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pharmaceutics
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Biological studies on cyclodextrins

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Abstract: In recent years, our knowledge of the biological effects of cyclodextrins has grown significantly. Cellular actions of cyclodextrins originate in their ability to form complexes with lipophilic biomolecules. Cyclodextrins can target different types of molecules according to their size, for instance, alpha-cyclodextrins form complexes with phospholipids, while beta-cyclodextrins can bind cholesterol or prostaglandin E2. Due to their interactions with the main membrane constituents, cyclodextrins can affect the barrier function of biological barriers or influence the function of membrane proteins. Nevertheless, cyclodextrins can enter the cells by endocytosis and affect the intracellular cholesterol storage. Based on these findings, 2-hydroxypropyl-beta cyclodextrin (HPBCD) received the orphan designation for the treatment of Niemann-Pick disease, type C. The endocytosis of cyclodextrins works in different cell types and can be applied in the delivery of drugs into the cells. Tissue distribution and pharmacokinetics of cyclodextrins could be further characterized by imaging techniques. Radiolabeled HPBCD and randomly methylated beta-cyclodextrin (RAMEB) were used to study their in vivo behavior by positron emission tomography recently. Interestingly RAMEB accumulation was detected in prostaglandin E2 (PGE2) positive tumors. These findings can promote further research and application of cyclodextrins in inflammation and tumor diagnosis or targeting. The presentation aims to give an overview of the main biological effects of cyclodextrins and the recent results of this research field.

Keywords: cyclodextrins, cellular effects, biological barriers, endocytosis, cholesterol

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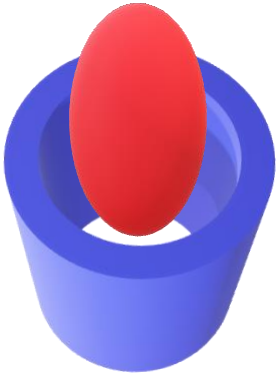
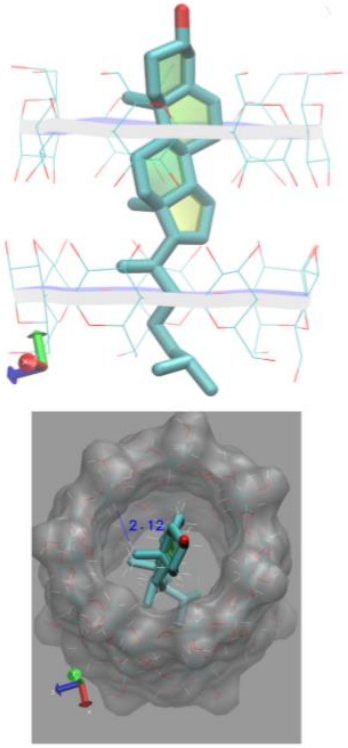
Cyclodextrins

Host-guest interactions



β-cyclodextrin + cholesterol



Beilstein J. Org. Chem. 2018, 14, 838-848.

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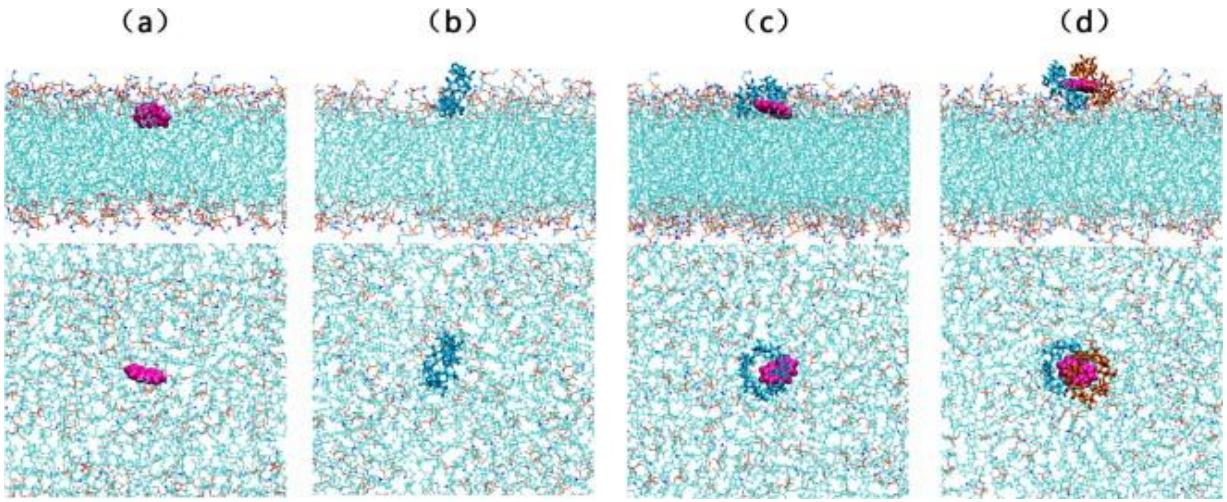
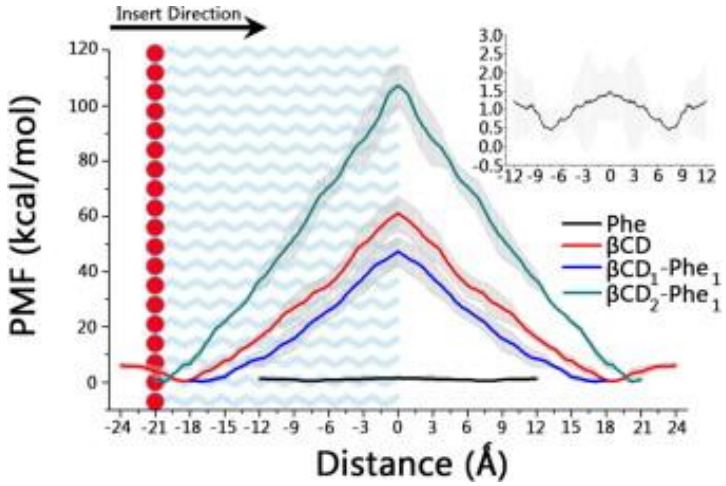
Cyclodextrin – cell membrane interactions



Planar view

Vertical view

Phenantrene β-CD β –CD_1 – Phe_1 β –CD_2 – Phe_1



Phe – no energy barrier
β-CD – high energy barrier

Ren B et al. J Hazard Mater, 2015, 281:148-156.

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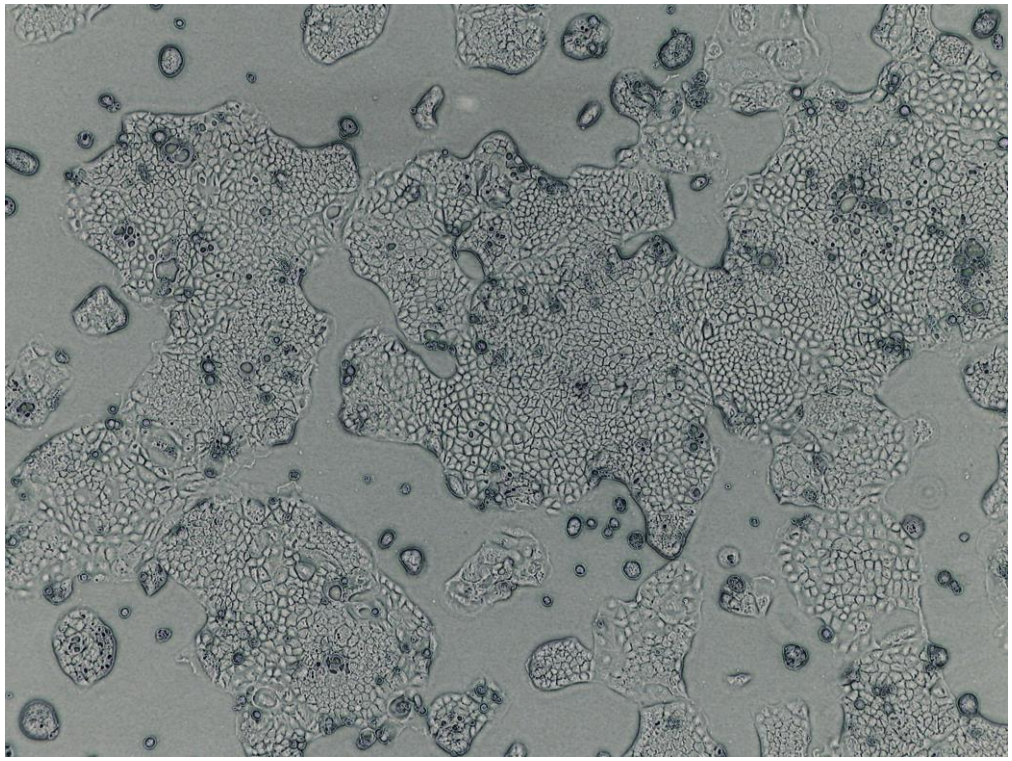
1. *In vitro* biological studies

