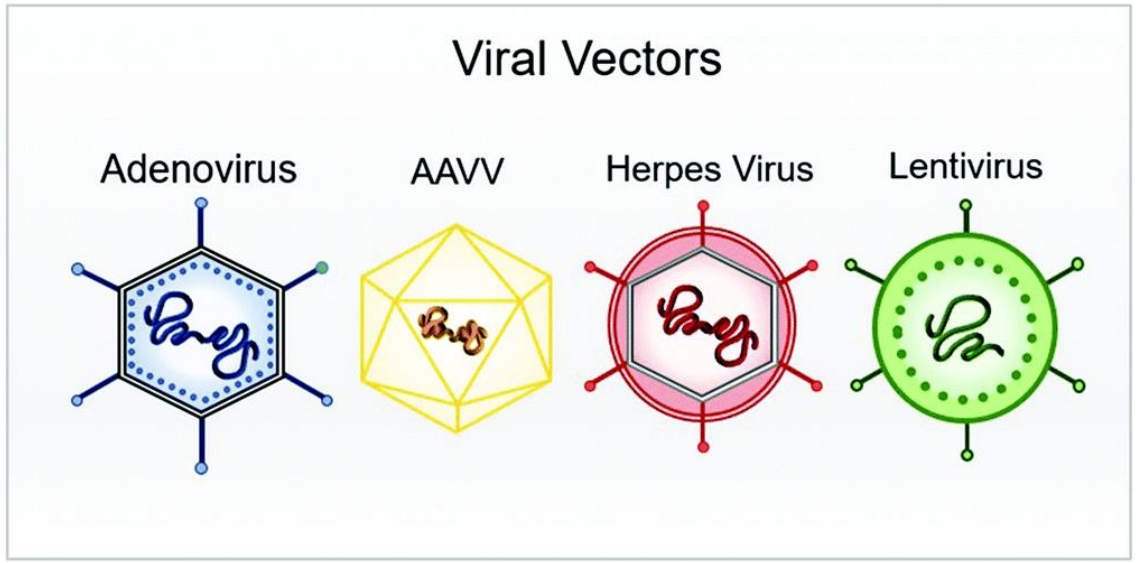
CARBOHYDE
REVOLUTION OF
CYCLODEXTRINS

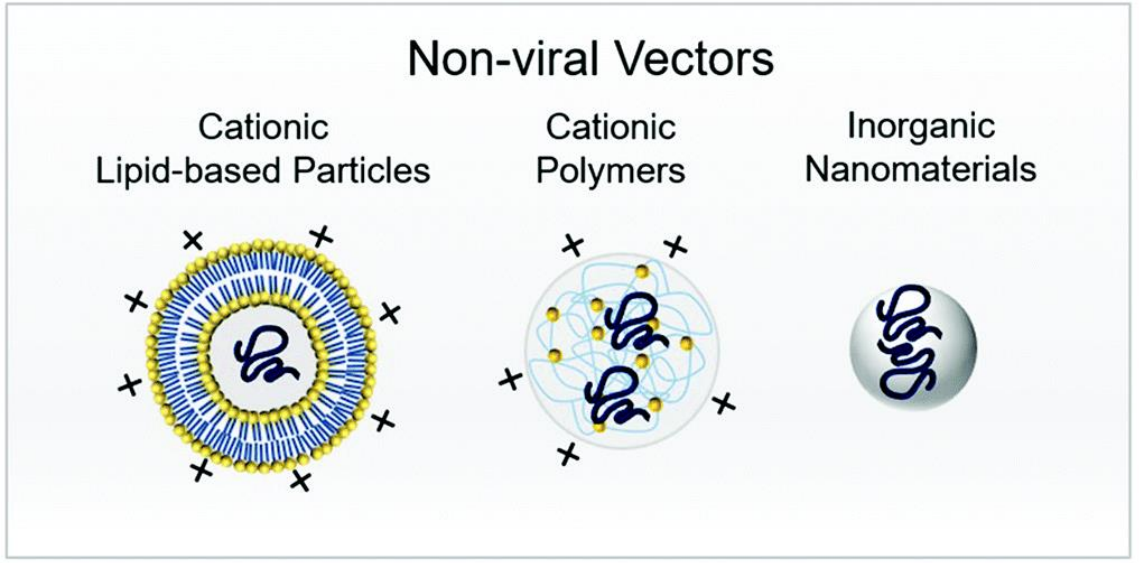
Cyclodextrins as Gene Delivery Systems

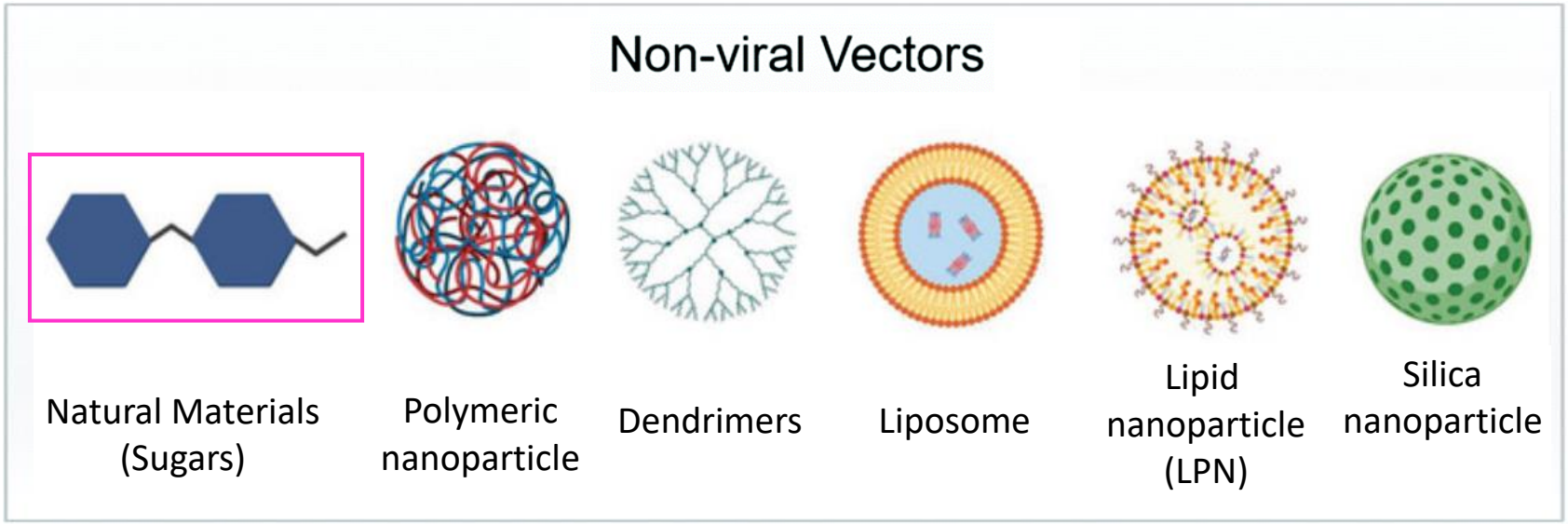
OUTLINES

- General concepts
- Cationic Polymers
- CDs as Pure Vectors
- Conclusions

Gene Delivery Systems





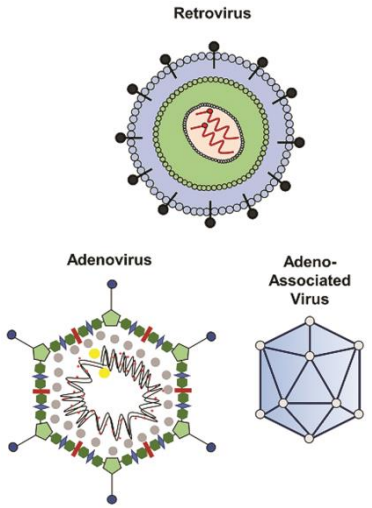


R. M. Haley, Drug Delivery and Translational Research, 10, 661-677 (2020)

Overcoming Limitations

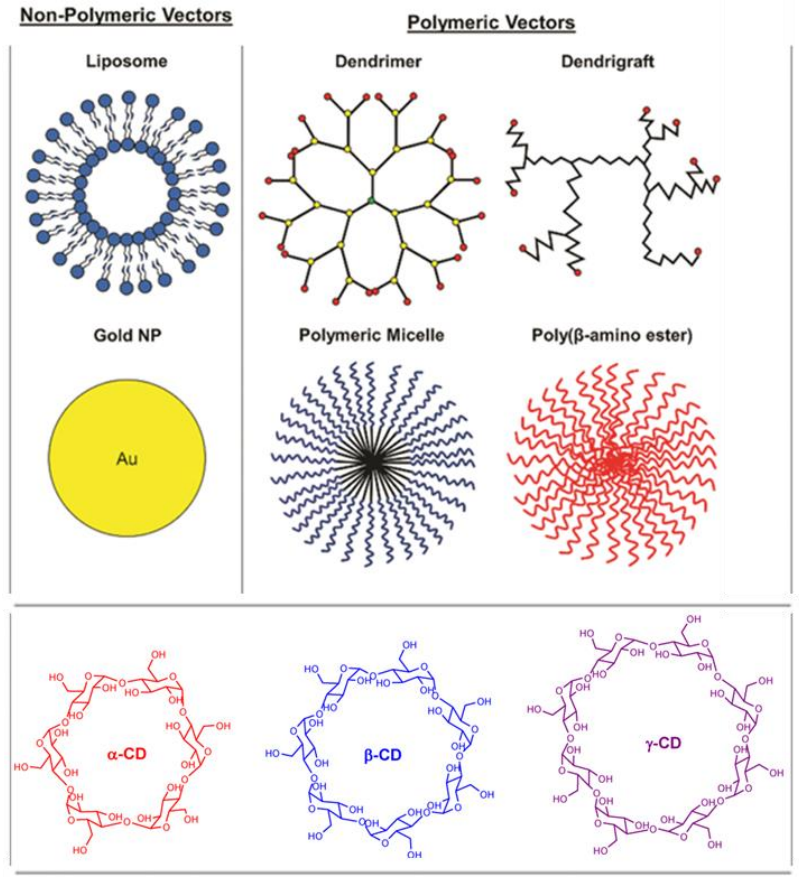
Viral vectors

- High immunogenicity
- Pre-existing immunity
- Limited cargo Capacity
- Safety concerns (viral replication, insertional mutagenesis)
- High cost
- Inhibited by some drugs (anti-coagulants)



Non-viral vectors (*Pro*)

- Better biocompatibility
- Lower immunogenicity
- Lower risk of pre-existing immunity
- Large payload capacity
- Safer (no viral replication, no insertional mutagenesis)
- Greater versatility



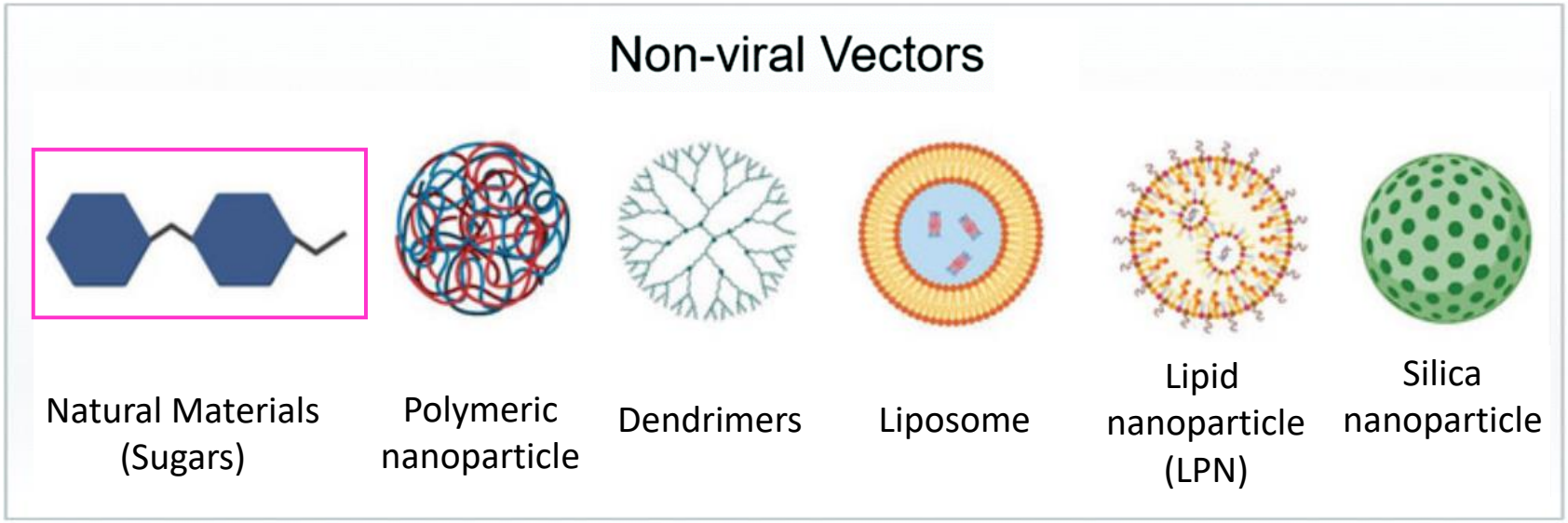
Non-viral vectors (*Contra*)

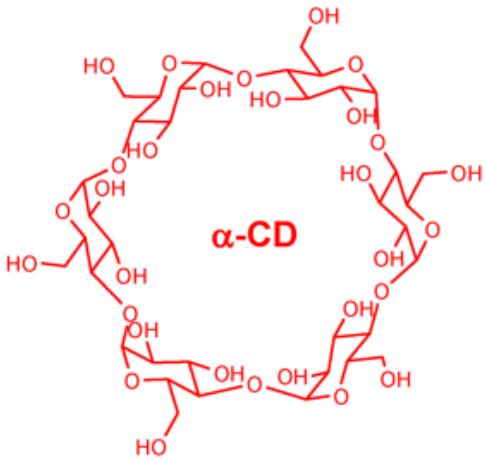
- Low transfection efficiency
- Still exhibit toxicity or immunogenicity
- Issues with reproducibility
- Shorter duration of gene expression
- Poor in-vivo stability

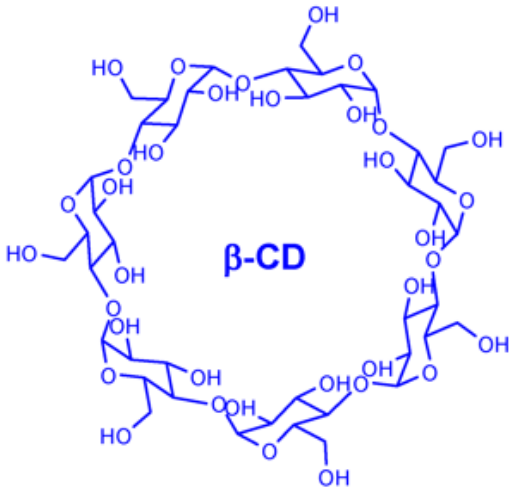
B. Caffery, et al., Nanomaterials 2019, 9(1), 105

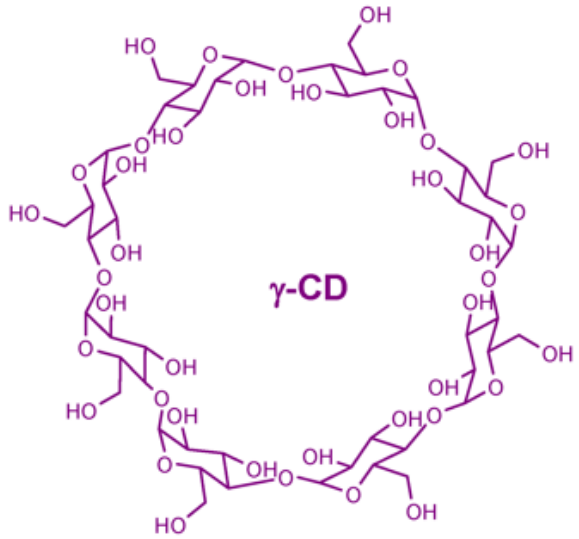
R. M. Haley, Drug Delivery and Translational Research, 10, 2020, 661-677

Why CDs?





