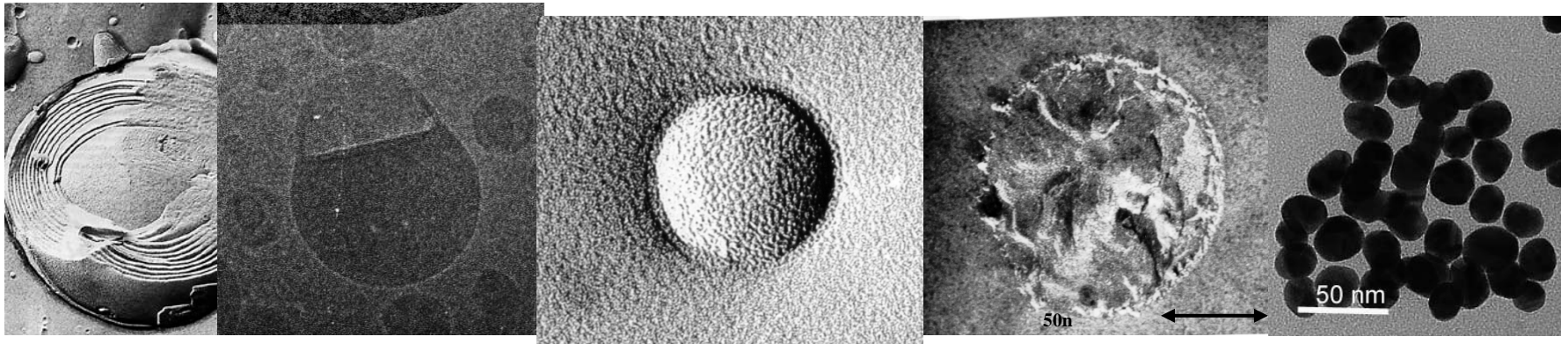
MACROPHAGE CAPTURE



LIVER AND SPLEEN ENDOTHELIUM



NANOCARRIER'S BIODISTRIBUTION

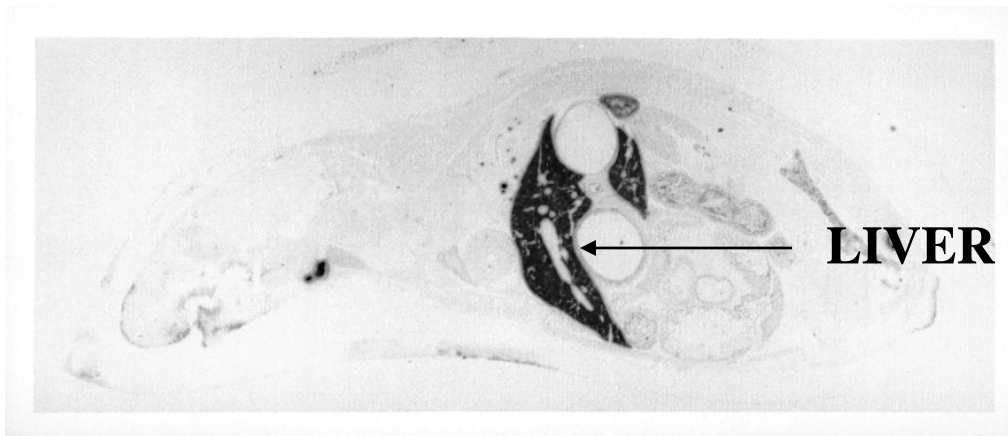


Liposome « Janus » vesicle

nanosphere

nanocapsule

USPIO

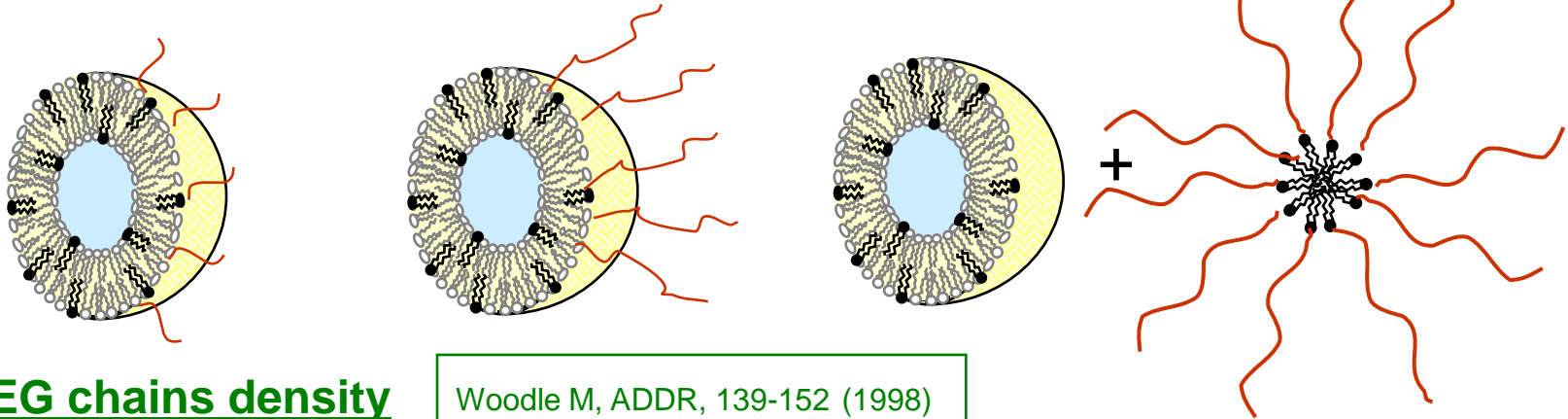


COMMENT EMPÊCHER LA CAPTURE HEPATO-SPLENIQUE?

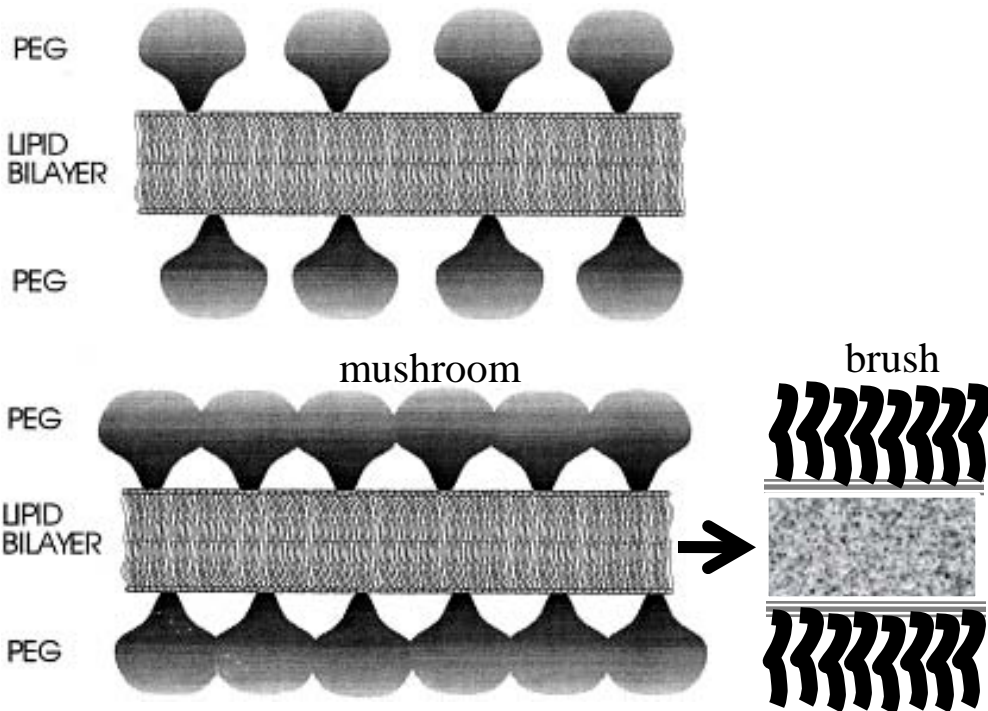
Dysopsonisation et répulsion stérique

IMPORTANT PARAMETERS

PEG chains length (between 2,000 and 5,000 Mw)



PEG chains density

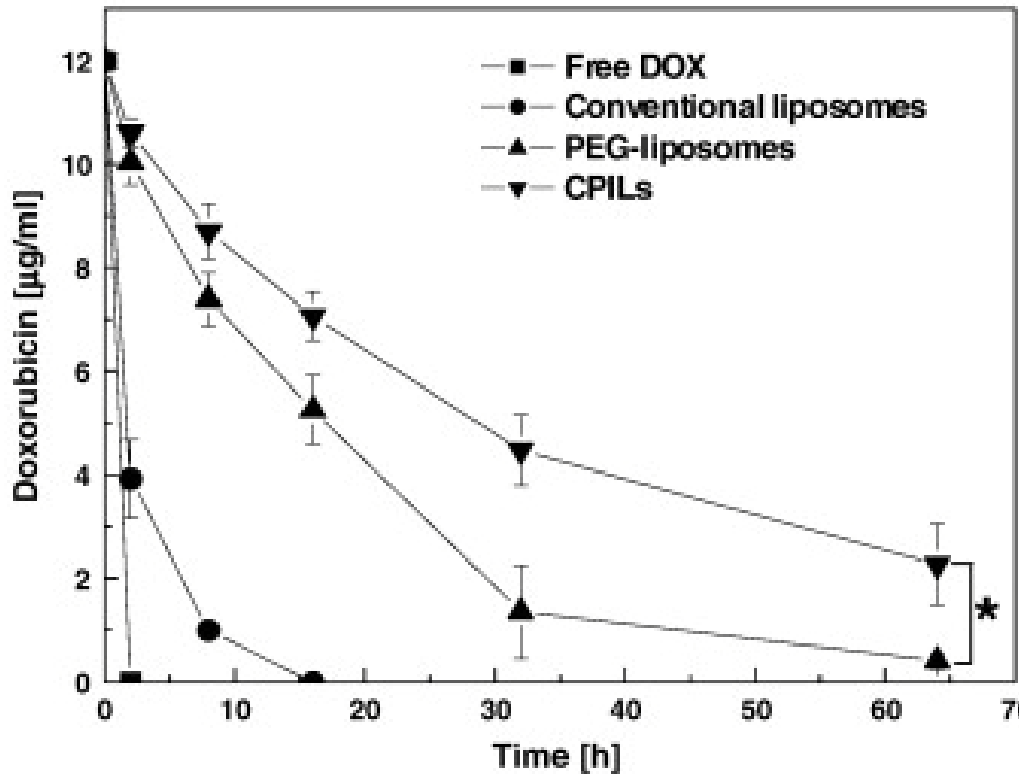
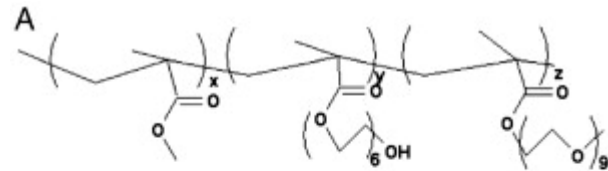


(A) Low grafting density, or mushroom regime, where PEG chains are independent

(B) increased grafting density at transition between mushroom and brush regimes where PEG chains begin to encounter neighbors.

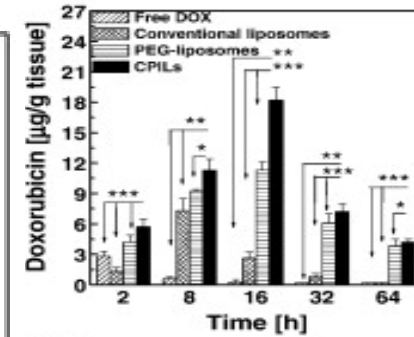
LIPOSOMES ENRICHED WITH COMBLIKE PEG-PMMA POLYMERS

Han et al, JCR, 120, 161-168 (2007)

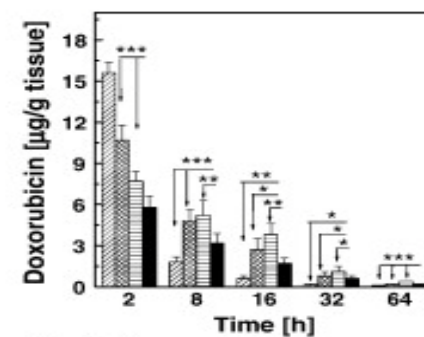


comblike polymer decorated liposomes (CPILs) is composed of hydrophobic MMA and the hydrophilic PEG derivatives

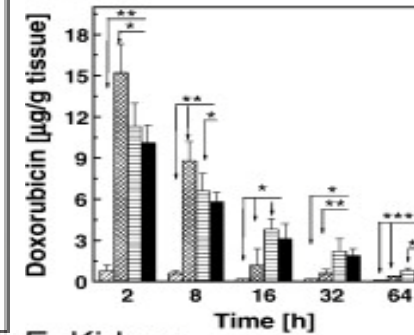
A: Tumor



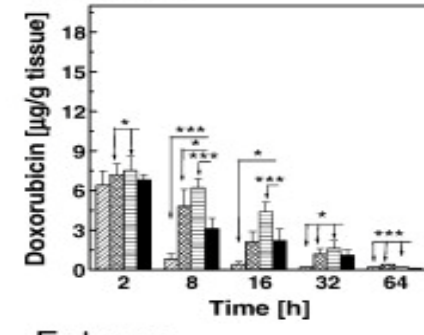
B: Heart



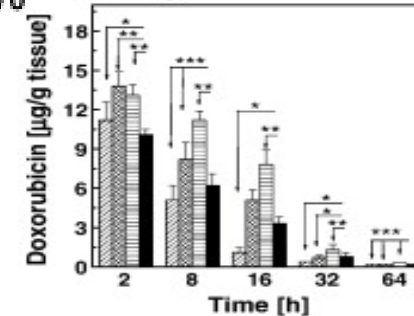
C: Liver



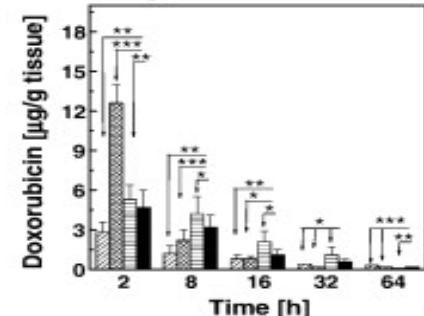
D: Spleen



E: Kidney



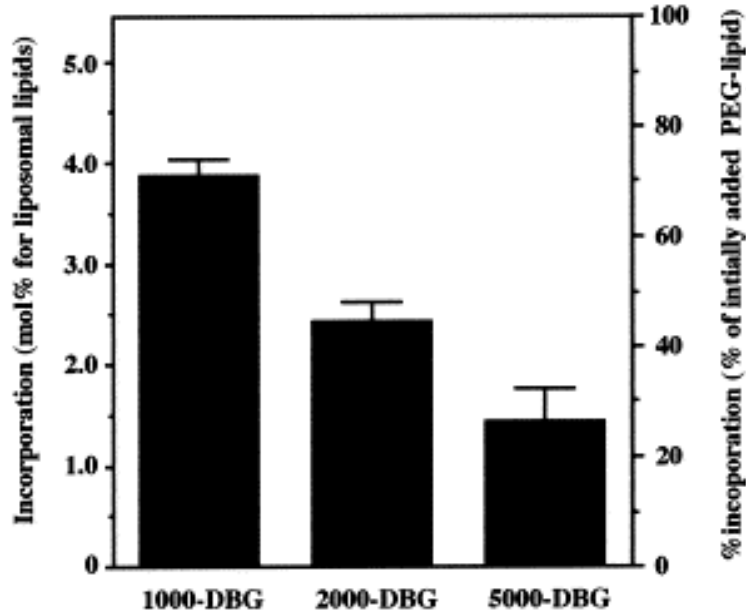
F: Lung



HOW TO STILL INCREASE PEGYLATION ?

