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蛋白质与蛋白质组学

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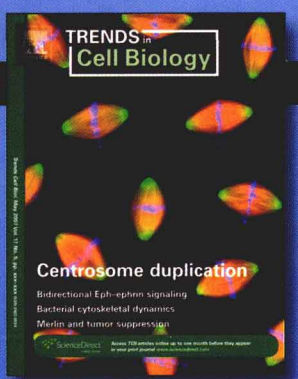

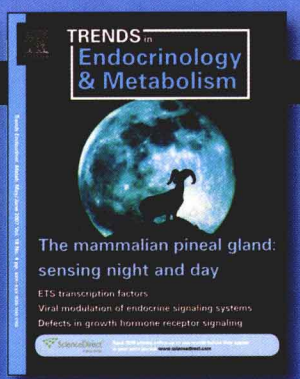

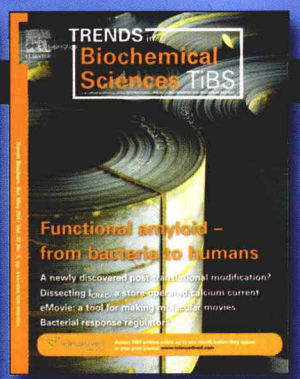

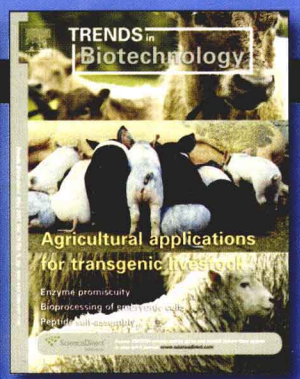

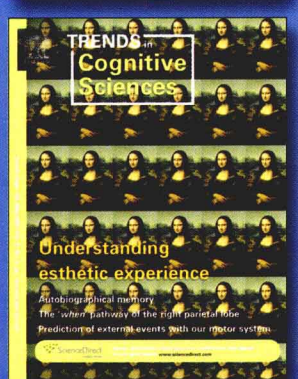

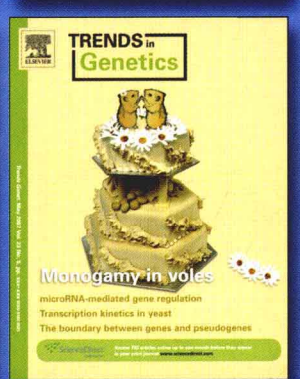

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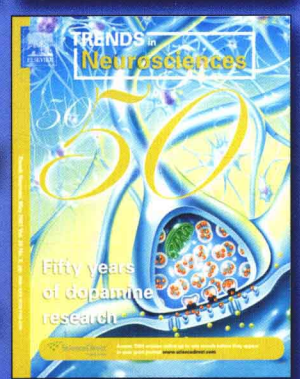

