SYNTHETIC MACROMOLACULAR MODULATORS OF ANTI-TUMOR DRUGS

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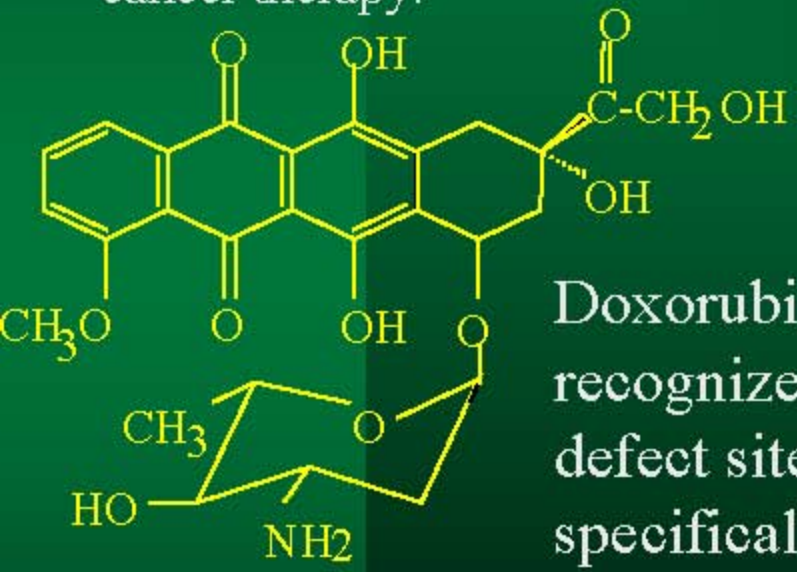
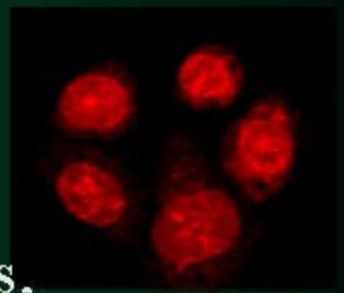
Doxorubicin

Doxorubicin (DOX) – one of the most widely used drugs of the first line of cancer therapy.



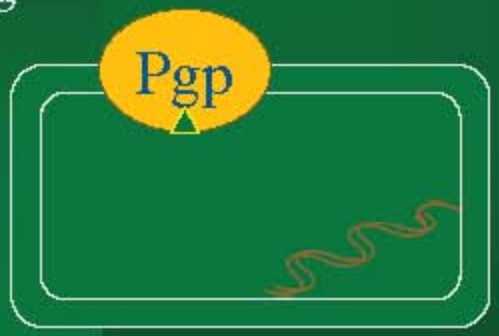
Doxorubicin can intercalate double helix of genomic DNA with high affinity



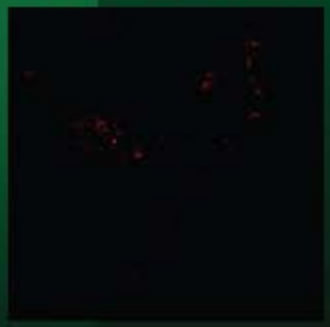
Doxorubicin-containing sites are recognized by nuclear enzymes as defect sites. Therefore these enzymes, specifically topoisomerase II, cut double helix at these sites, resulting in the accumulation of double strand cuts in the genomic DNA. This leads to the initiation of apoptotic cascade and finally kills the cell.

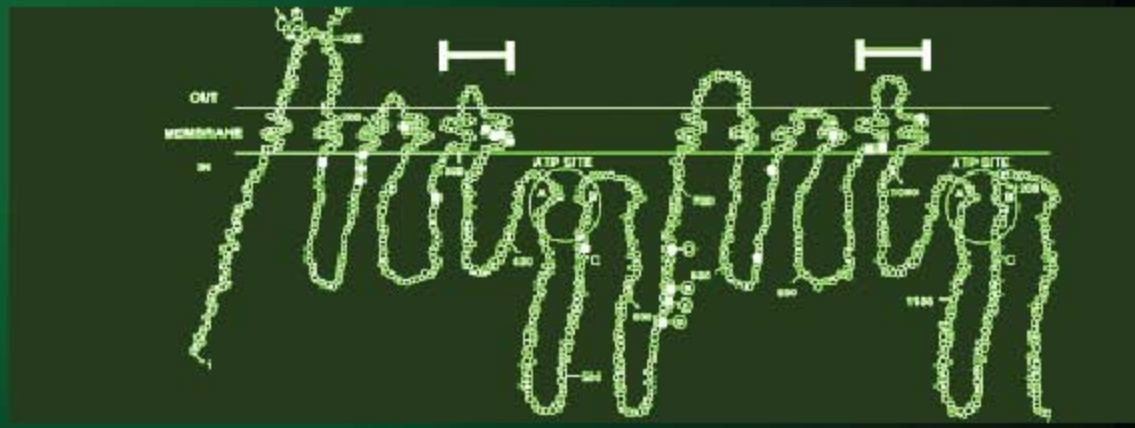
P-glycoprotein mediated efflux of drugs from multi-drug resistant cells.

▼ - Drug



Multi-drug resistant cell

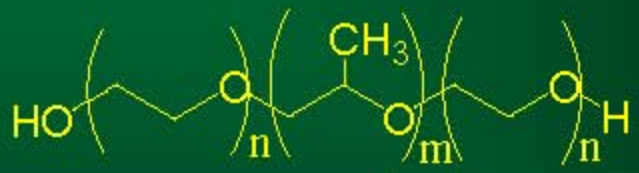


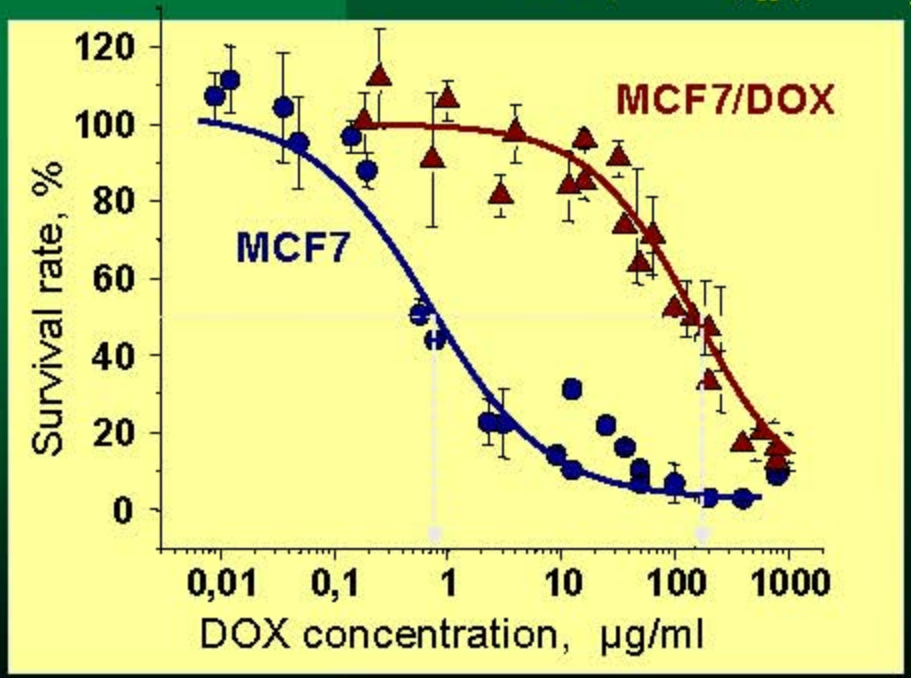


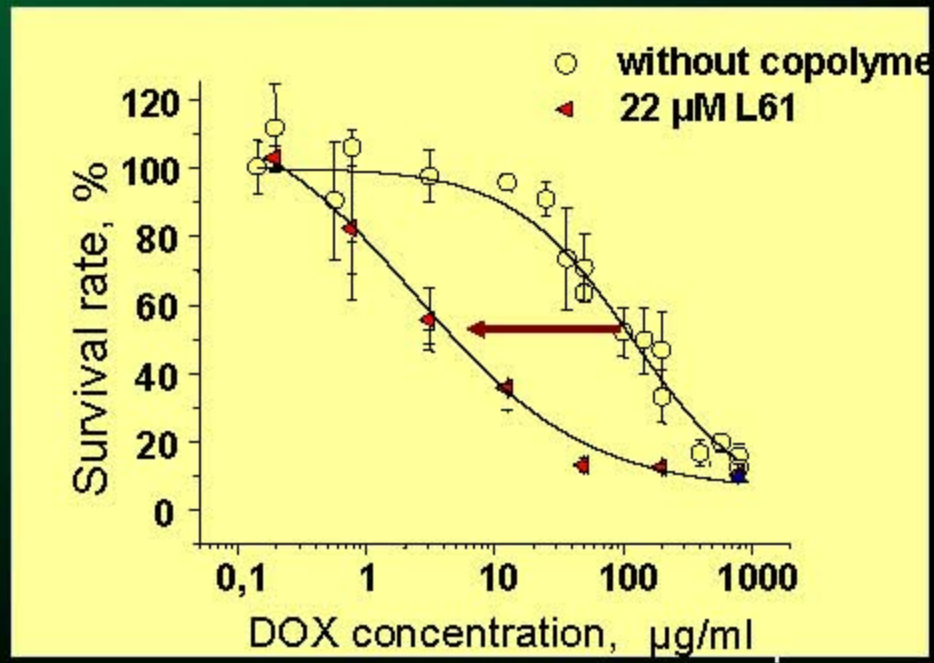
P-glycoprotein

Influence of the copolymers on Doxorubicin cytotoxicity

Addition of DOX simply mixed with ethylene oxide and propylene oxide tri-block copolymers, Pluronics, to the multi-drug resistant cells results in the increase in its cytotoxicity by nearly two orders of magnitude