PJ. Photos, et al., J Control Rel., 90, 323-334, 2003

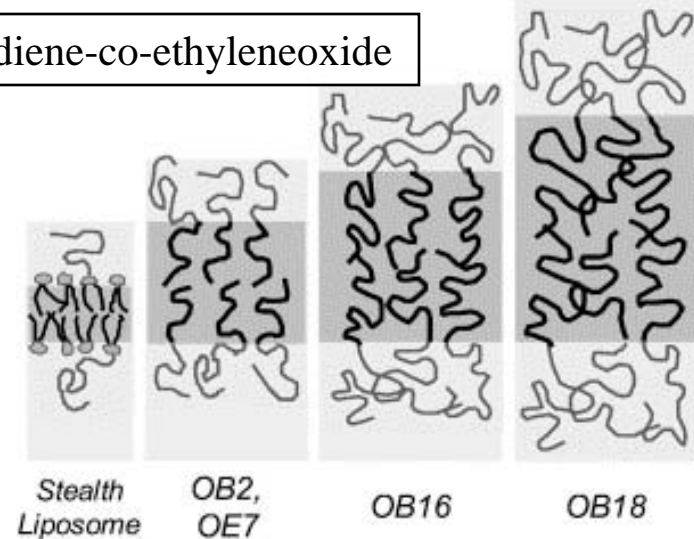
Effect of PEG chain length on incorporation into liposome membranes



Shimada K et al., Internat. J. Pharm., 203 (2000)

Amphiphile	M_n (g/mol)	PEG length (Da)	$f_{\text{hydrophilic}}$ (v/v)	d_{core} (nm) (± 1 nm)	
SOPC	C_{18} phospholipid	790	0	0.31	3
PEG-DSPE	C_{18} phospholipid- EO_x	2790-5790	2000-5000	0.80-0.90	3
OB2	EO_{26} - BD_{46}	3600	1200	0.28	9.6
OE7	EO_{40} - EE_{37}	3900	1840	0.39	8.0
OB16	EO_{50} - BD_{54}	5200	2300	0.37	10.6
OB18	EO_{80} - BD_{130}	10,400	3680	0.29	14.8

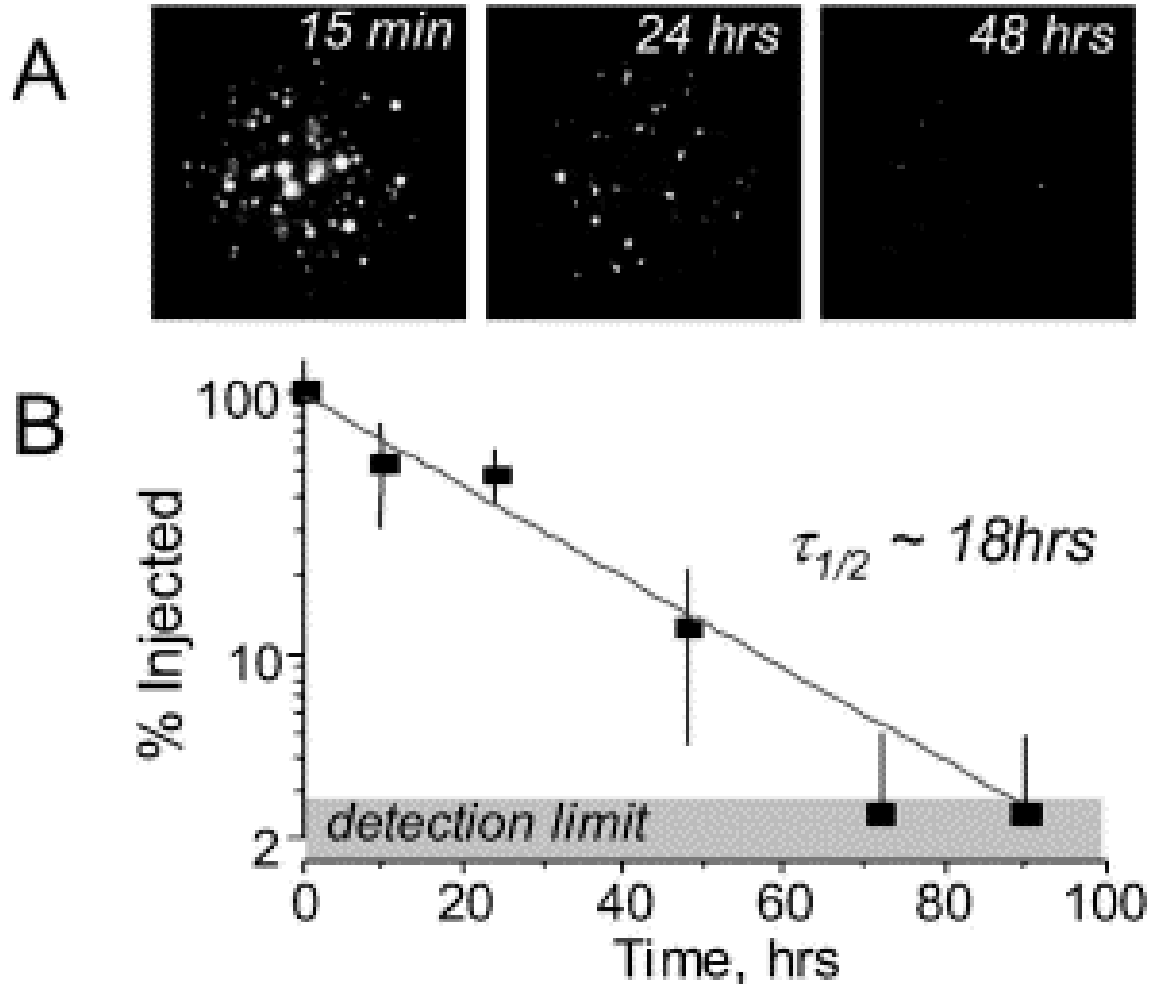
Polybutadiene-co-ethyleneoxide



Stealth liposomes contain a small percentage of lipid molecules (5–10%) that are covalently modified with PEG chains of 2–5 kDa. The 'OB' and 'OE' polymersome membranes consist of diblock copolymers with a PEG chain on every molecule. This effectively gives 100% **PEGylated** vesicles.

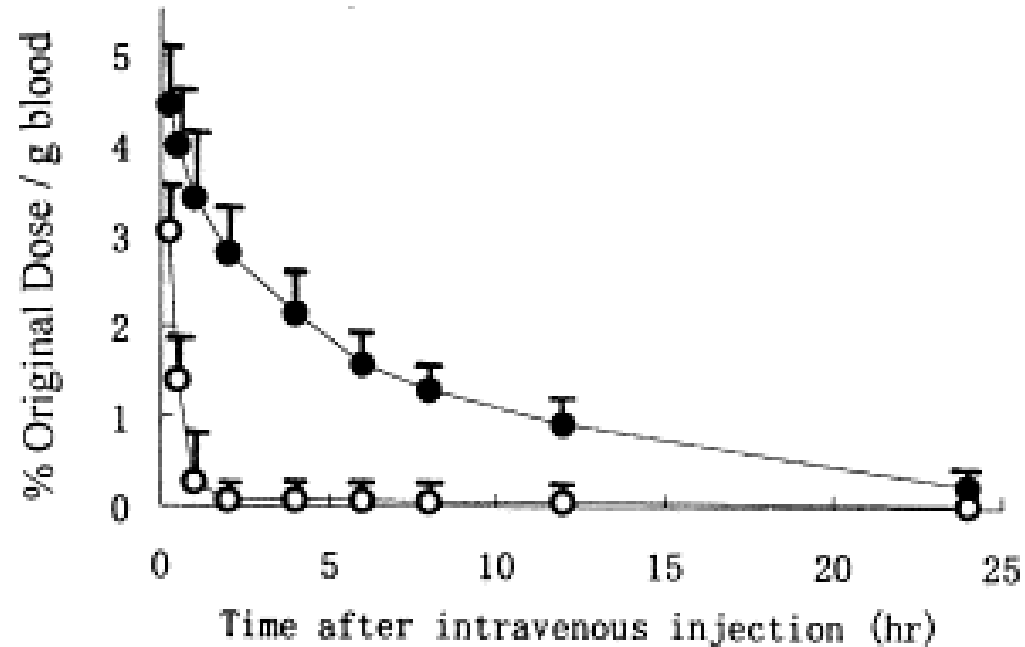
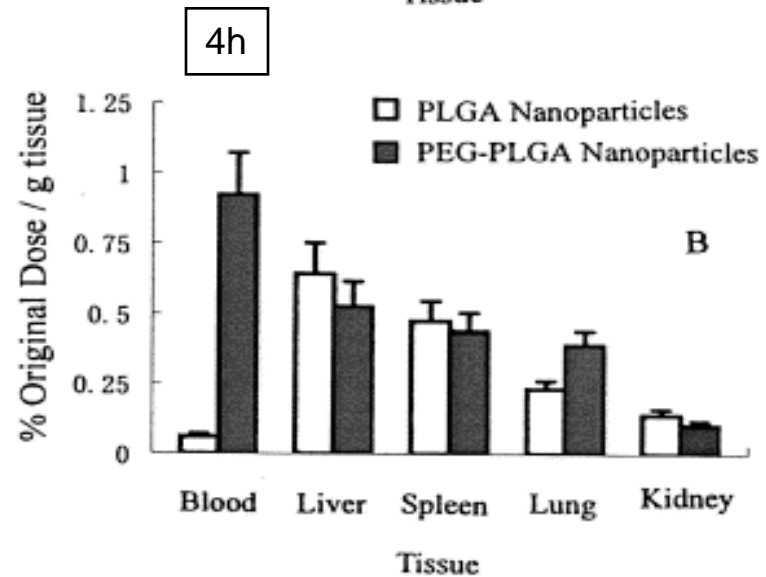
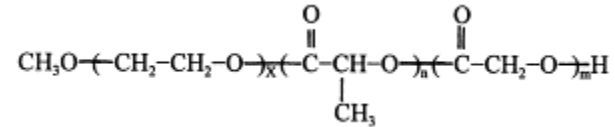
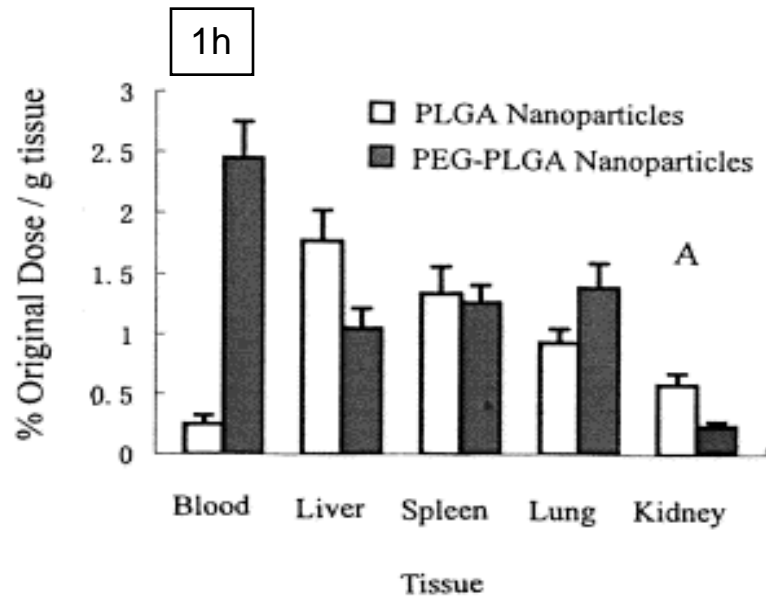
« STEALTH » POLYMEROSOMES

