論文 / 著書情報
Article / Book Information

題目(和文)	シャペロン機能カチオン性高分子による脂質膜のDNA駆動形態制御
Title(English)	DNA-commanded Morphological Control of Lipid Membranes Chaperoned by Cationic Copolymers
著者(和文)	ZHANGWANCHENG
Author(English)	Wancheng Zhang
出典(和文)	学位:博士(学術), 学位授与機関:東京工業大学, 報告番号:甲第12597号, 授与年月日:2023年9月22日, 学位の種別:課程博士, 審査員:丸山 厚,金原 数,清尾 康志,秦 猛志,堤 浩
Citation(English)	Degree:Doctor (Academic), Conferring organization: Tokyo Institute of Technology, Report number:甲第12597号, Conferred date:2023/9/22, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	Life Science and Technology 系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 (Philosophy) Doctor of
学生氏名： Student's Name	Zhang Wancheng	審査員主査： Chief Examiner	Maruyama Atsushi

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Phospholipids, fundamental components of biological membranes, are amphiphilic molecules that can spontaneously assemble into bilayer structure with their hydrophilic head groups facing water and lipidic tails embedded in the middle. In addition to their biocompatibility, the low molecular weight provides the bilayers with high fluidity. It makes other substances easy to incorporate into bilayers, allowing for flexible formulations. These advantages have attracting increasing attention to phospholipid-based nanodevices such as liposomes for applications including drug delivery, biosensing and bio-machinery. Lipid-based 2D nanomaterials, such as nanosheets and nanodiscs, share the above advantages, while possessing unique interfacial properties. However, forming stable lipid nanosheets is challenging because they spontaneously convert into micelles or vesicles to minimize the contact of hydrophobic moieties with the aqueous medium. Although some strategies have been developed to prepare stable nanosheets such as the use of amphiphilic substances, all confronted issues such as bio-incompatible and time-consuming preparation procedures, low yields or loss of flexibility.

The author's group previously reported a facile strategy for the preparation of lipid nanosheets utilizing the electrostatically assembled complex of the anionic peptide E5 and its cationic copolymer chaperone polyallylamine-*graft*-dextran (PAA-*g*-Dex), which can fracture the membranes of giant unilamellar vesicles (GUVs) and convert them into nanosheets with cell-sized surface within tens of seconds. Moreover, polyanions, exemplified by poly(vinylsulfonic acid) (PVS) can dissociate E5/PAA-*g*-Dex complexes, leading to the destabilization of lipid membranes and their conversion back to vesicle morphology. DNA is also a polyanion with a negatively charged phosphate backbone, so it can shield the positive charges of copolymer from E5, leading to disassembly of E5/copolymer complexes. It can therefore potentially drive the sheet-to-vesicle conversion of lipid membranes. Besides, the programmability of DNA sequence grants versatile design of DNA nanostructures and high selectivity. Henceforth, the utilization of DNA as a specific factor to command the morphological conversion of lipid membranes can further expand the application of lipid-based nanodevices.

In this dissertation, the loop of vesicle-sheet-vesicle conversion of lipid membrane has been investigated under the influence of DNAs. Chapter 1 reviews the major functions of membranes composed of phospholipids as an important component of cells, leading to the flourishing development of lipid nanotechnology for biological and medical research and applications. The

principles of formation of lipid nanosheets, an emerging member of lipid nanotechnology, are introduced, and their convertible property is elucidated. Chapter 2 revisits nanosheet formation and presents a dual-anchoring strategy of E5 and PAA-*g*-Dex on lipid membranes. This strategy enhances nanosheet stability against disrupting factors like dilution, making them more suitable than previous non-anchoring system for real applications. Taking advantage of this, measurements such as flow cytometry that requires highly diluted samples could be utilized to analyze the properties of lipid nanosheets. As for DNA-driven sheet-to-vesicle conversion, double-stranded DNA proves more effective than single-stranded DNA in dissociating E5/copolymer complexes and converting lipid nanosheets, indicating DNA nanostructures' significant role. But the conversion has no specificity, making them hard to be controlled under complex environments. Additionally, the sheet-to vesicle conversion requires large amounts of DNAs of several micromolar concentration, especially for dual anchoring system due to high stability, which limits the further application of the nanosheets. Chapter 3 demonstrated the preparation of a DNA-specific cationic copolymer based on the conjugate of cationic copolymer and peptide nucleic acid (PNA) through copper-free strain-promoted azide-alkyne cycloaddition, advancing the research in Chapter 2. The PNA strand can recognize and harvest complementary DNA sequence (cDNA) to neutralize the cation of the copolymers. The cascade of switch-OFF of chaperone function of the copolymer, function of E5 and sheet-to-vesicle conversion of lipid membrane can be controlled by cDNA with high responsiveness and low DNA usage in nano-molar scale. It showed much higher effectiveness than the use of non-complementary sequence, presumably due to higher affinity promoted by base-pair than mere electrostatic force. Furthermore, the hybridized DNA can be further removed by toehold displacement, allowing the copolymer to become positively charged and able to chaperone E5 again. As such, the restored peptide/copolymer complexes further fractured lipid membrane to smaller sizes, as observed by transmission electron microscopy. These results proved PNA-copolymer conjugates as promising tool for gene targeted delivery, biosensing and artificial cells. Chapter 4 concludes the dissertation. Also, the author highlights the usefulness of the convertible lipid membrane and envisions several perspectives for future research and application.

Overall, although certain experimental and theoretical details require more comprehensive investigation, the converting capability of the lipid membranes in response to DNA sequences and structures allows flexible, efficient, and smart control of structure of lipid bilayer, which are promising solutions to biocompatible lipidic devices, drug delivery systems and artificial cells. Meanwhile, the DNA-commanding strategy in this dissertation is not confined to the control of E5 peptide. It can be potentially employed to other nanodevices chaperoned by cationic copolymer, extending its scope to a broader range of applications.