ISSN 1007-7626
CN 11-3870/Q
中国生物化学与分子生物学报 http://cjbmb.bjmu.edu.cn
Chinese Journal of Biochemistry and Molecular Biology
2018 年 1 月
34(1):13~17

·Special Visiting to Nobel Prize Winner·

DOI: 10.13865/j.cnki.cjbmb.2018.01.02

The Era of Cross-Disciplinary Research for Medical Advances Is Coming—Briskly A Conversation with JOACHIM FRANK

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Perhaps most exciting, combining scientific acumen and engineering ingenuity will facilitate the creation and accumulation of knowledge on human biology today. The Nobel Prize in Chemistry this year was awarded to three biophysicists: Jacques Dubochet, Joachim Frank, and Richard Henderson, for their breakthrough contributions to developing cryo-electron microscopy (cryo-EM) for the high-resolution structure determination of biomolecules in solution. The application of this technique has opened the human eyes to the structures of proteins and eventually unravel the mysteries of life's mechanisms.

Dr. Joachim Frank described himself as a very visually oriented person paying attention to very small details. Photo Credits to Zhuo Li

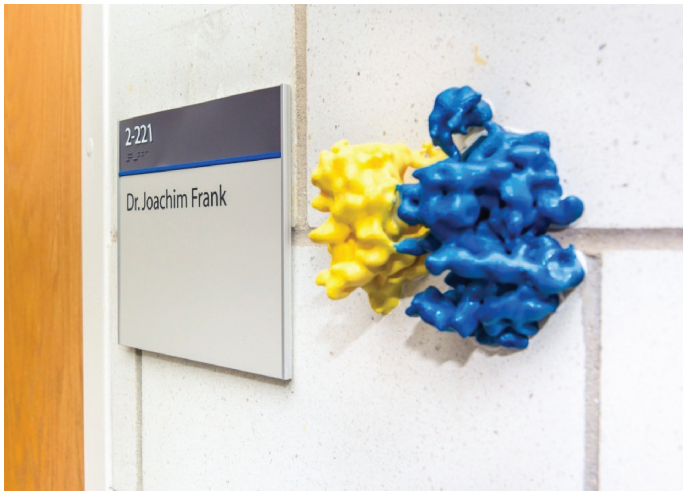
The Nobel Prize Committee for Chemistry singled out the fact that Dr. Joachim Frank developed the image analysis methods to allow many 2D images from ensembles of biomolecules in solution to be put together and produce a 3D image from them.

In this approach, determining the relative orientations and angles between the individual molecules is the most challenging problem. These difficulties made the accomplishments of Dr. Joachim Frank truly remarkable.

Dr. Joachim Frank (b.1940), born and educated in Germany, is a scientist and writer. He is also a photographer who has three published portfolios. He received degrees equivalent to Bachelor and Master degree in physics, and a PhD in Biophysics. In his doctoral and postdoctoral research, he developed methods of digital image analysis as applied to electron microscopy and also worked on problems of electron optics.

When he was a senior research scientist at the Wadsworth Center in Albany, New York, Dr. Frank developed the single-particle reconstruction approach and applied it to the investigation of the mechanism of translation on the ribosome. In 1977, he conceived the random conical method, a strategy for determining the angles of molecules lying randomly on the grid. Computer programs that realized this idea and brought it to fruition by 1987 were written by Michael Radermacher, his first postdoctoral student. Over the years, using cryo-EM and single-particle reconstruction methods, and flexible fitting of X-ray structures available since 2000, the dynamics of the decoding and translocation mechanisms were revealed.

In 2008 he moved to take on his current position as a Professor of Biochemistry and Molecular Biophysics and of Biological Sciences at Columbia University. Dr. Frank and his family are going to Stockholm in Sweden for the Nobel Prize ceremony and celebration in December. Recently before their departure I interviewed him in his lab at Columbia University Medical Center. Our conversation has been edited for length and clarity.



The Front Door of Dr. Joachim Frank's lab at Columbia University Medical Center

Dr. Joachim Frank and I, at the time of the interview in November of 2017

JC (Joy Cheng): Oh hello! My name is Joy Jiayi Cheng, I'm a bioengineer. I am the Co-founder of Dental and Craniofacial Regeneration Foundation. Congratulations on receiving the prestigious Nobel Prize in Chemistry! It is a privilege interviewing with you.

