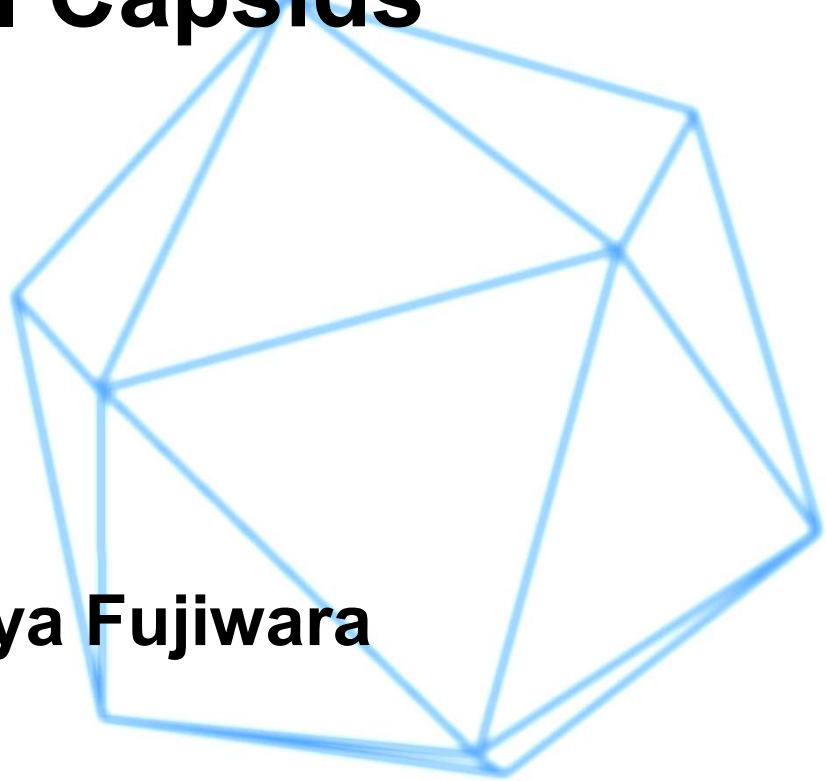
De Novo Designed Antimicrobial Capsids



2018/12/22 Takuya Fujiwara

Contents

1. Introduction

    - the structure and function of capsids

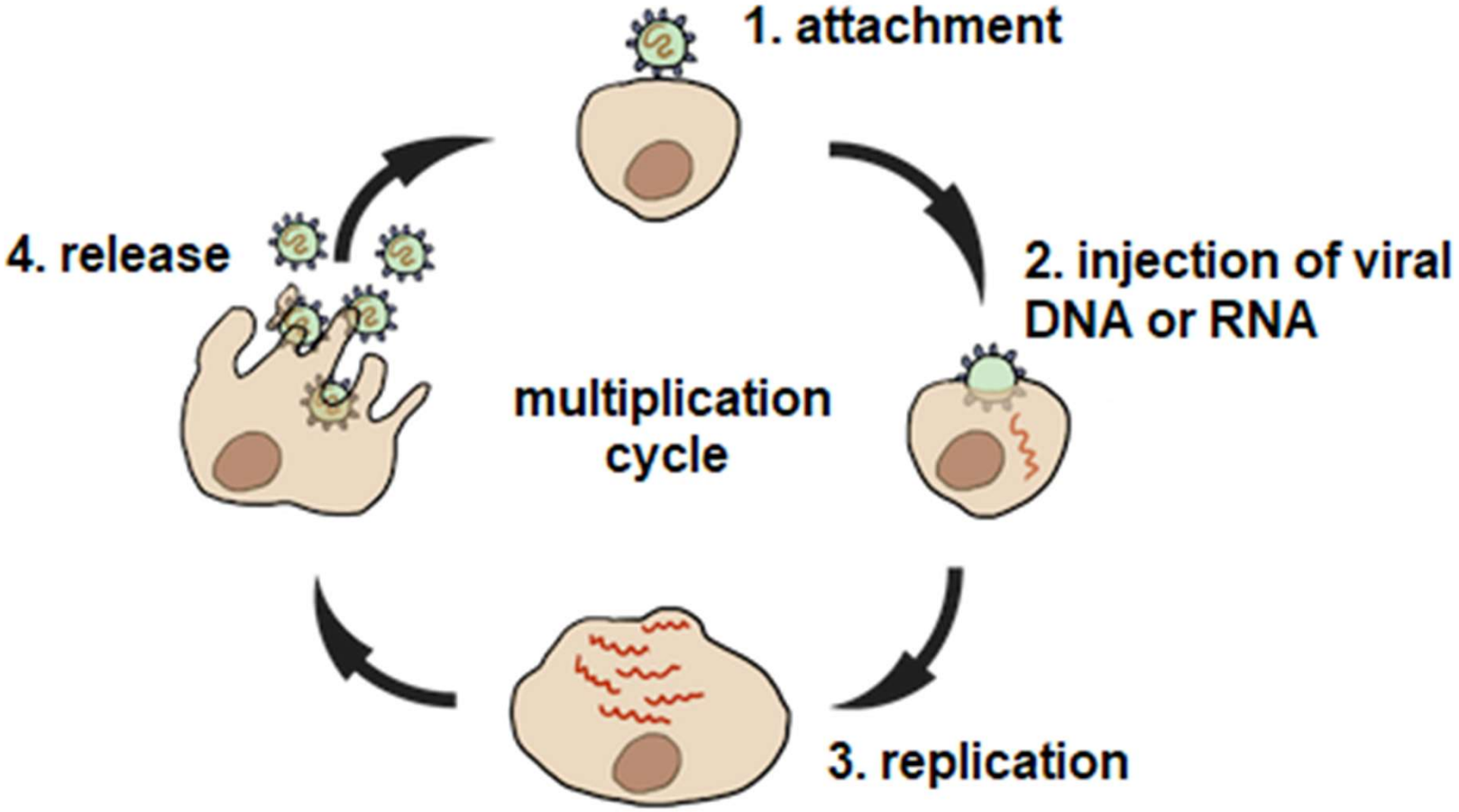
2. Main topic: “Antimicrobial peptide capsids of de novo design”

    Emiliana. D. S.; Hasan. A.; Baptiste. L.; Nilofar. F.; Angelo. B.; James. E. N.; Nicola. M.; Santanu. R.; Jonathan. R. B.; Alexander. R. Y.; Bart. W. H.; Maxim. G. R. *Nat. Commun.* 2017, *8*(1), 2263.

3. Future perspective: My proposal

Contents

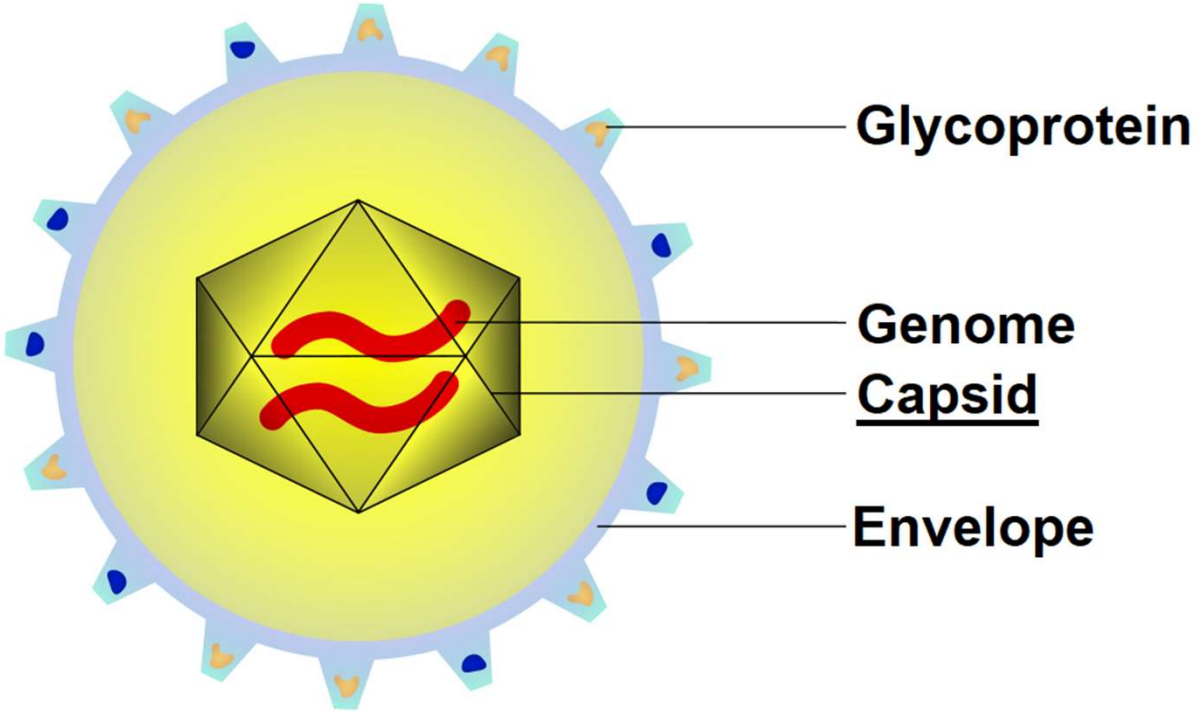
Multiplication of Virus



Viruses cannot multiply by themselves
Instead, viruses have the host cells replicate their genomes

Image is retrieved from https://www.shmoop.com/microorganisms-viruses/viral-entry.html

What is Capsids?



