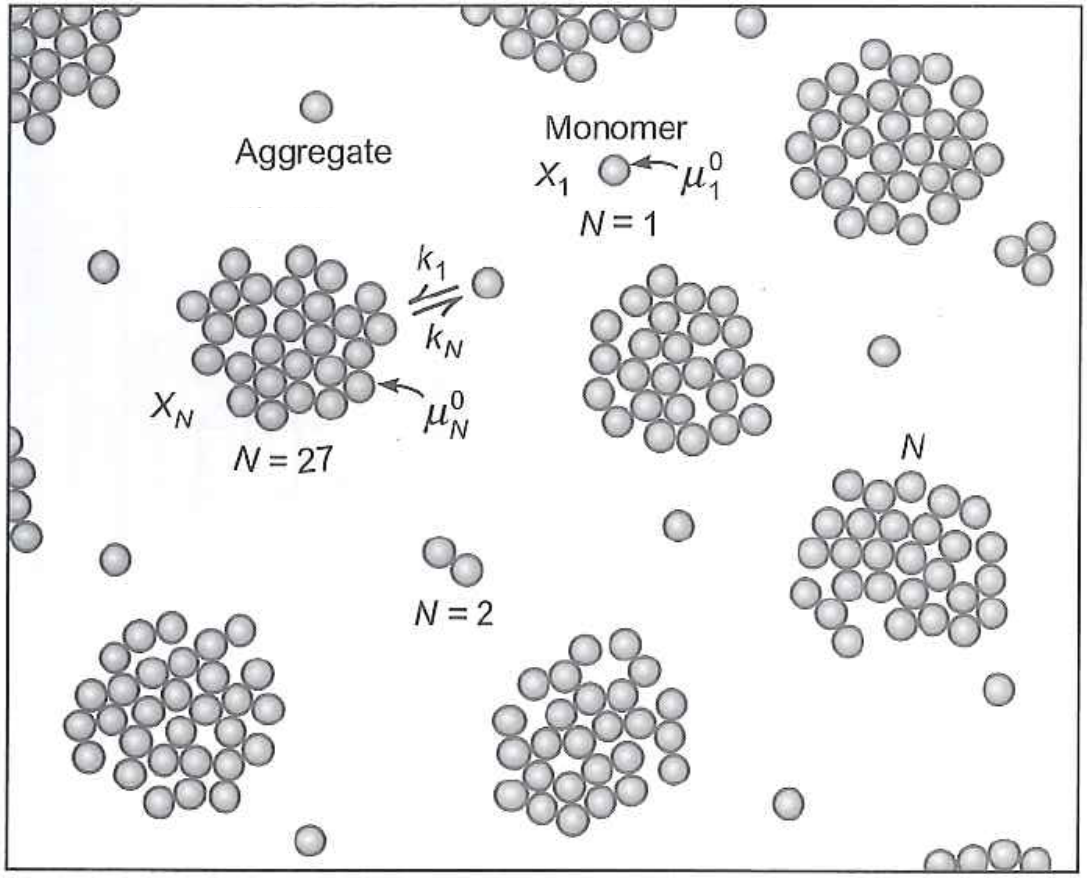
Self-assembly/precipitation

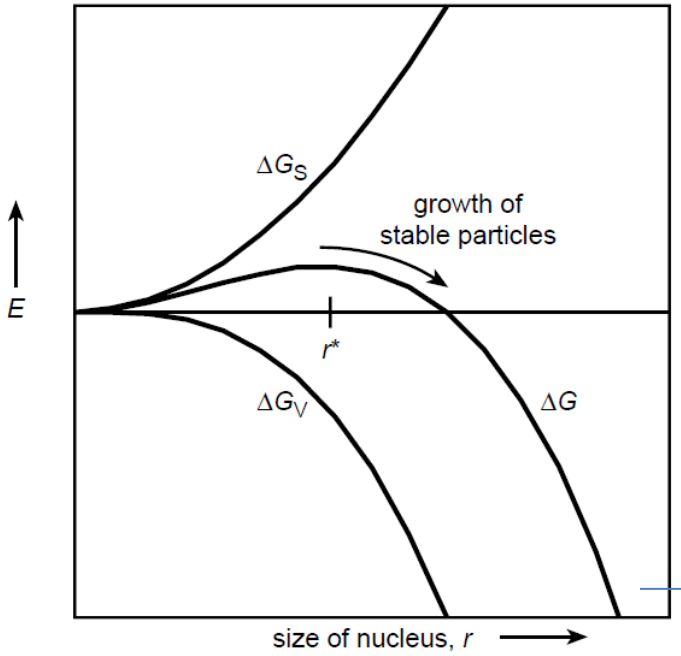
Molecules that want to stay together



Self-assembly: individual molecules group together, driven by intermolecular forces to form associated structures

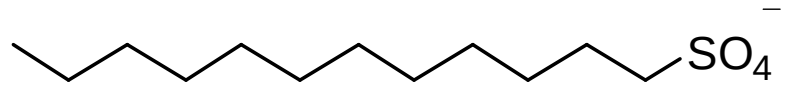
Nanoparticles growth

$$\Delta G = -\frac{4}{V}\pi r^3 k_B T \ln(S) + 4\pi r^2 \gamma$$

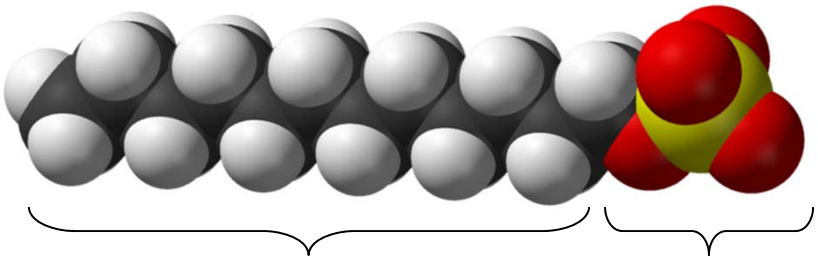


Aggregation/precipitation should stop at infinite radius

Surfactants



(Sodio) DodecilSolfato (SDS)



TENSIOATTIVO

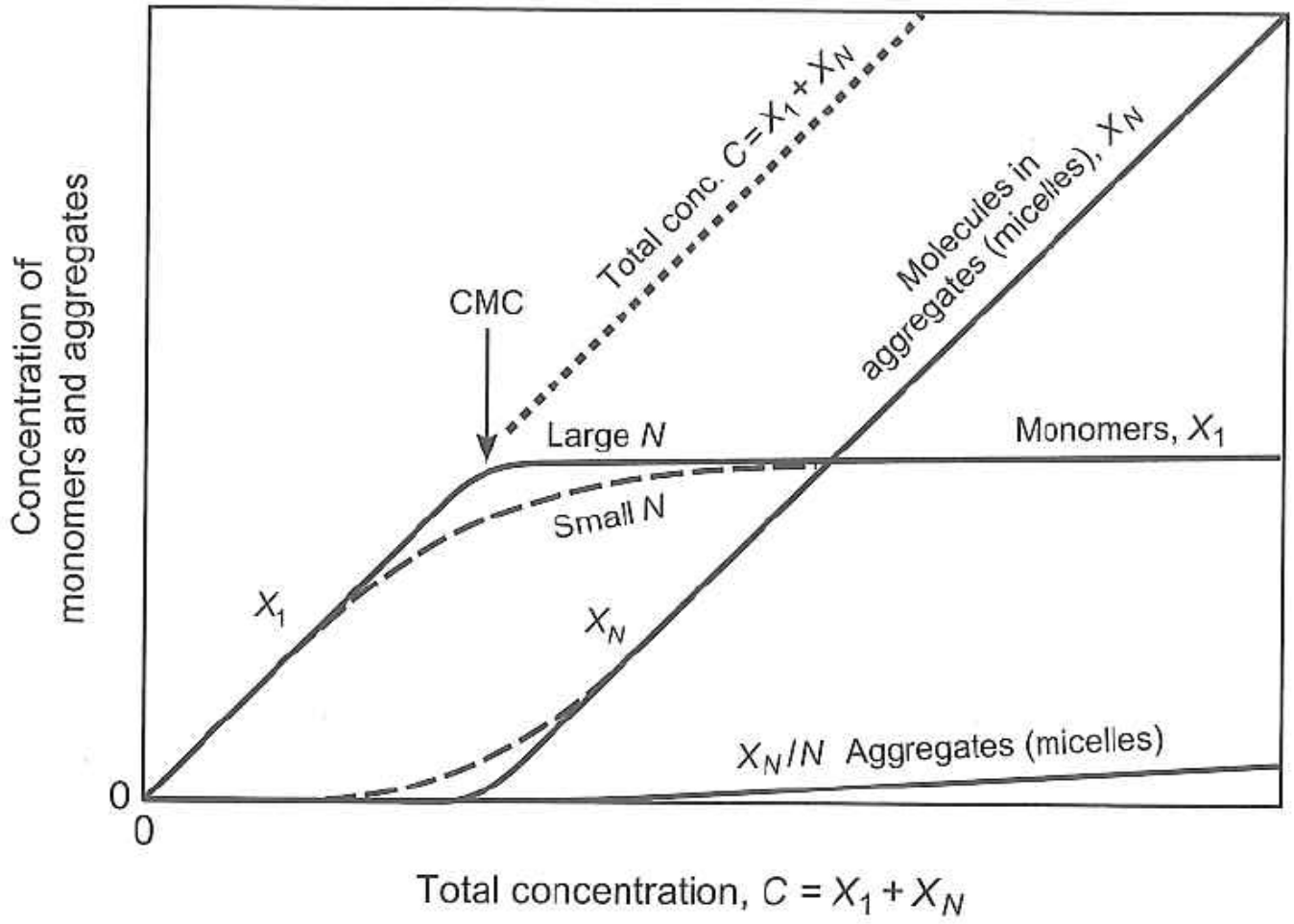
coda idrofobica **testa polare**

Insoluble aggregation

soluble repulsion

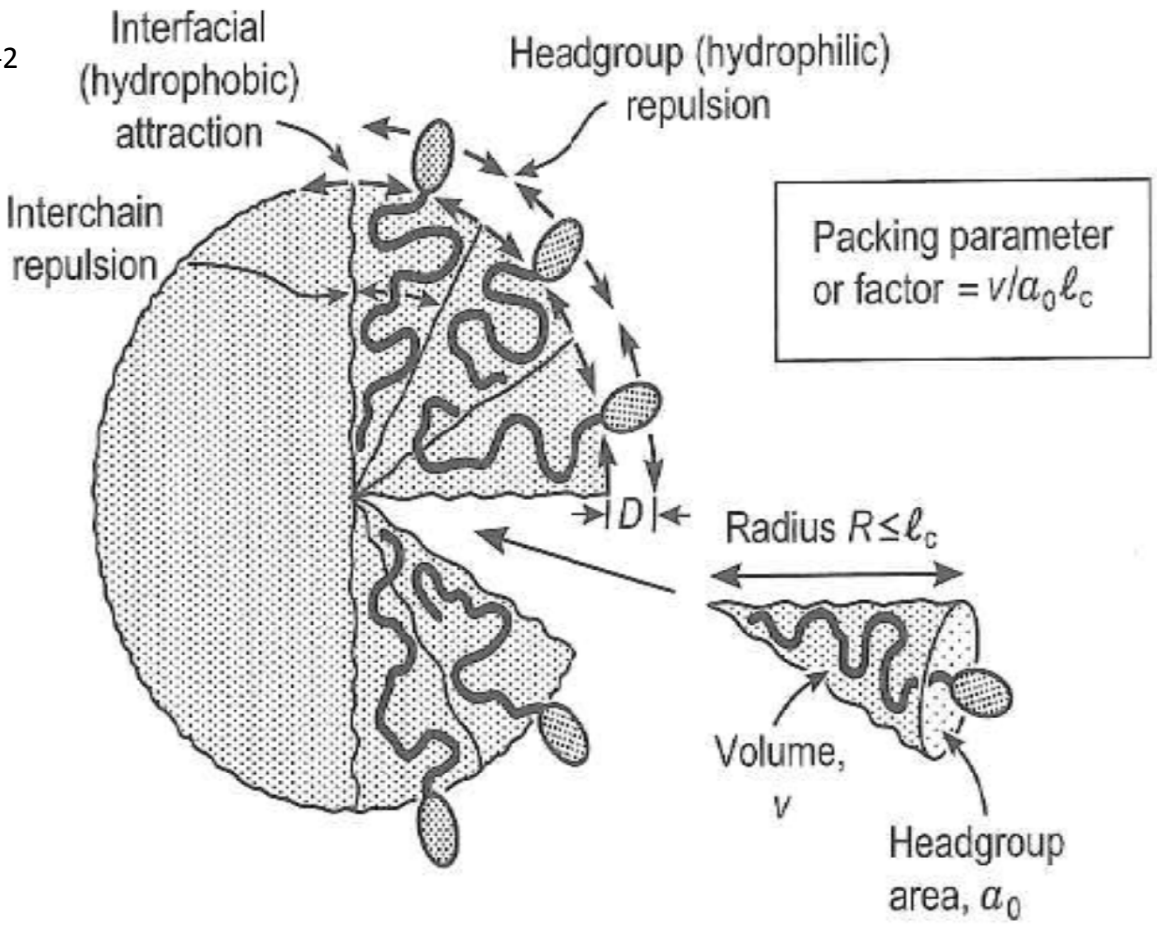
Self-assembly

Critical micelle concentration (CMC)



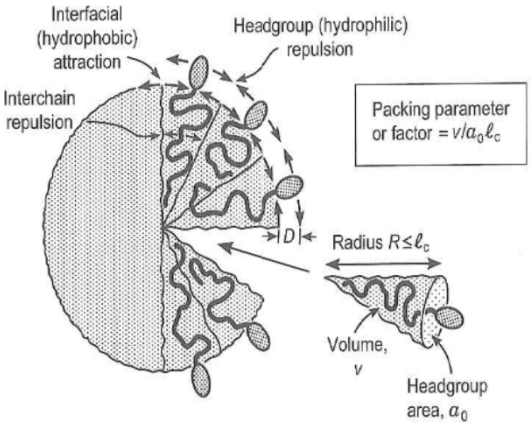
Micelles

20-50 mJ m^{-2}



Micelles

Optimal headgroup area



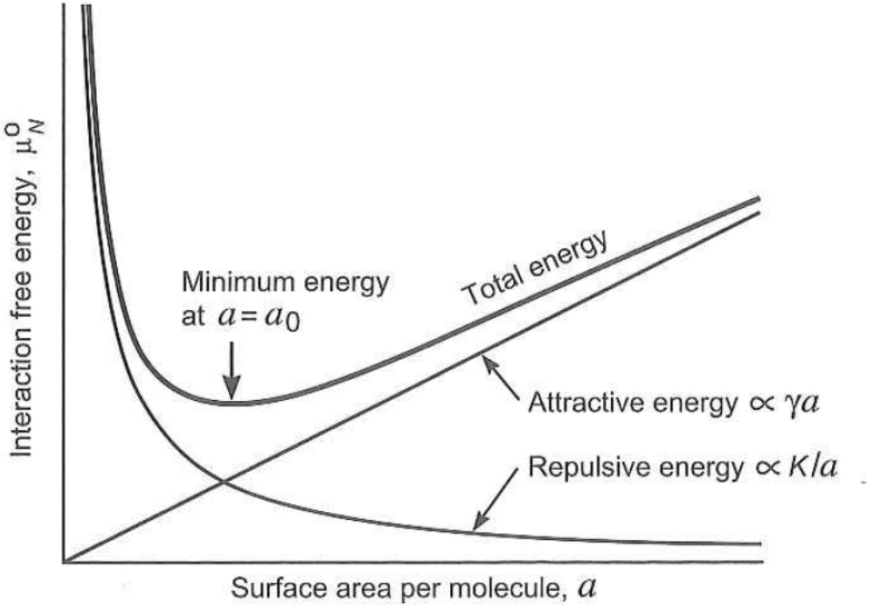
$$\mu_N^0 = \gamma a + \frac{K}{a}$$

Repulsive contribution

Attractive contribution

$$\mu_N^0(min) = 2\gamma a_0 \qquad a_0 = \sqrt{\frac{K}{\gamma}}$$

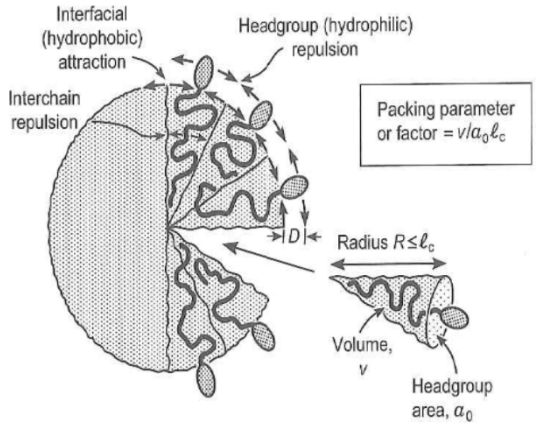
$$\mu_N^0 = 2\gamma a_0 + \frac{\gamma}{a}(a - a_0)^2$$



Micelles

Spherical micelles

To have a spherical micelle a_0 must be sufficiently large and v sufficiently small to allow the micelle radius to not exceed l_c. For a spherical micelle of radius R and mean aggregation number M we have:



$$M = \frac{4\pi R^2}{a_0} = \frac{4\pi R^3}{3v}$$

$$R = \frac{3v}{a_0}$$

$$\frac{v}{a_0 l_c} < \frac{1}{3}$$

$$l_c < l_{max} \approx (0.154 + 0.1265n)\ nm$$

$$v \approx (27.4 + 26.9n) \times 10^{-3}\ nm^3$$

$$SDS, M = 74$$

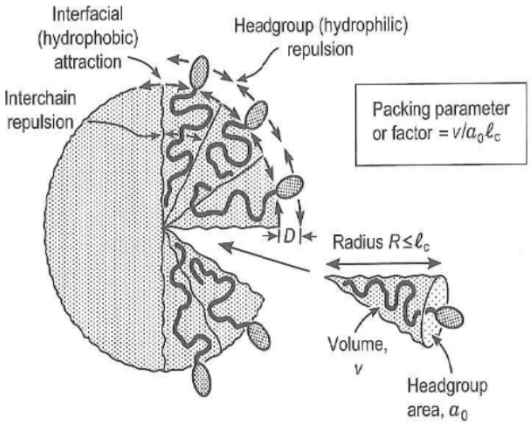
$$v \approx 0.352\ nm^3$$

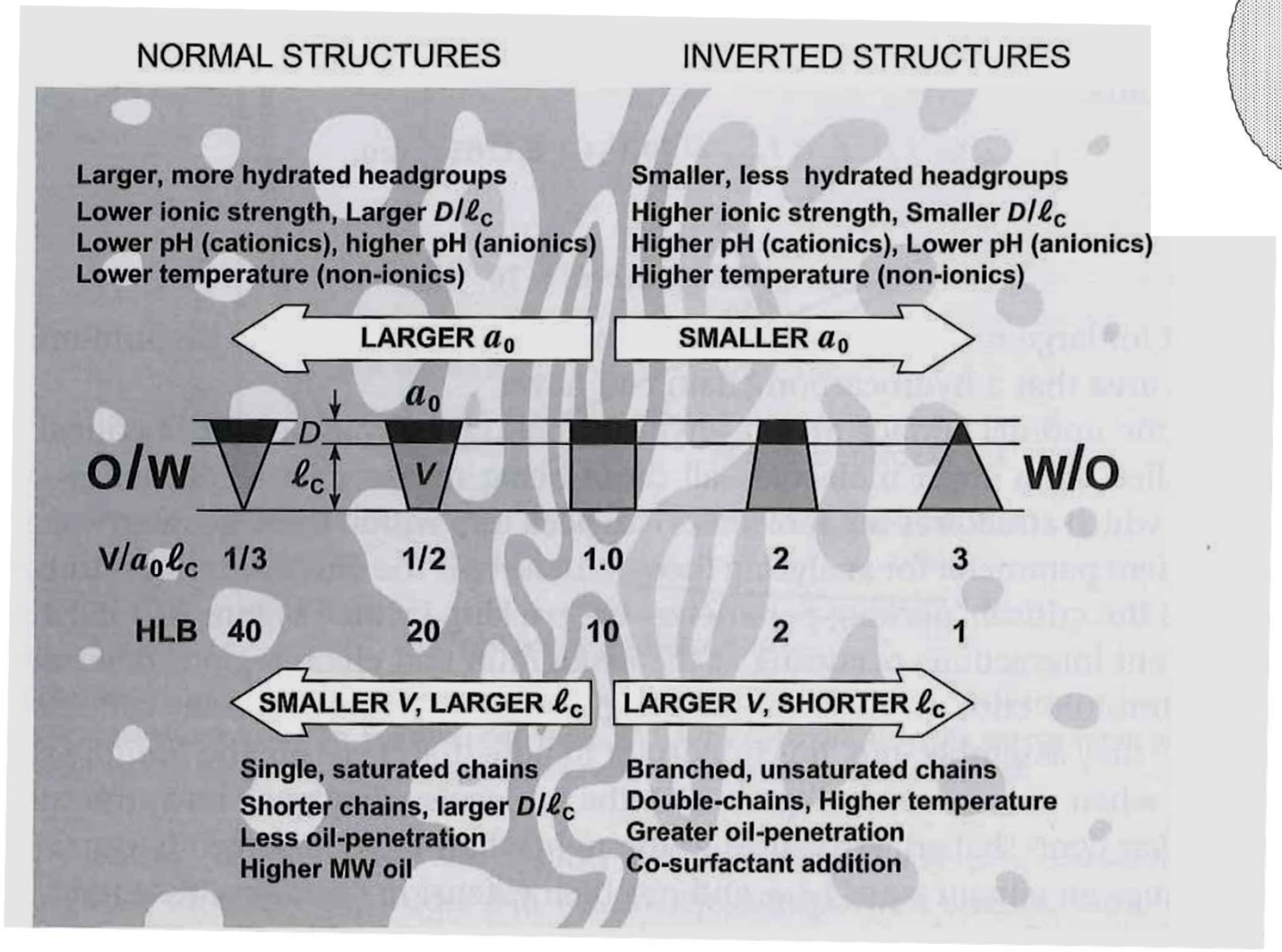
$$R = 1.84\ nm \qquad a_o = 0.57\ nm^2$$

$$l_c = 1.67\ nm \qquad \frac{v}{a_0 l_c} = 0.37$$

Micelles

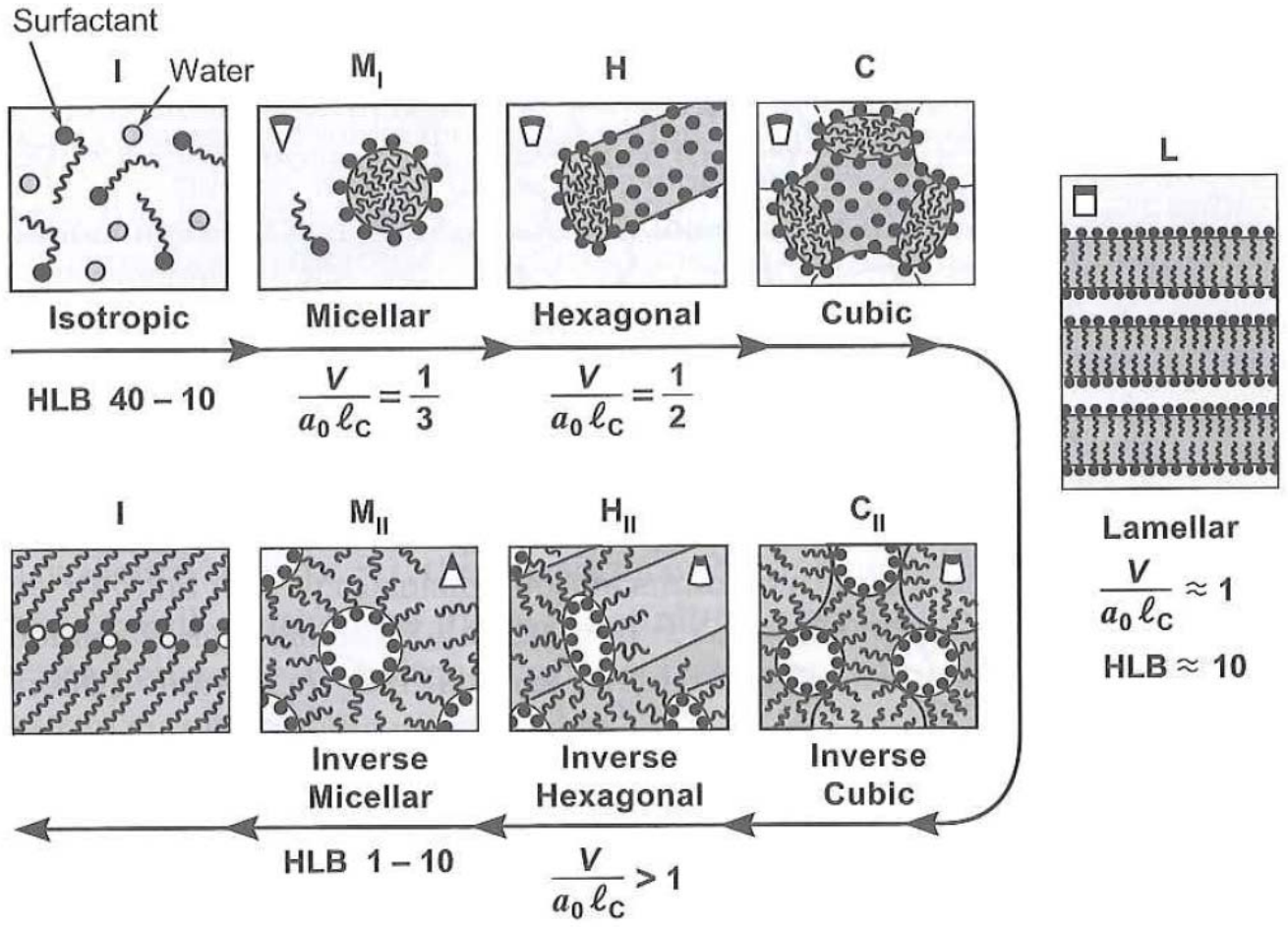
Packing parameter



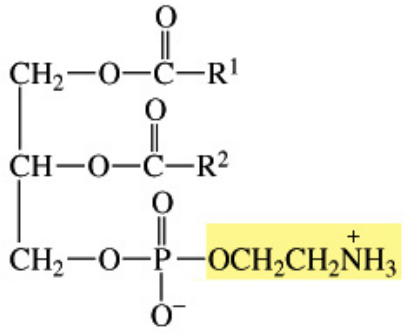


Micelles

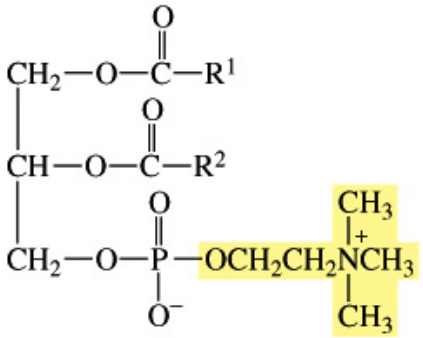
Sperical micelles



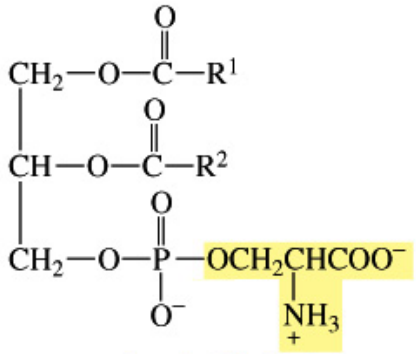
Tensioattivi bicoda



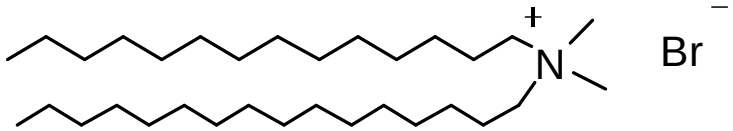
a phosphatidylethanolamine
a cephalin



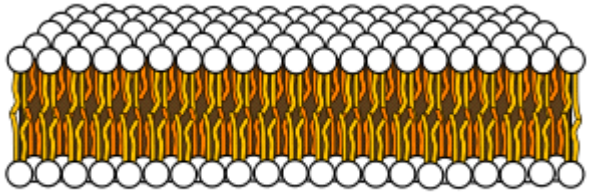
a phosphatidylcholine
a lecithin



a phosphatidylserine

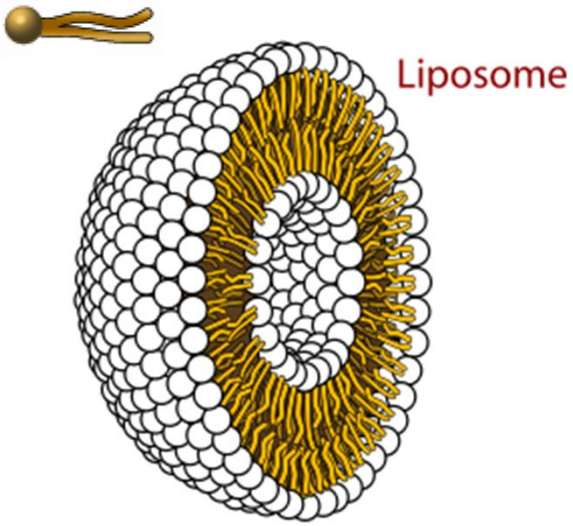


Double layers

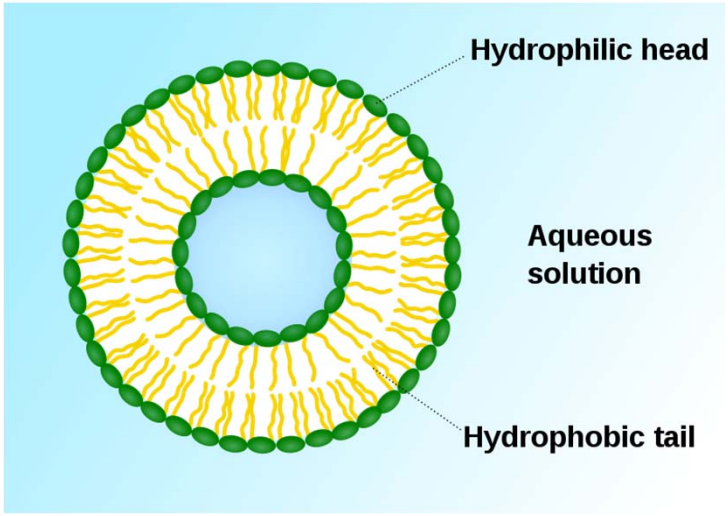


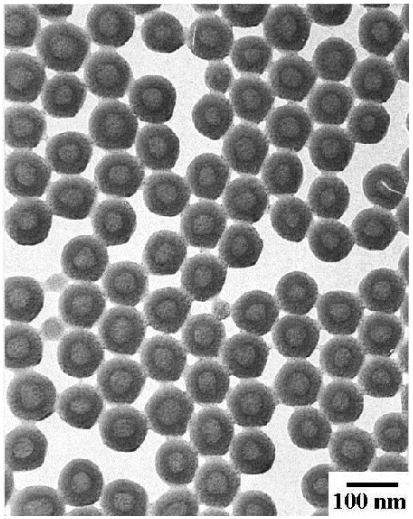
Doppio strato

Se un tensioattivo possiede due code idrofobiche, queste rendono la sua struttura cilindrica, favorendo un impaccamento a doppio strato

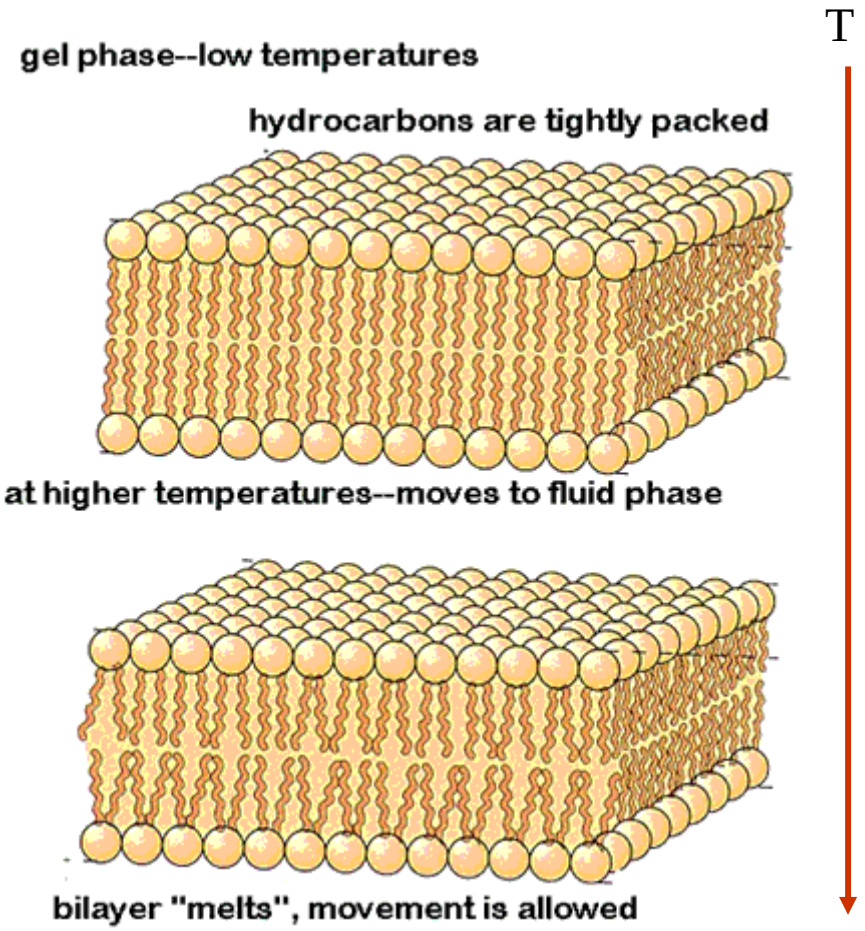


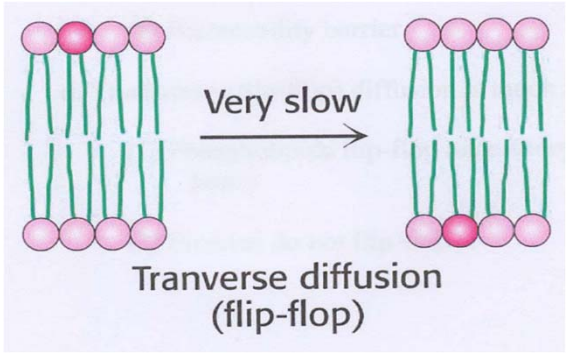
Vescicola/liposoma



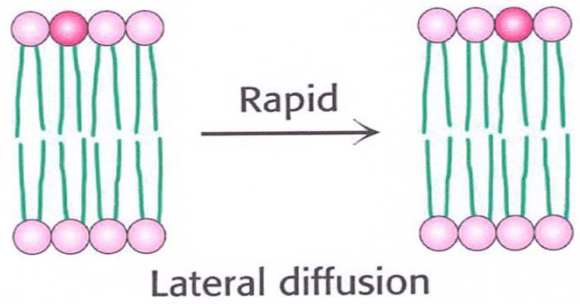


Vesicles and liposomes





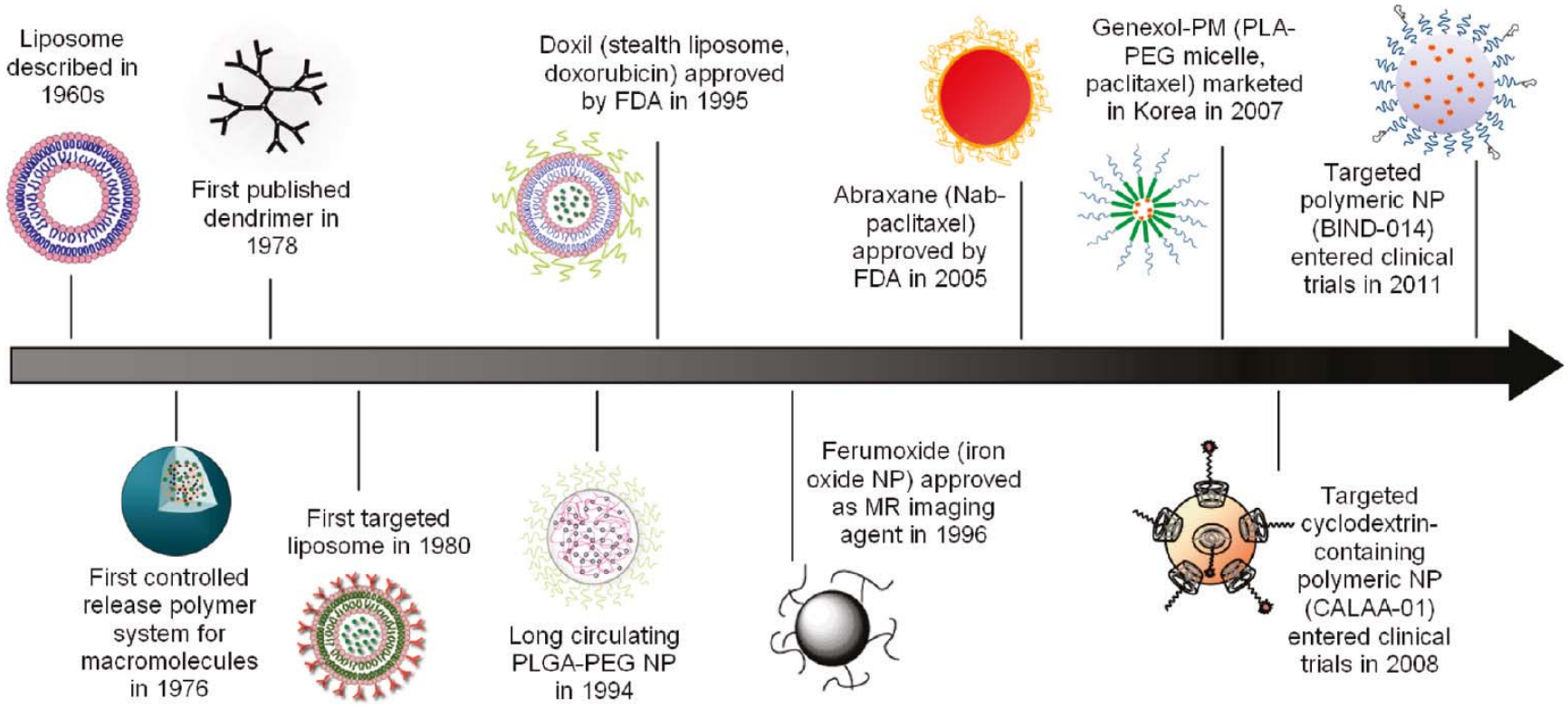
Il passaggio di un tensioattivo da una parte del doppio strato alla parte opposta è sempre molo lento. Il movimento dalla stessa parte dello strato è rapido.



