Revisiting the Correlation between Elastic Mechanics and the Fusion of Lipid Membranes

Probing the causing of diseases by X-ray scattering.

Lipid membrane fusion is a process whereby two membrane-bound entities merge into a single entity, with the initially discrete membranes and their enclosed contents mixed together (**Fig. 1**). This biological process is vital for living organisms. Many cellular events, such as the release of neurotransmitters, the invasion of enveloped viruses and the conception for sexual reproduction, involve membrane fusion. Given the ubiquity and importance of fusion in cellular activities, any abnormality in fusion can be pathogenic. Understanding the mechanism of fusion hence bears a biomedical relevance, whether it is to develop therapeutic means to target diseases related to fusion abnormality or to improve the preventive measures against viral invasions.

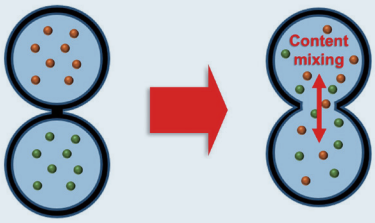


Fig. 1: Schematic representation of fusion of a lipid membrane.

A fusion entails lipid membranes undergoing several structural transformations. Before fusion, lipid membranes assume a planar conformation known as a bilayer structure (**Fig. 2(a)**). After an initiation of fusion, lipid membranes transform through complicated, curved conformations in a series (such as the stalk structure illustrated in **Fig. 2(b)**) before resuming the bilayer structure at the end of the process. Effecting these structural transformations consumes energy. The energy required for fusion, and thus the fusion potency of a lipid membrane, can be diminished or increased on tuning the lipid composition. This compositional dependence is widely believed to arise from the compositional dependence of the spontaneous curvature (C_0), an elastic property of a lipid membrane quantifying its tendency to form curved conformations (a larger C_0 indicates a stronger tendency). The currently prevalent view is that increasing C_0 by tuning the lipid composition decreases the energies of the curved structures, and thus depresses the energies required for fusion and boosts the fusion potency of a lipid membrane. Whereas the effect of varying C_0 on fusion has been confirmed and extensively studied, the notion that varying C_0 decreases the energies of the curved structures remains speculative. More strikingly, another key elastic property of a lipid membrane, the bending modulus, K_{cp} (which measures the stiffness of a membrane), had rarely been investigated for its influence on fusion, even though it plays a key role in determining the energy required to transform lipid membranes.

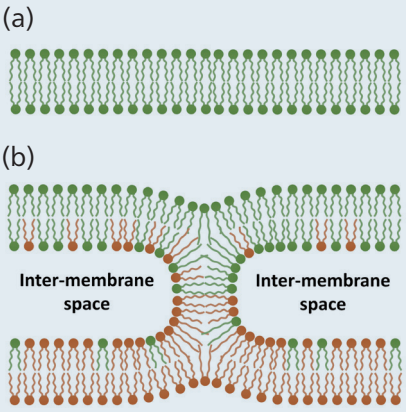


Fig. 2: Schematic representations of membrane conformations (a) bilayer structure, and (b) stalk structure. [Reproduced from Ref. 1]

In a recent article published by Yi-Fan Chen[1] (National Central University) and his colleagues investigated the correlations among C_0, K_{cp} and the fusion potency of lipid membranes, seeking to address the lingering questions regarding the fusion mechanism. The team began the investigation by measuring C_0 and K_{cp} for membranes composed of various lipid species. The measurements were performed with small-angle X-ray scattering and diffraction (SAXS and SAXD, respectively) techniques. With the high-quality X-rays delivered at **TLS 13A1** and **TLS 23A1** and the method developed in other work of the group,[2] the authors obtained high-quality SAXS and SAXD data, determined the detailed structures of the membranes under varied conditions and thereby inferred the values of the two elastic properties. Based on these C_0 and K_{cp} data for various lipid species, the authors prepared mixed lipid membranes (*i.e.*, membranes composed of more than one lipid species) in two series: one series comprised membranes with disparate K_{cp} but essentially identical C_0, whereas the other series consisted of membranes with disparate C_0 but marginally different K_{cp}. By fixing either elastic property, investigating the