Examination of β-cyclodextrin derivatives on cells



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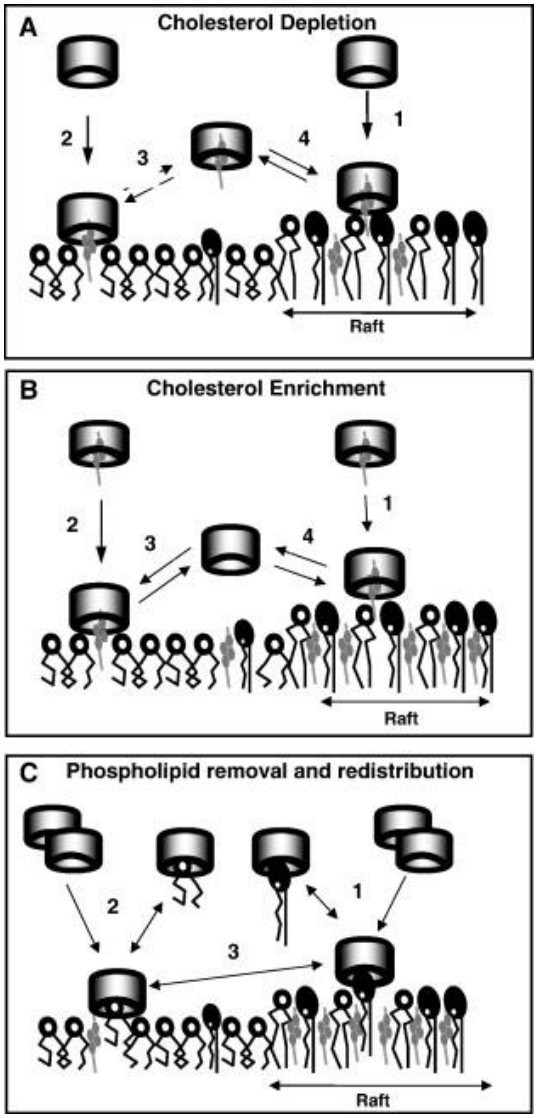
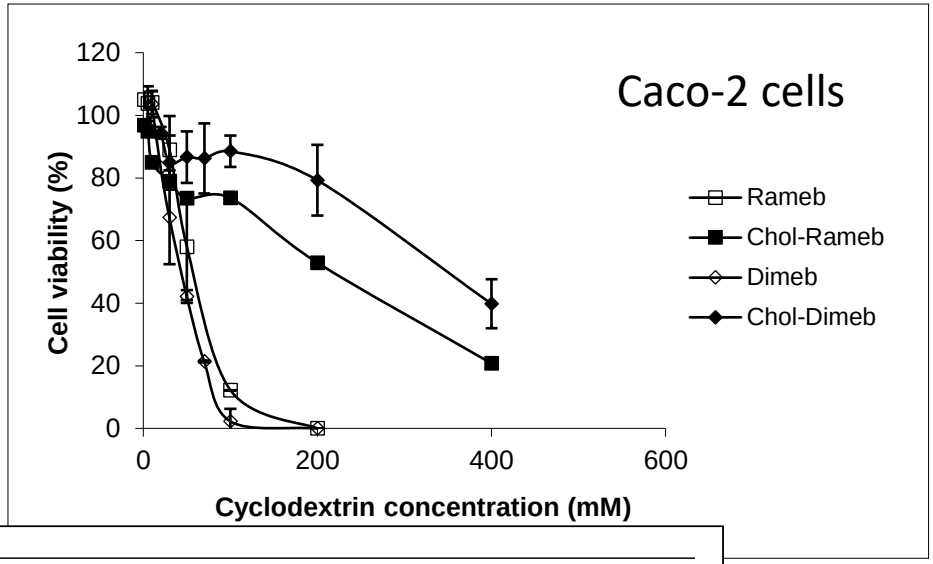
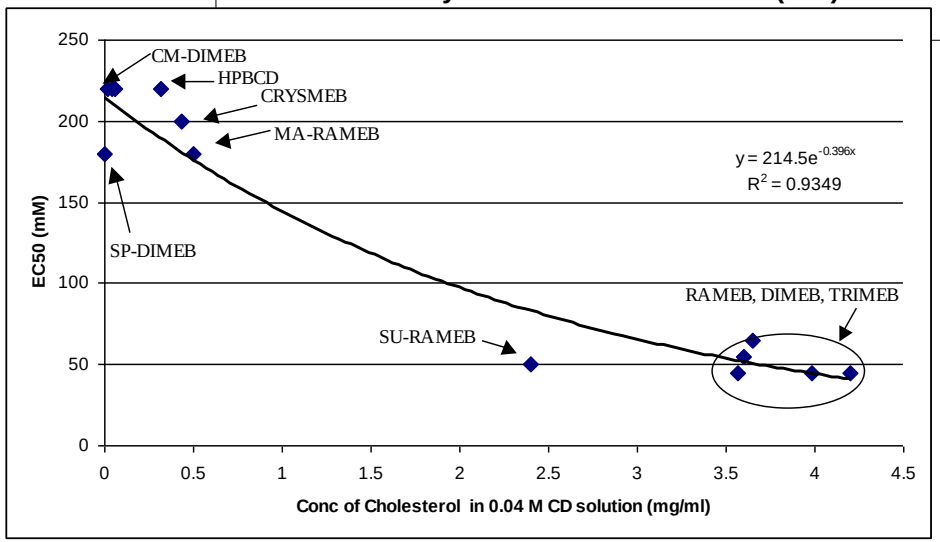
Cyclodextrin – cell membrane interactions
Cellular effects of β-cyclodextrins - Cytotoxicity



Zidovetzki R., Levitan I. BBA-Biomembranes, 2007, 1768:1311-1324





Kiss, T., Fenyvesi F., et al., European Journal of Pharmaceutical Sciences, 2010, 40,(4): 376-380

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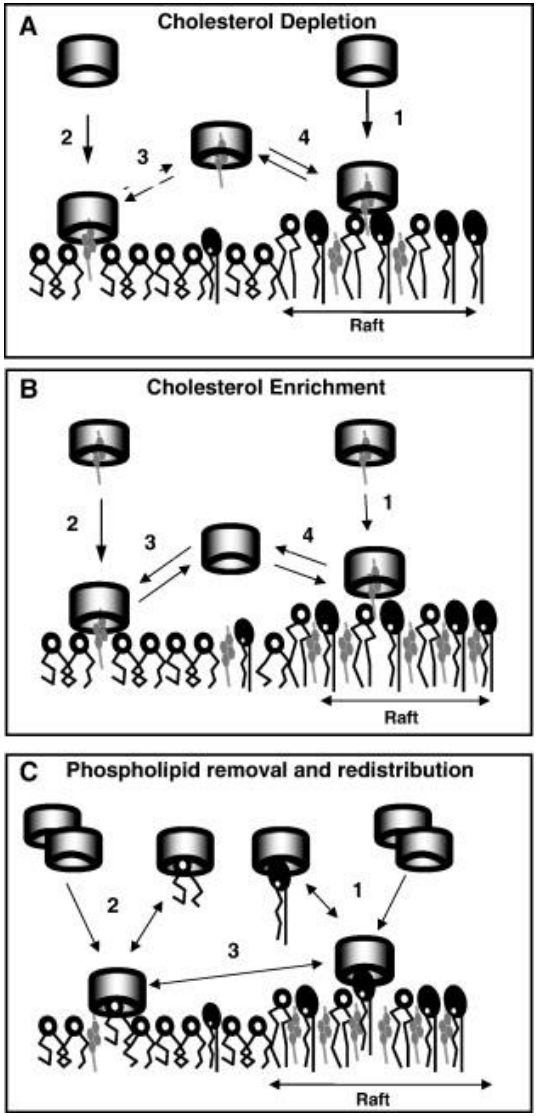
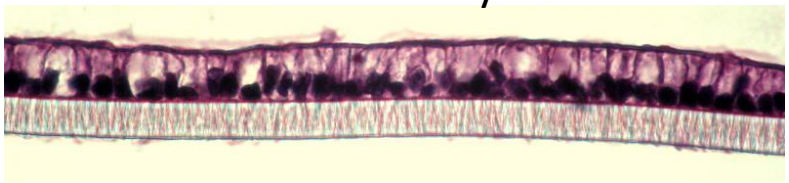
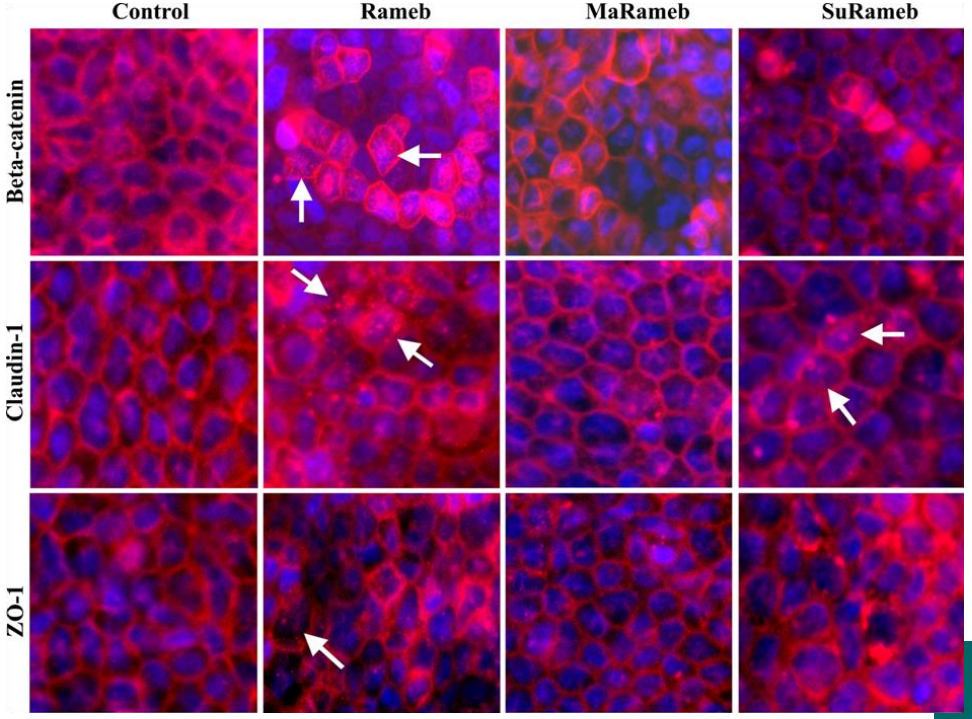
Cyclodextrin – cell membrane interactions
Cellular effects of β-cyclodextrins – Tight Junctions



