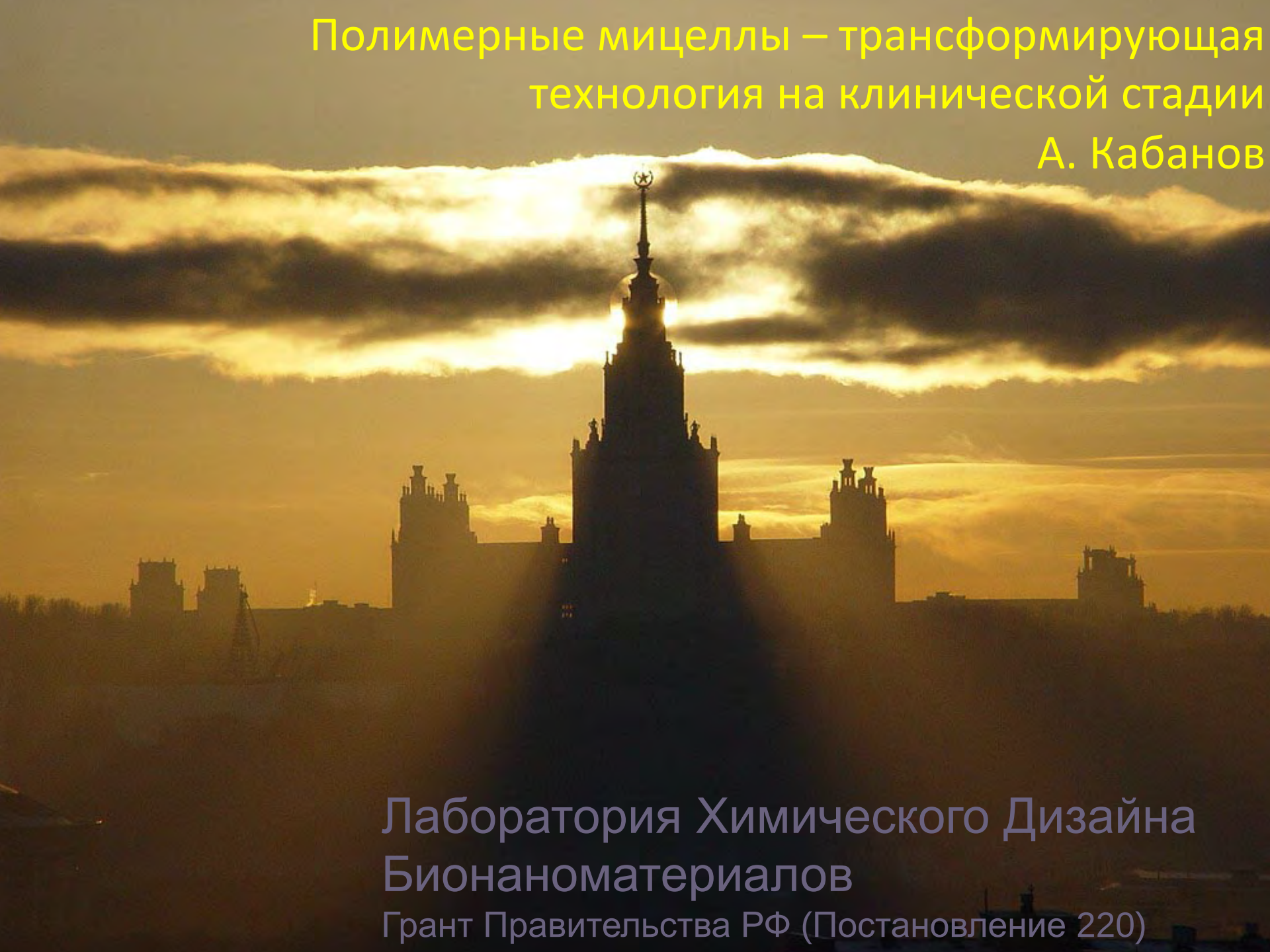
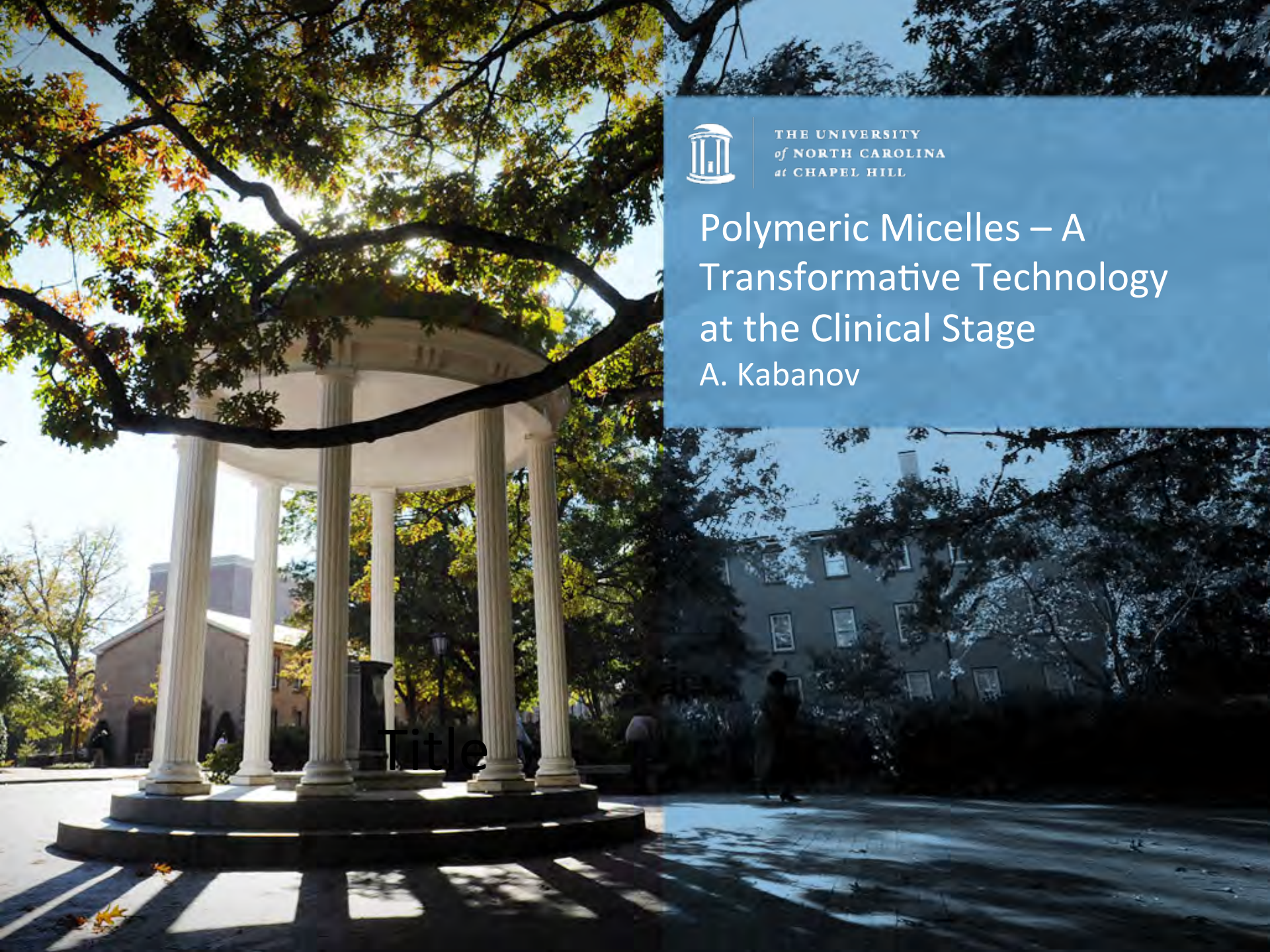
Полимерные мицеллы – трансформирующая технология на клинической стадии

А. Кабанов



Лаборатория Химического Дизайна Бионаноматериалов

Грант Правительства РФ (Постановление 220)


THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL
Polymeric Micelles – A Transformative Technology at the Clinical Stage
A. Kabanov
Title

Необходимость направленной доставки лекарств

Мировой рынок лекарств
2010 - $ 825+ млрд 2013 - $ 975+ млрд

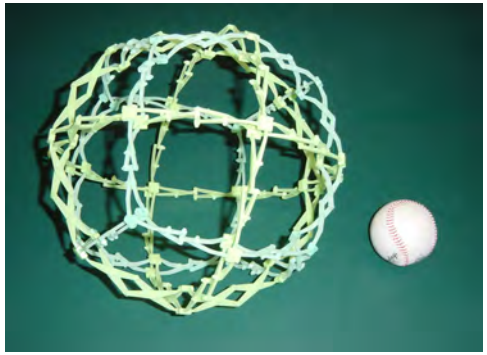
Основные проблемы решаемые доставкой лекарств

- Вещество не растворяется
- Не попадает в организм при «удобном» введении
- Выводится из организма перед тем как оно подействует
- Расщепляется в организме, что приводит к снижению активности и (часто) к увеличению токсичности
- Взаимодействует с нецелевыми клетками и системами организма
- Плохо проникает в целевые ткани и клетки
  - Плохая «гидродинамика» в опухолях
  - Целевые клетки отделены биологическим барьером (например, гематоэнцефалическим)
  - Клеточные мембраны и системы транспокта
- Лекарственная устойчивость

Идея Контейнера

Загрузка и Разгрузка

Контейнер и лекарство

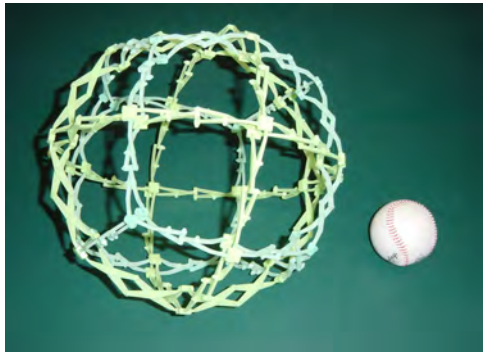


Kabanov & Batrakova, *Cur. Pharm. Design*, 2004, 10: 1355

Идея Контейнера

Загрузка и Разгрузка

Контейнер и лекарство



Загрузка

Kabanov & Batrakova, *Cur. Pharm. Design*, 2004, 10: 1355

Идея Контейнера

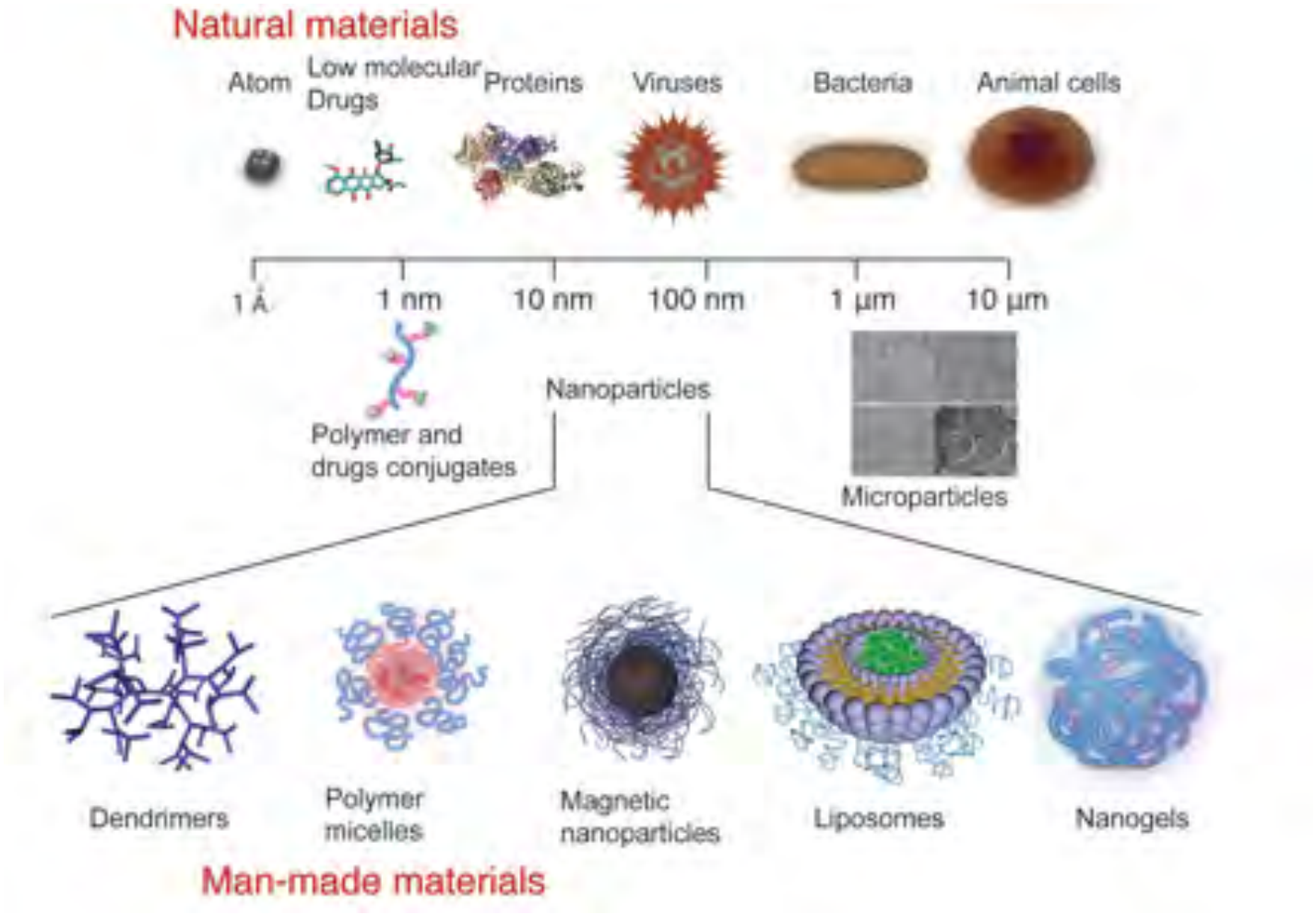
Загрузка и Разгрузка

Путь к мишени

Разгрузка

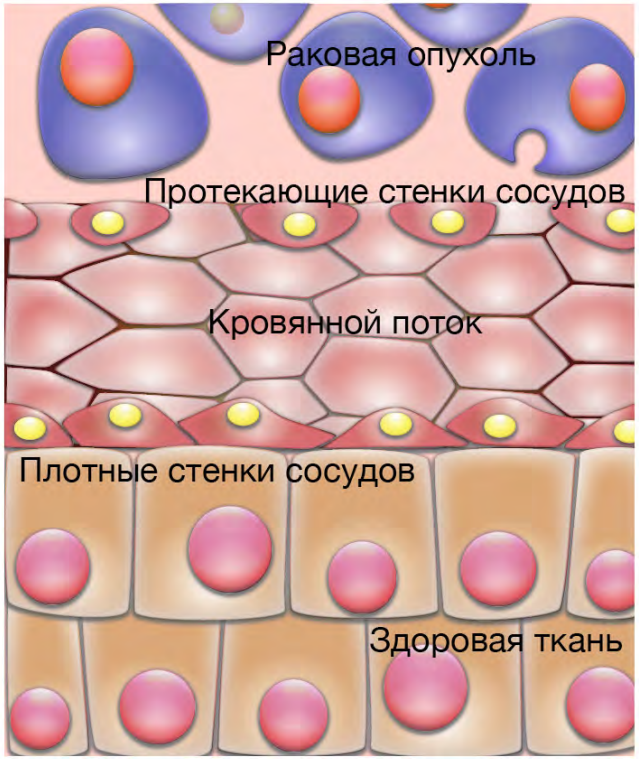
Kabanov & Batrakova, *Cur. Pharm. Design*, 2004, 10: 1355

Size matters….



