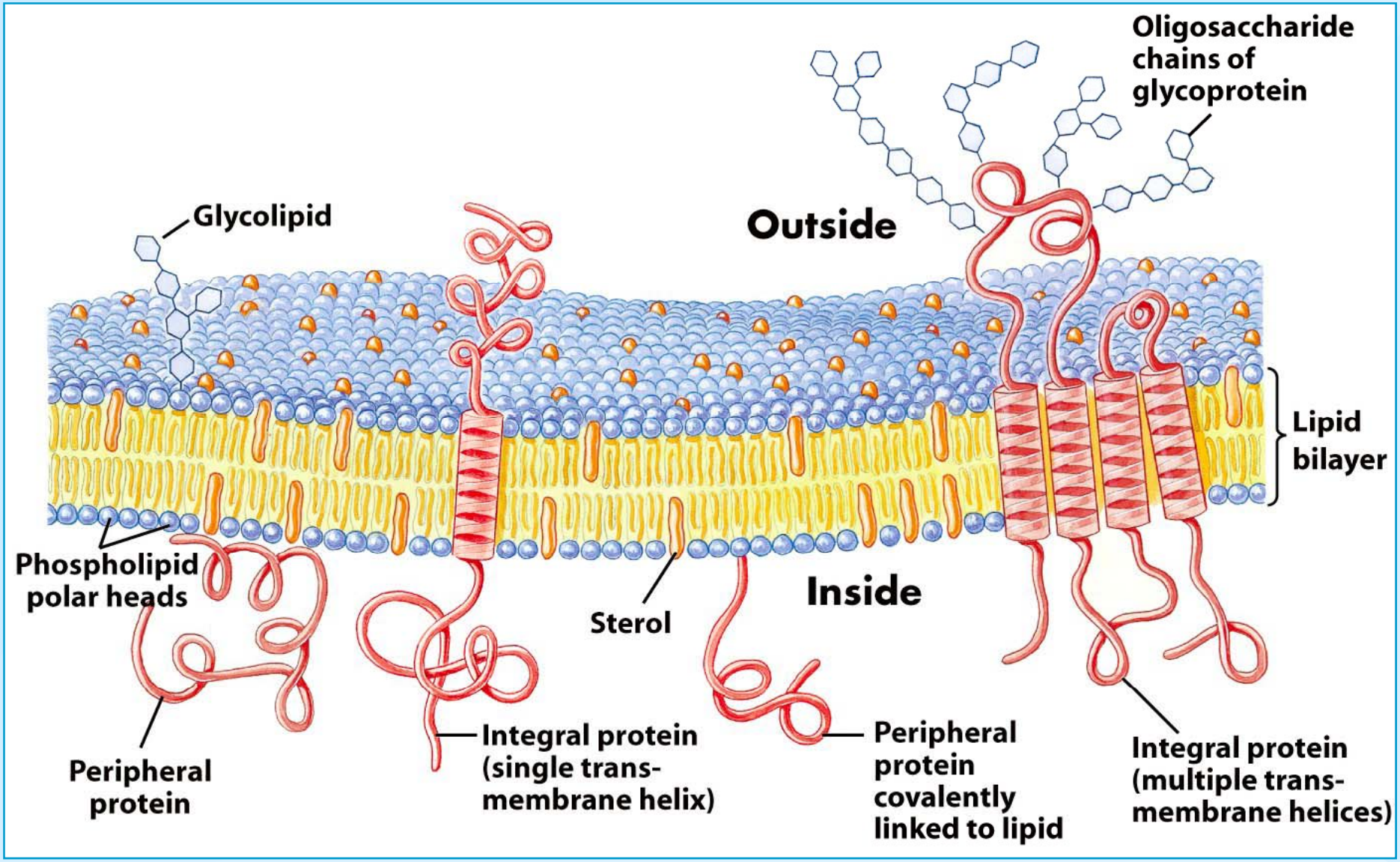
VPP PROJEKTS Nº 7
DNS, RNS, proteīnu, peptīdu un mazmolekulāro medicīnisko preparātu piegādes sistēmas izstrādāšana

Amfifīli pašasociējošies azīni nukleīnskābju un mazmolekulāro medicīnisko preparātu piegādei

15.11.2011.

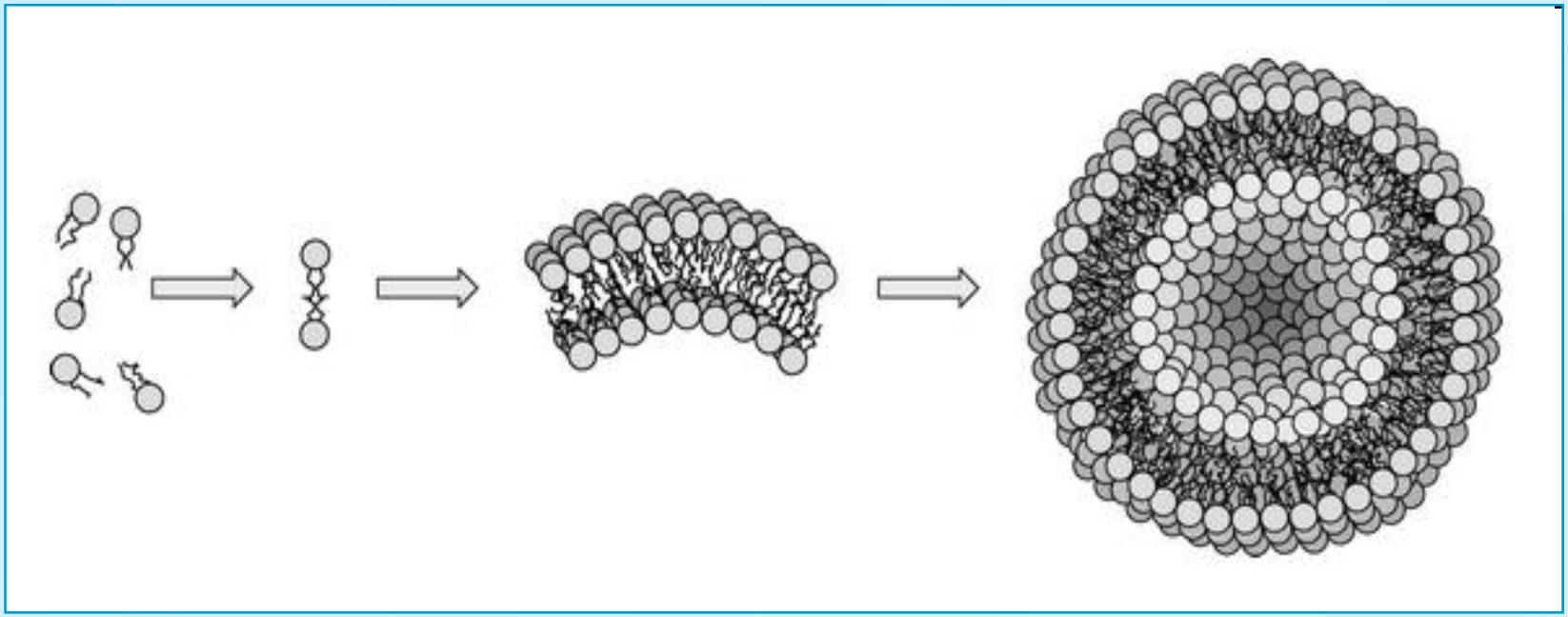

Oligosaccharide chains of glycoprotein
Glycolipid
Outside
Lipid bilayer
Phospholipid polar heads
Sterol
Inside
Peripheral protein
Integral protein (single trans-membrane helix)
Peripheral protein covalently linked to lipid
Integral protein (multiple trans-membrane helices)