Zidovetzki R., Levitan I. BBA-Biomembranes, 2007, 1768:1311-1324

Caco-2 monolayers





Fenyvesi F et. al., J Pharm Sci. 2011;100(11):4734-44

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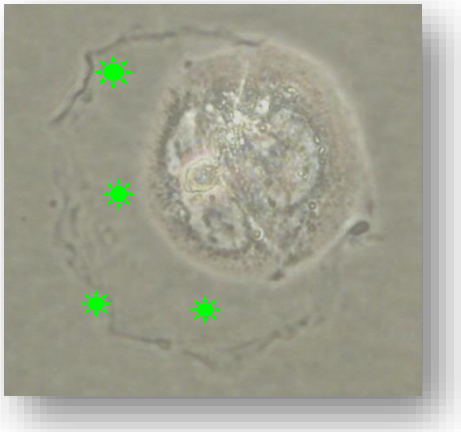
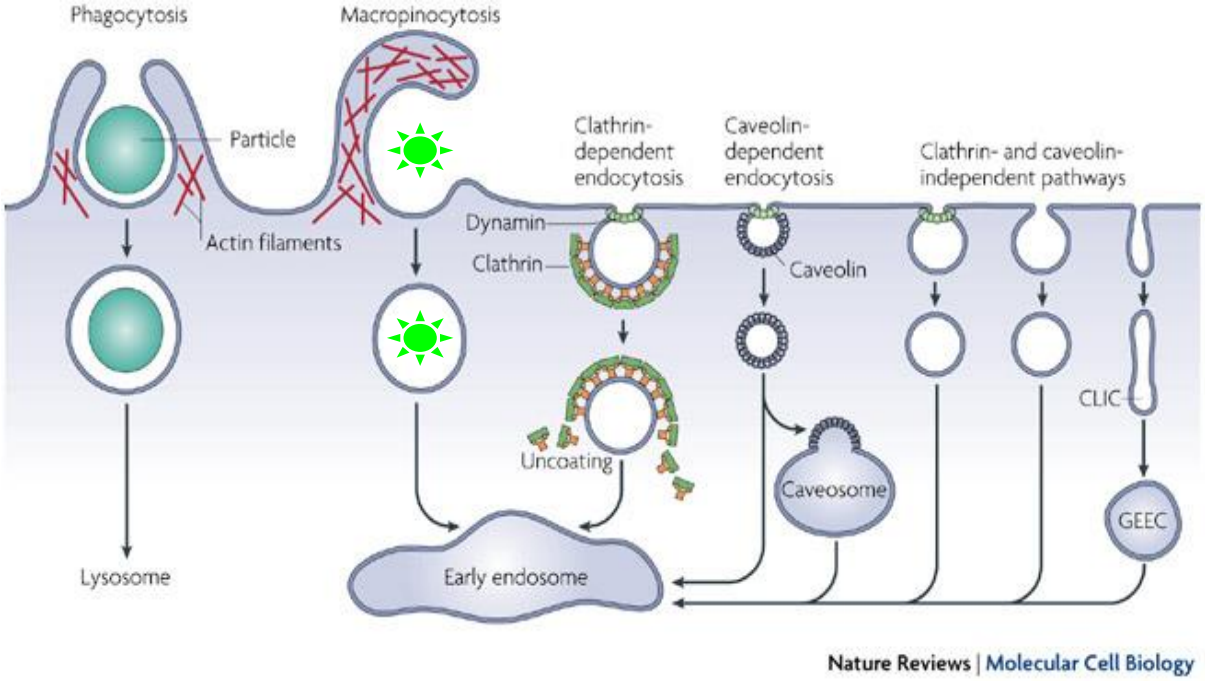
Cyclodextrin – cell membrane interactions - Endocytosis





Lopez, S. & Arias, C. (2010) How Viruses Hijack Endocytic Machinery. *Nature Education* 3(9):16

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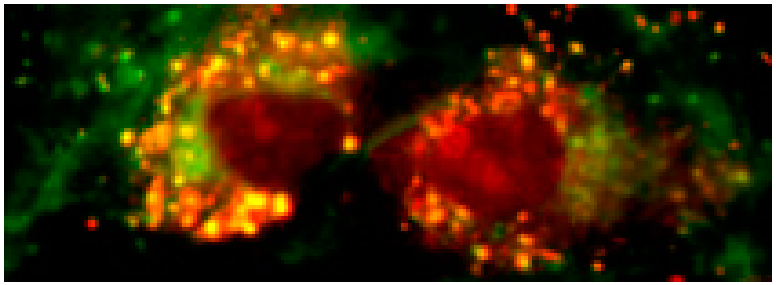
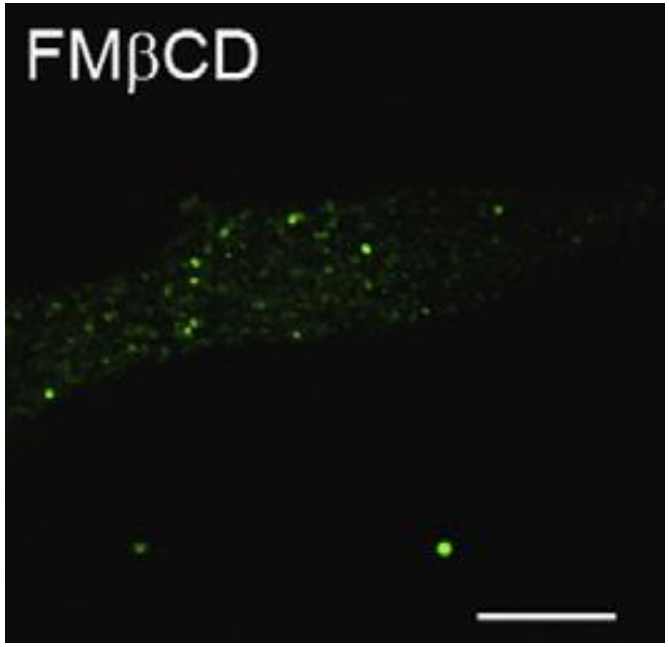
Cyclodextrins enter the cytoplasm

Niemann-Pick disease type C :
Lysosomal choesterol storage disorder



Rosenbaum et al. 2009. PNAS
Endocytosis of beta-cyclodextrins is responsible for cholesterol reduction in Niemann-Pick type C mutant cells



Plazzo et al. 2012. Chemistry and Physics of lipids
Uptake of a fluorescent methyl-β-cyclodextrin via clathrin-dependent endocytosis

Application of hydroxypropyl-beta-cyclodextrin in the treatment of Niemann-Pick disease type C as an orphan drug

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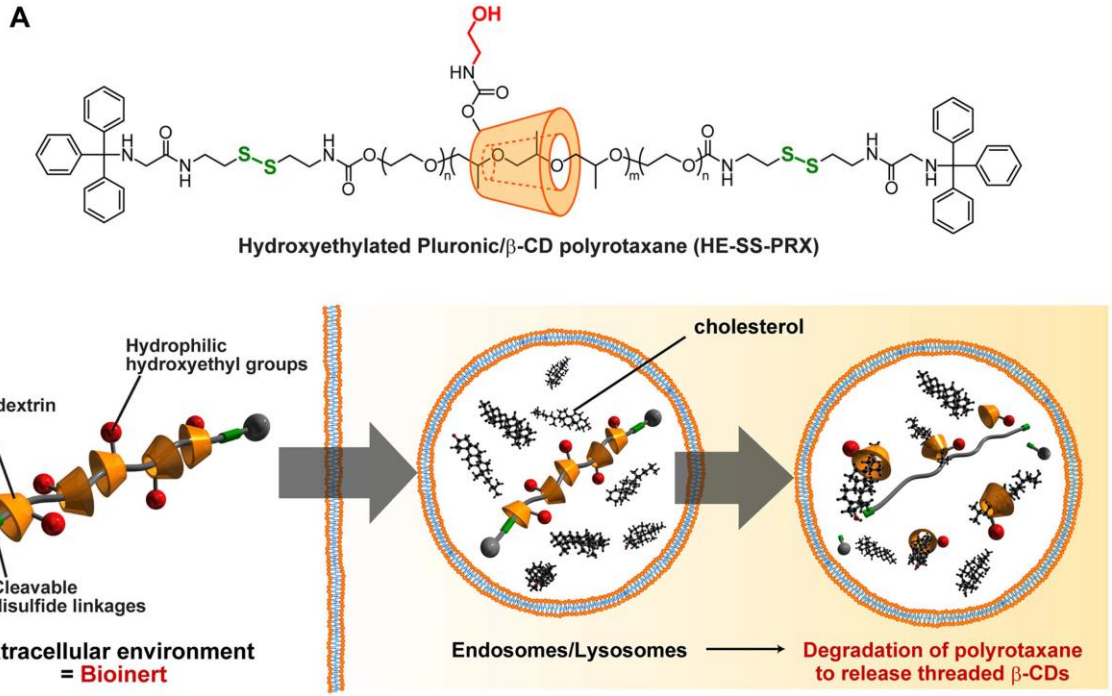
Lysosomal-specific cholesterol reduction by Polyrotaxanes



Tamura A. and Yui N., Sientific Reports, 2014, 4:4356.

Cylodextrins can act from inside the lysosomes.

Are cyclodextrins able to reach the cytoplasm of other cell types?

