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Efficient delivery of structurally diverse protein cargo into mammalian cells by a bacterial toxin

Anick Auger[1], Minyoung Park[2], Greg L Beilhartz[2] and Roman A Melnyk [2]
[1]Inception Sciences, Canada
[2]The Hospital for Sick Children, Canada

Given the vast array of applications for protein-based tools and therapeutics inside cells, there is great interest in developing safe and efficient protein delivery platforms that direct biologics into cells. To date, numerous approaches been investigated to facilitate protein entry into the cytoplasm of cells, however, though each capable of delivering protein cargo into cells to varying degrees, general mechanism-based limitations exist for these platforms. In particular, selectivity and/or efficiency remain elusive features for most platforms owing to their shared nonspecific mode of interaction with membranes. Protein toxins, which use host cell-surface receptors to initiate entry into cells, are attractive vectors to consider given their natural tendency to delivery proteins into specific cells with high efficiency. The paucity of development efforts for toxins as protein delivery vectors stem from early studies, which suggested that delivery was restricted to a select few cargo that were largely unfolded prior to translocation and that the cargo itself greatly diminished the efficiency of translocation of the system. Through careful engineering of the platform, we show that neither of these assertions is true. We show that the diphtheria toxin platform is capable of delivering proteins that are over 100 kDa in size and of varying structures and stability with exquisite efficiency. In fact, to our surprise, we found that diphtheria toxin could deliver the hyper-stable passenger protein mCherry, which we calculated to have a melting temperature greater than 90 degrees under the translocation conditions, suggesting that even folded proteins could be delivered into cells. Through a rigorous set of experiments we trace the misleading early results to effect of cargo on the readout of translocation, rather than the efficiency of translocation. We also provide functional evidence that the delivered cargo is functional. Using a-amylase as cargo we show that cytosolic glycogen is degraded in a dose and time dependent manner.

roman.melnyk@sickkids.ca