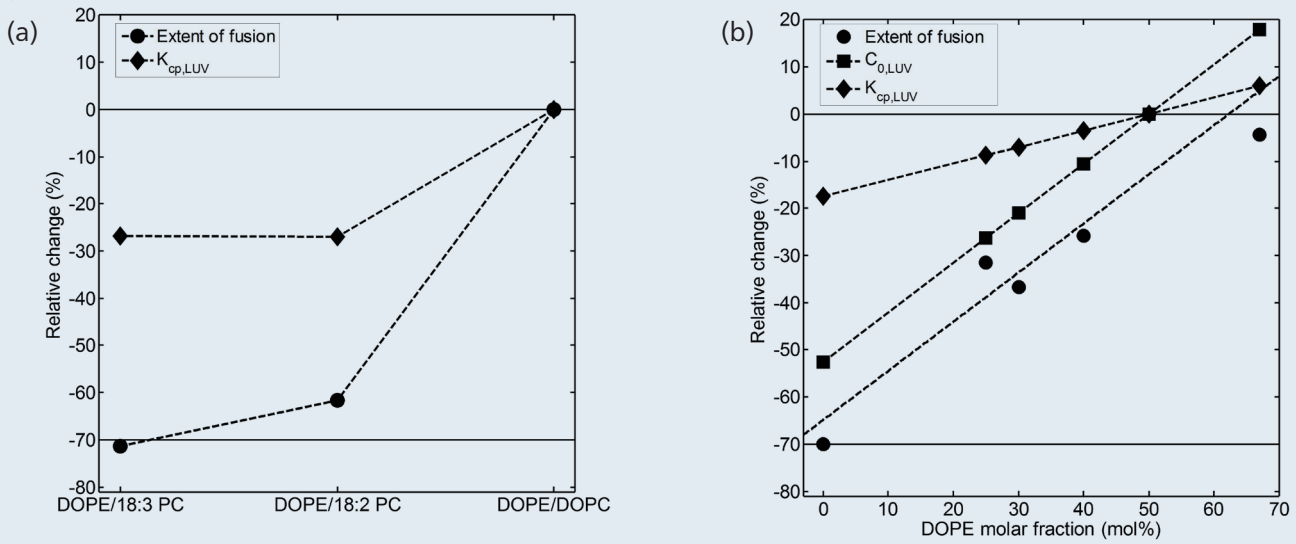


Fig. 3: Correlations among C_0, K_{cp} and fusion potency for (a) the series of membranes with C_0 varying significantly, and (b) the series of membranes with very different K_{cp}. [Reproduced from Ref. 1]

variation in the fusion potency within a series allowed the authors to differentiate the contribution of one elastic property from that of the other.

For the membranes in the series in which C_0 was varied significantly, the authors observed the expected correlation between C_0 and fusion potency, *i.e.*, a larger C_0 led to an increased fusion potency (**Fig. 3(a)**), but close thermodynamic analysis of the data revealed that what promoted fusion when C_0 was varied was not the decreased energy of the curved structures but the energetic elevation of the bilayer structure before fusion. Rather than stabilizing the curved structures to facilitate fusion, raising C_0 hence destabilized the bilayer structure before fusion, and consequently made fusion more energetically appealing. This finding transcended conventional wisdom and shed new light on the roles of proteins in coordinating fusion in cells.

A more astonishing observation arose when lipid membranes in the other series were examined for their fusion behavior. As expected, varying K_{cp} significantly affected the fusion potency of a lipid membrane; this experimentally observed correlation was the first of its kind, to the best of the authors' knowledge. Instead of promoting fusion as one might expect, making lipid membranes softer (*i.e.*, decreasing K_{cp}), however, counterintuitively suppressed fusion substantially; in some cases, fusion even ceased completely (**Fig. 3(b)**). A profound thermodynamic analysis of the data indicated that the suppression in the fusion potency did not arise from changes in any known factor considered to be crucial for fusion. The result therefore pointed to the existence of a yet-to-be-recognized mechanism that was, at least, as equally important for fusion as the known factors, and revealed the necessity to modify the decades-old view on fusion, which evolved around C_0 and the associated energies for fusion.