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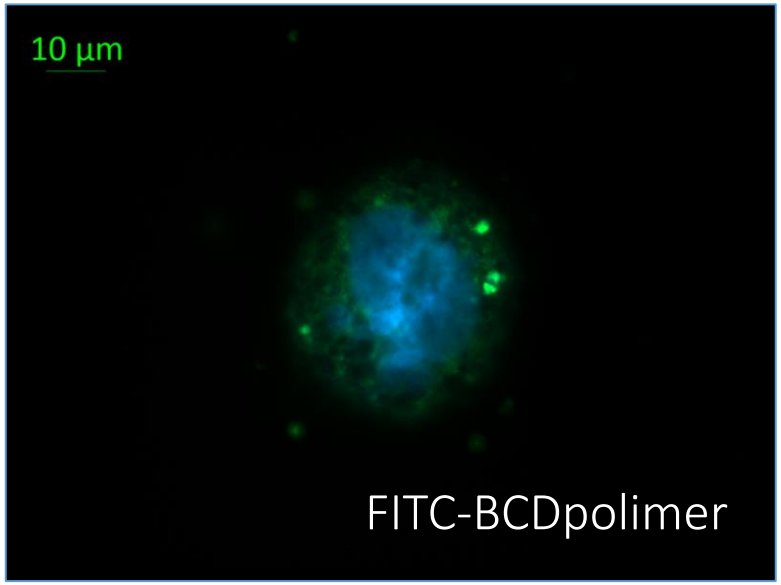
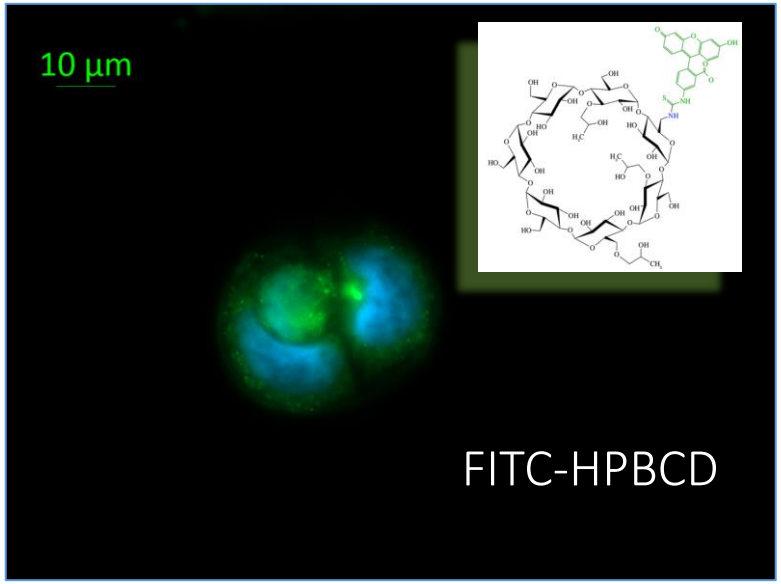
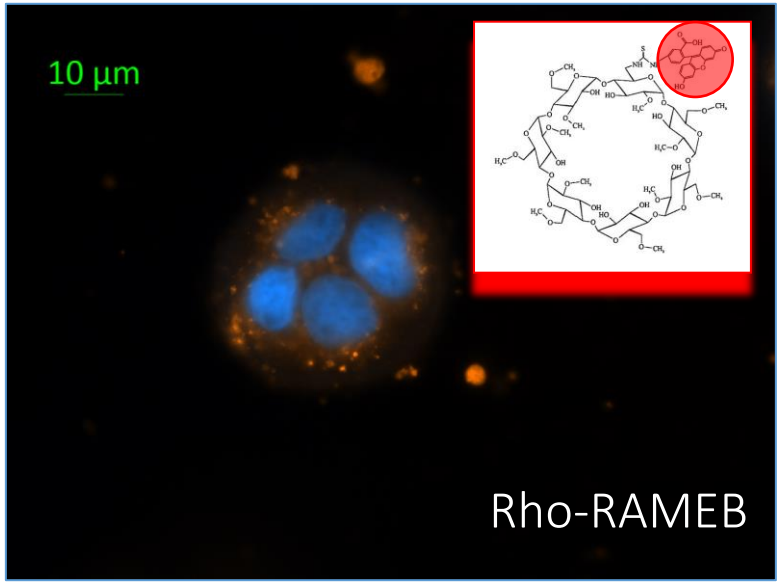
Fluorescent cyclodextrins in the cytoplasm of Caco-2 cells

50 µM, 30 min, 37 °C







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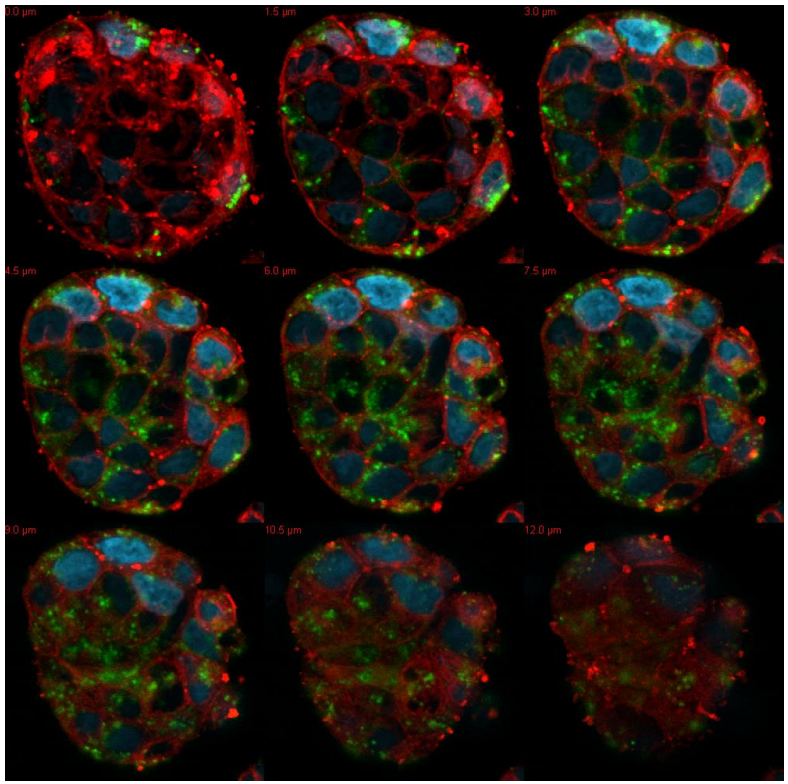
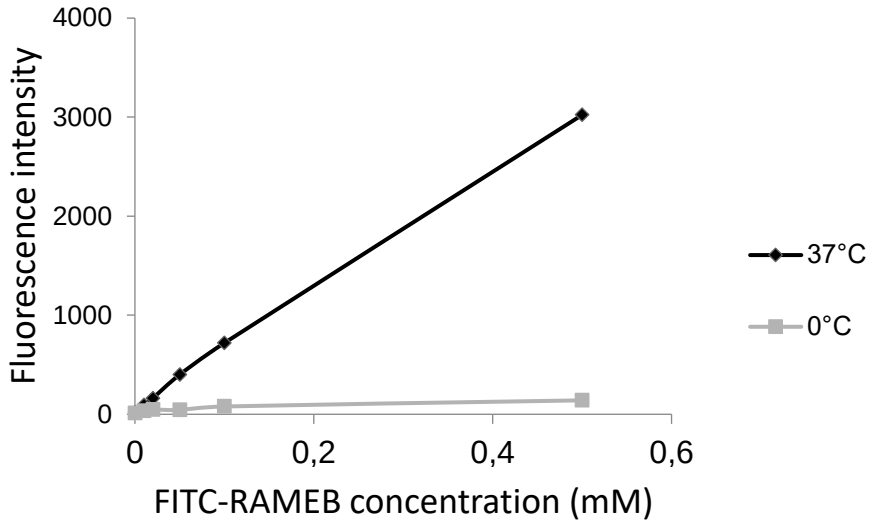
Intracellular FITC-RAMEB accumulation in Caco-2 cells





Fenyvesi F. et al., (2014) PLoS ONE 9(1): e84856.

Green – FITC-RAMEB
Blue – nucleus
Red – cell membrane

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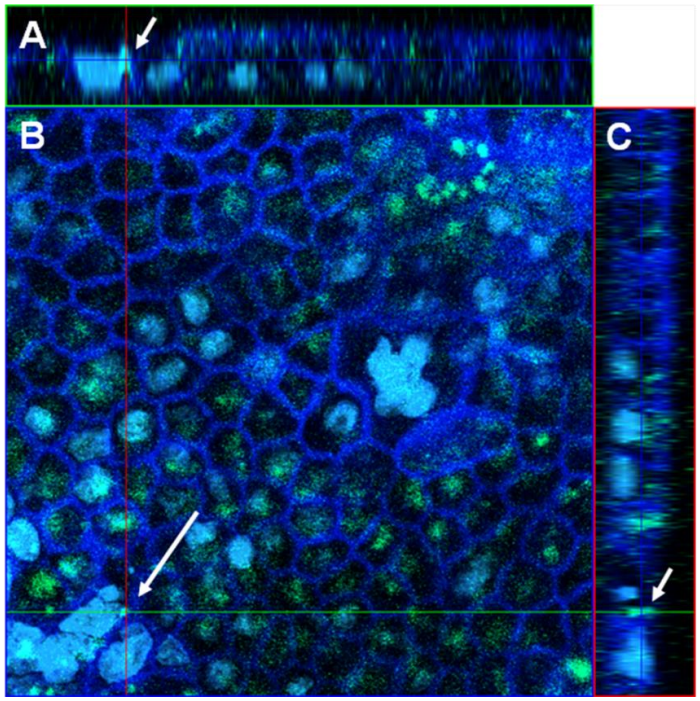
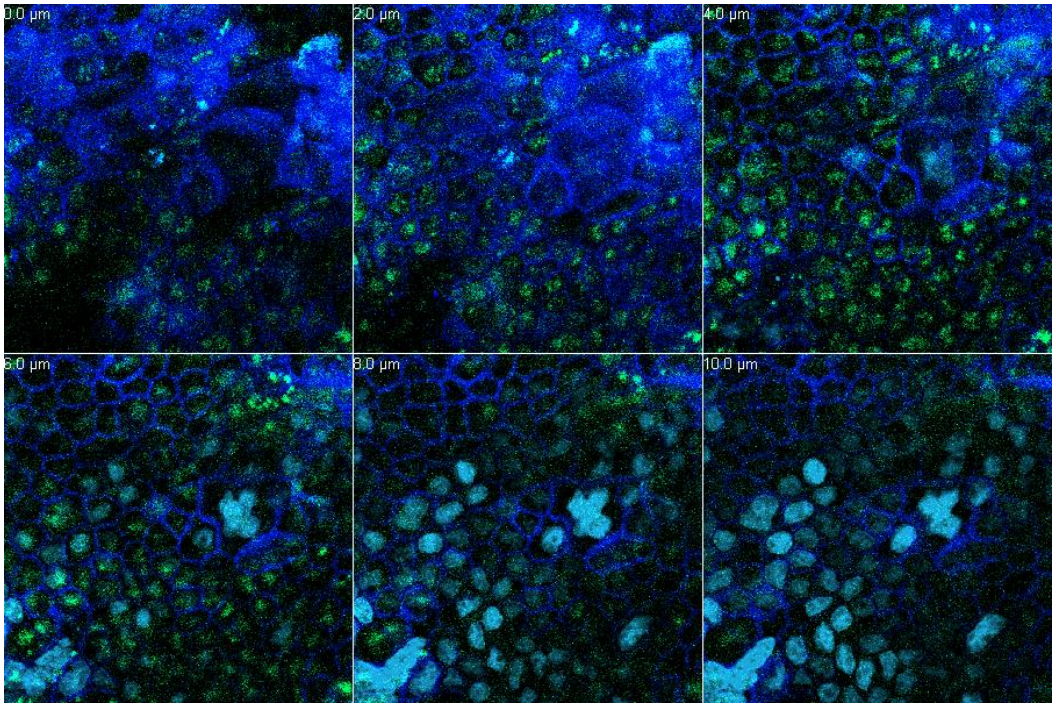
Intracellular FITC-RAMEB accumulation in Caco-2 cell layer





Green – FITC-RAMEB
Pale Blue – nucleus
Blue – cell membrane

Fenyvesi F. et al., (2014) PLoS ONE 9(1): e84856.

