孙飞研究组
2021年度工作报告

中国科学院生物物理研究所生物大分子国家重点实验室

2021

目录

年度工作总结

2021 年是孙飞研究组成立后的第十六年，在前三个五年发展阶段（学习、成长、成熟）基础上进入了第四个发展阶段（2021—2025），在这个阶段研究组目标产出更多更高水平研究成果，科研成果显示度和影响力显著提升，科研能力达到新高度。本阶段的研究主线包括：（1）原位结构生物学技术和应用；（2）超快冷冻电镜技术和应用；（3）三维构造生物学技术和应用；（4）物理生物学初步探索。在逐步围绕主线推进前沿研究的同时，将充分发挥研究组在冷冻电镜技术方面的特长与国内外相关课题组围绕重要生物大分子复合体（特别是膜蛋白质复合体）的结构功能研究展开合作。

围绕新的五年发展阶段的研究主线和目标，研究组在年初制定了年度工作计划，确定了年度工作重点、重点攻关课题、稳步推进课题和新的课题探索，做出了年度项目申请规划和学术交流计划，明确了科研突破、文章发表、专利申请和人才培养等任务目标。

2021 年，研究组科研成果丰硕。在冷冻电镜技术方法开发方面，与生物成像中心合作研发成功针对生物组织样品的原位冷冻电镜样品制备技术 VHUT-cryoFIB，发明了新型样品承载零件，综合高压冷冻技术、冷冻修块技术、冷冻聚焦离子束减薄技术和冷冻电子断层三维重构技术，实现了对菠菜叶片、小鼠骨骼肌、心肌和肝脏等组织的原位冷冻电镜成像，并解析了核糖体的原位结构。开发了基于疏水蛋白的新型冷冻电镜支出膜技术，解决了在运用冷冻电镜单颗粒三维重构技术过程中所遇到的气液界面问题，利用该技术有效解决了气液界面所导致的过氧化氢酶（Catalase）和流感病毒血凝素（Hemoglutinin A）的取向优势问题，此外利用该技术可以获得更高质量的冷冻样品，解析更高分辨率的结构，适用分子量范围广，最小可以到 64kDa 以下，有望在未来的冷冻电镜研究中获得广泛的应用。

在膜蛋白复合体结构功能研究方面，研究组与中科院上海药物所吴蓓丽 / 赵强研究组合作解析多个不同状态（非激活态、激活中间态、同源二聚体、异源二聚体等）的代谢型谷氨酸受体 mGlu2/mGlu7 的冷冻电镜结构，揭示了这类谷氨酸受体的信号转导模式，促进了对该类受体在中枢神经系统中功能调控机理的认识。与德国马普

生物物理所的 Hartmut Michel 教授合作，利用冷冻电镜单颗粒技术解析了超嗜热菌 Aquifex aeolicus 细胞色素 c 氧化酶的 3.4 埃分辨率结构，发现该细胞色素 c 氧化酶呈现一个独特的同源二聚体结构，在二聚体界面处形成了新的氢醌结合位点，从而解释了为什么该细胞色素 c 氧化酶能够同时氧化细胞色素 c 和氢醌，从进化的角度进一步丰富了人们对细胞色素 c 氧化酶结构功能的认识。

在膜动态分子机理研究方面，研究组与哈佛大学医学院 Victor 教授研究组合作研究了细胞内体运输途径包被蛋白 Sorting nexin 1（SNX1）诱导细胞膜变形重塑的分子机理，利用冷冻电镜螺旋三维重构技术解析 SNX1 包被脂质体小管的高分辨率三维结构，发现 SNX1 以二聚体作为最小组装单元排列在细胞膜表面，其 BAR 结构域与 PX 结构域之间的一段两性螺旋直接参与了细胞膜的重塑，通过结构分析和比较，提出了 Retromer–SNX1 衣被复合体的上膜组装分子过程和 SNX1 诱导细胞膜重塑分子机理的模型。

在原位冷冻电镜结构研究方面，研究组与北京大学张传茂教授研究组合作，优化冷冻电镜样品制备流程、数据收集策略和图像处理算法，利用冷冻电镜单颗粒技术解析了爪蟾卵母细胞核膜孔复合体 NPC 在核膜原位状态下其外周环（即胞质环和核质环）的亚纳米分辨率（8 埃）三维结构，并进一步利用最新的深度学习结构计算软件 AlphaFold2 搭建了爪蟾卵母细胞 NPC 外周环的接近完整的准原子模型，鉴定和搭建出一系列新的组成亚基（Nup358，Nup214，Nup205，Nup93，ELYS 等），为下一步研究 NPC 的动态组装奠定了重要结构基础。

此外，研究组也积极面向人类健康重大需求，围绕新冠防控基础研究需求，积极与国内相关研究组合作，在新冠病毒原位结构、侵染机理、多肽阻断抑制剂等方面取得了突出的研究成果，为未来新冠防控工作做出了贡献。新冠病毒表面布满了刺突状的 spike 蛋白，主要负责识别人体表面受体从而介导病毒感染，是疫苗和药物的主要设计靶点之一。研究组与中科院生物物理所王祥喜团队合作，解析了 β – 丙内酯灭活的新冠病毒表面 spike 蛋白 postfusion 状态下的精细原位结构特征，发现了其具有特殊的寡聚化行为。这种寡聚行为将显著增强 spike 蛋白在病毒膜上的局部丰度，有利于病毒感染中融合孔的形成。研究组与复旦大学陆路研究组联合攻关，解析了 EK1 多肽靶向结合新冠病毒 spike 蛋白的 HR1 结构域的高分辨率结构，详细阐述和评价了 EK1 作为泛冠状病毒融合抑制剂的作用机理，为该药物推向临床使用提供了重要的实验数据。此外，研究组还与复旦大学陆路研究组合作，解析了新冠 B.1.1.7 突变体 spike 蛋白和 ACE2 受体结合的高分辨率复合体结构，发现病毒不同突变位点以多种方式增强病毒感染效率的分子机制，加深了人们对新冠病毒突变体特性的认识。

在人才培养方面，朱赟副研究员成功升级为项目正高级研究员，朱春梅同学顺利通过答辩获得理学博士学位，台林华荣获 2021 年博士研究生国家奖学金，殷国良荣获 2021 年硕士研究生国家奖学金，朱国梁荣获 2021 年朱李月华优秀博士生奖学金（非西部）和 2021 年博士研究生所长奖学金。在队伍建设方面，实验助理苏瑞刚高级实验师光荣退休，新招聘实验助理刘甜和实验助理实习生杜梦琪。

在对外学术交流方面，参加国际国内学术会议 29 人次，应邀在国际国内学术会议上做学术报告 11 场次；针对合作项目组织了项目与课题的进展汇报与讨论，邀请领域专家做学术报告 2 场次。在内部学术交流活动方面，2021 年度研究组主要开展了以下学术交流活动：组织了 16 个专题讲座；组织 58 次文献阅读报告会，集中分享与解读了 54 篇文献，分享参加国际国内学术会议的收获与体会报告 4 次；组织 9 次研究组月交流会，遴选进展显著、有阶段性成果的课题进行专题汇报与深入讨论。此外，组织了重点课题交流会、三个研究小组自主召开的研究小组周交流会；还组织了研究组年度中期工作总结以及年度工作总结等一系列学术活动。

经过研究组全体师生一年来的共同努力和全力攻关，研究组基本完成了年初制定的工作计划，共发表研究论文 11 篇（其中通讯作者 10 篇），申请发明专利 3 项，成功组织申请科技部国家重点研发计划项目 1 项，成功参与申请科技部国家重点研发计划项目 1 项。研究组网站改版完成，研究组制度建设进一步修订完善，研究组实验技术手册编纂初步成形。研究组重点攻关课题取得明显突破，稳步推进课题和新探索课题取得显著进展，为 2022 年取得重要科研产出奠定了坚实基础。

2022 年，研究组将进一步围绕原位结构生物学技术应用、超快冷冻电镜技术应用和三维构造生物学技术应用主线展开深入研究，目标在原位冷冻电镜数据处理、超大蛋白质机器原位结构功能、蛋白质光激活瞬态结构等方面取得高水平的研究成果。

胸怀天下、心无旁骛、勇于创新、追求卓越、超越自我！祝愿研究组的全体师生在 2022 年取得新的更大的成绩！

孙飞

二零二一年十二月冬至

主要研究进展

2021 年度，研究组在冷冻电镜方法学和重要蛋白质的结构研究方面陆续取得了多项研究成果，并在新冠病毒的感染机制和药物开发方面也取得了重要的研究进展。主要阶段性研究成果如下：

1 冷冻电镜技术研发

• 原位样品制备的全新流程 VHUT-cryo-FIB

近年来，冷冻电子断层成像技术得到了快速的发展。相较于传统的单颗粒结构解析方法，冷冻电子断层成像技术能够在原位水平观察到生物大分子的高分辨率结构，在细胞、组织层面得到更丰富的信息。但是，在冷冻电子断层成像技术的原位样品制备方面仍然存在瓶颈：1）考虑到电子平均自由程的限制，细胞或者组织样品需要被制备成约 200 纳米厚的冷冻薄片。在过去几十年的时间里主要是通过冷冻切片来达到这个要求，但是冷冻切片在切割时会不可避免地对样品造成损伤，导致在后续的数据收集以及图像处理过程中出现问题；2）在处理组织样品时，由于组织样品不似传统样品那样薄，因此传统的投入式冷冻方法不能有效地冷冻样品。

针对这些问题，研究组与生物物理所成像中心联合开发了一套命名为 VHUT-cryo-FIB (Vibratome-High-pressure freezing-Ultramicrotome Trimming-cryo-FIB) 的工作流程，能够有效地制备用于冷冻电子断层成像的含水组织切片。该流程包括如下步骤：用振颤切片机切割新鲜的组织样品、高压冷冻、用冷冻超薄切片机进行预切、冷冻聚焦离子束减薄以及数据收集。这套流程的优势在于：1）高压冷冻能够有效地冷冻震颤切片机切割后的组织样品，不会出现冷冻速率低导致的冷冻不充分的现象；2）冷冻切片机的预切可以将样品区域暴露出来，有效地提高后续冷冻聚焦离子束切割的精度与效率；3）冷冻聚焦离子束切割样品相较于冷冻切片不容易产生切割损伤

目前，研究组已经用 VHUT-cryo-FIB 处理了多种组织样品，包括小鼠肌肉、肝脏以及菠菜叶等，都能够观察到结构保存完好、信息丰富的组织样品切片。并且还对菠菜叶以及肝脏样品中的核糖体进行了进一步的结构解析，成功获得了

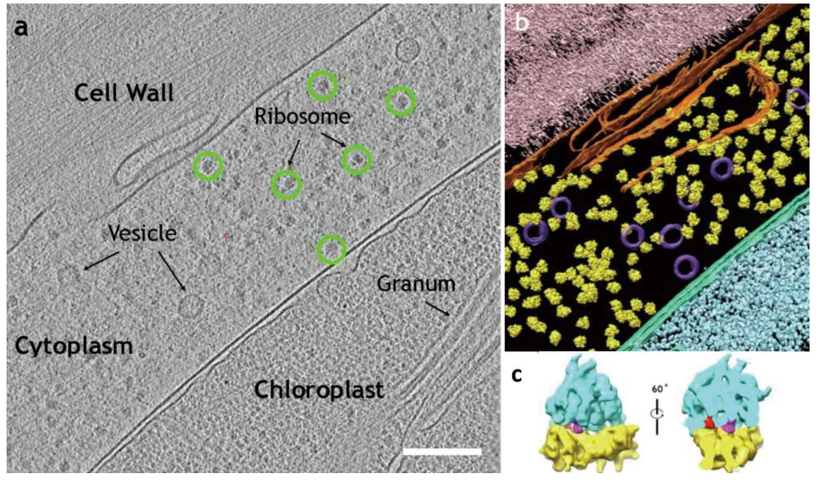

图 1. Cryo-FIB 方法制备的冷冻含水切片样品，Cryo-ET 方法解析菠菜叶片和小鼠肝样品中核糖体三维结构。a 菠菜叶片重构后的细胞内部结构，其超微结构包括细胞壁、细胞质、叶绿体、囊泡、核糖体和基粒。b 三维渲染后的图 a，黄色，核糖体；淡粉色，细胞壁；橙色，细胞膜；紫色，囊泡；绿色，叶绿体膜；青色，叶绿体基质。菠菜核糖体的原位结构，大亚基蓝色的，小亚基黄色。c Subtomo average 得到小鼠肝脏核糖体的原位结构 (左)，与通过单粒子重构得到小鼠胞质核糖体密度图 (右)。d 小鼠肝脏核糖体原位结构密度图，大亚基和小亚基分别为蓝色和黄色。红色 (P/E 位点) 和紫色 (A/P 位点) 显示了两个 tRNAs。Scale bar:150nm。