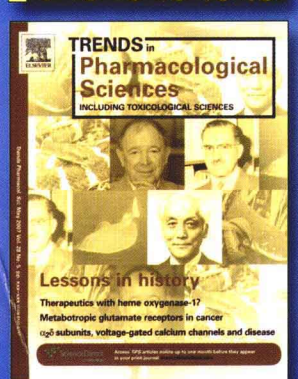

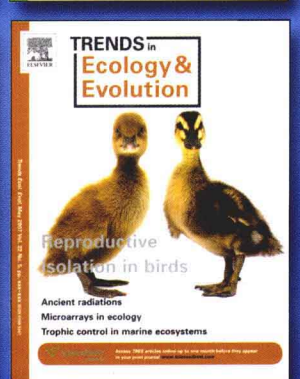

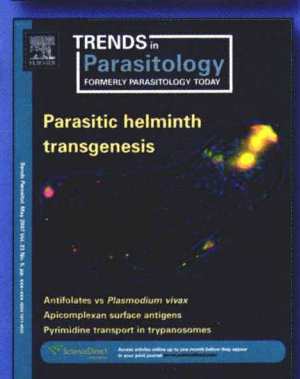

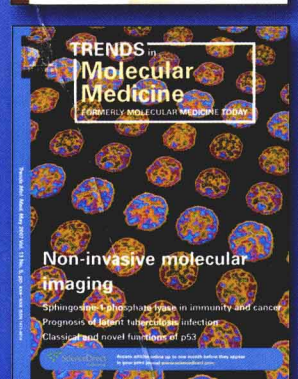

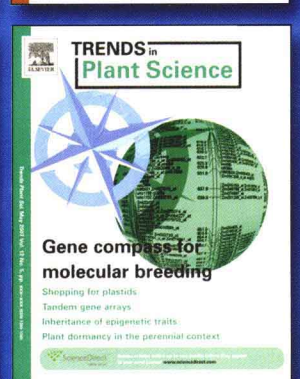

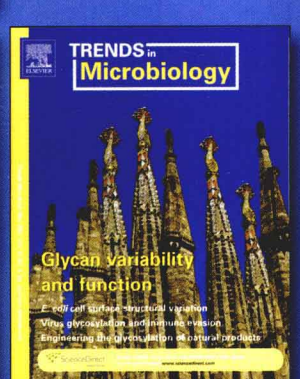

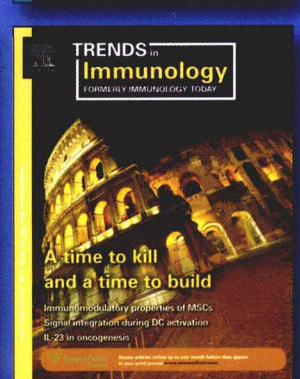

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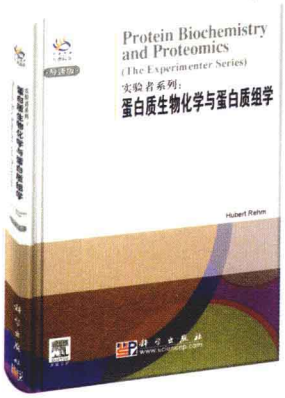

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The Experimenter Series: Protein Biochemistry and Proteomics

《实验者系列:蛋白质生物化学与蛋白质组学》（导读版）

原著：Hubert Rehm
导读：沈世华（中科院植物所）

在实验中，你是否严格遵循标准流程却一无所获？你是否想改进你的工作？你想更多地了解某一研究领域的各种方法吗？或许“实验者”丛书将对你有所帮助。

本书是“实验者”系列中的一本，从权威人士的视点，为你讲述蛋白质生物化学和蛋白质组学研究的新方法。你能够充分体会到作者饱尝了实验室的挑战带给他们的激动和沮丧。有了这些技巧和诀窍，你的实验成功的几率将更高。但这本颇有价值的实验室手册并非只是一部方法集锦：

* 它为你指明走出实验困境的道路，培养你适时选择正确实验的直觉。

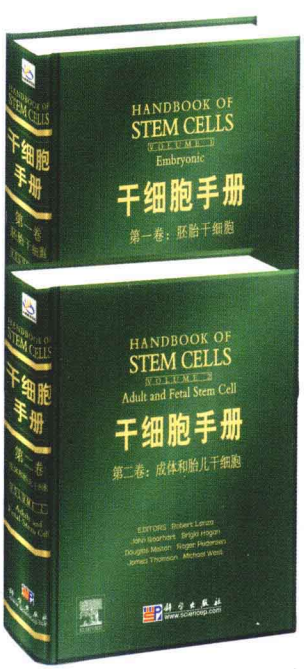
* 它简洁明了地概括了常规方法（例如柱层析，凝胶电泳）的步骤，还列出了不同方法的优缺点。

* 它详细介绍了配体结合实验、抗体生产和微序列分析方面的进展。

* 它论述了一些特殊方法，例如膜蛋白的溶解和重组，糖蛋白分析，或寡聚体蛋白质亚基的确定。

* 其中一节对蛋白质组学进行了导向性说明。

本书适用于生化与分子生物学、功能基因组学、蛋白质组学等生命科学相关领域的研究生和科研人员参考使用。



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Handbook of Stem Cells

《干细胞手册》（导读版）

（第一卷：胚胎干细胞；第二卷：成体和胎儿干细胞）
原著：Robert Lanza 等
译注：裴雪涛　教授（军事医学科学院）等

干细胞领域的新发现对科研和社会的影响越来越深远，其最终将产生针对肿瘤、心脏病、糖尿病，以及影响人类健康的许多其他疾病的新的治疗手段。

《干细胞手册》这部书分为上下两册内容，整合了该领域必备的生物学知识、手段、方法、研究等，以及国际专家对于每一个特定的器官系统相关知识发展现状的介绍。干细胞领域的所有主题无一例外地被收录其中，包括基础生物学/机制、早期发育、外胚层、中胚层、内胚层、方法（例如如何分离和培养动物和人胚胎干细胞的具体描述）、针对特定人类疾病的干细胞的应用、法规与伦理，等等。它们凝集了12位编辑和超过300位学者和科学家的共同努力，正是他们开拓性的工作使得我们对干细胞有了精确的理解。