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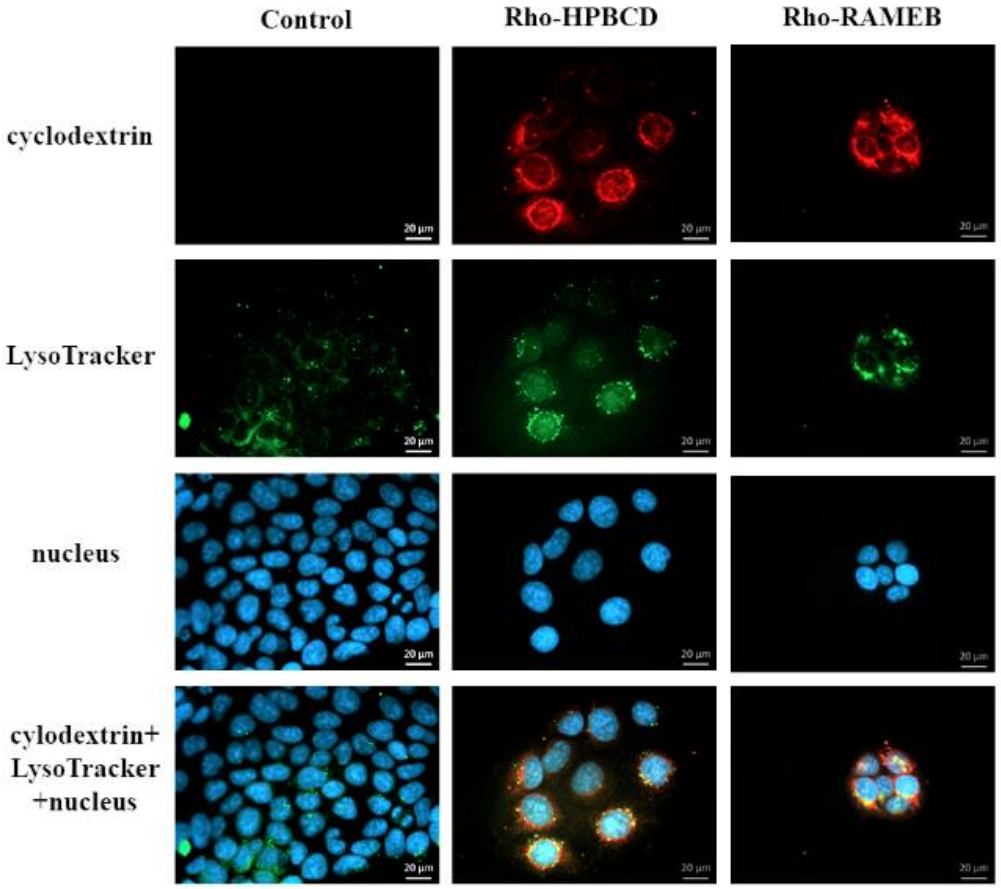
Intracellular localization of cyclodextrins



Detection of lysosomes
by fluorescence microscopy
in Caco-2 cells

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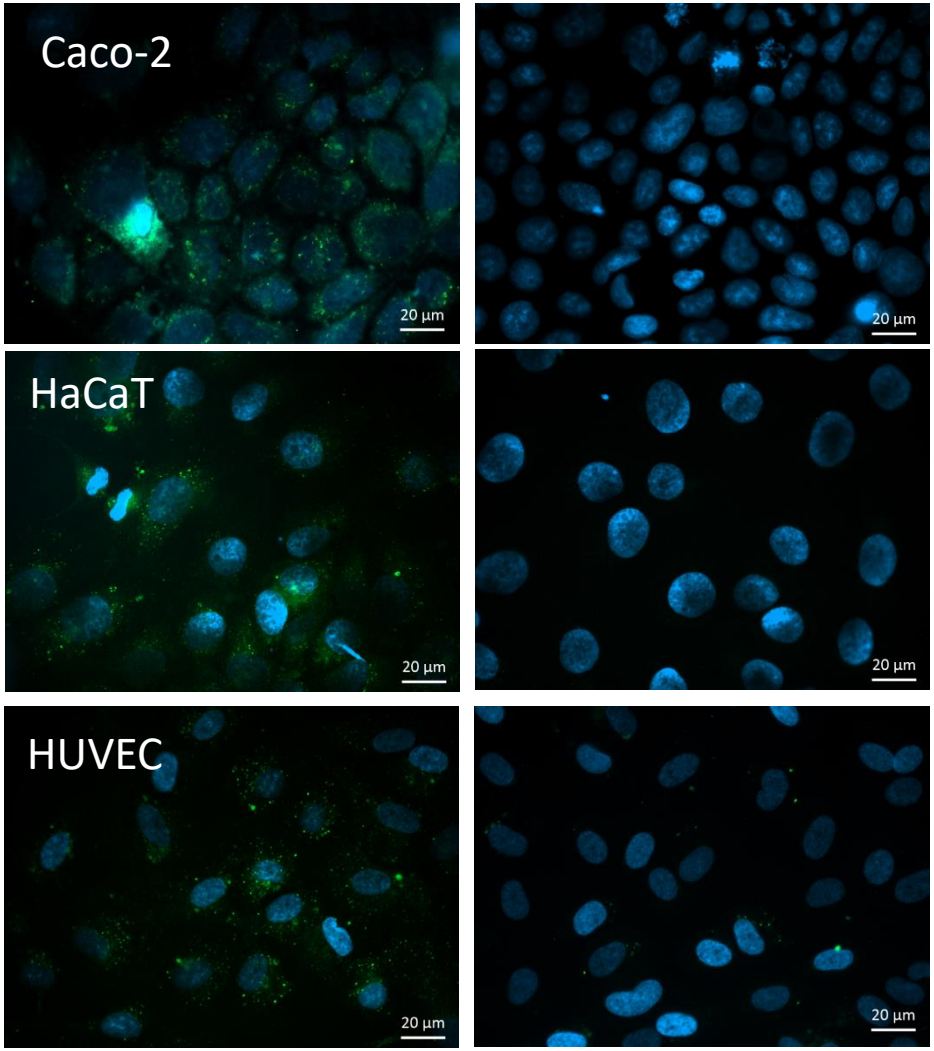
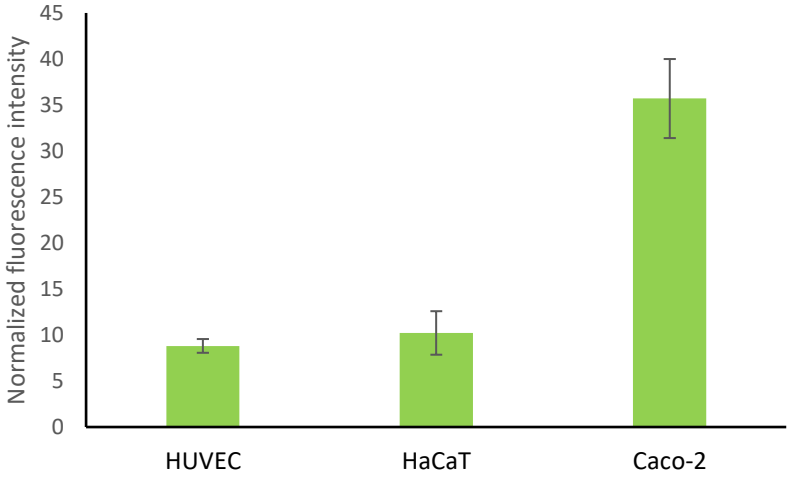
Intracellular FITC-HPBCD accumulation in different cell lines





50 µM, 30 min, 37 °C, flow cytometry

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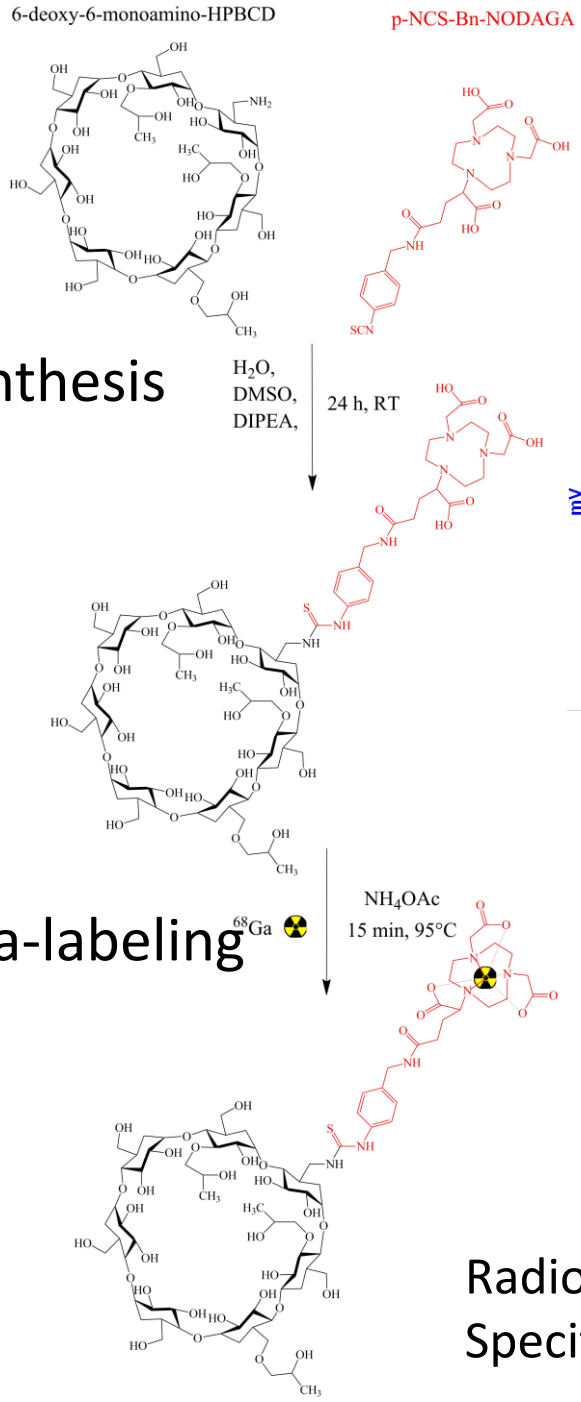
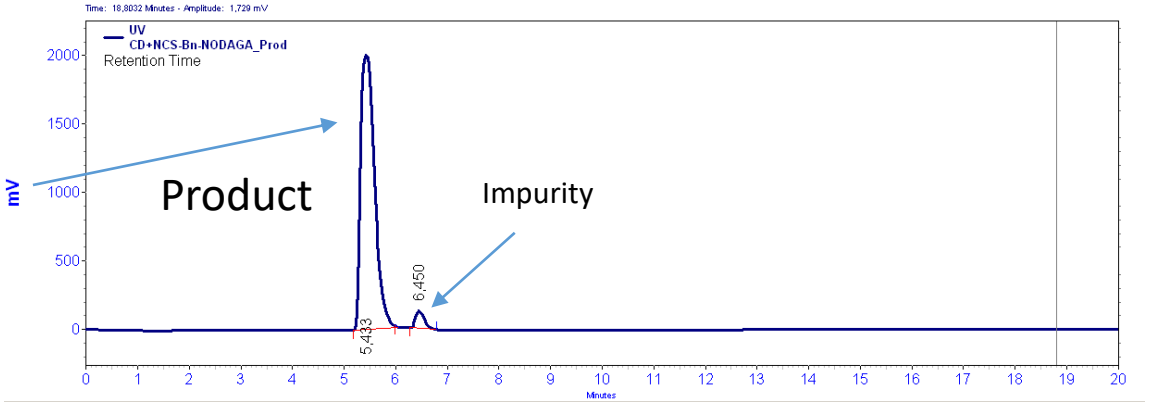
DEBRE

