"ωφελέειν, εἰ μη βλάπτειν"

'benefit and do not harm'

National & Kapodistrian University of Athens

Pharmaceutical Nanotechnology

Liposomes as drug delivery systems

Biopysical and Thermodynamical considerations of their metastable phases

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What is nanotechnology ? Milestones

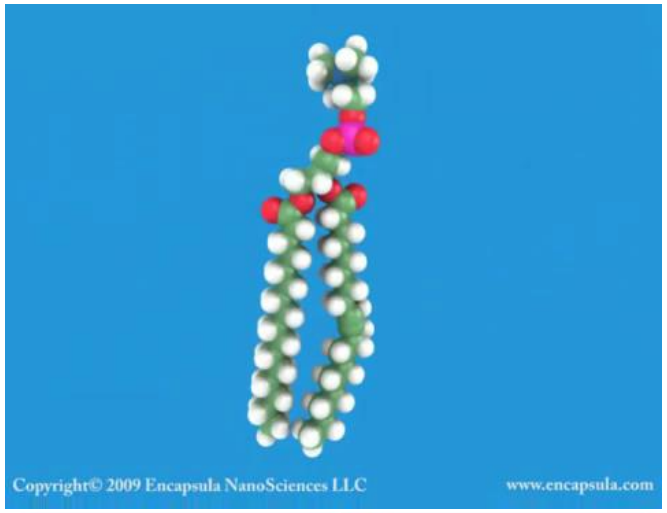
- Nanotechnology is multidisciplinary scientific field that deals with the development and use of materials with a dimension equal to one billionth of a meter (1nm = 10^{-9}m).
- The first report of nanotechnology was from Richard Feyman
- Prof. Nario Tanaguchi was the first to introduce the term nanotechnology from the University of Sciences in Tokyo, in 1974.
- In 1995, FDA approved Doxil (liposomal doxorubicin) to cure cancer.
- In 2012 the first report on bio-mimetic drug delivery nano systems was published
- In 2015 ThermoDox (thermosensitive liposomal doxorubicin) was evaluated in clinical trials (Phase III).
- In 2015 FDA approves Onivyde (liposomal irinotecan) for advanced pancreatic cancer
- In 2017 FDA approves Vyxeos, (liposomal cytarabine + dounorubicin) [Jazz Pharmaceuticals, Inc] for Acute Myeloid Leukemia (AML)

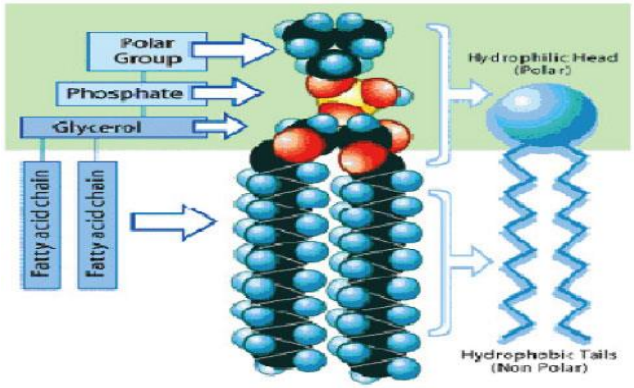
[44 mg daunorubicin and 100 mg cytarabine encapsulated together in liposomes. The volume of reconstituted Vyxeos required for each dose is calculated based on the daunorubicin dose (mg/m2) using body surface area. Since Vyxeos is a fixed-dose combination, and dosing based on the daunorubicin component, the corresponding cytarabine dose is included and does not need to be calculated].

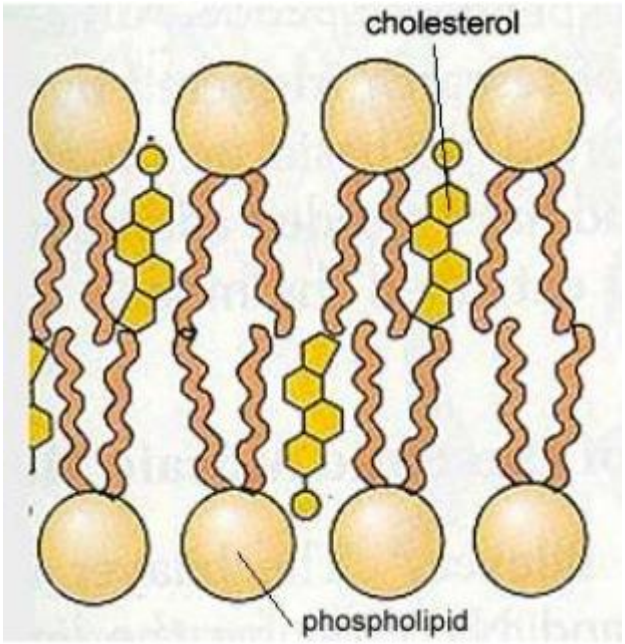
Ref. Saladin Nanotechnology for the development word. *Chaos Solition Fractals* 30 (4): 769-773, 2006

Ref. C. Demetzos '*Pharmaceutical Nanotechnology. Fundamentals and practical applications*' 2016, Springer

Phospholipids are the basic molecules from which lipidic bilayers consist







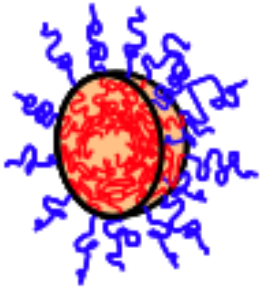
Cholesterol is a fundamental biomolecule that affects the lyotropism of liquid crystalline state of matter of lipidic bilayer. The lyotropic effect is a concentration dependent phenomenon

WHICH IS THE DRIVING FORCE FOR PRODUCING LIPID BILAYERS ?

SELF – ASSEMBLY

WHAT IS SELF – ASSEMBLY ?

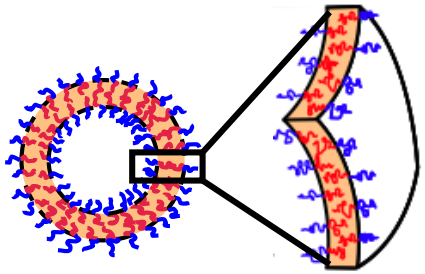
Self-assembly of bio structures



- "Self-assembly" is not synonymous with "formation." (Whitesiedes and Grzybowski, 2002).

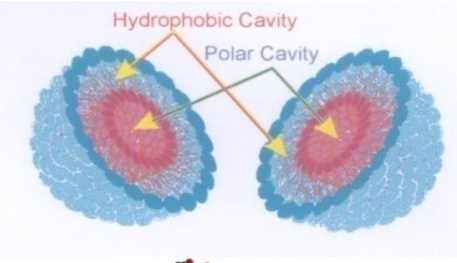
- **Self – assembly is the procedure by which individual compounds that contain enough information, can build an organized structure.**

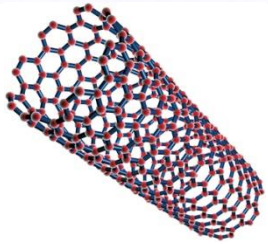
- The self-assembly process of amphiphilic molecules is of fundamental interest and is important in many applications,, nanomaterial synthesis, drug delivery, pharmaceutical formulation, and other dispersant technologies.



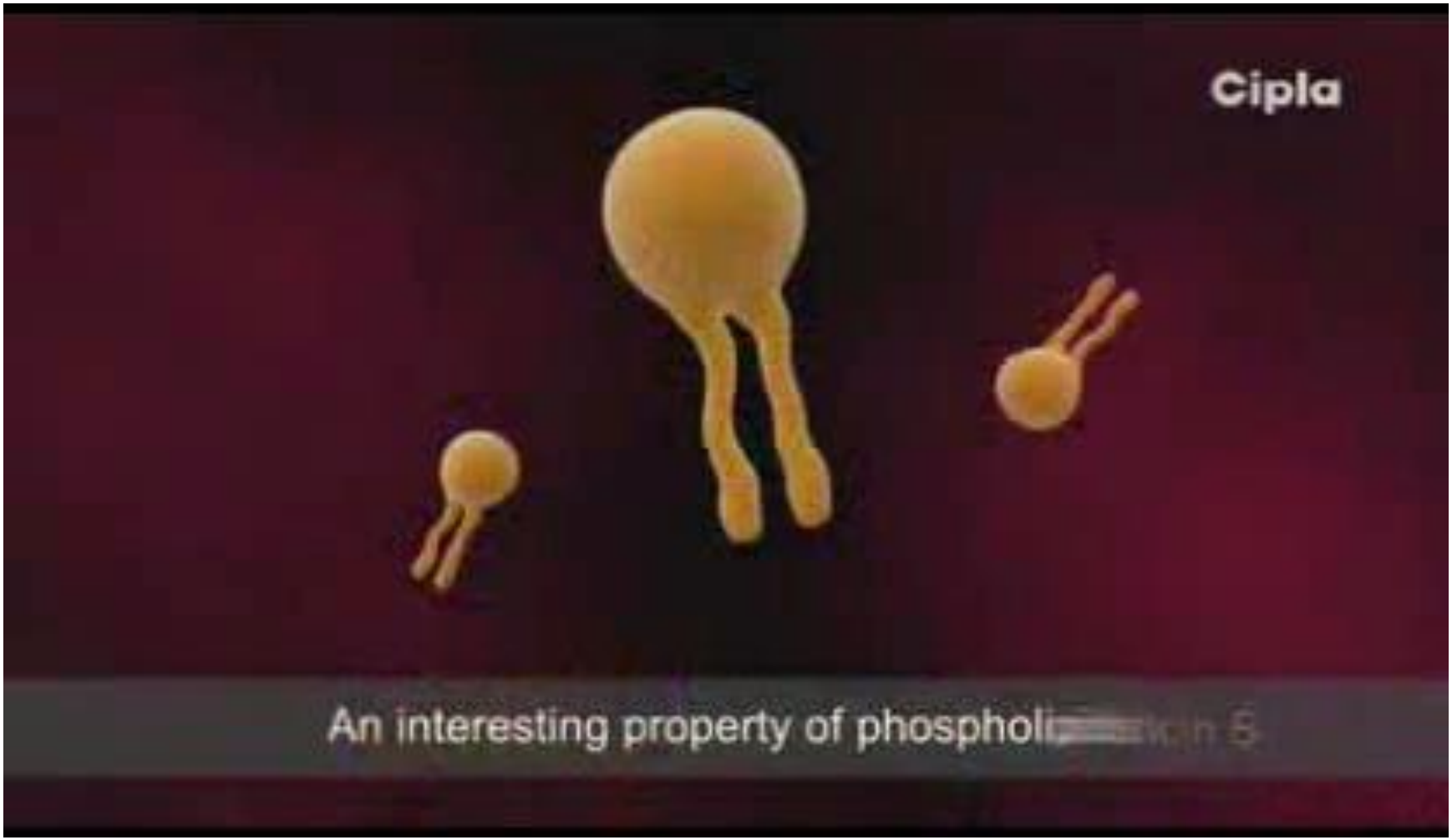
Particles can self-assemble as a result of their intermolecular forces. As systems look to minimize their free energy, self-assembly is one option for the system to achieve its lowest free energy thermodynamically

This *in situ* approach needs bio-organization process that '*promotes thermodynamic criteria governing phase transitions that are the mechanistic basis for their 'smartness'. There are no logic algorithms on board, no decision –making or rationalizing framework and no intellectual capacities*. [Ref. *Int. J. of Pharmaceutics* 454 , 521-524, 2013 by D. Grainger].



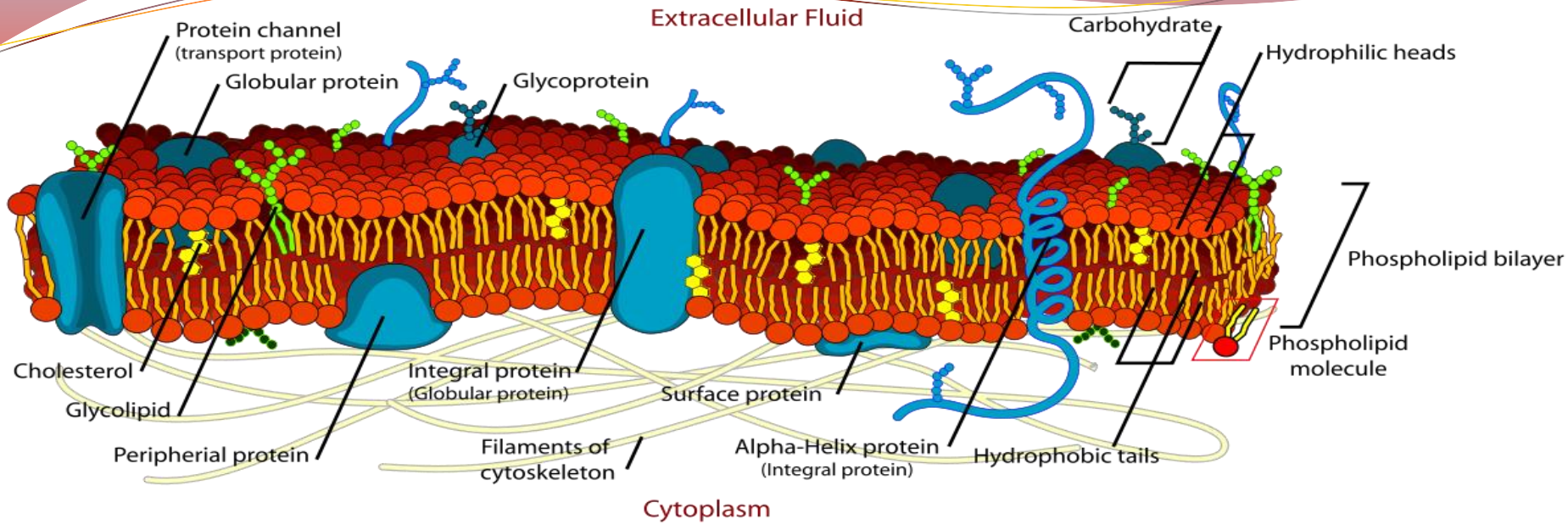


SELF ASSEMBLY PROCESS OF PHOSPHOLIPIDS FOR PRODUCING PHOSPHOLIPID BILAYERS.



