

Nanoparticles for Targetted Drug Delivery Systems and Construction of Vaccines



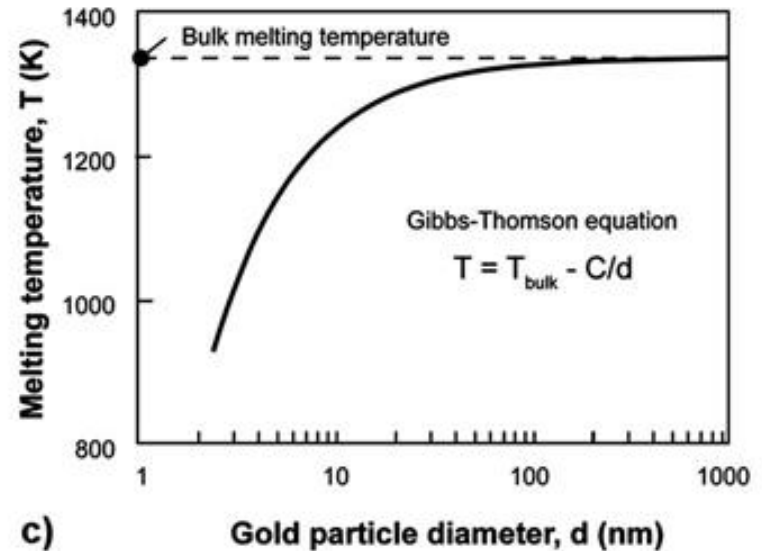
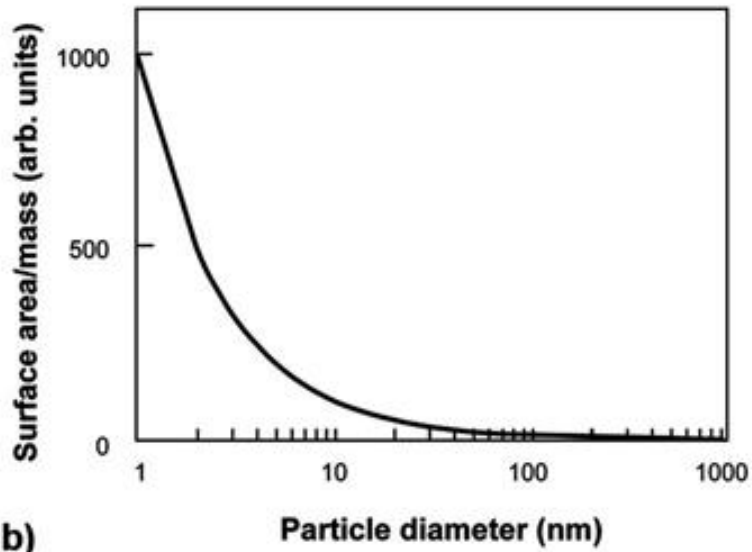
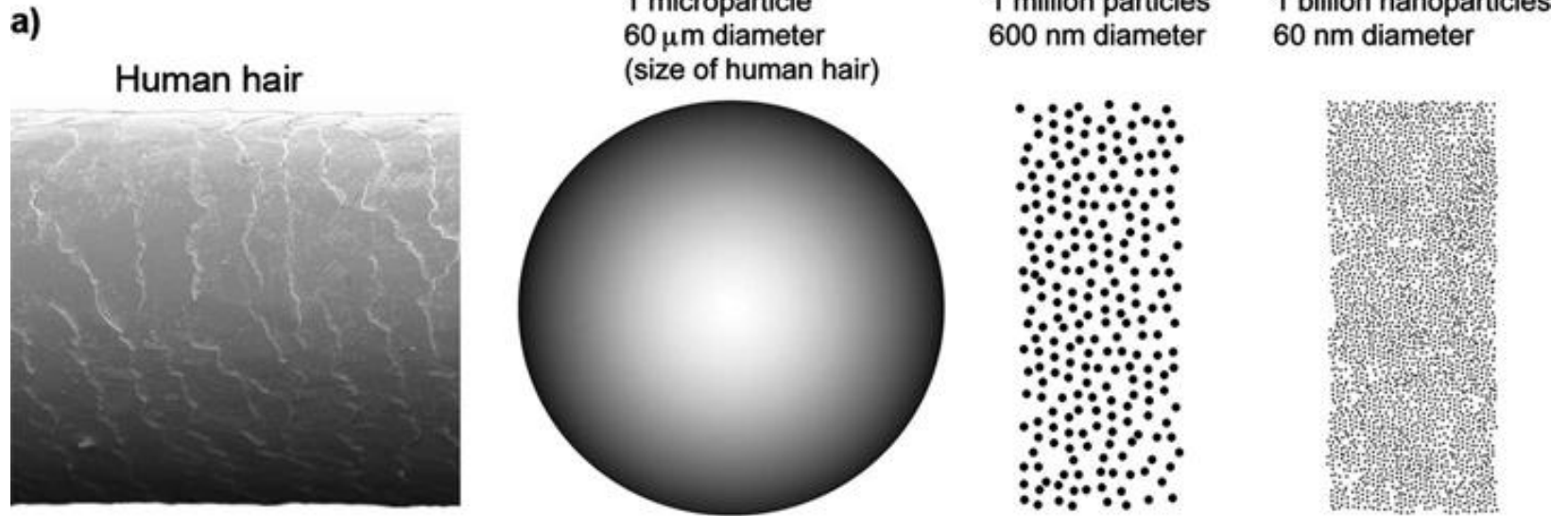
RNDr. Jaroslav Turánek, CSc.
Výzkumný ústav veterinárního lékařství

*„V neustálém cyklu proměn malé
se stává velkým a velké
nepatrným“*

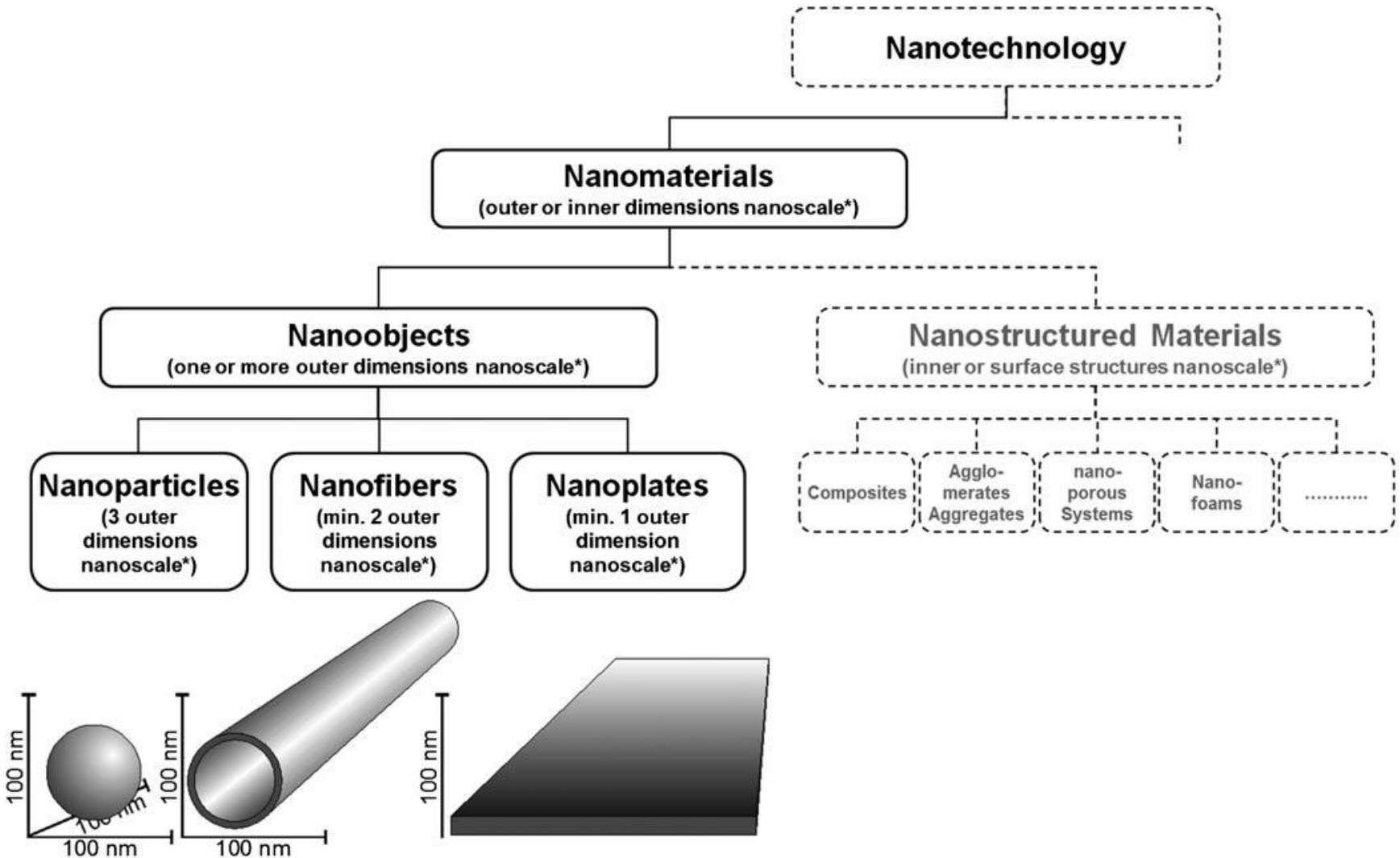
Lao‘c



Microparticles versus nanoparticles



Nanoobjects



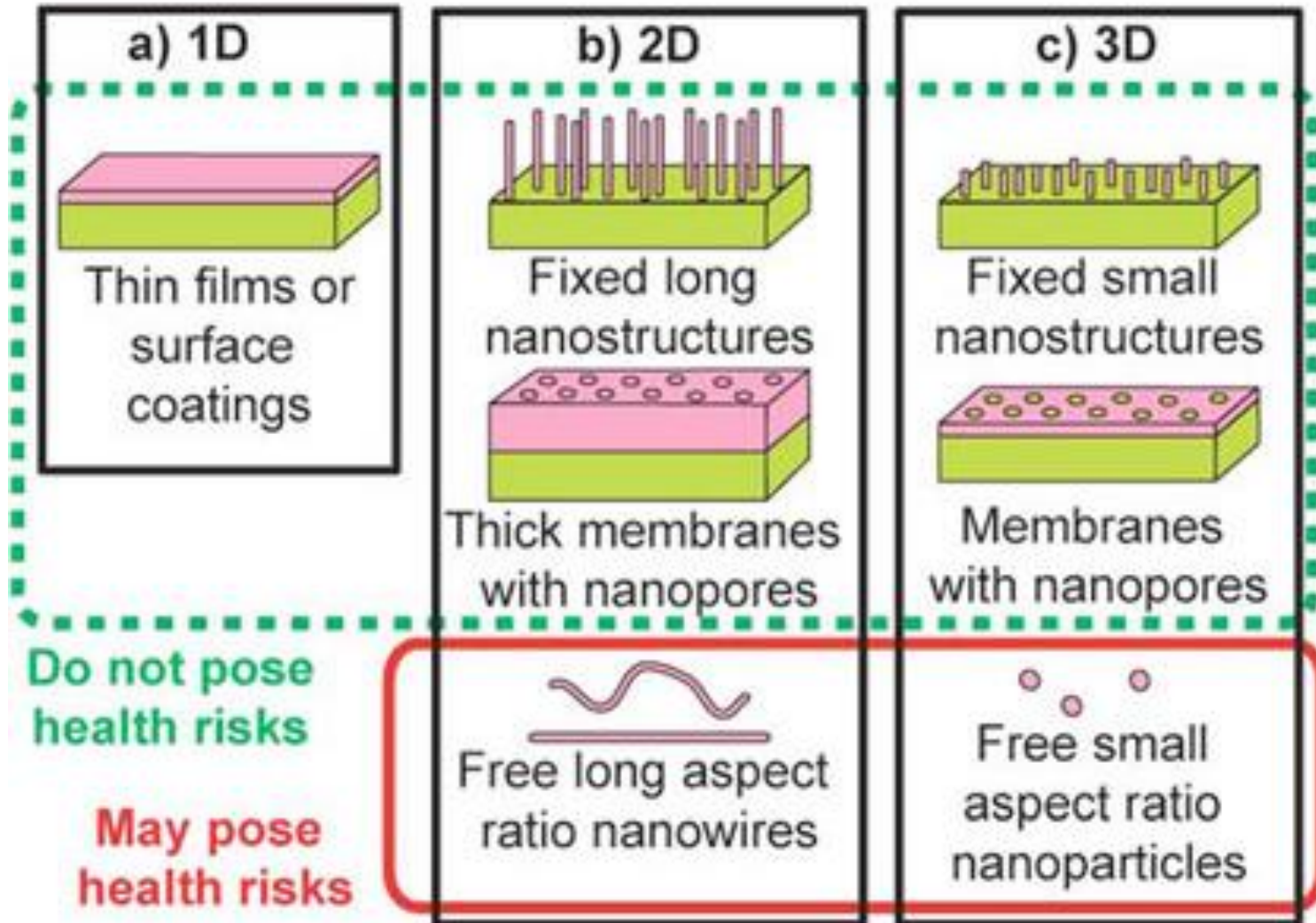
The ISO definition of nanoobjects. Included as nanoobjects are nanoparticles (nanoscale in all three dimensions), nanofibers (nanoscale in two dimensions), and nanoplates or nanolayers (nanoscale only in one dimension). * Nanoscale: a size of between 1 and 100 nm.

Main characteristics of Nanoparticles

- * Chemical composition, purity, impurities
- * Particle size and size distribution
- * Specific surface
- * Morphology (crystalline/amorphous, shape)
- * Surface chemistry, coating, functionalization
- * Degree of agglomeration/aggregation and particle size distribution under experimental conditions (for example, media with/without proteins)
- * Water solubility (differentiation between soluble, metastable, and biopersistent nanomaterials)
- * Surface reactivity and/or surface load (zeta potential).

NANOPARTICLE CLASSIFICATION

1. Dimensionality



Moderní farmakologie je založena na dogmatu interakce molekul léčiva s biologickými molekulárními strukturami organismu.

Tato interakce vyvolává následné děje, které vedou k žádoucímu terapeutickému efektu. Je zřejmé, že dochází také k dějům, které vedou k nežádoucím účinkům a toxickým projevům léčiva. Tyto účinky jsou souhrnně označovány jako vedlejší účinky léčiva. Pro porovnání terapeutických a vedlejších účinků léčiva se používá termínů jako **maximální tolerovatelná dávka (MTD)** a **terapeutický index léčiva**.

Výše uvedené dogma lze vyjádřit dvěma rovnicemi, které popisují interakci léčiva a struktury na kterou působí.

$$K_{\text{ter}} = [L] \cdot [S_{\text{ter}}] / [SL]$$

$$K_{\text{tox}} = [L] \cdot [S_{\text{tox}}] / [SL]$$

L – molekula léčiva

S – molekulární struktura, na kterou léčivo působí s terapeutickým výsledkem (ter) nebo toxickým výsledkem (tox)

K – rovnovážná konstanta tvorby komplexu léčiva L a molekulární struktury S.

Selektivita interakce makromolekul jako základ moderní molekulární farmakologie

1. Klasická terapeutika – interakce ligand - protein (enzym – inhibitor)

konstanta stability komplexu $K = 10^{-5} - 10^{-6} - 10^{-8} \text{ M}$

Blokování funkce proteinu

2. Immunoterapeutika – interakce protilátka – ligand (imunotoxiny, cílené nanočástice)

konstanta stability komplexu $K = 10^{-7} - 10^{-9} - 10^{-11} \text{ M}$

Blokování funkce receptoru nebo ligandu, destrukce antigenu buněčnými nebo molekulárními mechanismy, cílení farmak – zvýšení účinnosti klasických farmak

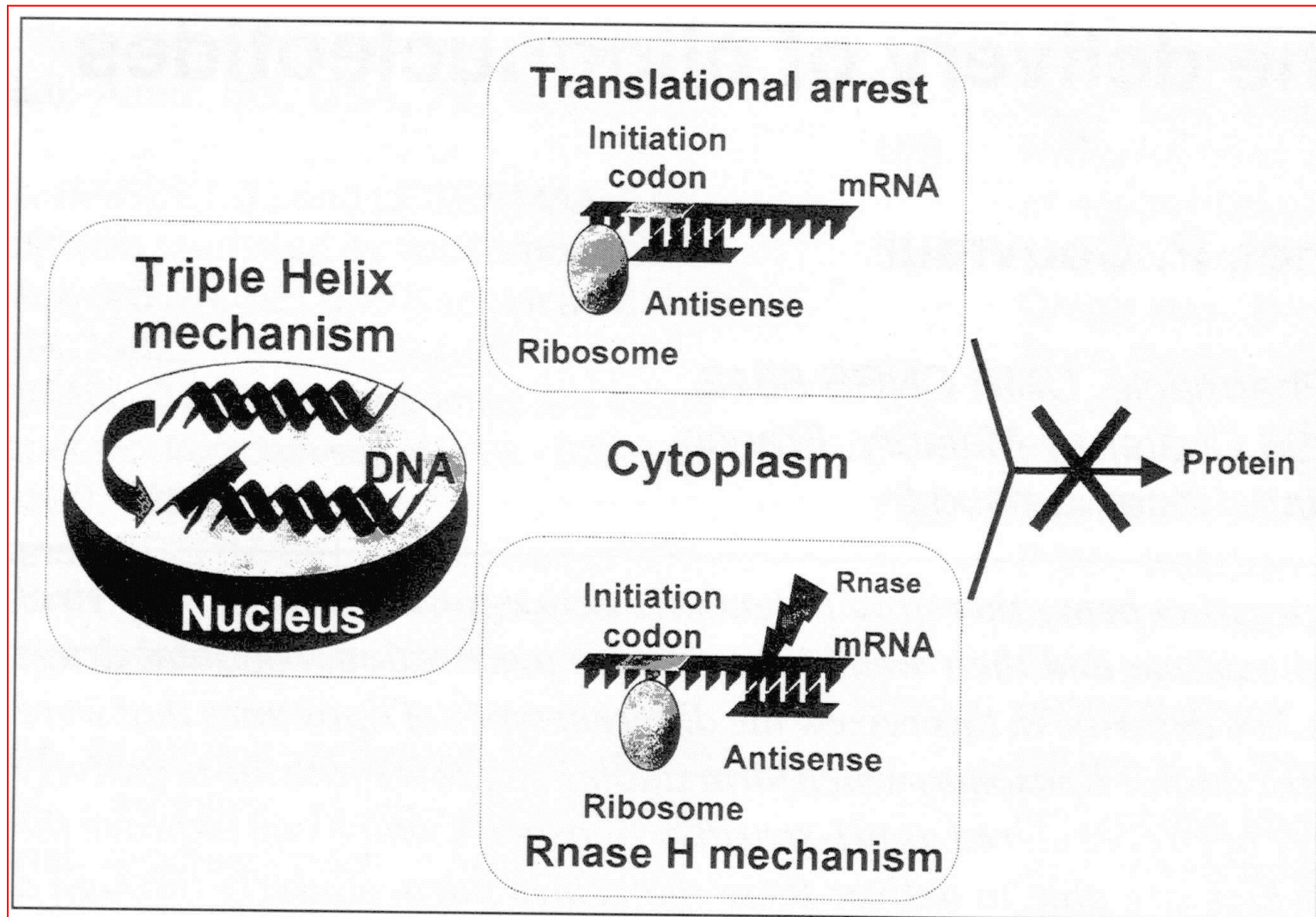
3. Oligonukleotidové sekvence – siRNA, antisense oligonukleotidy

extremně vysoká konstanta stability komplexu $K = 10^{-11} - 10^{-12} - 10^{-15} \text{ M}$

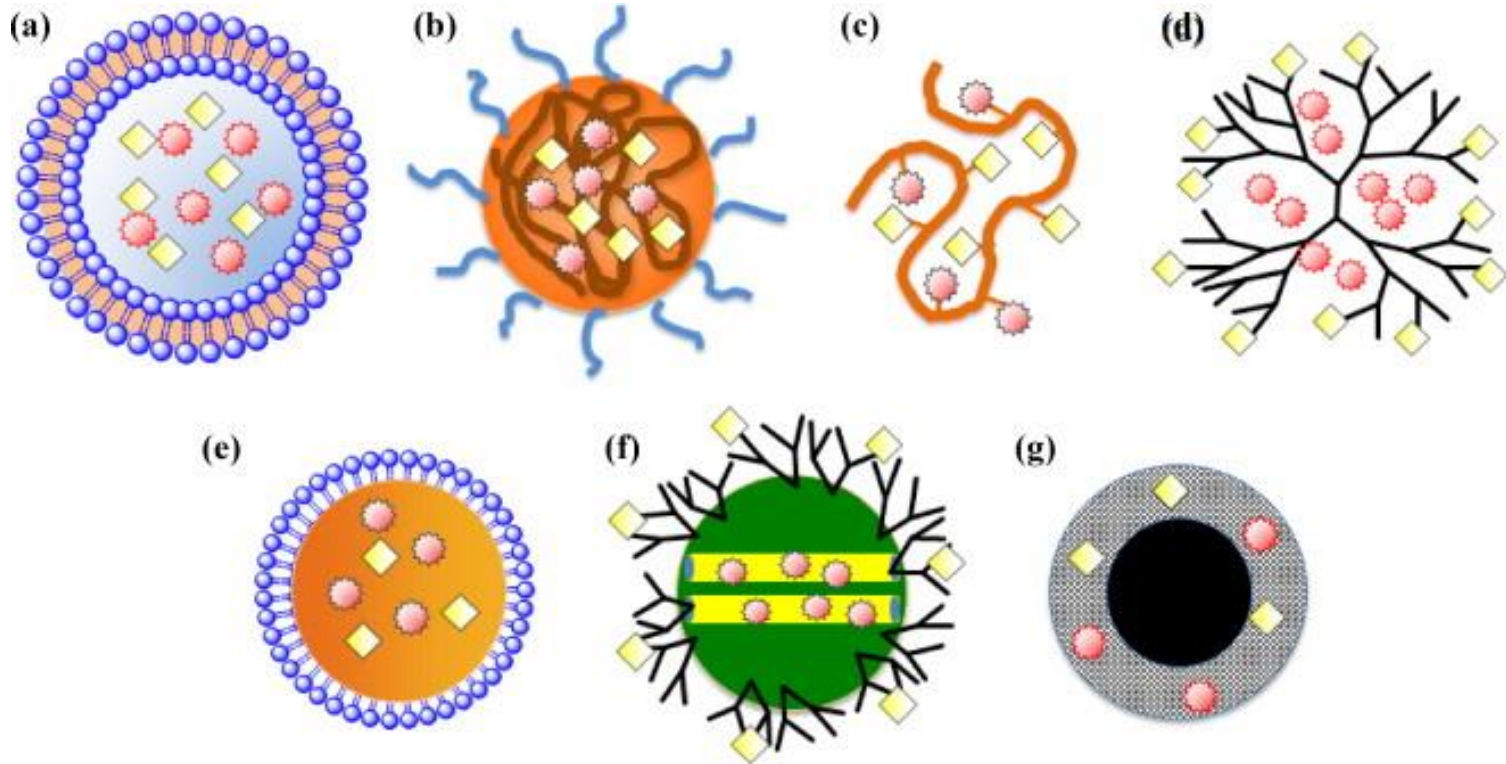
Blokování transkripce nebo translace, extrémní selektivita pro vyřazení genového produktu

4. Vnesení nového genu – forma plasmidového vektoru – využití pro zvýšení selektivity postupů **1-3**.

Inhibice genových produktů na různé úrovni



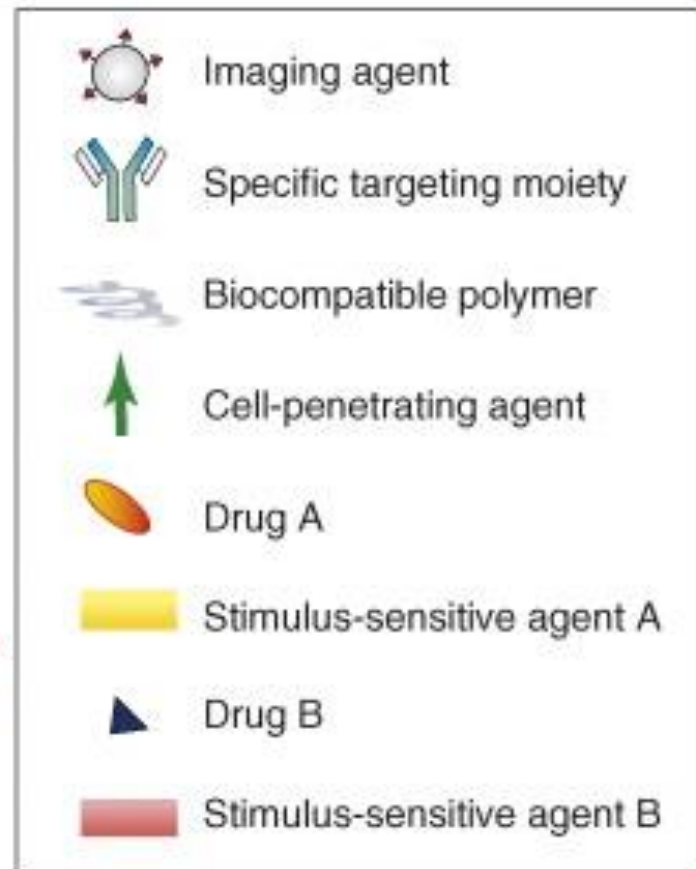
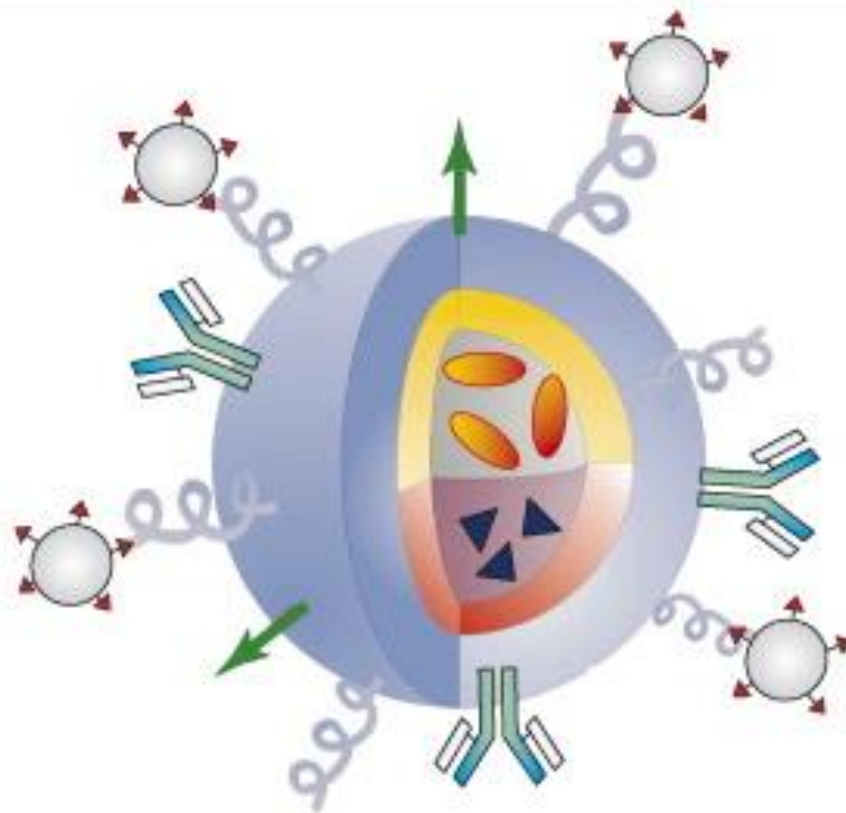
Nanoparticle based drug delivery systems