Capsids:

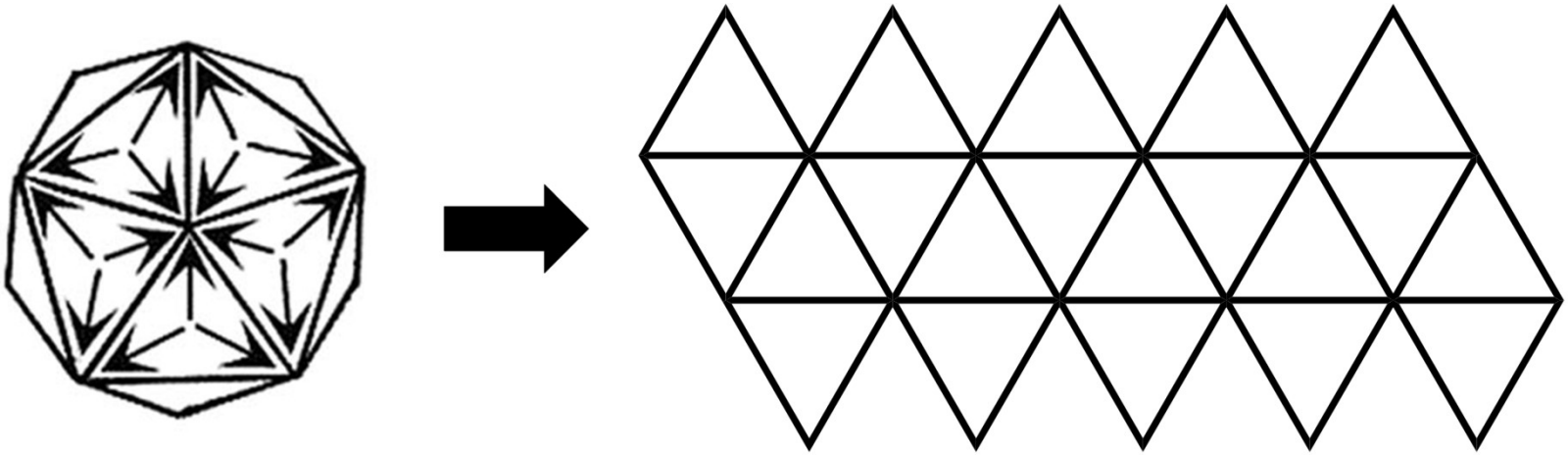
- **enclose and shield the virus genome (DNA or RNA)**
- **consist of several oligomeric subunits made of protein**
- **Icosahedral structures are most common**

Image is retrieved from https://en.wikipedia.org/wiki/Capsid

Icosahedral Structure of Capsids (1)

Icosahedron consists of 20 triangular faces and 12 fivefold vertexes
Each triangle is subdivided into three proteins

Therefore, the simplest capsid would be made up by a total of
(20x3 =) 60 proteins → *T* = 1 capsid

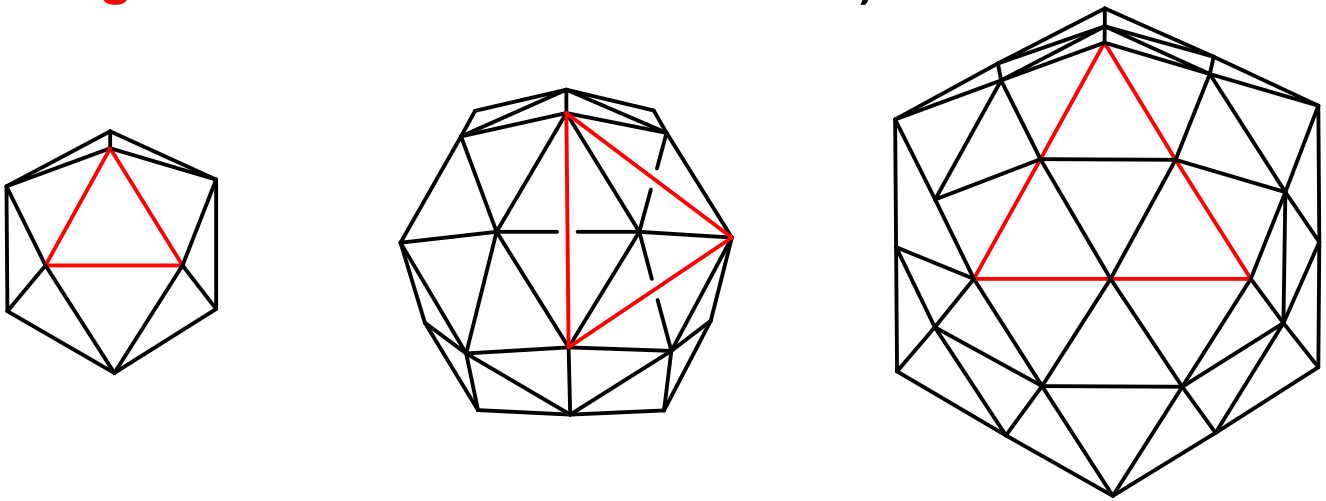


(each arrow represents one protein)

Icosahedral Structure of Capsids (2)

How many proteins compose each face is reflected in Triangulation number *T*

Each capsid is divided into 20*T* triangle (shown in black)
(Red triangle is one face of icosahedron)



T = 1 *T* = 3 *T* = 4

Feasible triangulation number *T* is found in a simple equation:

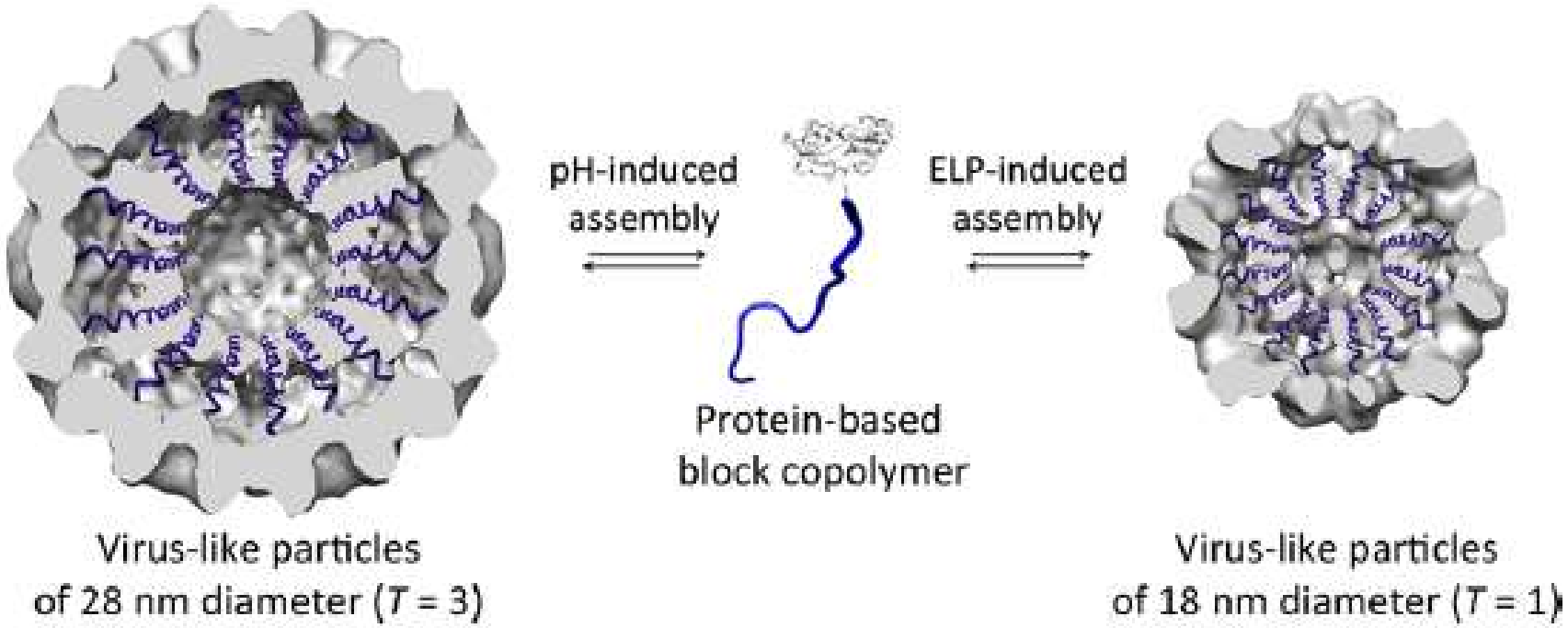
$$T = k^2 + k \times h + h^2$$

Where k and h are any integers with no common factor

Icosahedral Structure of Capsids (3)

Capsids have structural plasticity:

- The size variations are reflected in different *T* numbers and these conformation are in equilibrium
(example of cowpea chlorotic mottle virus is shown below)



