

2nd

STSM CONFERENCE

Lodz, Poland

Evaluation of cationic carbosilane dendrimers as nanovectors of therapeutic nucleic acids for cancer nanomedicine

Short-term Scientific Mission 7th Call; 1.8.-31.10.2022

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Dr. Tomáš Strašák

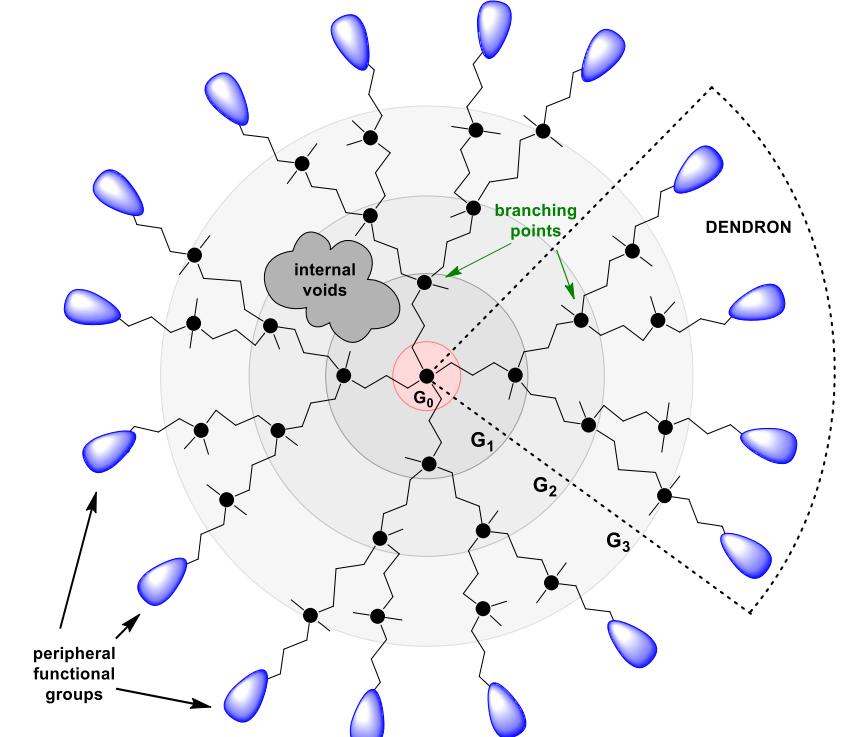
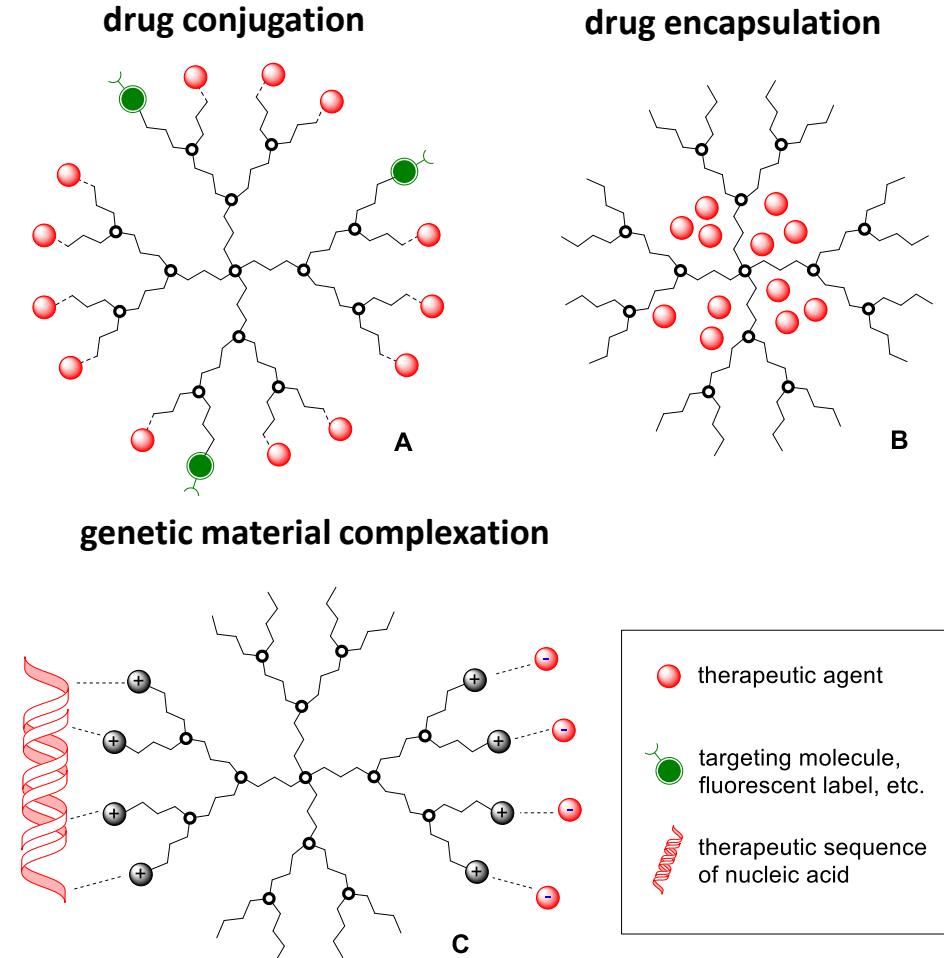
Dep. of general biophysics, University of Lodz, Poland