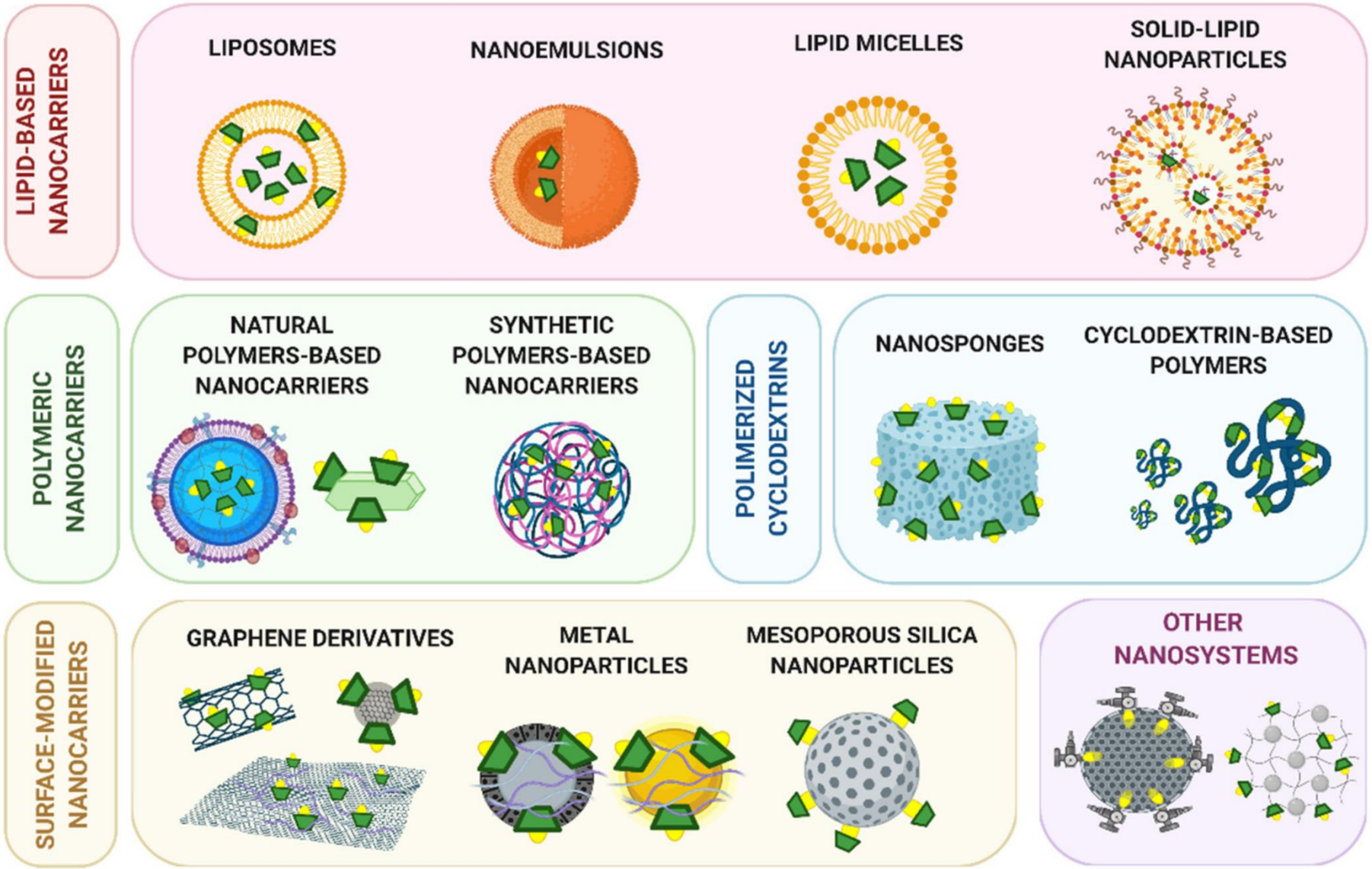




E. W. Kavanagh, et al., Advance Health Care materials, 11, 7, 2022, 2102145

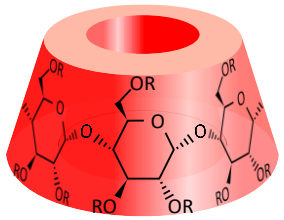
CD-modified Nanomaterials for Drug Delivery

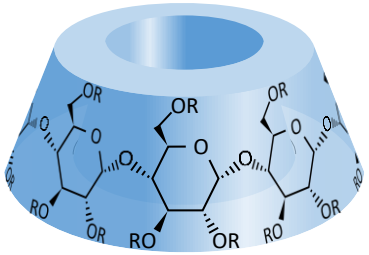
VERSATILITY

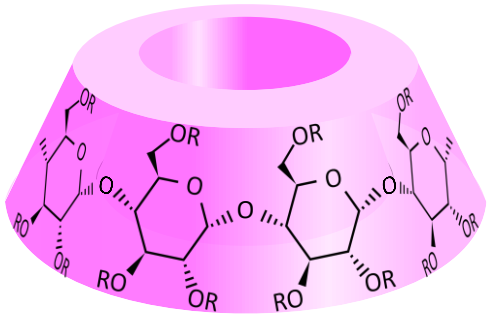


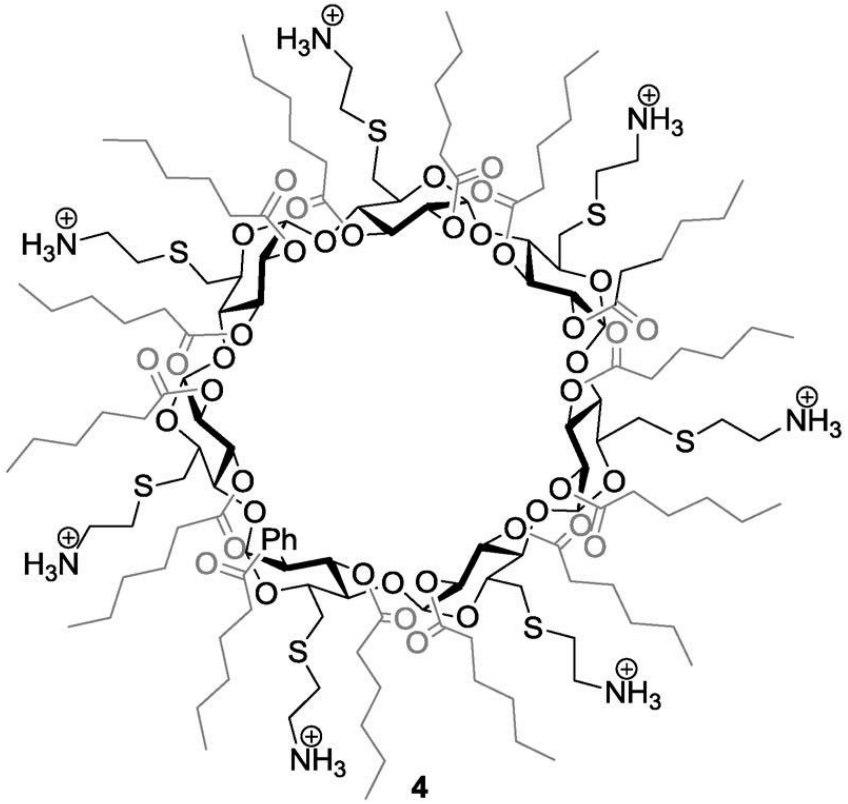
D. Andres Real, et al., Pharmaceutics 2021, 13(12), 2131

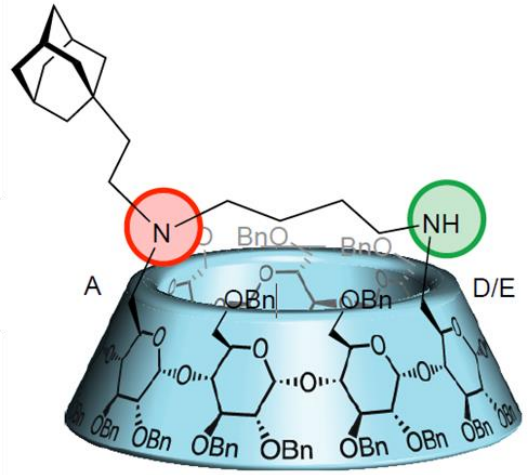
What about Safety of CDs?

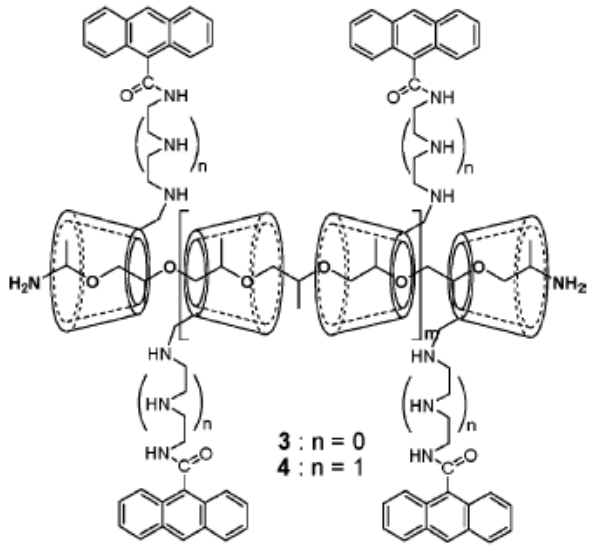






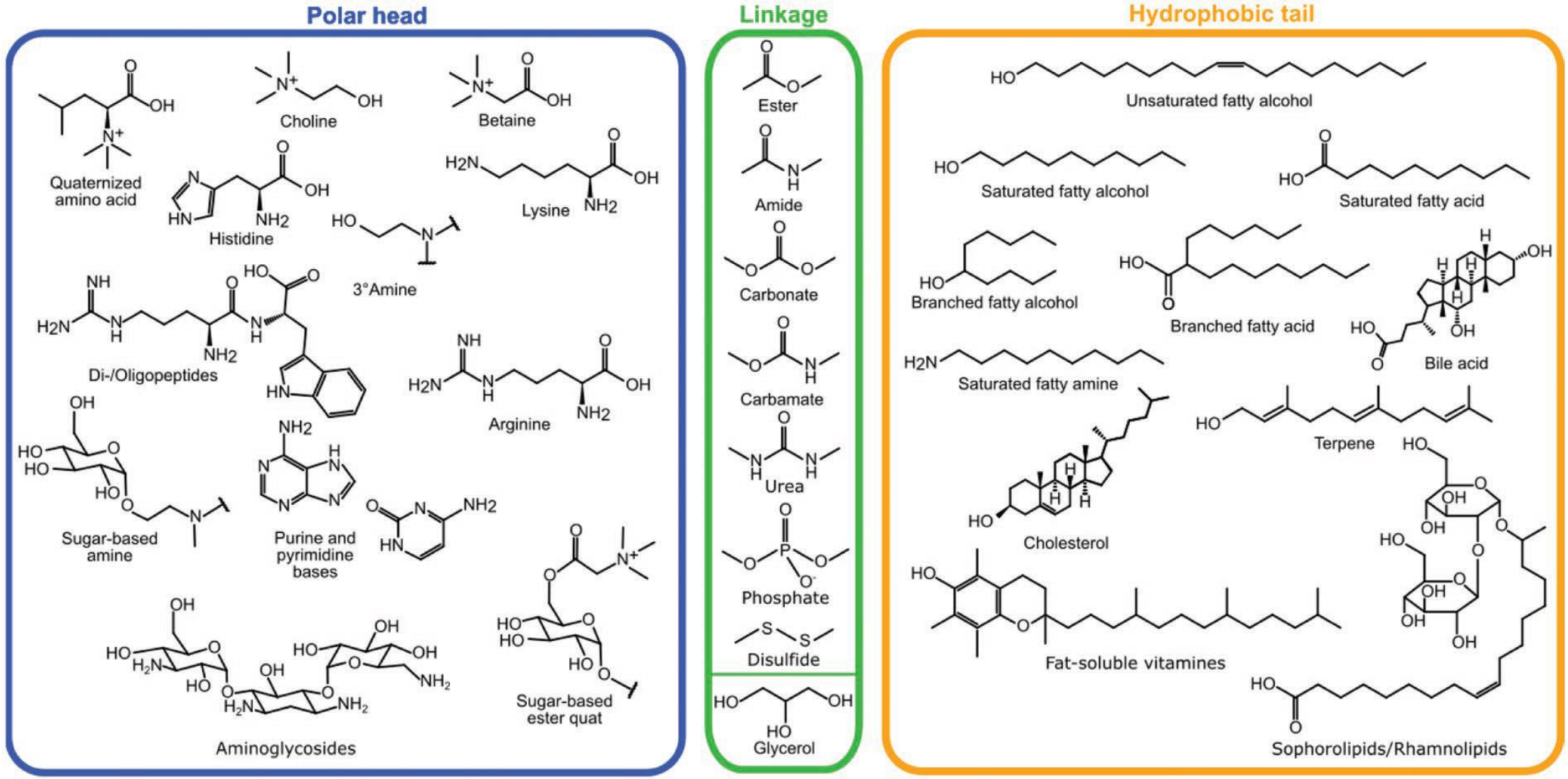






Cyclodextrins are naturally occurring compounds derived from starch and are generally considered biocompatible and safe

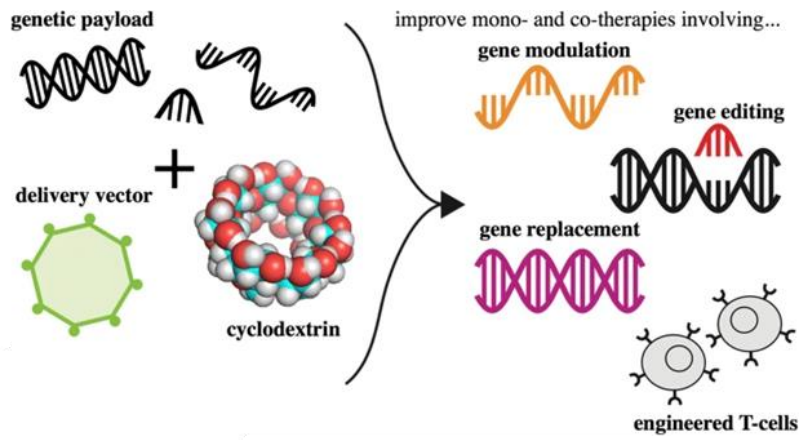
Roadmap for Safer Pharmaceutical Excipients



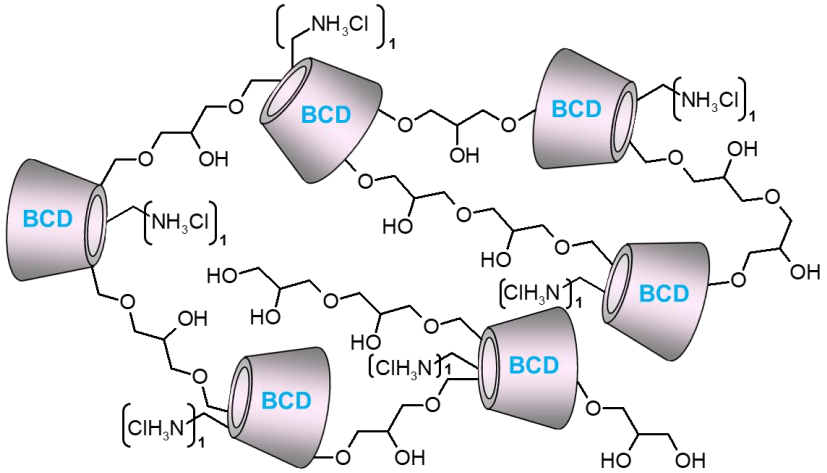
A. M. Jörgensen, et al., Small, 2023, 2206968, 1-24