2. *In vivo* biological studies

Chemical synthesis and radiolabeling of ^{68}Ga-labeled **NODAGA-HPBCD**





Purification by preparative RP-HPLC
- Purity > 98% (HPLC)

Radiochemical purity > 98% =HPLC)
Specific activity was 17.62±2.43 GBq/µmol

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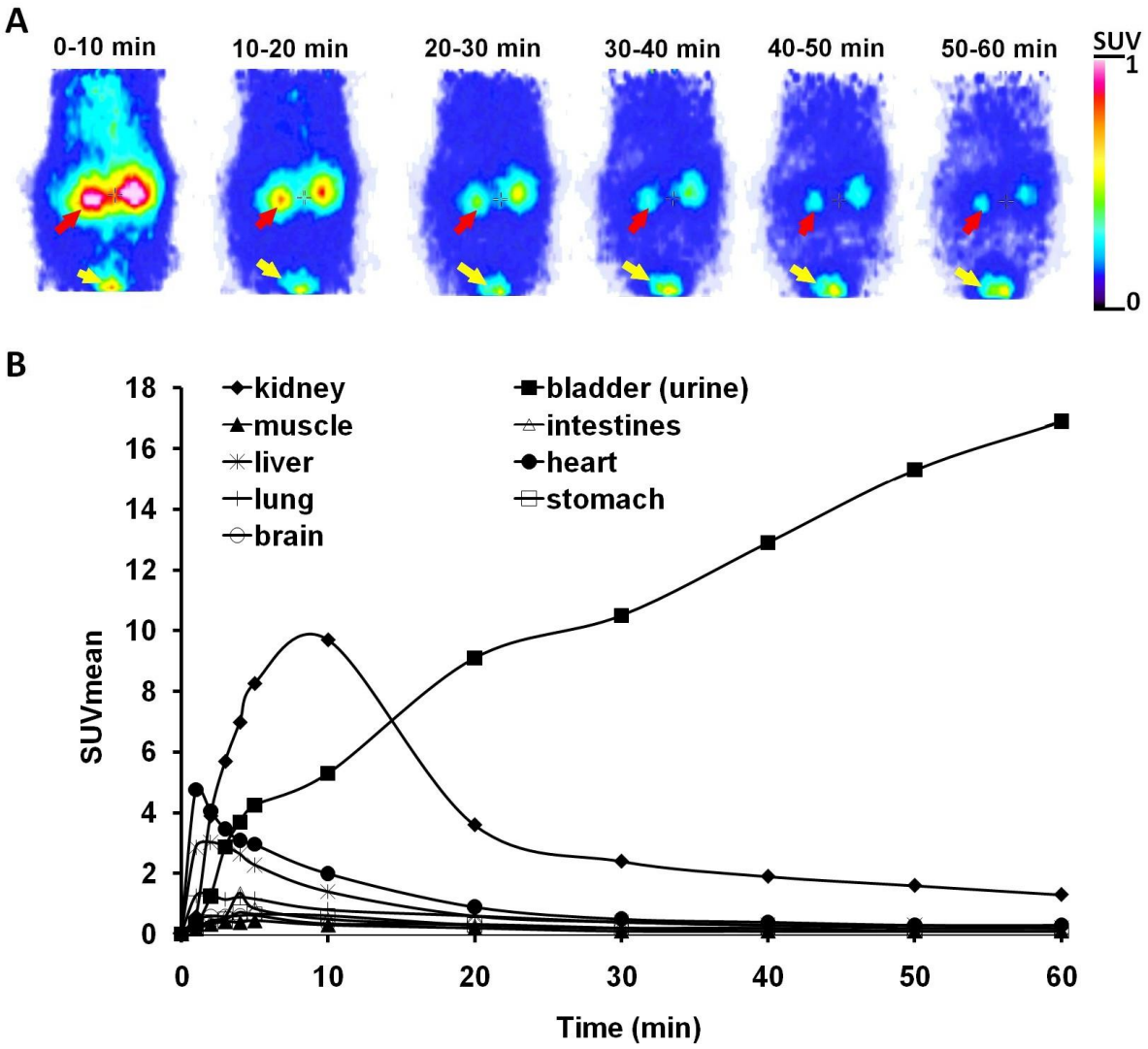
In vivo PET imaging

BALB/c mice, 6.3±0.2 MBq of ^{68}Ga-NODAGA-HPBCD i.v.



Hajdu I. et al., Eur J Pharm Sci. 2019; 128:202-208.

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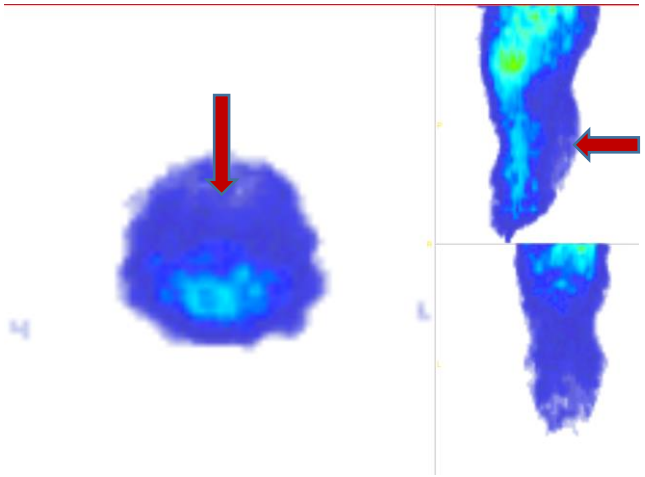
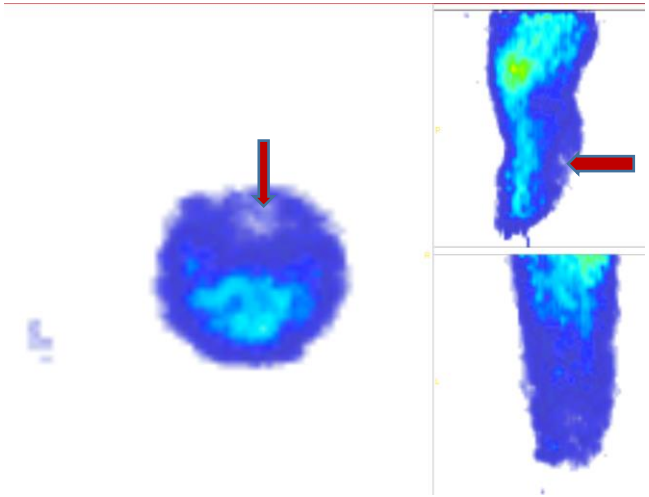
BRAIN

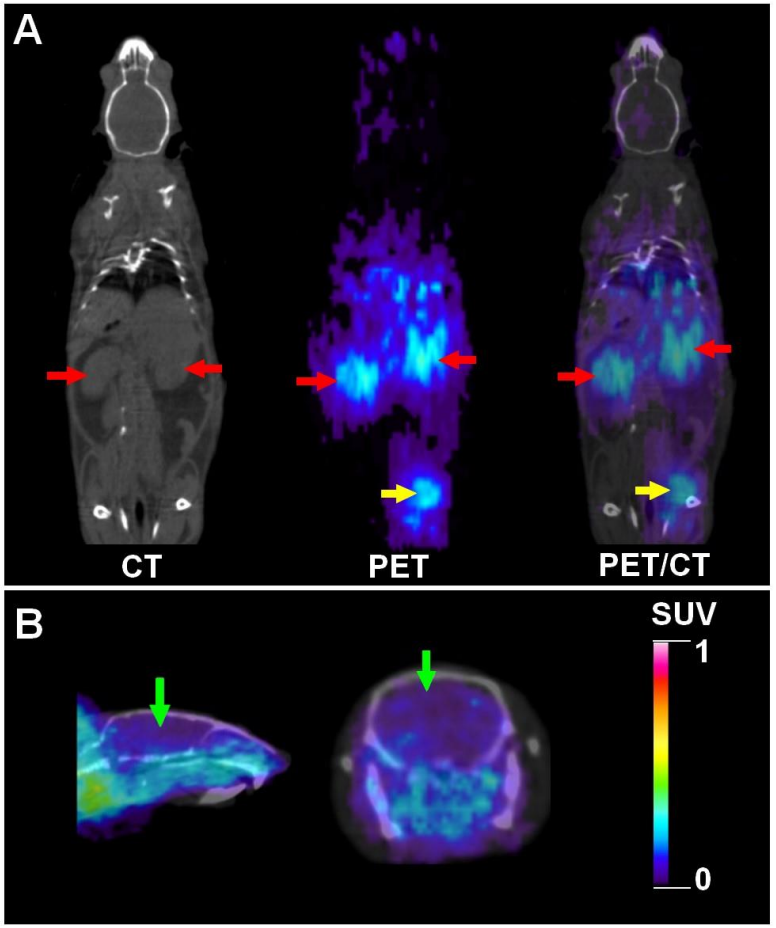
10 min post injection



15 min post injection



PET/CT 90 min



Hajdu I. et al., Eur J Pharm Sci. 2019; 128:202-208.