◆ and ● represent different types of drugs

Liposomy

Multifunctional nanoparticle



Application of nanoparticles in medicine

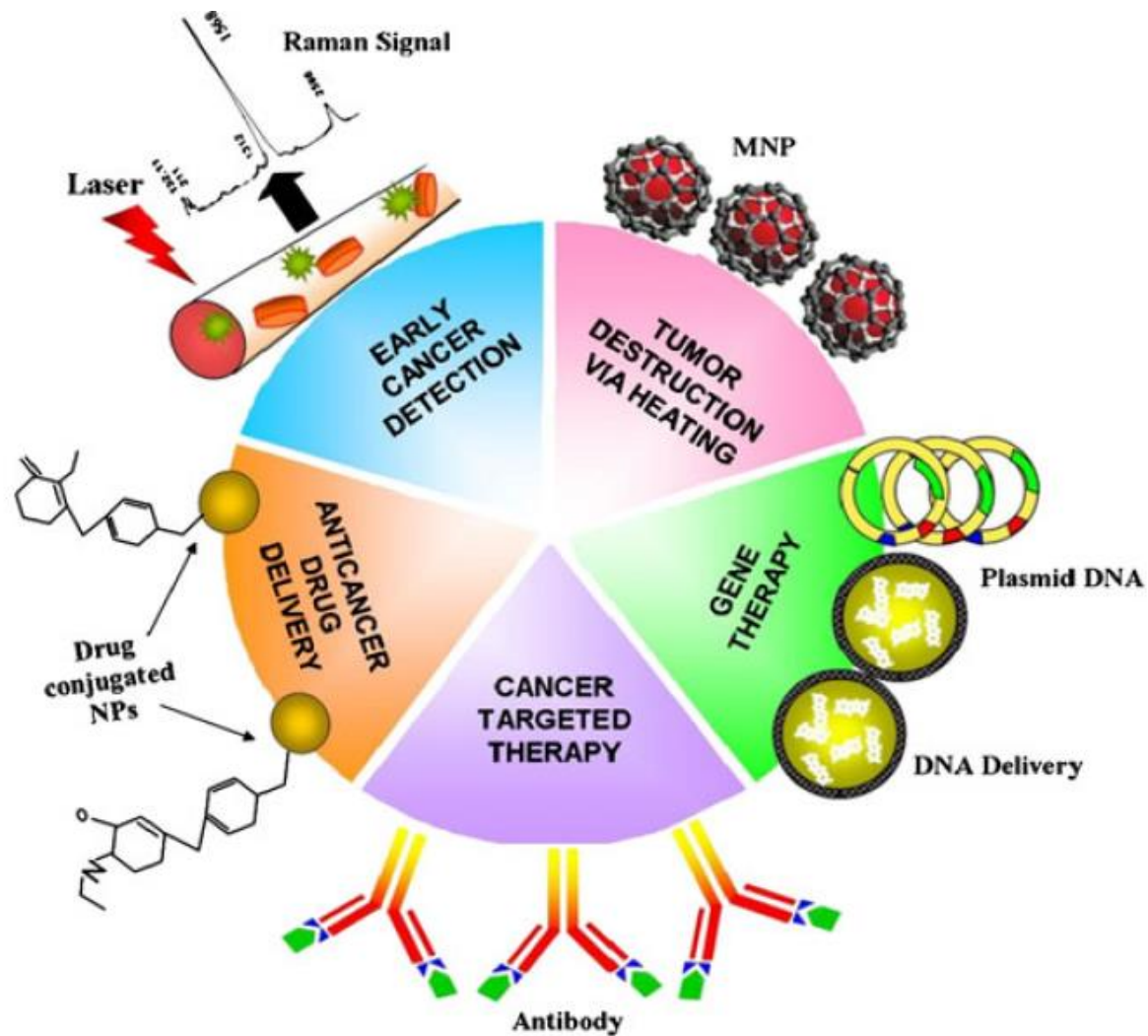
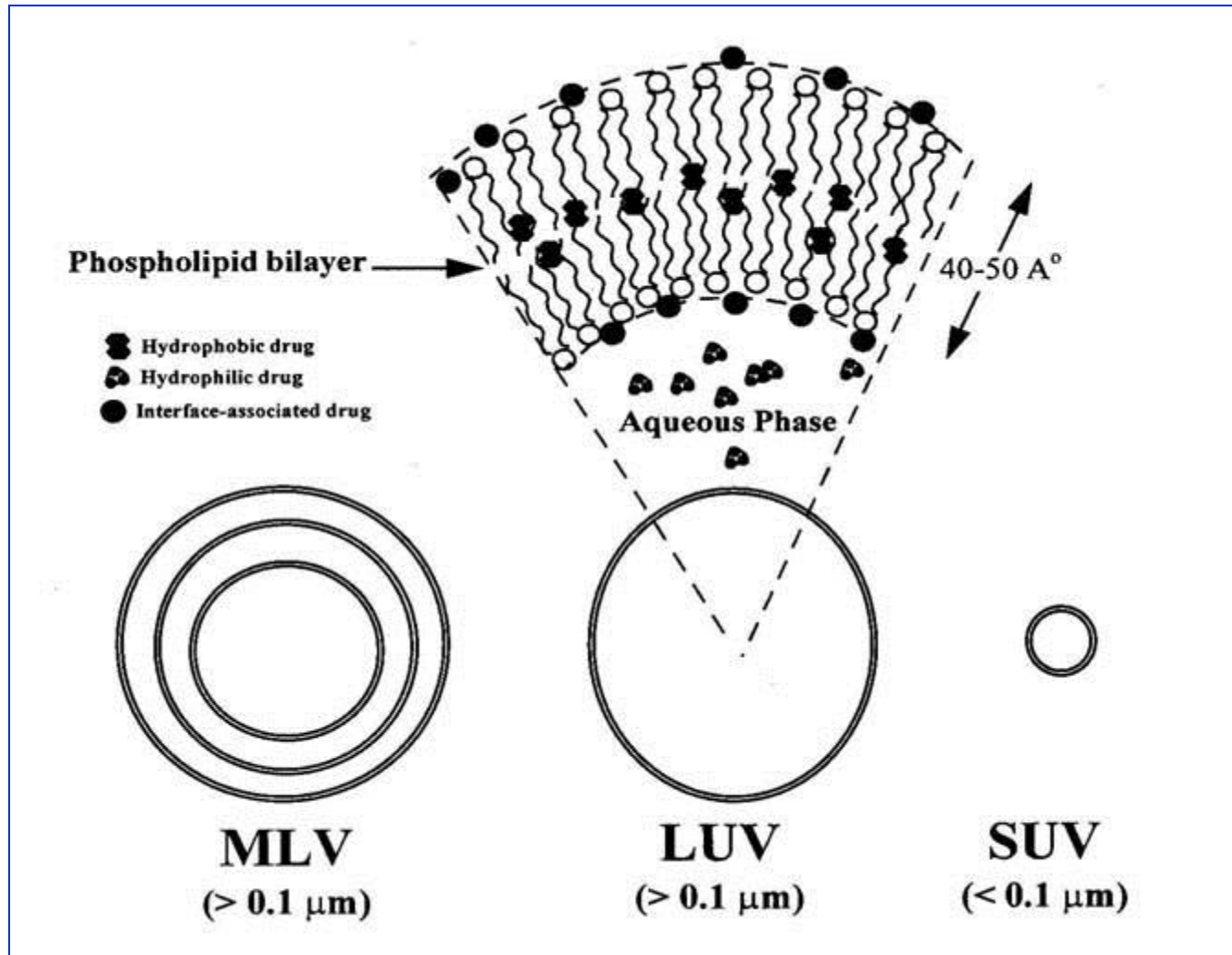


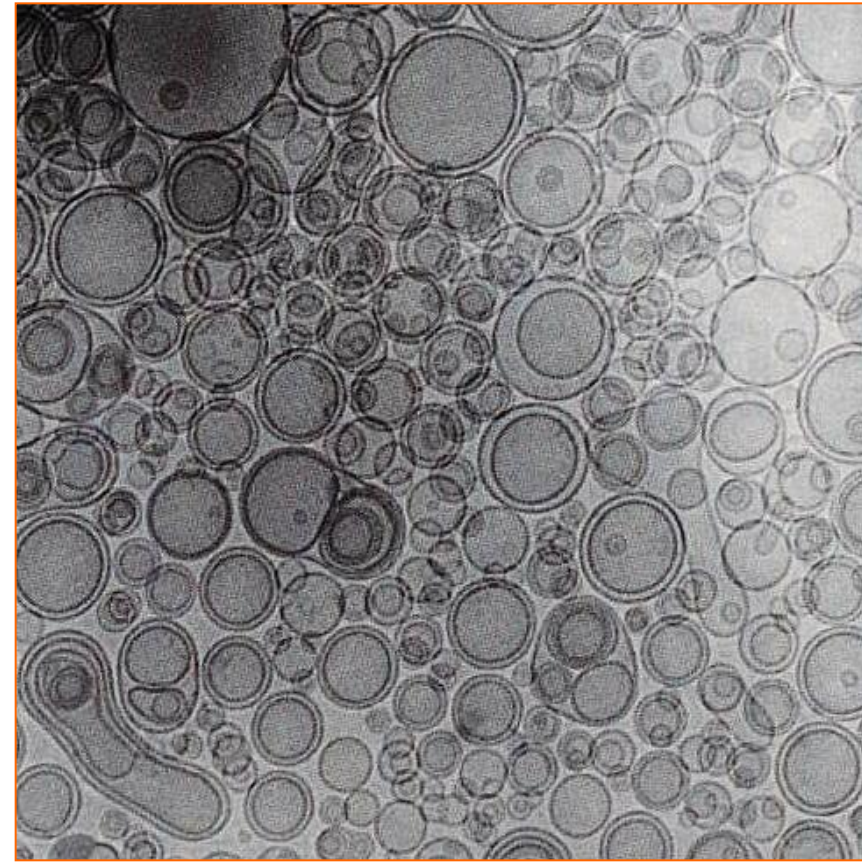
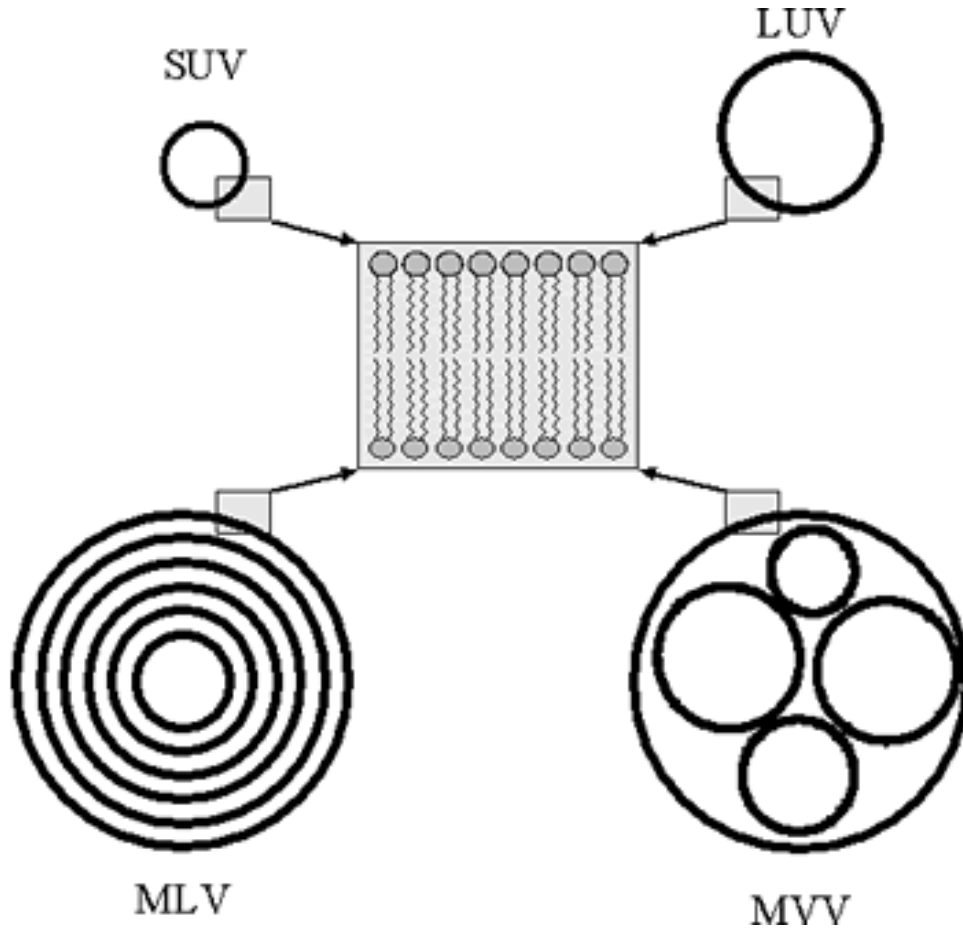
Figure 1. Schematic diagram showing nanotechnology-based approaches for cancer research related to early cancer detection, anti-cancer drug delivery, cancer-targeted therapy, gene therapy and tumor destruction via hyperthermia.

Morfologická klasifikace liposomů



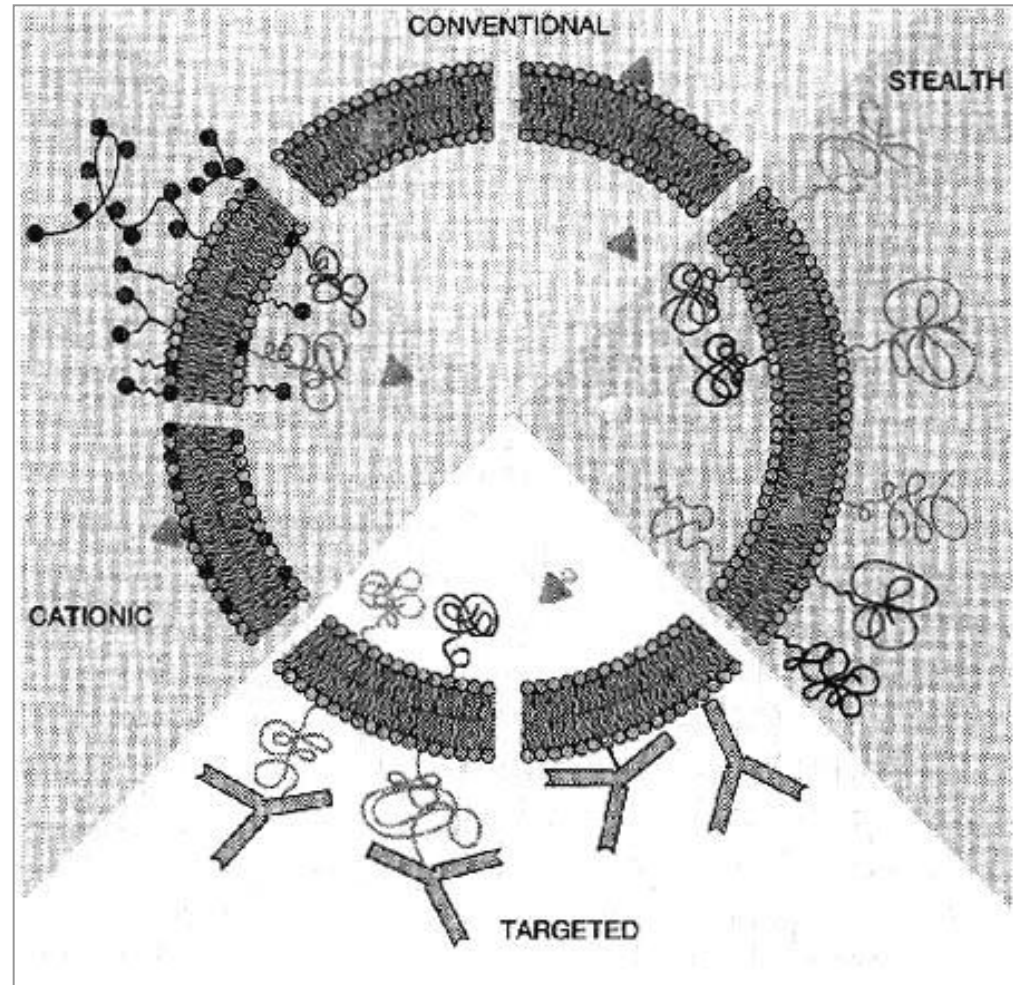
Morphology of liposomes

Viewed by cryoelectron microscopy



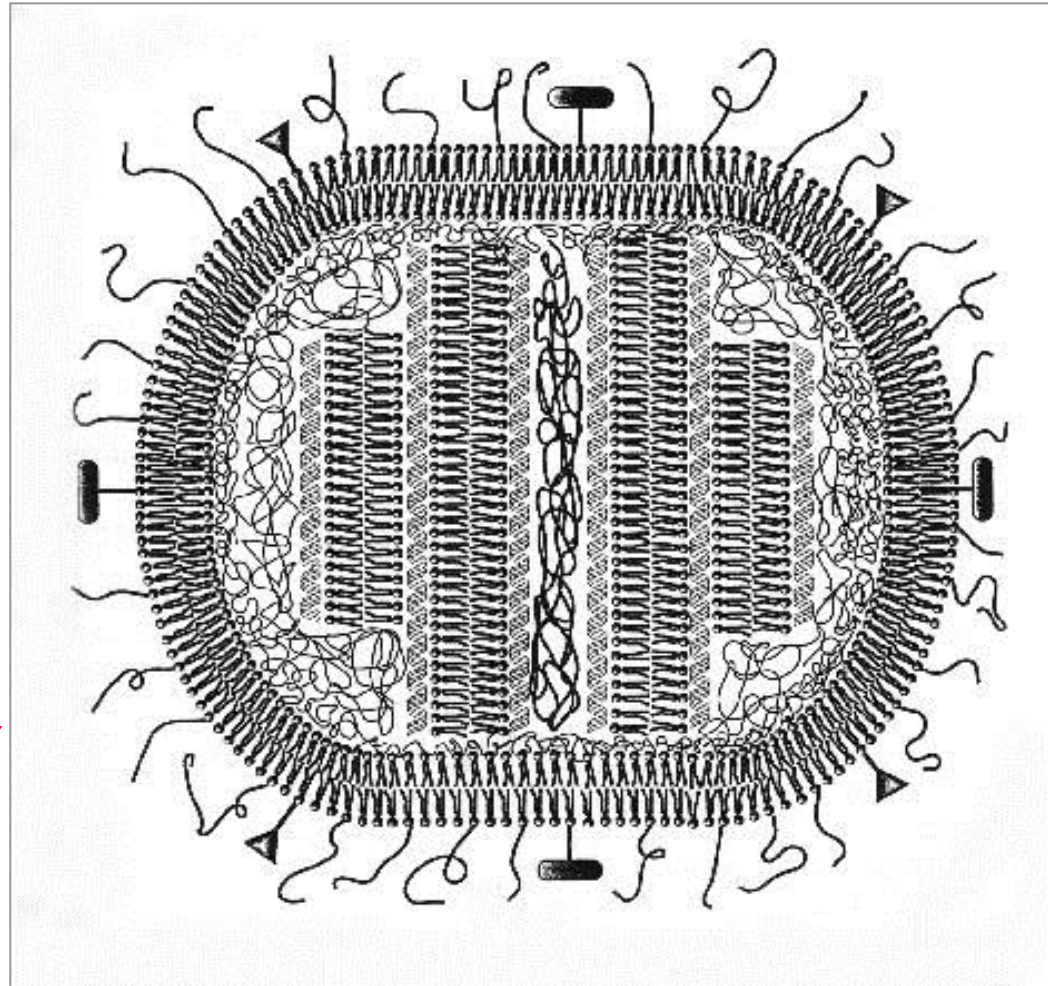
Typy liposomů podle jejich funkce

- Konvenční liposomy
nespecifické interakce s
prostředím, nestabilita v
séru
- Stericky chráněné
liposomy
dlouhodobá cirkulace
- Cílené liposomy
specifické interakce přes
navázaný ligand
- Kationické liposomy
schopnost interakce s
negativně nabitou DNA



Syntetický virus

- plně syntetická částice
- DNA kondenzovaná pomocí kationických liposomů nebo polyaniontů
- na povrchu molekuly pro selektivní cílení nebo fúze
- produkce v bezbuněčném systému
- funkčně připomíná živou atenuovanou virovou vakcínu, nízká bezpečnostní rizika



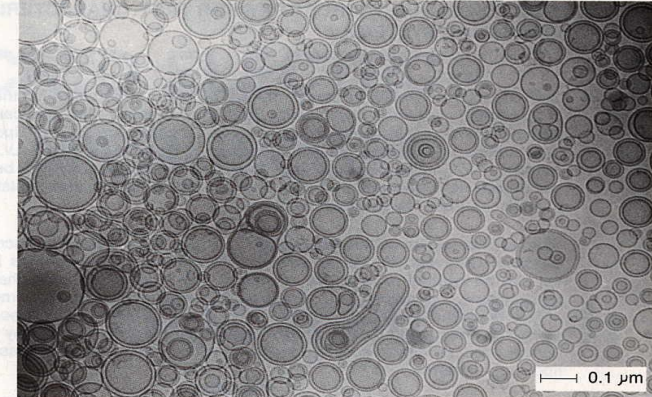
Příprava liposomů

- **Primární procesní metody** – produkce liposomů
 - Hydratace lipidního filmu
 - Metoda Proliposom-liposom
 - Lyofilizace z terc-butanolu
 - Odstranění detergentu
- **Sekundární procesní metody** – změna morfologie a distribuce velikosti
 - Vysokotlaková homogenizace
 - Mikrofluidizace
 - Extruze přes polykarbonátové filtry
 - Sonication
- **Finální adjustace liposomálního preparátu** – stabilizace skladovací formy
 - Sterilní filtrace
 - Lyofilizace

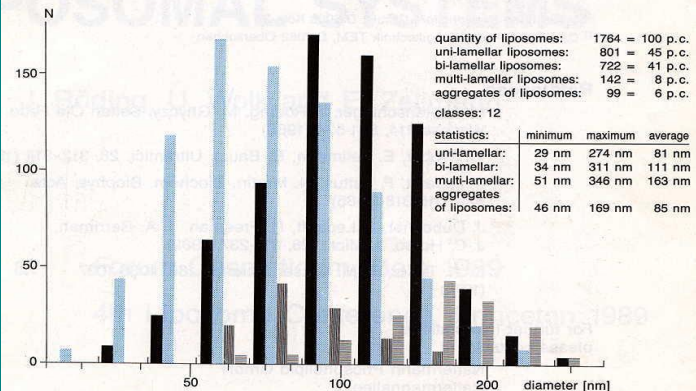
Distribuce velikosti liposomů

- DLS (Dynamic Light Scattering)
- SLS (Static Light Scattering)
- Průtoková cytometrie (Flow Cytometry)
- Elektronová mikroskopie
- GPC (Gel Permeation Chromatography)
- AFM (Atomic force microscopy)
- MLSA (Multi-angle Laser Scattering Analysis)

CLASSIFICATION

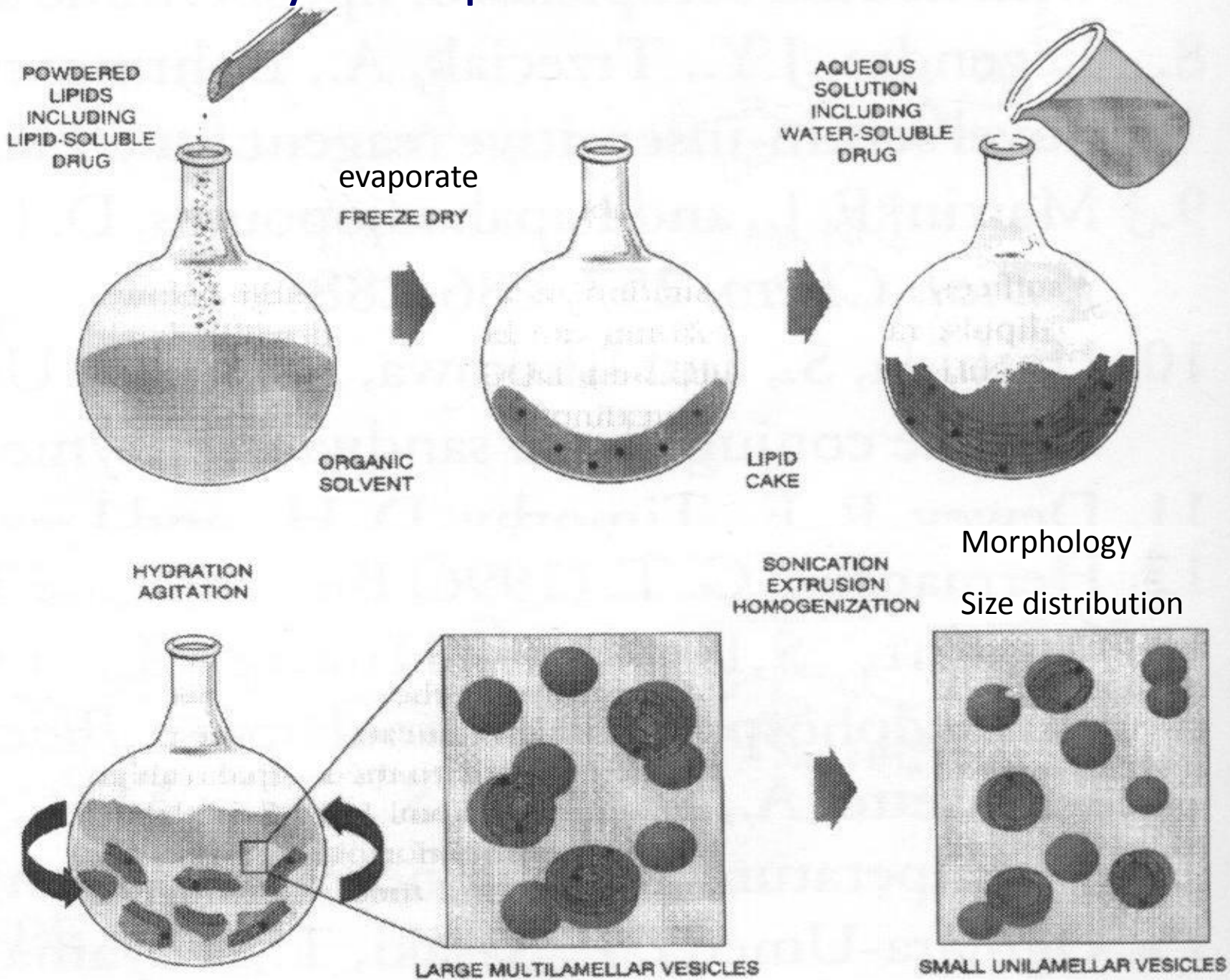


2 Filtered brightfield images at $\Delta E = 0\text{eV}$, accelerating voltage = 80 kV, table temperature: 108 K (-165°C) of a dispersion of liposomes consisting of natural soya-phospholipids in a physiological milieu, pH = 6,5



Histogram of distribution of sizes of uni-lamellar (▨), bi-lamellar (■), multi-lamellar (▩) liposomes and aggregates of liposomes (▧).