CDs in Gene Therapy Applications

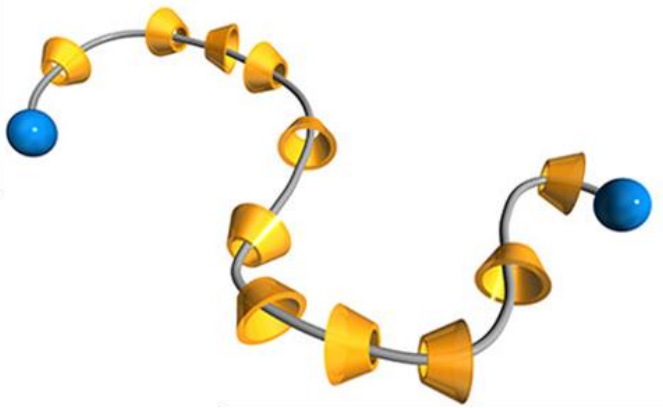
CDs as Formulation Enhancers



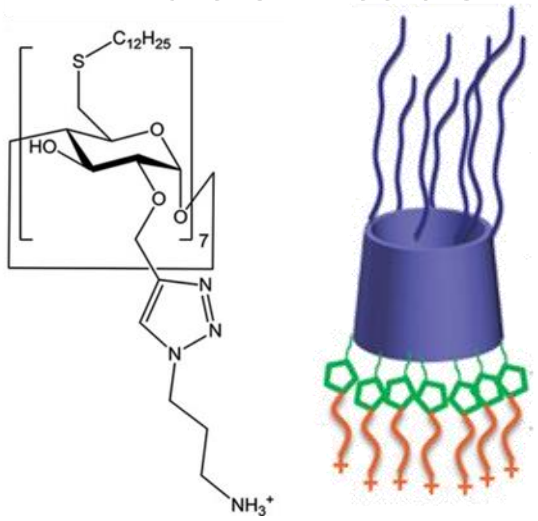
Cationic Polymers



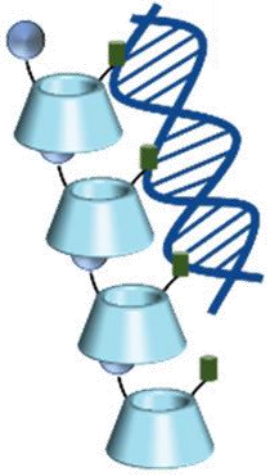
Polyrotaxanes



Pure CD vectors



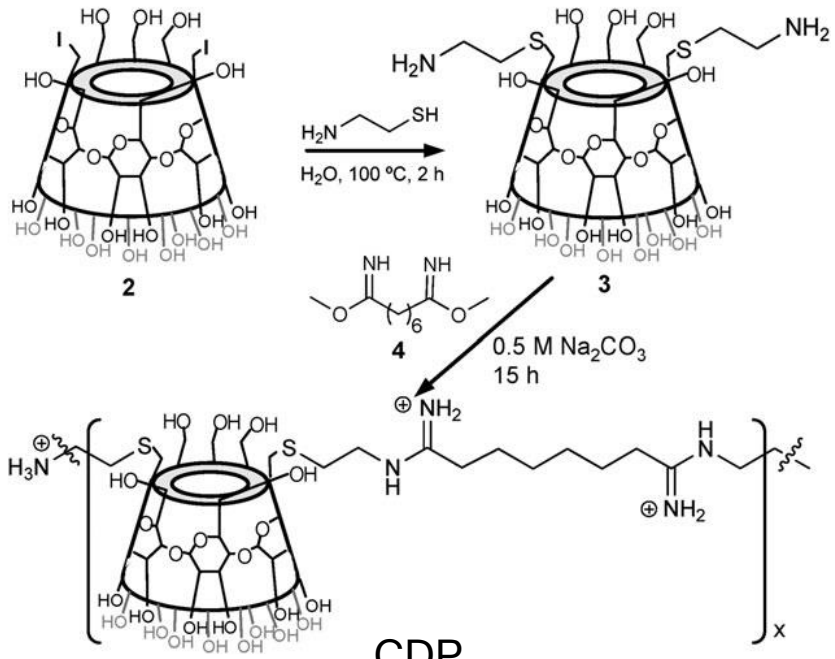
CD for supramolecular assembly



OUTLINES

- General concepts
- Cationic Polymers
- CDs as Pure Vectors
- Conclusions

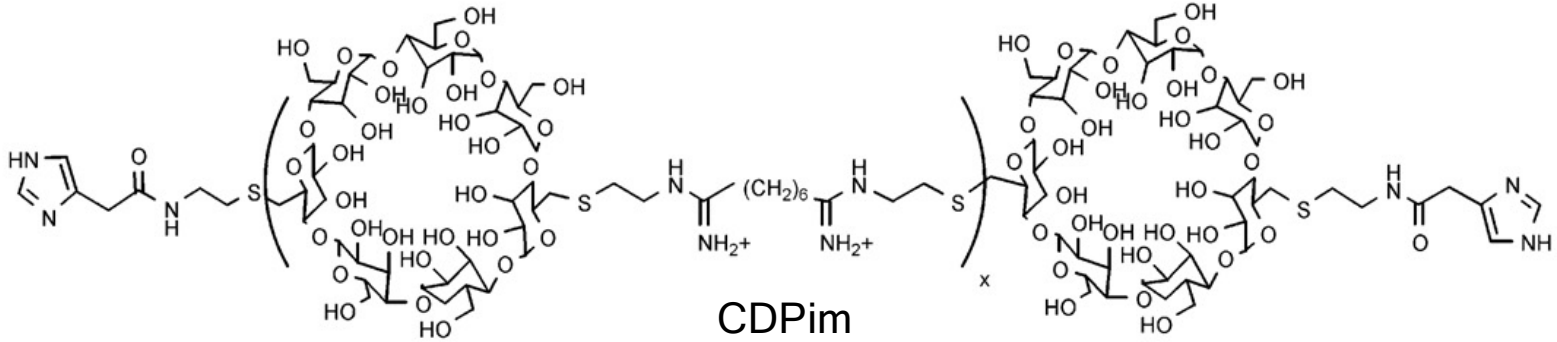
Cationic Polymers



CDP

Nanometric polyCDplexes (100-150 nm) with in vitro cell transfection efficiency comparable to polyethyleneimine (PEI) and Lipofectamine, but lower toxicity.

Structure-activity relationship: low molecular weight polymers (ca. 10 kDa, degree of polymerization, DP 5-8), CD units sufficiently spaced from amidine cationic centers.

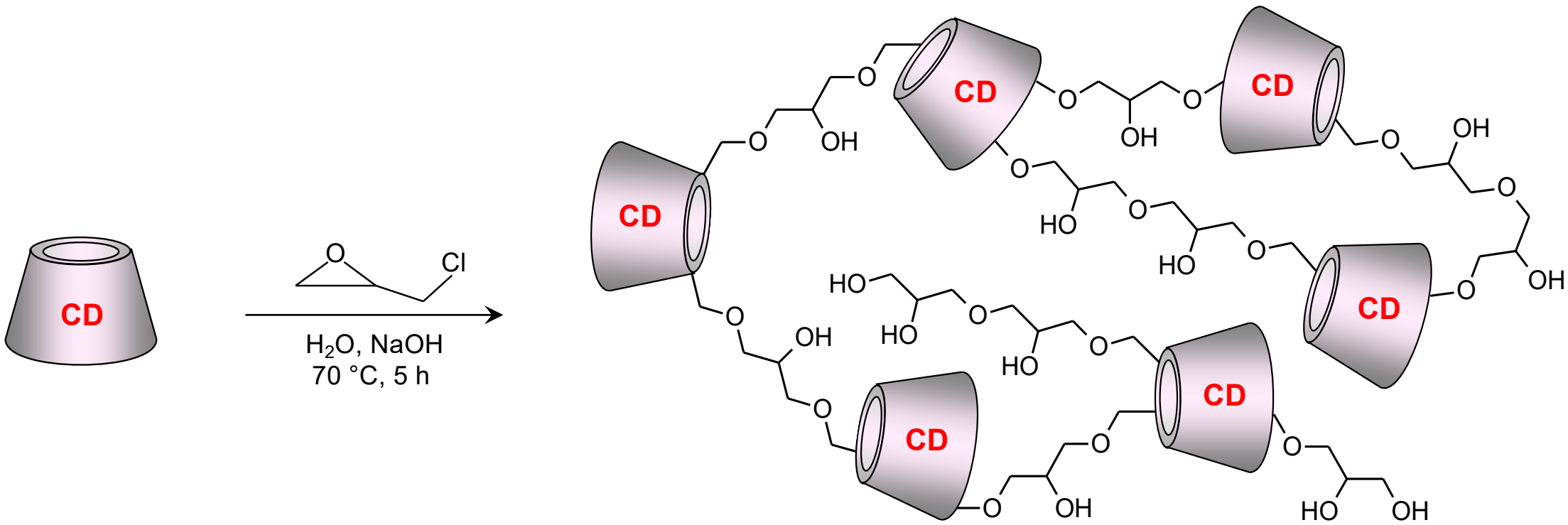


CDPim

Significant improvement in delivery efficiency by modifying the polymer endings with imidazole groups. Imidazole moieties should impart buffering capacity to CDP-gene polyCDplexes that would prove instrumental for efficient endosomal release by virtue of the ‘‘proton sponge’’ effect.

S. Mishra et al., Journal of Controlled Release 116 (2006) 179-191

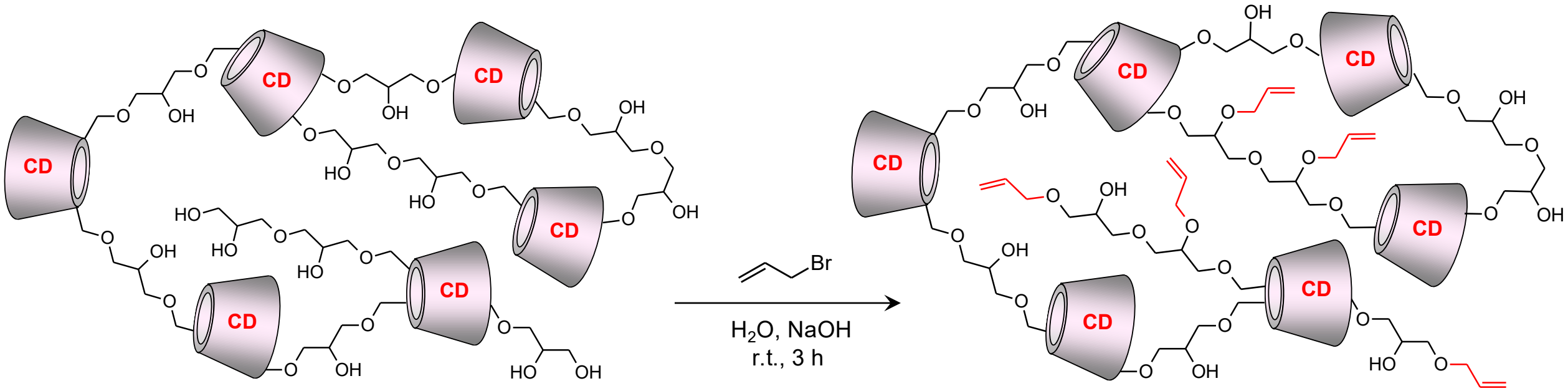
Epichlorohydrin Cross-linked CD Polymers



- Controlled molecular weight
- Established degree of crosslinking
- Highly biocompatible material
- Low toxicity
- Useful for combination therapy

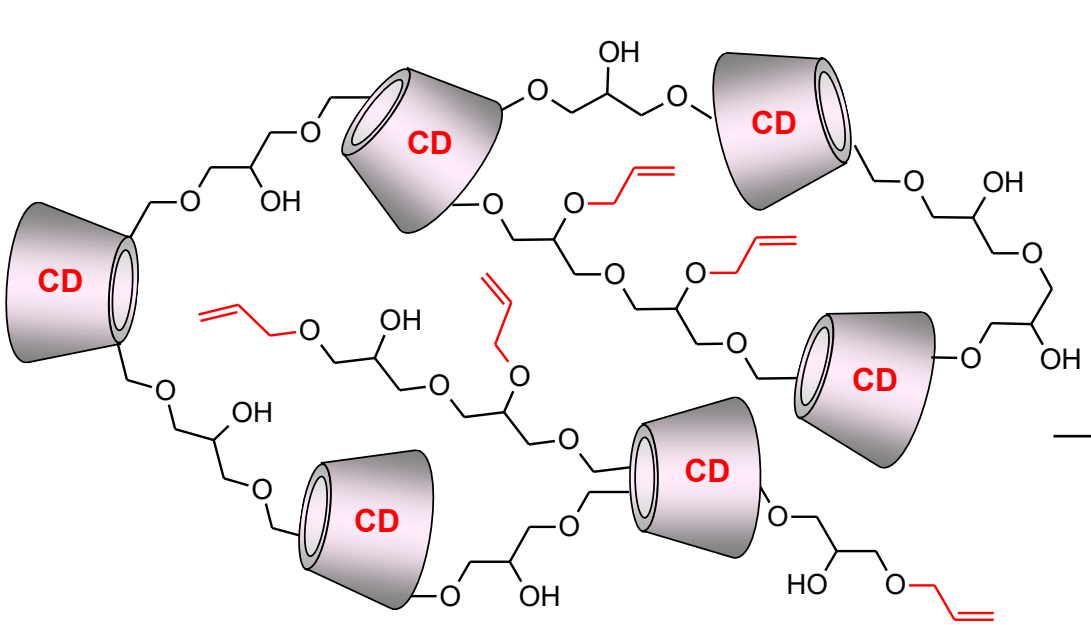
- Immunogenicity to be assessed
- Reduced cyclodextrin functionality
- Characterization is challenging
- Environmental impact

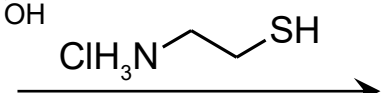
Partially Allylated CD-based Polymers

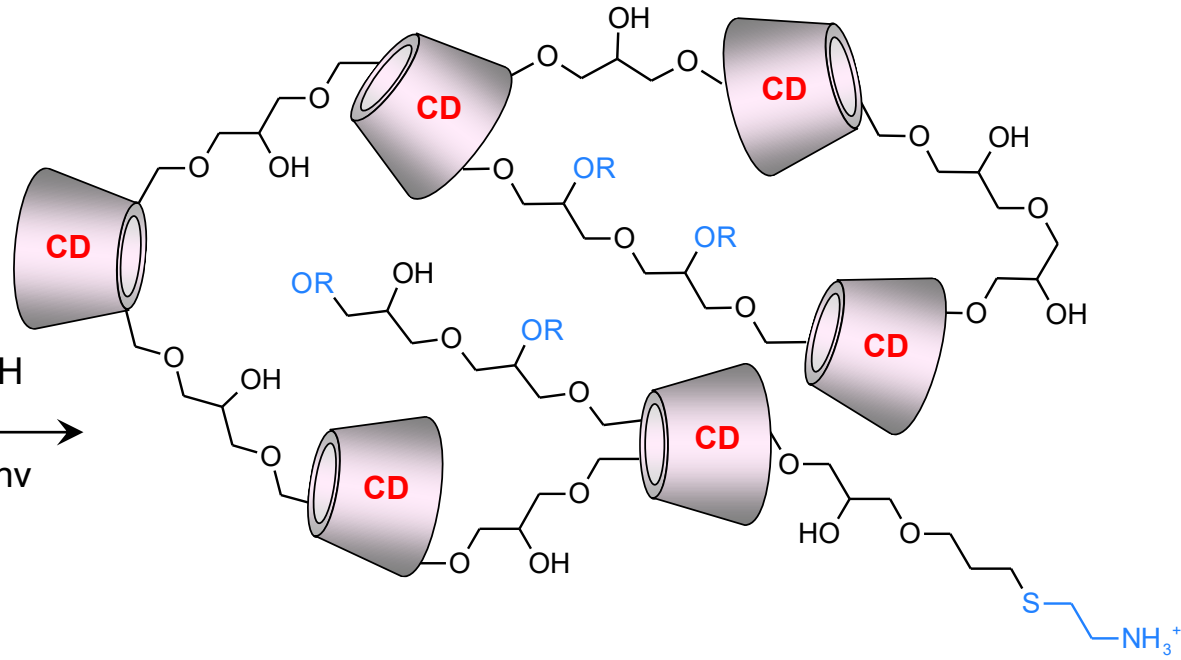


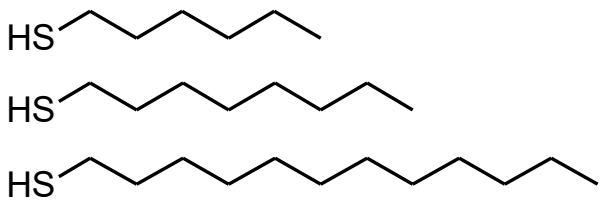
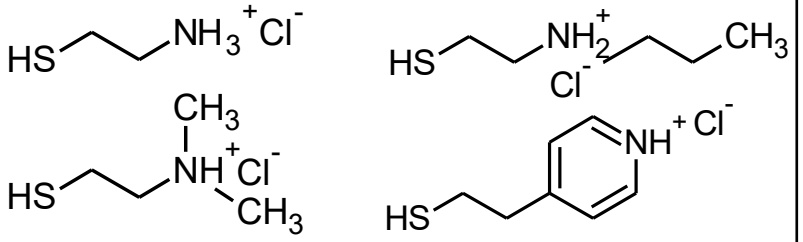
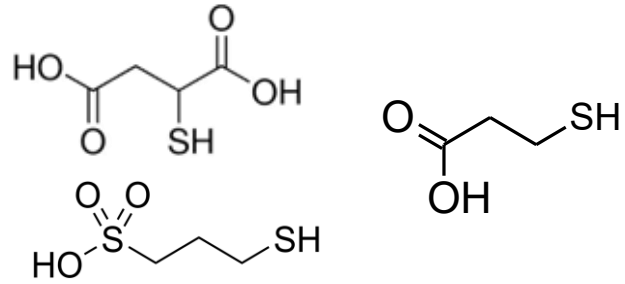
- Random derivatization
- Controlled degree of substitution
- Versatile intermediate