Host institution:
Prof. Anna Janaszewska
Prof. Barbara Klajnert-Maculewicz

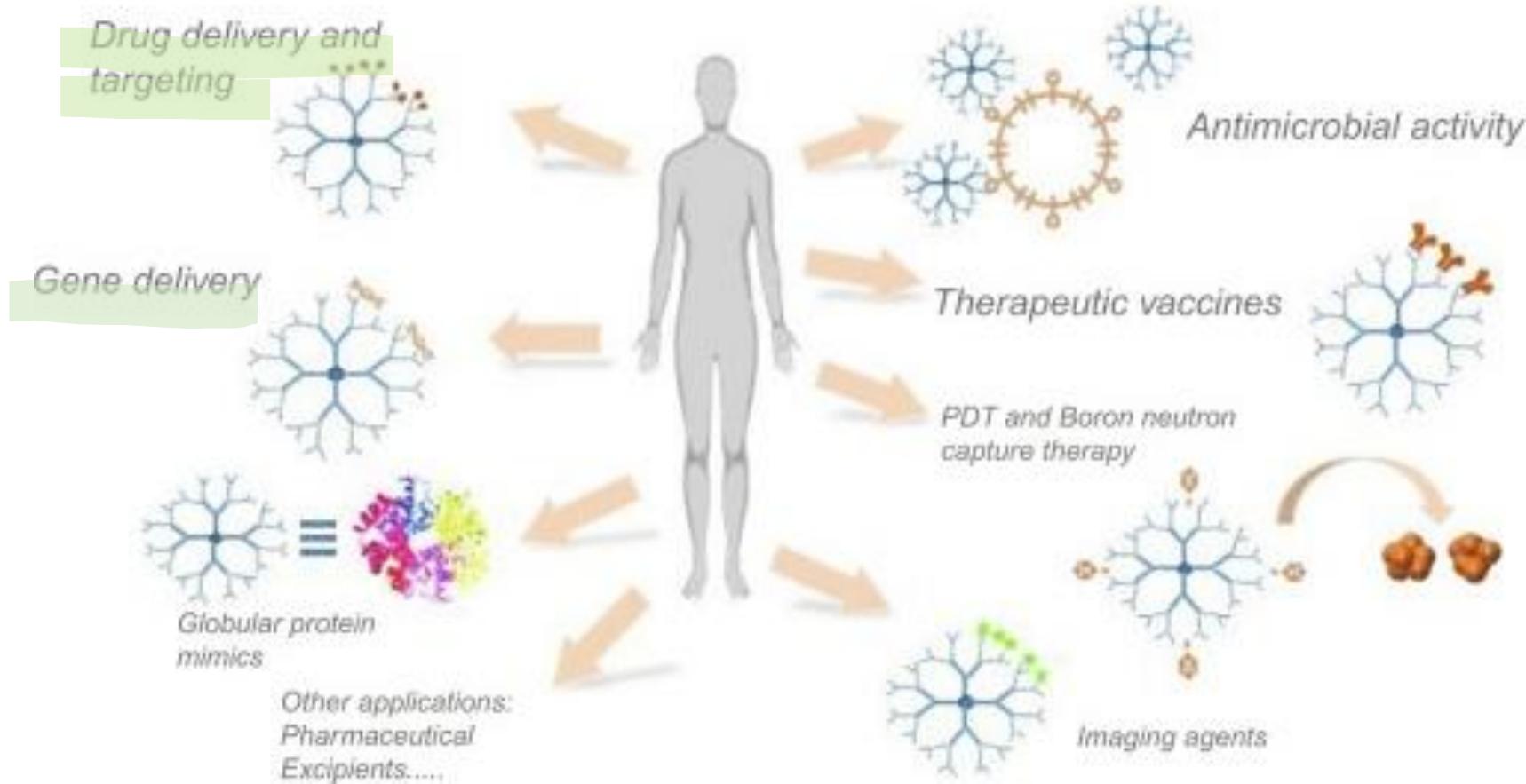


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Dendrimers: versatile platform for drug and gene delivery



Dendrimers in the context of nanomedicine



Toxicity?

... and how is it toxic compared to other (dendritic) systems???

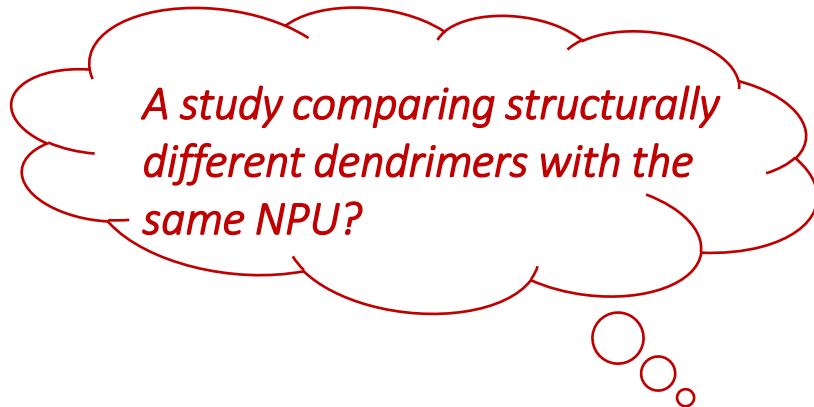
Efficiency?

... and how is it efficient compared to other (dendritic) systems???