Механизмы адресной доставки нанолекарств

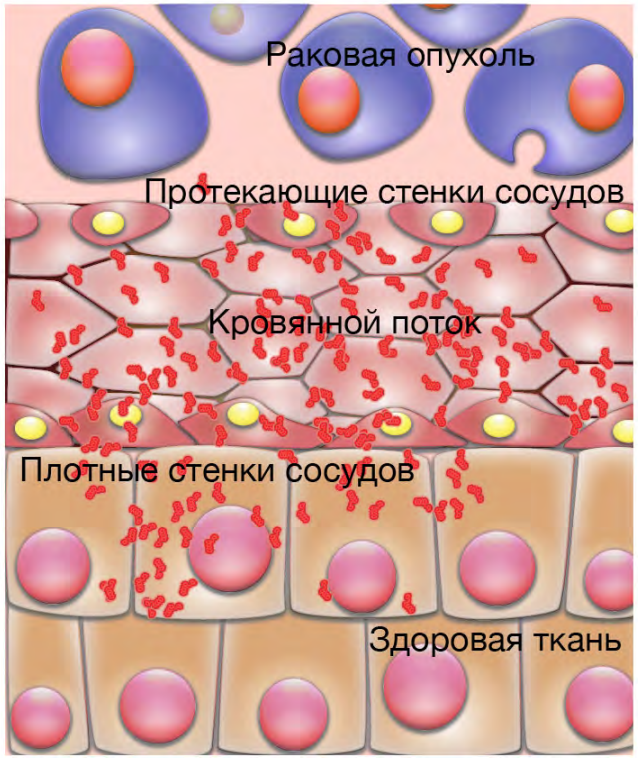
Пассивная доставка



UNC ESHELMAN SCHOOL OF PHARMACY

Механизмы адресной доставки нанолекарств

Пассивная доставка

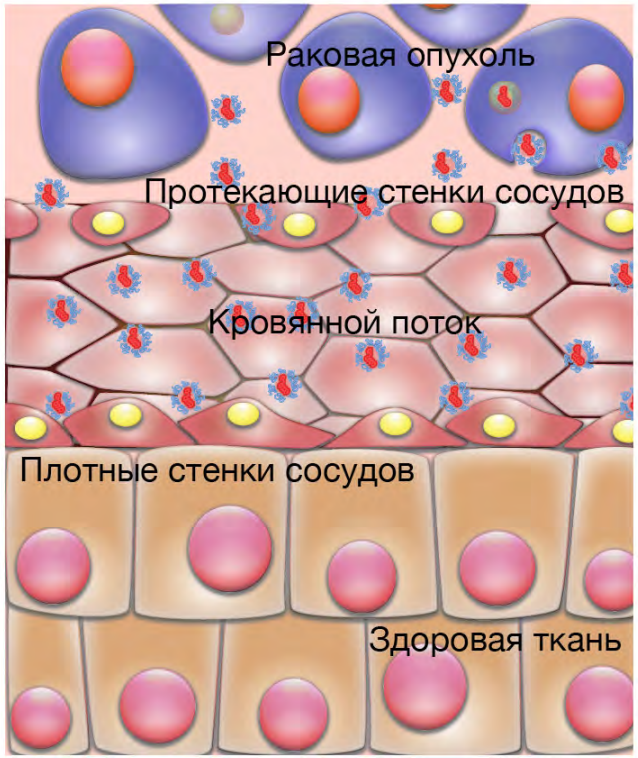


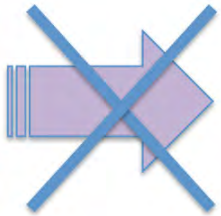
Выведение через почки

UNC ESHELMAN SCHOOL OF PHARMACY

Механизмы адресной доставки нанолекарств

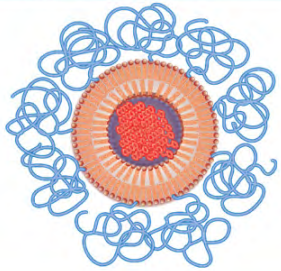
Пассивная доставка





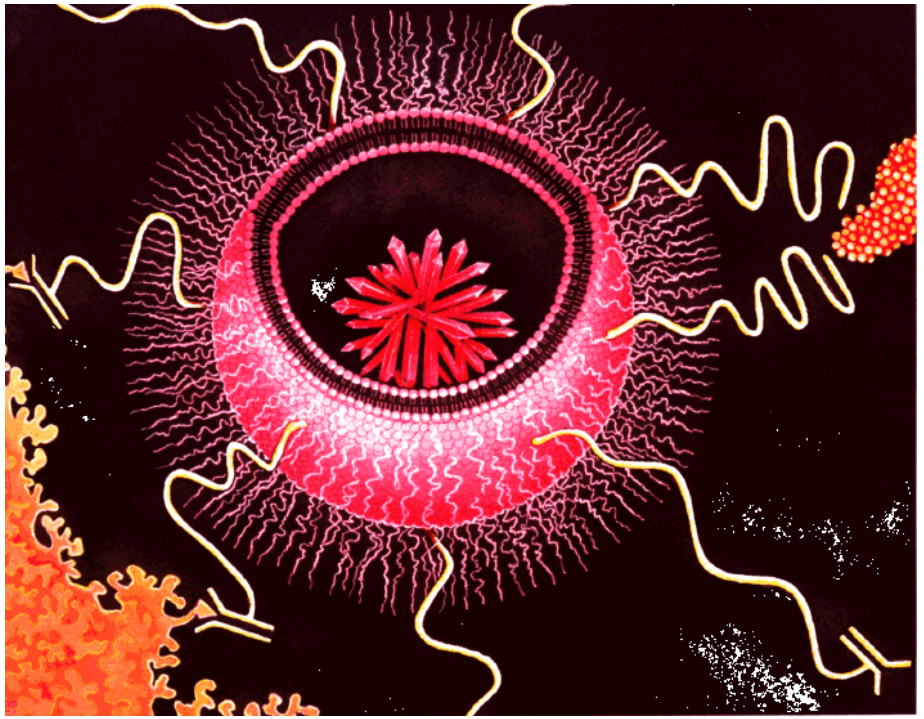
Нет выведения - размер выше предела

Препарат Доксил ($235M/год США)



UNC ESHELMAN SCHOOL OF PHARMACY

Модификация поверхности
“Stealth” эффект



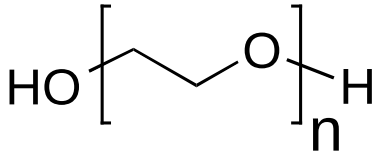
- Во избежание взаимодействия с белками, клеткам и другими компонентаи биологической среды

ПЭГилирование

Frank F. Davis

Abraham Abuchowski

- Модификация наночастицы или макромолекулы полиэтиленгликолем (ПЭГ, PEGylation).



1970

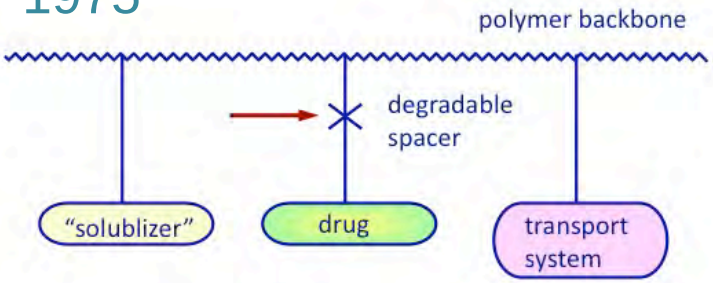
- Растворимость в воде, дисперсионная устойчивость, “stealth” эффект.
- Повышает биодоступность, время циркуляции, снижает токсичность, защищает от расщепления ферментами

Adagen (PEG-adenosine deaminase – SCID, 1990)
Oncaspar (PEG-L-asparaginase – ALL, 1994)
Pegintron (PEG-INFα - hepatitis C and B, 2000)
Doxil/Caelyx (dox-PEGliposome, cancer, 2001)
PEGASYS (PEG-INFα - hepatitis C & B, 2001)
Somavert: (PEG-hGH – Acromegaly, 2002)
Neulasta (PEG-recomb. methionyl hGSF – cancer chemotherapy induced neutropenia, 2002)
Macugen/Pegaptanib (PEG-antiVEGF aptamer - musc. Distrophy, 2004)
Mircera (PEG-EPO - anemia, 2007)
Cimzia/Certolizumab Pegol (PEG-Mab – RA & Crohn's disease, 2008)
Pegloticase (Krystexxa) (PEG-uricase – 2010)
Omontys/PEGinesatide (PEG-EPO analog - anemia, 2012)

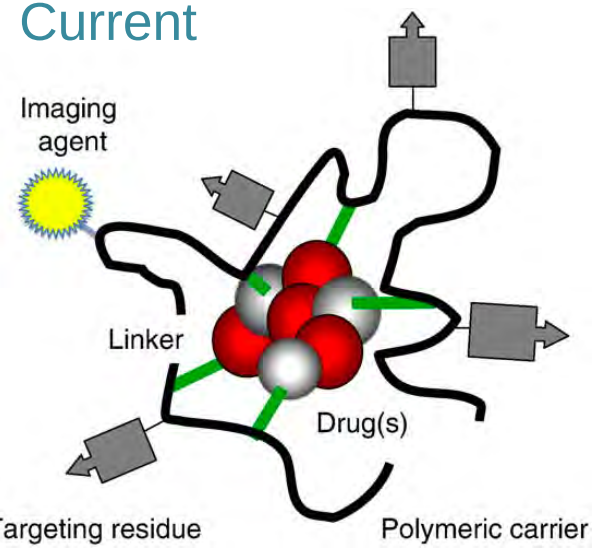
Конъюгаты полимера и лекарства

H. Ringsdorf

1975



Current



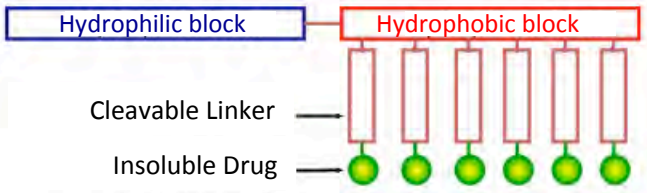
Конъюгаты полимера и лекарства на рынке или в клинических испытаниях

Polymer–drug conjugates				
CT-2103; Xyotax™; Opaxio®	Poly glutamic acid (PGA)-paclitaxel	Cancer-NSCLC, ovarian, various other cancers and combinations	iv	Phase II/III
Prolindac®	HPMA-copolymer-DACH platinate	Cancer-melanoma, ovarian	iv	Phase II
NKTR-102	PEG-irinotecan	Cancer-metastatic breast	iv	Phase II
PEG-SN38	Multiarm PEG-camptothecan derivative	Cancer-various	iv	Phase II
NKTR-118	PEG-naloxone	Opioid-induced constipation	oral	Phase II
XMT-1001 (Fleximer® technology)	Polyacetal-camptothecin conjugate	Cancer-various	iv	Phase I

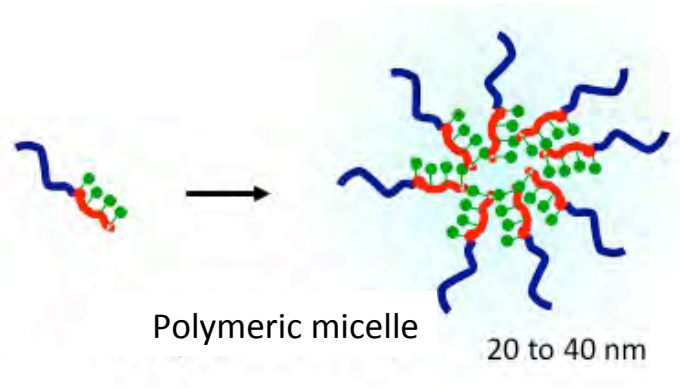
Polymeric Micelles as Drug Carriers

1984

In Germany



H. Ringsdorf



1990

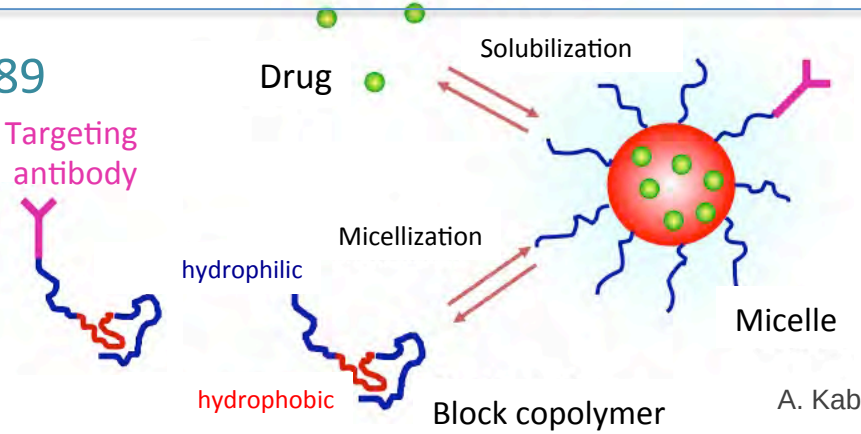
In Japan

K. Kataoka

T. Okano

M. Yokoyama, et al. (1990) *Cancer Res.*, 50: 1693

1989



In then Soviet Union

Small size ca. 10-50 nm
Narrow size distribution ~ 0.1

A. Kabanov et. al *FEBS Lett.*, 1989, 258: 343

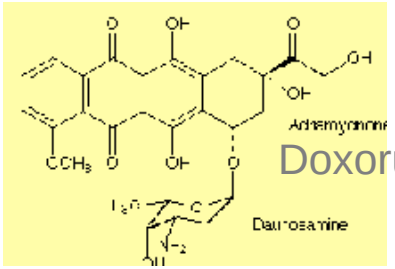
Polymeric Micelles at the Clinical Stage

No	Name	Drug	Goal	Stage	Cancer
1	SP1049C	doxorubicin	anti-MDR, anti-CSC	Phase III pending	Inoperable metastatic esophagus, upper GI
2	NK-911	doxorubicin	EPR	Phase II	Various solid
3	NC-6300/ K-912	epirubicin	EPR, pH-sensitive	Phase I	Breast nos metastatic recurrent
4	NK-105	paclitaxel	EPR	Phase III	Breast nos metastatic recurrent
5	NK-012	SN-38	EPR	Phase II	Advanced, metastatic triple negative breast
6	NC-6004	cisplatin	EPR	Phase III	Pancreatic, solid, NSCLC
7	NC-4016	DACH-platin	EPR	Phase I	Metastatic, colorectal
8	Genexol-PM	paclitaxel	solubilization	Approved	Breast, NSCL

Yokoyama et. al. *J Exp Clin Med* 2011, 3:8, updated

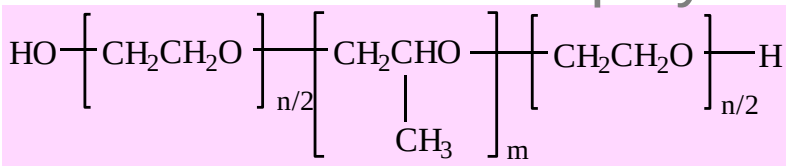
Clinical Trials of SP1049C: Pluronic Polymer Micelle Doxorubicin

SUPRATEK PHARMA INC.



