Membrane protein-embedded Enveloped Virus Replicas

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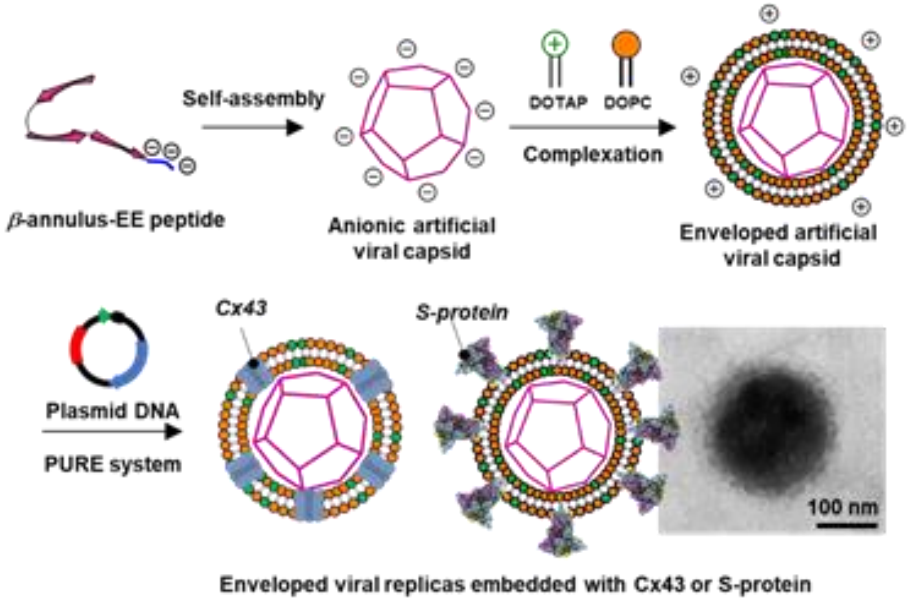
Progressive development of nano-architectures, self-assembled from rationally designed peptides, have also enabled the construction of viral capsid-like nanocapsules consisting of peptides. We previously found that a 24-residue β-annulus peptide, which is involved in the formation of the dodecahedral inner skeleton of the tomato bushy stunt virus, spontaneously self-assembles into a hollow artificial viral capsid with a size range of 30−50 nm.[1] Recently, we have succeeded in constructing the enveloped viral capsid by complexing cationic lipid bilayer with anionic artificial viral capsid self-assembled from β-annulus-EE peptides (INHVGGTGGAIMAPV AVTRQLVGSEE).[2]

Membrane protein connexin-43 (Cx43) is a four-fold transmembrane protein that forms gap junction structures to transport molecules between cells. Here, we constructed enveloped artificial viral replica embedded with Cx43 by using cell-free protein expression system and demonstrated molecular transport from the viral replica into Cx43-expressing liposomes and HepG2 cells through gap junctions.[3] The embedding of Cx43 on the enveloped artificial viral capsid was evaluated by detection with anti-Cx43 antibody using fluorescence correlation spectroscopy and transmission electron microscopy. When Cx43-embbeded viral replicas encapsulated with fluorescent dye TMR were added to Cx43-expressing HepG2 cells, transports of fluorescent dye into them were observed by confocal laser scanning microscopy. These results indicated that we succeeded in selectively transporting small molecules from Cx43-embedded viral replicas into Cx43-expressing cells via gap junctions.

SARS-Cov-2, which is a kind of enveloped virus, has spike proteins (S proteins) on its envelope surface. The S protein binds to the ACE2 receptor displayed on the host cell surface to enter the virus into the cell. We also constructed the enveloped viral replica embedded with S protein derived from SARS-Cov-2 using cell-free protein expression. In addition, we evaluated the binding of the viral replica to the ACE2 receptor using flow cytometry.



Enveloped viral replicas embedded with Cx43 or S-protein

References

[1] Matsuura, K. *Chem. Commun.,* **2018***, 54,* 8944 (Feature Article).
[2] Furukawa, H., Inaba, H., Inoue, F., Sasaki, Y., Akiyoshi, K., Matsuura, K. *Chem. Commun.*, **2020**, *56*, 7092.
[3] Furukawa, H., Inaba, H., Sasaki, Y., Akiyoshi, K., Matsuura, K. *RSC Chem. Biol.*, **2022**, *3*, 231.