P.J. Photos, et al., J Control Rel., 90, 323-334, 2003



TISSUE DISTRIBUTION OF [125I]BSA IN PLGA and PEG-PLGA NANOPARTICLES

Y. Ping-Li et al., J. Control. Rel., 71, 203-211, 2001

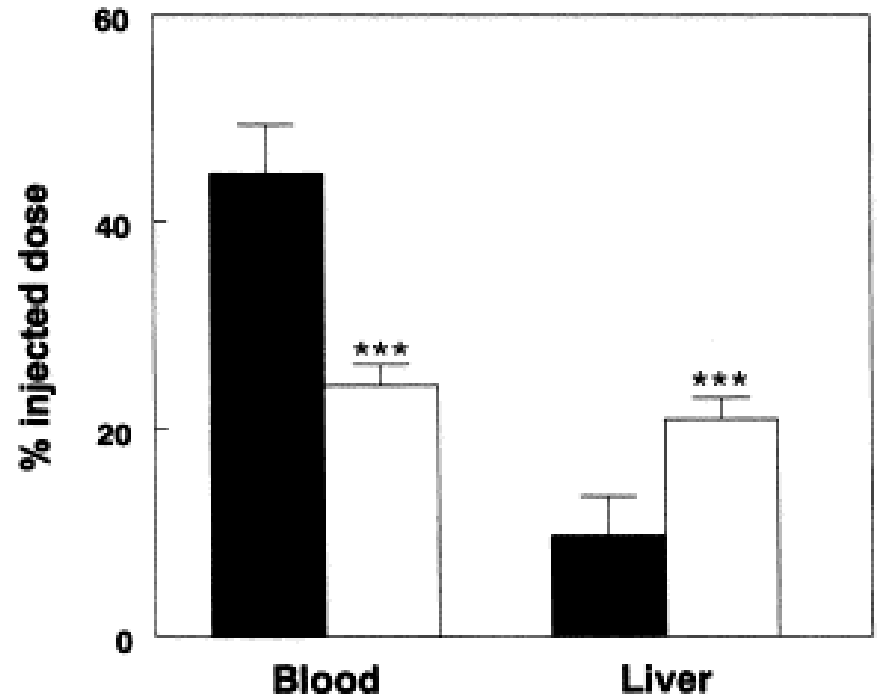
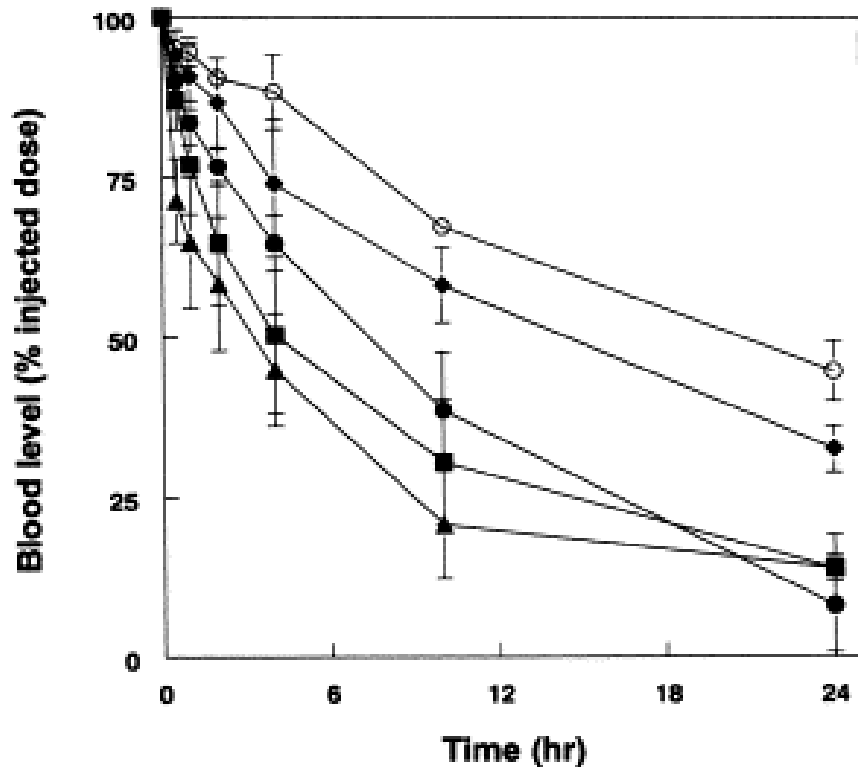


EFFECT OF REPEATED ADMINISTRATION ON BLOOD CLEARANCE

Tatsuhiro Ishida et al., J. Control. Rel., 88, 35-42, 2003

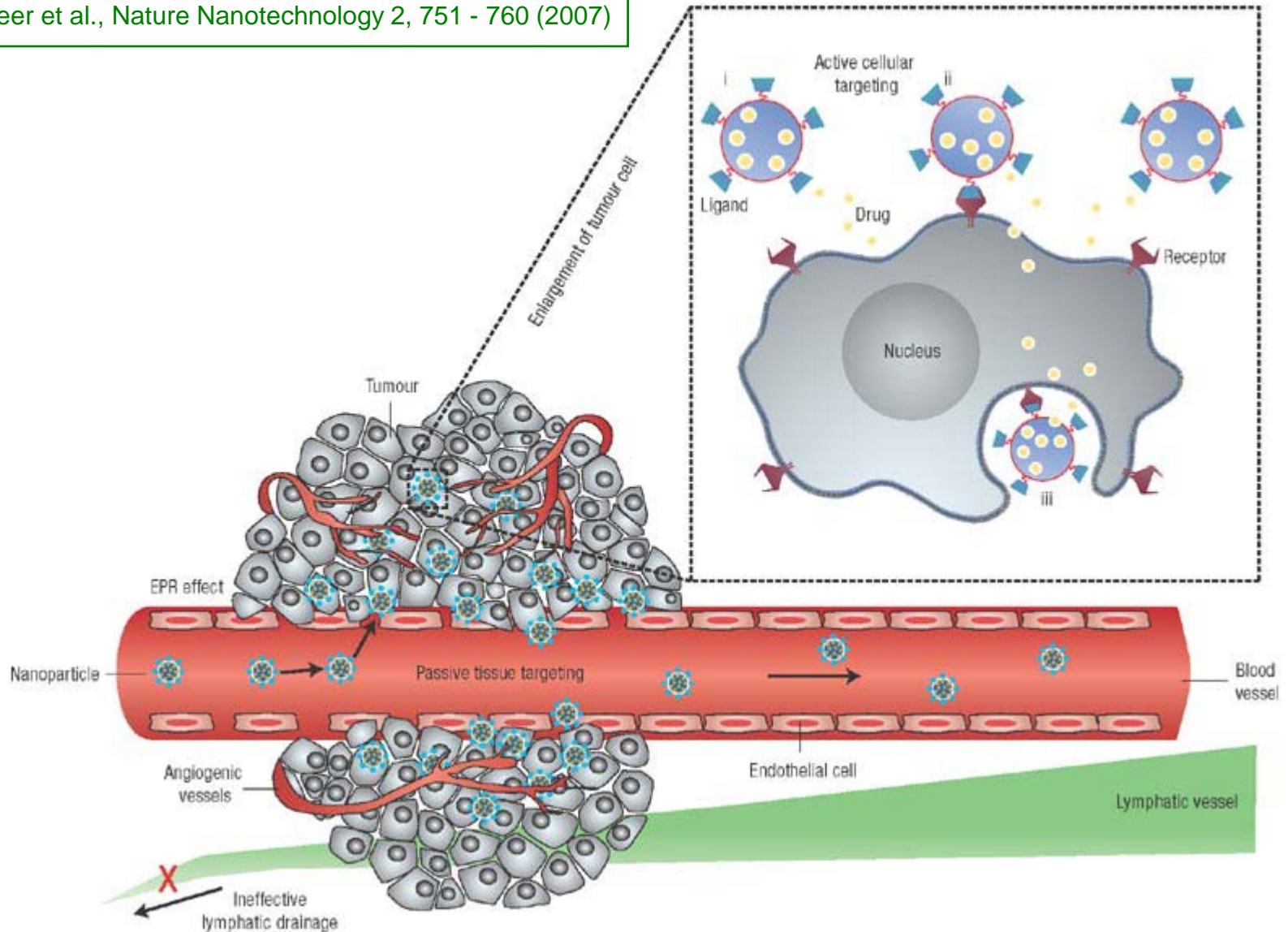
PEGylated liposomes in rats. Control (○), second dose at 3 (●), 5 (▲), 7 (■), and 10 (◆) days post-injection.

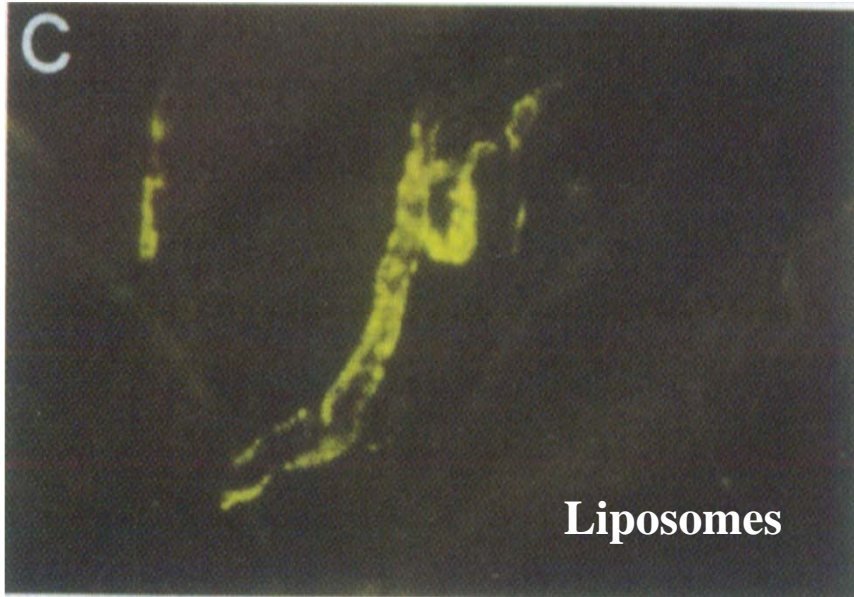
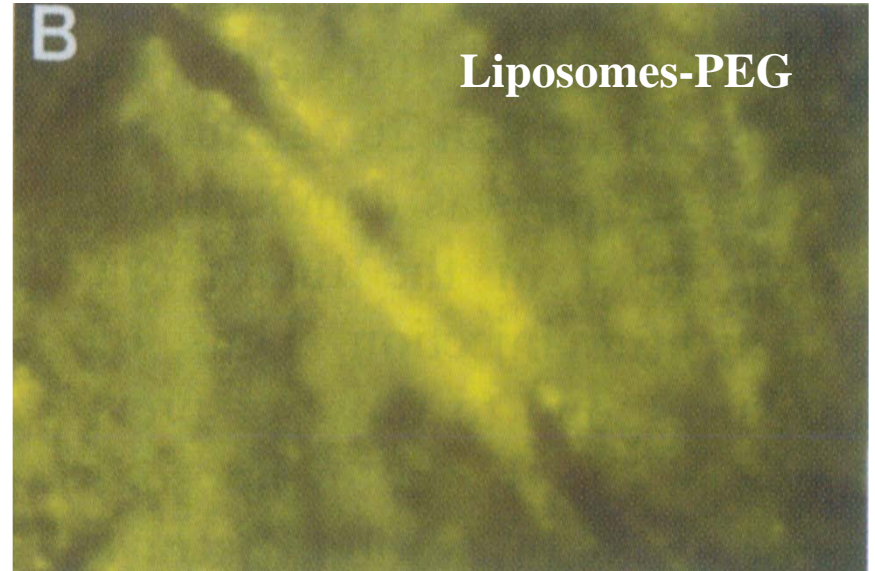
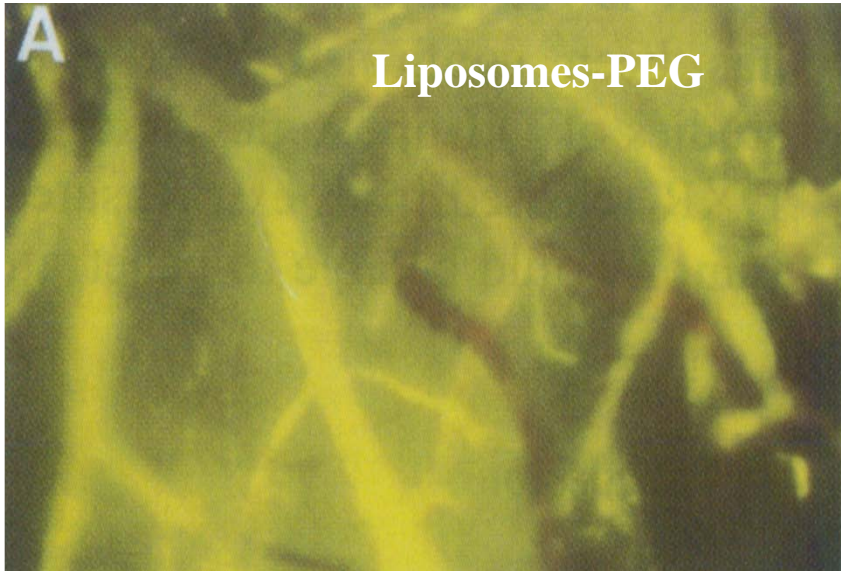
Role of a humoral serum factor in the clearance of a second dose of PEGylated liposomes. Rats were transfused with 1 ml of either normal serum (closed column) or pretreated serum (open column). Immediately after transfusion, [³H]CHE-labeled PEGylated liposomes were injected iv.