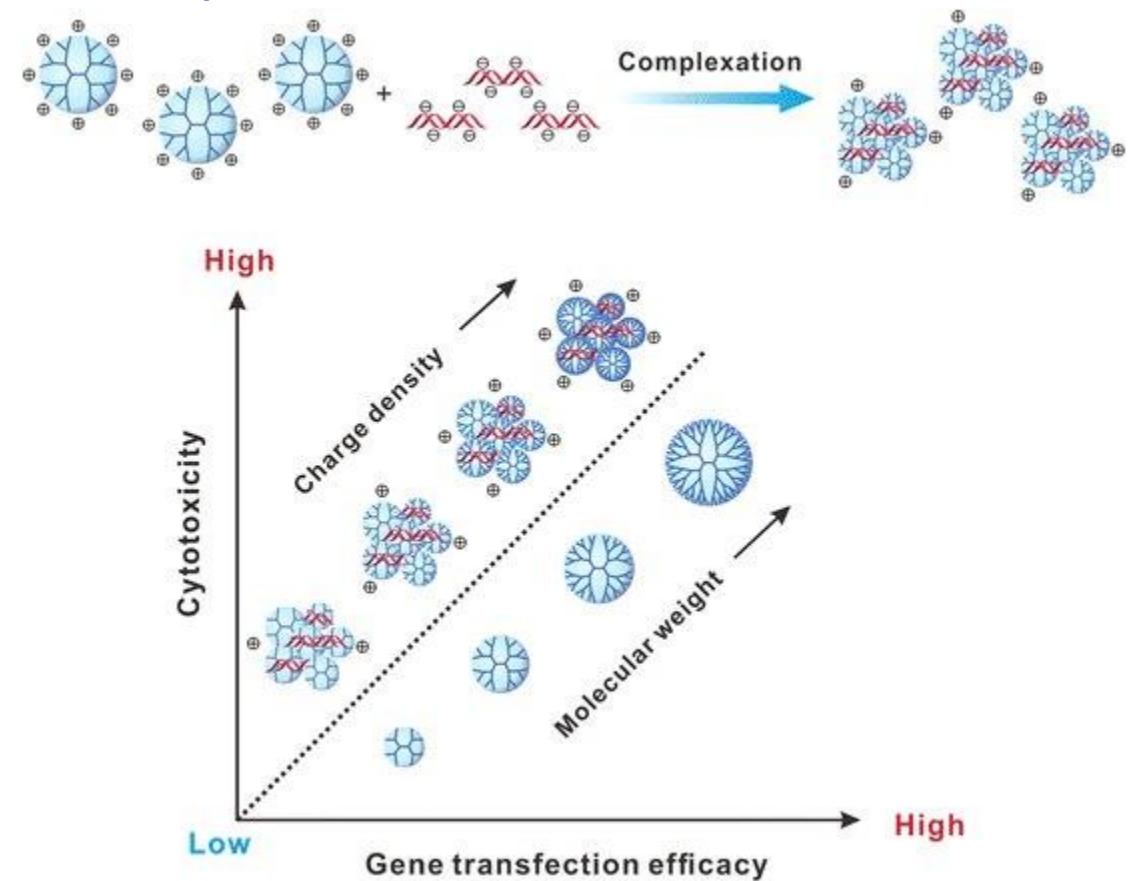
Conditions?

Nanovectors for gene delivery

Toxicity and efficiency is generally pre-determined by ...

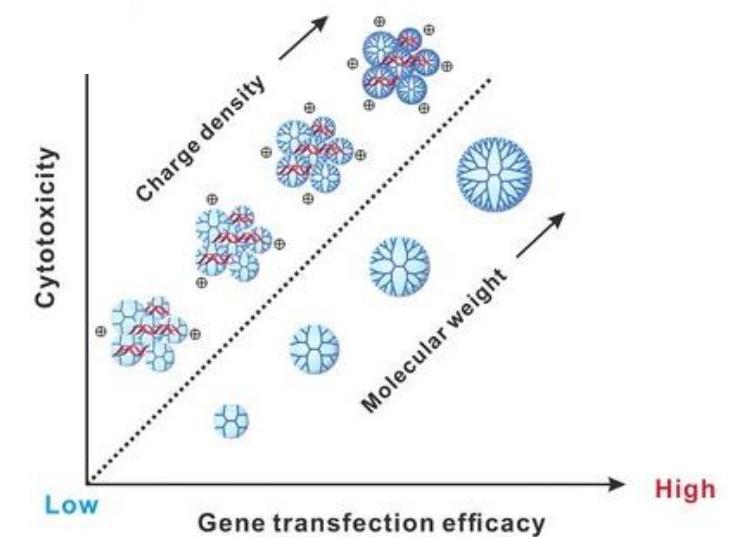
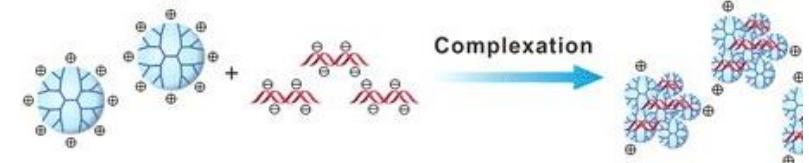


- ... the chemical **composition** and **structure**
- ... on the size/molecular weight of the molecule, resp. on the **charge density**, which is further determined..
 - by the **number of peripheral cationic units (NPU)** and **size of the molecule**

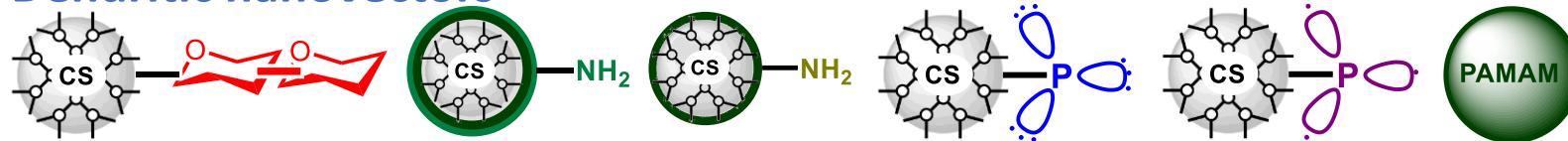


Goal of the study

- A pool of structurally different types of dendritic nanovectors
4 structural types, 20 compounds in total
- Biophysical and biological characterization under the same conditions
- **The first direct inter-structural comparison of the cytotoxicity and transfection efficiency of dendritic nanovectors**