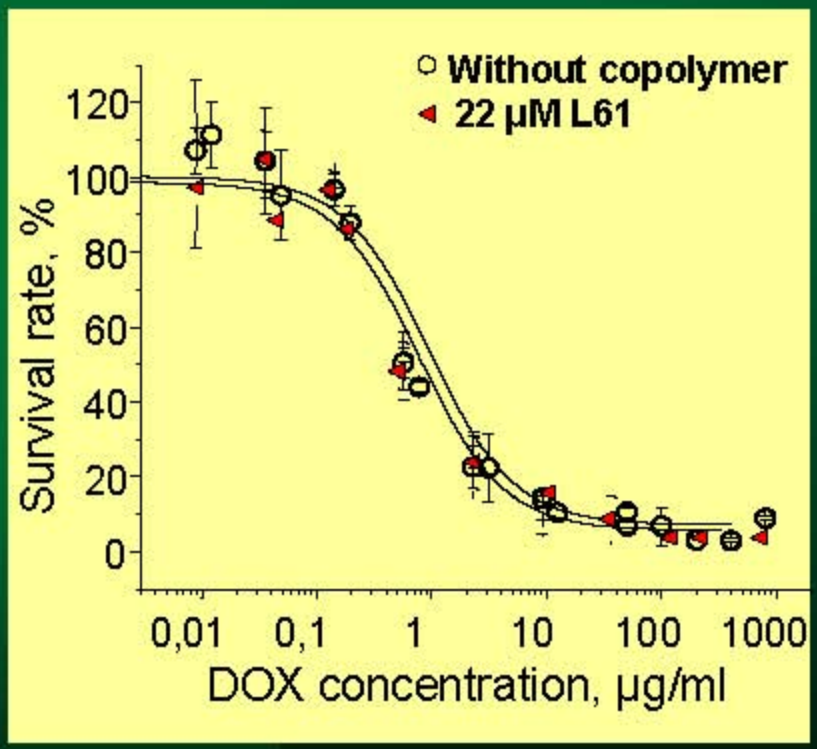




Outline

- Main reasons for the copolymer-induced sensitization of MDR cells
- Correlation between chemisensitizing activity of the copolymers and their effect on the properties of model membranes
- Physico-chemical basis for the disturbing activity of synthetic copolymers in lipid membranes
- Role of macromolecular architecture of amphiphilic copolymers for their chemisensitizing activity

Whether copolymers can facilitate DOX influx into the cells which do not contain P-glycoprotein?



The copolymers do not influence DOX toxicity towards tumor cells which do not contain P-glycoprotein.

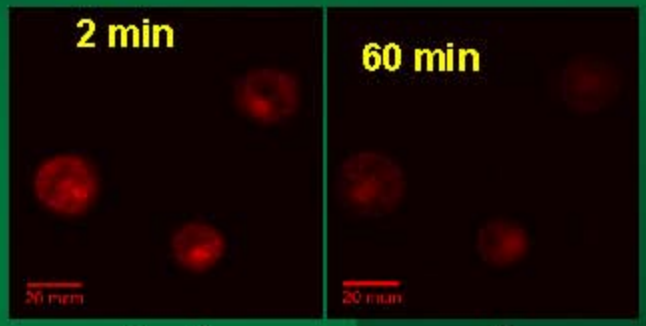
1) DOX accumulation is not limited by the transmembrane diffusion step, which can be influenced by Pluronics.

2) The effect of copolymers is mediated by their suppression of P-gp-mediated efflux

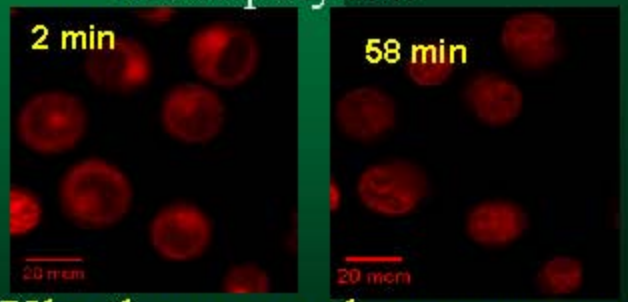
Effect of the copolymers on DOX efflux from MDR cells (i.e. on enzymatic activity of P-glycoprotein)

MDR cells → DOX (large amount) → DOX in cellular vesicles

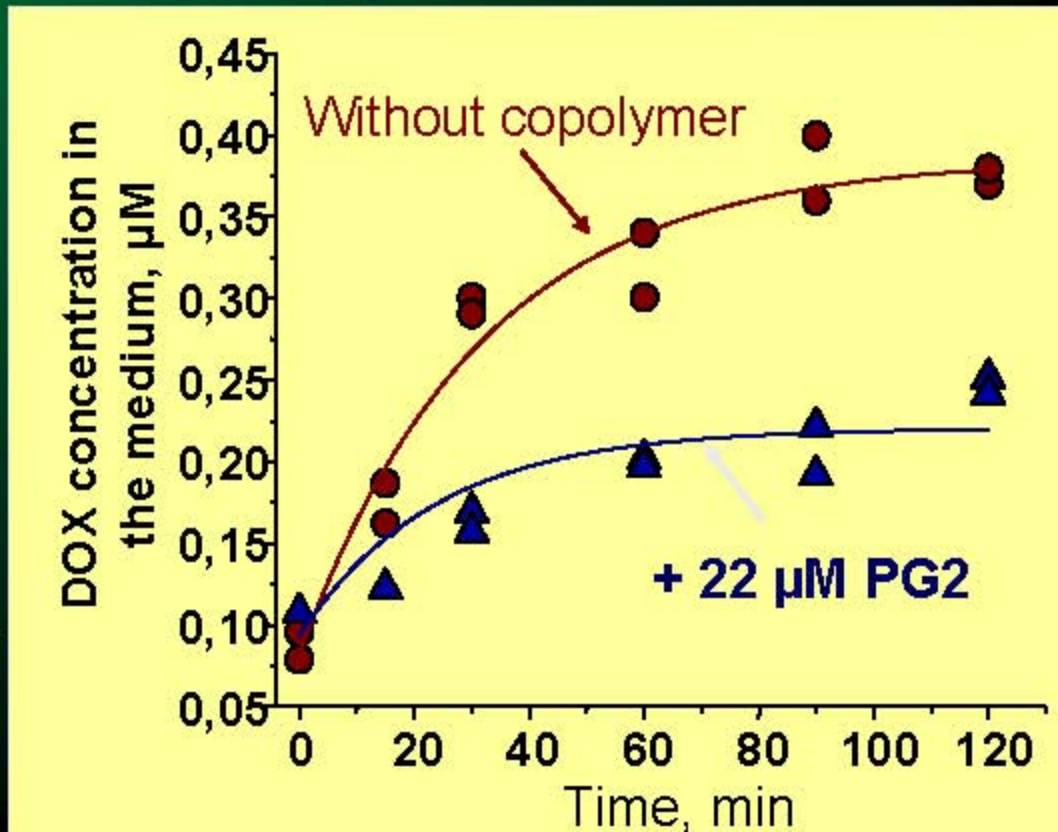
Without copolymer



In the presence of the copolymer

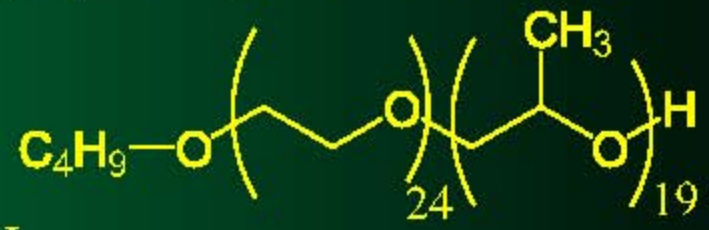


Whether copolymers can directly interact with P-glycoprotein?



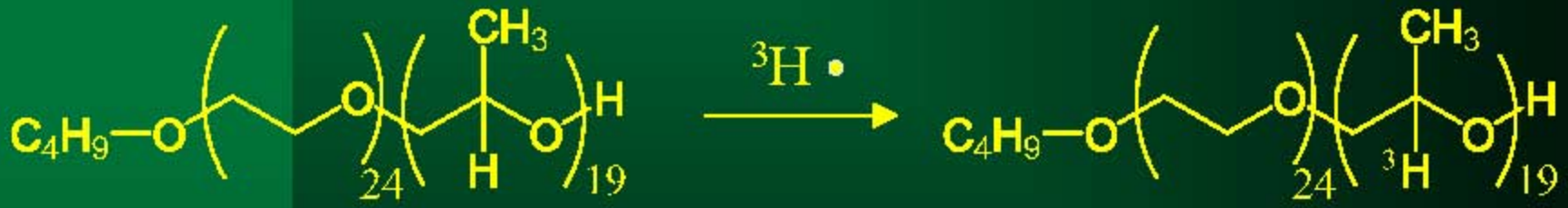
Whether copolymers can directly interact with P-glycoprotein?