ENHANCED PERMEABILITY AND RETENTION EFFECT [« EPR »]

Dan Peer et al., Nature Nanotechnology 2, 751 - 760 (2007)



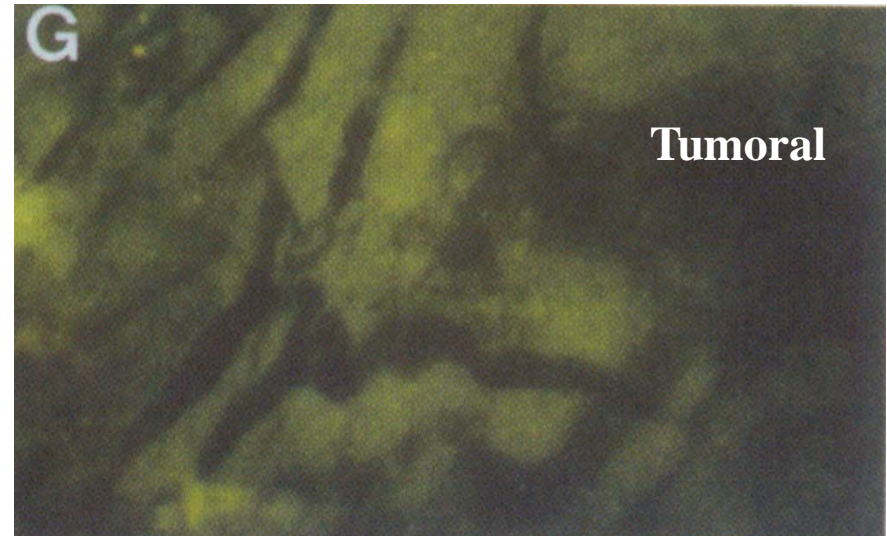
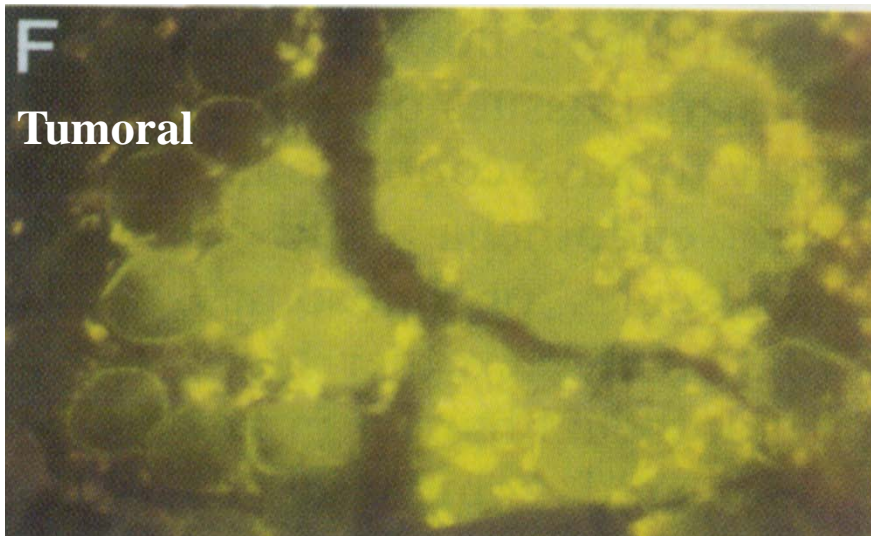
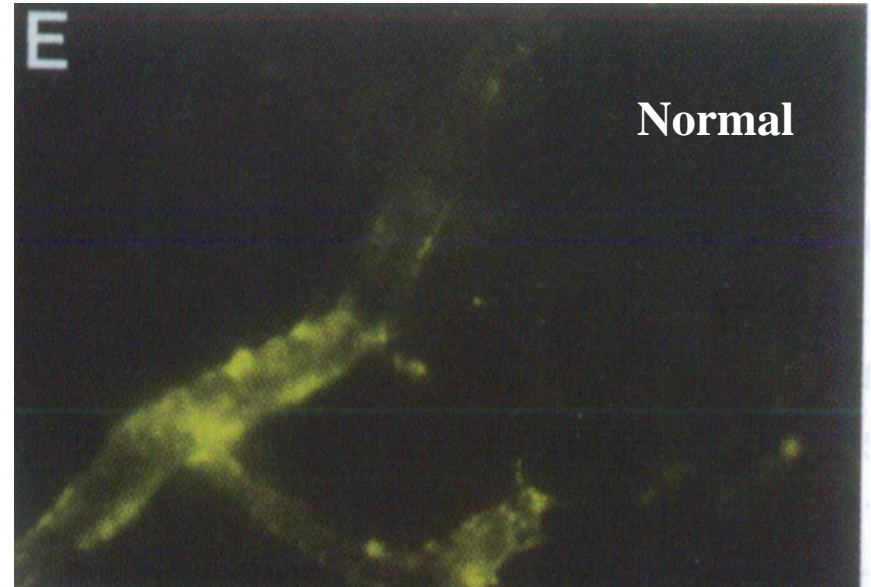
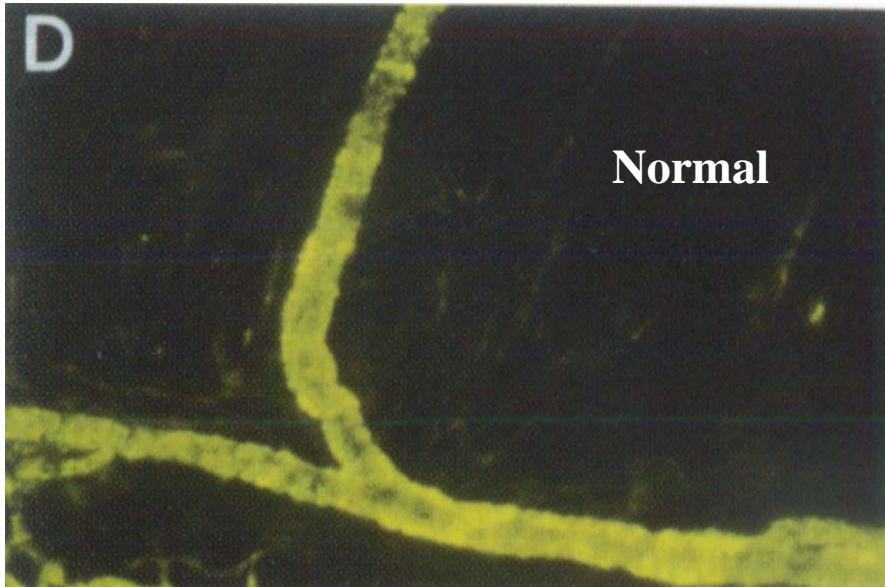


« EPR » EFFECT

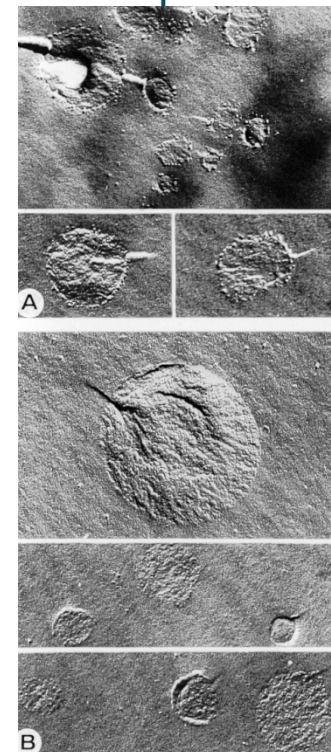
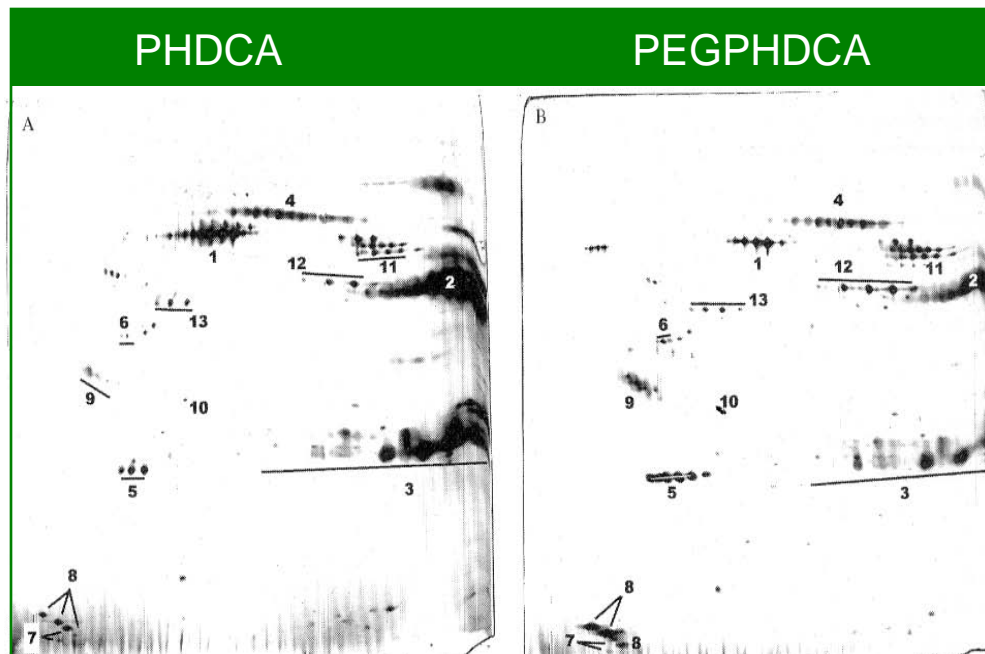
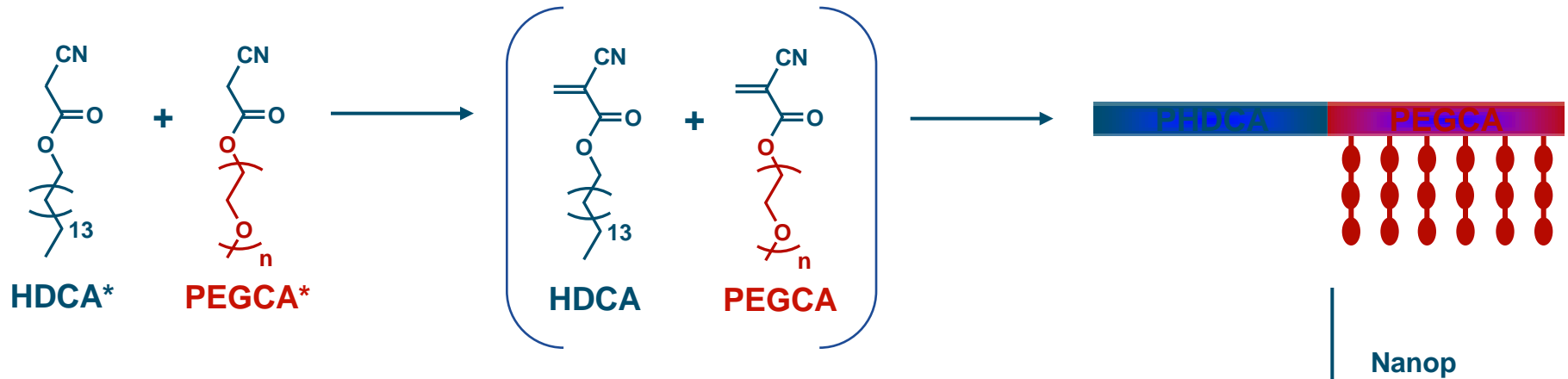
S. Unezaki, et al., Int. J Pharm, 1996

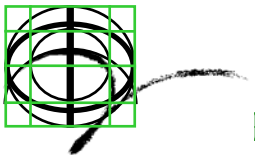
« EPR » EFFECT

S. Unezaki, et al., Int. J Pharm, 1996



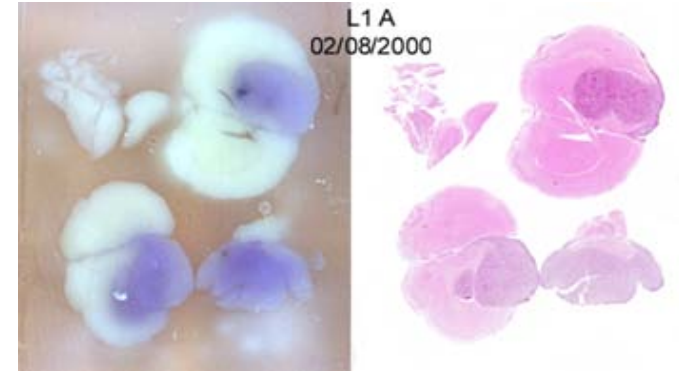
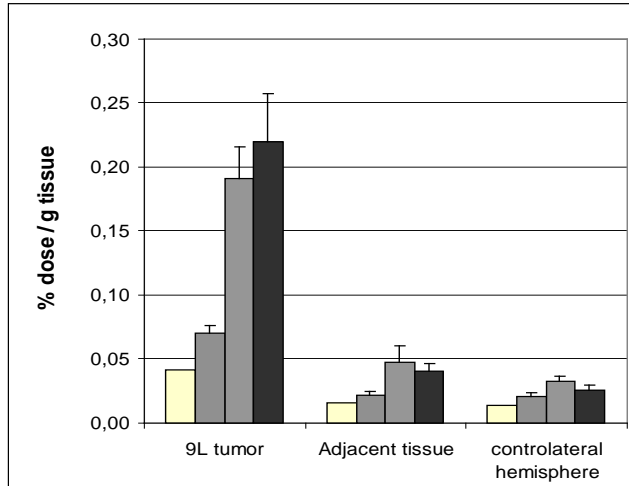
STEALTH NANOCARRIERS (PEG-PHDCA Nanoparticles)