菠菜胞质核糖体（34Å）和小鼠肝脏胞质核糖体（18Å）的原位三维结构（图 1）。这些结果证明 VHUT-cryo-FIB 方法可以广泛应用于各种生物组织样品的冷冻含水切片制备，为原位结构生物学研究提供有力的样品制备方法。

该项研究成果以“VHUT-cryo-FIB, a method to fabricate frozen hydrated lamellae from tissue specimens for in situ cryo-electron tomography”为题于 2021 年 6 月 24 日发表在 *Journal of Structural Biology [213(2021) 107763]* 上。

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VHUT-cryo-FIB, a method to fabricate frozen hydrated lamellae from tissue specimens for *in situ* cryo-electron tomography

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ABSTRACT

Cryo-electron tomography (cryo-ET) provides a promising approach to study intact structures of macromolecules *in situ*, but the efficient preparation of high-quality cryosections represents a bottleneck. Although cryo-focused ion beam (cryo-FIB) milling has emerged for large and flat cryo-lamella preparation, its application to tissue specimens remains challenging. Here, we report an integrated workflow, VHUT-cryo-FIB, for efficiently preparing frozen hydrated tissue lamella that can be readily used in subsequent cryo-ET studies. The workflow includes vibratome slicing, high-pressure freezing, ultramicrotome cryo-trimming and cryo-FIB milling. Two strategies were developed for loading cryo-lamella via a side-entry cryo-holder or an FEI AutoGrid. The workflow was validated by using various tissue specimens, including rat skeletal muscle, rat liver and spinach leaf specimens, and *in situ* structures of ribosomes were obtained at nanometer resolution from the spinach and liver samples.

1. Introduction

With the mature and rapid development of single-particle electron cryomicroscopy, structural biology has entered a new era with an increasing number of supra-macromolecular complexes and membrane protein complexes whose structures are well resolved and studied. A new direction has emerged involving the study of the structures of these complexes *in situ* without purifying them from their native environment and how these complexes locate and interact with their cellular environment, providing deeper insights into their physiological functions. Technological developments related to electron cryo-microscopy (cryo-EM) provide another opportunity for high-resolution *in situ* structural study. Cryo-electron tomography (cryo-ET) can reveal considerable rich information on organelle and macromolecular complexes in their natural cellular environment (Lucic et al., 2005).

Due to the limited penetration (mean free path) of electrons at the current accelerating voltage of the modern microscope, a thin (~200 nm) cryosection of cell or tissue specimen must be prepared, representing a persistent bottleneck in *in situ* cryo-ET studies. For decades, thin cryosections of cells and tissues have been prepared primarily via cryosectioning by cryo-ultramicrotomy (Al-Amoudi et al., 2004; Hsieh et al., 2006; McDowall et al., 1983). However, cutting artifacts are inevitable with cryo-ultramicrotomy, causing severe problems in subsequent data collection and image analysis, which limits the widespread application of cryo-ET in structural biology and cell biology (Al-Amoudi et al., 2005; Rigort et al., 2010).

Cryo-focused ion beam (cryo-FIB) milling has emerged recently as an alternative method to prepare high-quality cryo-lamellae of cells for *in situ* structural study (Marko et al., 2006). Lamellae can be milled to 50 ~ 300 nm thickness without producing the artifacts typically caused by

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• 用疏水蛋白膜作为支持膜的透射电镜载网

近年来，由于硬件和图像处理算法上的技术突破，使得冷冻电镜技术可以解析生物大分子结构至近原子分辨率，来阐明生物大分子发挥的功能机制。冷冻样品制备即利用冷冻剂如乙烷，丙烷或者两者的混合物将生物样品快速冷冻在无定型冰中，此时样品处于近生理状态，且成像时能够减轻辐射损伤对样品高分辨率结构信息的破坏。当用普通载网如 Quantifoil，GIG 进行冷冻样品制备时，常常会遇到以下问题：蛋白颗粒不进孔，样品更喜欢呆在厚冰处，严重的取向优势，气液界面导致蛋白质变性等问题。这些问题使得一些重要生物大分子高分辨率结构无法获得，导致许多结构生物学研究组需要花费不少时间与精力在样品优化上。

目前，冷冻电镜结构解析的瓶颈在样品制备阶段已成为共识，开发解决上述遇到的问题的技术方法将会推动冷冻电镜在结构生物学领域的发展，提高重要生物大分子复合物结构解析的通量与效率。

疏水蛋白是一类由高等丝状真菌分泌的小分子量蛋白质（7-15kDa），可在菌丝和孢子表面的形成两亲性蛋白膜，在真菌不同发育阶段中起到关键作用。疏水蛋白作为两亲性蛋白质，其表面展现出明显的疏水和亲水性区域，使得疏水蛋白成为表面活性最强的蛋白质之一。所有的疏水蛋白皆可在两相界面如气液界面、固液界面自组装成紧密两亲性纳米蛋白膜。疏水蛋白 HFB 的这些独特的性质使其在诸多生物技术研究中得到广泛的应用，如乳化、药物递送、组织工程、生物传感器、医学成像、蛋白质纯化、固定化修饰等。根据自组装蛋白膜的特性，疏水蛋白被分为 I 型和 II 类。一般来说，I 型疏水蛋白可自主装形成

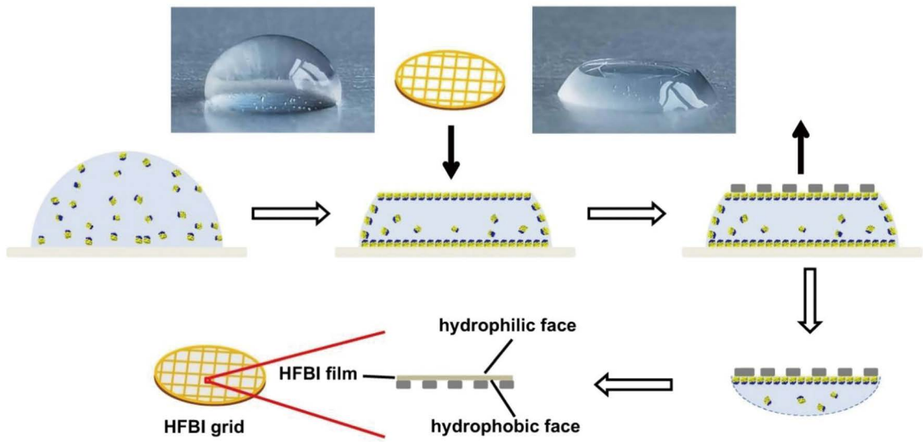

图 2. 疏水蛋白 HFBI 载网制备流程图

具有棒状结构的蛋白膜，只在强酸如纯三氟乙酸（TFA）或甲酸中解聚；而 II 类疏水蛋白可自组装形成高度有序的结构，容易被 2%SDS 和 60% 乙醇解聚。

疏水蛋白 HFBI 是一类由瑞氏木霉生产的 II 型疏水蛋白，可以在气液界面处自组装成单层、高度有序的二维晶体结构，将 HFBI 在毕赤酵母中异源表达，并进行大规模发酵和纯化，可以用于后续疏水蛋白载网制备。

研究组与南开大学生命科学学院乔明强研究组合作开发了一种用疏水蛋白 HFBI 膜作为支持膜的电镜载网，用于冷冻电镜样品制备中，并申请了国家发明专利（申请号：202110576212.4）。研究结果表明，该载网能够制出更薄的冰，解决气液界面和严重的取向优势问题等，提升了冷冻样品制备成功率，目前用该载网已成功解析了 8 个蛋白质及其复合物高分辨率结构，重构的分辨率最高可达 2Å 以下（图 2）。

该项研究成果以“A cryo-electron microscopy support film formed by 2D crystals of hydrophobin HFBI”为题，于 2021 年 12 月 14 日发表在 *Nature Communications* 上。

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A cryo-electron microscopy support film formed by 2D crystals of hydrophobin HFBI

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Cryo-electron microscopy (cryo-EM) has become a powerful tool to resolve high-resolution structures of biomacromolecules in solution. However, air-water interface induced preferred orientations, dissociation or denaturation of biomacromolecules during cryo-vitrification remains a limiting factor for many specimens. To solve this bottleneck, we developed a cryo-EM support film using 2D crystals of hydrophobin HFBI. The hydrophilic side of the HFBI film adsorbs protein particles via electrostatic interactions and sequesters them from the air-water interface, allowing the formation of sufficiently thin ice for high-quality data collection. The particle orientation distribution can be regulated by adjusting the buffer pH. Using this support, we determined the cryo-EM structures of catalase (2.29 Å) and influenza haemagglutinin trimer (2.56 Å), which exhibited strong preferred orientations using a conventional cryo-vitrification protocol. We further show that the HFBI film is suitable to obtain high-resolution structures of small proteins, including aldolase (150 kDa, 3.28 Å) and haemoglobin (64 kDa, 3.6 Å). Our work suggests that HFBI films may have broad future applications in increasing the success rate and efficiency of cryo-EM.

