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PROTECTION



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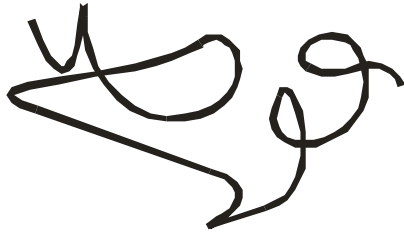
Sugar-modified PPI dendrimers as carriers of anti-leukemia drugs

Barbara Klajnert-Maculewicz

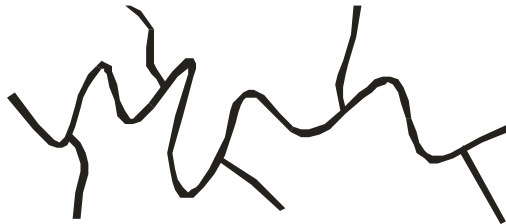
Department of General Biophysics

University of Lodz

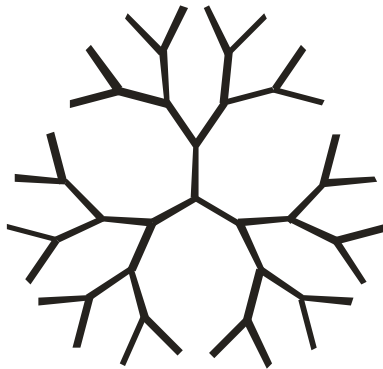




Linear polymer
1930s



Branched polymer
1960s



Dendrimer
1980s



dendrimer - gr. *dendron*
arborol - lat. *arbor*
cascade molecule



D. Tomalia

G. Newcome

F. Vögtle



↑
dendrimer

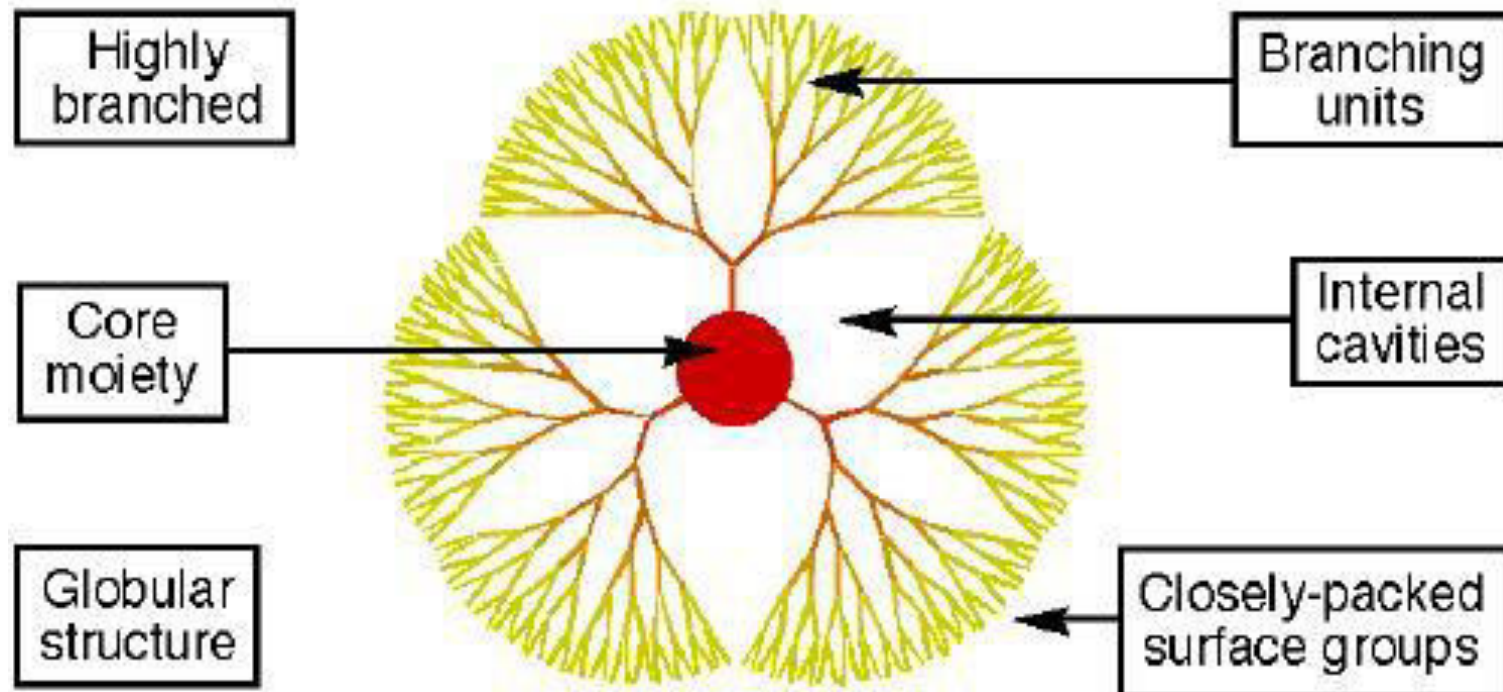
↑
cascade molecule

↑
arborol

dendrimer - gr. *dendron*
arborol - lat. *arbor*
cascade molecule



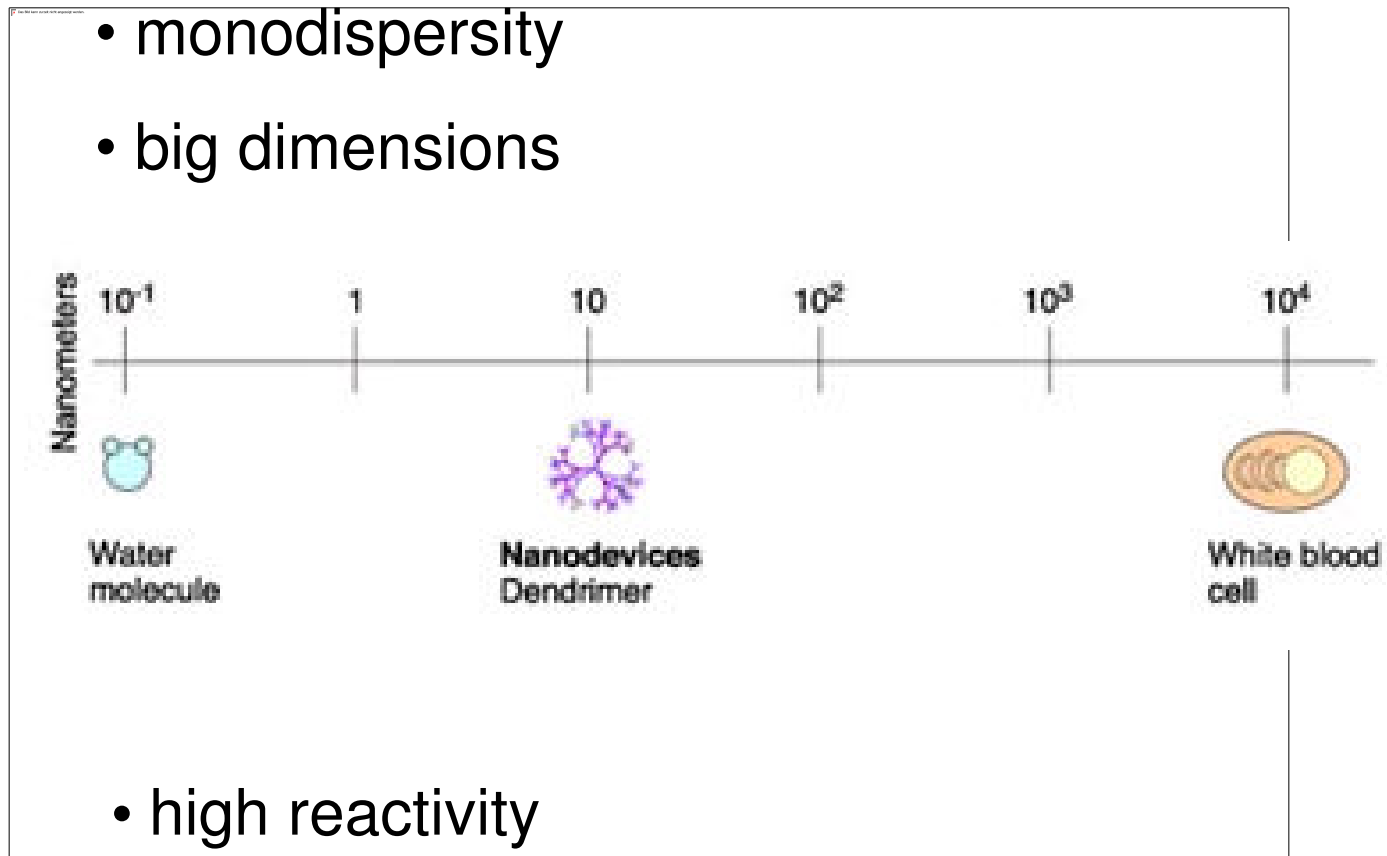
The Dendritic Structure



- globular shape

- monodispersity

- big dimensions



- high reactivity

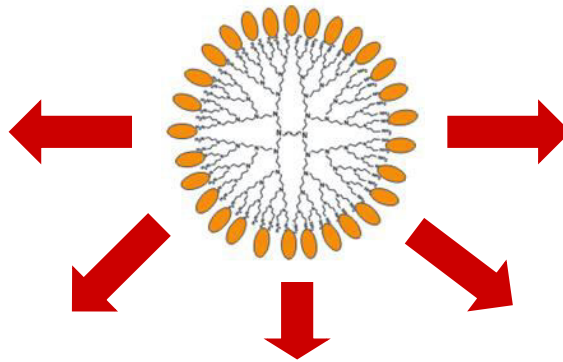
- good solubility



DRUG DELIVERY



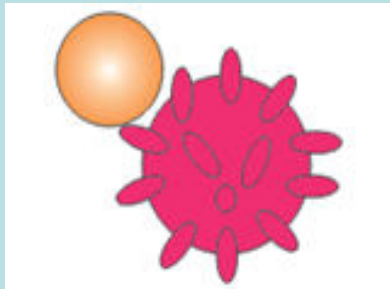
ANTI-MICROBIAL ACTIVITY