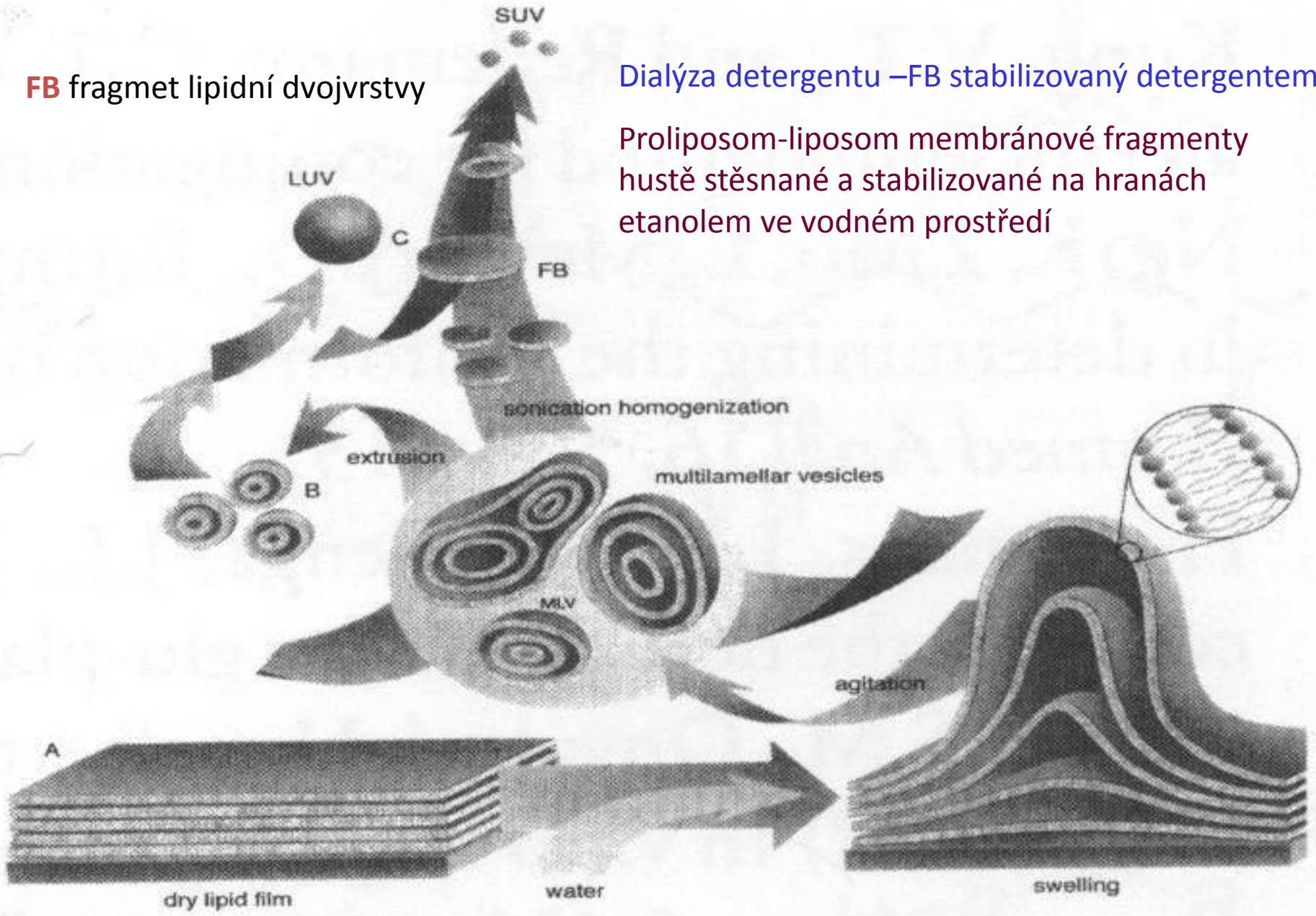
Hydratace lipidního filmu



FB fragment lipidní dvojvrstvy

Dialýza detergentu –FB stabilizovaný detergentem

Proliposom-liposom membránové fragmenty
hustě stěsnané a stabilizované na hranách
etanolem ve vodném prostředí

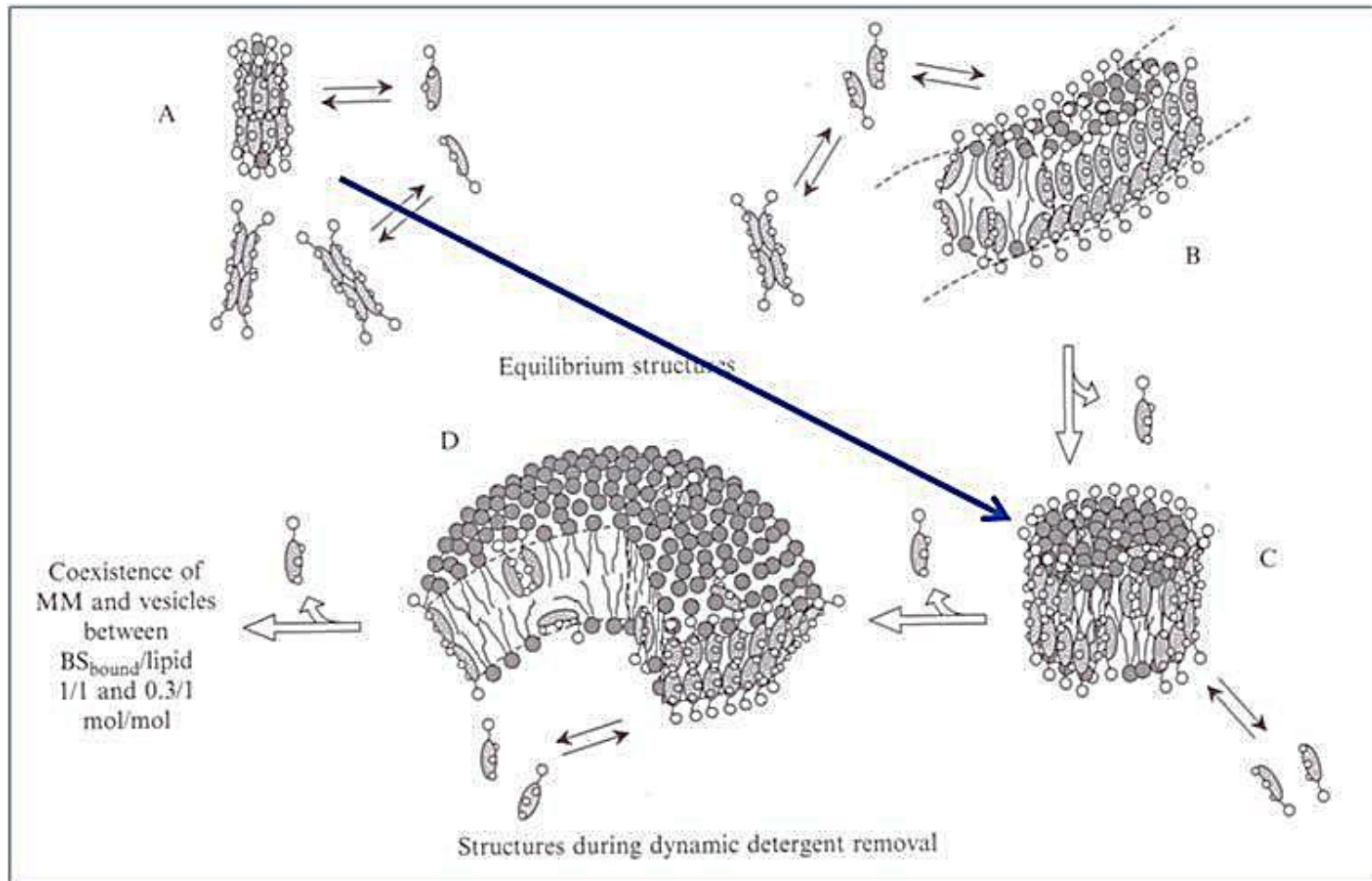


Koncept fragmentu dvojvrstvy navržený D.Lasicem

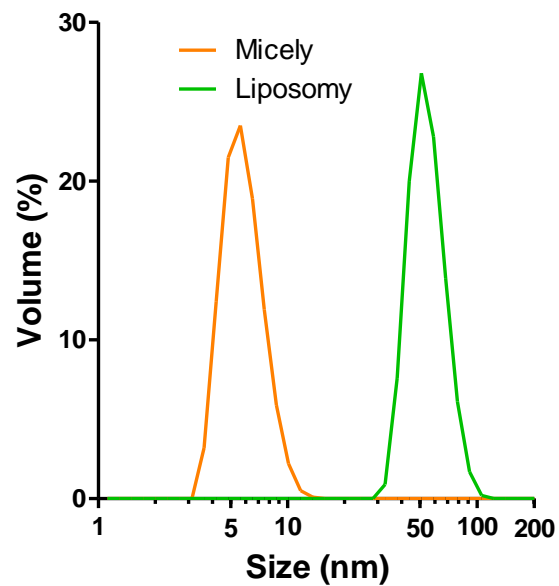
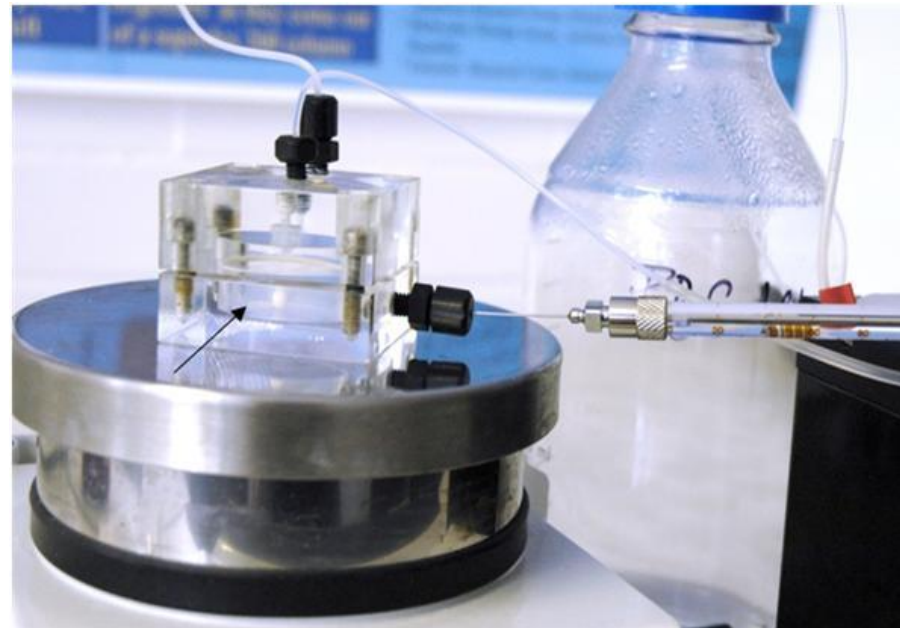
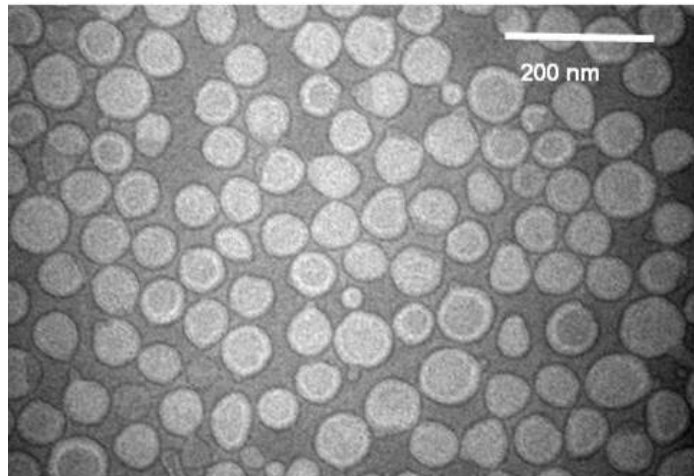
Příprava lipidního filmu



Princip metody odstranění detergentu



Zařízení pro metodu odstranění detergentu pomocí dialýzy





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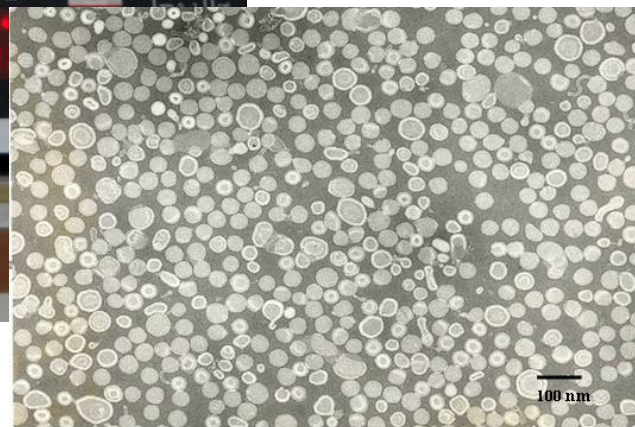
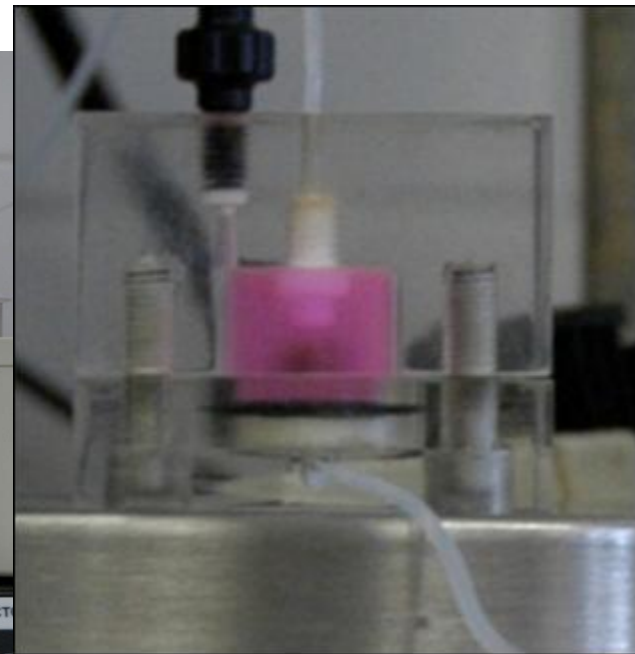


Immobilization of histidine-tagged proteins on monodisperse metallochelation liposomes: Preparation and study of their structure

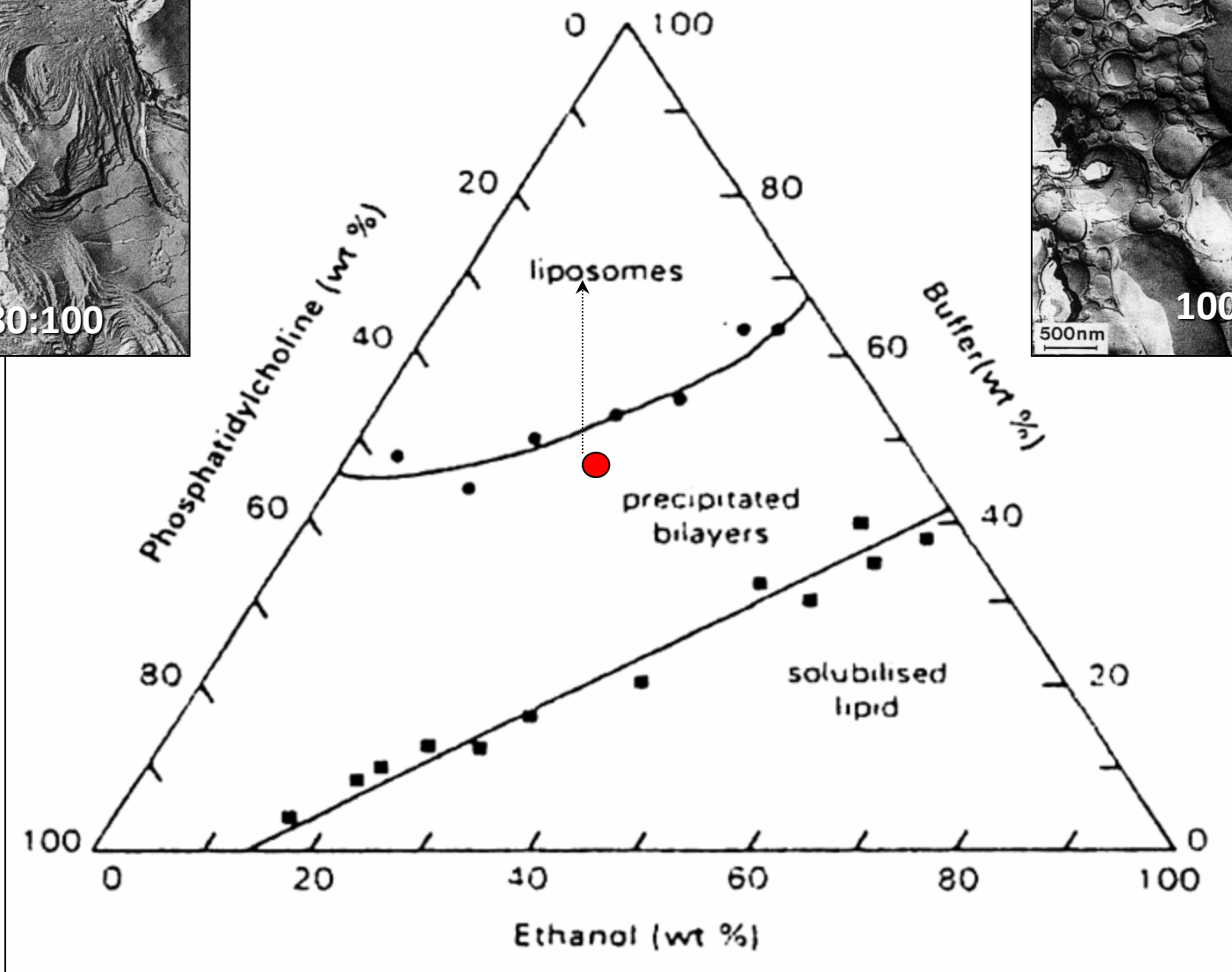
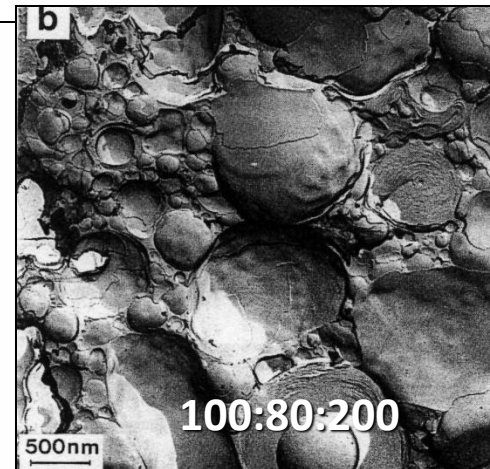
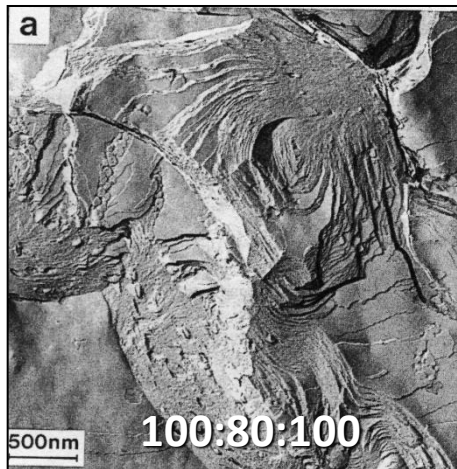
Josef Mašek^a, Eliška Bartheldyová^a, Zina Korvasová^a, Michaela Škrabalová^a, Štěpán Koudelka^a, Pavel Kulich^a, Irena Kratochvílová^b, Andrew D. Miller^c, Miroslav Ledvina^d, Milan Raška^e, Jaroslav Turánek^{a,*}

Příprava monodispersních liposomů pomocí odstranění detergentu ultrafiltrací

Míchaná průtoková ultrafiltrační cela



PROLIPOSOME- LIPOSOME Method



Metoda Proliposom-liposom



J. Turánek et.al. Preparation of Sterile Liposomes by Proliposome-Liposome Method. *Methods in Enzymology* 2003, **367**, 110-125

Mikrofluidizace a vysokotlaká homogenizace



Mikrofluidizér M-110L (Microfluidics)

HP Homogenizátor B30 (Avestin)

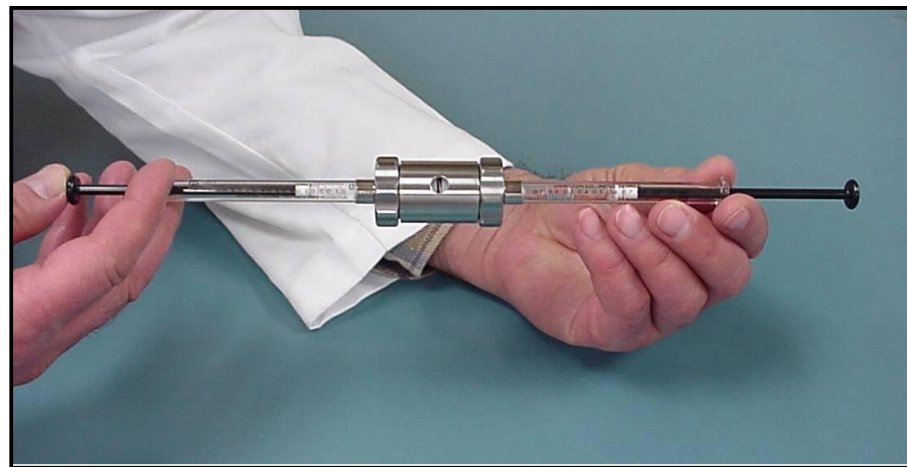
Příprava SUV pomocí ultrazvukové homogenizace



Lipex – zařízení pro extruzi
poháněné stlačeným
dusíkem



Ruční extrudér LiposoFast



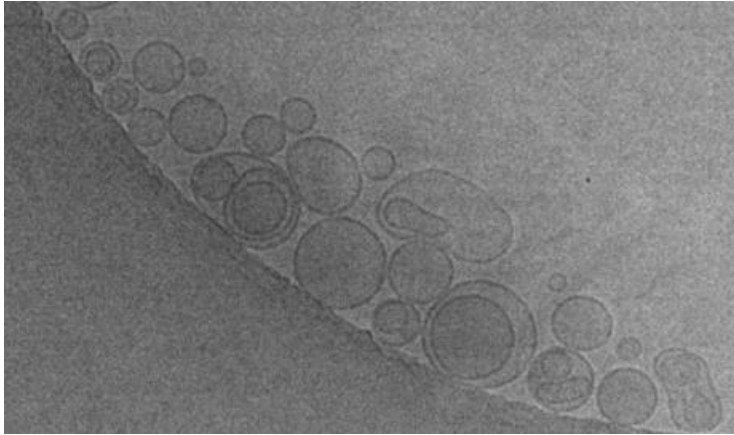
Příprava liposomů vysokotlakou extruzí



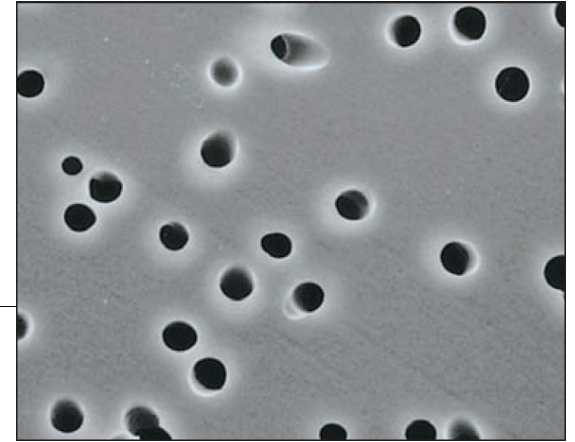
J. TURANEK. Fast-Protein Liquid-Chromatography System as a Tool for Liposome Preparation by the Extrusion Procedure **ANALYTICAL BIOCHEMISTRY** 218 (2): 352-357 1994