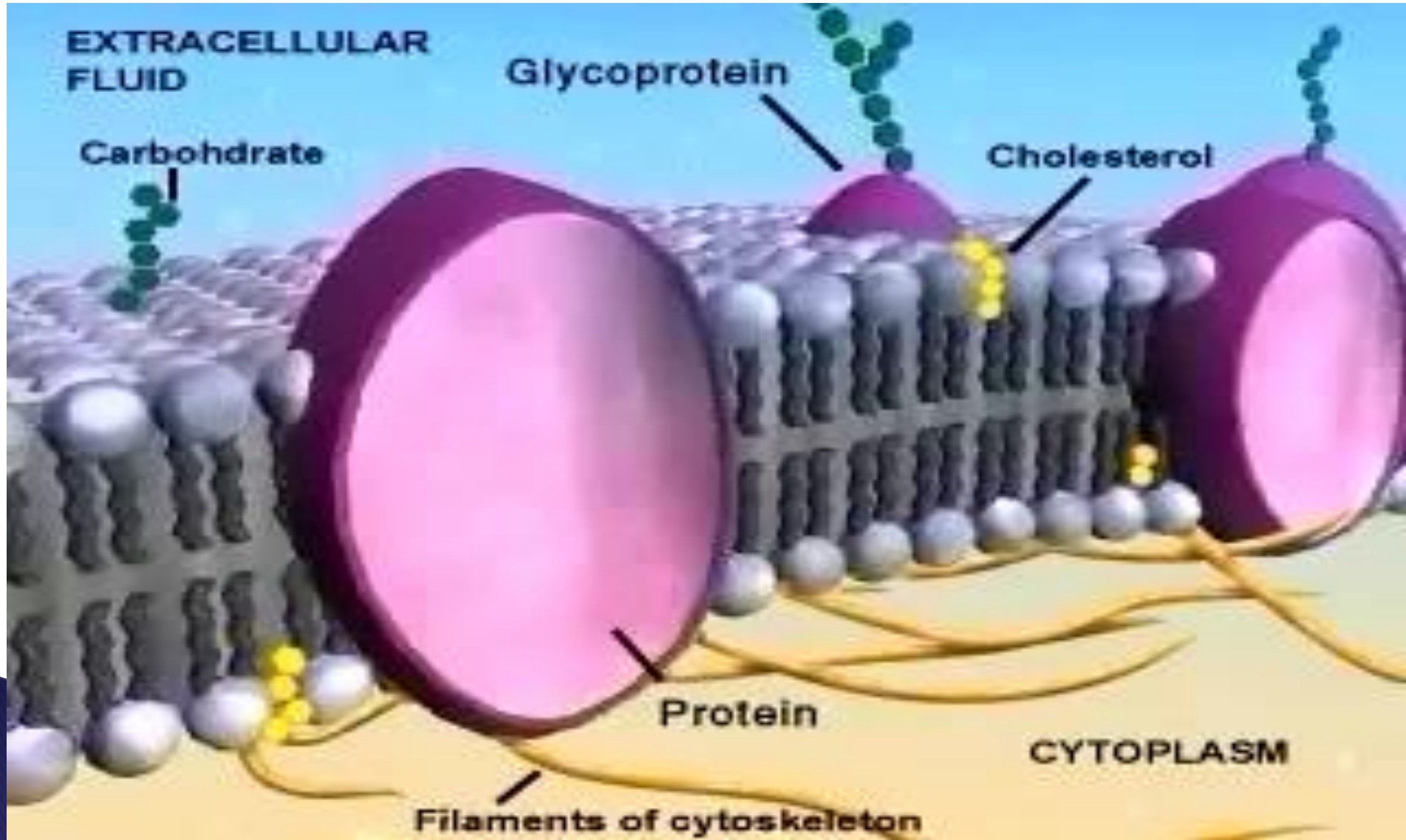
THE SELF ASSEMBLY PROCESS IS BASED ON THE PHYSICOCHEMICAL CHARACTERISTICS OF THE INITIAL BIOMATERIALS

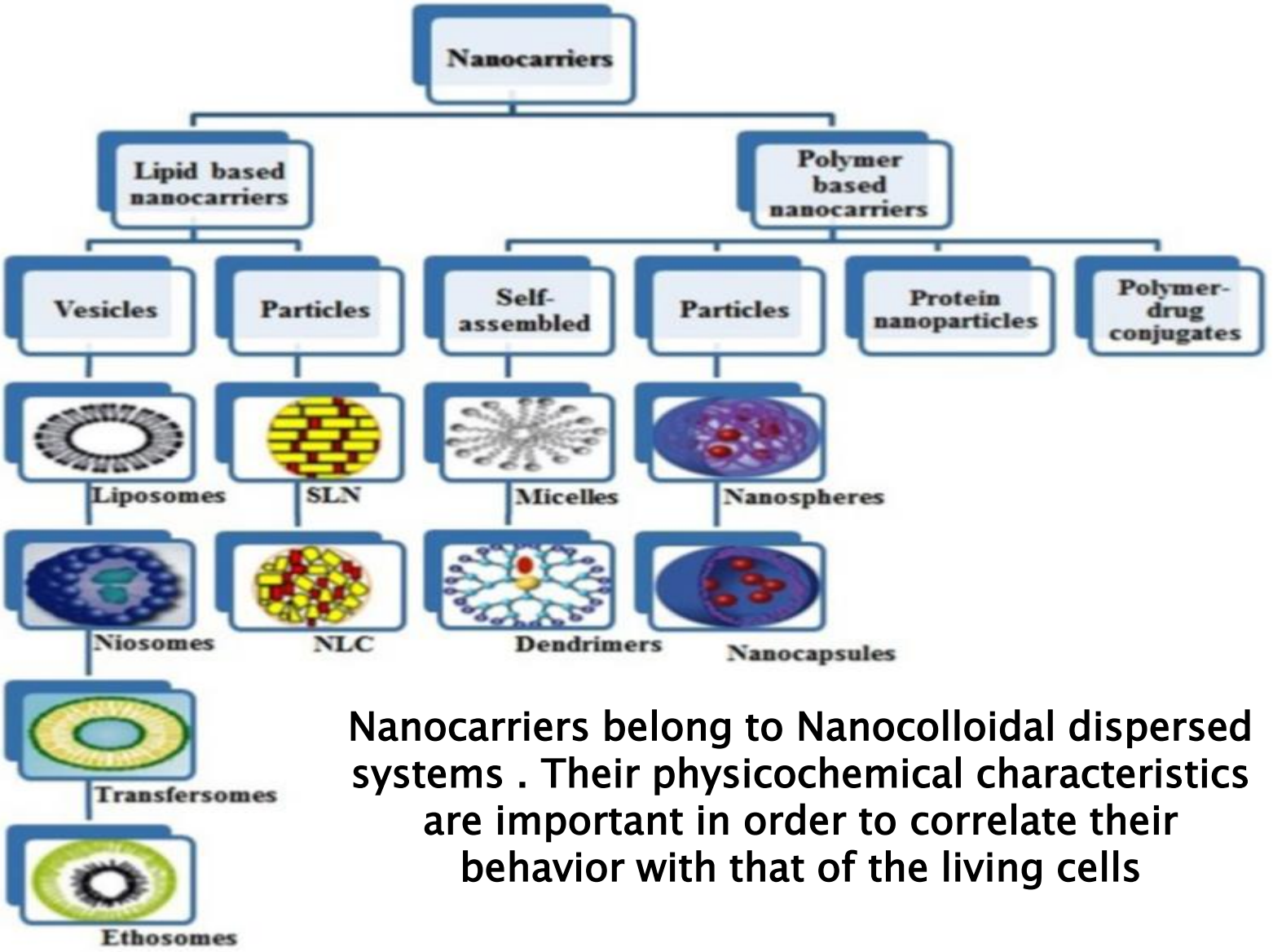
CELL MEMBRANE



Plasma membrane lipid composition by weight percent of mammalian red blood cells						
	species					
lipid	pig	human	cat	rabbit	horse	rat
cholesterol	26.8	26.0	26.8	28.9	24.5	24.7
phosphatidylcholine	13.9	17.5	18.7	22.3	22.0	31.8
sphingomyelin	15.8	16.0	16.0	12.5	7.0	8.6
phosphatidylethanolamine	17.7	16.6	13.6	21.0	12.6	14.4
phosphatidylserine	10.6	7.9	8.1	8.0	9.4	7.2
phosphatidylinositol	1.1	1.2	4.5	1.0	0.2	2.3
phosphatidic acid	0.2	0.6	0.5	1.0	0.2	0.2
lysophosphatidylcholine	0.5	0.9	0.2	0.2	0.9	2.6
glycosphingolipids	13.4	11.0	11.9	5.3	23.5	8.3
Source: From Thomas E. Andreoli et al., *Membrane Physiology* , 2nd ed. (1987), Table I, chapter 27.						

Nature promotes complex systems as 'living systems' . This concept promotes plethora of **metastable phases** and *clustering effects* which are processes of high quality (i.e signalling transduction) that take place within the lipid bilayers of cell membranes. (Ref. Binder eta al., Angew. Chem. Ind. Ed. , 42, 5802-27, 2003)



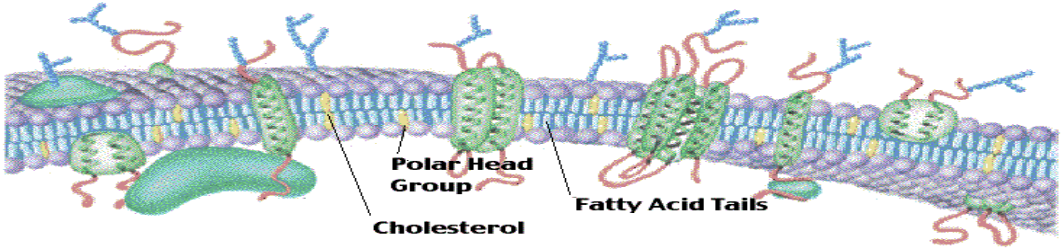


Nanocarriers belong to Nanocolloidal dispersed systems . Their physicochemical characteristics are important in order to correlate their behavior with that of the living cells

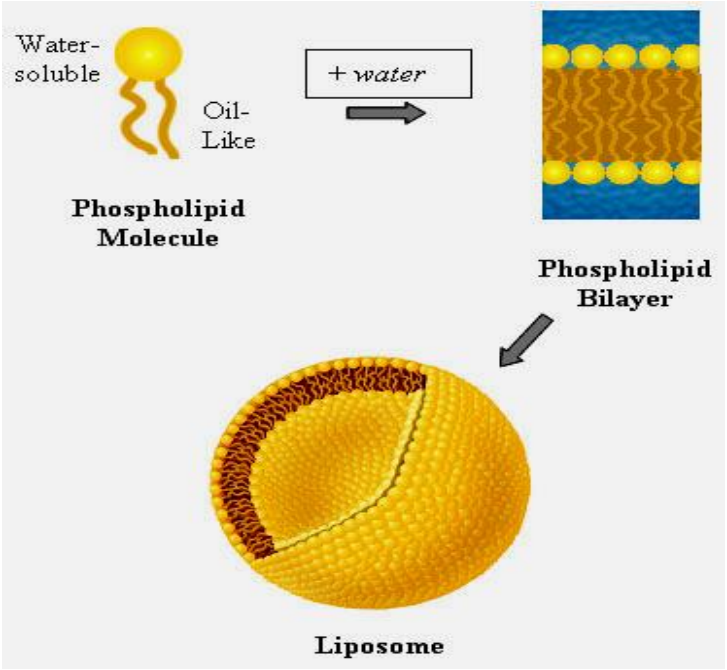
M. Estanqueiro et al. / Colloids and Surfaces B: Biointerfaces 126 (2015)631–648

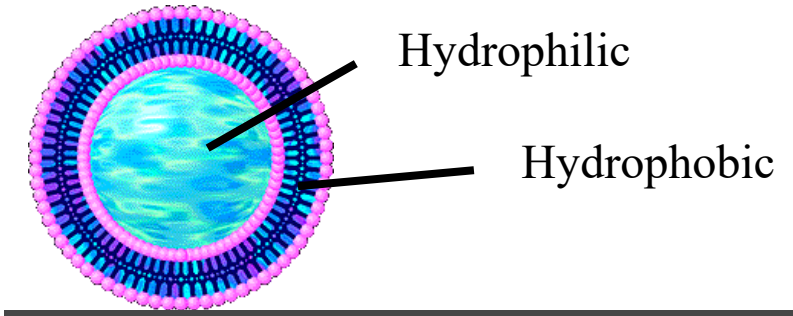
LIPIDIC NANOCARRIERS IN PHARMACEUTICS

What are liposomes ?



