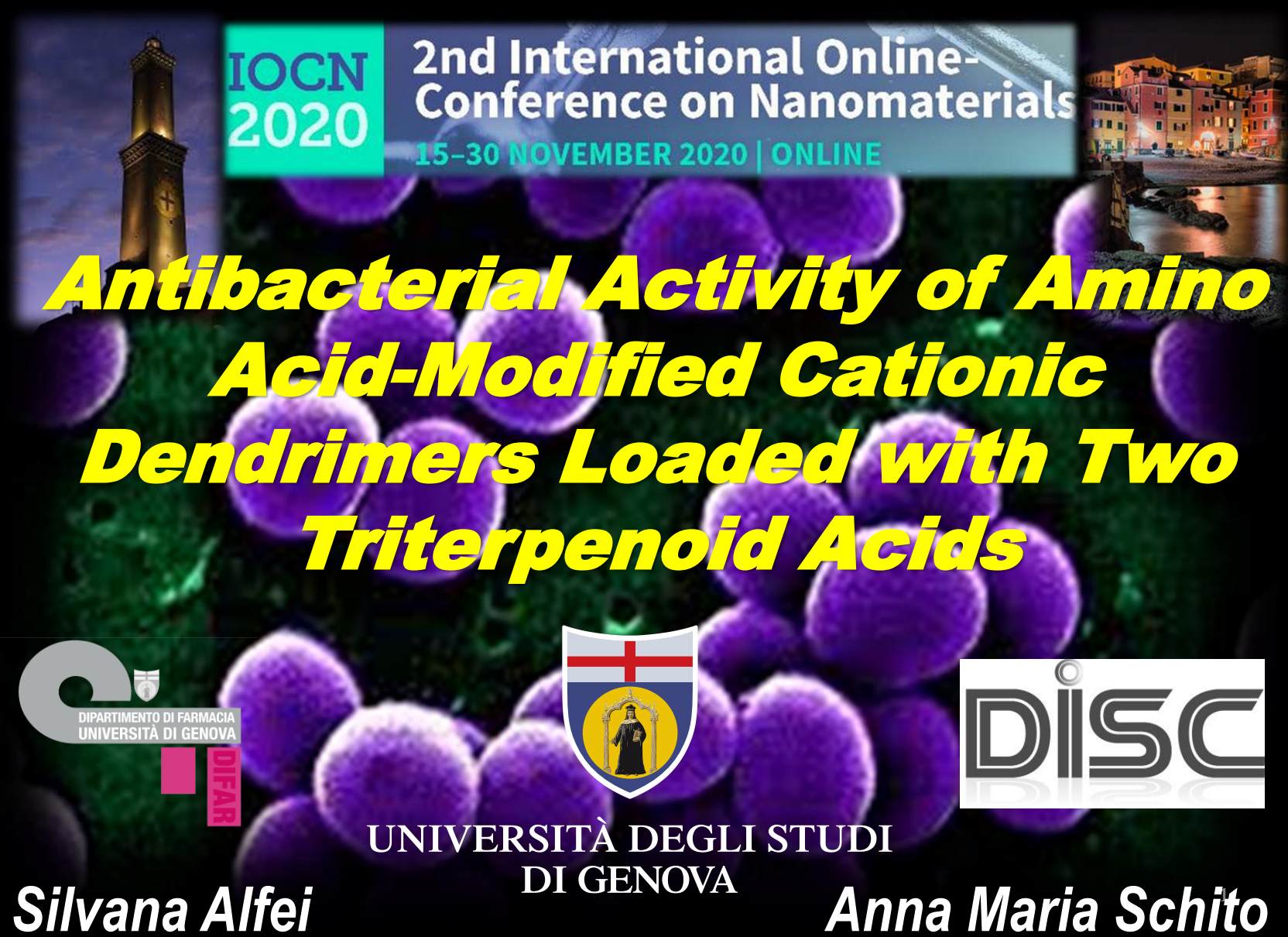
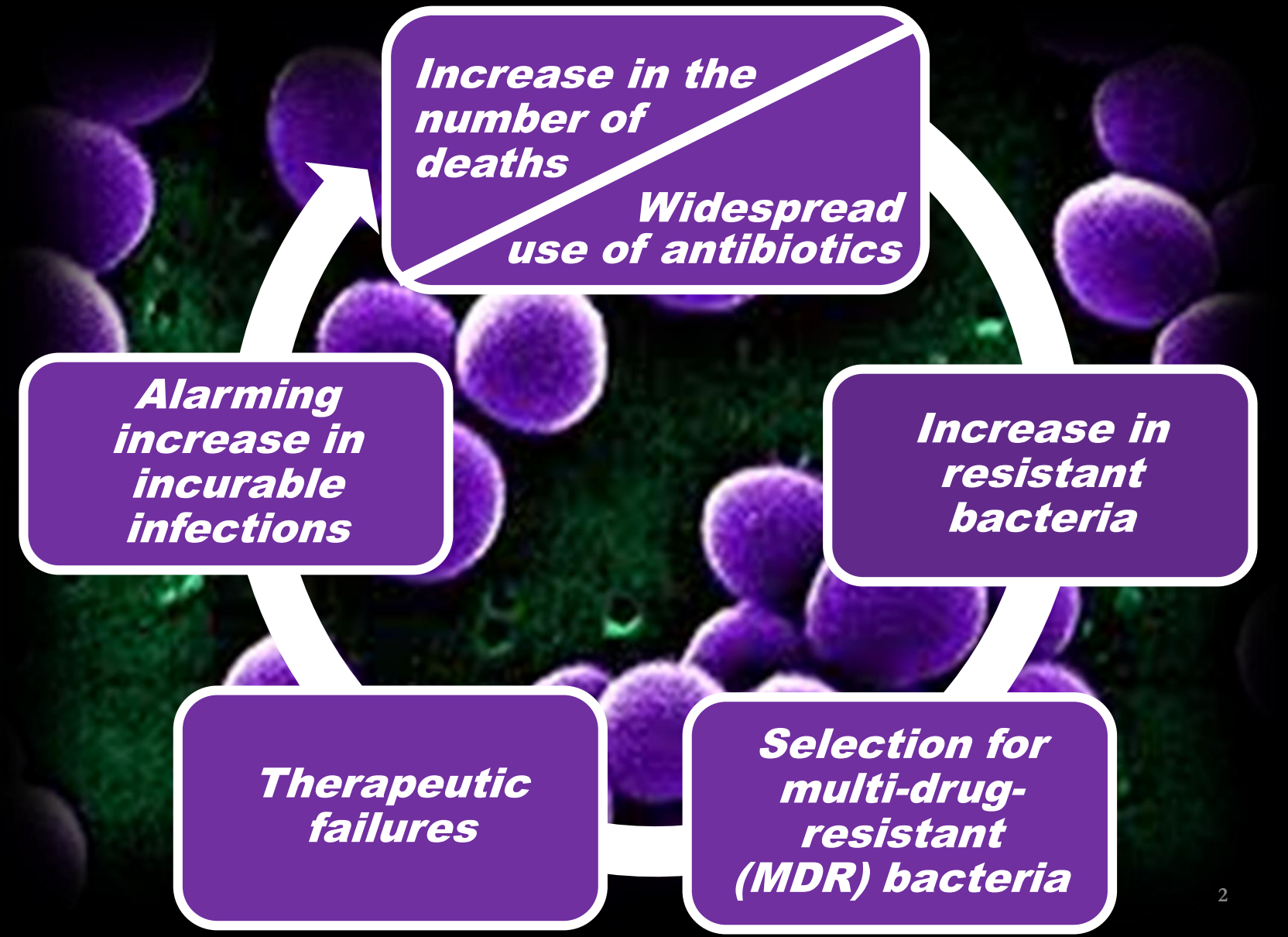