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2 膜蛋白复合体结构功能研究

• 代谢型谷氨酸受体结构揭示 C 类 GPCR 二聚化及功能调控机制

代谢型谷氨酸受体（Metabotropic glutamate receptor，mGlu）属于 C 类 G 蛋白偶联受体（G protein-coupled receptor，GPCR）家族，是人体内最重要的神经递质受体之一。目前在人体内共发现了 8 种代谢型谷氨酸受体（mGlu1-8），其功能涉及学习、记忆、情绪以及疼痛感知等，是阿尔兹海默症和精神分裂症等疾病的治疗靶点。然而，迄今尚无靶向这类受体的药物成功上市，因此其结构与功能研究对精神神经系统疾病的新药创制具有重要意义。与其它类型的 GPCR 相比，mGlu 等 C 类 GPCR 具有独特的激活机制，受体必须形成同源或异源二聚体才能行使功能。但是，长期以来不同类型二聚化对于受体活性的调控机制和该类受体如何激活 G 蛋白等细胞内效应蛋白一直不清楚。

研究组与中国科学院上海药物研究所赵强 / 吴蓓丽研究组等单位合作，利用冷冻电镜单颗粒技术，解析了多种人源代谢型谷氨酸受体处于不同功能和不同二聚化状态下的三维结构，包括处于非激活态的 mGlu2 同源二聚体、mGlu7 同源二聚体和 mGlu2-mGlu7 异源二聚体、处于激活中间态的 mGlu2 同源二聚体等。这是首次对代谢型谷氨酸受体从非活化到完全活化状态精细构象变化过程的全面阐释，并揭示了其同源和异源二聚体复杂的信号转导模式，为深入认识该类受体在中枢神经系统中的功能调控机理提供了重要的依据，对于全面认识 C 类 GPCR 的信号转导机制具有重大意义。

以往的研究表明，C 类 GPCR 二聚体通过调节两个亚基间的相对构象调控受体的功能。此次，通过解析不同代谢型谷氨酸受体的结构，发现不同受体以不同方式形成同源二聚体将各自构象稳定在非活化状态。与之前测定的 mGlu5 结构类似，mGlu7 的非激活态结构呈现一个完全开放的构象，两个亚基的跨膜结构域之间距离较远，没有直接接触。与此不同，mGlu2 二聚体中的两个跨膜结构域彼此靠近，通过各自的第四跨膜螺旋（TM4）形成紧密的相互作用。利用氨基酸突变和细胞信号转导实验，该研究证实 mGlu2 中的这一二聚体界面是该受体亚型特有的，对于稳定受体的非活性状态发挥着重要的作用。这一发现展示了该类受体功能调控模式的多样性。

与激动剂结合后，受体由非激活态向激活态转变。基于 mGlu2 分别处于非激活态、激活中间态和完全激活态的结构，首次完整阐释了代谢型谷氨酸受体在整个活化过程中的精细构象变化，为深入理解 C 类 GPCR 的激活机制提供了关键信息。受体胞外结构域与激动剂结合后，其构象由开放状态转变为闭合状态，带动跨膜结构域大幅度扭转，使两个亚基间的作用界面从 TM4-TM4 转换为 TM6-TM6 对称界面（图 3）。

此外，该研究还对 mGlu 异源二聚体的组装和功能调控机制进行了探索。通过对 mGlu2-mGlu7 异源二聚体开展结构研究，并结合细胞内信号转导、二硫键交联和荧光共振能量转移实验等多种

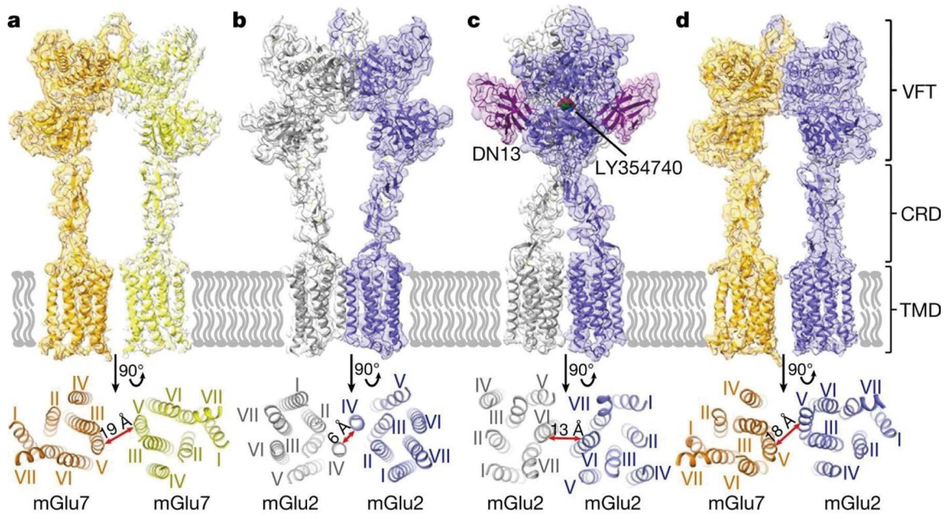

图 3. 代谢型谷氨酸受体结构示意图。代谢型谷氨酸受体在神经元兴奋中发挥关键作用，是神经精神系统疾病的重要治疗靶点。图中从左上到右下依次为非激活状态的 mGlu7 同源二聚体、mGlu2 同源二聚体、处于激活中间态的 mGlu2 同源二聚体和非激活状态的 mGlu2-mGlu7 异源二聚体。红色箭头表示最近的跨膜螺旋对之间的距离。

技术手段，发现在该异源二聚体中 mGlu7 对于二聚体组装和信号转导发挥主导作用。这是首次为 mGlu 异源二聚化研究提供的结构信息，对于进一步认识该家族受体异源二聚化分子调控机理奠定了坚实的基础。

该项研究成果以“Structures of human mGlu2 and mGlu7 homo- and heterodimers”为题，于 2021 年 6 月 16 日发表在 *Nature* 上。

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Article

Structures of human mGlu2 and mGlu7 homo- and heterodimers

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The metabotropic glutamate receptors (mGlus) are involved in the modulation of synaptic transmission and neuronal excitability in the central nervous system[1]. These receptors probably exist as both homo- and heterodimers that have unique pharmacological and functional properties[2–4]. Here we report four cryo-electron microscopy structures of the human mGlu subtypes mGlu2 and mGlu7, including inactive mGlu2 and mGlu7 homodimers; mGlu2 homodimer bound to an agonist and a positive allosteric modulator; and inactive mGlu2–mGlu7 heterodimer. We observed a subtype-dependent dimerization mode for these mGlus, as a unique dimer interface that is mediated by helix IV (and that is important for limiting receptor activity) exists only in the inactive mGlu2 structure. The structures provide molecular details of the inter- and intra-subunit conformational changes that are required for receptor activation, which distinguish class C G-protein-coupled receptors from those in classes A and B. Furthermore, our structure and functional studies of the mGlu2–mGlu7 heterodimer suggest that the mGlu7 subunit has a dominant role in controlling dimeric association and G-protein activation in the heterodimer. These insights into mGlu homo- and heterodimers highlight the complex landscape of mGlu dimerization and activation.

The mGlus contain a large extracellular domain composed of the Venus flytrap domain (VFT) that binds agonist and a cysteine-rich domain (CRD) that is connected to a seven-helical transmembrane domain (TMD), which is responsible for G-protein coupling[4]. It has previously been acknowledged that homodimerization is mandatory for the function of the mGlus, which makes these receptors complex allosteric proteins with two subunits that influence each other[3]. In addition, there is increasing evidence that suggests that the subunits of different mGlus can associate to form several types of heterodimer, which adds to the complexity of function modulation in this receptor family[2,4]. However, conformational differences between the mGlu homo- and heterodimers and how these dimers control mGlu function remain unclear. We therefore performed single-particle cryo-electron microscopy (cryo-EM) and X-ray crystallography studies to solve structures of mGlu2 and mGlu7 in both homo- and heterodimeric organizations, which provide insights into mGlu activity modulation.

Structure determination of mGlu2 and mGlu7

For the structure determination of inactive mGlu2 and mGlu7 homodimers, we purified a modified mGlu2 or mGlu7 in the presence of its selective negative allosteric modulator (NAM) (NAM563[5] for mGlu2 and MMPIP[6] for mGlu7) (construct design and function characterization are described in Methods) (Extended Data Fig. 1a–l). We obtained stable protein of mGlu2 bound to agonist by co-purifying another modified receptor with the agonist LY354740[7], the positive allosteric modulator (PAM) JNJ-40411813[8] and the nanobody DN13 (which acts as an mGlu2 PAM[9]) (Extended Data Fig. 1e, i, n–q). To solve the structure of the mGlu2–mGlu7 heterodimer, we co-purified an FK506 binding protein (FKBP)-tagged mGlu2 and a rapamycin binding fragment (FRB)-tagged mGlu7 in the presence of NAM563 and MMPIP (Extended Data Fig. 1r, Supplementary Fig. 1). We determined the inactive structures of mGlu2, mGlu7, mGlu2–mGlu7 and the agonist- and PAM-bound mGlu2 structure by cryo-EM at overall resolutions of 3.6 Å, 4.0 Å, 3.9 Å and 3.1 Å,

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• 揭示一种特殊血红素 – 铜终端氧化酶利用两种电子供体的分子机制

血红素 – 铜终端氧化酶家族蛋白是一类重要金属蛋白酶，负责高效催化电子从细胞色素 *c* 或氢醌传递给氧分子，将氧气还原为水。细胞色素 *c* 氧化酶属于血红素 – 铜终端氧化酶家族成员，之前的研究显示，它一般只能使用细胞色素 *c* 作为电子供体。不同物种间的细胞色素 *c* 氧化酶结构上存在显著差异，包括亚基数目、亚基 I/II 的跨膜螺旋数、质子通道以及氧气通道结构特征等等，研究这些细胞色素 *c* 氧化酶的不同结构可以揭示该蛋白的进化趋势和发挥不同生物学功能的结构基础。

Aquifex aeolicus 是细菌最古老的分支、最嗜热的菌之一，最适生长温度 85–95℃。它广泛分布在世界各地的高温液体环境中，包括陆地和海洋，能适应多种极端环境。在 2012 年，Hartmut Michel 课题组首次报道了该嗜热菌有一种独特的细胞色素 *c* 氧化酶，既能氧化细胞色

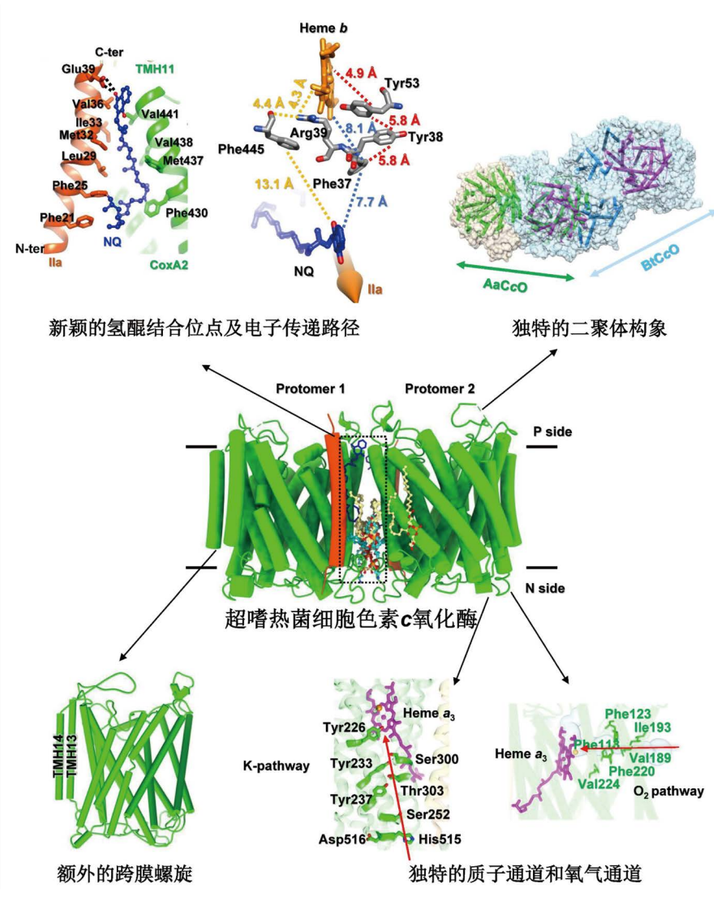

图 4. 超嗜热菌细胞色素 *c* 氧化酶冷冻电镜三维结构。独特的二聚体构象允许其既能氧化细胞色素 *c* 又能氧化氢醌，额外的跨膜螺旋、质子通道和氧气通道等新颖结构特征揭示了细胞色素 *c* 氧化酶在极端环境下的进化适应性。