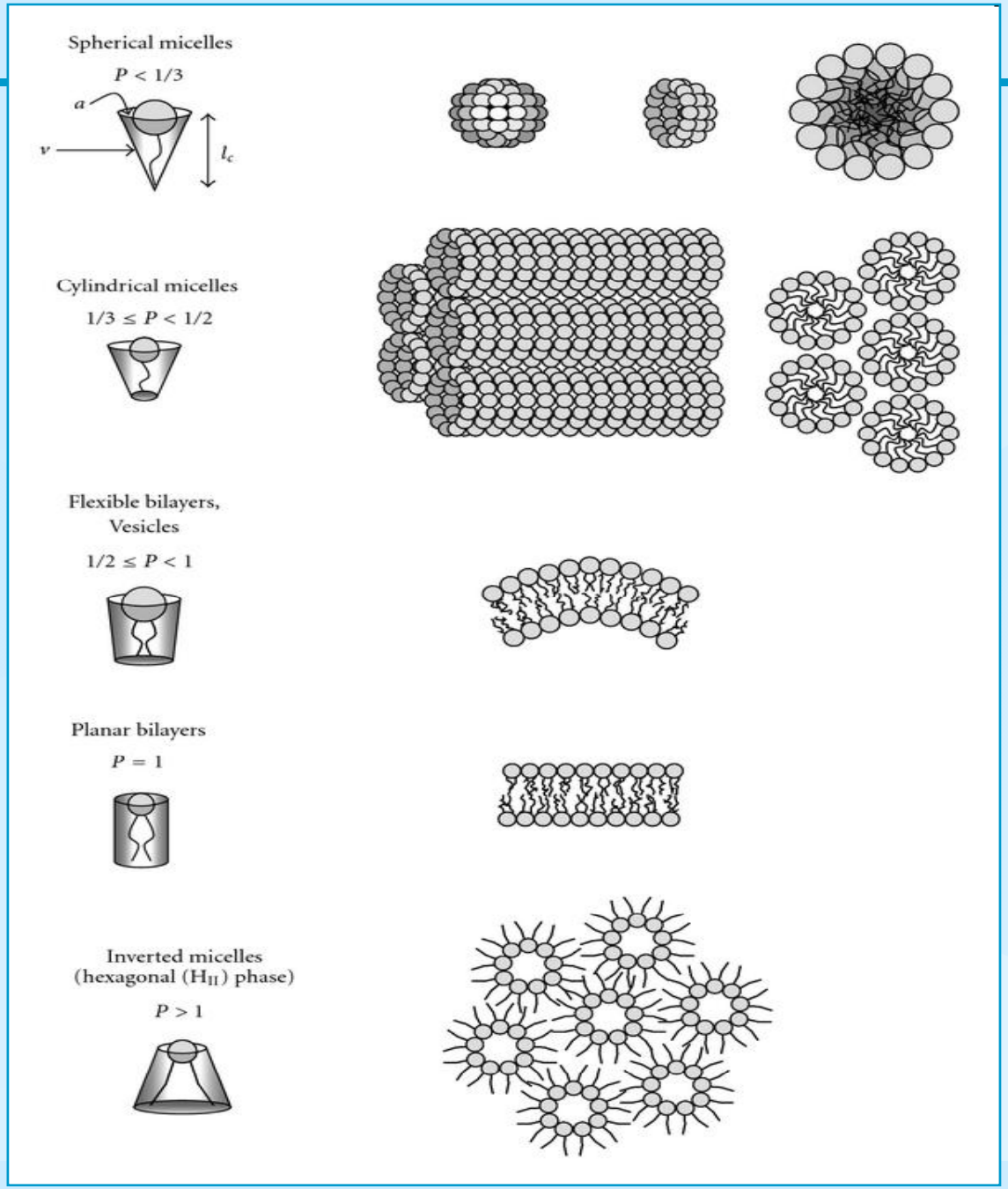
Lipids : **Membrane Composition and Architecture**

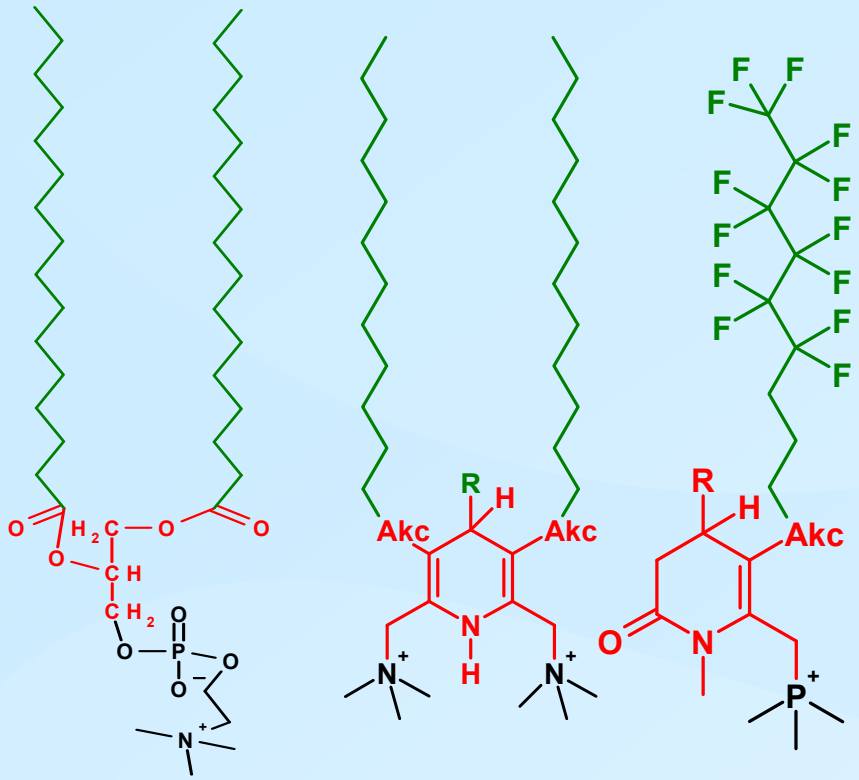
Biological membranes play many critical roles:

- insulation from environment (**chemical**, electric, temperature)
- compartmentalization
- communication and signaling
- selective transport
- cell motility and deformability
- architectural organization of complex protein networks
- (e.g. energy production -> photosynthesis and cellular respiration
- cell division and proliferation

Certain amphipathic lipid molecules in aqueous solution spontaneously form leaflets, then bilayer membranes, and eventually liposomes. Balazs,2011




Spherical micelles
P < 1/3
a
v
l_c
Cylindrical micelles
1/3 ≤ P < 1/2
Flexible bilayers,
Vesicles
1/2 ≤ P < 1
Planar bilayers
P = 1
Inverted micelles
(hexagonal (H_{II}) phase)
P > 1