Dendritic nanovectors



LEGEND

cationic
carbosilane
glycoDDMs

hybrid carbosilane-PAMAM
dendrimers

carbosilane
phosphonium-terminated DDMs

PAMAMs

4 NPU

8 NPU comp. 18

16 NPU comp. 1

32 NPU comp. 2

64 NPU

comp. 6

comp. 7

comp. 8

comp. 11

comp. 14

comp. 9

comp. 10

comp. 11

comp. 13

comp. 14

comp. 12

comp. 13

comp. 14

comp. 15

comp. 16

comp. 19

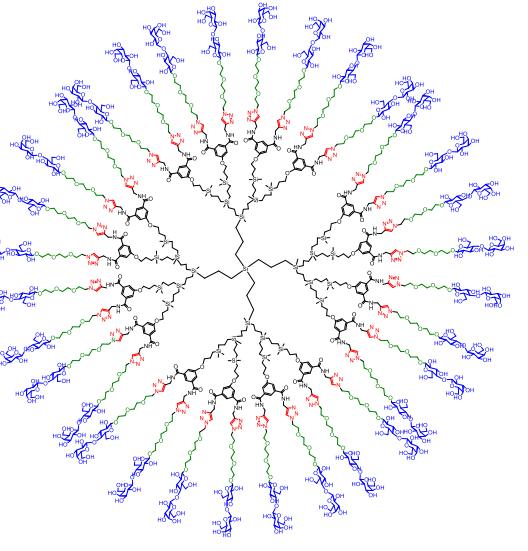
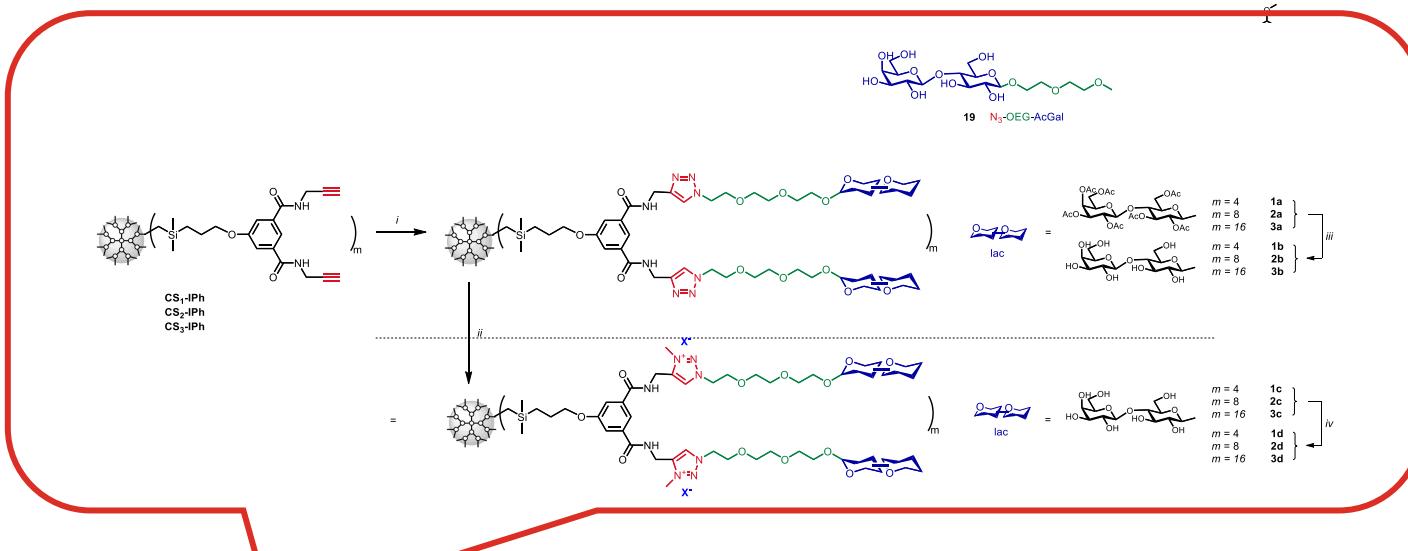
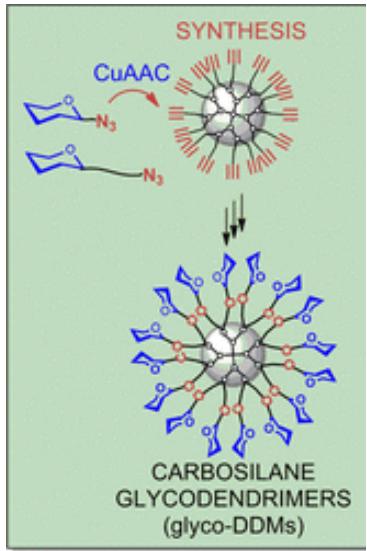
comp. 20

