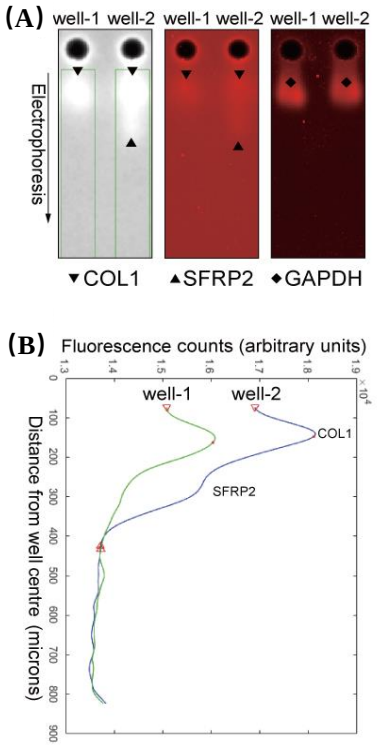
复旦大学王久存教授课题组于*British Journal of Dermatology*杂志上在线发表题为 **"ACKR1+ Endothelial Cells Mediate Leukocyte Infiltration and Synergize with SFRP2/ASPN+ Fibroblasts to Promote Skin Fibrosis in Systemic Sclerosis"** 的文章 (Y. Huang, et al. J. Wang, British Journal of Dermatology, 2024, ljae286)。在研究过程中，王教授课题组利用国重平台的**八通道全自动可加热式组织处理器GentleMACS、高端流式细胞分选仪BD FACSAria Fusion和单细胞蛋白质表达定量分析系统Milo**，建立了一套优化的人类皮肤组织单细胞处理体系，该系统可同时处理8例组织样本，提高样本处理效率的同时减少了研究的批次效应；通过流式分选获得高质量的目的细胞，提高了单细胞测序的数据质量；结合单细胞蛋白质表达定量分析系统，实现了单细胞测序结果的蛋白质表达水平验证。



This study performed single-cell sequencing on skin tissues of normal people and scleroderma patients in the Chinese population and constructed a single-cell transcriptome map of fibrotic skin tissue.

We used the eight-channel fully automatic heatable tissue processor GentleMACS and the high-end flow cytometer BD FACSAria Fusion on the National Key Laboratory of Clinical Oncology to prepare single-cell samples. The independent heating module and program control system of GentleMACS can process 8 different tissue samples simultaneously or separately, and the standardized program control system can ensure the high reproducibility of experimental results. The equipment uses closed, sterile disposable sample separation tubes, and the specially designed rotor and stator on the tube cap can gently dissociate the sample to obtain a single cell suspension that retains complete antigen epitopes and high activity. Combined with the BD flow cytometer, cell debris, dead cells and doublets can be removed to obtain cell samples that meet the machine standards.

In addition, we also used Milo, the single-cell protein expression quantitative analysis system, to verify the transcriptome data results at the single-cell protein level. The system uses a microfluidic western blot chip, which collects single cells through a single-cell microwell design, lyses the cells in situ for protein electrophoresis, and then captures the proteins in situ. Western blot is used to verify the direct hybridization of antibodies and fluorescently labeled secondary antibodies. After the scanner scans the chip, the Scout software performs in-depth quantitative analysis of the scanning results.

图1 硬皮病患者皮肤成纤维细胞中SFRP2及I型胶原的单细胞蛋白表达水平。
(A) 通过 Milo单细胞蛋白印迹法测量一个硬皮病患者皮肤成纤维细胞中 SFRP2、COL1 和GAPDH的蛋白质表达水平。
(B) 孔 1 和孔 2 的荧光值随孔中心的距离而变化情况。使用 Scout 分析数据 (https://www.bio-techne.com/resources/instrument-software-download-center)。

八通道全自动可加热式组织处理器
GentleMACS
负责老师：吴海
仪器位置：G107
电话：31246713

高端流式细胞分析仪
BD FACSAria Fusion
负责老师：沈素芹
仪器位置：E01
电话：31246566

单细胞蛋白质表达定量分析系统
Milo
负责老师：张齐翔
仪器位置：G107
电话：31246713

本研究对中国人群中正常人和硬皮病患者的皮肤组织进行了单细胞测序，构建了纤维化皮肤组织的单细胞转录组图谱。我们利用了国重平台的**GentleMACS和BD FACSAria Fusion**进行单细胞样本的制备。GentleMACS独立的加热模块及程序控制系统可同时或分别处理8个样本，标准化的程序控制系统可保证实验结果的高度可重复性。设备使用封闭、无菌的一次性样本分离管，配合管盖上特殊设计的转子和定子可温和解离样本，获得保留完整抗原表位及高活性的单细胞悬液。结合BD流式细胞分选仪，可去除细胞碎片、死细胞和黏连体，进而获得符合上机标准的细胞样本。