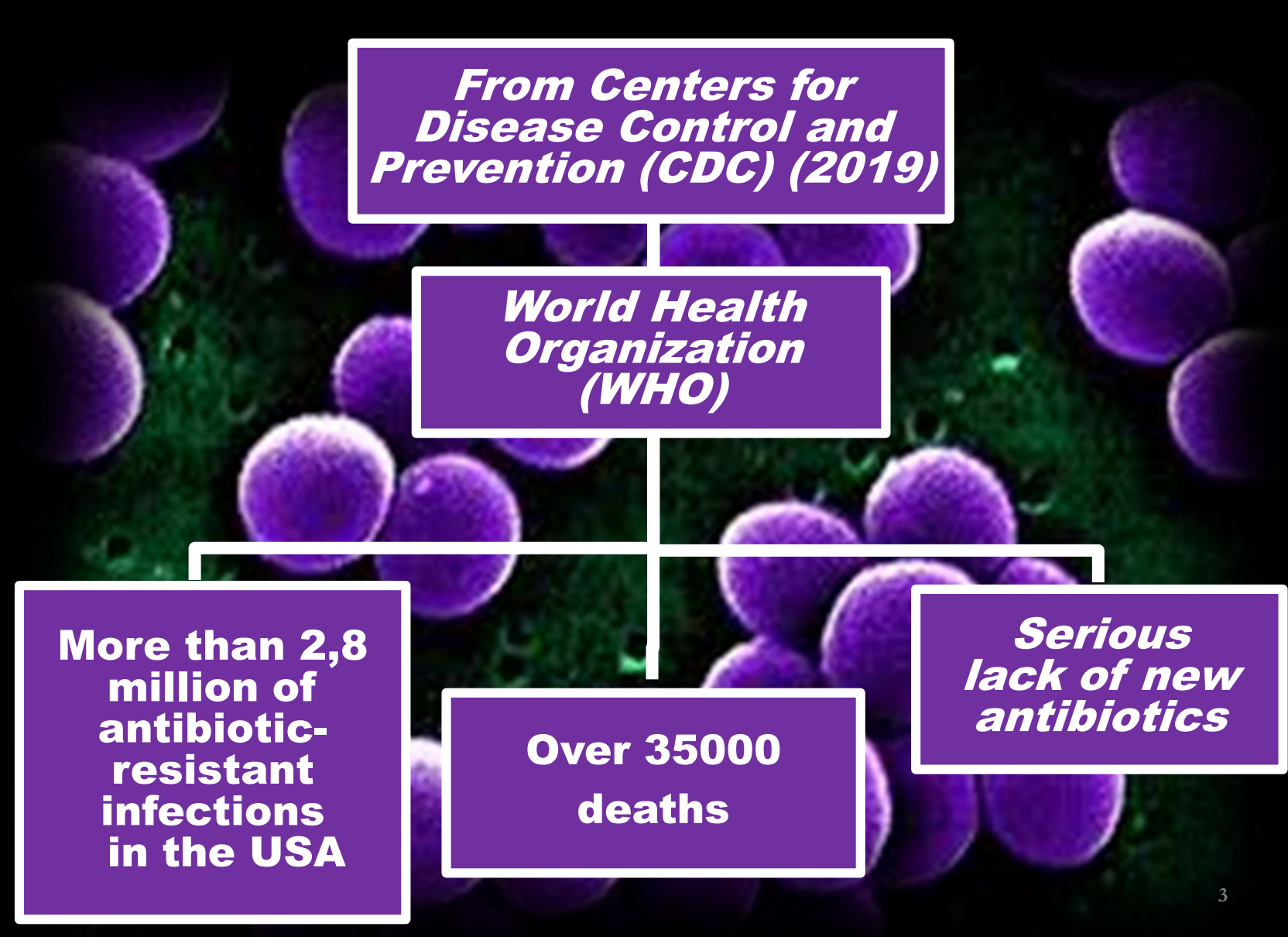
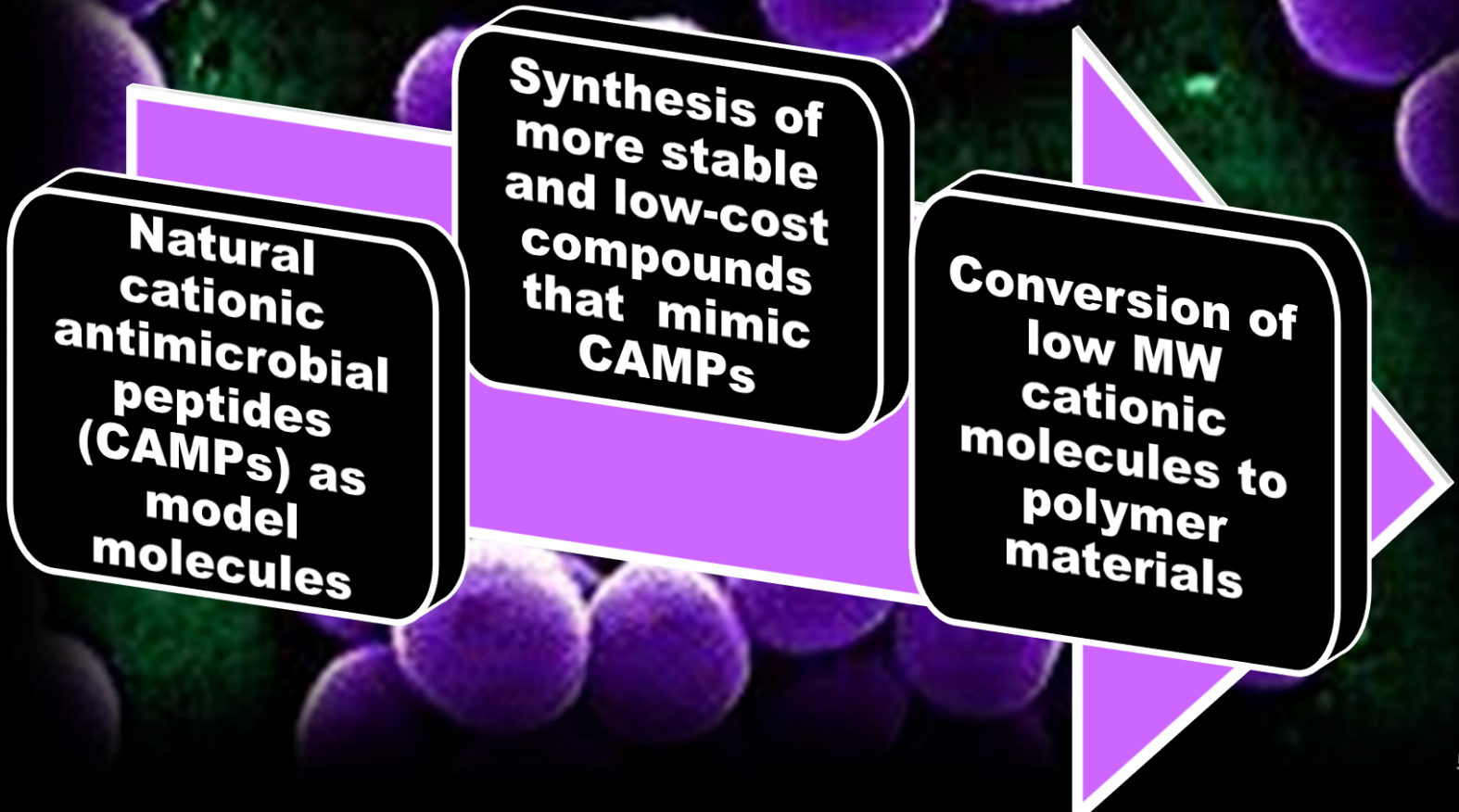