JF (Joachim Frank): Oh thank you very much!

JC: This year the Nobel Prize is awarded to cryo-EM as a powerful tool for solving structures of molecules in solutions. Why do we need to solve the structures of macromolecules and their complexes to atomic resolution in solutions?

JF: Well, as you know, there is a powerful method for solving structures of molecules that can be crystallized: the method of X-ray crystallography. But the problem is, there are many many molecules that cannot be crystallized. So those that cannot be solved in this way can now be solved by cryo-EM. In the single-particle method, molecules are completely detached from each other; they are completely "single". They are exactly as they are in solution. Alright? So you can think of single-particle cryo-EM as a new, complementary way of solving structures – one that shows these structures in their native form. X-ray crystallography left a very big gap in structure research. It was left because of the difficulty to get molecules crystallized, or, even if they could be crystallized, the crystals still did not diffract high resolution which means they are too much disordered to get information from that.

JC: The Nobel Prize has been awarded to the field of structural biology many times since 1962, why does it continue to be awarded to this field?

JF: Well, you know, chemistry and biochemistry is really about the relationship and interaction between molecules. And in order to say something about life functions we need to determine these interactions. For this to happen, we need to know their structure. So I think, as time goes on, structural biology, or a comprehensive knowledge of biomolecular structure, becomes more and more important in biology.

JC: Other than the three Laureates, are there other pioneers who have also contributed a lot to cryo-EM?

JF: There are many many. I don't really know whether I should single any one out. That's very difficult. Because they have contributed to the answers to so many different technical questions, they made astonishing discoveries, and so forth. So it's very difficult to say. I do want to say that the EM community has been sort of very happy about the selection. People I spoke to, and people who sent me messages felt that the different aspects of the whole development were very well represented.

JC: Do you think the development of cryo-EM is finished? What should people do next?

JF: Well, it's not… the development is not finished, and neither is the application of the existing technique. Even with the existing technique, I think it will take years to apply it, to… to all the molecules that we want to know the structure of. There are many channel structures, receptors… These are all proteins that are most happy in membrane, in a lipid environment. They all are very hard to solve by X-ray crystallography. And there are so many of them. They are so important in medicine. It will take years to get the structures of all these proteins.

Now as far as the development is concerned, I think, this is far from complete. The cameras, for instance, I've been told, will become more powerful than they are now, meaning it will get even easier reach atomic resolution than it is now, and faster. We will see the development of at least semi – automated ways of solving structures. And then there are also developments that are completely in the infancy now.

One of them is time-resolved cryo-EM to look at structures that are very short-lived, structures that only live for some number of milliseconds, you know, maybe 20 milliseconds, 100 milliseconds. So if you use ordinary cryo-EM, then the whole step of specimen preparation takes too long to observe any of those structures.

Another development that I'm particularly interested in is to use the data that we collect from cryo-EM in a more exhaustive way than it has been done before. Right now the maximum likelihood algorithms used single out the conformational states that are most often encountered. However, there is actually a continuum of states that are left out by this approach. There is now a new kind of analysis that allows us to map all existing states.

Besides, Dr. Frank also discussed with me the most important criteria for him to select his PhD students. "I'm looking for evidence of very independent thinking and intelligence. That's sometimes very hard. Recommendation letters don't tell you what 'specimen' you're dealing with. An interview is very important to me." said Dr. Joachim Frank with a smile. "I'm looking for diversity to keep balance. Because this is a sort of an interdisciplinary lab, we'll need influx from computer science, math, biochemistry… many different areas."

There aren't many medical advances that have not taken advantage of sophisticated tools. After a brief interview, I was left imagining the potential impact on the biotechnological and pharmaceutical field. The technology developed and application of cryo-EM will revolutionize how drug is screened, designed and modified. It can be used to help visualize exquisite protein structures, from antibiotic-resistant proteins to the surface of HIV virus etc., and numerous intelligently designed novel drugs can be born to "fix" the abnormal proteins causing diseases. Thanks to scientists and engineers from a variety of backgrounds' innovation and commitment to excellence, this field is being propelled into the future.