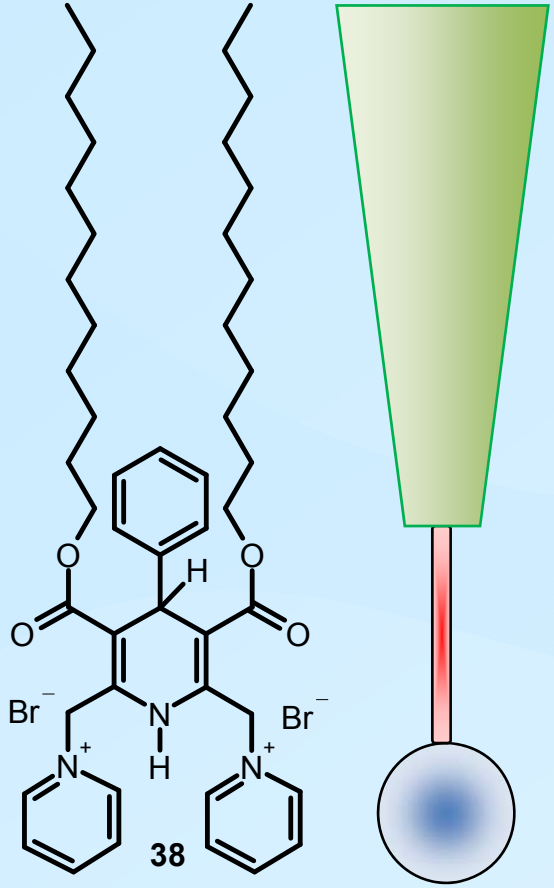
DPPC DHP THP_n

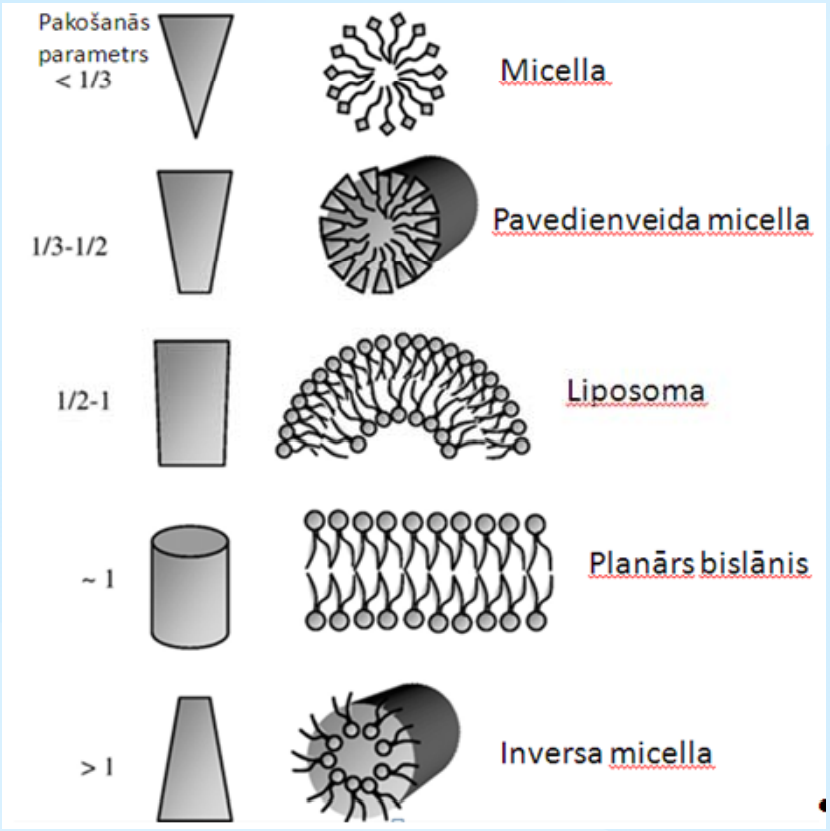
Hidrofobā, lipofīlā daļa

Savienotājposms

Hidrofīlā, jonogēnā daļa

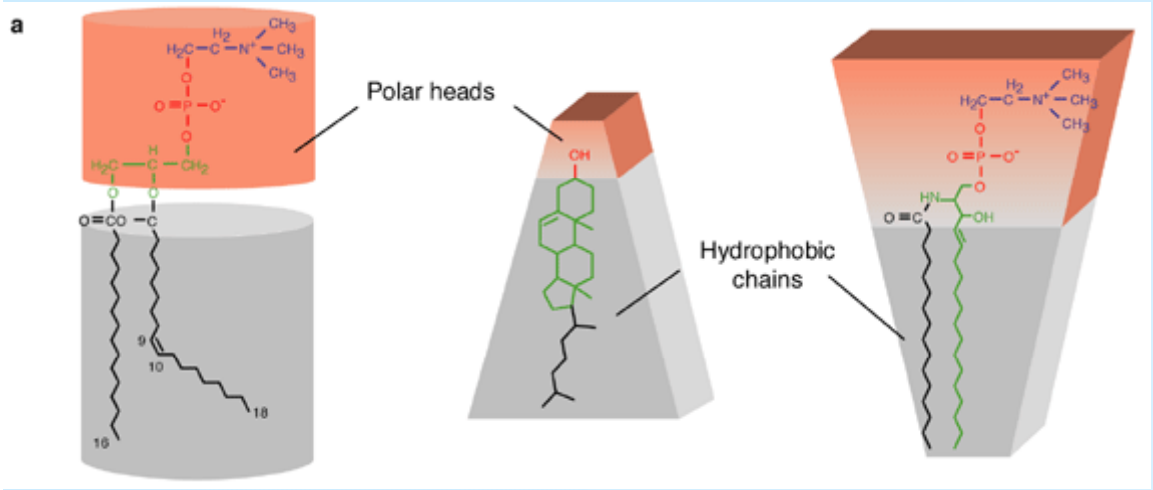
Nanoagregātu veidi

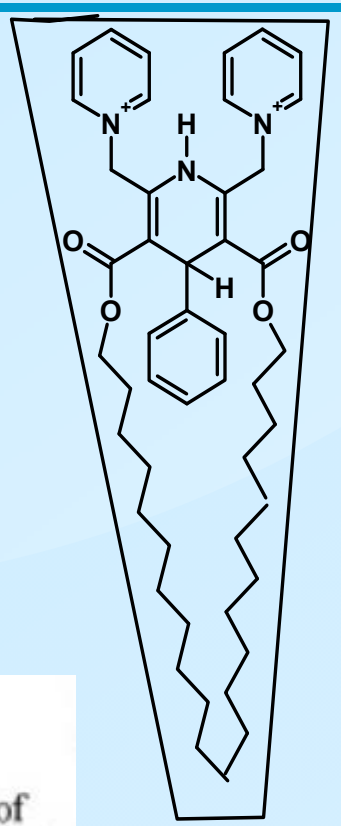


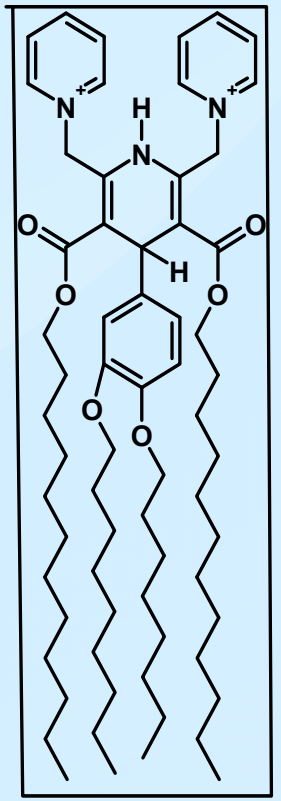


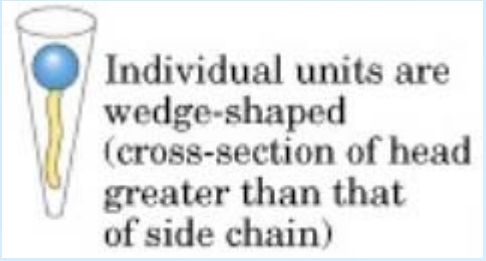
$$p = \frac{v}{a \times l}$$

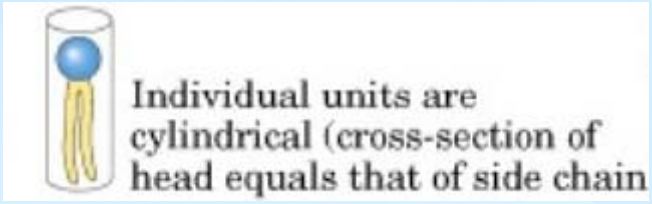
l – hidrofobās alkilķēdes garums
v – tilpums, ko aizņem molekulas hidrofobā daļa
a – molekulas polārās daļas šķērsgriezuma laukums