这两本书将成为学生和科研人员必备的全面的参考书。

美国国家科学院主席，一版再版、享誉世界的经典名著《细胞的分子生物学》（Molecular Biology of the Cell）的主编 Bruce Alberts 教授为本书作序。

New Focus in Life Sciences

蛋白质与蛋白质组学

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蛋白质与蛋白质组学

8 New light for science: synchrotron radiation in structural medicine

Trends in Biotechnology, Volume 24, Issue 11, November 2006, Pages 500-508

Thomas L-M. Sorensen, Katherine E. McAuley, Ralf Flaig and Elizabeth M.H. Duke

科学新亮点：结构医学中的同步辐射

大分子晶体学是一种获得大分子详尽三维结构信息非常有用的方法。使用同步X射线的大分子晶体学已经在基础研究和应用研究两个方面均做出了有意义的贡献，其中也包括为了应对重大疾病，以结构为基础的药物设计。新的第三代同步加速器对产生的X射线束在质量和亮度上都做出了实质性的改进。一些重要类型的大分子，例如膜蛋白（包括许多受体），以及大分子复合物，都很难得到足够的量，也不易结晶，这阻碍了使用大分子晶体学方法对其进行分析。最新的同步加速器产生的极亮的X射线允许极小晶体的使用，对这些以前难于对付的结构的解析必将由此迎来快速发展的时期。

17 Relative quantification in proteomics: new approaches for biochemistry

Trends in Biochemical Sciences, Volume 31, Issue 8, August 2006, Pages 473-484

Richard D. Unwin, Caroline A. Evans and Anthony D. Whetton

蛋白质组学中的相对定量法：生物化学的新方法

质谱和蛋白阵列技术的新近发展为从小样本细胞材料中获得系统的蛋白质组信息提供了机会。样品的相对定量可以通过使用凝胶或不使用凝胶的方法获得。而特殊技术的改进有助于绝对定量。本文将双向凝胶电泳相对定量的方法与其它非凝胶的方法（如肽的同位素标记，用必需氨基酸的同位素化合物形式对活细胞进行预标记，以及蛋白阵列平台等）进行了比较。此外，也使用了流式细胞仪的方法。所有这些技术都可以在一个较宽的范围内精确测定蛋白或生物标记物量的水平。

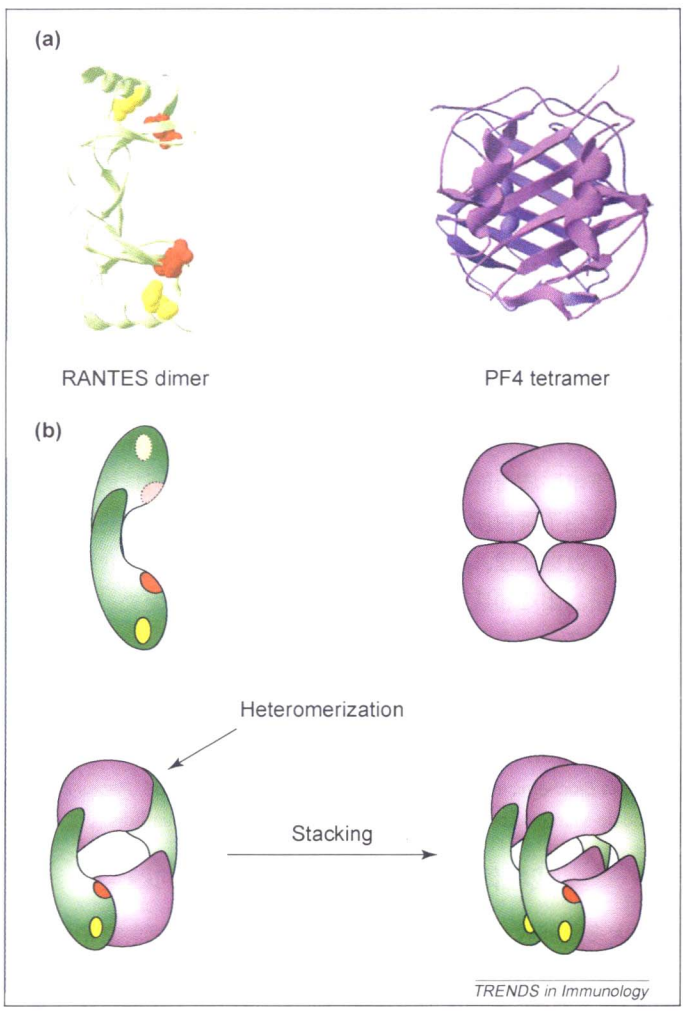
29 Fine-tuning leukocyte responses: towards a chemokine 'interactome'