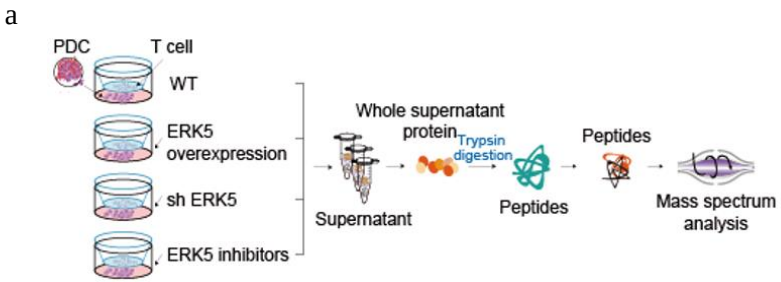
此外，我们还运用**Milo**对转录组数据结果在单细胞蛋白水平上进行验证。该系统采用微流控westernblot芯片，通过单细胞微孔设计，采集单细胞，原位裂解细胞进行电泳，之后进行蛋白质原位捕获，使用westernblot验证抗体及荧光标记二抗直接杂交，扫描仪进行芯片扫描后，Scout软件可对扫描结果进行深度定量分析。

超速流式细胞分选仪在泛黑色素瘤多组学研究中的应用

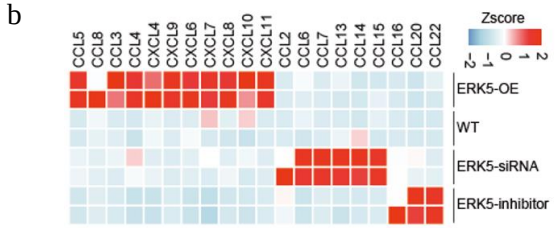
复旦大学丁琛教授课题组于*Cell Discovery*杂志上发表题为“**Proteogenomic insights into the biology and treatment of pan-melanoma**”的文章 (H. Xiang, et al. C. Ding, Cell Discovery, 2024 July; 10(1):78. doi: 10.1038/s41421-024-00688-7)。在研究过程中，丁琛教授课题组利用超速流式细胞分选仪分选T细胞，并将T细胞与病人来源的肿瘤细胞在不同条件下进行共培养，然后提取全蛋白进行质谱检测。进一步将T细胞和黑色素瘤细胞在不同条件下进行共培养，测定细胞培养上清液中的IFN-γ表达。最后结果验证了我们的假设，ERK5的激酶活性的上调可能使得更多T细胞被招募到肿瘤微环境中，从而增加黑色素瘤患者对免疫治疗的敏感性。