GENE CARRIERS



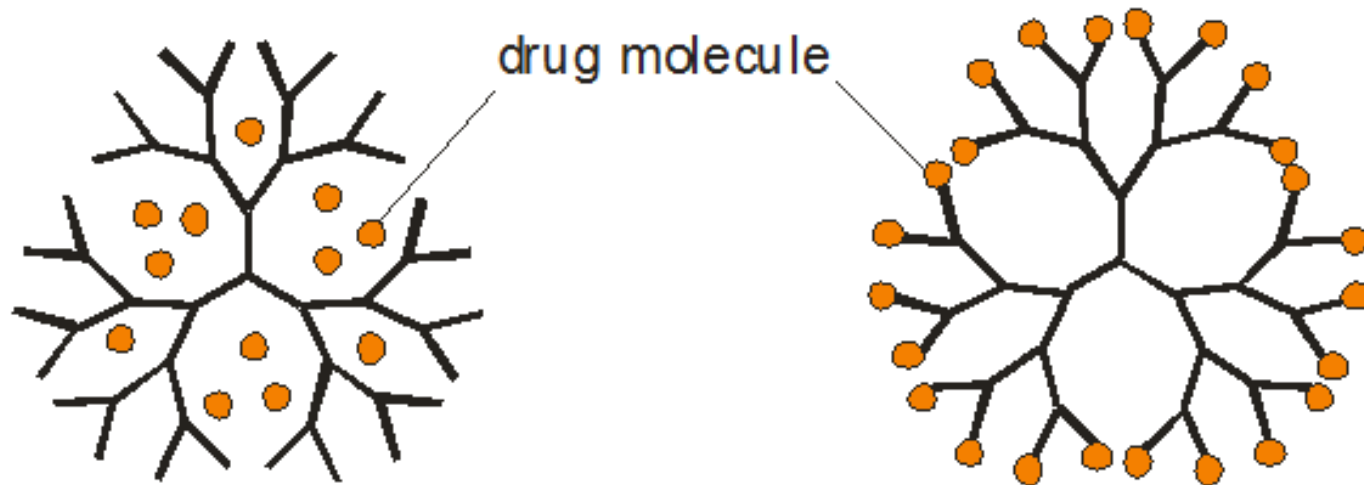
ANTI-HIV ACTIVITY

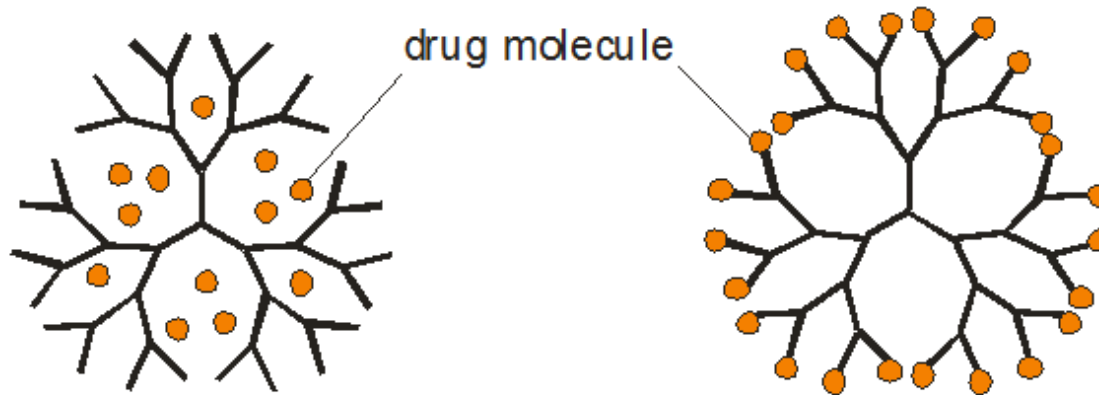


ANTI-PRION ACTIVITY



Dendrimers as drug carriers





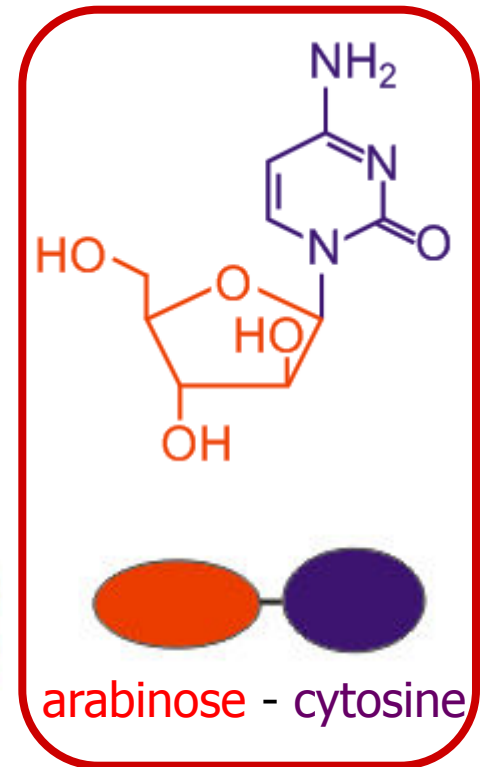
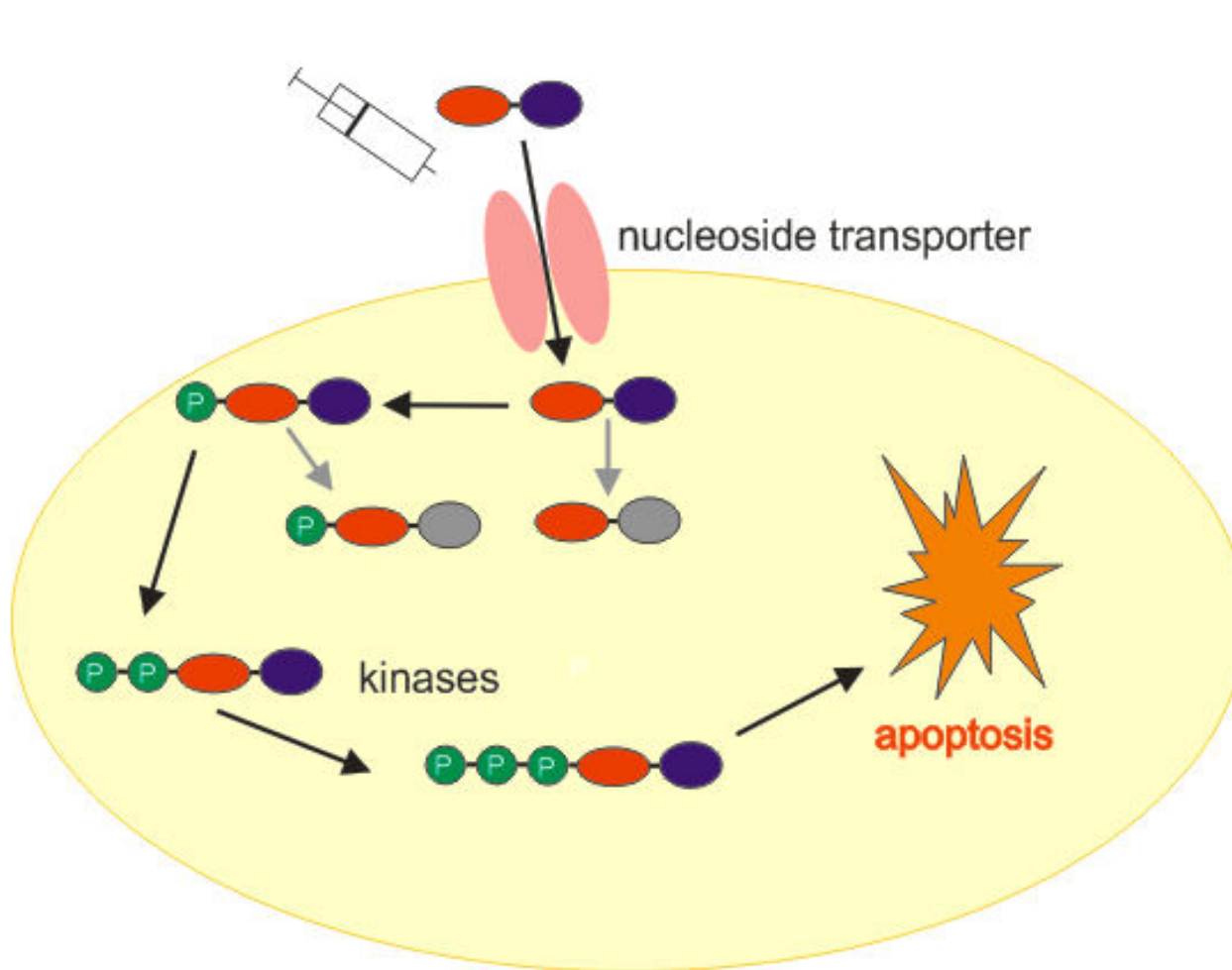
- prolonging the circulation time in blood
- protecting unstable drugs from the environment
- enhancing solubility of poorly soluble drugs
- achieving a controlled release and tissue targeting
- one molecule of dendrimer is capable to carry drugs at high density
- slower release than for a free drug



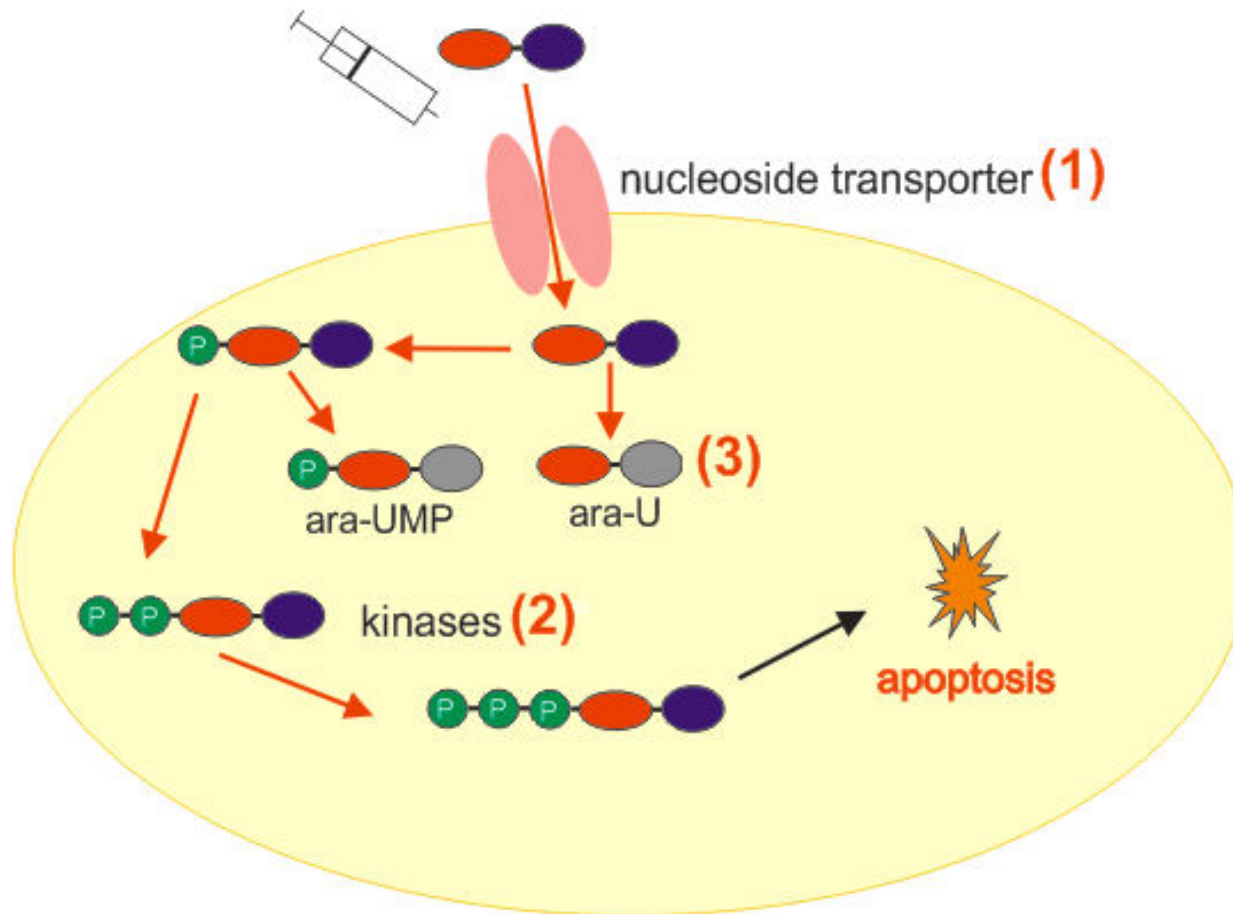
Physiological nucleoside analogues (NAs)



Cytarabine is used to treat different forms of leukemia, including acute and chronic myelogenous (AML and CML) and acute lymphocytic leukemia (ALL).



Drug resistance mechanisms

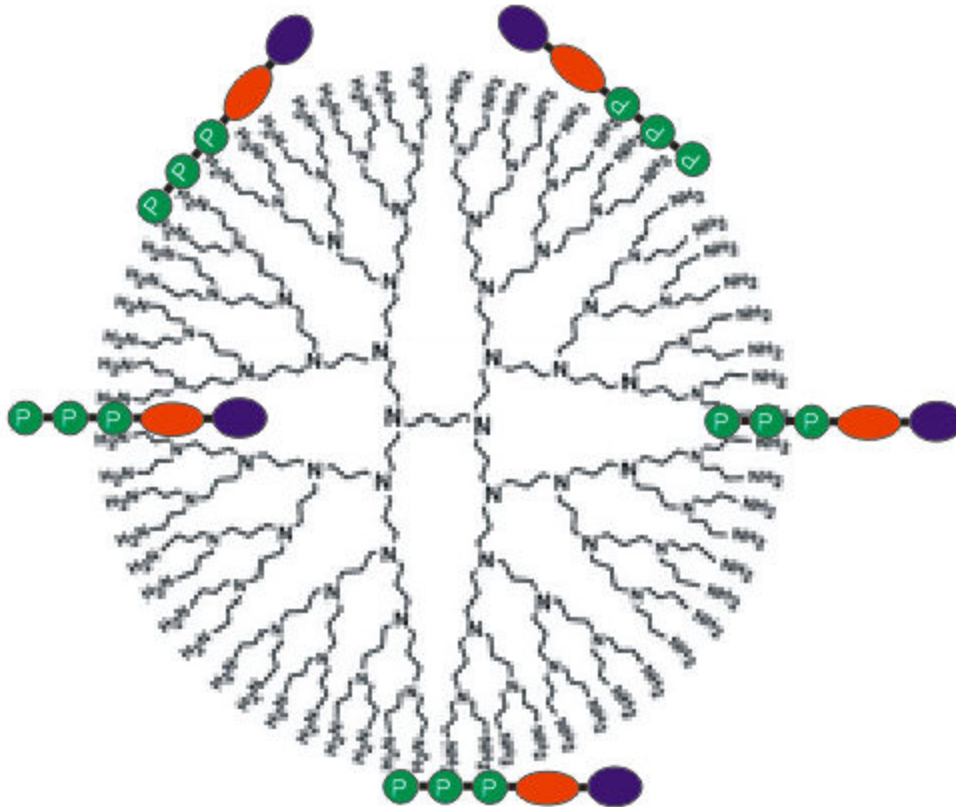


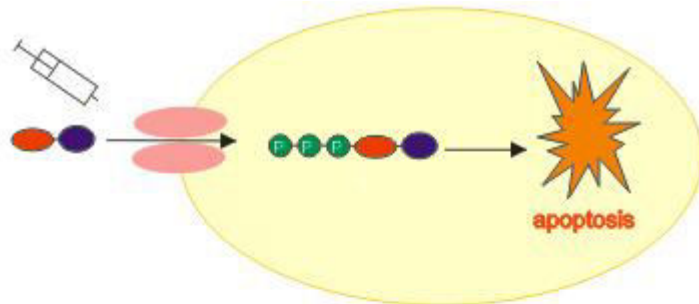


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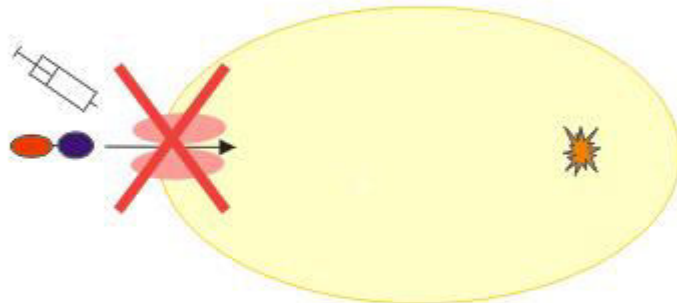