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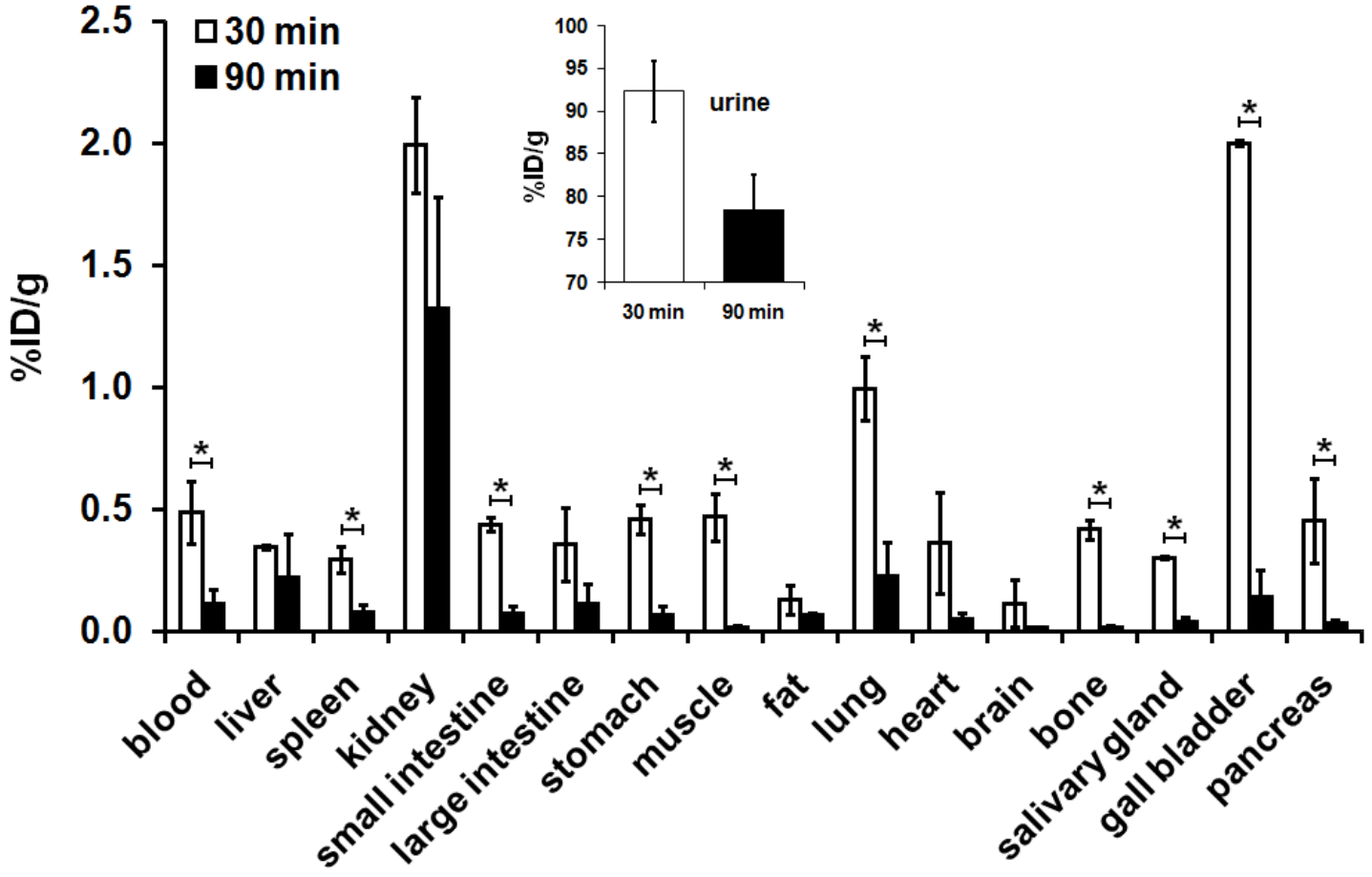
Ex vivo
biodistribution studies
NODAGA-HPBCD



Hajdu I. et al., Eur J Pharm Sci. 2019; 128:202-208.

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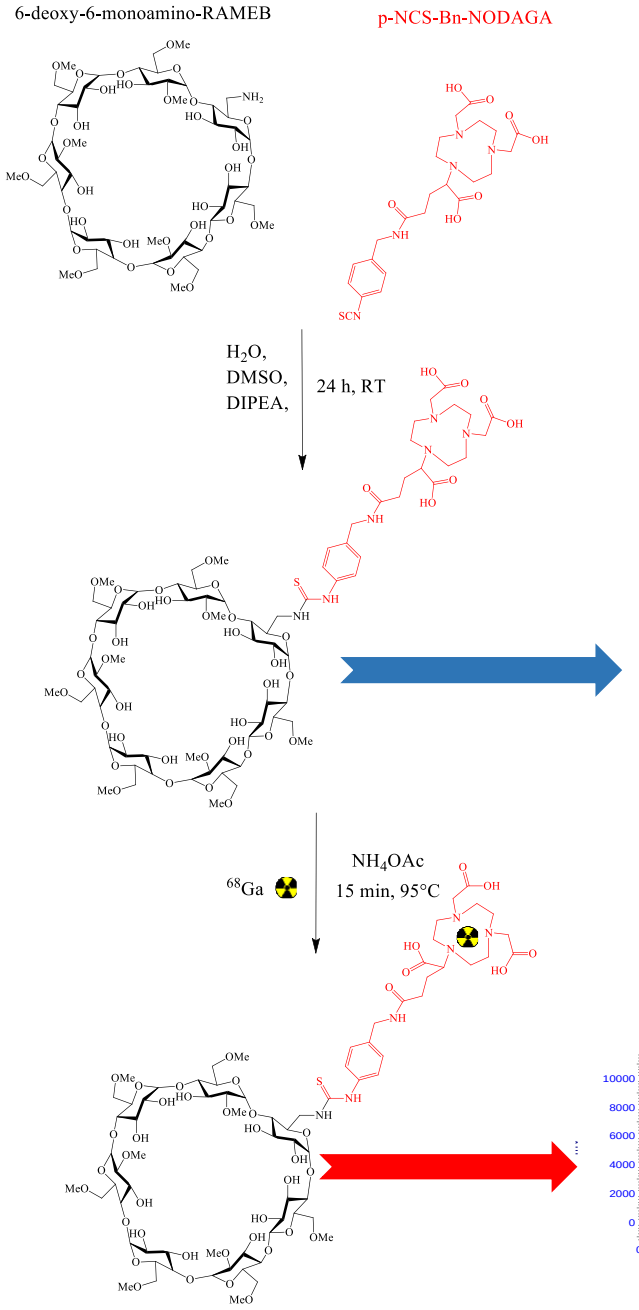
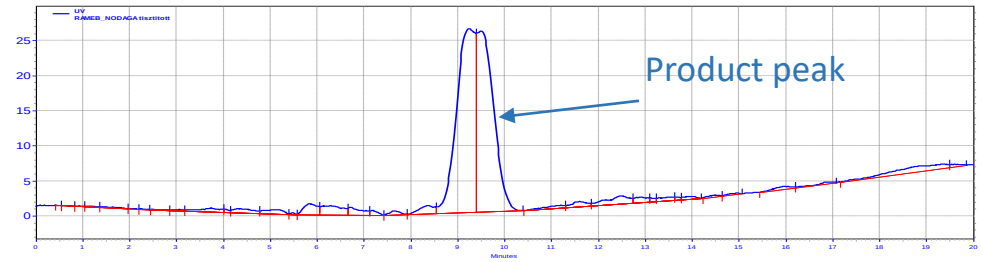
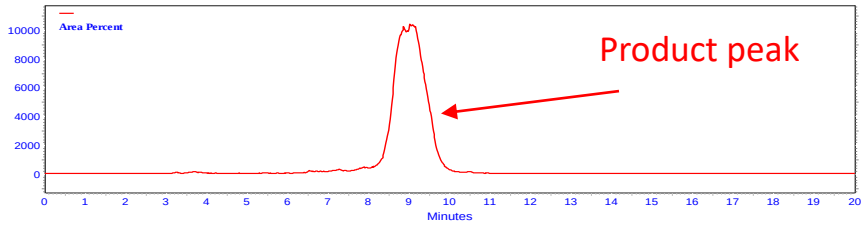
Chemical synthesis and radiolabeling of ^{68}Ga labeled **NODAGA-RAMEB**



HPLC chromatogram (UV) of HPLC purified NODAGA functionalized RAMEB (NODAGA-RAMEB)



Radio HPLC chromatogram of ^{68}Ga-labeled NODAGA-RAMEB (^{68}Ga-NODAGA-RAMEB)