流式细胞分析仪
BD FACS Calibur
负责老师：沈素芹

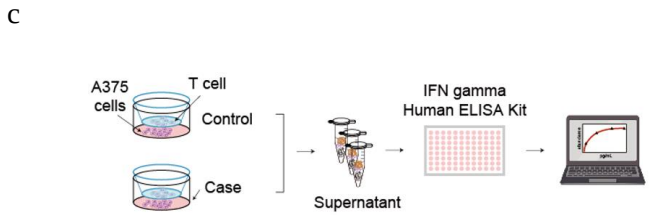
超速流式细胞分选仪
MoFlo-XDP
负责老师：刘龙江



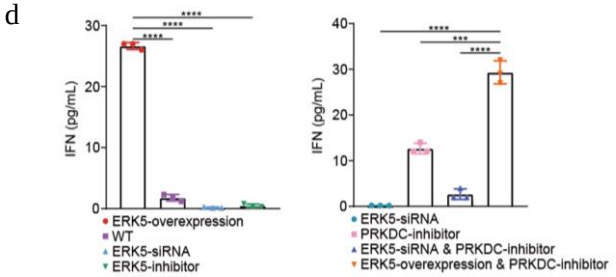
a. PDC细胞和T细胞共培养后进行质谱检测的实验流程图 。



b.热图展示了趋化因子在不同培养条件下的表达情况。



c.黑色素瘤细胞和T细胞共培养后进行酶联免疫吸附测定的实验流程图 。



d.箱型图展示了黑色素瘤细胞与T细胞在不同培养条件下的上清中IFN-γ含量情况。

Using the high-speed flow cytometry assay and coculturing them with melanoma cells under different conditions, it was found that chemokines such as CCL5 and CXCL4 involved in T cell recruitment were significantly upregulated in the ERK5 overexpression group. This suggests that upregulation of ERK5 kinase activity may lead to more T cells being recruited into the tumor microenvironment, thereby increasing the sensitivity of melanoma patients to immunotherapy. Further coculture melanoma cells with T cells under different conditions, and then collect the cell supernatant for enzyme-linked immunosorbent assay. The results showed that the IFN-γ content significantly increased in the supernatant of the ERK5 overexpression group. Meanwhile, the IFN-γ content significantly increased in the supernatant of the ERK5 overexpression and simultaneous PRKDC inhibition groups. These results suggest that PRKDC may reduce the sensitivity of melanoma patients to immunotherapy by promoting DNA damage repair in melanoma.

利用超速流式细胞分选仪分选T细胞，并将T细胞与黑色素瘤细胞在不同条件下进行共培养，发现参与T细胞招募CCL5、CXCL4等趋化因子在ERK5过表达组中显著上调，这提示了ERK5的激酶活性的上调可能使得更多T细胞被招募到肿瘤微环境中，从而增加黑色素瘤患者对免疫治疗的敏感性。进一步将黑色素瘤细胞与T细胞在不同条件进行共培养，然后收集细胞上清进行酶联免疫吸附测定。结果显示，与野生型组和ERK5抑制组相比，IFN-γ含量在ERK5过表达组的上清中显著上升。同时，与ERK5抑制组和PRKDC抑制组相比，IFN-γ含量在ERK5过表达和同时PRKDC抑制组的上清中显著上升。这些结果提示，PRKDC可能通过促进黑色素瘤的DNA损伤修复，从而降低黑色素瘤患者对免疫治疗的敏感性。

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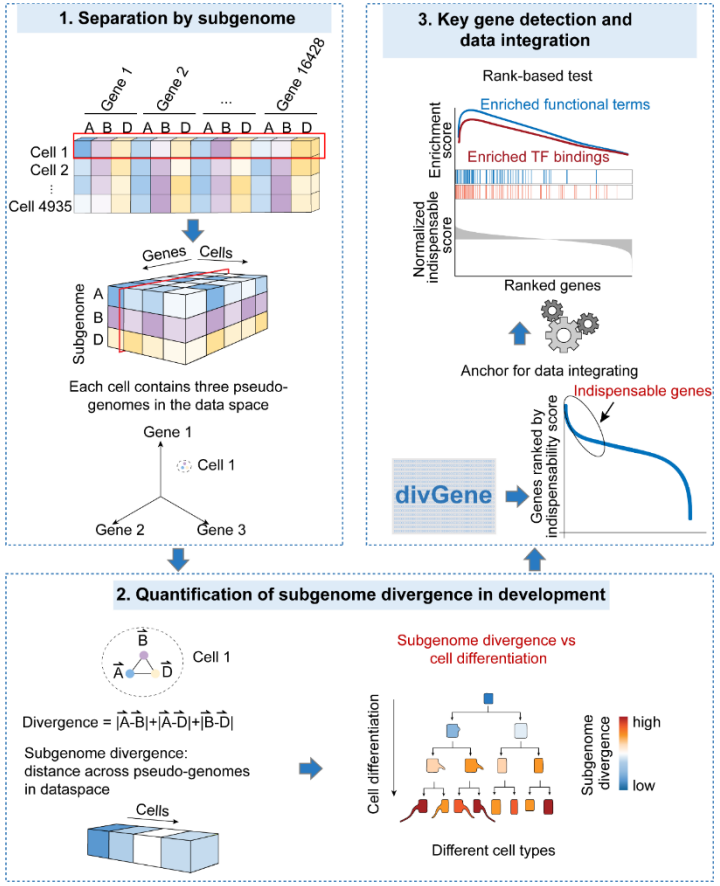
多倍体单细胞数据整合与发育核心基因排序的定量计算框架

复旦大学张一婧研究员课题组于*Molecular Biology and Evolution*杂志上发表题为“**A quantitative computational framework for allopolyploid single-cell data integration and core gene ranking in development**”的文章（Meiyue W. et al. *Molecular Biology and Evolution*, 2024, doi:10.1093/molbev/msae178）。在研究过程中，课题组利用国重平台的**Covaris ME220聚焦超声仪**，超声破碎小麦组织细胞基因组DNA，用于后续建库测序。该设备利用聚焦超声波技术，能够在极短的时间内产生高度局部化的机械剪切力，从而高效、高通量、非接触式地将大分子DNA切割成特定大小的片段，且具有很好的可重复性。