Block copolymer of ethylene oxide and propylene oxide, REP

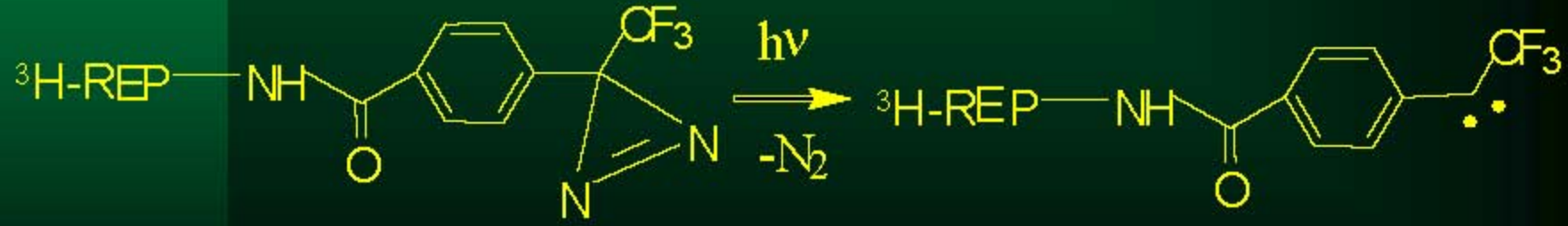


Tritium labeling via heat atom bombardment :

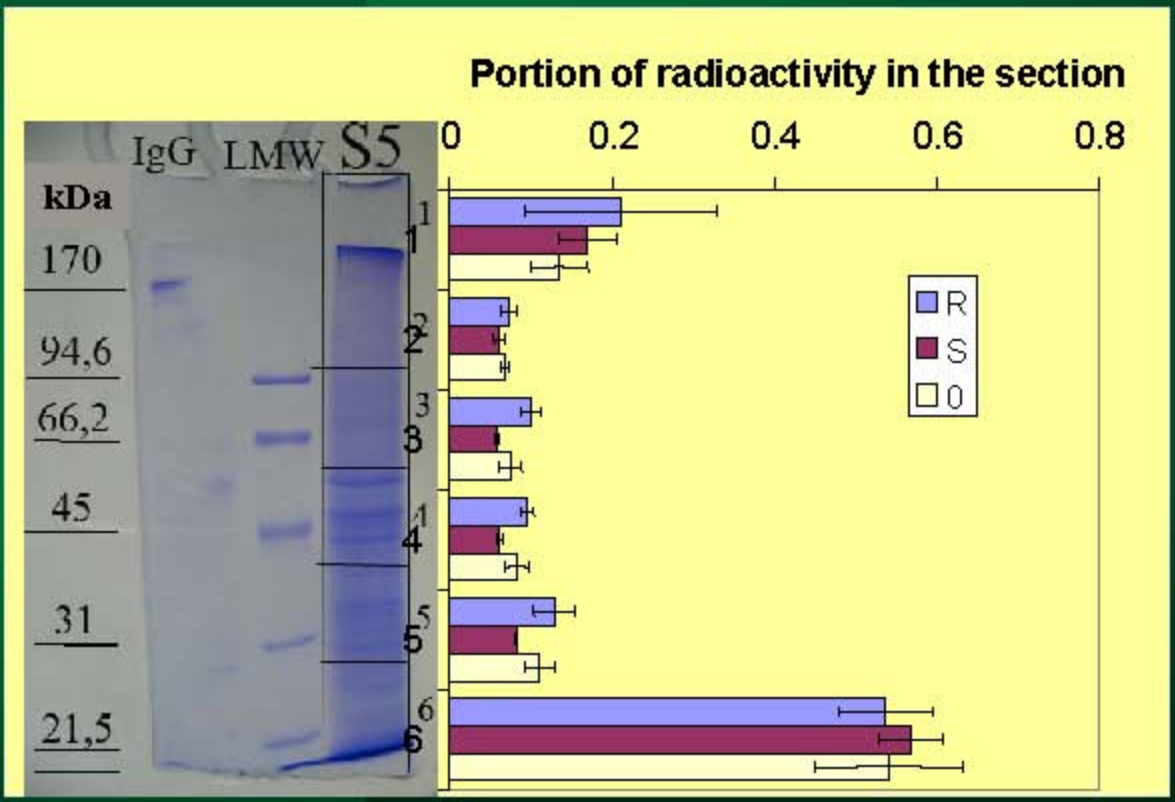
$^3H_2 \xrightarrow{W,\ 600K} 2\,^3H\bullet$



Photolytic activation:



We were unable to confirm direct interaction betwe P-glycoprotein and block copolymer containing photoreactive trifluoromethyldiazirine.



SDS-PAAGE analysis of protein fraction of MDR cells treated with ^{3}H-labeled photoreactive block copolymer in 7% PAAG.

Left panel. Bars and digits in the right indicate the scheme of gel cutting for further measuring of radioactivity.

Right panel. Distribution of radioactivity along the gel lanes. Abscissa depicts the portion of radioactivity of the corresponding gel section.

R – samples from MDR-cells

S – samples from non-MDR cells

0 – control samples (without light)

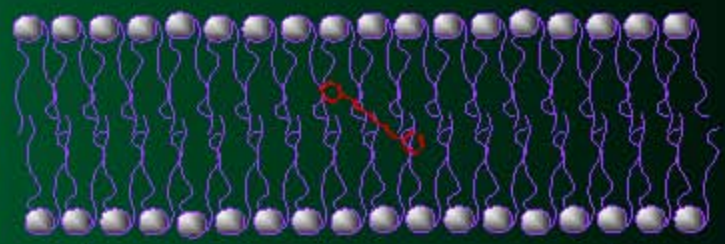
P-glycoprotein inhibition by amphiphilic copolymers is indirect

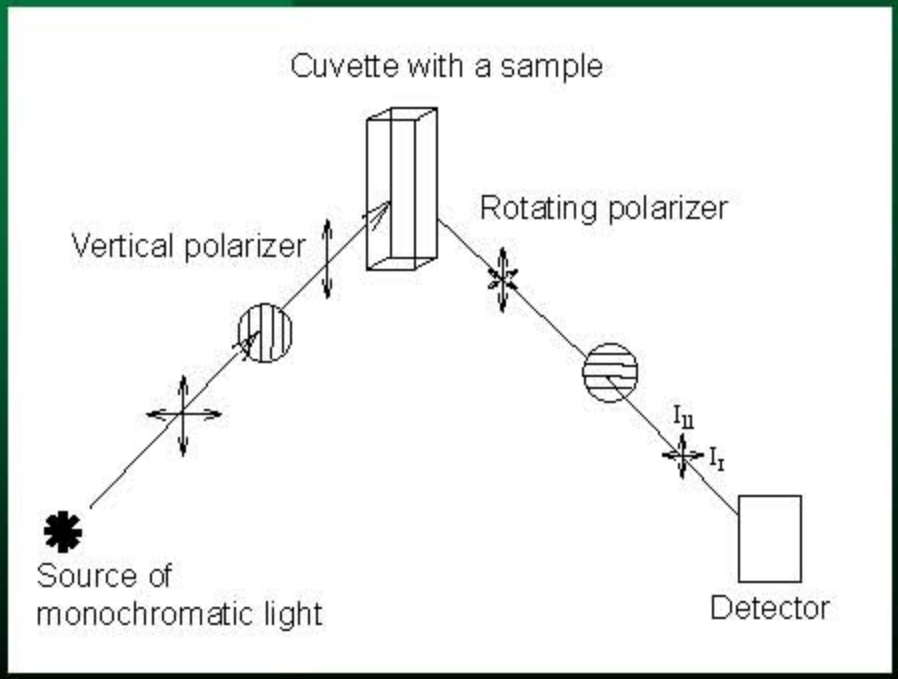
- Whether P-glycoprotein activity decreases due to the changes in the structure of lipid bilayer ?

Interaction of the copolymers with lipid membranes

Influence on the membrane microviscosity

1,3,5-Diphenylhexatriene

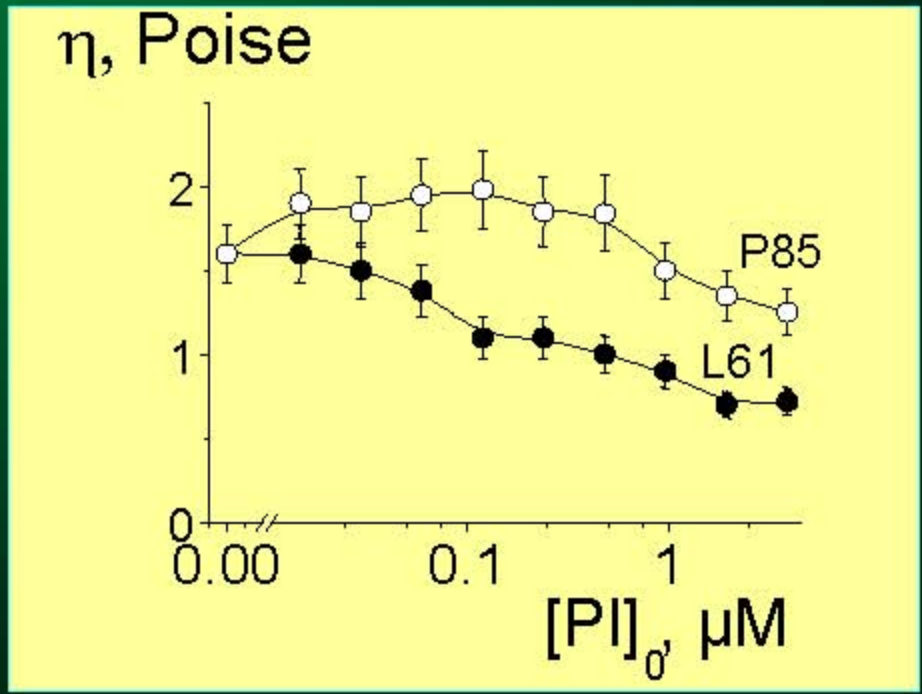




$$r = \frac{I_{II} - I_{\perp}}{I_{II} + 2I_{\perp}}$$

$$\frac{r_0}{r} = 1 + \frac{RT\tau}{V\eta}$$

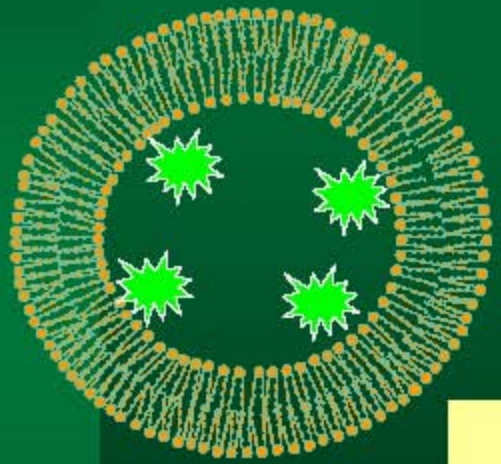
Interaction of Pluronic copolymers with cells decreases membrane microviscosity