IOCN 2020
2nd International Online-Conference on Nanomaterials
15–30 NOVEMBER 2020 | ONLINE
Antibacterial Activity of Amino Acid-Modified Cationic Dendrimers Loaded with Two Triterpenoid Acids
DIPARTIMENTO DI FARMACIA
UNIVERSITÀ DI GENOVA
DIFAR
DISC
UNIVERSITÀ DEGLI STUDI
DI GENOVA
Silvana Alfei
Anna Maria Schito


Increase in the number of deaths
Widespread use of antibiotics
Increase in resistant bacteria
Selection for multi-drug-resistant (MDR) bacteria
Therapeutic failures
Alarming increase in incurable infections

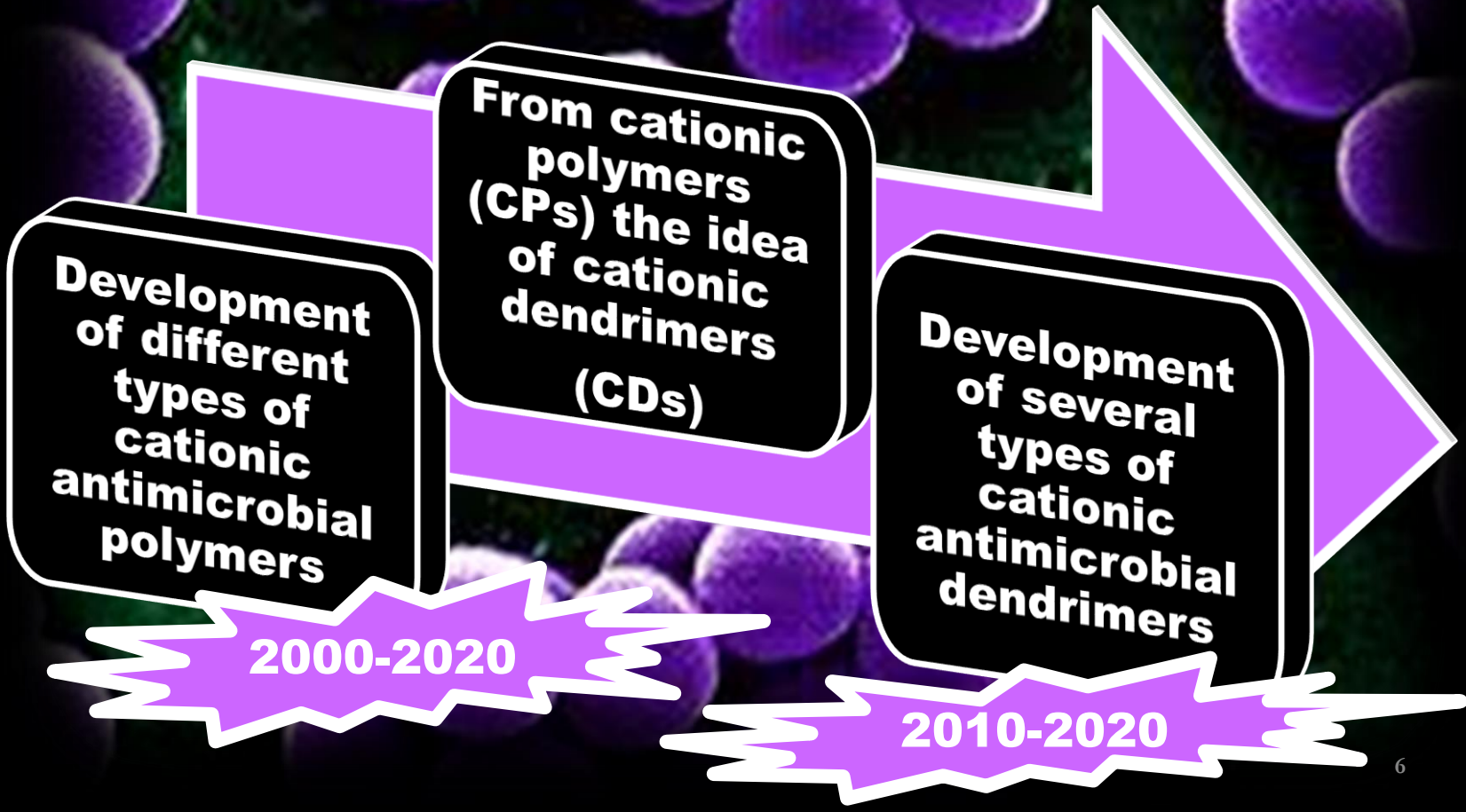

From Centers for Disease Control and Prevention (CDC) (2019)
World Health Organization (WHO)
More than 2,8 million of antibiotic-resistant infections in the USA
Over 35000 deaths
Serious lack of new antibiotics


Hard work
is needed to
develop new
antimicrobial
agents

A Current Strategy Involves The Following Steps

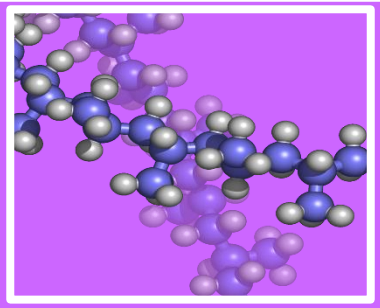


Further Developments



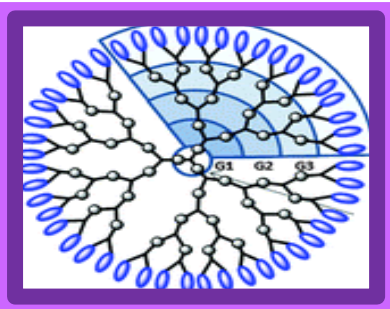
Advantages Provided by Macromolecular Structure

CPs



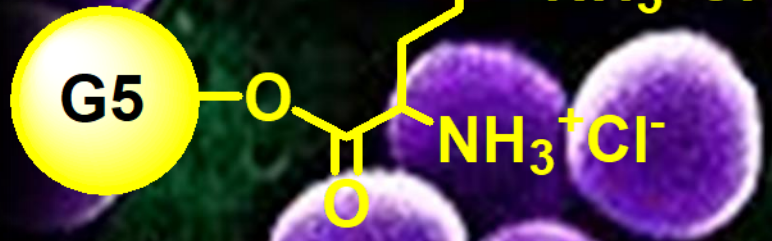
- More long-term activity
- Limited residual toxicity
- Chemical stability
- Non-volatility
- No permeation through the skin thanks to macromolecular structure and high MW