Extruze liposomů

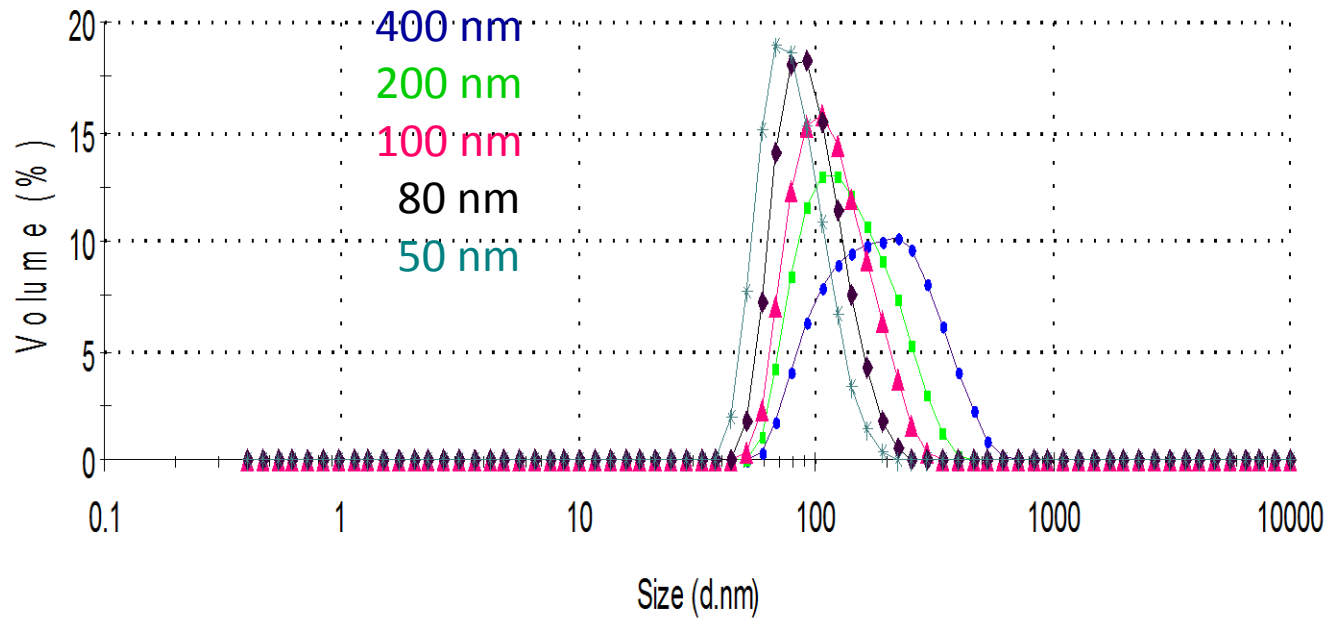
Kryoelektronová mikroskopie



SEM polykarbonátového filtru



Size Distribution by Volume



Zetasizer Nano ZS, Malvern

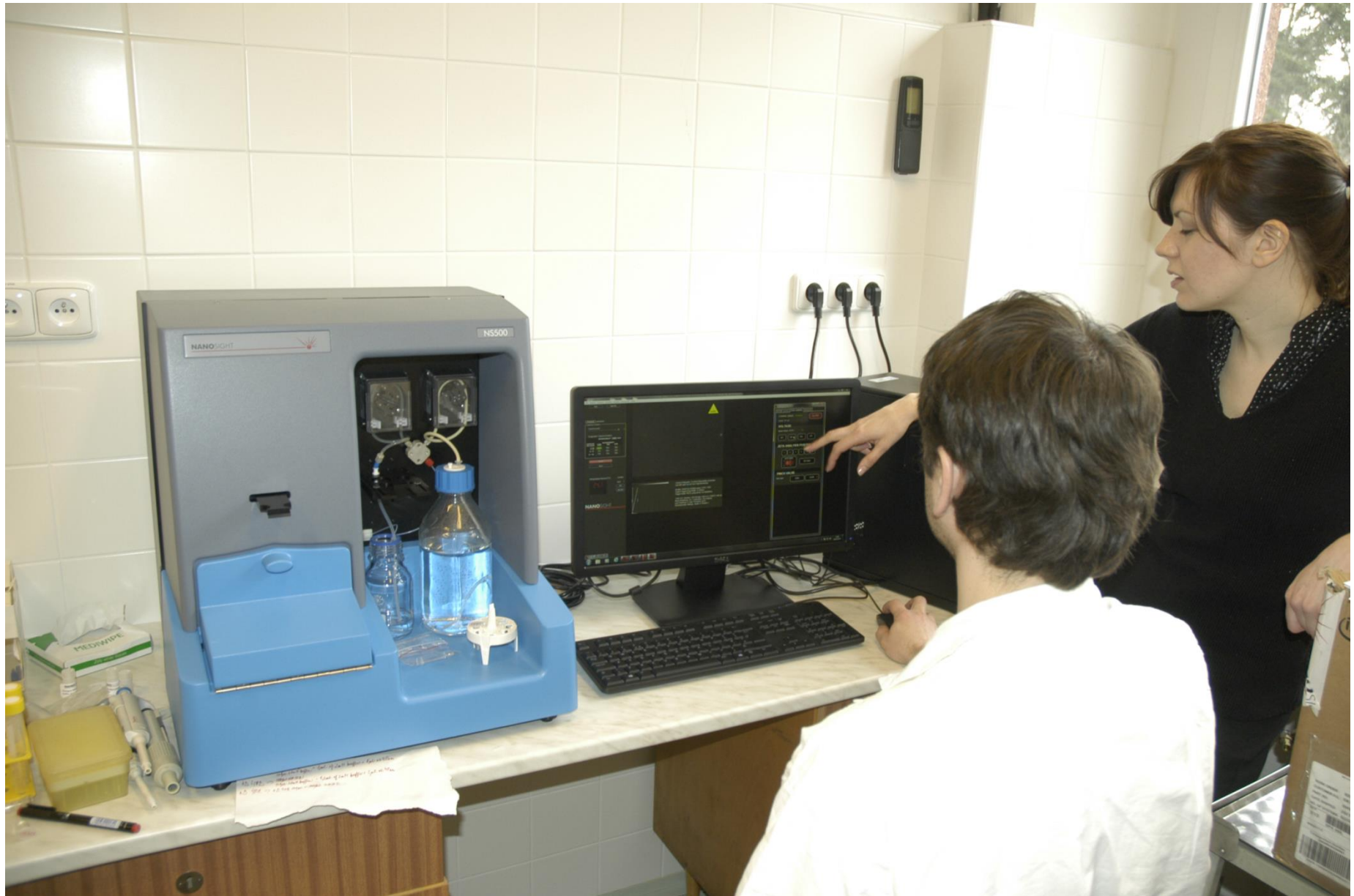


Parameter	Value
Sizing range	0.6 nm to 6 μ m Diam
Concentration range	0.1 mg/mL Lys to 30w%
Min sizing sample volume	12 μ L
Min zeta sample volume	0.75 mL
Temperature control	2 to 90 $^{\circ}$ C
Conductivity range	0 to 200 mS/cm
Laser	3 mW 633 nm HeNe
Detector	APD

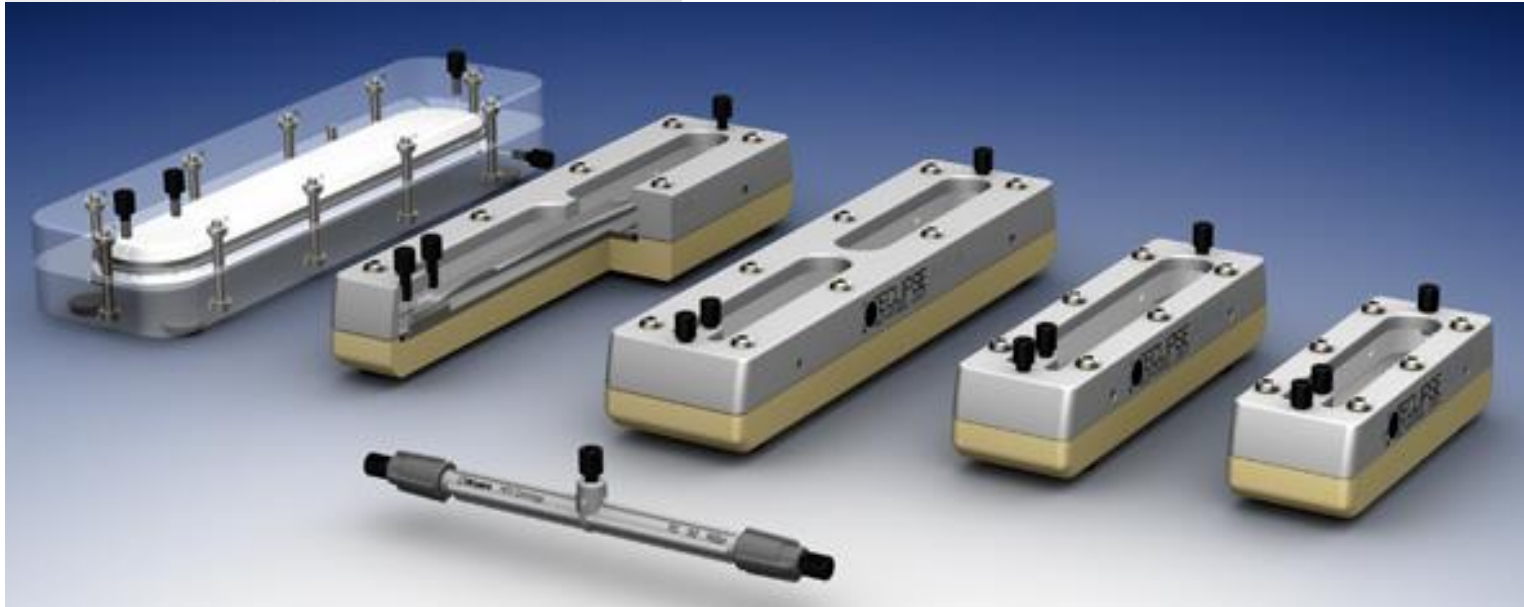


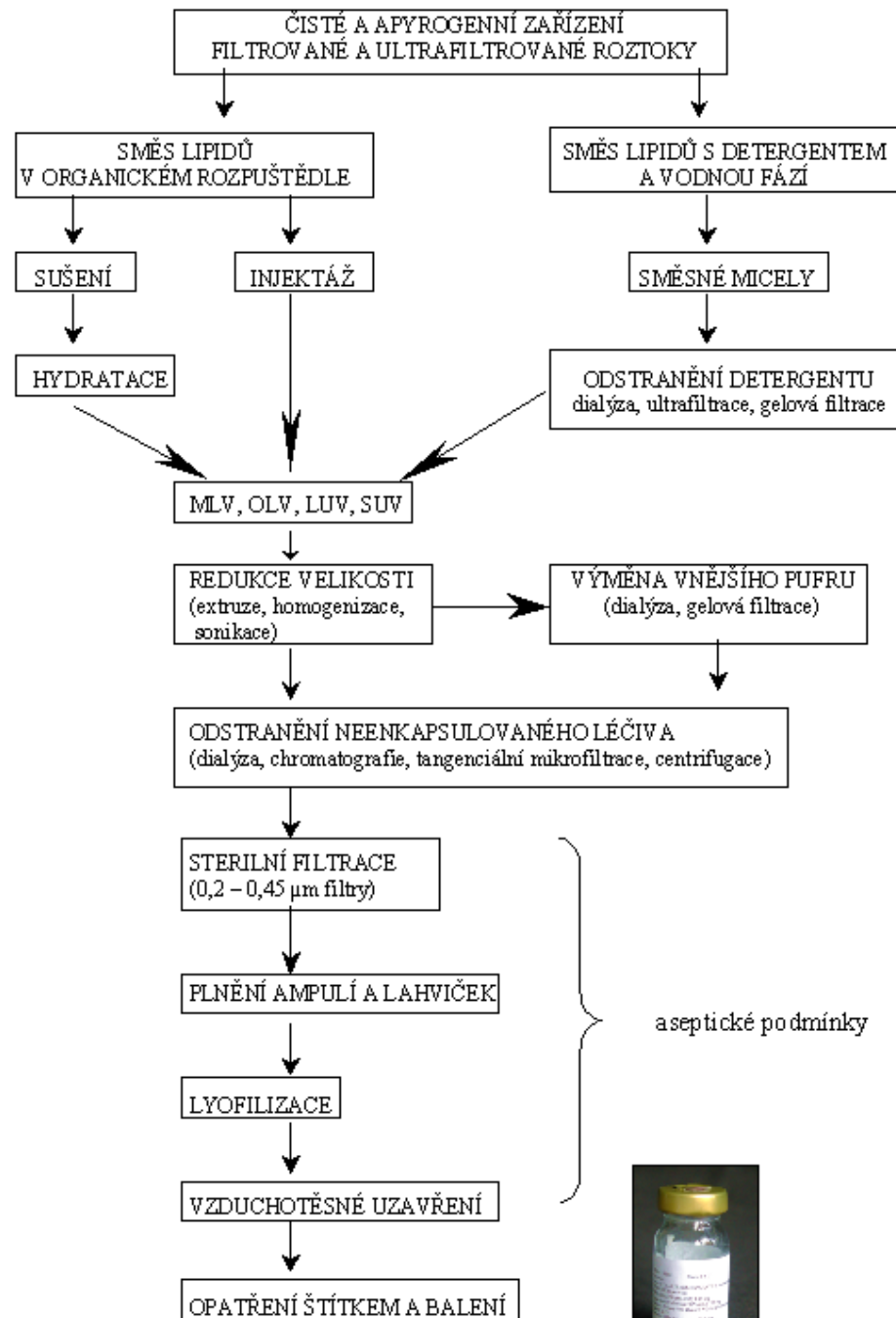
- ❖ Crystal screening
- ❖ Protein & polymer characterization
- ❖ CMC measurements
- ❖ Drug delivery systems
- ❖ Formulation stability
- ❖ Biological assemblies
- ❖ Virus & vaccine characterization
- ❖ Macromolecular critical points

NanoSight 500



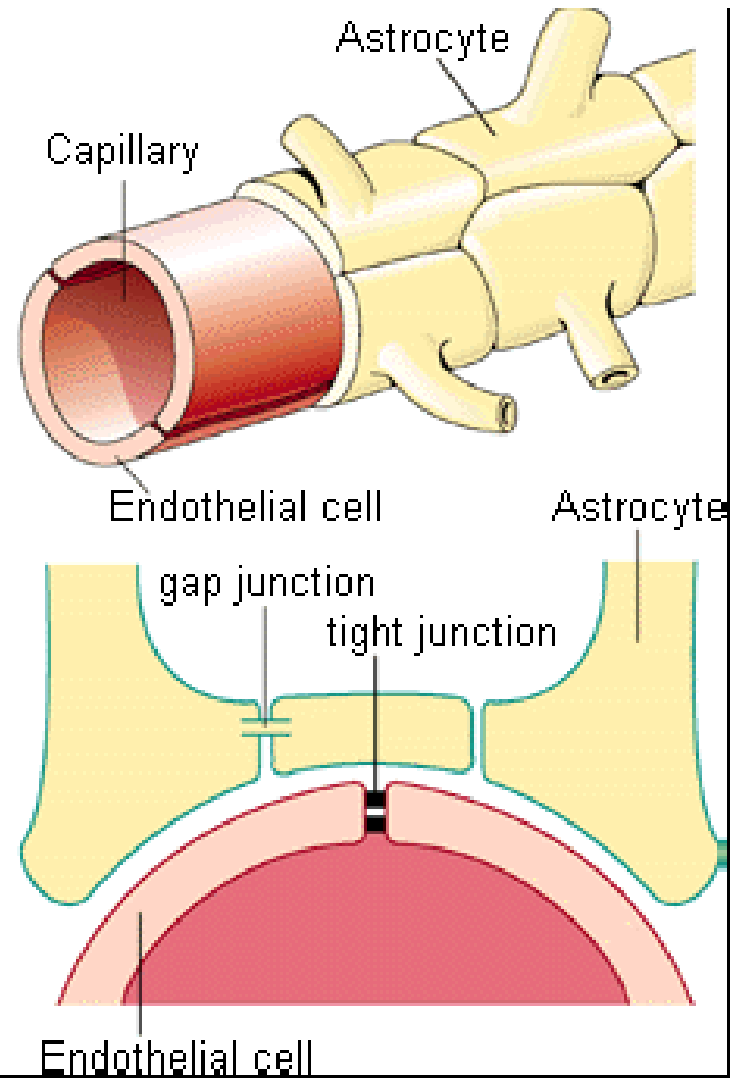
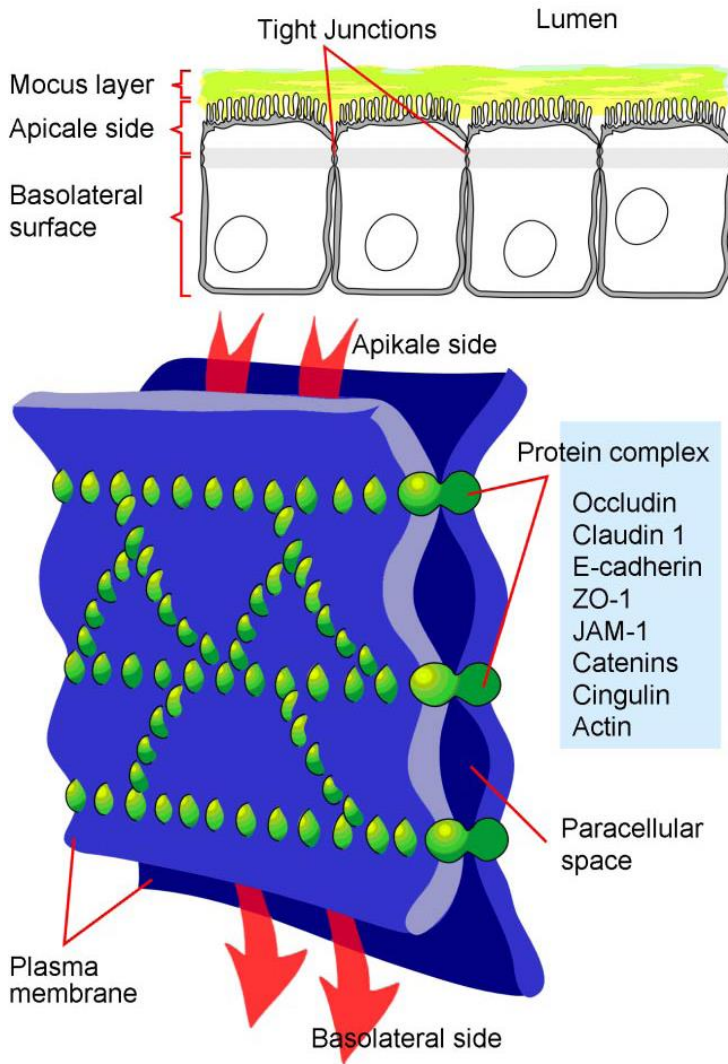
Fiel Flow Fractionation



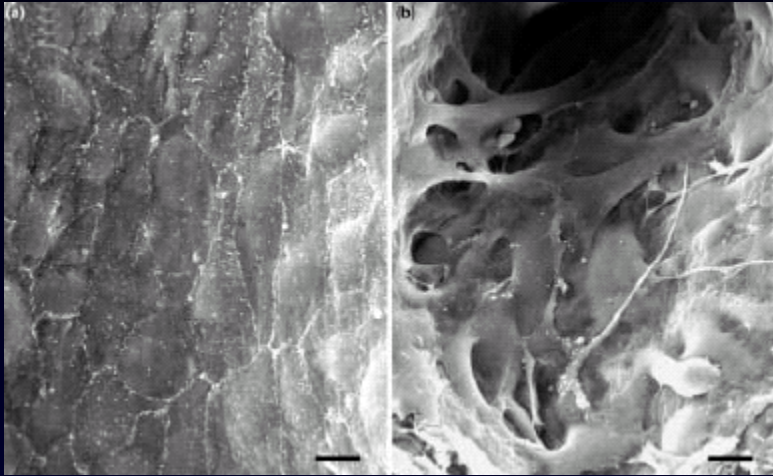


Nanocarriers and body compartments

Tight junction

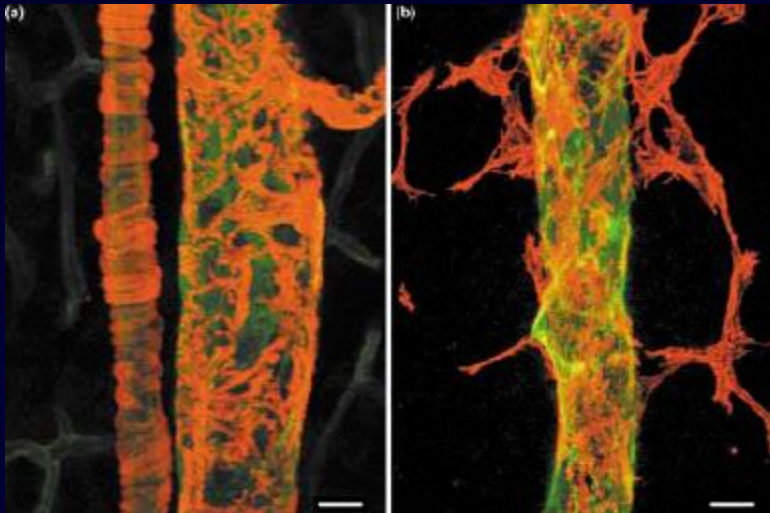


Normal vs tumour blood vessels



SEM images of the luminal surface of blood vessels in normal breast tissue and in mammary carcinomas.

