BMB Reports - Manuscript Submission

Manuscript Draft

Manuscript Number: BMB-16-174

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Article Type: Perspective (Invited Only)

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Extracellular vesicles as novel carrier for therapeutic molecules

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Keywords: Extracellular vesicles, Exosome, Protein drug, Drug delivery, Light-induced protein-protein interaction

Abbreviations: EV, Extracellular vesicles; EXPLOR, Exosomes for protein loading via optically reversible protein-protein interaction; MVB, Multivesicular bodies; CRY2, cryptochrome 2; CIB1, CRY-interacting basic-helix-loop-helix 1; CIBN, a truncated version of CIB1

Perspective to: Nambin Yim et al (2016) Exosome engineering for efficient intracellular delivery of soluble proteins using optically reversible protein-protein interaction module. Nature communications, 22;7:12277. doi: 10.1038/ncomms1227

Abstract

Extracellular vesicles (EVs) are natural carriers of biomolecules that play central roles in cell-to-cell communications. Based on this, there have been many attempts to use EVs as therapeutic drug carriers. Various macromolecules, from chemical reagents to nucleic acids, were successfully loaded into EVs; however, loading of proteins with high molecular weight has been huddled with several problems. Purification of recombinant proteins is expensive and time consuming, and easily results in modification of proteins by physical or chemical forces. And the loading efficiency of conventional methods is too low for most of proteins. We have recently proposed a new method so-called exosomes for protein loading via optically reversible protein-protein interaction (EXPLORs) to overcome the limitations. Because EXPLORs are produced by actively loading of intracellular proteins into EVs using blue light without protein purification steps, we demonstrated that EXPLOR technique significantly improves the loading and delivery efficiency of therapeutic proteins. We further showed the potential of EXPLOR technology as a novel platform of biopharmaceuticals by successful delivery of several functional proteins such as Cre recombinase into the target cells *in vitro* and *in vivo*.

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All multicellular organisms developed various ways for exchange of various biomolecules including not only small chemicals but also nucleic acids and proteins. As major carriers of biomolecules, extracellular vesicles (EVs) play central roles in intercellular communication. EVs are spherical nano-size particles surrounded by lipid bilayer, and further classified based on the physical and biochemical properties. Representatively, microvesicles derived from plasma membrane have the size of 100 – 1000 nm, and exosomes originated from intracellular multivesicular bodies (MVBs) have the size of 50 – 200 nm. EVs can be simply isolated by ultracentrifugation, size exclusion chromatography or ultrafiltration, and can be easily distinguished by size measurement in primary and detection of surface markers such as tetraspanins in secondary.

EV-based drug delivery was originally attempted for small chemical drugs such as curcumin, a well-known anti-inflammatory drug after loading into exosomes (Dongmei Sun et al. Molecular therapy, 2010; 18(9); 1606-1614. doi: 10.1038/mt.2010.105). EV-based drug delivery has been shown to solve several major problems such as non-specific biodistribution and short half-life of drugs in the systemic circulation. Drug–loaded EVs can be produced by simple incubation of EVs with chemical drugs in room temperature, and various drugs including doxorubicin, porphyrins, and paclitaxel were successfully loaded and delivered by EV. On the one hand, the first biopharmaceutical for EV-based delivery was a short interfering RNA (Lydia Alvarez-Erviti et al. Nature biotechnology. 2011; 29(4); 341-345. doi:10.1038/nbt.1807). The authors transferred siRNAs into exosomes using an electroporation method, and showed that the nucleic acids–loaded exosomes can deliver siRNAs to the brain in mice. After then, not only siRNA but also various nucleic acids such as microRNAs, messenger RNAs and plasmid DNAs were successfully loaded into the exosomes and delivered *in vitro* and *in vivo*.

