Export of active enzymes by cell-penetrating peptides in synthetic cell systems

Joseph Heili, Jose Gomez-Garcia, Aaron Engelhart, Kate Adamala

Department of Genetics, Cell Biology and Development, University of Minnesota

Synthetic minimal cells are a class of small liposome bioreactors that hold vast potential in the areas of origins of life research, as well as biotechnology.

One hurdle to overcome in the use of these liposome bioreactors is how to efficiently export cell contents to the outer environment. Cell penetrating peptides (CPPs) have shown promise as membrane-translocating chaperones.

Here we sought to determine whether CPPs could carry a protein cargo across the membrane of a synthetic minimal cell, and verify the efficacy of the cargo post translocation. We investigated the efficiency of CPP-mediated transport by comparing fluorescence inside and outside liposome populations expressing CPP-tagged mClover, a green fluorescent protein, as well as liposome populations expressing non-CPP-tagged mClover. We found that the CPP-tagged mClover was able to exit the liposomes, while the non-tagged protein remained within the liposomes. To verify the continued functionality of the CPP-tagged cargo, we tagged T7 RNA polymerase and used that enzyme in transcription reactions post-membrane translocation. Finally, to investigate the method of CPP-mediated membrane translocation, we introduced calcein dye to the outer environment surrounding the liposomes, and tracked its movement while CPP-tagged firefly luciferase exited the liposomes. We found that while firefly luciferase was exported efficiently from the liposomes, calcein was not able to enter.

These findings suggest CPP-tagged proteins transit membranes by passive diffusion, as opposed to pore formation. These results demonstrate it is possible to develop tools to export proteins created within liposome bioreactors which may prove to be important in areas such as drug delivery or environmental remediation.