**Human paraneoplastic antigen Ma2 (PNMA2) forms icosahedral capsids that can be engineered for mRNA delivery**

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**Presenter:** Yi-Chen Tsai **Date/Time:**2024/09/12,15:00-16:00

**Commentator:** Shainn-Wei Wang **Location:** Room 601 , Med College Building

**Background:** Current gene delivery modalities, such as lipid nanoparticles and viral vectors, are effective but faces challenges, including limitations on cargo size, immunogenicity, difficulties in achieving tissue-specific targeting, and obstacles in scalable production. Natural gene delivery systems in human, such as endogenous retrotransposon-derived proteins, offer potential alternatives to address these limitations. Certain paraneoplastic antigens, which associate with autoimmune responses in cancer, can self-assemble into icosahedral capsid-like structures resembling retroviral capsids. These structures could be promising for mRNA delivery due to their stability and ability to protect genetic material. Thus, the capsid-forming properties of PNMA2 present a novel opportunity to develop efficient and targeted mRNA delivery systems that emulate viral mechanisms while being engineered for therapeutic purposes.

**Objective:** To investigate PNMA2's self-assembly into icosahedral capsids, engineer these capsids for mRNA delivery, evaluate their stability and efficiency, and explore their therapeutic potential in gene therapy and vaccines.

**Results:** A number of paraneoplastic antigens PNMAs were found to be multimers and can be secreted from human cells. Among these PNMNs, PNMA2 could form non-enveloped icosahedral capsid efficiently but did not naturally encapsidate nucleic acids. The structure of PNMA2, which was predicted by AlphaFold and resolved by cryo-EM, was further leveraged to engineer with a single-stranded RNA binding domain from CCMV for an oligomerized ePNMA2 particle with RNA packaging ability. Preliminary studies indicated that recombinantly purified ePNMA2 could successfully self-assembled into stable icosahedral capsids *in vitro*, with a triangular number T=1 for encapsulating and protecting mRNA. These ePNMA2 assemblies could efficiently deliver *Cre* mRNA into cells, particularly with cell penetrating peptide LAH4. Collectively, *Cre* mRNA containing ePNMA2-LAH4 assemblies facilitated reporter GFP intracellular translation, with enhanced stability, low toxicity, and good biocompatibility.

**Conclusion:** PNMA2 capsids effectively encapsulate and deliver mRNA, offering a novel platform for mRNA-based therapies like vaccines and gene therapy. While promising, further optimization is needed for clinical use, highlighting the system's potential for broader therapeutic