FUNCTIONALIZATION OF ENVELOPED PROTEIN NANOCAGES VIA DESIGNED TRANSMEMBRANE PROTEINS

00163

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BACKGROUND

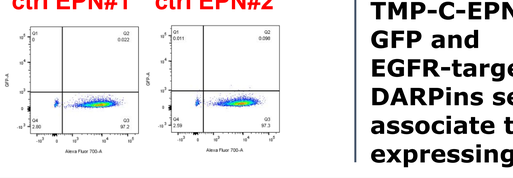
Extracellular vesicles (EVs) are an exciting tool for therapeutic use, but engineering EVs remains challenging. To enable precise engineering of EVs, we developed Enveloped Protein Nanocages (EPN): EVs generated by designed proteins that induce their own release from cells inside cell-derived membrane envelopes. EPN are a genetically addressable platform that can be engineered to introduce new functions. Here we describe methods that functionalize EPN membranes via incorporation of designed and natural Transmembrane Proteins (TMP)

- Enveloped Protein Nanocages (**EPN**) are a versatile, robust, and modular platform for engineering Extracellular Vesicles (EV)
- EPN may be pseudotyped, allowing for passive incorporation of Transmembrane Proteins (TMP) from producer cell membranes
- Transmembrane Protein Conjugated EPN (**TMP-C-EPN**) are formed by covalent conjugation of a designed TMP to the cage via the **SpyCatcher-SpyTag** system (Zakeri et al. 2012)
- EPN conform to designed models, are the correct size and architecture, and can be directed to specific target cells via conjugation of a designed DARPin-TMP

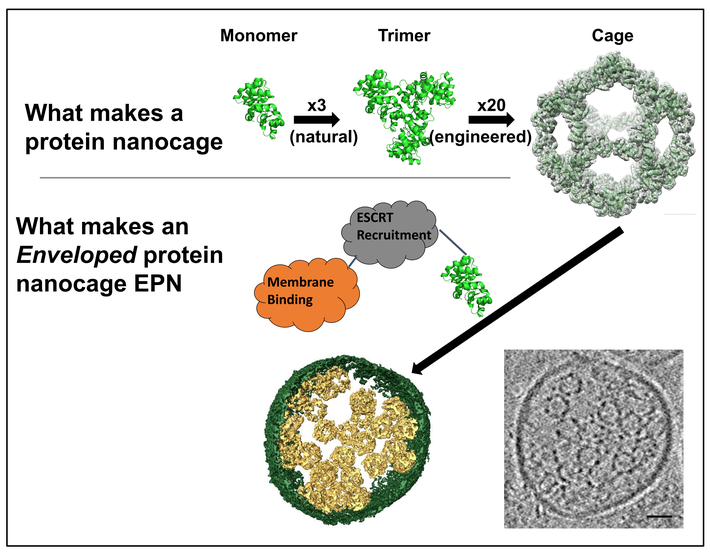
Ongoing Projects

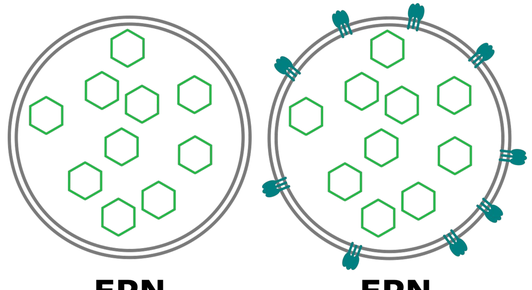
EPN as vaccines

Free trimer vs EPN-based



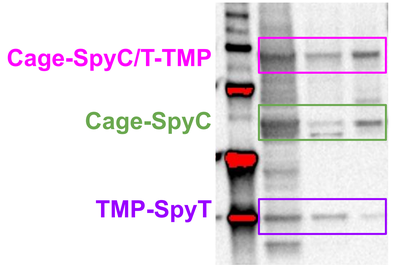
Is there an immunological difference?

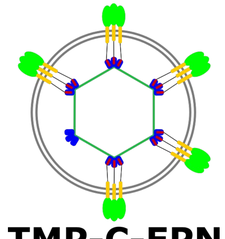


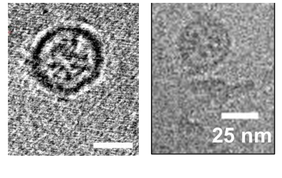


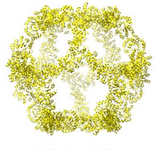
Designed TMP are covalently bonded to the cage scaffold, via SpyCatcher-SpyTag bond, guaranteeing presentation. Diameter of the EPN is lowered, with the membrane wrapped more tightly around the cage scaffold. The covalent cage-TMP linkage allows for monodisperse "monocage" EPN.

Direct and efficient *in-vivo* conjugation of **cage** and **designed TMP** for a **single covalently-linked protein**

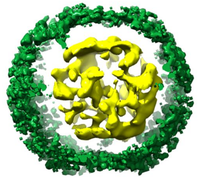








PDB: 5KP9



EPN as targeted nanoparticles

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