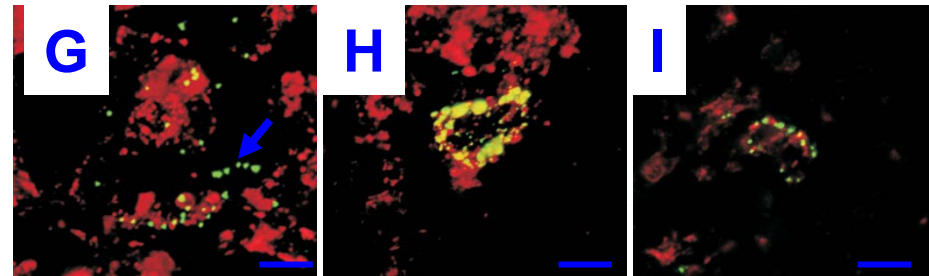
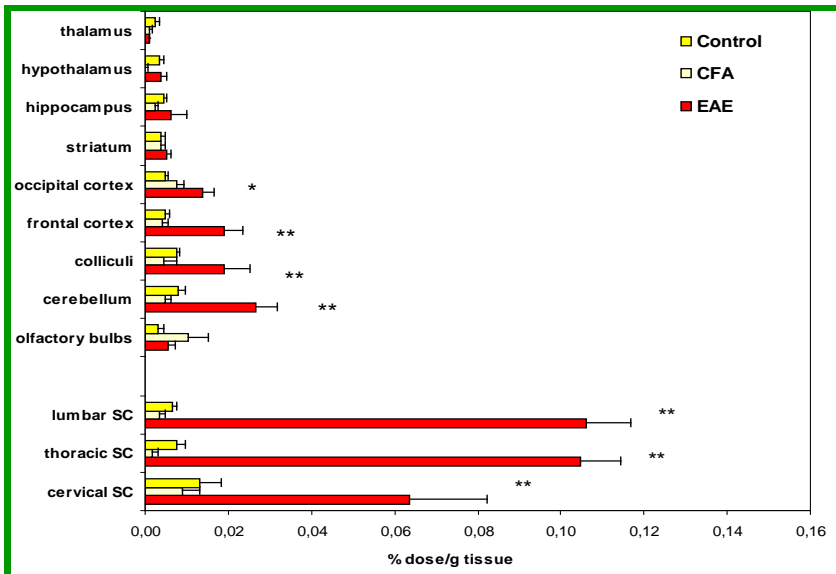
RAIN TRANSLOCATION OF PEG-PACA NANOSPHERES AND BHE DISRUPTION

9L GLIOMA



Brigger et al., *J. Pharmacol. Exp. Ther.*, **303**, 928-936 (2002)

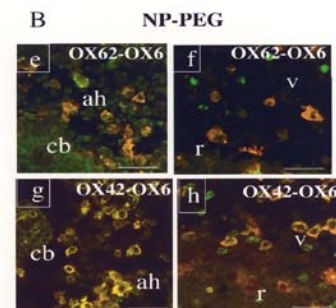
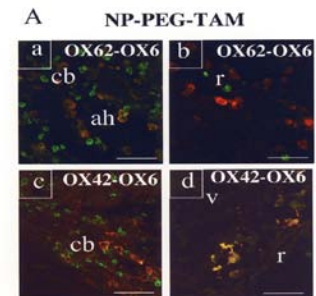
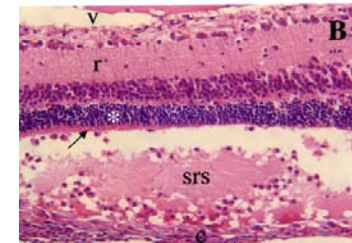
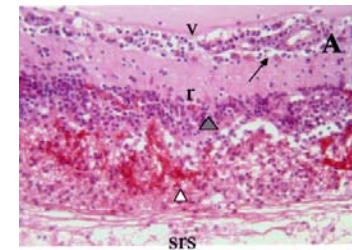
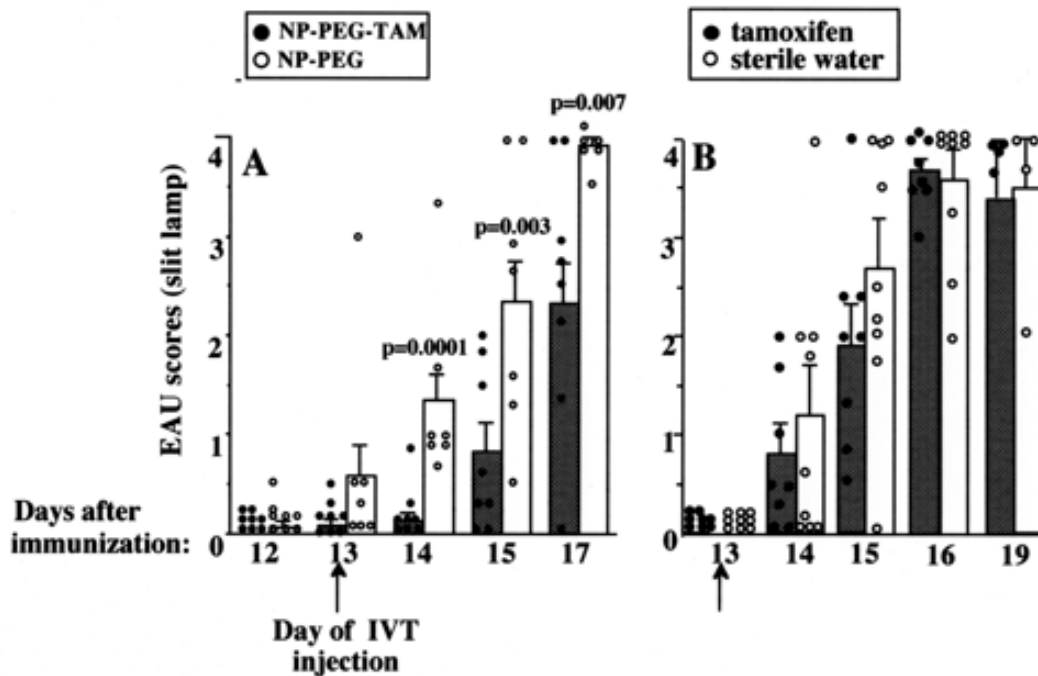
EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS



Calvo, et al., *Europ. J Neurosc.*, **15**, 1317-1326 (2002)

PEG-PACA NANOSPHERES FOR THE TREATMENT OF AUTOIMMUNE UVEITIS

Y. de Kozak et al., *Europ J Immunol.*, **34**, 3702-3712 (2004)

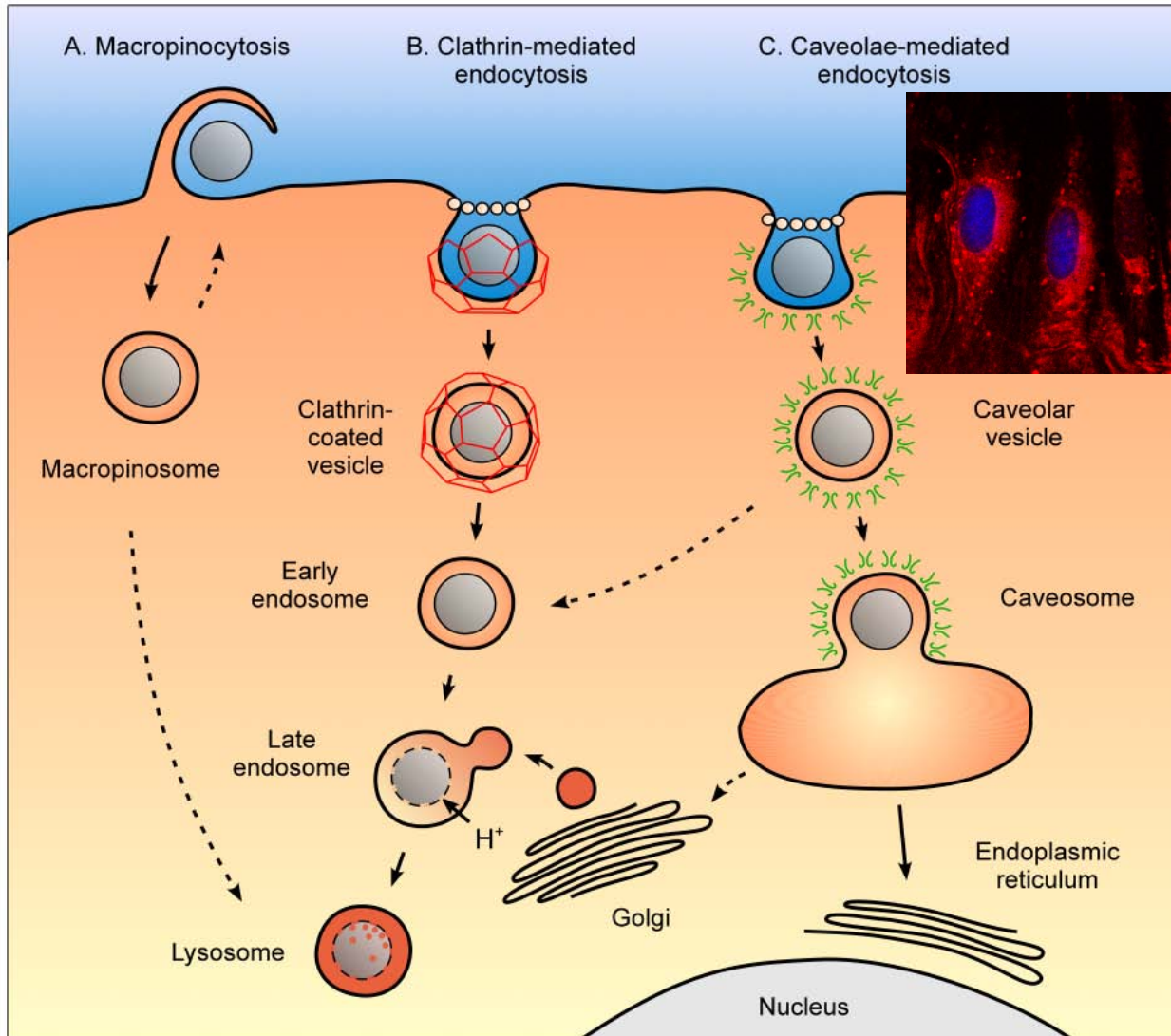


CONSIDERATIONS TOXICOLOGIQUES

- Rechercher les phénomènes d'agrégation susceptibles d'entraîner des phénomènes thromboemboliques à l'administration
- N'utiliser que des matériaux biodégradables et biocompatibles → risques de toxicité par thésaurismose
- Surveiller tous les processus liés à la capture par le RES
- Problématique de l'activation du complément

TRAFIC INTRACELLULAIRE

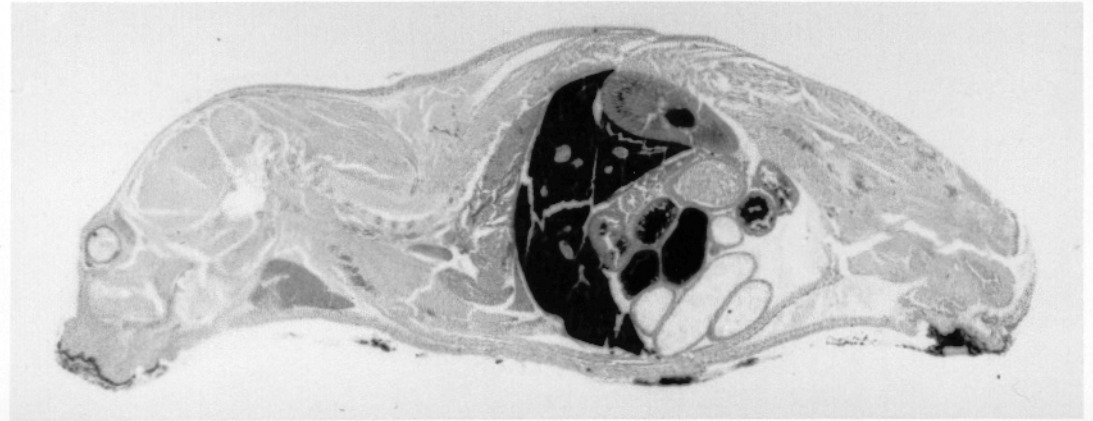
H. Hillaireau and P. Couvreur, CMLS, 2009



REALISATION DU BILAN METABOLIQUE/DISTRIBUTION



Cage à métabolisme



Autoradiographie

Utilisation exclusive de matériaux biodégradables et biocompatibles:

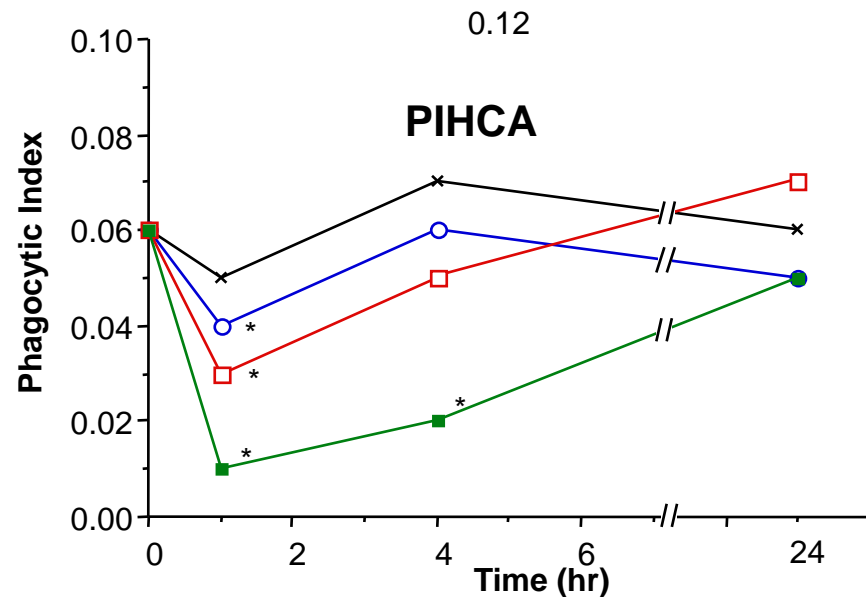
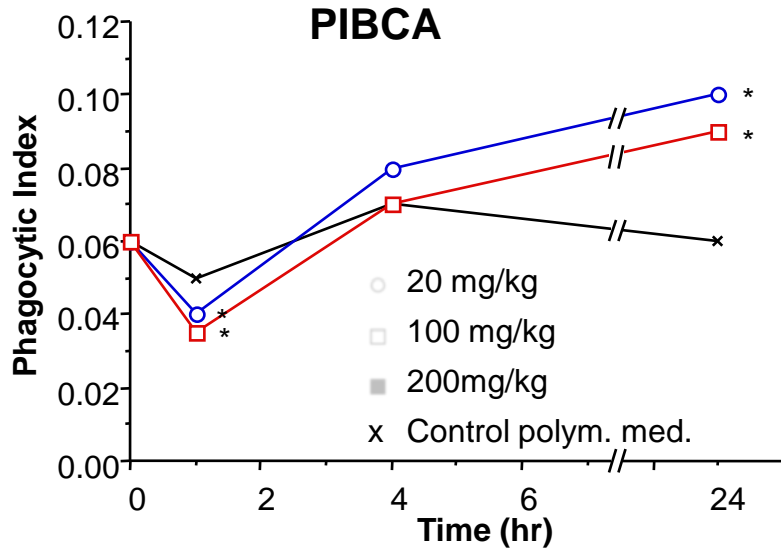
- Phospholipides et autres lipides naturels
- Polymères synthétiques (PLAGA et PACA)
- Polymères naturels (albumine, polyaminoacides, etc.)

