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HOW LARGE MOLECULES CAN ENTER CELL

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Various endocytic pathways have evolved to tightly regulate the vital internalization of large molecules into cells (see Figure 1). However, viruses can hijack these processes to enter their hosts. After the interaction between the virus and membrane receptors, the plasma membrane is bent and wrapped around the virus. Once the wrapping is completed, the virus is internalized in the endosome. We have shown that such wrapping could be a spontaneous process, i.e., not requiring ATP, and its efficacy depends on the virus size, shape, and coverage of binding sites[1,2]. This pathway is not limited to viruses and could be utilized by nanoparticles and other drug carriers. Later in the cell, viruses need to release their content into the cell. This release was previously assumed to occur via tiny pores/openings observed in non-enveloped RNA virus structures. However, such a release would be slow, requiring the unwinding of putative double-stranded segments and enabling genome degradation. We have recently combined cryo-electron microscopy and computer simulations to demonstrate an alternative release mechanism in which the capsid cracks open, and the genome rapidly releases via a large opening[3,4]. This release was triggered by decreased pH *in vitro*, and self-reassembled capsids were found to occasionally miss one or few capsid-protein pentamers after the release. The shape and extent of the opening were determined to depend primarily on the interaction range between the pentamers[5]. These findings uncover molecular details of virus entry and genome release that could be utilized in the development of antiviral drugs or nanoparticles for drug delivery.

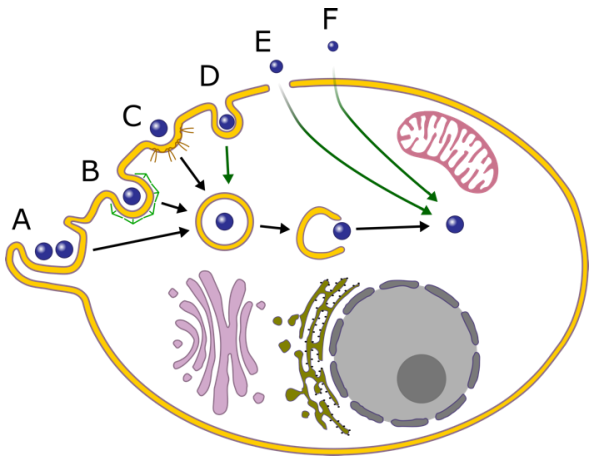

Fig. 1. **Illustration of internalization pathways of large molecules in cells.** A-C) endocytosis including phagocytosis and clathrin and calveolin mediated endocytosis D-F) passive internalization via D) clathrin and calveolin independent path, E) transport via membrane pores, and F) direct permeation.

An alternative pathway for large molecules to enter the cell is via large transmembrane pores (see Fig. 1E). Such pores do not occur spontaneously but could be formed and stabilized by proteins/peptides. We developed a computational approach to formulate the design guidelines and generate de novo peptides able to form such transmembrane pores with few nanometers in diameter[6]. The guidelines for pore-forming peptides were verified on several examples by fluorescent dye leakage experiments using lipid vesicles and atomic force microscopy with a supported lipid membrane. The advantage of our approach is the identified role of each residue, which could be used for fine-tuning the peptides/pores to specific applications. We demonstrated this fine-tuning on the generation of antimicrobial peptides that are able to kill even antibiotic-resistant bacteria while having low toxicity for human cells. Such peptides could be a good starting point for developing new antibiotics. Similar fine-tuning can be performed for other medical and biotechnological applications.

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REFERENCES

1. Vácha R., Martinez-Veracoechea F. J., Frenkel D.: Nano Lett. *11* (12), 5391 (2011).
2. Schubertová V., Martinez-Veracoechea F.J., Vácha R.: Soft Matter *11*, 2726 (2015).
3. Buchta D., Füzik T., Hrebík D., Levdansky Y., Sukeník L., Mukhamedova L., Moravcová J., Vácha R., Plevka P.: Nat. Commun. *10*, 1138 (2019).
4. Škubník K., Sukeník L., Buchta D., Füzik T., Procházková M., Moravcová J., Šmerdová L., Přidal A., Vácha R., Plevka P.: Sci. Adv. *7* (1), eabd7130 (2021).
5. Sukeník L., Mukhamedova L., Procházková M., Škubník K., Plevka P., Vácha R.: ACS Nano *15* (12), 19233 (2021).
6. Rahul D., Kabelka I., Přibyl J., Vácha R.: bioRxiv, doi: 10.1101/2022.05.09.491086 (2022).