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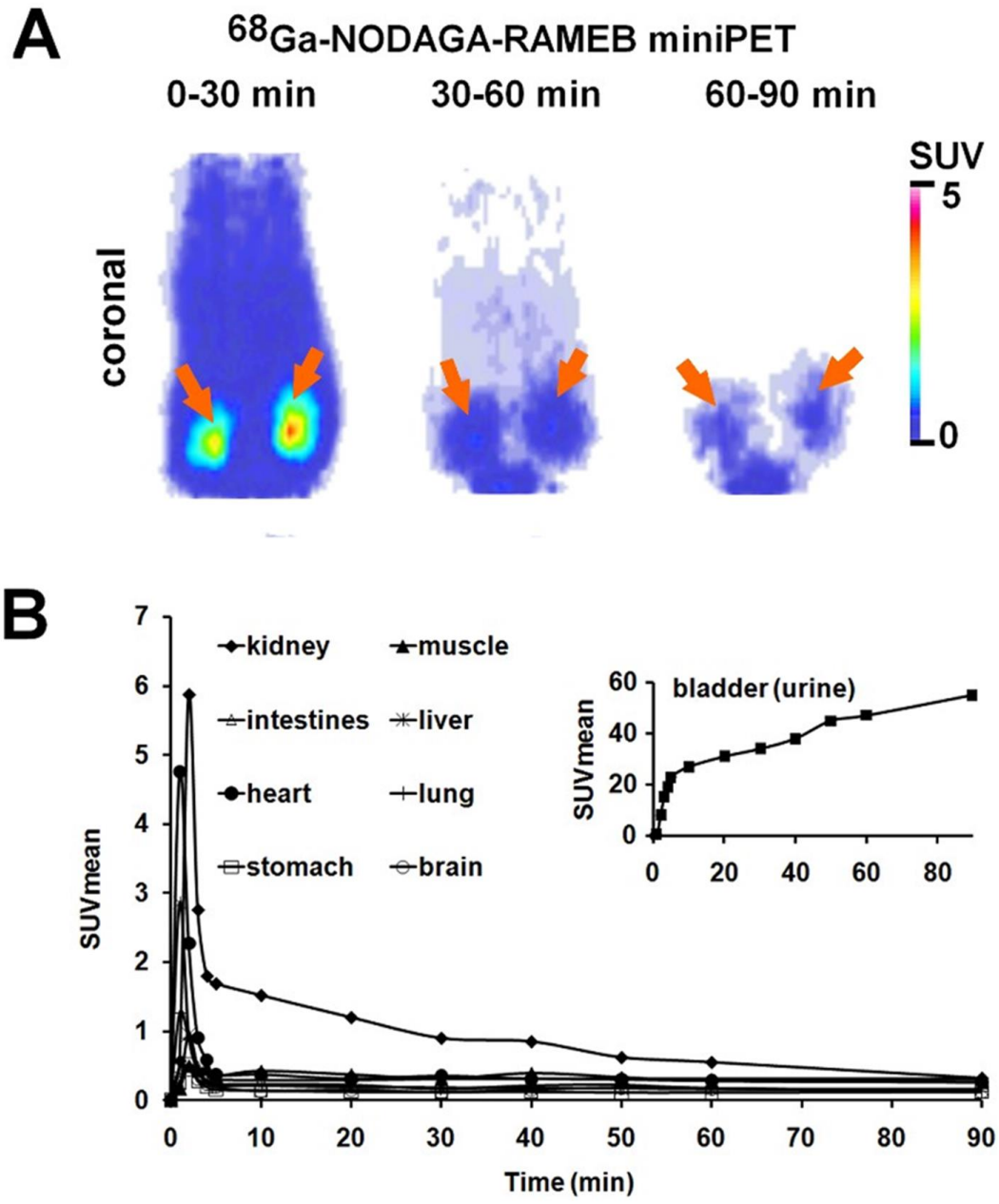

Trencsényi G. et al., Int J Pharm. 2020; 576:118954.

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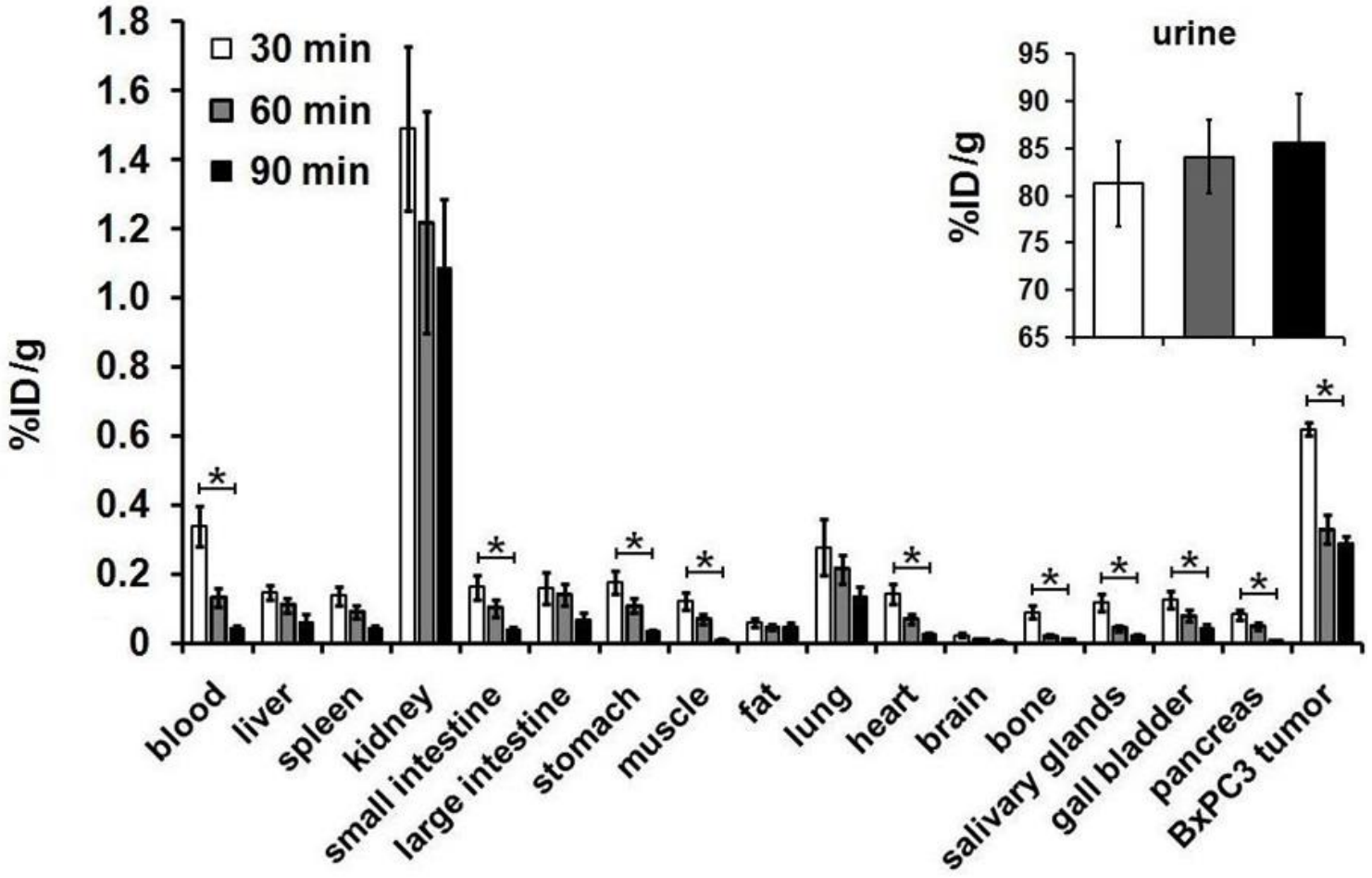
Ex vivo biodistribution - ^{68}Ga-NODAGA-RAMEB



Trencsényi G. et al., Int J Pharm. 2020; 576:118954.

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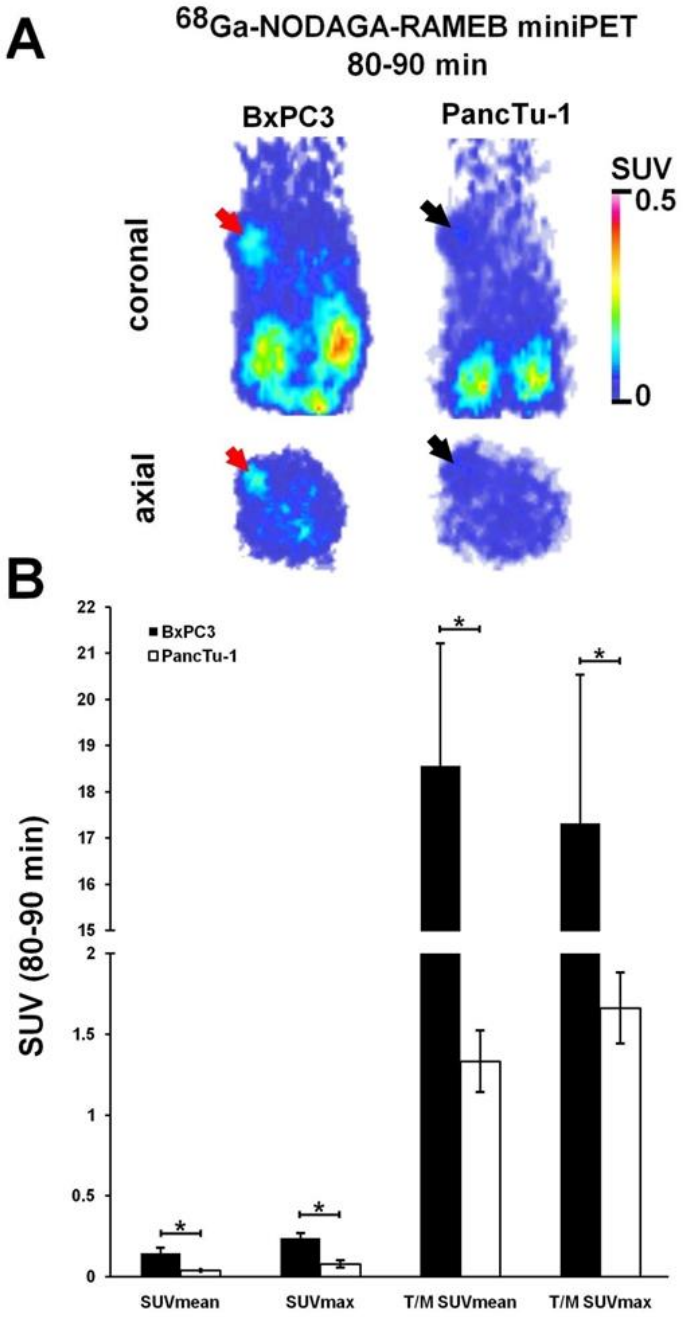
BxPC3
High expression of Prostaglandin E2 Receptor (EP2)



PancTu-1
Low expression of Prostaglandin E2 Receptor (EP2)

SUV - standardized uptake value
T/M - tumor-to-muscle (background) ratio

Trencsényi G. et al., Int J Pharm. 2020; 576:118954.

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Conclusions

- Cyclodextrins interact with biomolecules at different levels of biological systems
- Cyclodextrins enter the cytoplasm of different cell lines by endocytosis
- ^{68}Ga-labeled NODAGA-HPBCD and RAMEB have fast elimination and limited tissue distribution
- Unexpected distribution in the lung- but in accordance with toxicity data
- No uptake to the brain after i.v. administration
- Unexpected ^{68}Ga-labeled NODAGA-RAMEB tumor accumulation in PGE2 positive tumors

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Thank you for your kind attention!

Acknowledgments

- Cyclolab Ltd. Budapest, Hungary
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