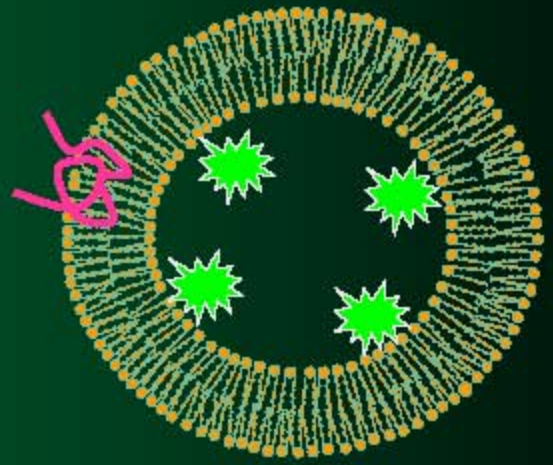


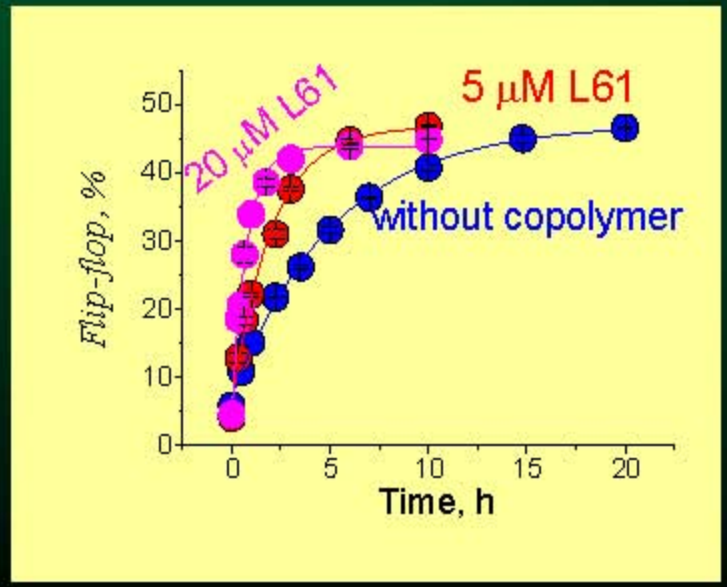
Studies of interaction of amphiphilic copolymers of different chemical nature and macromolecular architecture on the properties of model membranes

- Rate of transmembrane migration of lipids
- Doxorubicin permeation through lipid bilayer
- Formation of pores in lipid bilayer permeable for charged solutes

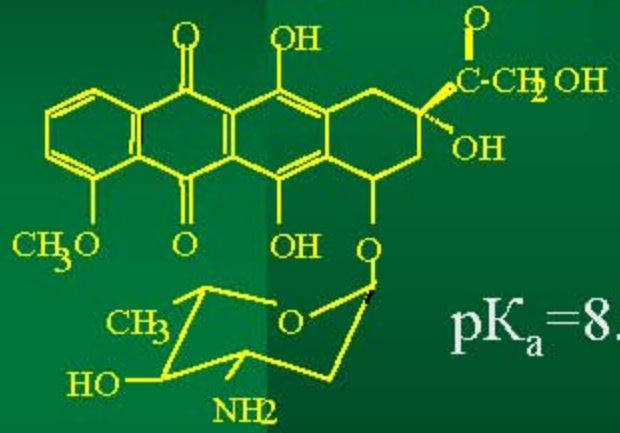
Influence of the copolymers on the rate of transmembrane movement of lipids in model membranes



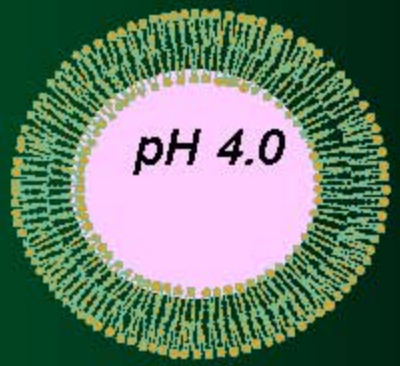




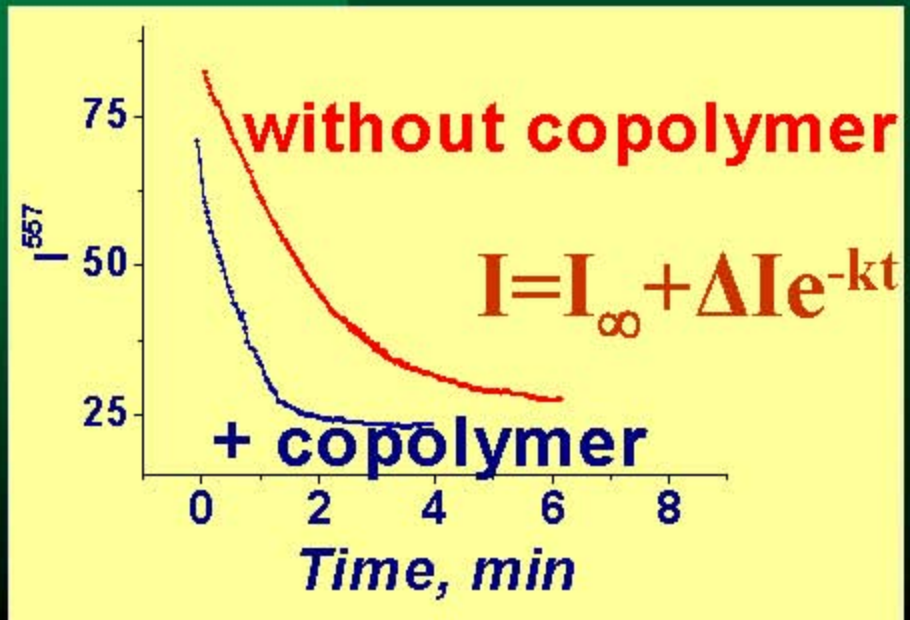
Acceleration of doxorubicin permeation through model lipid membrane