CONSIDERATIONS TOXICOLOGIQUES

- Rechercher les phénomènes d'agrégation susceptibles d'entraîner des phénomènes thromboemboliques à l'administration
- N'utiliser que des matériaux biodégradables et biocompatibles → risques de toxicité par thésaurismose
- Surveiller tous les processus liés à la capture par le RES et le foie
- Problématique de l'activation du complément

EFFECT ON PHAGOCYTOTIC FUNCTION

Urussano et al., J Biomed Mater Res., 31, 401-408 1996

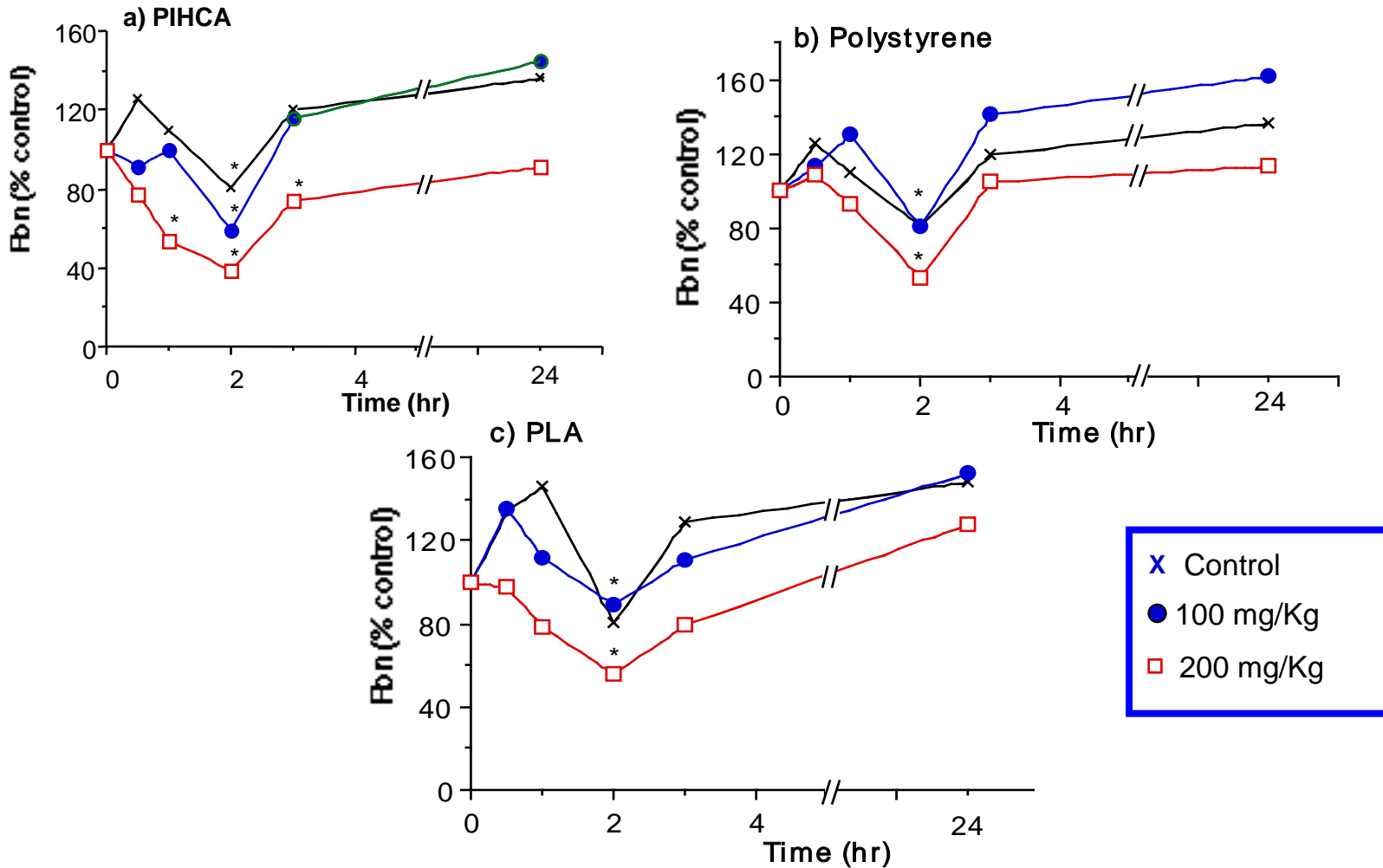


Injection of carbon particles by IV
 Blood sampling
 Analysis of carbon particles at 650
 nm

$$\text{Phagocytic Index} = \frac{\ln C_1 - \ln C_2}{t_2 - t_1}$$

PLASMA FIBRONECTIN AFTER IV ADMINISTRATION OF NANOPARTICLES

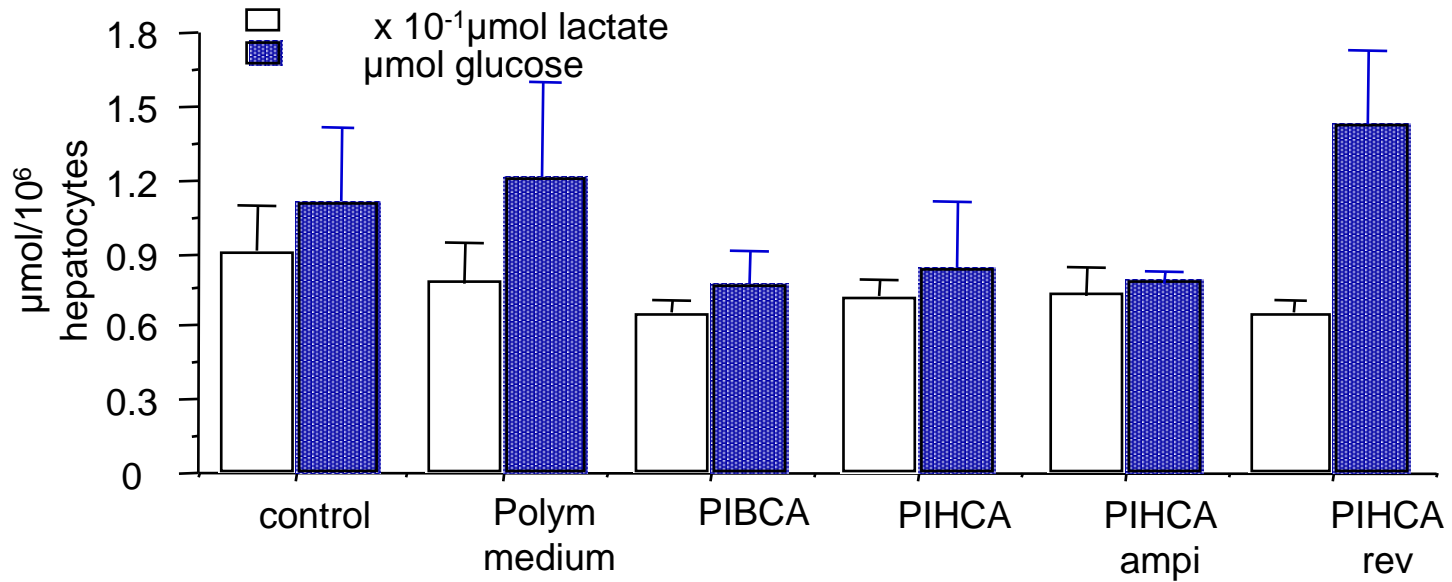
Urussono et al., J Biomed Mater Res., 31, 401-408 1996



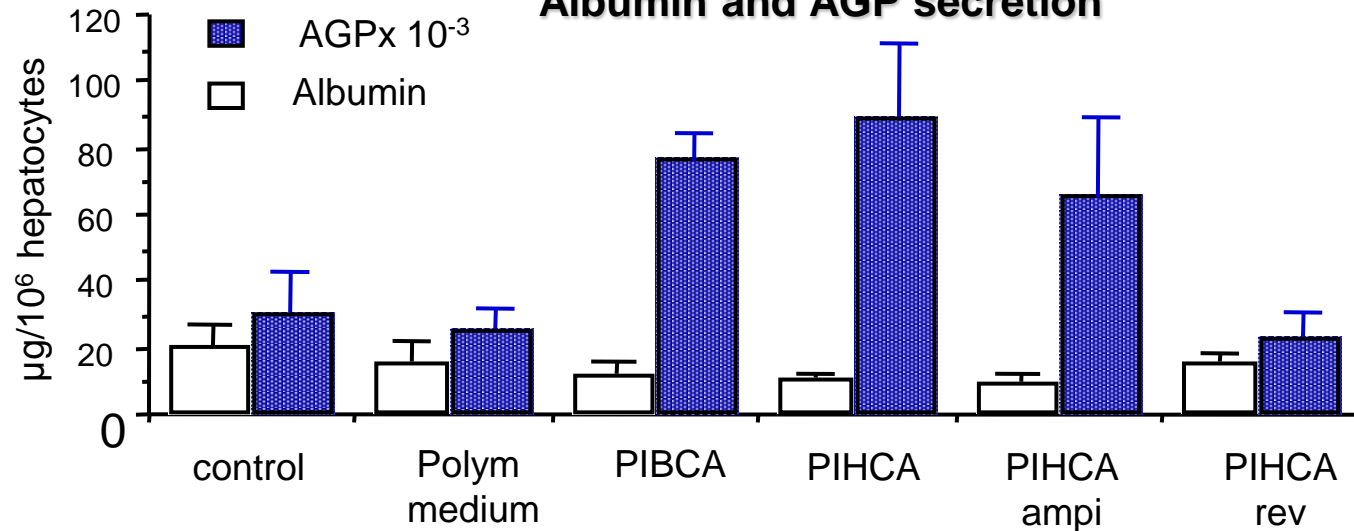
INVESTIGATION OF HEPATOCYTE RESPONSE TO NANOPARTICLES INJECTION

- ❖ Model: Rat
- ❖ Hepatocytes isolated by collagenase perfusion
- ❖ Treatment: Acute (1) or Chronic (repeated) administration
- ❖ Evaluation of glucose and lactate production from fructose metabolism
- ❖ Evaluation of albumin and α 1acid glycoprotein secretion

Glucose and Lactate synthesis from fructose



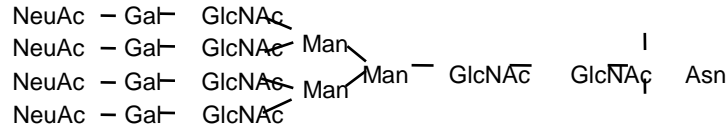
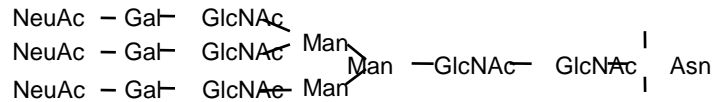
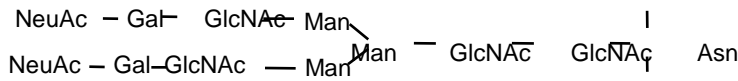
Albumin and AGP secretion