- Pseudo-Spherical vesicles with a phospholipid bilayer

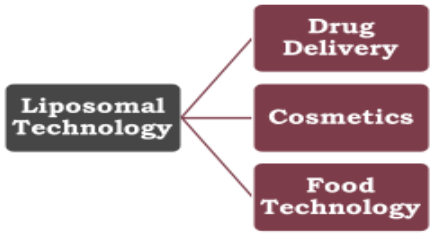




Liposomes in brief.....

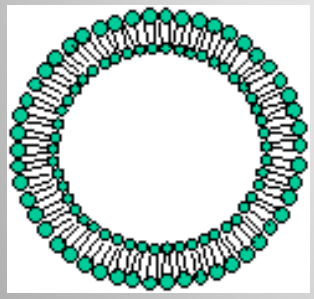
- Liposomes were first discovered by A.D. Bangham in 1960 in an attempt to create lipid – water systems that behave as biomembranes.

- Liposomes belong to the class of bio-colloids and could affect the physical properties (release, solubility, pharmacokinetics) or ADME profile of the encapsulated drug.

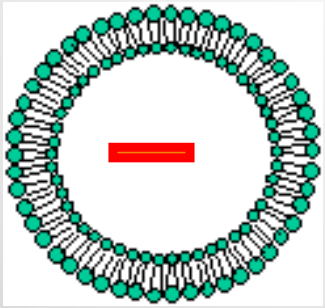


CONVENTIONAL LIPOSOMAL DRUG DELIVERY nanoSYSTEMS

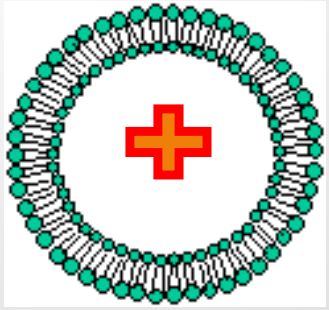
Simple liposomes



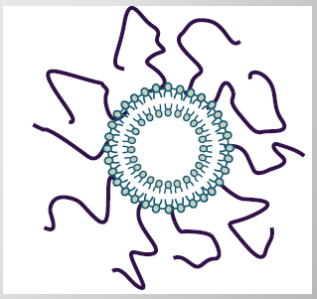
Anionic liposomes



Cationic liposomes



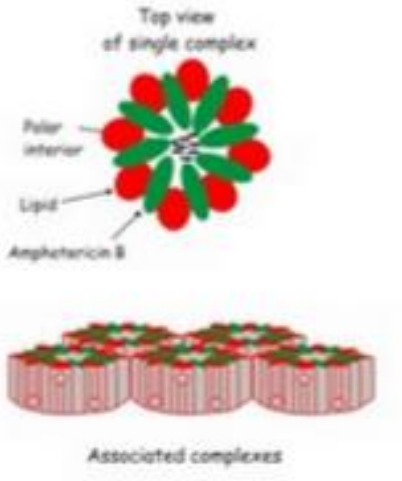
PEGylated (stealth) liposomes



Lipidic and Liposomal formulations of Amphotericin B

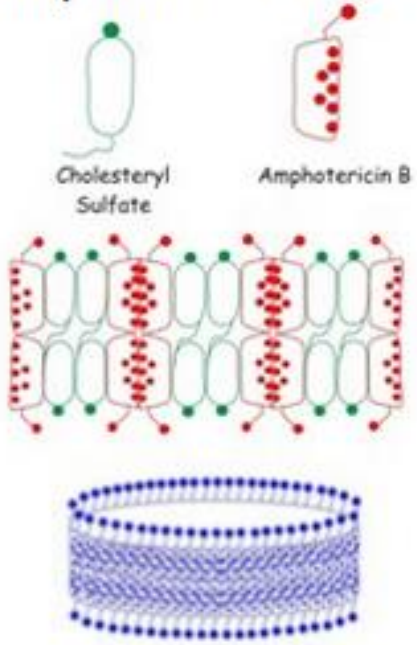
Amphotericin **B** **L**ipid **C**omplex

Abelcet ® ABLC

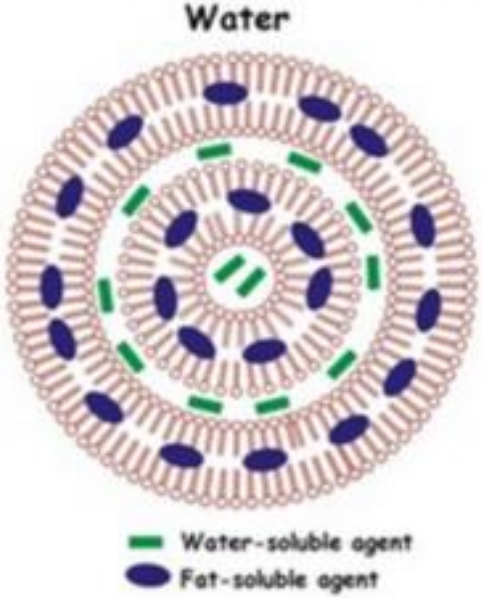


Amphotericin **B** **C**olloidal **D**ispersion

Amphotec ® ABCD



Ambisome ® L-AMB



Ribbon-like particles

Carrier lipids: DMPC, DMPG

Particle size (μm): 1.6-11

Disk-like particles

Carrier lipids: Cholesteryl sulfate

Particle size (μm): 0.12-0.14

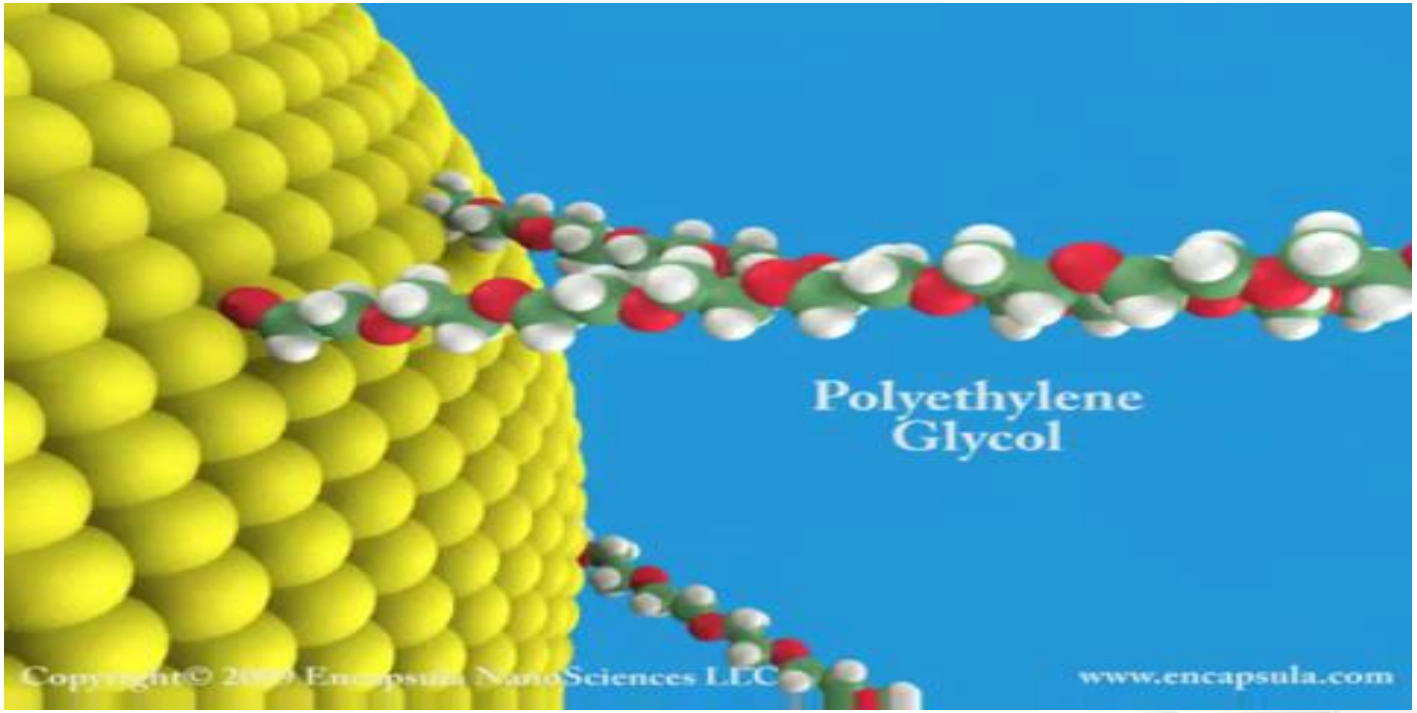
Unilaminar liposome

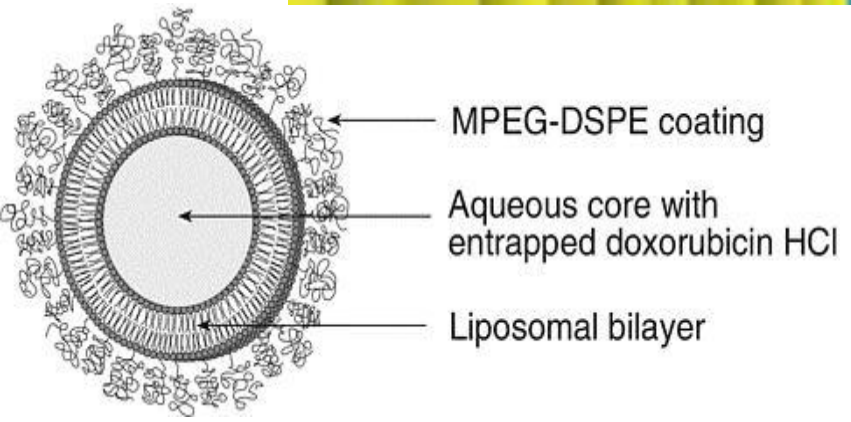
Carrier lipids: HSPC, DSPG, cholesterol

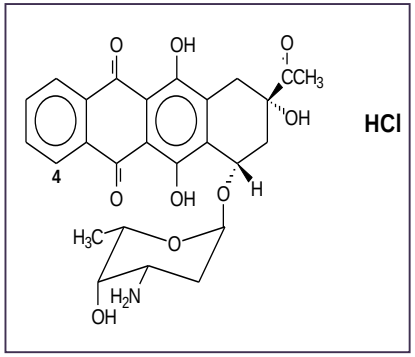
Particle size (μm) : 0.08

AMBISOME (LIPOSOMAL AMPHOTERICIN B)

STEALTH LIPOSOMAL FORMULATION







Σημειώσεις

Table 1. Clinically used liposome-based products.

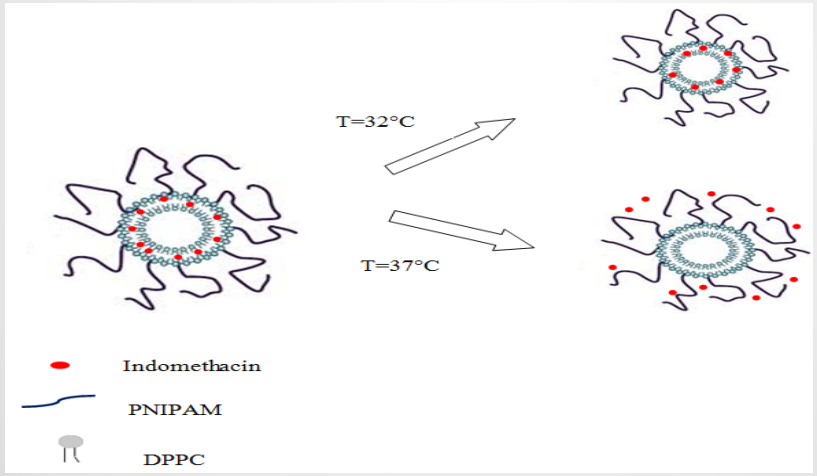
SN	Clinical Products (Approval Year)	Administration	Active Agent	Lipid/Lipid:Drug Molar Ratio	Indication	Company
1.	Doxil® (1995)	i.v.	Doxorubicin	HSPC:Cholesterol:PEG 2000-DSPE (56:39:5 molar ratio)	Ovarian, breast cancer, Kaposi's sarcoma	Sequus Pharmaceuticals
2.	DaunoXome® (1996)	i.v.	Daunorubicin	DSPC and Cholesterol (2:1 molar ratio)	AIDS-related Kaposi's sarcoma	NeXstar Pharmaceuticals
3.	Depocyt® (1999)	Spinal	Cytarabine/Ara-C	DOPC, DPPG, Cholesterol and Triolein	Neoplastic meningitis	SkyPharma Inc.
4.	Myocet® (2000)	i.v.	Doxorubicin	EPC:Cholesterol (55:45 molar ratio)	Combination therapy with cyclophosphamide in metastatic breast cancer	Elan Pharmaceuticals
5.	Mepact® (2004)	i.v.	Mifamurtide	DOPS:POPC (3:7 molar ratio)	High-grade, resectable, non-metastatic osteosarcoma	Takeda Pharmaceutical Limited
6.	Marqibo® (2012)	i.v.	Vincristine	SM:Cholesterol (60:40 molar ratio)	Acute lymphoblastic leukaemia	Talon Therapeutics, Inc.
7.	Onivyde™ (2015)	i.v.	Irinotecan	DSPC:MPEG-2000:DSPE (3:2:0.015 molar ratio)	Combination therapy with fluorouracil and leucovorin in metastatic adenocarcinoma of the pancreas	Merrimack Pharmaceuticals Inc.
8.	Abelcet® (1995)	i.v.	Amphotericin B	DMPC:DMPG (7:3 molar ratio)	Invasive severe fungal infections	Sigma-Tau Pharmaceuticals
9.	Ambisome® (1997)	i.v.	Amphotericin B	HSPC:DSPG:Cholesterol:Amphotericin B (2:0.8:1:0.4 molar ratio)	Presumed fungal infections	Astellas Pharma
10.	Amphotec® (1996)	i.v.	Amphotericin B	Cholesteryl sulphate:Amphotericin B (1:1 molar ratio)	Severe fungal infections	Ben Venue Laboratories Inc.
11.	Visudyne® (2000)	i.v.	Verteporphin	Verteporphin:DMPC and EPG (1:8 molar ratio)	Choroidal neovascularisation	Novartis
12.	DepoDur™ (2004)	Epidural	Morphine sulfate	DOPC, DPPG, Cholesterol and Triolein	Pain management	SkyPharma Inc.
13.	Exparel® (2011)	i.v.	Bupivacaine	DEPC, DPPG, Cholesterol and Tricaprylin	Pain management	Pacira Pharmaceuticals, Inc.
14.	Epaxal® (1993)	i.m.	Inactivated hepatitis A virus (strain RGSB)	DOPC:DOPE (75:25 molar ratio)	Hepatitis A	Crucell, Berna Biotech
15.	Inflexal® V (1997)	i.m.	Inactivated hemaglutinine of Influenza virus strains A and B	DOPC:DOPE (75:25 molar ratio)	Influenza	Crucell, Berna Biotech