CDs



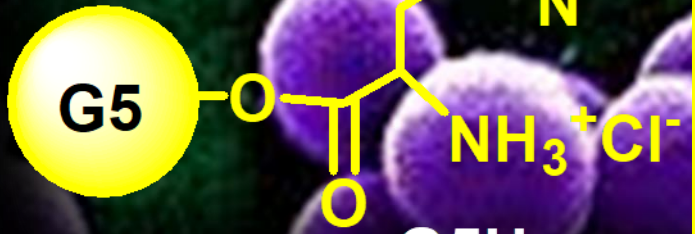
- Tree-like generational structure
- Symmetric spherical architecture
- Monodisperse macromolecules
- Nano dimensions
- Inner cavities to host drugs
- High number of peripheral functional groups

Our Previous Fifth Generation Cationic Dendrimers (G5CDs)



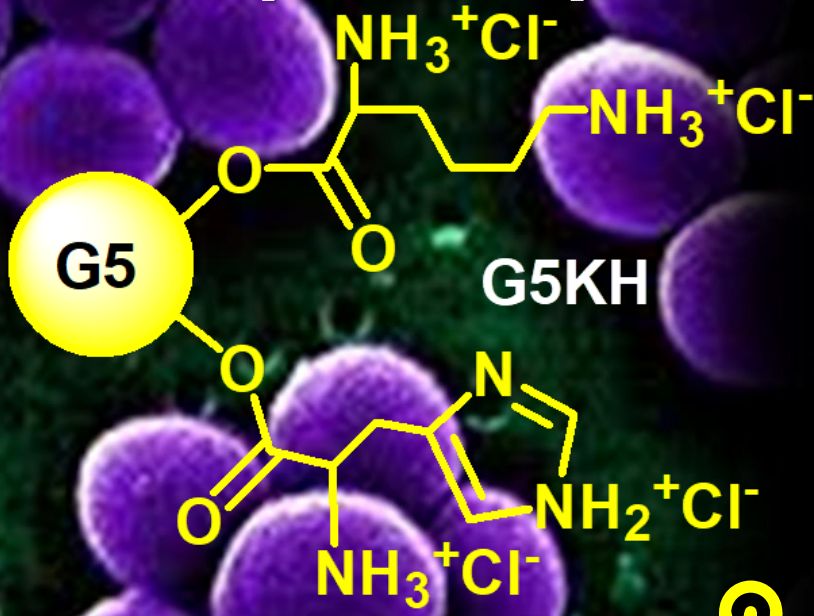
G5K

K=Lysine



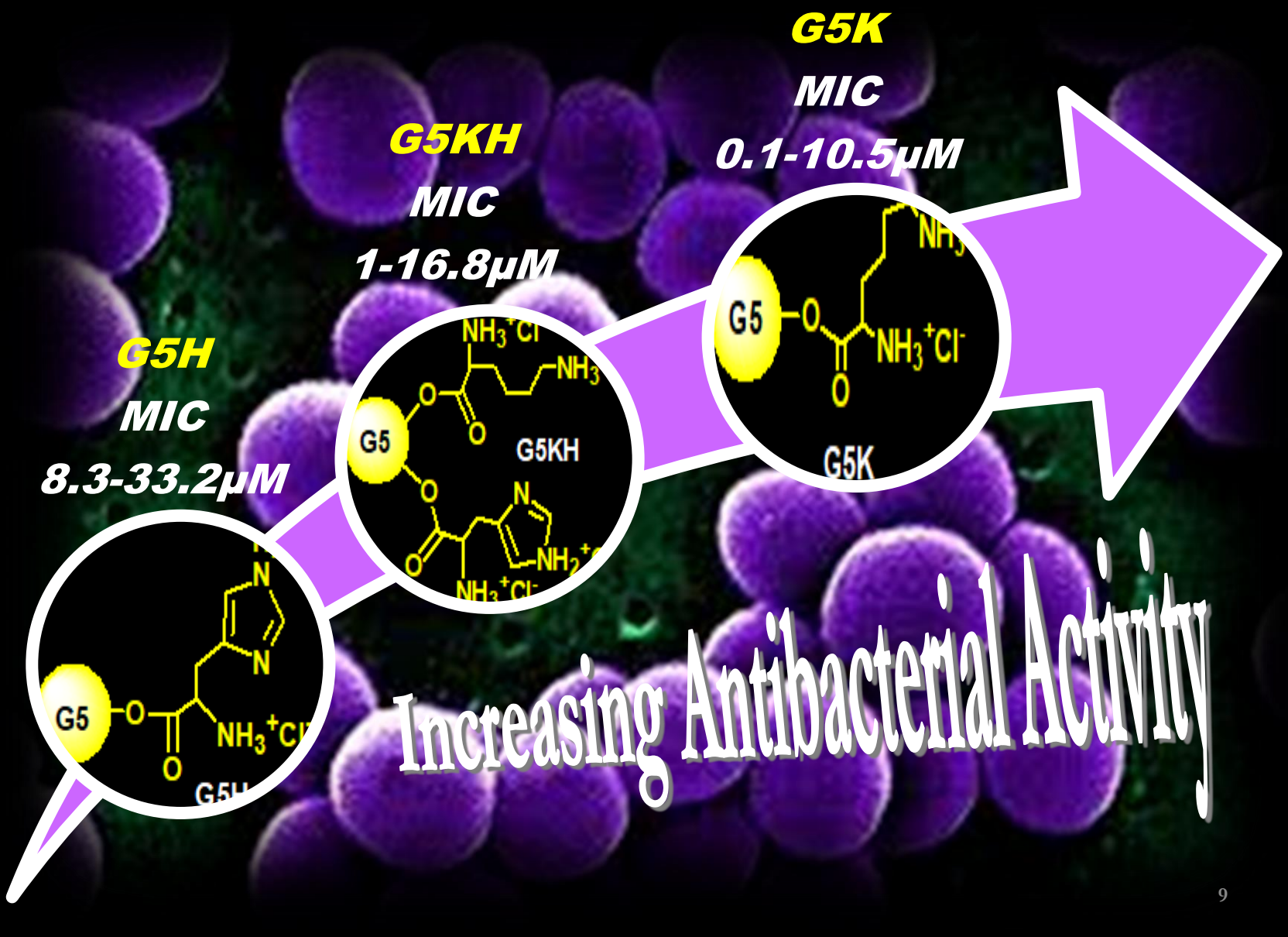
G5H

H=Histidine

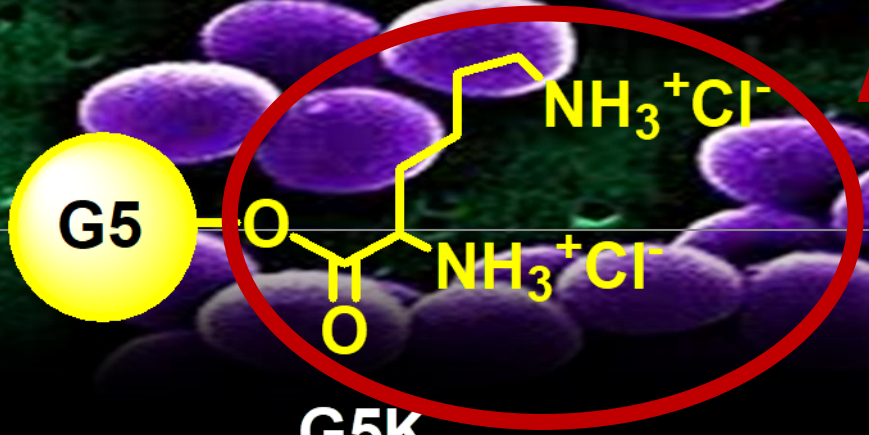


G5KH

Active Against Non-Fermenting Gram-negative Species

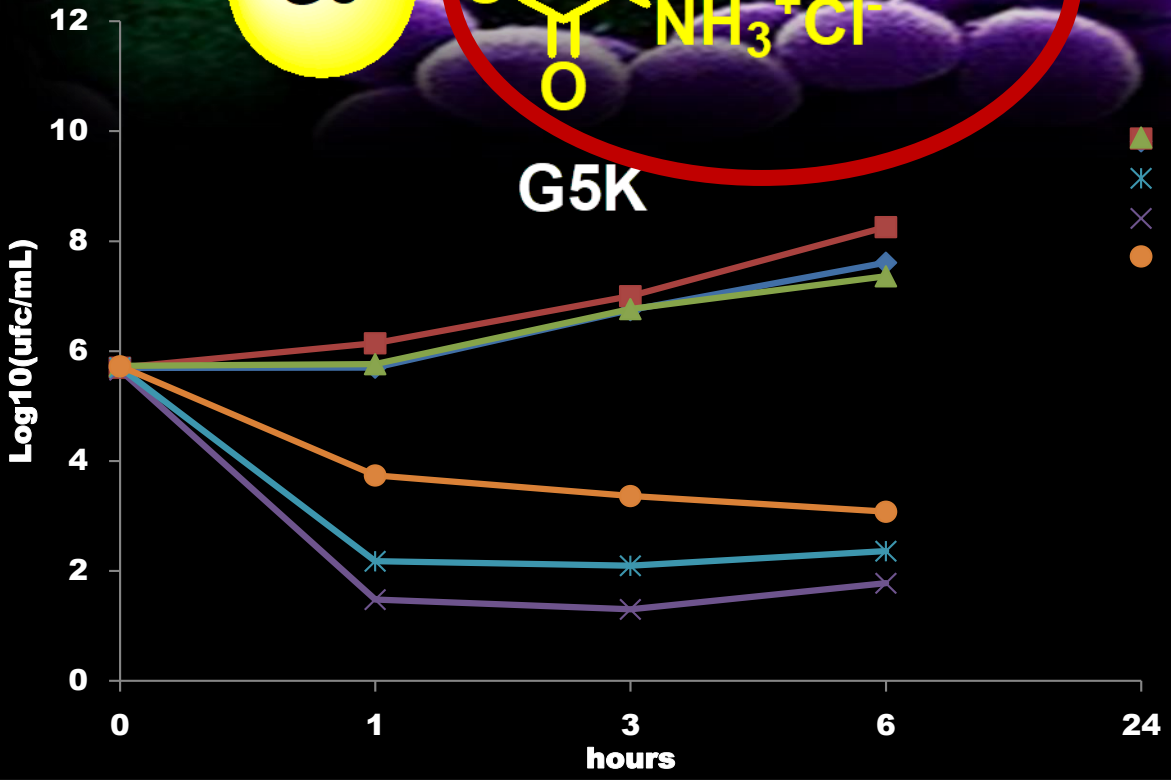

G5K
MIC
0.1-10.5μM
G5KH
MIC
1-16.8μM
G5H
MIC
8.3-33.2μM
G5
G5KH
G5K
Increasing Antibacterial Activity

Time Killing Profile of the most active dendrimer



G5K

Lysine was pivotal for potency



This new Study

Strategy

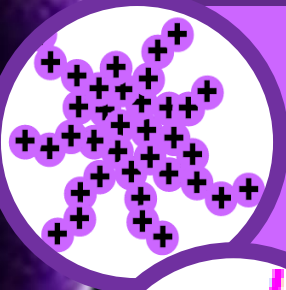
To use similar CDs(1)
To maintain lysine(2)
To include arginine(3)

Scope

To develop new antibacterial agents, active against other resistant bacterial species

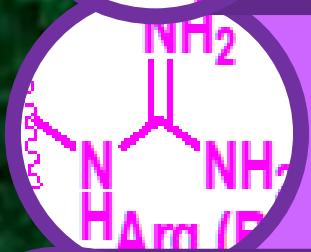