Mark. B. van E.; Joseph. C.-Y. W.; Inge. J. M.; Chenglei. L.; Adam. Z.; Roeland. J. M. N.; Jeroen. J. L. M. C.; Jan. C. M. van H. *J. Am. Chem. Soc.* **2012**, *134*, 18506-18509

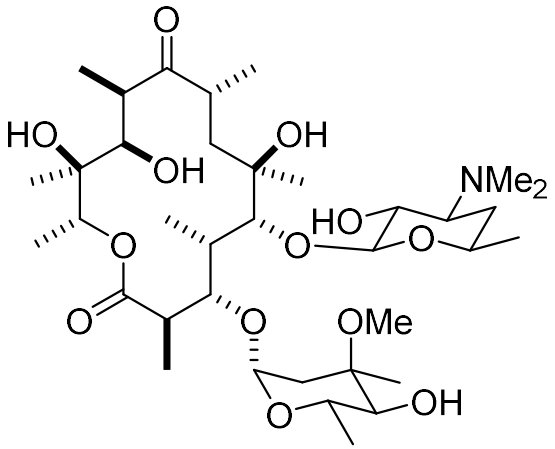
Contents

Antimicrobial Drug Strategy

Conventional approach:

<u>"targeting intercellular processes" strategy</u>

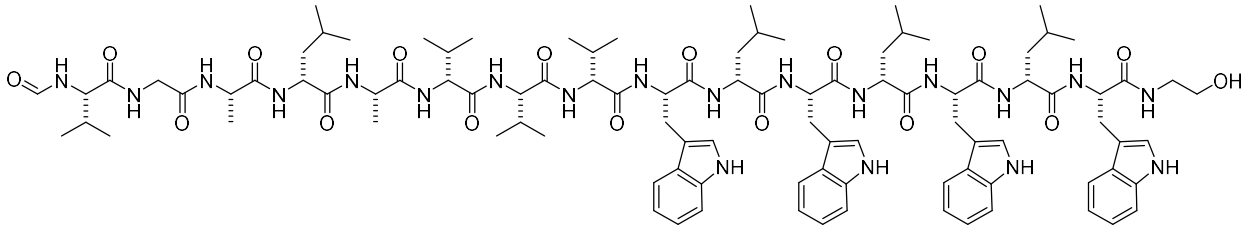
- **rapidly acquired resistance**
- **impermeable bacterial membranes**
- **resistance of quiescent bacteria**



erythromycin:
bind to 50S ribosomal subunit

<u>"disrupting membrane" strategy</u>

- **side effect to mammalian membrane (off-target)**
- **necessity of locally high concentration**



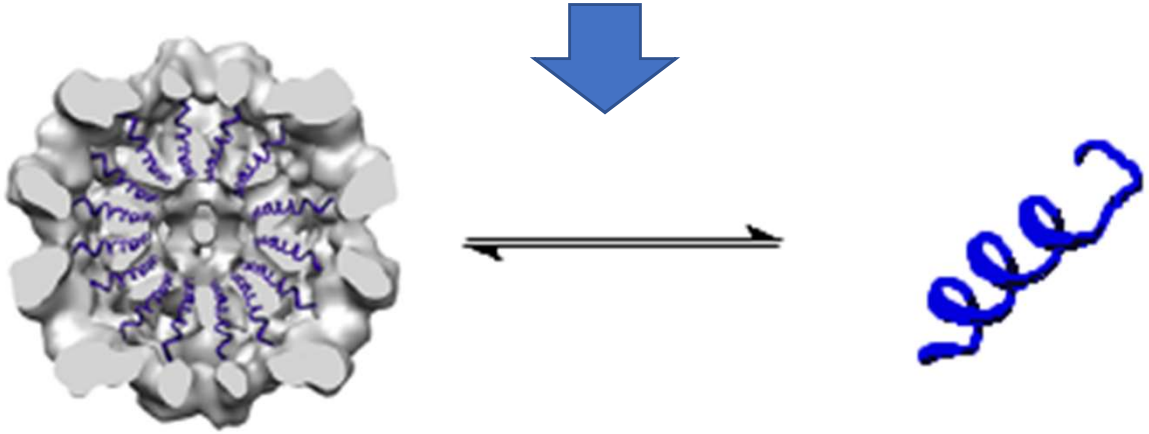
gramicidin A:
form an ion channel on microbial membrane

Author's Strategy

"disrupting membrane" strategy
- side effect to mammalian membrane (off-target)
- necessity of locally high concentration

+

Capsid itself is non-toxic to mammalian cells
Capsid conformations are in equilibrium



Inactive assembled state

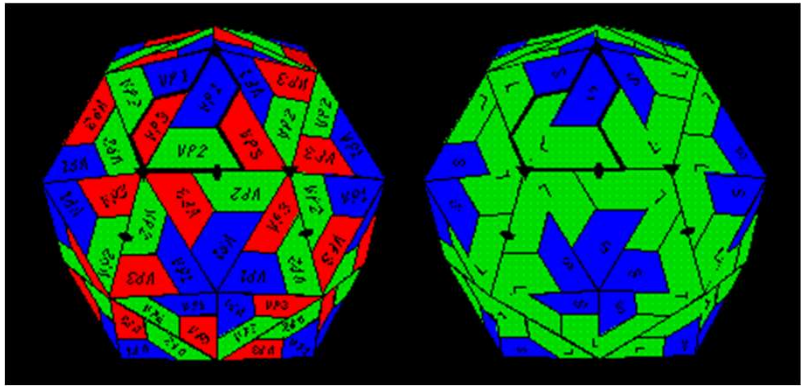
Active discrete state

- exist in oligomerized structure
- formed on the zwitterionic mammalian membranes

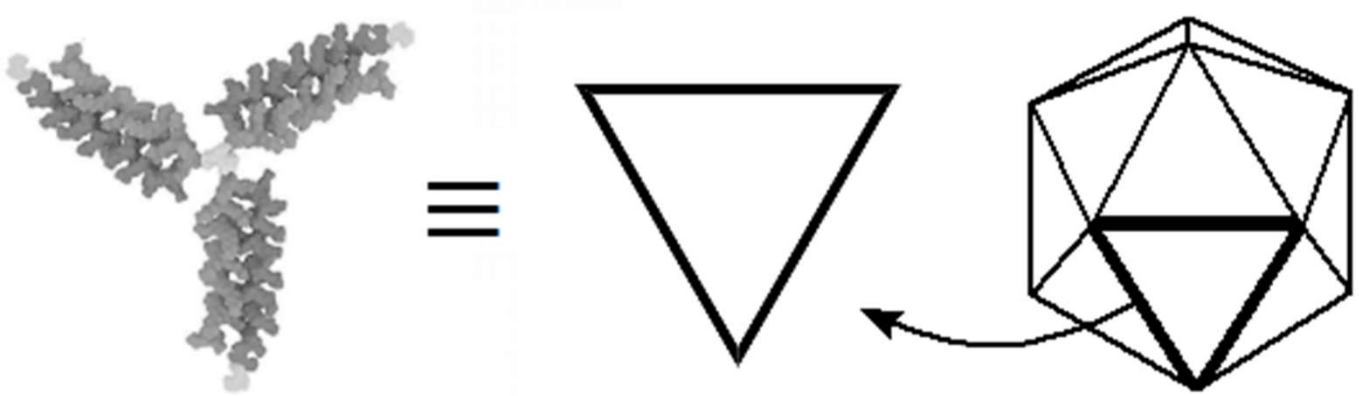
- exist in α-helical structure
- formed on the anionic microbial membranes

Design of Capsids (1)

In natural viral capsids,
several different proteins Interface
via precisely matched interactions,
which is difficult to emulate



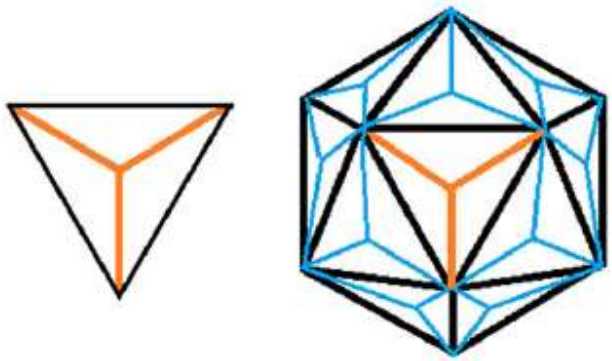
The single protein motif is designed:
one protein corresponds to one triangular face

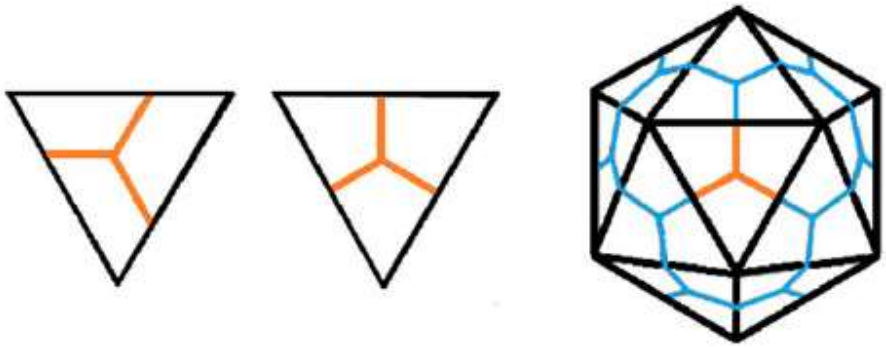


Caspar. D. L. D.; Klug. A. *Cold Spring Harbor Symp. Quant. Biol.* **1962**, *27*, 1-24.