Doxorubicin

2 Pluronic block copolymers



L-61 – 90% of POP, 10% of POE, Mm ~2,000Kd

F127 – 30% of POP, 70% of POE, Mm ~12,000Kd

Phase I results (Danson et al. *Br. J. Cancer,* 2004 90: 2085):

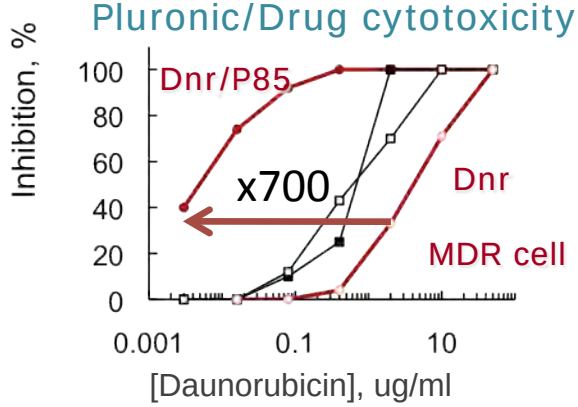
- 26 advanced stage IV cancer patients
- MTD = 70 mg/m²
- DLT = neutropenia at 90 mg/m²
- Plasma PK - linear, dose-proportional, terminal elimination phase - circa 50 hrs
- Toxicity profile similar to doxorubicin
- Activity observed in highly resistant cancer:
  - esophageal cancer

Phase II SP1049C-202-UK - open-label, phase II window (Valle et. al. *Invest New Drugs*. 2010)

- First line therapy, adenocarcinoma of the esophagus
- 21 patients (19 evaluable)
- 75 mg/m² of SP1049C i.v. every 3 weeks up to 6 cycles
- SP1049C appears active in monotherapy (RR 47%)
- Median survival 9.96 months
- Toxicity profile: predominantly haematological and cardiac

Pluronic-induced sensitization of multidrug resistant cells

Pluronic/Drug cytotoxicity



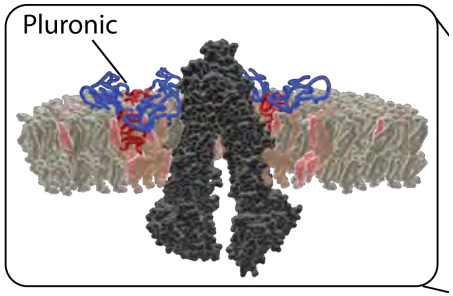
Alakhov et al. *Bioconjug. Chem.* 1995, 7:209-16

Inhibition of drug efflux systems

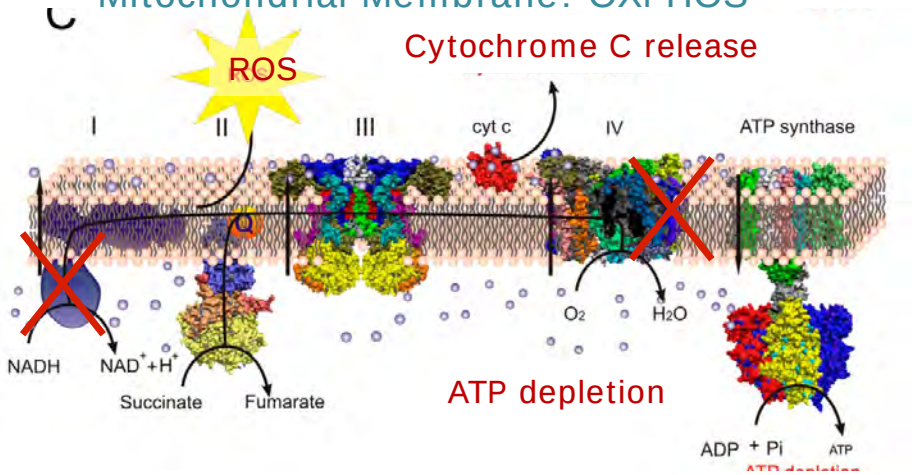
Membrane fluidization

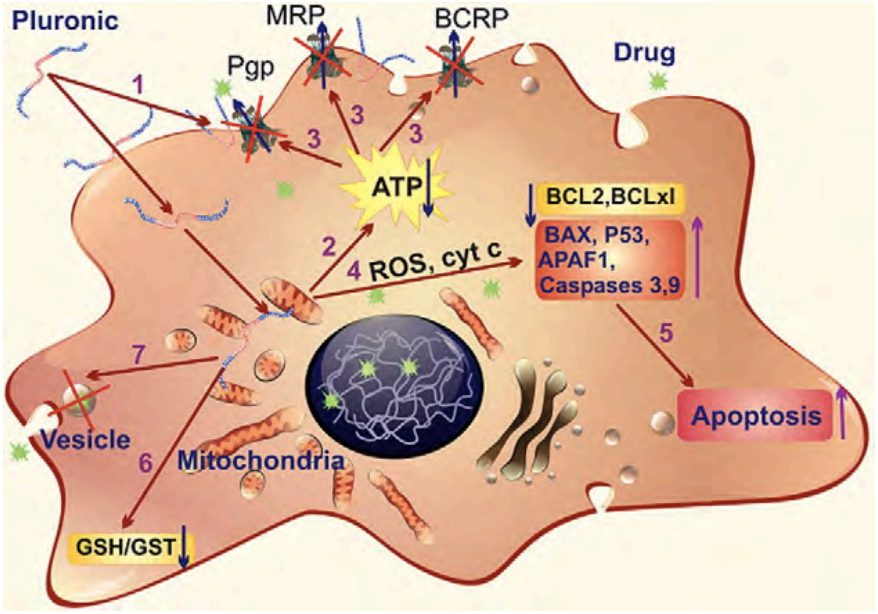
Lipid raft disruption

Pgp inhibition



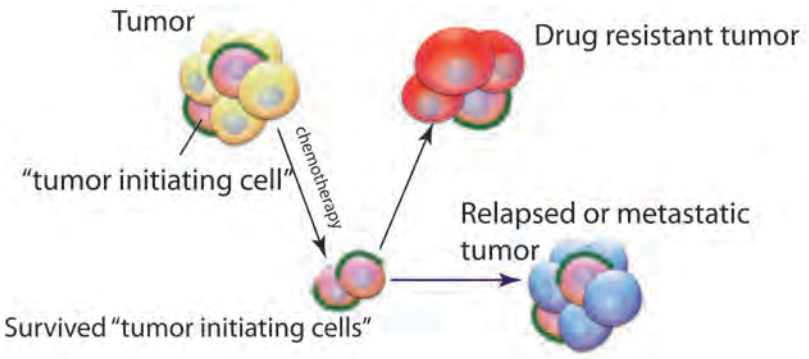
Mitochondrial Membrane: OXPHOS

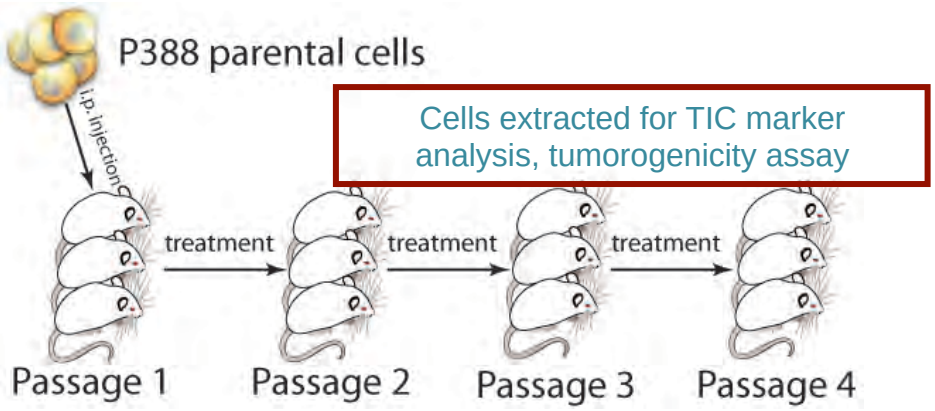




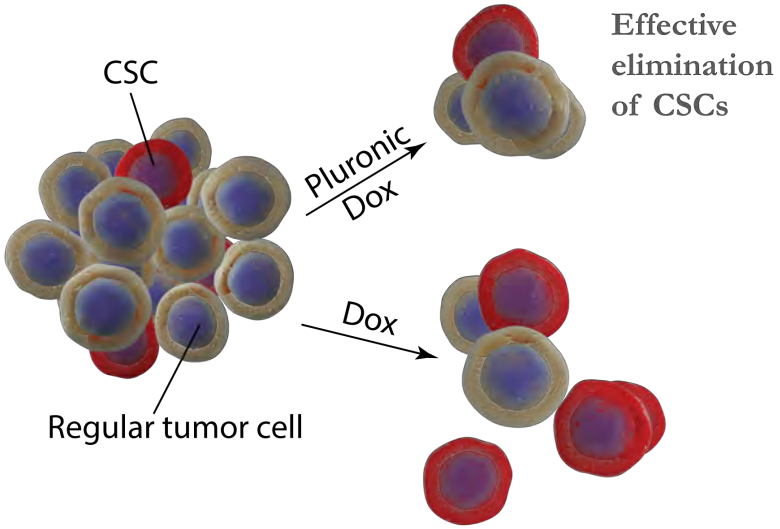
Batrakova & Kabanov, *J. Contr, Release* 2008, 130:98-106

SP1049C Effective Against Cancer Stem Cells (CSC)



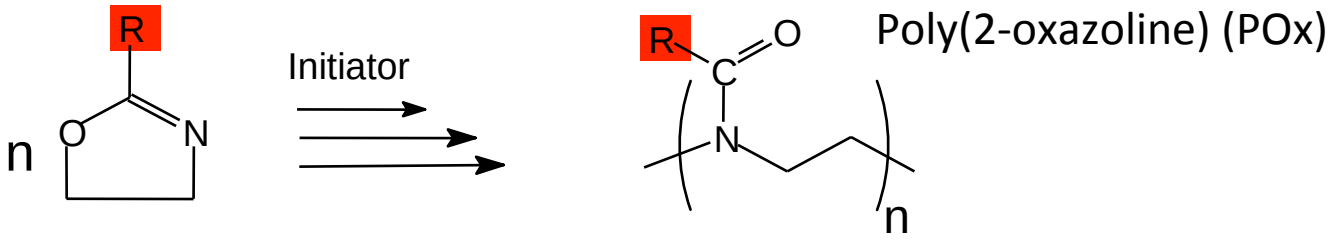


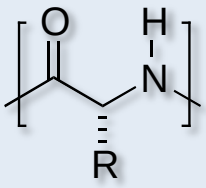
- Decreases colony formation
- Decreases tumor aggressiveness and increases survival (in new host animals, s.c.)
- Decreases tumor formation frequency
- Depletes CSC marker (CD133+)
- Attenuates Wnt/β-cathenin signaling pathway
- Prevents development of drug resistance ABCG2 (BCRP) & ABCB1 (Pgp)



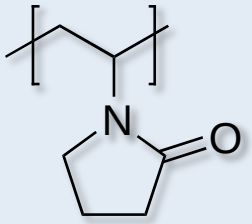
PLOS ONE Alakhova, D.Y., Zhao, Y. et al. PLOS One, 2013, 8(8): e72238

Poly(2-oxazoline)s





Polypeptide



PVP

(used as blood plasma expander since WWII)

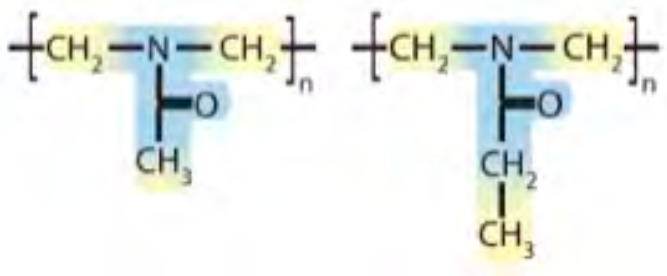
Living cationic ring-opening polymerization (LCROP):

- Strictly linear polymers
- Direct control of polymer chain length by $[M]_0$ / $[I]_0$ - ratio
- Low dispersity Đ = M_w/M_n ~ 1.05 – 1.3
- Quantitative introduction of terminal functional groups
- Introduction of pendant groups via functional monomers & polymer analog reactions
- Block, gradient and random copolymerization of different 2-oxazoline monomers control of amphiphilicity & distribution of functions

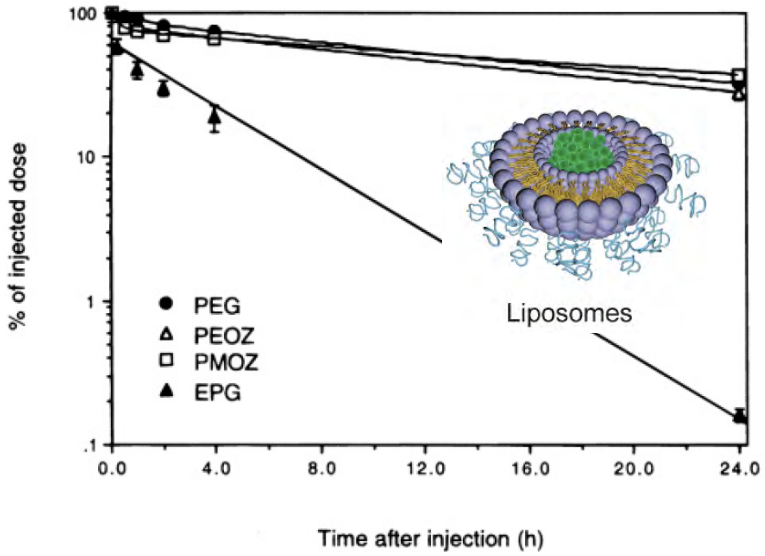
- "no side-reactions"
- limited degree of polymerization of about n ~150-200

- POx are miscible with most polymers and soluble in many solvents
- POx show a temperature dependent water-solubility:
  LCST that can be fine-tune between (0) … 4-95… (100)°C

POxylation: analogous to PEGylation

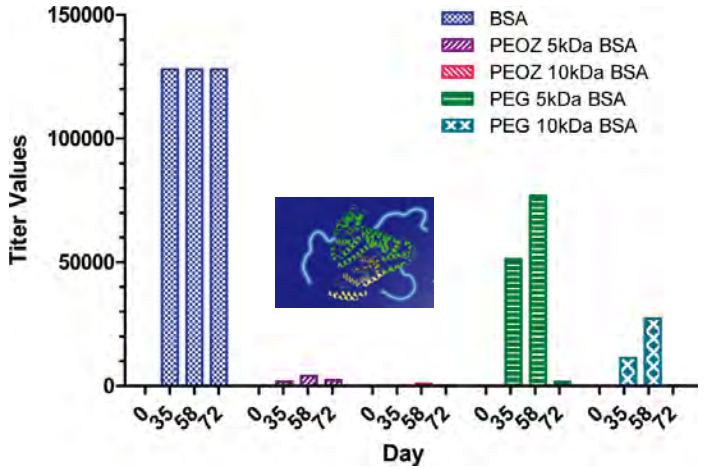


„Stealth Effect“ of POx liposomes



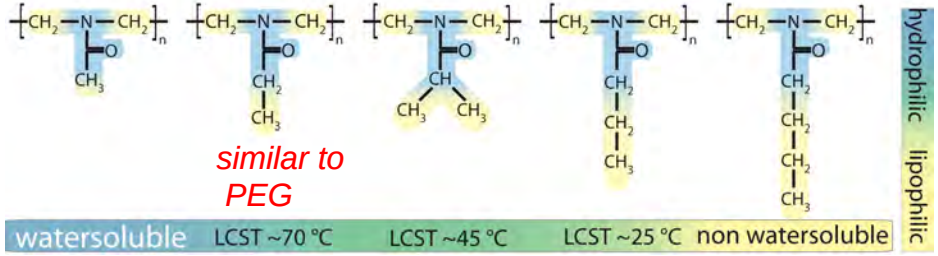
Woodle et al. *Bioconj. Chem.* **1994**, *5*, 493.