A bassa T le catene idrocarburiche sono completamente estese ed impaccate (**fase gel**), ad alta T le catene diventano più mobili (fase fluida). La transizione avviene ad una determinata T detta di transizione di fase.

Liposomes and nanoparticles in therapy

Historical timeline of clinical-stage nanoparticle technologies.



Liposomes and nanoparticles in therapy

While less attractive in scientific literature, liposomes represent the most diffused nanotehcnology used for therapy and diagnosis.

“To date, 11 liposomal drugs and 1 Nab drug have been approved by the FDA for a myriad of clinical applications, along with one polymeric micelle product for oncologic use in Korea. Three iron oxide NP products are also on the market for in vivo imaging use (recently withdrawn n.d.d.).”

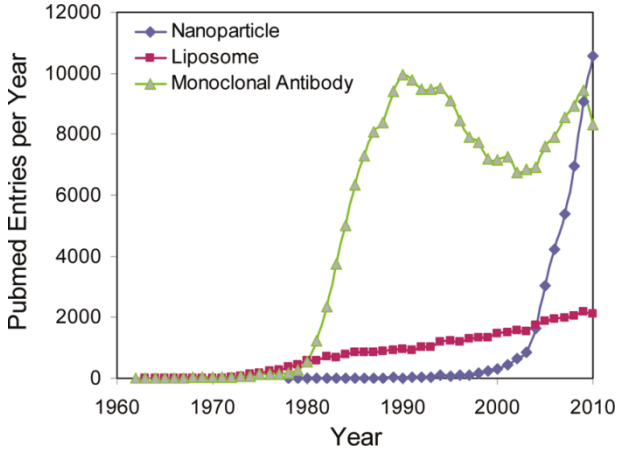
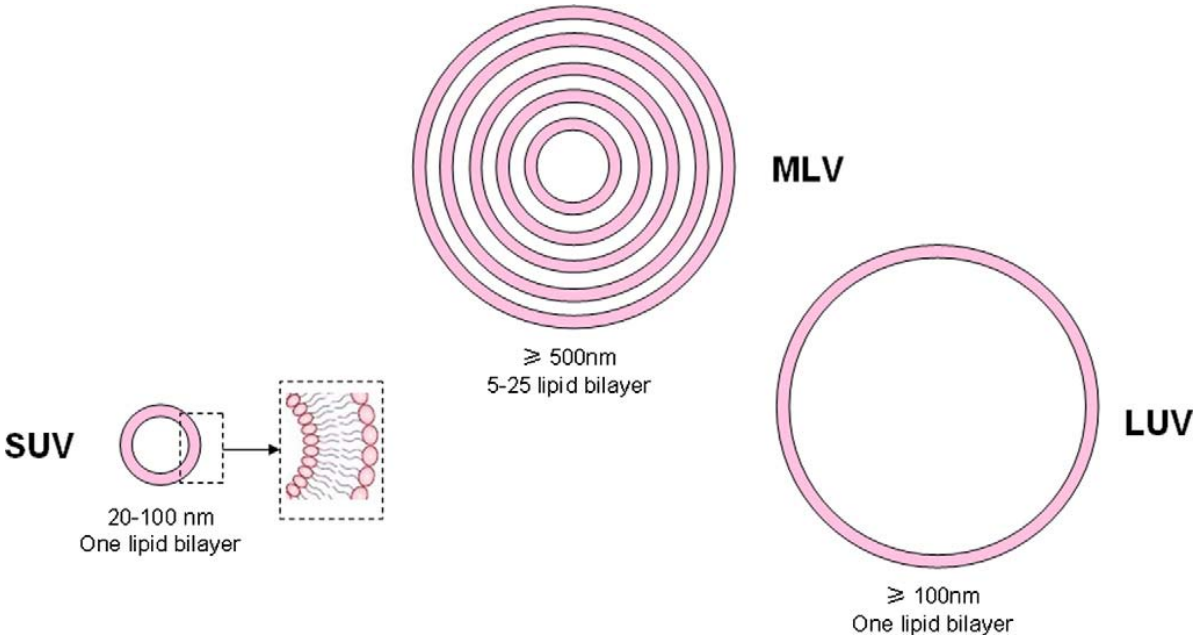


Table 2. Colloidal systems in development or on the market for oncology

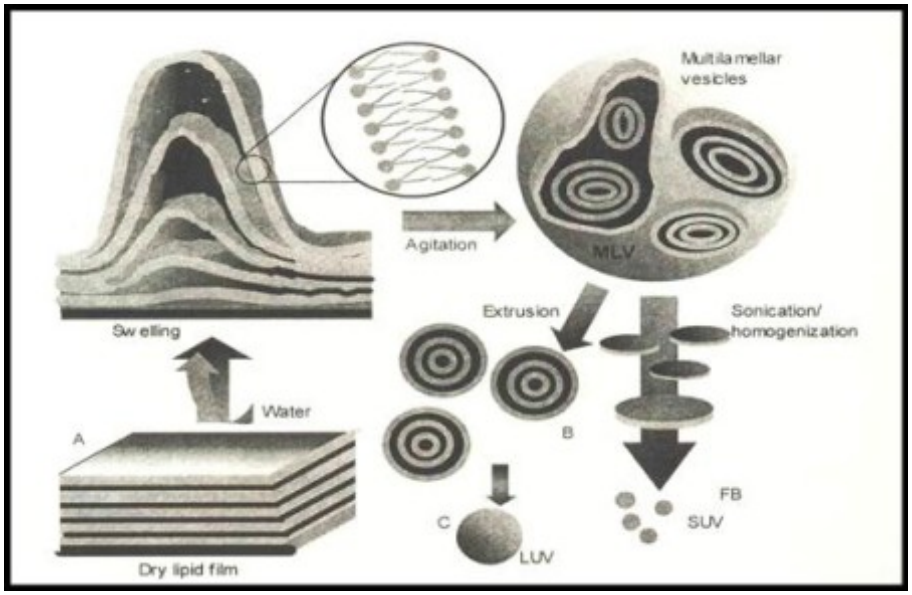
Drug	Product	Company	Formulation	Administration mode[a]	Application	Status	Refs
Paclitaxel	Abraxane	Abraxis Bioscience	NPs	i.v.	Head and neck, lung, ovarian, prostate, colon, and metastatic breast cancer	On the market	[74,75]
	Genexol-PM	Samyang Pharmaceuticals	Polymeric micelles	i.v.	Breast and lung cancer	On the market, USA phase II	[76]
	NK105	Kataoka’s team	Polymeric micelles	i.v.	Pancreatic, bile duct, gastric, colon cancer	Phase I	[20,27,77,78]
Doxorubicin	Doxil/Caelyx	Janssen Products	Liposomes	i.v.	Metastatic breast cancer, ovarian, Kaposi’s sarcoma	On the market	[1,20,76,79]
	Myocet	Cephalon	Liposomes	i.v.	Metastatic breast cancer	On the market	
	Livatag	Bioalliance	NPs	i.v.	Hepatocellular carcinoma	Phase II	
	NK911	Kataoka’s team	Polymeric micelles	i.v.	Solid tumors	Phase I	[20]
	SP1049C	Supratek pharma	Polymersomes	i.v.	Upper GI cancer	Phase II	[80]
	Thermodox	Celsion	Liposomes	i.v.	Carcinoma, hepatocellular, and recurrent chest wall breast cancer	Phase I to market depending on application	[81][b]
Cisplatin	NC-6004	Kataoka’s team	Polymeric micelles	i.v.	Colorectal, NSCLC, esophageal, pancreatic, melanoma, mesothelioma, renal cell, and hepatocellular cancer	Phase I	[20,27,78,82,83]
	SLIT cisplatin	Transave, Inc.	Liposome	Aerosol	Lung cancer, pulmonary metastases	Phase I/II	[2][c]
Camptothecin	NK-012 (encapsulation of a camptothecin derivative)	Kataoka’s team	Polymeric micelles	i.v.	Colon cancer, resistant tumors	Phase I	[27,78]
Daunorubicin	Daunoxome	NeXstar Pharmaceutica	Liposome	i.v.	Kaposi sarcoma	On the market	[1]
Annamycin	L-Annamycin	Callisto Pharmaceuticals	Liposomes	i.v.	Leukemia	Phase I/II	[2]
Docetaxel	Docetaxel-PNP	Samyang	NPs	i.v.	Advanced solid malignancies	Phase I	[2]
Irinotecan	NL CPT-11	University of California	Liposomes	i.v.	Recurrent high-grade gliomas	Phase I	[2]

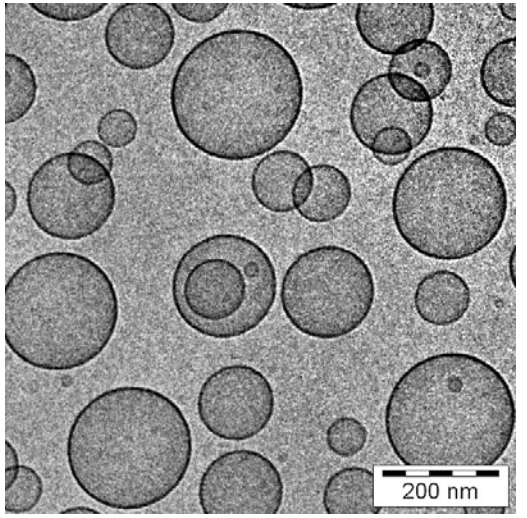
Vescicole e doppi strati

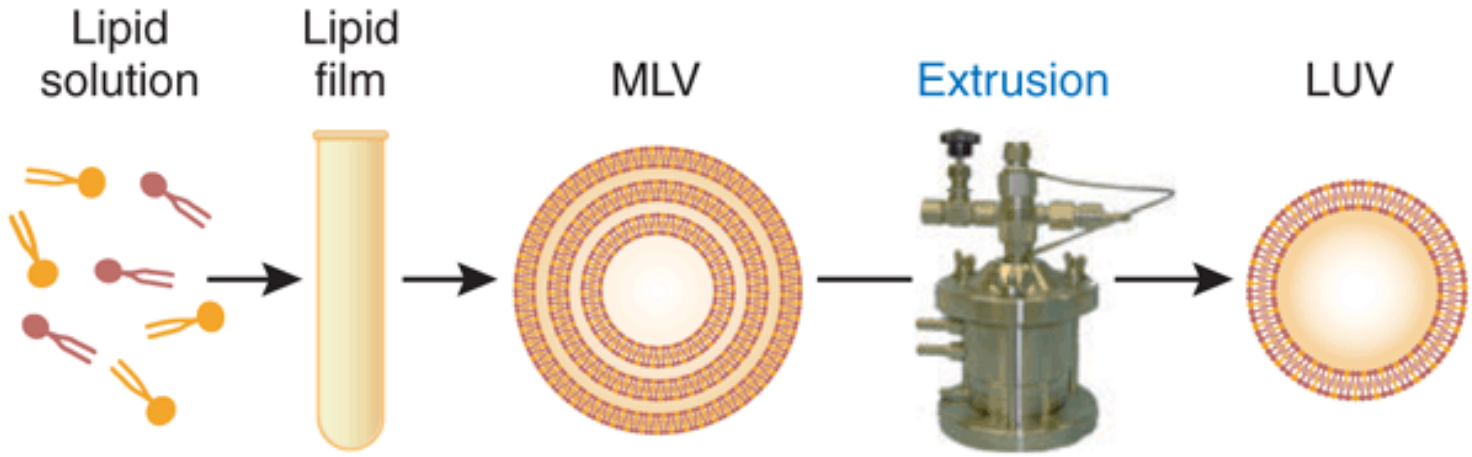


Liposomes: synthesis

Classical methods: sonication and extrusion



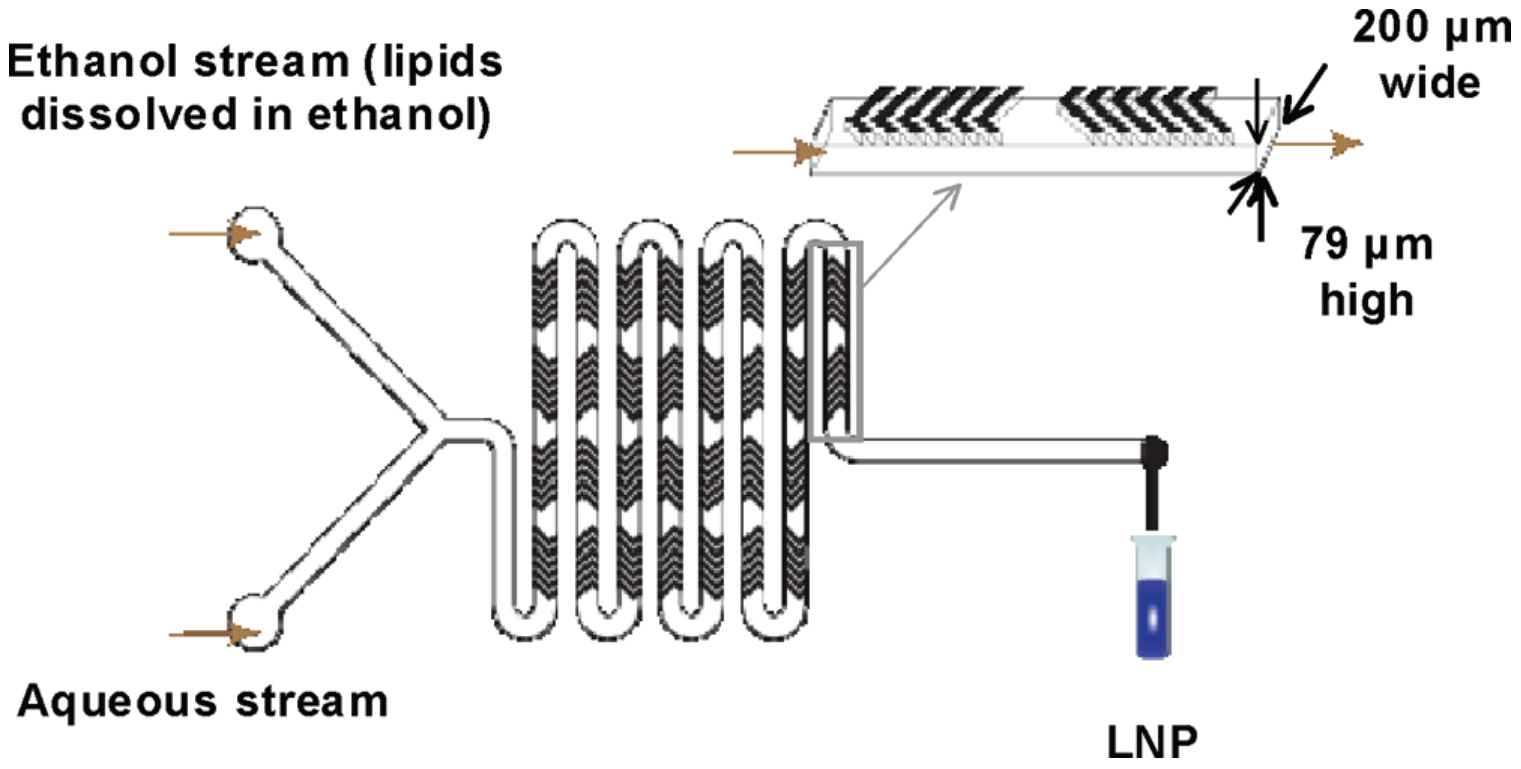




Liposomes: synthesis

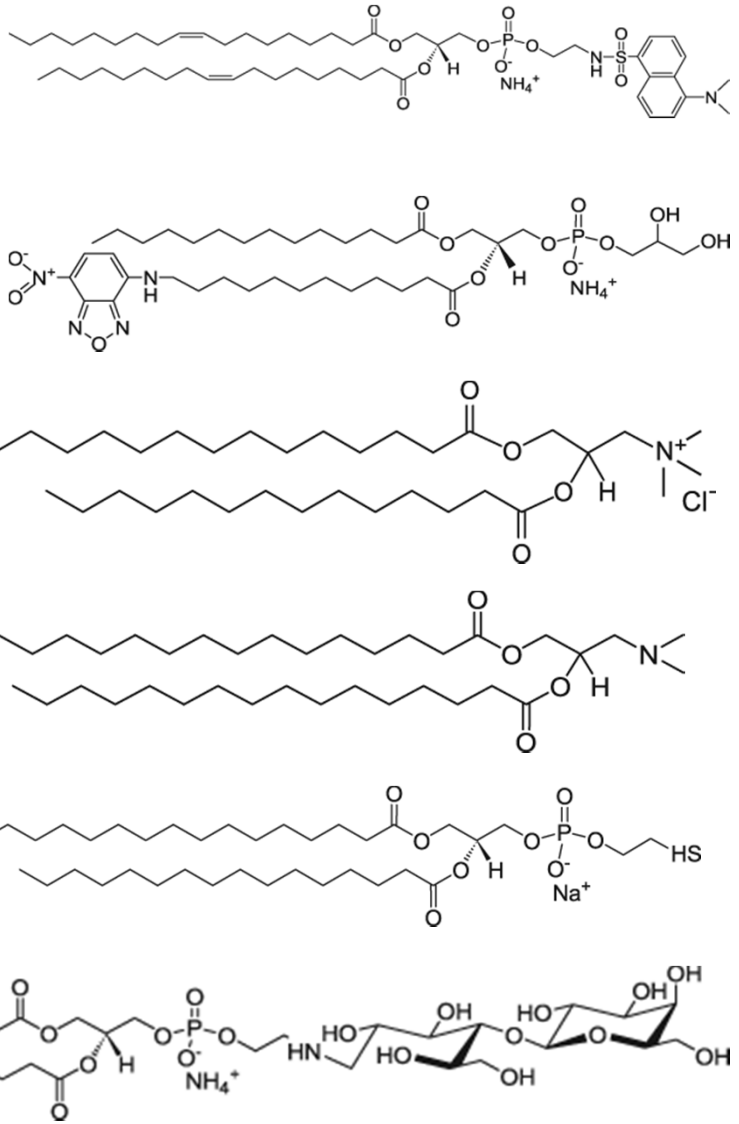
Classical methods: microfluidics

- Extrusion: large liposomes (down to 80-50 nm)
- Sonication: small liposomes (down to 20 nm). Contamination, degradation, scaling-up.
- Microfluidics: fast mixing prevents formation large aggregates.



Self-assembled system

Large library of building blocks



Evolution of liposomes for drug delivery

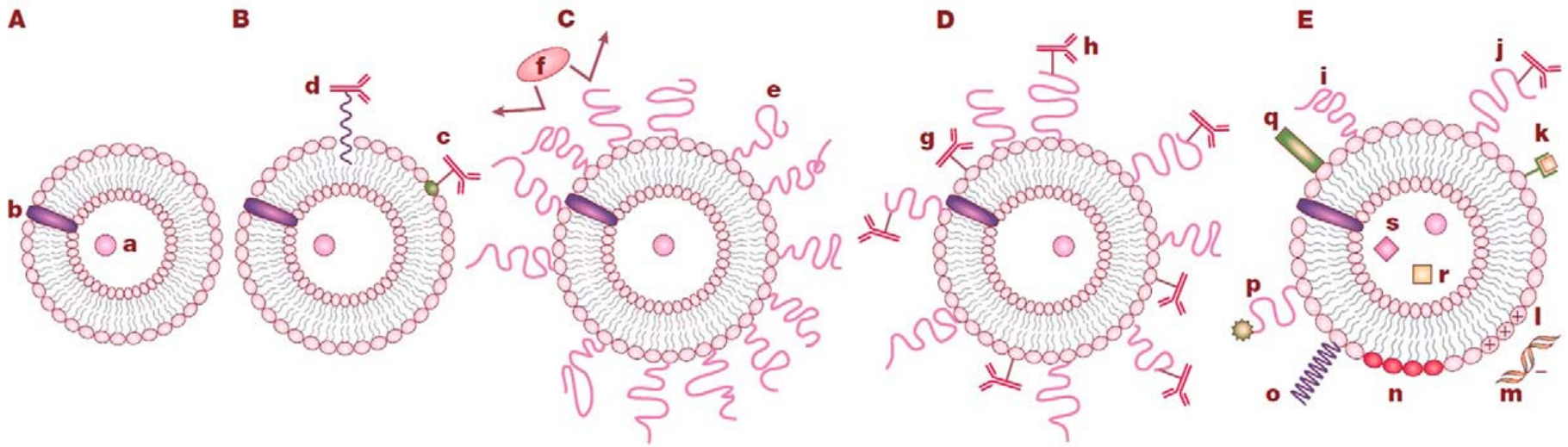
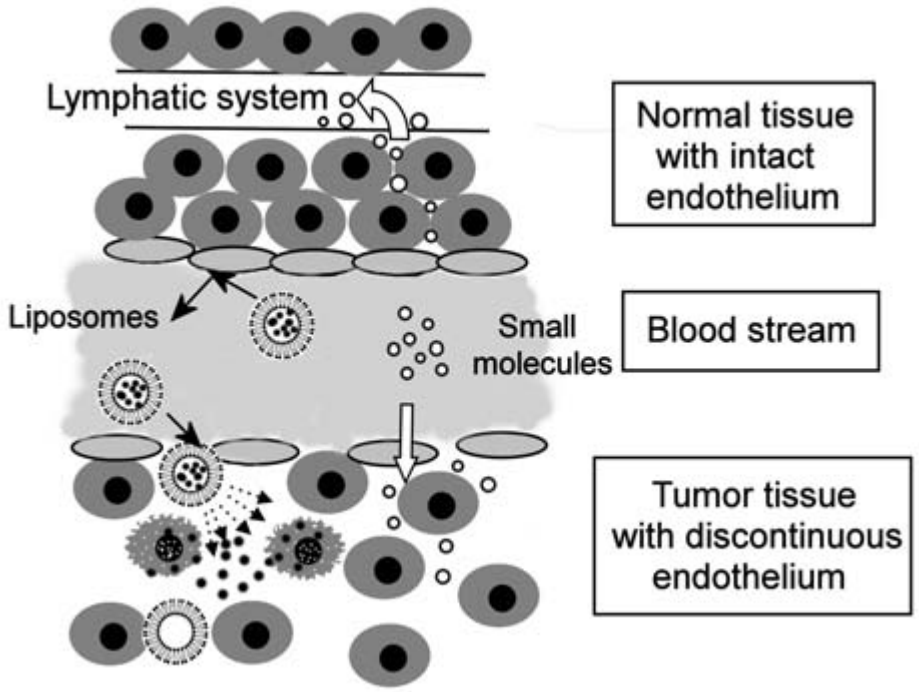


Fig. 2. Evolution of liposomes. (A) Early traditional liposomes with water soluble drug (a) entrapped into the aqueous liposome interior, and lipophilic drug (b) incorporated into the liposomal membrane. (B) Antibody-targeted immunoliposome with antibody covalently coupled (c) to the reactive phospholipids in the membrane, or hydrophobically anchored (d) into the liposomal membrane after preliminary modification with a hydrophobic moiety. (C) Long-circulating liposome grafted with a protective polymer (e) such as PEG, which shields the liposome surface from the interaction with opsonizing proteins (f). (D) Long-circulating immunoliposome simultaneously bearing both protective polymer and antibody, which can be attached to the liposome surface (g) or, preferably, to the distal end of the grafted polymeric chain (h). (E) New-generation liposome, the surface of which can be modified (separately or simultaneously) by different ways. Among these modifications are: the attachment of protective polymer (i) or protective polymer and targeting ligand, such as antibody (j); the attachment/incorporation of a diagnostic label (k); the incorporation of positively charged lipids (l) allowing for the complexation with DNA yielding lipoplex structures (m); the incorporation of stimuli-sensitive lipids (n); the attachment of a stimuli-sensitive polymer (o); the attachment of a cell-penetrating peptide (p); the incorporation of viral components (q). In addition to a drug, liposomes can be loaded with magnetic particles (r) for magnetic targeting and/or with colloidal gold, silver particles or fluorescent molecules (s) for microscopic analysis. Reproduced from 20: Torchilin VP. Nat Rev Drug Discov. 2005;4(2):145–160.

Liposome-based cancer therapy

Carrier design: EPR (Enhanced Permehatio and Retention) effect



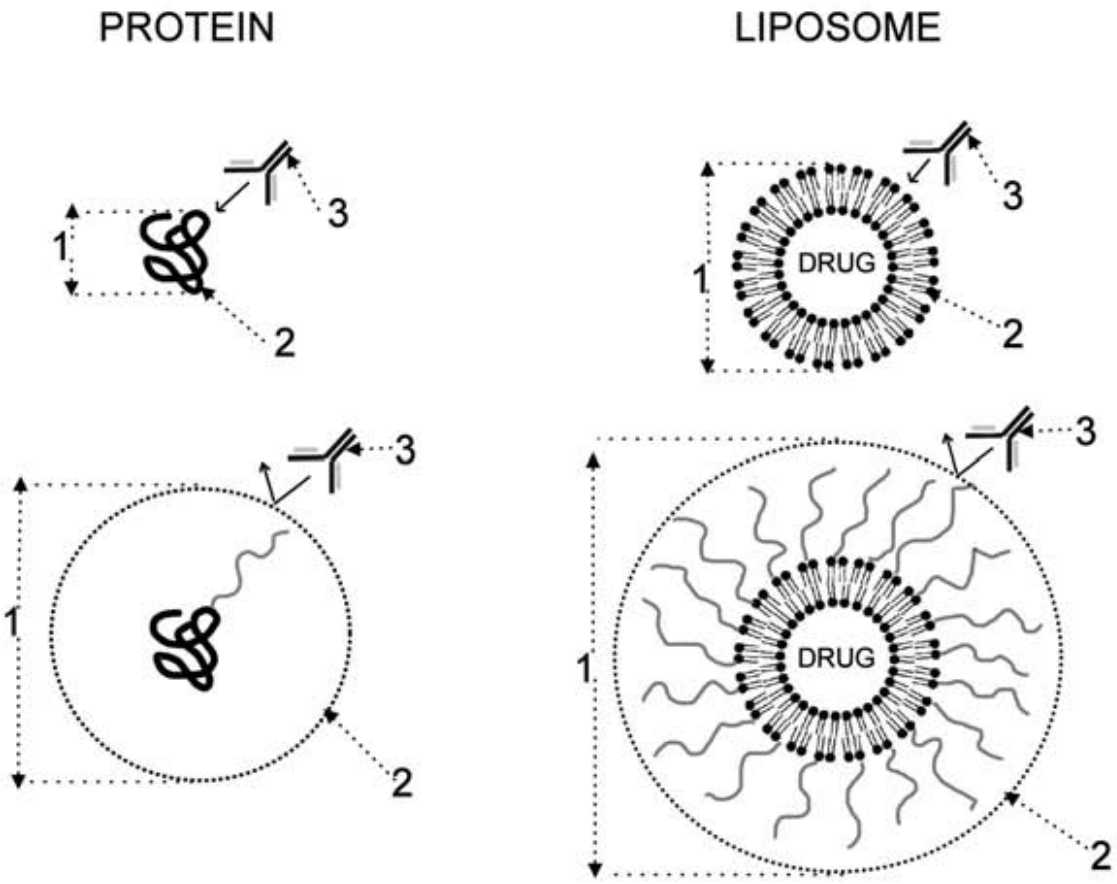
- Due to the leaky (immature) tumor vasculature and to the absence of lymphatic drainage
- Passive targeting effect: the drug accumulate in the tumor because it is large.
- Prolonged circulation times improve the effect.
- Optimal size: above 10 nm, below 200 nm to avoid spleen filters and renal clearance.