素 *c* 又能氧化氢醌，但催化机制和进化上的意义仍然未知。

研究组与德国马普生物物理研究所 Hartmut Michel 研究组经过多年合作，利用冷冻电镜单颗粒技术成功解析了 *A. aeolicus* 细胞色素 *c* 氧化酶的三维结构，分辨率达到了 3.4 埃（图 4）。它形成一个独特的同源二聚体，结合形式不同于以往报道的其它物种的细胞色素 *c* 氧化酶。在它的二聚体界面处，有两个底物氢醌分子（NQ）紧密结合在复合体上，形成一条潜在的电子传递链，使得整个细胞色素 *c* 氧化酶在氧化细胞色素 *c* 的同时又能氧化氢醌。这也表明这种独特的二聚化特征对于双底物催化是必要的。结构分析还证实，*A. aeolicus* 细胞色素 *c* 氧化酶为了适应低氧、高温环境，它的质子通道效率显著降低，而氧气通道效率增加。此外，与 *A. aeolicus* 呼吸链复合物 III 类似，它也有一些独特的热稳定性结构特征。这些研究成果进一步丰富了人类对于细胞色素 *c* 氧化酶的结构和功能的认识。这是该研究团队继成功解析 *A. aeolicus* 呼吸链复合物 III 结构的又一重要突破，有利于人们深入了解这一古老超嗜热菌的呼吸作用以及呼吸链复合物为适应极端条件采取的进化策略。

该项研究成果以“The unusual homodimer of a heme-copper terminal oxidase allows itself to utilize two electron donors.”为题，于 2021 年 5 月 6 日发表在 *Angew. Chem. Int. Ed. 2021, 60, 13323–13330* 上。

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The Unusual Homodimer of a Heme-Copper Terminal Oxidase Allows Itself to Utilize Two Electron Donors

Guoliang Zhu[+], Hui Zeng[+], Shuangbo Zhang[+], Jana Juli, Linhua Tai, Danyang Zhang, Xiaoyun Pang, Yan Zhang, Sin Man Lam, Yun Zhu,* Guohong Peng,* Hartmut Michel,* and Fei Sun**

Abstract: *The heme-copper oxidase superfamily comprises cytochrome c and ubiquinol oxidases. These enzymes catalyze the transfer of electrons from different electron donors onto molecular oxygen. A B-family cytochrome c oxidase from the hyperthermophilic bacterium Aquifex aeolicus was discovered previously to be able to use both cytochrome c and naphthoquinol as electron donors. Its molecular mechanism as well as the evolutionary significance are yet unknown. Here we solved its 3.4 Å resolution electron cryo-microscopic structure and discovered a novel dimeric structure mediated by subunit I (CoxA2) that would be essential for naphthoquinol binding and oxidation. The unique structural features in both proton and oxygen pathways suggest an evolutionary adaptation of this oxidase to its hyperthermophilic environment. Our results add a new conceptual understanding of structural variation of cytochrome c oxidases in different species.*

Introduction

In all respiring organisms electrochemical proton gradients drive the flux of protons back through the membrane via ATP-synthases, which produces adenosine-5′-triphosphate by attaching an inorganic phosphate to adenosine-5′-diphosphate. In aerobic organisms, the electrochemical proton gradient is generated by a series of proton translocation reactions in the respiratory chains. Cytochrome *c* oxidase (CcO) is the terminal enzyme in the respiratory chains of many aerobic organisms. It is located in the inner membrane of mitochondria and bacteria, and catalyzes the electron transfer from cytochrome *c* to molecular oxygen that is reduced to water. Studies on this integral membrane protein complex revealed that eight protons are taken up from the matrix side of mitochondrial membrane or from the bacterial cytoplasm (N-side), four protons are pumped across the membrane into the intermembrane space of mitochondria or the periplasm of gram-negative bacteria (P-side), while another four protons are used for water formation.[1]

CcO is a member of the heme- and copper-containing terminal oxidases (HCOs) superfamily,[2] which also includes ubiquinol oxidases (QOXs), for example, the well-studied cytochrome bo_3 from *Escherichia coli* (*E. coli*)[3] but not the cytochrome *bd* oxidases from the same bacterium.[4] HCOs are classified into three families, A, B and C, based on their amino acid sequences and proton transfer pathways.[5] They are multi-subunit complexes, for example, they possess 14 protein subunits in mammalian mitochondria[6] and 3 subunits in some bacteria.[7]

The conserved central catalytic subunit I contains two heme groups and a copper atom (Cu_B). The low-spin heme can be a heme *a* or a heme *b* in prokaryotes,[3,8] whereas only heme *a* has been found in mitochondrial cytochrome *c* oxidases.[9] The low-spin heme *a* in the A-family C*c*O from

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膜动态分子机制

• 细胞内体运输途径中 SNX 诱导膜变形分子机制

囊泡运输 (vesicle trafficking) 是真核细胞内膜性细胞器之间主要的物质运输方式，既是生命活动的基本过程，又是一个极其复杂的动态过程，涉及到众多蛋白分子和调控因子。包被蛋白 (coat proteins) 是囊泡运输的关键分子，它可以诱导细胞膜发生变形，并参与货物分子的分选识别从而形成囊泡。经典的包被蛋白主要有三类，即 COPI、COPII 和 Clathrin。不同于经典的包被蛋白，Sorting nexin 类蛋白分子 SNXs 是内体运输途径中的一类重要包被蛋白。已知 SNXs 参与 MI-CPR（cation- independent mannose 6-phosphate receptor）和 Semaphorin 4C 等膜蛋白受体的循环运输过程。尽管 SNXs 蛋白可以使细胞膜变形，但是具体变形分子机制尚不清楚。

研究组利用冷冻电镜螺旋三维重构技术解析了 SNX1 重塑细胞膜复合体的三维结构（图 5）。SNX1 包含 PX 结构域和 BAR 结构域。螺旋结构表明，SNX1 二聚体是最小的组装单元，它的凹面覆盖在细胞膜上，组装体的层与层之间主要由 BAR 结构域之间较短的侧面 – 侧面相互作用介导，层内主要由 BAR 结构域与 PX 结构之间作用介导。研究还发现 BAR 结构域与 PX 结构域之间的一段两性螺旋直接参与了细胞膜的重塑，该段两性螺旋的删除明显降低了 SNX1 与细胞膜的结合，严重影响了细胞膜的变形，提示该段双性螺

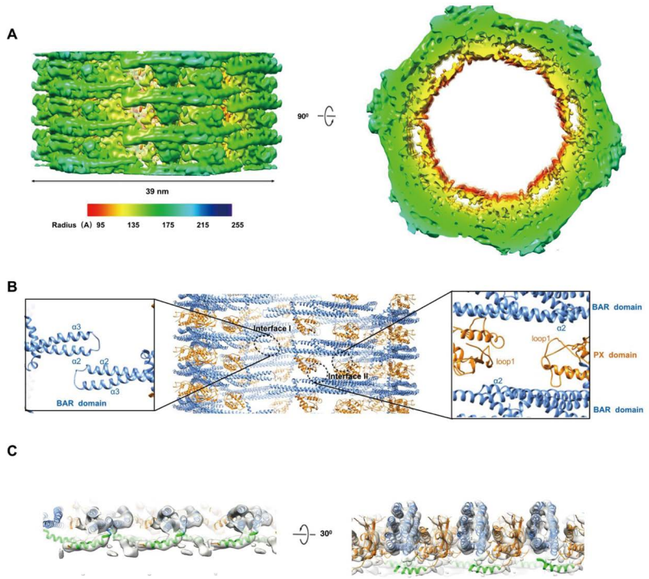

图 5. SNX1 重塑细胞膜复合体的冷冻电镜三维结构。A，SNX1 包被管状结构的冷冻电镜密度图；B，SNX1 在细胞膜上的组装模型；C，两性螺旋（绿色显示）与电镜密度相吻合。

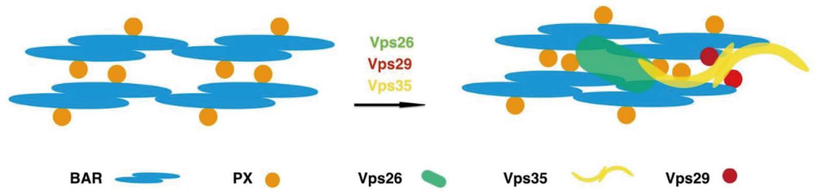

图 6. SNX1 膜结合状态组装体的构象变化模式图

旋是 SNX1 执行细胞膜重塑功能的关键因子。另外，研究发现 PX 结构域的 PI3P 结合位点对于细胞膜的结合和管状结构的形成也是必要的。

与 SNX1 晶体结构相比，膜结合状态的 SNX1 曲率变小了，推测是与细胞膜作用导致构象变化，从而形成延伸的二聚体结构排布在细胞膜上。与来自真菌中的同源 Sorting nexin 类包被蛋白复合体 retromer-vps5 相比，SNX1 二聚体与 Vps5 二聚体基本单元非常相似，但在组装方式上有所不同，主要表现在层间或者层内相邻的两个二聚体间的距离。Vps5 之间的距离短从而形成更紧密的组装体。

该项研究阐释了 SNX1 重塑细胞膜变形的分子机理——SNX1 主要通过 PX 结构域和一段双性螺旋与细胞膜结合，通过 BAR 结构域和 PX 结构域在细胞膜上形成螺旋组装体。该项研究还提示了 Retromer-SNX 衣被复合体的组装过程，即 SNX1 首先结合到细胞膜上，SNX1 二聚体的曲率从大变小，形成更延伸的状态，进一步 Retromer 结合到 SNX1 上，则形成一个更加紧凑的组装体（图 6）。

该项研究成果以“Structural insights into membrane remodeling by SNX1”为题，于 2021 年 3 月 3 日发表在 *Proc Natl Acad Sci U S A.* 上。

文章链接：

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Structural insights into membrane remodeling by SNX1

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The sorting nexin (SNX) family of proteins deform the membrane to generate transport carriers in endosomal pathways. Here, we elucidate how a prototypic member, SNX1, acts in this process. Performing cryoelectron microscopy, we find that SNX1 assembles into a protein lattice that consists of helical rows of SNX1 dimers wrapped around tubular membranes in a crosslinked fashion. We also visualize the details of this structure, which provides a molecular understanding of how various parts of SNX1 contribute to its ability to deform the membrane. Moreover, we have compared the SNX1 structure with a previously elucidated structure of an endosomal coat complex formed by retromer coupled to a SNX, which reveals how the molecular organization of the SNX in this coat complex is affected by retromer. The comparison also suggests insight into intermediary stages of assembly that results in the formation of the retromer-SNX coat complex on the membrane.

helical reconstruction | coat complex | membrane deformation | SNX1 | cryoelectron microscopy

Sorting nexins (SNXs) exist as a large family of proteins defined by the presence of a PX (phox homology) domain (1, 2). Members of this family have been found to act as coat proteins in endosomal pathways that include recycling from endosomes to the plasma membrane and retrieval from endosomes to the Golgi complex (3, 4). Defects in these transport processes is associated with various neurologic disorders including Alzheimer's disease, Parkinson's disease, and Down's syndrome (5, 6).

Coat proteins assemble into complexes on the membrane to initiate intracellular transport pathways by coupling two main functions: bending the membrane to generate transport carriers and binding to cargoes for their sorting into these carriers (7). Retromer, a trimeric complex consisting of Vps26, Vps29, and Vps35, has been found to couple with different SNXs to form multiple endosomal coat complexes, in which select members of the SNX family act in membrane deformation while retromer acts in cargo recognition (8–17). Recently, a detailed molecular view of this functional cooperation has been achieved by elucidating the structure of a retromer-SNX complex on the membrane (18).

Notably, it has been further discovered recently that an endosomal coat complex can be formed with only SNX members. SNX1/2 have been found to heterodimerize with SNX5/6 to form the endosomal SNX–BAR sorting complex for promoting exit 1 (ESCPE-1) complex, in which SNX1/2 are proposed to act in membrane deformation while SNX5/6 act in cargo recognition (19). As such, a key question has become whether SNX that acts in membrane deformation in this type of coat complex would be organized similarly on the membrane, as previously elucidated for SNX in the context of a retromer-SNX complex (18).