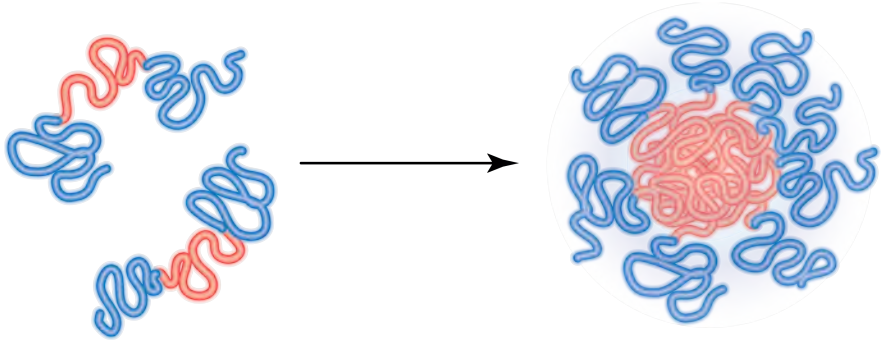
Low Immunogenicity (POx-BSA)



Viegas, et al. *Bioconjug. Chem.* **2011,** *22*, 976.

Poly(2-oxazoline)s: Water Solubility & Micellization



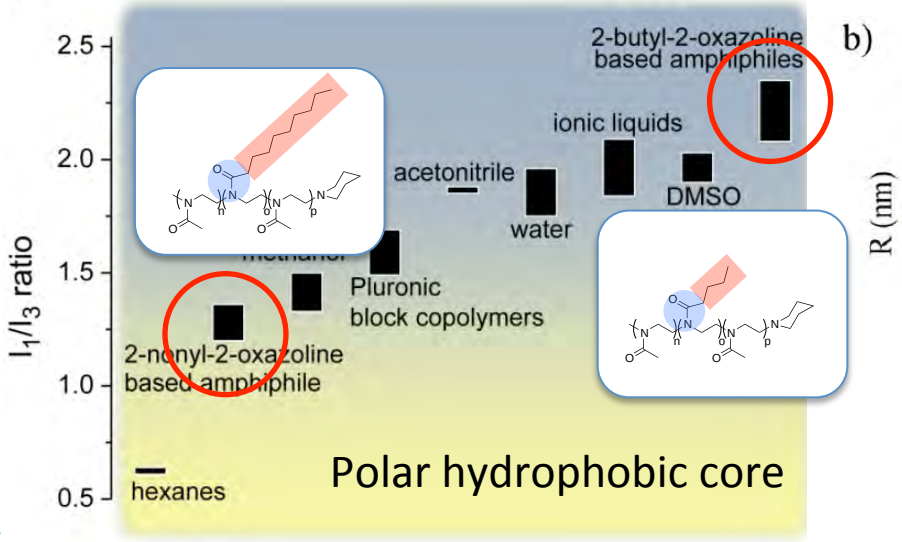


- Tuning by side chain
- Tuning by copolymerization

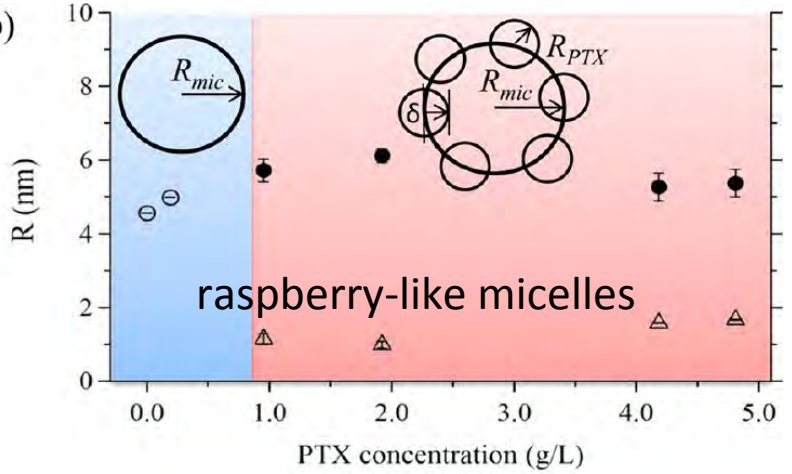
R. Luxenhofer, et al, *Biomaterials* **2010**, *31*, 4972

A. Schulz et al. ACS Nano 2014, 8, 2686

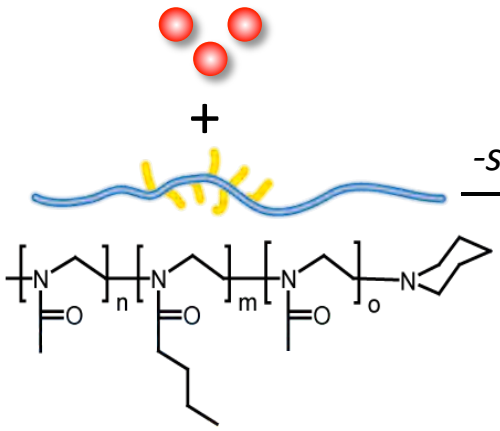
SANS measurements and fittings



SANS experiments and fittings

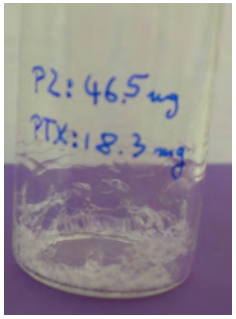


Formulation of Paclitaxel with POx



+ solvent

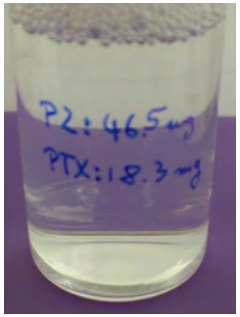
-solvent



film

+water

- water

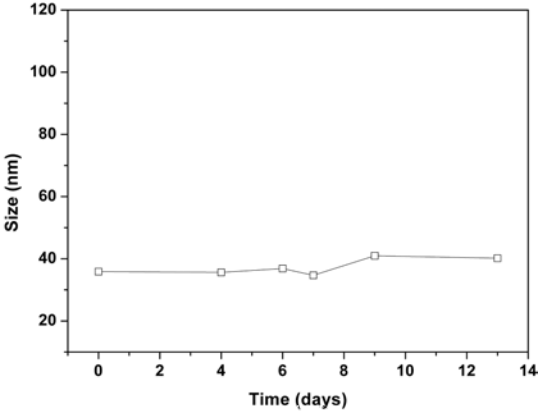


micellar solution

PTX loaded micelles:
- Stable, Small & Clear
- R_h = 12-22 nm
- Uniform: PD = 0.04-0.12

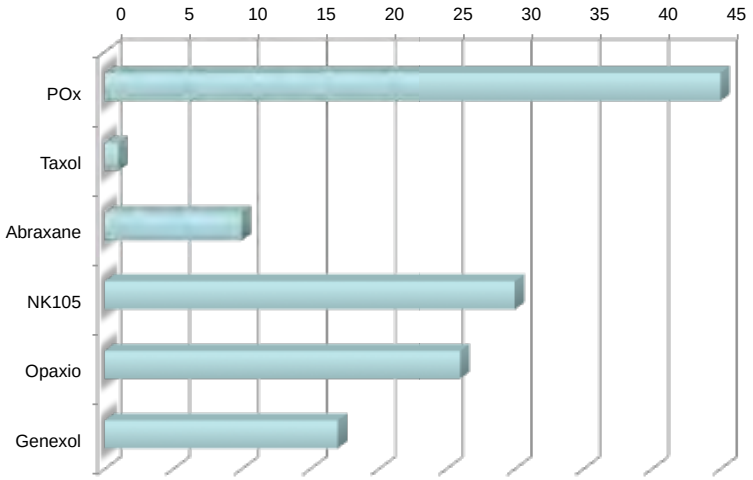
- Formulation e.g. with PTX is a stable micellar solution of small/defined micelles
- No further excipients needed (no cryoprotectants)
- Formulation can be fully reconstructed
- POx is thermally stable e.g. @ 200°C for 24 h (UV, NMR, GPC, IR identical)
- POx (POx/drug film) can be sterilized i.e. to remove endotoxines

Taxol day 1 **Taxol day 2** **POx-PTX day 2**

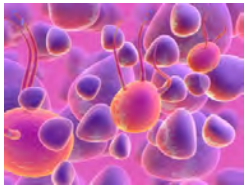


R. Luxenhofer, A. Schulz, C. Roques, S. Li, T. Bronich, E. Batrakova, R. Jordan, A. Kabanov, *Biomaterials* **2010**, *31*, 4972.
A. Kabanov, R. Jordan, R. Luxenhofer, PCT Appl. EP2009/004655

Unprecedented Capacity for PTX

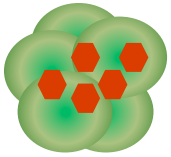


Drug loading capacity %(w/w)



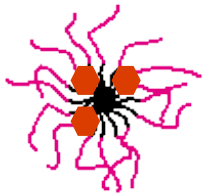
Taxol®

1%
1:100



Abraxane®

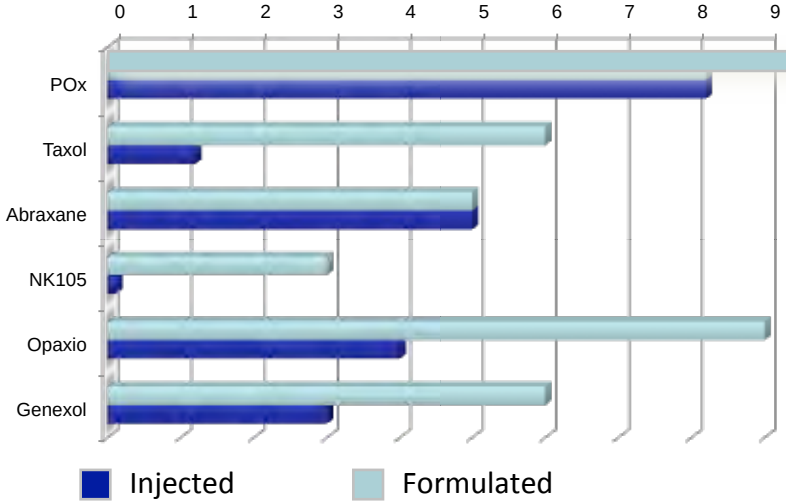
10%
1:10



POx micelles

45%
1:1

Drug concentration (mg/mL) in stable formulation



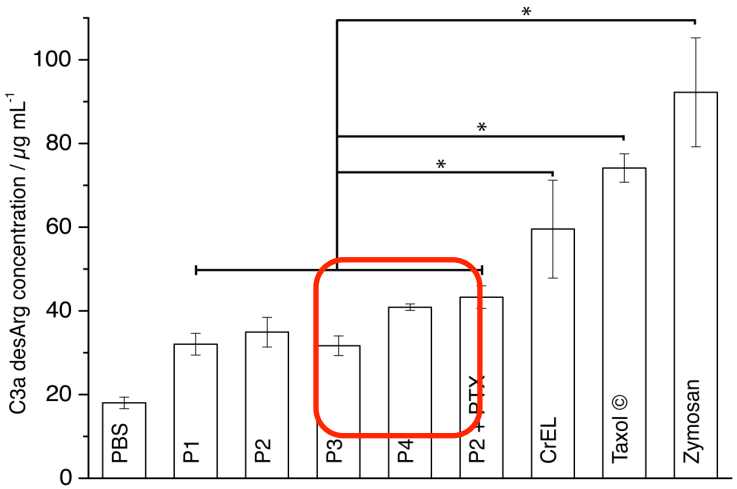
40 mg/mL PTX

- solubilized in 50 mg/mL POx
- solution stabile for at least 14 days
- solubility increase > *x 50.000*

A. Kabanov, R. Jordan, R. Luxenhofer, PCT Appl. EP2009/004655
R. Luxenhofer, A. Schulz, C. Roques, S. Li, T. Bronich, E. Batrakova, R. Jordan, A. Kabanov, *Biomaterials* **2010**, *31*, 4972