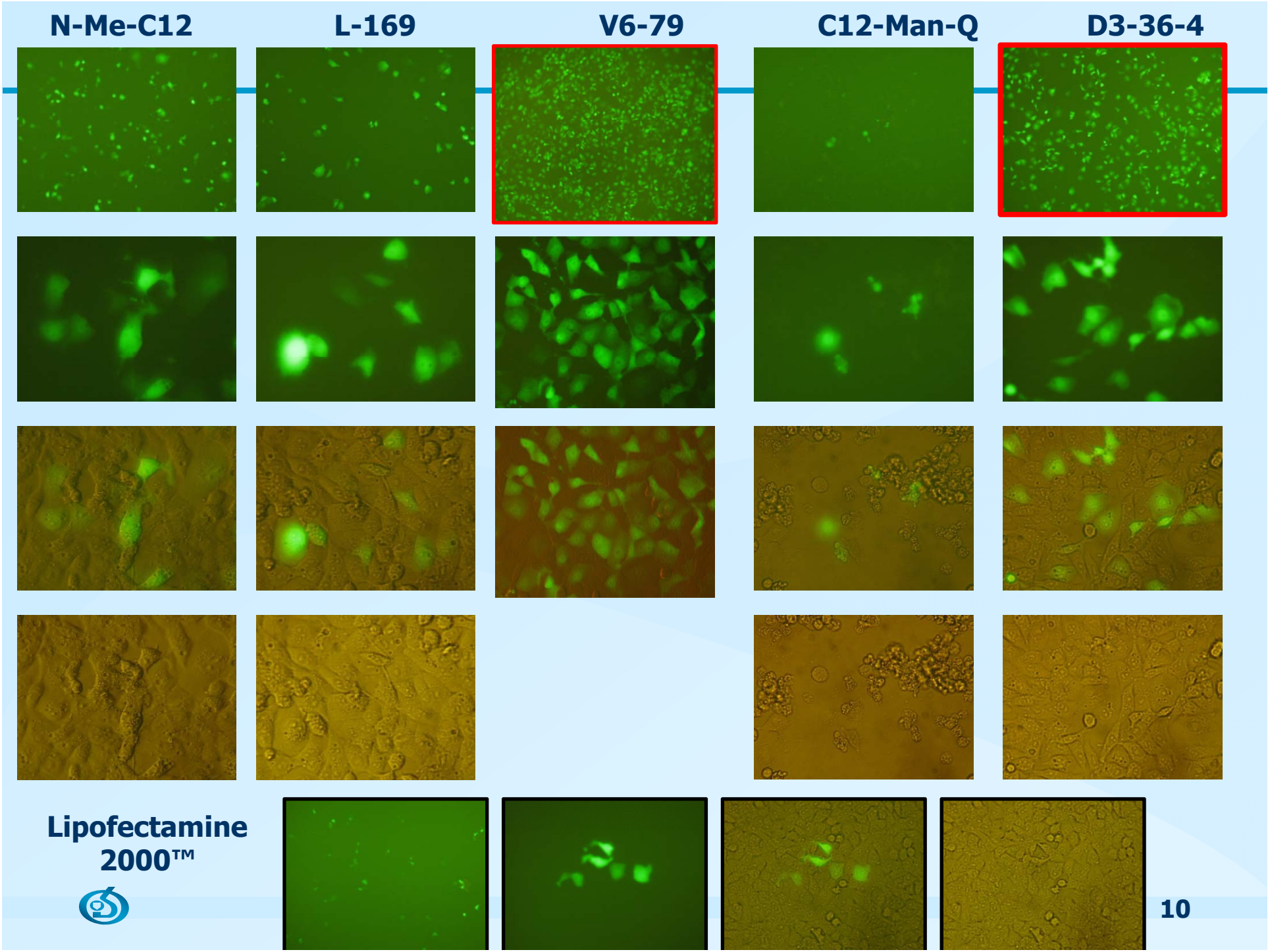




"Molecular shape of the cationic lipid controls the structure of cationic lipid/DOPE-DNA complexes and the efficiency of gene delivery"
Jarmila Misterová, Anno Wagenaar, Marc C. A. Stuart, Evgeny Polushkin, Gerrit ten Brinke, Ron Hulst, Jan B. F. N. Engberts, and Dick Hoekstra
J. Biol. Chem, 10.1074/jbc.M106199200,Submitted on July 3, 2001

RNS sintezēts *in vitro* no pSFV1/EGFP plazmīdas.

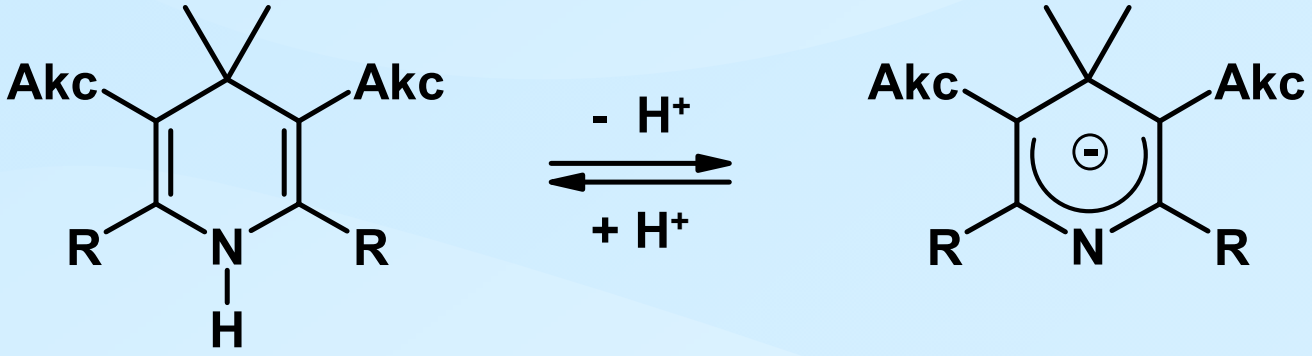
BHK-21 šūnu līnija
D-47
N-Me-C12
L-169
BA-31
V6-79
C12-Man-Q
D3-36-4
Kontrole: Lipofectamine 2000™


N-Me-C12
L-169
V6-79
C12-Man-Q
D3-36-4
Lipofectamine 2000™

Dihidropiridīni

Fizikāli-ķīmiskās īpašības

- cikla NH grupai konjugācijas dēļ ir ļoti vājas bāziskās īpašības, bet var būt visai izteiktas skābes īpašības ar protona disociāciju un anjona veidošanos, sevišķi spācīgu elektronakceptoru aizvietotāju klātbūtnē



- DHP atvasinājumiem ir fluorescences īpašības – atkarībā no konjugācijas sistēmas (hipsohroma vai batohroma, hipsfluora vai batofluora nobīde), koplanaritātes un mikrovides polaritātes un viskozitātes (kvantu iznākums)

Dihidropiridīni

Ķīmiskās īpašības

- redoksreakcijas (dzīvības procesu pamatreakcija):
  - enerģijas sagāde
  - enerģijas uzglabāšana
  - enerģijas izlietošana
- piedalīšanās elektronu un ūdeņraža pārnesē
  - iespējamās antiradikāļu un antioksidantu aktivitātes

Dihidropiridīnu struktūra – privileģēta

(nosaka bioloģisko aktivitāti)

Piemīt **pleiotropas** bioloģiskās īpašības (daudzpusīgas, diversificētas, atkarībā no aizvietotāju elektroniskajām un stēriskajām īpašībām)

Dihidropiridīnu struktūra – privileģēta

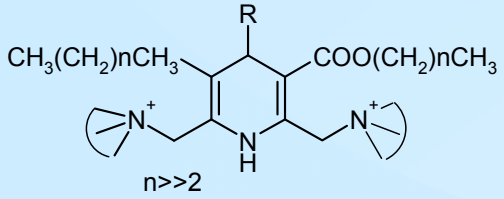
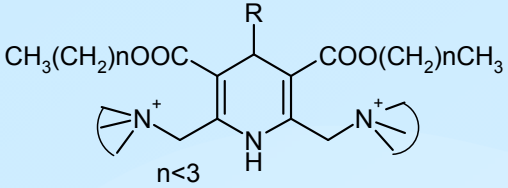
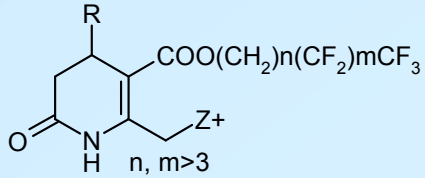
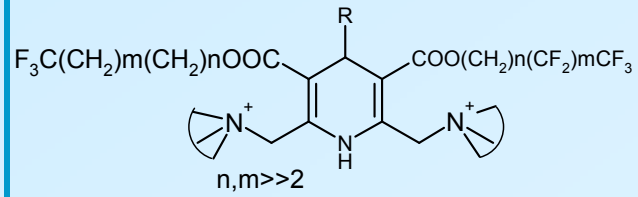
Sekas:

1,4-DHP sistēma kā privileģēta var saistīties ar dažādiem receptoriem (atkarībā no aizvietotāju dabas), tādēļ

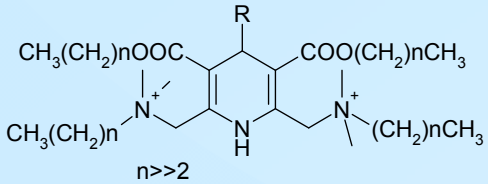
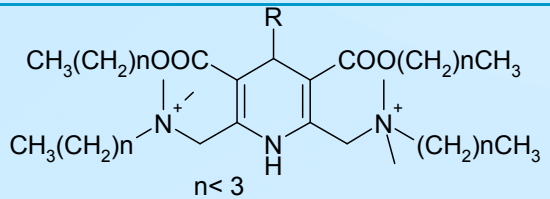
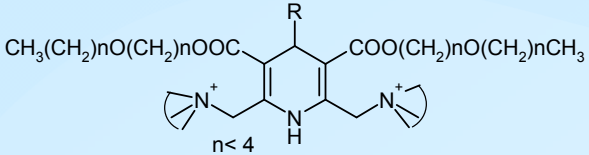
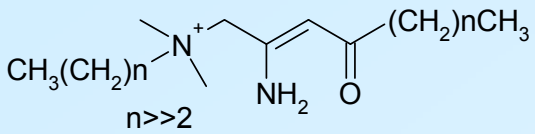
1) transportformai var piemist pašai savas bioloģiskās īpašības;

2) nanodaļiņas veidojošie savienojumi var kalpot kā adrese – sekmēt nanodaļiņu selektīvu sadalījumu organismā.