异源多倍体单细胞数据整合及发育过程中核心基因排序的定量

Polyploidization drives regulatory and phenotypic innovation. How the merger of different genomes contributes to polyploid development is a fundamental issue in evolutionary developmental biology and breeding research. Recent single-cell sequencing techniques enabled probing differentiation at the cellular resolution. However, analyzing single-cell data suffers from high error rates due to high-dimensionality, noise, and sparsity, and the errors stack up in polyploid analysis due to the increased dimensionality of comparisons between subgenomes of each cell, hindering deeper mechanistic understandings. Here, we developed a quantitative computational framework, pseudo-genome divergence quantification (pgDQ), for quantifying and tracking subgenome divergence directly at the cellular level. pgDQ produces robust results and is insensitive to data dropout and noise, avoiding high error rates due to multiple comparisons of genes, cells, and subgenomes. A statistical diagonostic approach is proposed to identify genes that are central to subgenome divergence during development, which facilitates the integration of different data modalities, enabling the identification of factors and pathways that mediate subgenome-divergent activity during development.

八通道全自动可加热式组织处理器
GentleMACS

Covaris ME220 Focused-ultrasonicator
Covaris ME220聚焦超声仪

我们使用**Covaris ME220聚焦超声仪**来对小麦基因组进行超声打断，用于后续RNA建库测序。简单的载物平台、便捷的软件设置、清晰的可视化操作界面，使得操作者可以在快速培训后对仪器进行熟练使用。一次可以放入多个样品，并对每个样品进行不同设置，满足对样品处理高通量的需求。非接触式聚焦超声技术，保证处理温和精准。

在常规的超声破碎中，受限于探头体积，需要大量的样品起始量，而Covaris ME220聚焦超声仪因为是非接触式精准聚焦，因此仅需要微量样品，大大减轻了样品收集的压力。不仅如此，Covaris ME220聚焦超声仪还能通过设置固定参数实现将大分子DNA切割成特定大小的片段，使得实验稳定可重复。

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供稿人:张一婧课题组　撰稿人:应松倍　编辑:刘鹤华

抗SARS-CoV-2三价蛋白药物的设计与验证

复旦大学黄强教授课题组于 *Journal of Nanobiotechnology* 上发表了题为“**Computational design and engineering of self-assembling multivalent microproteins with therapeutic potential against SARS-CoV-2**”的文章 (2024, 22:58)。在研究过程中，该课题组利用重点实验室的**ÄKTA avant 蛋白纯化系统**成功纯化和表征了所设计的的靶向新冠病毒Spike蛋白的自组装三价微型蛋白，并验证了它们的自组装能力；另外，还使用了**200 kV 冷冻电子显微镜 Glacios**，通过单颗粒冷冻电镜技术解析了所设计的三价纳米抗体束与Spike的复合物结构，证实了其能够同时结合Spike上三个受体结合结构域 (RBD)，并将Spike锁定在“3-RBD-up”构象，从而抑制人受体ACE2与RBD的结合。