Increased safety

Complement activation



R. Luxenhofer, et al. *Biomaterials* 2010, 31, 4972.

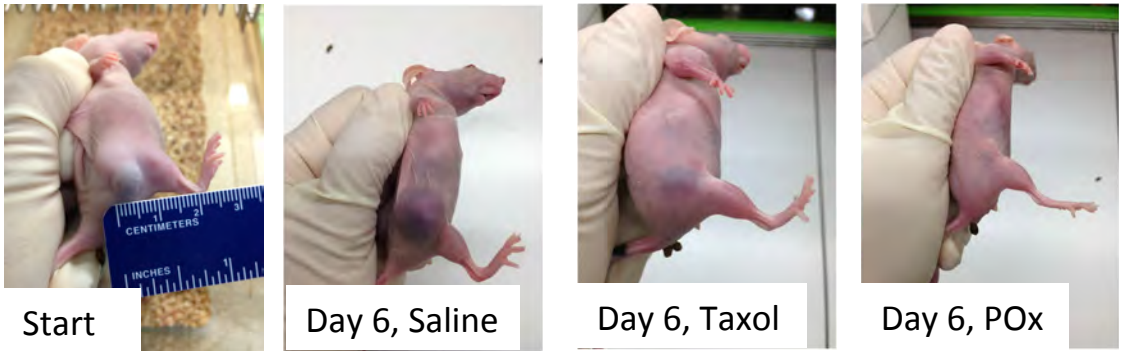
MTD in NCI nude mice 4 X every 4 days

Formulations	1 Dose (mg/kg)	Animal death	Weight loss
Taxol	20	0/3	3.47%
	25	1/3	Animal Death
Abraxane	90	0/3	Weight gain
	120	1/3	Animal Death
PTX-loaded POx Micelles	80	0/3	Weight gain
	100	0/3	Weight gain
	120	0/3	Weight gain
	150	0/3	Weight gain
	200	2/3	>15%

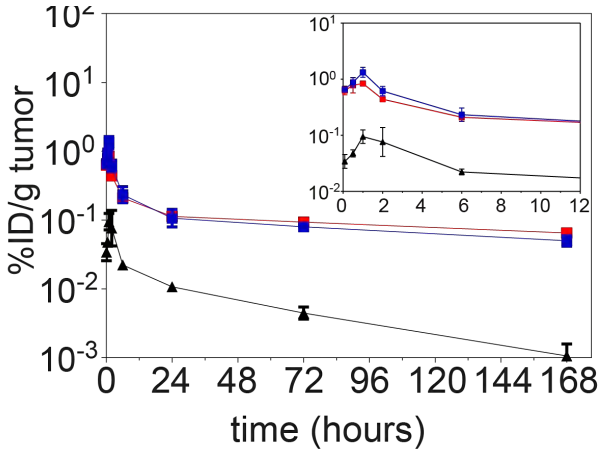
- Taxol: 20mg/kg (x 4)
- Abraxane: 90mg/kg (x 4)
- POx-PTX: 150mg/kg (x 4)
- POx: > 1000mg/kg (x 4)

PK and Efficacy of PTX/POx vs. Taxol & Abraxane

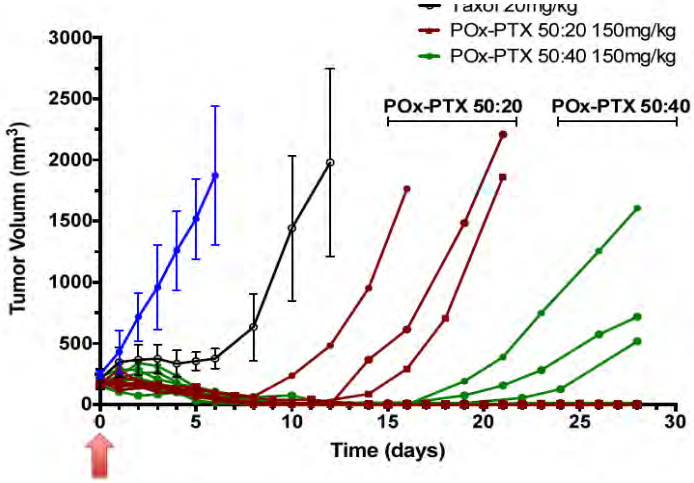
Flank model A2780 ovarian cancer in NCI nude mouse

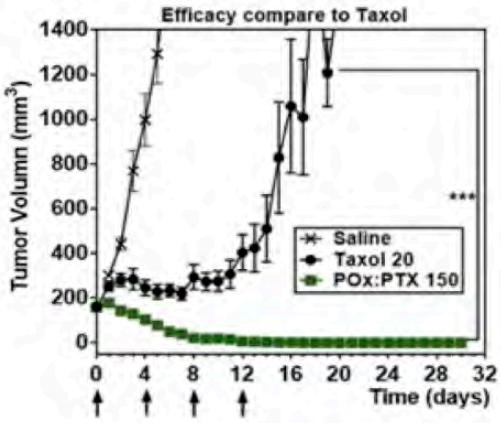


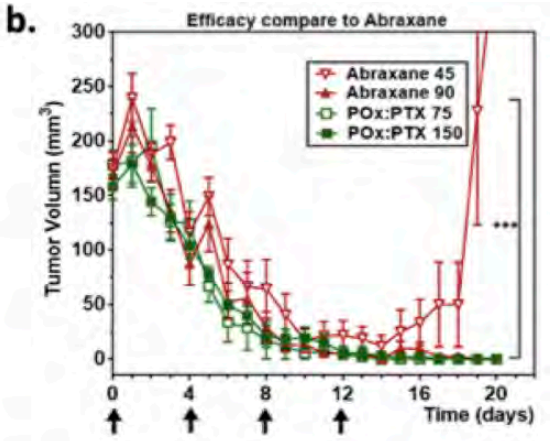
Drug concentration in tumor



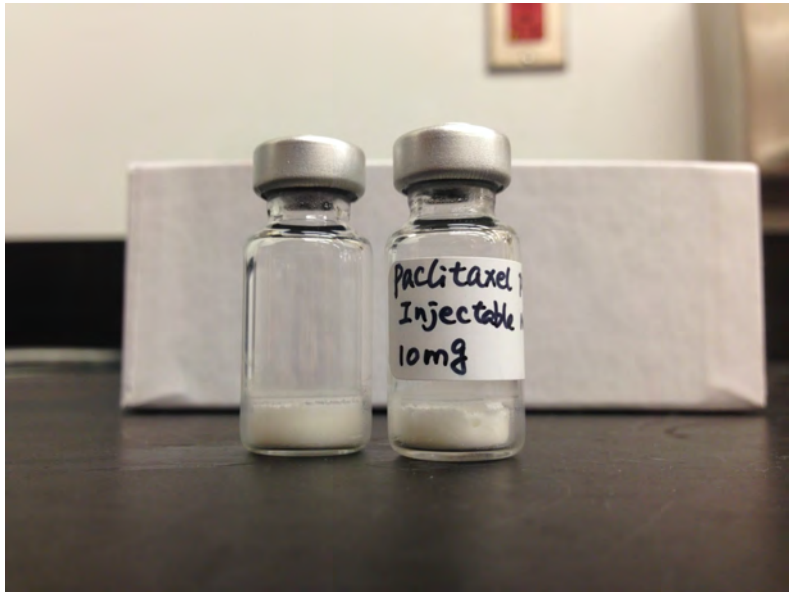
Tumor growth inhibition

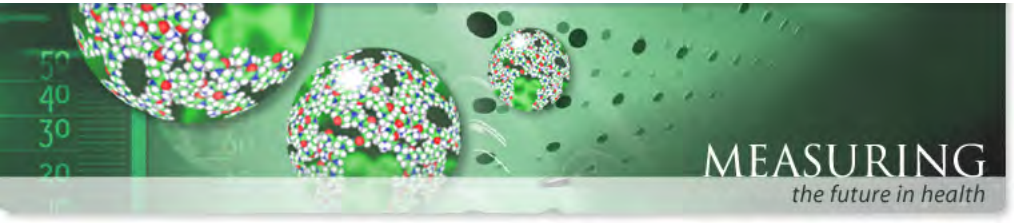






- Samples submitted to NCI/NCL for characterization.
- 10 mg PTX/vial; POx: PTX = 1.25:1





NANOTECHNOLOGY CHARACTERIZATION LABORATORY

Nanotechnology Characterization Laboratory
P.O. Box B, Bldg. 469, 1050 Boyles St.
Frederick, MD 21702-1201
Phone: (301) 846-6939; Fax: (301) 846-6399
http://ncl.cancer.gov

May 18, 2012

Alexander Kabanov, Ph.D.
DRC1 Room 1013
985830 University of Nebraska Medical Center
Omaha, NE 68198-5830

Subject: NCL Acceptance for Characterization

Dear Dr. Kabanov:

The Nanotechnology Characterization Laboratory is pleased to inform you that the nanotech strategy described in your Part II presentation entitled, "Amphiphilic poly(2-oxazoline)s as high capacity drug delivery systems", has been accepted for characterization.

Since you are currently exploring active pharmaceutical ingredients (APIs) besides paclitaxel for your delivery system, NCL characterization of the current product (Poxol) will have to be limited, not for lack of the project's merit, but because we would like to reserve NCL resources for follow-on products with greater clinical potential. We are therefore sending you this letter offering NCL resources with respect to physicochemical and in vitro characterization and such in vivo studies as are mutually determined to be relevant for future development of the platform. We offer to collaboratively agree upon a research plan which will be relevant to your critical path, keeping in mind NCL's limited resources for characterization of this formulation. The NCL reserves the right to cease characterizing your technology at any time, if that option is determined to be in the best interests of NCI.

It will be necessary to execute a materials transfer agreement (MTA) before you submit material. Once this agreement is executed, we would like to have a preliminary telephone discussion of NCL capabilities with respect to your particles. After this discussion, we will provide a draft schedule of NCL experiments (research plan) and invite your input. If you have any questions about NCL protocols or capabilities, please do not hesitate to ask.

Please also keep us informed on any developments with respect to testing other APIs and API combinations in the POx platform.

Sincerely,

Anil K. Patri, Ph.D.
Deputy Director

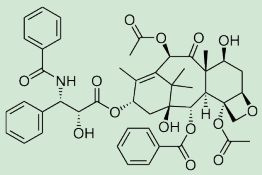
size, PDI distribution, release, endotoxin levels, stability, drug concentration and drug loading,
in vitro stability in human serum

SAIC Frederick

UNC ESHELMAN SCHOOL OF PHARMACY

We have data on 20+ compounds already

paclitaxel



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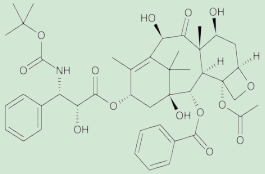
docetaxel



logP=2.5

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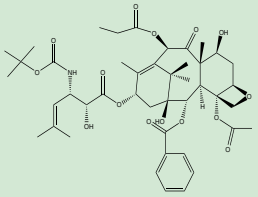
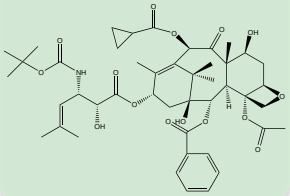
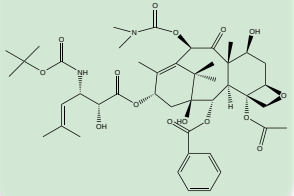


SB-T-1214



SB-T-1216



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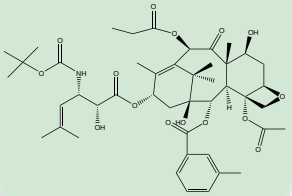

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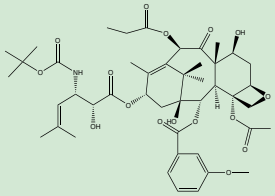

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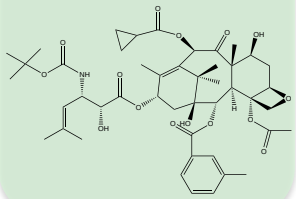
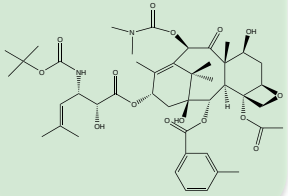


SB-T-121602



17-AAG

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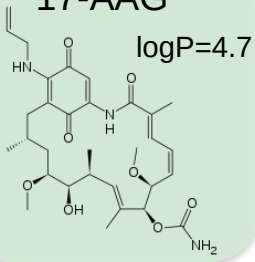
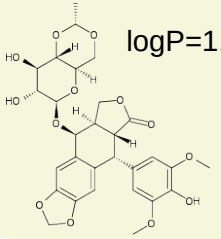


etoposide

logP=1.0



bortezomib

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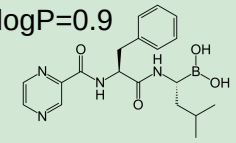
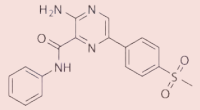
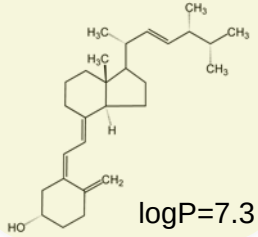


VE-821



ergocaliceferol



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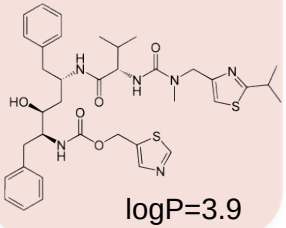
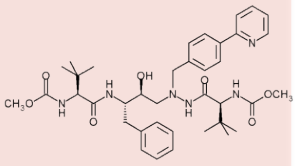
ritonavir



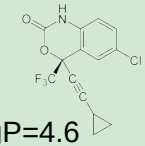
logP=3.9

atazanavir



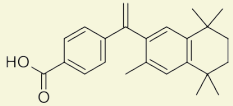
logP=4.5

efavirenz



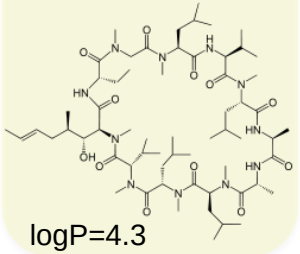
logP=4.6

bexarotene



logP=6.9

cyclosporine A



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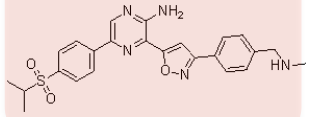
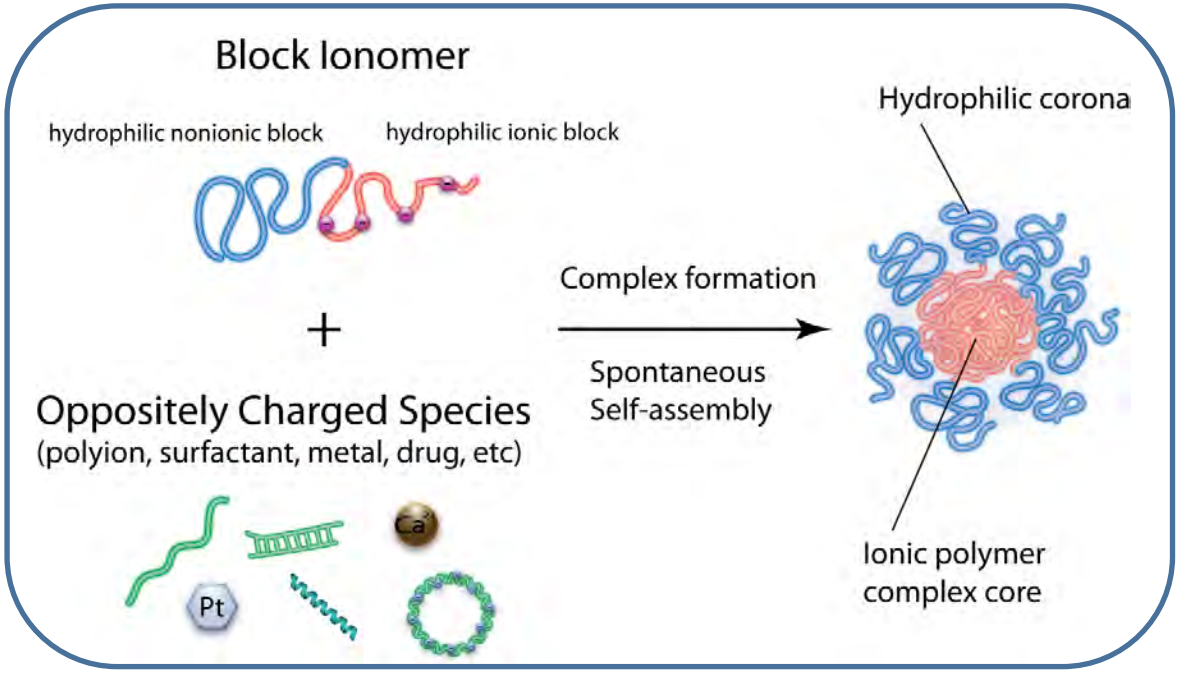
VE-822