Trends in Immunology, Volume 27, Issue 6, June 2006, Pages 268-273

Christian Weber and Rory R. Koenen

对白细胞应答的微调：相关的趋化因子的“相互作用组”

不断扩大的趋化因子家族调节了白细胞的激活，协助将它们投送到炎症部位，并在免疫监视中协调其运输。趋化因子与其螺旋受体的结合和功能被认为是受到多方面因素的控制，其中包括它们与细胞表面蛋白聚糖的相互作用、寡聚体的形成、天然存在的拮抗剂以及蛋白酶解的加工等。最近的研究表明，趋化因子间的嗜异相互作用明显



地改变了它们的生物学活性。从这些研究中，我们能够开始窥探到这一新的调节机制的结构基础。本文中，我们提出了一个功能性的“相互作用组”（interactome）的概念，它们是在特定微环境中，由不同嗜异的趋化因子-趋化因子相互作用构成的。这个模型可以说明不同趋化因子产生的信号如何被整合，以便共同控制白细胞的应答。

35 Clinical proteomics: searching for better tumour markers with SELDI-TOF mass spectrometry

Trends in Pharmacological Sciences, Volume 27, Issue 5, May 2006, Pages 251-259

Judith Y.M.N. Engwegen, Marie-Christine W. Gast, Jan H.M. Schellens and Jos H. Beijnen

临床蛋白质组学：利用 SELDI-TOF 质谱系统寻找更好的肿瘤标志物

最近，癌症研究的焦点已经从人类基因组遗传信息拓展到蛋

白质表达分析。因为蛋白质组更精确地反映了细胞、组织或有机体的状态，人们期盼从蛋白质组中得到更多关于癌症诊断和治疗的标志物。一些蛋白质组新技术特别适合于寻找新的生物标志物。表面增强激光解吸电离 - 飞行时间 - 质谱（SELDI-TOF）技术常被用来寻找作为癌症（如卵巢癌、乳腺癌、前列腺癌及结肠癌）生物标志物的新蛋白。不过人们越来越认识到，应该像强调蛋白质的起源和特征一样，强调这些蛋白质分子标志的重现性和有效性。通过这些努力，蛋白质谱将会有助于我们更好地优化疾病的诊断和治疗程序。

44 Protein identification and expression analysis using mass spectrometry

Trends in Microbiology, Volume 14, Issue 5, May 2006, Pages 229-235

Eugene Kolker, Roger Higdon and Jason M. Hogan

质谱用于蛋白质鉴定和表达分析

特定条件下表达于整个机体的蛋白质的鉴定和定量是高通量蛋白质组学的主要关注点。先进的蛋白质组学方法促进了新的生物学相关数据和有力的假设的产生。在这里我们介绍哪些蛋白质组研究能在一个普通装备的实验室中完成，哪些不能。我们还讨论了最普遍的基于串联质谱的方法，并着重介绍了如何得到可信的结果。文中分步介绍了蛋白质组的实验方法，包括样品制备、消化、标记、液相层析、数据处理、数据库检索和统计分析等。我们还介绍了蛋白质组分析的困难和瓶颈，并讨论了需要进一步改善之处。另外，文章还描述了一些高通量的、以蛋白质组学为基础的微生物研究方法。

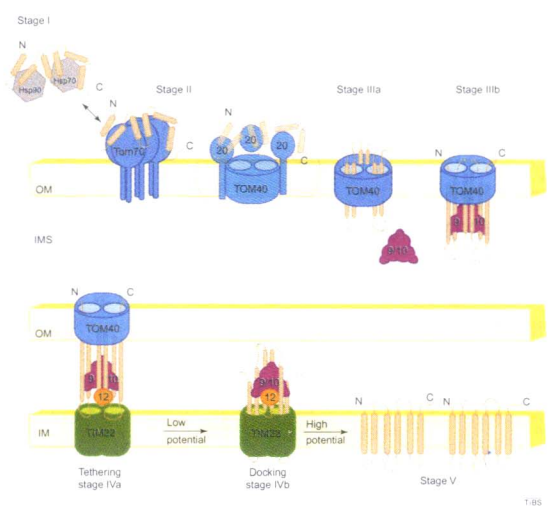
51 Translocation of mitochondrial inner-membrane proteins: conformation matters