Ionic / amphiphilic CD-based Polymers

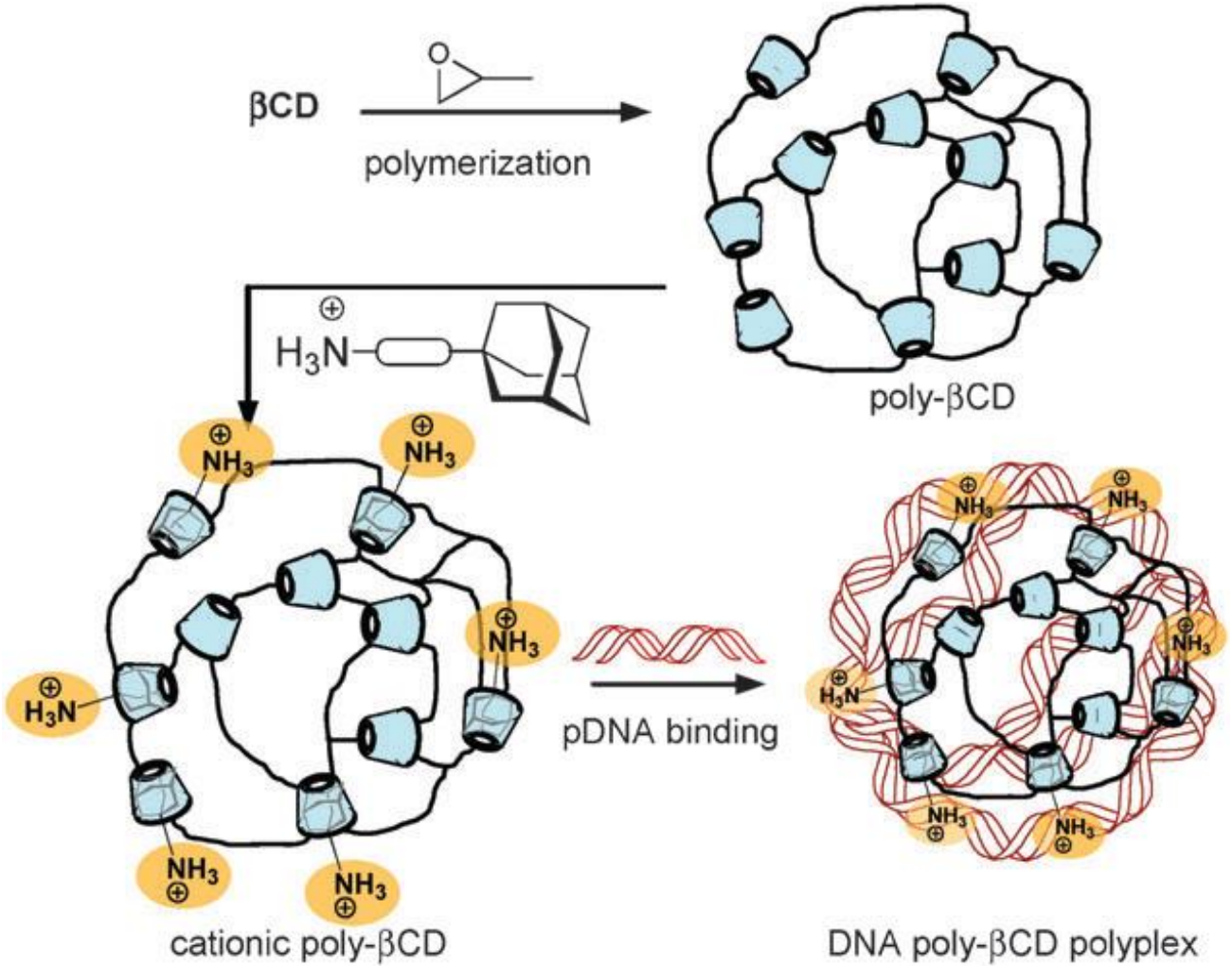


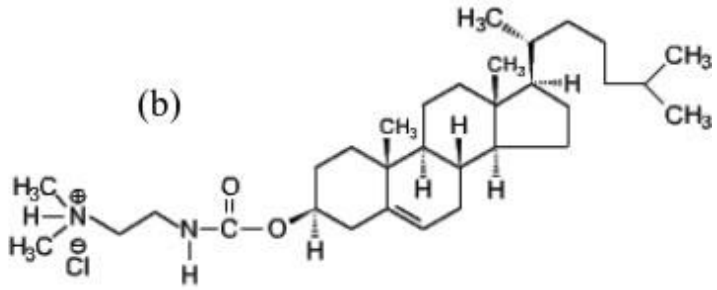




Apolar	Cationic	Anionic
		

Cationic Polymers

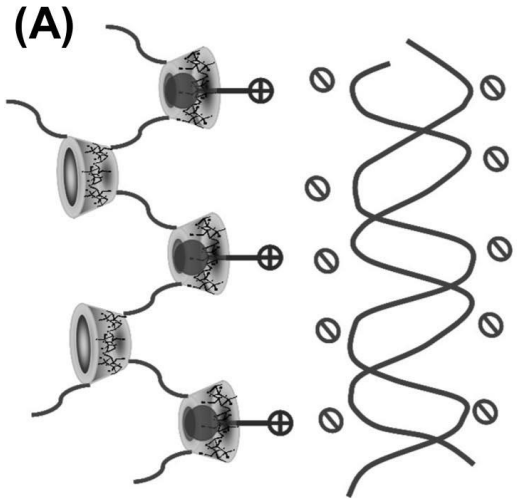


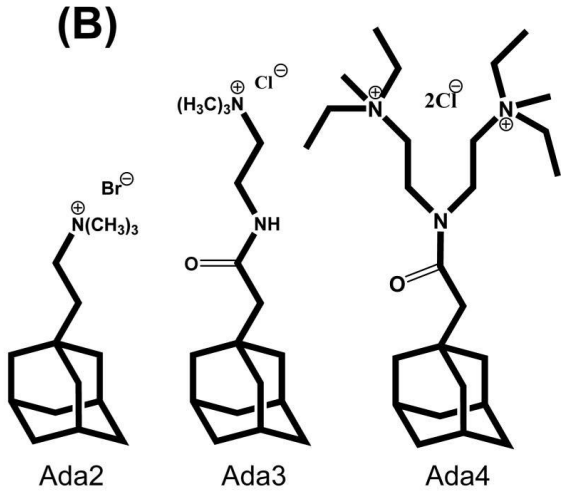


Neutral epichlorohydrin-cross-linked BCD polymer and cationic compounds having high affinity for the BCD cavity (e.g. positively charged adamantane or cholesterol derivatives)

C. O. Mellet, et al., Chem. Soc. Rev., 2011, 40, 1586-1608

Cationic Polymers



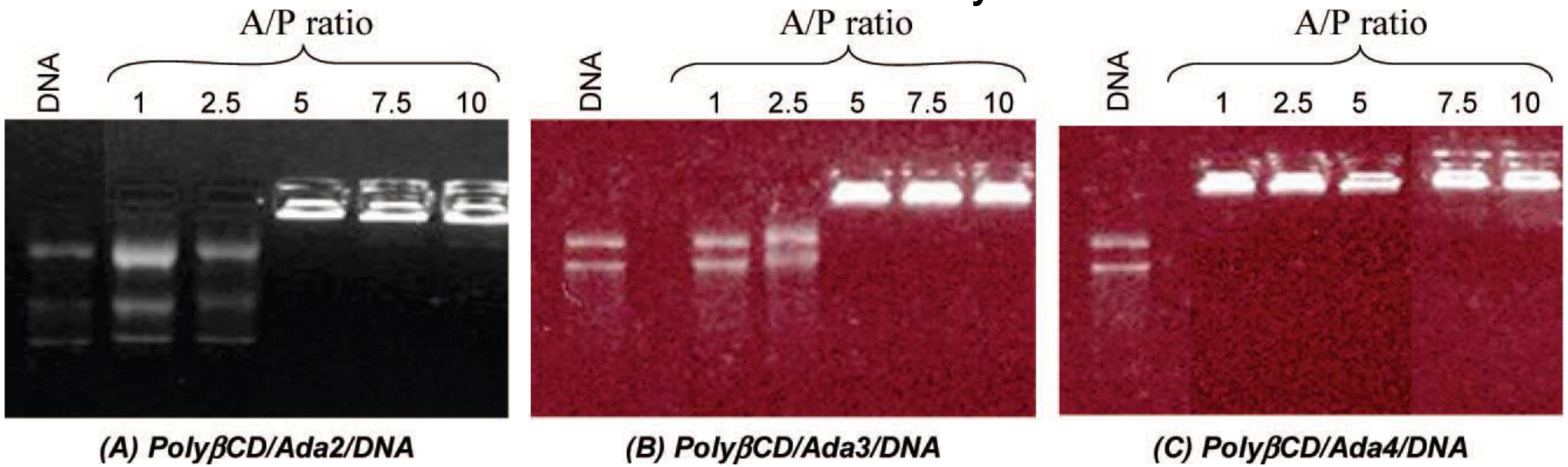


Fluorescence measurements

Table 1. Logarithm of the Binding Constants (Determined with a SD of 10%) for Different Cyclodextrin Derivatives (βCD, HPβCD, and polyβCD) to the Adamantyl Connectors (Ada2, Ada3, and Ada4) and Their Variation with the Addition of NaCl

	Ada2	Ada3	Ada4
$\log(K_{\beta CD})$	4.95	5.56	4.89
$\log(K_{HP\beta CD})$	3.95	3.98	3.18
+ NaCl 150 mM	*4.04*		*3.11*
$\log(K^{app}{}_{poly\beta CD})$	3.04	2.85	2.18
+ NaCl 150 mM	*3.72*		

DNA retardation assay

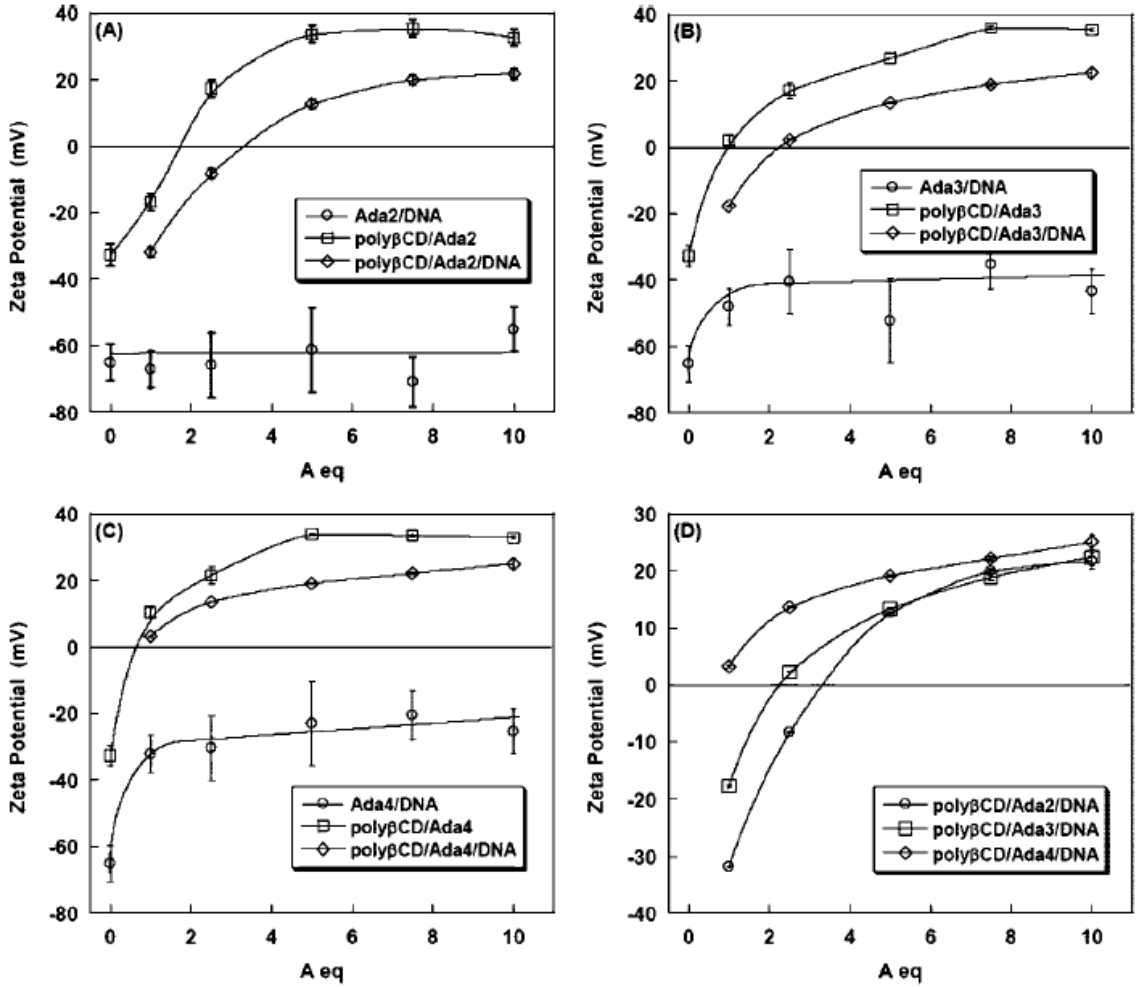


(A) PolyβCD/Ada2/DNA *(B) PolyβCD/Ada3/DNA* *(C) PolyβCD/Ada4/DNA*

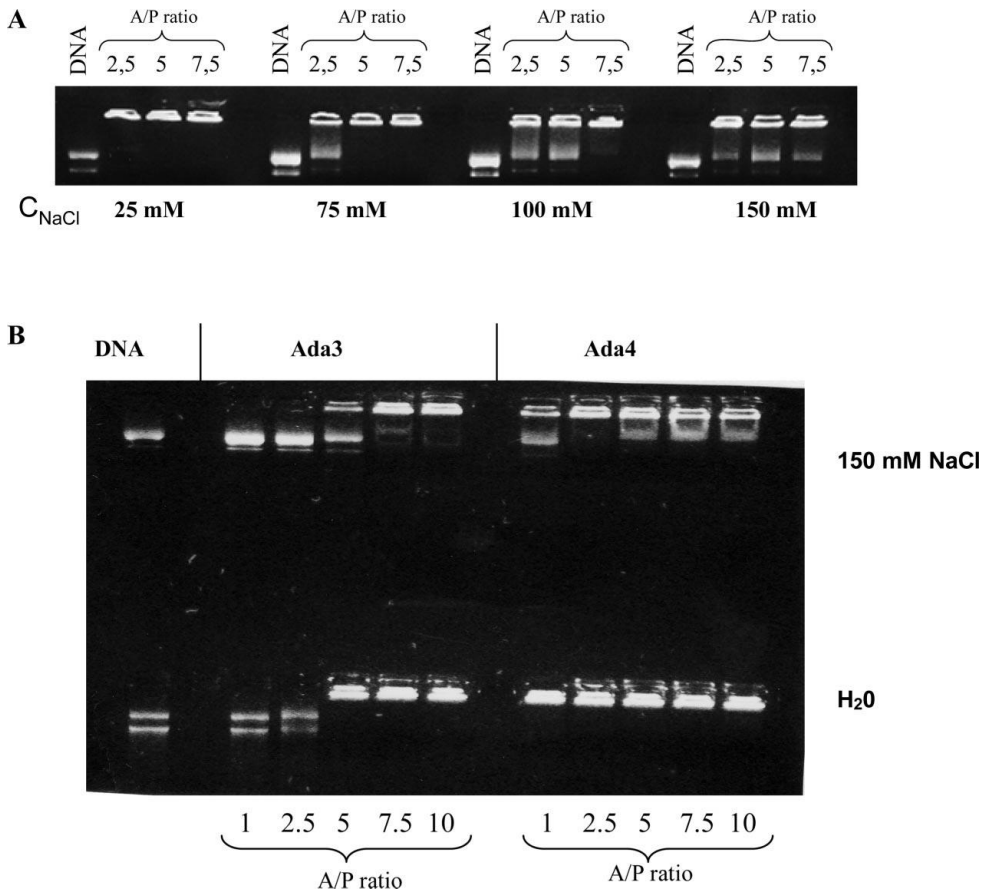
V. Burckbuchler, et al., Bioconjugate Chem. 2008, 19, 2311-2320

Cationic Polymers

Zeta Potential



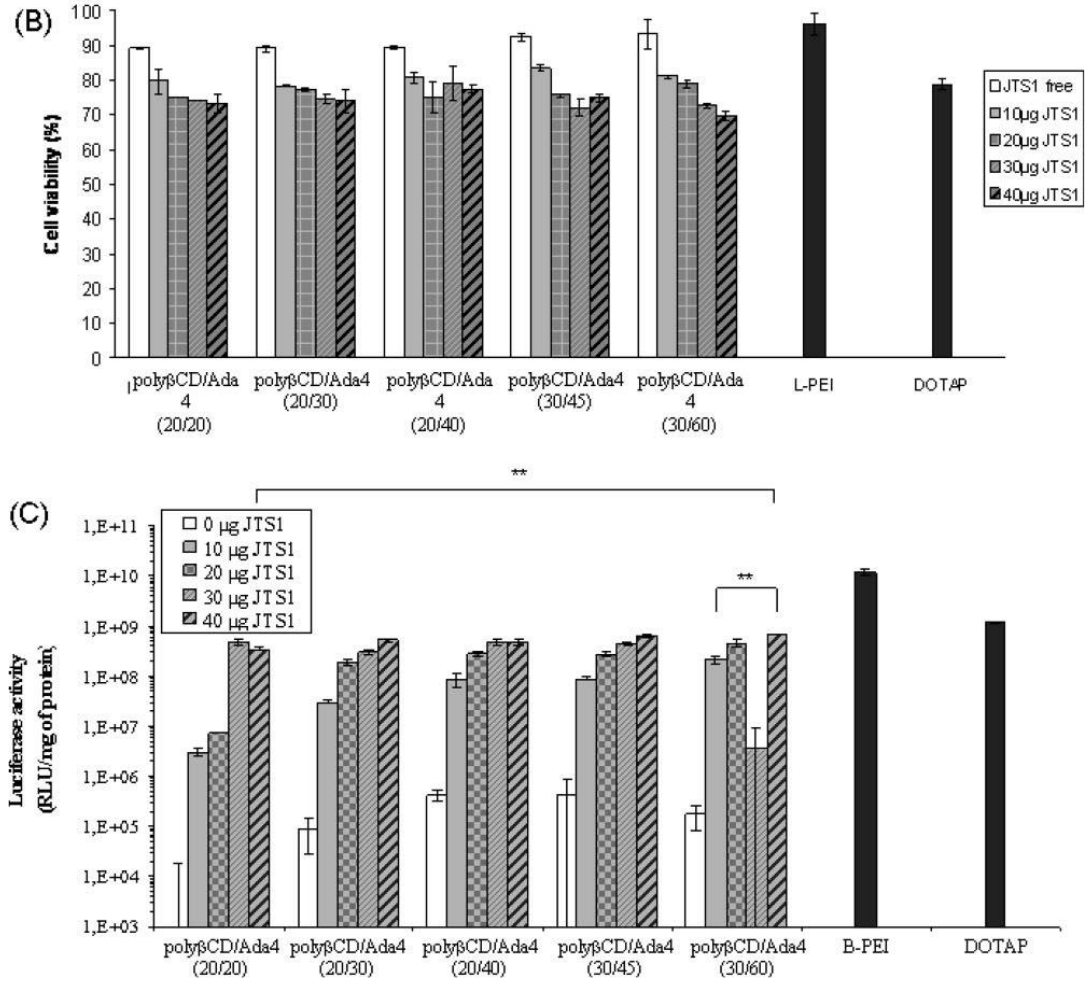
Salt Effect of Polyplexes stability



V. Burckbuchler, et al., Bioconjugate Chem. 2008, 19, 2311-2320

Cationic Polymers

In-vitro Transfection and Cytotoxicity



- Synthesis minimized
- Very versatile approach
- Easier DNA-complexation tunability
- Further properties can be included (apolar chains, targeting moieties, etc.)