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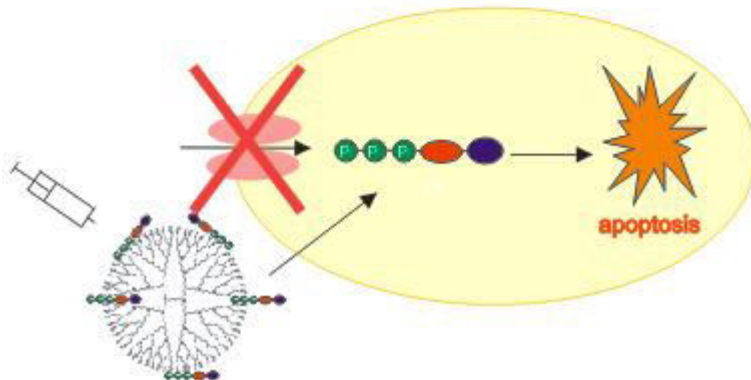




Conventional therapy with ara-C



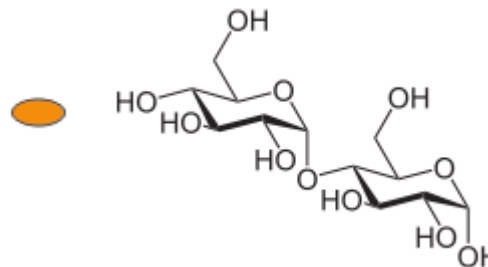
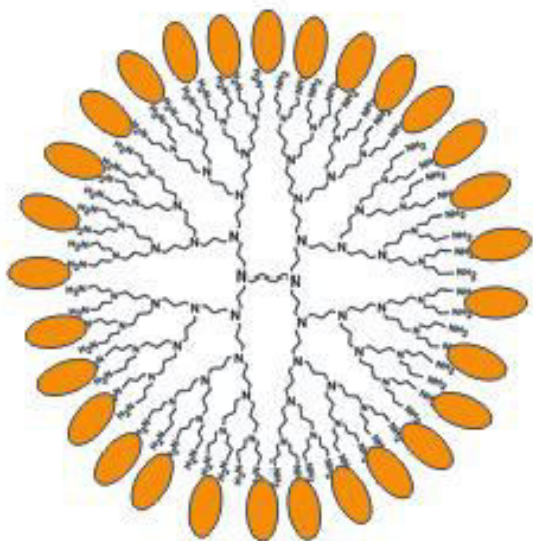
Drug resistance



Dendrimers as carriers of ara-CTP



Maltose-modified PPI dendrimer

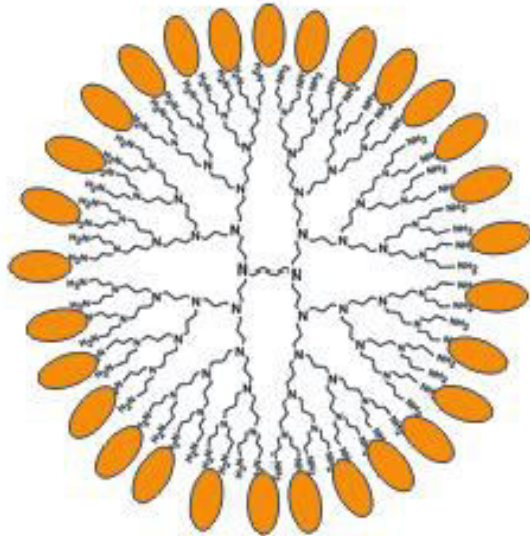


PPI-OS-Mal G5 (approx. 30% maltose)

Dr. Dietmar Appelhans

Synthesis: Fischer et al. *Biomacromolec.* **10** (2009) 1314-1325

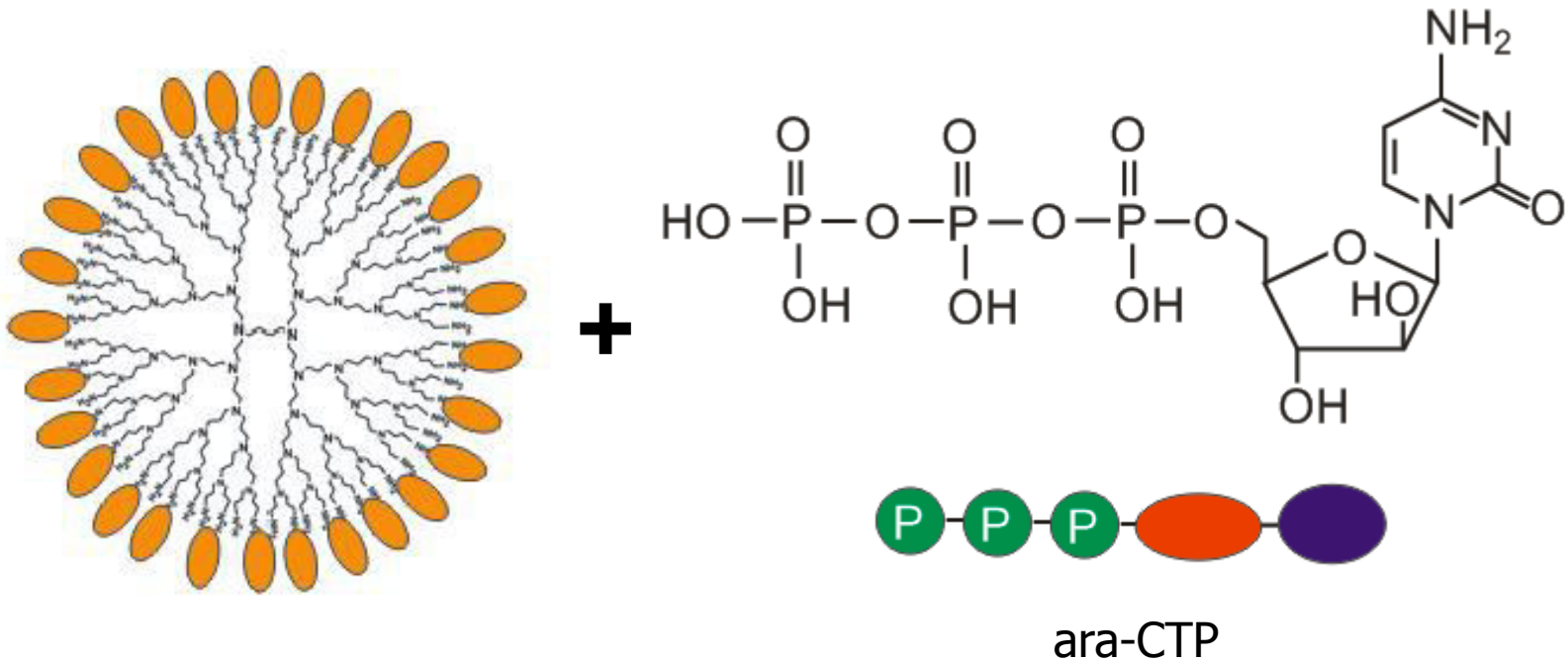
Advantages of sugar modification



- non-hemolytical ^{1, 2}
- do not cause platelets aggregation ²
- low toxicity *in vitro* ³
- low toxicity *in vivo* ⁴
- retained activity ^{1, 5, 6}

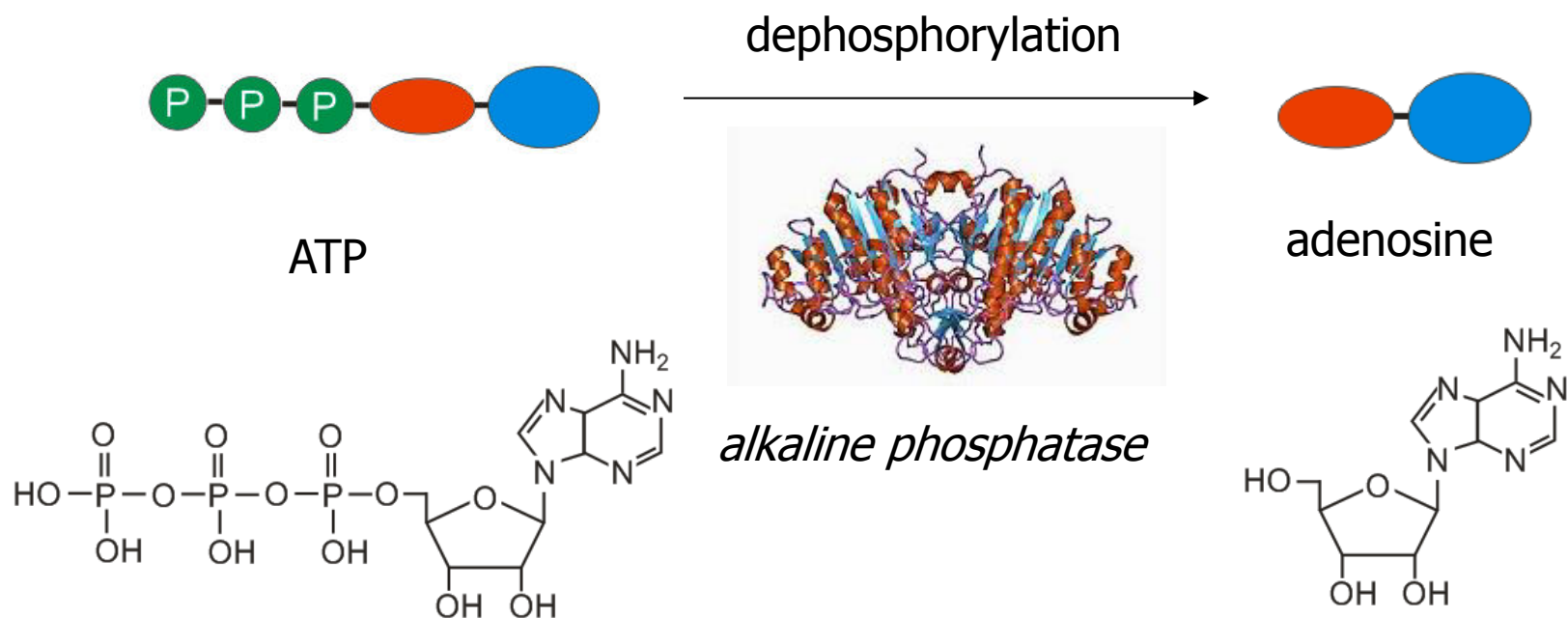
1. B. Klajnert et al. Chem. Eur. J. **14** (2008) 7030-7041
2. B. Ziemia et al. J. Biomed. Mater. Res. A. **100** (2012) 2870
3. A. Janaszewska et al. New J. Chem. **36** (2012) 428-437
4. B. Ziemia et al. J. Biomed. Mater. Res. A **99** (2011) 261-268
5. M. Fischer et al. Biomacromolecules **11** (2010) 1314-1325
6. M. Ciołkowski et al. Colloid Surface B **95** (2012) 103-108

complexes:

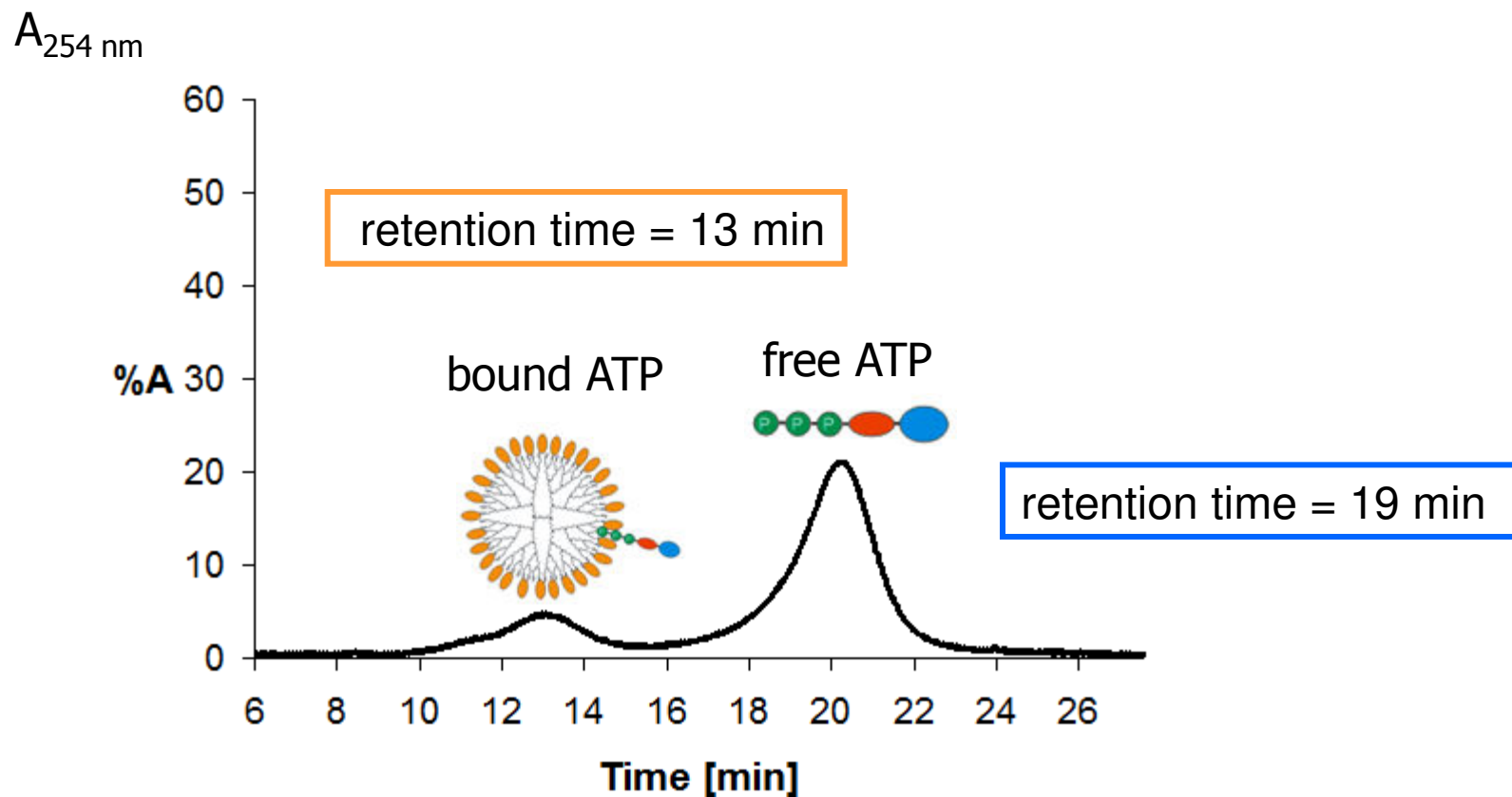


- dendrimer and ara-CTP form complexes (ara-CTP/dendrimer=10)
- complexes are stable
- ara-CTP is protected against enzymatic degradation

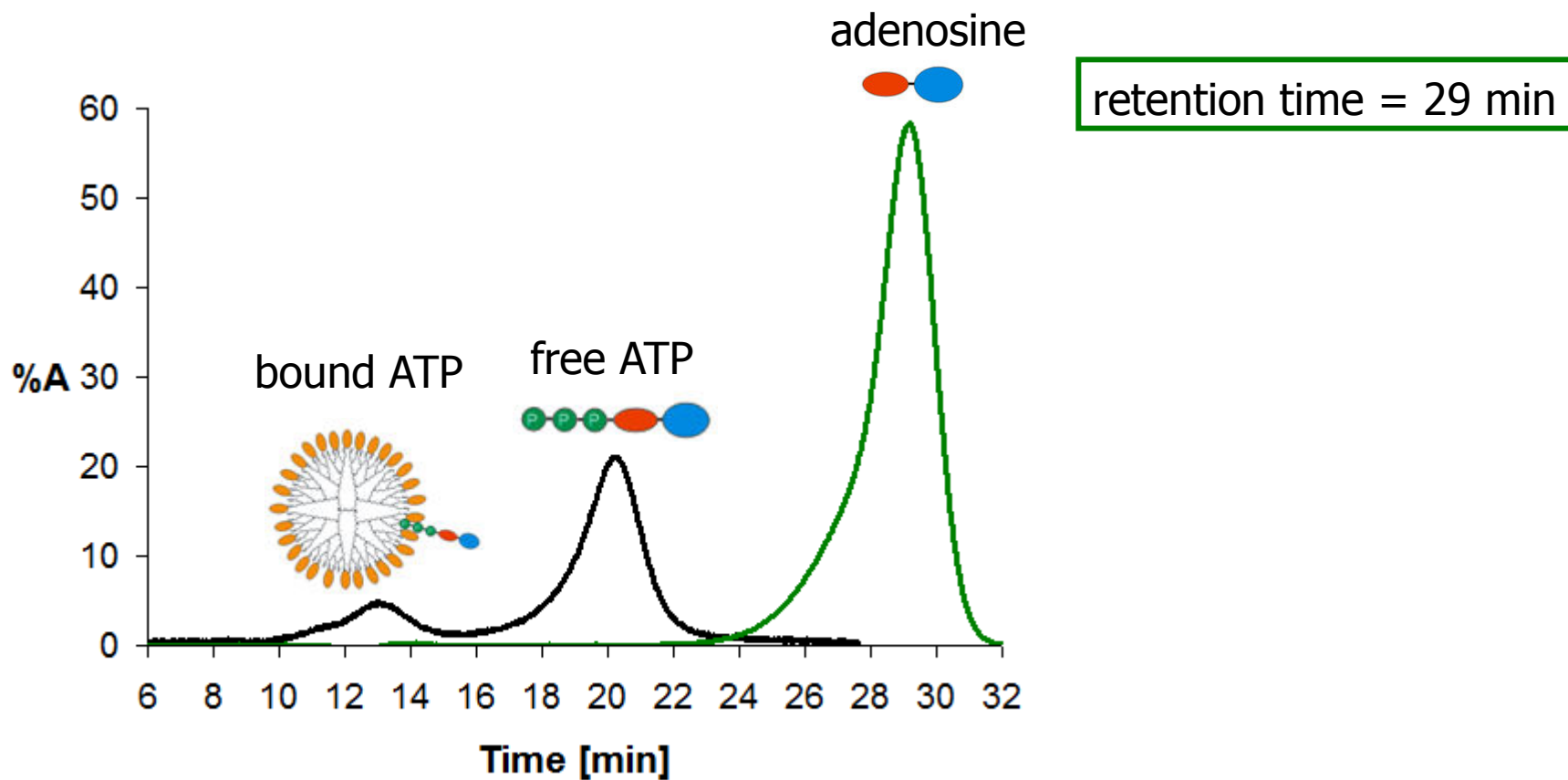
Protection against enzymatic degradation – FPLC method



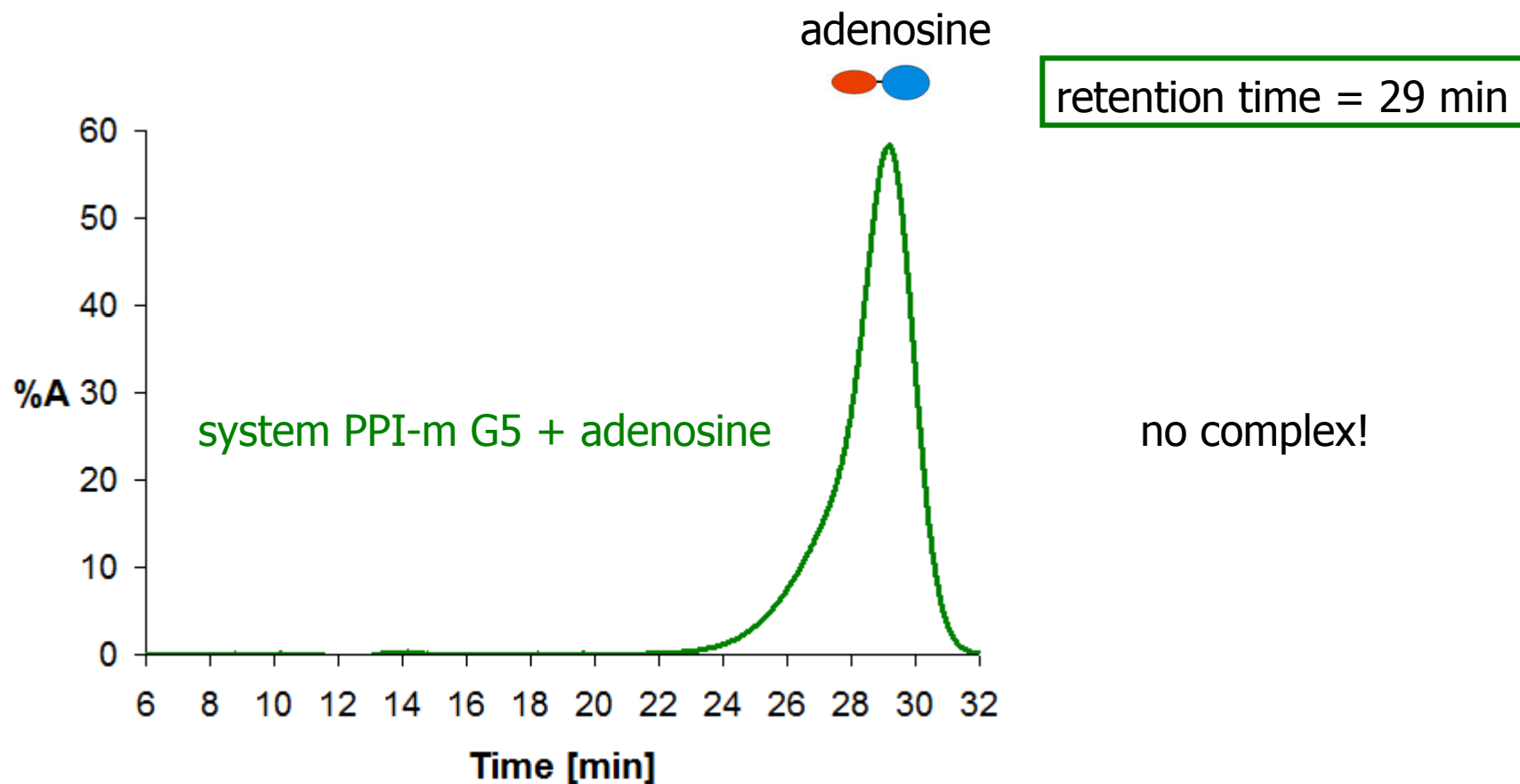
Protection against enzymatic degradation – FPLC method