pK_a=8.6



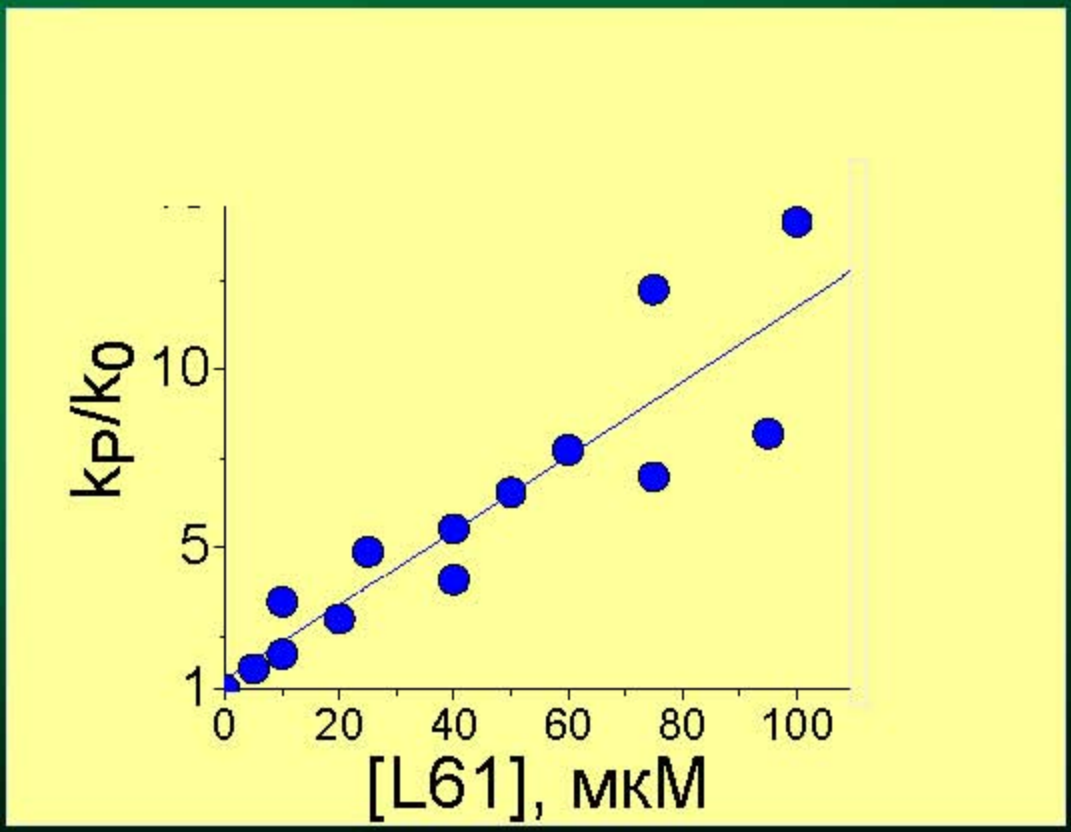
pH 7.0



Effect of copolymer = k_P / k_0

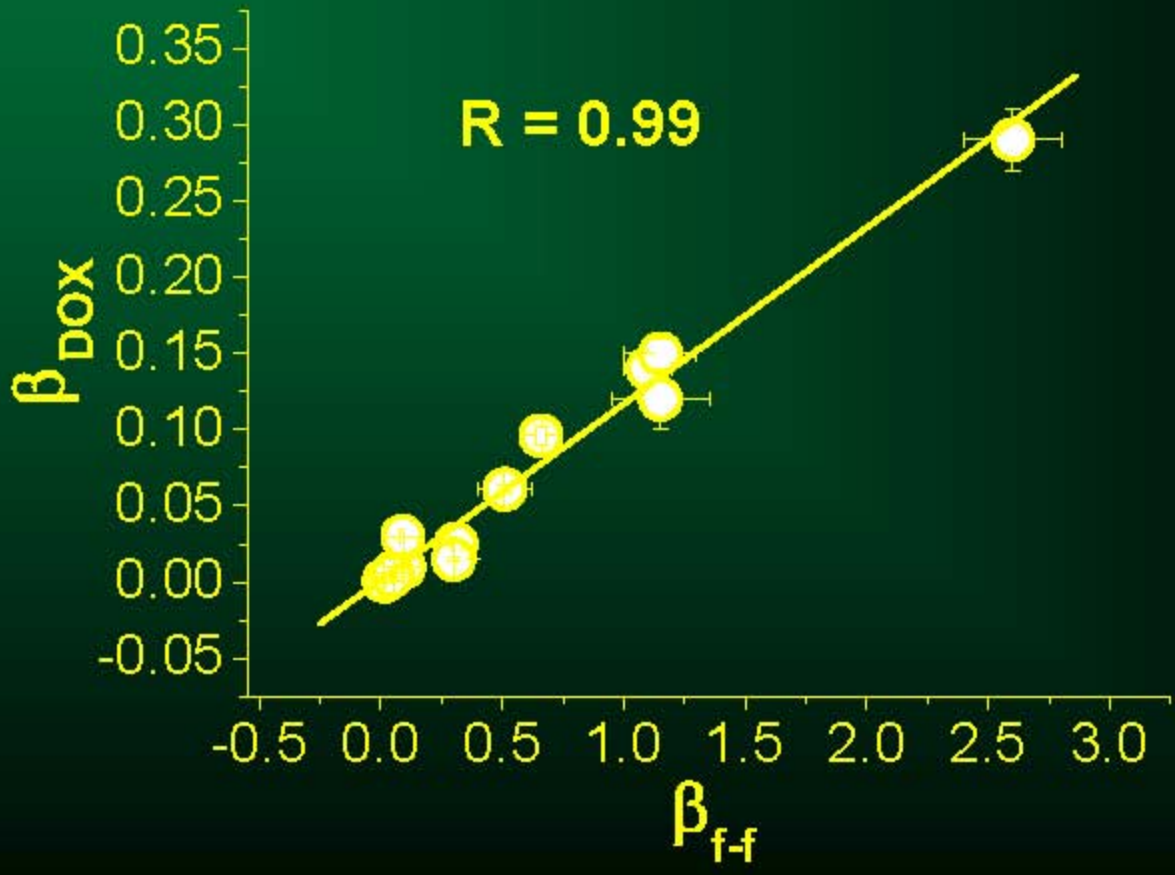
Acceleration of doxorubicin permeation through model lipid membrane

$$\frac{k_P}{k_0} = 1 + \beta_{DOX} C_0$$

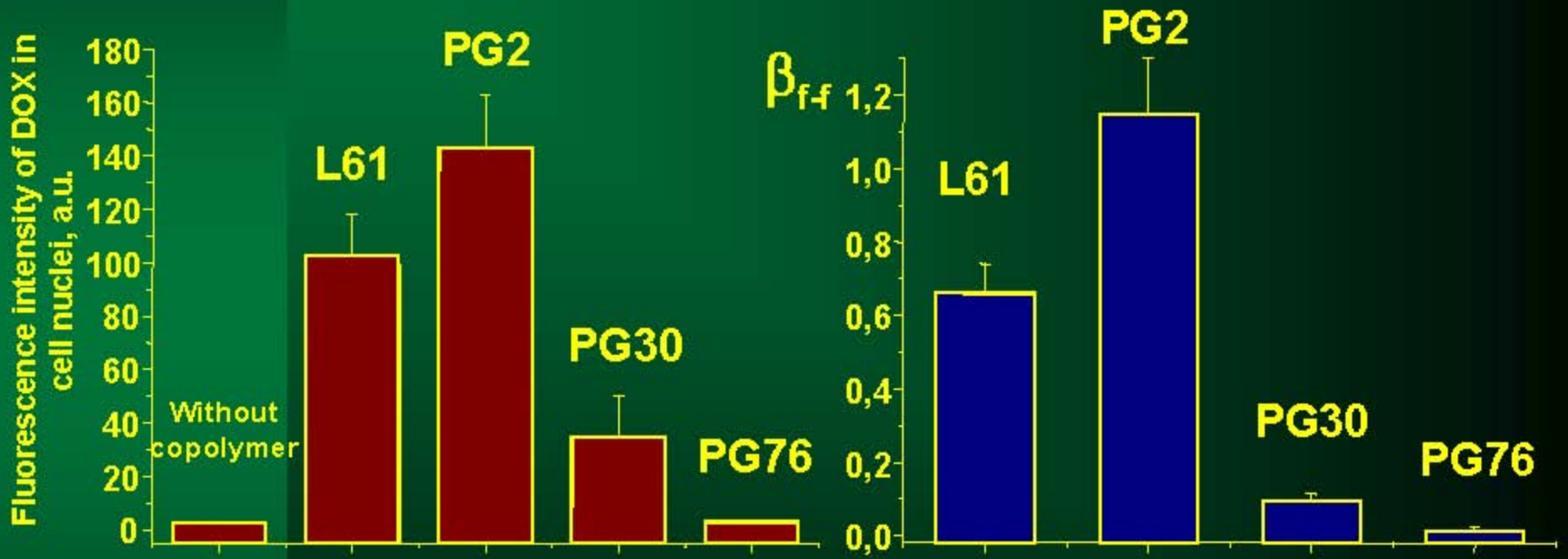


β_{DOX} – concentration independent ability of the copolymer to accelerate DOX permeation

Correlation between copolymer ability to facilitate doxorubicin permeation and its effect on the rate of flip-flop in model membranes

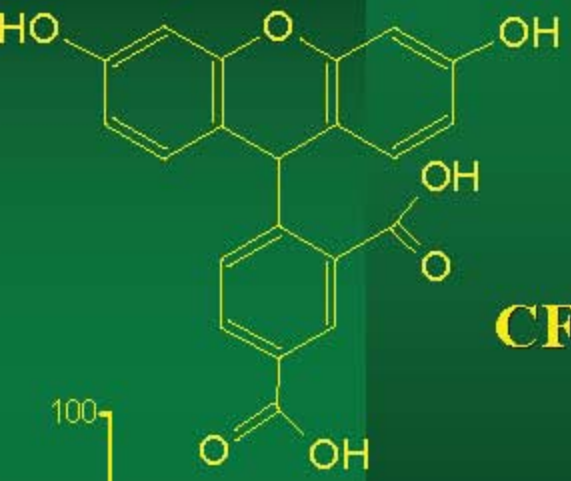


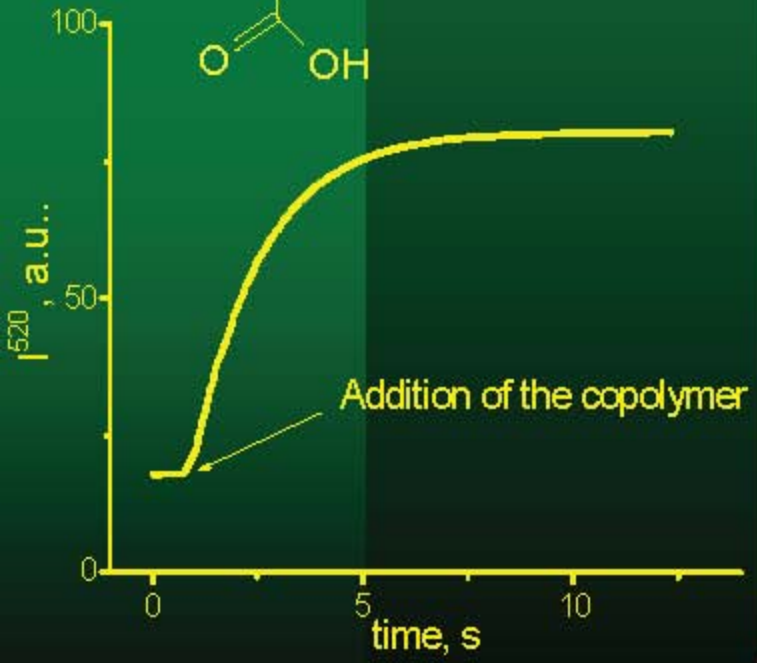
Comparison of the copolymer effect on doxorubicin accumulation in MDR cells and its effect on lipid flip-flop in liposomes.



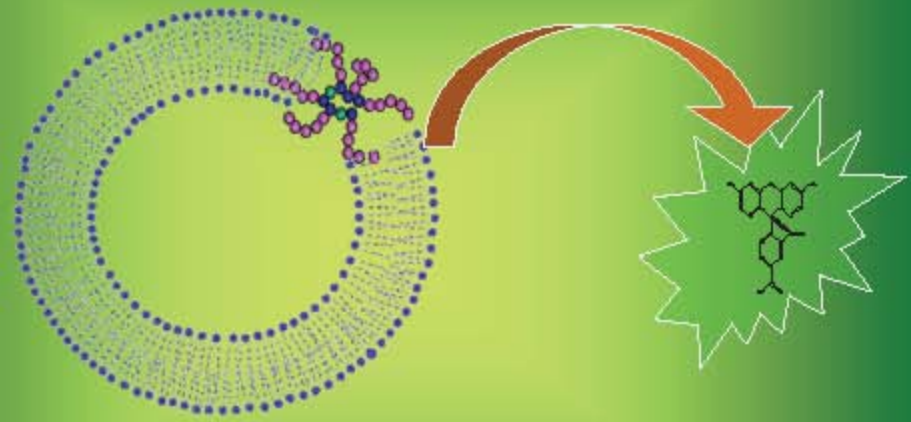
Effect of the copolymers on DOX accumulation in MDR cells and their effect on lipid flip-flop in liposomes are described by similar regularities