- Cavities accessibility
- Low endosomal escape
- Immunogenicity and toxicity concerns

V. Burckbuchler, et al., Bioconjugate Chem. 2008, 19, 2311-2320

Cationic Polymers – Conclusions

- Highly customizable, both in size and the type of genetic material
- Polyplexes hold and protect the genetic material during delivery
- They can also be engineered to have long shelf lives
- By proton sponge effect, polyplex escapes endosome and deliver its genetic cargo within the cell
- Useful for combination therapy

- Low uptake and efficiency
- Toxicity and immunogenicity

OUTLINES

- General concepts
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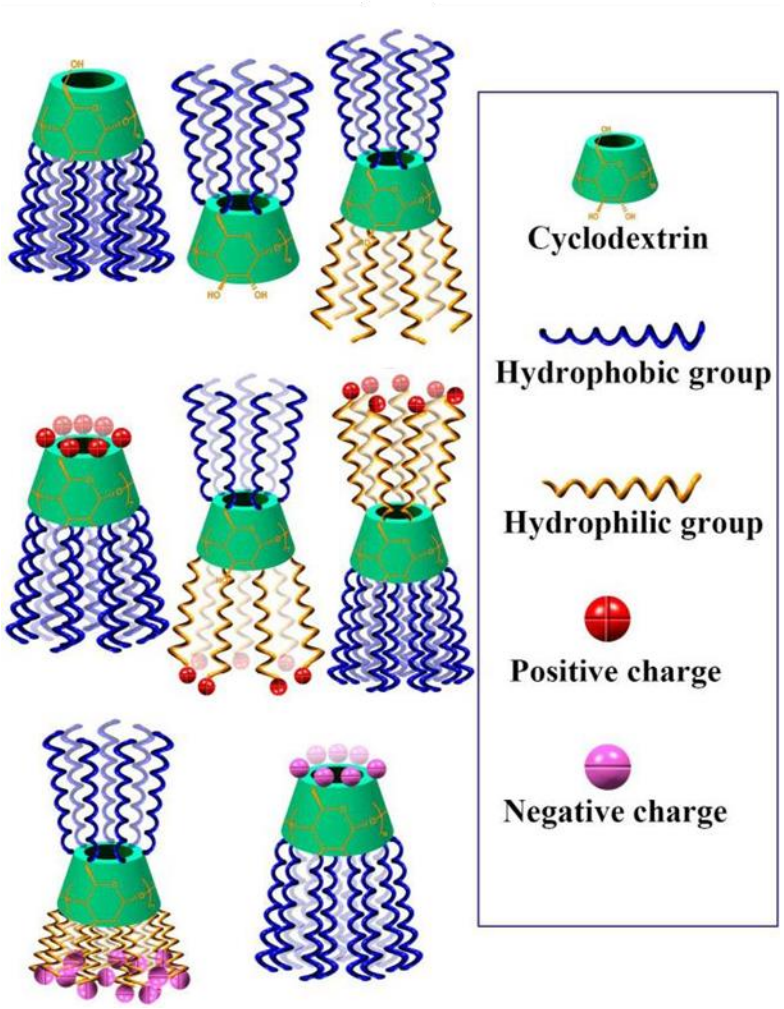
- Conclusions

CDs as Pure Vectors

Amphiphilic Cyclodextrins



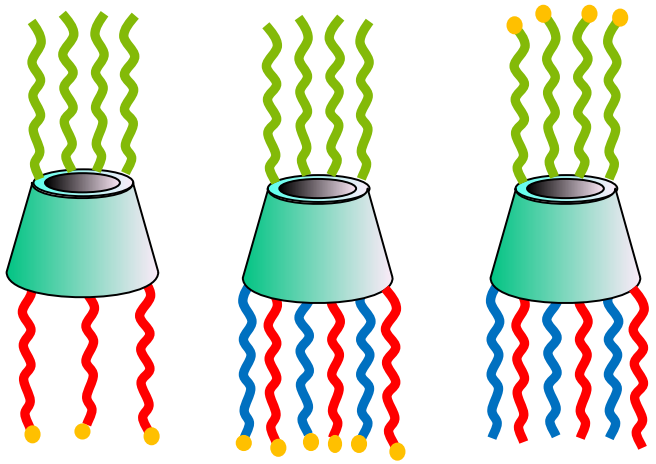
Applications

Solubilizers
Stabilizers and Emulsifiers
Detergent Additives.
Analytical Chemistry

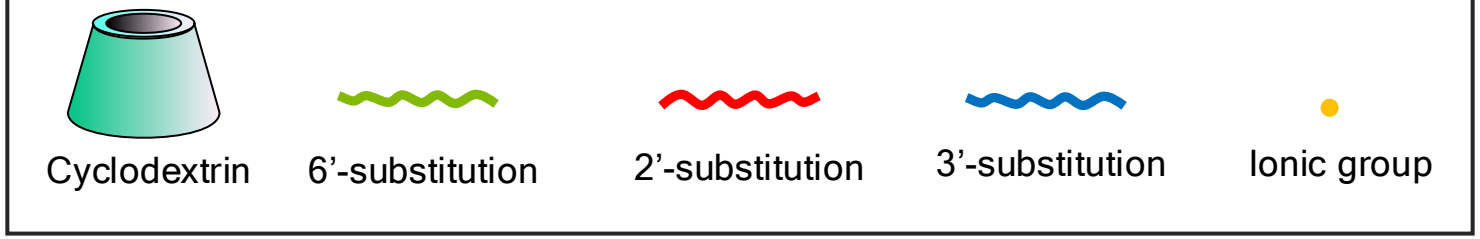
Drug Delivery
Gene Delivery
Tissue Engineering
Wound Healing
Diagnostic Imaging

J. Zhang, et al., Advanced Drug Delivery Review, 65, 9, 2013, 1215-1233

Cationic Amphiphilic CDs as Pure Vector



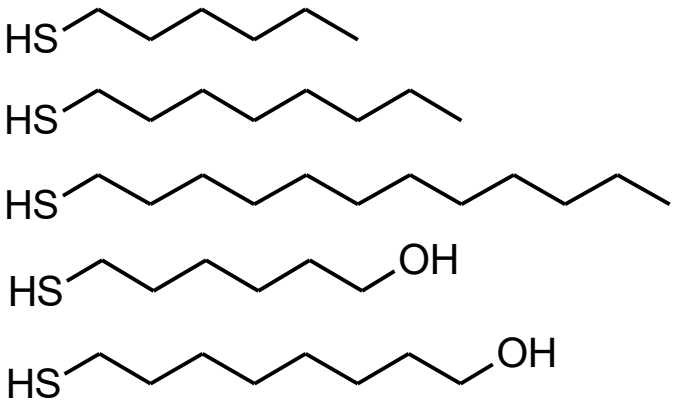
- Primary and secondary side can be easily modified
- Different type of hydrophobic chain
- High variability in the linker
- High variety of cationic groups
- Scale-up assessed



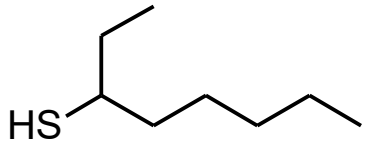
Cationic Amphiphilic CDs by CarboHyde

6’-substitution

Linear thioalkyl chain

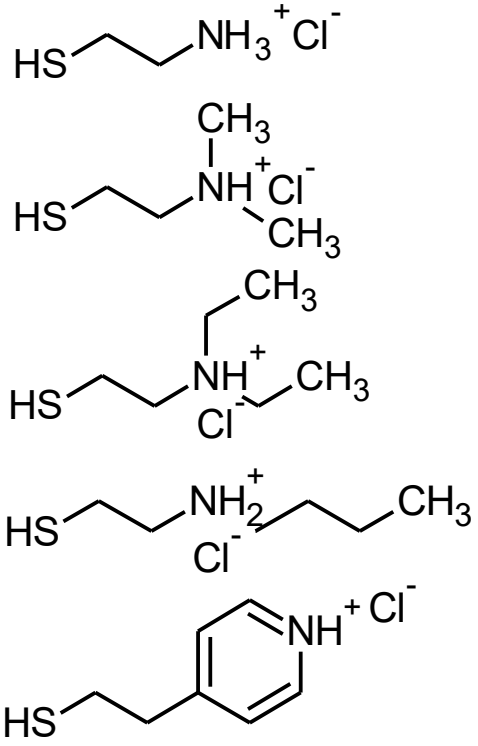


Branched thioalkyl chain

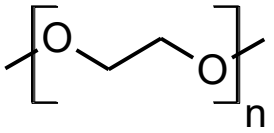


2’/3’-substitution

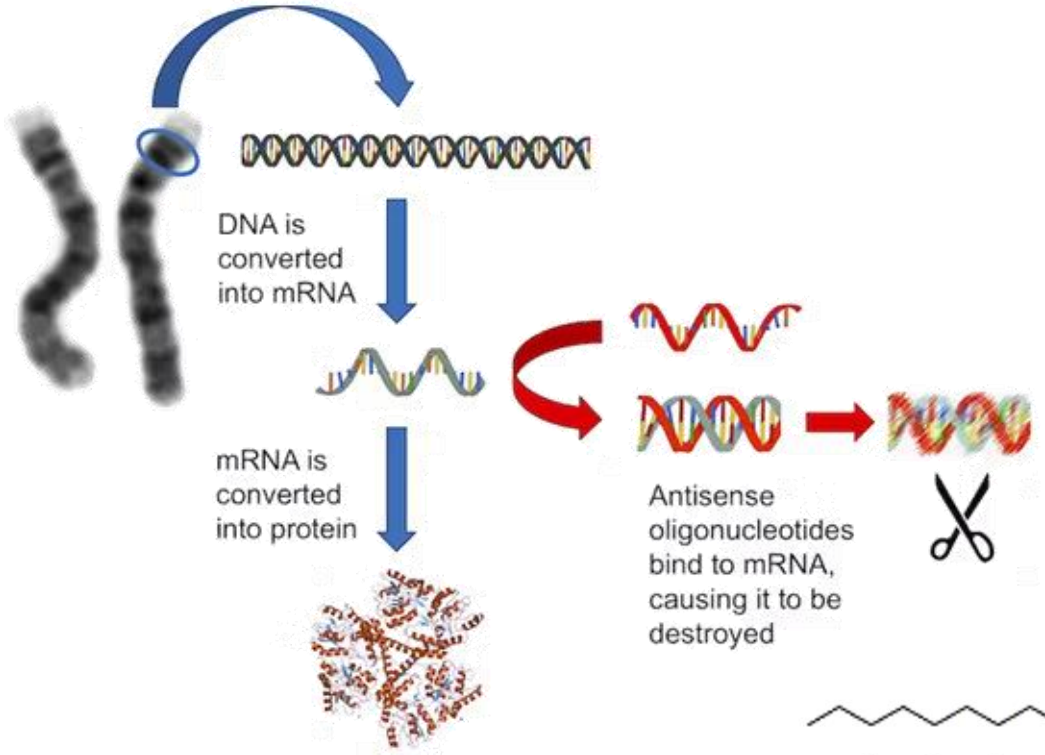
Ionic compounds

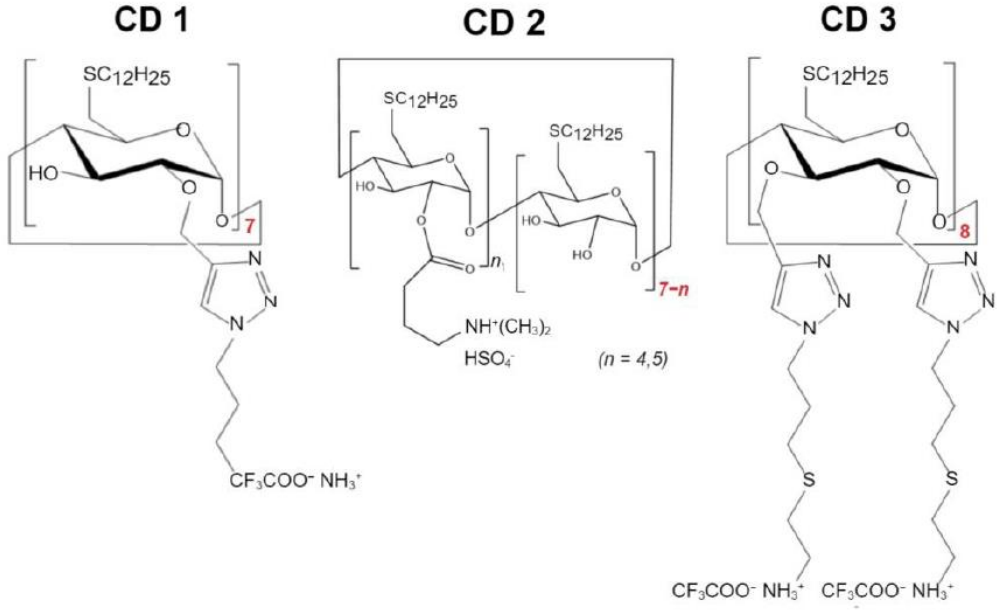


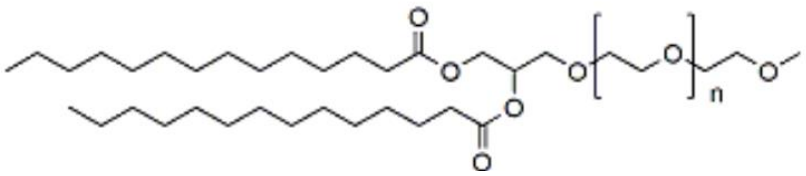
Spacer



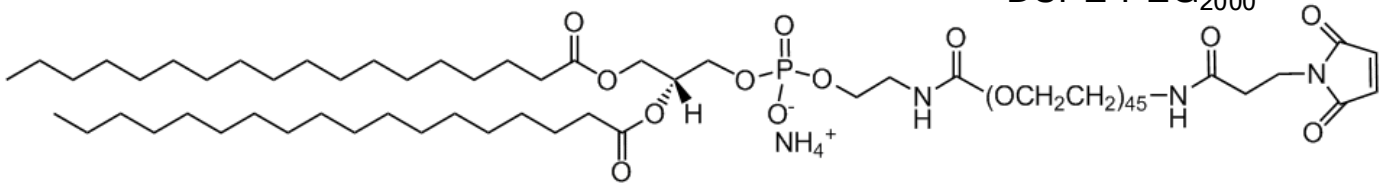
CDs-NP for Targeting Huntingtin with ASO