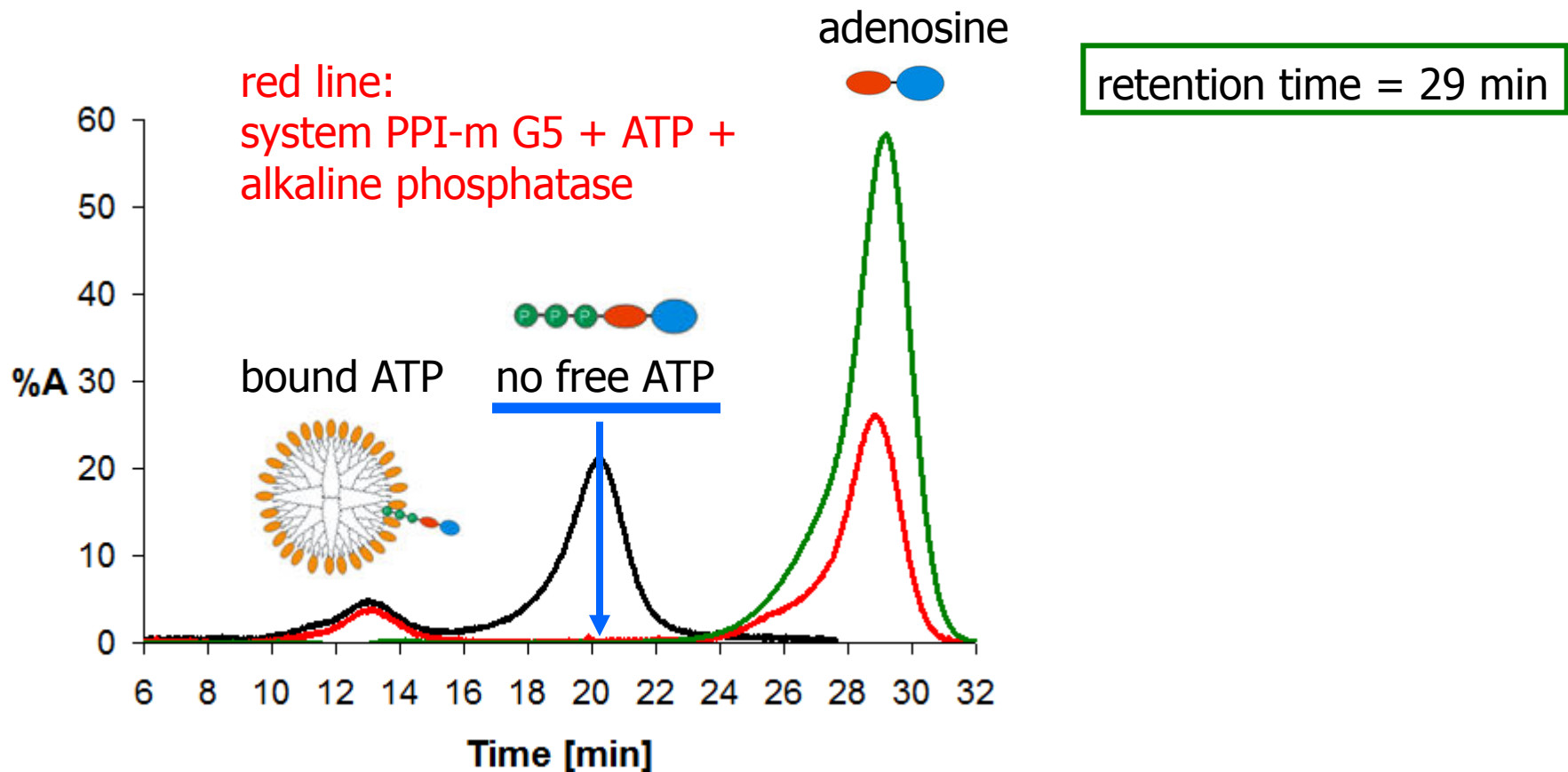
Protection against enzymatic degradation – FPLC method



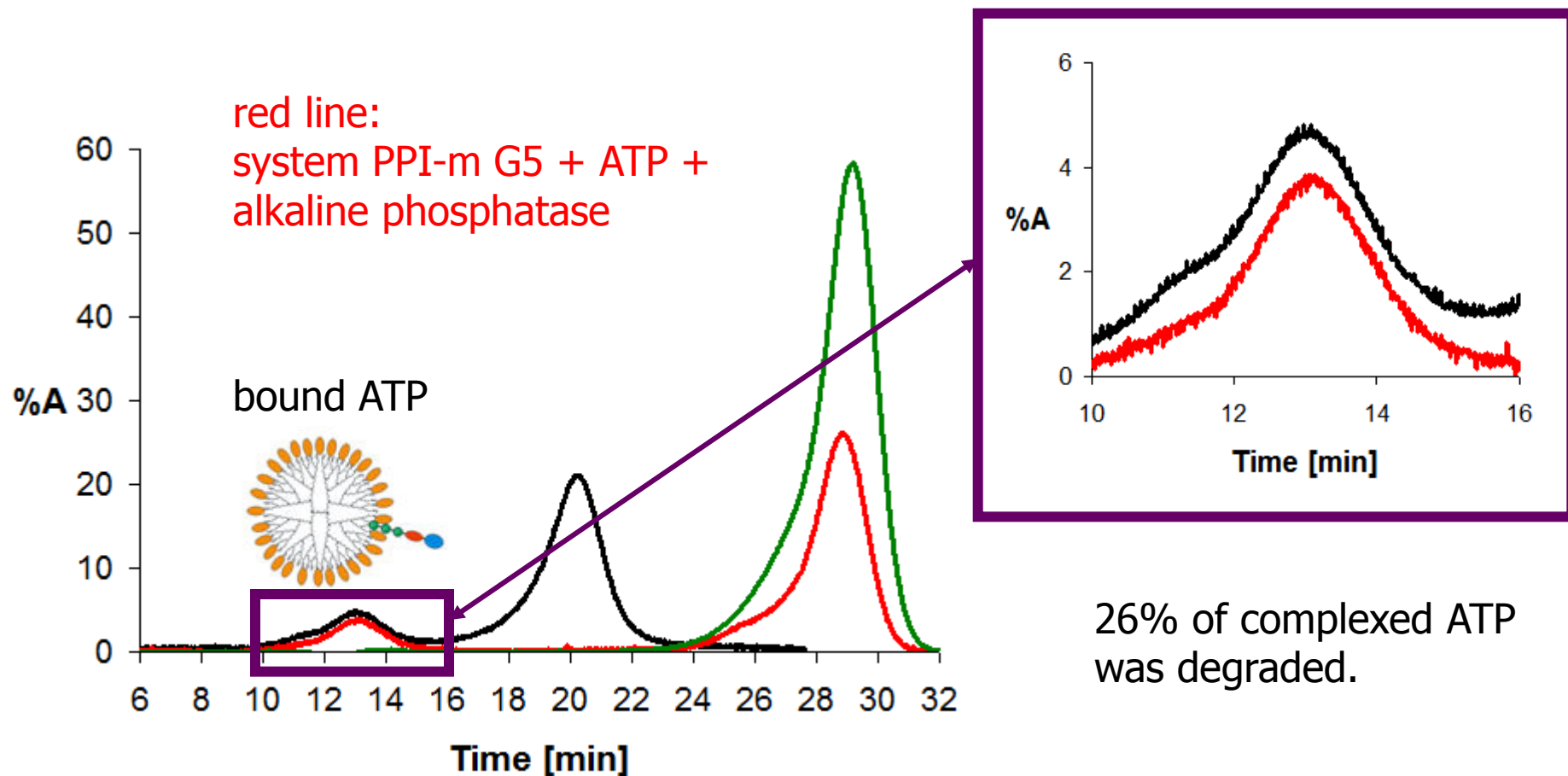
Protection against enzymatic degradation – FPLC method



Protection against enzymatic degradation – FPLC method

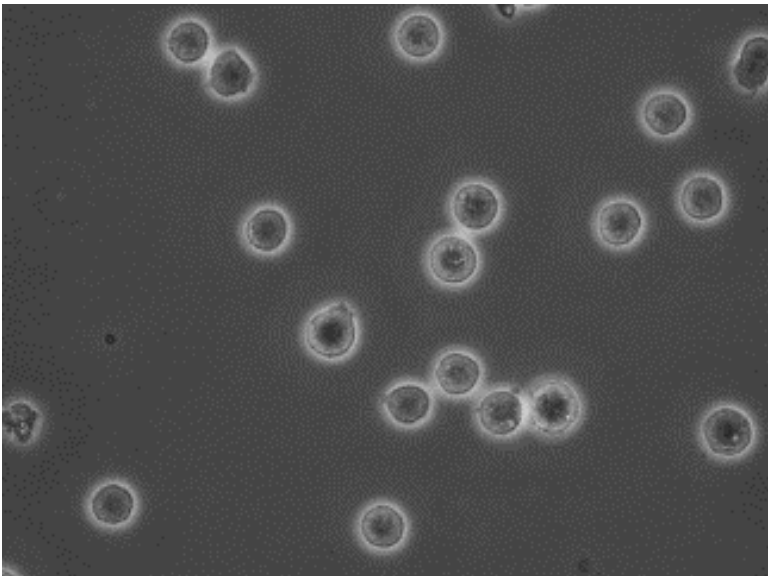


Protection against enzymatic degradation – FPLC method



leukemia cell line:

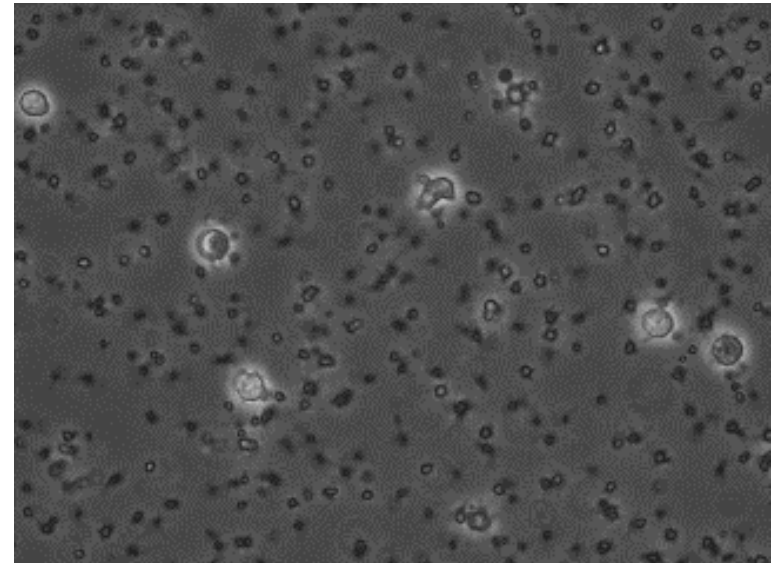
1301



human acute lymphoblastic T cells

control cells:

PBMC

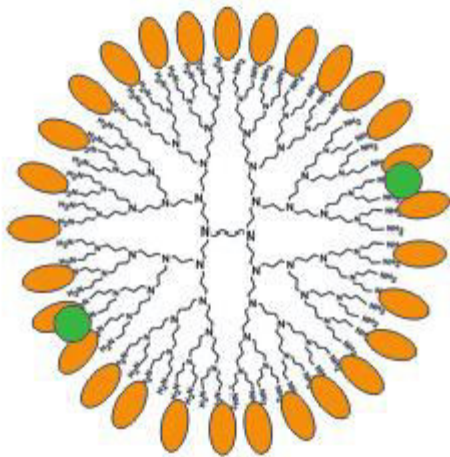


peripheral blood mononuclear cells

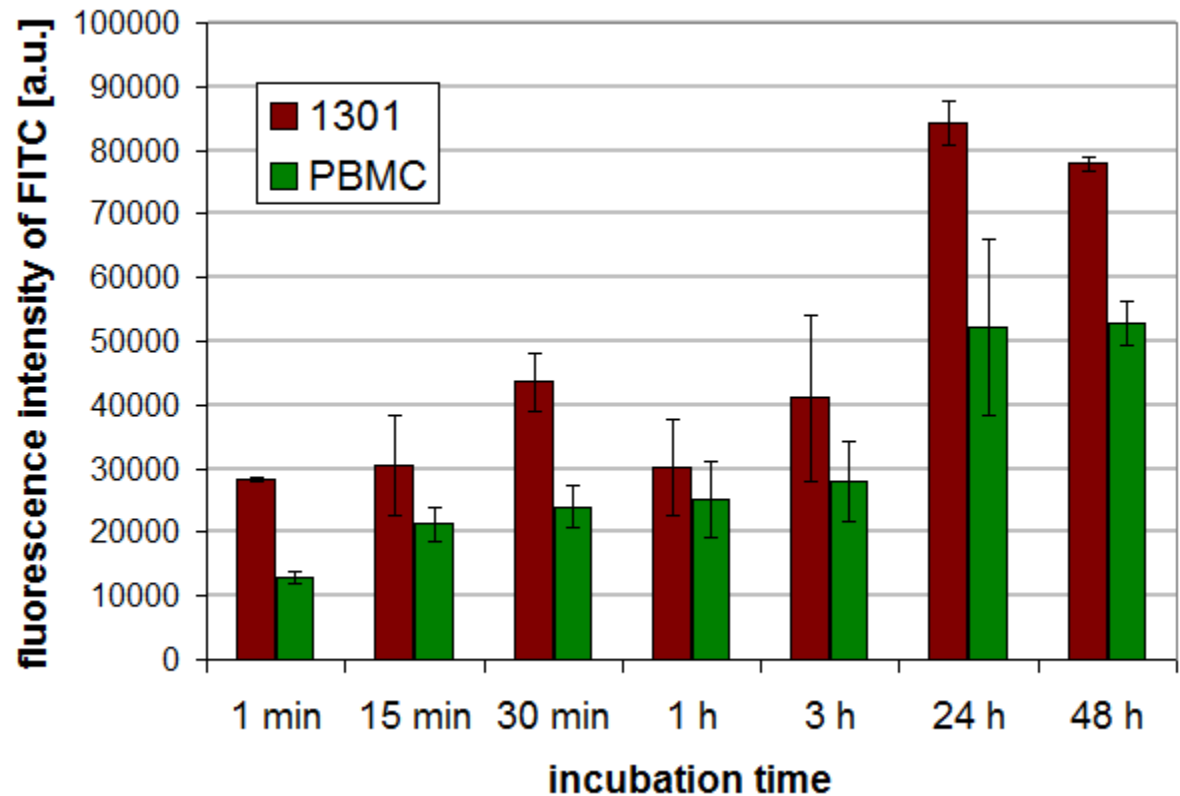


Internalization of dendrimers into cells

Flow cytometry



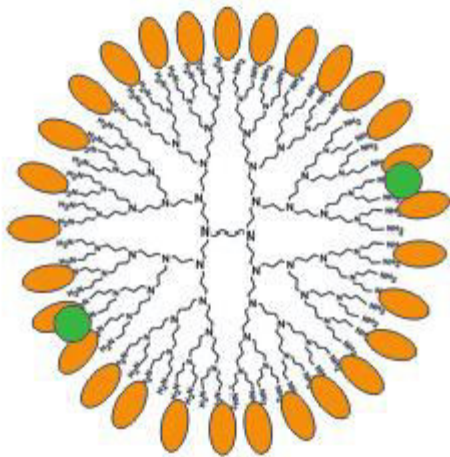
● fluorescein (FITC)