Several methods for intracellular protein delivery using EVs have also been tried around the same time (Fig. 1). Unlike chemical drugs or nucleic acids, proteins cannot passively penetrate cellular membrane. Furthermore, proteins are easily modified by physical and chemical environment, limiting the applicability of EV-based delivery of proteins with therapeutic potentials. In 2011, one group reported that highly oligomeric proteins can be

targeted to exosomes by plasma membrane anchors (Shen B et al. J Biol Chem. 2011; 286; 14383-14395. doi: 10.1074/jbc.M110.2086600). Based on this principle, they developed the EV targeting technology and finally commercialized the method now called as XPACK technology (System Biosciences, Mountain View, CA, USA). The caveat of this method is that targeting proteins should be anchored to the exosome membrane, thus limiting the subcellular localization of delivered proteins to the cellular membrane of the recipient cells. Another group recently proposed alternative methods for passive loading of recombinant proteins into exosomes *ex vitro* (Matthew J. Haney et al. Journal of Controlled Release. 2015; 207; 18-30. doi:10.1016/j.jconrel.2015.03.033). They tried to load catalases into exosomes using various methods including simple incubation, freeze-thaw, sonication, and extrusion and demonstrated successful delivery of recombinant intracellular proteins. Since these loading processes were based on mechanical dispersion, this method cannot be applied for most of unstable proteins with therapeutic potential.

Exosomes for protein loading via optically reversible protein-protein interaction (EXPLORs) are novel protein carriers developed for overcoming the limitations of conventional EV-based protein delivery. The EXPLOR technology was designed that the protein drug can be actively and transiently docked into the exosomes by blue light illumination. For that, cryptochrome 2 (CRY2), a photoreceptor of *Arabidopsis thaliana* that can bind to CRY-interacting basic-helix-loop-helix 1 (CIB1) by blue light wavelength, was conjugated to intracellular protein drug, and exosome-associated tetraspanin protein CD9 was conjugated to CIBN (a truncated form of CIB1). Once the cargo proteins are introduced into the exosomes via endogenous biogenesis, they can be detached from CD9-conjugated CIBN by removal of the illumination source, resulting in release into the intraluminal space of the exosomes and efficient delivery to the cytosolic compartment of target cells. Thus, not only EXPLOR technology showed a dramatic increase of the loading efficiency in exosomes compared to the previous protein loading systems such as passive loading of recombinant proteins into exosomes *ex vitro* or XPACK, but also it showed a high efficiency of cytosolic protein delivery. Specifically, we successfully demonstrated the phenotype changes in various cell types and *loxP-STOP-loxP-eNpHR3.0-eYFP* transgenic mice with EXOPLORs loaded with Bax, super-repressor IκB (a S32A and S36A mutant form of IκB) and Cre recombinase.

In conclusion, we have recently developed a novel EV–based protein carrier EXPLOR that can deliver therapeutic proteins efficiently *in vitro* and *in vivo* using active targeting induced by blue light. Since EXPLOR does not require the steps for purifying proteins, it is simple and cost-effective. Additionally because EXPLOR adopts an endogenous active protein loading process instead of exogenous passive loading, most of intracellular proteins such as transcription factors, signal transducers, and enzymes can be targets for EXPLOR-based therapeutics with high efficiency. These advantages will overcome the limitations of the previous protein drug delivery, opening a new paradigm for future biopharmaceuticals.

Acknowledgements

This research was supported by KAIST Institute for the BioCentury, Cancer Metastasis Control Center (Grant Number: N11160071).