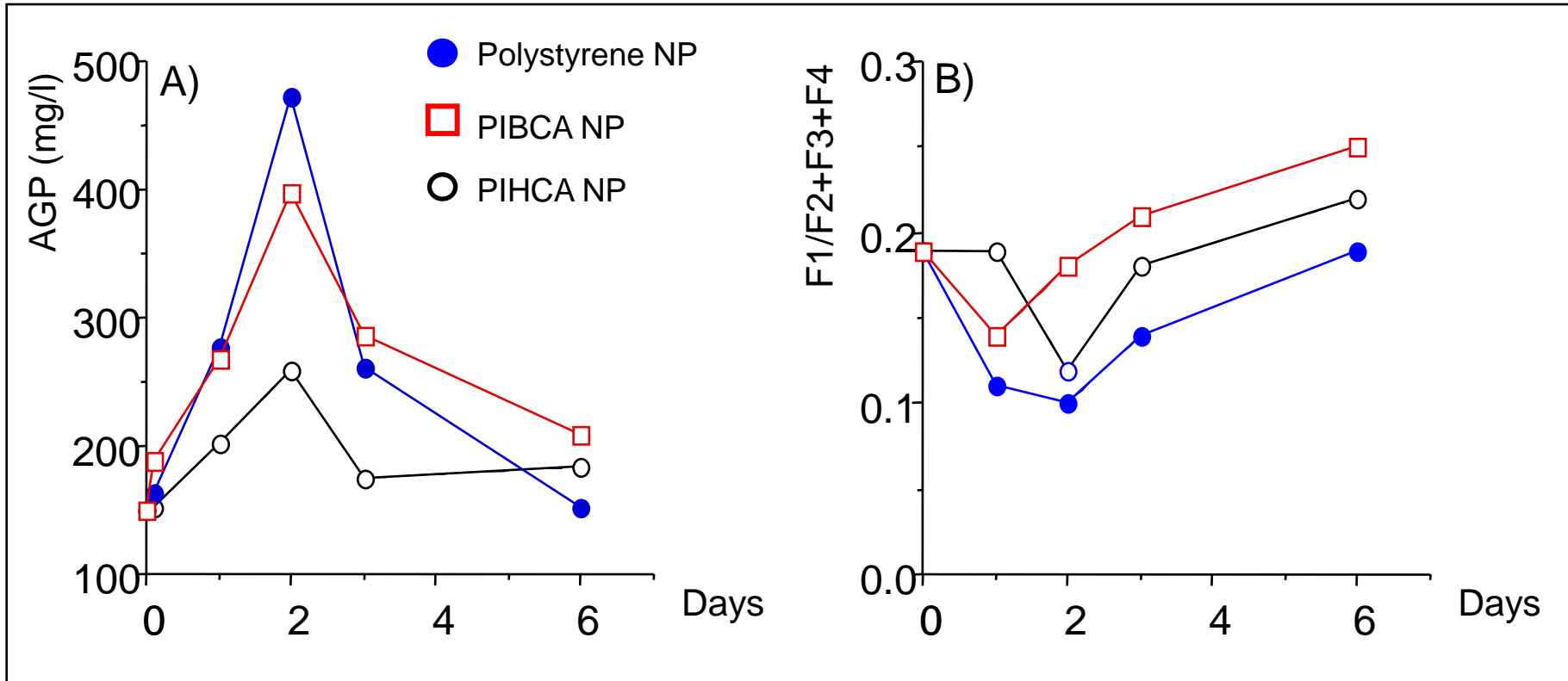
STRUCTURE OF α 1 GLYCOPROTEIN

(Crossed immunoaffinoelectrophoresis with Concanavalin A)

Fernandez-Urrusuno et al., Toxicol Appl Pharmacol., 130, 272-279 1995

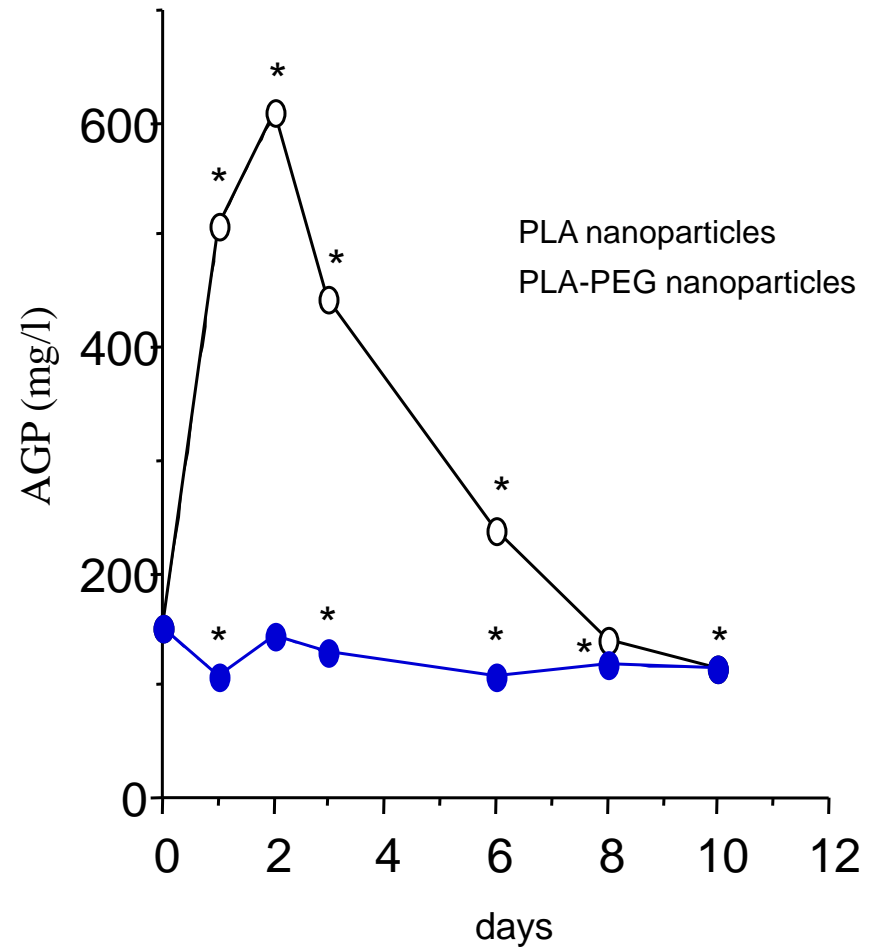
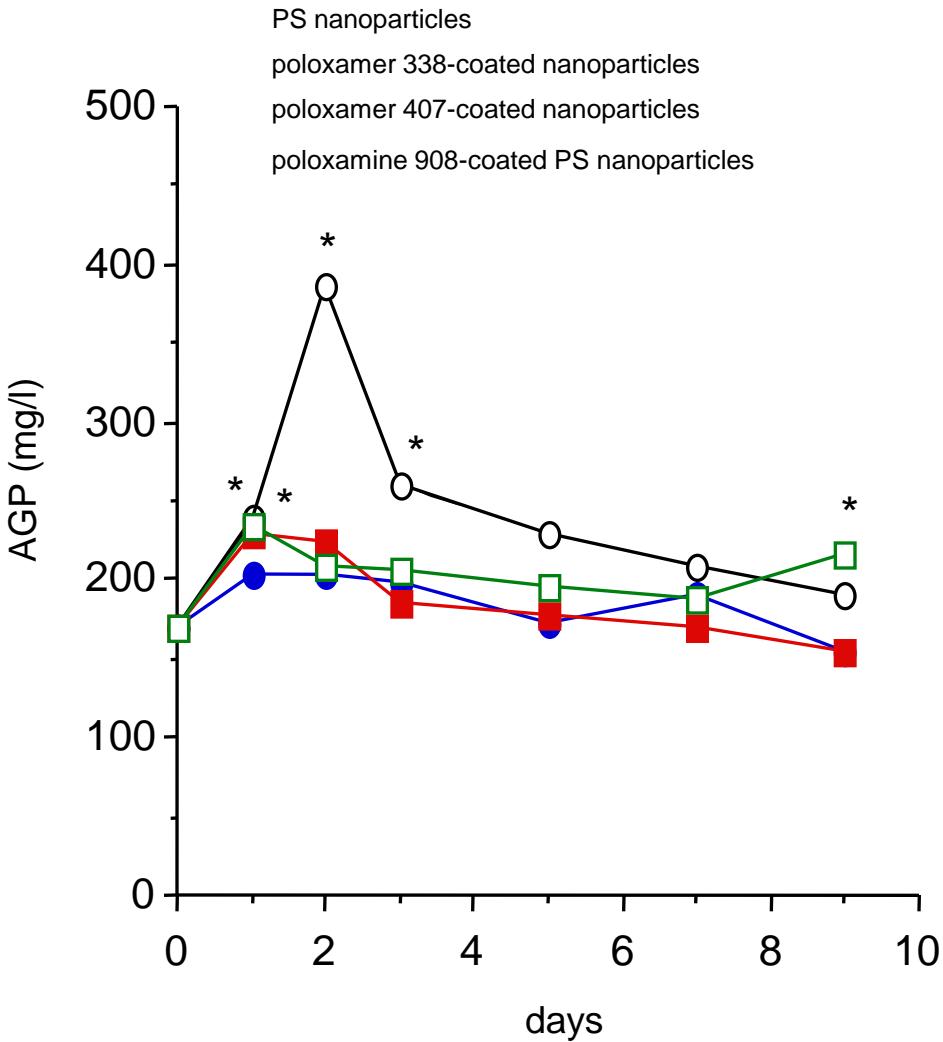


- **Inflammation**
- **Liver deficiency**
- bi-antennary AGP (F4)**
- tri- tetra-antennary AGP (F1)**
- hyposialylation**



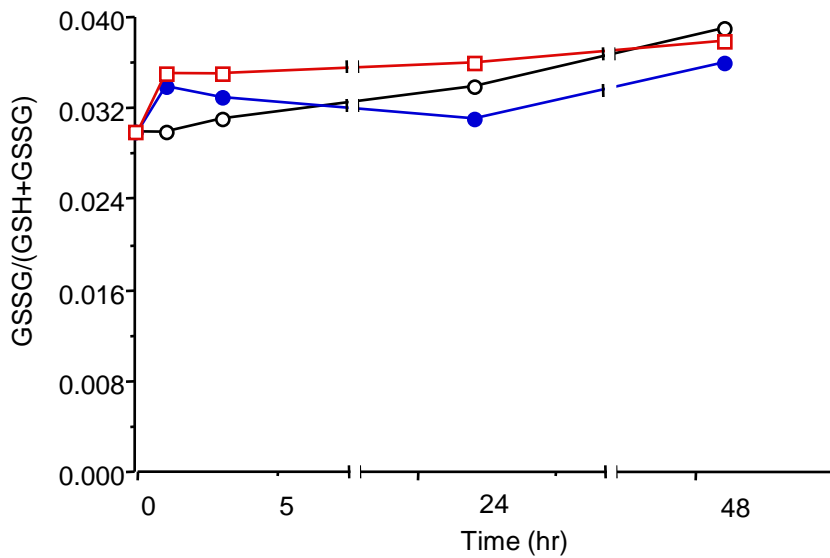
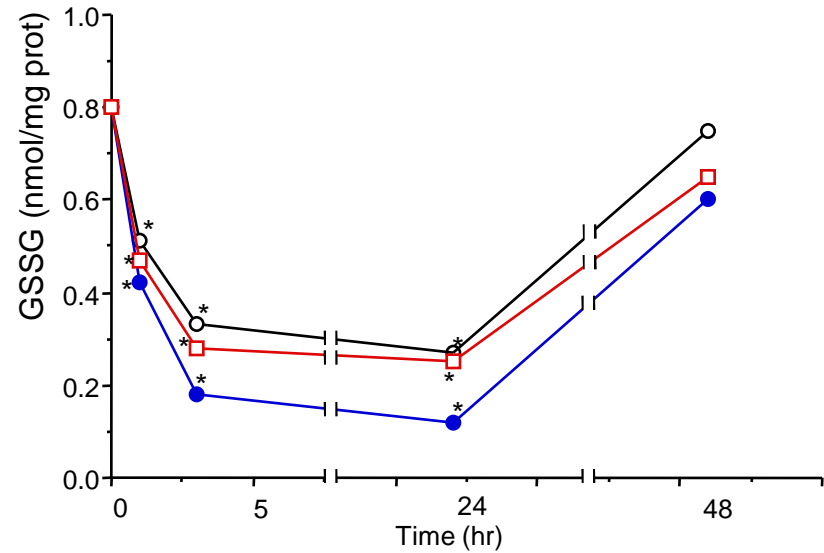
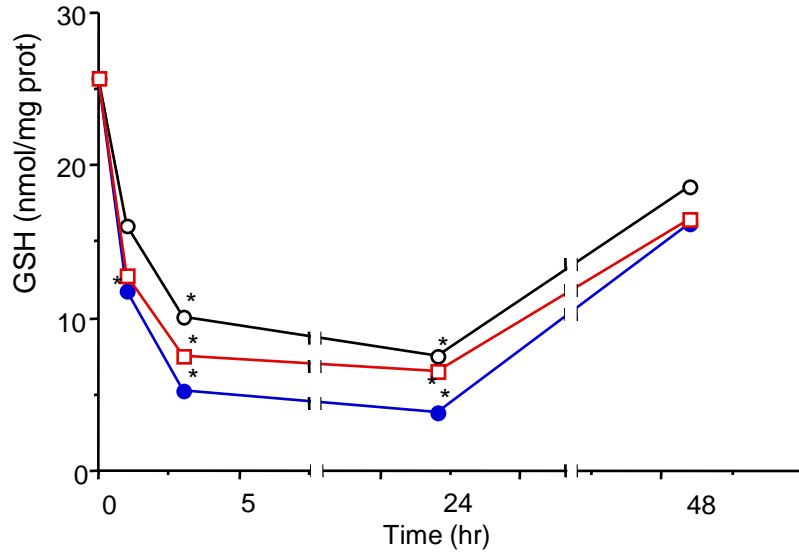
INFLUENCE OF SURFACE PROPERTIES ON INFLAMMATION RESPONSE