To include some compound known for being active against different bacterial species(4)

Uppercase Notes Explanation



(1) Dendrimers similar to G5CDs were chosen because, thanks to their high cationic character, they would have acted as membrane disruptors

(2) Lysine (K) was preferably retained because it was found to be essential for providing CDs with high antibacterial potency



(3) Arginine (R) was included because, as reported, compounds containing cationic guanidine are especially active against Gram-positive bacteria

(4) We hypothesized that the presence of compounds known to be active on bacterial species other than Gram-negative ones could help redirect the activity towards other targets.

A Previous Study Considered Helpful

The strategy

CDs including those containing arginine have been used to trap UOA

Its Scope

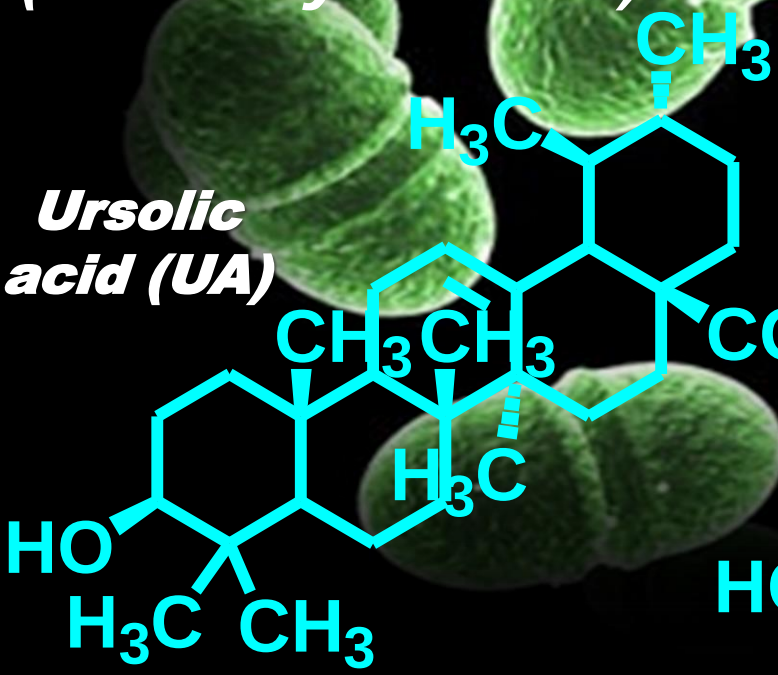
To make a mixture of extracts of ursolic and oleanolic acids (UOA) water-soluble and administrable in vivo