One of the best characterized mechanisms of membrane deformation involves proteins that possess the BAR (Bin/Amphiphysin/Rvs) domain. This domain has been shown to undergo homodimerization to form a banana-shaped structure, which can impart membrane curvature through a scaffolding mechanism that involves electrostatic interactions between the positive charges lining the concave side of the curved BAR dimer and the negative charges that line the surface of the membrane bilayer. In some cases, the BAR domain can deform the membrane through a second mechanism, which involves the formation of an amphipathic helix that inserts into one leaflet of the membrane bilayer to generate bilayer asymmetry in driving membrane curvature (20, 21).

Besides the PX domain, SNX1 also possesses a BAR domain. However, studies have found that its BAR domain is not sufficient in driving membrane deformation. Instead, the PX domain as well as the linker region between the BAR and PX domains are also needed (22, 23). As such, a key goal has been to achieve a better understanding of how the various parts of SNX1 contribute to its ability to deform the membrane.

Structural studies, such as those involving crystallography and single-particle electron microscopy (EM), have been advancing a molecular understanding of coat proteins (24), including components of endosomal coats (17, 19, 22, 25–27). Notably, however, these approaches solve protein structures in solution, but the functional form of coat proteins involves their association with the membrane. In this study, we have pursued cryo-EM to reveal how SNX1 is organized on the membrane to explain its ability to deform the membrane. The result advances a molecular understanding of how an endosomal coat that contains only SNXs generates transport carriers. Moreover, by comparing our SNX1 structure to the previously solved retromer-SNX structure (18), we delineate the extent to which the molecular organization of SNX on the membrane is affected by the presence of retromer. This comparison also suggests insight into intermediary stages of coat assembly that form the retromer-SNX complex on the membrane.

BIOPHYSICS AND COMPUTATIONAL BIOLOGY

Results

Membrane Binding and Tubulation by SNX1. We initially generated liposomes that contain the major phospholipids of organellar

Significance

Coat proteins play a central role in the intracellular transport pathways by coupling two main functions: bending the membrane to generate transport carriers and binding to cargoes for their sorting into these carriers. Studies thus far have mostly solved the structure of coat proteins in solution, but their functional form requires assembly on the membrane into protein complexes. Here, we have pursued cryo-EM to reveal in molecular detail how SNX1 assembles on the membrane to deform the membrane. When compared to a previously solved retromer-SNX complex, our elucidation also suggests how retromer affects SNX in this complex as well as the intermediary stages of this coat assembly.

Author contributions: X.P., V.W.H., and F.S. designed research; Y.Z., X.P., J.L., and J.X. performed research; Y.Z., X.P., J.L., V.W.H., and F.S. analyzed data; and Y.Z., X.P., V.W.H., and F.S. wrote the paper.

The authors declare no competing interest.

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4 蛋白质机器的原位结构研究

• 核膜孔复合体外周环的结构研究

细胞核是真核生物细胞中最大的细胞器，在细胞核膜上坐落有沟通细胞核质和胞质物质和能量运输的孔道，称为核孔复合体。从胞质侧到核质侧，核孔复合体可以分为胞质纤维，胞质环，内环，腔内环，核质环和核篮。核孔复合体整体呈现为环绕核孔中央孔道的准八重对称空心圆柱状结构，在高等真核生物（如人、爪蟾等）中由约 30 种、1000 多个不同大小的蛋白质亚基构成，分子量可达 100 兆道尔顿以上。解析核孔复合体的高分辨结构对了解其组装机制和真核生物的起源都有重大意义。但由于其巨大的分子质量和与生理功能相适应的高度动态性，目前核孔复合体依旧缺乏可靠的高分辨率原子结构模型，限制了对其结构和生理功能的深入研究。2020 年，西湖大学施一公团队利用冷冻电镜单颗粒分析技术和断层成像技术研究了非洲爪蟾核孔复合体的胞质环及腔内环的结构特征，可以在大多数胞质环组件中识别和定位二级结构元素，加深了人们对 NPC 组装机制的理解。

研究组联合北京大学张传茂研究组，针对核孔复合体的样品特性，进一步发展了冷冻电镜单颗粒 w 分析技术，收集了爪蟾卵母细胞核膜在不

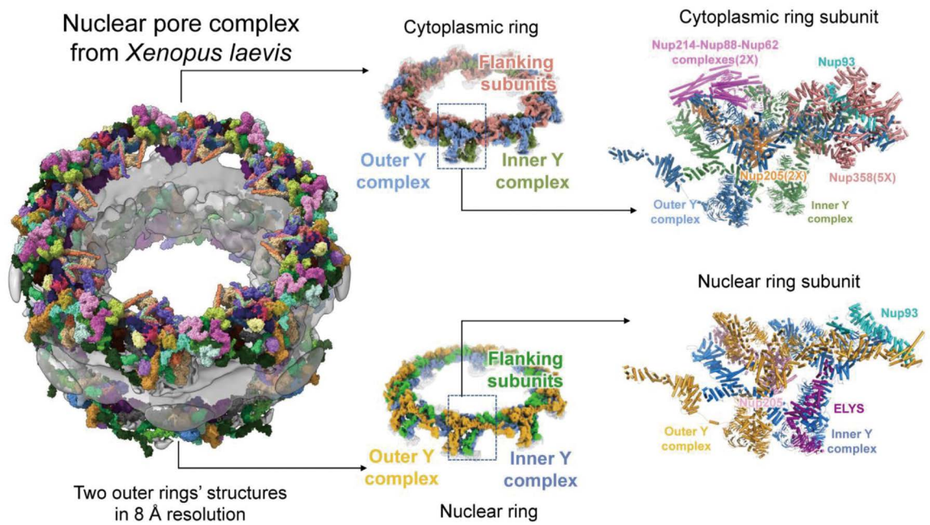

图 7. 爪蟾核孔复合体外环的精细结构研究揭示了众多蛋白质亚基的组成和相互作用模式

同倾转角度下的图像数据，从中挑选出了包含各种取向的核孔复合体进行三维重构计算。最终的数据处理结果显示，相比于前人利用电子断层成像进行重构的结果，这种数据收集和处理方法可以达到更高分辨率，其中大部分蛋白质亚基可以达到二级结构分辨率水平。对于核孔复合体的外环（即胞质环和核质环）的不同区域，可以达到 8 埃左右的整体分辨率。

在此基础上，借助于目前准确率最高的蛋白质三维结构预测软件 AlphaFold2，研究人员预测了爪蟾核孔复合体所有核孔蛋白的全长三维结构，并依据高质量的三维重构结果，针对核孔复合体外环的密度图进行模型搭建和结构修正，获得了目前最完整的 NPC 外环结构模型。这个结构不仅补足了核孔复合体外环结构骨架——Y 复合体缺失的部分结构（图 7），还在外环上鉴定出一系列发挥重要功能的核孔蛋白亚基，阐明了这些未知组分的精细结构及组装形式。在胞质环上的每个不对称单元上，鉴定出 Nup358 五元蛋白复合物，发现两个 Nup214 复合体两两相接构成信使核糖核蛋白出核平台，一个 Nup93 蛋白发挥桥接作用连接胞质环两个 Y 复合体的茎部区域，以及两个 Nup205 蛋白分别发挥稳定胞质环结构的功能。在核质环的每个不对称单元上，鉴定出一个 Nup205 发挥稳定核质环结构的功能，一个 ELYS 蛋白发挥起始有丝分裂后期核孔复合体组装的功能，一个 Nup93 发挥桥接作用连接核质环 Y 复合体的茎部区域的功能。这些结构信息可以为未来核孔复合体的组装研究提供了重要的参考数据。

该项研究成果以“8 Å structure of the outer rings of the Xenopus 3 laevis nuclear pore complex obtained by 4 cryo-EM and AI”为题，投稿至 *Protein Cell*，并于 2021 年 12 月 16 日被 *Protein Cell* 正式接收。

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Protein & Cell

RESEARCH ARTICLE

8 Å structure of the outer rings of the *Xenopus laevis* nuclear pore complex obtained by cryo-EM and AI

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ABSTRACT

The nuclear pore complex (NPC), one of the largest protein complexes in eukaryotes, serves as a physical gate to regulate nucleocytoplasmic transport. Here, we determined the 8 Å resolution cryo-electron microscopic (cryo-EM) structure of the outer rings containing nuclear ring (NR) and cytoplasmic ring (CR) from the *Xenopus laevis* NPC, with local resolutions reaching 4.9 Å. With the aid of AlphaFold2, we managed to build a pseudoatomic model of the outer rings, including the Y complexes and flanking components. In this most comprehensive and accurate model of outer rings to date, the almost complete Y complex structure exhibits much tighter interaction in the hub region. In addition to two copies of Y complexes, each asymmetric subunit in CR contains five copies of Nup358, two copies of the Nup214 complex, two copies of Nup205 and one copy of newly identified Nup93, while that in NR contains one copy of Nup205, one copy of ELYS and one copy of Nup93. These in-depth structural features represent a great advance in understanding the assembly of NPCs.

KEYWORDS nuclear pore complex, cryo-EM, *Xenopus laevis*, AlphaFold2, nuclear ring, cytoplasmic ring

INTRODUCTION

In eukaryotes, a double-layered membrane encloses a large organelle called the nucleus to separate genetic materials from the cytoplasm (Lin and Hoelz, 2019). The nuclear pore complex (NPC) fuses the inner and outer nucleus membrane (INM and ONM) to form the sole gateway mediating cargo transfer between the nucleoplasm and cytoplasm (Hoelz et al., 2011; Hampoelz et al., 2019). NPCs are formed by approximately 30 different kinds of nucleoporins (Nups) with multiple copies in an eightfold symmetrical assembly. Each NPC contains approximately 550 proteins in fungi or approximately 1000 proteins in vertebrates (Rout et al., 2000; Cronshaw et al., 2002; Ori et al., 2013). NPCs have a cylindrical appearance throughout their overall organization, where a central channel mediating bidirectional nucleocytoplasmic cargo transfer exists (Hinshaw et al., 1992; Akey and Radermacher, 1993). Three scaffold rings, including an inner ring (IR), a cytoplasmic ring (CR) and a nuclear ring (NR), anchored onto the nuclear envelope (NE), provide bases for other functional parts, such as the cytoplasmic filament, nuclear basket, and permeability barrier (Beck et al., 2004; Beck et al., 2007; Maimon et al., 2012; Bui et al., 2013).

Detailed structural information is necessary for a mechanistic understanding of NPC functions, but it has long been

Linhua Tai, Yun Zhu and He Ren have contributed equally to this work.