i.v. (intravenous); i.m. (intramuscular); HSPC (hydrogenated soy phosphatidylcholine); PEG (polyethylene glycol); DSPE (distearoyl-sn-glycero-phosphoethanolamine); DSPC (distearoylphosphatidylcholine); DOPC (dioleoylphosphatidylcholine); DPPG (dipalmitoylphosphatidylglycerol); EPC (egg phosphatidylcholine); DOPS (dioleoylphosphatidylserine); POPC (palmitoyloleoylphosphatidylcholine); SM (sphingomyelin); MPEG (methoxy polyethylene glycol); DMPC (dimyristoyl phosphatidylcholine); DMPG (dimyristoyl phosphatidylglycerol); DSPG (distearoylphosphatidylglycerol); DEPC (dierucoylphosphatidylcholine); DOPE (dioleoly-sn-glycero-phophoethanolamine).

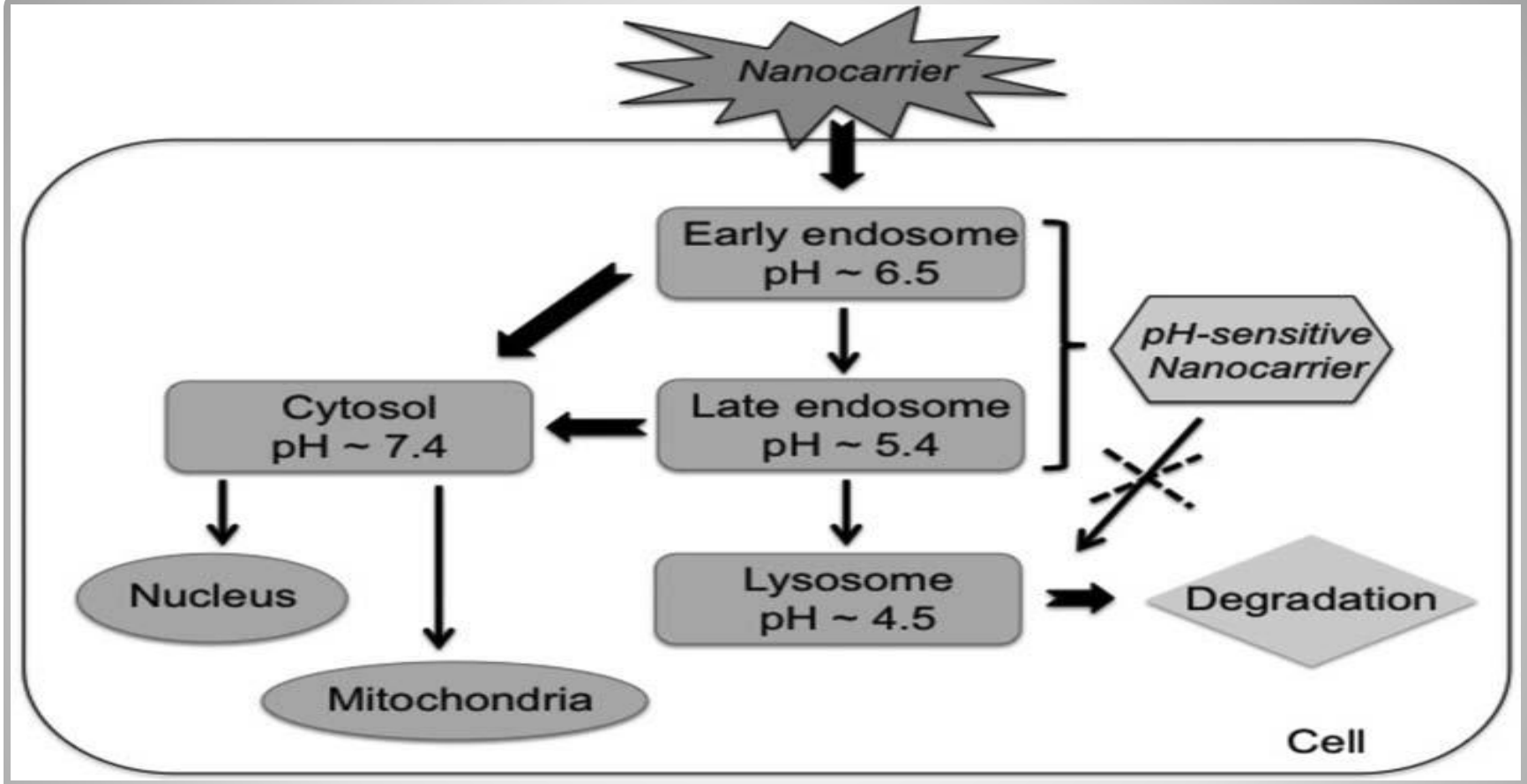
Ref. Liposomal Formulations in Clinical Use: An Updated Review by Upendra Bulbake †, Sindhu Doppalapudi †, Nagavendra Kommineni and Wahid Khan , *Pharmaceutics* 9, 12, 2017

Advanced Liposomal Drug Delivery nanoSystems

- **STIMULI - RESPONSIVE LIPOSOMAL nanoSYSTEMS**



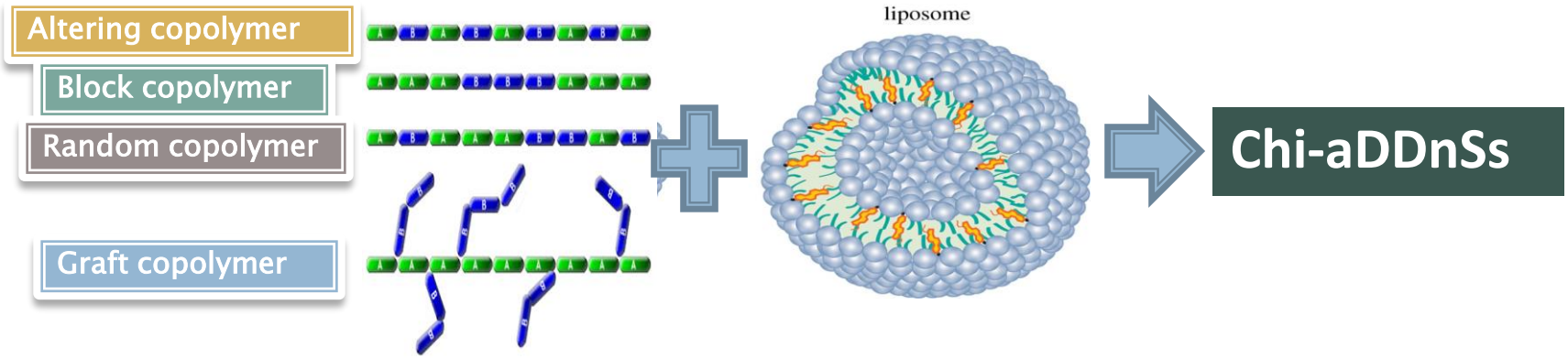
- **CHIMERIC/MIXED LIPOSOMAL nanoSYSTEMS (Chi-aDDnSs)**



Scheme of the intracellular trafficking of a nanocarrier after cell uptake. The nanocarrier with pH-sensitive properties can undergo several processes that result in selective targeting to the cytosol, nucleus or other subcellular organelles

Ref. Nanotechnology Approaches to Target Endosomal pH: A Promising Strategy for an Efficient Intracellular Drug, Gene and Protein Delivery by Daniele Rubert Nogueira, Montserrat Mitjans and M. Pilar Vinardell*in Drug Delivery Letters 2014

MIXED/CHIMERIC (polymer and Liposome) NANOCARRIERS IN PHARMACEUTICS



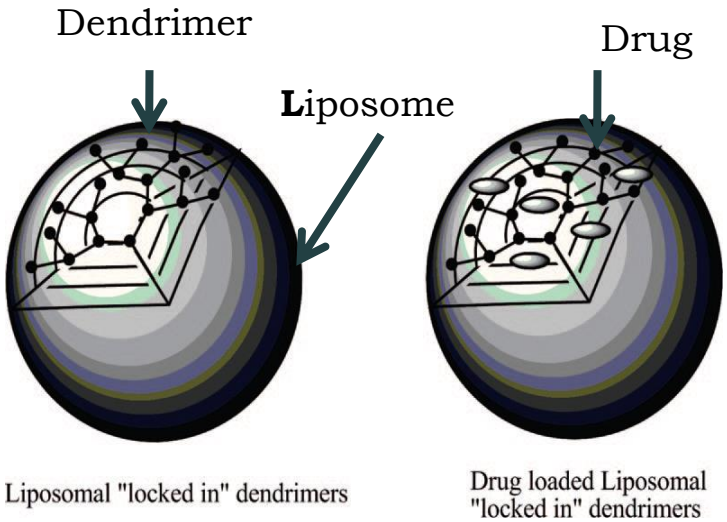
1) **Polymers are materials that are widely used in the pharmaceutical industry in various technological formulations** and in bioactive molecule delivery system coating.
2) Polymer behavior is directly related to their chemical structure. Also, their abilities depend on the way that monomers are connected to each other.
3) Polymers can have linear or branched chains that may cross each other. Copolymers are composed of more than one monomer and develop new polymers with completely new properties.

Chimeric Chi- aDDnS

Liposomes + Pamam + Doxorubicin

Liposomes + Pamam + Methotraxate

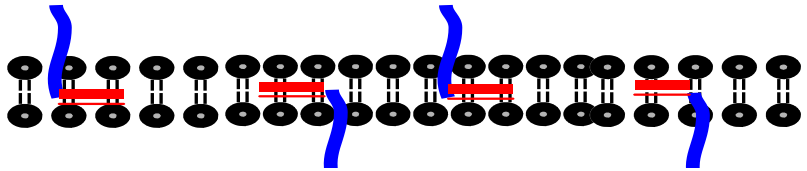
Ref. Klopade et al.,Int. J. Pharm., 2002



Ref. 1. Papagiannaros, Demetzos et al., *Int. J. Pharm.* 2005

2. Papagiannaros, Demetzos, National Patent, 2006

3. Gardikis, Demetzos et al.,, J.Pharm.Sc., 2010



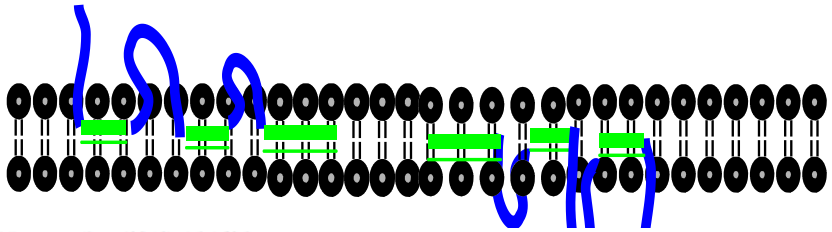
Soft Matter RSCPublishing

PAPER

PEO-*b*-PCL–DPPC chimeric nanocarriers: self-assembly aspects in aqueous and biological media and drug incorporation†

Cite this: *Soft Matter*, 2013, 9, 4073

Natassa Pippa,[ab] Eleni Kaditi,[a] Stergios Pispas*[a] and Costas Demetzos[b]



J Nanopart Res (2013) 15:1685
DOI 10.1007/s11051-013-1685-3

RESEARCH PAPER

DPPC/poly(2-methyl-2-oxazoline)-grad-poly(2-phenyl-2-oxazoline) chimeric nanostructures as potential drug nanocarriers

Natassa Pippa · Eleni Kaditi · Stergios Pispas · Costas Demetzos

BASIC SCIENTIFIC TOOLS FOR STUDYING LIPOSOMAL PHOSPHOLIPIDIC MEMBRANES

The **Biophysics** and **Thermodynamics** are considered as the basic scientific elements for studying artificial cell membranes and provide projection of the behavior of nano systems as artificial cell models.