Figure 1

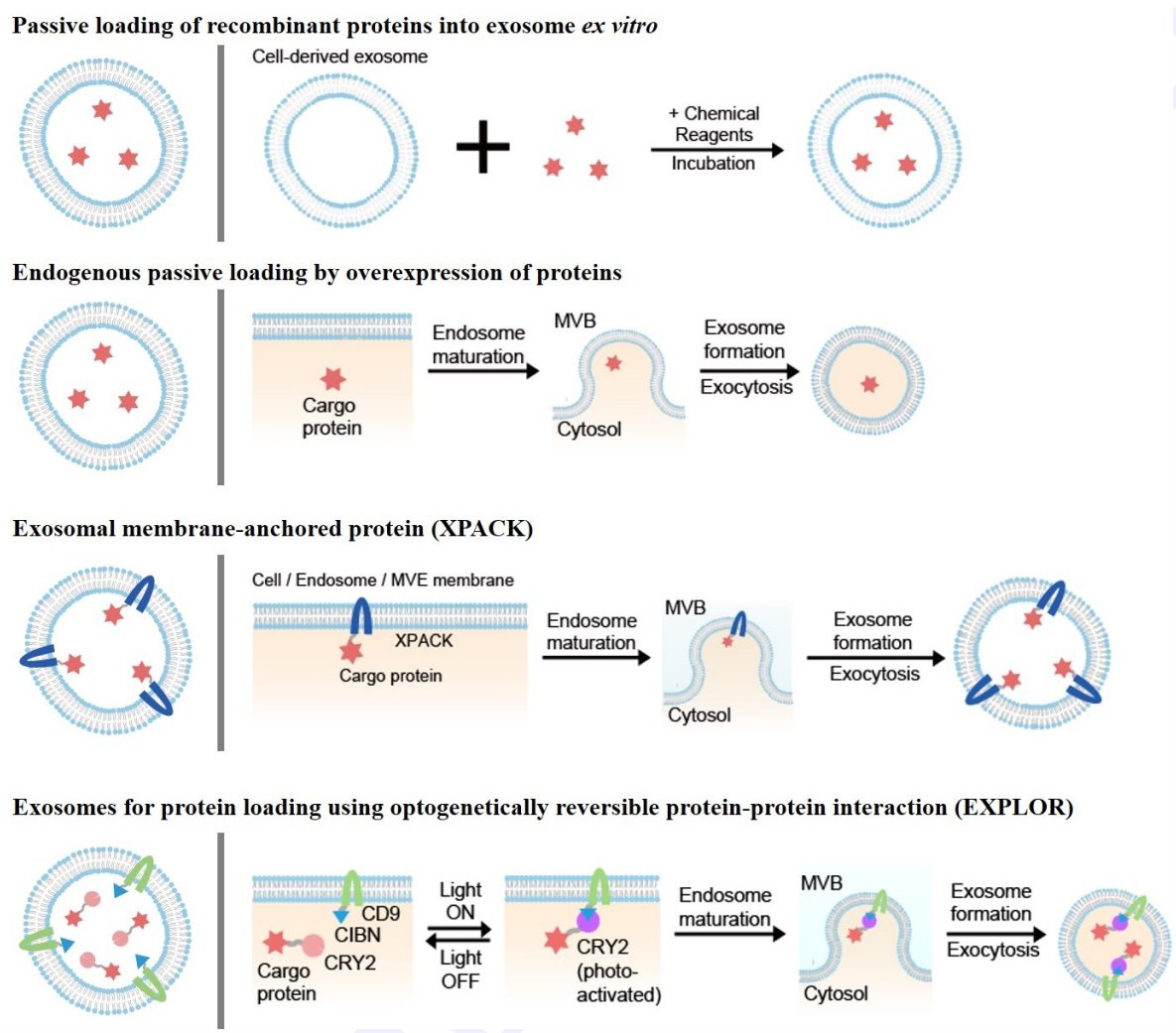

Figure Legend

Methods for loading proteins into exosomes. Passive loading of recombinant proteins into exosome *ex vitro*: recombinant proteins can be loaded into exosomes by various methods such as simple incubation, freeze-thawing, sonication, or extrusion. Endogenous passive loading by overexpression of proteins: proteins are passively loaded into exosomes in exosome producing cells overexpressing target proteins with low possibilities. Exosomal membrane-anchored protein (XPACK): proteins are attached to the inner layer of exosomal membrane, thus can be conjugated with exosomes along natural exosome biogenesis. Exosomes for protein loading using optogenetically reversible protein-protein interaction (EXPLOR): proteins are actively loaded into exosomes under light illumination. The interaction is reversible, thus proteins can be detached from exosome membrane as free forms in the lumen of exosomes.