Results

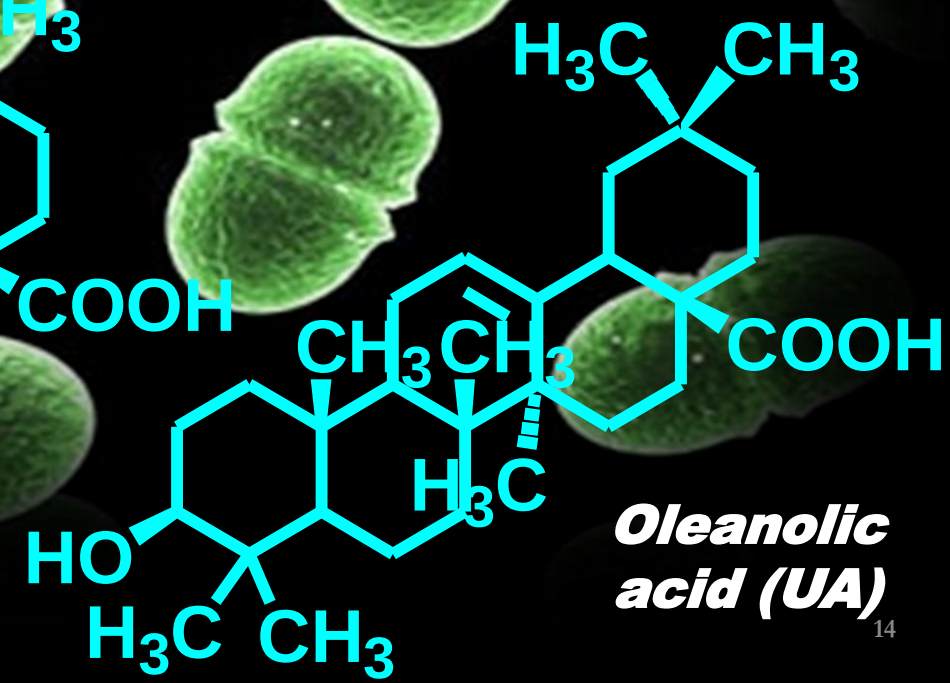
Six cationic dendrimers were obtained, loaded with UOA and soluble in water

Structures of Compounds Entrapped in CDs (UOA)

UA and OA are natural occurring triterpenoid acids known for having antibacterial properties particularly against Gram-positive species. A crude extract of UOA was kindly provided by Prof. Angela Bisio (University of Genoa).

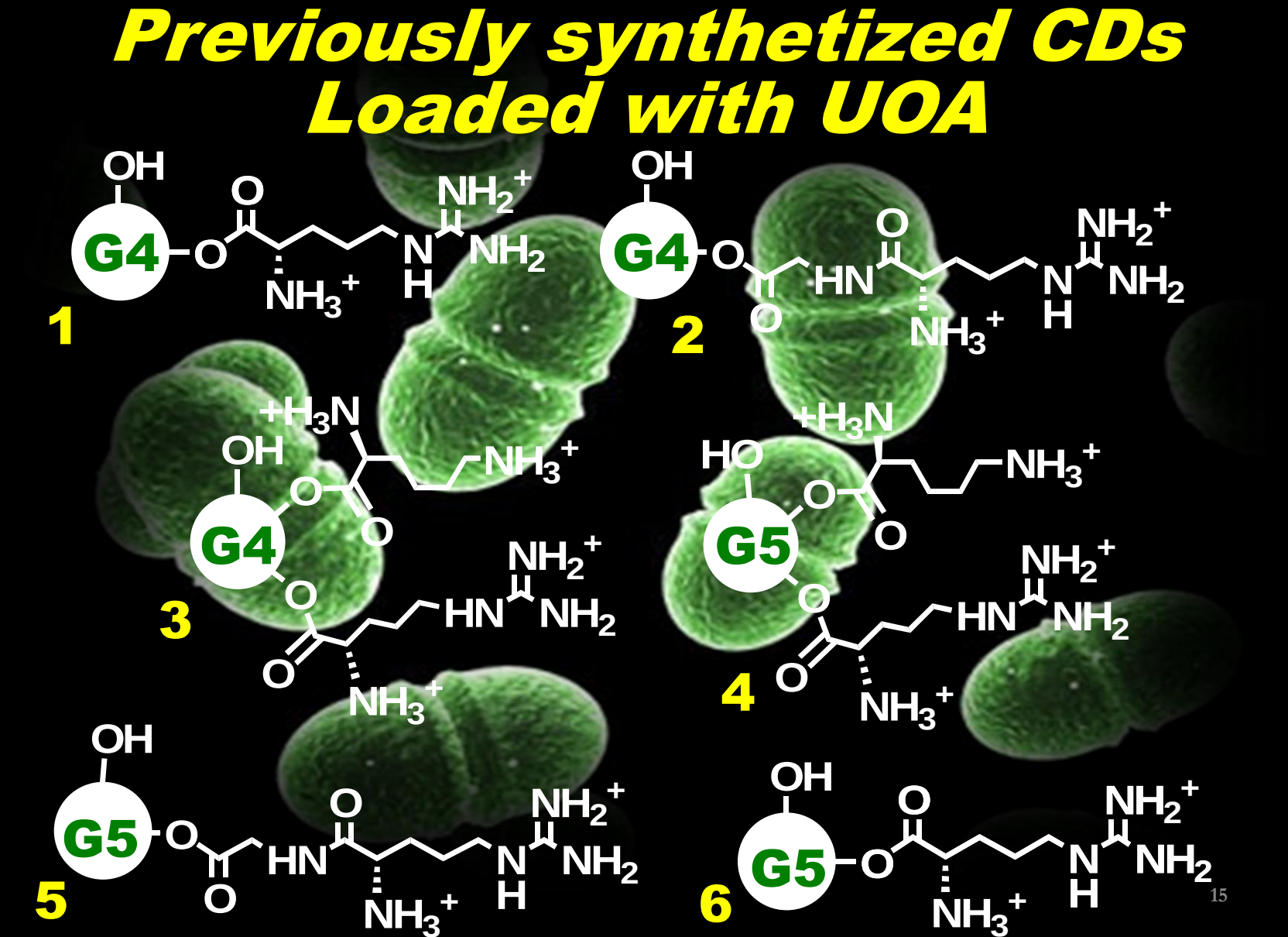


Ursolic acid (UA)