Design of Capsids (2)

Two subunit arrangements within a triangle are possible:
“Starburst” or “Honeycomb”





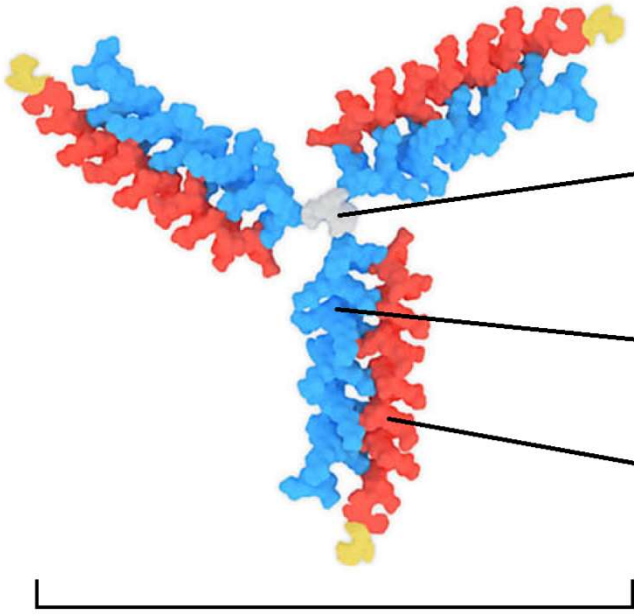
Starburst
- propagate 5 or 6-fold at its termini
- tight packing

→ <u>suitable</u>

Honeycomb
- propagate 2-fold at its termini
- hollow vertices

→ unsuitable

Design of Capsids (3)



a triskelial hub

triskelion

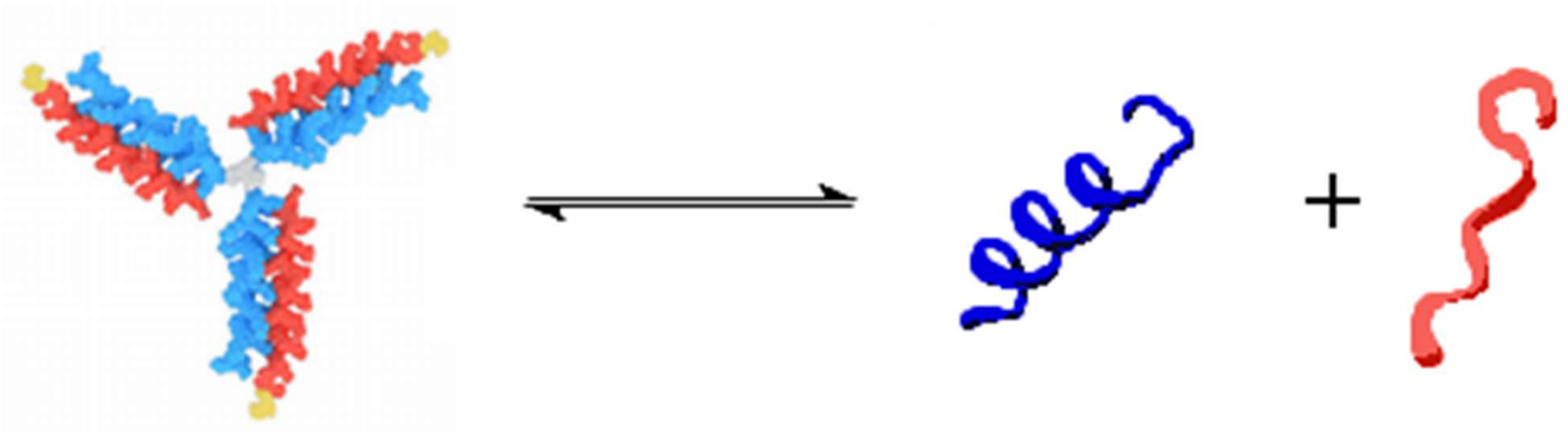
C_1 (+) strand
(antimicrobial without C_1 (-) strand)

C_1 (-) strand

C_3-subunit
= three C_1 (+) strand conjugating onto a triskelial hub
+ three C_1 (-) strand

Image is retrieved from https://fr.wikipedia.org/wiki/Fichier:Triskele-Symbol-spiral.svg

Design of Capsids (4)



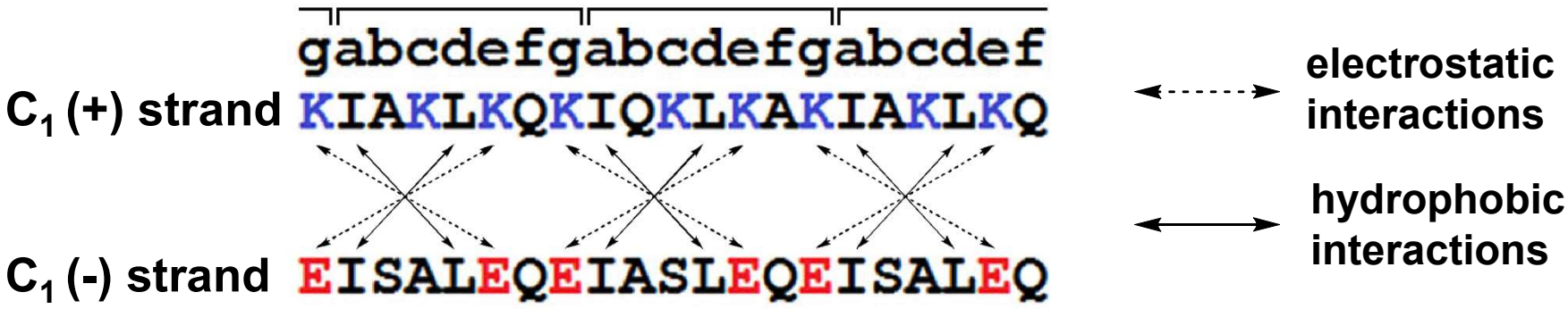
Inactive assembled state
(C_1 (+) strand + C_1 (-) strand)

- coiled-coil formation
  (3.5 residues per turn)
- stable in neutral condition

Active discrete state
(C_1 (+) strand) **(C_1 (-) strand)**

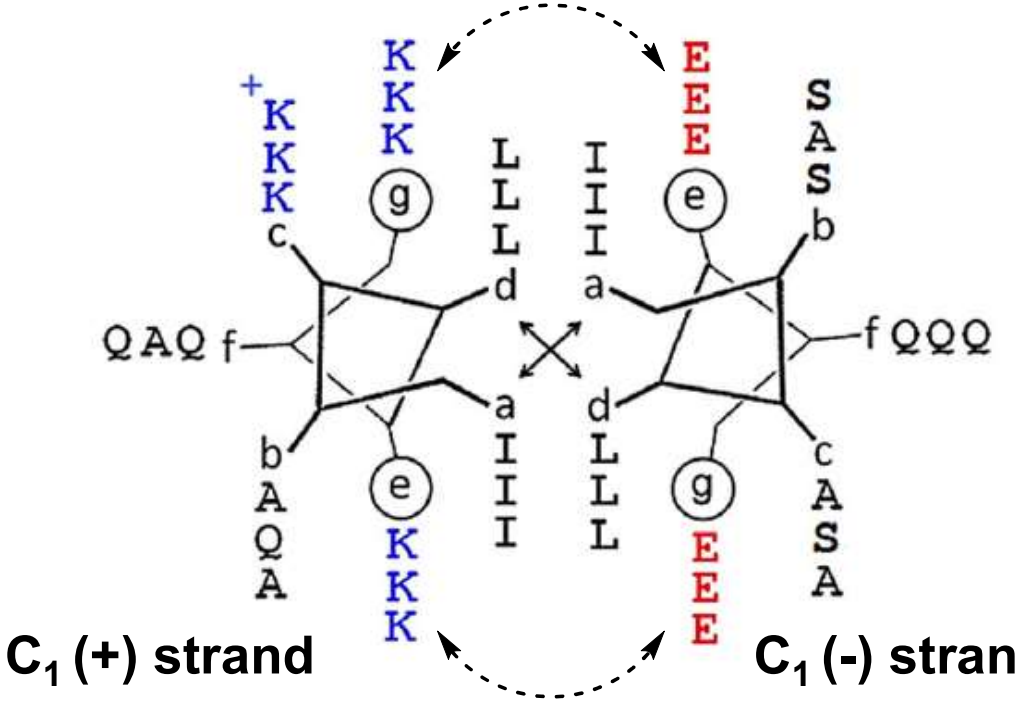
- α-helical structure
  (3.6 residues per turn)
- bind to anionic microbial membrane