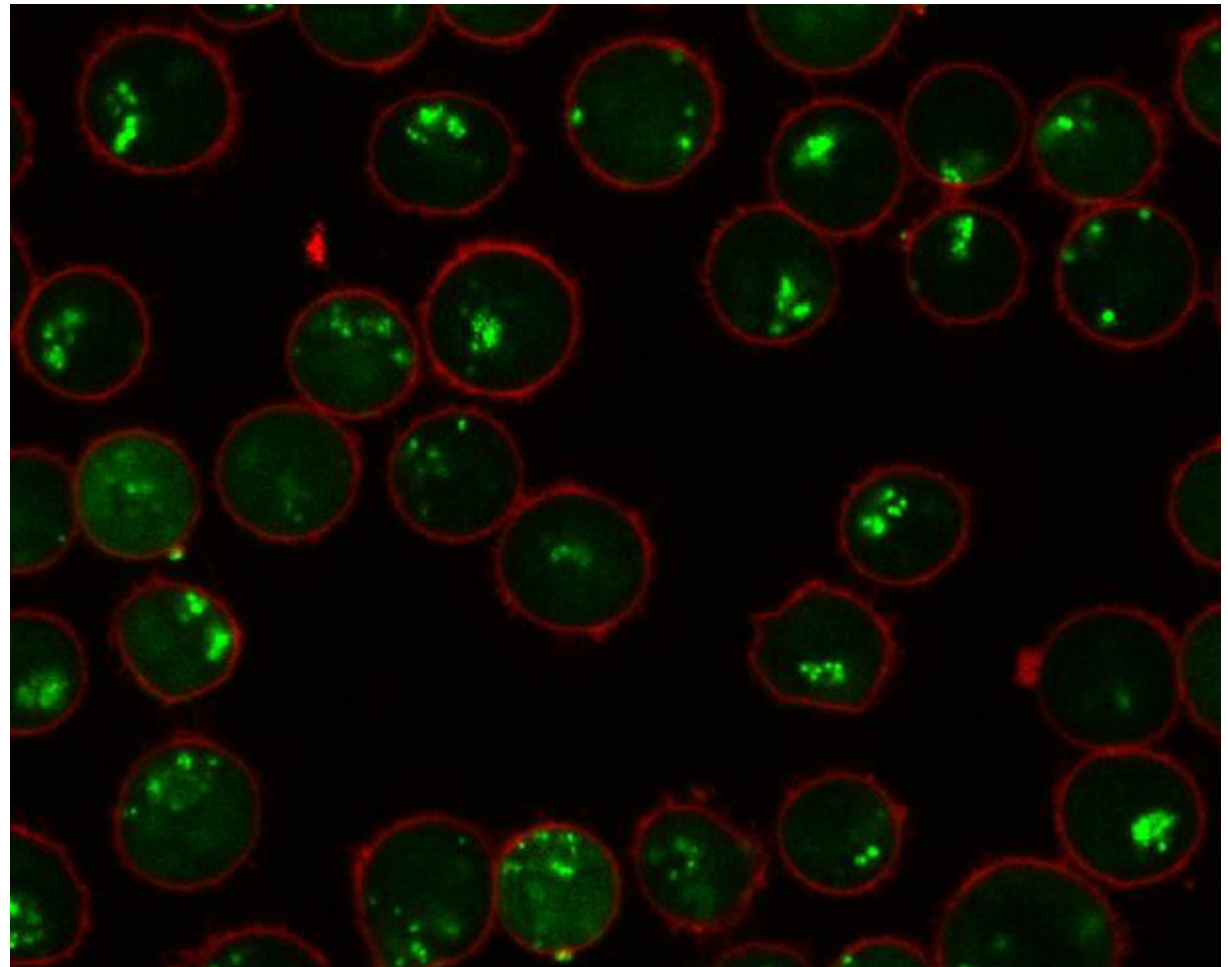


Internalization of dendrimers into 1301 cells

*Confocal
microscopy*



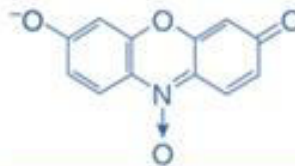
● fluorescein (FITC)





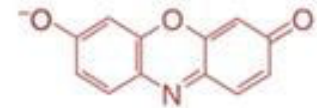
Cytotoxicity Alamar Blue assay

Cytotoxicity test with resazurin - in live cells it is reduced to resorufin:



Resazurin
Blue and weakly fluorescent

Reduction by
Metabolically Active Cells



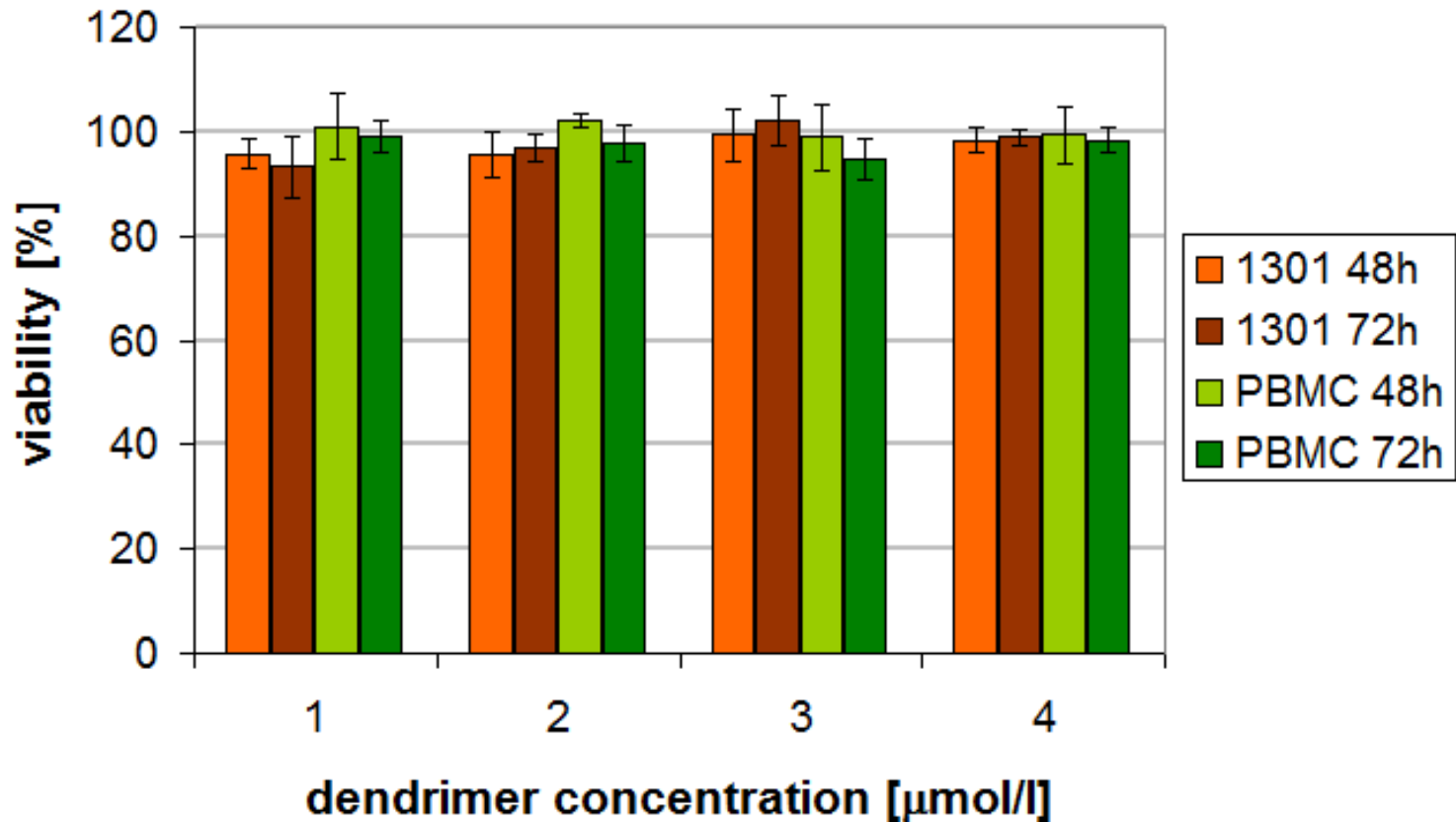
Resorufin
Red and highly fluorescent

$$\lambda_{\text{exc}} = 530 \text{ nm}$$

$$\lambda_{\text{em}} = 590 \text{ nm}$$

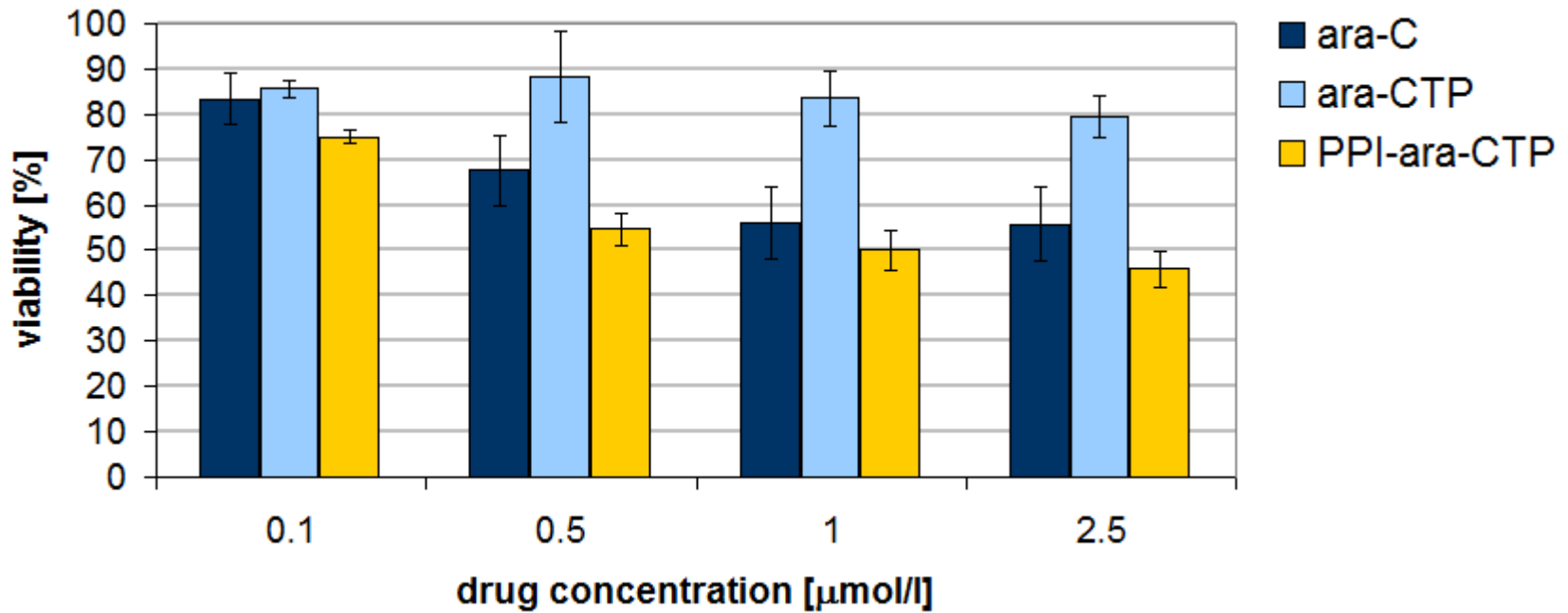


Dendrimer cytotoxicity



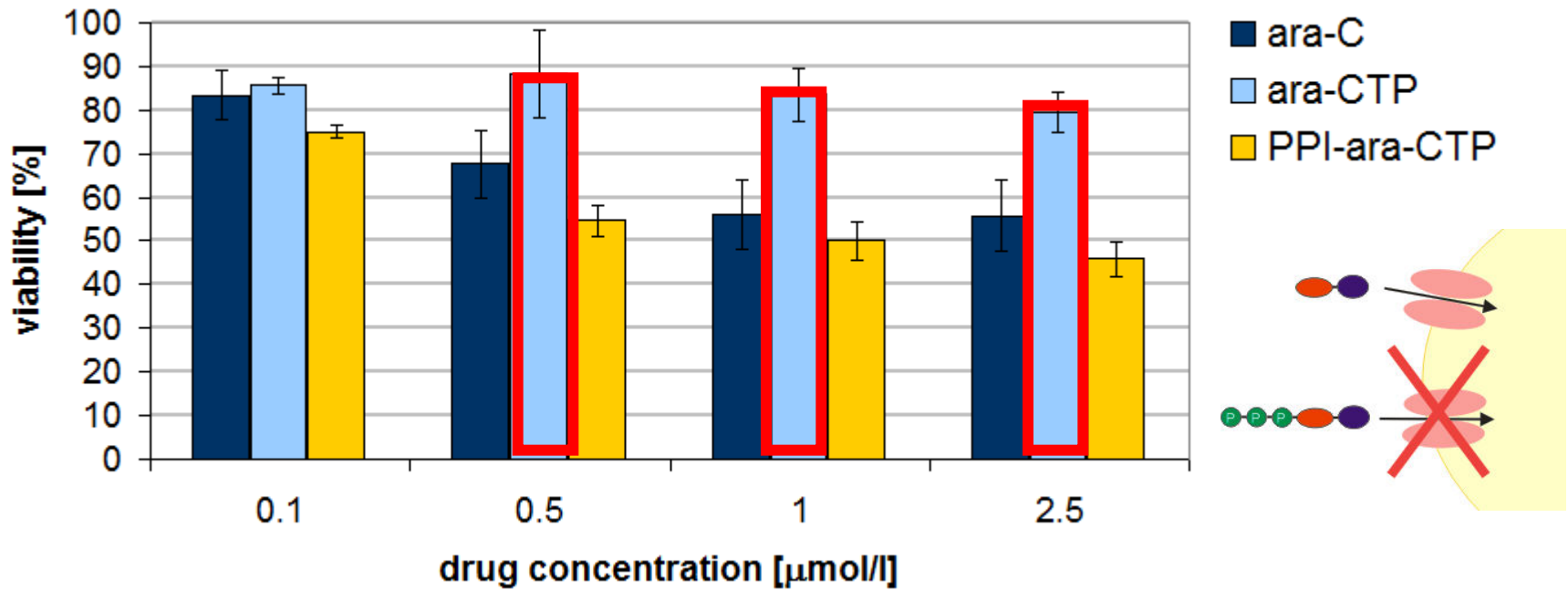


Cytotoxicity - 1301 48h



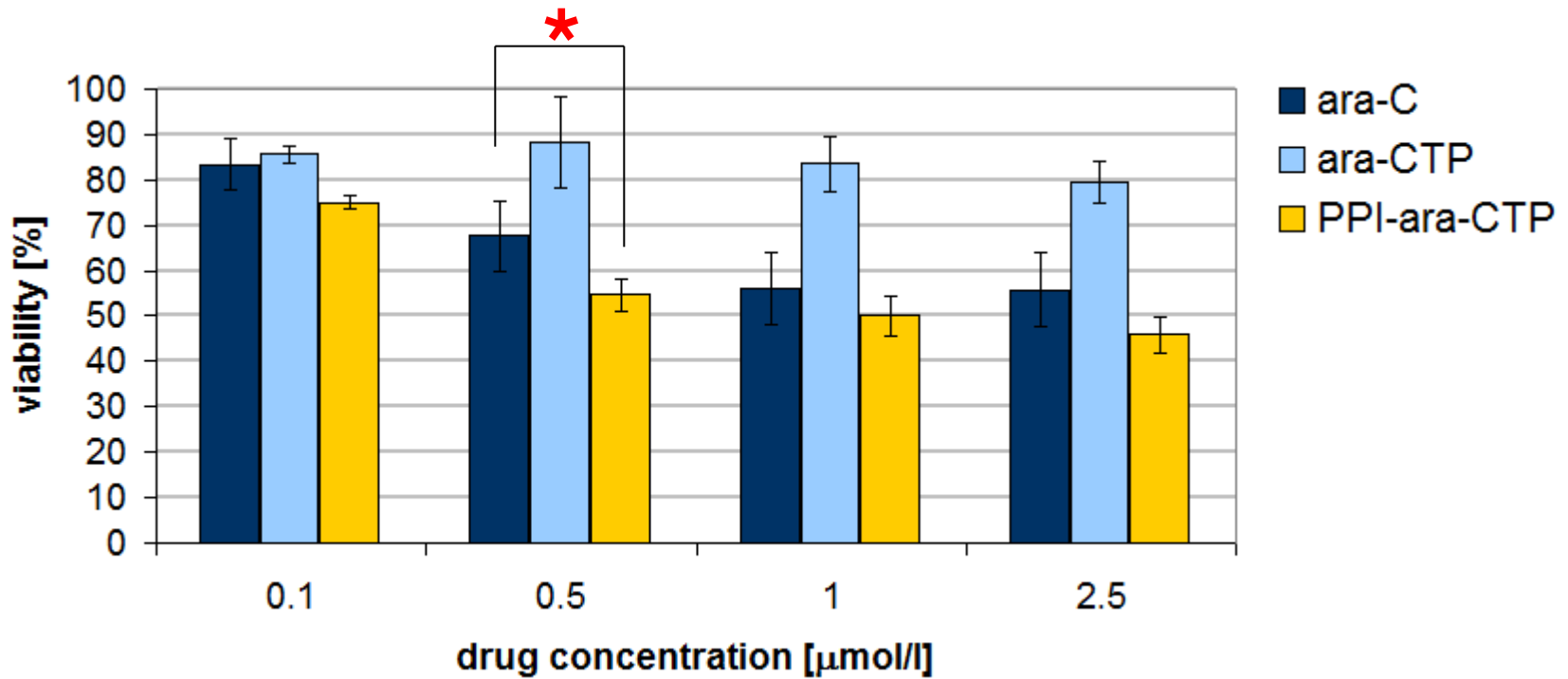


Cytotoxicity - 1301 48h



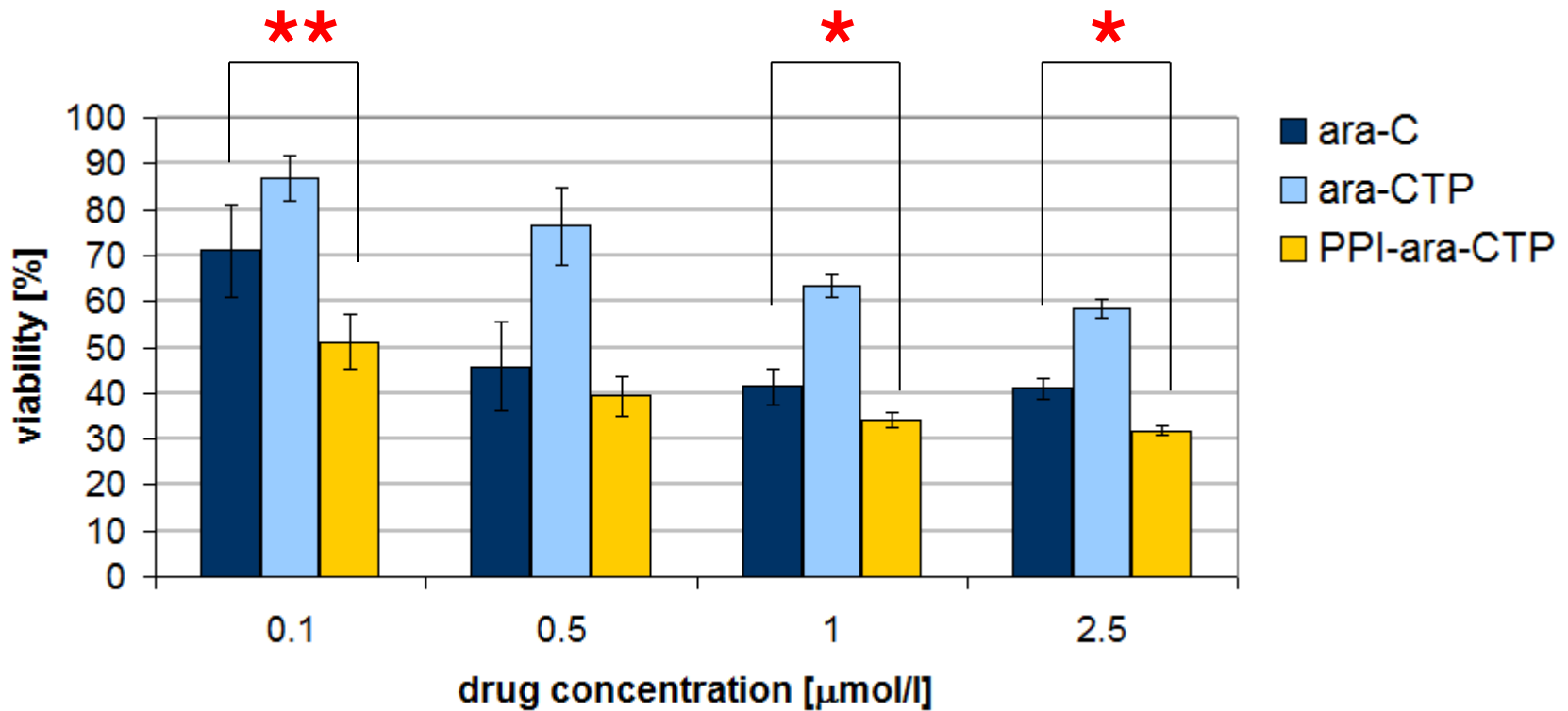


Cytotoxicity - 1301 48h



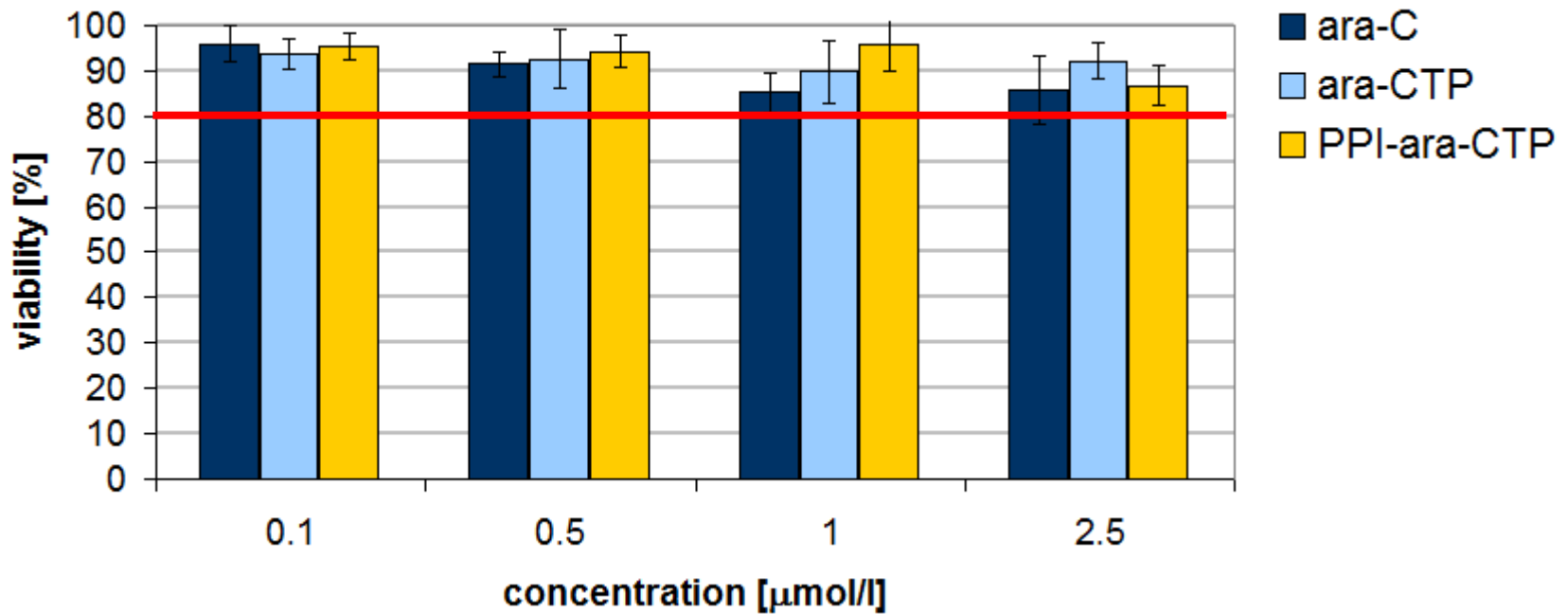


Cytotoxicity - 1301 72h



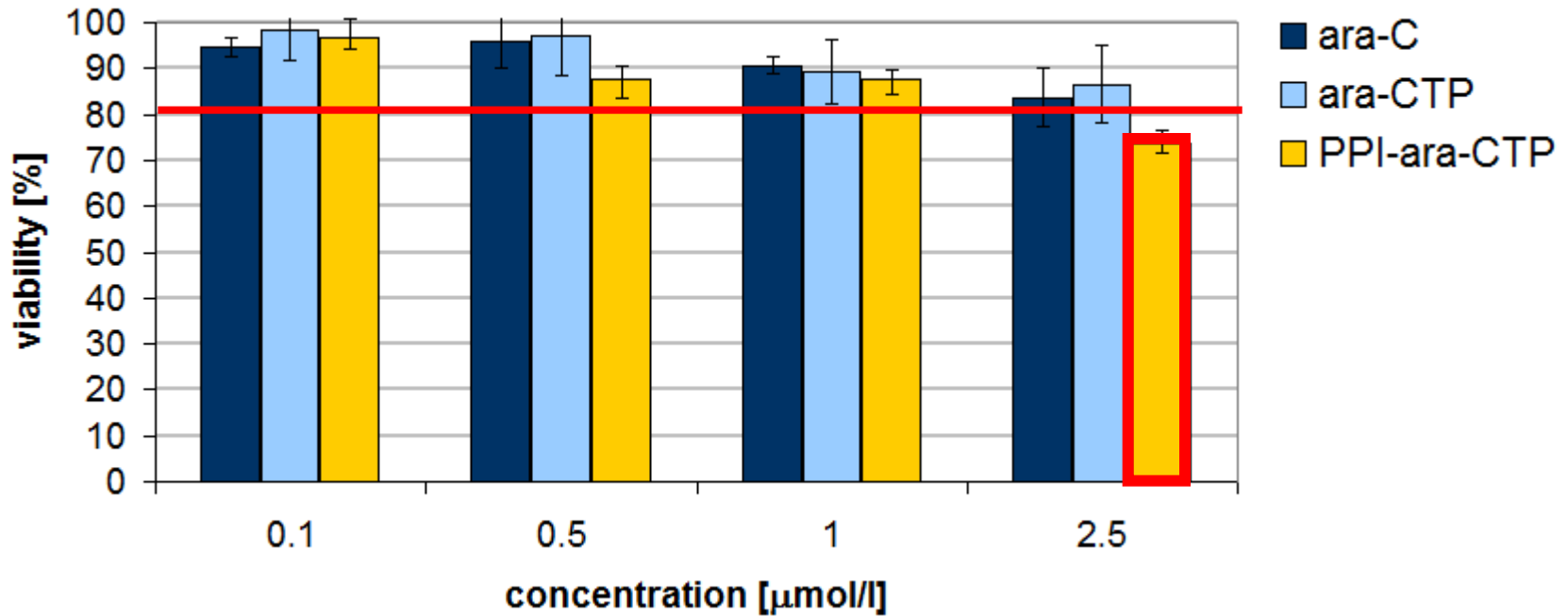


Cytotoxicity - **PBMC** 48h



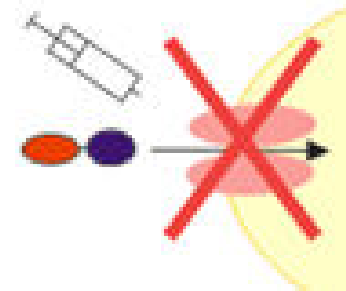
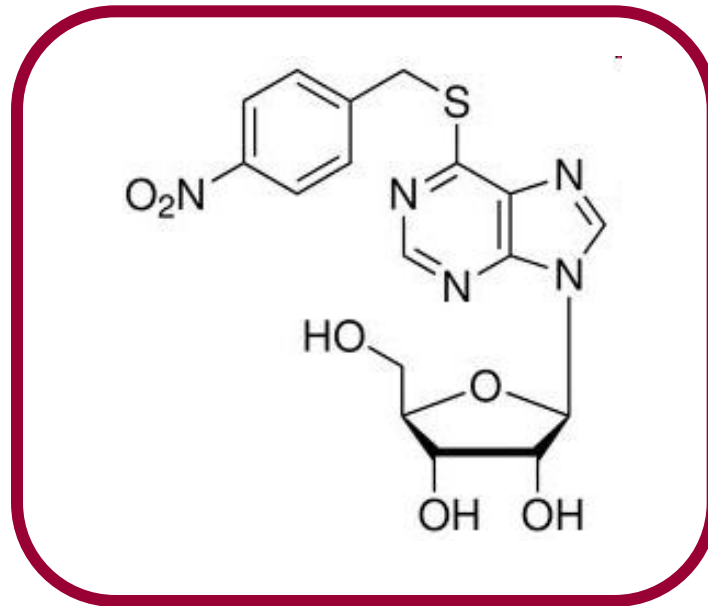


Cytotoxicity - **PBMC** 72h





Inhibitor of nucleoside transporters



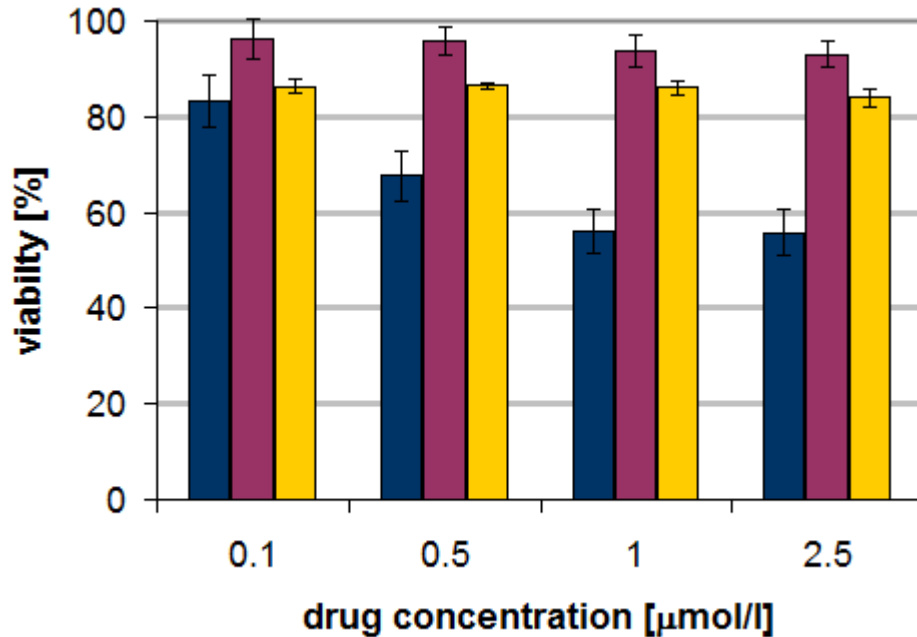