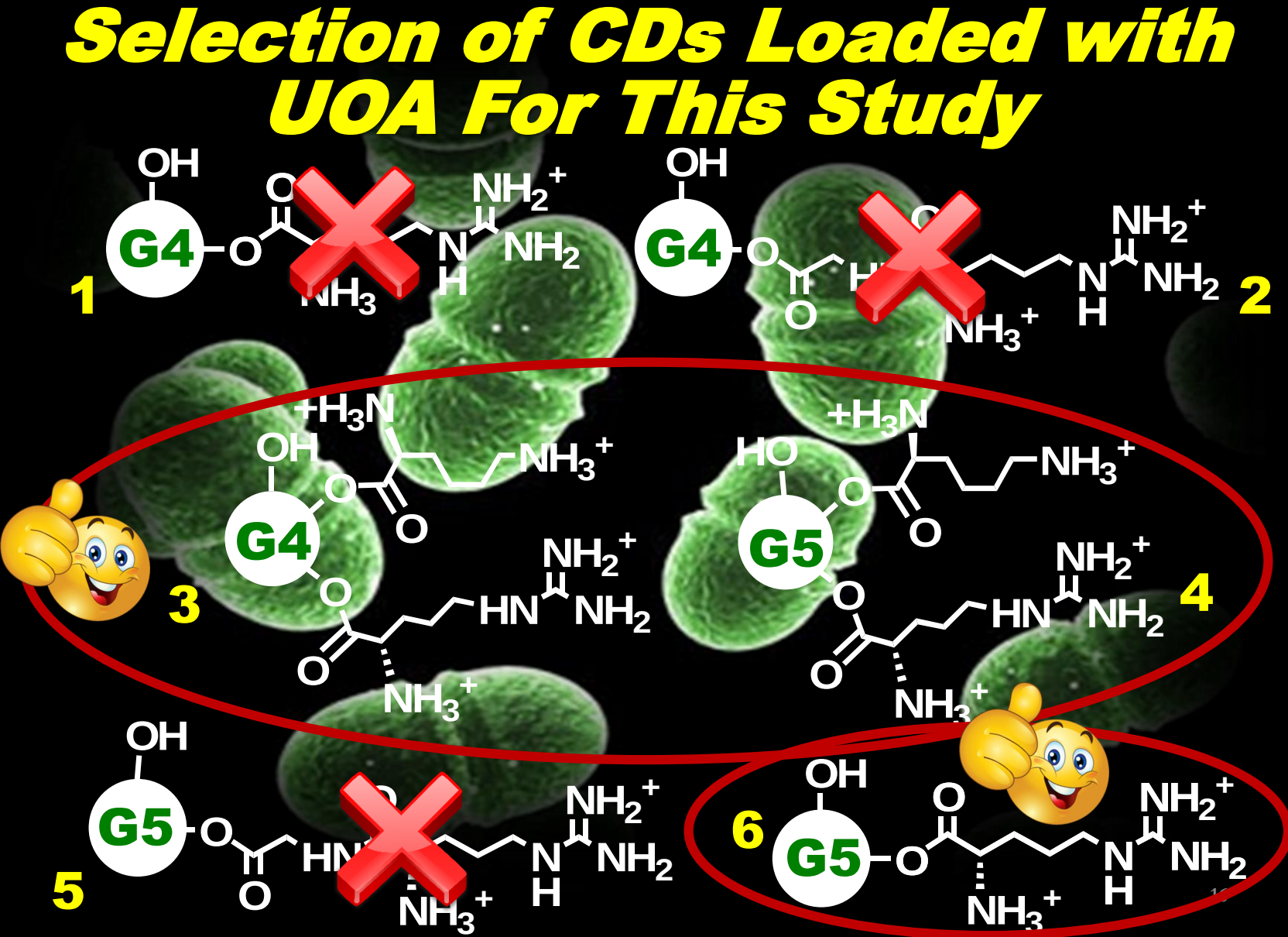


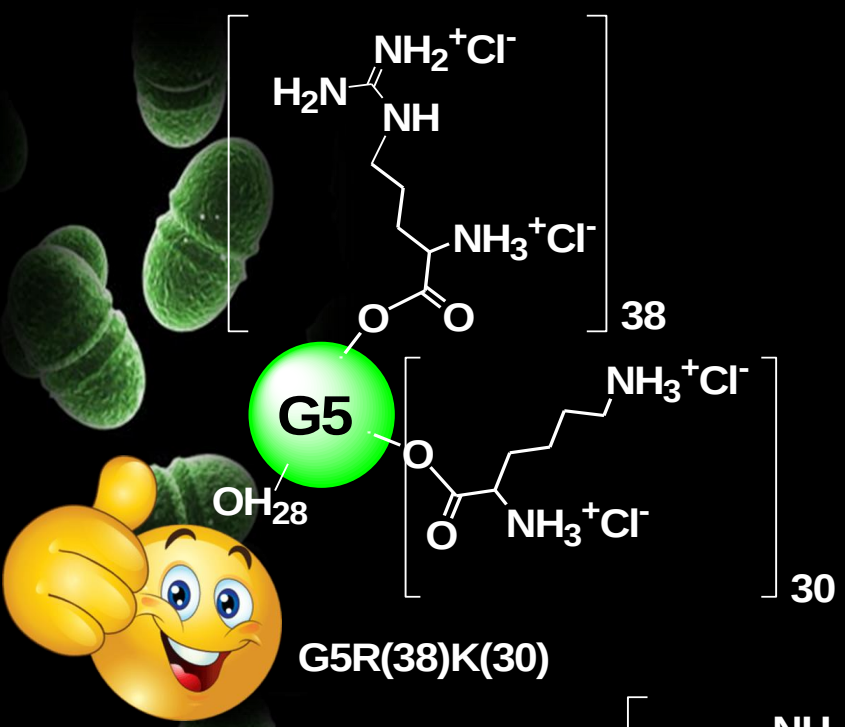
Oleanolic acid (UA)

Previously synthetized CDs Loaded with UOA

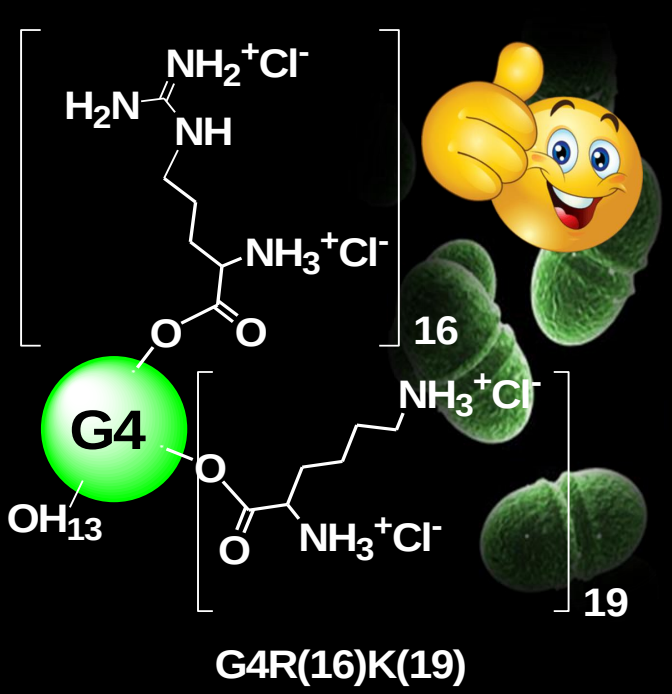


Selection of CDs Loaded with UOA For This Study



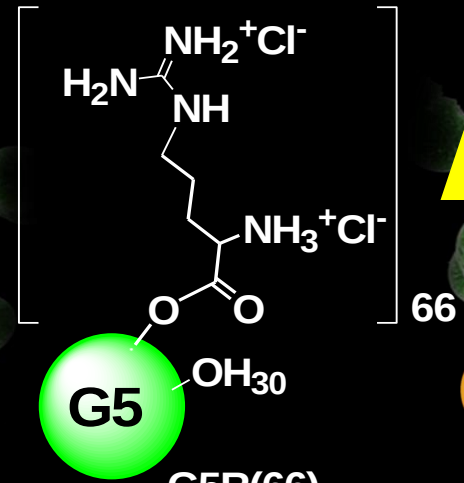


G5R(38)K(30)



G4R(16)K(19)

Selected Dendrimers



G5R(66)