Solubilization: **Green** – excellent;
yellow – so-so (ordinary); **red** - poor

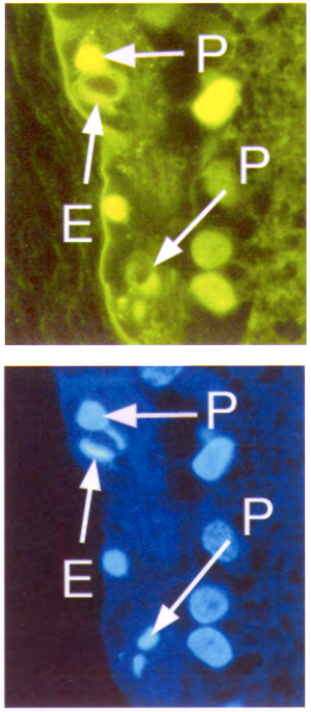
Micelle Formation as a Result of Polyion Complexation

Block ionomer complexes (BIC)



Kabanov et al. *Bioconj. Chem*. 1995, 6: 639
Kataoka et al. *Macromolecules* 1996, 29: 8556
Wolfert et al. *Hum. Gene Ther.* 1996, 7: 2123

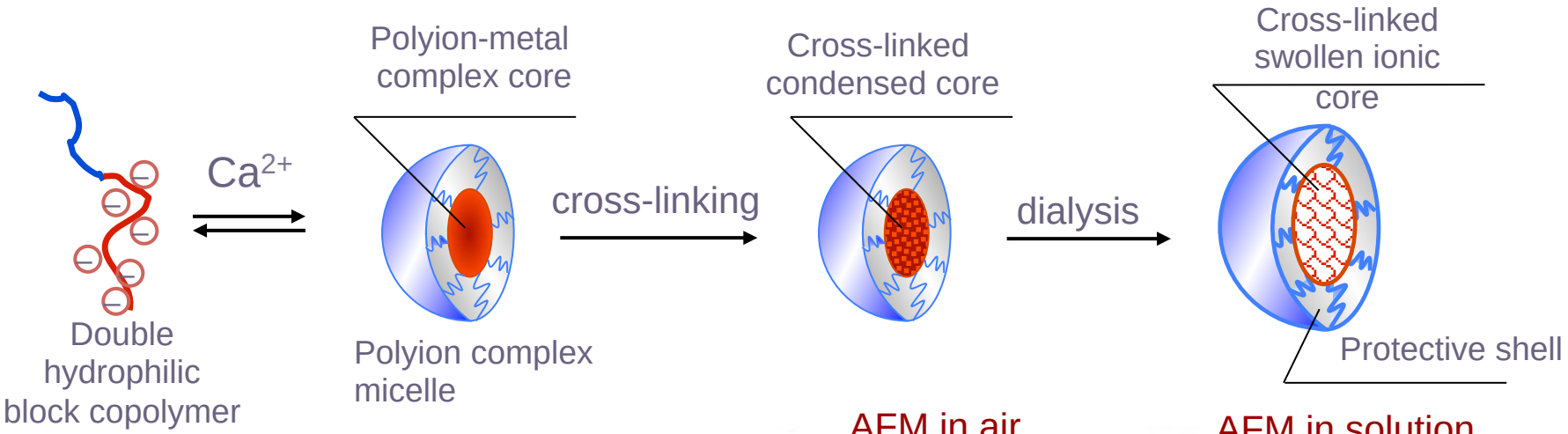
Oligo delivery to retina

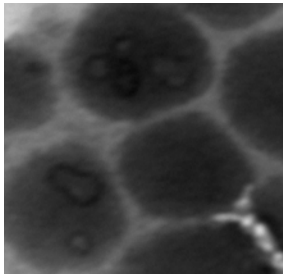


- FITC
- DAPI

Roy et al. *Nature Biotech*. 1999, 17: 476

Polymer Micelles with Cross-Linked Ionic Core (cl-Micelles) = NanoGELS





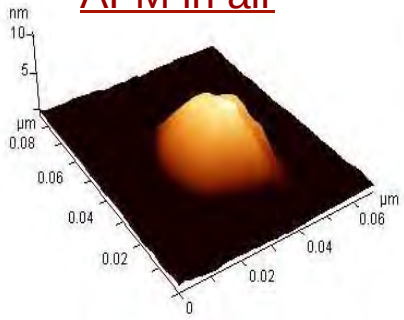
Shell thickness

TEM
7.82 + 0.24 nm (N=69)

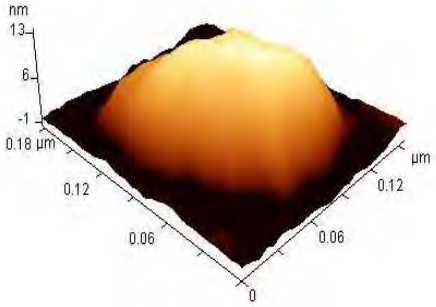
Model of extended polymer brush

L ≅ 6.2 nm

AFM in air



AFM in solution



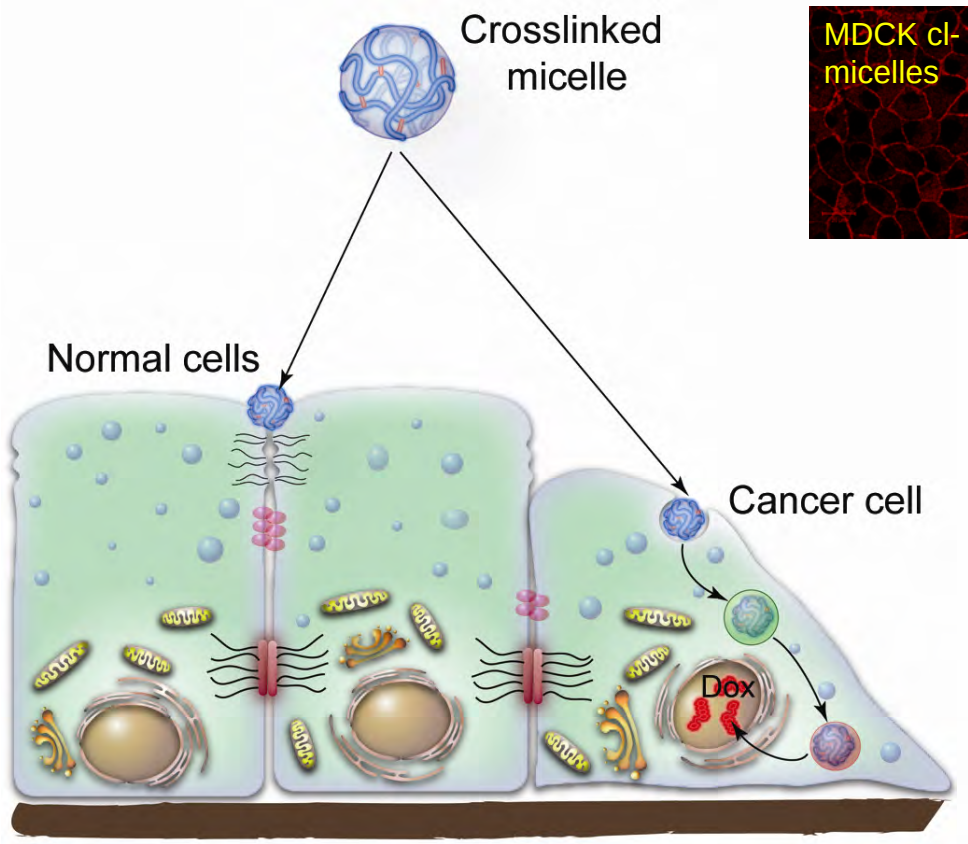
D_d = 19.64 ± 0.67 nm

D_{sw} = 55.0 ± 3.3 nm

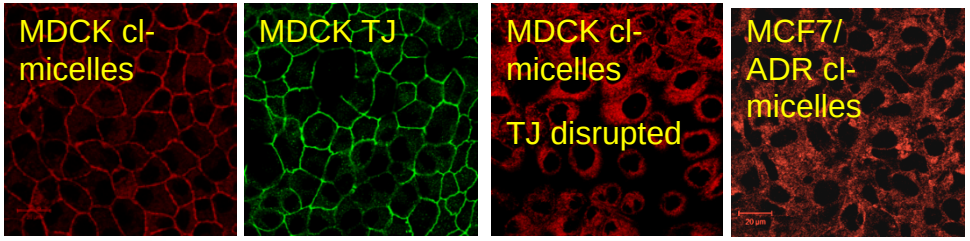
Equilibrium swelling ratio 14.5

Bronich et. al. *J. Am. Chem. Soc.* 2005, 127(23): 8236

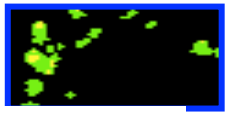
Differential Trafficking of cl-NanoGels in Normal and Cancer Epithelial Cells



TJ and cell uptake

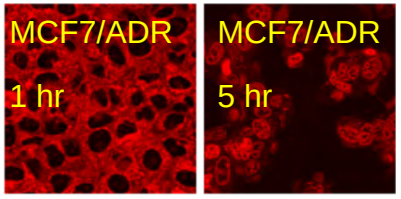


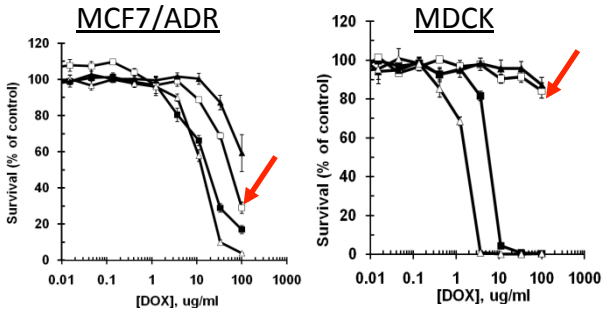
Final destination lysosomesca



lysotracker, 30 min

pH-sensitive cl-micelles/Dox

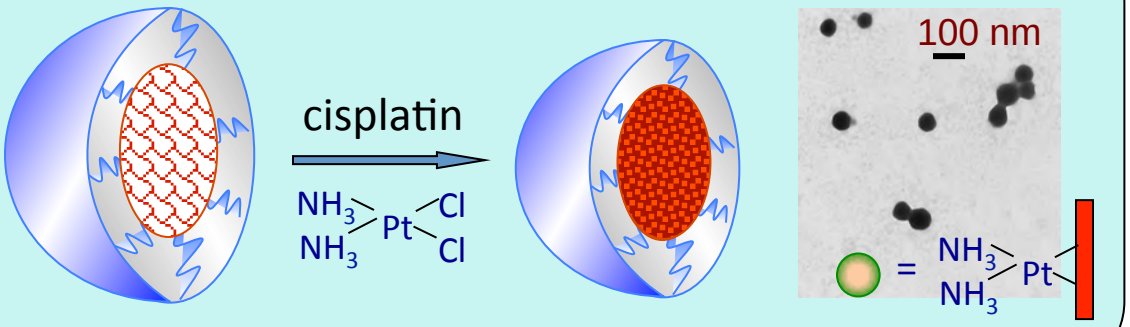




G. Sahay, et al. Biomaterials, 2009, 31:923-33

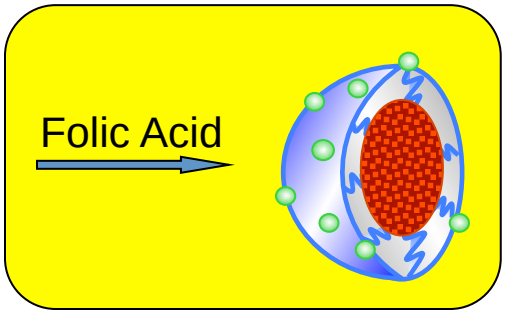
Delivery of Cisplatin to Tumors Via Folate-Targeted NanoGels

m.w. ≈ 2 x 10^8 Da +90-95 % H_20 Chain flexibility



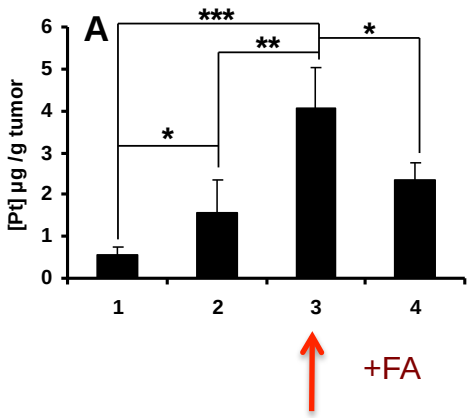
Bronich et. al. *J. Am. Chem. Soc.* 2005, 127(23): 8236

