# (SD2022-010) Method for transmembrane protein semisynthesis and reconstitution in lipid membranes

Tech ID: 32859 / UC Case 2021-Z08-1

## BACKGROUND

Cellular lipid membranes are embedded with transmembrane proteins crucial to cell function. Elucidating membrane proteins' diverse structures and biophysical mechanisms is increasingly necessary due to their growing prevalence as a therapeutic target and sheer ubiquity in cells. Most biophysical characterization strategies of transmembrane proteins rely on the tedious overexpression and isolation of recombinant proteins and their reconstitution in model phospholipid bilayers.

Unfortunately, membrane protein reconstitution depends on the use of denaturing and unnatural detergents that can interfere with protein structure and function. We have developed a detergent-free method to reconstitute transmembrane proteins in model phospholipid vesicles and GUVs. Additionally, transmembrane proteins are difficult to express in cells due to the extreme insolubility of their transmembrane domain. By incorporating a synthetic transmembrane peptide into liposomes and simply expressing soluble portions of transmembrane proteins in cells, we can use this semisynthetic ligation strategy to more easily construct functional transmembrane proteins and reconstitute them into liposomes for biophysical and biochemical studies.

Inteins can be found contiguously or non contiguously within some proteins. Non-contiguous inteins are called "split inteins". Inteins can be thought of as a type of protein intron which splices itself out of proteins. When non-contiguous inteins find and bind to each other, they are then able to excise themselves resulting in the ligation of their respective exteins. Split intein pairs (C-intein and N-intein) can be attached to proteins of interest in synthetic and cellular systems to ligate protein sequences together.

## TECHNOLOGY DESCRIPTION

Researchers from UC San Diego have developed a novel method for the semisynthesis of transmembrane proteins in lipid membranes.

This method will improve biochemical, biophysical, and pharmacological research on TM proteins as it will expedite the production of reconstituted proteins anchored by synthetic or native TM domains in giant unilamellar vesicles (GUVs).

By utilizing split intein ligations, they were able to semisynthesize single pass transmembrane proteins on liposomes and giant unilamellar vesicles (GUVs). By covalently linking a glycosylated mammalian protein to

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**OTHER INFORMATION**

**KEYWORDS**
therapeutics, high throughput screening, transmembrane protein reconstitution

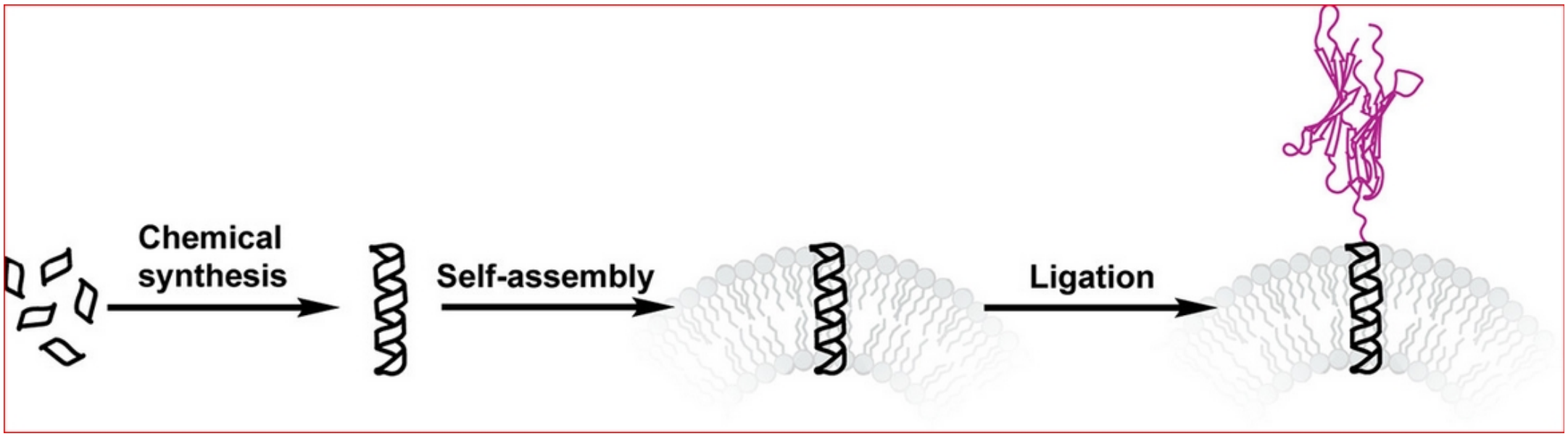
**CATEGORIZED AS**
- Medical
  - Research Tools
  - Screening
  - Therapeutics

**RELATED CASES**
2021-Z08-1

a TM domain anchored in the phospholipid membrane while retaining its extracellular binding functions, they have created a reconstitution system for cell biologists to model extracellular interactions.

Split inteins are natural or engineered protein trans-splicing domains. By leveraging the biorthogonality and chemoselectivity of split inteins, overexpressed soluble domains are ligated to synthetic transmembrane peptides to build semisynthetic membrane proteins directly on phospholipid vesicles. This one-pot method bypasses the painstaking expression of recombinantly expressed integral membrane proteins and the multistep process of detergent-based protein reconstitution, making it easier to study these important biomolecules in an isolated system.

In this invention, a synthetic transmembrane (TM) peptide, of a natural or unnatural sequence, fused to a C-intein construct is synthesized via solid phase peptide synthesis. A soluble protein or soluble domain of a transmembrane protein is expressed in cells as a recombinant protein-N-intein fusion. The TM peptide is incorporated into liposomes by making a phospholipid (DOPC) + TM peptide film and hydrating it in water or buffer. Multilamellar vesicles with incorporated TM peptide are made via simple hydration while GUVs with incorporated TM peptide are made via electroformation. The soluble protein-intein fusion is added to the peptide-loaded vesicles and the ligation reaction proceeds on the phospholipid membrane: split intein association results in an N to S acyl shift. A transthioesterification results in the formation of the branched intermediate. Succinimide formation releases both inteins and a final S to N acyl shift results in the ligated extein product (in this invention, a transmembrane peptide fused with soluble proteins or protein domains) with a native peptide bond. Subsequent SDS-PAGE, microscopy, and mass spectra of the product verifies that the reaction has taken place.




## APPLICATIONS

Expression and reconstitution of functional membrane proteins. High throughput drug screening of transmembrane (TM) proteins in model membranes.

This method will improve biochemical, biophysical, and pharmacological research on TM proteins as it will expedite the production of reconstituted proteins anchored by synthetic or native TM domains in giant unilamellar vesicles (GUVs).

## INTELLECTUAL PROPERTY INFO

Patent-pending technology. Worldwide rights available. UC San Diego is seeking partners for commercial development.

## RELATED MATERIALS


- Podolsky KA, Masubuchi T, Debelouchina GT, Hui E, Devaraj NK. In Situ Assembly of Transmembrane Proteins from Expressed and Synthetic Components in Giant Unilamellar Vesicles. ACS Chem Biol. 2022 May 20;17(5):1015-1021. doi: 10.1021/acschembio.2c00013. Epub 2022 Apr 28 - 04/28/2022



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