Savienojumu citotoksiskā aktivitāte uz šūnu līnijām HT-1080 (cilvēka fibro-sarkoma) un MG-22A (peļu hepatoma) un citotoksicitāte uz normālām šūnām

	HT-1080		MG-22A		3T3
	IC_{50} CV μg/ml	IC_{50} MTT μg/ml	IC_{50} CV μg/ml	IC_{50} MTT μg/ml	LD_{50} mg/kg
 n>>2	3	3	6	3	1482
 n<3	*	*	*	*	>2000
 n, m>3	3	3	2	3	618
 n,m>>2	**	**	**	**	>2000

Savienojumu citotoksiskā aktivitāte uz šūnu līnijām HT-1080 (cilvēka fibro-sarkoma) un MG-22A (peļu hepatoma) un citotoksicitāte uz normālām šūnām

	HT-1080		MG-22A		3T3
	IC_{50} CV μg/ml	IC_{50} MTT μg/ml	IC_{50} CV μg/ml	IC_{50} MTT μg/ml	LD_{50} mg/kg
 n>>2	3	3	10	10	840
 n< 3	**	**	**	**	2425
 n< 4	**	**	*	*	>2000
 n>>2	3	2	2	3	485

Secinājumi: augstu citotoksisko aktivitāti 1,4-DHP nosaka garas alkilesteru ķēdes 3.,5. vietās un piridīnija vai trialkilamonija katjoni 2.,6. vietās. Polifluoralkilesteru grupu saturoši 1,4-DHP neuzrāda citotoksisko aktivitāti un ir nekaitīgi. (Precīzas struktūras netiek norādītas, varētu tikt patentētas)

Reversal of Multidrug Resistance in Murine Lymphoma Cellsby Amphiphilic Dihydropyridine Antioxidant Derivative

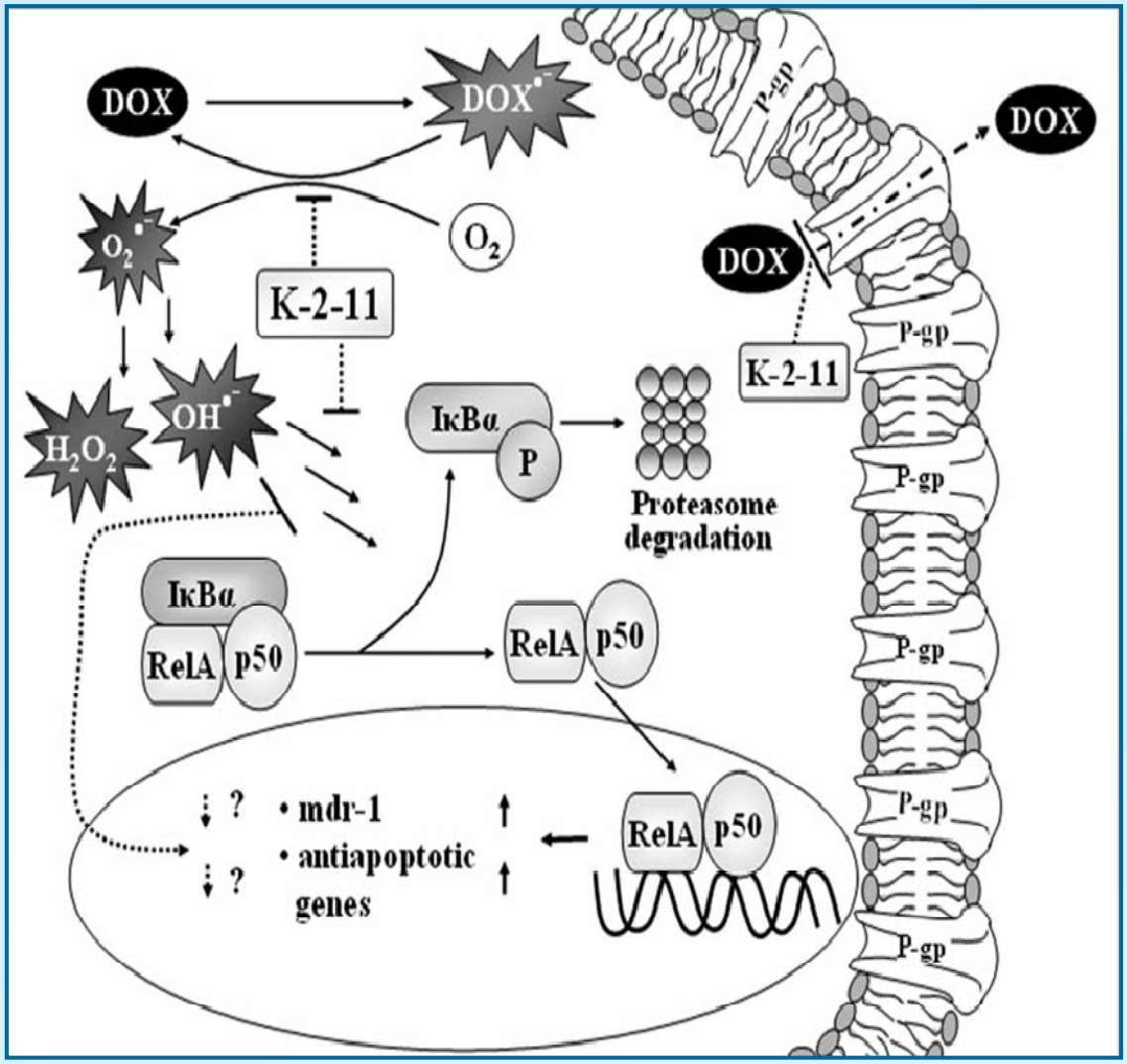
M.Cindric, A.Cipak, J.Serly, A.Plotniece, M.Jaganjac, L.Mrakovcic, T.Lovakovic, A.Dedic, I.Soldo, G.Duburs, N.Zarkovic, J.Molnár *Anticancer Research*. 30: 4063-4070 (2010)

Abstract. Background: Multidrug resistance, the principal mechanism by which cancer cells develop resistance to chemotherapy drugs, is a major factor in the failure of many forms of chemotherapies. **Aim**: The aim of the study was to investigate the effect of K-2-11 on the reversal of multidrug resistance. Materials and **Methods**: The effects of amphiphilic dihydropyridine derivative K-2-11 were tested on MDR1-expressing mouse lymphoma cells and their parental control. The effects of K-2-11 with and without doxorubicin were studied by determination of cell viability, cell proliferation and production of reactive oxygen species.

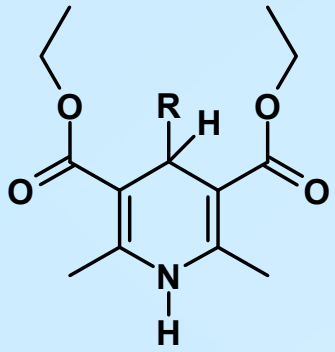
Results: K-2-11 caused complete reversal of multidrug resistance of the MDR cells, being much more efficient than the positive control verapamil. Accordingly, the cytotoxic effects of doxorubicin were enhanced by K-2-11, both in the MDR and in parental cell line, while K-2-11 alone did not affect cell viability. K-2-11 also acted as an antioxidant, reducing the cellular generation of reactive oxygen species.

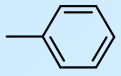
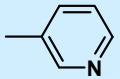
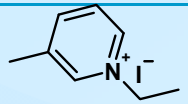
Conclusion: Our results indicate the high potential of K-2-11 as a novel antioxidant with potent MDR-blocking ability that should be studied further for development in adjuvant anticancer treatments.