Amino Acid Sequence



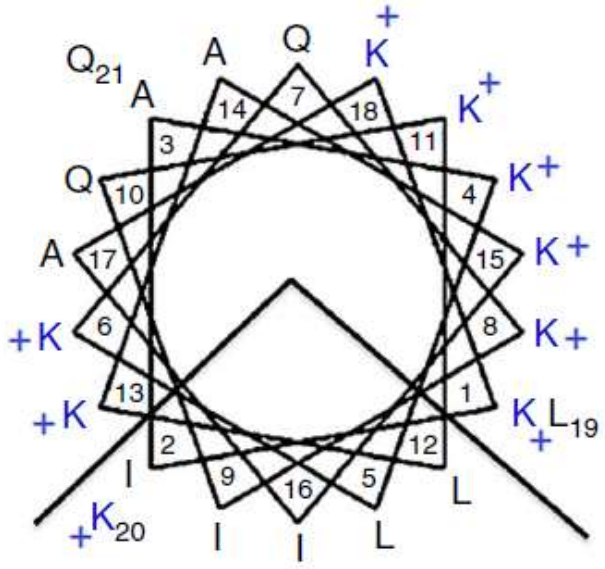
Inactive assembled state

coiled-coil formation

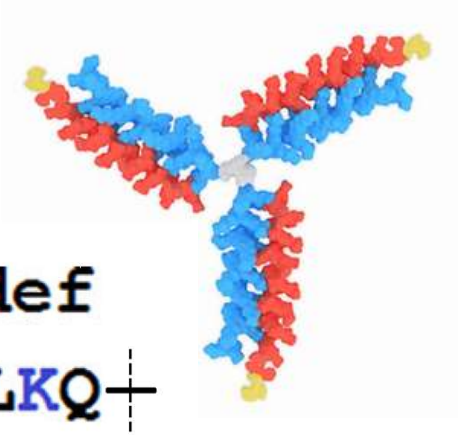


Active discrete state

α-helical structure



An Overview of C_3-subunit



Components of triangular C_3-subunit

C_1 (+) strand

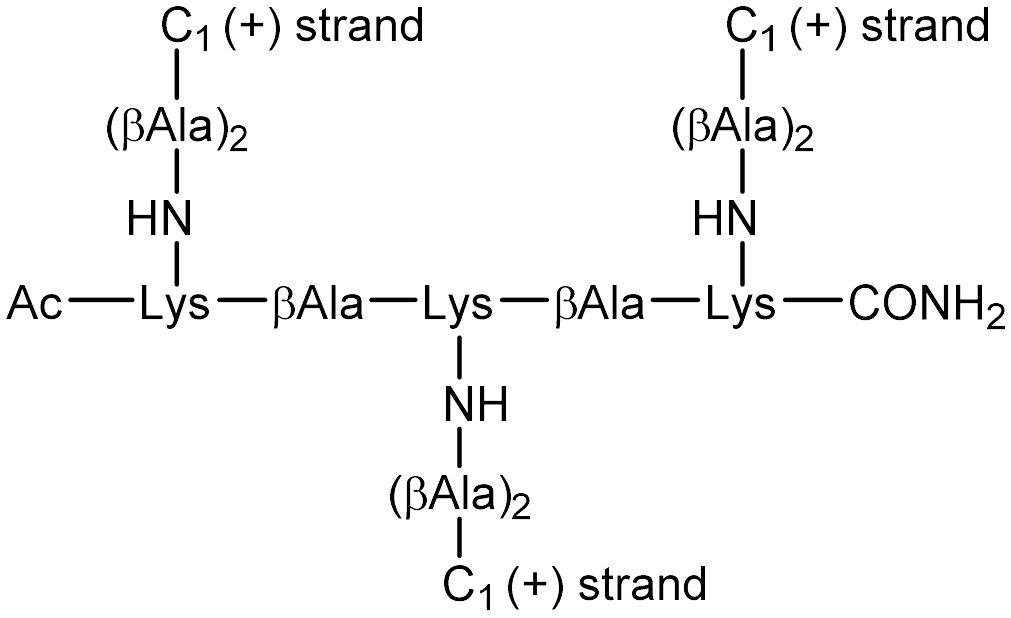
C_1 (-) strand

gabcdefgabcdefgabcdef

KIAKLKQKIQKLKAKIAKLKQ

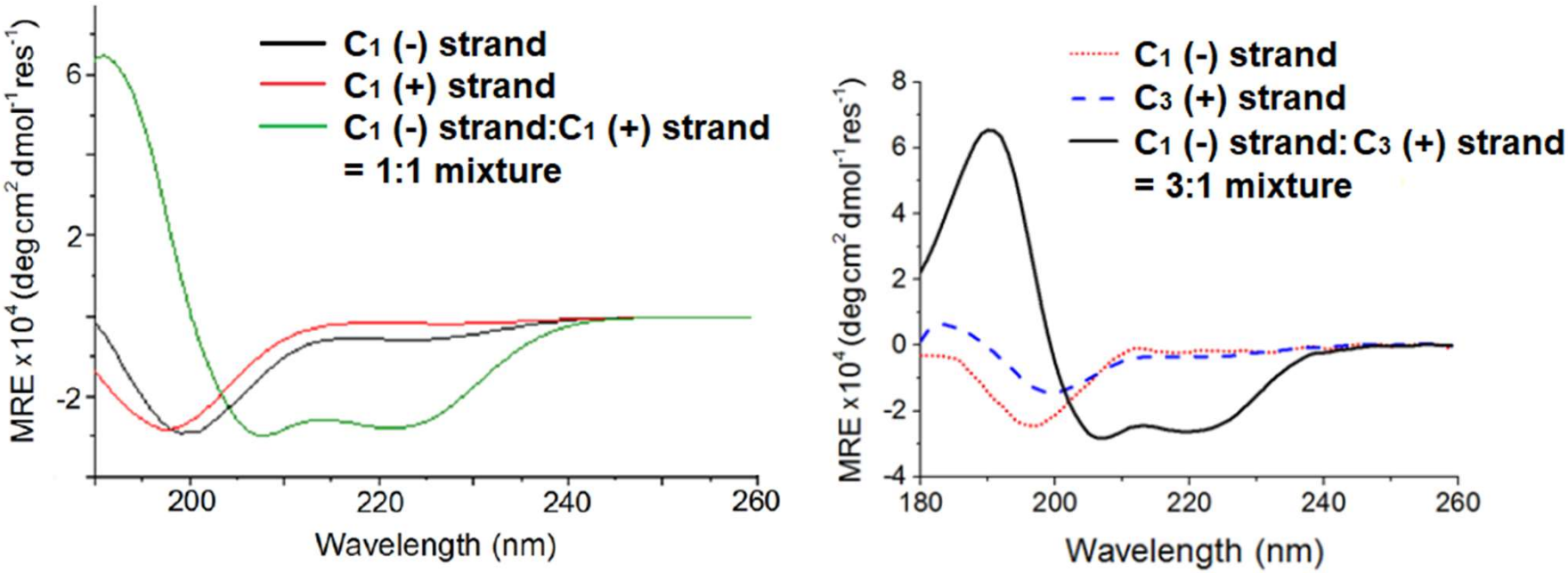
ac-CβAEISALEQEIASLEQEISALEQ

C_3 (+) strand



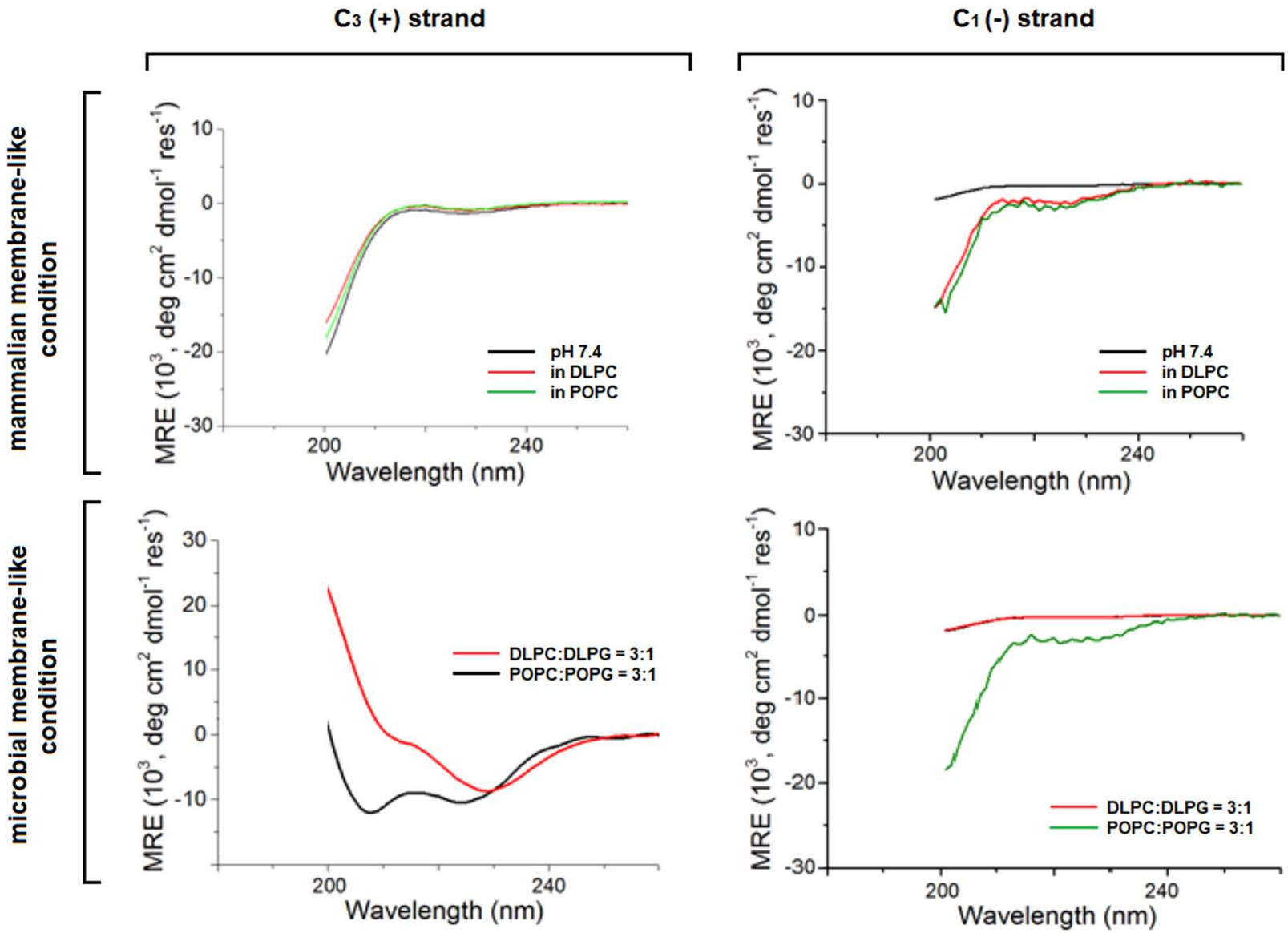