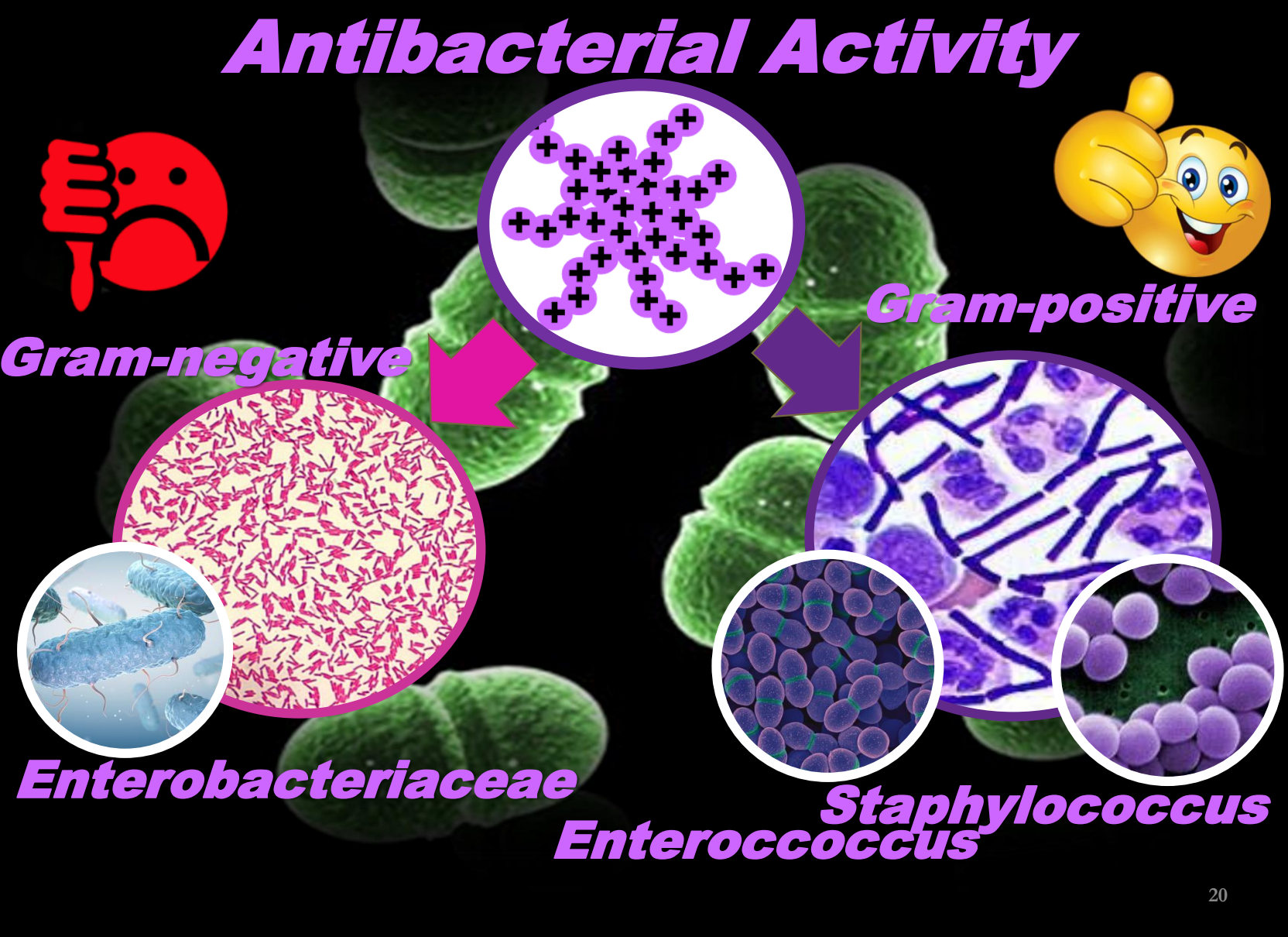
Reasons for This Selection

Not Selected Dendrimers	Selected Dendrimers	Reasons
1		Not containing K G4CD
2		Not containing K G5CD
	3	K-containing G4CD
	4	K-containing G5CD
5		Only R-containing G4CD
	6	Only R-containing G5 CD

Main Features of Selected Dendrimers

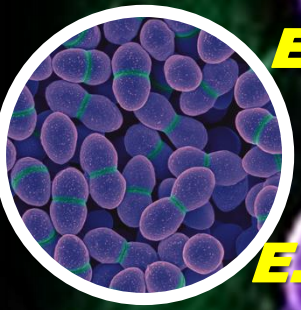
Features	G4R(16)K(19)	G5R(38)K(30)	G5R(66)
Arginine, lysine, hydroxyls units	16, 19, 13	38, 30, 28	66, 0, 30
UOA moles *per* dendrimer mole	4	8	3
UOA loading % (wt/wt)	12.6	12.7	5.0
Cationic groups	70	136	132
Molecular Weight	14600	29300	27400
UOA released after 24 h (%)	75.2	65.9	65.2
Z-potential (mV)	24.8 ± 0.2	31.8 ± 0.1	34.0 ± 0.6
Z-Ave size (nm)	24.9 ± 1.1	20.3 ± 3.1	16.1 ± 2.1
UOA released by complexes after 24h (µg/10 mg)	75.5	65.9	65.2

Antibacterial Activity



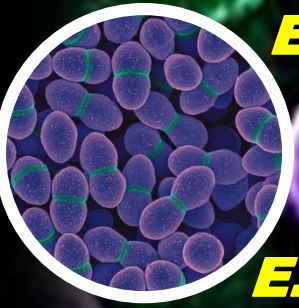
MIC (μM) of CDs loaded with UOA

	G5R	G4RK	G5RK
S. aureus MRSA	9.3-18.7	17.5-35.1	4.4-8.7
	Free UOA 35.0-70.1		
S. epidermidis MRSE	4.7-9.3	8.8-17.5	2.2-4.4
	Free UOA 35.0-70.1		



E. fecalis VRE	9.3-18.7	2.2-4.4	0.5-1.1
	Free UOA 8.8-17.5		
E. faecium VRE	9.3-18.7	2.2-4.4	0.5-1.1
	Free UOA 4.4-8.8		

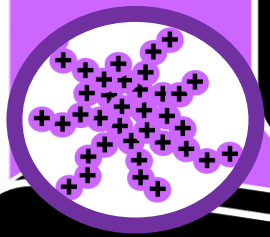
UOA does not contribute to the antibacterial activity observed



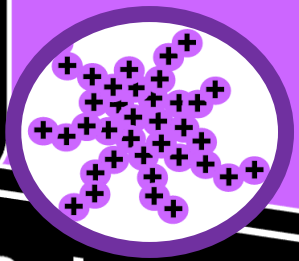
	MIC values of UOA (μM)	Max UOA released (μM)
S. aureus MRSA	35.0-70.1	Too Low / 1.8-8.5
S. epidermidis MRSE	35.0-70.1	Too Low / 0.9-4.2
E. fecalis VRE	8.8-17.5	Too Low / 0.3-7.4
E. faecium VRE	4.4-8.8	Too Low / 0.3-7.4

Conclusions

K is essential for obtaining potent antimicrobial CDs and R for directing activity towards Gram-positive species

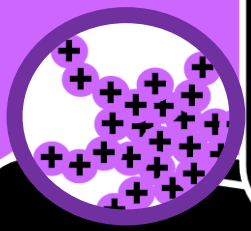


The R/KCDs tested in this study displayed remarkable antibacterial activity against 12 strains of 4 Gram-positive species



The observed antibacterial activity is attributable only to CD carriers and not to the presence and release of UOA

G5RK CD, harmonizing a high cationic character with a balanced R/K content, showed very high antimicrobial effects (MICs = 0.5-8.7 µM)



G5RK CD could represent a promising alternative to several conventional antibiotics now plagued by the burden of widespread resistance

The Future

More in-depth investigations are underway to better characterize the bioactivity of the developed CDs

Silvana Alfei & Anna Maria Schito

DIPARTIMENTO DI FARMACIA
UNIVERSITÀ DI GENOVA
DIFAR

DISC

IOCN 2020