Effect of copolymers to induce leakage of ionic dye carboxyfluorescein

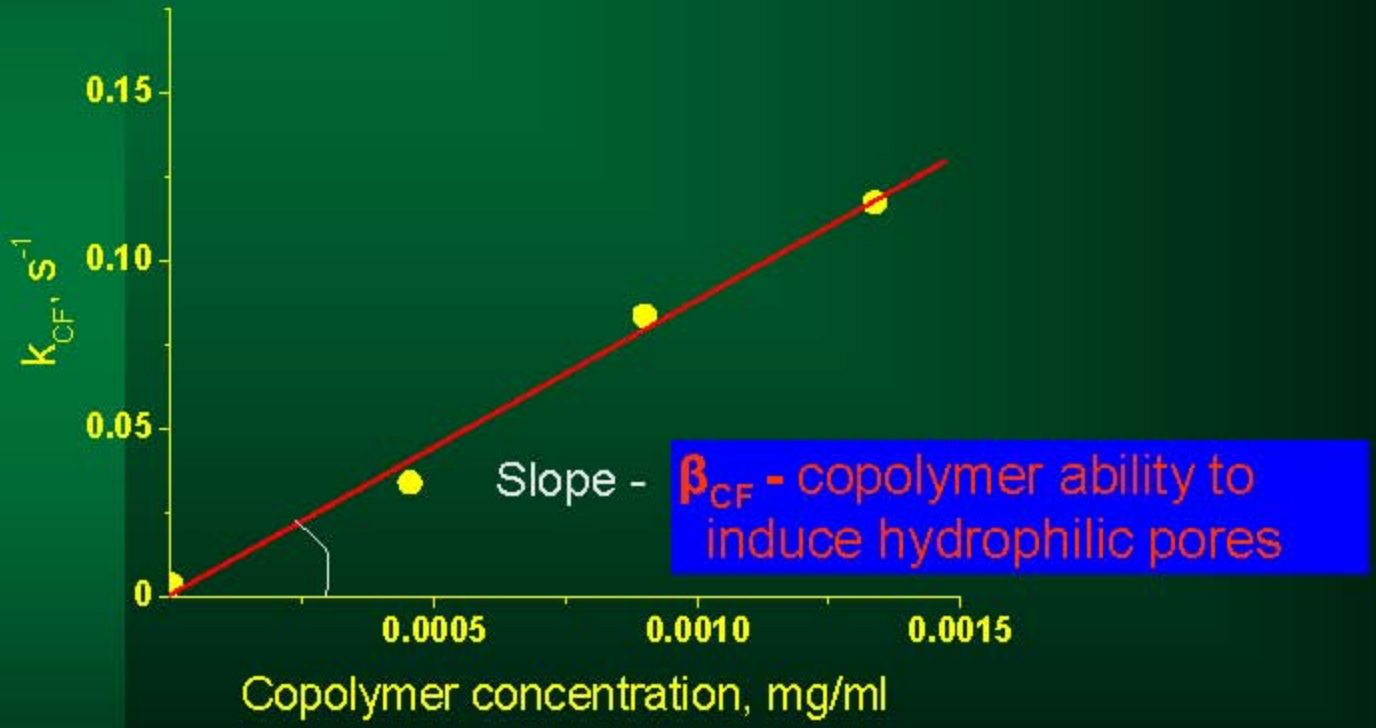




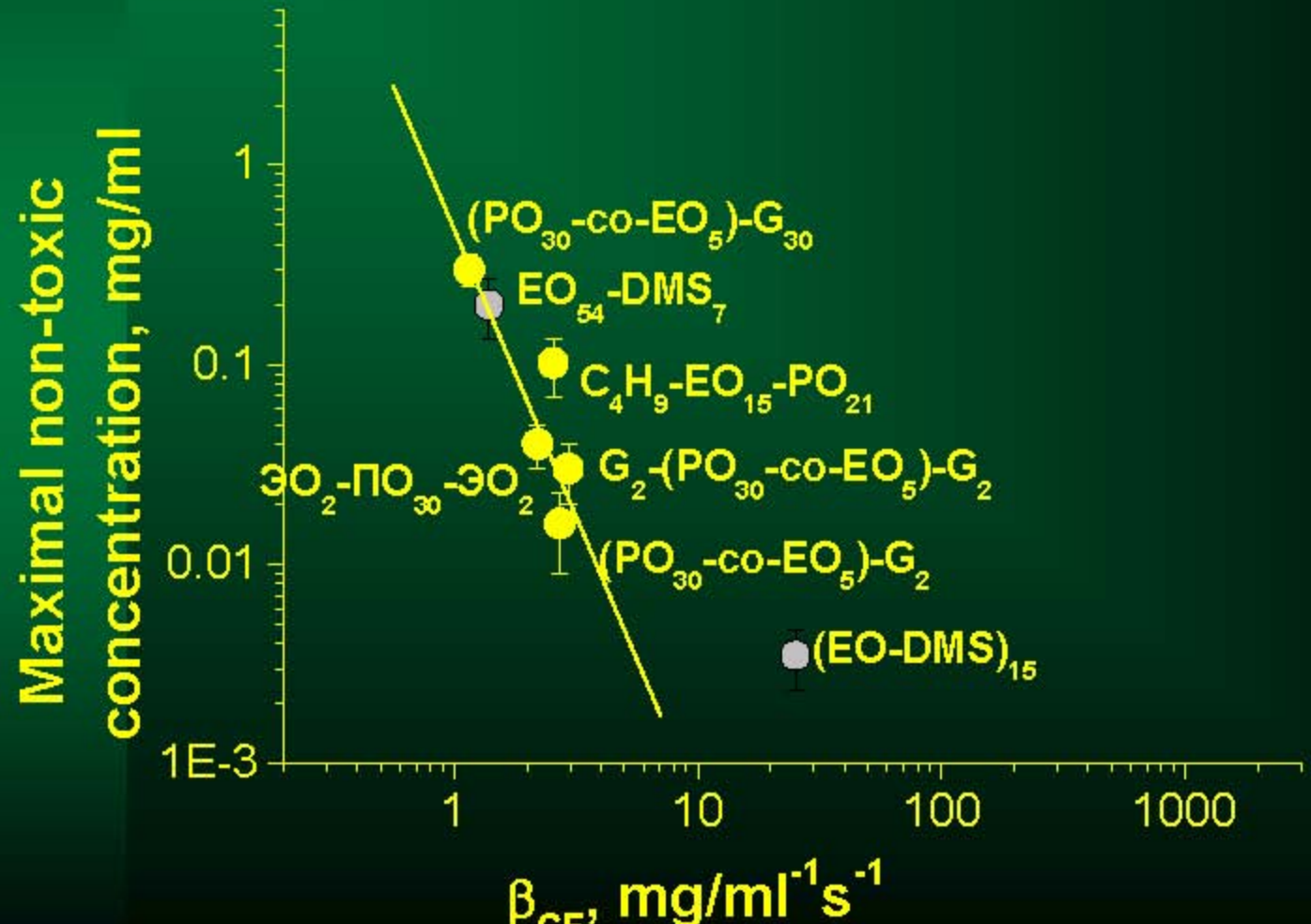
Leakage of CF indicates formation of hydrophilic pores



Copolymers accelerated CF leakage in a concentration dependent manner

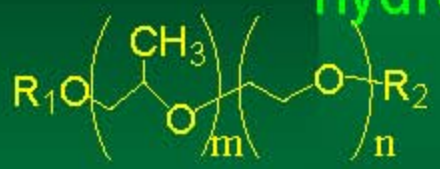


Ability of the copolymer to form hydrophilic pores correlates with its cytotoxicity

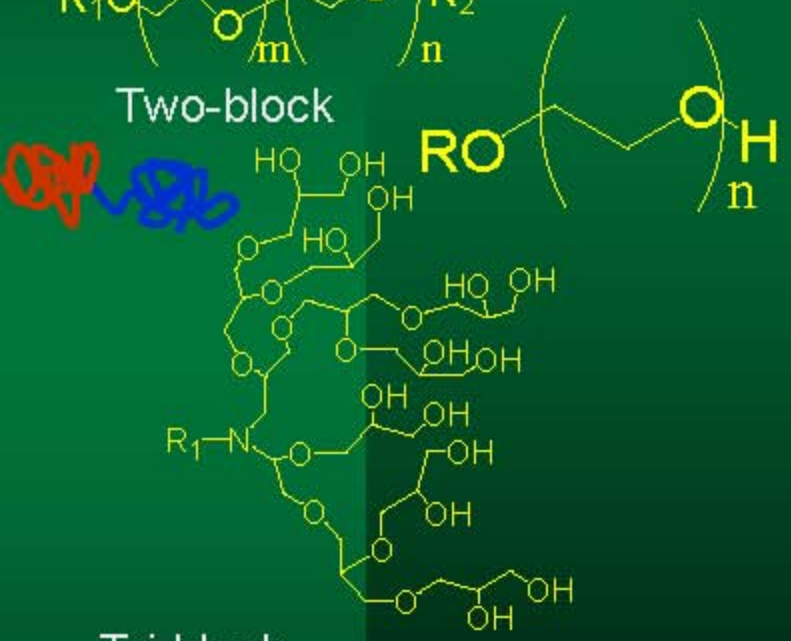


Copolymers which disturbed lipid bilayer packing

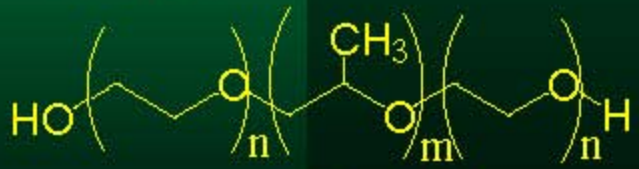
Copolymers with a single hydrophobic block