The synergy regarding the biophysical behavior of artificial biomembranes and of cell biology has promoted nanoparticulate systems as drug delivery nano-platforms, while their thermotropic behavior can be correlated with cell functionality

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DOI: 10.1208/s12249-015-0321-1

Mini-Review

Biophysics and Thermodynamics: The Scientific Building Blocks of Bio-inspired Drug Delivery Nano Systems

Costas Demetzos[1,2]

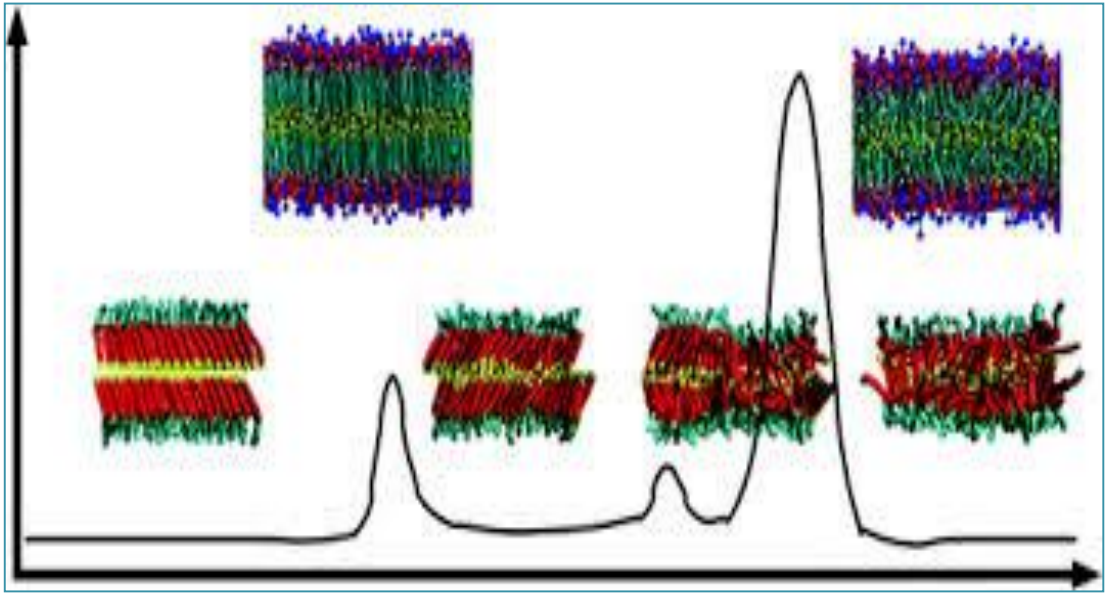
Received 9 March 2015; accepted 7 April 2015; published online 22 April 2015

Abstract. Biophysics and thermodynamics are considered as the scientific milestones for investigating the properties of materials. The relationship between the changes of temperature with the biophysical variables of biomaterials is important in the process of the development of drug delivery systems. Biophysics is a challenge sector of physics and should be used complementary with the biochemistry in order to discover new and promising technological platforms (*i.e.*, drug delivery systems) and to disclose the 'silence functionality' of bio-inspired biological and artificial membranes. Thermal analysis and biophysical approaches in pharmaceuticals present reliable and versatile tools for their characterization and for the successful development of pharmaceutical products. The metastable phases of self-assembled nanostructures such as liposomes should be taken into consideration because they represent the thermal events can affect the functionality of advanced drug delivery nano systems. In conclusion, biophysics and thermodynamics are characterized as the building blocks for design and development of bio-inspired drug delivery systems.

KEY WORDS: biophysics; drug delivery nano systems; pharmaceutics; thermal analysis; thermodynamics.

Thermodynamics is the fundamental scientific element that could efficiently be used for studying and analyzing the behavior of artificial biological membranes that could be correlated with biological networks and create scientific platforms for the system therapeutics concept.

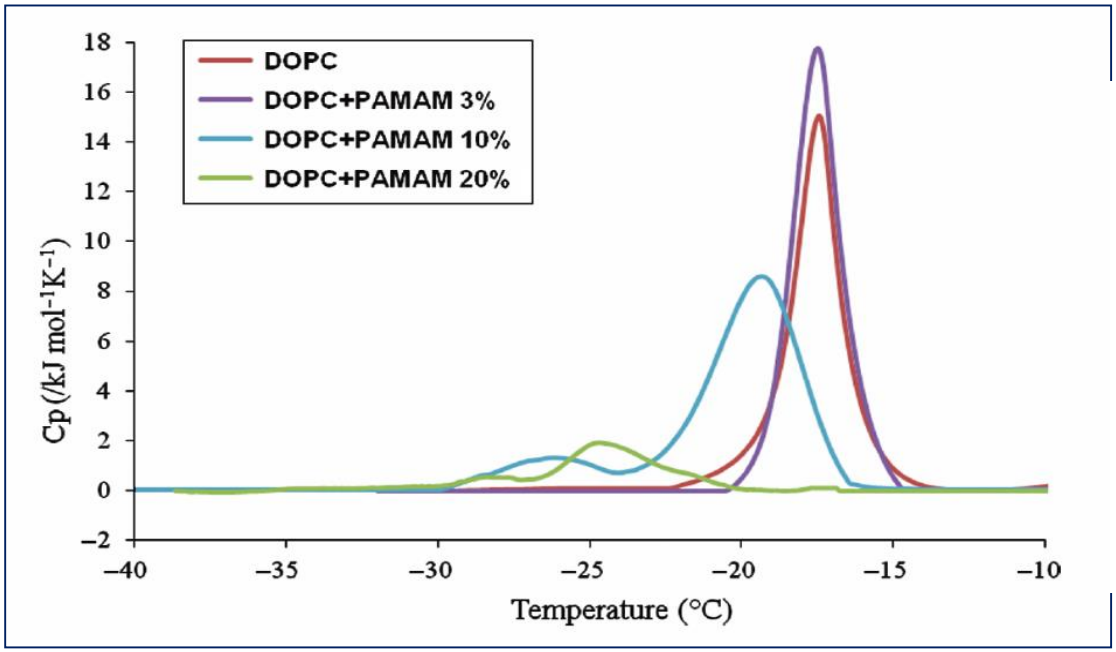
Liquid crystalline phases of phospholipids



Ref. Koynova R., Caffrey M., Phases and phase transition of the phosphatidylocholines, Biochim. Biophys. Acta 1376, 91-145, 1998

Applications of DSC on Lipid Bilayers and on Liposomes

Liposomes Incorporating Bioactive Compounds and Biomaterials – PAMAMDendrimer

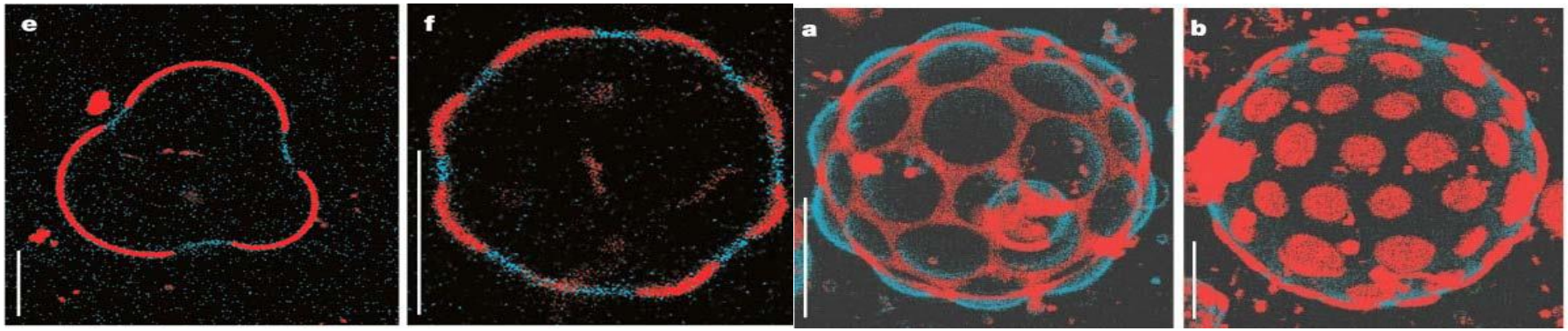


Journal of
Nanoscience and Nanotechnology
Vol. 11, 3764–3772, 2011

A New Chimeric Drug Delivery Nano System (chi-aDDnS) Composed of PAMAM G 3.5 Dendrimer and Liposomes as Doxorubicin's Carrier. *In Vitro* Pharmacological Studies

Konstantinos Gardikis[1], Dimitrios Fessas[2], Marco Signorelli[2], Konstantinos Dimas[3], Chrisiida Tsimplouli[3], Maksim Ionov[4], and Costas Demetzos[1,*]

[1] *University of Athens, School of Pharmacy, Department of Pharmaceutical Technology, Panepistimioupolis, Zografou, 15771, Athens, Greece*
[2] *Università di Milano, DISTAM, via Celoria 2 - 20133 Milano, Italy*
[3] *Division of Pharmacology-Pharmacotechnology, Foundation for Biomedical Research, Academy of Athens, 11527, Athens, Greece*
[4] *Department of General Biophysics, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland*



These are metastable phases that coexist in a non-equilibrium state

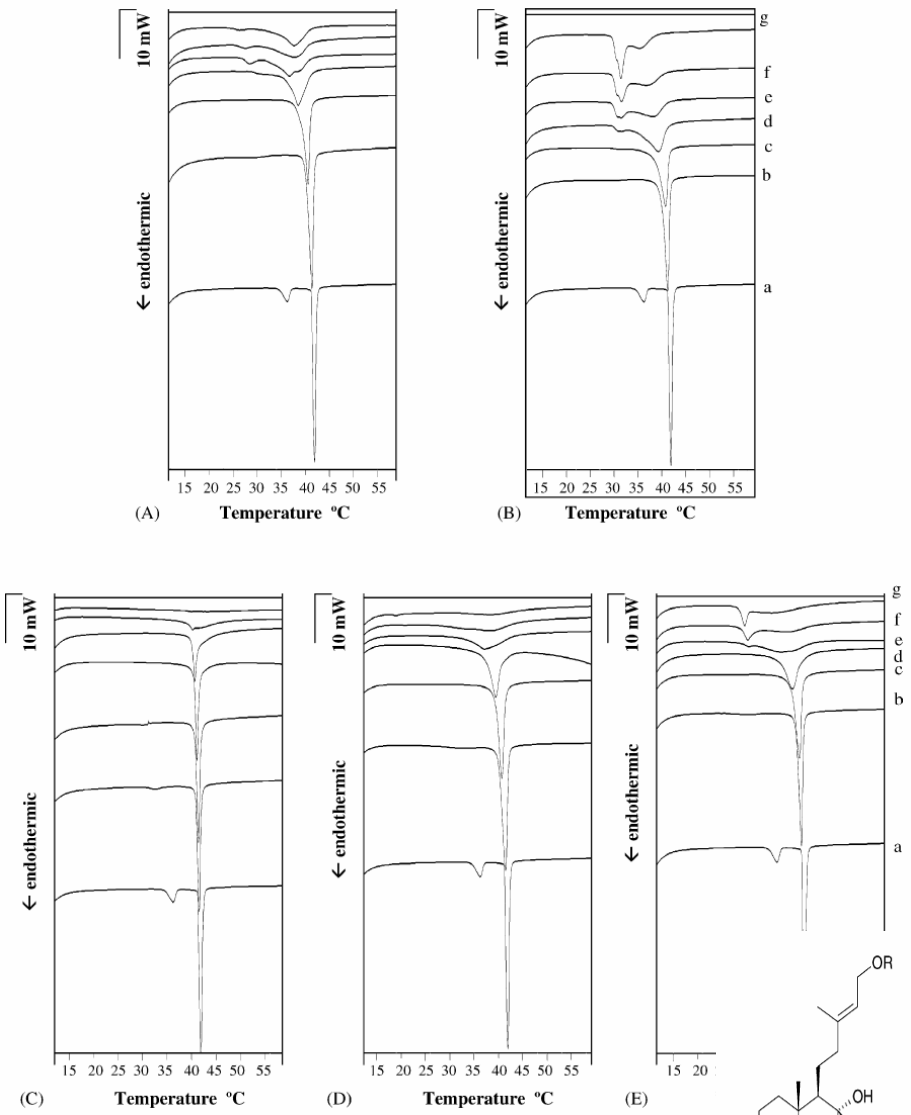
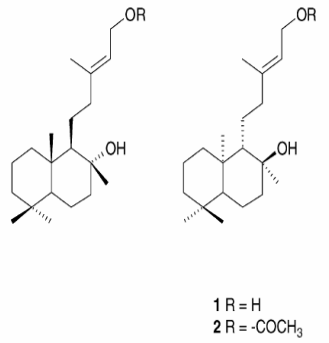


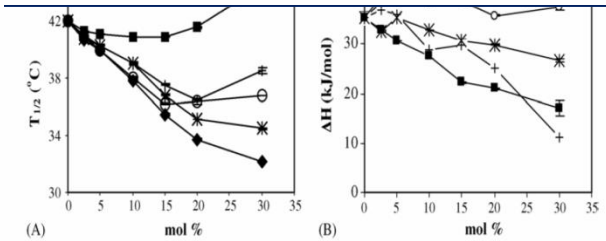
Fig. 2. DSC thermograms of fully hydrated bilayers of DPPC with varying amounts (a, 0 mol%; b, 2.5 mol%; c, 20 mol%; g, 30 mol%) of compound **1** (A), compound **2** (B), cholesterol (C), equimolar mixture of cholesterol a mixture of cholesterol and compound **2** (E).