蛋白纯化工艺开发系统
ÄKTA avant
负责老师：刘鹤华
仪器位置: A201
电话：31246713

200kV 冷冻电子显微镜
Glacios
负责老师：周德健
仪器位置：E101
电话：31246568

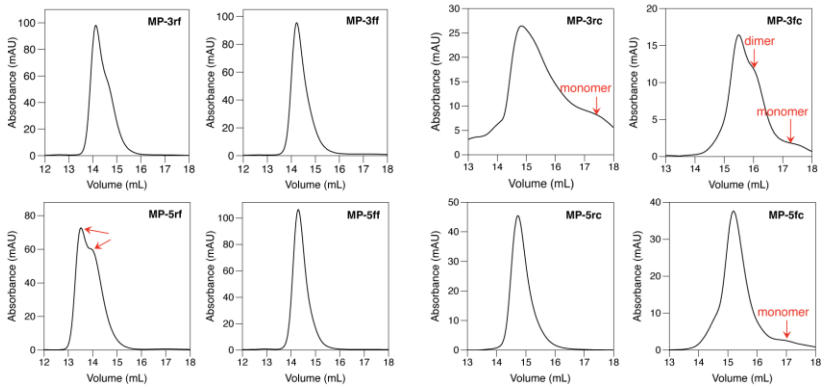

图1 纯化后的三价微型蛋白的分子筛图谱

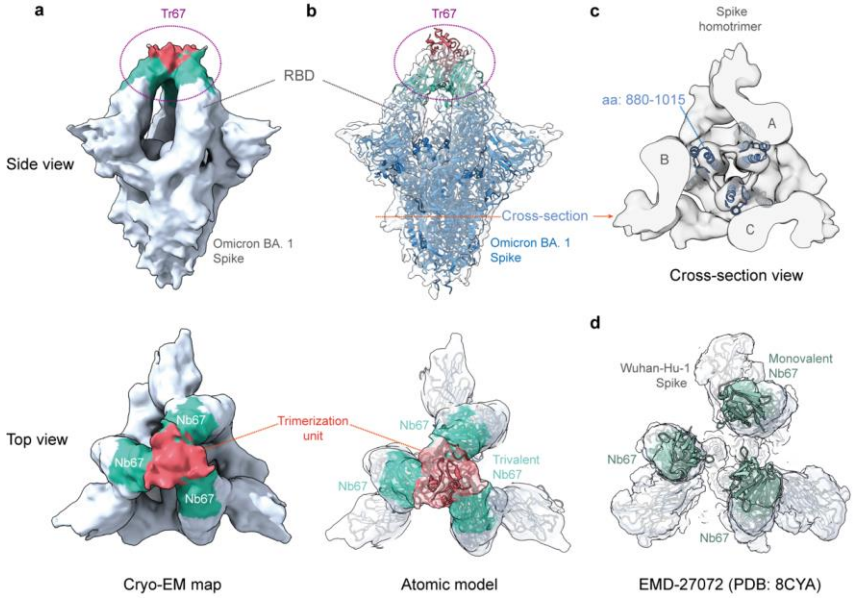

图2 三价纳米抗体束Tr67与SARS-CoV-2（Omicron BA.1）Spike蛋白复合物的冷冻电镜结构。(a) “3-RBD-up”构象下的Tr67-Spike复合物的电镜密度图。(b) 复合物原子模型与电镜密度图的拟合。(c) Spike蛋白茎部的横截面视图。(d) 文献报道的三个独立的Nb67与SARS-CoV-2 (Wuhan) Spike蛋白的复合物结构。

This study developed a computational method for designing self-assembled multivalent microproteins targeting SARS-CoV-2 Spike protein, and thereby successfully designed trivalent microproteins with therapeutic potential: MP-5ff, and Tr67. We successfully purified the designed trivalent proteins using the ÄKTA avant system and verified their self-assembly ability. In addition, structural analysis by cryo-EM confirmed that the designed trivalent nanobody cluster Tr67 could simultaneously bind to all three receptor binding domains (RBDs) of Spike and lock Spike in the “3-RBD-up” conformation. Tr67 showed potent neutralizing activity against major Omicron variants, including XBB.1 and XBB.1.5. Therefore, this study provides a new approach for the rapid and efficient development of protein drugs with broad-spectrum neutralizing activity against SARS-CoV-2 and other pathogenic coronaviruses..

本研究开发了一种计算方法，用于设计靶向SARS-CoV-2刺突蛋白的自组装多价微蛋白，并成功设计出了具有治疗潜力的三价微型蛋白：MP-5ff和Tr67。我们利用 **ÄKTA avant系统**成功纯化出了所设计的三价蛋白，并验证了它们的自组装能力。另外，通过**冷冻电镜**进行结构分析，证实了所设计的三价纳米抗体束Tr67能同时结合Spike的三个受体结合结构域（RBD），并将Spike锁定在“3-RBD-up”构象。Tr67对主要Omicron变异株都表现出很强的中和活性，包括XBB.1和XBB.1.5。所以，本研究为迅速快速开发具有广谱中和能力的抗SARS-CoV-2和其他致病性冠状病毒的蛋白药物提供了新的途径。