comp. 15

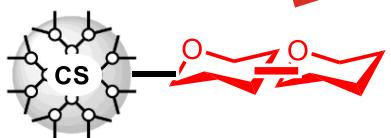
comp. 16

comp. 17

Pool of dendrimers



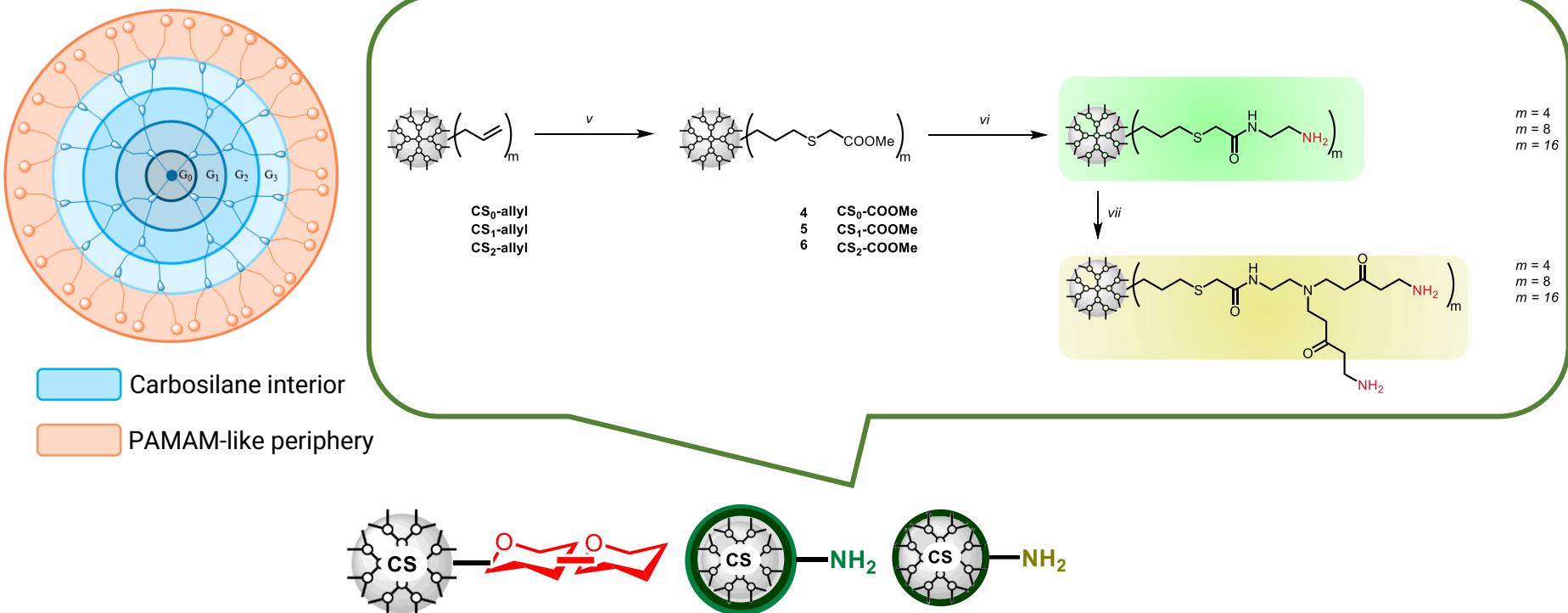
Biomacromolecules 2022, 23, 1, 276–290
(My STSM No.1)



LEGEND

cationic carbosilane glycoDDMs	hybrid carbosilane-PAMAM dendrimers	carbosilane phosphonium-terminated DDMs	PAMAMs
4 NPU			
8 NPU	comp. 18	comp. 6	comp. 9
16 NPU	comp. 1	comp. 3	comp. 12
32 NPU	comp. 2	comp. 4	comp. 10
64 NPU		comp. 5	comp. 13
		comp. 8	comp. 11
		comp. 7	comp. 14
		comp. 11	comp. 15
		comp. 10	comp. 16
		comp. 12	comp. 17
		comp. 13	comp. 19
		comp. 14	comp. 20

Pool of dendrimers



BSc. Susanne Krkoška



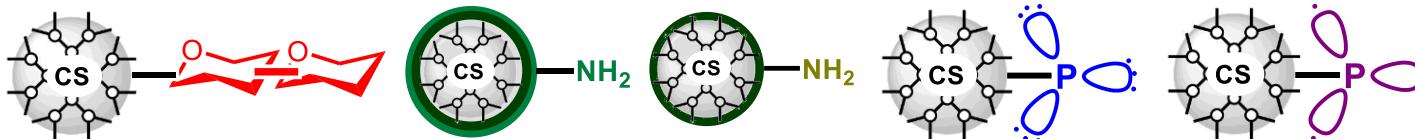
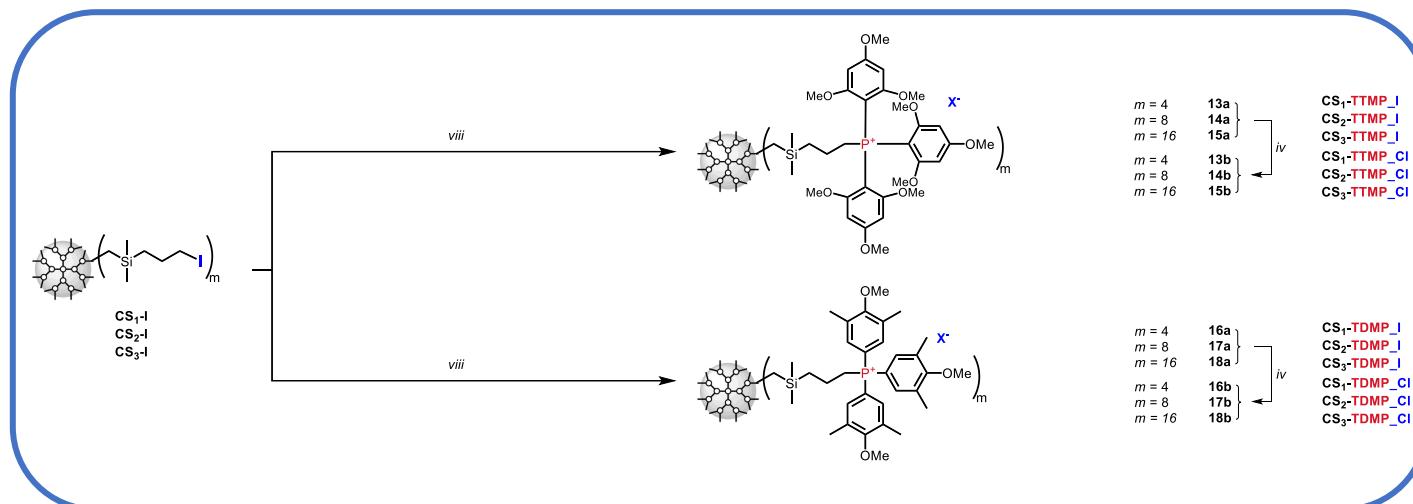
I FGEND

The legend is organized into four main sections:

- cationic carbosilane glycoDDMs**: Contains entries for 4 NPU (comp. 18), 8 NPU (comp. 1), 16 NPU (comp. 2), 32 NPU (comp. 5), and 64 NPU.
- hybrid carbosilane-PAMAM dendrimers**: Contains entries for comp. 3, 4, 6, 7, 10, and 11.
- carbosilane phosphonium-terminated DDMs**: Contains entries for comp. 9, 12, 13, 14, 15, 16, 17, and 19.
- PAMAMs**: Contains entries for comp. 19, 20, and 21.