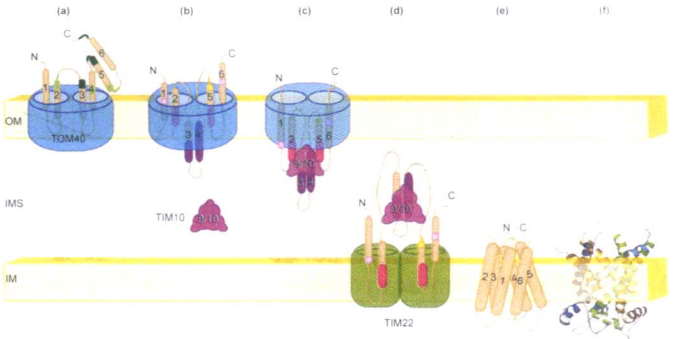
Trends in Biochemical Sciences, Volume 31, Issue 5, May 2006, Pages 259-267

Carine de Marcos-Lousa, Dionisia P Sideris and Kostas Tokatlidis

线粒体内膜蛋白质的转位：构象问题

大多数线粒体内膜的蛋白质形成时并没有前序列，它们的靶向定位依赖于目前认识还不充分的肽链内部的片段。尽管蛋白质组学已经鉴别了输入蛋白的许多组分，但是人们对疏水输入底物的性质仍了解得很少。最近的研究支持了有关这些膜蛋白的几个原则：首





先，它们在转位子中组织成为部分组装的形式；其次，它们呈递着不连续的靶向信号；第三，它们诱导转位酶亚基构象的改变，从而介导输入蛋白的“按需组装”。这些蛋白质穿越外膜，通过膜间空间，并靶向定位到内膜时，需要的能量可能由涉及输入组分的构象改变所提供，这些组分似乎具有天然的未折叠结构。这种结构柔韧性可能使得某些转位酶的亚基更擅长驱动蛋白质进入细胞的过程。

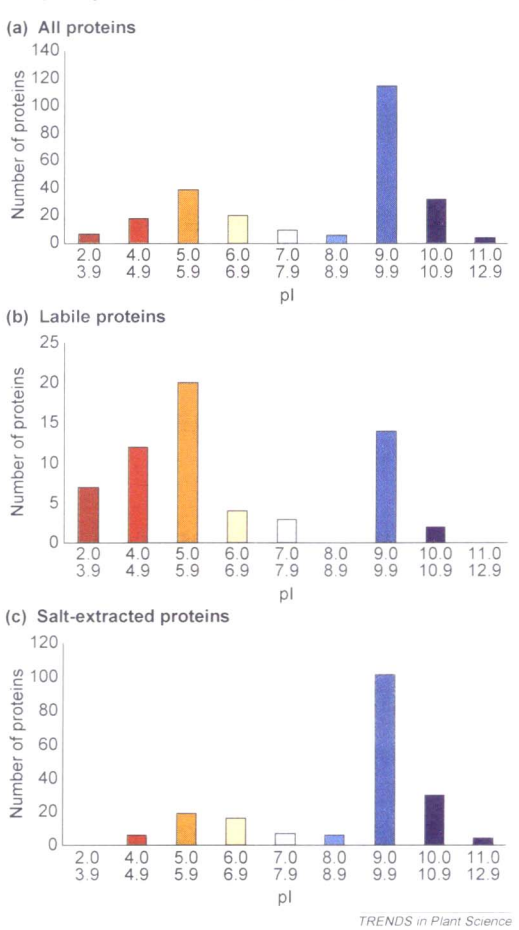
60 Cell wall proteins: a new insight through proteomics

Trends in Plant Science, Volume 11, Issue 1, January 2006, Pages 33-39

Elisabeth Jamet, Hervé Canut, Georges Boudart and Rafael F. Pont-Lezica

细胞壁中的蛋白质：蛋白质组学的新视角

细胞壁蛋白质是植物细胞壁的基本组成物质，它们参与细胞壁组分的改变、胞壁结构、信号传送，以及与细胞表面的质膜蛋白质的相互作用。将蛋白质组学方法应用于细胞壁区室时引发了一些重要的问题：有没有仅针对细胞壁蛋白质组学的技术问题？在拟南芥的细胞壁中可发现什么样的蛋白质？其中有预想之外的蛋白吗？迄今为止在细胞壁蛋白质中已经鉴定到了哪些类型的翻译后修饰？本综述的目的是，讨论至今利用蛋白质组学方法已得到的实验结果，以及在将来的研究中一些新的挑战性问题。



67 Development and application of proteomics technologies in *Saccharomyces cerevisiae*

Trends in Biotechnology, Volume 23, Issue 12, December 2005, Pages 598-604
Annemieke Kolkman, Monique Slijper and Albert J.R. Heck