Targeting

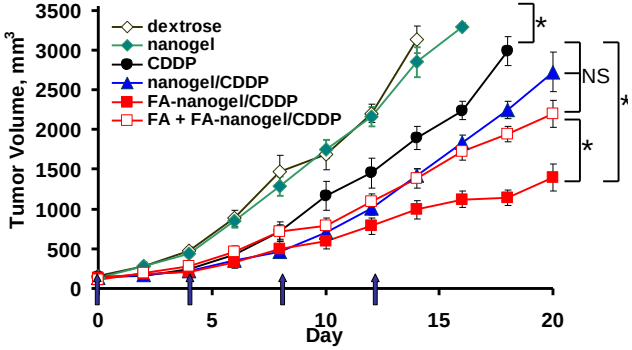


Targeting in FR Expressing Ovarian Carcinoma A2780

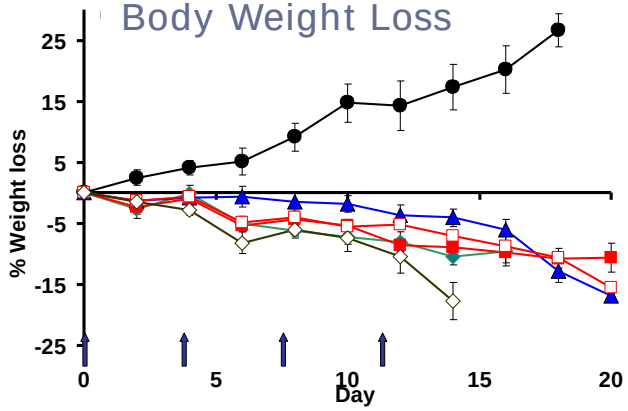
Pt Accumulation in Tumor



Tumor Volume

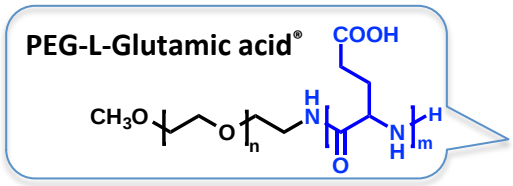


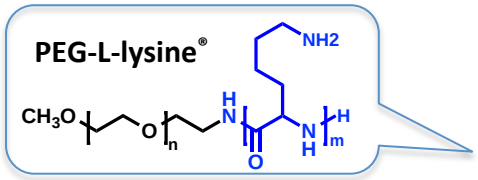
Body Weight Loss

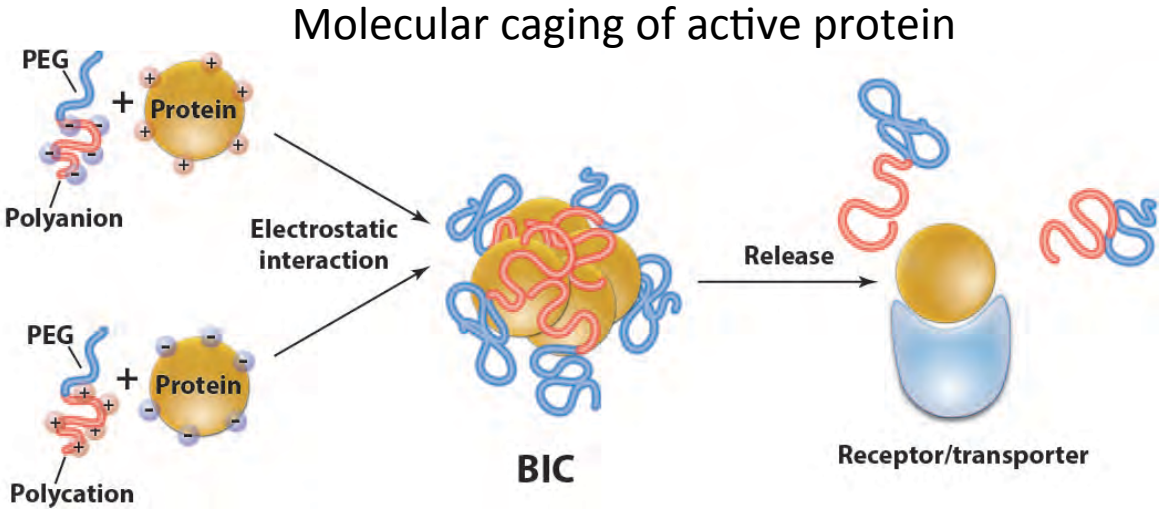


N. Nukolova, et al. Biomaterials, 2011, 32 (23), 5417-5426

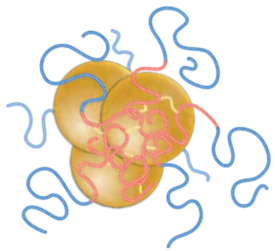
Proteins in Block Ionomer Complexes (BIC)







Manickam, D. S., et al. (2012) Journal of Controlled Release 162, 636-645; Gaydess, A., et al. (2010) Chemical Biological Interactions 187, 295-8; Rosenbaugh, E., et al. (2010) Biomaterials 3, 5218-26

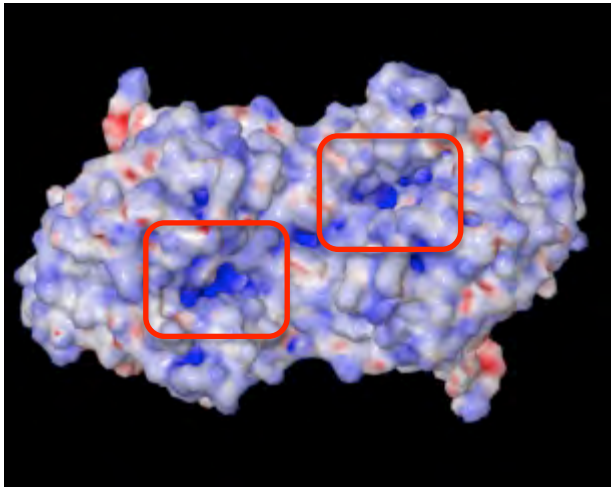


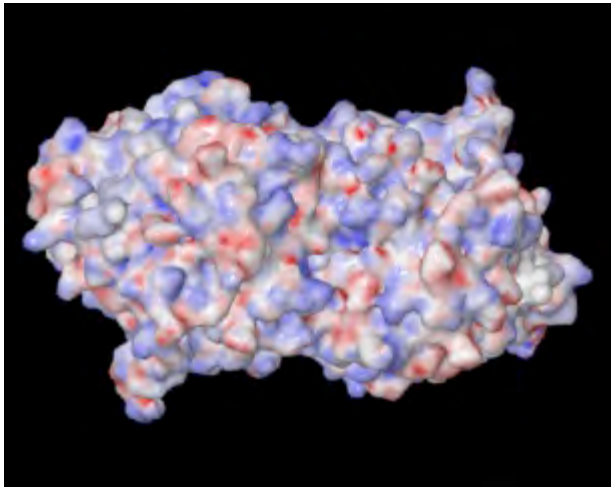
Nanozyme

Summary

- Readily scalable self-assembly process
- Forming stable, biologically active nano-sized particles
- Applicable to positively charged or negatively charged proteins
- Improved peripheral or brain PK
- Efficacy tested in animal models (hypertension, stroke, Parkinson's disease, influenza, uveitis, organophosphate poisoning, spinal cord injury)

CATALYTIC SCAVENGER ORGANOPHOSPHATE HYDROLASE (OPH)

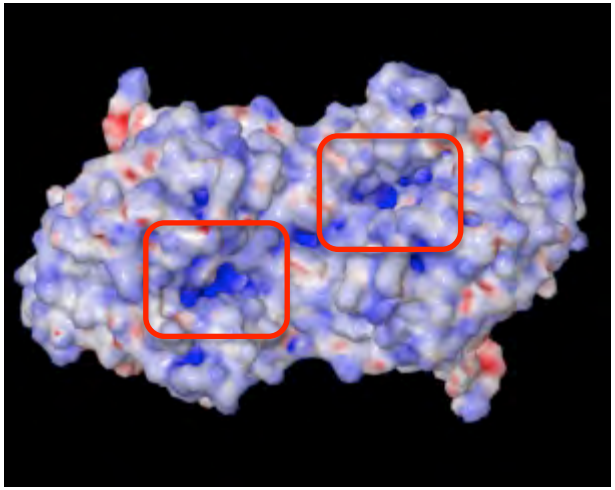


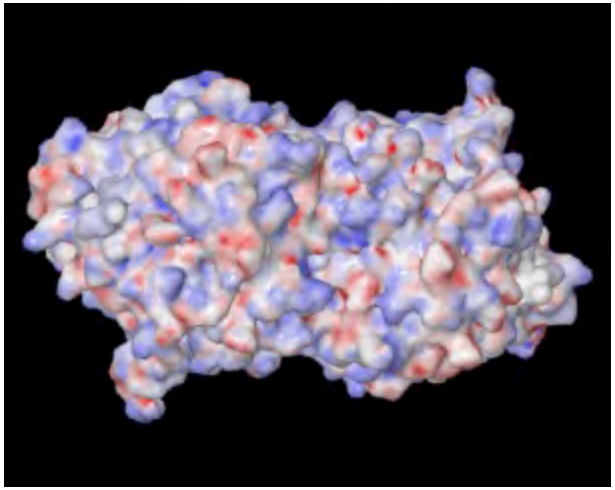


Dimer 72 kDa

OPH distribution of charges
blue = -
red = +

CATALYTIC SCAVENGER ORGANOPHOSPHATE HYDROLASE (OPH)





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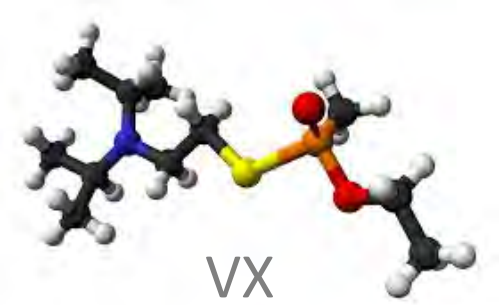
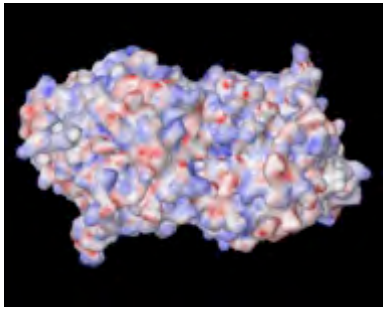
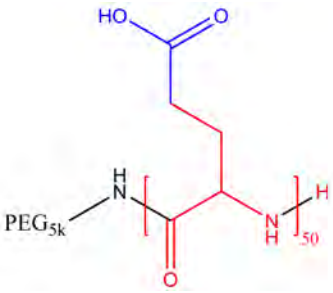


VX

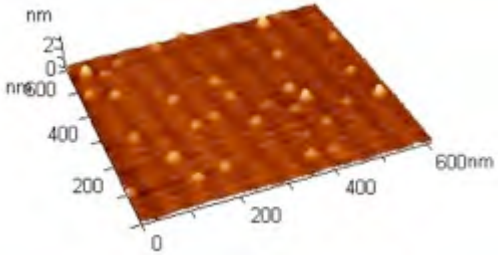
Ohio Natl. Guard

NANOZYME TECHNOLOGY: nano-OPH

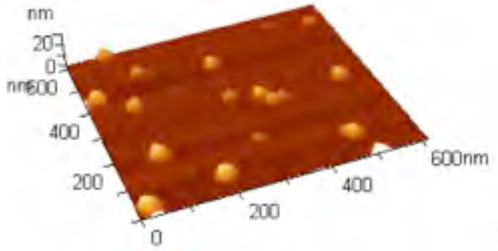




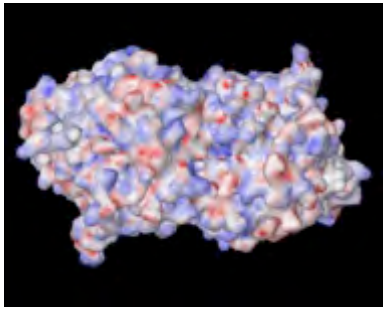
OPH
5-10 nm

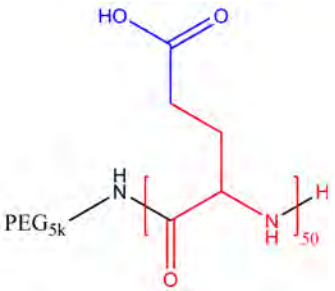


nano-OPH ($Z_{-/+}$=0.5)
50±10 nm

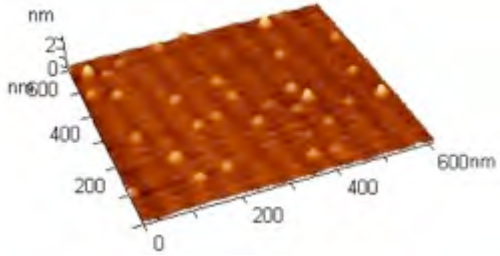


NANOZYME TECHNOLOGY: nano-OPH

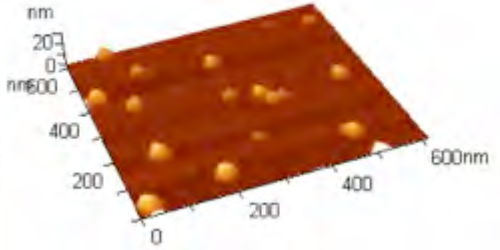




OPH
5-10 nm



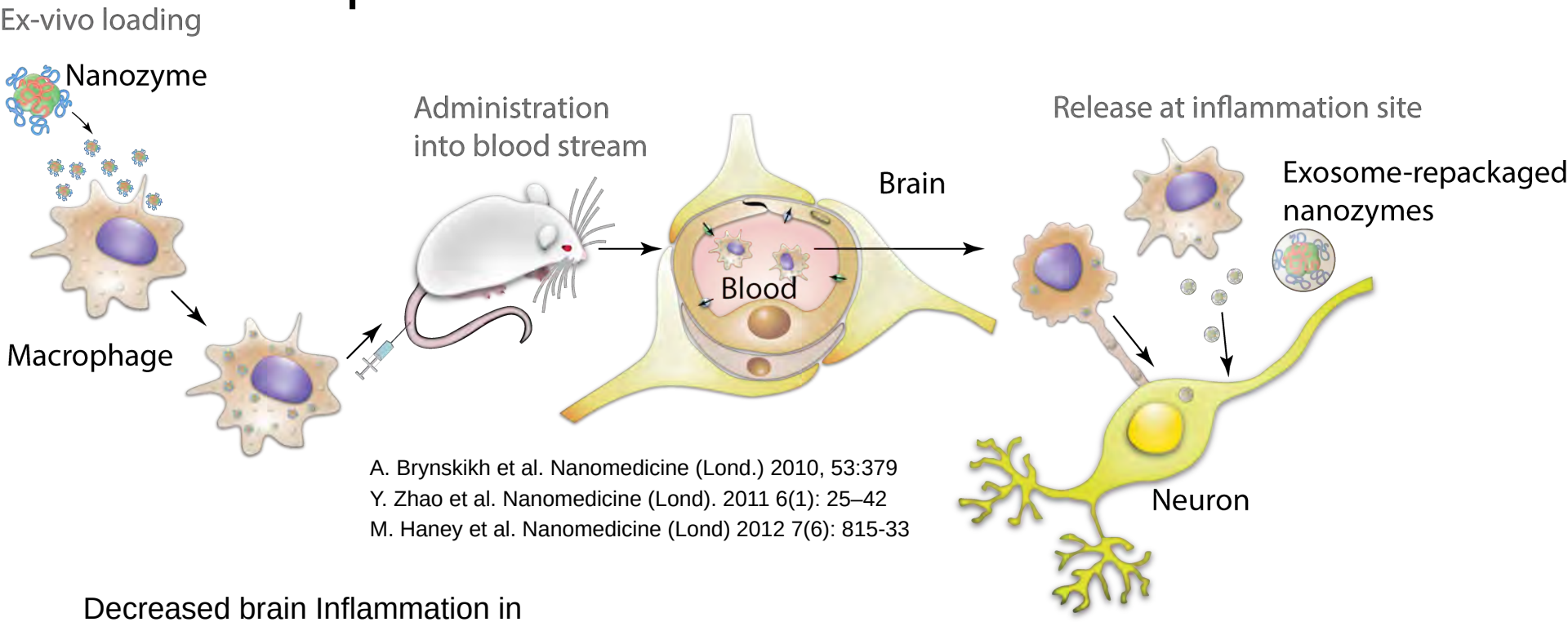
nano-OPH ($Z_{-/+}$=0.5)
50±10 nm



Summary

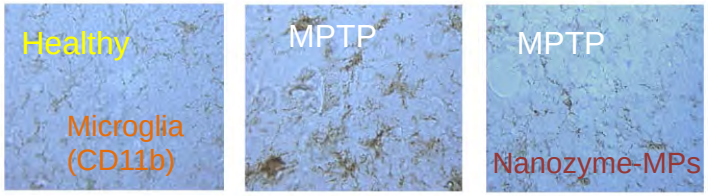
- Excellent storage (2 years in solution)
- Thermo-stable
- Little immune response
- Long circulation (15-20 h)
- Effective when added before exposure (20 h)
- Effective intramuscularly & transbucally