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• 新冠病毒 Spike 蛋白原位结构研究

目前，新冠病毒的感染仍在全球蔓延，死亡人数不断增多，给人类健康和全球经济都造成了巨大的威胁。了解新冠病毒的结构特征，尤其是精细的原位结构，对于疫苗和药物的开发都极为重要。新冠病毒表面布满了刺突状的 spike 蛋白，主要负责识别人体表面受体从而介导病毒感染，是疫苗和药物的主要设计靶点之一。研究组与合作团队的前期研究发现，靶向 spike 蛋白融合后构象状态的多肽制剂可以有效阻断新冠病毒感染（Cell Research，2020；STTT，2021），但是这个靶点的准确原位结构特征尚不为人知。因此，研究组利用冷冻电镜断层重构技术，收集了 β-丙内酯灭活的新冠病毒粒子的连续倾转数据。数据处理结果发现，病毒表面存在有数量相当的

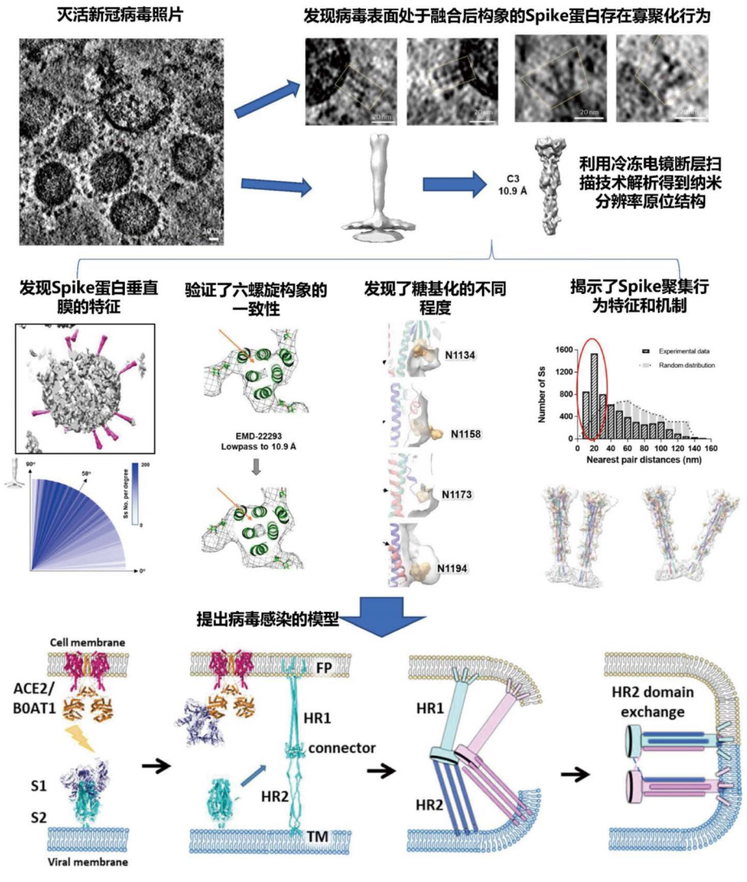

图 8. 新冠病毒 spike 蛋白 postfusion 状态的原位结构研究

prefusion 状态和 postfusion 状态的 spike 蛋白，其中 postfusion 状态的蛋白更倾向于表现出与病毒膜垂直的状态。虽然每个病毒上 postfusion 的 spike 蛋白数目不太多，但是统计数据发现很多蛋白之间的距离小于 20nm，显示出一定程度的寡聚行为。

通过进一步的子体积平均计算，研究人员计算得到了 10.9 埃分辨率的 postfusion 状态的 spike 原位结构。结构中可以看到蛋白跨膜区的位置，也揭示了药物靶点六螺旋结构域的准确原位结构。结构中共观察到 7 个糖基化位点，其密度的强弱与相应位点糖基化的程度相关。通过将蛋白结构与断层重构体相互对照，研究人员推测出两种不同的 spike 寡聚化机制，即平行排列的状态可能是通过相邻蛋白之间 HR2 结构域的交换来寡聚，而分叉排列的状态可能是通过 FP 结构域或者 TM 结构域的相互结合来寡聚。这些寡聚行为都将显著增强 spike 蛋白在病毒膜上的局部丰度，将有利于病毒感染中融合孔的形成（图 8）。这些原位结构信息可以为靶向 spike 的抗病毒药物和疫苗的开发提供重要的参考数据。

该项研究成果以“Nanometer-resolution in situ structure of the SARS-CoV-2 postfusion spike protein”为题，于 2021 年 11 月 15 日发表在 *Proc Natl Acad Sci U S A.* 上。

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Nanometer-resolution in situ structure of the SARS-CoV-2 postfusion spike protein

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The spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mediates membrane fusion to allow entry of the viral genome into host cells. To understand its detailed entry mechanism and develop a specific entry inhibitor, in situ structural information on the SARS-CoV-2 spike protein in different states is urgent. Here, by using cryo-electron tomography, we observed both prefusion and postfusion spikes in β-propiolactone–inactivated SARS-CoV-2 virions and solved the in situ structure of the postfusion spike at nanometer resolution. Compared to previous reports, the six-helix bundle fusion core, the glycosylation sites, and the location of the transmembrane domain were clearly resolved. We observed oligomerization patterns of the spikes on the viral membrane, likely suggesting a mechanism of fusion pore formation.

cryo-electron tomography | postfusion state | SARS-CoV-2 | spike protein | subtomogram analysis

Over the past two decades, several zoonotic coronavirus (CoV) diseases have emerged and posed a devastating threat to global public health and the economy, such as severe acute respiratory syndrome (SARS) (1), Middle East respiratory syndrome (MERS) (2), and COVID-19 (3). As of this writing, COVID-19 has more than 229 million confirmed cases and has caused 4.7 million deaths worldwide, with rapidly increasing numbers. This pneumonia epidemic was caused by a novel coronavirus named SARS coronavirus 2 (SARS-CoV-2), a β-coronavirus, with a genomic sequence that is closely related to SARS-CoV. SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA virus with an ∼30-kb genome (4). Given the current pandemic situation, understanding the structure of SARS-CoV-2 as well as its infection process is very important for vaccine development and drug discovery.

The SARS-CoV-2 genome encodes three viral surface proteins: the spike (S) glycoprotein, envelope (E) protein, and membrane (M) protein. During the infection process, the trimeric S glycoprotein is cleaved by host proteases (4, 5) to produce two functional subunits: The N-terminal S1 subunit is responsible for receptor recognition, and the C-terminal S2 subunit is responsible for membrane fusion (6). Mediated by receptor binding and proteolytic activation, the S1 subunit falls off, and the S2 subunit undergoes extensive and irreversible conformational changes to insert its hydrophobic fusion peptide (FP) into the target cell membrane. Subsequently, two heptad repeat regions of the S2 subunit, heptad repeat 1 (HR1) and heptad repeat 2 (HR2), form a stable six-helix bundle (6-HB) fusion core to bring together the viral and cellular membranes, leading to colocalization of the FP and the transmembrane (TM) region at the same end to form the fusion pore (7). Thus, the S protein is one of the major targets for developing vaccines and antiviral drugs.

After the outbreak of COVID-19, the in vitro structures of SARS-CoV-2 S in the prefusion state were promptly solved using single-particle cryo-electron microscopy (cryo-EM) (8, 9) and X-ray crystallography (7, 10, 11). Soon afterward, the in situ structures of S in the prefusion state were revealed by cryo-electron tomography (cryo-ET) and cryo-subtomogram averaging (cryo-STA) (12–14), uncovering the distribution of different conformational states as well as the native glycosylation sites. However, how the S protein is activated to induce membrane fusion with its host is less understood. The structure of S in the postfusion state would provide an important clue to investigate the fusion mechanism. The high-resolution structure of recombinant S in the postfusion state has been reported by Cai et al. (15), but this in vitro study failed to determine how the postfusion S proteins organize on the membrane. Previous in situ studies (12, 13, 16) explored this question but yielded limited information, due to the poor quality of the density map. In addition, we previously showed that the recombinant 6-HB fusion core of S in the postfusion state would be an effective target for the design of viral fusion inhibitors (7), which needs to be further validated by a higher-resolution structure and glycosylation information of in situ S in the postfusion state.

In the present work, we utilized cryo-ET and cryo-STA to study the structure of SARS-CoV-2 viruses that were inactivated by β-propiolactone (BPL). We solved the in situ structures of S in both the prefusion and postfusion states with resolutions of 12.9 and 10.9 Å, respectively. In addition to

Significance

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a severe threat to public health and the global economy. Its spike protein is responsible for the membrane fusion and is thus a major target for vaccine and drug development. Our study presents the in situ structure of the spike protein in the postfusion state with higher resolution, giving further insights into the design of a viral entry inhibitor. Our observation of the oligomerization states of spikes on the viral membrane implies a possible mechanism of membrane fusion for viral infection.

Author contributions: Z.R., X.W., F.S., and Y.Z. designed research; L.T., G.Z., M.Y., L.C., X.X., G.Y., C.Q., and Y.Z. performed research; L.T., G.Z., C.C., and Y.Z. analyzed data; and L.T., G.Z., F.S., and Y.Z. wrote the paper.

The authors declare no competing interest.

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5 新冠病毒相关研究

• 泛冠状病毒融合抑制剂的结构功能研究及临床前评价

2021 年 7 月，研究组与复旦大学陆路教授等科研团队联合攻关，以“Structural and functional basis for pan-CoV fusion inhibitors against SARS-CoV-2 and its variants with preclinical evaluation” 为题在《Signal Transduction and Targeted Therapy》杂志上发表了重要的研究成果。该研究从结构和功能的角度详细阐述和评价了泛冠状病毒融合抑制剂的作用机理，为该药物推向临床使用提供了重要的实验数据。

新冠病毒大流行对公共卫生和经济构成了全球威胁。不断出现的 SARS-CoV-2 变体对抗病毒药物和疫苗的开发提出了重大挑战。2020 年的研究中，研究组与复旦大学团队的研究成果已经发现 EK1 和 EK1 的胆固醇偶联衍生物 EK1C4 具有广泛而显著的抗病毒活性。接下来的研究又发现，作为泛冠状病毒融合抑制剂，他们在肺源性和肠源性细胞系（Calu-3 和 Caco2）中也有很好的抗病毒效果。针对最近出现了新冠流行株和重要的突变体病毒，比如 B.1.1.7(Alpha)、B.1.1.248(Gamma)、N417T、E484K、N501Y

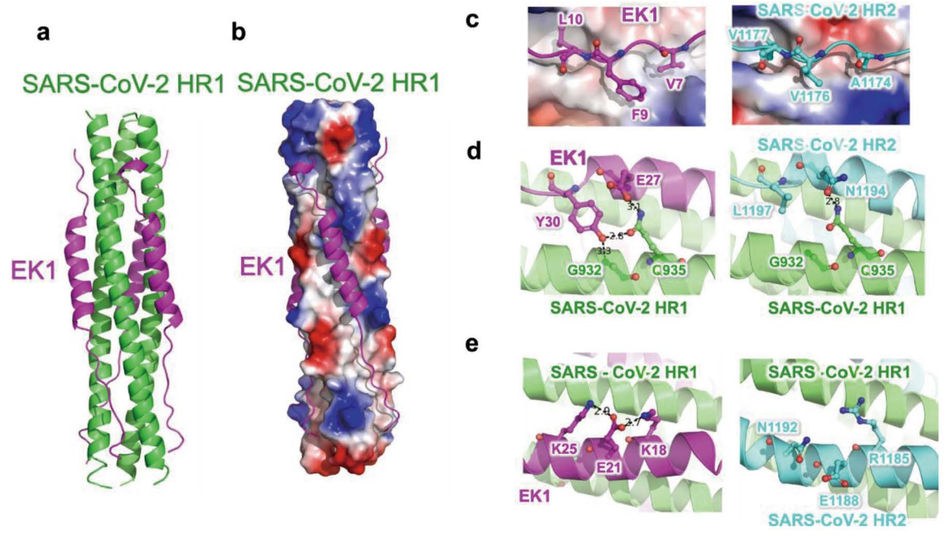

图 9. SARS-CoV-2-HR1 和 EK1 多肽形成 6-HB 的结构

和 D614G 等，EK1 和 EK1C4 都显示出了很好的抑制活性。

进一步，研究组成功解析了 EK1 多肽靶向结合新冠病毒 S 蛋白的 HR1 结构域的高分辨率结构，发现 EK1 以反平行的方式嵌入两个相邻 HR1 螺旋之间形成的凹槽中，形成一个 6-HB 结构模拟物，与融合后状态下的病毒三聚体 HR1-HR2 复合物一样紧密组装（图 9）。EK1 通过占据病毒 HR1 三聚体上 HR2 基序的结合位点，从而阻止了病毒 6-HB 的形成和随后的膜融合。而且，通过 EK1 多肽上的亲水残基的可变构象，EK1 多肽可以适应不同冠状病毒 HCoV 的 HR1 基序中的多个残基差异，从而保持对 HR1 的高亲和力和对不同 HCoV 的高抑制活性。最后，通过使用 SD 大鼠模型、比格犬模型、豚鼠模型和兔子模型进行了 EK1 多肽的体内毒性试验，发现 EK1 肽具有良好的安全性。