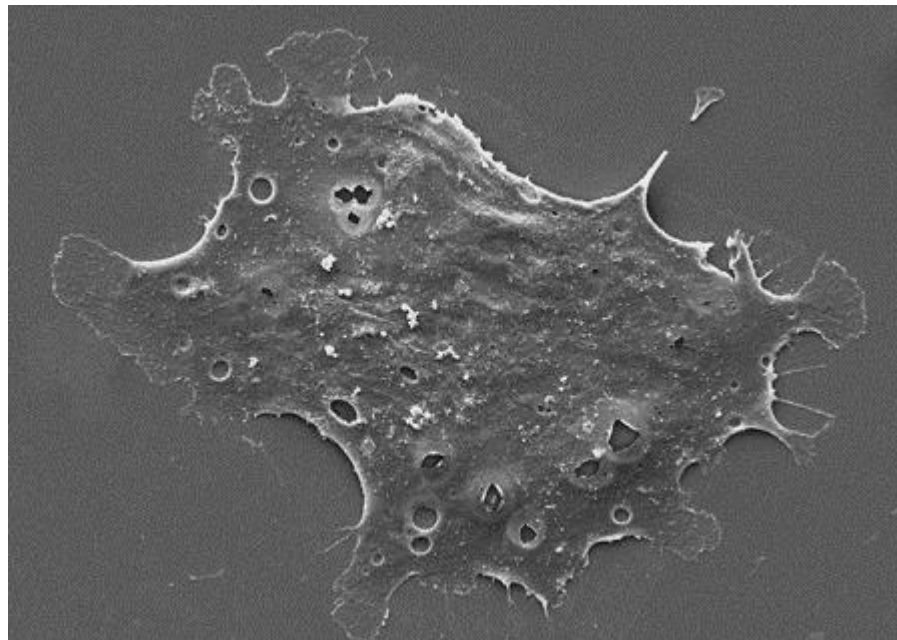
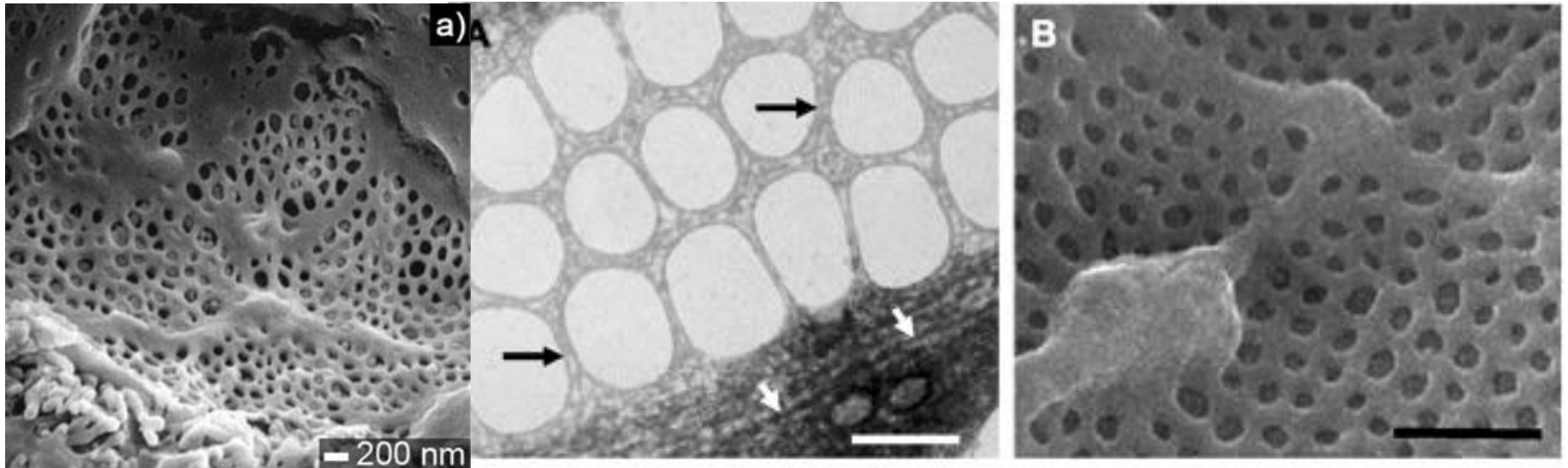
Normal EC - rather uniform in size, all in contacts with each other; tumour ECs - deformed, separated from each other, and overlapping each other, multiple cellular projections apparent as well



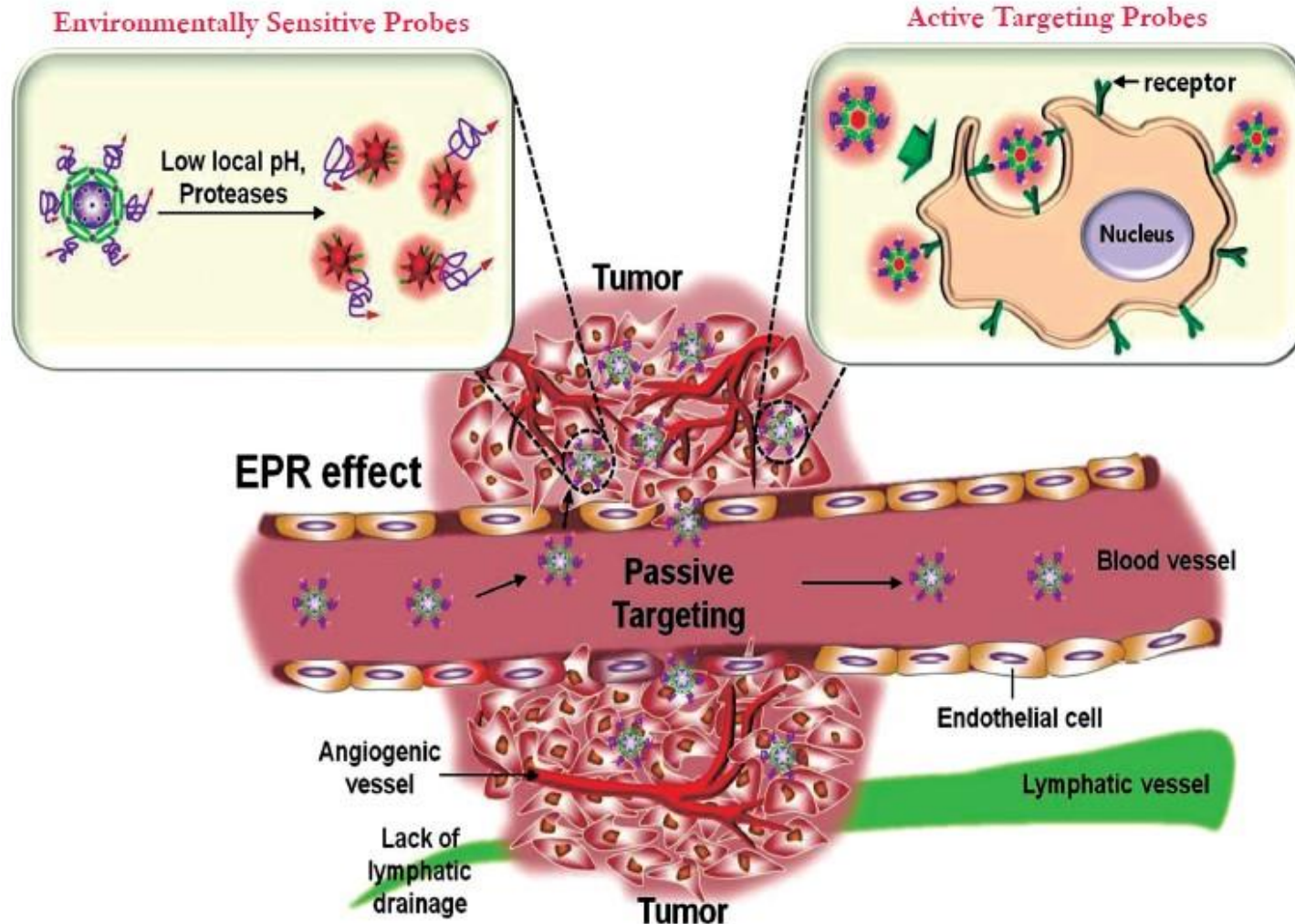
Confocal microscopic images of pericytes on normal venule and a venule on tumour surface

Pericytes visualised by immunostaining for α -smooth-muscle-actin (red), ECs by staining for the EC marker, CD31 (green). Normal pericytes well organised, adhering closely to endothelium, those on 'tumour' vessel disorganised, loosely adhering to the blood vessel.

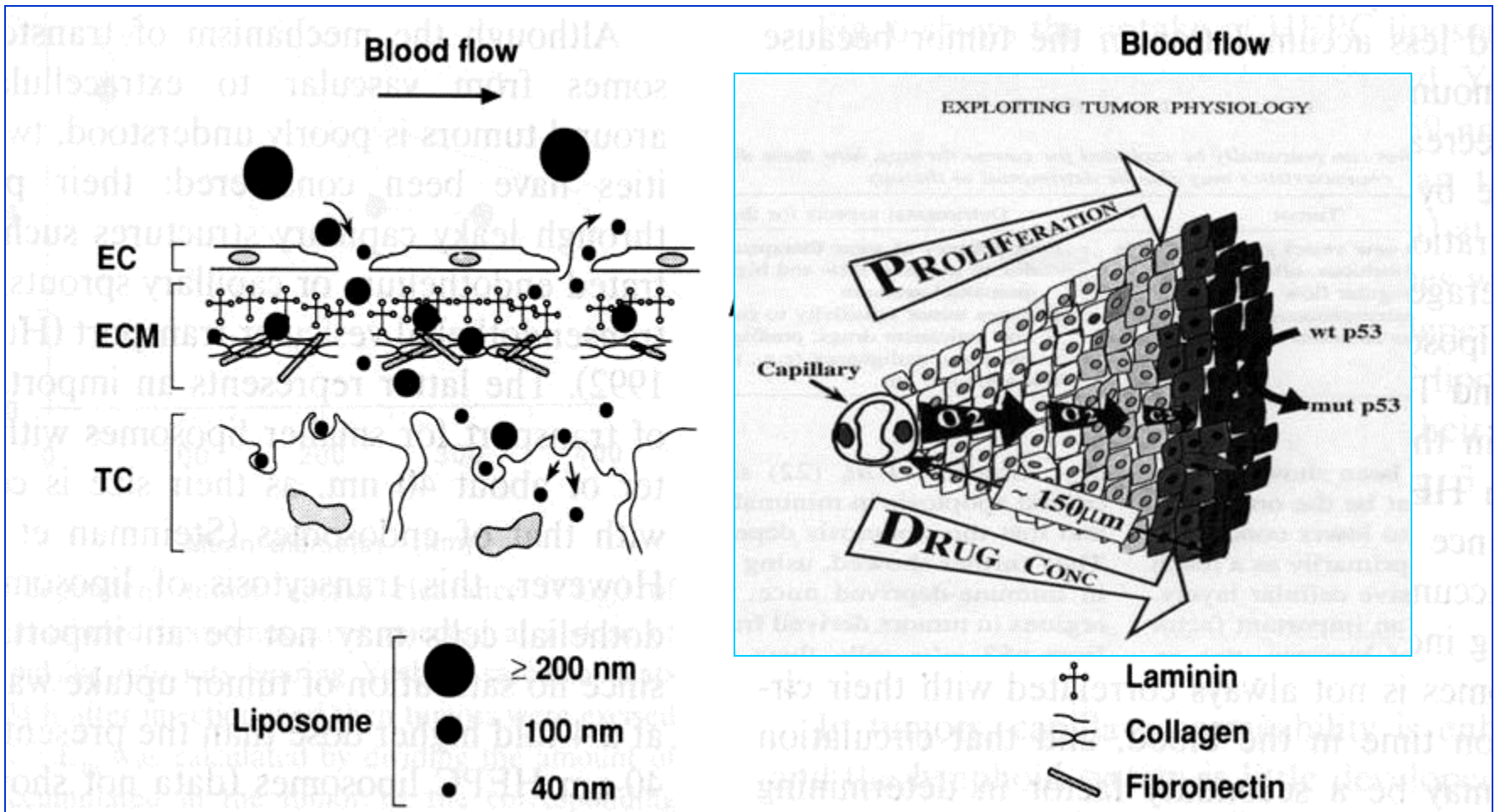
Liver and kidney fenestrations



Principle of EPR Effect

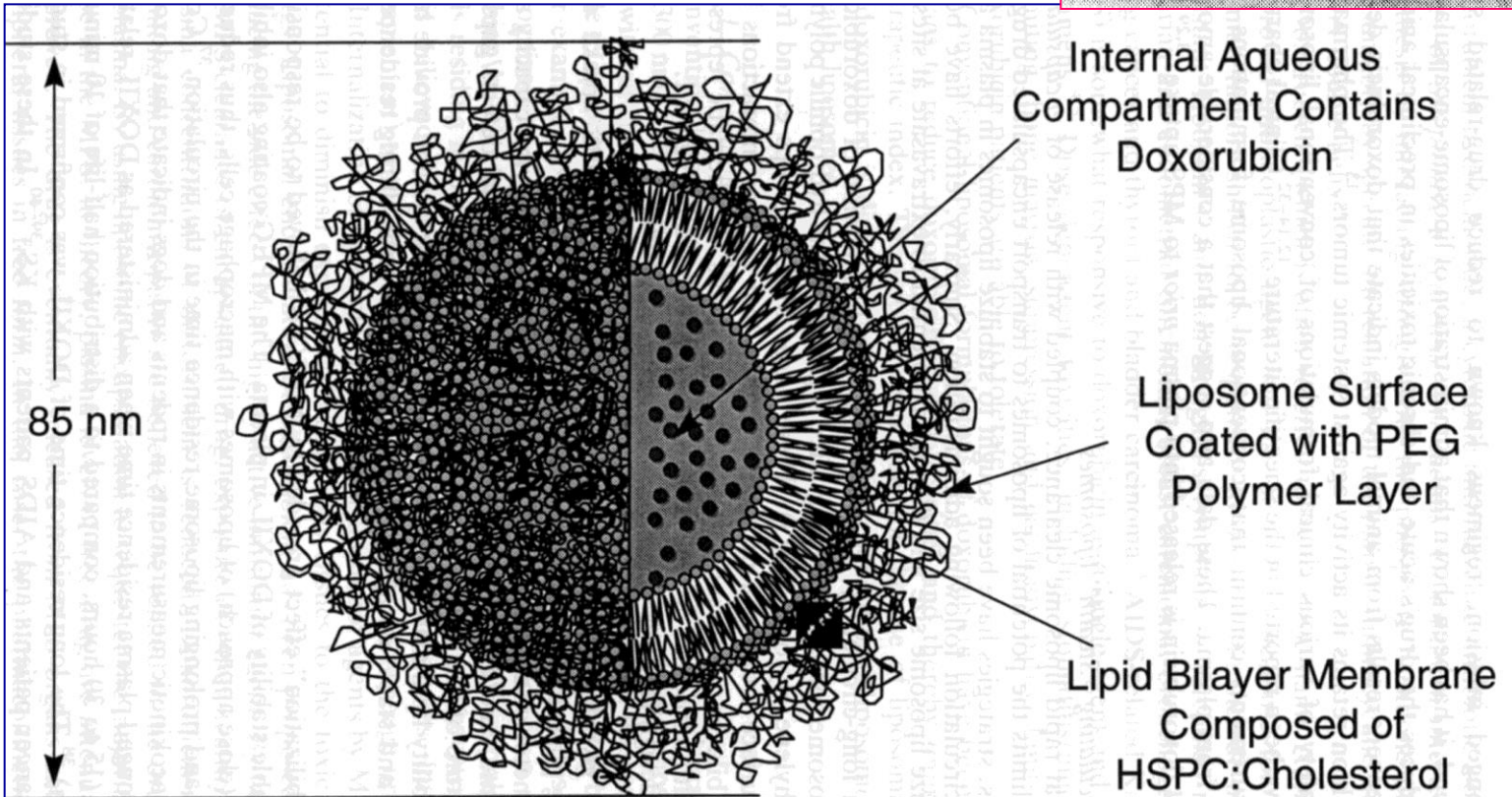
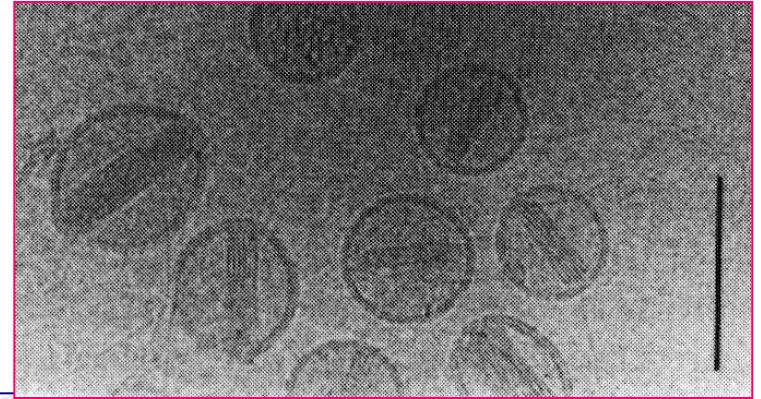


Penetration of liposomes through epithelial fenestrations - Passive targeting of liposomes



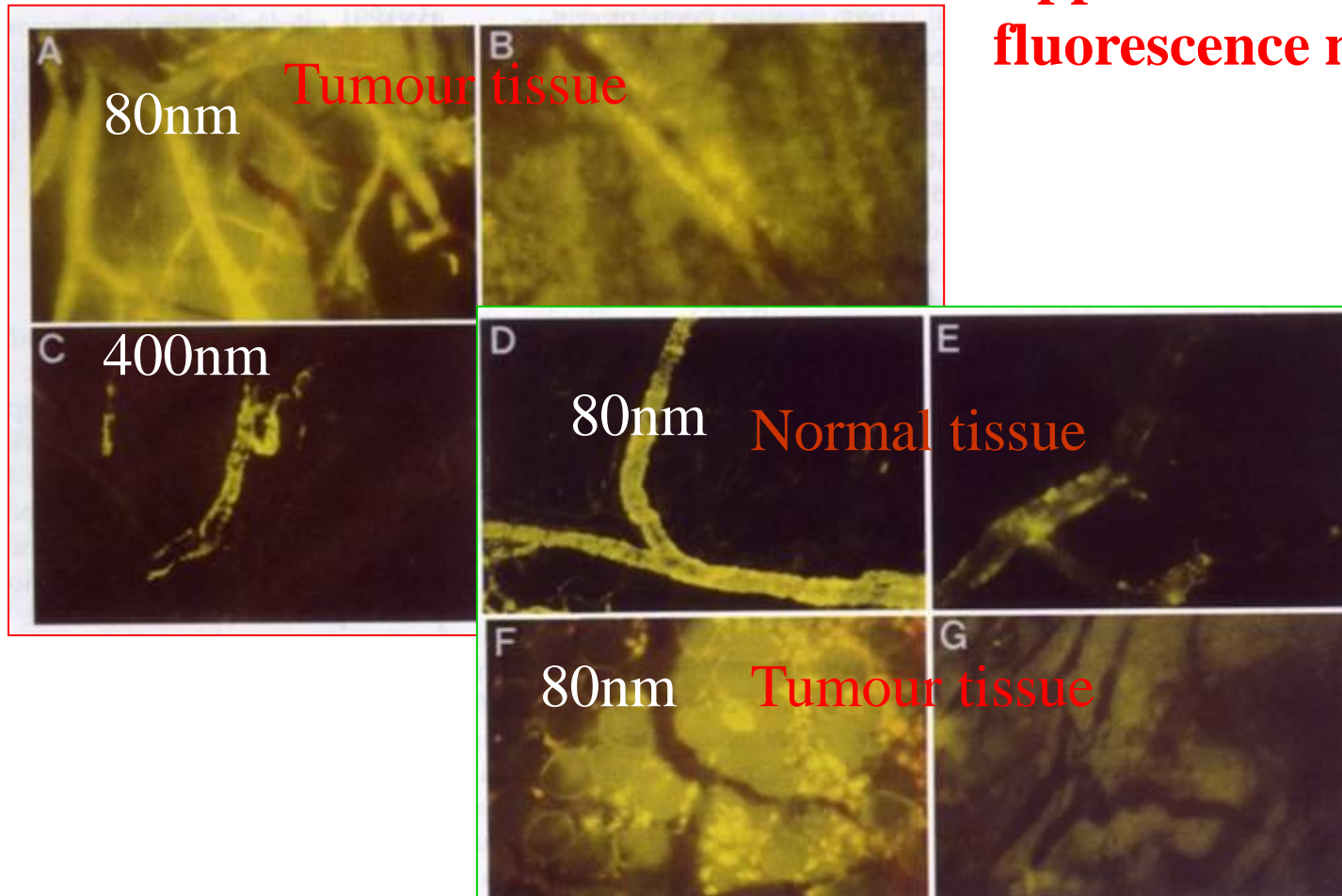
Stealth liposomes

Doxorubicin

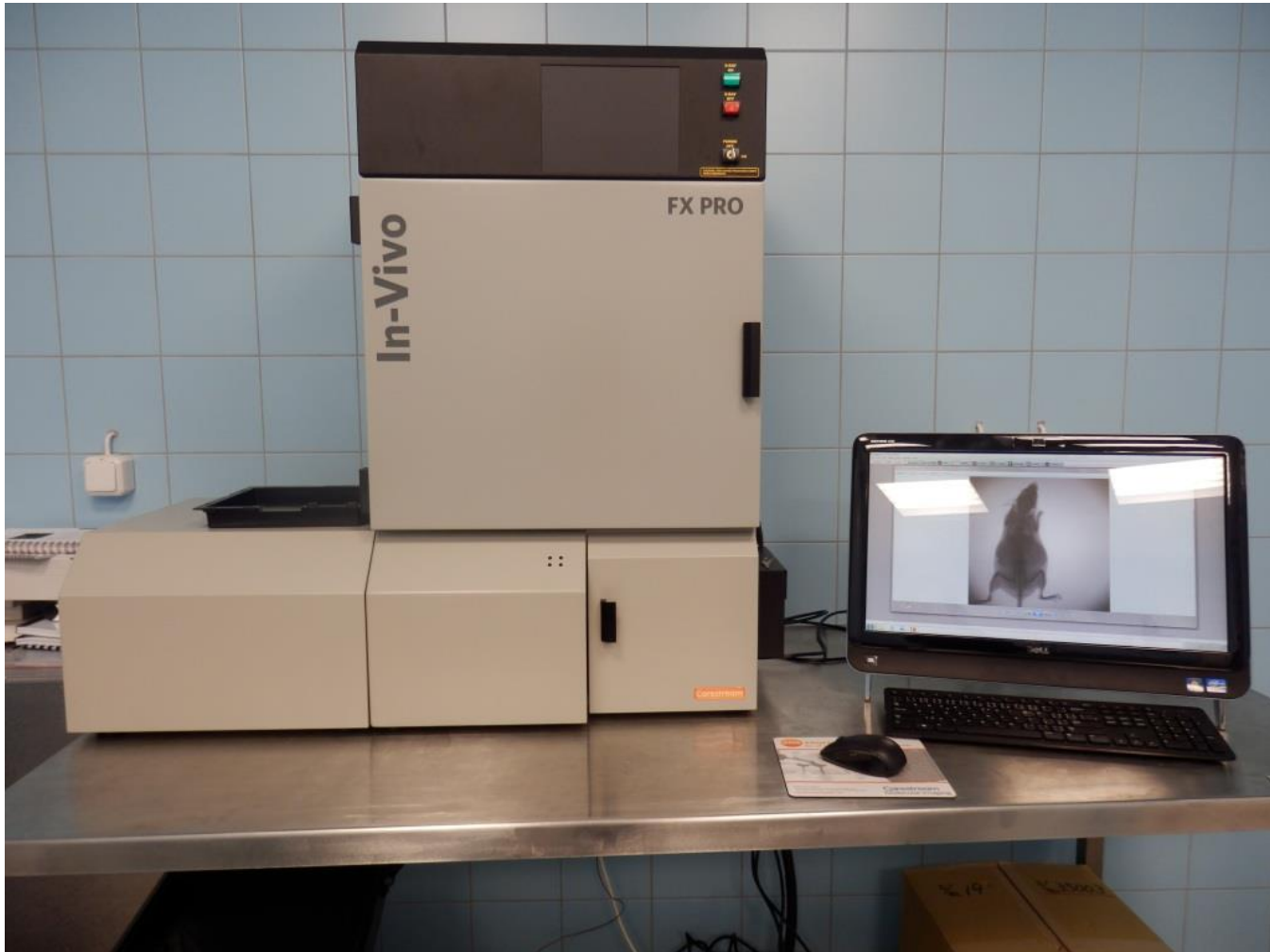


Penetration of Stealth liposomes into normal and tumour tissues

Application of *in vivo* fluorescence microscopy

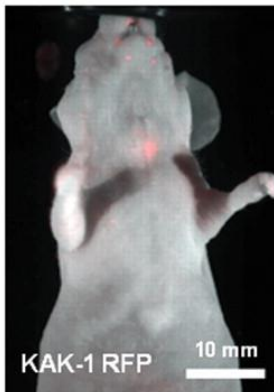
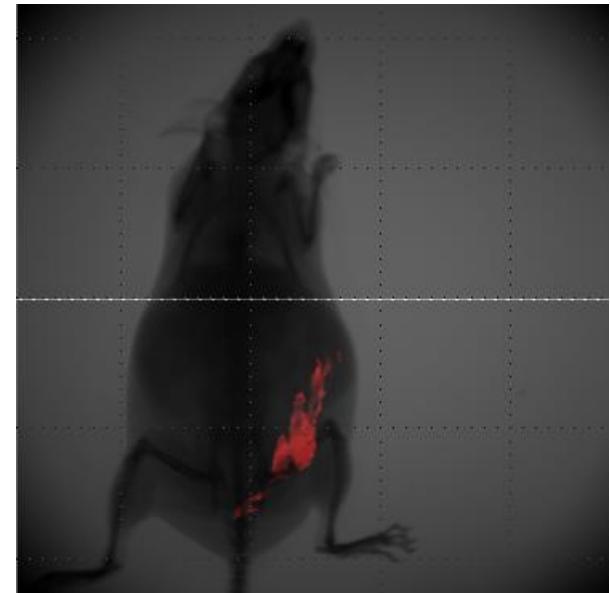
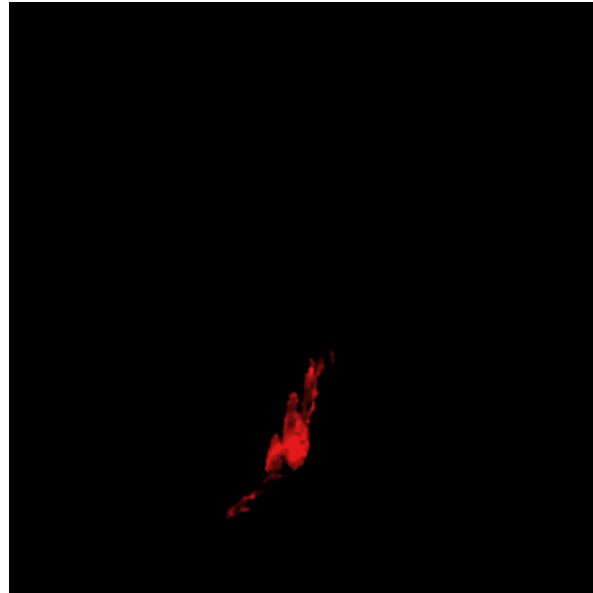
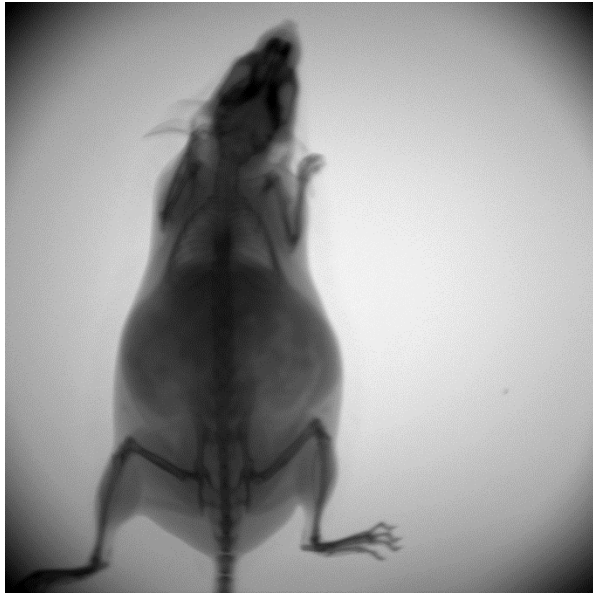


- In-vivo imaging

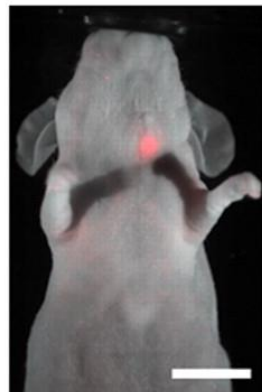


In-vivo imaging

- s.c. aplikace liposomální suspenze (100ul; 5mg lipidů/ml; 0,4mol % Lyssamin-Rhodamin B) fluorofor: Lyssamin-Rhodamin B