蛋白质组学在酿酒酵母中的技术进展和应用

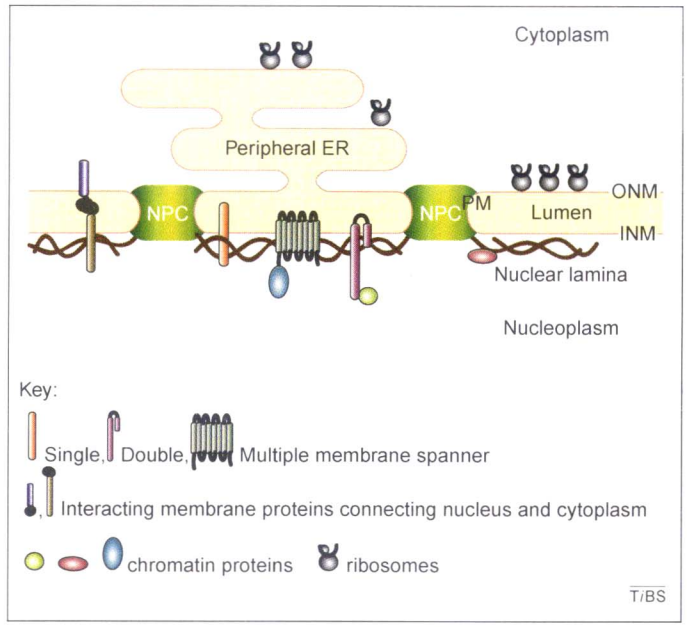
蛋白质组学的研究关注细胞、有机体或组织中所有蛋白质的鉴定和定量。蛋白质组学在技术上是复杂的，因为它包涵了基因组表达的所有蛋白质的特征及功能分析。另外，由于蛋白质的表达水平高度依赖复杂的调节系统，因而蛋白质组也是高度动态变化的。本文集中介绍了两种主要的蛋白质组学方法，一种基于双向凝胶电泳技术，另一种基于液相色谱、质谱联用技术。另外，本文描述了这些技术的最新进展及其在定量蛋白质组学中的应用。酿酒酵母模式系统被认为是利于开展蛋白质组学研究的工具。文本还综述了有关酵母菌适应（营养）环境变化的研究。

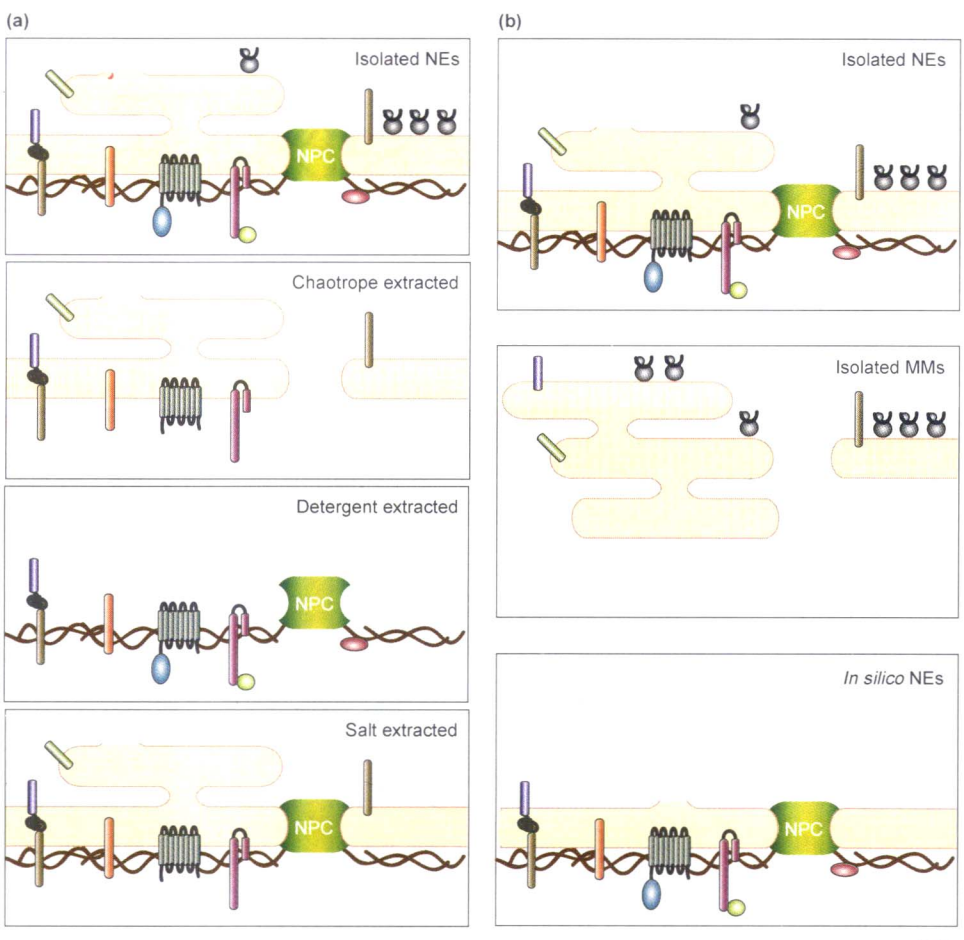
74 The nuclear membrane proteome: extending the envelope