Pool of dendrimers

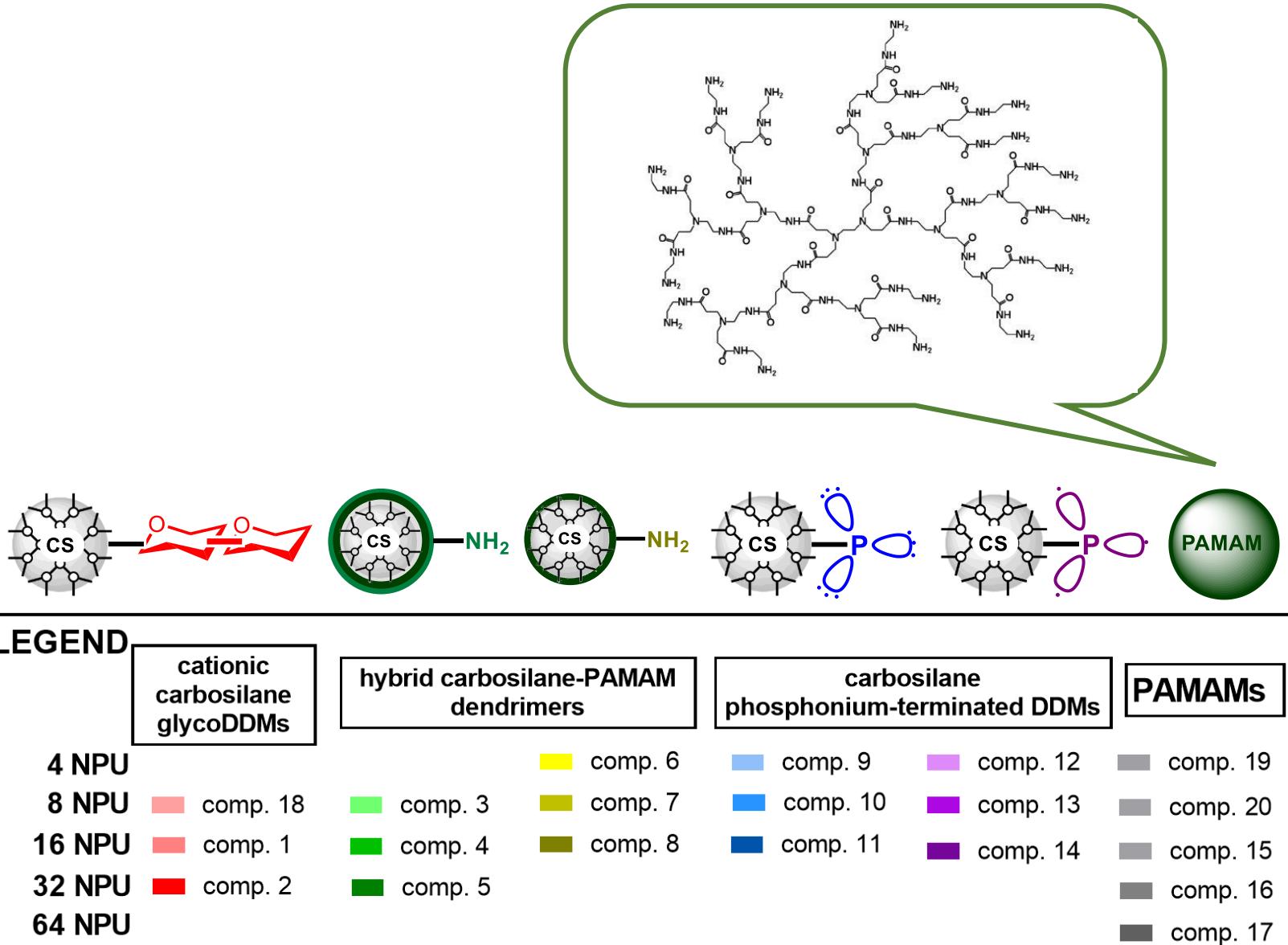
MSc. Wahid Tattan



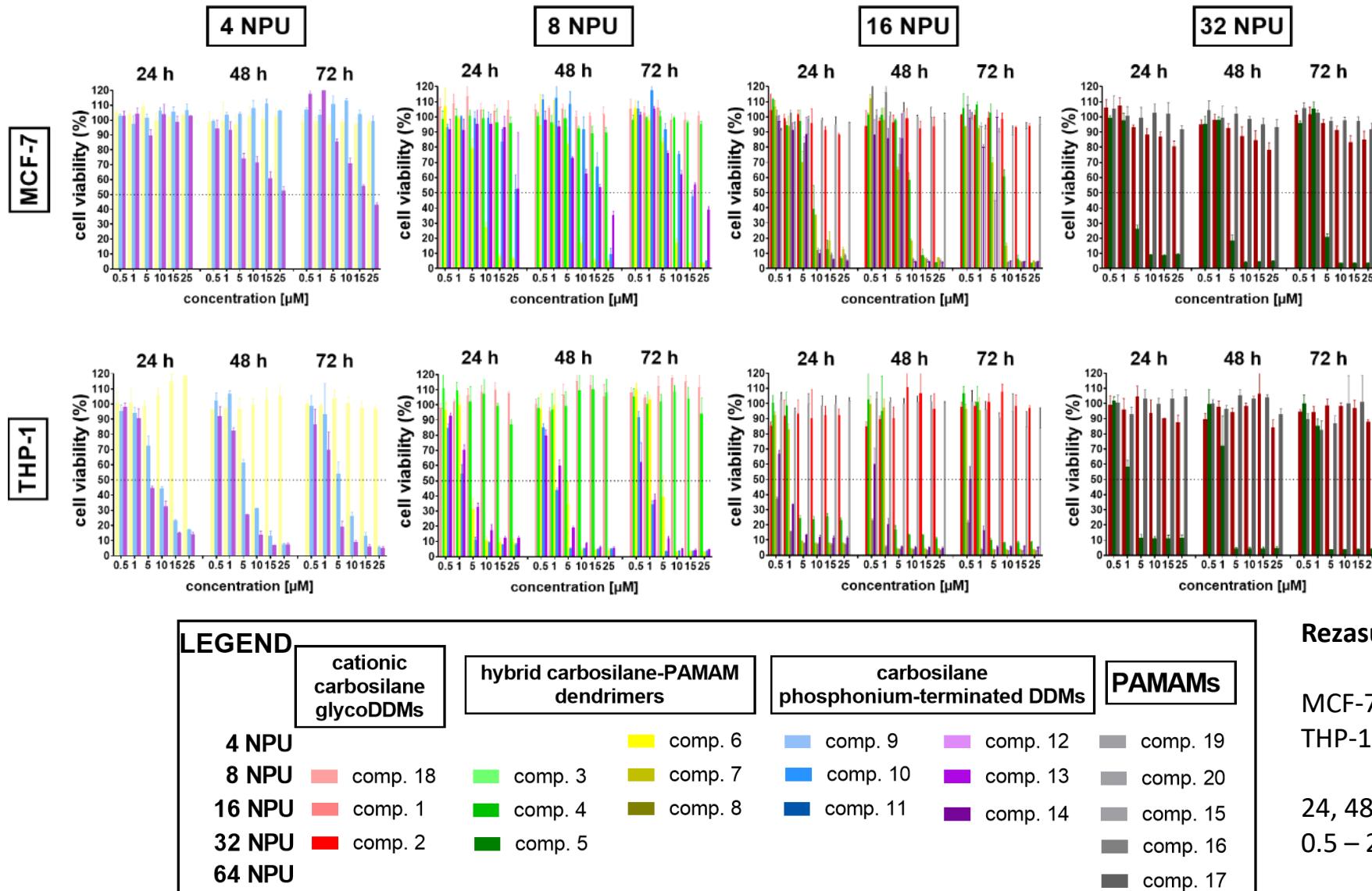
LEGEND

	cationic carbosilane glycoDDMs	hybrid carbosilane-PAMAM dendrimers	carbosilane phosphonium-terminated DDMs	PAMAMs
4 NPU				
8 NPU	comp. 18	comp. 3	comp. 6	comp. 9
16 NPU	comp. 1	comp. 4	comp. 7	comp. 12
32 NPU	comp. 2	comp. 5	comp. 8	comp. 10
64 NPU			comp. 11	comp. 13
				comp. 14
				comp. 19
				comp. 20
				comp. 15
				comp. 16
				comp. 17

Pool of dendrimers



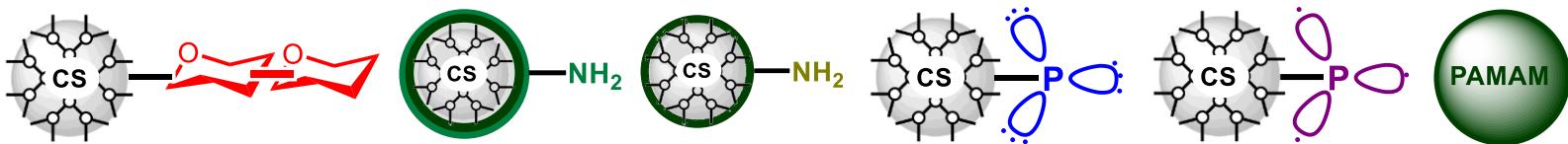
Cytotoxicity of dendrimers: influence of the structure and NPU



In vitro cytotoxicity – IC50 values

Rezasurin assay

24h



MCF-7: Human breast cancer

THP-1: human monocytic leukemia

LEGEND

cationic carbosilane glycoDDMs

hybrid carbosilane-PAMAM dendrimers

carbosilane phosphonium-terminated DDMs

PAMAMs

Dendrimer Size	Component	Color
4 NPU	comp. 6	Yellow
8 NPU	comp. 18	Red
16 NPU	comp. 1	Light Red
32 NPU	comp. 2	Dark Red
64 NPU	comp. 3	Green
	comp. 4	Dark Green
	comp. 5	Dark Green
	comp. 7	Yellow-Green
	comp. 8	Dark Yellow-Green
	comp. 9	Blue
	comp. 10	Dark Blue
	comp. 11	Dark Blue
	comp. 12	Purple
	comp. 13	Magenta
	comp. 14	Dark Magenta
	comp. 15	Grey
	comp. 16	Dark Grey
	comp. 17	Very Dark Grey

- ✓ CCS glyco-DDMs are biocompatible
 - ✓ Hybrid CS-PAMAM are very toxic except G1
 - ✓ CS phosp. DDM biocompatible with MCF-7
while toxic to THP-1
 - ✓ Low toxicity of PAMAMs up to G3
(lit. $153 \pm 3 \mu\text{M}$ for G2
 $120 \pm 3 \mu\text{M}$ for G3 after 24h)