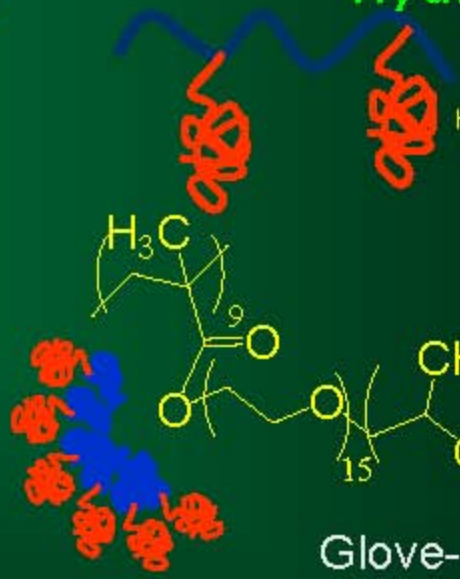
Two-block



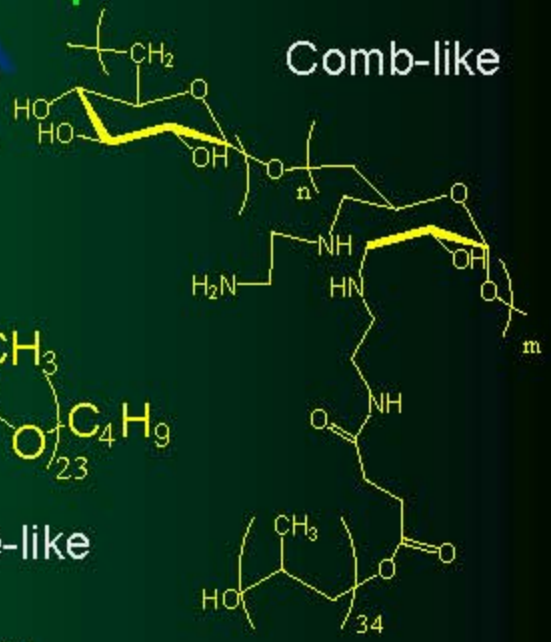
Tri-block



Copolymers with multiple hydrophobic blocks

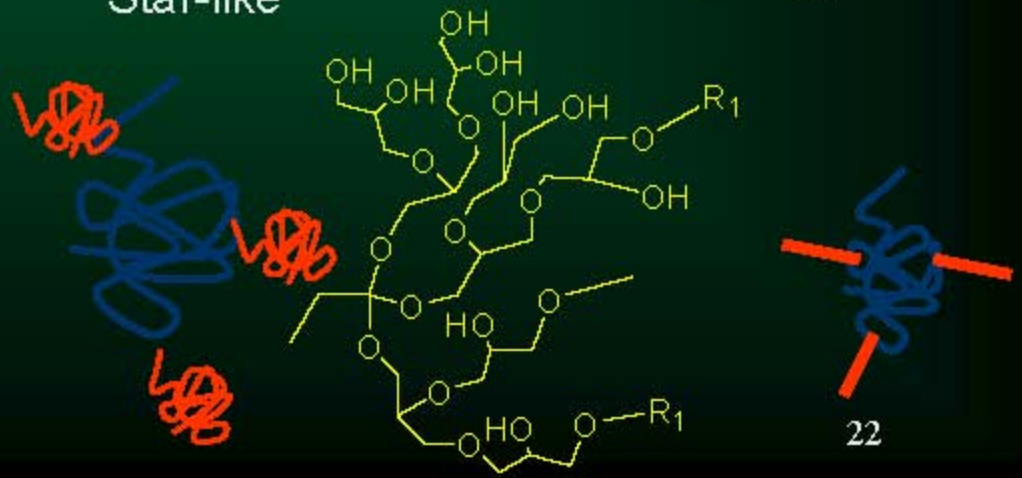


Glove-like



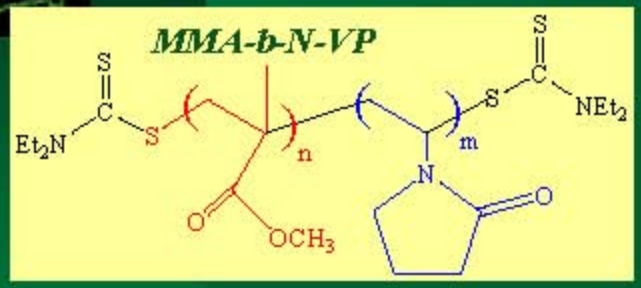
Comb-like

Star-like

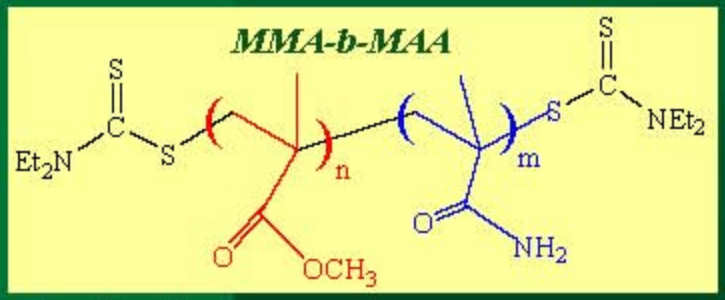


Copolymers that exhibited no or only marginal chemisensitizing activity

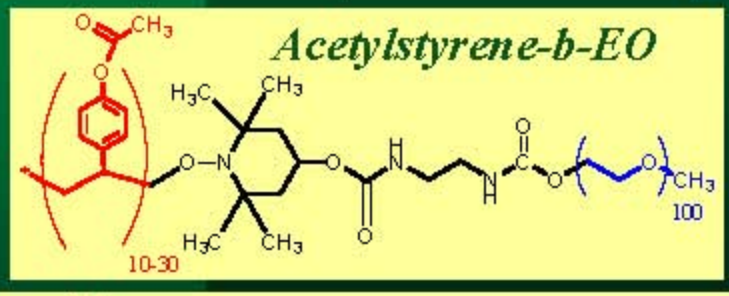
MMA-b-N-VP



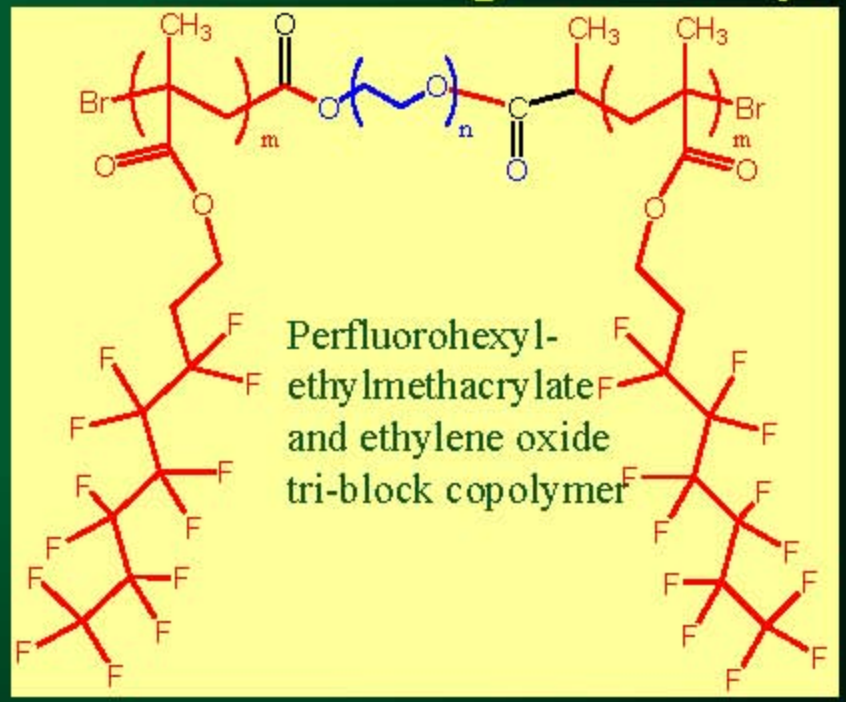
MMA-b-MAA



Acetylstyrene-b-EO



Hydroxystyrene-b-EO

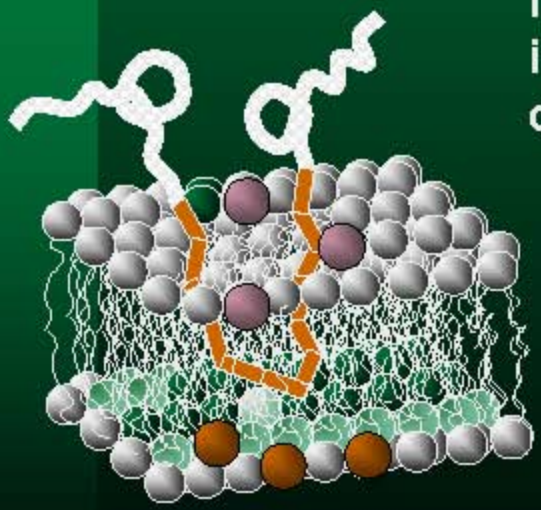


Perfluorohexyl-ethylmethacrylate and ethylene oxide tri-block copolymer

Physico-chemical basis for the disturbing activity of synthetic copolymers in lipid membranes

$\Delta\Delta G_{Water} < \Delta\Delta G_{Polymer}$ | $\Delta\Delta G_{Polymer} > 0$

$$\Delta\Delta G_o = 0 = \Delta\Delta G_{Water} + \Delta\Delta G_{Polymer\text{-}Lipid} + \Delta\Delta G_{Cavity} + \Delta\Delta G_{Polymer} + \Delta\Delta G_{Lipid}$$



Insertion of the copolymer hydrophobic block into lipid bilayer results in the restriction of its conformational mobility

These losses of conformational entropy result in the compensatory increase in the mobility of membrane components

High flexibility of the copolymer hydrophobic block – is important requirement to the structure of amphiphilic copolymer capable of disturbing membrane structure

Membrane disturbing copolymer should meet the following requirements

$$\delta = \frac{|\Delta\Delta G_{Polymer} + \Delta\Delta G_{Cavity}|}{|\Delta\Delta G_{Water}|}$$

$\delta > 1$ – membrane disturbance

$\delta < 1$ – membrane stabilization

Membrane disturbing ability of a copolymer would be observed if its hydrophobic block meets the following criteria:

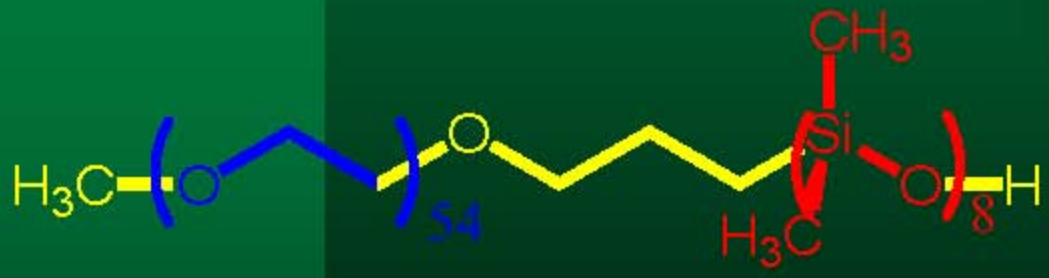
1) High flexibility

2) Poor thermodynamic compatibility with lipid bilayer

3) Large volume

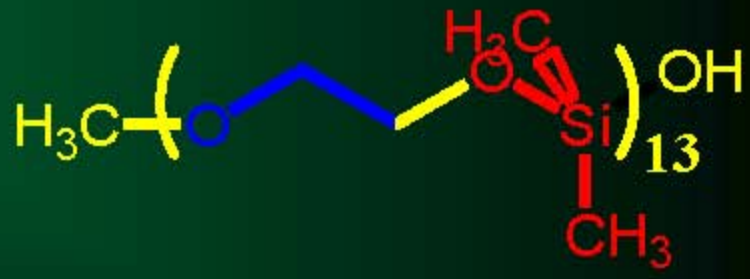
Polydimethylsiloxane completely meets all these criteria :

- It is very flexible
- It is poorly compatible with aliphatic hydrocarbons



PEO-b-PDMS

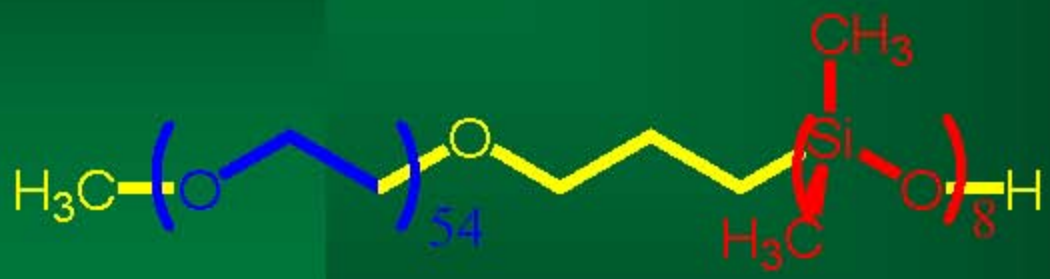
Mw = 3600

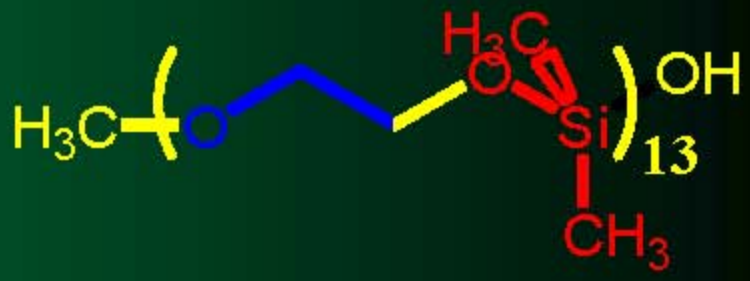


PEO-alt-PDMS

Mw = 1200

Comparison of the copolymers interaction with model membranes

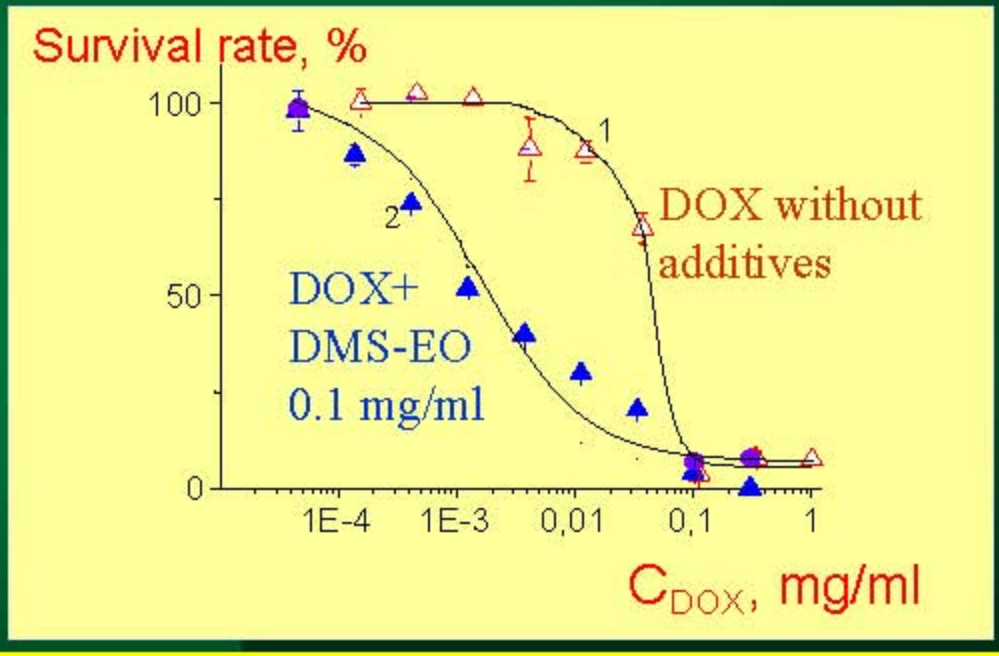




Sharp acceleration of flip-flop
Sharp acceleration of DOX permeation
Poor pore-forming ability

Poor acceleration of flip-flop
Poor acceleration of DOX permeation
Strong pore-forming ability

Influence of polydimethylsiloxane copolymers on multi-drug resistance of tumor cells

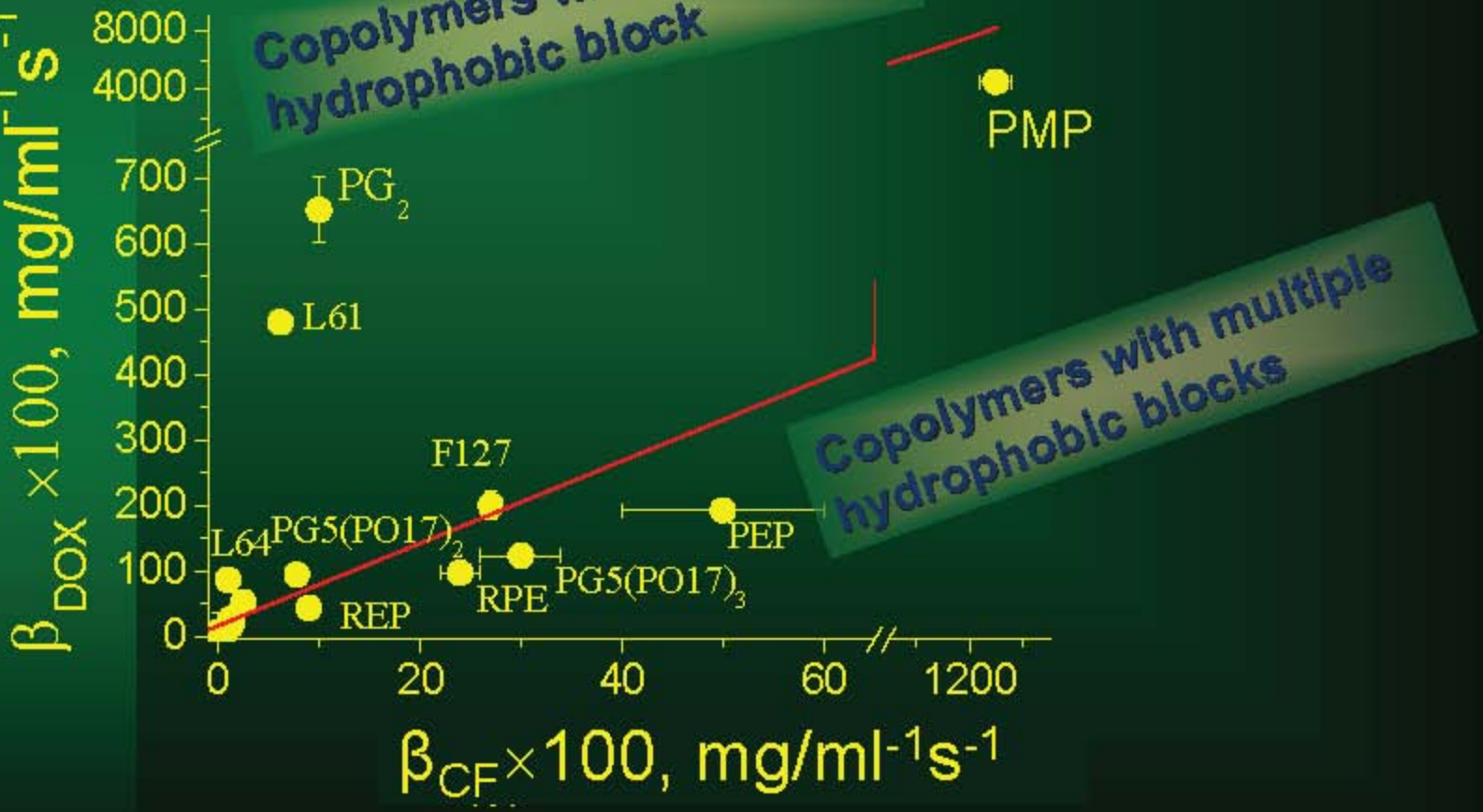


Alternating copolymer was highly toxic and did not exhibit any sensitization of MDR cells.

Block copolymer was poorly toxic and induced nearly 100-fold increase in the DOX toxicity towards MDR cells.

Macromolecular architecture of amphiphilic copolymer is an important factor determining its interaction with biological membranes

Comparison of β_{DOX} and β_{CF} for different copolymers



Conclusions

- Amphiphilic copolymers with flexible and poorly compatible with hydrocarbons hydrophobic block can disturb lipid part of biological membranes. This effect correlates with the copolymer ability to inhibit P-glycoprotein in MDR cells.

- Copolymers with a single hydrophobic block exhibit tendency to disturb lipid bilayer rather than form hydrophilic pores.

- In contrast, copolymers with multiple hydrophobic blocks form pores in lipid bilayers to the most extent.

Thank you for your attention!