Trends in Biochemical Sciences, Volume 30, Issue 10, October 2005, Pages 551-558
Eric C. Schirmer and Larry Gerace

核膜蛋白质组：拓展外被

通过蛋白质组学与细胞生物学的联姻，人们已经积累了很多亚细胞器的蛋白质资源。最近蛋白质组分析已经确认了许多新的可能在核被中穿越膜的蛋白质，而转录物组总体分析则提示核膜蛋白质组在不同的组织中显示了一些明显的变化。核被（特别是那些含有疾病相关的蛋白质核纤层蛋白 A 的何被）的蛋白质亚复合物组成的细胞类型特异性的差别，可能产生不同的功能；从而可以解释人类多种核被相关疾病的组织特异性。综合起来考虑，这些最新的研究结果显示核被的功能有着意料之外的复杂性。





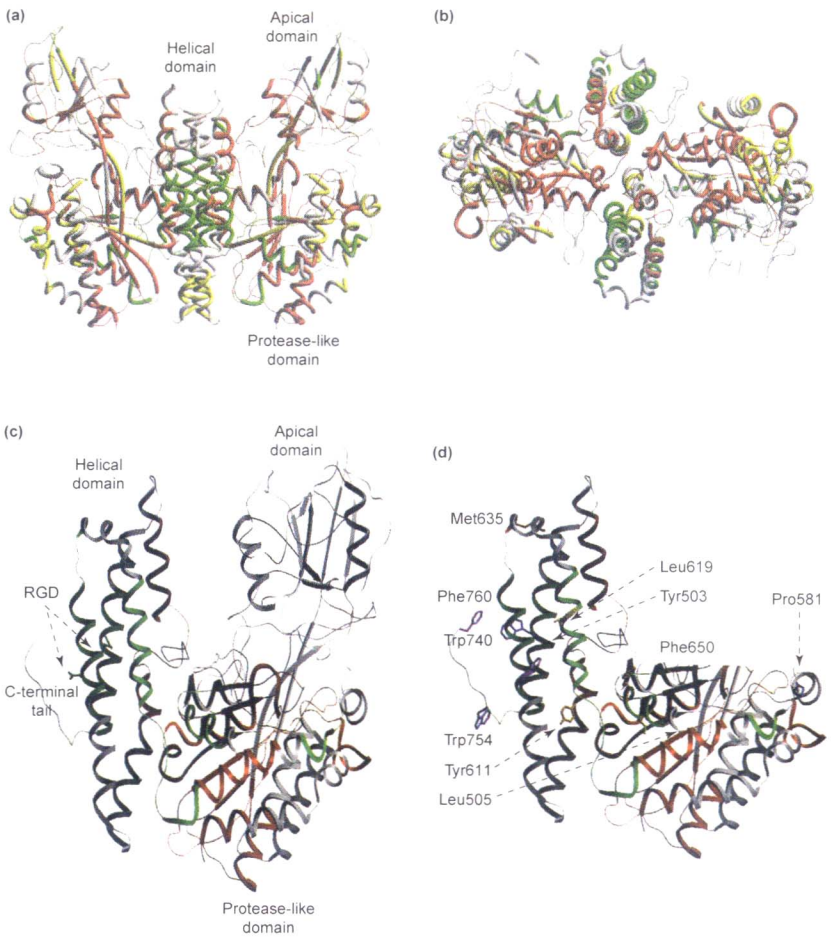
82 Structural proteomics of macromolecular assemblies using oxidative footprinting and mass spectrometry