C_3-subunit = C_3 (+) strand + three C_1 (-) strand

Behavior of the Designed Capsids (1)



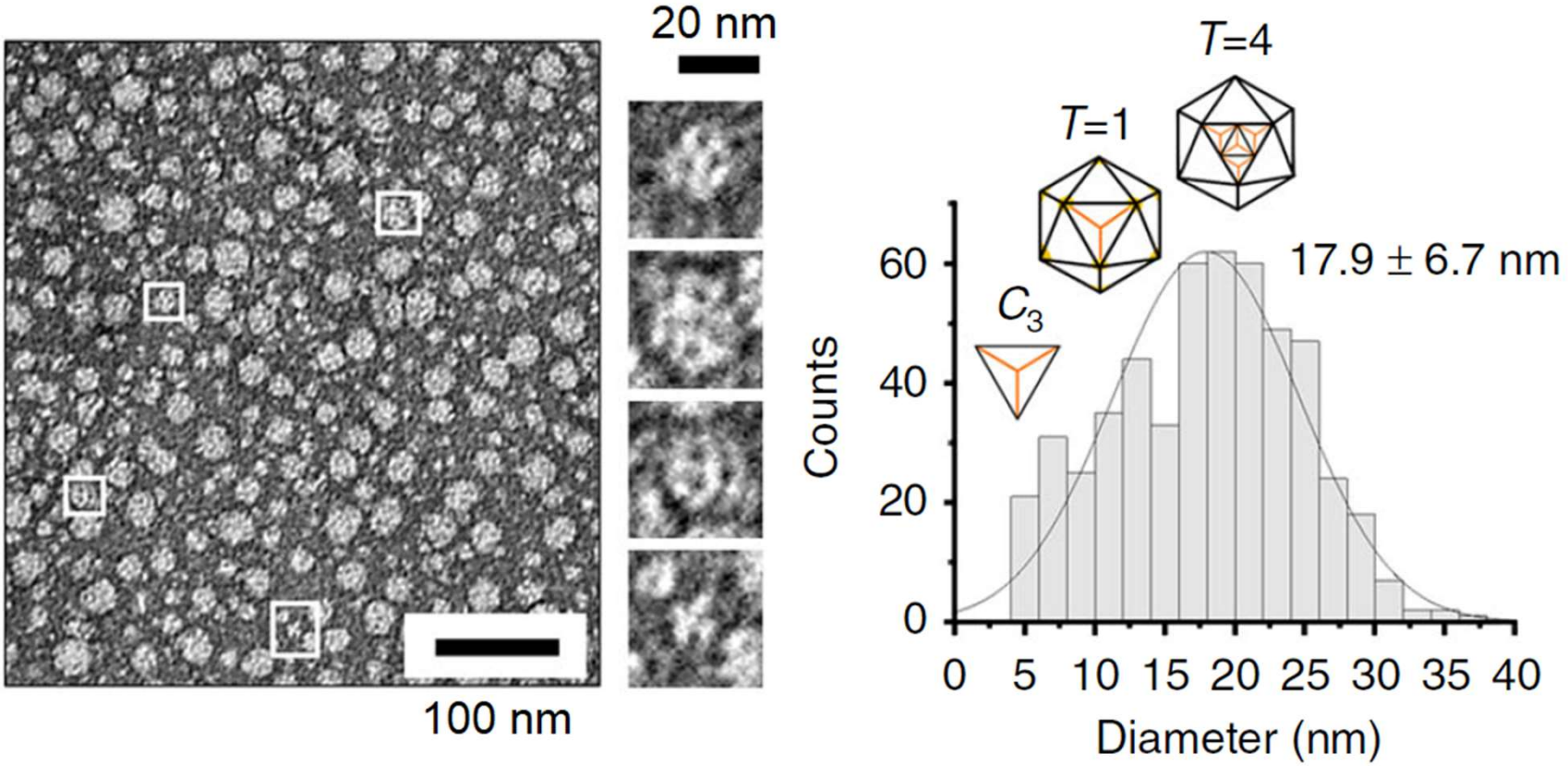
→ C_1 (+) strand and C_1 (-) strand interact along with structural change

Behavior of the Designed Capsids (2)



→ **regulated properly depending on the condition (mammalian/microbial)**

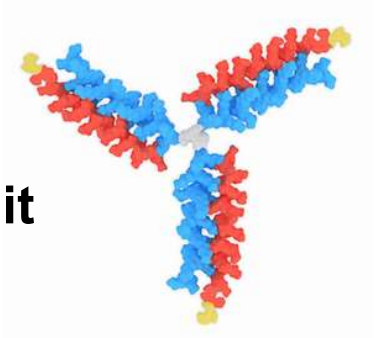
Structure of the Designed Capsids (1)

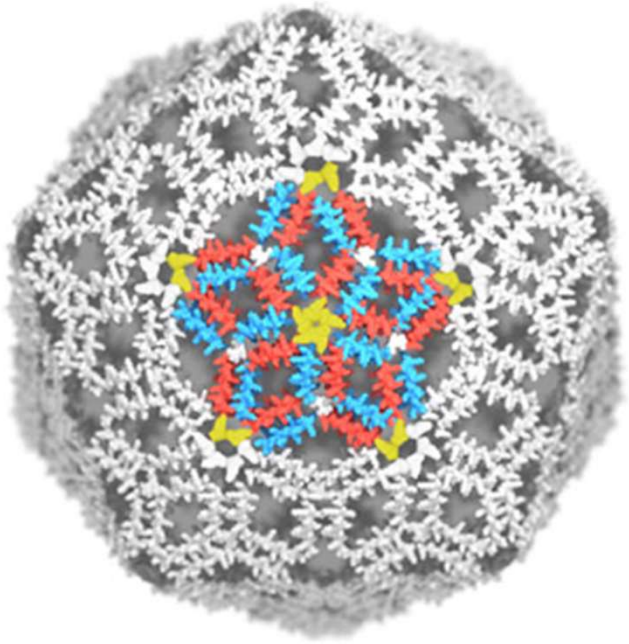


→ ***T*** **= 4 capsids are major**
(*T* = 3 capsids cannot be formed by only equilateral triangles)