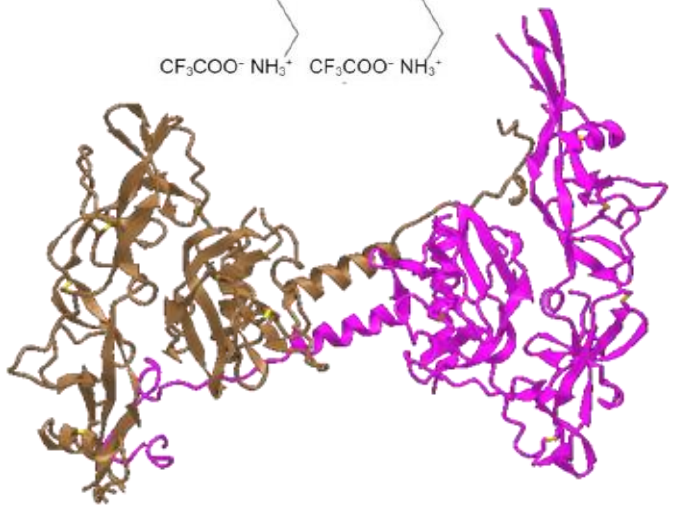




DSPE-PEG_{2000}



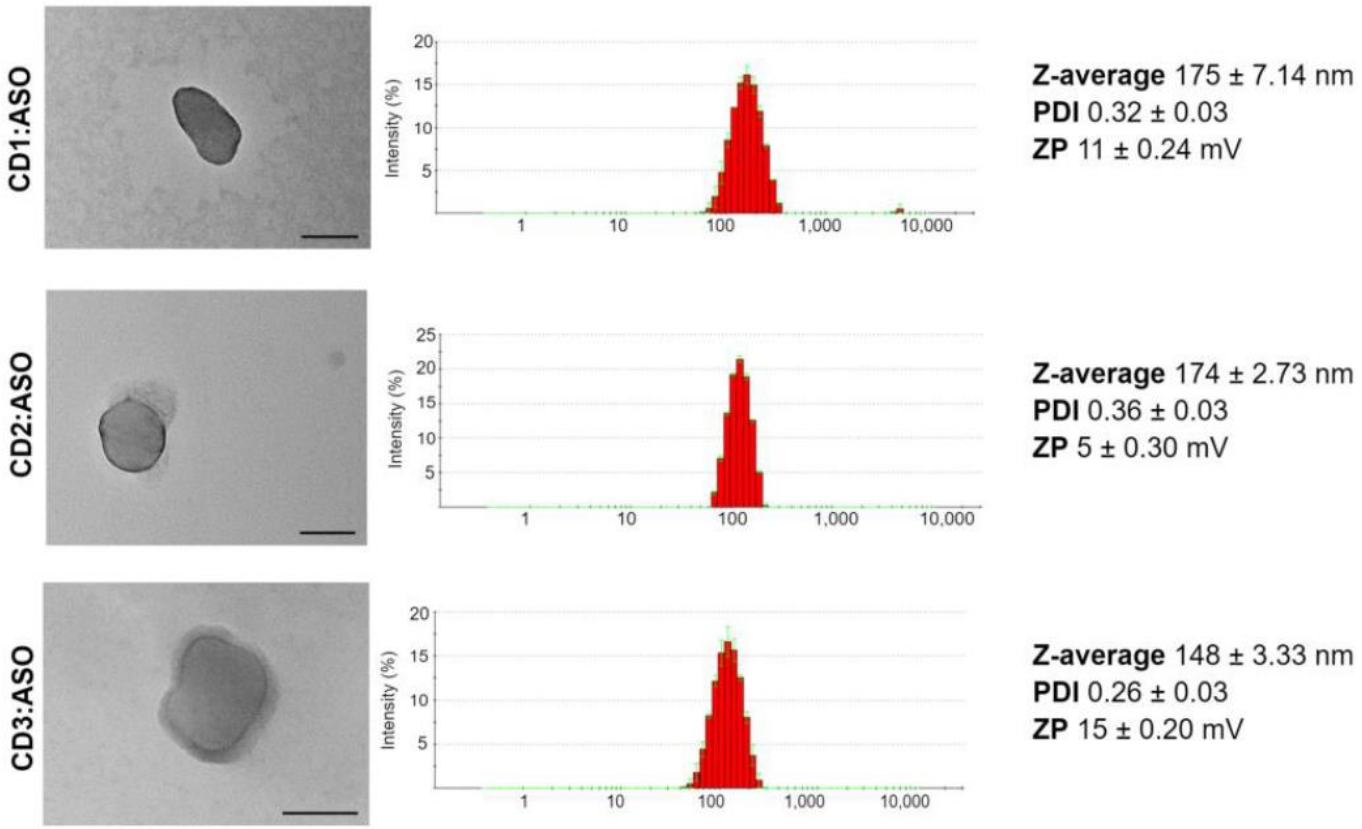
DSPE-PEG_{2000} Maleimide



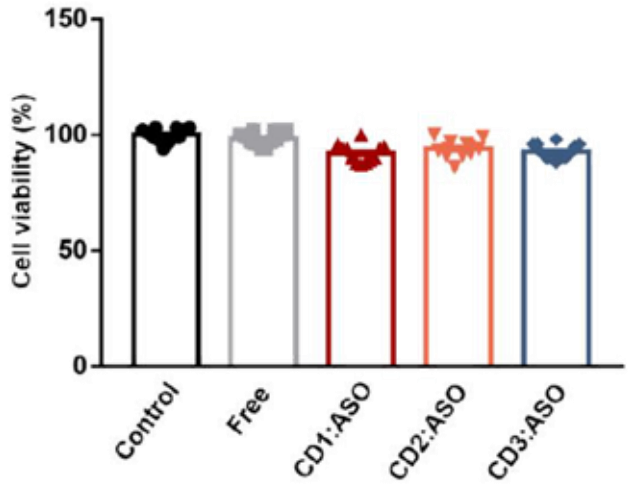
Rabies Virus Glycoprotein (RVG)

M. C. P. Mendonça, et. al Pharmaceutics 2023, 15, 520

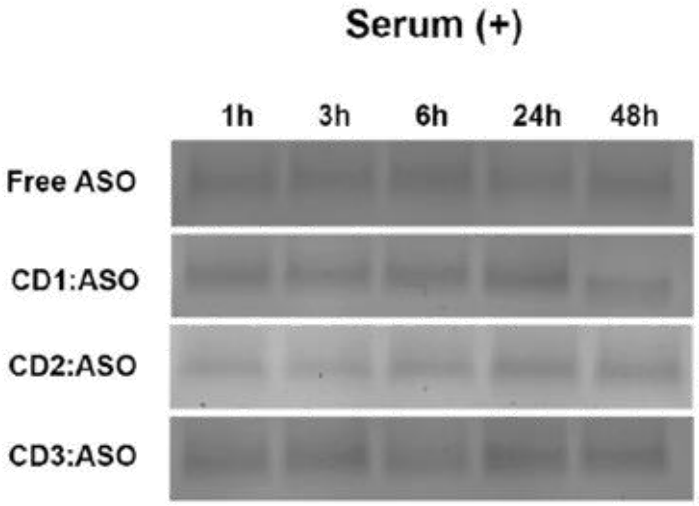
Selecting the best CDs:ASO Nanocomplexes



Physicochemical characterization of cyclodextrins (CDs):ASO nanocomplexes. (A) TEM of CDs:ASO nanocomplexes. (B) Dynamic Light Scattering histogram of the particle size distribution and physicochemical properties.



Cytotoxicity of CDs:ASO nanocomplexes on Striatum cells



Stability of (CDs):ASO nanocomplexes in presence of Serum

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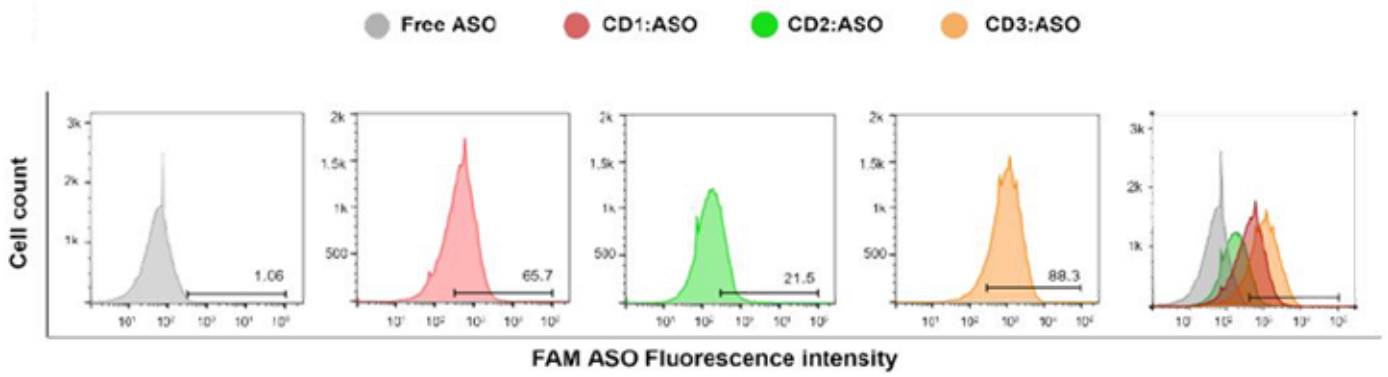
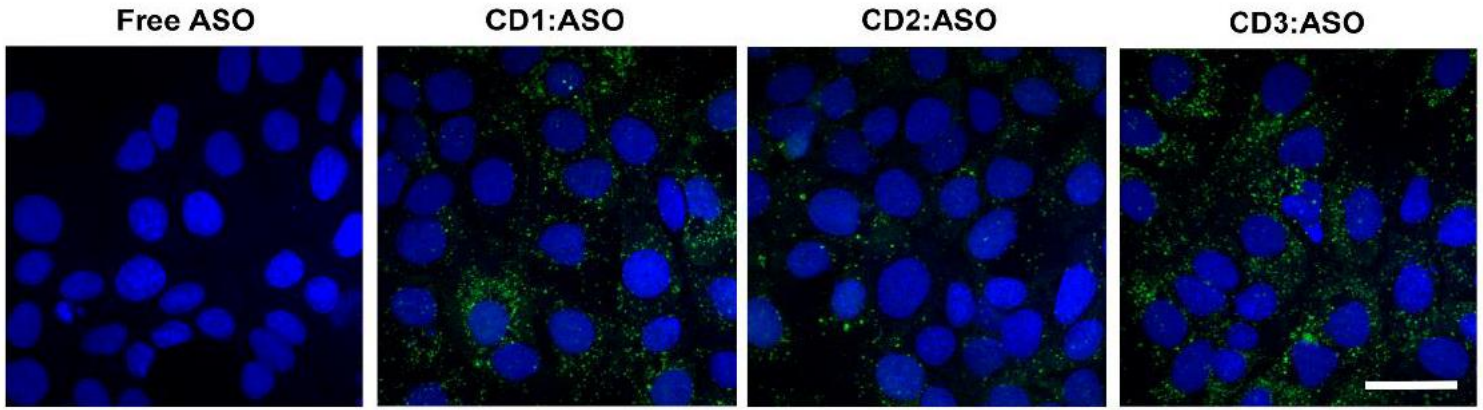
Selecting the best CDs:ASO Nanocomplexes

Cellular uptake of CDs:ASO nanocomplexes

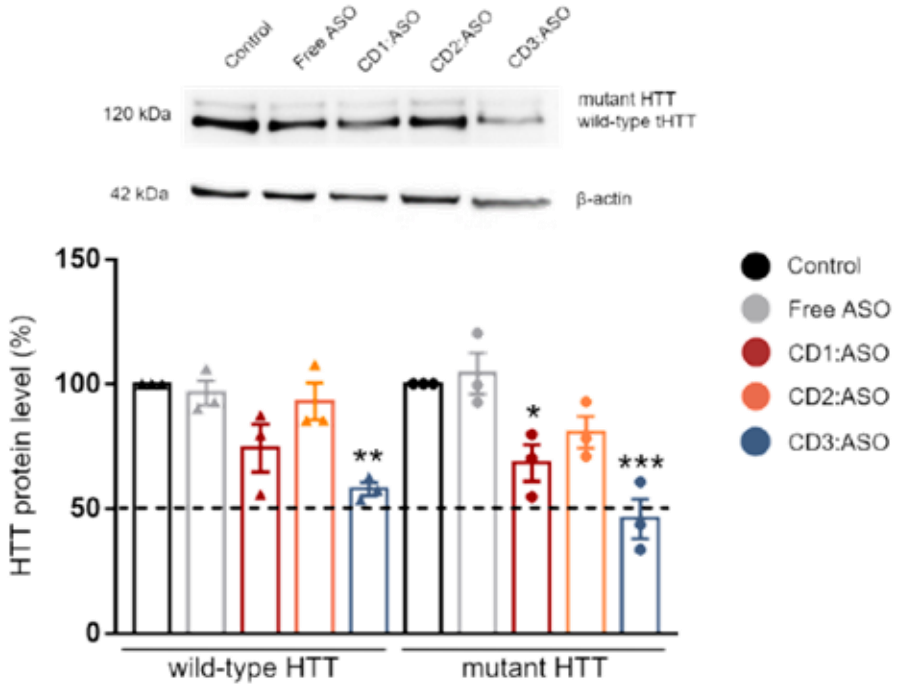


Representative flow cytometry histogram of CDs:ASO nanocomplexes internalization



Intracellular fluorescence of FAM-labelled ASO (green) and nuclei (blue)

Efficacy in a Neuronal In Vitro Model of HD



Evaluation of ASO efficacy after transfection with ASO alone or complexed with cyclodextrins.

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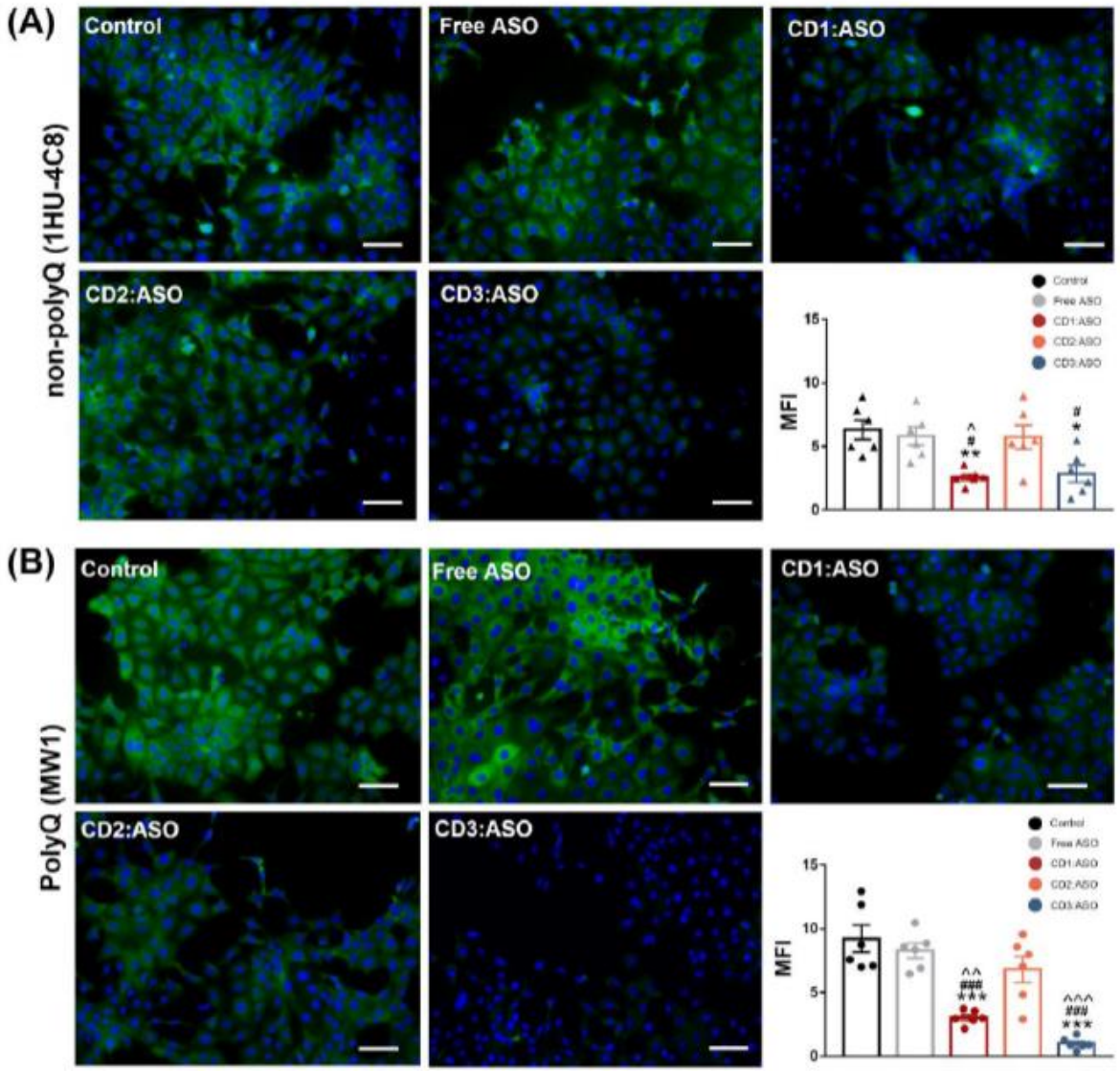
Selecting the best CDs:ASO Nanocomplexes



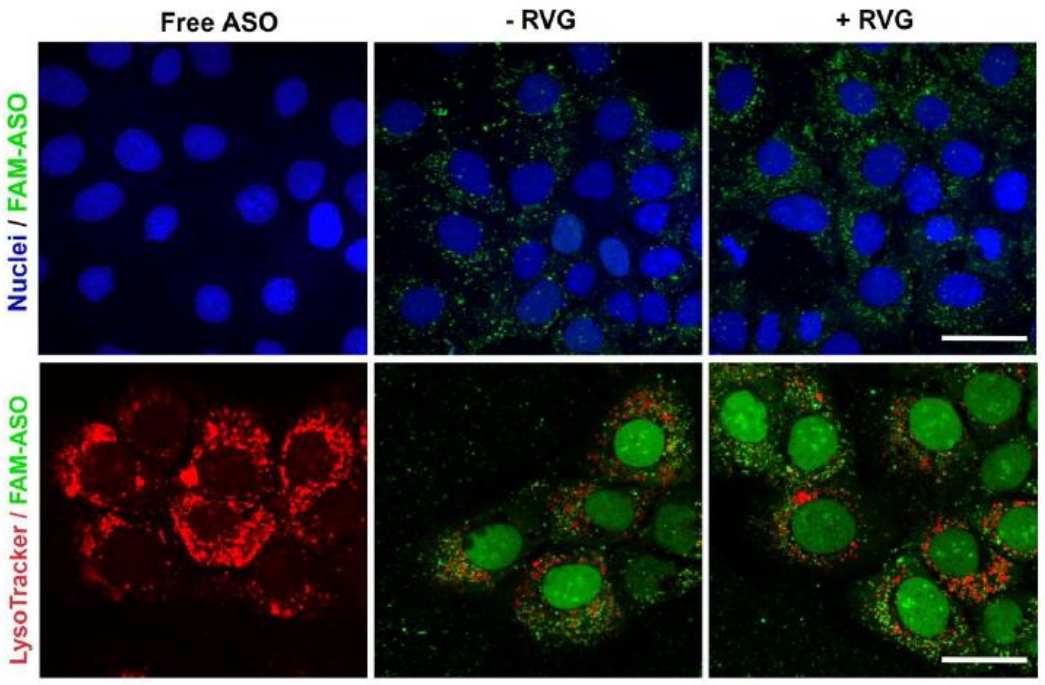
The expression levels of mutant and total HTT after transfection with ASO alone or complexed with CDs. Cells were treated with ASO for 72 h.
After this period, the cells were fixed and stained with polyQ (mutant) antibody and non-polyQ antibody.