Other tumor features:

- High interstitial pressure due to the lacking of lymphatic vessels
- Lower pH
- Poor in nutrients and oxygen

Liposome-based cancer therapy

Carrier design: stealth properties



Non-PEGylated

1 small molecular size
= rapid clearance by glomerular filtration

2 low hydrophilicity
= poor solubility

3 recognition by antibodies and proteases
= high immunogenicity, antigenicity and uptake by RES

PEGylated

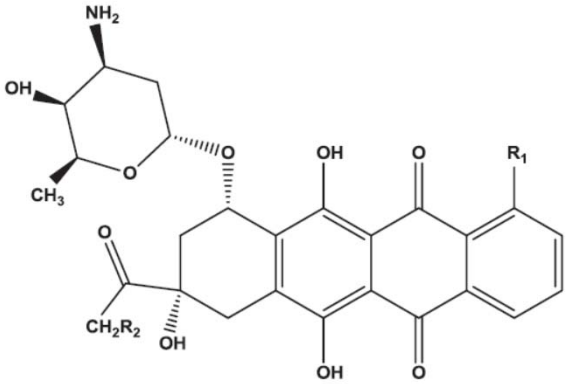
1 high molecular size
= decreased clearance and increased half-life

2 high hydrophilicity
= increased solubility

3 shielding against the recognition by antibodies and proteases
= low immunogenicity and antigenicity, RES evasion

DOXORUBICIN

and other anthracyclines



IDA: $R_1 = H, R_2 = H$
DOX: $R_1 = OCH_3, R_2 = OH$
DNR: $R_1 = OCH_3, R_2 = H$

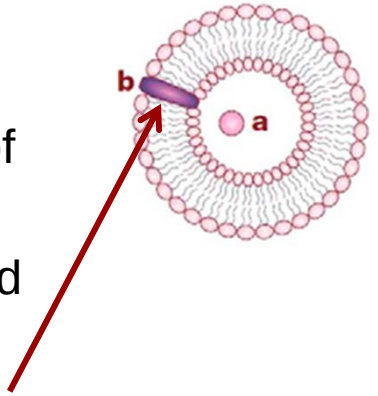
- Discovered in the ‘50 (in Italy, commercial name Adriamicin)
- Widely used for leukemia, Hodgkin’s lymphoma, bladder and breast cancers

- Kill selectively rapidly growing tissues by intercalating DNA during cell division
- Inhibits the enzyme topoisomerase II, preventing the relaxing of super-coiled DNA
- Forms iron-mediated free radicals that cause oxidative damage to DNA, proteins, and cell membrane lipids (in particular negatively charged cardiolipin present in mitochondria)

- Toxic for hair, gastrointestinal mucosa, and blood cells
- Severe cardiotoxicity (high amount of mitochondria)
- Multidrug resistance (MDR) due mainly to efflux proteins

First trials: OLV-DOX

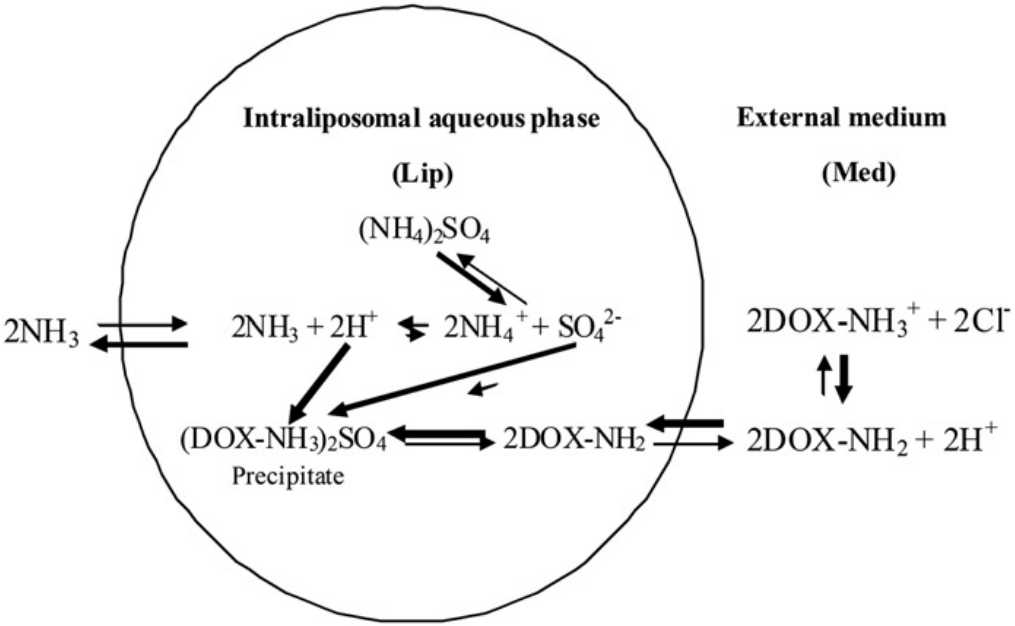
Carrier design: EPR effect

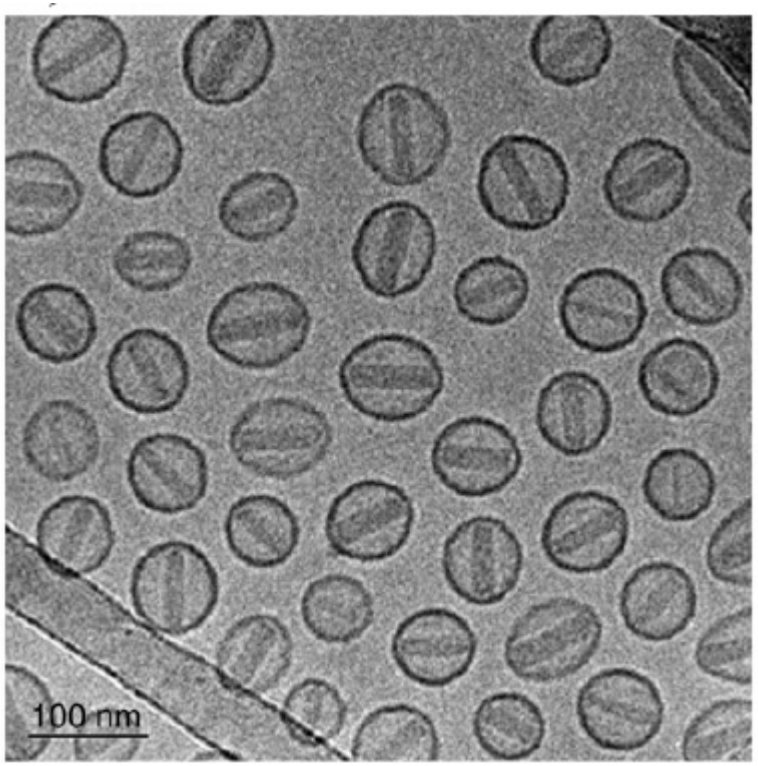


- Medium-size (300 nm) oligolamellar liposomes (OLV) composed of two low-T_m (fluid) phospholipids: the zwitterionic egg-derived phosphatidylcholine (EPC) and the negatively charged egg-derived phosphatidylglycerol (EPG)
- Doxorubicin was membrane associated and passively loaded during the lipid hydration
- Effective in mice
- Clinical trials with humans: higher maximum tolerated dose (MTD) than free DOX, no substantial therapeutic benefit
- Fast release due to high dilution (1:5 in mice, 1:3500 in humans)
- RES capture (negative charge)
- No extravasation (size)
- No uptake even by liver tumors

Solutions: drug loading

Remote loading





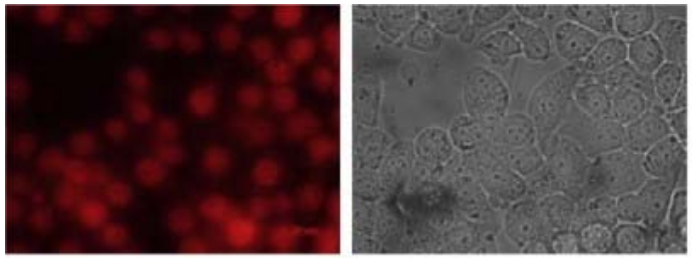
$[(NH_4)_2SO_4]_{lip} >> [(NH_4)_2SO_4]_{med}$ (1000-fold)

$pH_{lip} << pH_{med}$ (3 pH units)

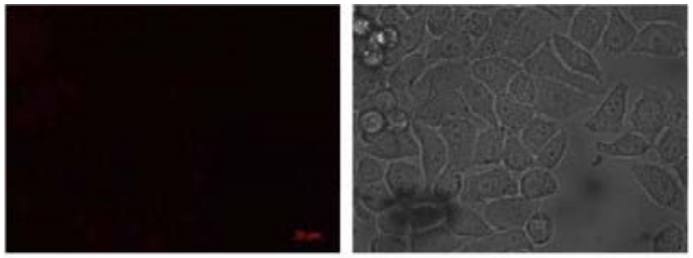
Encapsulation efficiency > 90%

Liposome-based cancer therapy

Carrier design: stealth properties

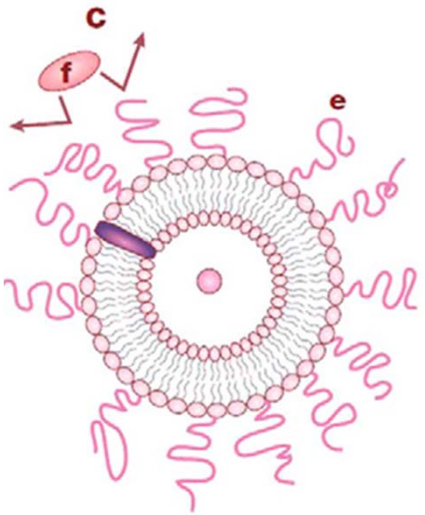


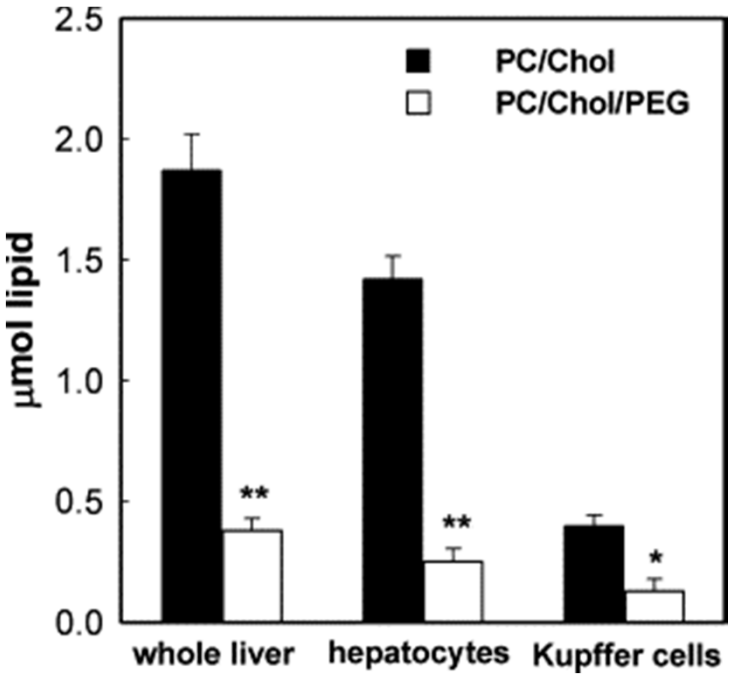
a Free Dox



b Caelyx

KB cells, treated with DOX 50 μM

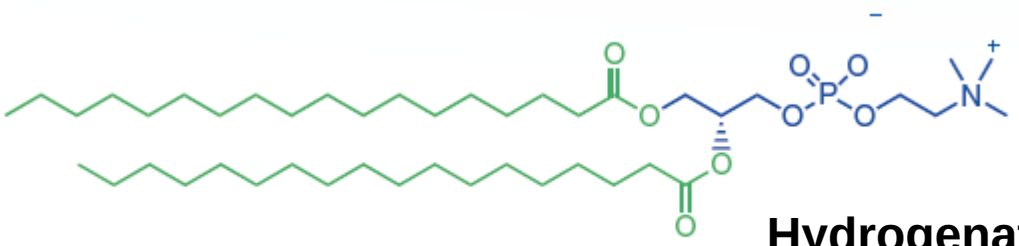




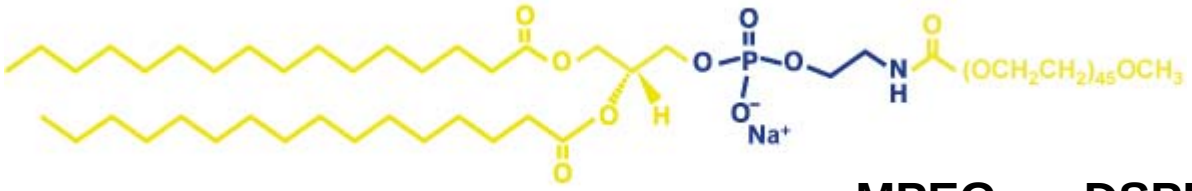
Rats, injected with PEGylated and non-PEGylated liposomes

Liposome-based cancer therapy

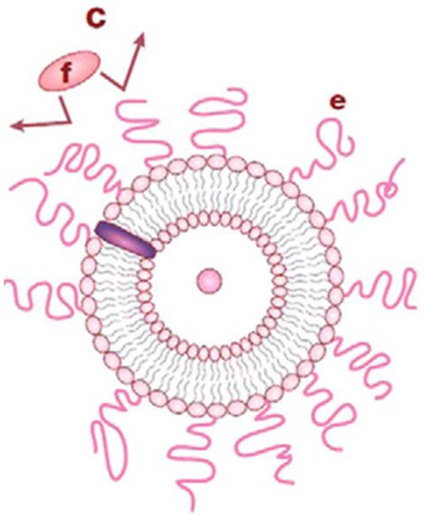
High T_m liposome



Hydrogenated soy PC



$MPEG_{2000}$-DSPE



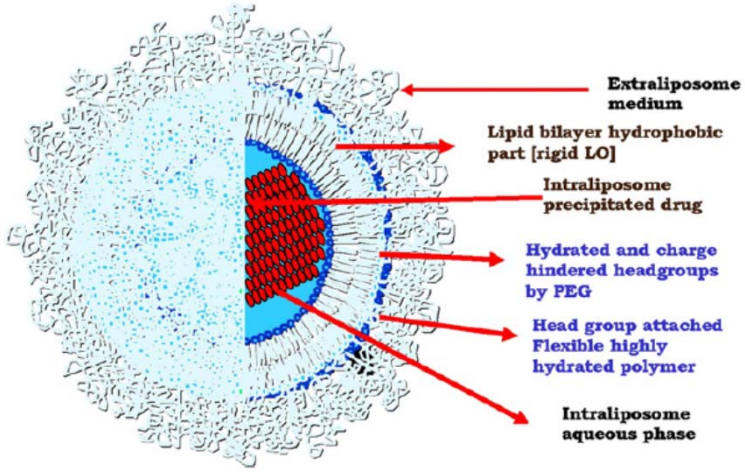
HsPC/Cholesterol/MPEG2000-DSPE 56.4:38:3

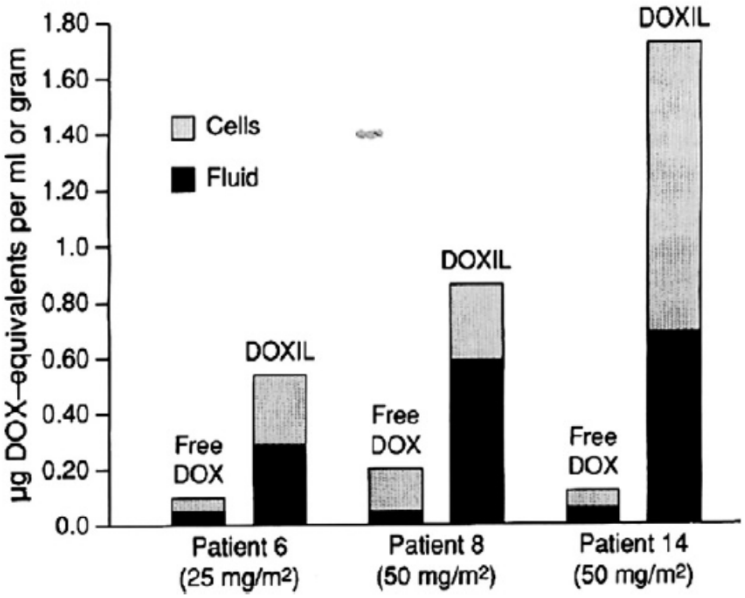
Drug to lipid ratio (weight): 0.126

DOXYL/CAELIX

PEGylated liposome-incapsulated doxorubicin

- Marketed in 1995 (Patent expired in 2010)
- The liposome technology changes the pharmacokinetics and biodistribution of Dox, allowing for longer circulation half-life (250-fold longer) and higher tumor concentration (4-16-fold higher).
- Clearance show a two phases profile with a minor fast (1-3 h) and a major slow one (30-90 h).
- Prolonged tumor peak (4 days)

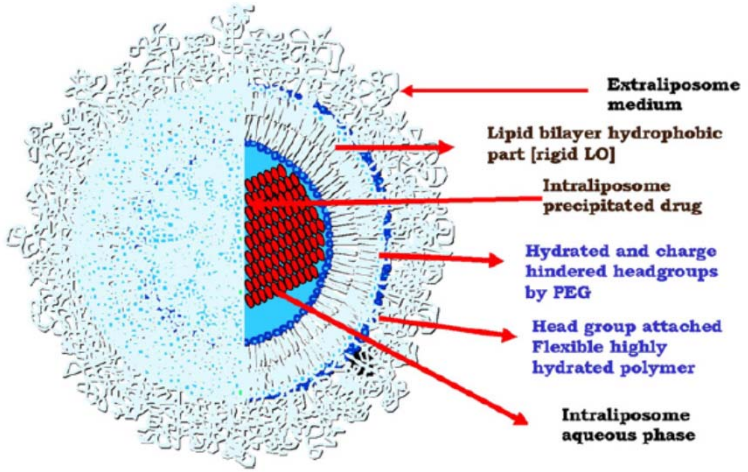




- 98% of circulating Dox in plasma is liposome-associated
- The presence of metabolites in urine demonstrates release in the tumor site (Dox is metabolized only intracellulary)
- Stealth liposomes associated cis-platinum is not released and ineffective.
- Most likely release mechanism is osmotic shock or pH gradient inversion or ammonia neutralization

DOXYL/CAELIX

PEGylated liposome-incapsulated doxorubicin

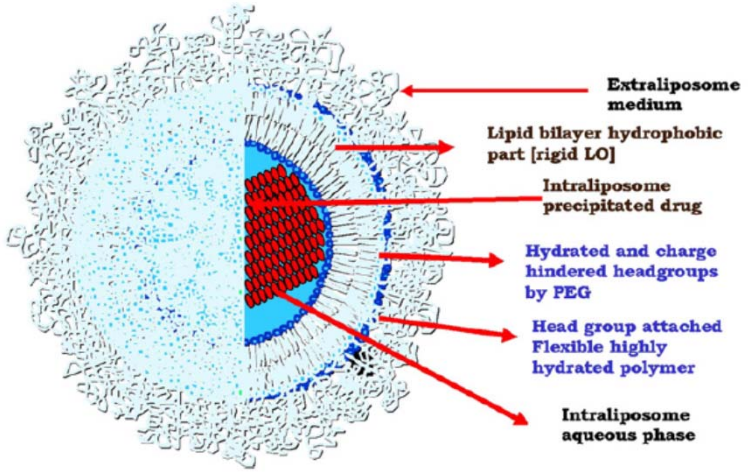


- DOXIL enhances the therapeutic index of Dox by reducing its cardiotoxicity, higher cumulative dose is allowed. Perform better than free DOX in several cases.
- Recurrent, platinum-resistent ovarian cancer can be treated with carboplatinum/DOXIL, DOXIL has an immunosuppressant activity that prevent anti-platinum Ig activity.
- DOXIL exhibits a new side effects: i) severe hand-foot syndrome (palmarplantar erythrodysesthesia), ii) complement activation-related pseudo-allergy (CARPA) during infusion.
- Due to these side effects, maximum tolerated dose (MTD) of DOXIL (50 mg/m^2 every 4 weeks) is lower than that of standard Dox (60 mg/m^2 every 3 weeks)
- Limitations lack of sustained release and narrow range of chemical payloads that can be compatible with this platform.

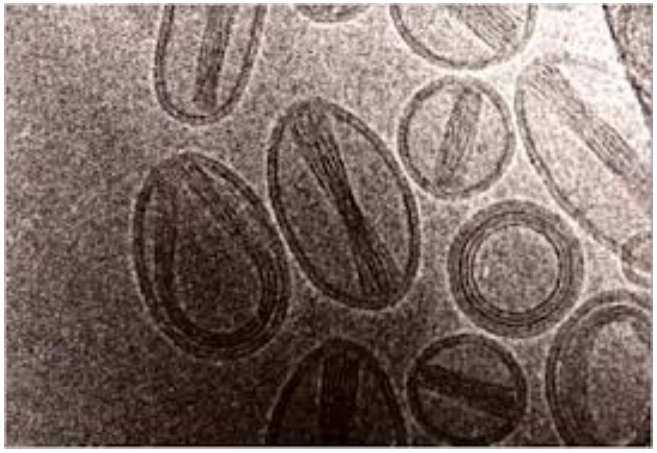
MIOCET

liposome-incapsulated doxorubicin

PC

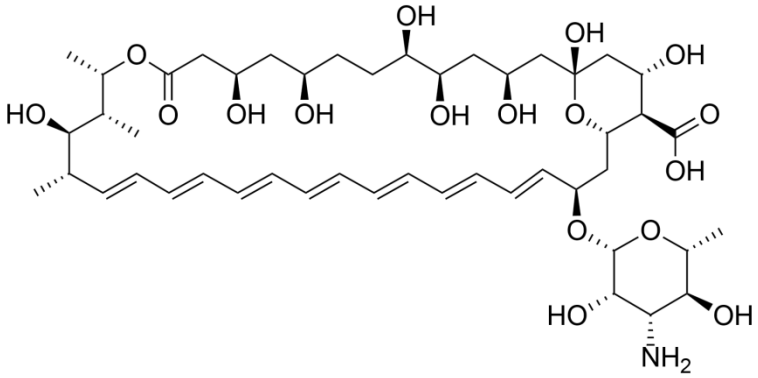


- Approved in 2000 in Europe
- Egg phosphatidylcholine/colesterol 1:1 (low T_m), 180 nm
- Lipid/drug 0.27, loading by pH gradient, internal precipitation of citrate salt
- Rapid uptake by MPS (Mononuclear Phagocitic System) followed by slow release, avoid plasma peaks and consequent cardiotoxicity
- 5-fold slower clearance than free Dox
- MTD, 75 mg/m^2 every three weeks
- Toxicity: myelosuppression



AmBisome®/Fungisome®

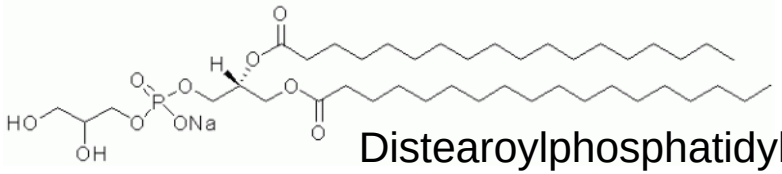
Liposome-incapsulated Amphotericin B



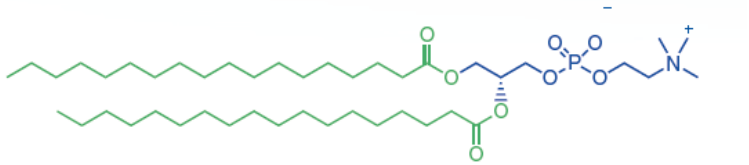
- Targets sterols (**ergosterol,** colesterol) in cell membrane causing membrane disruption (ion channels)
- Sterols are present both in fungal and mammalian cell membranes
- Severe infusion reaction (fever)
- Severe nephrotoxicity

AmBisome®/Fungisome®

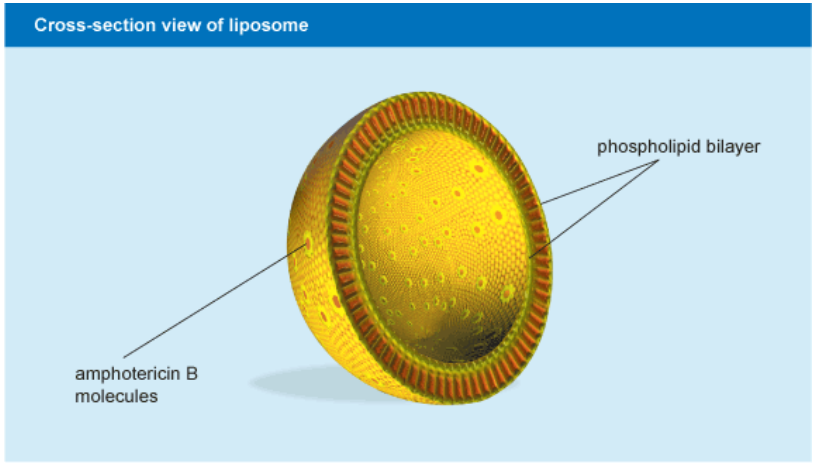
Liposome-incapsulated Amphotericin B



Distearoylphosphatidylglycerol



Hydrogenated soy phosphatidylcholine



PC/DSPG/Col 50:25:25 Drug loading 10% 80 nm

Apparently, Amphotericin B loaded liposome disrupt after interaction with fungal cell walls but not with mammalian cell walls (selective delivery)

Accumulation in tissues (included MPS) and slow release have been recently suggested.

Liposome-based drug formulations

Carrier design: active targeting

Table 1
Marketed liposomal and lipid-based products, plus a selection of products in clinical development.

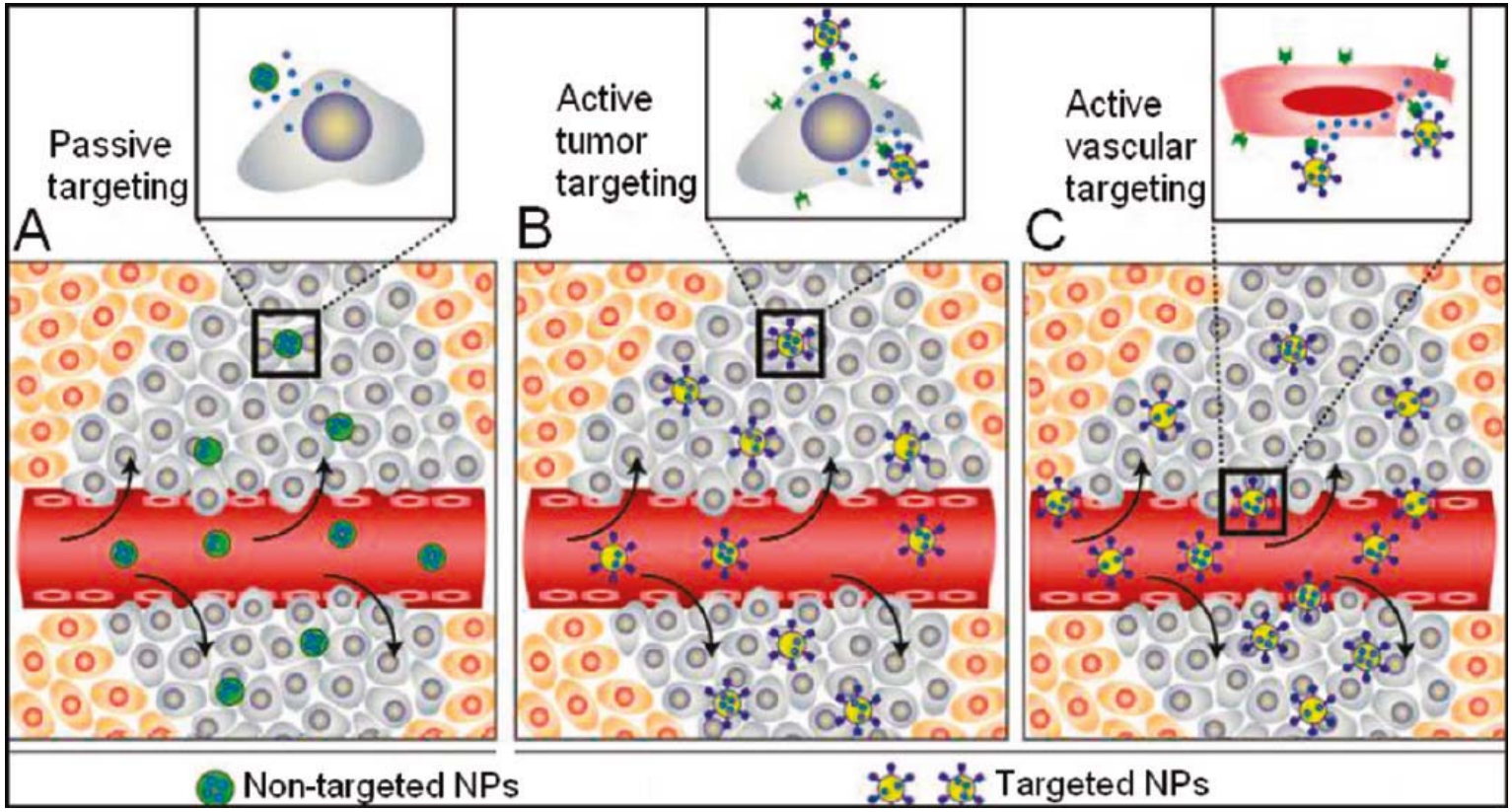
Product	Drug	Indications	Year approved	Reference
Approved products				
AmBisome (Gilead)	Amphotericin B	Fungal infections Leishmaniasis,	1990 (Europe), 1997 (USA), 2000	[255,256]
Doxil/Caelyx (Johnson & Johnson)	Doxorubicin	Kaposi's sarcoma Ovarian cancer Breast Cancer Multiple myeloma + Velcade	1995 1999 2003 (Europe, Canada) 2007	[93,257–259]
DaunoXome (Galen)	Daunorubicin	Kaposi's sarcoma	1996 (Europe), 1996 (USA)	[260]
Myocet (Cephalon)	Doxorubicin	Breast cancer + cyclophosphamide	2000 (Europe)	[261]
Amphotec (Intermune)	Amphotericin B	Invasive aspergillosis	1996	[262]
Abelcet (Enzon)	Amphotericin B	Aspergillosis	1995	[263]
Visudyne (QLT)	Verteporphin	Wet macular degeneration	2000 (USA), 2003 (Japan)	[250]
DepoDur (Pacira)	Morphine sulfate	Pain following surgery	2004	[264]
DepoCyt (Pacira)	Cytosine Arabinoside	Lymphomatous meningitis Neoplastic meningitis	1999	[265,266]
Diprivan (AstraZeneca)	Propofol	Anesthesia	1986	[267]
Estrasorb (King)	Estrogen	Menopausal therapy	2003	[268]
Lipo-Dox (Taiwan Liposome)	Doxorubicin	Kaposi's sarcoma, breast and ovarian cancer	2001 (Taiwan)	[269]
Marqibo (Talon)	Vincristine	Acute lymphoblastic leukemia	2012 (USA)	[270,271]

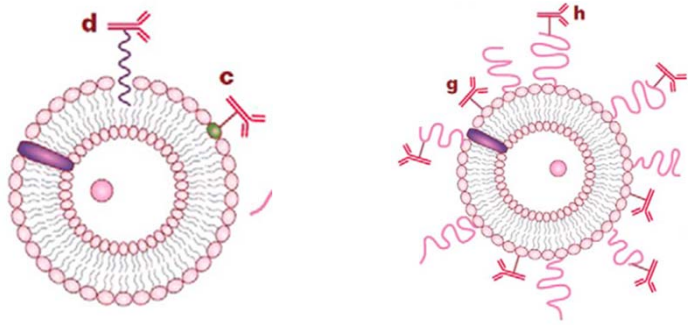
No PEG

sphingomyelin/cholesterol
Targets MPS

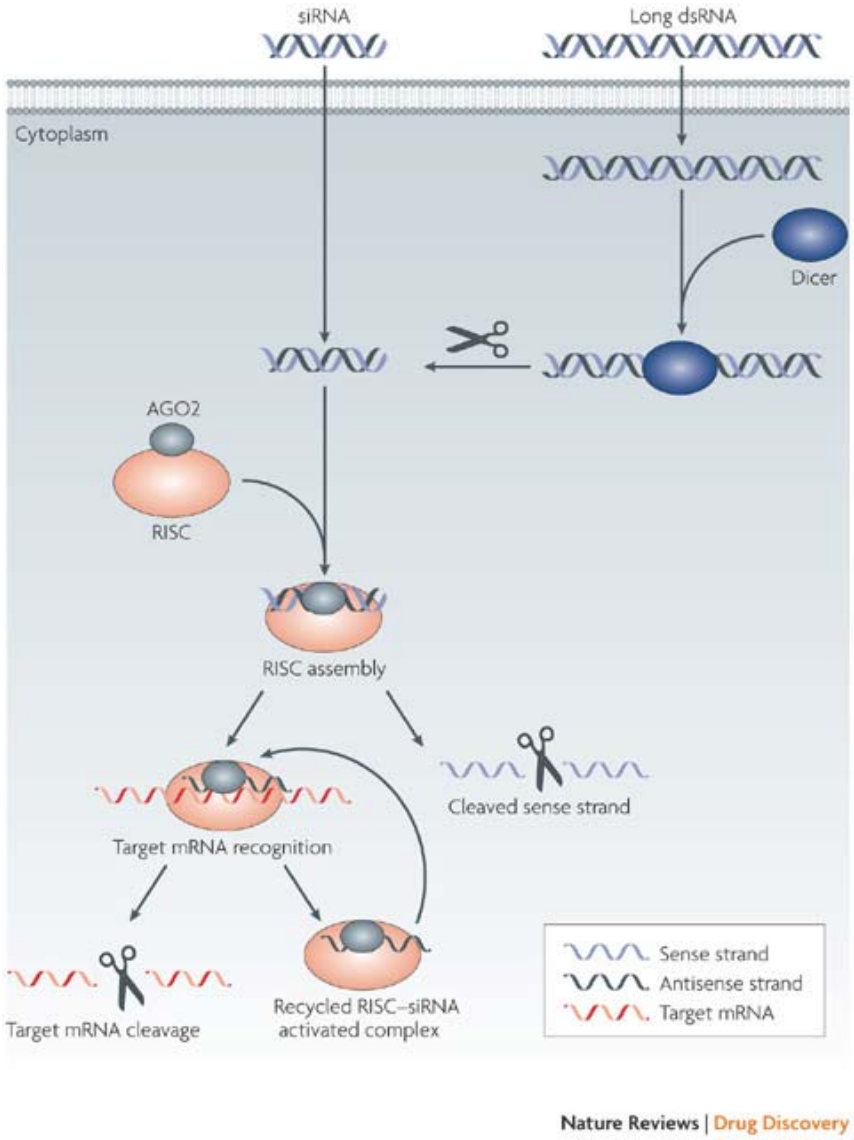
Liposome-based cancer therapy

Carrier design: active targeting



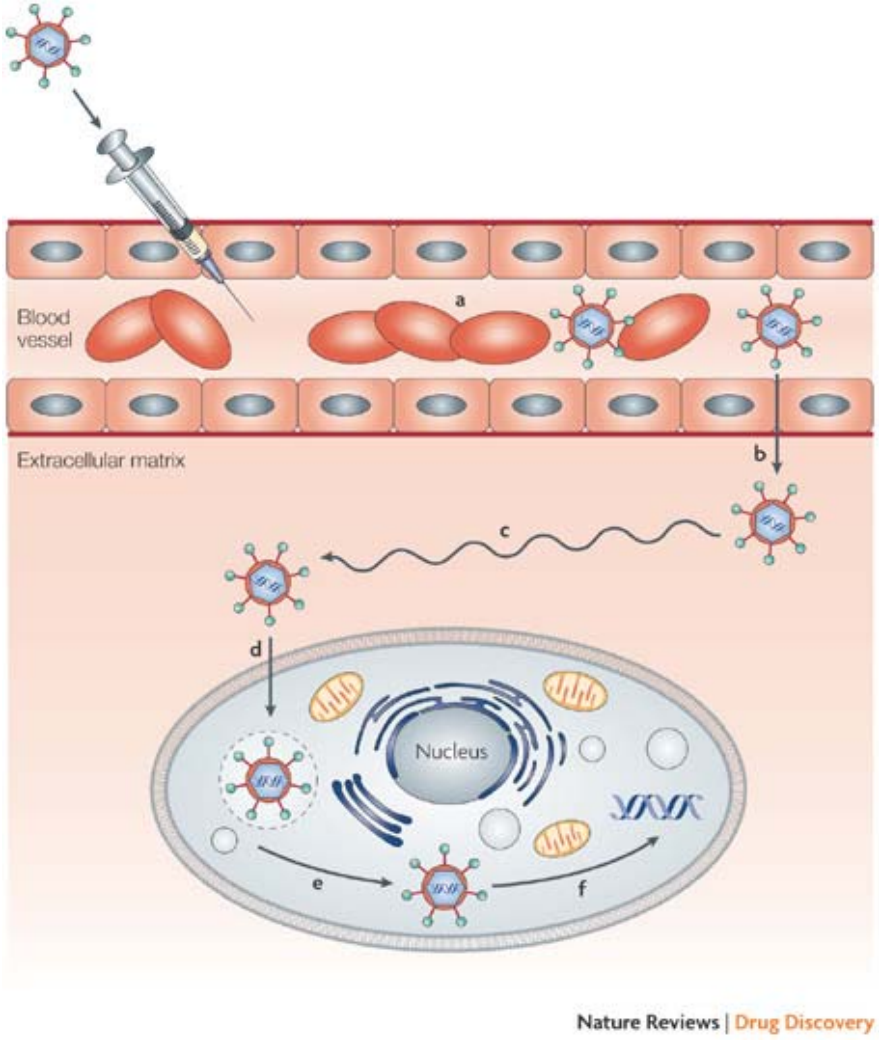


Evolutions: liposomes for siRNA delivery



- New therapeutic modality introduced in early 2000.
- siRNA, once entered in the cytoplasm, is incorporated into a protein complex called the RNA induced silencing complex (RISC)
- There, the siRNA sense strand is cleaved and the activated RISC seeks and cleave mRNA complementary to the siRNA antisense strand.
- therapeutic effect lasts for 3–7 days in rapidly dividing cells, and for several weeks in non-dividing cells
- broader therapeutic potential than typical small-molecule drugs, as any gene can in principle be silenced using the appropriate siRNA.

