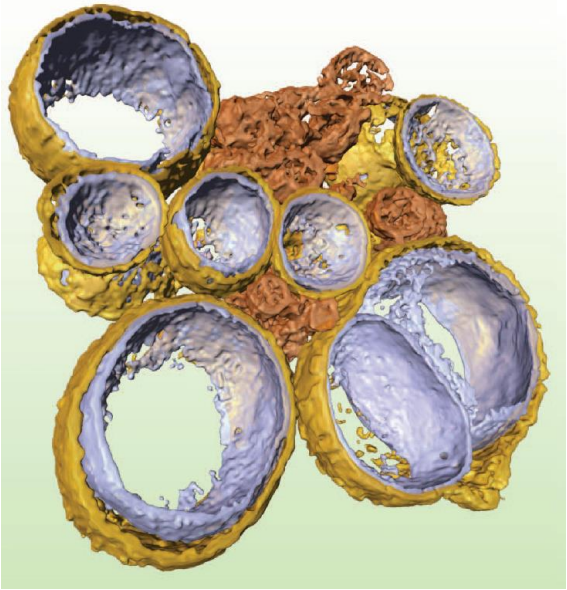
Reconstitution of viral double-membrane vesicles: a proof of principle for *in vitro* organellogenesis

In all extant cells, a biological membrane defines the boundary between living and inanimate matter. In addition, eukaryotic cells also possess a very elaborate internal system of membranes, which includes organelles with very different shapes such as the nuclear envelope, the endoplasmic reticulum and mitochondria. The specific shape of each organelle matters, because membrane shape defines and supports the organelle function. It is of critical importance to study how organelles are formed, how their shape is maintained and how it changes in different conditions, as in the case of diseases.

To try to answer these questions, one can directly study organelles within a living cell. However, this approach has limitations: the cell is a very complicated system and sometimes it can behave in an unexpected way. Moreover, we have only a limited awareness and control over the multitude of processes that occur in a cell.
An alternative (and complementary) approach is to start from purified components such as lipids and proteins and try to recreate biological systems in a more controlled way. Such *in vitro* reconstitution approach can be extremely useful to address specific questions that can't be answered by the *in vivo* approach. In the case of organelles, using an *in vitro* approach means that we need first of all to be able to reproduce the complex membrane shape of organelles in a controlled way, something that is not possible with current technologies.

This is precisely to aim of this project: to develop a new *in vitro* strategy to shape membranes into complex shapes, similar to those of organelles.
As a proof of principle, we will reconstitute a membrane organelle that is formed during a pathological conditions, namely coronavirus infection. Coronaviruses are able to rearrange the endoplasmic reticulum of the host cell creating double-membrane compartments where the virus can comfortably replicate its genome and thus create new viral particles to infect new cells. Such compartments, also called viral factories, are common to many viruses and we still know little about their origin and function. By reconstituting viral factories *in vitro*, we will be able to study how they form and how they work, potentially identifying new drug targets. Moreover, this strategy can be applied in the future to reconstruct more complex organelles, such as the nuclear envelope and the endoplasmic reticulum.



Reconstruction of double-membrane vesicles created by coronaviruses in the cytoplasm of the infected cells. The two membranes are colored in violet and yellow. These viral factories remain connected with their organelle of origin, the endoplasmic reticulum, by regions of less structured membrane (in orange).