5



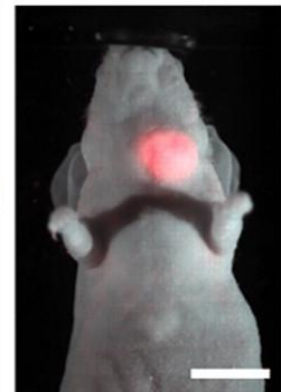
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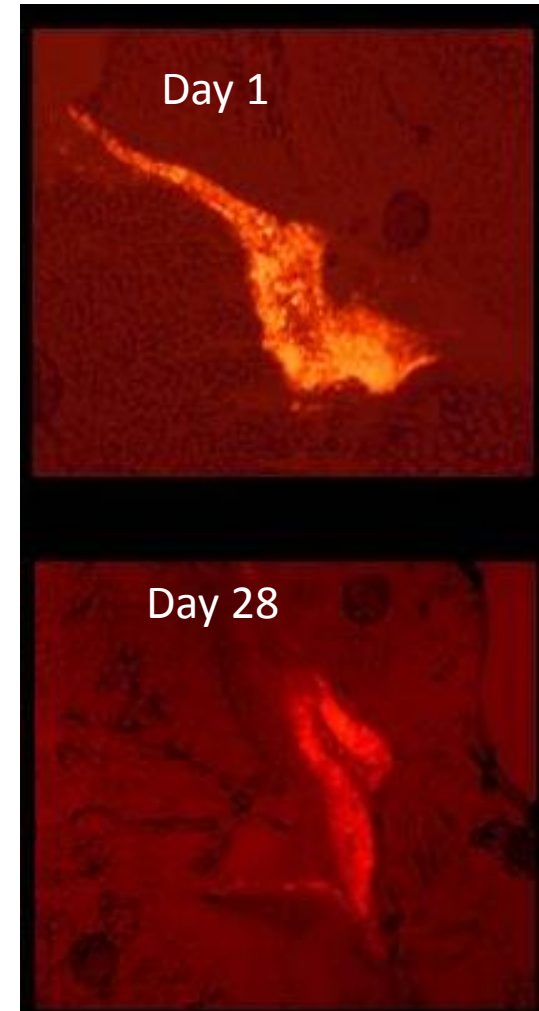
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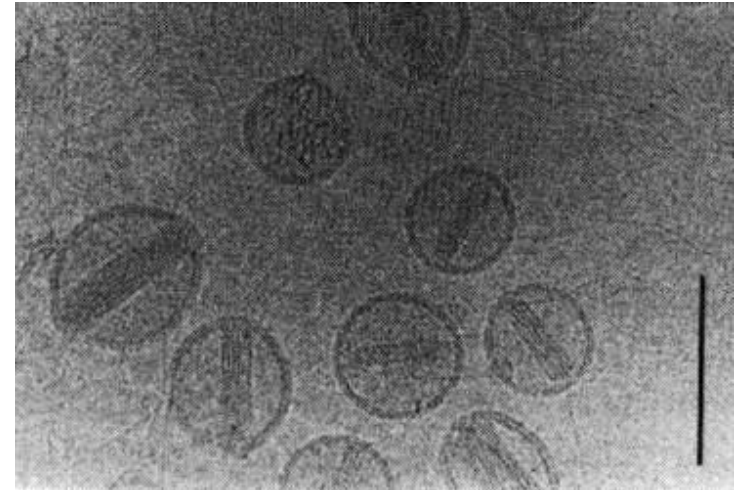
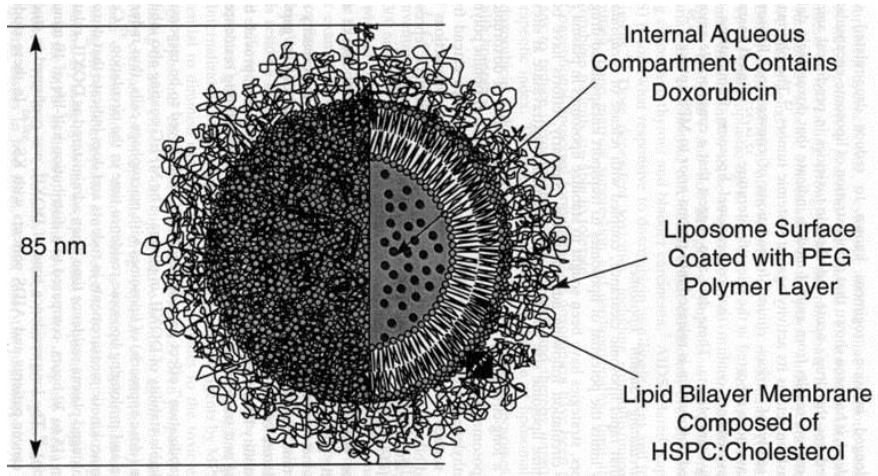
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Weeks after SOI

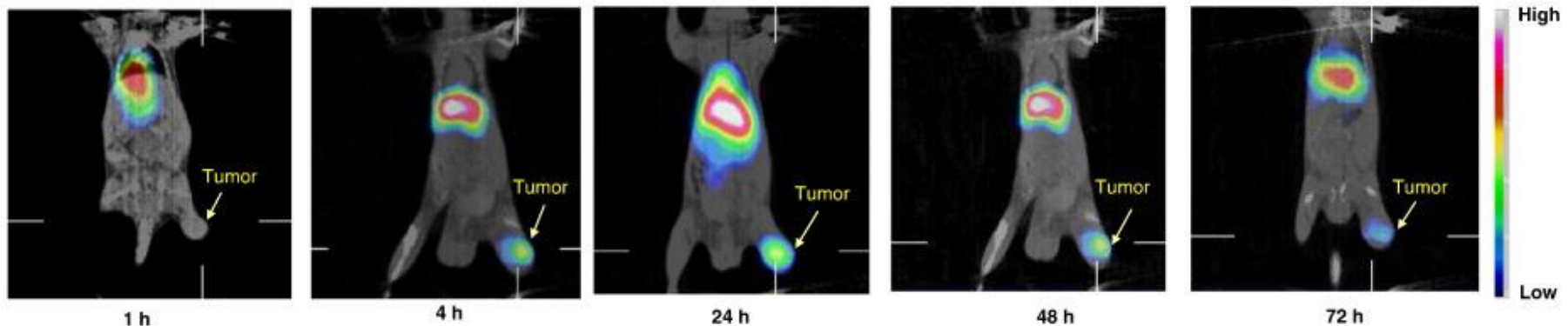
Visualizace liposom-DNA depotu fluorescenčním celotělným zobrazením



Kumulace isotopicky značených liposomů s doxorubicine v nádorové tkáni myši

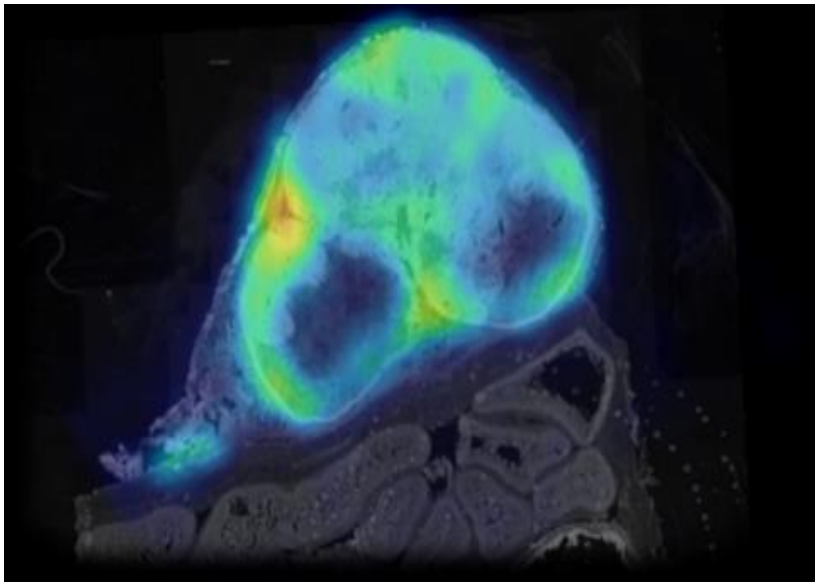
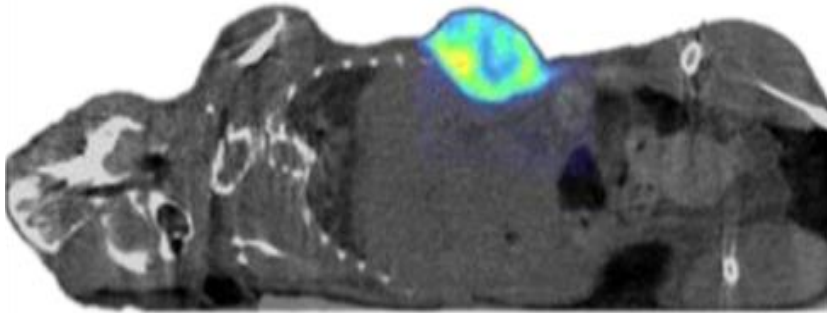


B ^{188}Re -DXR-liposome



Quantify heterogeneity of molecules within tumors

U-SPECT⁺/CT



JOURNAL OF PHARMACEUTICAL SCIENCES, VOL. 99, NO. 5, MAY 2010
Liposomes With High Encapsulation Capacity for Paclitaxel:
Preparation, Characterisation and In Vivo Anticancer Effect

Journal of Controlled Release 163 (2012) 322–334



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Journal of Controlled Release

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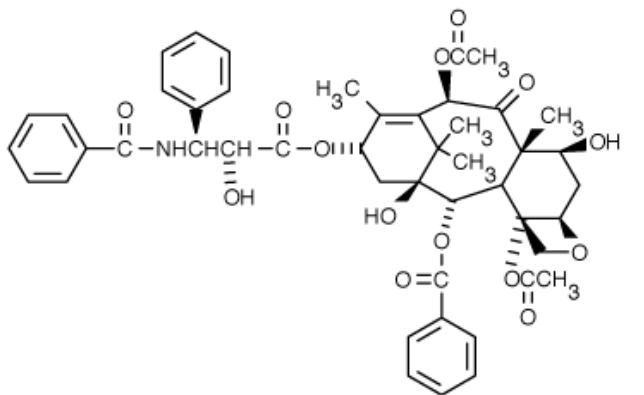
Review

Liposomal paclitaxel formulations

Štěpán Koudelka ^a, Jaroslav Turánek ^{a,b,*}

^a Department of Toxicology, Pharmacology and Immunotherapy, Veterinary Research Institute, Brno, Czech Republic

^b Department of Pharmacology, Faculty of Medicine, Masaryk University, Brno, Czech Republic



Paclitaxel



- This drug is approved for **ovarian and breast cancer treatment** and is one of the most exciting anticancer molecules currently available. Suitable drug formulation remains still a problem, because paclitaxel has a low therapeutical index owing to its **high lipophilic character and low solubility in water**.
- The commercially available injection preparation is a sterile solution of the drug in **Cremophor® EL**. Present-day, cancer chemotherapy with paclitaxel frequently causes **hypersensitivity reactions** in spite of suitable premedication (corticosteroids, anti-histamines). The major hurdles for successful therapy with paclitaxel are the availability of the drug and its delivery.

Drug delivery formulations of paclitaxel

- **Liposomes**, niosomes and micels
- Water-soluble prodrugs (increased solubility, problems with stability and activity)
- Lipophilic prodrugs (enhanced incorporation into lipid-based emulsion)
- Enzyme-activatable prodrugs conjugated with antibodies or albumin
- Parenteral emulsions
- Microspheres
- Inclusion complexes with cyclodextrins
- Nanocrystals.



Liposomal Paclitaxel

Increase in MTD, lower toxicity and higher efficiency was found for these preparations when compared with Cremophor based formulation of paclitaxel.

Maximum entrapment capacity for paclitaxel in these liposomal formulations was around 3 molar %.



Toxicity of PTX-liposomes

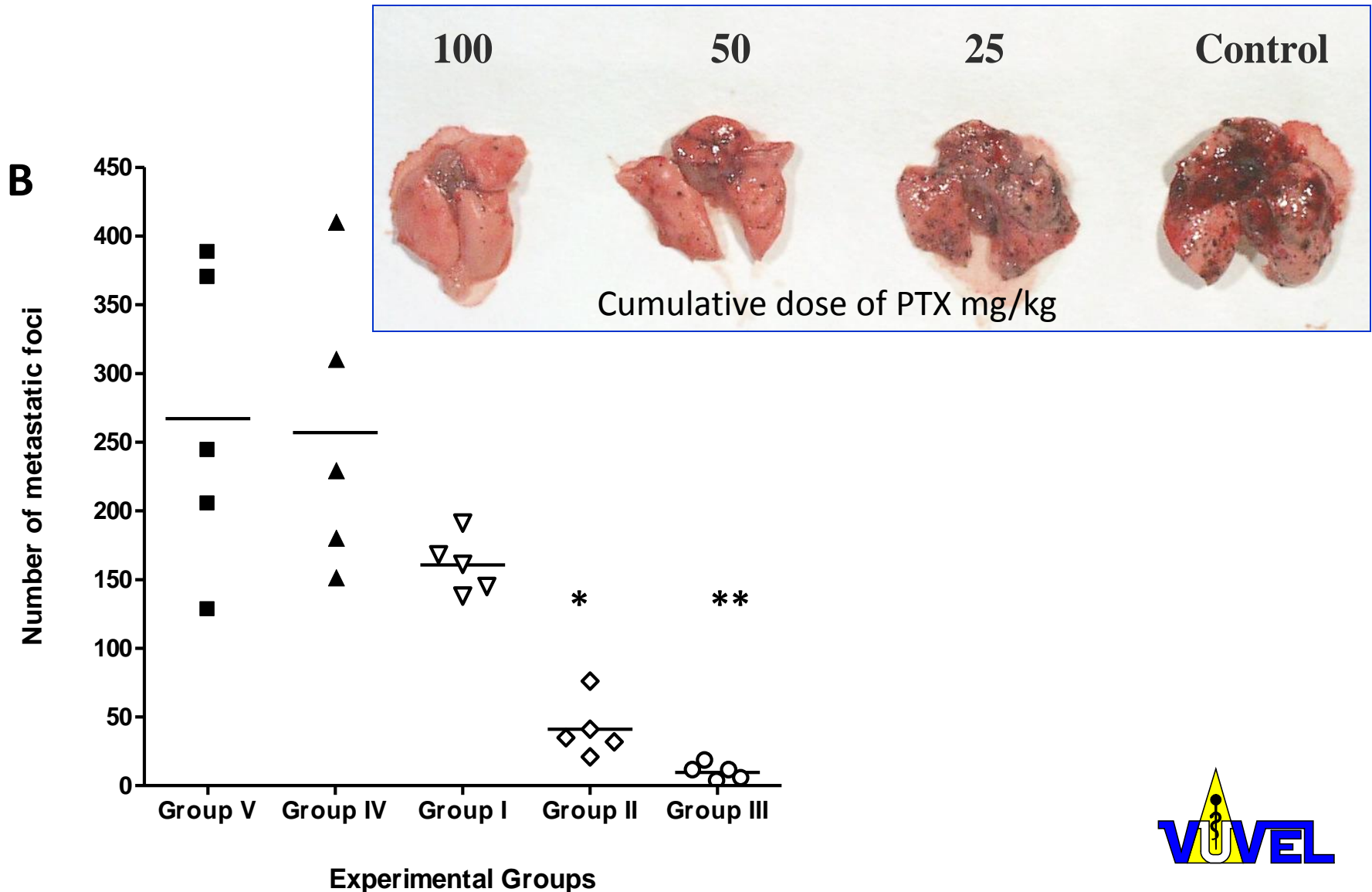
Liposomal PTX was applied to mice within 15 s by *i.v.* route via tail vein. The size of liposomes was about 200 nm. The dose of 200 μ l contained 0.41; 0.82 a 1.64 mg PTX/mouse (20.5, 41 and 82 mg PTX /kg), respectively.

No symptoms of toxicity were seen based on the Berlin test.

The highest dose of PTX used for the treatment of mice is about **100 mg/kg** (usually 10-25 mg/kg). Maximal cumulative doses of PTX used for experimental testing on mice models are 120 mg/kg (3-5 doses with an interval of 48 h, 30 mg/kg per single dose). In view of this fact, the side effects of our liposomal PTX are negligible in comparison to Taxol® (Paclitaxel solubilised in Cremophor, Bristol-Myers Squibb Co), whose toxic dose is about **20-25 mg/kg** (Cabanés A., et al. Comparative in vivo studies with paclitaxel and liposome-encapsulated paclitaxel. INTERNATIONAL JOURNAL of ONCOLOGY. 1998, 12, 1035-1040).



Expanses of lung metastases of B16F10 melanoma cells in mice treated with PTX-liposomes



Lyophilised Liposome-Based Formulations of α -Tocopheryl Succinate: Preparation and Physico-Chemical Characterisation
Štěpán KOUDELKA, Josef MAŠEK, Jiří NEUZIL, Jaroslav TURÁNEK

Toxicology and Applied Pharmacology 237 (2009) 249–257

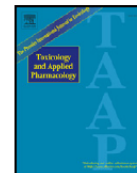


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Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap



Liposomal formulation of α -tocopheryl maleamide: *In vitro* and *in vivo* toxicological profile and anticancer effect against spontaneous breast carcinomas in mice

Jaroslav Turánek ^{a,*}, Xiu-Fang Wang ^b, Pavlína Knötigová ^a, Štěpán Koudelka ^a, Lan-Feng Dong ^b, Eva Vrublová ^a, Elahe Mahdavian ^c, Lubomír Procházka ^a, Smink Sangsura ^c, Antonín Vacek ^a, Brian A. Salvatore ^c, Jiri Neuzil ^{b,d,*}



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Oncogene. Author manuscript; available in PMC 2009 April 14.

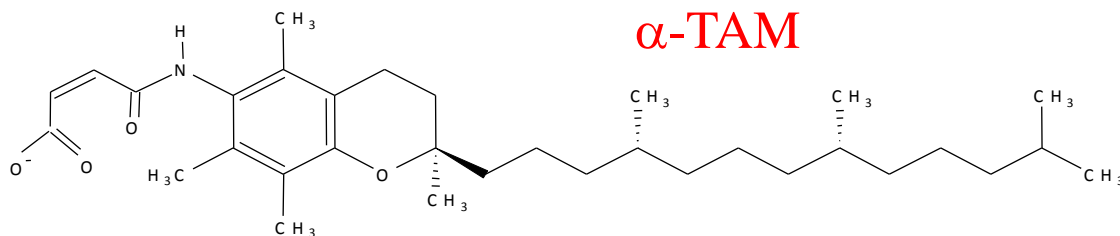
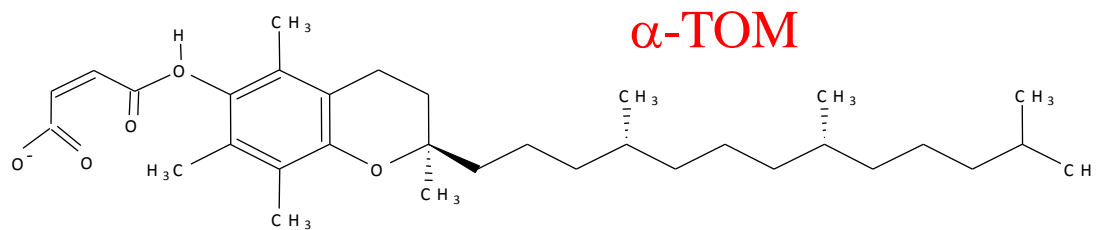
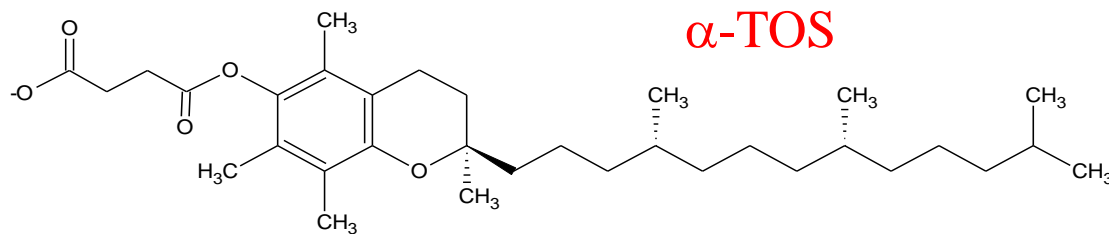
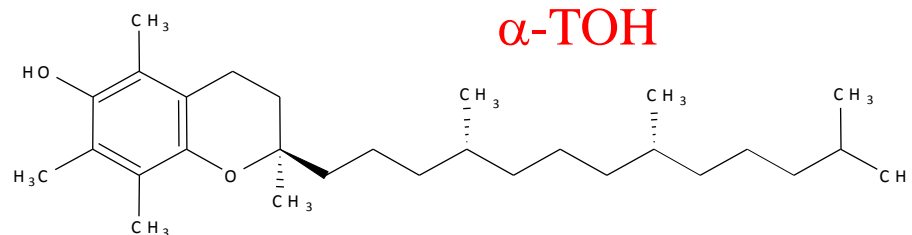
Published in final edited form as:

Oncogene. 2008 July 17; 27(31): 4324–4335. doi:10.1038/onc.2008.69.

α -Tocopheryl succinate induces apoptosis by targeting ubiquinone-binding sites in mitochondrial respiratory complex II

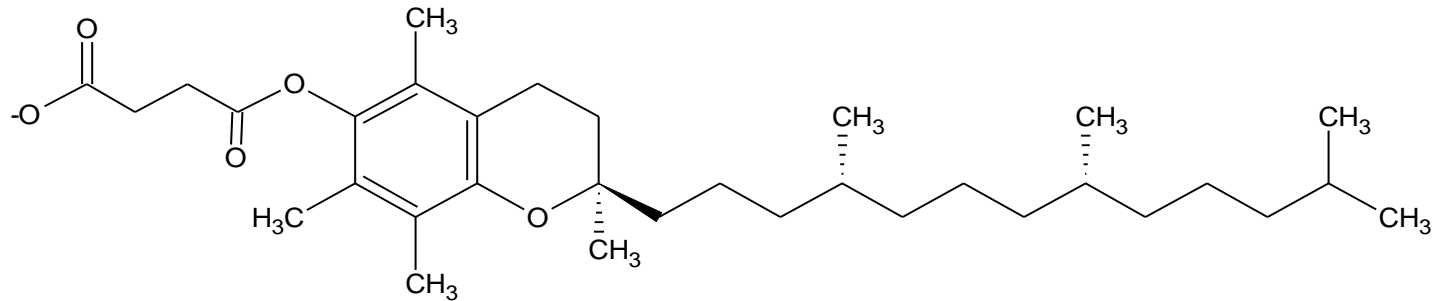
Lan-Feng Dong¹, Pauline Low², Jeffrey C. Dyason³, Xiu-Fang Wang¹, Lubomir Prochazka^{4,#}, Paul K. Witting⁵, Ruth Freeman¹, Emma Swettenham¹, Karel Valis⁶, Ji Liu¹, Renata Zobalova^{1,6}, Jaroslav Turanek⁴, Doug R. Spitz⁷, Frederick E. Domann⁷, Immo E. Scheffler⁸, Stephen J. Ralph^{2,*}, and Jiri Neuzil^{1,6,*}

New anticancer drugs based on derivatives of vitamine E

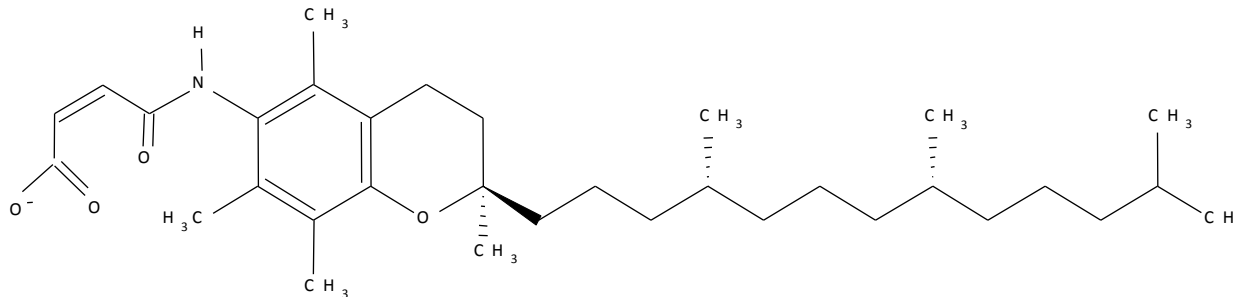


In vitro activity

Mechanism of action at cellular and subcellular levels



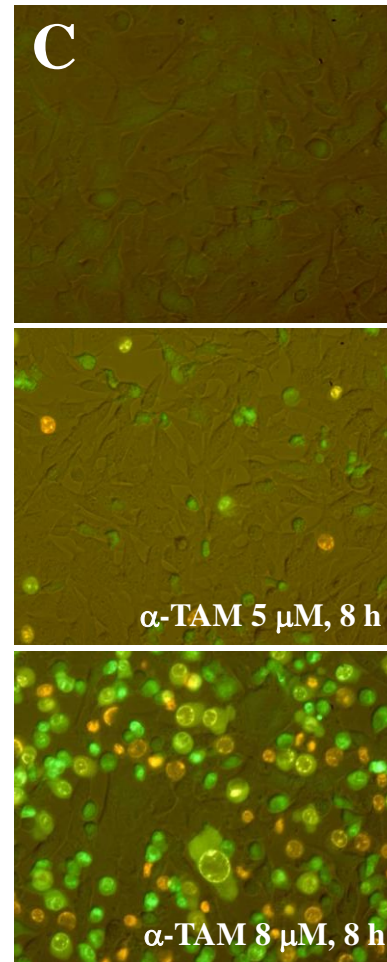
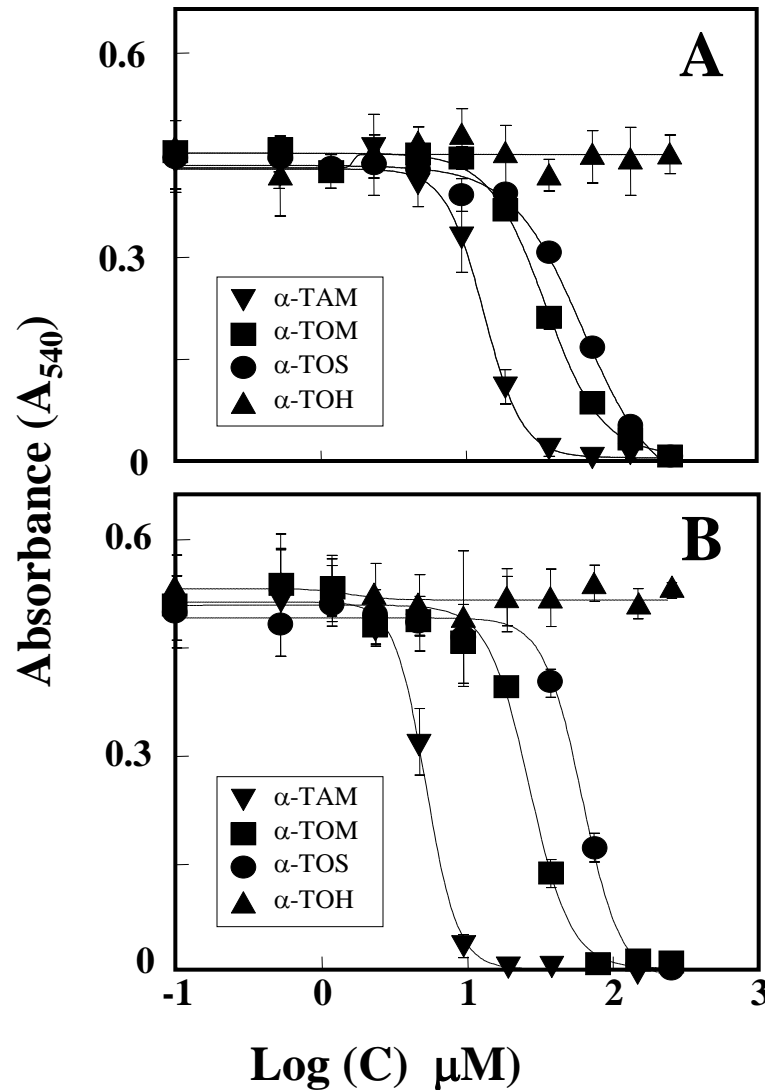
Ester bond in α TOS is cleavable by **nonspecific esterases** (decreased activity of this enzyme was found in **85%** of cancer cells)