该项研究成果以“Structural and functional basis for pan-CoV fusion inhibitors against SARS-CoV-2 and its variants with preclinical evaluation”为题，于 2021 年 7 月 29 日发表在 *Signal Transduction and Targeted Therapy (2021) 6:288* 上。

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https://doi.org/10.1038/s41392-021-00712-2

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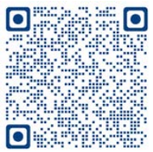

Signal Transduction and Targeted Therapy

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ARTICLE OPEN

Structural and functional basis for pan-CoV fusion inhibitors against SARS-CoV-2 and its variants with preclinical evaluation

Shuai Xia[1], Qiaoshuai Lan[1], Yun Zhu[2], Chao Wang[3], Wei Xu[1], Yutang Li[1], Lijue Wang[1], Fanke Jiao[1], Jie Zhou[1], Chen Hua[1], Qian Wang[1], Xia Cai[1], Yang Wu[1], Jie Gao[4], Huan Liu[4], Ge Sun[4], Jan Münch[5], Frank Kirchhoff[5], Zhenghong Yuan[1], Youhua Xie[1], Fei Sun[2,6]✉, Shibo Jiang[1]✉ and Lu Lu[1]✉

The COVID-19 pandemic poses a global threat to public health and economy. The continuously emerging SARS-CoV-2 variants present a major challenge to the development of antiviral agents and vaccines. In this study, we identified that EK1 and cholesterol-coupled derivative of EK1, EK1C4, as pan-CoV fusion inhibitors, exhibit potent antiviral activity against SARS-CoV-2 infection in both lung- and intestine-derived cell lines (Calu-3 and Caco2, respectively). They are also effective against infection of pseudotyped SARS-CoV-2 variants B.1.1.7 (Alpha) and B.1.1.248 (Gamma) as well as those with mutations in S protein, including N417T, E484K, N501Y, and D614G, which are common in South African and Brazilian variants. Crystal structure revealed that EK1 targets the HR1 domain in the SARS-CoV-2 S protein to block virus-cell fusion and provide mechanistic insights into its broad and effective antiviral activity. Nasal administration of EK1 peptides to hACE2 transgenic mice significantly reduced viral titers in lung and intestinal tissues. EK1 showed good safety profiles in various animal models, supporting further clinical development of EK1-based pan-CoV fusion inhibitors against SARS-CoV-2 and its variants.

Signal Transduction and Targeted Therapy (2021)6:288 ; https://doi.org/10.1038/s41392-021-00712-2

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19). SARS-CoV-2 belongs to the coronaviridae family, β-coronavirus genera, and shows close relationship with SARS-CoV.[1,2] As of 28 June 2021, SARS-CoV-2 had caused 180,817,269 confirmed cases and 3,923,238 deaths worldwide (https://covid19.who.int/). SARS-CoV-2 transmits mainly through the respiratory pathway and may cause severe respiratory symptoms, such as dyspnea and even pulmonary failure.[3] Additionally, SARS-CoV-2 RNA has been frequently detected in fecal samples from COVID-19 patients with gastrointestinal symptoms, including abdominal pain, diarrhea, vomiting, anorexia and nausea, suggesting that SARS-CoV-2 may also be transmitted through the fecal–oral pathway,[4,5] which further increases the difficulty in combating SARS-CoV-2 pandemic.

Additionally, as the global pandemic of SARS-CoV-2 presents different states of infectivity in different countries, many variants have emerged and spread worldwide. For example, the Alpha variant B.1.1.7 emerged in southeastern England in November 2020 and has quickly spread worldwide to become the dominant circulating SARS-CoV-2 variant in many countries.[6] Recently, B.1.1.248 (Gamma) and B.1.351 (Beta) variants with enhanced transmissibility and/or pathogenicity have also been reported and became dominant in South African variants and Brazilian, respectively.[7,8] Such SARS-CoV-2 variants have brought a more severe challenge to the prevention and control of the COVID-19 pandemic.

The SARS-CoV-2 spike (S) protein presents on the viral surface and plays a crucial role in mediating viral infection.[9] S protein can be divided into two subunits, S1 and S2. The S1 subunit contains the receptor binding domain (RBD), which is responsible for virus binding to the cellular receptor, human angiotensin-converting enzyme-2 (hACE2), and possibly other receptors, such as heparin and the tyrosine-protein kinase receptor UFO (AXL).[10,11] In the prefusion state, S1 subunits rest above S2 subunits, preventing conformational rearrangement of S2 subunits from a metastable prefusion conformation to a highly stable postfusion conformation.[12] Hence, the viral S1 subunit, especially the RBD, is exposed and vulnerable to host immunity. Indeed, under the pressure of host immunity, some mutations, which have occurred in the RBD, such as N501Y, K417N, and E484K, are found in numerous variants, including B.1.1.248 and B.1.351 dominant variants.[7,8] These mutations may play important roles in increasing viral fitness or

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• 揭示新冠病毒 B.1.1.7 突变体感染性增强的机理

SARS-CoV-2 病毒突变体相比与野生型病毒具有更高的传播能力，在突变体中 B.1.1.7 是首个引起大家关注的 SARS-CoV-2 突变体，于 2020 年 9 月 20 日在英国首次发现，并迅速成为当地主要的流行突变体。目前它已经蔓延到 90 多个国家，导致大约 1000 万人感染。

B.1.1.7 突变体的感染效率相比于野生型显著增强，然而关于 B.1.1.7 突变体传染性增强的机制的基础研究仍然缺乏。研究组与复旦大学陆路课题组展开合作，通过冷冻电镜技术解析了高分辨率的 B.1.1.7 突变体 Spike 蛋白和 ACE2 受体的复合物结构（图 10）。经过结构分析，发现 B.1.1.7 突变体 Spike 蛋白上的 10 个突变位点（69，70，144 位氨基酸缺失突变，N501Y，A570D，D614G，P681H，T716I，S982A，D1118H 氨基酸替换突变）以多种方式增强病毒的感染效率，包括增加 Spike 蛋白与 ACE2 的亲和力，增加 Spike 蛋白 prefusion 状态的稳定性，增加 Spike 蛋白 RBD 结构域“抬起”的几率，提高 furin 酶切效率等。增加了人们对新冠病毒突变体感染性增强的认识。

该项研究成果以“Structure-based evidence for the enhanced transmissibility of the dominant SARS-CoV-2 B.1.1.7 variant (Alpha)”为题，于 2021 年 11 月 9 日发表在 *Cell Discovery* 上。

文章链接：

https://doi.org/10.1038/s41421-021-00349-z

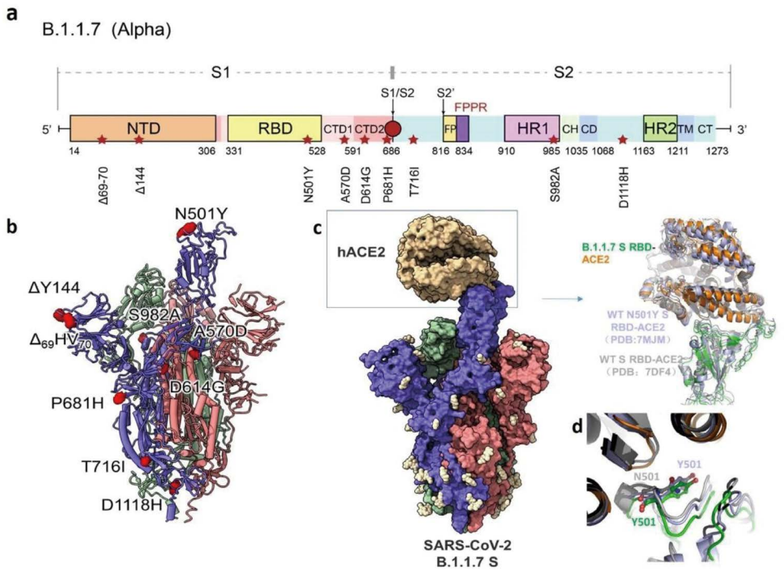

图 10. 新冠病毒 B.1.1.7 突变体结合 hACE2 受体的结构

Xia et al. *Cell Discovery* (2021)7:109
https://doi.org/10.1038/s41421-021-00349-z

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CORRESPONDENCE Open Access

Structure-based evidence for the enhanced transmissibility of the dominant SARS-CoV-2 B.1.1.7 variant (Alpha)

Shuai Xia[1], Zuoling Wen[2,3], Lijue Wang[1], Qiaoshuai Lan[1], Fanke Jiao[1], Linhua Tai[2,3], Qian Wang[1], Fei Sun[2,3,4], Shibo Jiang[1]✉, Lu Lu[1]✉ and Yun Zhu[2]✉

Dear Editor,

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has resulted in over 245 million infections and ~5 million deaths, severely threatening global public health. Moreover, numerous SARS-CoV-2 variants of concern (VOCs) with even higher transmissibility, such as B.1.1.7 (Alpha), B.1.351 (Beta), B.1. 617.2 (Delta), and C.37 (Lambda), are continuously emerging[1]. Monitoring these dominant SARS-CoV-2 variants and exploring the potential reason for their higher transmissibility are important for controlling the current COVID-19 pandemic. B.1.1.7, the first SARS-CoV-2 VOC, was first identified on September 20, 2020 in the United Kingdom (UK) and quickly became the locally dominant circulating mutant. Currently, it has spread to more than 90 countries, causing ~10 million infections (https://cov-lineages.org/global_report.html). Previous studies have reported that the B.1.1.7 variant shows a significant increase in the effective reproductive rate with increased secondary attack rate[2]. However, basic studies elucidating the mechanism underlying the increased infectivity of the B.1.1.7 variant are lacking. In particular, structural studies of the complex containing the B.1.1.7 mutant spike (S) protein and hACE2 receptor are not currently available. Therefore, it is still unclear whether the higher infectivity of the full-length B.1.1.7 mutant S protein is related to its increased receptor-binding affinity.

The rapid spread of B.1.1.7 has increased concern about those natural mutations in the S protein, including 69–70 deletion, 144 deletion, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H (Fig. 1a). Previous studies reported that the B.1.1.7 mutant S protein showed greater receptor-binding affinity than the wild-type (WT) S protein[3]. In contrast, some researchers found a reduced binding affinity between B.1.1.7 and hACE2[4,5]. These conflicting results challenge the assumption of “the higher infectivity of B.1.1.7 mediated by the enhanced receptor-binding affinity”. Therefore, it is necessary to reveal the structure of the B.1.1.7 S-hACE2 complex to fully understand the potential mechanism underlying the high infectivity of the B.1.1.7 variant.

To determine the structural basis for the enhanced infectivity mediated by the S protein of the dominant SARS-CoV-2 B.1.1.7 variant, we used cryo-electron microscopy (cryo-EM) single-particle analysis (SPA) to solve the structures of B1.1.7 S in the 1-RBD-up state and the B1.1.7 S-hACE2 complex (Supplementary Fig. S1 and Table S1) with resolutions of 3.7 and 4.1 Å, respectively, according to the gold standard Fourier shell correlation (FSC) coefficient of 0.143 (Supplementary Fig. S2). To improve protein stability for cryo-EM data processing, proline substitutions at K986 and V987 and a “GSAS” substitution at the furin cleavage site (S1/S2 site, R682 to R685) were introduced into the purified S protein, according to a previous report[6]. In the two structures, the most stable region with the highest local resolution is the helical bundle of the S2 subunit (Supplementary Fig. S2 and Video S1), consistent with the previous reports[3,6].