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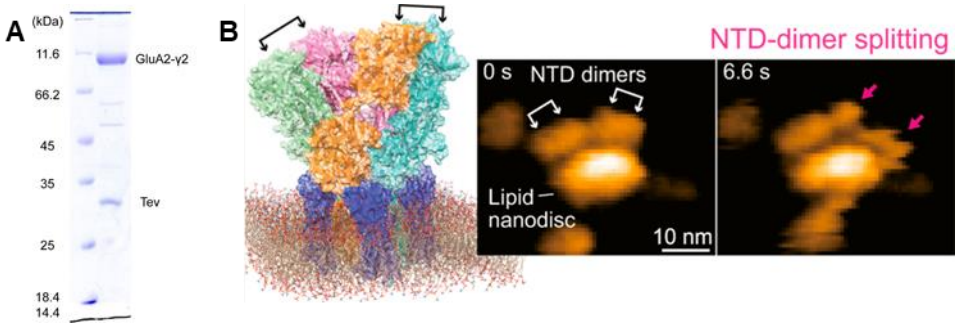
AMPA型离子型谷氨酸受体的首次HS-AFM结构解析

复旦大学服部素之研究员课题组于*ACS Nano*杂志上发表题为“**High-Speed Atomic Force Microscopy Reveals Fluctuations and Dimer Splitting of the 3N-Terminal Domain of GluA2 Ionotropic Glutamate Receptor-Auxiliary Subunit Complex.**”的文章 (Y. Z. et al, M. Hattori. *ACS Nano.* 2024)。在该研究中，服部教授课题组利用国重平台的**蛋白质微量纯化系统 AKTA Micro AKTA Micro 和Beckman Coulter Optima XPN-100**，完成了对AMPA型离子型谷氨酸受体（AMPAR）的纯化等实验，利用高速原子力显微镜发现了在AMPAR的N端结构域的结构波动与二聚体分裂现象，进一步揭示了N端结构域在AMPAR突触聚集中的关键作用，并为突触传递的基本过程提供了有价值的见解。

Beckman Coulter Optima XPN-100
超速离心机

蛋白质微量纯化系统
AKTA Micro AKTA Micro



AMPAR蛋白的HS-AFM结构解析 (A) 使用超蛋白质微量纯化系统纯化AMPAR样品；(B) AMPAR结构示意图和的HS-AFM结果

α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid glutamate receptors (AMPARs) enable rapid excitatory synaptic transmission by localizing to the postsynaptic density of glutamatergic spines. AMPARs possess large extracellular N-terminal domains (NTDs), which are crucial for AMPAR clustering at synaptic sites. However, the dynamics of NTDs and the molecular mechanism governing their synaptic clustering remain elusive.

Here, we employed high-speed atomic force microscopy (HS-AFM) to directly visualize the conformational dynamics of NTDs in the GluA2 subunit complexed with TARP γ2 in lipid environments. HS-AFM videos of GluA2-γ2 in the resting and activated/open states revealed fluctuations in NTD dimers. Conversely, in the desensitized/closed state, the two NTD dimers adopted a separated conformation with less fluctuation. This NTD-dimer splitting resulted in subunit exchange between the receptors and increased the number of interaction sites with synaptic protein neuronal pentraxin 1 (NP1). Moreover, our HS-AFM studies revealed that NP1 forms a ring-shaped octamer through N-terminal disulfide bonds and binds to the tip of the NTD. These findings suggest a molecular mechanism in which NP1, upon forming an octamer, is secreted into the synaptic region and binds to the tip of the GluA2 NTD, thereby bridging and clustering multiple AMPARs. Thus, our findings illuminate the critical role of NTD dynamics in the synaptic clustering of AMPARs and contribute valuable insights into the fundamental processes of synaptic transmission.

哺乳动物大脑中的快速兴奋性突触传递的主要介导蛋白，AMPA受体（α-氨基-3-羟基-5-甲基-4-异恶唑丙酸受体，AMPAR）定位于谷氨酸能突触的突触后致密区，介导快速兴奋性突触传递。AMPAR具有较大的细胞外N端结构域(NTD)，这对于AMPAR在突触位点聚集至关重要。然而，NTD的动力学和控制其突触聚集的分子机制仍然是难以捉摸的。在本研究中，利用国重平台的**蛋白质微量纯化系统 AKTA Micro AKTA Micro 和Beckman Coulter Optima XPN-100**，完成了对AMPA型离子型谷氨酸受体（AMPAR）的纯化等实验，使用高速原子力显微镜(HS-AFM)直接观察到了在脂质环境下AMPAR的GluA2亚基与跨膜AMPAR调节蛋白 (TARP γ2) 配合的NTD的构象动力学。这些发现揭示了一种分子机制，在形成八聚体后，NP1分泌到突触区域并结合到GluA2的NTD尖端，从而桥接和聚集多个AMPAR，从而解释了NTD在AMPAR突触聚集中的关键作用，并为突触传递的基本过程提供了有价值的见解。

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