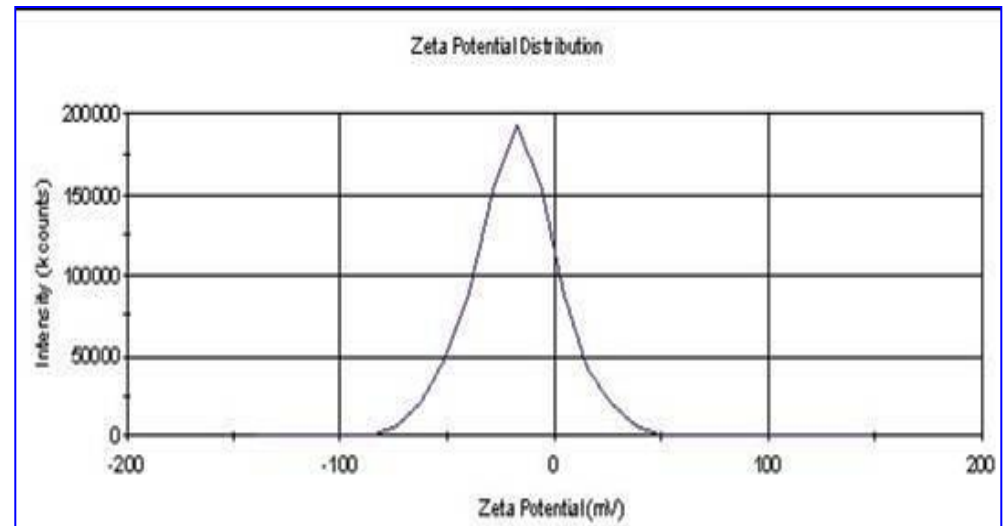
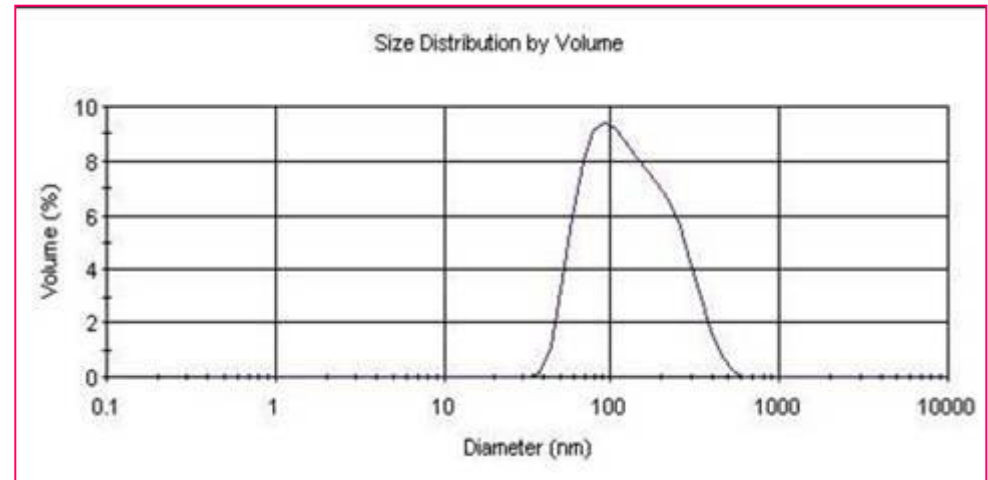
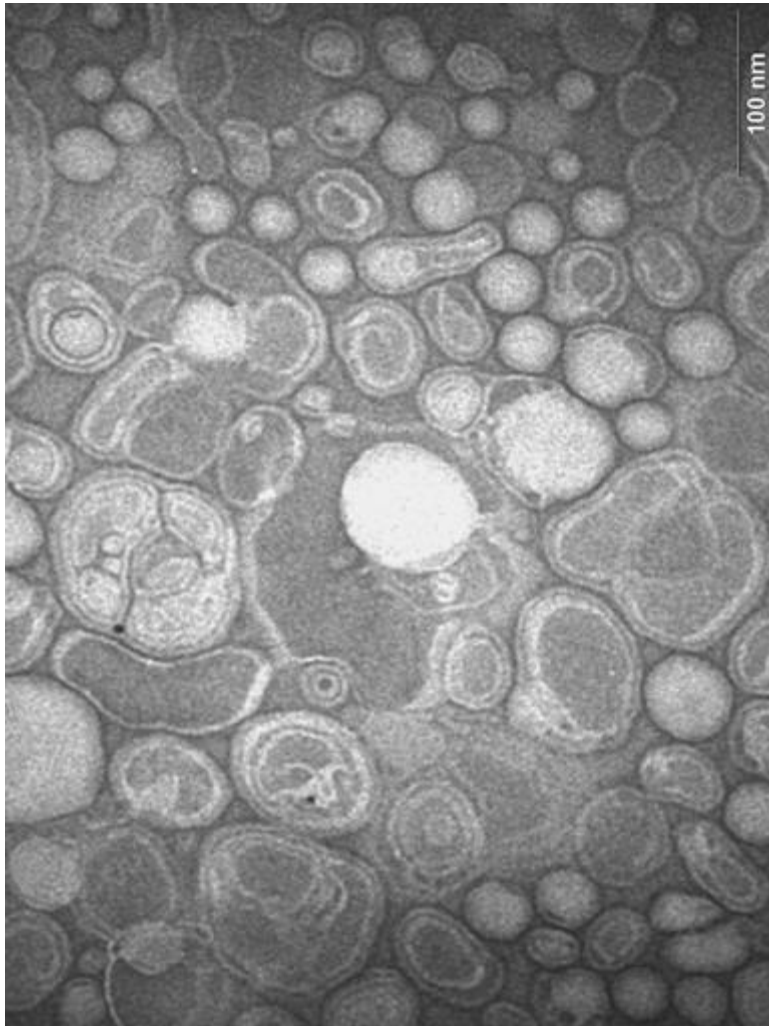
α TAM represents those derivatives which are resistant to degradation by **nonspecific esterases**

Higher cytotoxicity but also **serious side effects** (lethal neurotoxicity)

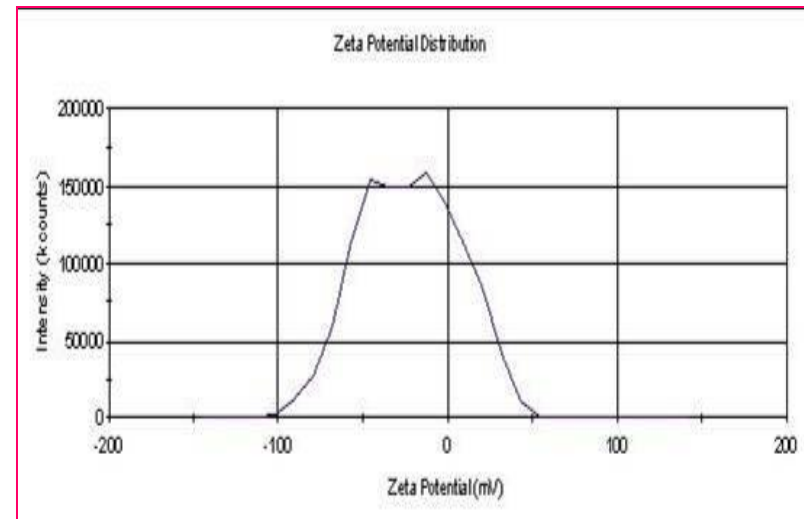
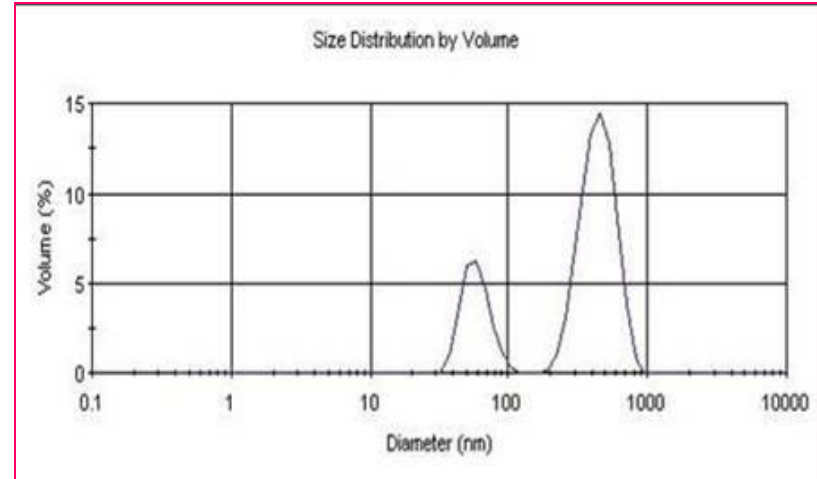
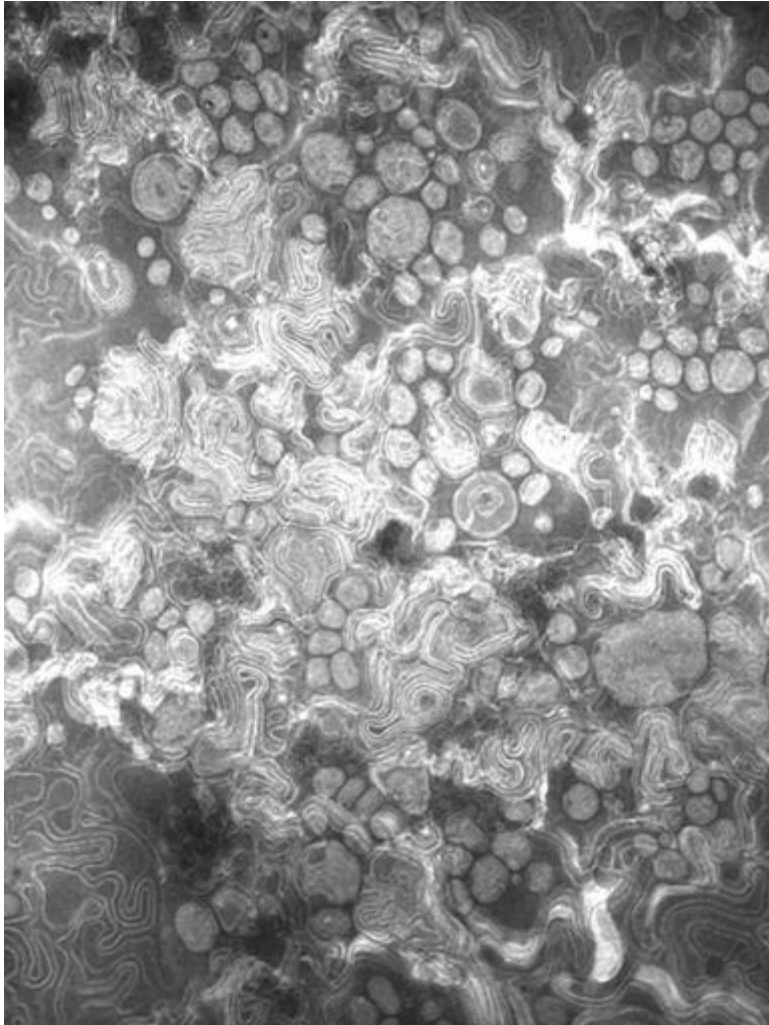
In vitro cytotoxicity – melanoma B16F10



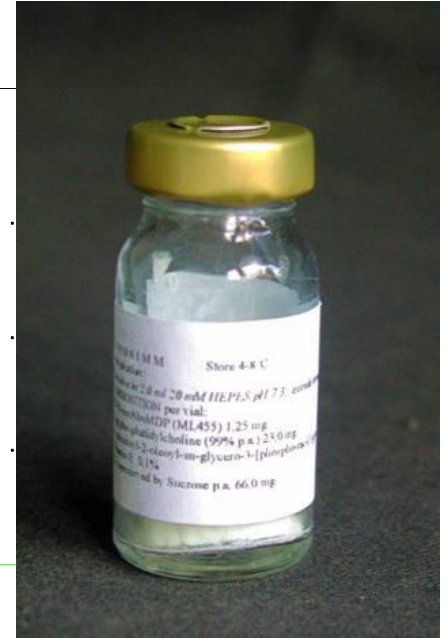
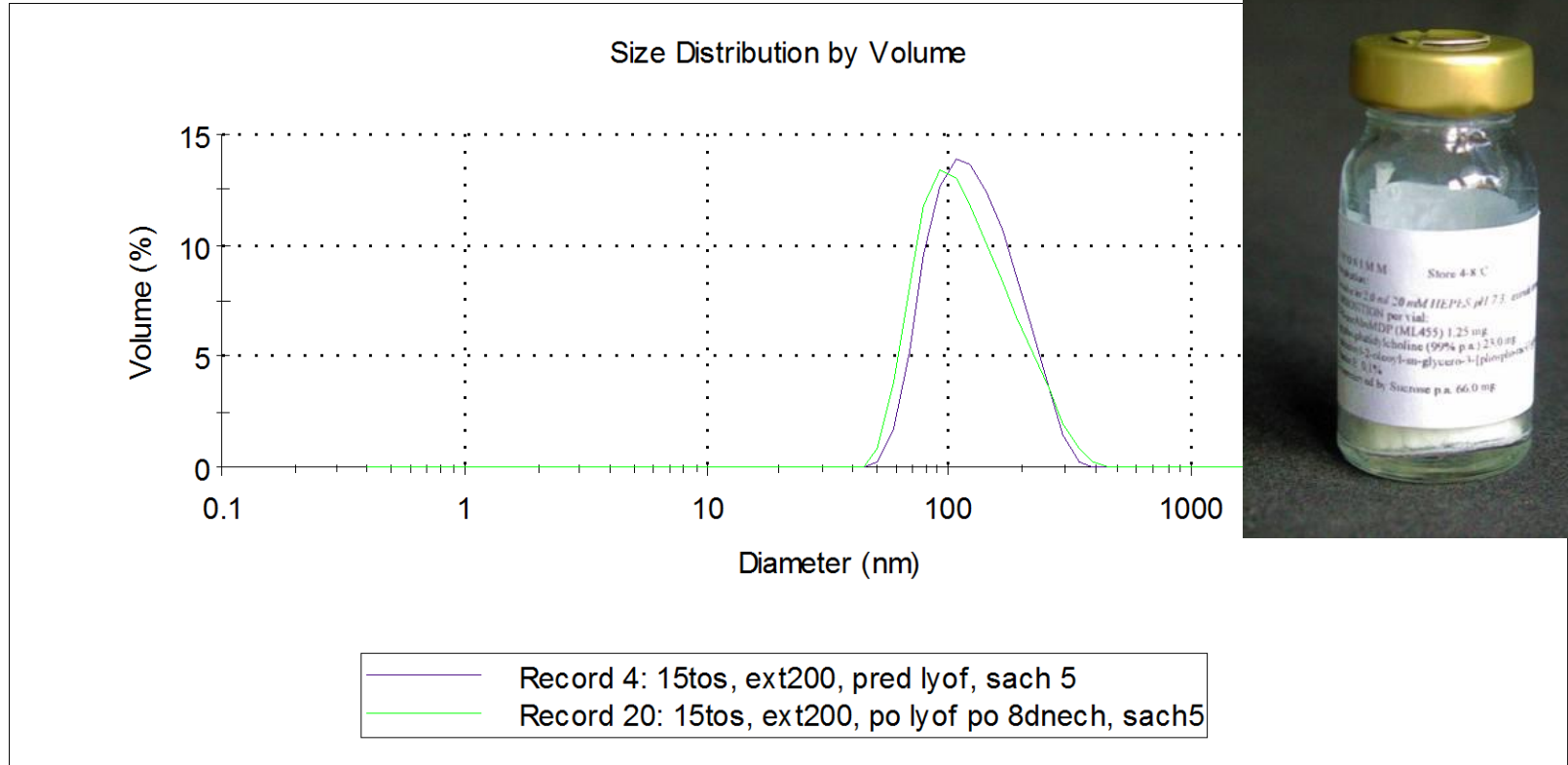
Liposomes 10% α TOS, extrusion through filters 200nm



Liposomes 20% α TOS, extrusion through 600nm



Stability of Lyophilised Liposomal Preparation of α TOS

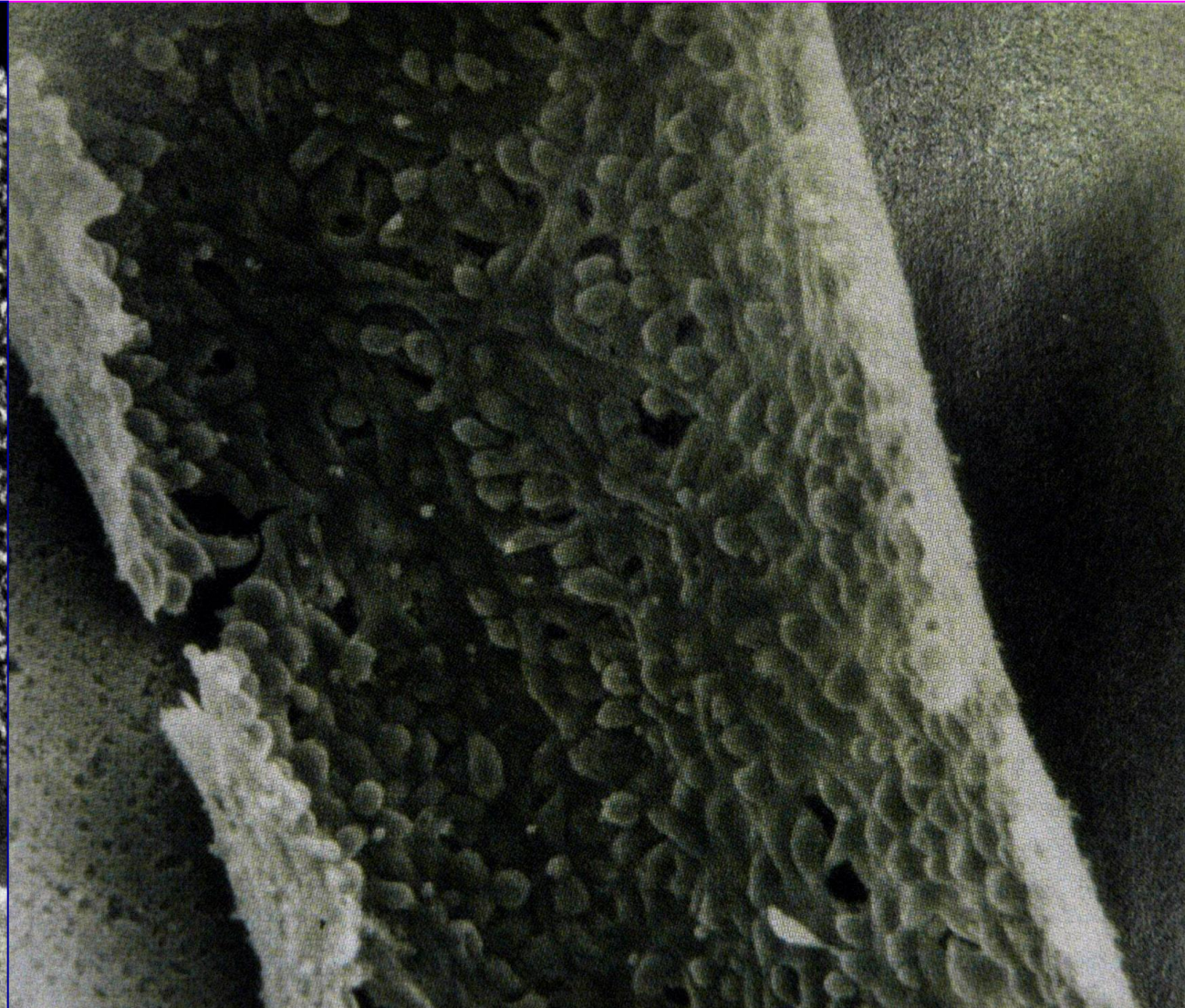
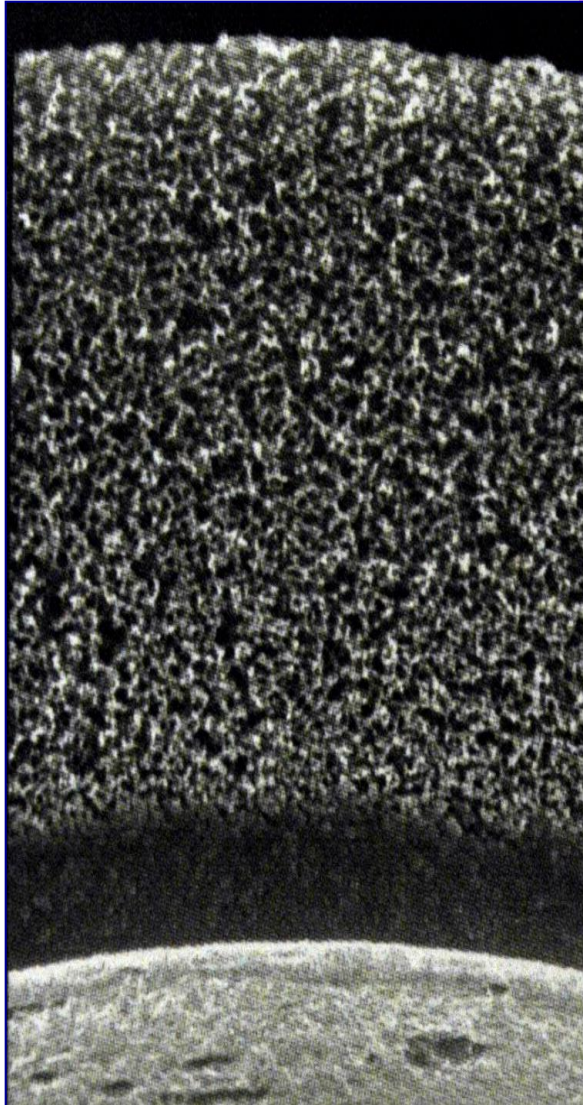


