Antibody Strategies for Membrane Protein Targets

- **Soma S.R. Banik, PhD**
  Research Scientist and
  Communications Specialist
- **Benjamin J. Doranz, PhD**
  President and Chief
  Scientific Officer
  Integral Molecular
  Philadelphia, PA

Membrane-embedded proteins, including G protein-coupled receptors (GPCRs) and ion channels, are currently the most important class of therapeutic targets, and antibodies directed against them are highly sought for therapeutic, diagnostic, and research purposes. Despite substantial interest in these targets, high-quality antibodies against membrane proteins have been challenging to generate using conventional means.

The most specific and potent antibodies against these proteins typically target conformationally-complex epitopes formed by the tertiary structure of the proteins. For most soluble protein targets these antibodies are generated by inoculating animals with purified, full-length protein. Unfortunately, this approach is not effective for most multiple-spanning membrane proteins, whose native structure normally depends on an intact lipid bilayer. As such, alternative and often innovative approaches have been developed to generate antibodies against membrane proteins. Conventional approaches for generating such antibodies include immunization with whole cells, reconstituted proteins, or peptides, but these approaches are difficult to apply to some of the most important membrane protein targets. Novel technologies, such as the use of virus-like particles presenting high concentrations of native membrane proteins (Lipoparticles), represent a promising approach to this problem.

Deriving antibodies against membrane proteins

Given the importance of membrane protein targets, private and academic laboratories have tried numerous approaches to raise antibodies against them (Table 1, page 16). The most common way of generating these antibodies is immunization with whole cells over-expressing the target protein. This allows membrane protein targets to be displayed in their native conformation without mechanical or detergent disruption. Typically, stable murine cells expressing a human membrane protein are used to immunize mice. Using this approach, a number of antibodies against highly expressing membrane proteins have been developed. Transiently-transfected cells, membrane preparations, and cells selected for the highest expression levels have been used to improve this approach, with varying degrees of success. However, for many important membrane protein targets the ability of cell-based immunogens to elicit high-quality antibodies has been limited by low membrane-protein expression, the abundance of non-specific proteins, and target protein toxicity during cell line selection.

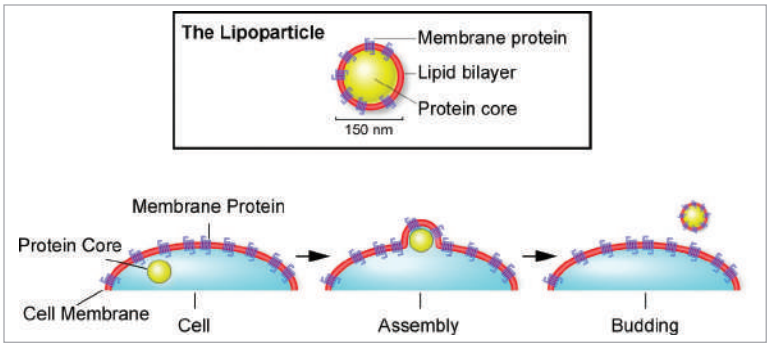

Figure 1. Production of Lipoparticles. Lipoparticles contain high concentrations of a target membrane protein in a virus-like particle assembled around a non-infectious core protein (Gag). Lipoparticles are harvested from producer cells overexpressing the membrane protein of interest. Because Lipoparticle formation is an active process mediated by Gag, the resulting particles are morphologically homogeneous. Lipoparticles do not contain cytoplasmic proteins or inverted membrane proteins that can result in an unfocused immune response. (Image: Integral Molecular)

Purified, reconstituted membrane proteins do not always maintain their native structure, but, when feasible, have resulted in highly significant monoclonal antibodies. For example, crystallography of the β2-adrenergic receptor (β2-AR) was enabled by a conformational antibody developed against the purified GPCR.[1] The β2-AR protein was detergent solubilized, reconstituted into phospholipid vesicles, and then used as an immunogen in mice. Extensive screening resulted in the isolation of a conformation-dependent antibody that stabilized a critical flexible region of the receptor, enabling its crystallization. Similarly, another conformation-dependent antibody played a crucial role in one of the first crystal structures of an ion channel, the KcsA

Immunogen	Native Conformation	Immunogen Concentration	Immunogen Purity
Purified Protein	-	+++	+++
Reconstituted Protein in Vesicles	+/-	+++	+++
Peptides	-	+++	+++
CLIPS Peptides	+/-	+++	+++
Cellular Over-expression	+	+	+
Lipoparticles	+	++	++

Table 1: Comparison of different immunogens used for the production of antibodies against complex membrane protein targets. (Source: Integral Molecular)

potassium channel.[4] The limitation of this approach is the requirement for purified, solubilized, and reconstituted membrane protein in its structurally intact form, which is a difficult requirement for many membrane proteins.

Although conformationally complex structures typically form the most important functional regions of proteins, simple peptides can be effective immunogens for deriving antibodies against linear epitopes. A novel variation of this approach, CLIPS (chemical linkage of peptides onto scaffolds), uses peptides to recreate individual conformational epitopes for immunization. The premise of CLIPS is to constrain linear epitope sequences using cysteine linkages to mimic specific structured conformations that better represent the native protein. Using CLIPS, conformational antibodies have been raised against follicle-stimulating hormone (FSH) and the GPCR CXCR7.[2] Although it requires predicting and recreating the structure of complex membrane proteins, this technique could prove useful if it can be broadly adapted to different membrane protein antigens.