Adoptive Cell-Mediated Transfer of Nanoparticles to Sites of Inflammation

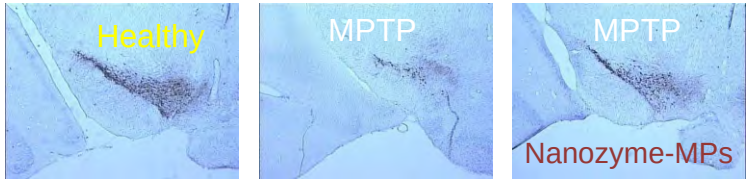


A. Brynskikh et al. Nanomedicine (Lond.) 2010, 53:379
Y. Zhao et al. Nanomedicine (Lond). 2011 6(1): 25–42
M. Haney et al. Nanomedicine (Lond) 2012 7(6): 815-33

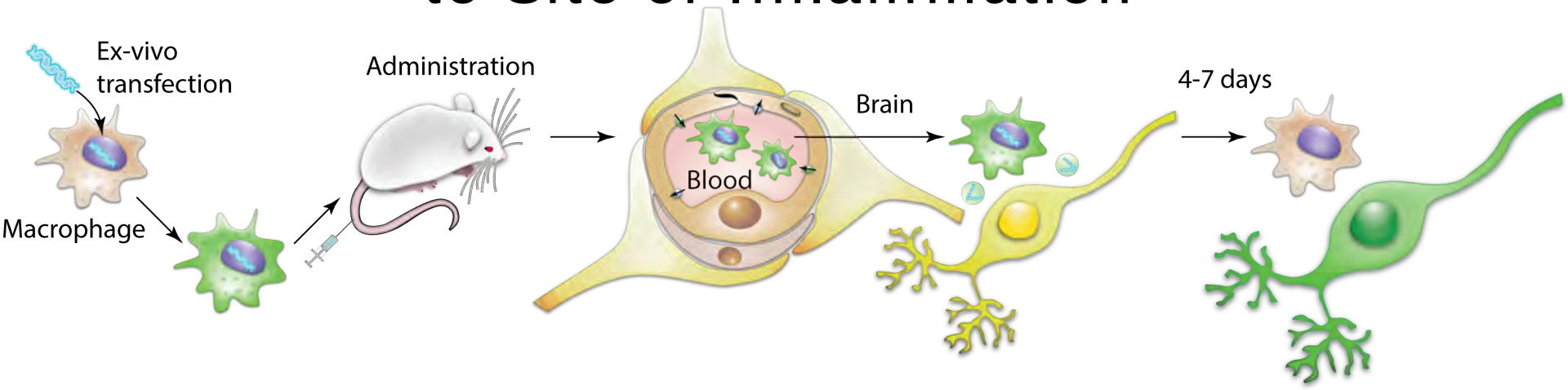
Decreased brain Inflammation in Parkinson's disease model



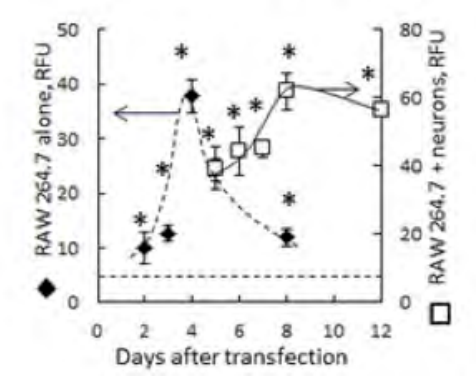
Neuron survival in Parkinson's disease model



Adoptive Cell-Mediated Gene Transfer to Site of Inflammation

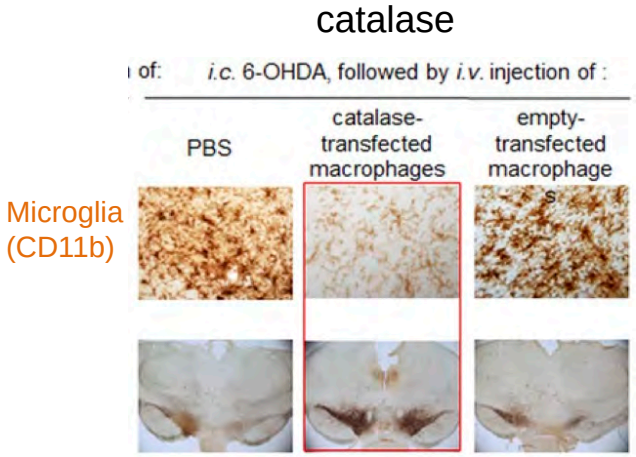


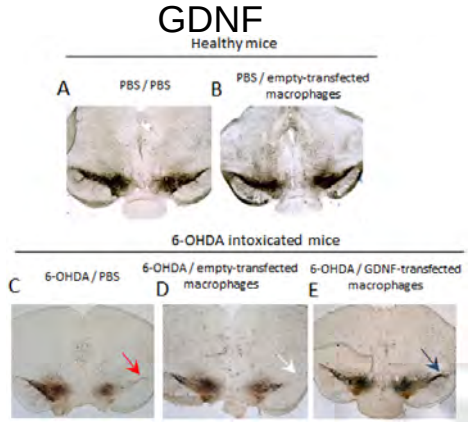
Gene transfer from macrophages to neuronal cells



Haney et al. *PLoS ONE* 2013 8(4): e61852

Anti-inflammatory and neuroprotective effects of transfected macrophages in Parkinson's disease mouse model





Zhao et al. *PLoS ONE* 2014 9(9): e106867

PLOS ONE

Идея Контейнера

Загрузка и Разгрузка

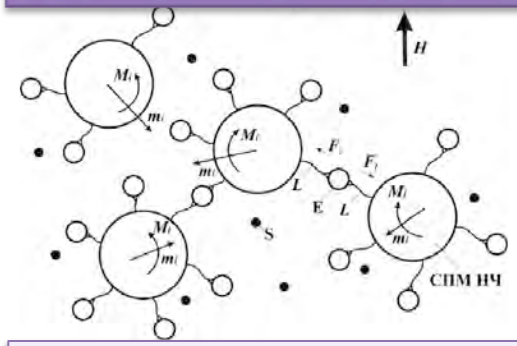
Путь к мишени

Разгрузка

Kabanov & Batrakova, *Cur. Pharm. Design*, 2004, 10: 1355

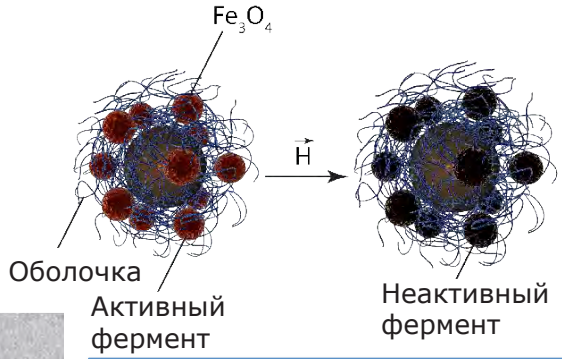
Наноматериалы с дистанционным управлением

Магнито-механическое воздействие на наноразмерном уровне

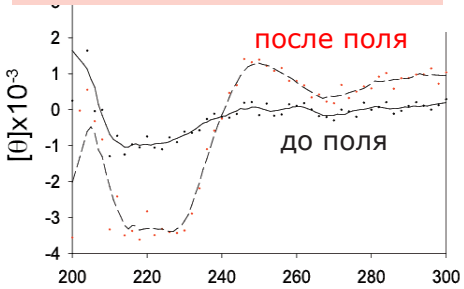


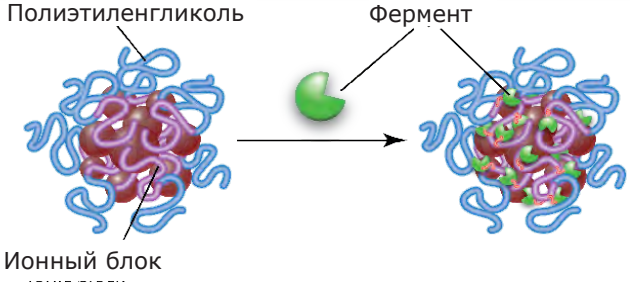
Ориентационное движение магнитных наночастиц в электромагнитном поле сверхнизкой частоты (десятки Гц) может оказывать силу в десятки и сотни пико-Ньютонов (пН) на молекулы, связанные с этими частицами. Такие силы могут «двигать» макромолекулы и рвать химические связи.

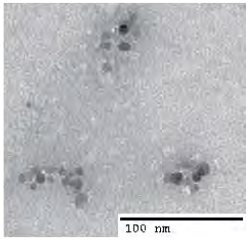
Чтобы “поймать” эти силы, молекулы фермента были использованы в качестве «репортеров». Они были прикреплены к наноразмерным частицам магнетита с полимерной оболочкой



Спектры КД





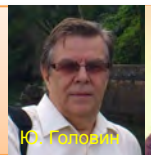


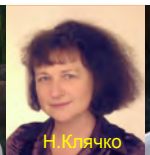
Впервые показано, что воздействие негреющих переменных электромагнитных полей сверхнизкой частоты меняет структуру и активность фермента

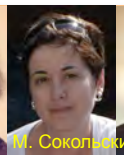
N. Klyachko, M. Sokolsky-Papkov, et.al. Angew. Chemie, 2012, accepted.

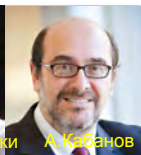
Таким образом, можно создать наноматериалы с электромагнитным «реле», которые в ответ на дистанционные радиоволновые сигналы могут совершать полезную работу - например, включать и выключать ферменты или высвобождать лекарства «по требованию»

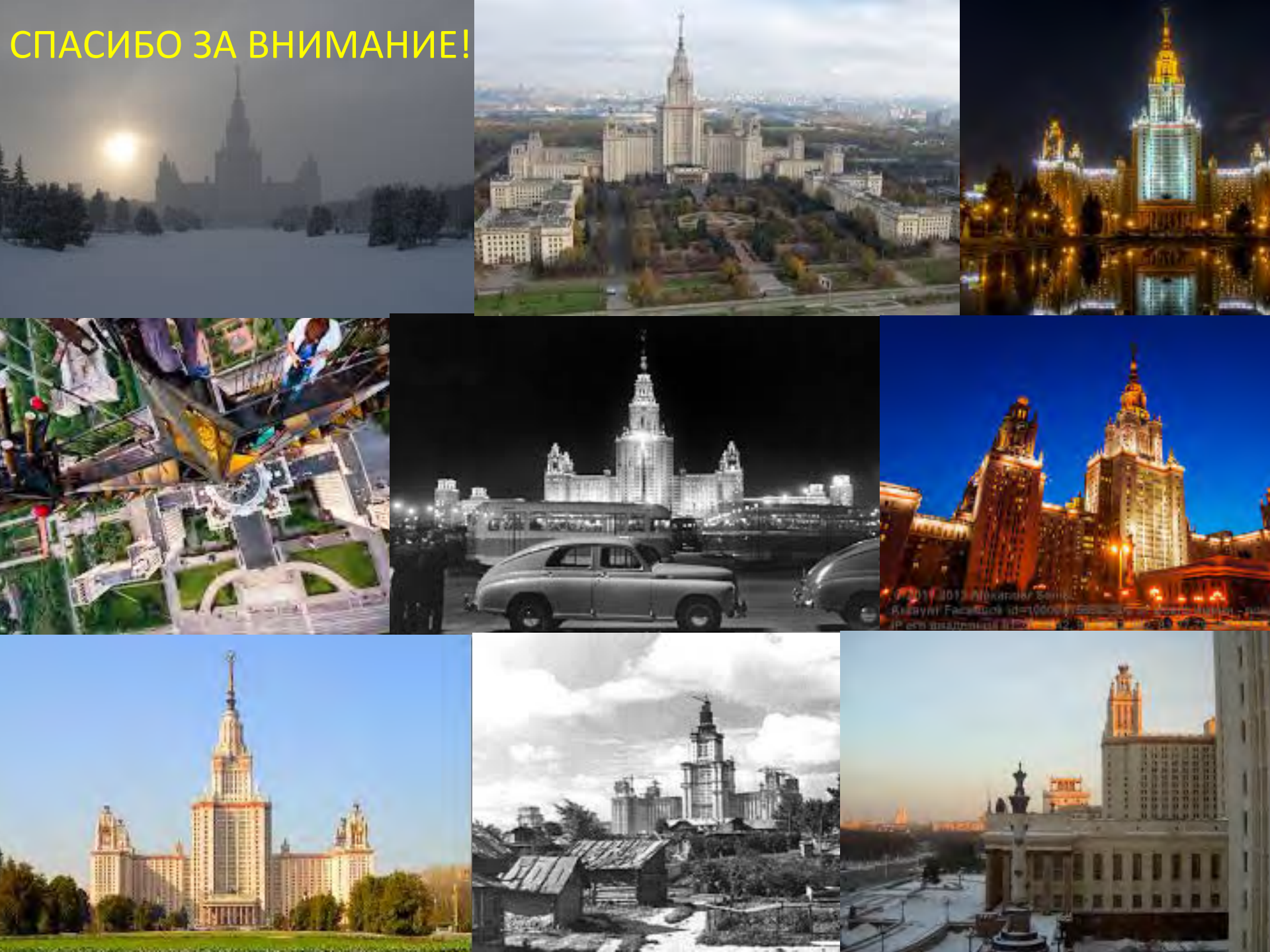
Лаборатория Химического Дизайна Бионаноматериалов
Ведущий ученый **Кабанов А.В.**
Тема научного исследования: **“Химия: химический дизайн бионаноматериалов для медицинских применений”**
Договор № 11G.34.31.0004 от “30” ноября 2010 г.










СПАСИБО ЗА ВНИМАНИЕ!