Representative confocal images of (A) non-polyQ antibody and (B) polyQ antibody after treatment with ASO alone or complexed with CDs. Both antibodies were stained with Alexa 488 (green), while the nucleus was stained with DAPI (blue).

M. C. P. Mendonça, et. al Pharmaceutics 2023, 15, 520

Improving the Delivery of CD3:ASO with RVG

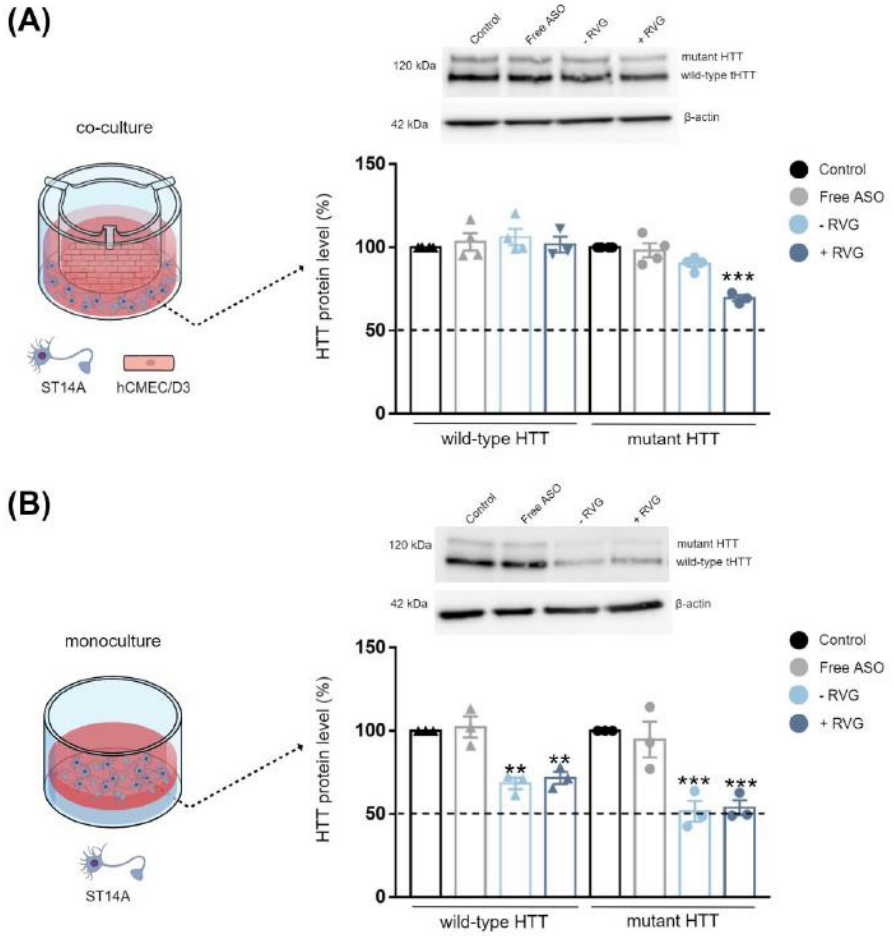
Cellular uptake and late endosome/lysosome escape of RVG-targeted CDs:ASO nanocomplexes



Representative confocal fluorescence images of ST14A cells treated with ASO alone or complexed with CDs for 6 h. Intracellular fluorescence of FAM-labelled ASO (green), nuclei (blue), and LysoTracker Red DND-99 (red). The yellow color indicates co-localization.

M. C. P. Mendonça, et. al Pharmaceutics 2023, 15, 520

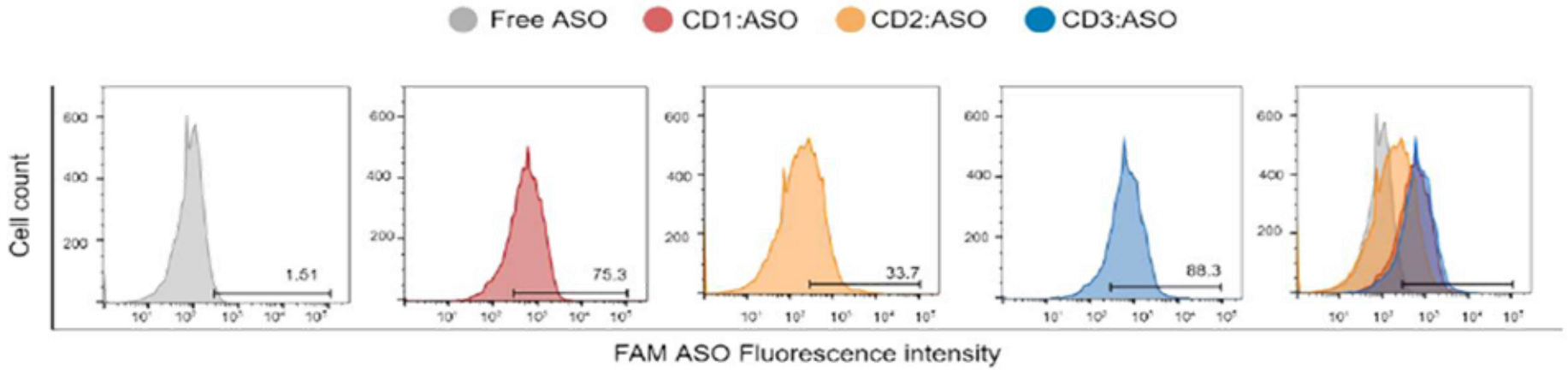
ASO efficacy after transfection with RVG-targeted and untargeted CDs in a co-culture and monoculture models

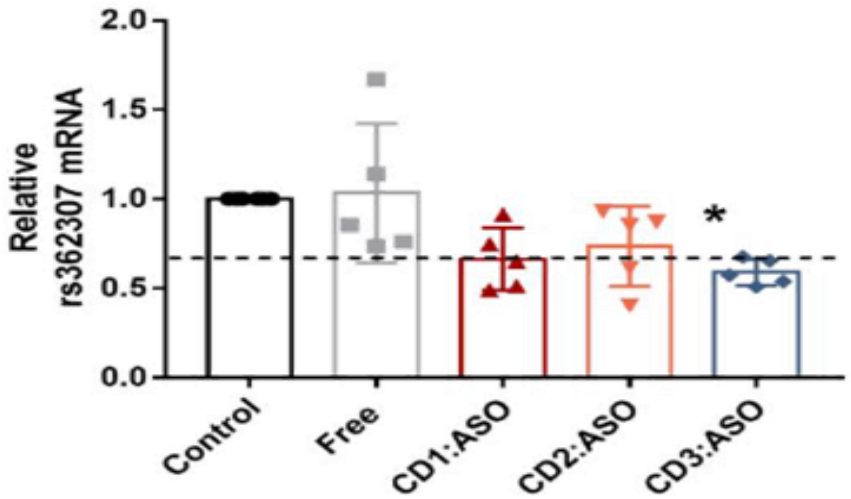


(A) the BBB co-culture model containing hCMEC/D3 cells in the apical compartment and ST14A cells in the basal compartment and (B) monoculture of ST14A cells.

HD Patient-Derived Fibroblasts

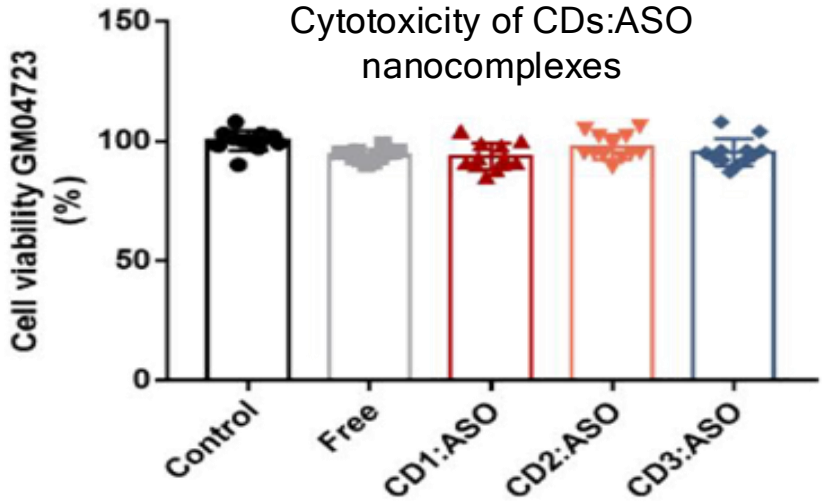
Cellular uptake of CDs:ASO nanocomplexes





Evaluation of ASO efficacy after transfection with ASO alone or CDs:ASO nanocomplexes

Cytotoxicity of CDs:ASO nanocomplexes



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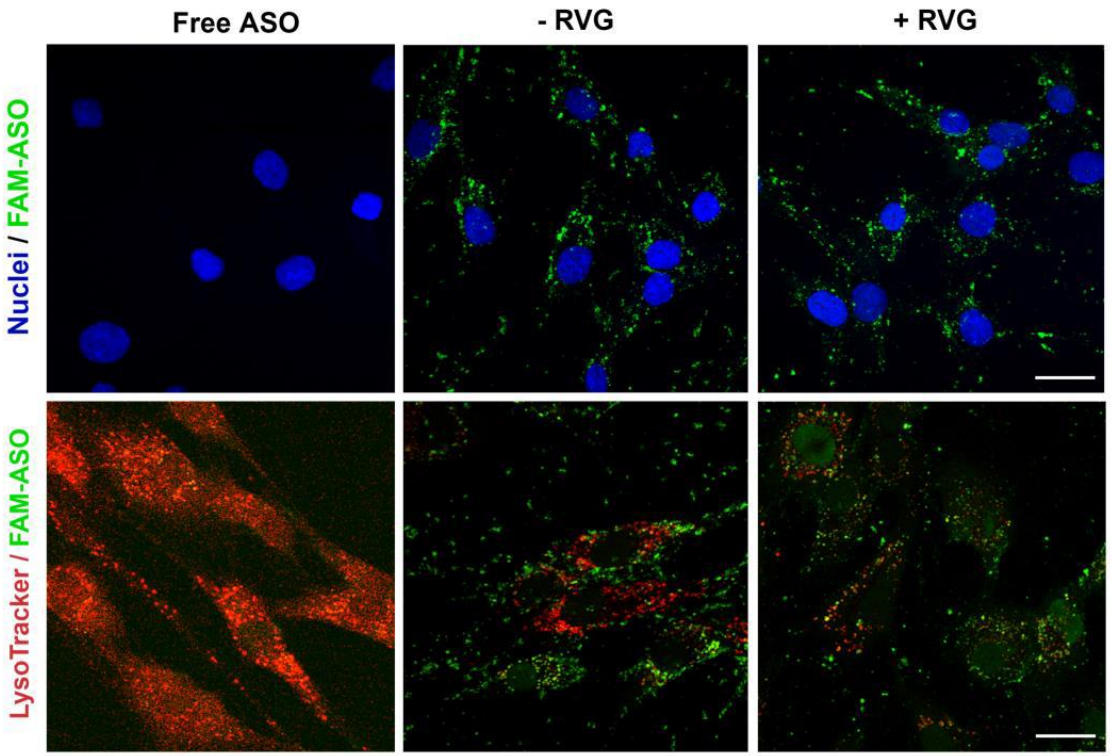
HD Patient-Derived Fibroblasts

Cellular uptake and late endosome/lysosome escape of RVG-targeted cyclodextrins CDs:ASO nanocomplexes.



Representative confocal fluorescence images of fibroblasts from an HD patient (CAG15/67) treated with ASO alone or complexed with CDs for 6 h. Intracellular fluorescence of FAM-labelled ASO (green), nuclei (blue), and LysoTracker Red DND-99 (red). The yellow color indicates co-localization.

ASO efficacy after transfection with RVG-targeted and untargeted CDs in a co-culture and monoculture models

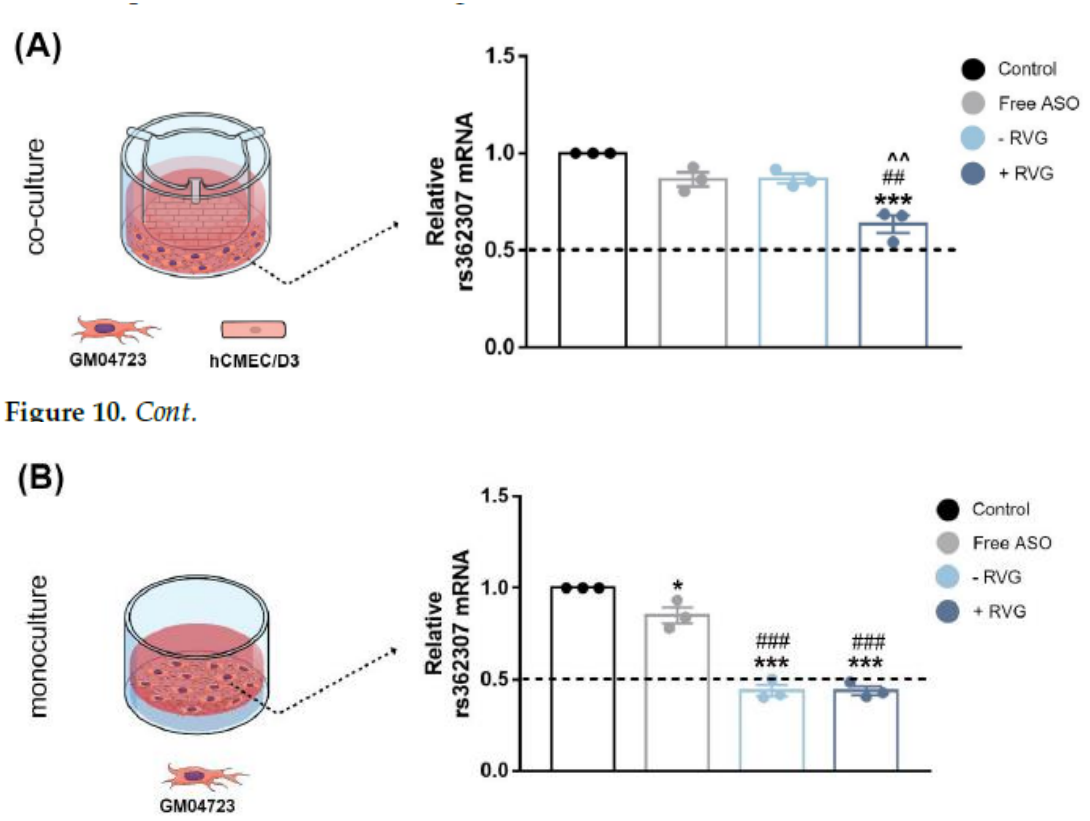


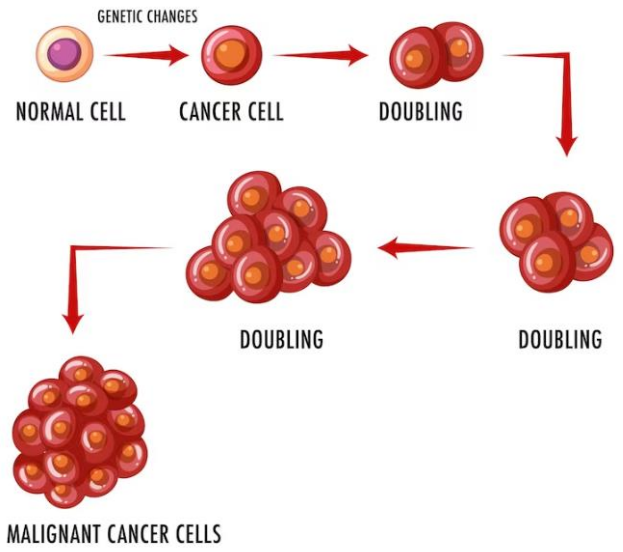
Figure 10. *Cont.*

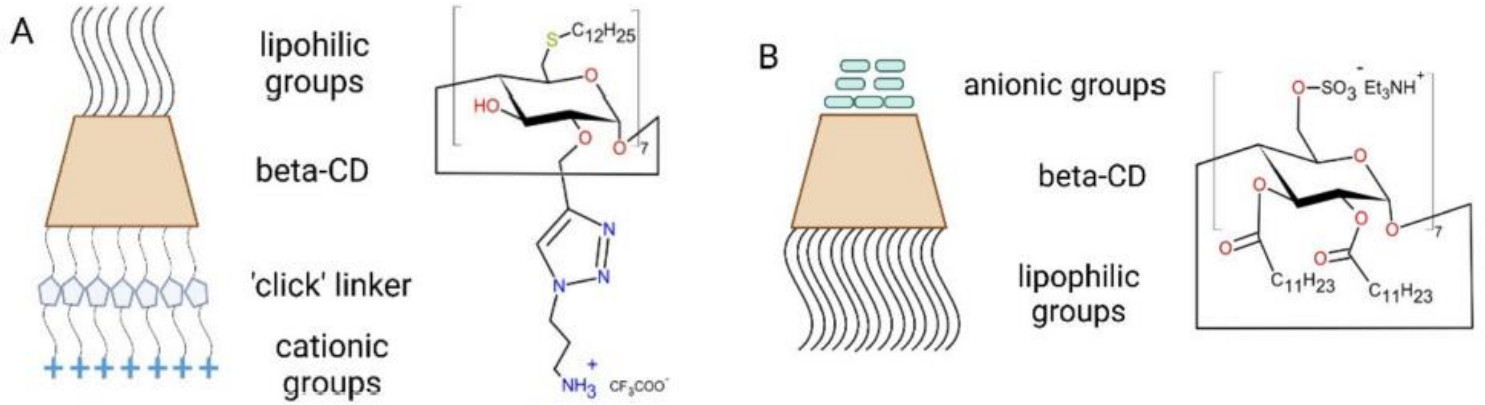
(A) the BBB co-culture model containing hCMEC/D3 cells in the apical compartment and HD-patient derived fibroblasts (CAG15/67) in the basal compartment and (B) monoculture of fibroblasts.