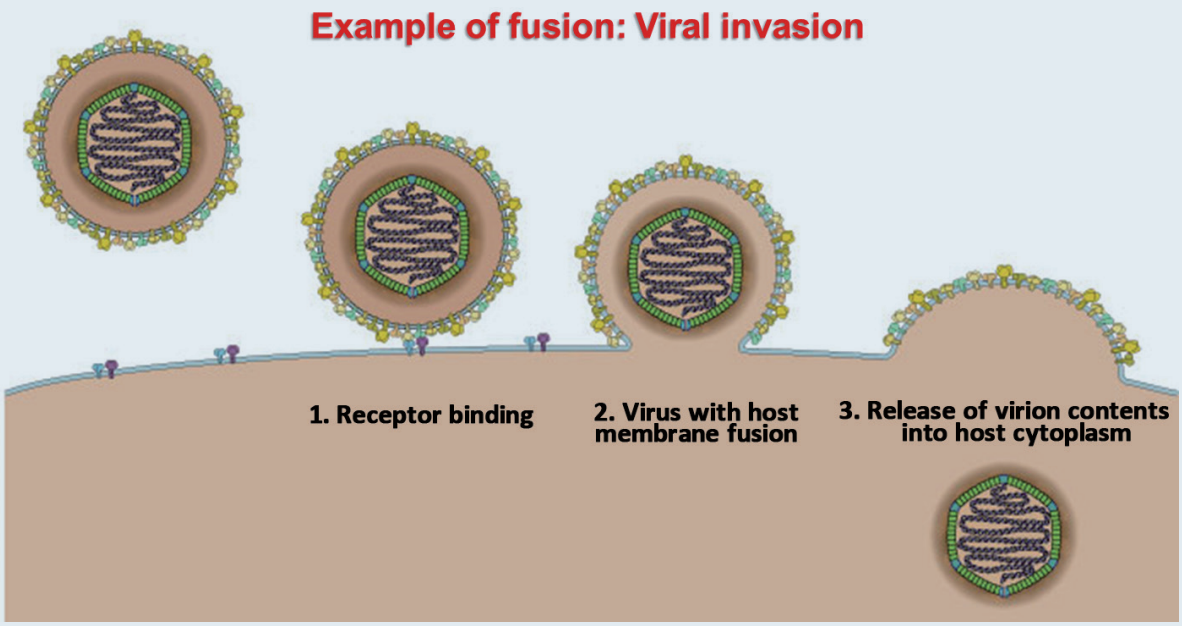
Overall, the two key findings of this work have reshaped both our understanding of the fusion mechanism and the biological functions of proteins involved in fusion. This progress would eventually lead to a new perspective on the fusion processes occurring in living organisms and thereby facilitate the development of drugs to treat or to prevent human diseases associated with fusion. (Reported by Yi-Fan Chen, National Central University)

This report features the work of Yi-Fan Chen and his co-workers published in Scientific Reports ***6****, 31470 (2016).*

TLS 13A1 SW60 – X-ray Scattering
TLS 23A1 IASW – Small/Wide Angle X-ray Scattering
- SAXS, WAXS
- Soft Matter, Biological Physics, Membrane Biophysics

| References |

1. Z.-A. Fan, K.-Y. Tsang, S.-H. Chen, and Y.-F. Chen, Sci. Rep. **6**, 31470 (2016).
2. Y.-F. Chen, K.-Y. Tsang, W.-F. Chang, and Z.-A. Fan, Soft Matter **11**, 4041 (2015).



Source: Swiss Institute of Bioinformatics

Designing a Starch-Based Active Coating with a pH-Responsive Property

Starch serves as a smart material for food preservation.

Food spoilage due to microbial growth or lipid oxidation occurs commonly in our daily life. The spoilage not only decreases the shelf life of foods but causes serious sickness if toxins are produced by microorganisms and ingested by consumers. The addition of preservatives and antioxidants is the most efficient way to prevent food spoilage from the growth of microorganisms and lipid oxidation. Currently, the direct addition of more than adequate amounts of preservatives and antioxidants in foods is commonly applied, but the spoilage of foods is a gradual process occurring over time during storage; the initial addition of more than necessary amounts of preservatives or antioxidants might be not the best way from concerns about safety, effectiveness and economics. Active package techniques, such as the inclusion of active compounds to prevent microbial growth and to retard lipid oxidation, have hence attracted great attention. A pH-responsive material can not only play the role of a carrier to carry the active compounds but also a controlled release of active compounds when the pH in foods alters during storage. In practice, the minimum dose of antioxidants carried by an active coating of starch film becomes released only when the pH decreases resulting from free fatty acids released through lipid hydrolysis. How to make the active compounds release from the starch matrix when really needed is a critical technique for the development and applications of a starch-based active coating with pH-responsive property.

Hsi-Mei Lai at Department of Agricultural Chemistry in National Taiwan University is famous in the field of starch chemistry and applications in both academia and food industry. Lai's group sought to design a pH-responsive starch-based thin film that can promise a quick response upon pH altering in food systems.[1-3] Charges on the modified starch molecules interact with the negative charges on the additive (citric acid in this case). In detail, the positive charges on the cationic starch (CS) interact with the negative charges on the citrate, which has three carboxyl groups (pK_a 3.14, 4.77 and 6.39), depending on the pH in the food systems.

As **Fig. 1** shows, there is no significant difference in the size of starch granules embedded in a CS thin film between pH 3.0 and 5.5 in NaCl solution (0.01 M) (**Figs. 1(a) and 1(b)**). Once citric acids are added to the CS starch film forming a solution, the citrate-CS