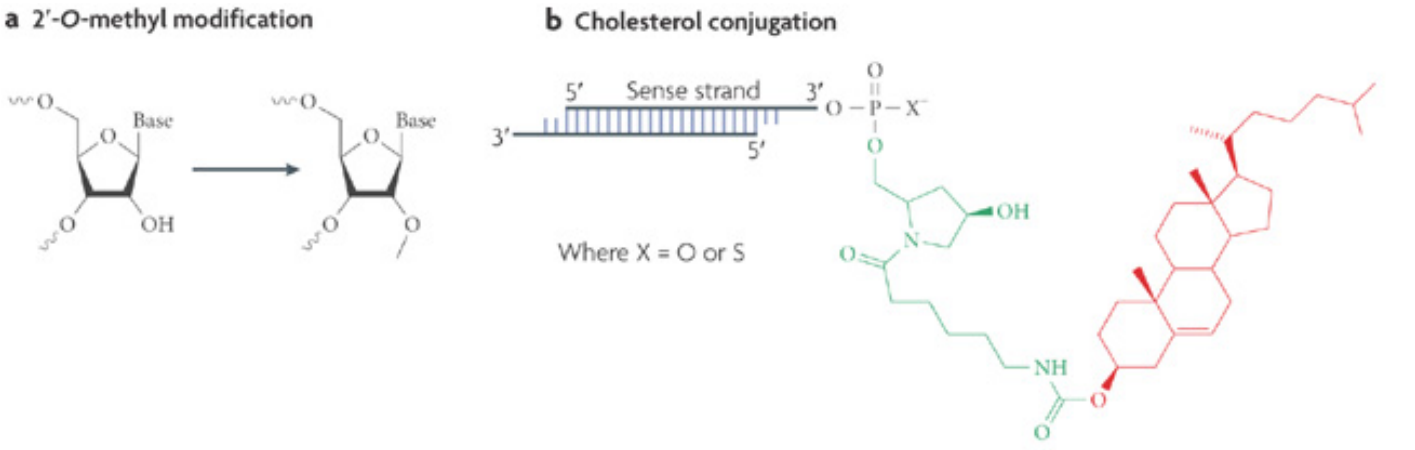
Obstacles in siRNA delivery



- *In vivo* degradation by nucleases.
- siRNA is too large (13 kDa) and negatively charged to cross membranes and enter cells
- Rapid plasma elimination.
- Renal and hemodynamic toxicity
- Immunogenicity

siRNA delivery

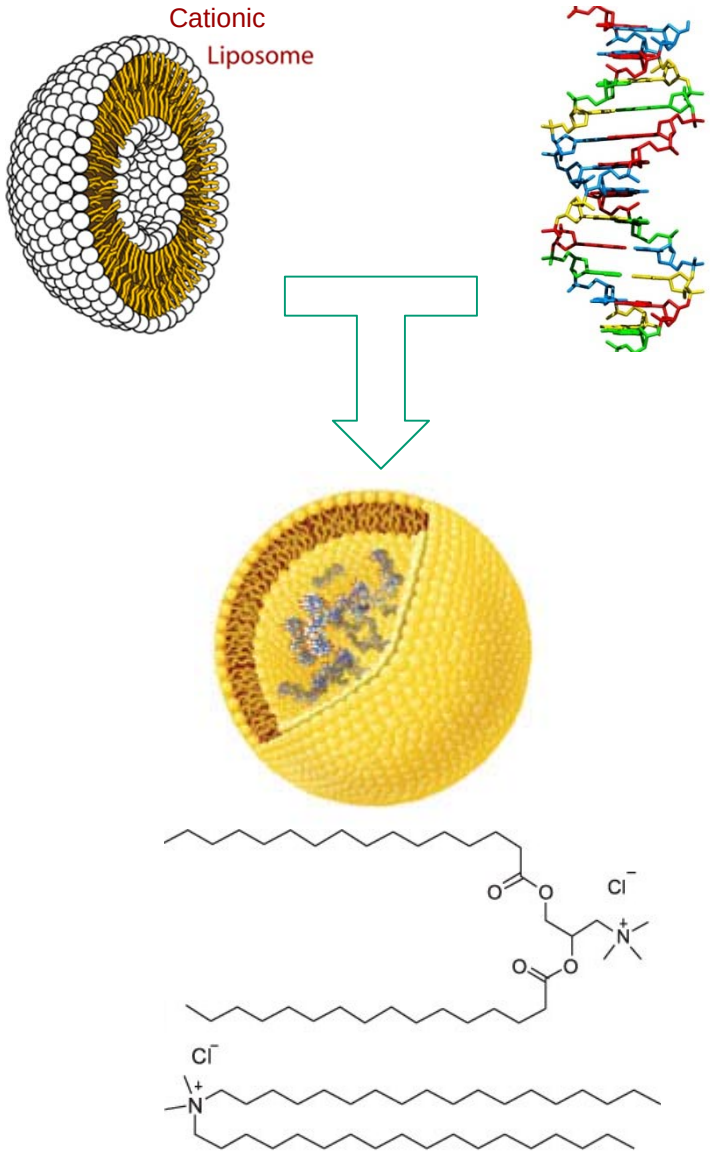
Solutions by RNA modification:



- Direct administration in the target tissue prevents rapid clearance
- Renal and hemodynamic toxicity is reduced by lowering dose and slowing down infusion.
- 2’-O-methylmodification prevents immune system activation and nuclease cleavage
- Cholesterol conjugation improves biodistribution by association of siRNA with albumin.

Liposomes for siRNA delivery

Delivery by cationic liposomes



Neutral lipids

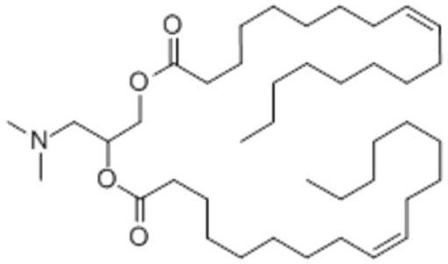
- ☹ Low encapsulation efficiency

Cationic lipids

- ☺ High encapsulation efficiency
- ☺ Increased cellular uptake in *in vitro* experiments
- 😐 Aggregate structure unclear
- ☹ In vivo proteins absorption
- ☹ Decreased uptake
- ☹ Increased clearance
- ☹ Complement activation

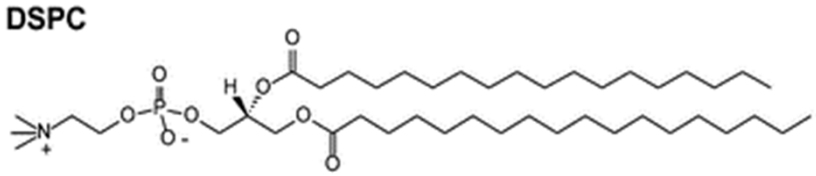
Evolutions: liposomes for siRNA delivery

pH sensitive phospholipids



DODAP

$pK_a = 6.6$



DSPC, CH, DODAP, PEG-CerC_{14} (25/45/20/10)

1. Dried lipid film + RNA in buffer (pH 4), extrusion, ion chromatography (pH 7).

⇨ 30% encapsulation efficiency, extrusion slow, 30% RNA and lipid loss

2. Dried lipid film + RNA in buffer (pH 4) + ethanol, extrusion, ion chromatography (pH 7).

⇨ 70% encapsulation efficiency, extrusion fast, >3% RNA and lipid loss

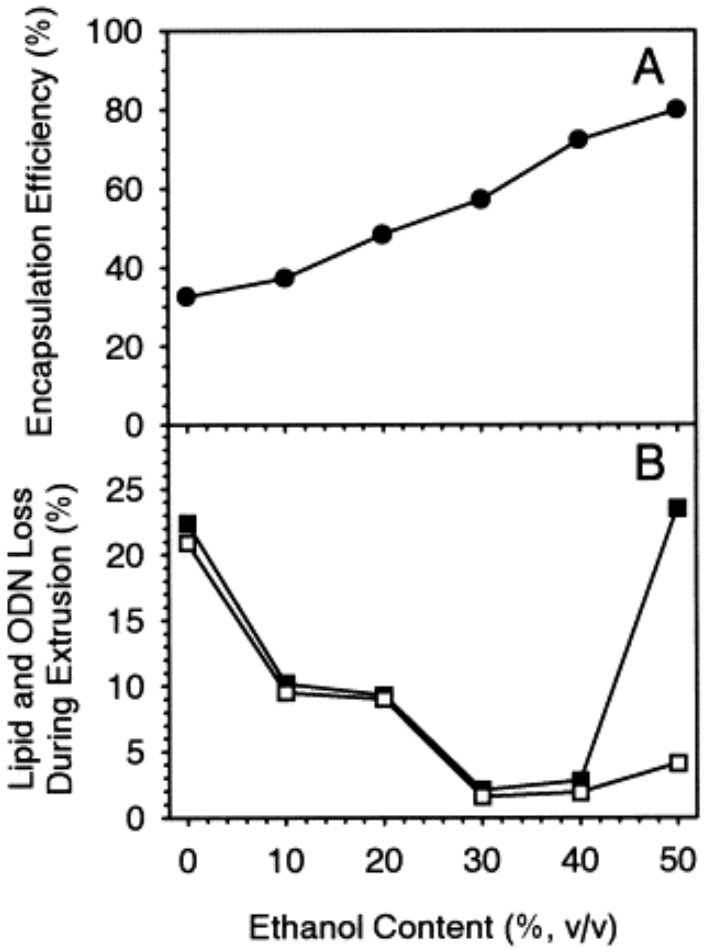
3. Ethanol lipid solution+ RNA in buffer (pH 4) , extrusion, ion chromatography (pH 7).

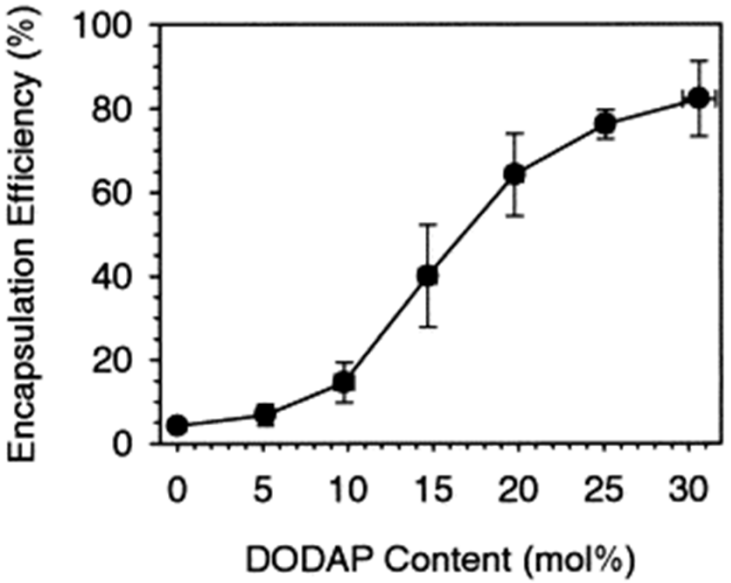
⇨ As above

C14 PEG phospholipid prevents liposomes aggregation during preparation/functionalization, but *in vivo* is released in 5-10 minutes after injection.

Liposomes for siRNA delivery

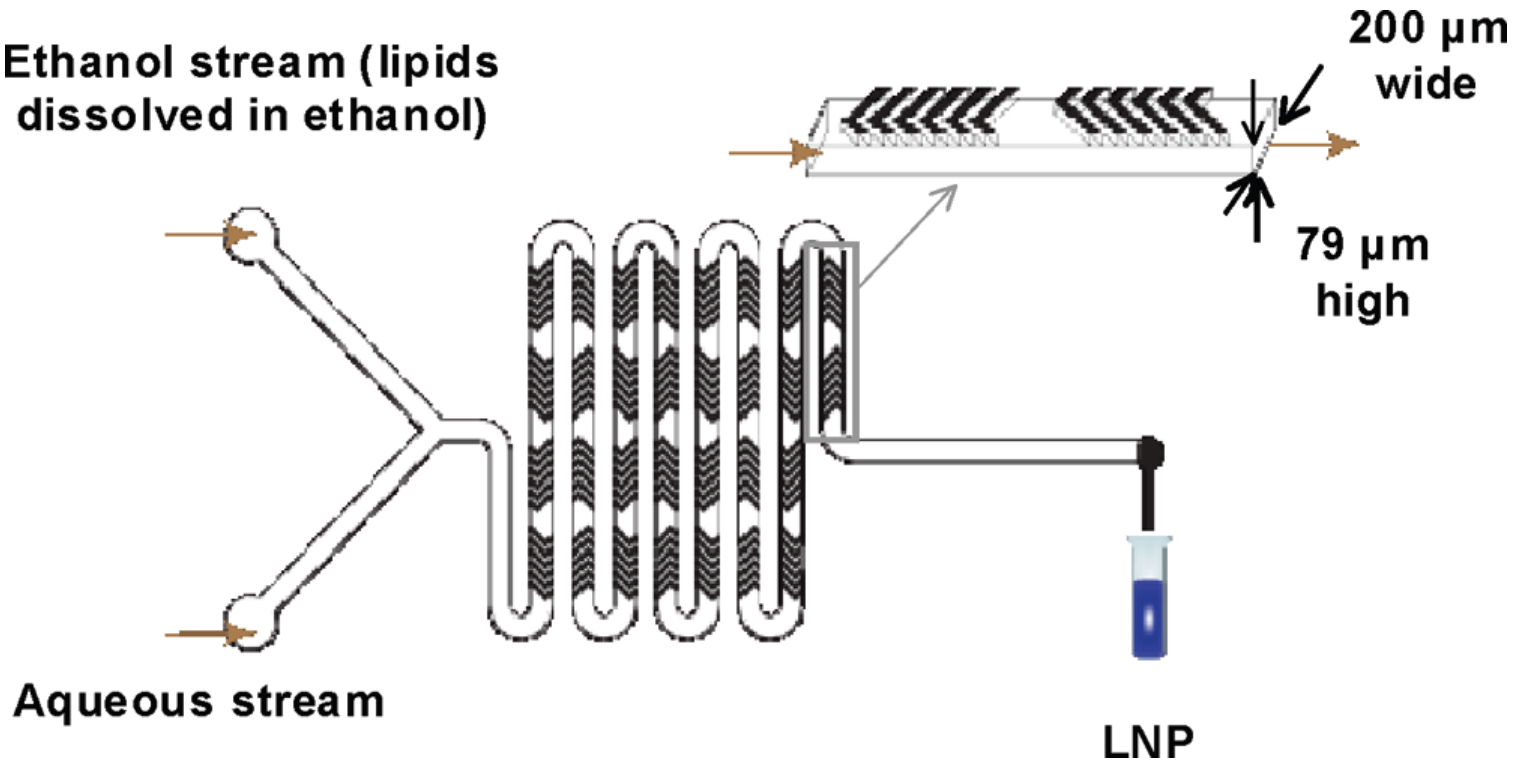
Encapsulation efficiency





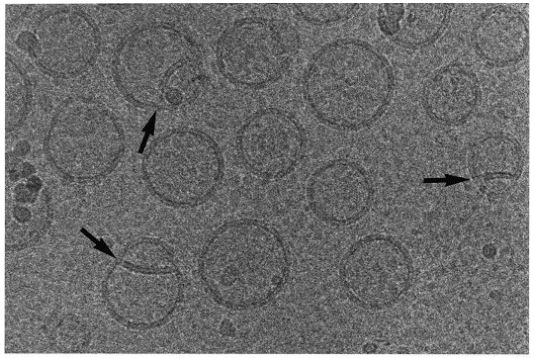
Liposomes: synthesis

Microfluidic synthesis

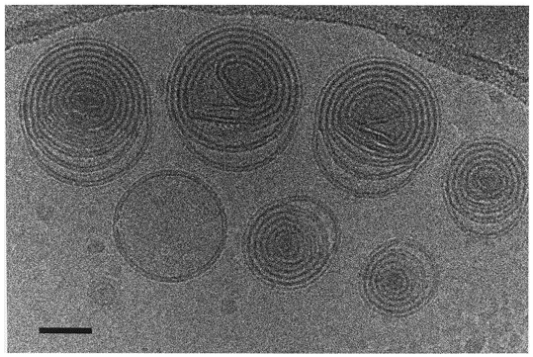


Liposomes for siRNA delivery

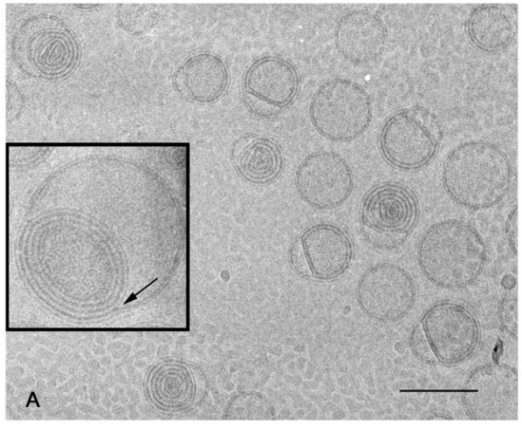
Size and morphology: the role of PEG



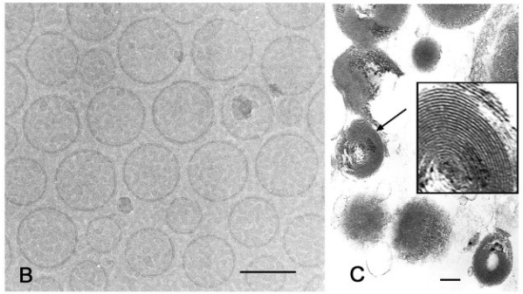
Low RNA content



High RNA content



RNA/ethanol/
PEG-Cer



No PEG-Cer/
no ethanol

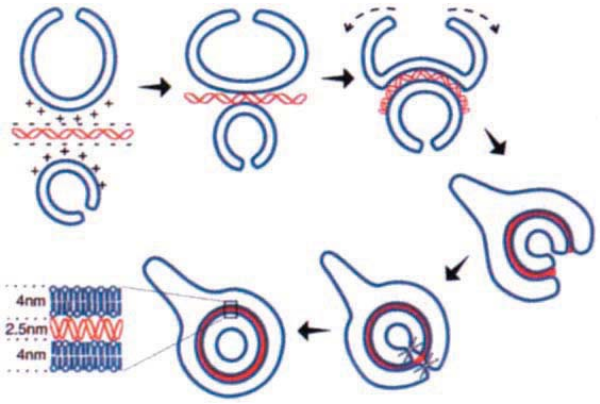
TABLE 2 Effect of PEG-Cer on entrapment

PEG-CerC$_{14}$ (mol %)	% Encapsulation	Average size and polydispersity (nm)
25% ethanol		
2.5	14 ± 1.5	125 ± 35 (108 ± 26)
10	5 ± 1	92 ± 18 (93 ± 18)
40% ethanol		
2.5	45.5 ± 3	131 ± 40 (108 ± 26)
5	51 ± 1.5	126 ± 36 (107 ± 22)
10	56.5 ± 2	100 ± 26 (93 ± 18)

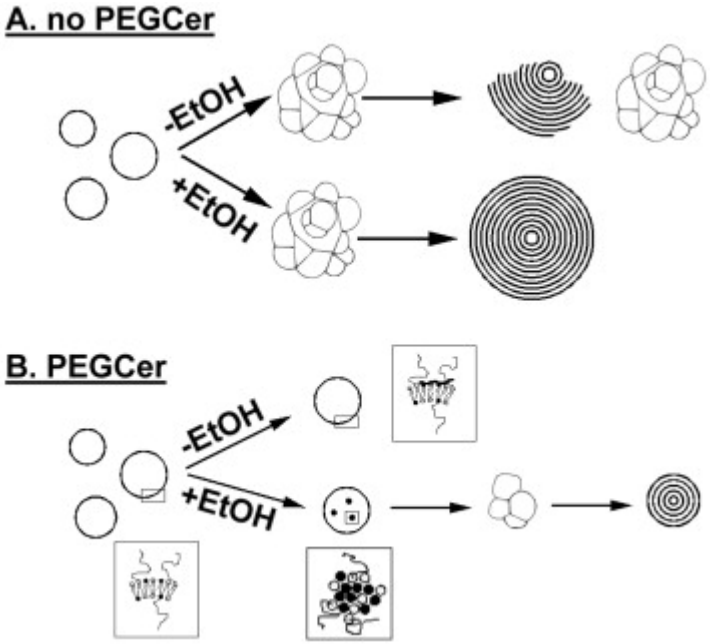
Increase in PEG-Cer lead to smaller and monodisperse particles

Liposomes for siRNA delivery

Encapsulation mechanisms



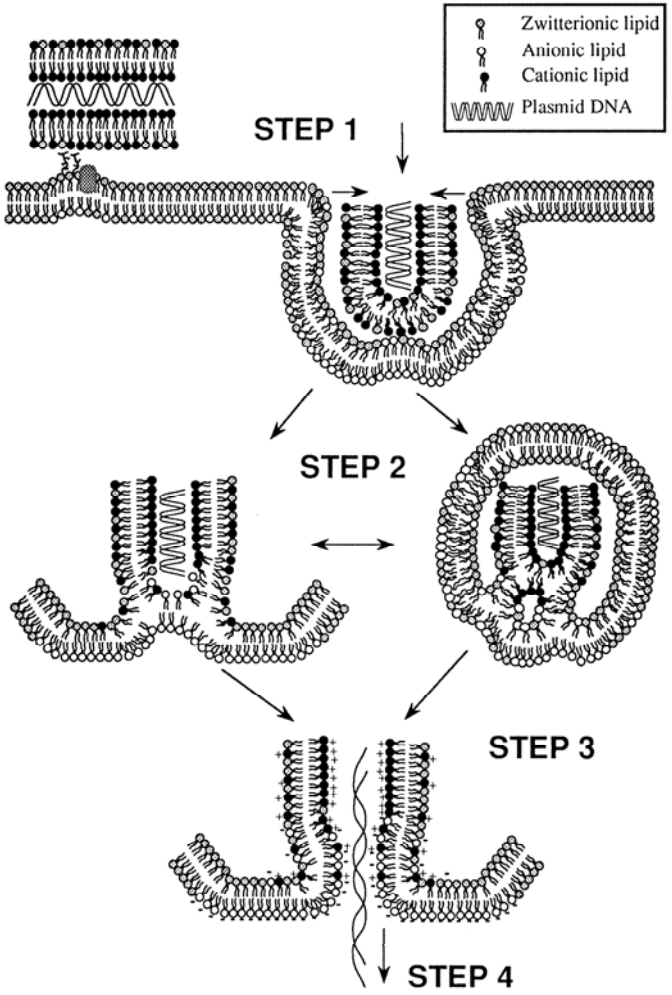
Polyanionic DNA induces aggregation and later fusion of cationic liposomes

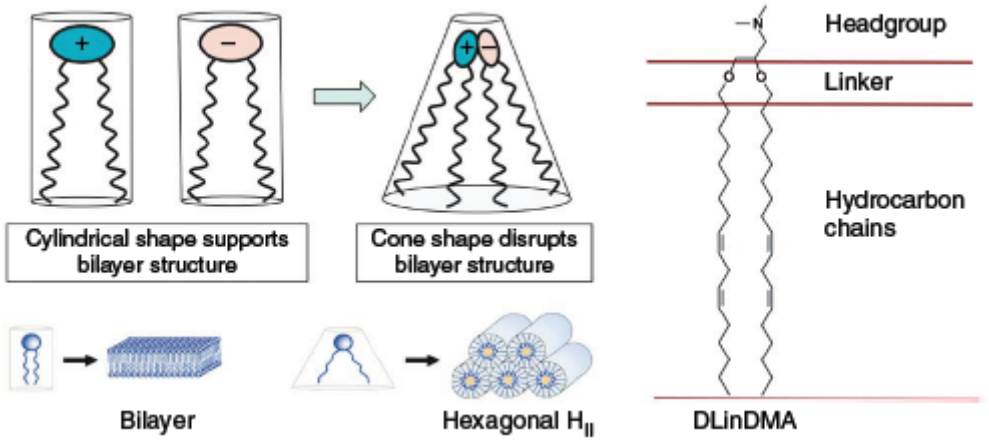


PEG-Cer prevents aggregation and encapsulation in the absence of ethanol
In the presence of ethanol end PEG-Cer small multilamellar aggregates are formed.
In the absence of PEG-Cer and ethanol large multilamellar aggregate form, which transform into large MLV with ethanol.

Liposomes for siRNA delivery

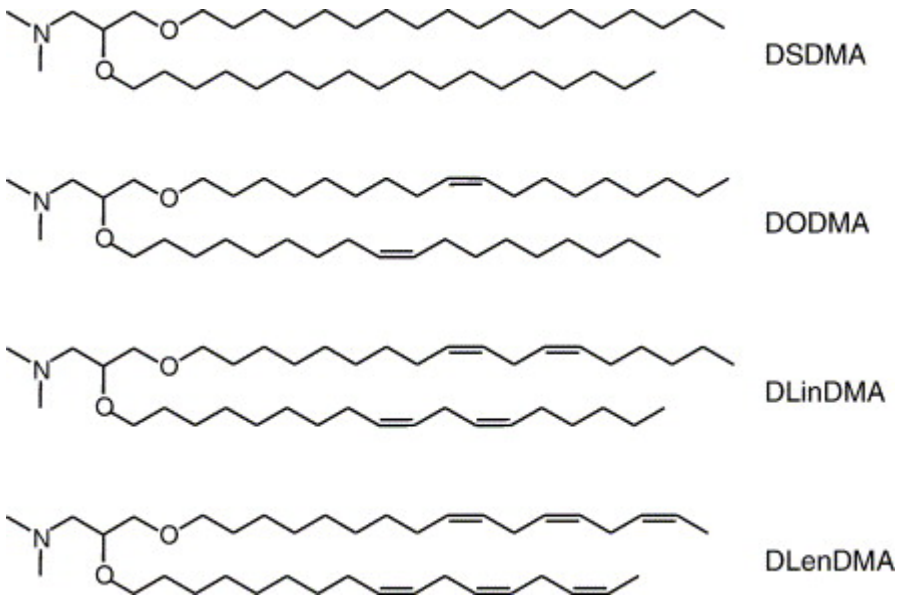
Endosome escape mechanism

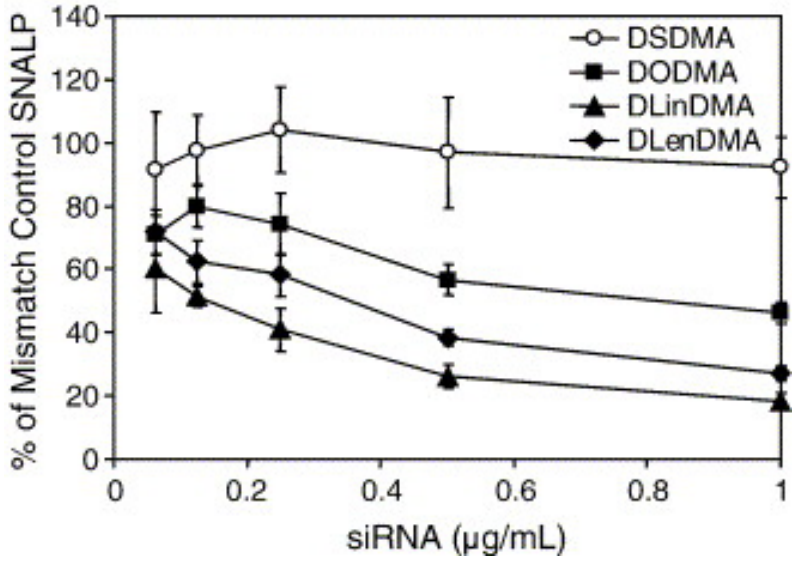




Liposomes for siRNA delivery

Optimizion: the alkyl chains

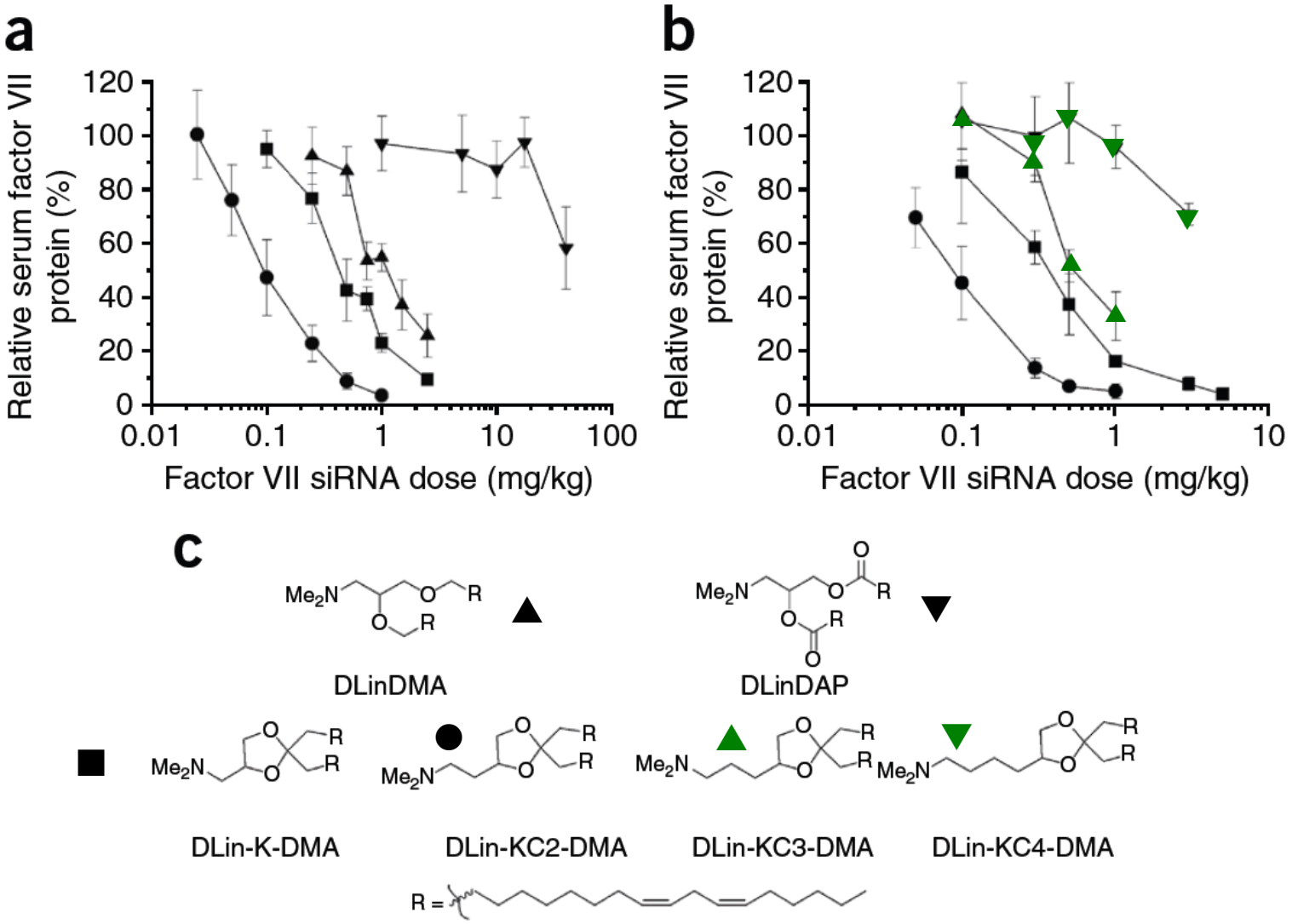




The presence of cis double bonds increase the surfactant tendency to form hexagonal lamellar phases