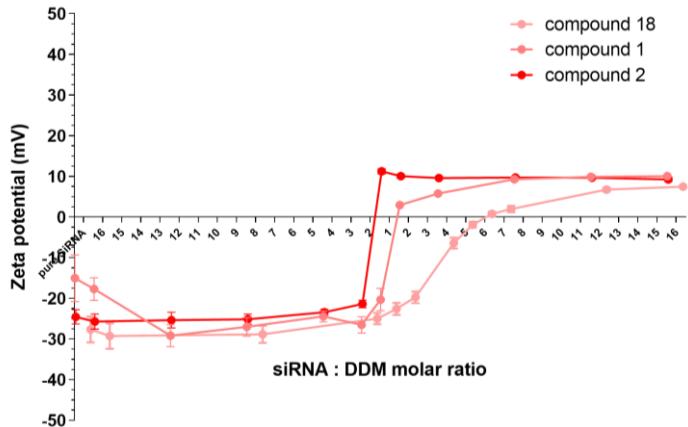
J. Health Science 2009, 5 (2), 169-177

IC₅₀ values (μM)

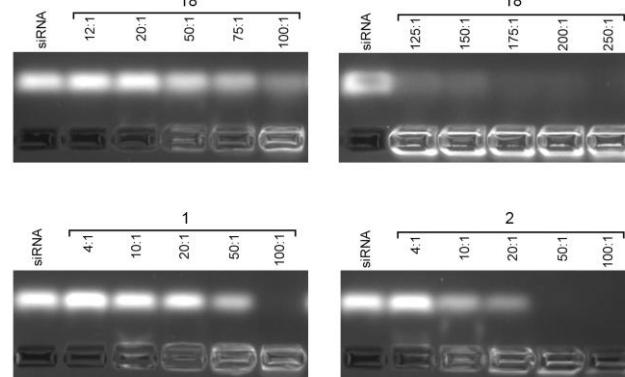
NPU	CCS glycoDDMs		Hybrid CS-PAMAM DDMs				CS phosphonium-terminated DDMs						PAMAMs		
	Zeta	GE*	MCF-7	THP1	MCF-7	THP1	MCF-7	THP1	MCF-7	THP1	MCF-7	THP1	MCF-7	THP1	
4					6	-*	-*	9	-*	7.8±0.8	12	-*	4.5±0.4	19	
8	18	-*	-*	3	-*	-*	7	6.0±1.4	3.2±0.6	10	54±14	1.3±0.2	13	109±28	2.6±0.2
16	1	195±50	-*	4	8.1±2.2	4.0±0.7	8	6.6±1.0	2.0±0.4	11	5.6±1.5	0.3±0.0	14	5.2±1.5	0.7±0.1
32	2	99±15	176±45	5	2.9±0.7	1.8±0.3								16	-*
64	*above 200 µM												17	13±1	50±6

Characterization of nanoparticles

Zeta potential measurement: DDM/siRNA complex ratio



Gel electrophoresis: Stability of the DDM/siRNA complexes



- ✓ The tested dendritic platforms form saturated complexes in much lower molar ratio compared to PAMAMs
 - ✓ ? Protection of the siRNA from nucleases?
 - ✓ Release of genetic material in acidic environment?

DDM : siRNA

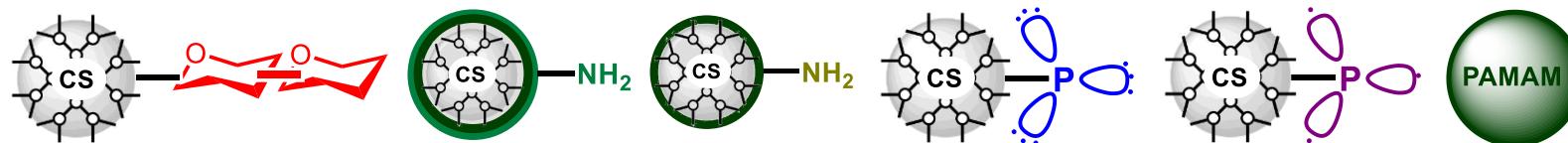
NPU	CCS glycoDDMs		Hybrid CS-PAMAM DDMs				CS phosphonium-terminated DDMs						PAMAMs					
	Zeta	GE*	Zeta	GE*	Zeta	GE*	Zeta	GE*	Zeta	GE*	Zeta	GE*	Zeta	GE*				
4					6	73.0	400	9	8.9	NA	12	10.3	50	19	x	50 000		
8	18	6.0	175	3	40.6	100	7	13.9	50	10	3.2	NA	13	4.8	20	20	800	4 000
16	1	1.9	100	4	3.6	50	8	8.6	16	11	0.7	NA	14	3.1	20	15	12.8	25
32	2	0.7	50	5	0.5	10									16	11.0	12	
64															17	0.1	5.1	

Summary

- ✓ Direct inter-structural comparison of the cytotoxicity of **20** dendritic nanovectors
- ✓ Nanoparticle characterization (DLS measurements, Zeta potential, gel electrophoresis)

...still „to do“:

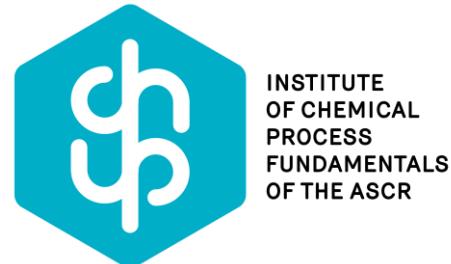
- DDM/siRNA complex cytotoxicity?
- transfection efficiency and cellular internalisation? /flow cytometry, confocal microscopy/
- gene silencing? /RT-PCR/



Acknowledgements

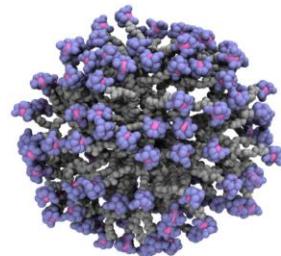


COST ACTION CA 17140
NANO2CLINIC
CANCER NANOMEDICINE - FROM THE
BENCH TO THE BEDSIDE



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MSc. Wahid Tattan
BSc. Susanne Krkoška*



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NANO2CLINIC

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