M. C. P. Mendonça, et. al Pharmaceutics 2023, 15, 520

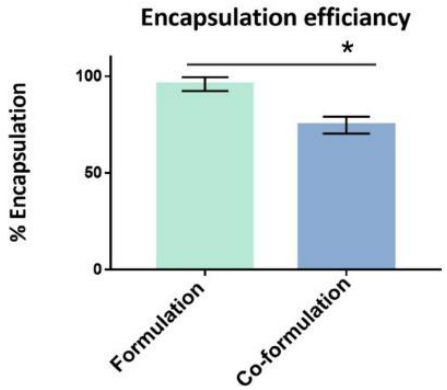
Co-Formulated-CDs for Treatment of AML

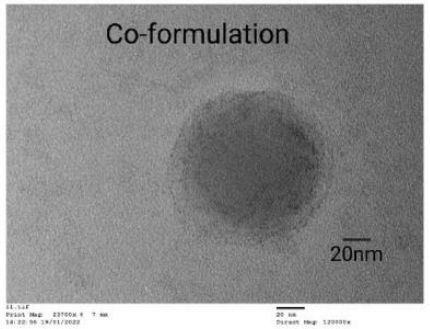
Cancer development process

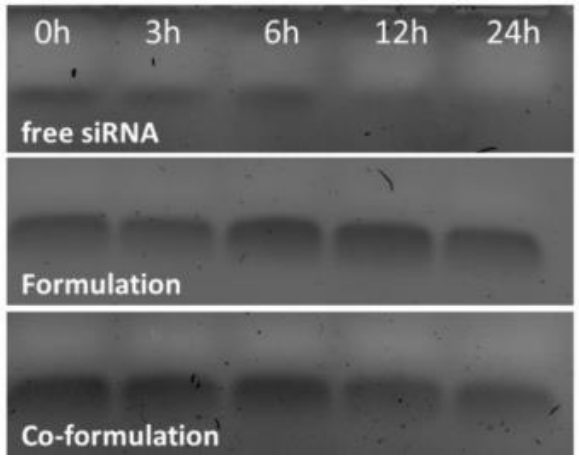




Reduction in the cationic charge density is likely to extend the in-vivo circulation time and minimize potential aggregation and toxicity.

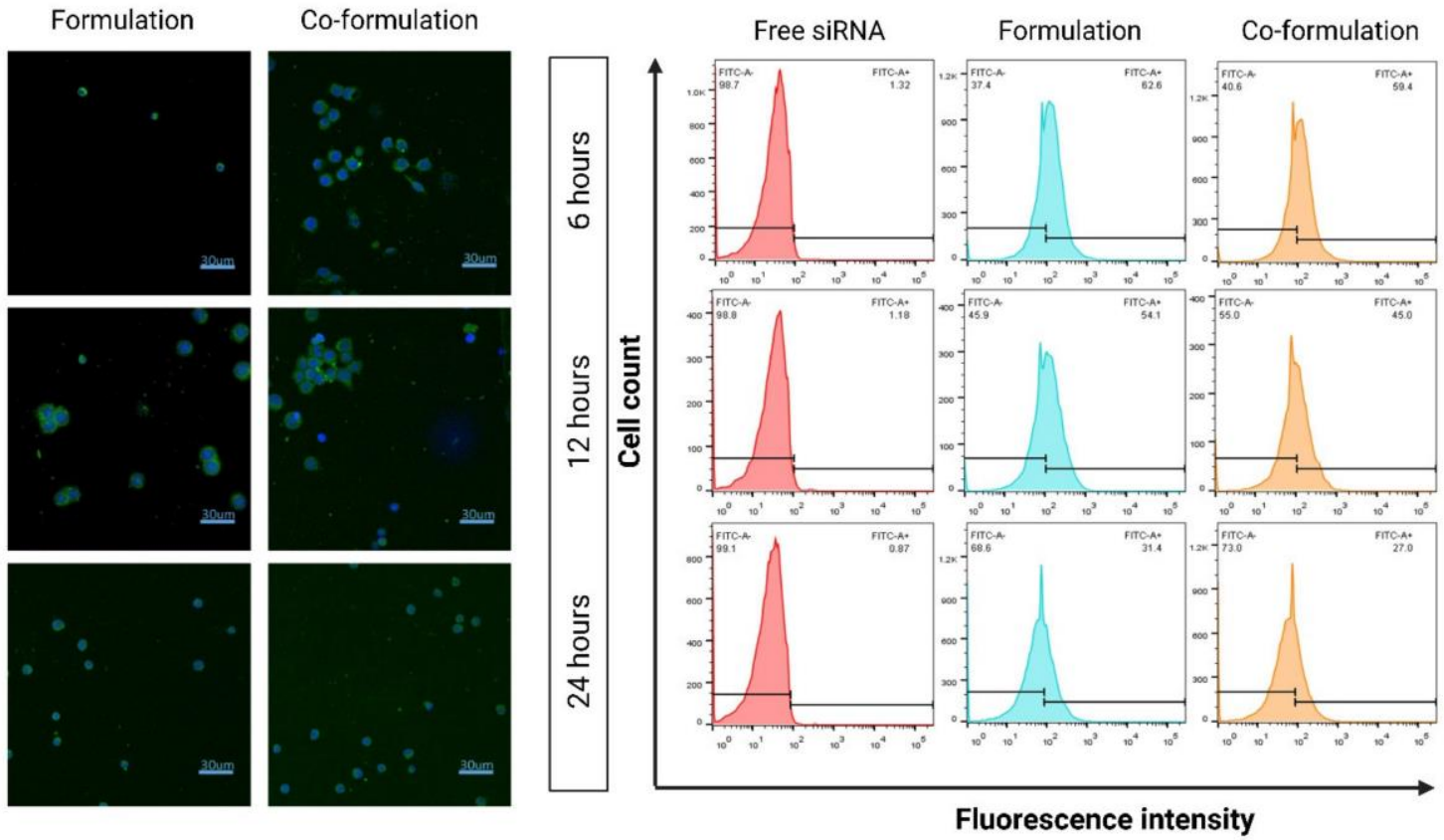






A. Kont, et. al, Int. J. Mol. Sci. 2022, 23, 9791

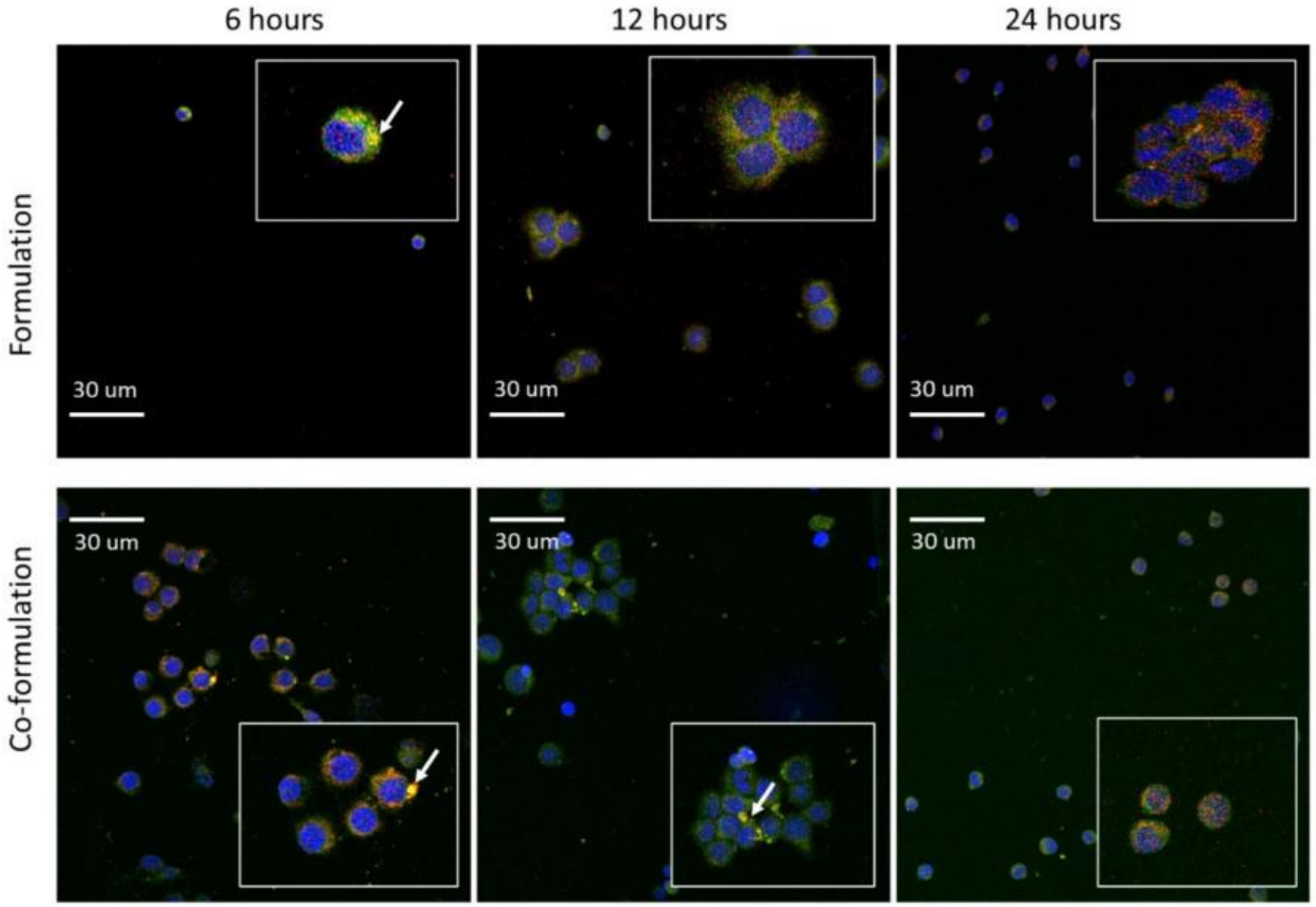
Cellular Uptake of the Co-Formulated CDs



The relatively lower surface charge of the co-formulation did not alter the cellular uptake indicating that charge alone is not the sole driver for internalization. In contrast, the amphiphilicity of the CDs promoting a lipophilic interaction at cellular level may also be a significant factor.

A. Kont, et. al, Int. J. Mol. Sci. 2022, 23, 9791

Endosomal Release of the Co-Formulated CDs



After 12 h, late-endosomal/lysosomal release was seen with the formulation, but not with the co-formulation which still showed co-localization. Endo-lysosomal escape for the co-formulation appeared mainly to occur at the later time of 24 h.

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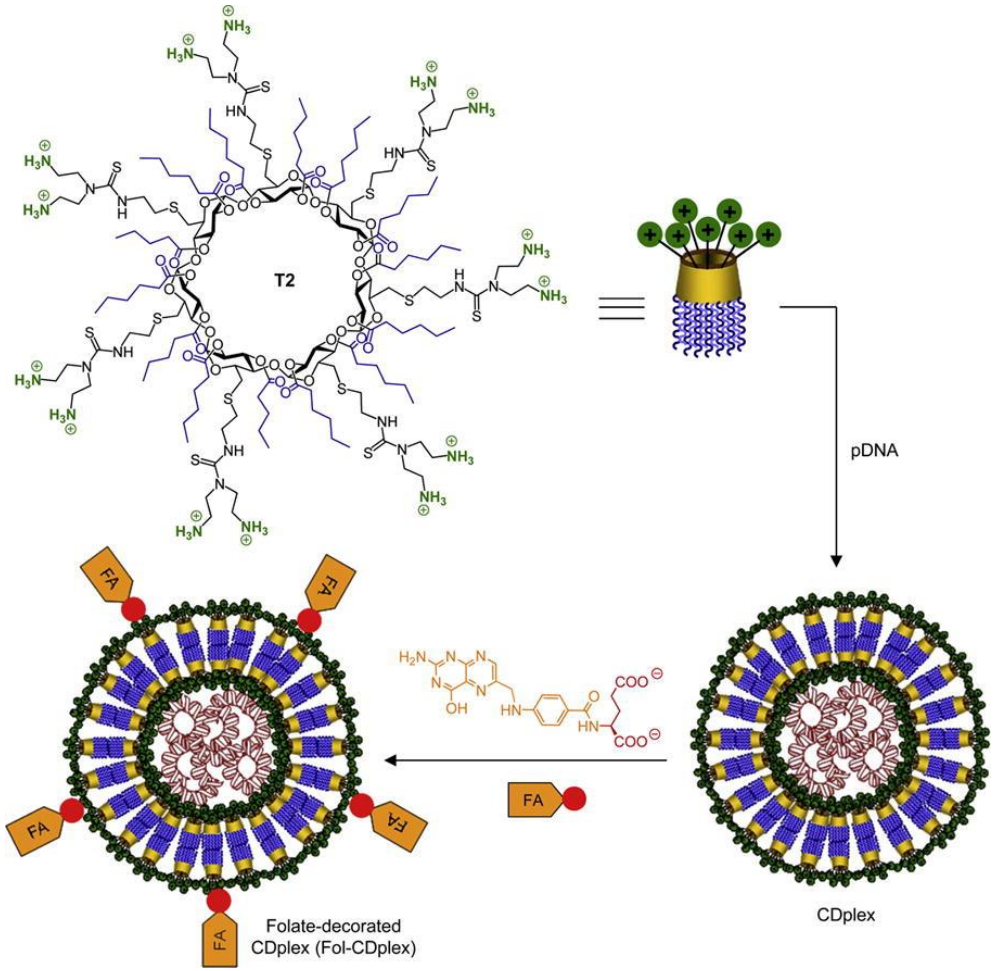
CDs as pure Vectors

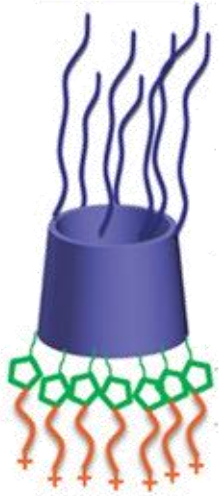
- Enhanced cellular uptake
- Targeted gene delivery
- Potential for controlled release
- Good stability and protection of genetic material
- High structural versatility

- Complexity of synthesis
- Batch-to-batch variability
- High cost
- Immunogenicity and toxicity concerns
- Limited clinical translation

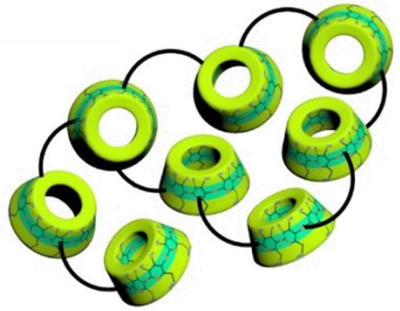


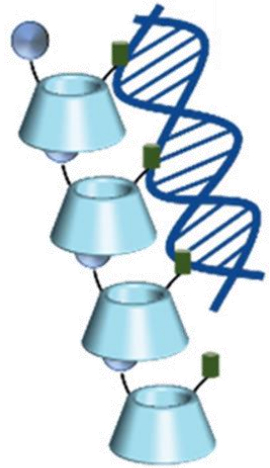
C. Aranda, et al., European Journal of Pharmaceutics and Biopharmaceutics, 85, 3, Part A, 2013, 390-397

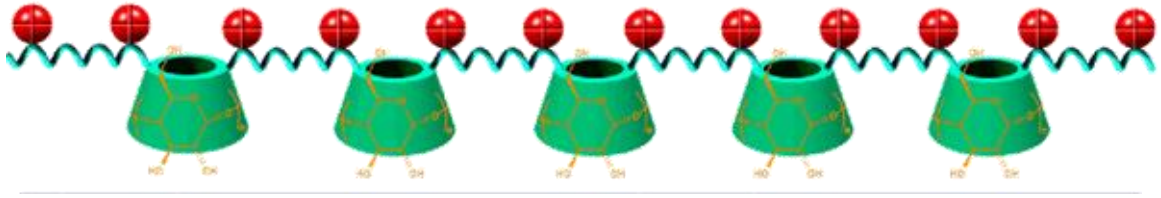
Conclusions



- CDs are the most versatile substrate for the design of gene delivery systems
- CDs-based systems ar promising in terms of toxicity and immunogenicity
- CDs-based systems are ideal for combination therapy
- CDs-based constructs hold and protect the genetic material effectively
- CDs-based system are suitable for prolonged/sustained release
- CDs-based architectures can be engineered to have long shelf lives
- CDs-based architectures are amenable for tropism-modification strategies







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info@carbohyde.com