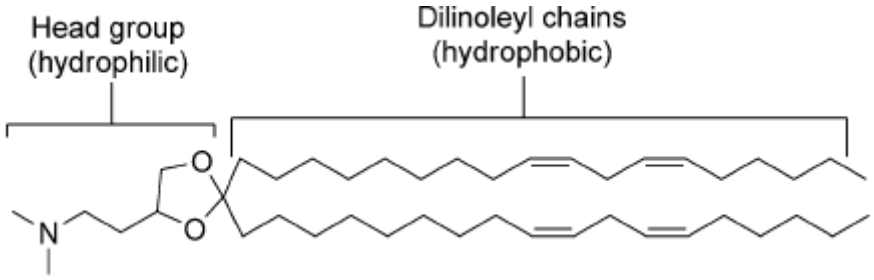
Liposomes for siRNA delivery

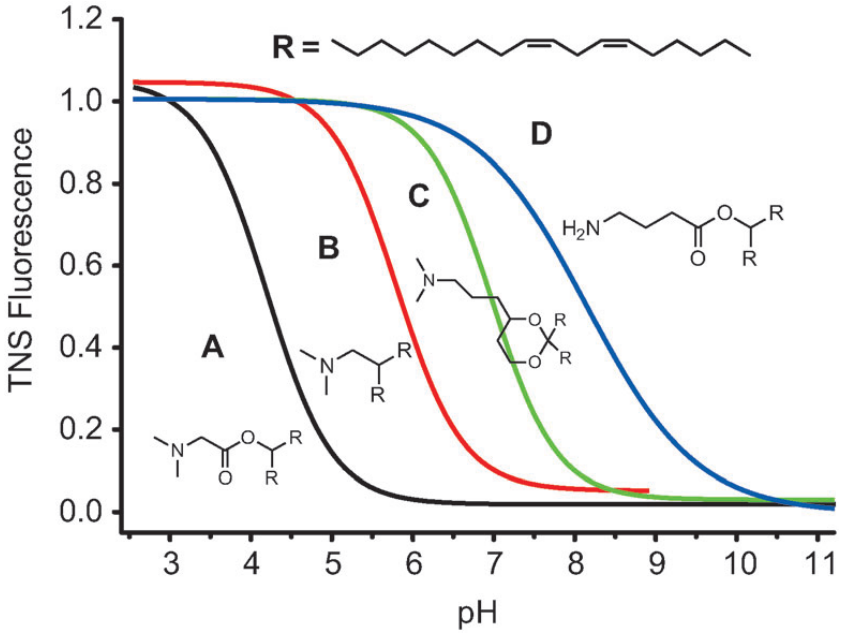
Optimization: the linker

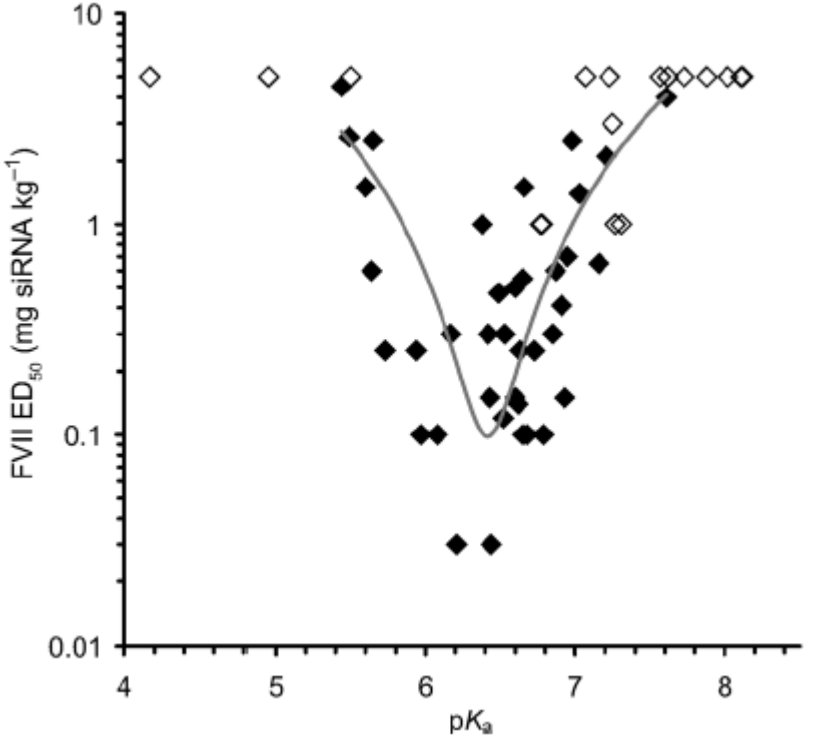


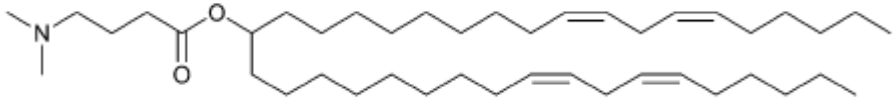
Liposomes for siRNA delivery

Optimization: the pH sensitive unit



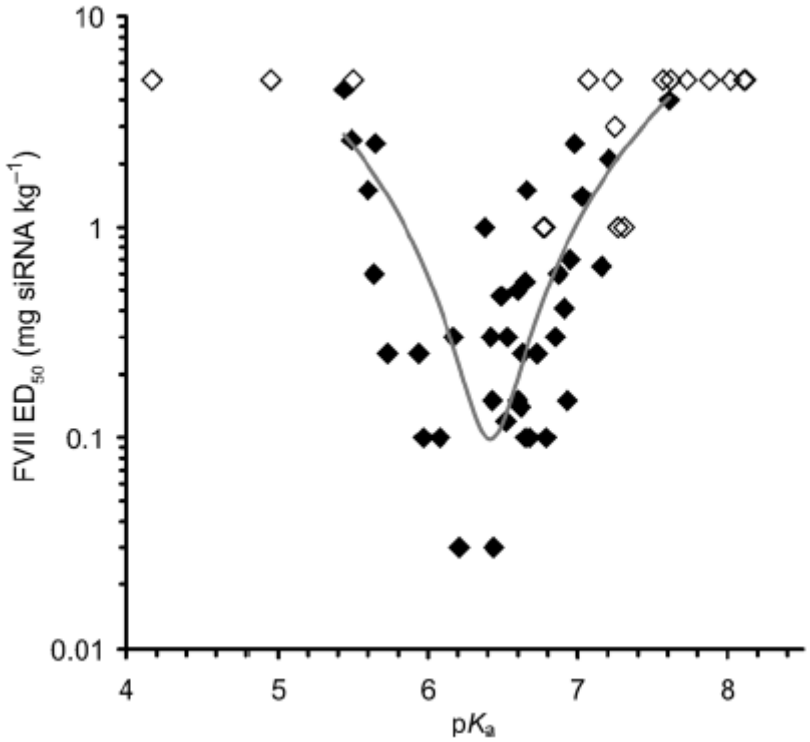


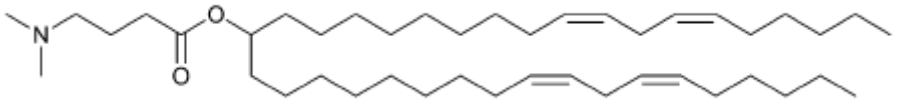




Evolutions: liposomes for siRNA delivery

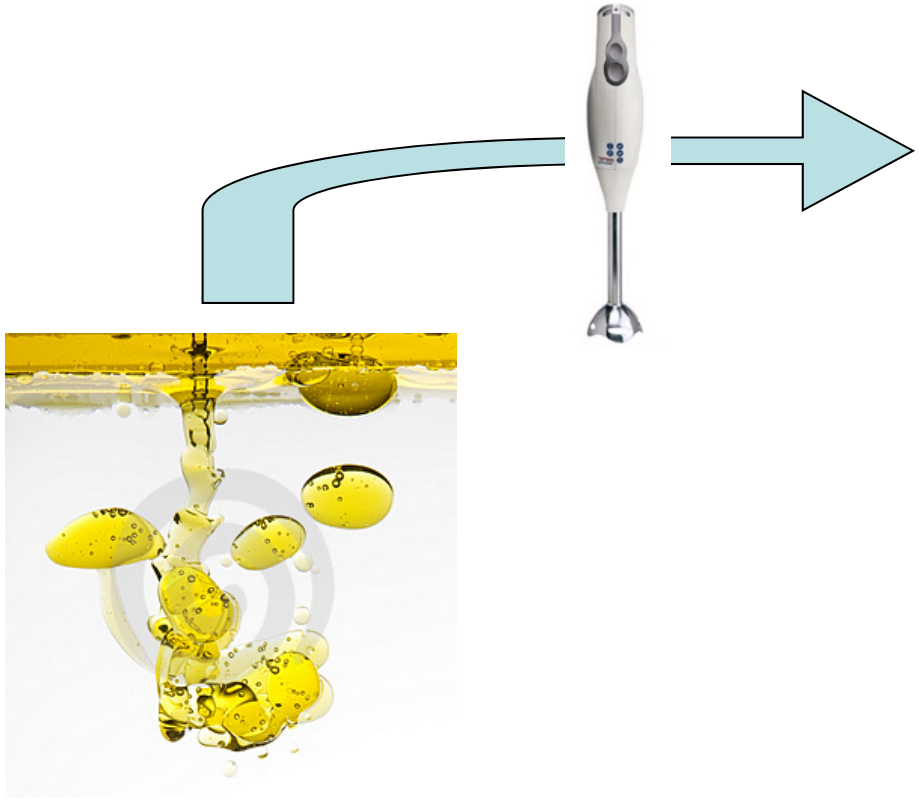
pH sensitive phospholipids





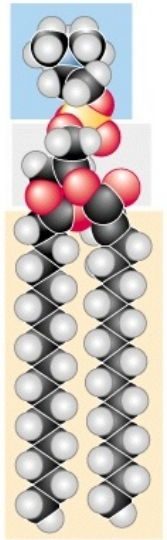
- pKa 6.44 is the optimal compromise between having a full positive charge in the liposomes (pH 5) and no charge at pH 7.4.
- Positive charge helps interaction with negatively charged cellular membranes favoring liposomial membrane disruption.
- Neutral charge reduces opsonization and favors the adsorption of lipoprotein ApoE.
- ApoE is the targeting agent toward hepatocytes.

Microemulsioni: stabilizzare la superficie

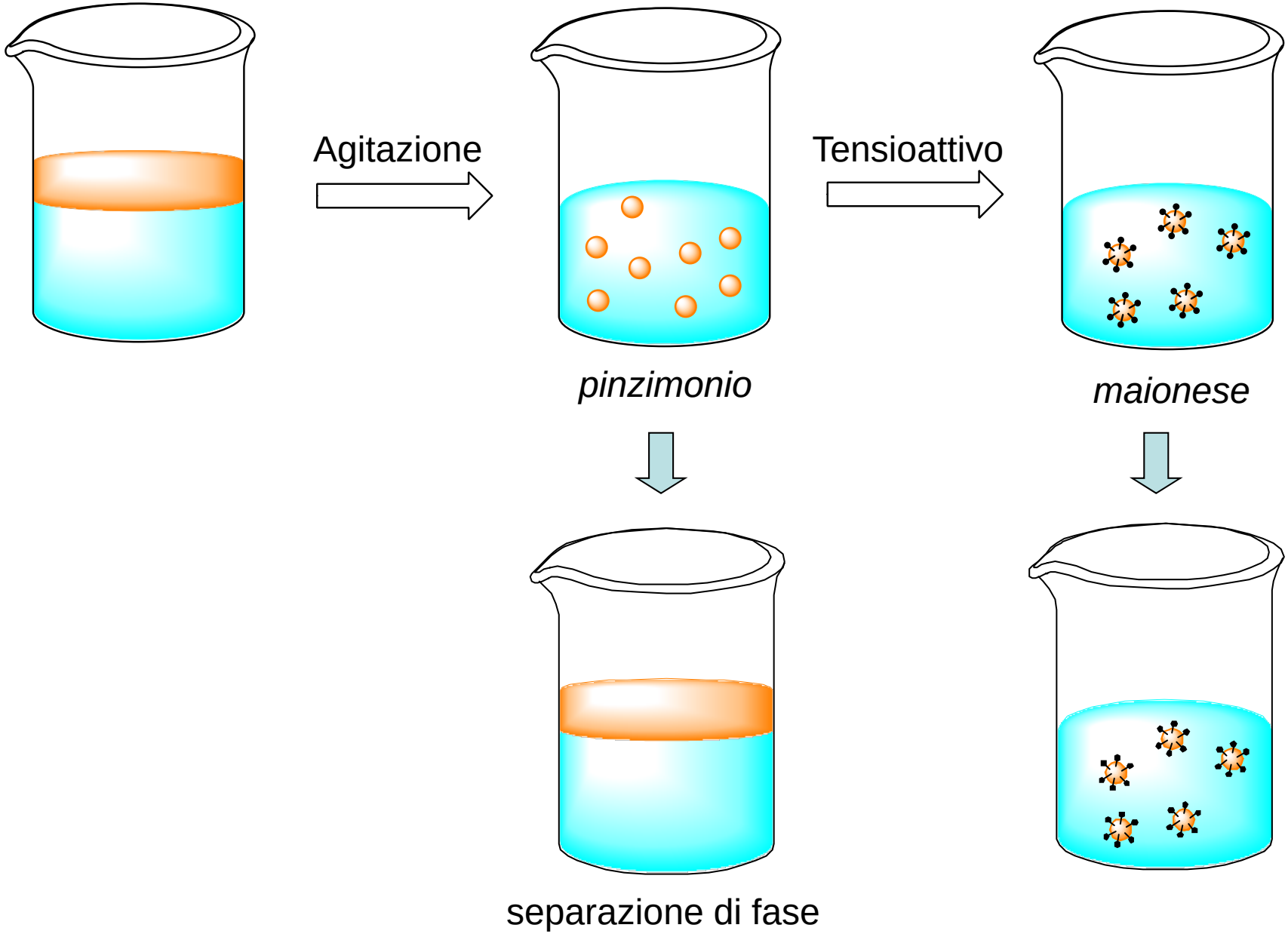


pinzimonio

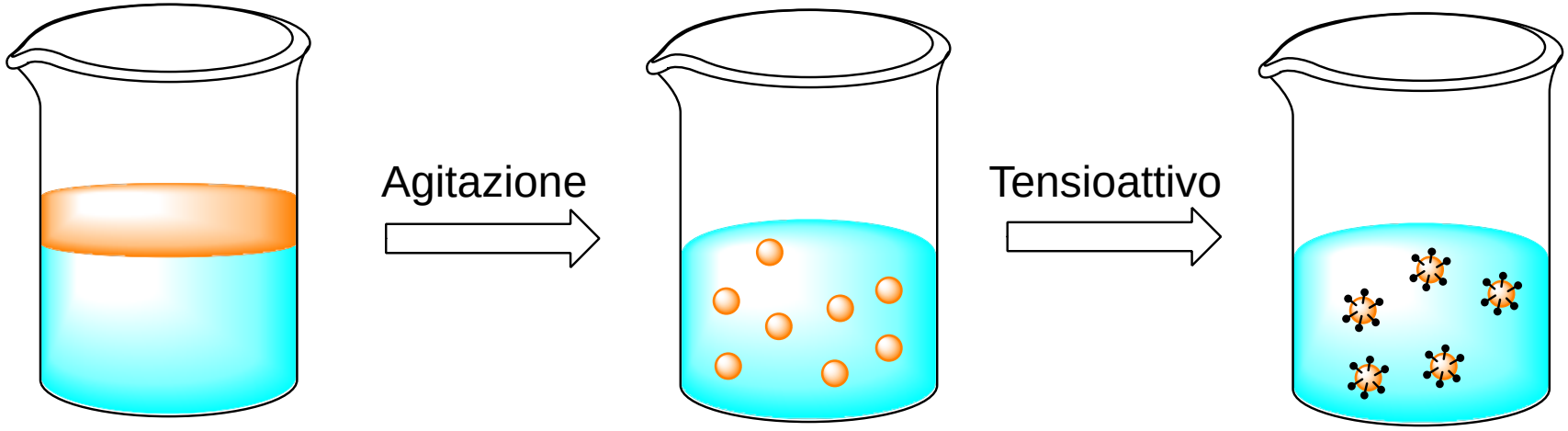
maionese



Microemulsioni: stabilizzare la superficie



Microemulsioni: stabilizzare la superficie



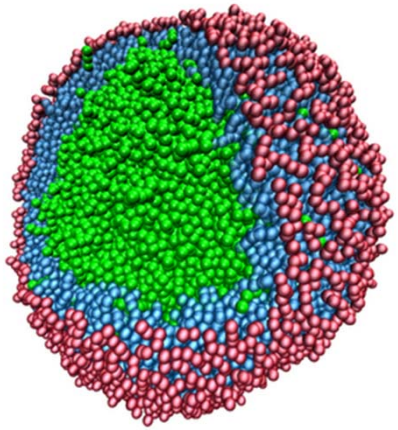
Un intervento meccanico (agitazione, sonicazione) può separare la fase immiscibile in goccioline anche piccolissime (fino a 50 nm).

La superficie totale della fase idrofobica diventa molto elevata, inoltre la zona superficiale è instabile a causa della repulsione acqua-olio (= tensione superficiale).

Ricombinandosi, le gocce diminuiscono l'area totale della fase immiscibile.

Il tensioattivo stabilizza l'emulsione impedendo la ricombinazione delle gocce: effetto **sterico** ed **elettrostatico**. Solitamente non è richiesta energia per formare la microemulsione, mentre è necessaria per formare un'emulsione.

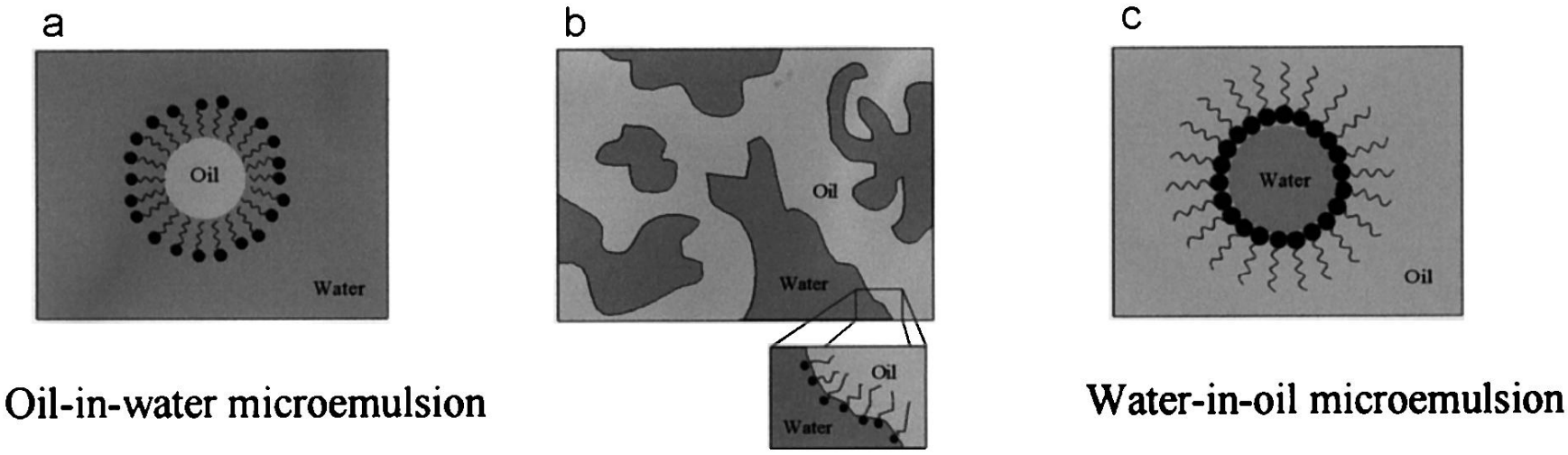
Microemulsioni: stabilizzare la superficie



Microemulsions are thus defined as ‘a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution.’

Danielsson and Lindman, 1981

“the system **contains** some and definite microstructure, in other words there is a definite boundary between the oil and water phases at which the surfactant is located”.

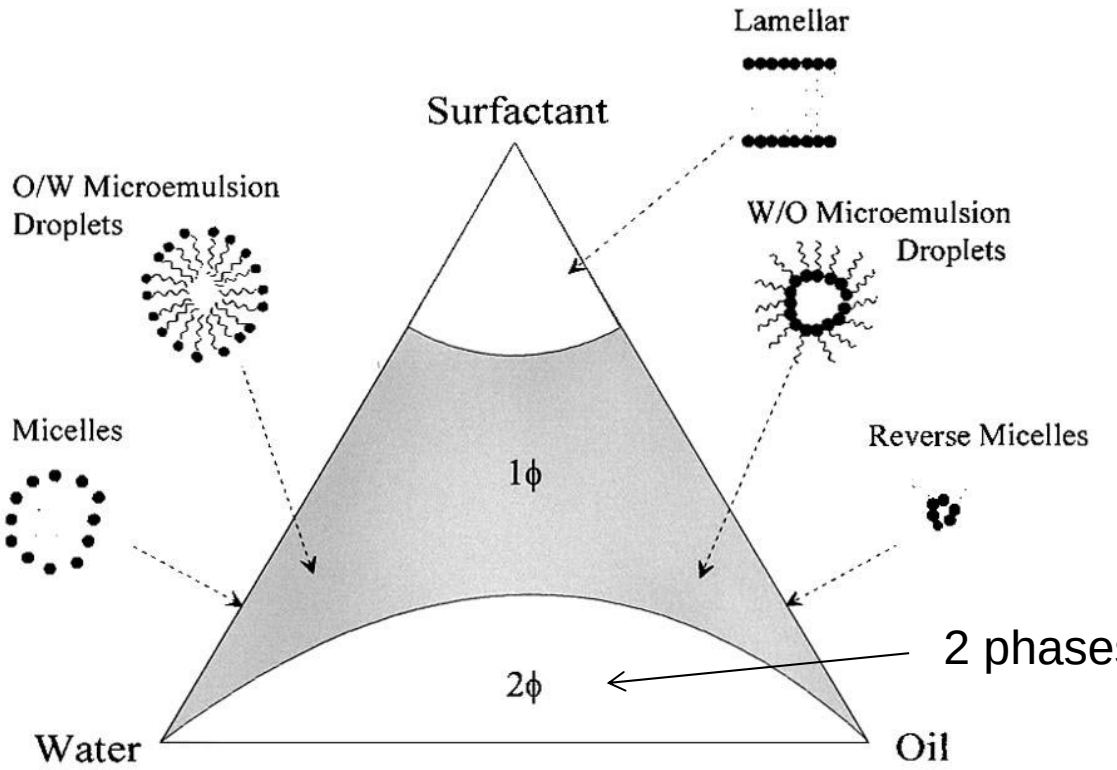


Oil-in-water microemulsion

Water-in-oil microemulsion

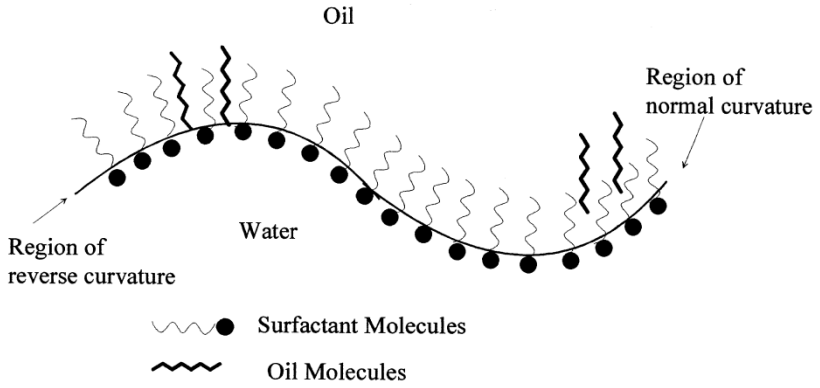
Bicontinuous microemulsion

Microemulsioni: stabilizzare la superficie

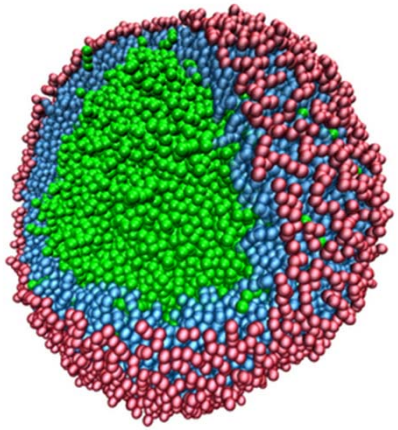


Depending on the components used, microemulsion existance may be confined to a very narrow composition range.

Water exposure of the oil phase is greater in regions of reverse curvature.

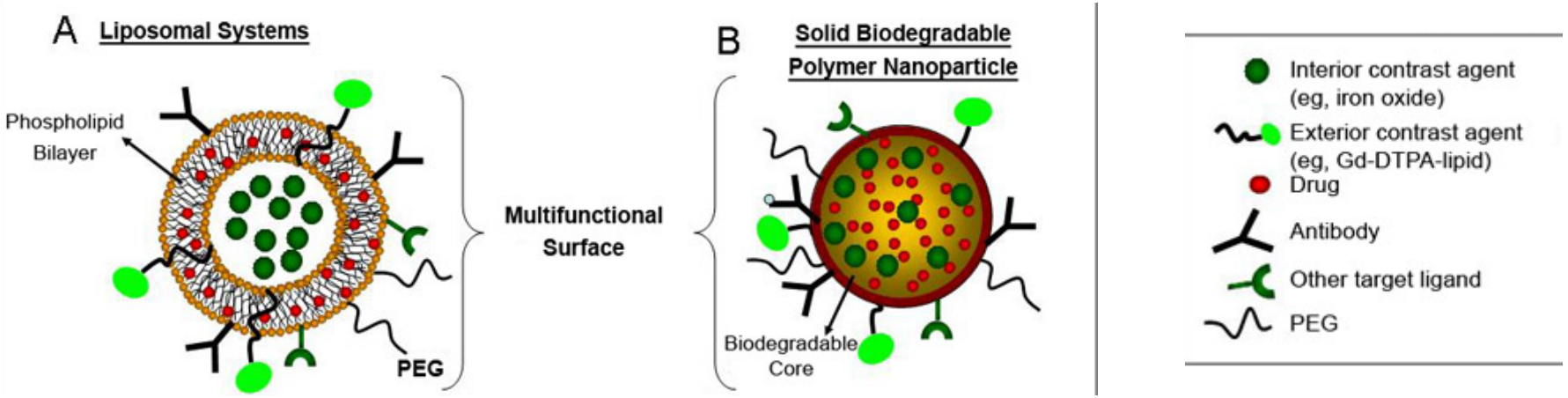


Microemulsioni: stabilizzare la superficie



Il tensioattivo può agire anche da funzionalizzante, impartendo capacità stealth, di targeting attivo, ecc.

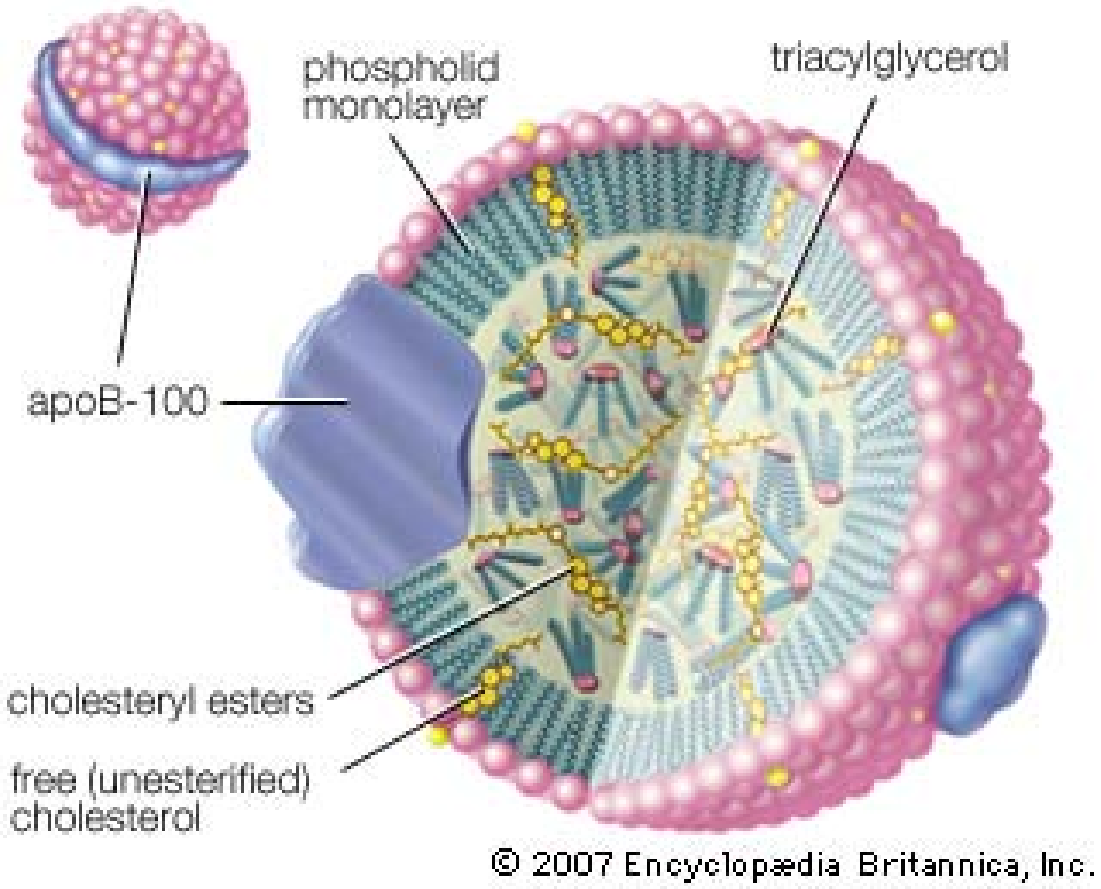
Molecole di farmaco possono essere caricate nella fase idrofobica.



Vantaggi

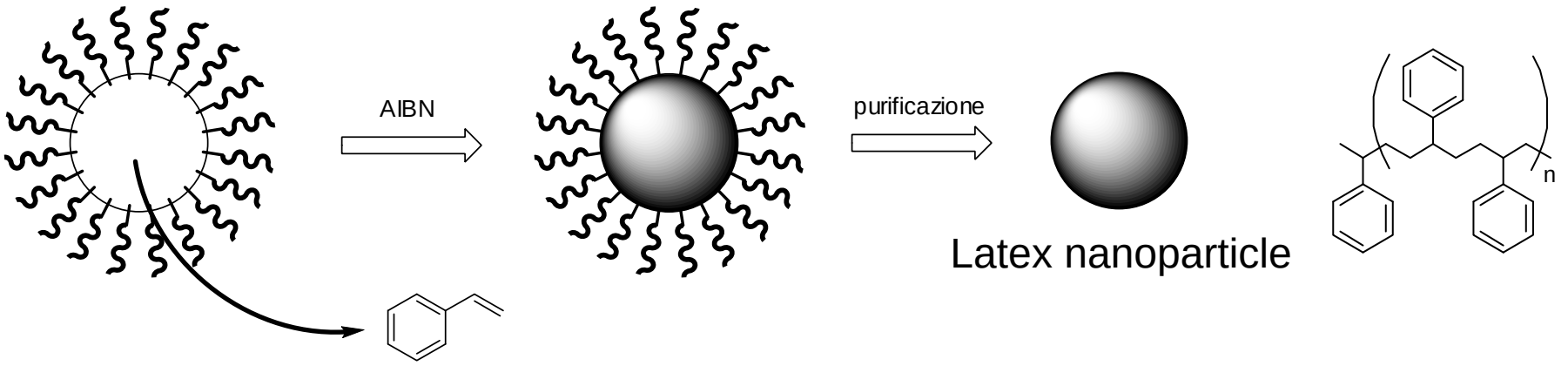
- Nanoparticelle più grandi delle micelle
- Maggior capacità di carico di molecole idrofobiche rispetto a micelle e liposomi

Microemulsioni in natura: lipoproteine



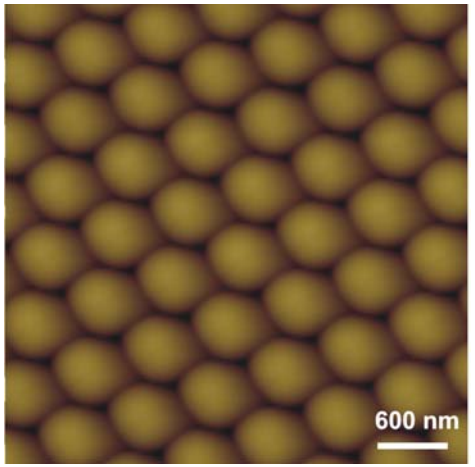
Microemulsioni: nanoreattori

All’interno di microemulsioni (anche a fase inversa) possono essere sintetizzati altri tipi di nanosistemi



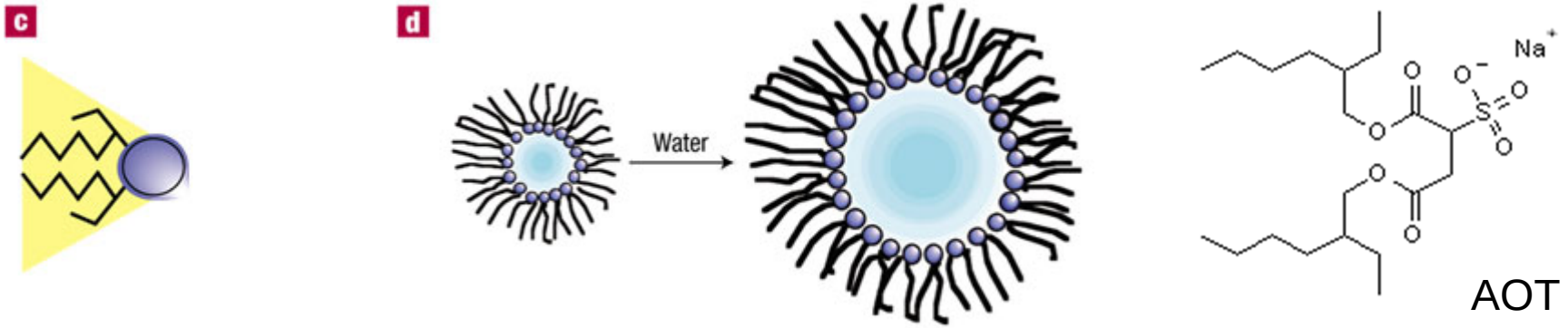
Latex nanoparticle

Se le molecole che compongono/sono contenute nella fase idrofobica vengono indotte a polimerizzare si possono ottenere nanoparticelle polimeriche.



Microemulsioni: nanoreattori

Emulsioni a fase inversa (water in oil, w/o), sono piccole goccioline d'acqua in un solvente organico stabilizzate da un tensioattivo.

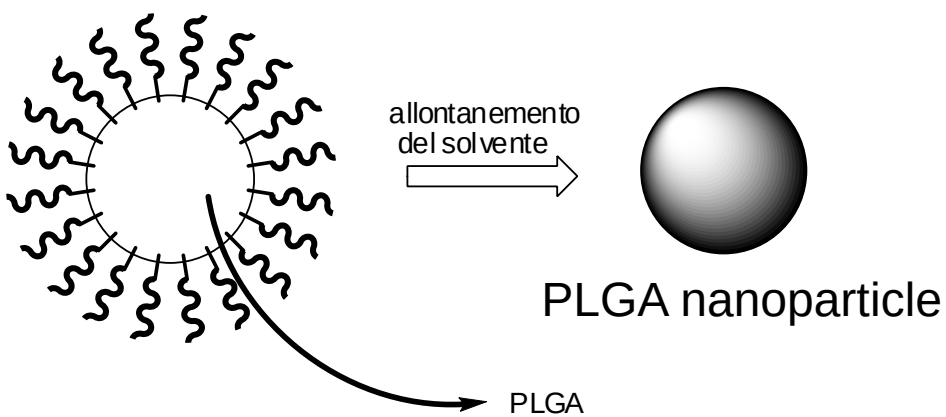


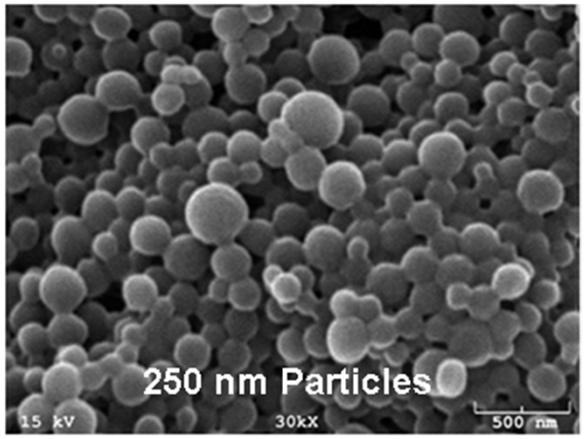
Le dimensioni dipendono essenzialmente dalla quantità di acqua e di tensioattivo aggiunta.

Non sono utili in campo biomedico, ma possono essere un utile nanoambiente in cui crescere diversi tipologie di nananosistemi.

Microemulsioni: nanoreattori

All’interno di microemulsioni (anche a fase inversa) possono essere sintetizzati altri tipi di nanosistemi





Se la fase idrofobica è costituita da una **soluzione**, in genere di un *polimero* o un *farmaco*, in un solvente organico, l’allontanamento del solvente lascia come residuo nanoparticelle organiche.

Microemulsioni: nanoreattori

L'allontanamento del solvente organico può avvenire in diversi modi:

- evaporazione
- diffusione in acqua

Evaporazione

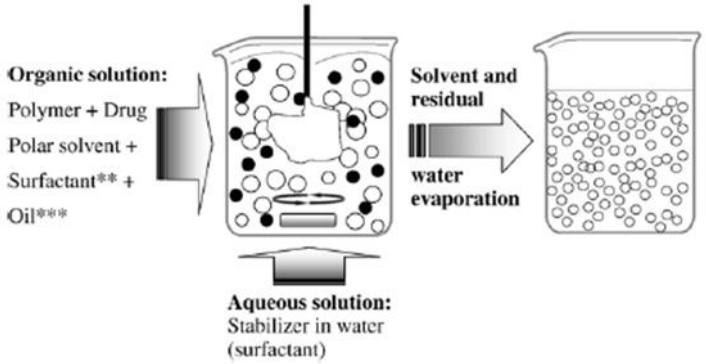


Fig 2. Schematic representation of the solvent displacement technique. **Surfactant is optional. ***In interfacial deposition method, a fifth compound was introduced only on preparation of nanocapsules.