Last but not the least, I would like to reuse Joachim Frank's own words here: "Glancing over the peripatetic history of the single-particle reconstruction method, from its obscure beginning in 1978 to its present wide acceptance makes me realize that the technique has succeeded in a way nobody imagined. For one, I would not have predicted that a molecule, after the harsh treatment of freeze-plunging and without support of companions in a crystal matrix, would allow us to see molecular detail at its very periphery."

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Joy is a bioengineer and research scientist with a Bachelors in Pharmaceutical Sciences, dual Masters in Pharmacology & Biotechnology and a PhD candidate in Dentistry. She is a member of ORS (Orthopaedic Research Society), NYAM (New York Academy of Medicine) and TERMIS (Tissue Engineering and Regenerative Medicine International Society). She's the co-founder of New York State registered Dental and Craniofacial Regeneration Foundation expected to lead state-of-the-art scientific education, research and clinical translation on reconstruction of dental, oral, maxillofacial and craniofacial tissues for better treatments and cures.
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·独家专访·

与诺贝尔化学奖得主 Joachim Frank 对话：跨学科研究促进医学发展的时代已经到来

本刊特约记者 程佳祎[1),2)]
翻译 傅子赦[1)]，秦 珂[2)] 审校 尹长城[3)]
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我们高兴地看到，科学前沿与工程学技巧的结合将加强人类生物学知识的创造和积累。2017 年度诺贝尔化学奖授予了三位生物物理学家：Jacques Dubochet，Joachim Frank 和 Richard Henderson，以表彰他们的突破性贡献——发展冷冻电子显微镜技术（cryo-EM）用于测定溶液中生物分子的高分辨率结构。这项技术使得人们能够领略蛋白质的结构，并有望最终揭开生命活动机制的奥秘。

诺贝尔化学奖委员会认为 Joachim Frank 博士对图像分析处理方法做出了贡献。使用该方法可以将在溶液中的生物分子的电子显微镜二维图像经过处理转化成三维结构图像。确定群体分子的相对取向和角度是单颗粒图像分析处理方法的最具挑战性的步骤，因此 Joachim Frank 博士在该领域所取得的成就更加难能可贵。

Joachim Frank 博士 1940 年出生于德国锡根，在德国接受教育。他既是一位科学家，也是一位作家，同时还是一位摄影师，出版了三部个人作品集。他拥有物理学学士与硕士学位、生物物理学博士学位。在博士和博士后研究中，他开发了应用于电子显微镜的数字图像分析方法，并研究了电子光学的相关问题。Joachim Frank 博士在纽约州奥尔巴尼市沃兹沃斯中心做高级研究科学家时，研发了单颗粒重建方法，并将其应用于核糖体翻译机制的研究中。1977 年，建立了随机圆锥倾斜法，用于确定分子随机分布的角度。1987 年，他的这一设想通过他的第一位博士后 Michael Radermacher 编写的计算机程序得以实现，之后又不断加以完善。通过多年来连续不断的积累和努力，用冷冻电镜和单颗粒重建的方法（2000 年后电镜中又结合了柔性拟合的 X 射线结构）揭示了翻译过程的解码和易位的机制。自 2008 年起，Joachim Frank 博士在纽约哥伦比亚大学担任生物化学与分子生物物理学系和生物科学系教授。

2017 年 11 月，Joachim Frank 博士在哥伦比亚大学医学中心的实验室接受了我的独家专访。以下谈话内容，为了方便阅读，在篇幅与表述上做了某些调整。

程佳祎：您好！我叫 Joy Jiayi Cheng，是一名生物工程师，也是纽约州注册的牙齿与颅面再生基金会的联合创始人。恭喜您获得了诺贝尔化学奖！我很荣幸能与您面谈。

JF（Joachim Frank）：非常感谢！

程佳祎：今年诺贝尔奖授予了用于解析溶液中分子结构的冷冻电镜技术。我们为什么需要研究大分子和大分子复合物在溶液中的原子分辨率结构呢？

JF：你可能知道，X 射线晶体学已经是一种非常有效的方法，用于解析那些能够形成晶体的分子结构。但问题是，很多分子并不能形成晶体。那些不能形成晶体的分子结构，现在可以通过冷冻电镜的方法来解析。在“单颗粒”方法中，（在快速冷冻状态下）分子相互之间完全是分开的、单一分散的，和它们在水溶液中的状态是一样的，你明白吧？所以你可以把冷冻电镜想作是一种新的和 X 射线晶体学互补的解析结构的方法，可以让你观测到自然状态下的结构。X 射线晶体学在结构生物学领域留下了一个很大的空缺。由于很多分子难以结晶，即使它们可以结晶，但是晶体衍射达不到高分辨率，这意味着虽然分子能够形成晶体，但是它们的排列仍然不够有序，不能够得到高分辨率结构的信息。