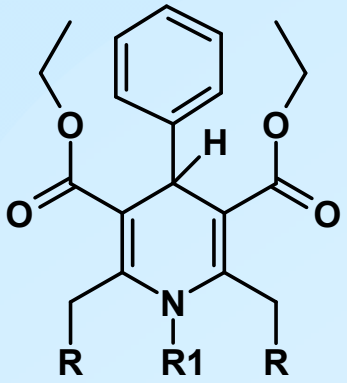
Reversal of Multidrug Resistance in Murine Lymphoma Cellsby Amphiphilic Dihydropyridine Antioxidant Derivative

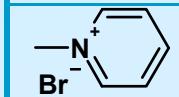
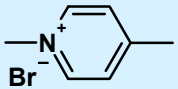
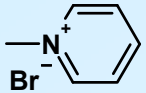


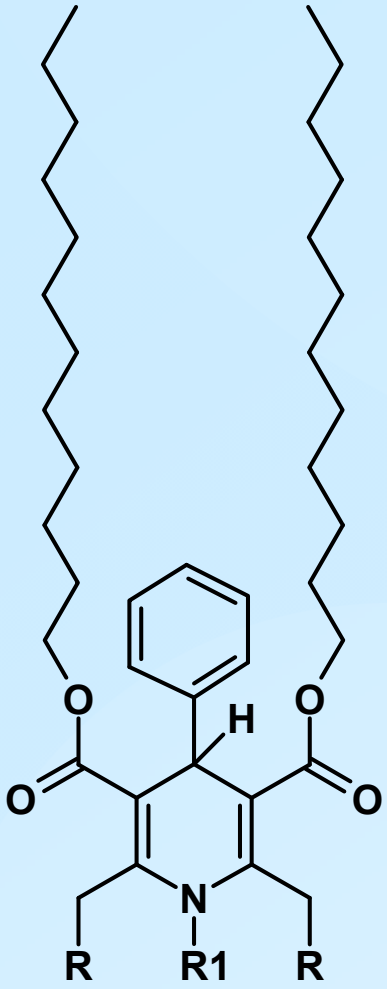
Proposed mechanism of K-2-11 action on cancer cells. Doxorubicin (DOX) reduction generates doxorubicin semiquinone, a free radical capable of reducing molecular oxygen and generating superoxide, thereby initiating ROS production. ROS activates NF-κB through activation of kinases, causing IκBα phosphorylation and releasing RelA/p50 dimer that binds DNA, thus inducing overexpression of MDR1 and antiapoptosis genes. This cascade results in an increase of P-gp, leading to chemoresistance of cancer cells. When K-2-11 is added, P-gp activity is blocked, making cancer cells chemosensitive due to doxorubicin retention in cells. In addition, K-2-11 suppresses ROS increases, thus preventing NF-κB activation that could consequently lead to a normal expression of MDR1 and antiapoptosis genes, restoring chemosensitivity of cancer cells

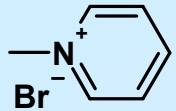
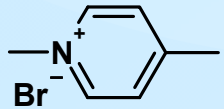
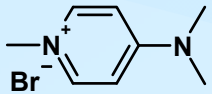
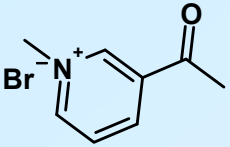
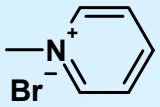


R	ARA(%)	Comments
H	40.6±1.8	Unsubstituted in position 4 1,4-DHP (2,6-dimethyl-3,5-diethoxycarbonyl-1,4-DHP or diethone) possesses significant antiradical activity. Insertion of phenyl- or pyridylgroups almost completely abolishes ARA.
	0	
	0	
	8.5±1.1	

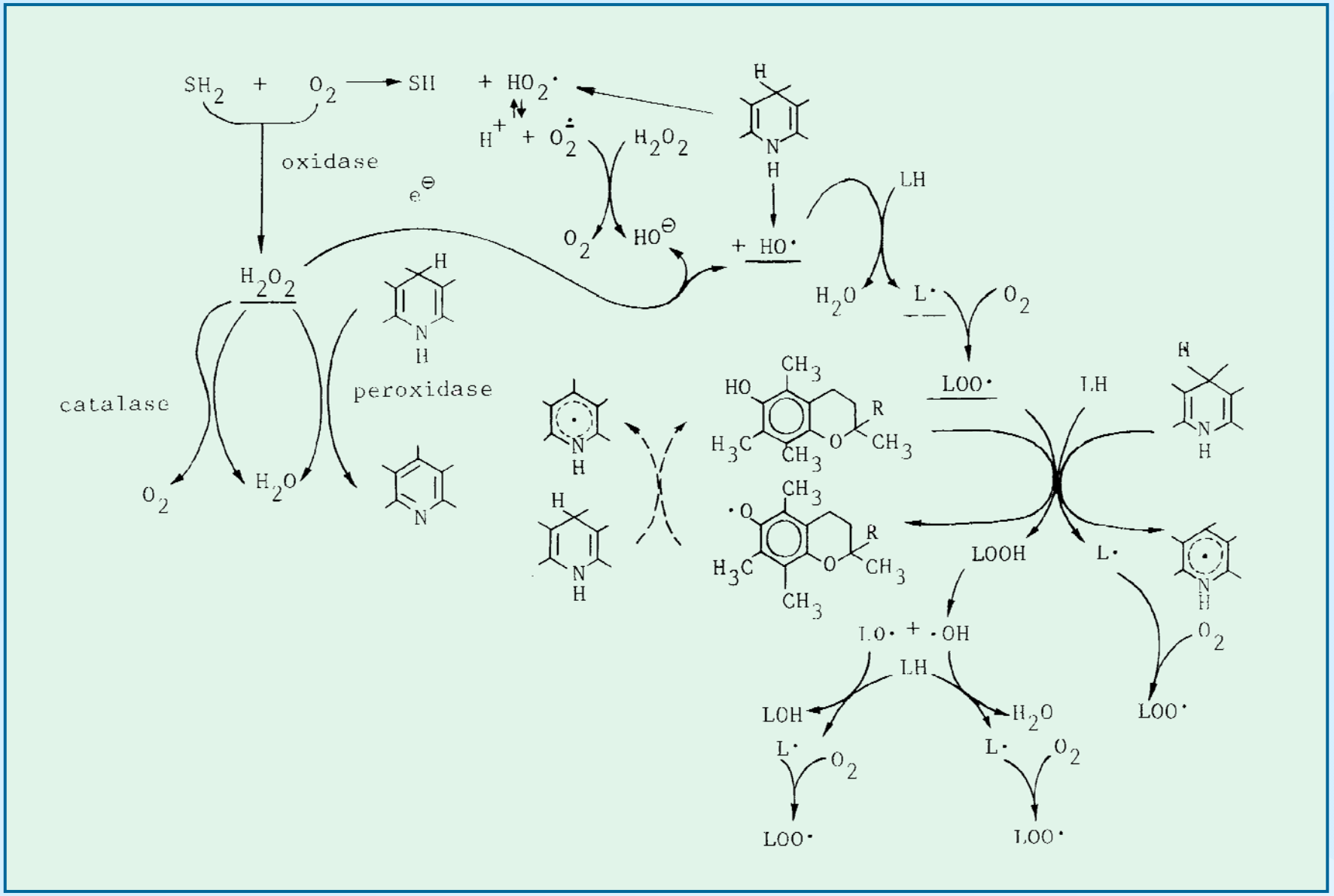


R	R1	ARA(%)	Comments
H	H	0	4-Phenyl-3,5-diethoxycarbonyl-2,6-dimethyl-1,4-DHP and its 2,6-dibromomethyl derivative almost lack ARA. Insertion of pyridinio groups in 2,6-methyl substituents leads to significant ARA. It depends on presence of N-H atom: its substitution diminishes ARA significantly.
Br	H	0	
	H	58.4±1.6	
	H	44.3±1.8	
	CH_3	8.0±0.2	



R	R1	ARA(%)	Comments
H	H	0	4-Phenyl-3,5-didodecyloxycarbonyl-1,4-DHP derivative possessing 2,6-dimethyl substituents lacks ARA. The same is for compounds possessing 2,6-bisbromomethyl substituents. On the contrary appropriate 2,6-dipyridiniomethyl derivative has significant ARA: close to known antioxidant diethone (diludine). In case of substituted pyridinio groups it is possible to tune ARA of compounds. N-methyl derivative has very low ARA.
Br	H	0	
	H	39.5±0.3	
	H	41.2±2.6	
	H	27.5±0.2	
	H	35.9±2.3	
	CH_3	7.3±0.4	

DHP involvement in free radical reactions



Modulation of cellular defense processes in human lymphocytes in vitro by a 1,4-dihydropyridine derivative

N.I. Ryabokona, N.V. Nikitchenko, O.V. Dalivelya, R.I. Goncharova, G. Duburs, M. Konopacka, J. Rzeszowska-Wolny *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 679 (2009) 33–38

The study revealed dual effects of AV-153 on cellular defense systems against endogenously generated DNA damage: the compound *per se* simultaneously reduces DNA strand breaks and stimulates apoptosis, with a maximal efficiency of 76% and 42%, respectively; in contrast, after genotoxic stress (2 Gy of gamma-radiation) AV-153 reduces DNA strand breaks, the number of MN cells and apoptotic cells in a similar dose-dependent manner. A maximal efficiency of 67% was found for reduction of DNA strand breaks, while for MN cells and apoptotic cells the efficiencies were, respectively, 47% and 44%. While limited in number, these preliminary studies show the direct correlation between the efficiency of AV-153 in reduction of radiation-induced DNA breaks and MN cells on one side, and in reduction of apoptosis on the other. It suggests that the major target of the compound's action on genotoxic stress is DNA repair, followed by reduction of the number of damaged cells entering apoptosis.