Diffusione

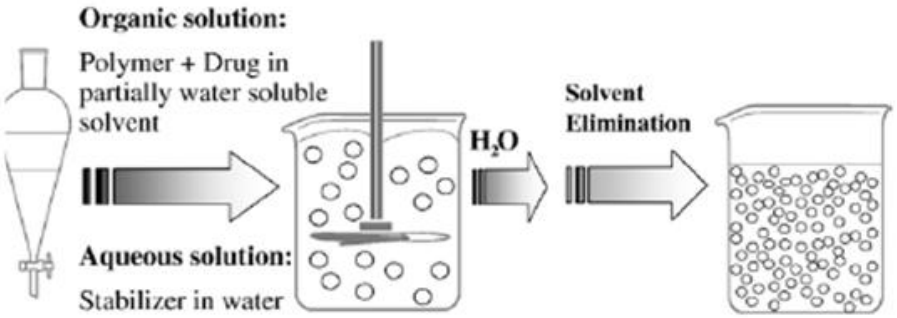


Fig 3. Schematic illustration of the ESD technique.

Diffusione

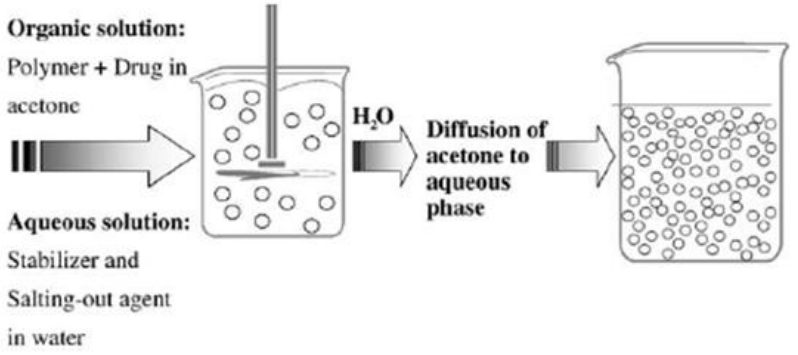
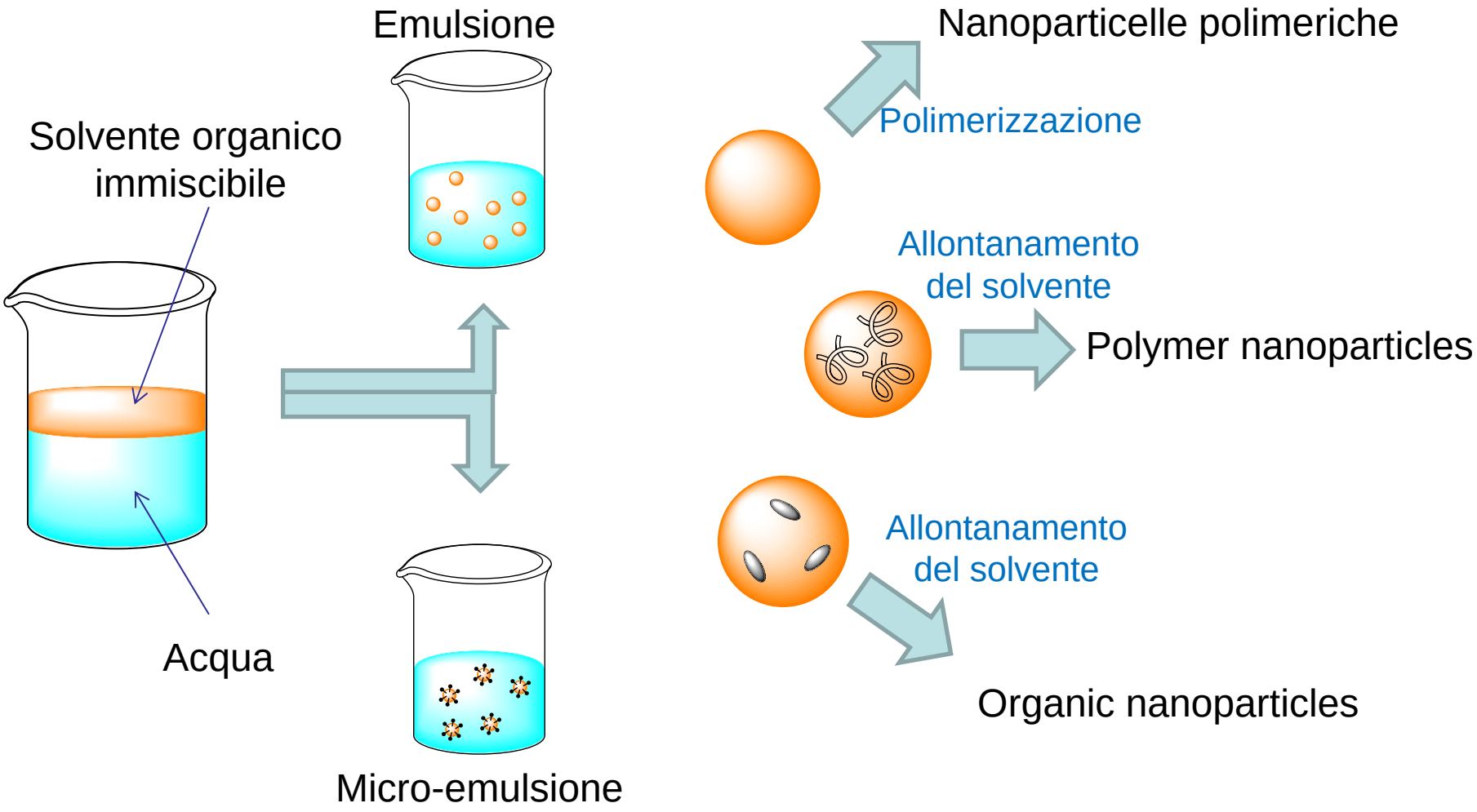


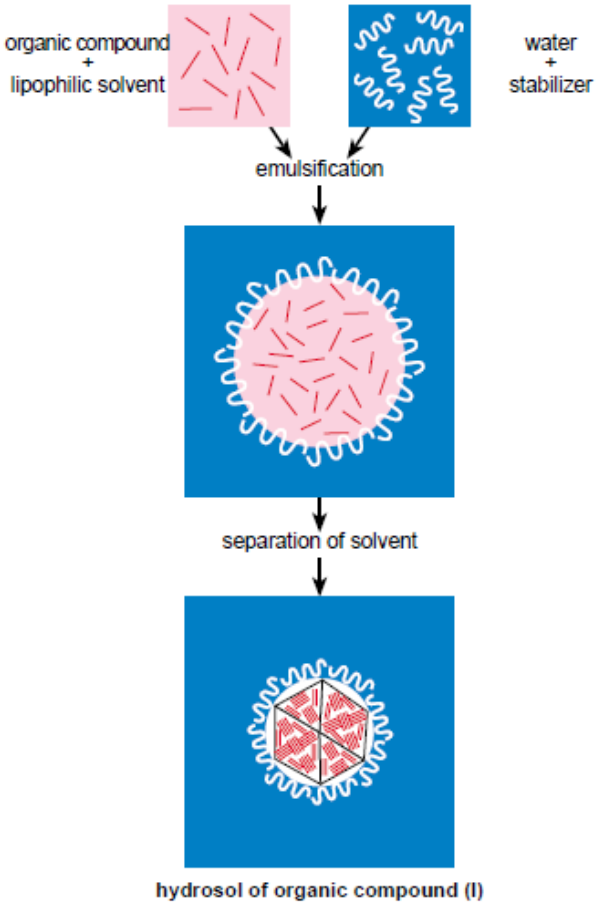
Fig 4. Schematic of the salting-out technique.

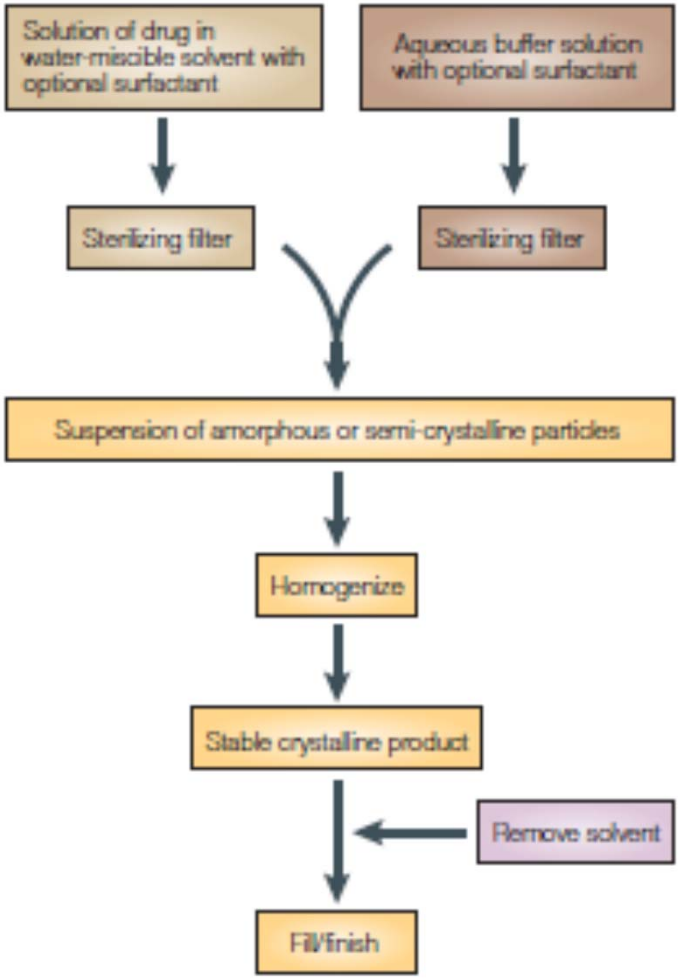
Nanoparticelle organiche da emulsioni



Nanoparticelle di molecole organiche

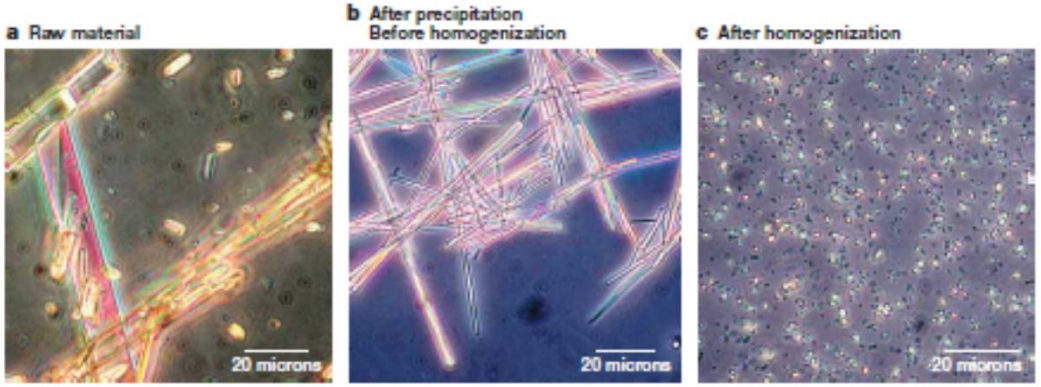
Usando le tecniche di microemulsione e omogeneizzazione, le nanoparticelle possono essere preparate anche con piccole molecole organiche, ad esempio farmaci

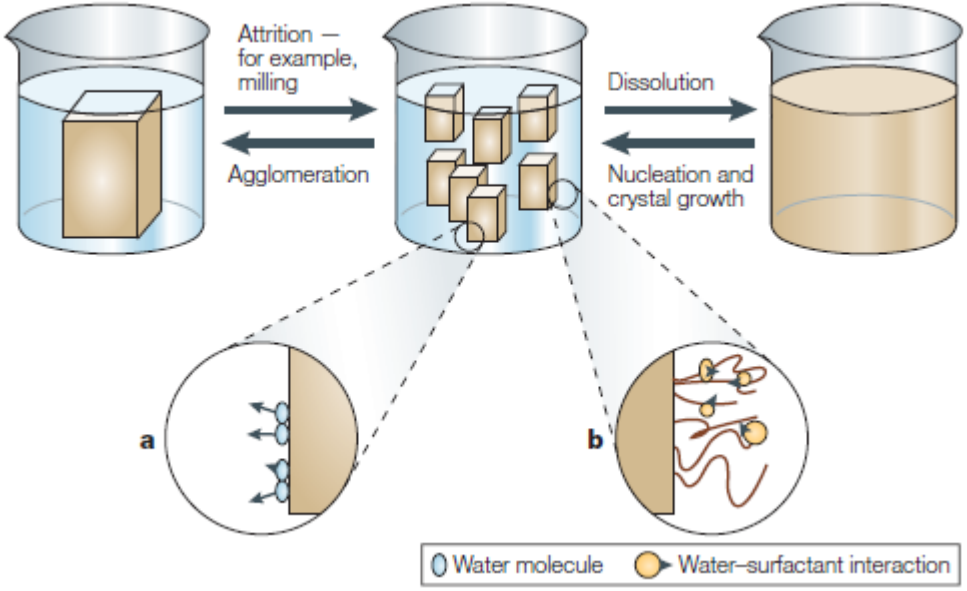


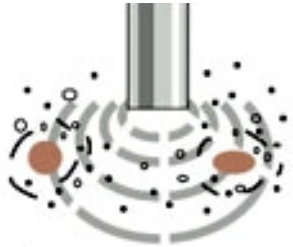


Nature Reviews, 2004, 785

Nanoparticelle di molecole organiche

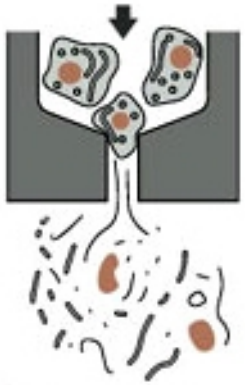




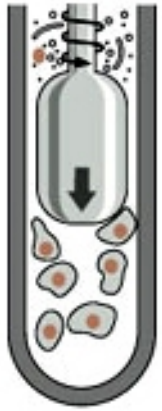


1 break cells with high frequency sound

2 use a mild detergent to make holes in the plasma membrane



3 force cells through a small hole using high pressure



4 shear cells between a close-fitting rotating plunger and the thick walls of a glass vessel

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Nanoparticelle di molecole organiche

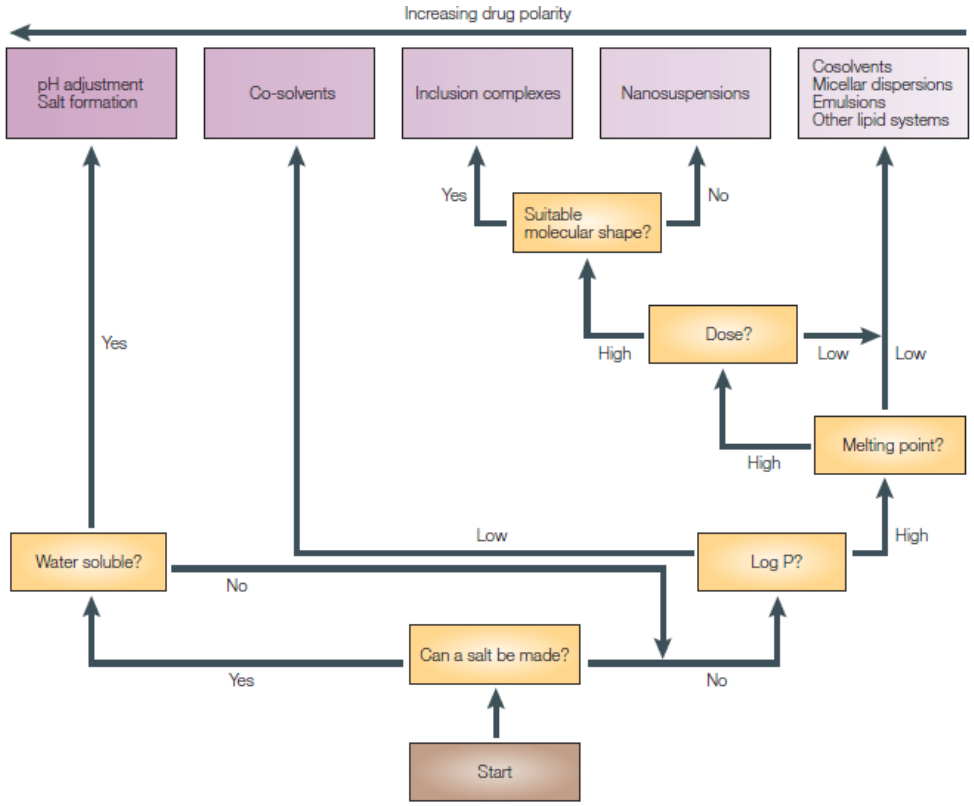

Table 1 | **Benefits of nanosuspensions**

Physicochemical characteristic	Potential benefits
Increased drug amount in dosage form without harsh vehicles (extreme pH, co-solvents)	Intravenous: reduced toxicity, increased efficacy
Reduced particle size: increased drug dissolution rate	Oral: increased rate and extent of absorption, increased bioavailability of drug: area under plasma versus time curve, onset time, peak drug level, reduced variability, reduced fed/fasted effects. Pulmonary: increased delivery to deep lung
Solid state: increased drug loading	Reduced administration volumes; essential for intramuscular, subcutaneous, ophthalmic use
Solid state: increased stability	Increased resistance to hydrolysis and oxidation, increased physical stability to settling
Particulate dosage form	Intravenous: potential for intravenous sustained release via monocyte phagocytic system targeting, reduced toxicity, increased efficacy. Oral: potential for reduced first-pass hepatic metabolism

Table 2 | **Solid-particulate-nanosuspension-based formulations in development and in the market**

Drug	Indication	Drug delivery company	Pharma company	Route	Status
Paclitaxel	Anticancer	American BioScience	American Pharmaceutical Partners	Intravenous	Phase III
Undisclosed multiple	Anti-infective	Baxter NANOEDGE	Undisclosed	Oral/ intravenous	Preclinical to Phase II
Undisclosed	Anticancer	Baxter NANOEDGE	Undisclosed	Intravenous/ oral	Preclinical to Phase I
Rapamune	Immuno-suppressant	Elan Nanosystems	Wyeth	Oral	Marketed
Emend	Anti-emetic	Elan Nanosystems	Merck	Oral	Marketed
Cytokine inhibitor	Crohn's disease	Elan Nanosystems	Cytokine PharmaSciences	Oral	Phase II
Diagnostic Agent	Imaging agent	Elan Nanosystems	Photogen	Intravenous	Phase I/II
Thymectacin	Anticancer	Elan Nanosystems	NewBiotics./Ilex Oncology	Intravenous	Phase I/II
Fenofibrate	Lipid lowering	SkyePharma	Undisclosed	Oral	Phase I
Busulfan	Anticancer	SkyePharma	Supergen	Intrathecal	Phase I
Budesonide	Asthma	Elan Nanosystems	Sheffield Pharmaceuticals	Pulmonary	Phase I
Silver	Eczema, atopic dermatitis	NUCRYST	Self-developed	Topical	Phase I
Calcium phosphate	Mucosal vaccine adjuvant for herpes	BioSante	Self-developed	Oral	Phase I
Insulin	Diabetes	BioSante	Self-developed	Oral	Phase I

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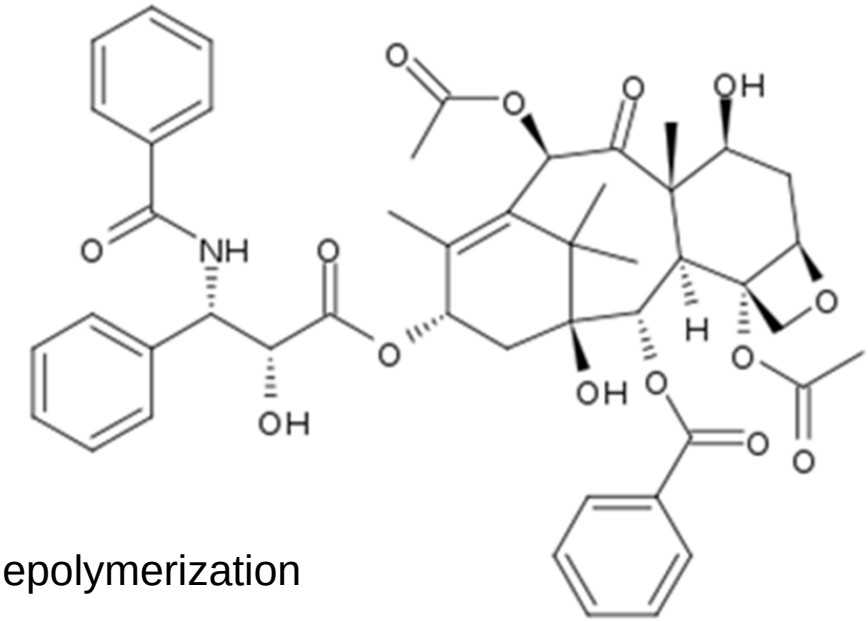
Paclytaxel

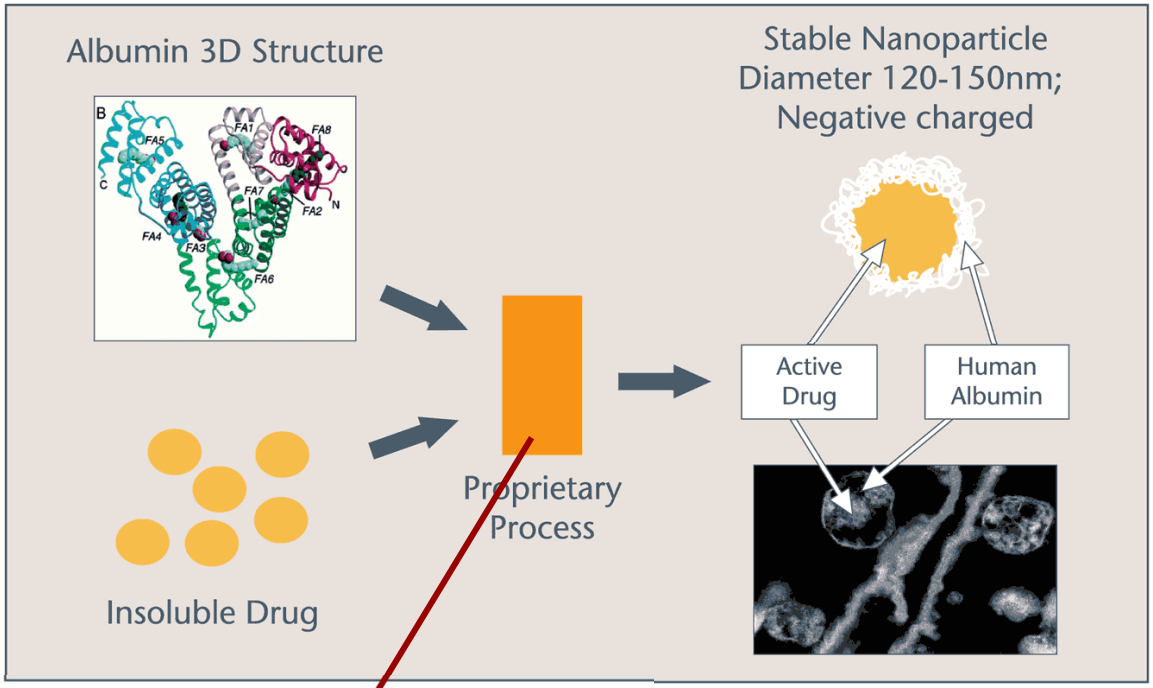


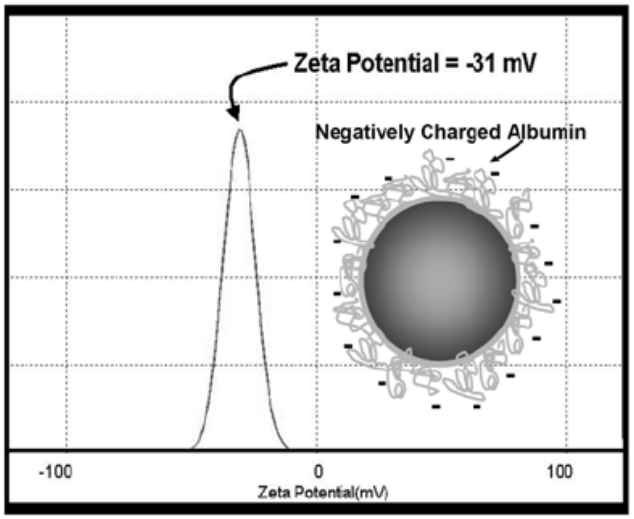
- Discovered in the ‘70
- It act as mitosis inhibitor due to microtubles depolymerization

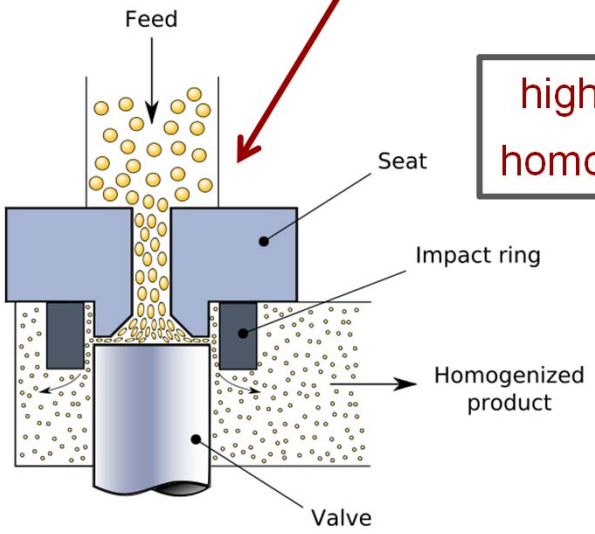
- Cornerstone drug for many tumours and leukemia
- Insoluble in water, delivered dissolved in castor oil derivative, Cremophor EL, and ethanol
- High toxicity arising from the solvent

- Docetaxel is a similar derivative administered in Tween-80 micelles.

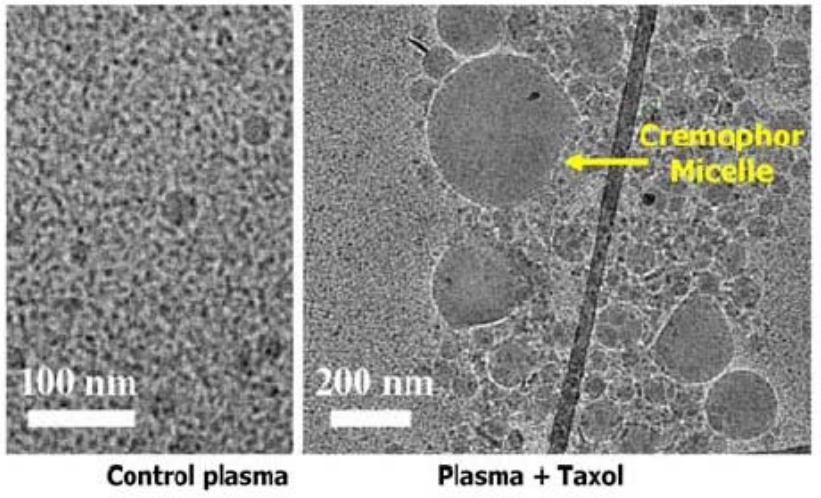
Abraxane



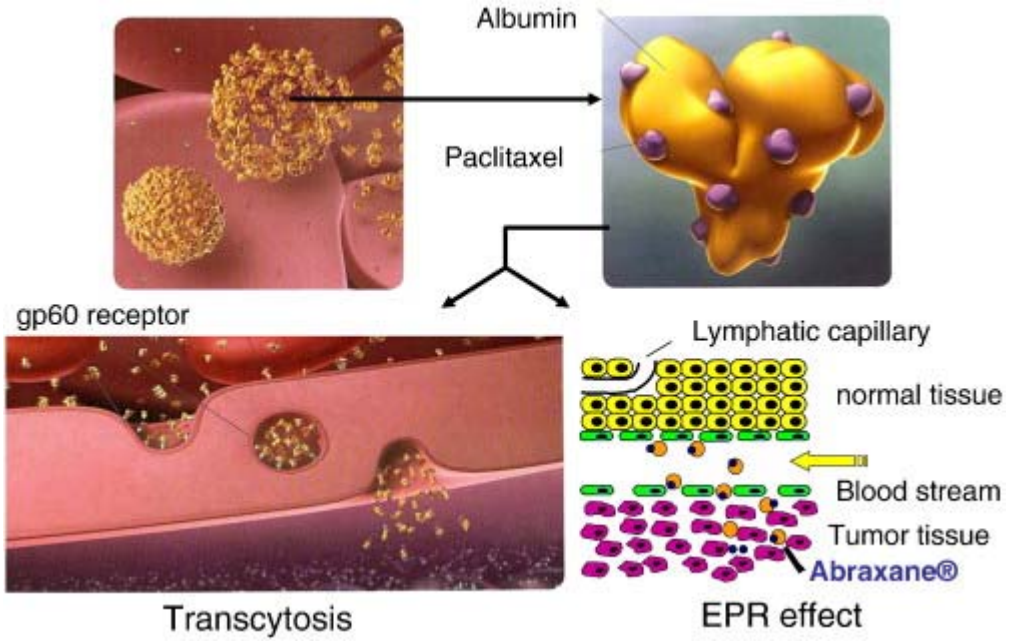


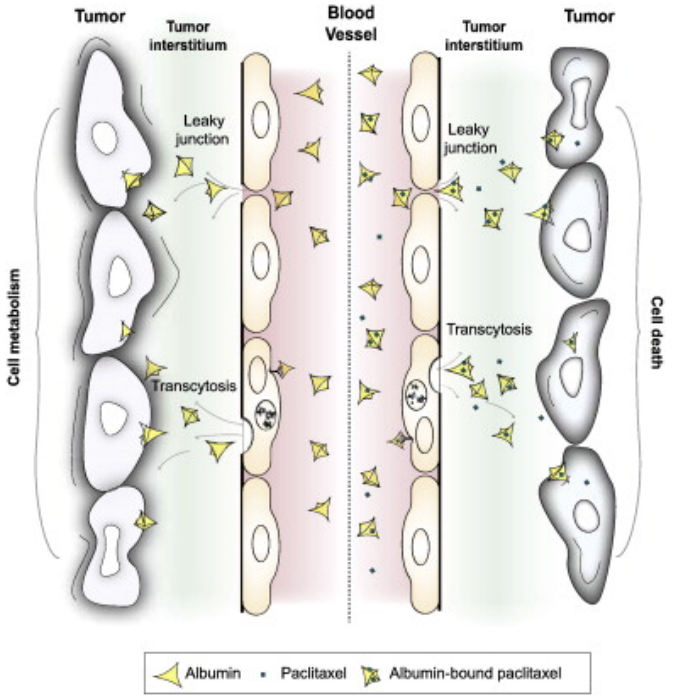


high-pressure homogenization



Abraxane





When compared to standard paclitaxel, Abraxane demonstrated significantly higher tumor response rates (33% vs 19%) and longer times to tumor progression (23.0 vs 16.9 weeks) among metastatic breast cancer patients who have failed combination therapy

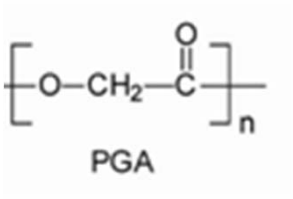
Nabs rapidly disassociate into individual albumin molecules and then circulate with paclitaxel (Ptxl), thus minimally altering the circulation half-life and biodistribution profiles of Ptxl, at difference with Cremophore which preventes Ptxl albumin binding.

the Nab technology significantly improved the MTD of Ptxl from 175 to 260 mg/m^2 every 3 weeks by enabling the exclusion of the toxic formulation excipient, Cremophor.

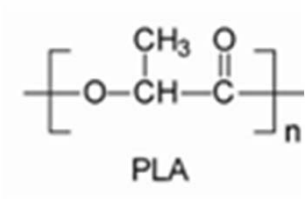
Target the cd60 receptor on epithelial cells

Nab tecnology is ineffective with Docetaxel

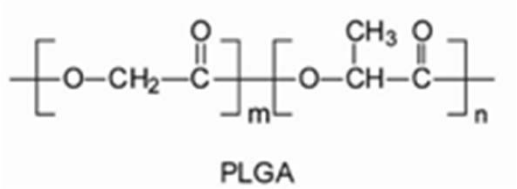
Nanoparticelle di PLGA



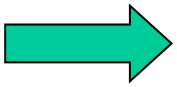
acido poliglicolico
idrofilico

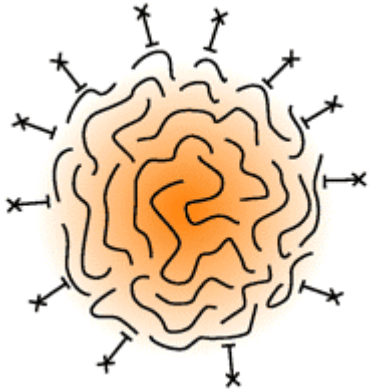


acido polilattico
idrofobico

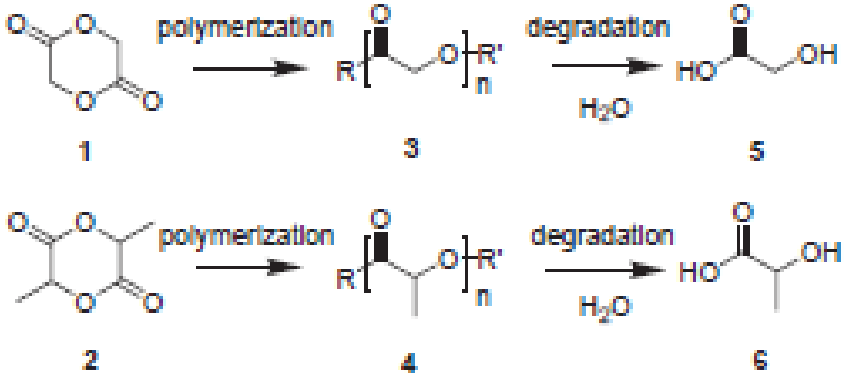


copolimero polilattico-co-glicolico





PGA e PLA sono due **polimeri biodegradabili**: in presenza di acqua i gruppi esterei vengono idrolizzati ed il contenuto della particelle rilasciato.