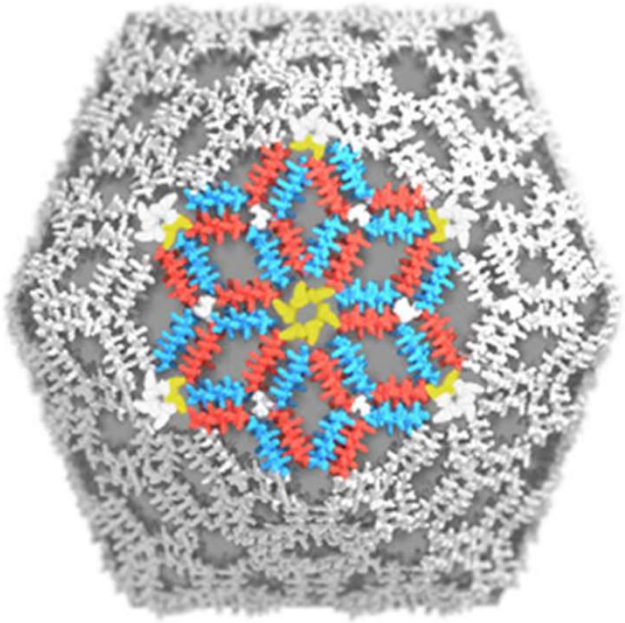
Structure of the Designed Capsids (2)

model of $T = 4$ capsid assembled from C_3-subunit





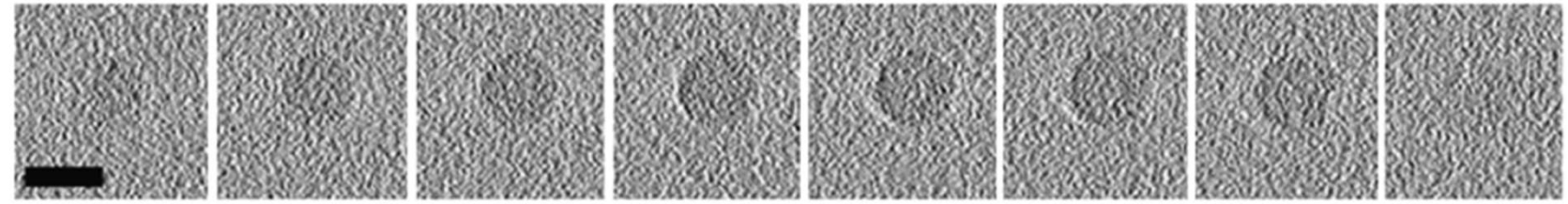
around 5-fold vertex



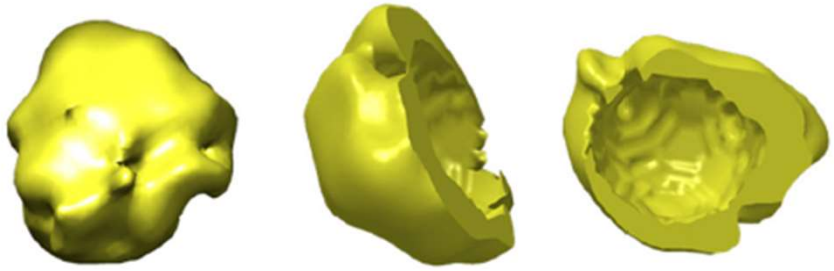
around 6-fold vertex

Structure of the Designed Capsids (3)

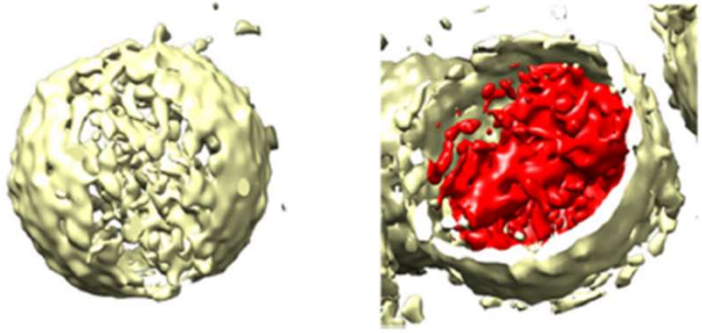
representative cryo-electron tomography for an assembled capsid



3D rendering



3D structure of the designed capsid assembled into spherical, hollow shells



HIV (EMDB 1155)

Antimicrobial Activity

Cell	Peptide[a]					
	C_3 (+) strand	C_3-capsid	Cecropin B	Daptomycin	Polymyxin B	Gramicidin S
	Minimum inhibitory concentrations, μM[b]					
P. aeruginosa (ATCC27853)	3	12	<2	>100	<1	>40
S. aureus (ATCC6538)	50	>100	>100	<8	<50	<20
E. coli (K12)	1.5	3	<1	>100	<1	<20
B. subtilis (ATCC6633)	1.5	3	>50	<8	1.5	>40
S. enterica (ATCC700720)	3	3	3	>50	<1	<10
E. faecalis (OG1X)	50	>50	<25	>100	>100	ND
K. pneumoniae (NCTC 5055)	>12	<25	<1	>50	<1	ND
	(LC_{50}), μM[c]					
Human erythrocytes	>250[d]	>250	>250	>250[d]	UD	>20

All tests were done in triplicates
UD undetectable, ND not determined
[a]C_1 (−) strand was inactive (>250 μM)
[b]Total peptide concentration
[c]Median (50%) cell death compared to untreated cells
[d]Haemolysis of <10% observed at higher concentrations

antimicrobial activity was observed

- **MIC of C_3-capsid is a total concentration of individual components, so the actual active concentration is reduced to 1/4**
- **partial antagonistic action of C_1 (-) strand is inevitable**

- **weak activity toward Gram-positive bacteria is maybe due to a thicker peptidoglycan layer which induces the disassembly of the capsids**

Antimicrobial Activity

Cell	Peptide[a]					
	C_3 (+) strand	C_3-capsid	Cecropin B	Daptomycin	Polymyxin B	Gramicidin S
	Minimum inhibitory concentrations, μM[b]					
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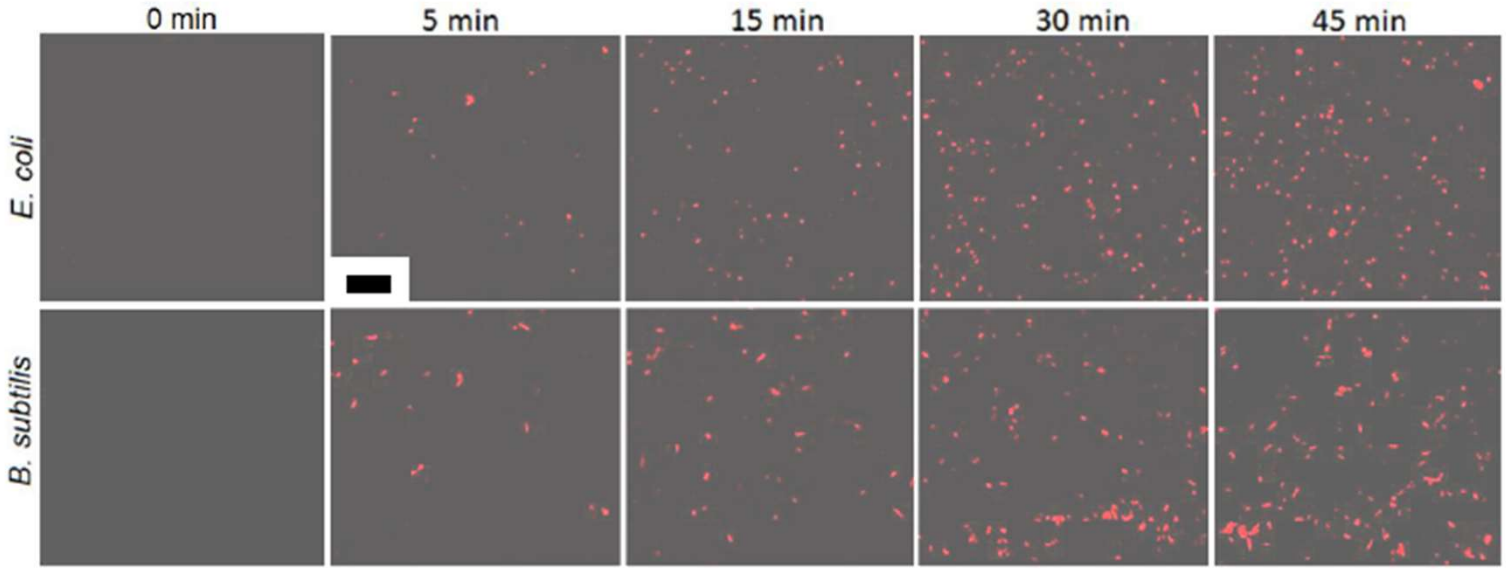
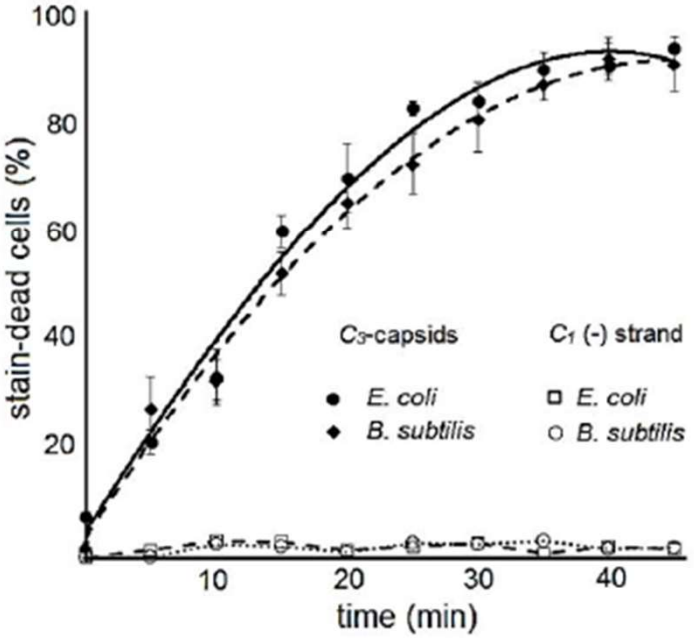
B. subtilis (ATCC6633)	1.5	3	>50	<8	1.5	>40
S. enterica (ATCC700720)	3	3	3	>50	<1	<10
E. faecalis (OG1X)	50	>50	<25	>100	>100	ND
K. pneumoniae (NCTC 5055)	>12	<25	<1	>50	<1	ND
	(LC_{50}), μM[c]					
Human erythrocytes	>250[d]	>250	>250	>250[d]	UD	>20