NBMPR - nitrobenzylmercaptapurine



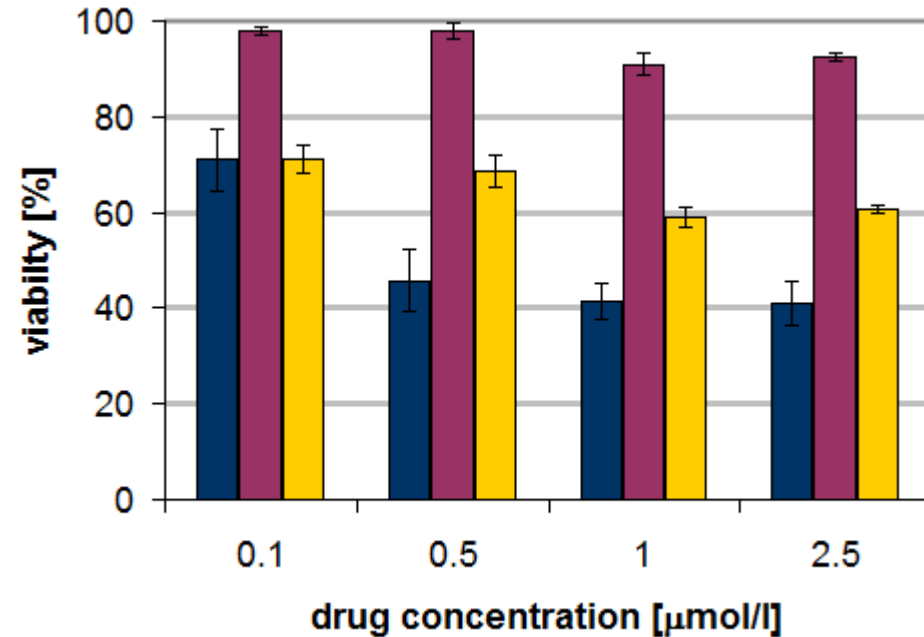
Inhibitors of NT in 1301

- ara-C
- ara-C+NBMPR
- PPI-ara-CTP+NBMPR

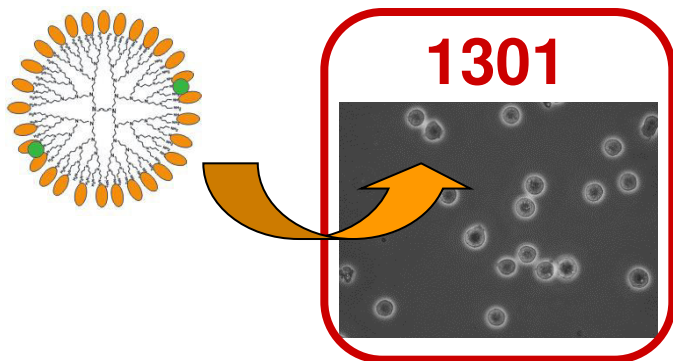
48 h



72 h



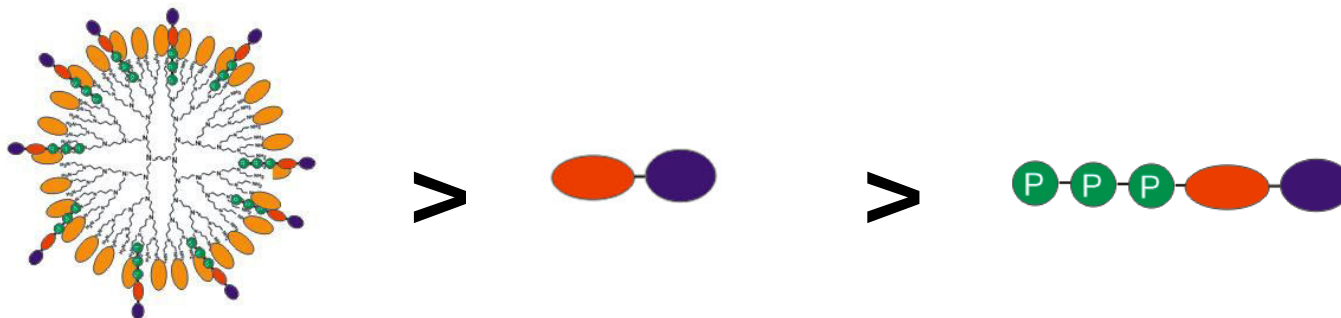
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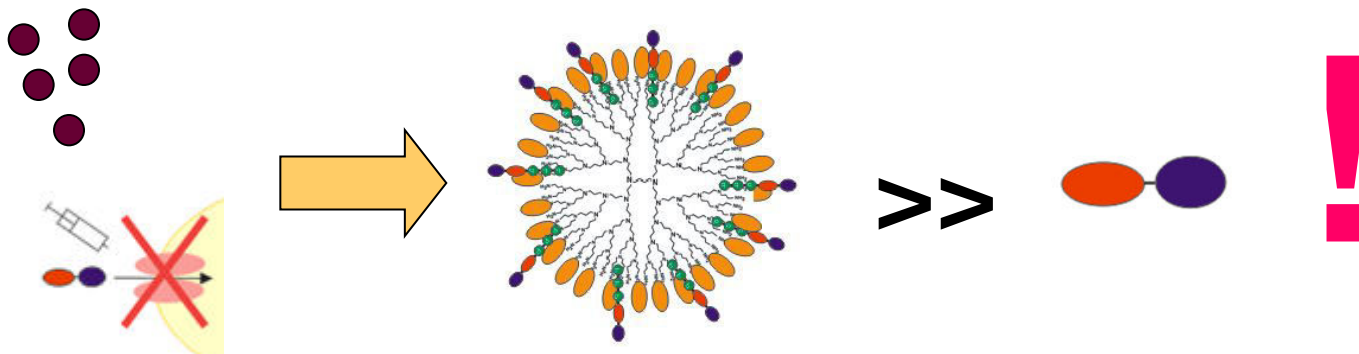
CONCLUSIONS:

2.

Anti-leukemia activity:



3.





Acknowledgements



Dr. Dietmar Appelhans
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für Polymerforschung
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PhD student
University of Lodz
Poland



**INNOVATIVE
ECONOMY**
NATIONAL COHESION STRATEGY



EUROPEAN UNION
EUROPEAN REGIONAL
DEVELOPMENT FUND



TEAM project „Biological properties and biomedical applications of dendrimers”



Thank you.



Faculty of Biology and Environmental Protection,
Lodz, Poland