Nanoparticelle di PLGA

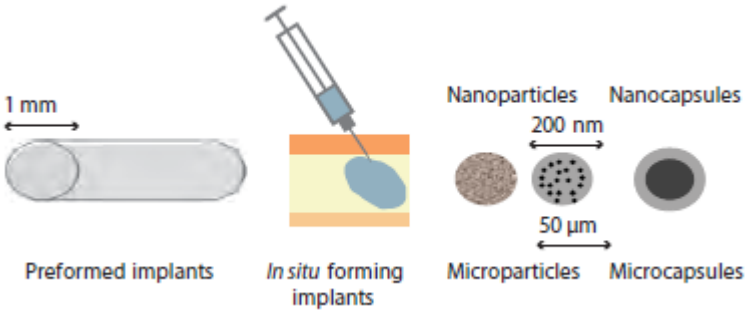


Table 1. Key parameters and corresponding effects on RESOMER® properties.

Parameter	Influence
Molecular Weight	High M_w increases the degradation time
Ratio Lactide/Glycolide	Polymers with one monomer degrade more slowly. Degradation times: PLA > PGA > PLGA 50:50
Stereochemistry	L-PLA: semicrystalline D,L-PLA: amorphous
Blockage of Acidic Endgroups	Polymers with free -COOH groups are more hydrophilic (e.g., R503H compared to R503)
PEGylation	Increase in hydrophilicity, change of degradation and release behavior

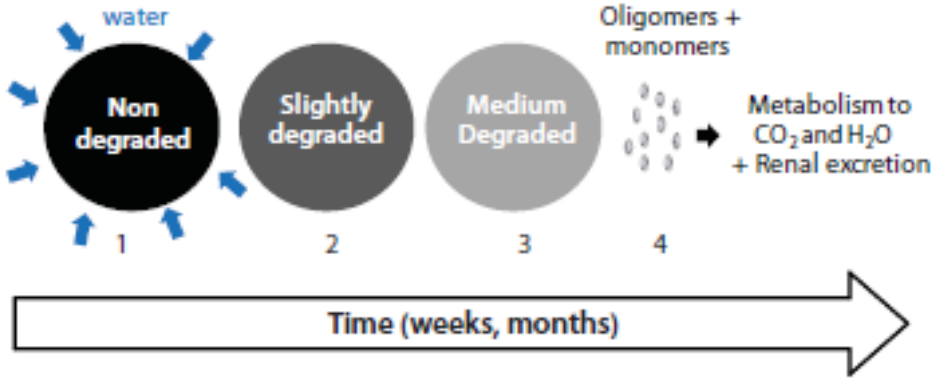


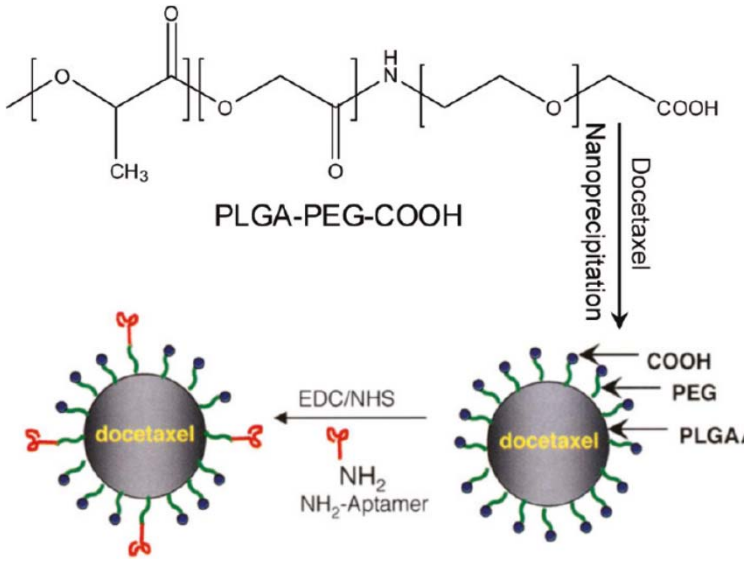
Figure 2. The biofate of RESOMER® polymers includes several steps: (1) Wetting and water diffusion, (2) Decrease of the molecular weight = Polymer degradation (3) Mass loss = Polymer erosion and (4) Renal excretion or metabolism to carbon dioxide and water.

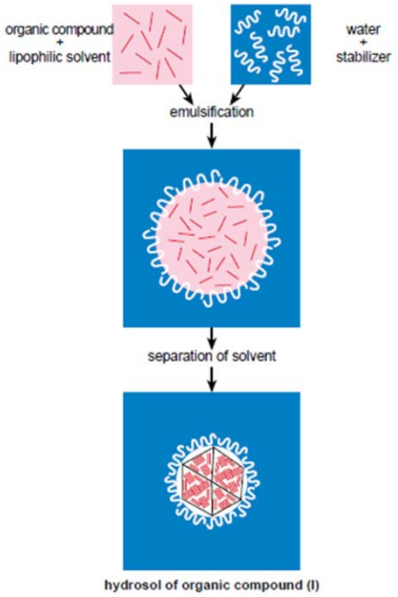
La velocità di degradazione viene controllata variando il **rapporto PLA/PGA**: maggiore il numero di unità idrofiliche (PGA), più veloce l'idrolisi.

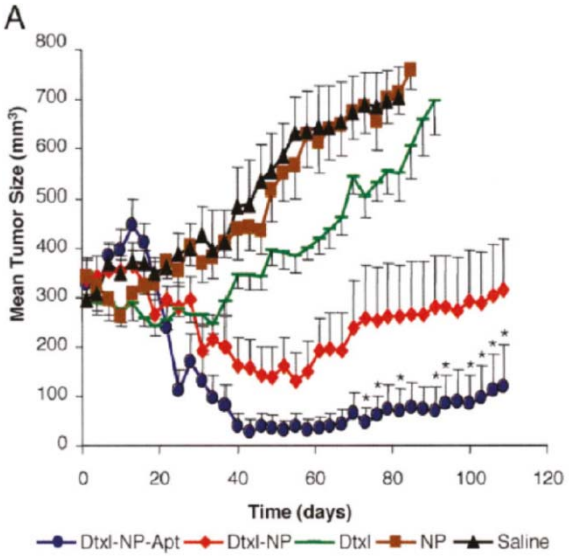
Enzimi esterasi presenti nei tessuti biologici accelerano la degradazione.

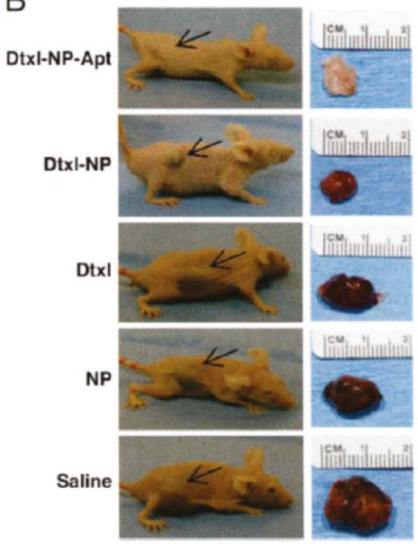
Polymeric nanoparticles

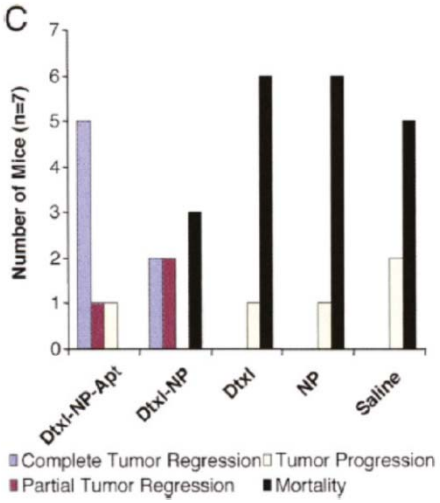
A10 Apt, which binds to the extracellular domain of prostate specific membrane antigen (PSMA)

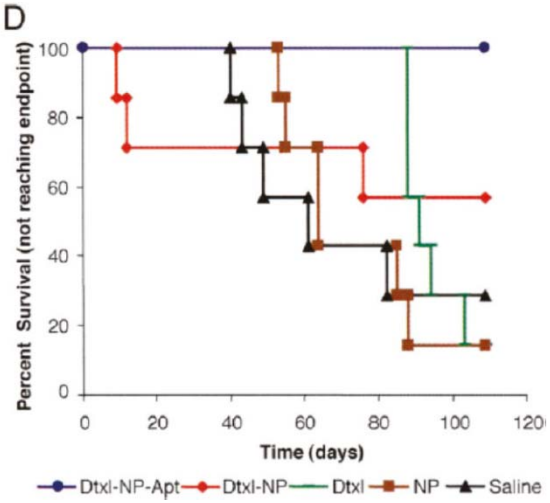






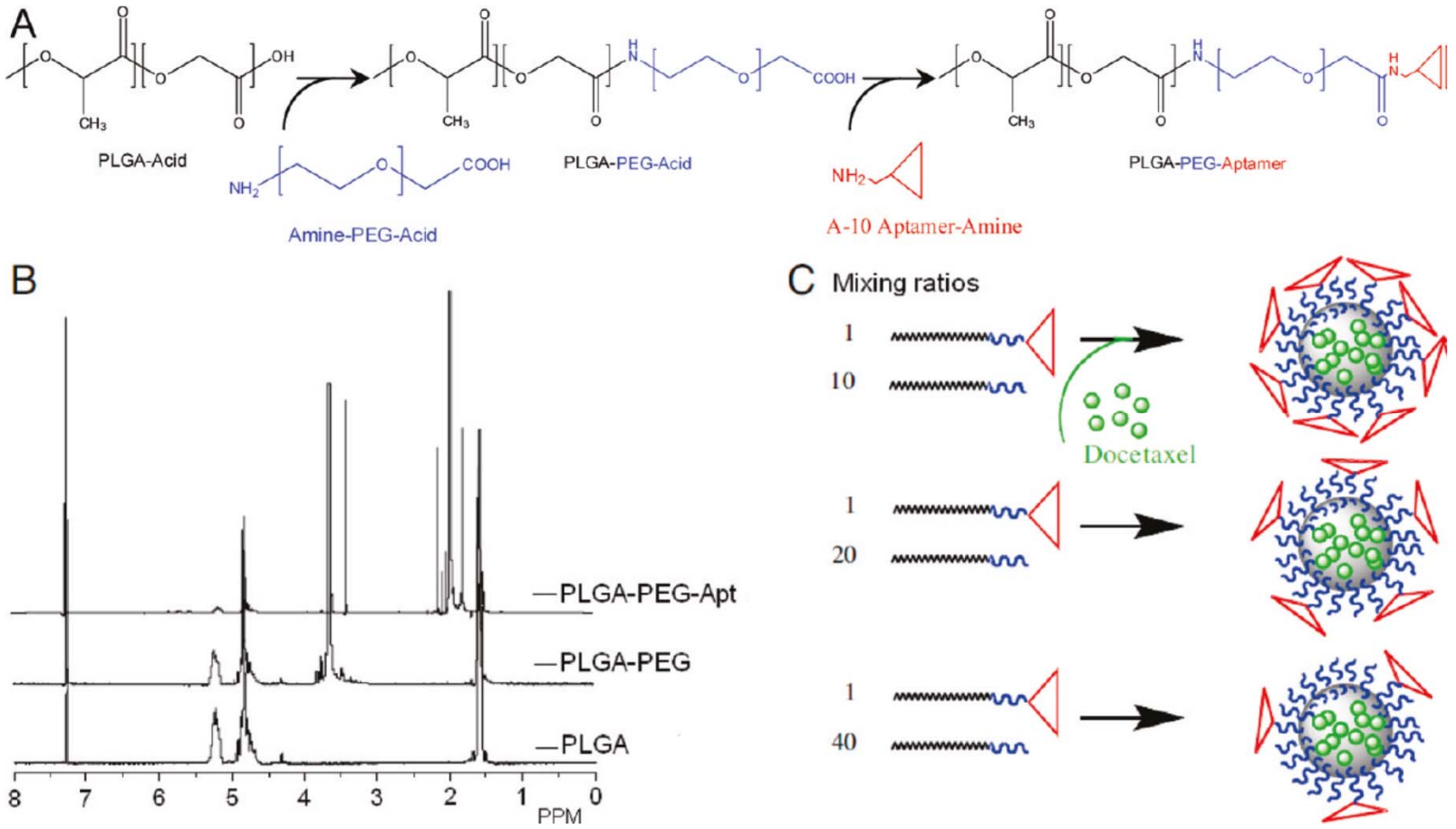






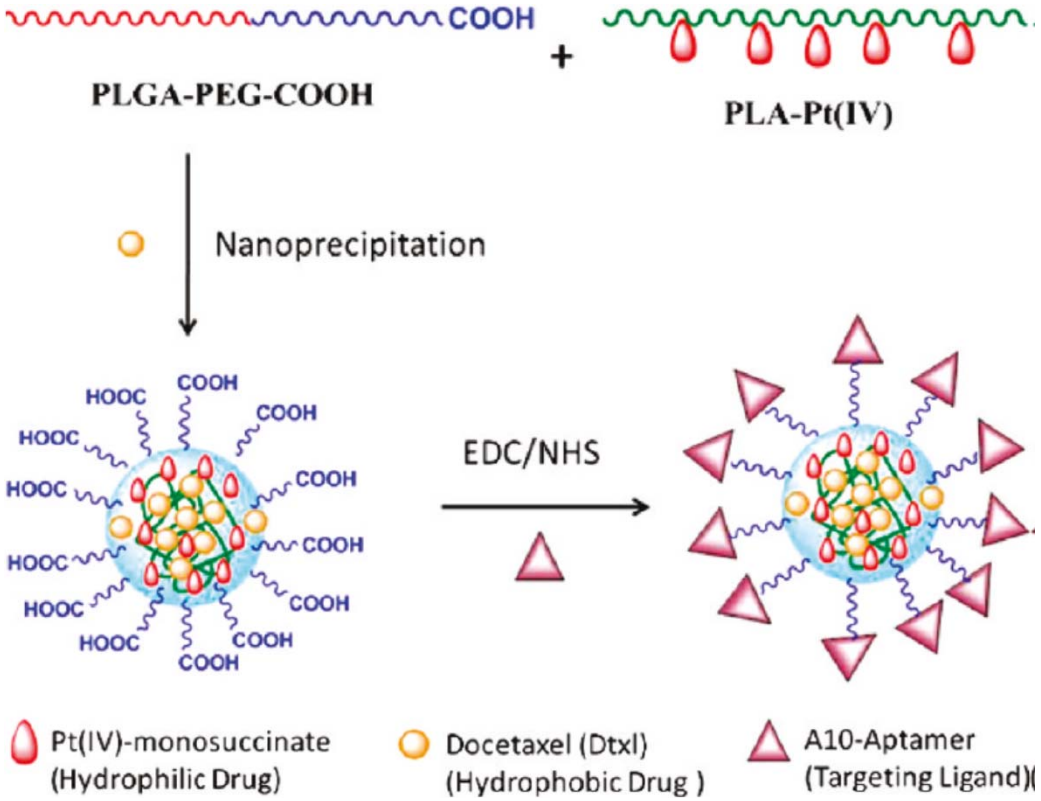
Polymeric nanoparticles

Pre-functionalization



Polymeric nanoparticles

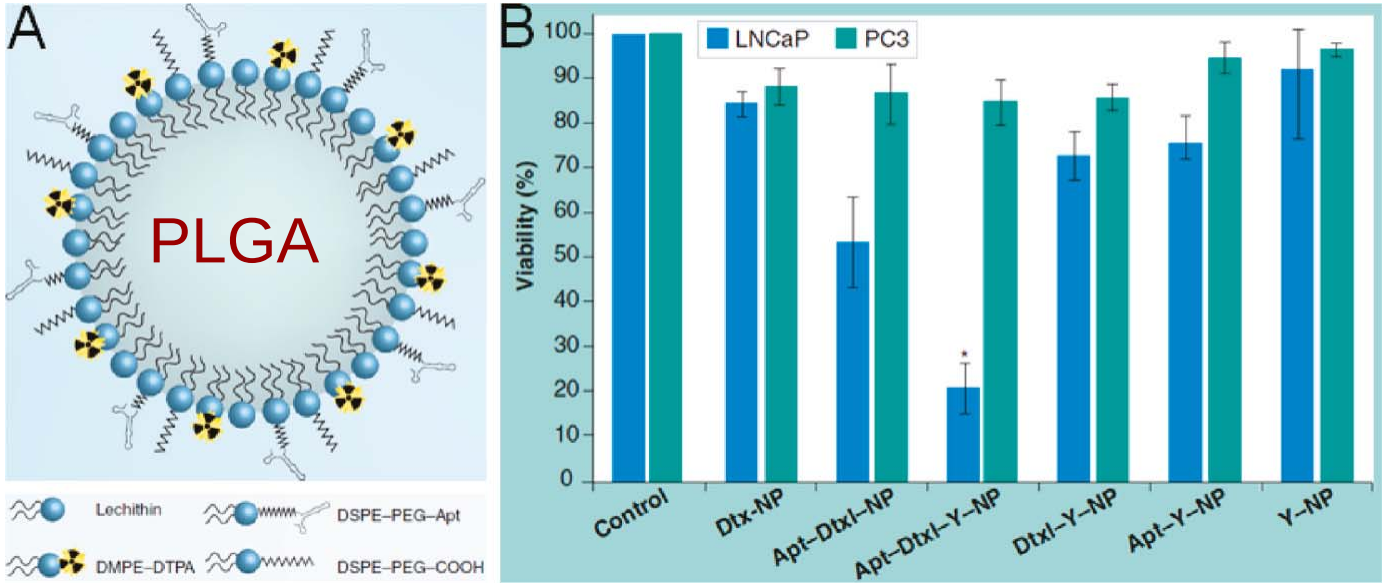
Multi-drug delivery



(1) delivery of a correct drug ratio to the target-of-interest for synergistic therapeutic effects
(2) suppression of drug resistance
(3) control of each drug exposure in a temporal manner.
In vitro studies demonstrate that the Apt-targeted, dual-drug encapsulated NPs are ~10 and 5.5 times more cytotoxic than PLA-Pt-NP-Apt and Dtxl-NP-Apt respectively

Polymeric nanoparticles

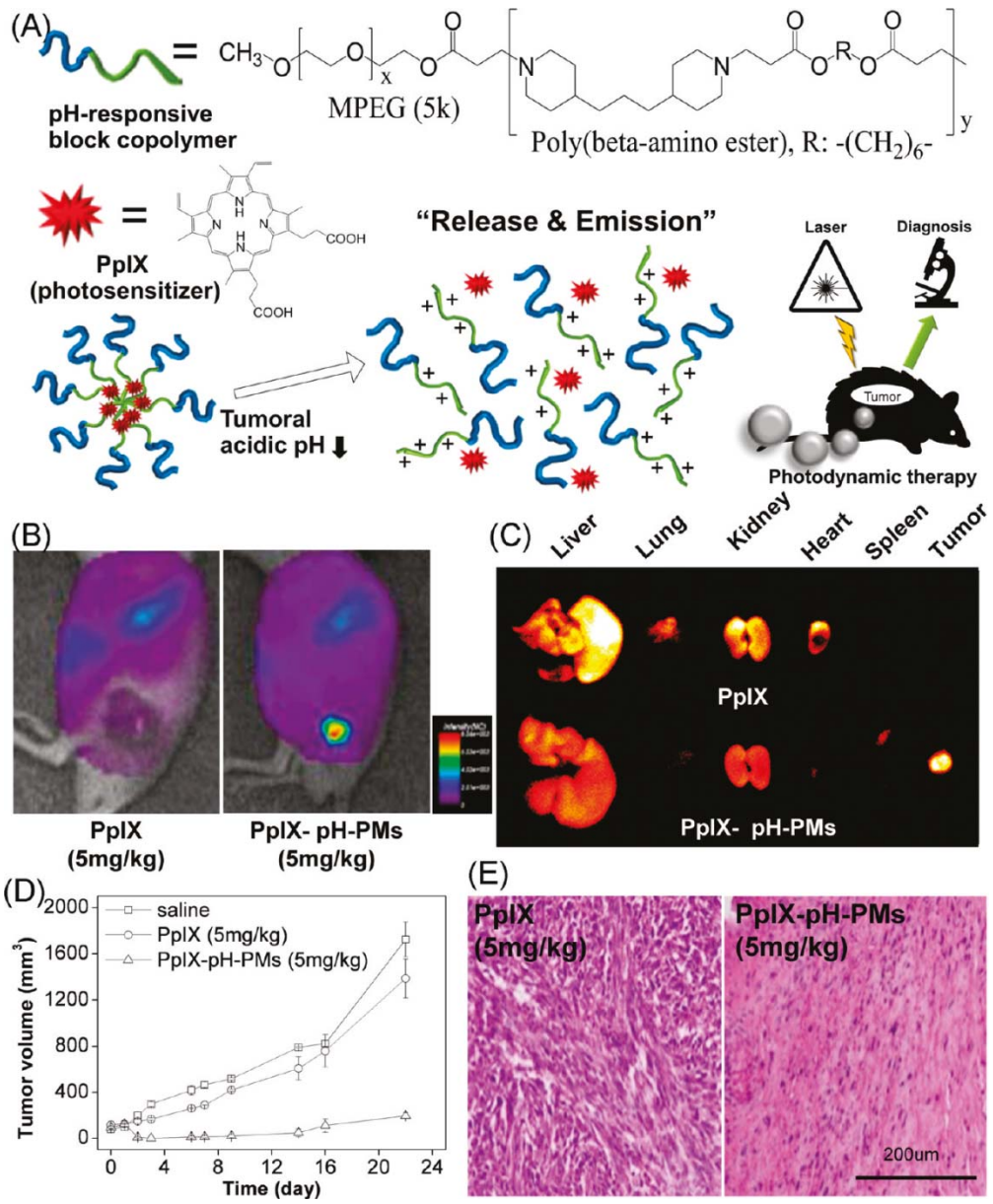
Multi-drug delivery



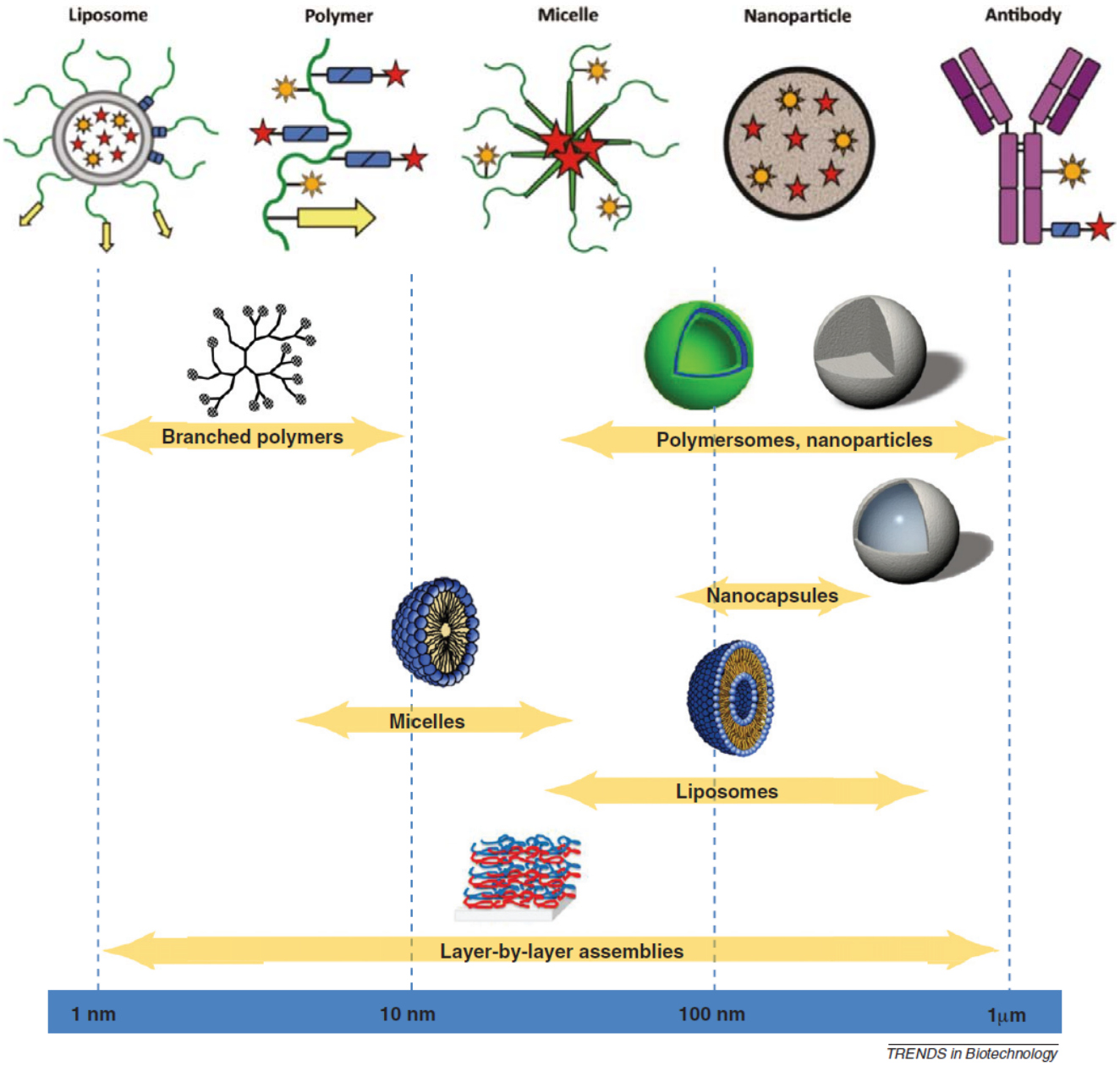
higher drug loading and slower drug release, which are mainly attributed to the existence of a lecithin monolayer at the interface of the PLGA core and PEG shell

Polymeric nanoparticles

pH responsive



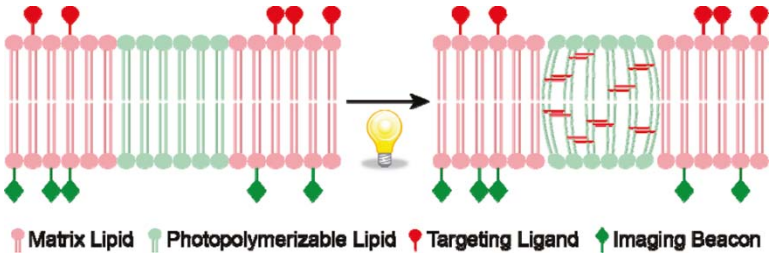
«Soft» nanoparticles

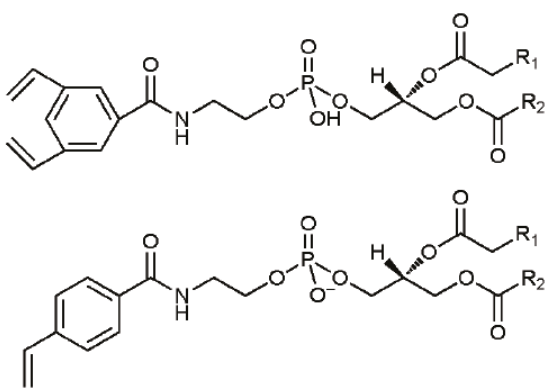


«Soft» nanoparticles

Nanocarrier	Preparation methods	Advantages	Drawbacks
Liposomes	Film casting and rehydration of this film + extrusion	Can encapsulate both hydrophobic and hydrophilic drugs Lower toxicity Functionalization of the surface is possible	Fair stability Poor batch-to-batch reproducibility Difficulties in sterilization Low drug loading
Polymersomes	Solvent switch method Film casting and rehydration of the film	Can encapsulate both hydrophobic and hydrophilic drugs Lower toxicity Possibly stimuli-responsive May help in MDR	
Polymeric micelles	Direct organization or controlled aggregation in a solvent	Prolonged blood circulation time Adequate size for EPR Efficient for hydrophobic drugs Lower toxicity Possibly stimuli-responsive May help for MDR Simple preparation Functionalization of the surface is possible	Not good for hydrophilic drugs Obtained by self-assembly, mostly spherical shape
Layer-by-Layer systems	LbL	Can be applied to a huge variety of surfaces (tissue engineering)	Ionic nature of the vector
Solid lipid NPs	Freezing of an emulsion of lipids heated above melting point of lipids	Most established Biocompatible, biodegradable Flexibility of size and surface manipulation Higher efficacy, lower toxicity vs non-liposomal formulation Possibly stimuli-responsive	Poor stability Poor batch to batch reproducibility Sterilization difficulties Low drug loading Release of drug not always well controlled Presence of possibly toxic co-solvents
Polymer NPs/capsules	NPs: polymerization of monomers by emulsion process H/E or starting from existing polymers, nanoprecipitation, gelification or emulsion process Nanocapsules: Interfacial polymerization of monomers or phase inversion process with emulsions of polymers	Simplicity May help for MDR Shape, size, and mechanical properties can be tuned Controlled release is possible	Colloidal stability not always good Possible residual chemicals from process
Dendrimers	Convergent or divergent synthesis	Carrier solidity Highly functionalized surface Prolonged pharmacodynamic profile	Tedious preparation Some are toxic

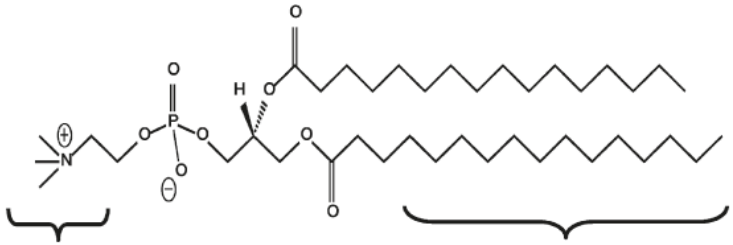
Evolutions: polymerizable liposomes





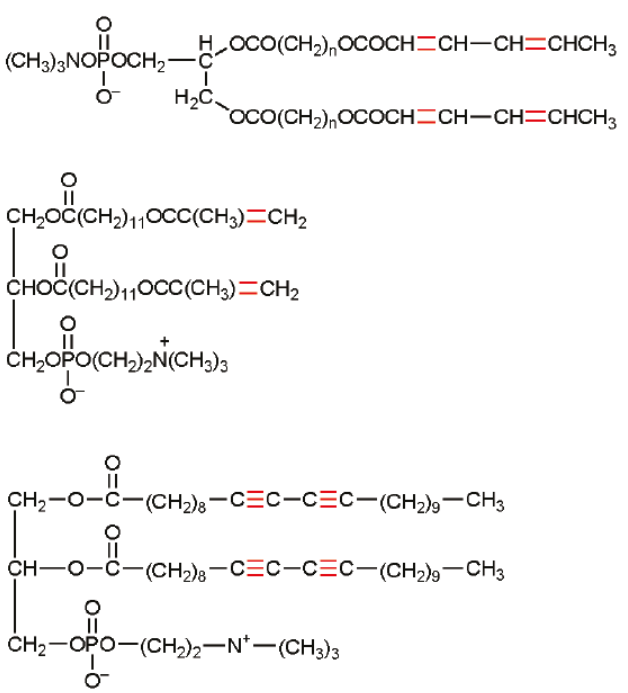
Free Radical Initiated Polymerization

Sites for Chemical Modifications in Phospholipids (photoreactive lipids)