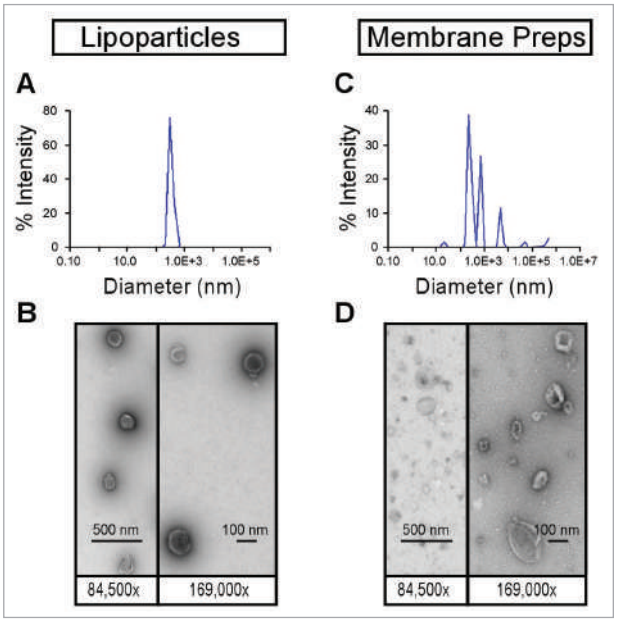

Figure 2. Purity and homogeneity of Lipoparticles vs. membrane preparations. Dynamic light scattering (A and C) and electron microscopy (B and D) confirm that Lipoparticles are free of contaminating cellular debris that occurs with membrane preparations. Dynamic light scattering measures the size dispersion of particles in solution, while electron microscopy enables visualization of these contaminants. The purity and homogeneity of Lipoparticles explains their improved immunogenicity. (Image: Integral Molecular)

The lipoparticle

Integral Molecular has taken a different approach to the challenge of presenting native membrane protein immunogens by developing the Lipoparticle.[3] Lipoparticles are virus-like particles that incorporate high concentrations of target membrane proteins in their native conformation. Lipoparticles are produced from mammalian cells by co-expressing the retroviral structural core polyprotein, Gag, along with a desired membrane protein. Gag core proteins self-assemble at the plasma membrane, where they bud off and capture target membrane proteins (Figure 1, page 14). Lipoparticles are approximately 150 nm in diameter, so are readily suspended in aqueous solutions that can be used for inoculation. Because membrane proteins within Lipoparticles are

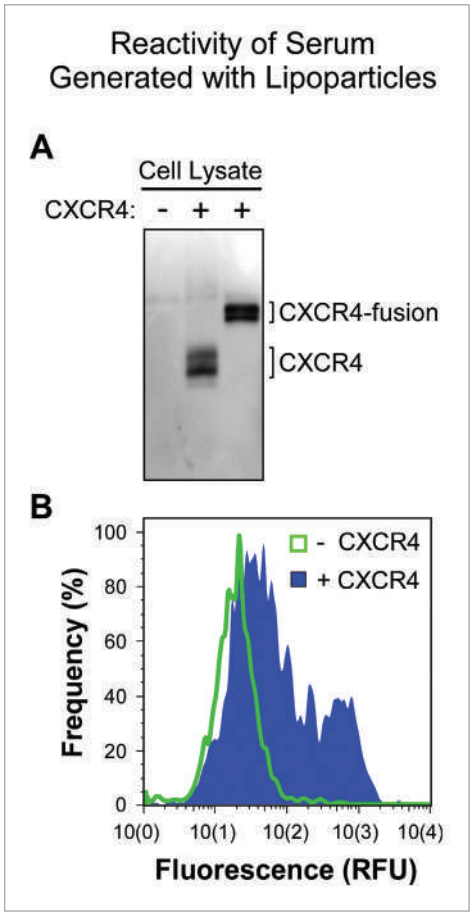

Figure 3. High-titer antibody production using Lipoparticles. A) Western blot reactivity using anti-CXCR4 serum (1:500) derived using CXCR4-Lipoparticles. Lysates were prepared from cells with or without CXCR4 using a different cell type than was used for Lipoparticle production. B) Staining of cells transiently transfected with either CXCR4 or a control plasmid using CXCR4-Lipoparticle antiserum (1:200), as analyzed by flow cytometry. The specific staining of CXCR4-positive cells is indicative of the high-titer immune response against the GPCR elicited by CXCR4-Lipoparticles. (Image: Integral Molecular)

derived directly from the cell surface without mechanical disruption or detergents, the native structure and orientation of the membrane proteins are retained. Unlike traditional sources of membrane proteins (Figure 2), Lipoparticles do not contain cytoplasmic proteins or inverted membrane proteins that can result in an unfocused immune response. Typical concentrations of specific membrane proteins in Lipoparticles are 50-200 pmol/mg, approximately 10 to 100 fold more concentrated than in cells or membrane preparations. As a result, Lipoparticles represent a high quality immunogen.

Lipoparticles are currently being used to produce antibodies against a number of different classes of membrane proteins, including GPCRs, ion channels, and structurally-complex single-transmembrane proteins. For example, to generate antibodies targeting the GPCR CXCR4, purified Lipoparticles incorporating the receptor were used to immunize naïve mice. Sera from these mice demonstrated strong immunoreactivity against CXCR4 by both Western blot and flow cytometry (Figure 3). The 50% reactivity titer of CXCR4-Lipoparticle antiserum against cells expressing CXCR4 was greater than 1:500 by flow cytometry, representing a highly reactive immune response against the membrane protein. These data demonstrate that Lipoparticles can be used to generate specific high-titer immune responses to conformationally-intact membrane proteins. The development of new technologies, such as the Lipoparticle, for manipulating membrane proteins in their native structure offers the opportunity to develop high-quality antibodies against previously intractable protein targets. ■

Soma S.R. Banik's most current research both at Integral Molecular and elsewhere has focused on viral drug targets. Benjamin J. Doranz has been studying viruses and membrane proteins for the past 20 years.

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