程佳祎：为什么诺贝尔奖自从 1962 年后，多次授予结构生物学领域，为什么这个领域一直受到青睐？

JF：我想化学和生物化学真正研究的是分子之间的关系及其相互作用。如果想阐释生命活动的功能，

我们需要研究生物分子之间的相互作用。为了这一点,我们需要了解结构。所以我想在未来,结构生物学或者关于结构的知识在生物学领域将变得越来越重要。

程佳祎:除了这三位诺贝奖得主外,还有哪些人也对冷冻电镜领域做出了贡献呢?

JF:实在太多了。我都不知道我能否将他们一一列举出来,这实在太难了。因为很多人对解答不同的技术性问题都做出了贡献,他们有如此卓越的发现……所以这实在太难回答了。我想说的是,在电镜领域中,大家对我们三位被选为诺奖得主还是很高兴的。他们认为这代表了整个发展过程的不同方面。

程佳祎:你觉得冷冻电镜的发展接近尾声了么?研究者应该继续做什么?

JF:并没有结束,发展没结束,应用也没结束,我甚至认为,还需要很多年才能将这项已经存在的技术应用到所有我们想了解结构的分子上,有那么多的通道、受体!它们都是一些在细胞膜、在磷脂环境中才稳定的蛋白质,它们的结构难以用 X 射线晶体学方法来解析,它们数量庞大,在医学研究领域上又十分重要,这需要花上很多年才能将它们的结构一一解析出来;至于这项技术本身的发展,我觉得任重而道远,例如电子检测器的发展,我知道它将会变得更加有效,这意味着将来解析原子分辨率的速度将会比现在更快,半自动化或者自动化解析结构也是该方法发展的未来;另外还有很多发展方向现在处于萌芽阶段,其一就是时间分辨冷冻电镜,它可以用来观测转瞬即逝的结构,存在的时间段在毫秒级别,比如可能是 20 毫秒或者 100 毫秒,相比而言,如果你使用普通冷冻电镜就观察不到这些存在的结构,那是因为整个样品制备的过程花费了很长的时间。另外一个发展方向我很感兴趣,那就是如何利用收集到的数据。我们利用冷冻电镜收集到了比以往更加详尽全面的信息。目前所用的最大似然算法只是选出最常见的构象,但是这种方法忽略了构象的变化是连续的,现在我们有了一种新的分析方法能够绘制出所有存在的状态。

简短的采访中,我们还谈到了挑选博士生的重要标准。“我最看重的是独立思考能力和智力。但有时这非常难以确定,因为推荐信没法告诉你申请者真实的样子,所以我觉得面试非常重要。”Joachim Frank 博士微笑着说,“我力求保持实验室里人员的多样性。我的实验室是一个跨学科实验室,聚集了来自计算机、数学、生物化学等各个领域的人才,力求保持全面。”

医学的进步离不开尖端工具的应用。可以想象冷冻电镜技术将给生物制药行业带来一场变革。把这项技术应用于各种结构复杂的蛋白质研究中,比如造成抗生素耐药性的蛋白质、艾滋病病毒表面(抗原)……许多新药就可以被巧妙地设计出来用于“修理”导致疾病的异常蛋白。我深深地感受到来自不同领域科学家和工程师的合作创新和追求卓越正把这个领域推向激动人心的未来。

在文章最后,我想引用 Joachim Frank 博士自己的话来作为结语:“纵观单颗粒重建方法的漫长发展史,从 1978 年初的晦涩难懂到现在被广泛接受认可,让我意识到这项技术的成功是无人能够预料到的。至少其中有一点我无法预料到,一个分子,经过粗糙的冷冻处理,没有在晶体中的支撑,即可在人眼前呈现出全方位的精细的分子结构。”

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(图片摄影:李卓光)