Head group Polymerization *(refs 33-37,70)*

Photo-polymerization *(Refs 12, 14, 17, 18, 30, 32, 39, 40,42,44,46)*



Fatty Acyl Chain Modified Polymerization

Evolutions: polymerizable liposomes

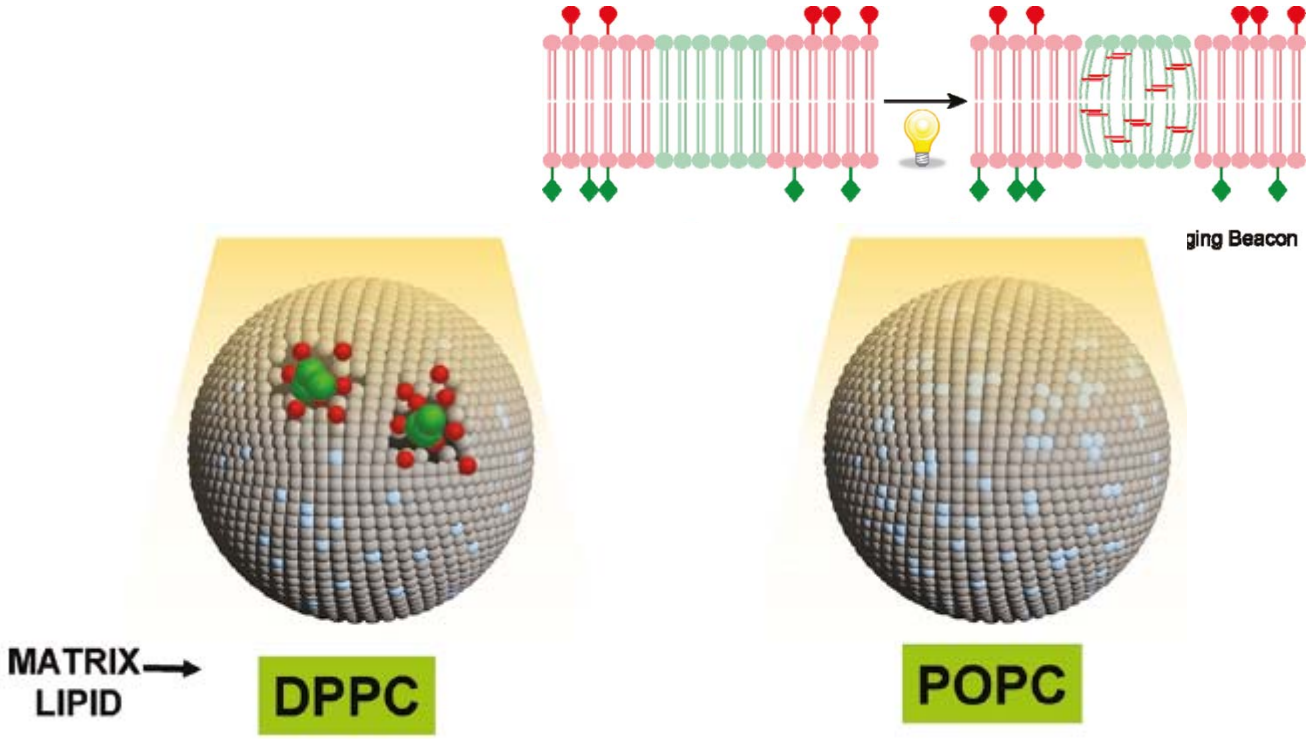
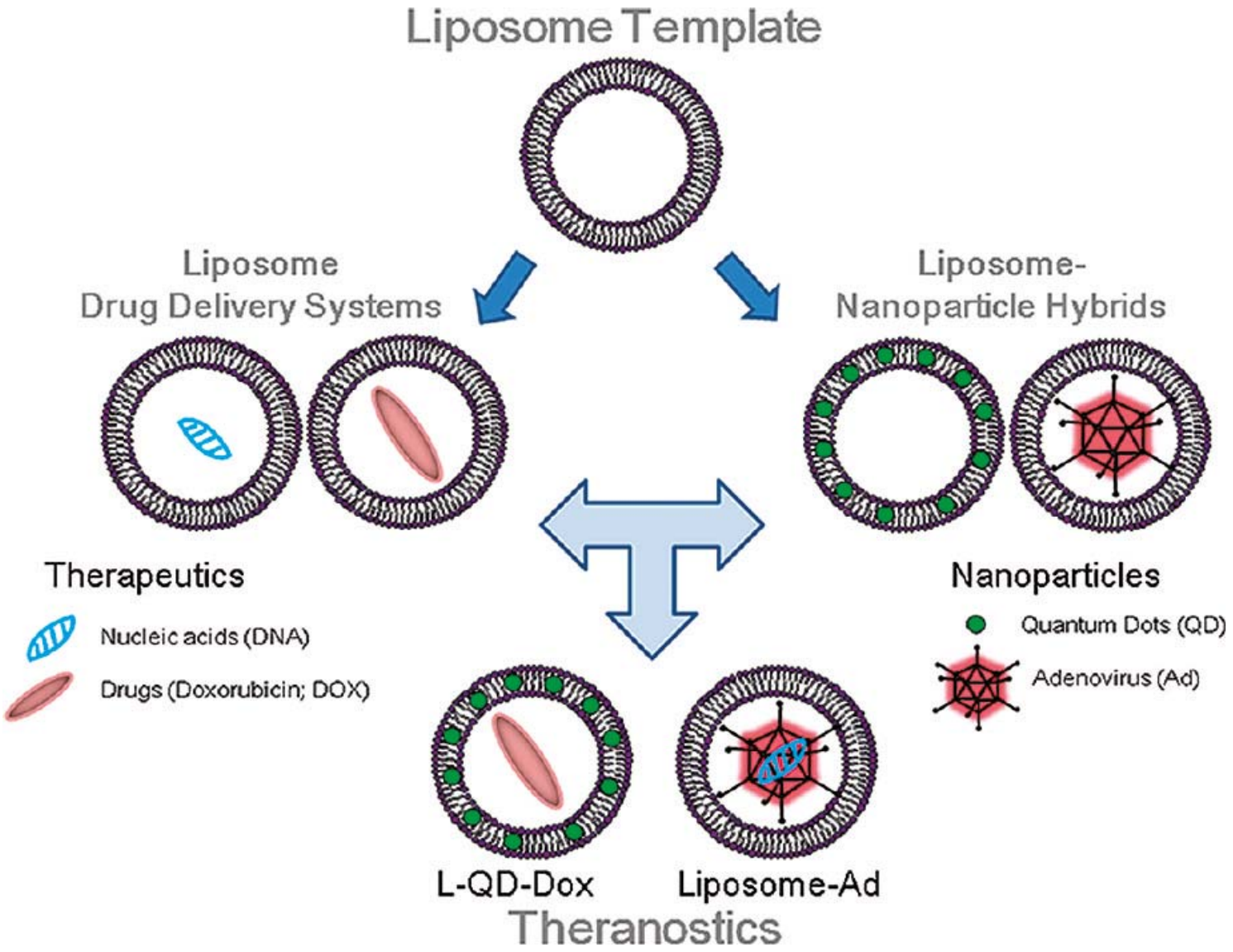
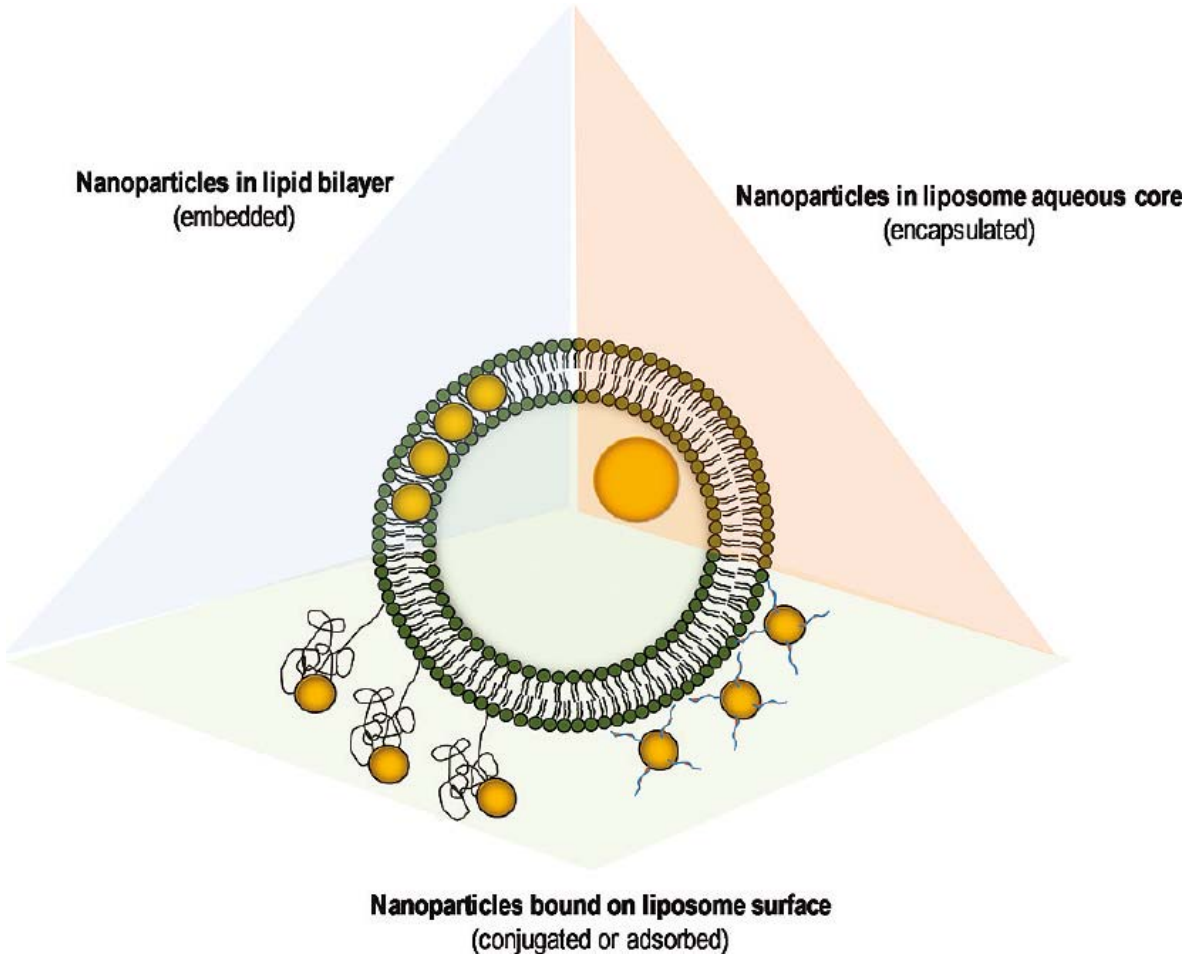


FIGURE 3. Cartoon depicting effect of bulk (matrix) lipids on self-assembly of a polymerizable lipid, $DC_{8,9}PC$ in the lipid bilayers. Gray, matrix lipid (left panel, DPPC (T_m, 41 °C); right panel, POPC (T_m, −2 °C)). Blue, light-activated $DC_{8,9}PC$ (T_m, 44 °C). $DC_{8,9}PC$ clustering in DPPC results in light-induced activation of molecules (shown in blue) that leads to $DC_{8,9}PC$ polymerization. This results in release of drugs (green) or imaging molecules (red). Right panel, $DC_{8,9}PC$ is not clustered in POPC molecules; light treatment results in activation of $DC_{8,9}PC$, but no polymerization and hence no release of contents. Adapted from refs 12 and 20.

Evolutions: nanoparticles liposome hybrids

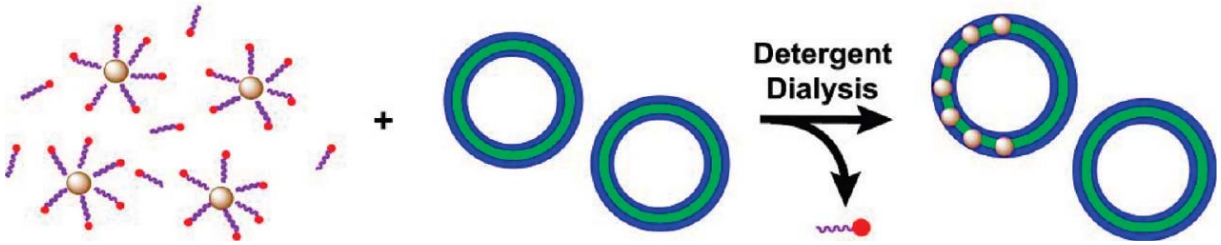


Evolutions: nanoparticles-liposome hybrids

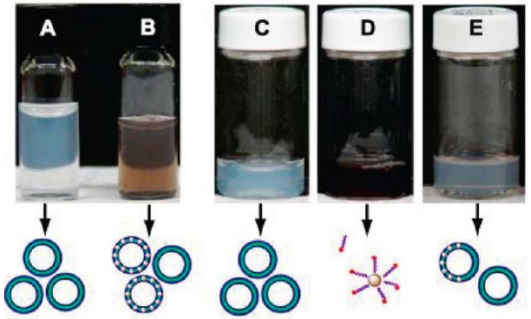


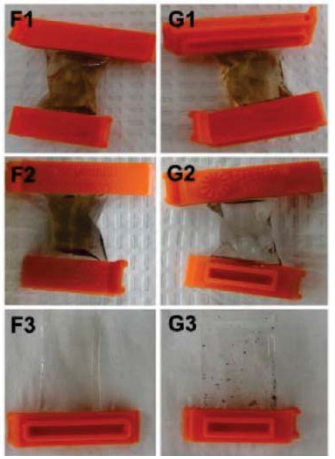
Embedding nanoparticles in the double layer

- 50 nm POPC liposomes
- 1.8 nm gold nanoparticles coated with 1-dodecanethiol



Co-extrusion

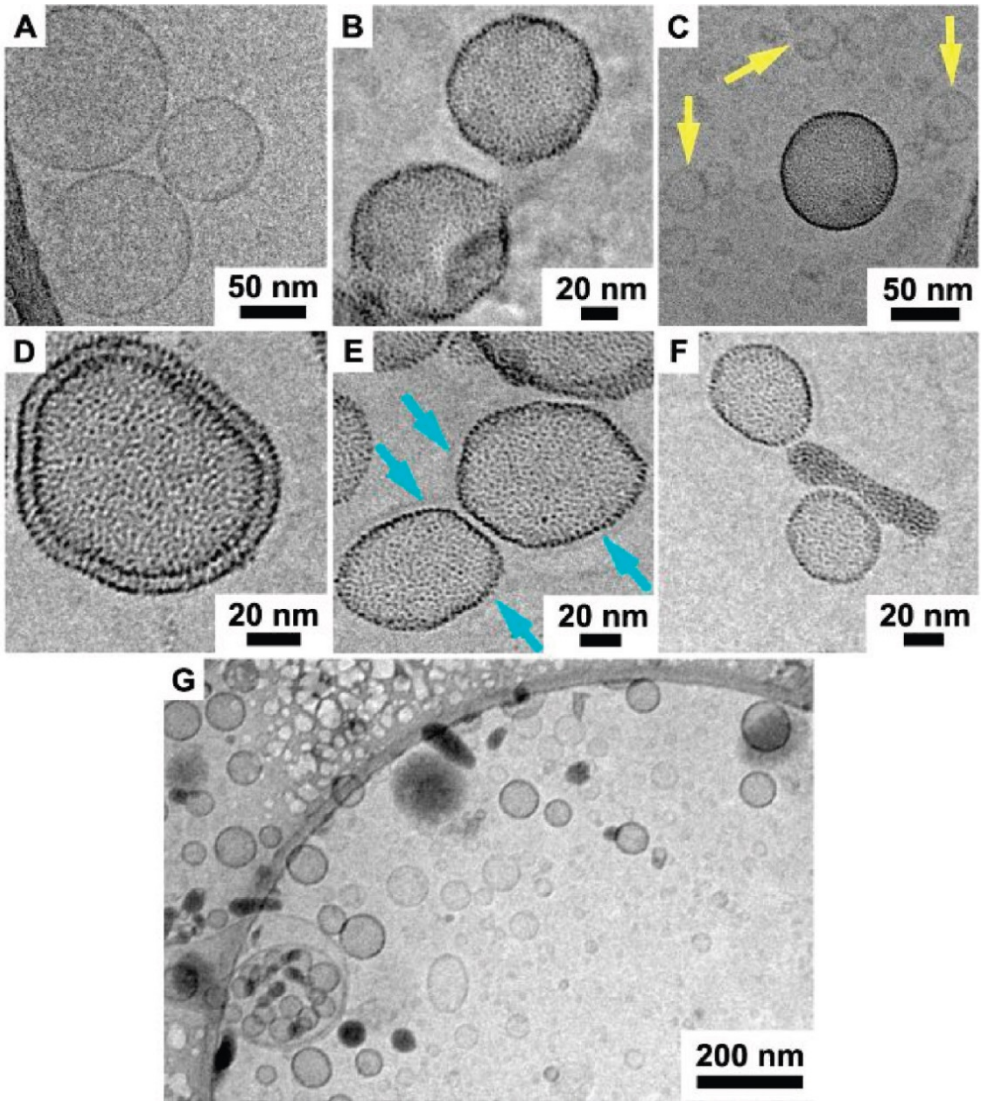




Dyalisis

Embedding nanoparticles in the double layer

Co-extrusion

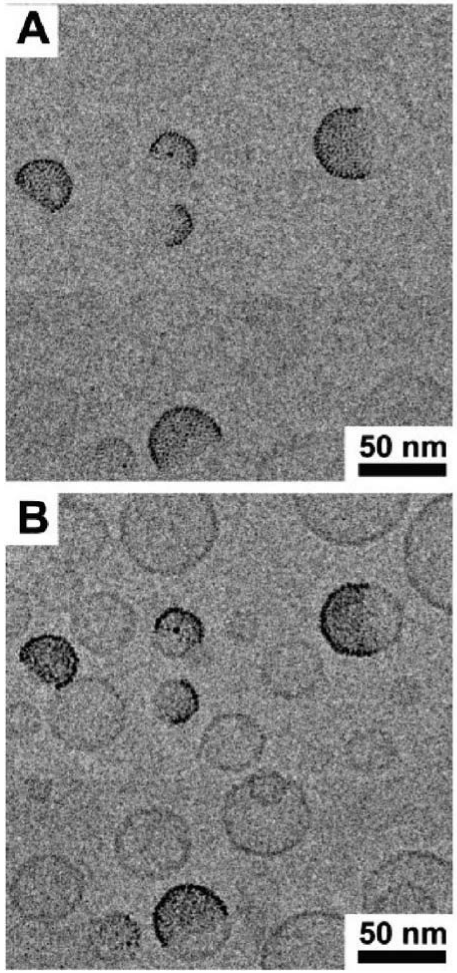


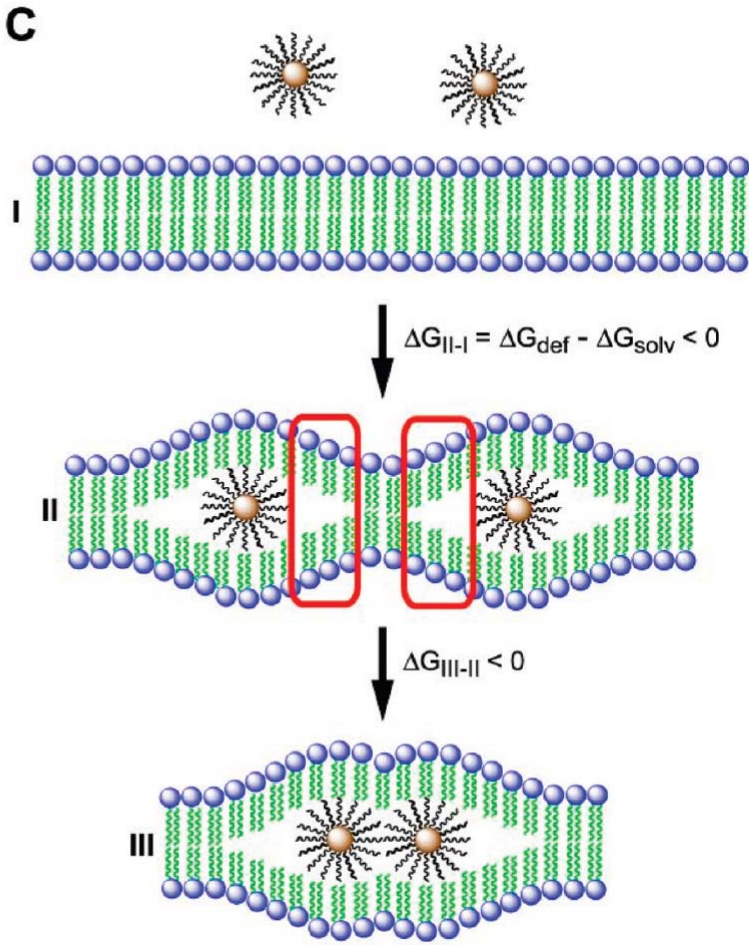
Lipid:np 1500:1

Lipid:np 100:1

Embedding nanoparticles in the double layer

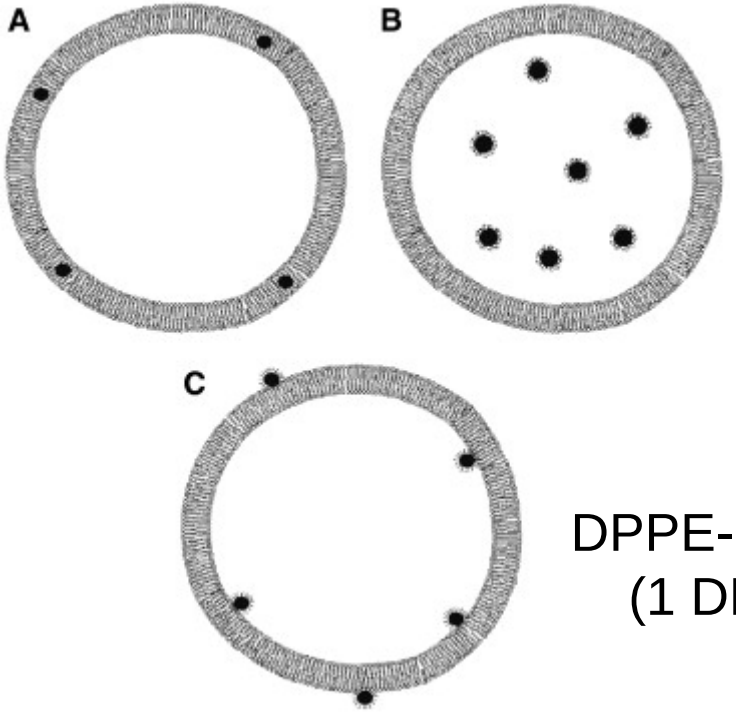
Dialysis





Phototriggered drug release

1-hexanethiol

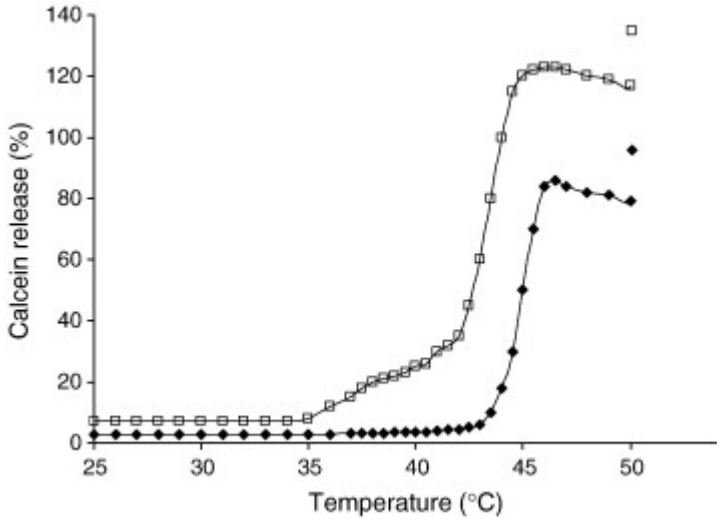


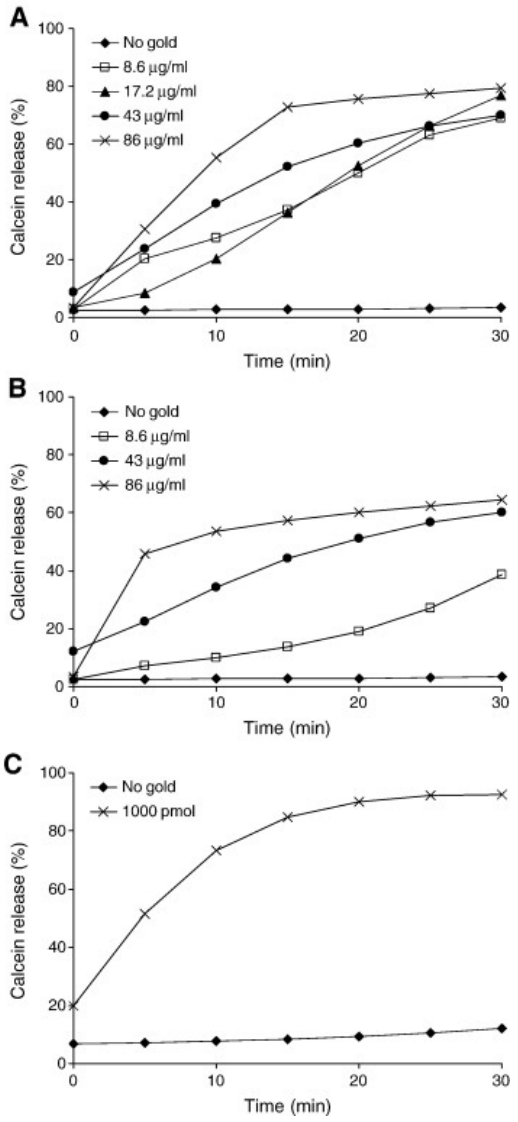
Mercaptosuccinate

DPPE-Nanogold®-particles
(1 DPPE per particle)

Prepared by co-extrusion

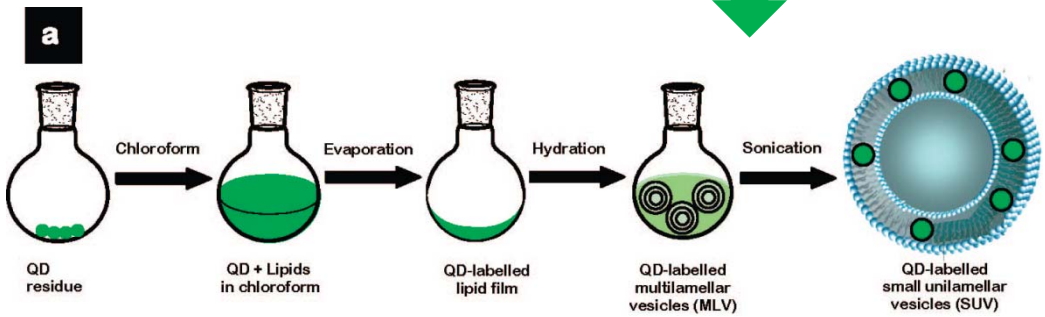
Phototriggered drug release

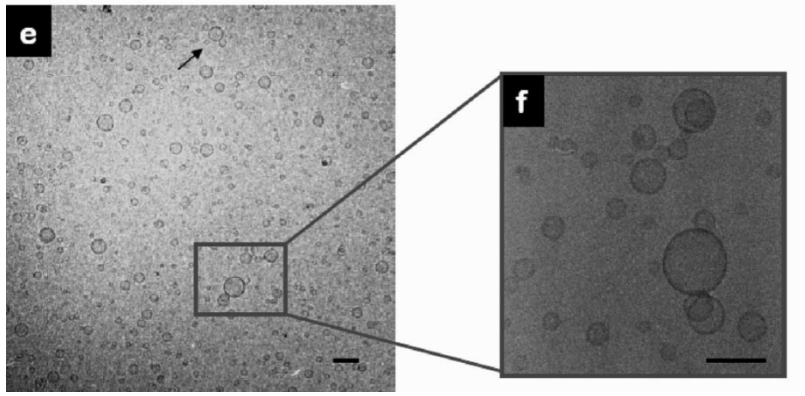




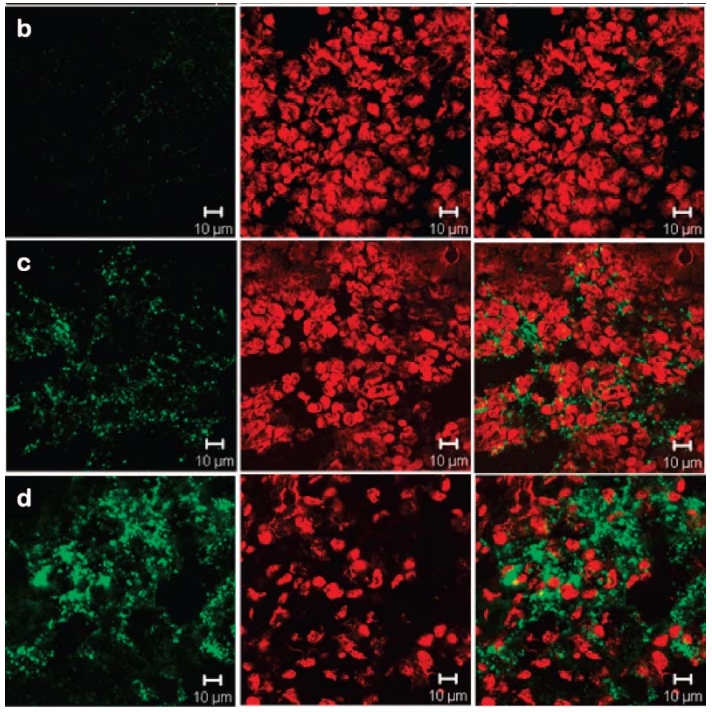
Nanoparticles-liposome hybrids

Bilayer Embedded NP		Nanoparticle type	Lipid composition	Hybrid average diameter	Hybrid Functionality	Theranostic activity	Ref
	Gold	Dodecanethiol coated Au NP (2nm)	PC	50-60nm	Cell membrane probe	No	19
	Gold	Hexanethiol capped Au NP (2.5nm)	DSPC:DPPC	200-500nm	UV light-induced drug release	No	20
	Gold	Stearylamine coated Au NP (3-4nm)	DPPC	20-200nm	Stabilize liposome membrane	No	21
	Iron	Oleic acid coated SPIO (5nm)	DPPC	150-200nm	Radiofrequency-induced drug release	No	22
	QD	TOPO-capped CdSe QD (2-4nm)	DMPC:DOTAP:DPPE-PEG$_{2000}$	20-100nm	QD solubilization Cell labeling *in vitro*	No	23
	QD	TOPO-capped CdSe/ZnS QD (2-4nm)	DOTAP:DOPE:Chol DSPC:Chol:DSPE:PEG$_{2000}$	80-100nm	Cell labeling *in vitro* and *in vivo* Cell imaging and drug delivery	Yes (Doxorubicin)	24,25





Xenografted tumor slices (gree: QD, red: nuclei)



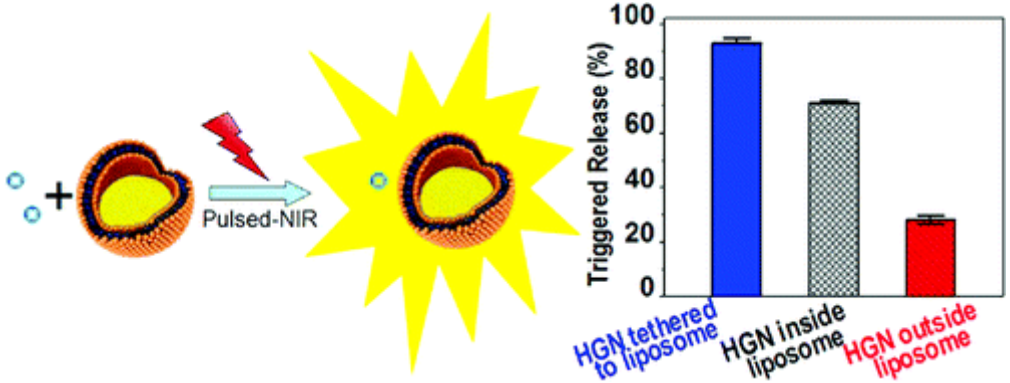
DSPC 5 m

DOTAP 5 m

DOTAP 24 h

Nanoparticles-liposome hybrids

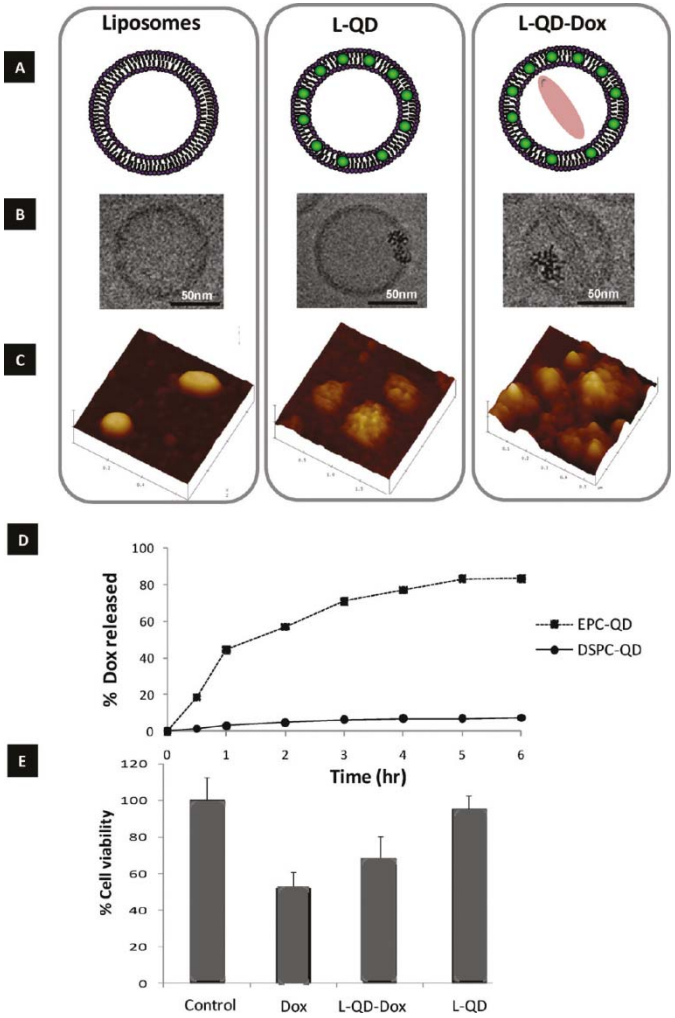
Encapsulated NP	Gold	Gold nanoshell (100nm)	PC:Chol:DPPE-PEG_{2000}	N/A	Phototherapy-induced hyperthermia	No	26
		Hollow gold nanoshell (30-40nm)	DPPC	400-500nm	Laser-induced drug release	No	27
	Ceramic	$Y2O3:Er^{+3}$ (150nm)	DPPC:Chol:DPPG	500nm	NIR imaging	No	28
	QD	COOH-PEG-QD (25nm)	DOPC:DC-Chol DSPC:Chol:DSPE-PEG_{2000}	80-100nm	Cell labeling and imaging Tumor targeting	No	29,30
	Iron oxide	Magnetite (Fe_3O_4)	TMAG:DLPC: DOPE	N/A	Cell sorting and gene delivery	No	31
		Dextran Magnetite (Fe_3O_4) (5-10nm)	SPC:Chol:PS	N/A	Targeted drug delivery	No	32
		Citrate stabilized Maghemite (γFe_2O_3) (7.7nm)	EPC: DSPE-PEG_{2000}	200nm	MRI imaging	No	33
	Lipid	DSPC:Chol liposomes (50nm,200nm)	DPPC, DSPC	0.3-2μm	Drug delivery	No	34
	Polystyrene	Sulphate and amidine polystyrene NP (100-300nm)	DODAB, DODAC, DHP, PC	100-200nm	Nanoparticle stabilization Biosensor constructs	No	10,35



Nanoparticles-liposome hybrids

		Nanoparticle type	Lipid composition	Hybrid average diameter	Hybrid Functionality	Theranostic activity	Ref
Surface conjugated NP 	QD	Streptavidin-QD	DOTAP:DOPE:$DSPE_{2000}$-biotin	100nm	Multicolor cell imaging	No	36
		Carboxylated CdSe/ZnS Qd chemically linked to amine functionalized PEG_{2000}-DSPE (4nm)	DSPC:Chol:DSPE-PEG_{2000}	200nm	Imaging and therapeutic modalities	Yes (Doxorubicin)	37
	Gold	Citrate coated Au NP (13nm)	EYPC:DDAB EYPC:DSPE-PEG_{2000}	200nm	Increase liposome colloidal stability	No	38
		DPPE-Nanogold (1.4nm)	DPPC:Chol	90 nm	Drug delivery and imaging system	No	39
		DPPE-Nanogold (1.4nm)	DSPC:DPPC	200-500nm	Light-induced drug release	No	20
		Hollow gold nanoshell (30-40nm)	DPPC	400-500nm	Laser-induced drug release	No	27
		PEG-maleimide-functionalized Au NP (64nm)	SOPC:DOPE	120-620nm	Cell membrane probe	No	40
Surface adsorbed/complexed NP 	QD	DNA-QD conjugate	Lipofectamine2000	N/A	Cell labeling and gene delivery	Yes (pDNA)	41
		PEG-QD	Lipofectamine2000	N/A	Co-delivery of siRNA and QD	Yes (siRNA)	42
	Gold	COOH-Au (4nm)	EPC:DOTAP	92nm	Stimuli-responsive (acid) NP-stabilized liposomes	No	43
		Hydrophilic Au NP (300nm aggregates)	DPPC:DOTAP:Chol	5µm	NIR-induced drug release	No	44
		DDAB coated Au NP (9nm)	DOTAP, Lipotap	N/A	Gene delivery	Yes (pDNA)	45
	Polystyrene	COOH- polystyrene NP (20nm)	DLPC	N/A	Liposome stabilization	No	46

Nanoparticles-virus hybrids



- sterically stabilized (DSPE-PEG-containing) L-QD exhibited prolonged blood circulation compared to cationic L-QD hybrids.
- cationic L-QD hybrids showed high transient lung accumulation post-injection

Nanoparticles-virus hybrids

- Viruses are attracting a great interest for their nanosize and ease of functionalization taking advantage of bioconjugation reactions with surface amines (K) or cystein (S).
- Virus are an optimal choice for DNA delivery but the present major drawbacks.
- rapid blood clearance (requiring multiple administrations),
- tissue toxicity (liver in the case of Ad),
- activation of severe and complex immune responses
- PEGylation reduce RES activation but also transfection efficecy