Table 1
Calorimetric parameters

Sample (x = mol%)	T_{onset} (°C)	S.D.	T_m (°C)	S.D.	$T_{1/2}$ (°C)	S.D.	ΔH (kJ/mol)	S.D.
DPPC	41.28	0.16	41.67	0.15	41.96	0.17	35.48	1.14
DPPC/cholesterol (x=2.5)	40.72	0.01	41.31	0.02	41.24	0.01	32.79	0.08
DPPC/cholesterol (x=5)	40.57	0.01	41.15	0.01	41.09	0.01	30.87	0.02
DPPC/cholesterol (x=10)	40.35	0.01	40.84	0.00	40.86	0.01	27.56	0.13
DPPC/cholesterol (x=15)	39.87	0.00	40.49	0.00	40.90	0.00	22.24	0.24
DPPC/cholesterol (x=20)	38.64	0.03	40.18	0.03	41.55	0.02	21.10	0.14
DPPC/cholesterol (x=30)	38.83	0.89	41.20	0.02	44.48	0.43	17.21	1.51
DPPC/**1** (x=2.5)	40.02	0.01	41.07	0.02	40.86	0.01	38.64	0.06
DPPC/**1** (x=5)	38.96	0.04	40.39	0.00	39.98	0.01	39.28	0.10
DPPC/**1** (x=10)	35.77	0.12	38.38	0.02	38.05	0.01	38.56	0.17
DPPC/**1** (x=15)	32.45	0.18	36.52	0.09	36.20	0.05	38.74	0.20
DPPC/**1** (x=20)	31.03	0.31	37.69	0.11	36.37	0.19	35.83	0.09
DPPC/**1** (x=30)	33.61	0.04	37.50	0.06	36.76	0.02	37.52	0.66
DPPC/Chol/**1** (x=1.25+1.25)	39.91	0.02	41.21	0.04	40.95	0.01	37.04	0.10
DPPC/Chol/**1** (x=2.5+2.5)	39.22	0.02	40.47	0.01	40.27	0.01	35.98	0.11
DPPC/Chol/**1** (x=5+5)	37.31	0.03	39.27	0.03	39.06	0.02	28.88	0.23
DPPC/Chol/**1** (x=7.5+7.5)	32.84	0.10	37.07	0.12	37.52	0.04	29.84	0.12
DPPC/Chol/**1** (x=10+10)	28.75	0.22	38.54	0.14	36.49	0.03	25.21	0.15
DPPC/Chol/**1** (x=15+15)	31.71	0.49	38.58	0.09	38.52	0.18	11.06	0.33
DPPC/**2** (x=2.5)	39.76	0.01	40.99	0.02	40.71	0.00	39.81	0.17
DPPC/**2** (x=5)	38.64	0.08	40.60	0.05	39.99	0.05	38.88	0.21
DPPC/**2** (x=10)	34.33	0.24	39.19	0.08	37.76	0.06	39.14	0.29
DPPC/**2** (x=15)	29.68	0.26	31.39	0.06	35.45	0.09	40.19	0.22
DPPC/**2** (x=20)	29.55	0.01	31.45	0.07	33.65	0.11	40.57	0.09
DPPC/**2** (x=30)	29.83	0.23	31.31	0.02	32.17	0.02	39.82	0.04
DPPC/Chol/**2** (x=1.25+1.25)	40.04	0.01	40.96	0.00	40.79	0.00	32.71	0.01
DPPC/Chol/**2** (x=2.5+2.5)	39.08	0.03	40.63	0.03	40.29	0.02	35,41	0.24
DPPC/Chol/**2** (x=5+5)	36.63	0.05	39.25	0.05	38.98	0.02	32.91	0.10
DPPC/Chol/**2** (x=7.5+7.5)	30.21	0.31	36.85	0.20	36.82	0.04	30.80	0.22
DPPC/Chol/**2** (x=10+10)	28.63	0.07	30.14	0.04	35.11	0.03	29.96	0.12
DPPC/Chol/**2** (x=15+15)	28.27	0.10	29.56	0.04	34.48	0.04	26.72	0.29

T_{onset}, temperature at which the thermal effect starts; T_m, temperature at which heat capacity (ΔC_p) at constant pressure, is maximum; $T_{1/2}$, ich the transition is half completed; ΔH, transition enthalpy normalised per mole of DPPC; **1**, labd-13(E)-ene-8α,15-diol; **2**, E)-ene-8α-ol-15-yl-acetate.





and ΔH (J/mol DPPC) values of DPPC bilayers vs. concentrations of compound **1** (○), **2** (♦), cholesterol (■), equimolar mixture (+) and equimolar mixture of cholesterol/**2** (★).

DSC thermograms of fully hydrated bilayers of DPPC with varying amounts (a: 0 mol%, b: 2.5 mol%, c: 5 mol%, d: 10 mol%, e: 15 mol%, f: 20 mol%, g: 30 mol%) of compound 1 (A), compound 2 (B), cholesterol (C), equimolar mixture of cholesterol and compound 1 (D) and equimolar mixture of cholesterol and compound 2 (E). (Adapted with permission from Elsevier, from *Chem Phys Lipids,* 2005 138, 1-11. Labdane-type diterpenes: thermal effects on phospholipid bilayers, incorporation into liposomes and biological activity. Matsingou, C; Hatziantoniou, S; Georgopoulos, A; Dimas, K; Terzis, A; Demetzos, C.)

Metastable phases play an important role in the behavior of lipid membranes. Topics touched upon include the experimental detection of domains, their composition, domain induction, properties of rafts (a special form of domain), and **the relationship of metastable phases to human diseases**.

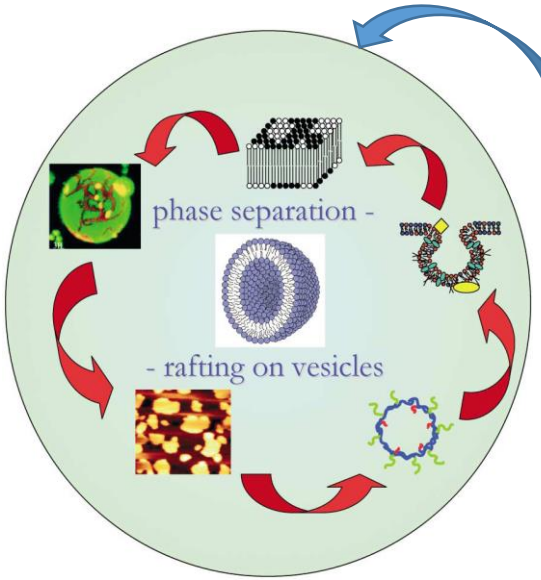
The metastable phases, can cause biophysical and thermodynamic abnormalities that could be defined as ' ***biophysical disease factors***'

'***Pharmaceutical Nanotechnology. Funtamentals and practical applications'***,

by

Costas Demetzos

Springer, 2016



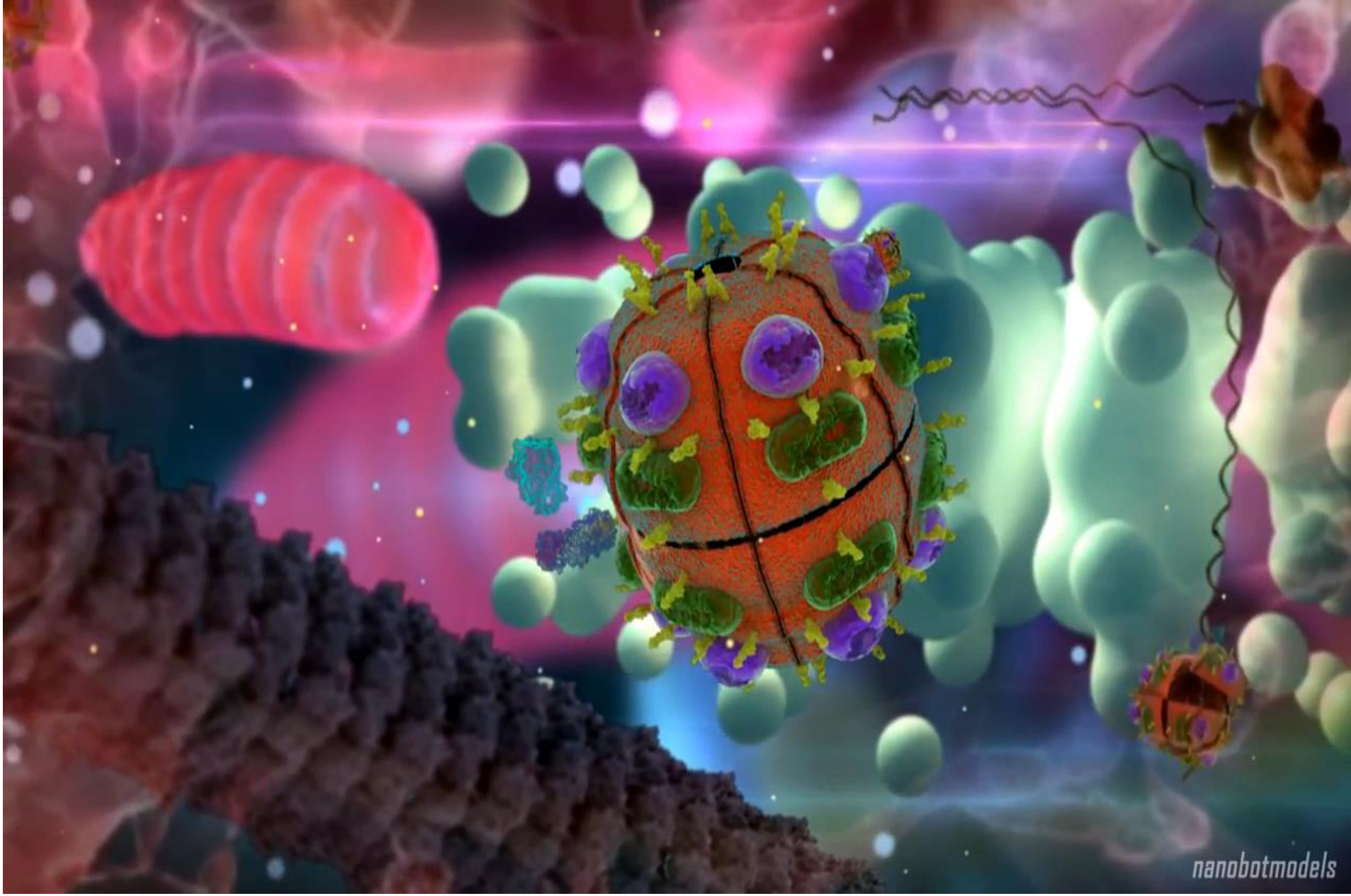
Domains and Rafts in Lipid Membranes

by

Wolfgang H. Binder,* Veronique Barragan, and Fredric M. Menger

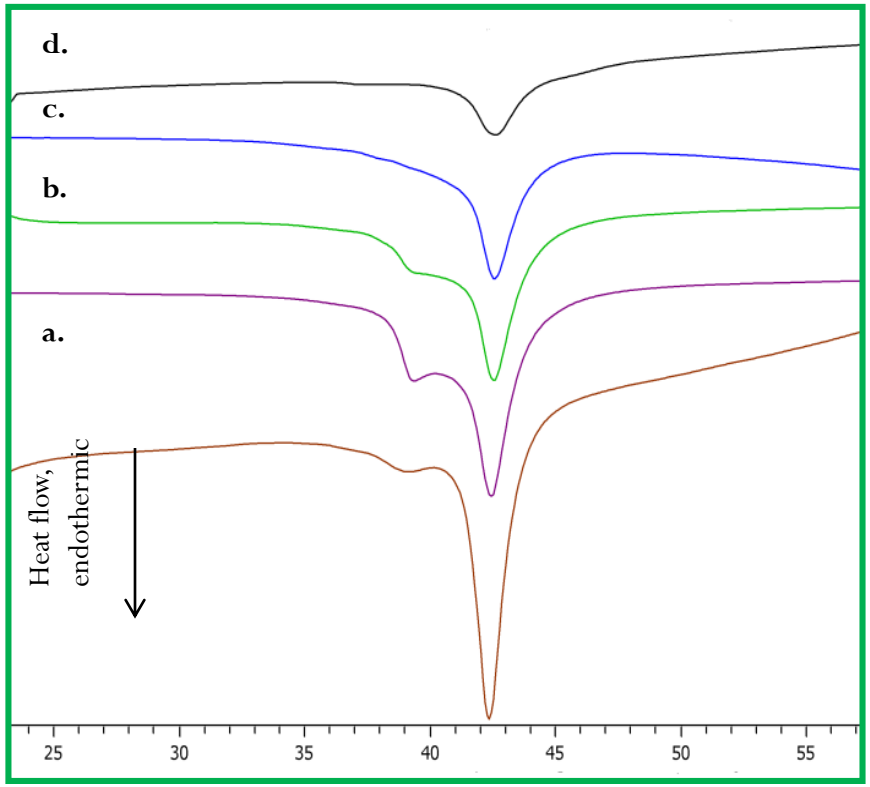
Angewandte Chemie

Keywords:
block copolymers · domains · lipid membranes · rafts · vesicles

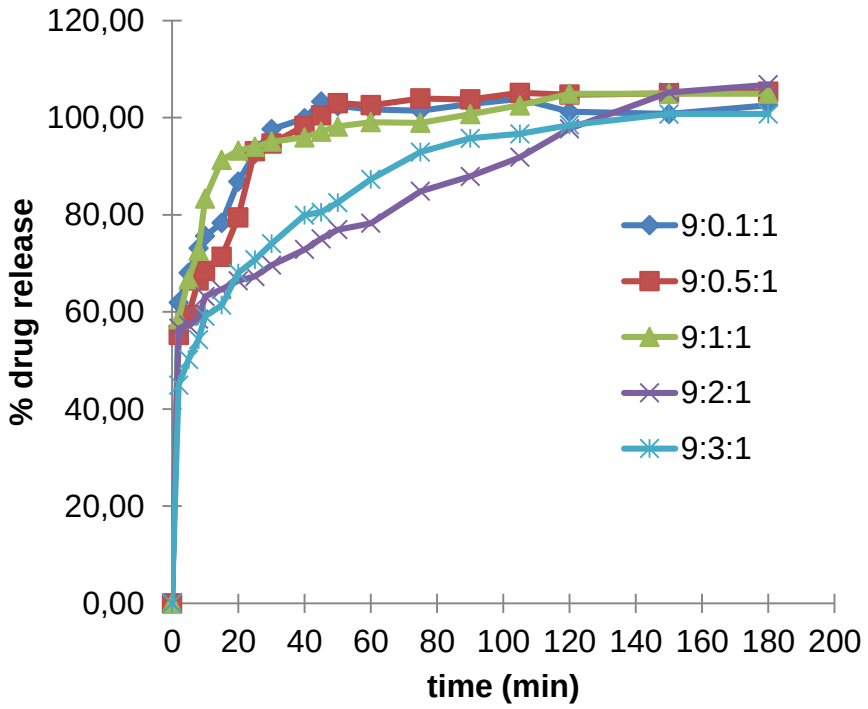

nanobotmodels

Correlation of the thermotropic behaviour of *chimeric* liposomal nanosystems incorporating drug with their release profile.