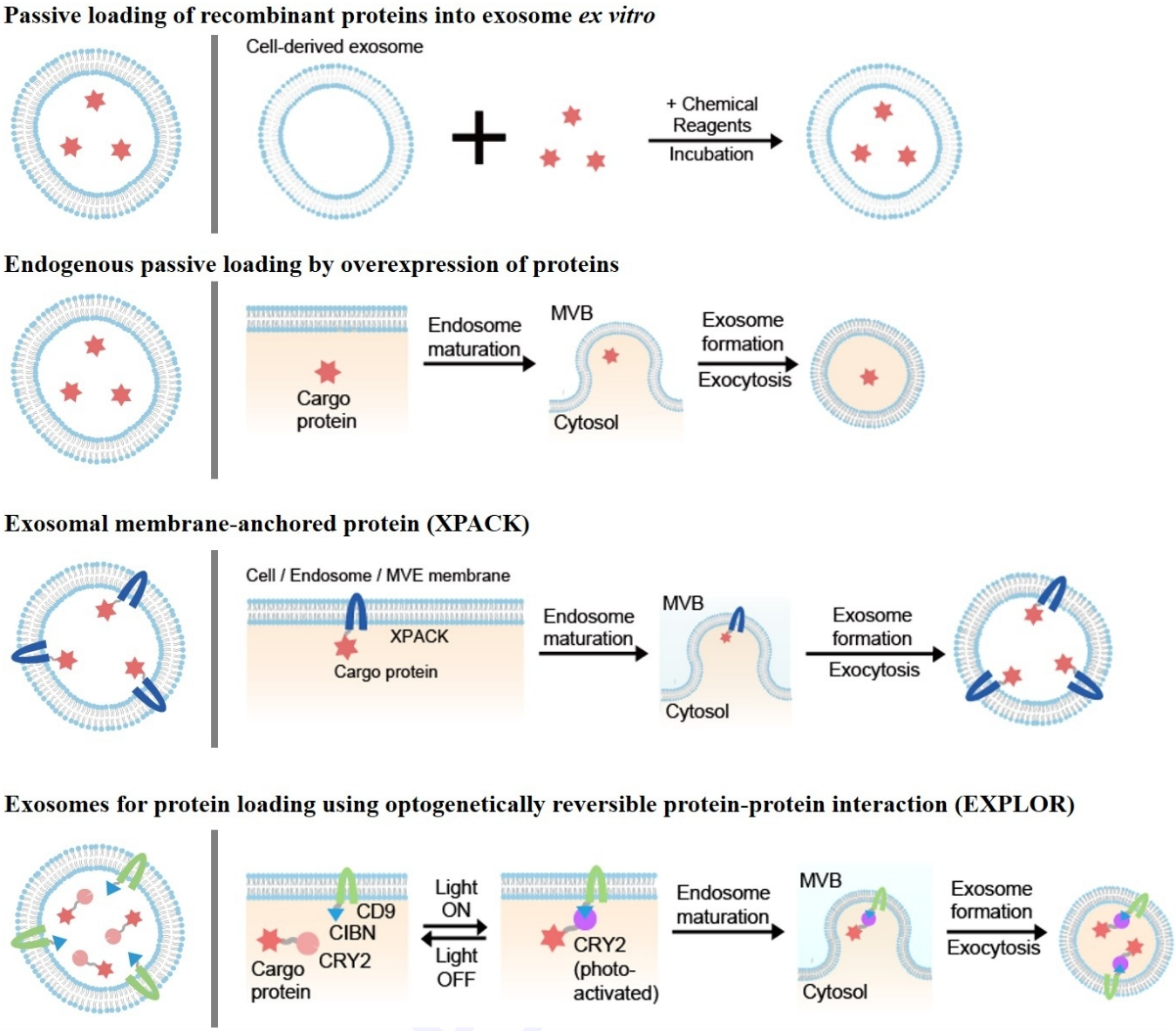

Fig. 1