Saccharose:Lipid Molar Ratio 5:1

Hollow fibers implants with tumour cells



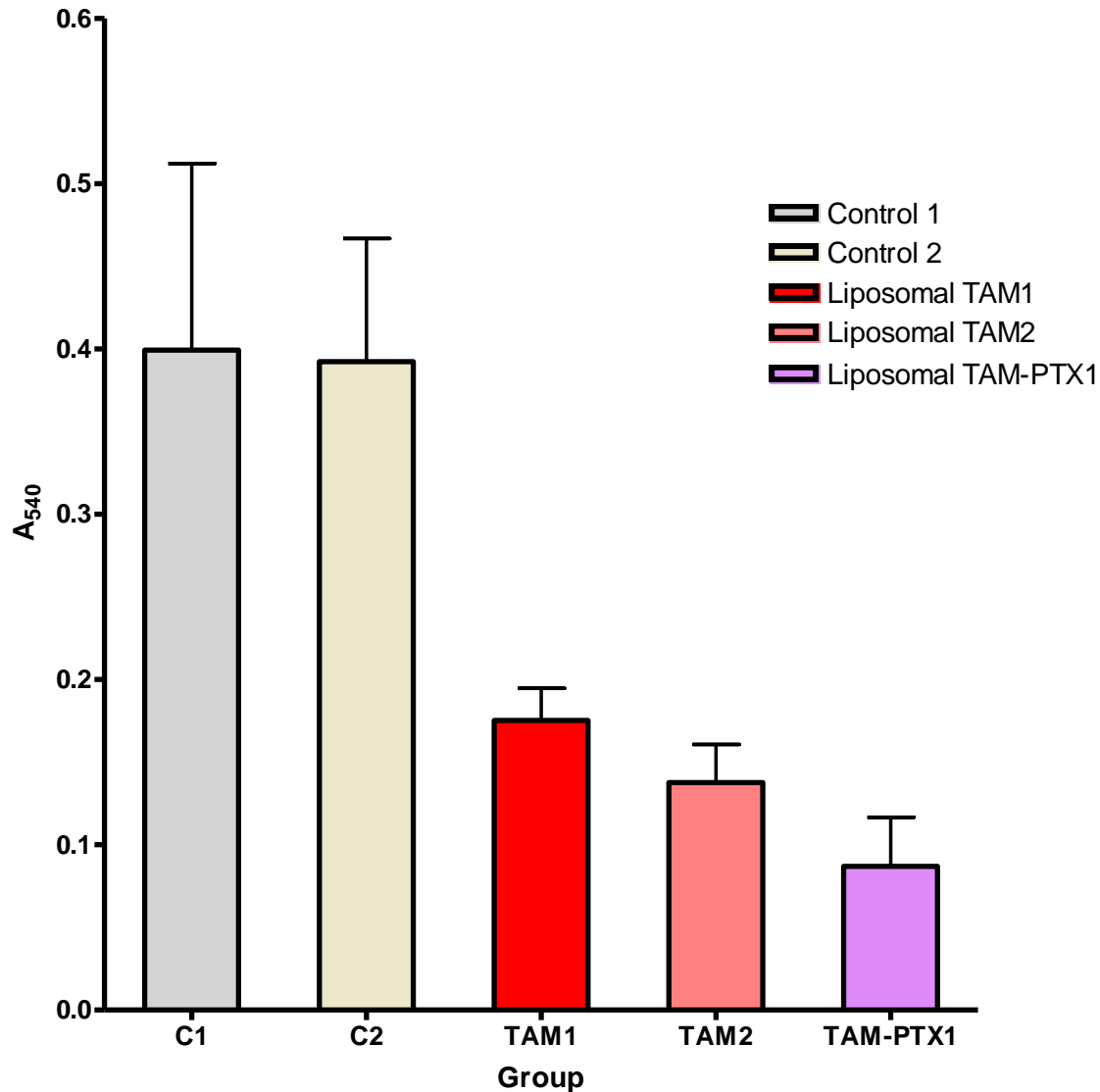
Hollow Fibres



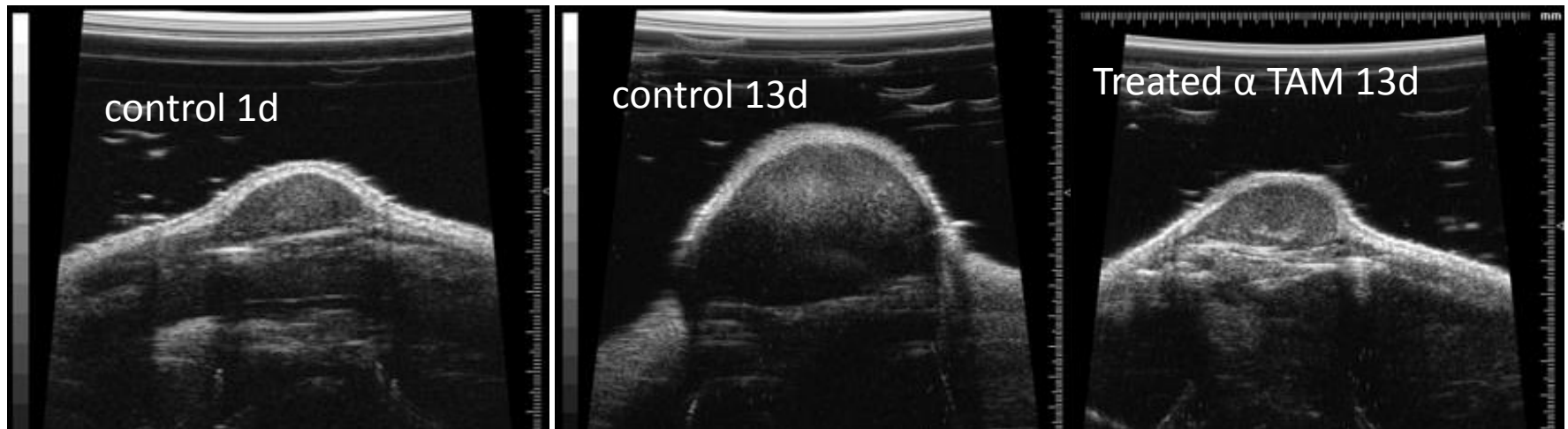
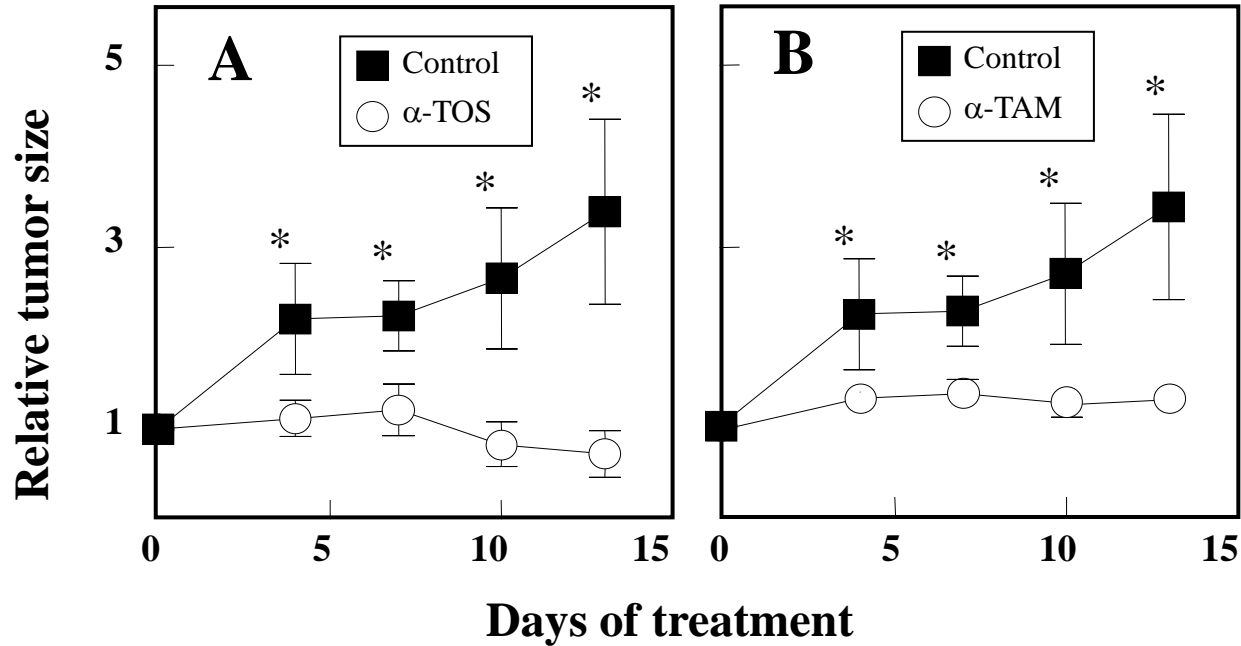
Postoperative Cure



The effect of liposomal TAM and liposomal TAM-PTX on survival of B16F10 melanoma cells in hollow fibres implanted to C57/Black mice (female, age of 8-9 months)



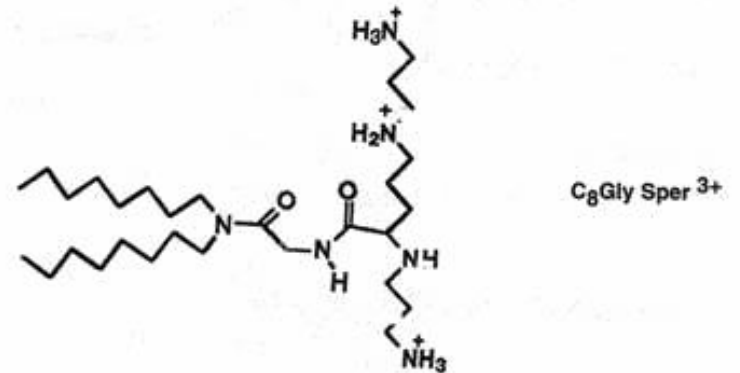
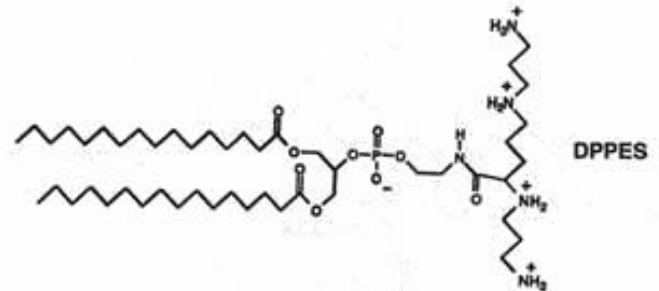
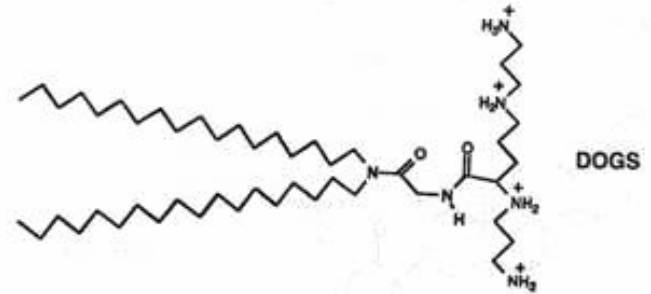
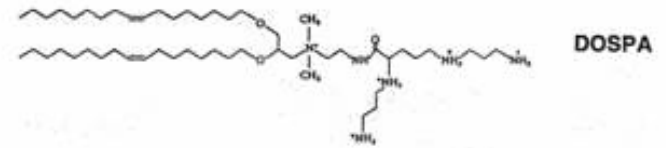
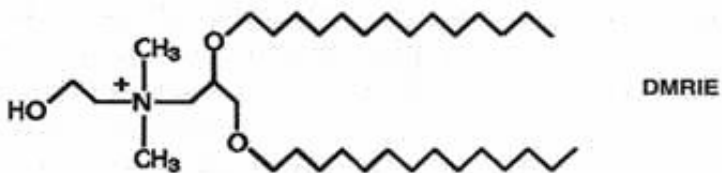
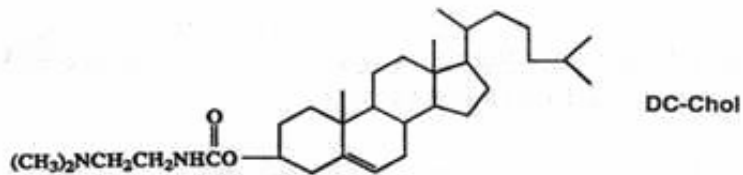
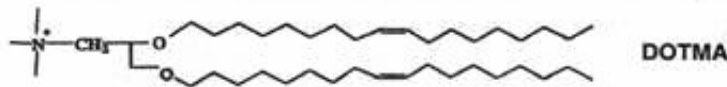
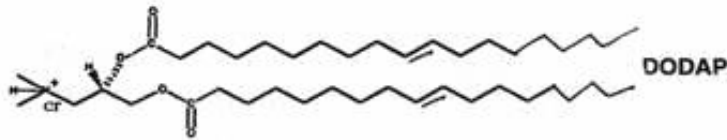
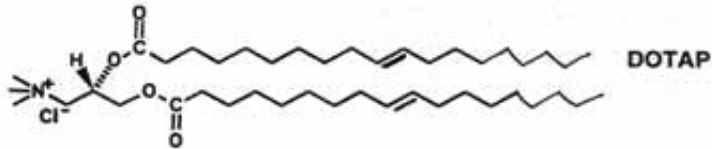
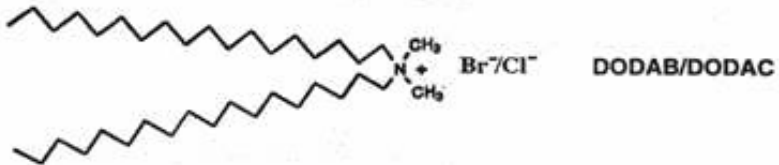
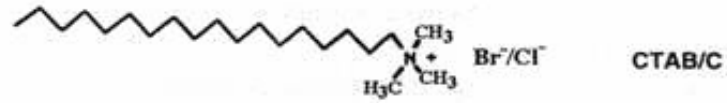
Liposomal VE analogs suppress breast carcinomas in Transgenic FVB/N *c-neu* mice with spontaneous breast carcinomas



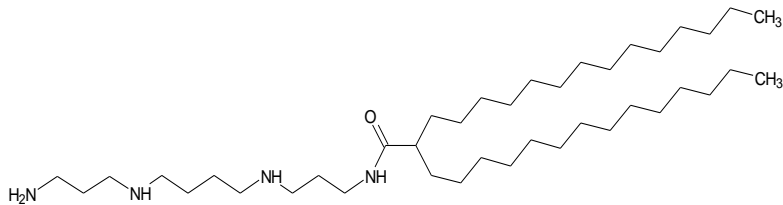
Kationické liposomy

- elektrostaticky interagují s negativně nabitou DNA za tvorby komplexů (lipoplexy)
- interakce komplexů s povrchovými proteoglykany buňky
- vstup do buněk pomocí endocytózy nebo fagocytózy
- po injekci mohou být přijímány APC buňkami infiltrujícími místo injekce
- ochrana DNA před degradací nukleázami

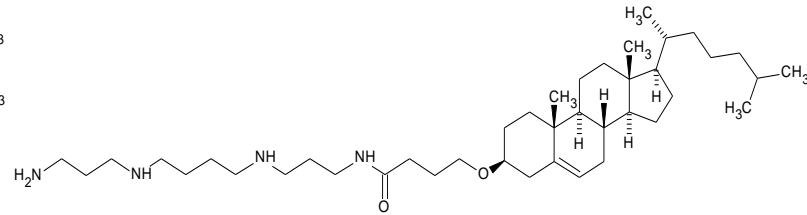
Kationické lipidy



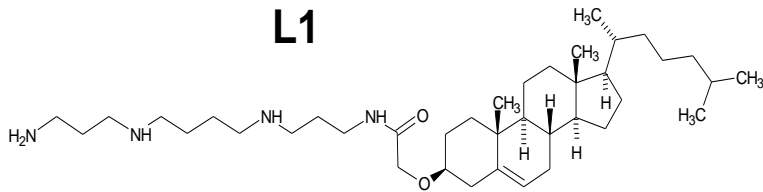
Kationické lipidy



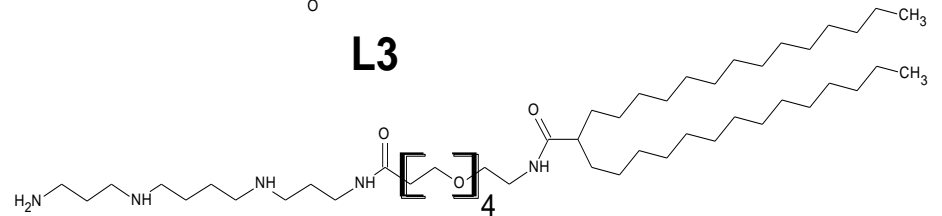
L1



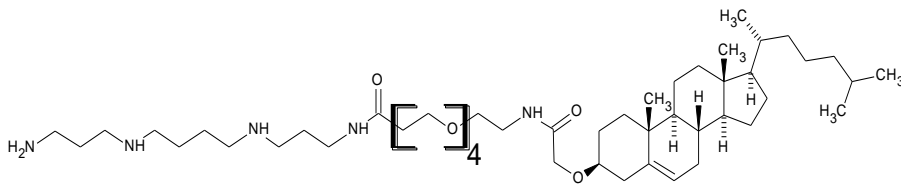
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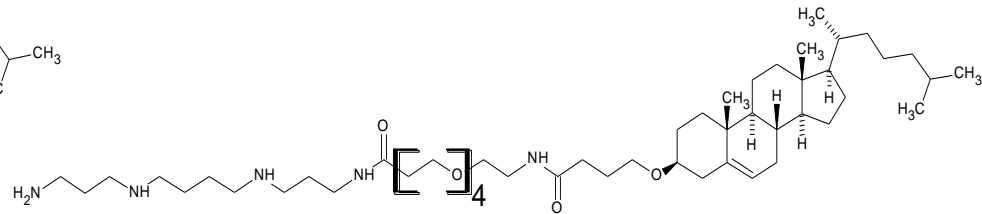
L2



L4



L5



L6

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Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Antiviral effect of HPMPC (Cidofovir®), entrapped in cationic liposomes: *In vitro* study on MDBK cell and BHV-1 virus

Zina Korvasová ^{a,1}, Lukáš Drašar ^{b,1}, Josef Mašek ^a, Pavlína Turánek Knotigová ^a, Pavel Kulich ^a, Ján Matiašovic ^a, Kamil Kovařík ^a, Eliška Bartheldyová ^a, Štěpán Koudelka ^a, Michaela Škrabalová ^a, Andrew D. Miller ^d, Antonín Holý ^c, Miroslav Ledvina ^{c,**}, Jaroslav Turánek ^{a,*}

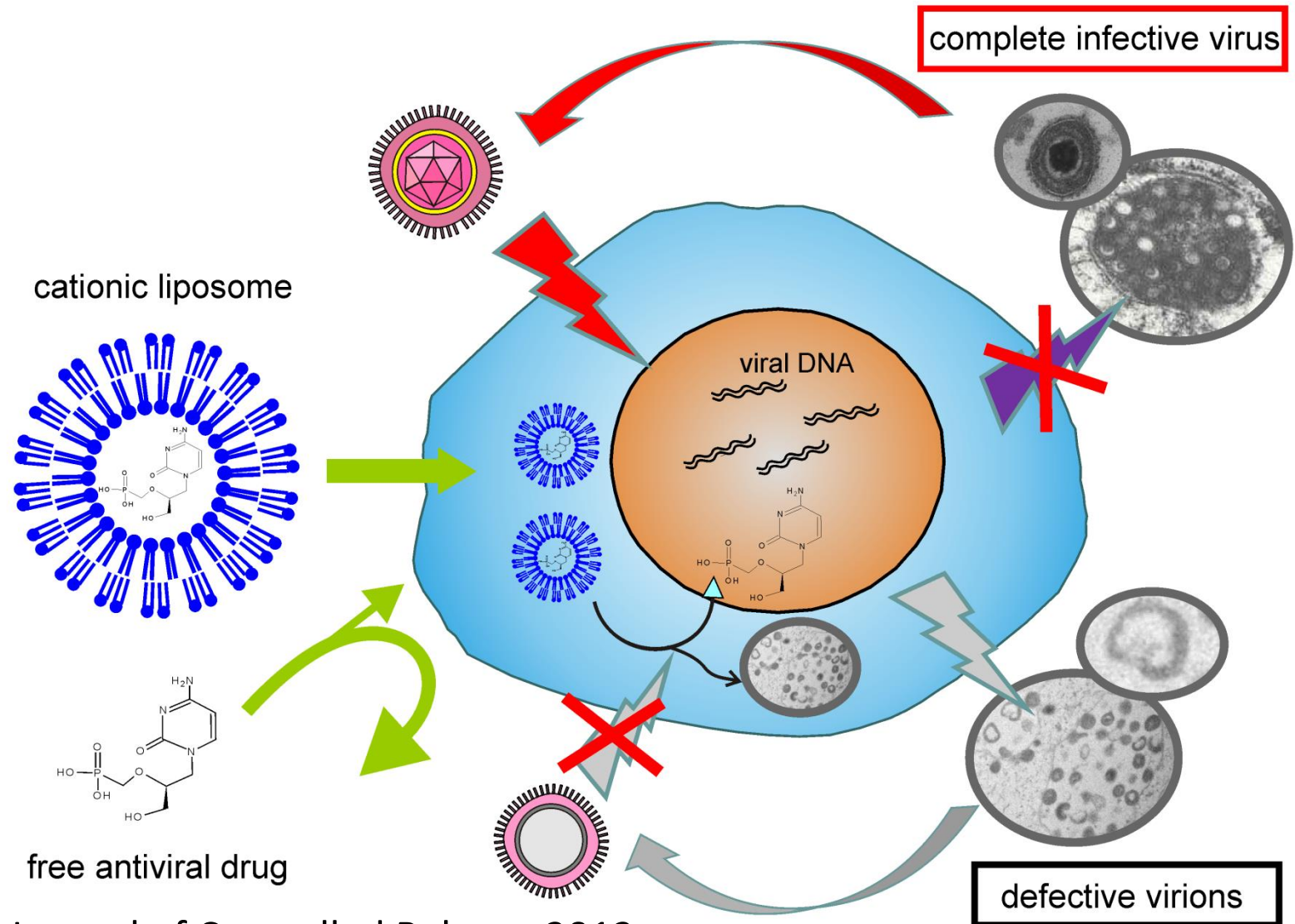
^a Veterinary Research Institute, Department of Toxicology, Pharmacology and Immunotherapy, Brno, Czech Republic

^b Institute of Chemical Technology, Department of Chemistry of Natural Compounds, Prague, Czech Republic

^c Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic

^d Imperial College London, Imperial College Genetic Therapies Centre, Department of Chemistry, London, UK; King's College London, Institute of Pharmaceutical Science, London

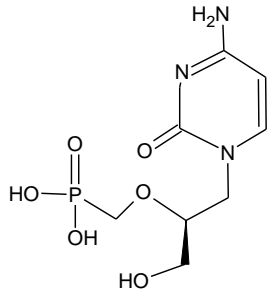
Kationické liposomy jako nosiče léčiv



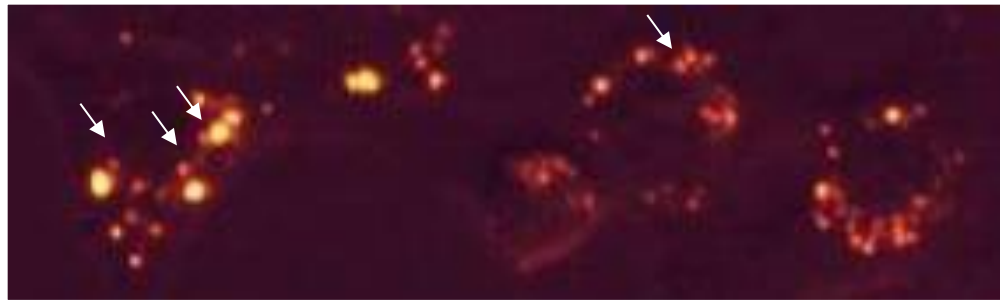
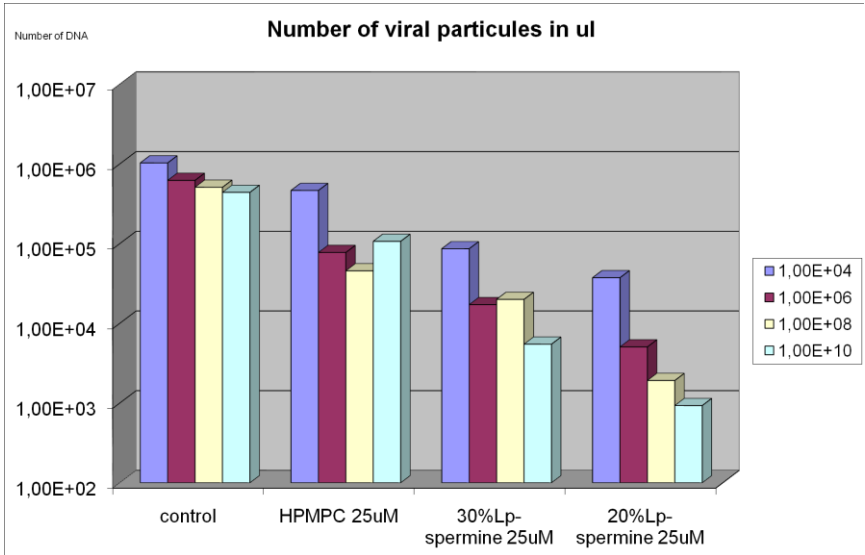
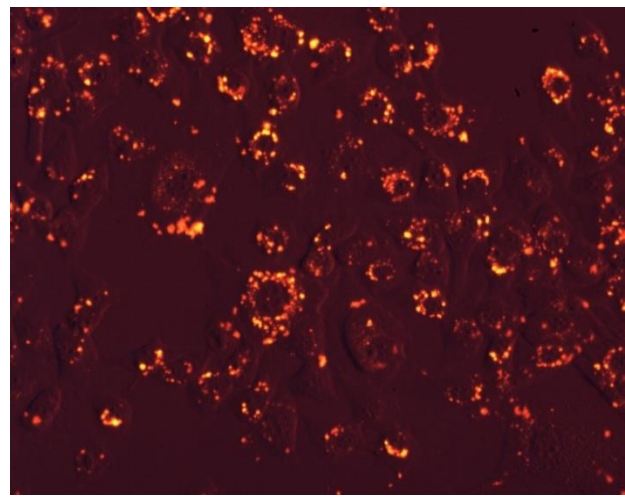
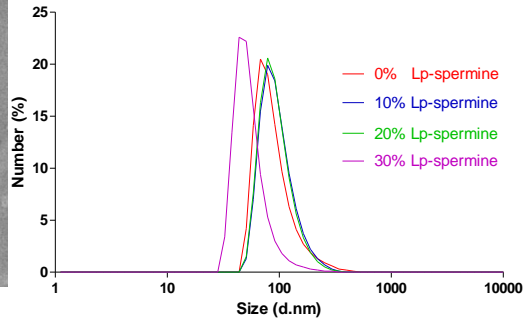
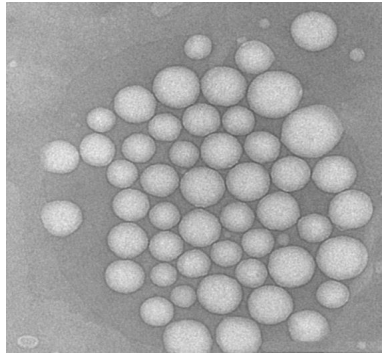
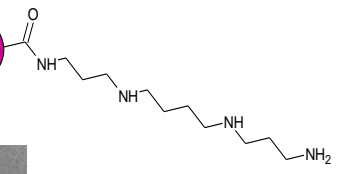
Liposomální antivirotika – BHV-1 *in vitro* model na MDBK buněčné linii

HPMPC (S)-1-(3-hydroxy-2-phosphonylmethoxy propyl)cytosine

(Cidofovir®)



Bulky hydrophobic ligand



Inhibice virové replikace (TEM)