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These authors contributed equally: Shuai Xia, Zuoling Wen, Lijue Wang

文章与专利

1 发表论文

本年度研究组与合作研究团队在 *Nature*，*Natl Acad Sci U S A.*，*Angew. Chem. Int. Ed.*，*Signal Transduction and Targeted Therapy*，*Nature Communications* 等国际学术刊物上发表研究成果性论文 12 篇

1. Linhua Tai[#], Guoliang Zhu[#], Minnan Yang[#], Lei Cao, Xiaorui Xing, Guoliang Yin, Chun Chan, Chengfeng Qin, Zihe Rao, Xiangxi Wang[*], **Fei Sun**[*], and **Yun Zhu**[*]. Nanometer-resolution in situ structure of the SARS-CoV-2 postfusion spike protein. *Proc Natl Acad Sci U S A.* 2021 Vol. 118 No. 48 e2112703118. https://doi.org/10.1073/pnas.2112703118

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2 专利

本年度申请国家发明专利 2 项，申请美国发明专利 1 项，获得新型实用专利授权 1 项

申请专利

申请号	名称	类别	发明人	申请人	申请日
202110576212.4	用疏水蛋白膜作为支持膜的电镜载网及其制备方法	国家发明专利	范宏成 孙飞 王波 乔明强 朱赟	中科院生物物理研究所、南开大学	2021.5.26
202111106412.X	一种无接触式冷冻电镜样品制备装置及方法	国家发明专利	范宏成 朱博玲 陈永圣 黄小俊 孙飞	中科院生物物理研究所	2021.9.22
PCT/CN2021/131404	一种无接触式冷冻电镜样品制备装置及方法	美国发明专利	范宏成 朱博玲 陈永圣 黄小俊 孙飞	中科院生物物理研究所	2021.11.18

授权专利

专利号	名称	类别	发明人	申请人	授权日
ZL202021846827.1	一种用于冷冻成像用样品台	实用新型专利	孙磊 张建国 张丹阳 季刚 孙飞	中科院生物物理研究所	2021.3.2

新立项项目

国家重点研发计划项目

主持（项目负责人：孙飞）

号码：2021YFA1301500
名称：超大蛋白质机器结构分析前沿技术
时间：2021/12-2026/11

国家重点研发计划青年科学家项目

参加（子任务负责人：张艳）

号码：2021YFF0704300
名称：面向海量冷冻电镜数据的高分辨原位结构智能解析软件平台
时间：2021/12-2026/11

学术活动

1 开展学术交流

2021 年 1 月，召开了“孙飞研究组 2020 年度工作总结暨 2021 年度工作计划会议”，并特邀部分课题合作者参加会议，就研究组 2020 年度开展的课题进行了逐一汇报与梳理，就 2021 年度的工作计划进行了讨论与安排。

2021 年 5 月，孙飞研究员作为项目负责人，在中国科学院大连化学物理研究所组织召开了中国科学院 B 类战略性先导科技专项“生物大分子跨尺度结构研究前沿技术”项目 2021 年度工作会议会议，强化项目研究目标，以及项目下属各层级的责任、任务分工及年度目标；梳理与总结项目四 12 个子课题的研究进展、实施过程中遇到的问题与困难；提出并讨论合作需求，联合攻关，共同解决相关科学问题；强化项目四的组织运行、财务、档案等管理措施。本次会议还对项目未来的发展和实施进行了详细的布属和规划，提高了项目科研团队的凝聚力，为项目今后的顺利实施和团队之间的密切合作奠定了坚实的基础。各个课题和子课题按照项目的原定计划顺利开展实验，目前进展一切顺利，成果突出。

2021 年 8 月，召开了“孙飞研究组 2021 上半年工作总结会议”，对照年度工作计划，对本年度开展的课题研究进展进行了中期汇报总结与鞭策。

2021 年 9 月，基于合作课题研究进展需要，邀请中国科学院大学人工智能学院杨戈教授研究团队召开了“基于深度学习神经网络的冷冻断层扫描图像 (cryo-ET) 目标颗粒的自动挑选的课题进展讨论会”，双方就合作课题近期研究进展进行了汇报与梳理，就下一步工作计划进行了充分讨论与安排。

2021 年 10 月，邀请中国科学院重庆绿色智能技术研究院超分辨光学研究中心主任王化斌研究员来访，开展合作交流，并做题为“太赫兹生物检测”（Terahertz technology in biological research）的生物大分子国家重点实验室学术报告。

2021 年 11 月，邀请美国圣犹达儿童研究医院孙吉教授做题为“Structural analysis of the human full-length LRRK2”的学术报告（线上会议）

2021 年 1 月至 12 月，研究组集中组织了 58 次文献阅读报告会，集中分享与解读了 54 篇文献，分享外出参加学术会议的收获与体会报告 4 次。并每月在“FeiLab”微信公众号上推送一次文献汇总的简报，包括 *Cell*、*Nature*、*Science* 等杂志文章和 *Nature Methods*、*Journal of Structural Biology*、*Ultramicroscopy* 等经典方法学杂志的文章。

2021 年 1 月至 12 月，共组织了 9 次研究组月交流会，根据研究小组组长推荐，由研究组组长确定，从研究组所开展的课题中遴选进展显著、有阶段性成果的课题，由课题第一负责人进行专题汇报，并进行深入讨论。

本年度，研究组开展了系列专题讲座。包括：

《晶体学课程》系列专题讲座（共四次专题讲座，讲授人：孙飞）

《冷冻电镜结构生物学综述》专题讲座（讲授人：朱国梁）

《冷冻电镜蛋白质样品制备》专题讲座（讲授人：朱春梅）

《哺乳动物表达系统简介》专题讲座（讲授人：翟宇佳）

《傅里叶变换》专题讲座（讲授人：台林华）

《电子显微成像原理》专题讲座（讲授人：殷国良）

《cryo-EM 的应用》专题讲座（讲授人：殷国良）

《电镜成像与 CTF 调制》专题讲座（讲授人：范宏成）

《冷冻电镜成像技术和要素》专题讲座（讲授人：温作令）

《单颗粒分析（上）》专题讲座（讲授人：徐嘉树）

《单颗粒分析（下）》专题讲座（讲授人：岐晨）

《质量光度仪的使用科普》专题讲座（讲授人：翟宇佳）

《Relion 4.0》视频专题集体学习（主持人：台林华）

针对研究组 2021 年度的 5 个年度重点课题，分别召集承担年度重点课题研究的相关成员，召开重点课题交流会，汇报研究进展，有针对性地深入讨论所遇到的问题，分析和总结下一步的工作方案，推动年度重点课题的研究进度。

研究组下设三个研究小组，每周各自组织一次研究小组周交流会，促进研究小组成员之间交流，督促课题研究进展。整理研究小组周交流会议纪要，供研究组组长掌握与跟进相关课题进展情况，并作为科研档案留存。

2021 年 12 月，分别组织召开了孙飞研究组研究生 2021 年度工作考核、孙飞研究组工作人员 2021 年度工作考核。

2 参加学术会议

2021 年 4 月

第十五届中国科学仪器发展年会（ACCSI2021）& 首届中国电镜产业发展论坛（无锡）

参会人员：孙飞研究员

2021 全国太赫兹生物物理分会年会（天津）

参会人员：孙飞研究员、朱赟副研究员、王宇清博士

报告题目：Three dimensional electron microscopy technology in life sciences（孙飞）

北京市 2021 年度激光共焦超高分辨显微学学术研讨会（北京）

参会人员：孙飞研究员

中国科学院生物物理研究所生物大分子国家重点实验室学术年会（北京）

参会人员：孙飞研究员

报告题目：高分辨率生物显微成像技术创新（孙飞）

2021 年 7 月

2021 年中国科学技术大学 – 中国科学院生物物理研究所双边研讨会（合肥）

参会人员：孙飞研究员

报告题目：CryoEM technology development orientating in situ structural study（孙飞）

第十九届中国暨国际生物物理大会（合肥）

参会人员：孙飞研究员、王宇清博士、殷国良（博士生）、孙文浩（硕士生）、王子岩（硕士生）

第三届国际遗传与表观遗传精准医疗论坛

参会人员：孙飞研究员

报告题目：生物三维电镜技术与应用（孙飞）

2021 年 8 月

2021 BioEM Talks Series Registration（线上会议）

参会人员：孙飞研究员

报告题目：Infrastructure and technology development in Center for Biological Imaging, IBP, CAS（孙飞）

2021 年 9 月

第七届全国冷冻电子显微学与结构生物学专题研讨会（深圳）

参会人员：孙飞研究员、朱赟副研究员、台林华（博士生）、徐嘉树（博士生）、朱春梅（博士生）、朱国梁（博士生）、岐晨（硕士生）、杭雨墨（硕士生）

报告题目：Technology development of sample preparation for high resolution cryo-electron microscopy（大会特邀报告，孙飞）

Nanometer resolution in situ structure of SARS-CoV-2 post-fusion spike（专题报告，朱赟）

2021 年广州病原体结构与临床应用产业峰会（广州）

参会人员：孙飞研究员

第十四期科学仪器发展高层沙龙“加快冷冻电镜研发攻关与产业布局”（北京）

参会人员：孙飞研究员

2021 年 10 月

2021 年全国电子显微学学术年会大会（东莞）

参会人员：孙飞研究员、顾桐年博士、范宏成（博士生）

报告题目：扫描透射电子显微技术在生命科学研究中的应用（孙飞）

2021 中国生物材料大会（上海）

参会人员：朱赟副研究员

报告题目：重组胶原蛋白的三螺旋结构表征方法研究（朱赟）

四川大学华西生物治疗国重“冷冻电镜与生物医药”研讨会（成都）

参加人员：朱赟副研究员

报告题目：冷冻电镜原位结构解析与生物医药（朱赟）

2021 年 11 月

The 2021 International Symposium of Multiscale Biomolecular Imaging and Computing（线上会议）

参会人员：孙飞研究员

报告题目：Structure of outer ring of Xenopus laevis nuclear pore complex solved by cryoEM and AI（孙飞）

奖励与荣誉

台林华

荣获
2021 年博士研究生
国家奖学金

荣获
2021 年硕士研究生国家奖学金
中国科学院大学三好学生
中国科学院大学优秀学生干部
“中国科学院第十七届公众科学日”优秀科普志愿者
中国科学院生物物理研究所“建党一百周年歌咏比赛”
优秀组织者

殷国良

荣获
2021 年朱李月华优秀博士生奖学金
（非西部）
2021 年博士研究生所长奖学金

朱国梁

荣获
2021 年中国科学院大学
三好学生

范宏成

团建活动

1 奥森跑步

奥森跑步活动为研究组的传统体育活动，旨在督促研究组全体成员养成坚持运动，强身健体，劳逸结合的良好习惯，在运动中培养大家互帮互助的团队合作精神。奥森跑步通常安排在研究组的月交流会后，由孙飞研究员带领大家去奥林匹克森林公园跑步。跑步结束后，由孙老师自费请大家聚餐，让大家在享受美食的过程中，愉快交流，增进情感。

2 秋游活动

为进一步加强师生交流，增进团队凝聚力和向心力，孙飞研究组于 2021 年 10 月 30 日在雁栖小镇组织举办了秋游轰趴活动和真人 CS 活动。告别喧嚣，放飞心情，赏旖旎风光，享盎然秋意；开展真人 CS 对抗赛；一起玩台球、桌游、游戏机等诸多轰趴休闲活动……大家乐在其中，使生活的压力、工作中积累的负面情绪都得到了积极的宣泄，再次以饱满的热情投入到工作与生活中，砥砺前行。

心无旁骛 勇于创新
超越自我 追求卓越

2021