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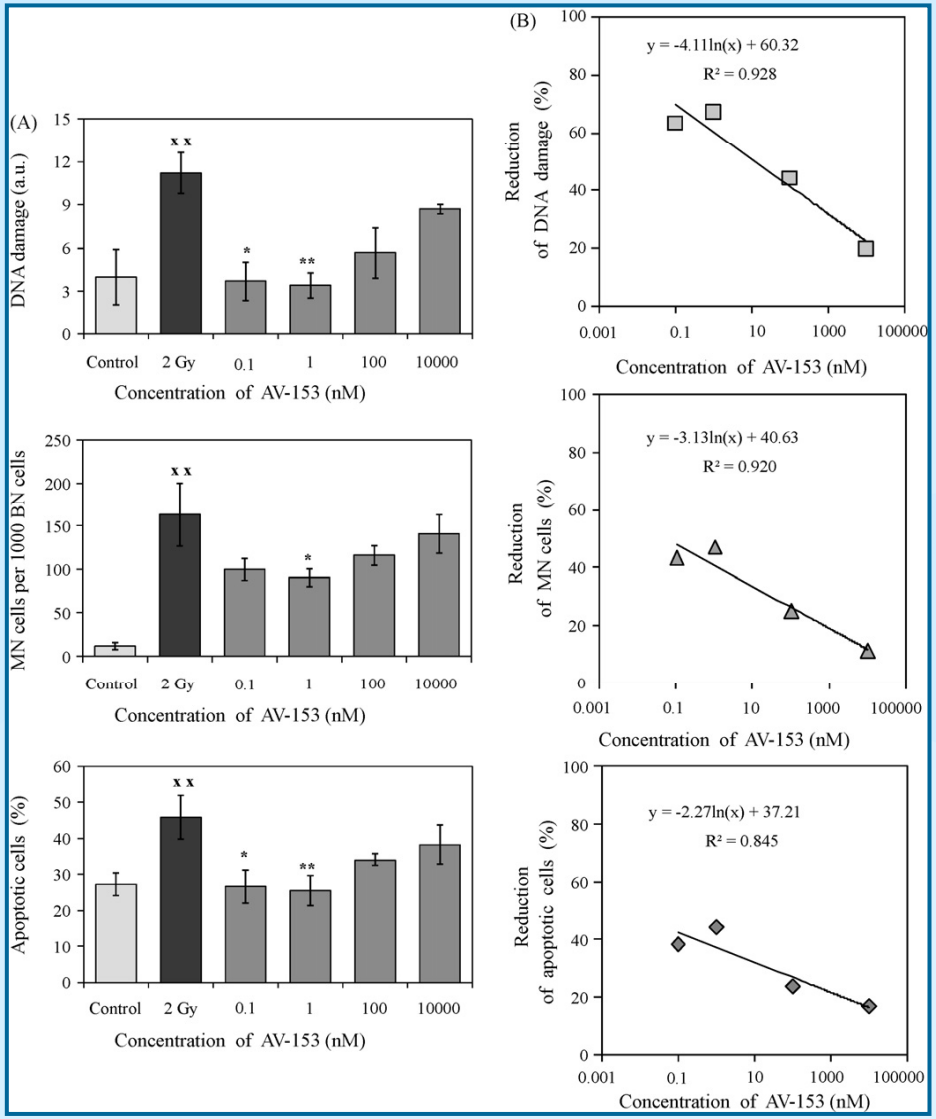
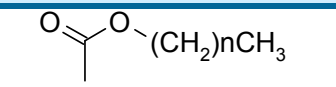
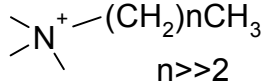
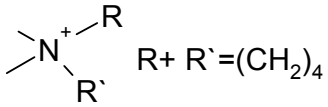
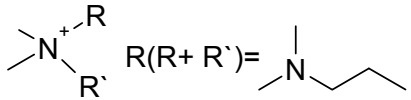
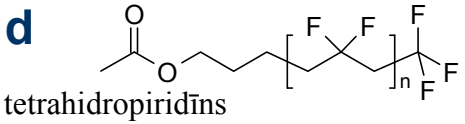
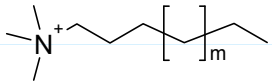


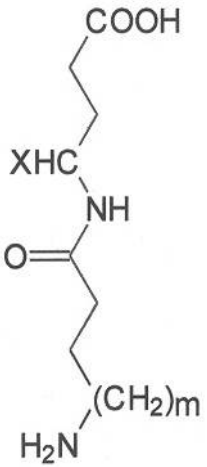
(A) Effect of different concentrations of AV-153 on the radiation-induced levels of DNA strand breaks, MN cells and apoptosis in human lymphocytes; $^{xx}P < 0.01$ when compared with the control level, $^{*}P < 0.05$ and $^{**}P < 0.01$ when compared with radiation-induced level (Student's *t*-test). (B) Concentration-dependent reduction of the endpoints (regression analysis).

Citotoksiskā aktivitāte uz šūnu līnijām HT-1080 un MG-22A un toksicitāte uz normālām šūnām

 linkers hidrofīlā daļa n>>2	HT-1080		MG-22A		3T3
Hidrofīlā daļa vai hidrofīlā daļa ar otru lipofīlo ķēdi	IC_{50} CV mkg/ml	IC_{50} MTT mkg/ml	IC_{50} CV mkg/ml	IC_{50} MTT mkg/ml	LD_{50} mg/kg
a  n>>2	~3	~2	2 vai 25	1 vai 28	500÷700
b 	<1	<1	1	1	~300
c 	2	2	1	1	200÷300
d  tetrahidropiridīns 	3	30	26	30	900

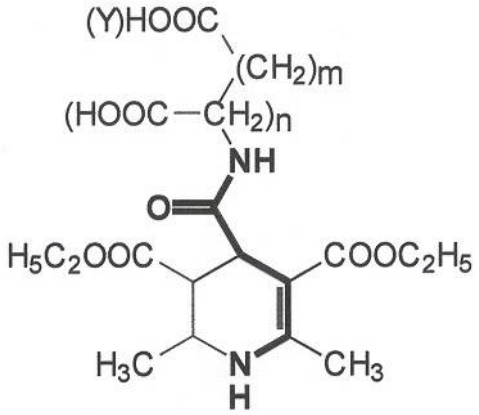
Peptidomimētiķi

Dipeptīdi



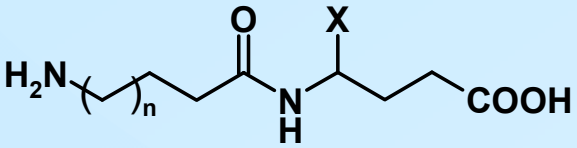
m = 1	X = H	Abu-Abu	
m = 0	X = H	β-Ala-Abu	
m = 1	X = CO_2H	Abu-Glu	
m = 3	X = H	ε-Aka-Abu	

Peptidomimētiķi

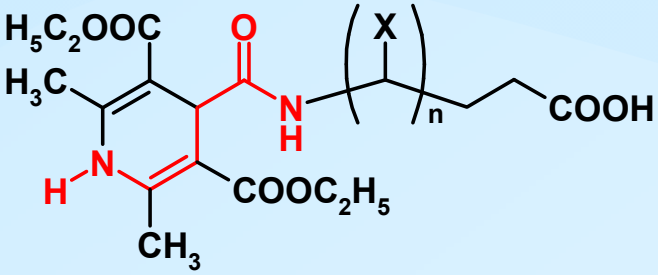


n = 0,	m = 1,	Y = COOH	DHI-Gly	*Glycipyrone*
n = 0,	m = 2,	Y = COOH	DHI-β-Ala	*Alapyrone*
n = 0,	m = 3,	Y = COOH	DHI-Abu	*Gammapyrone (GAP)*
n = 0,	m = 2,	Y = SO_2OH	DHI-Taurine	*Taropyrone*
n = 1,	m = 1,	Y = COOH	DHI-Asp	*Aspapyrone*
n = 1,	m = 2,	Y = COOH	DHI-Glu	*Glutapyrone (GLP)*