Fernandez-Urrusuno et al., Toxicol Appl Pharmacol., 130, 272-279 1995



EFFECT OF IV INJECTION OF PIBCA AND POLYSTYRENE NANOPARTICLES ON REDUCED (GSH) AND OXIDIZED (GSSG) GLUTATHIONE

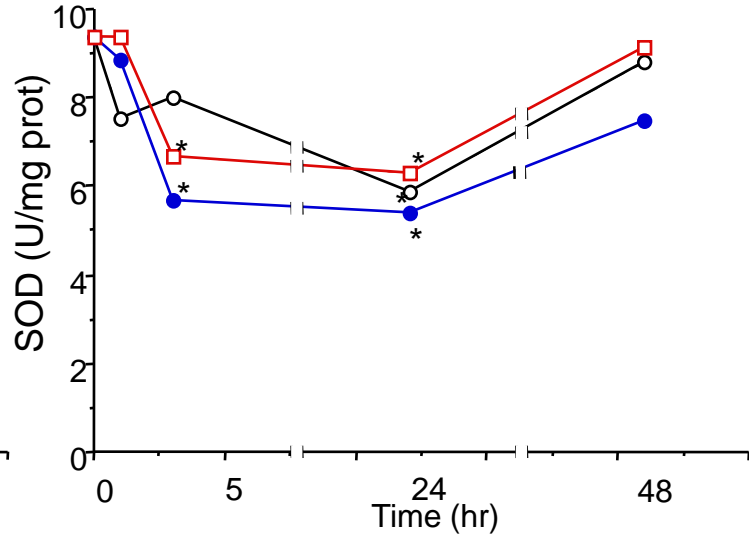
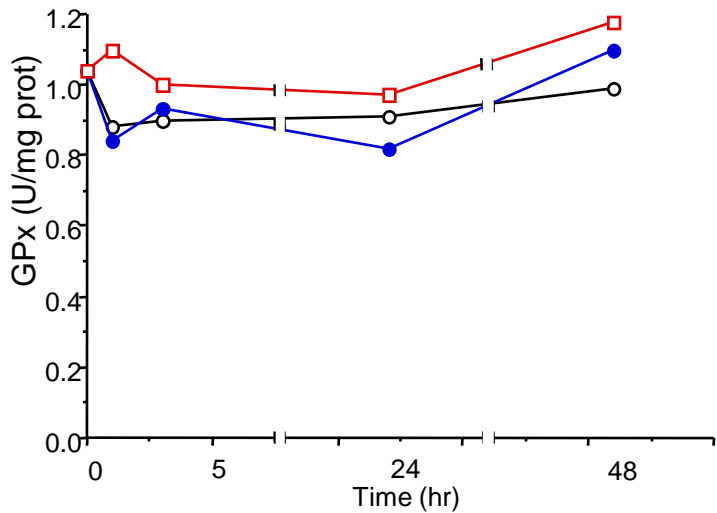
Fernandez-Urrusuno et al., Biomaterials, 18, 511-517, 1997



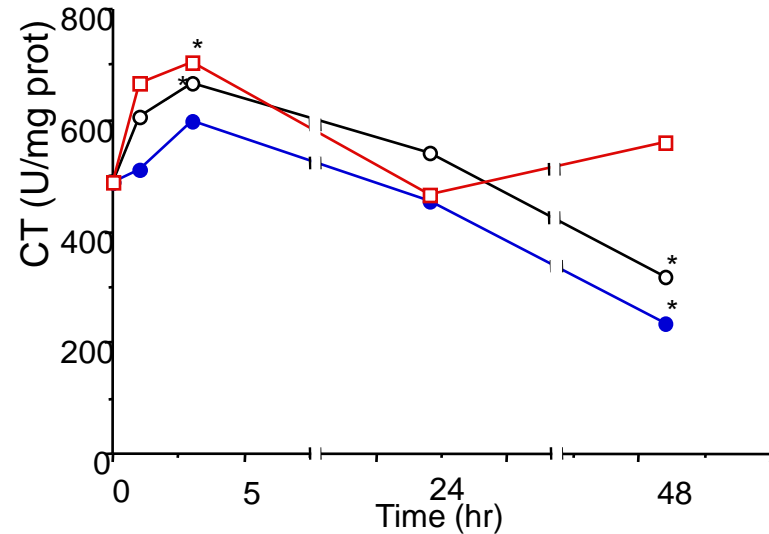
PIBCA NP 20mg/kg
PIBCA NP 100mg/kg
PS NP 100mg/kg

EFFECT OF IV INJECTION OF PIBCA AND POLYSTYRENE NANOPARTICLES ON GLUTATHION PEROXYDASE (GPx), SUPEROXIDE DISMUTASE (SOD) and CATALASE (CT)

Fernandez-Urrusuno et al., Biomaterials, 18, 511-517, 1997



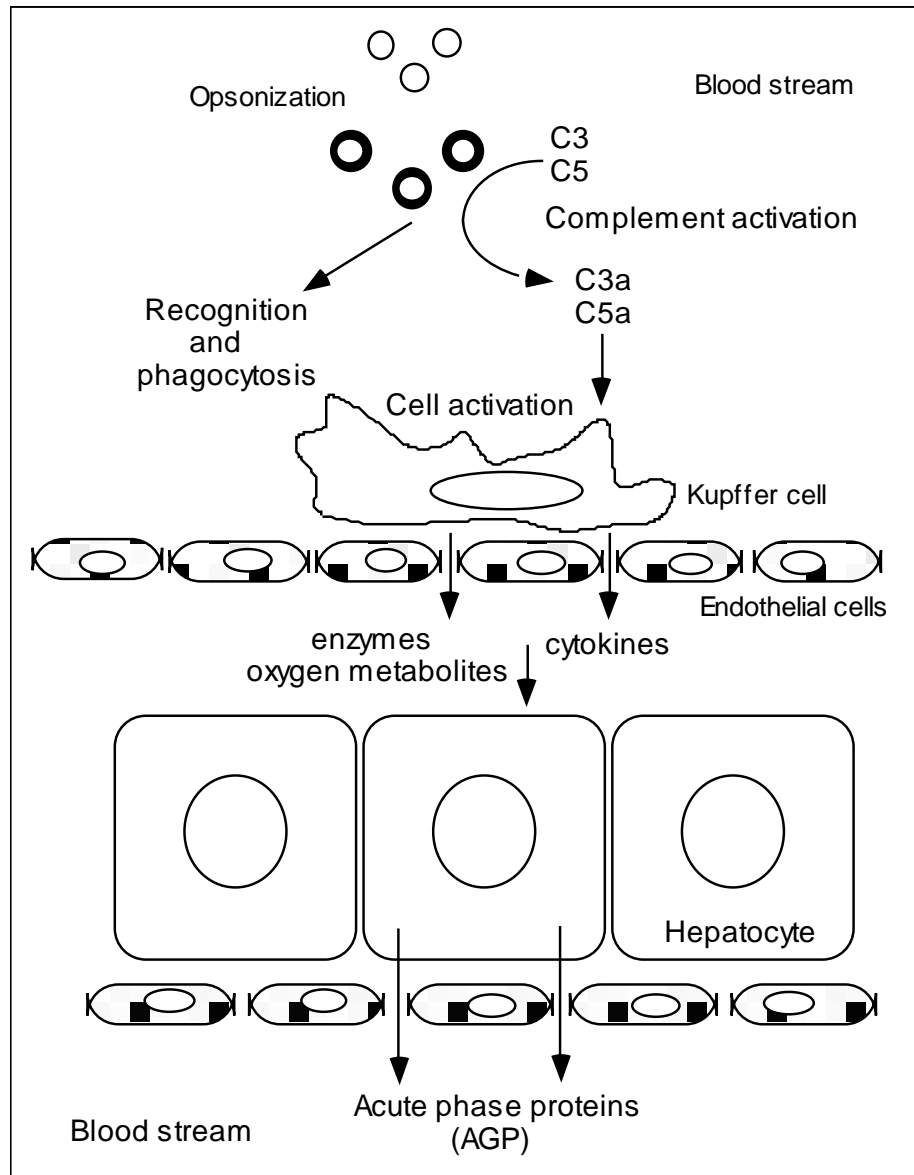
PIBCA NP 20mg/kg
 PIBCA NP 100mg/kg
 PS NP 100mg/kg



ABSENCE OF LIVER INSUFFICIENCY
ABSENCE OF IRREVERSIBLE HEPATOCYTES DAMAGE
ABSENCE OF LIPID PEROXYDATION BUT
INFLAMMATORY PROCESS

MECHANISM OF INFLAMMATORY RESPONSE TO NANOPARTICLES

Fernandez-Urrusuno et al., Toxicol Appl Pharmacol., 130, 272-279 1995



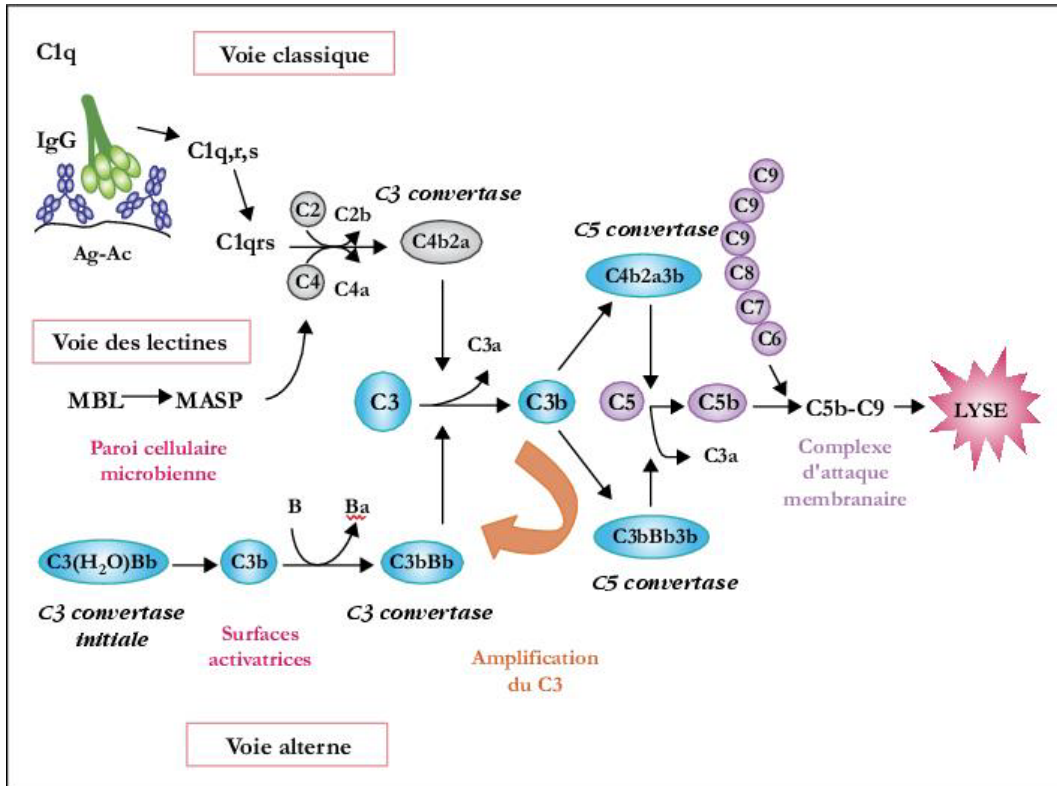
EFFECT ON PHAGOCYTOTIC FUNCTION

- ❖ Ability of MPS to remove colloidal carbon was diminished after single injection of PACA nanoparticles
- ❖ Diminution of uptake may be due to reduction of opsonins and very likely to cytotoxicity versus Kupffer cells.

CONSIDERATIONS TOXICOLOGIQUES

- Rechercher les phénomènes d'agrégation susceptibles d'entraîner des phénomènes thromboemboliques à l'administration
- N'utiliser que des matériaux biodégradables et biocompatibles → risques de toxicité par thésaurismose
- Surveiller tous les processus liés à la capture par le RES et le foie
- **Problématique de l'activation du complément**

C ACTIVATED RELATED PSEUDO-ALLERGY



Les manifestations :

l'hypo/hypertension, le mal de tête, l'apparition de nausées, de la fièvre etc.

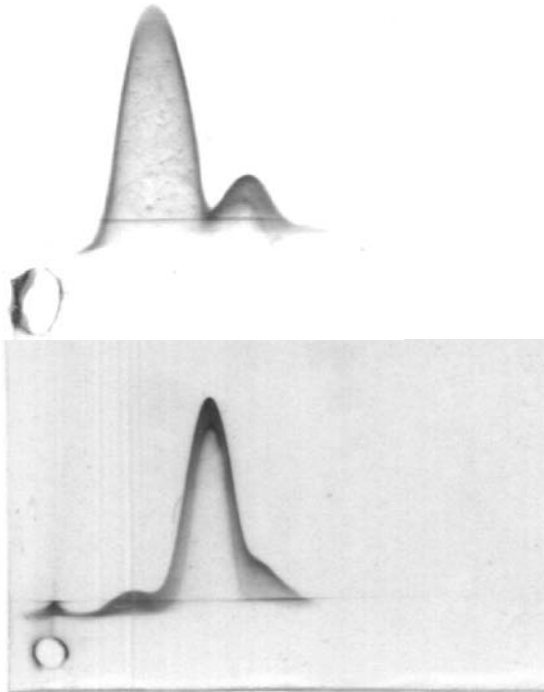
Les caractéristiques:

- apparaît dès la première administration,
- est imprévisible et non détectable par les tests d'allergie classiques
- peut être fatal.

Le CARPA est associé à 6 des 10 principaux médicaments issus des biotechnologies et TOUS les nanomédicaments semblent présenter des réactions pseudo-allergiques de type CARPA

La cause: production d'anaphylatoxines (ie C3a et C5a) → production de cytokines pro-inflammatoires.

QUELQUES ELEMENTS DE CONSENSUS CONCERNANT LES ESSAIS CLINIQUES DES NANOMEDICAMENTS



- INTERROGATION DU PATIENT ET DE SES ANTECEDENTS ALLERGIQUES
- PREMEDICATION AUX CORTICOIDES
- INJECTION LENTE OU EN PERFUSION
- TESTS D'ACTIVATION DU COMPLEMENT SUR LE SERUM DU PATIENT
- NECESSITE DE DEVELOPPER DES TESTS PREDICTIFS

CONCLUSION

- L'utilisation des nanotechnologies pour le médicament répond aux mêmes critères que ceux qui président à la mise sur le marché de nouveaux médicaments à savoir le rapport bénéfice/risque
- Elles sont donc soumises à aux essais toxicologiques habituels en plus des investigations qui relèvent des aspects spécifiques aux systèmes particulières (étude de la clearance, de l'activation du complément, de la fonction hépatique, de l'agrégation en milieu sérique, du bilan métabolique etc.)