All tests were done in triplicates
UD undetectable, ND not determined
[a]C_1 (−) strand was inactive (>250 μM)
[b]Total peptide concentration
[c]Median (50%) cell death compared to untreated cells
[d]Haemolysis of <10% observed at higher concentrations

MIC assess the activities in relatively longer time scales (~24h)
In order to assess them in shorter time scales (~minutes), bacterial viability assay and poration monitoring was conducted

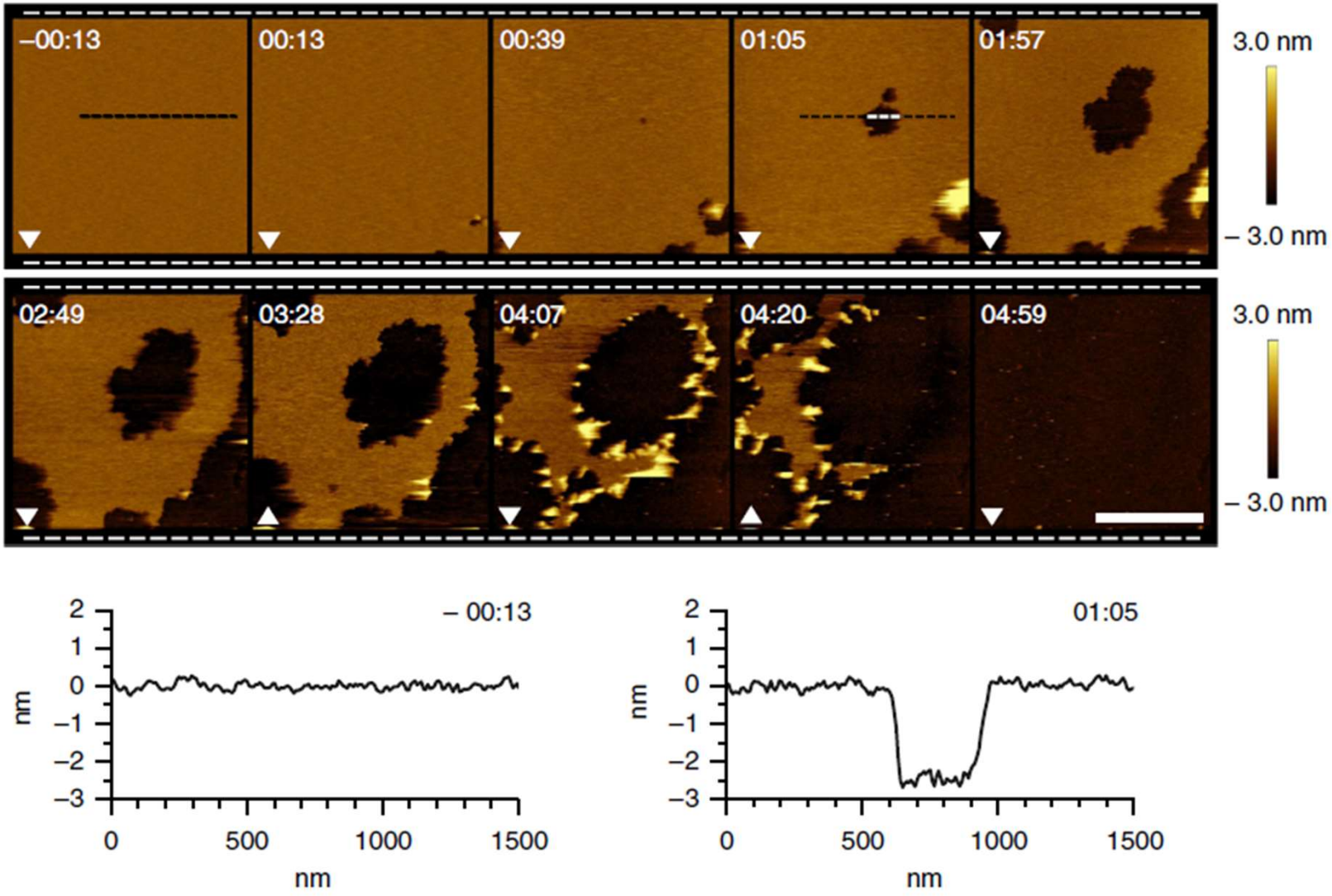
Bacterial Viability Assay





The capsids used at the MIC (3μM) showed rapid antimicrobial activity

Real Time Monitoring

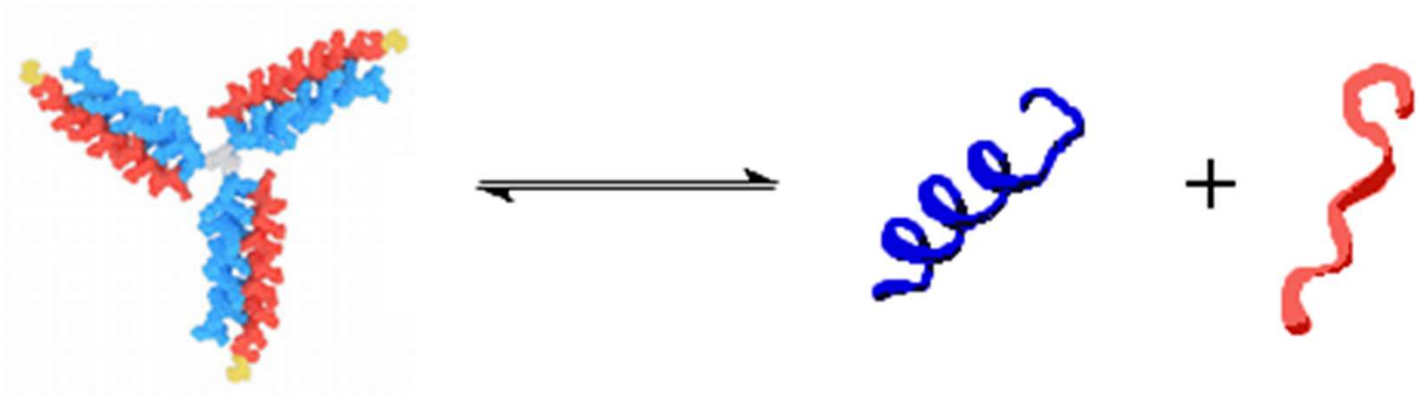


→ **Pore was formed in about one minutes**

Summary

・capsids-like antimicrobial peptide is devised to overcome the weak point of “disrupting membrane” strategy: off-target and local high concentration

・Designed capsids are regulated precisely depending on the condition



Inactive assembled state

Active discrete state

・Capsids showed strong and rapid activity against Gram-negative bacteria by poreting the phospholipid membranes

Contents

1. Introduction
   - the structure and function of capsids

2. Main topic: “Antimicrobial peptide capsids of de novo design”

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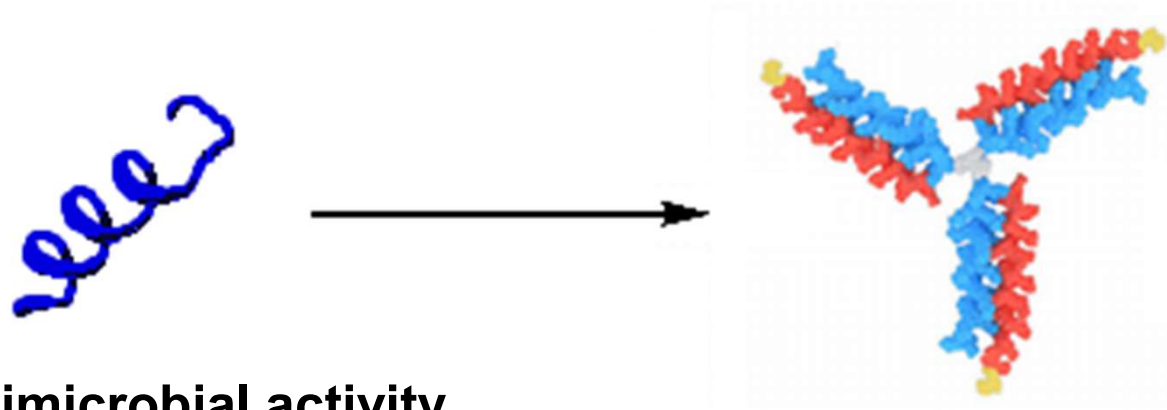
3. Future perspective: My proposal

Future Perspective

・for more potent activity

- the designed capsids showed antimicrobial activity without cytotoxicity, but its antimicrobial activity is not strong enough to be compatible with other antimicrobial peptides

develop new antimicrobial capsids based on the peptide
which have potent antimicrobial activity with strong cytotoxicity



potent antimicrobial activity
with strong cytotoxicity

weakened cytotoxicity?