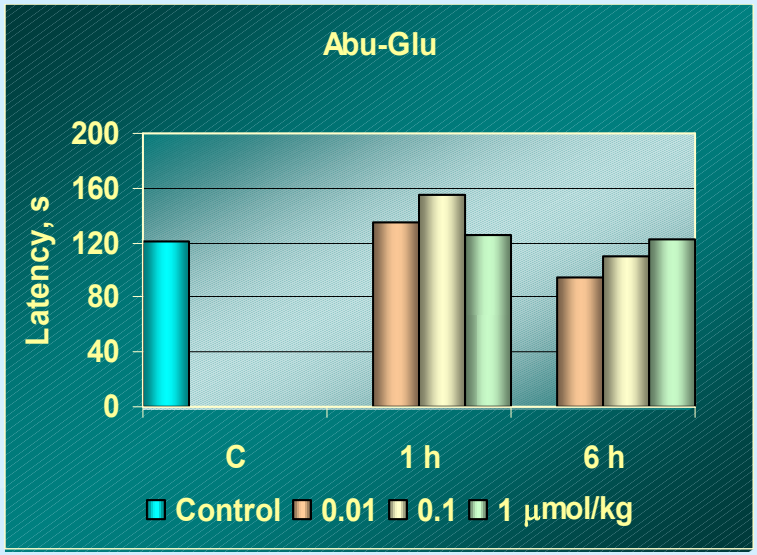
Influence of Abu-Glu and Glutapyrone on passive avoidance response in BALB/c mice

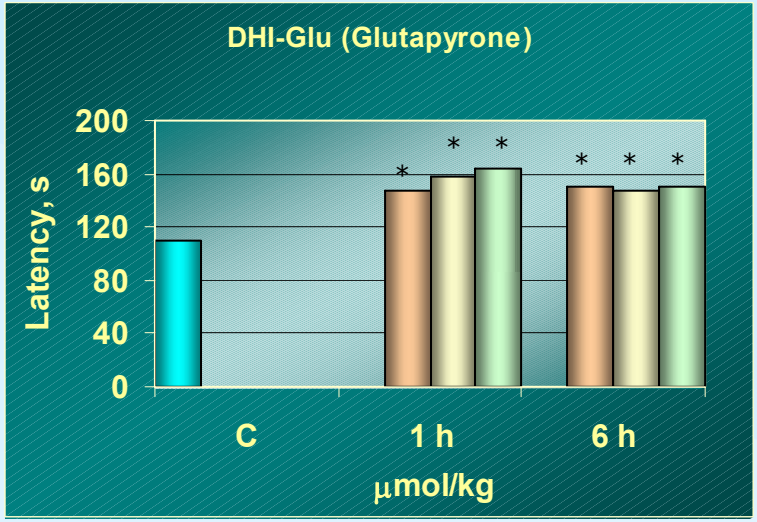


m=1; X=COOH Abu-Glu



n=1; X=COOH DHI-Glu





* P<0.05 *vs* intact control (C), Student's test

DQAsomes as Carriers of Pro-apoptotic Drugs

Dysregulation of the apoptotic machinery is generally accepted as an almost universal component of the transformation process of normal cells into cancer cells and a large body of experimental data demonstrates that mitochondria play a key role in the complex apoptotic mechanism. Consequently, any therapeutic strategy aimed at specifically triggering apoptosis in cancer cells is believed to have potential therapeutic effect. Several clinically approved drugs such as VP-16 (etoposide), arsenite and vinorelbine, as well as an increasing number of experimental anticancer drugs (reviewed by Constantini *et al.*), such as betulinic acid, lonidamine, ceramide and CD437 have been found to act directly on mitochondria, resulting in triggering apoptosis. In order to maximize the therapeutic potential of such anticancer drugs, which are known to act at or inside mitochondria, the use of **DQAsomes as a mitochondria-specific drug** delivery system has been proposed.

DQAsomes as Carriers of Pro-apoptotic Drugs

Hypothetically, DQAsome-based anticancer chemotherapy entails features which would make it putatively superior to conventional chemotherapeutic approaches on the cellular, as well as the subcellular level:
1) the delivery of drugs known to act directly on mitochondria may trigger apoptosis in circumstances in which conventional drugs fail to act, because endogenous, "upstream of mitochondria" apoptosis induction pathways are disrupted;
2) transporting the cytotoxic drug to its intracellular target could overcome multi-drug resistance by hiding the drug inside the delivery system until it becomes selectively released at the particular intracellular site of action, i.e. mitochondria;
3) **many carcinoma cells**, including human breast adenocarcinomaderived cells, **have an elevated plasma membrane potential** relative to their normal parent cell lines in addition to the higher mitochondrial membrane potential.
They could provide the basis for a double-targeting effect of DQAsomes, i.e. on the cellular level (normal cells vs. carcinoma cells), and on the sub-cellular level (mitochondria versus nucleus).

Strategies in the design of nanoparticles for therapeutic applications

Robby A. Petros* and Joseph M. DeSimone‡ *NATURE REVIEWS | Drug Discovery*, 2010, 9, 615-627

Engineered nanoparticles have the potential to revolutionize the diagnosis and treatment of many diseases; for example, by allowing the targeted delivery of a drug to particular subsets of cells. However, so far, such nanoparticles have not proved capable of surmounting all of the biological barriers required to achieve this goal. Nevertheless, advances in nanoparticle engineering, as well as advances in **understanding the importance of nanoparticle characteristics such as size, shape and surface properties** for biological interactions, are creating new **opportunities** for the development of nanoparticles for therapeutic applications.

Organelle-specific targeting

Tools and principles for effective organelle targeting are emerging, such as those for targeted delivery to the nucleus, cytosol, mitochondria, peroxisomes and endosomes/lysosomes. Delivery to mitochondria is largely based on electrostatic interactions between the engineered nanoparticle and the mitochondrial membrane, which has a membrane potential of 130–150 mV. This potential is lower than other membranes in the cell and can be exploited by grafting cationic species, such as **triphenylphosphonium cations**, to the surface of the carrier. This strategy was shown to be effective at delivering hydroxypropylmethacrylamide polymer conjugates to mitochondria *in vitro*. Peptide ligands provide an alternative method for targeting mitochondria, which was shown recently by the successful targeting and localization of peptide-targeted quantum dots in mitochondria.

General nanoparticle characteristics

Current findings indicate that **particle shape** is just as important, if not more so, than size in controlling key aspects of both these phenomena. For example, a recent report investigated the effect of particle size and shape on the rate of particle internalization in HeLa cells using non-spherical particles. A clear correlation between the shape and size of the particles on the rate of internalization was observed. Furthermore, it was shown that particles with similar volumes but different shapes were internalized at vastly different rates. It was also shown recently that the geometry of interaction between a cell and a particle can induce or inhibit internalization, and that **shape has a significant impact** on biodistribution.

Ietekme uz dzīvības procesiem

Transporta (pārvietošanas) procesi
-saistīti ar nehomogēno (anizotropo) vidi
-telpas (šūnas, citoplazmas) kompartmentalizācija
-vielu transports – asimilācija, disimilācija
-biostruktūru pašasociācija
-kļūdaino gēnu papildināšana vai nomaiņa

Regulē:
-ārstniecības vielu, gēnu terapijas aģentu nogādi šūnā
-kavē ārstniecības vielu izvadīšanu no šūnas
-jonu transportu
---- Ca^{2+} pa L-, T- vai N-tipa potenciālatkarīgajiem kanāliem
---- K^{+} kanālu aktivatori
---- Cl^{-} kanālu modulatori

Perspektīvas

Konstruēt molekulas ar **iekodētu informāciju** par nanoagregātu mērķtiecīgu **pašuzbūvi**.

Sintētiskā bioloģija:

- dabīgo membrānu modeļu veidošana no sintētiskajiem amfifīlajiem savienojumiem
- sintētisko lipīdu membrānās iekļauto enzīmu specifiskuma un aktivitātes modifikācija