The *chimeric* liposomal nanosystems is composed of phospholipids (DPPC) and of a block co-polymer (MPOx). The lyotropic effect of the nanosystem (biophysical behaviour) is driven by the percentage composition of the polymeric guest



DSC heating scans of DPPC:MPOx **a.** 9:0 **b.** 9:0.1 **c.** 9:05 **d.** 9:1 and **e.** 9:3 molar ratio liposomes.



Ref. Pippa N., Dokumetzidis A., Pispas S., Demetzos C., et al. Intern. Journal of Pharmac 2014.

The cartoon represents the structural organization of the lipidic bilayer

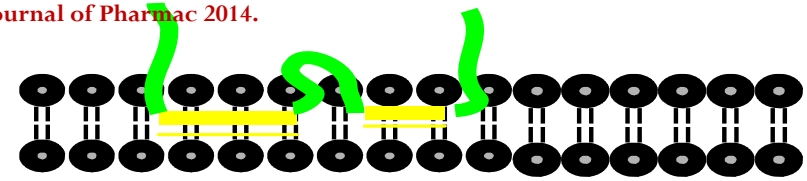


Table 2. Liposomal formulations present in clinical trials.

SN	Products	Administration	Active Agent	Lipid Composition	Indication	Company
Phase III						
1.	Arikace	Aerosol delivery	Amikacin	DPPC and cholesterol	Lung infections	Transave Inc.
2.	Stimuvax	s.c.	Tecemotide	Cholesterol, DMPG, DPPC	Non-small cell lung cancer	Oncothyreon Inc.
3.	T4N5 liposomal lotion	Topical	T4 endonuclease V	Egg lecithin	Xeroderma pigmentosum	AGI Dermatics Inc.
4.	Liprostin	i.v.	Prostaglandin E-1 (PGE-1)	Unknown	Restenosis after angioplasty	Endovasc Inc.
5.	ThermoDox	i.v.	Doxorubicin	DPPC, Myristoyl stearyl phosphatidylcholine and DSPE-*N*-[amino(polyethylene glycol)-2000]	Hepatocellular carcinoma and also recurring chest wall breast cancer	Celsion
6.	Lipoplatin	i.v.	Cisplatin	DPPG, soy phosphatidyl choline, mPEG-distearoyl phosphatidylethanolamine lipid conjugate and cholesterol	Non-small cell lung cancer	Regulon Inc.
Phase II						
7.	Aroplatin	i.v.	Platinum analogue cis-(trans-R,R-1,2-diaminocyclohexane) bis (neodecanoato) platinum (II)	DMPC and DMPG	Metastatic colorectal cancer	Agenus Inc.
8.	Liposomal annamycin	i.v.	Semi-synthetic doxorubicin analogue annamycin	DMPC and DMPG	Relapsed or refractory acute myeloid leukaemia	Aronex Pharmaceuticals
9.	SPI-077	i.v.	Cisplatin	Soybean phosphatidylcholine, cholesterol	Lung, head and neck cancer	Alza Corporation
10.	OSI-211	i.v.	Lurtotecan	HSPC and cholesterol	Ovarian, head and neck cancer	OSI Pharmaceuticals
11.	S-CKD602	i.v.	Potent topoisomerase I inhibitor	Phospholipids covalently bound to mPEG	Cancer	Alza Corporation
12.	LE-SN38	i.v.	Irinotecan's active metabolite	DOPC, cholesterol and cardiolipin	Advanced colorectal cancer	NeoPharm Labs Ltd.
13.	LEP-ETU	i.v.	Paclitaxel	DOPC, cholesterol and cardiolipin	Cancer	NeoPharm Labs Ltd.
14.	Endotag-I	i.v.	Paclitaxel	DOTAP: DOPC: Paclitaxel	Breast and pancreatic cancers	Medigene
15.	Atragen	i.v.	All-trans retinoic acid	DMPC and soybean oil	Hormone-resistant prostate cancer, renal cell carcinoma and acute myelogenous leukaemia	Aronex Pharmaceuticals

Ref. Liposomal Formulations in Clinical Use: An Updated Review by Upendra Bulbake †, Sindhu Doppalapudi †, Nagavendra Kommineni and Wahid Khan , *Pharmaceutics*, 9, 12, 2017

Table 2. *Cont.*

SN	Products	Administration	Active Agent	Lipid Composition	Indication	Company
Phase I						
16.	LEM-ETU	i.v.	Mitoxantrone	DOPC, cholesterol and cardiolipin	Various cancers	NeoPharm Labs Ltd.
17.	Liposomal Grb-2	i.v.	Antisense oligodeoxynucleotide growth factor receptor bound protein 2 (Grb-2)	Unknown	Hematologic malignancies	Bio-Path holdings
18.	INX-0125	i.v.	Vinorelbine tartrate	Cholesterol and sphingomyelin	Advanced solid tumours	Inex Pharmaceuticals
19.	INX-0076	i.v.	Topotecan	Cholesterol and sphingomyelin	Advanced solid tumours	Inex Pharmaceuticals
20.	TKM-080301	Hepatic intra-arterial administration	PLK1 siRNA	Unique LNP technology (formerly referred to as stable nucleic acid-lipid particles or SNALP)	Neuroendocrine tumours	Tekmira Pharmaceuticals
21.	Atu027	i.v.	PKN3 siRNA	AtuFECT01	Pancreatic cancer	Silence Therapeutics
22.	2B3-101	i.v.	Doxorubicin	Glutathione PEGylated liposomes	Solid tumours	2-BBB therapeutic
23.	MTL-CEBPA	i.v.	CEBPA siRNA	SMARTICLES® liposomal nanoparticles	Liver cancer	MiNA Therapeutics
24.	ATI-1123	i.v.	Docetaxel	Protein stabilizing liposomes (PSL™)	Solid tumours	Azaya therapeutic
25.	LiPlaCis	i.v.	Cisplatin	The lipid composition of the LiPlasomes is tailored to be specifically sensitive to degradation by the sPLA2 enzyme	Advanced solid tumours	Oncology Venture
26.	MCC-465	i.v.	Doxorubicin	DPPC, cholesterol and maleimidated palmitoyl phosphatidyl ethanolamine; immunoliposomes tagged with PEG and the F(ab′)2 fragment of human monoclonal antibody GAH	Metastatic stomach cancer	Mitsubishi Tanabe Pharma Corporation
27.	SGT-53	i.v.	p53 gene	Cationic lipids complexed with plasmid DNA encoding wild-type p53 tumour suppressor protein	Various solid tumours	SynerGene Therapeutics
28.	Alocrest	i.v.	Vinorelbine	Sphingomyelin/cholesterol (OPTISOME™)	Breast and lung cancers	Spectrum Pharmaceuticals

DMPG (Dimyristoyl phosphatidylglycerol); DPPC (Dipalmitoyl phosphatidylcholine); DPPG (Dipalmitoyl phosphatidylglycerol); DMPC (dimyristoyl phosphatidylcholine); HSPC (hydrogenated soy phosphatidylcholine); PEG (polyethylene glycol); mPEG (methoxy polyethylene glycol); DOPC (dioleoylphosphatidylcholine); DSPE (distearoyl-sn-glycero-phosphoethanolamine); i.v. intravenous.

Ref. Liposomal Formulations in Clinical Use: An Updated Review by Upendra Bulbake †, Sindhu Doppalapudi †, Nagavendra Kommineni and Wahid Khan , *Pharmaceutics* 9, 12, 2017

FINAL Conclusions

Liposomes are considered as attractive drug delivery nanosystems and they are also used as nanotechnological platforms in many application (cosmetics, food technology, etc)

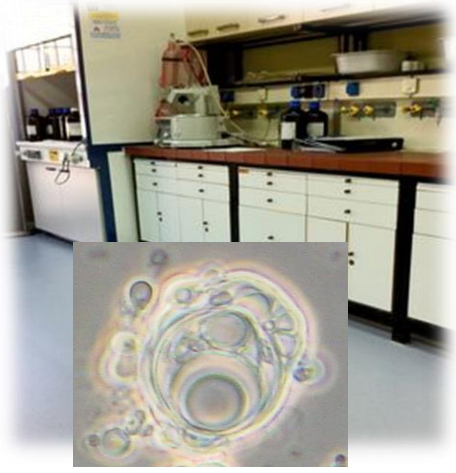
The behavior of bio-membranes as highly complex bio-systems, as well as of artificial lipidic membranes, leads to the formation of **metastable phases.**

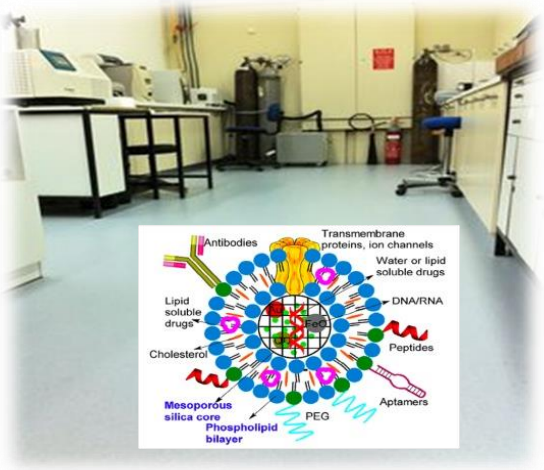
By mimicking biological functions and by 'reproducing' the metastable phases of living cells by constructing artificial bio-systems at nano-dimension (i.e. **liposomes**) we can create 'smart' bio-nanosystems that are able to mimic living cell membranes and can be used in pharmaceutical formulations.

Ref. 1. Costas Demetzos *'Pharmaceutical Nanotechnology. Funtamendals and practical Applicatio*n', 2016, Springer
2. W. H. Binder, V. Barragan, and F. M. Menger in the Journal of *Angew. Chem. Ind. Ed*, 2003, 42, 5802-5827

Laboratory of Pharmaceutical Nanotechnology University of Athens, Greece

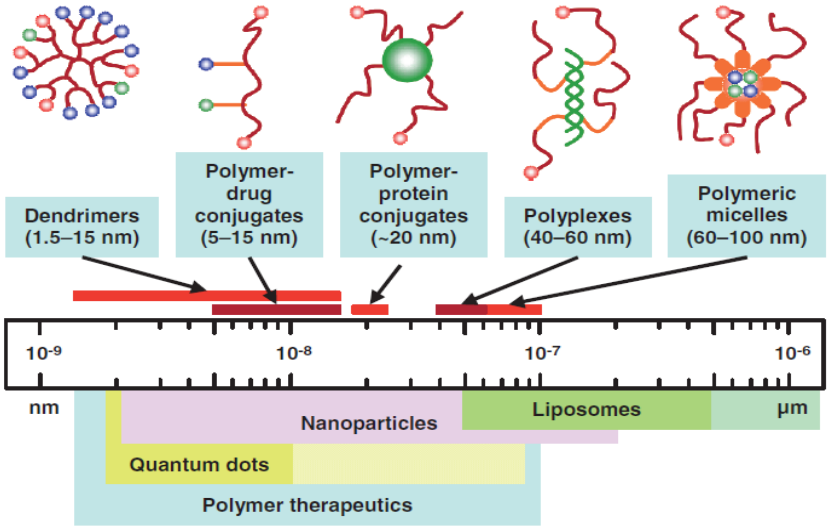
http://nanopharmlab.gr/index.php/en/



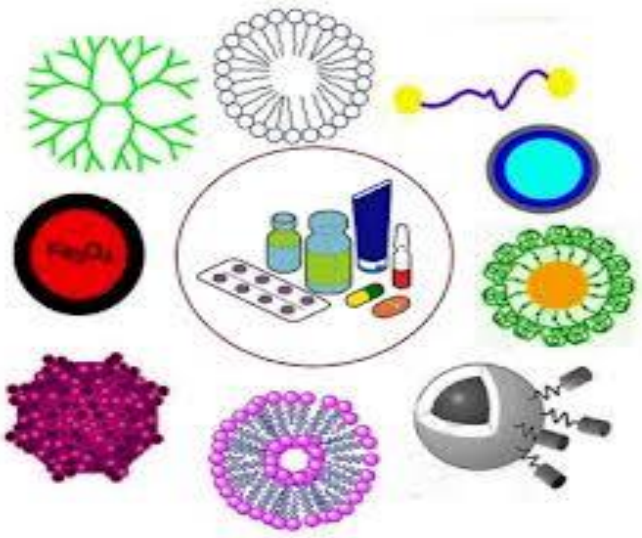


Research Interests:

- Pharmaceutical nanotechnology: nanocarriers/nanovehicles
  - Liposomes
  - Lipid nanoparticles
  - Polymersomes
  - Micelles
  - Nanoemulsions
  - Niosomes
  - Hydrogels
  - Liquid crystals
  - Chimeric /mixed systems



- Physicochemical characterization
  - Thermal Analysis
  - Dynamic Light Scattering
  - Electrophoretic Light Scattering
- Drug Release studies
  - Drug release Kinetics



SUCCESS STORIES :

- More than **150 publications** in the field of pharmaceutical nanotechnology (2002-2017)
- More than **300 presentations and invited lectures** into scientific meetings and International Congresses
- More than **10 patents** in the field of nanocarriers
- **European Research projects:** **in progress**

  in collaboration with 6 European laboratories
- **International and National Awards**
- National and European Scholarships

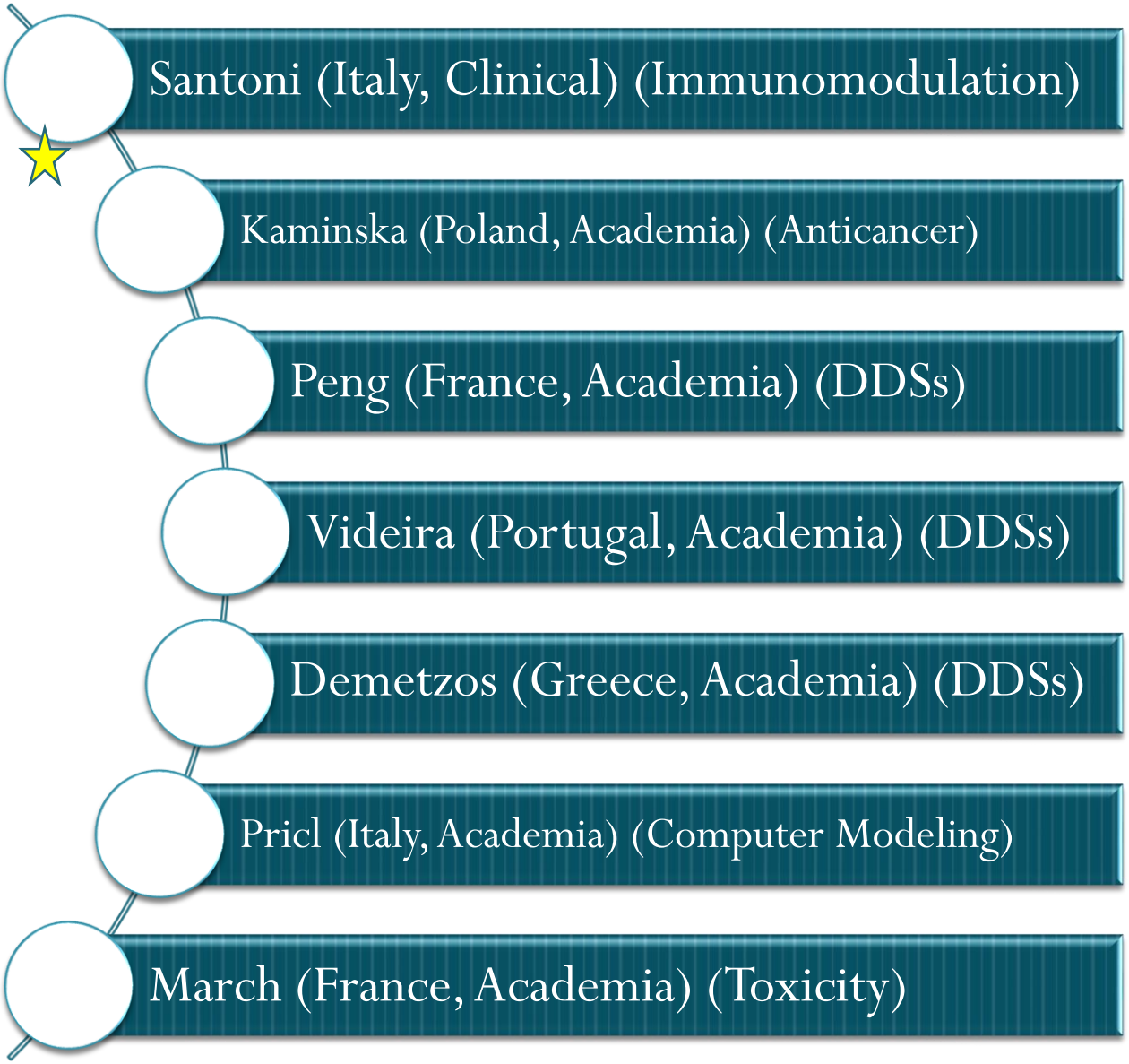
  of the members of the Lab
- **Collaboration with Pharmaceutical Industries**

NANOGLIO
NANOTECHNOLOGY BASED IMMUNOTHERAPY FOR GLIOBLASTOMA
EURONANOMED 2

EURONANOMED II
Joint Transnational Call for Proposals (2016) for "EUROPEAN INNOVATIVE RESEARCH & TECHNOLOGICAL

DEVELOPMENT PROJECTS IN NANOMEDICINE"
Nanotechnology based immunotherapy for glioblastoma (NANOGLIO)

7 Partners


Santoni (Italy, Clinical) (Immunomodulation)
Kaminska (Poland, Academia) (Anticancer)
Peng (France, Academia) (DDSs)
Videira (Portugal, Academia) (DDSs)
Demetzos (Greece, Academia) (DDSs)
Pricl (Italy, Academia) (Computer Modeling)
March (France, Academia) (Toxicity)

SUCCESS STORIES :

COLLABORATION WITH PHARMACEUTICAL INDUSTRIES

- Complete Physicochemical characterization (stability studies)
- Design and Development of nanocarriers
- Encapsulation of bioactive substances into nanocarriers
- Preparation of the dossier of the final product/final formulation
- Education of the opinion leaders
- Dissemination: papers and international conferences

- Generic medicines: liposomal amphotericin B
- Kits with biopolymers
- Cosmeceuticals: cerasomes
- Nutraceuticals: liposomal vitamins and liposomal natural products

Advanced Science, Engineering and Medicine
Vol. 9, 1–7, 2017
www.aspbs.com/asem

Evaluation of the Physicochemical Characteristics of Liposomal Formulations of *Dr. Formulas'* Food Supplements

Natassa Pippa[1], Nikolaos Fikioris[2], and Costas Demetzos[1,*]

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Physicochemical Characteristics of Liposomal Formulations of Doctor's Formulas' Food Supplements in Biorelevant Dispersion Media

Natassa Pippa[1], Nikolaos Fikioris[2], and Costas Demetzos[1,*]

[1] *Department of Pharmaceutical Technology, Faculty of Pharmacy, PanepistimioupolisZografou 15771, National and Kapodistrian University of Athens, Athens, Greece*
[2] *In Touch Health, 11 Merlin, Athens, Greece*

Peer-review papers on the scientific excellence of the final formulations:

Case Report

Efficacy of a New Heparan Sulfate Mimetic Dressing in the Healing of Foot and Lower Extremity Ulcerations in Type 2 Diabetes: A Case Series

The International Journal of Lower Extremity Wounds
2016, Vol. 15(1) 63–67

DOI: 10.1177/1534734616629302
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Nikolaos Papanas, MD[1], Costas Demetzos, PhD[2], Natassa Pippa, PhD[2], Efstratios Maltezos, MD[1], and Nicholas Tentolouris, MD[2]

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Vol. 7, 1–5, 2015
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Advanced Science, Engineering and Medicine
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Cerasomes as Innovative Excipients in Cosmetic Product "Pregnaderm Extreme Hydration Body Cream": A Physicochemical Study

Natassa Pippa[1], Grigoris Mountrichas[2], Ioulia Tseti[2], and Costas Demetzos[1,*]

[1] *Faculty of Pharmacy, Department of Pharmaceutical Technology, University of Athens, Panepistimioupolis, Zografou, Athens 15771, Greece*
[2] *Intermed Pharmaceutical Laboratories SA, 27 Kaliftaki Str., Kifissia 14564, Greece*

Innovative Excipients and Formulation Platforms in Cosmetic Product Series for Acne (ACNOFIX®): The Physiochemical Characteristics of Cosmeceutical Vehicle

Natassa Pippa[1], Ioulia Tseti[2], and Costas Demetzos[1,*]

[1] *Faculty of Pharmacy, Department of Pharmaceutical Technology, University of Athens, Panepistimioupolis, Zografou, Athens 15771, Greece*
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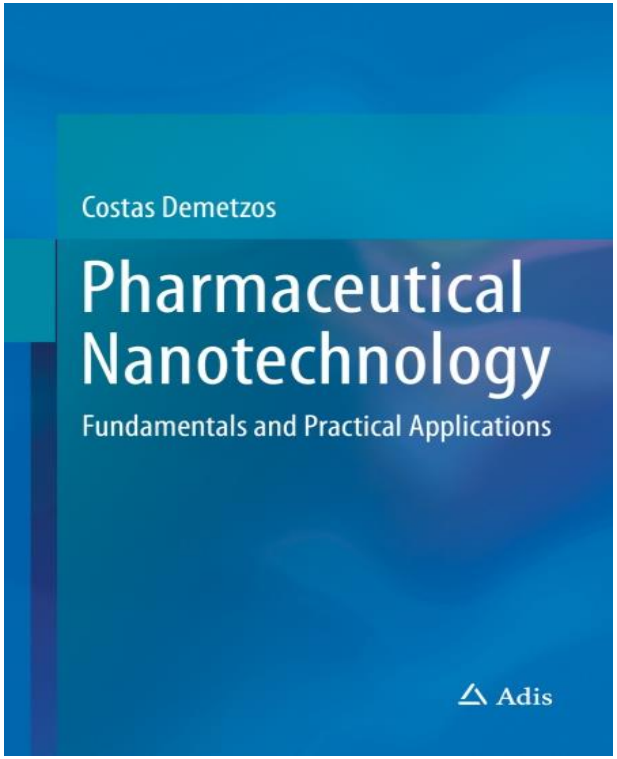
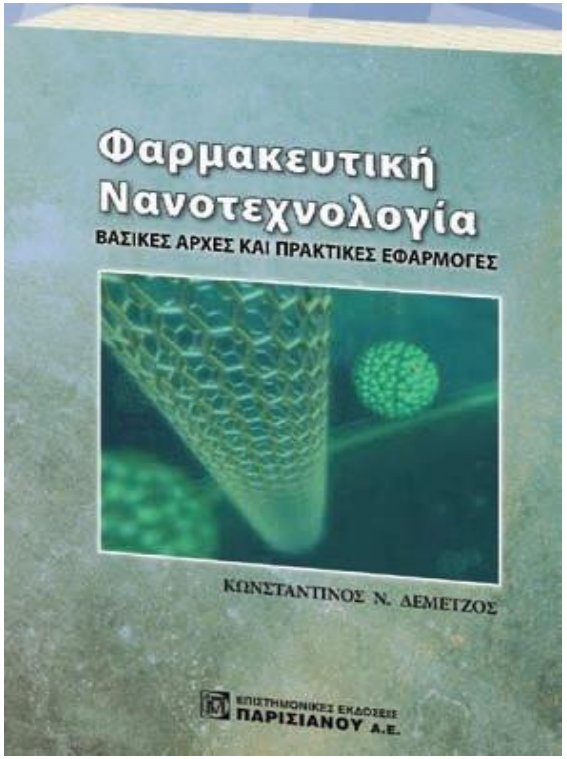
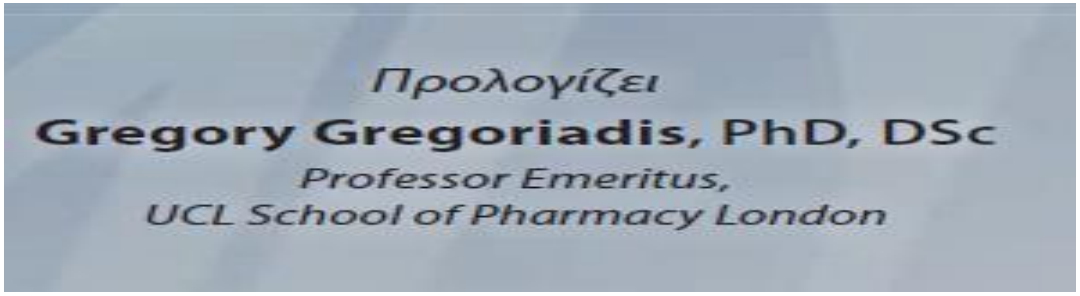




"(…) It has been therefore a great personal pleasure to write the Preface of the present book by Professor Costas Demetzos. His monograph, 'Pharmaceutical Nanotechnology', is a unique publication (…)"



Thank you for your kind attention

Vincent van Gogh
Branch of almond – tree in flowers, 1890