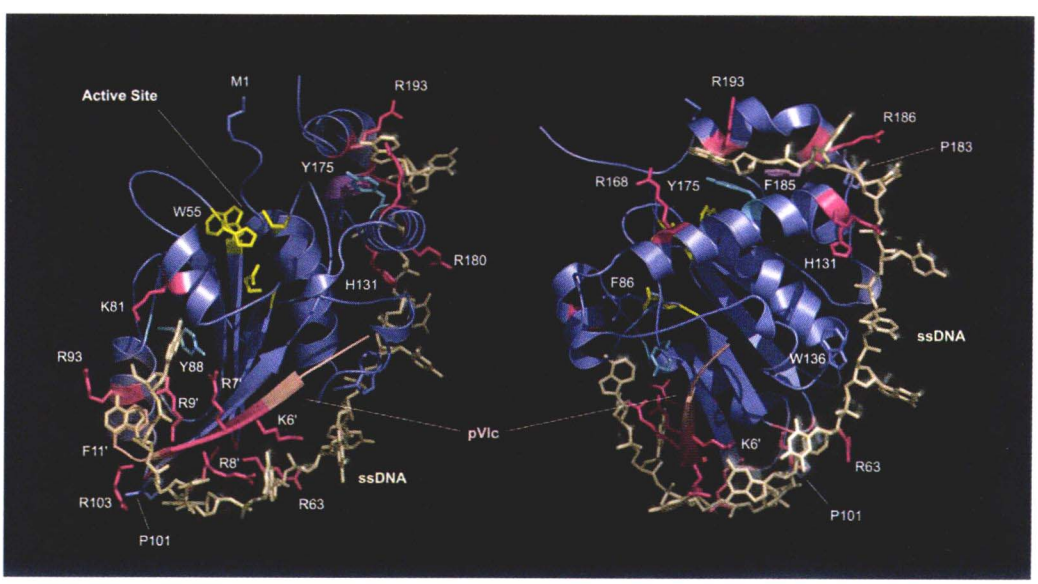
Trends in Biochemical Sciences, Volume 30, Issue 10, October 2005, Pages 583-592

Jing-Qu Guan and Mark R. Chance

利用氧化印迹和质谱法研究大分子组装的结构蛋白质组学

了解大分子的组成、结构、动力学及装配是当前生物学最活跃的研究领域。利用质谱的羟自由基介导的蛋白印迹法可以确定大分子的结构和装配，并可通过共价修饰试剂测量氨基酸侧链基团的活性从而确定大分子在溶液中的构象变化。蛋白质经活性氧氧化后，再经特定的蛋白酶消化，可以产生用于质谱分析的多肽。蛋白侧链活性的精确测定可以联用定量液相色谱和质谱，其中大分子探针中的侧链位置可以用串联质谱识别。此外，配合使用印迹数据和计算机建模的方法，是检验和修正大分子及其复合物结构模型的强有力的新方法。





92 Making the most of affinity tags

Trends in Biotechnology, Volume 23, Issue 6, June 2005, Pages 316-320
David S. Waugh

亲和标签的批量制作

蛋白质物理化学性质的多样性使得对其进行高通量分析变得很困难。因此，亲和标签已经成为启动结构和功能蛋白质组学不可或缺的工具。亲和标签最初是被用来推动重组蛋白质的检测和纯化的，但是最近几年人们清楚地认识到，亲和标签可以对其融合对象的产率、溶解度，乃至折叠产生积极的影响。然而，没有单一的亲和标签是适用于所有这些参数的，每种都有各自的优点和弱点。因此，若要在高通量装置中应用全能亲和标签，组合标签可能是唯一的方法。

97 Proteomics of the *Drosophila* immune response

Trends in Biotechnology, Volume 22, Issue 11, November 2004, Pages 600-605
Ylva Engström, Olga Loseva and Ulrich Theopold

果蝇免疫应答的蛋白质组学

果蝇基因组测序的完成使得人们可以通过蛋白质组学的方法对一些复杂过程（例如对微生物的固有免疫防卫）进行研究。微生物感染导致应答的激活，这些应答涉及到翻译和翻译后水平的改变。蛋白质组学是测定蛋白表达、定位和转录后修饰中这些改变的一个工具。最近，一些研究已经报道了果蝇免疫应答的全基因组分析，包括了转录组和蛋白质组水平两个方面，使得人们更全面地了解果蝇的免疫。在这篇综述中，我们叙述和比较了用于这些分析的蛋白质组学技术，并讨论了果蝇免疫应答的差别蛋白谱方面得到的结果。

103 High-throughput cell-free systems for synthesis of functionally active proteins

Trends in Biotechnology, Volume 22, Issue 10, October 2004, Pages 538-545
Alexander S. Spirin

用于功能活性蛋白质合成的高通量无细胞体系

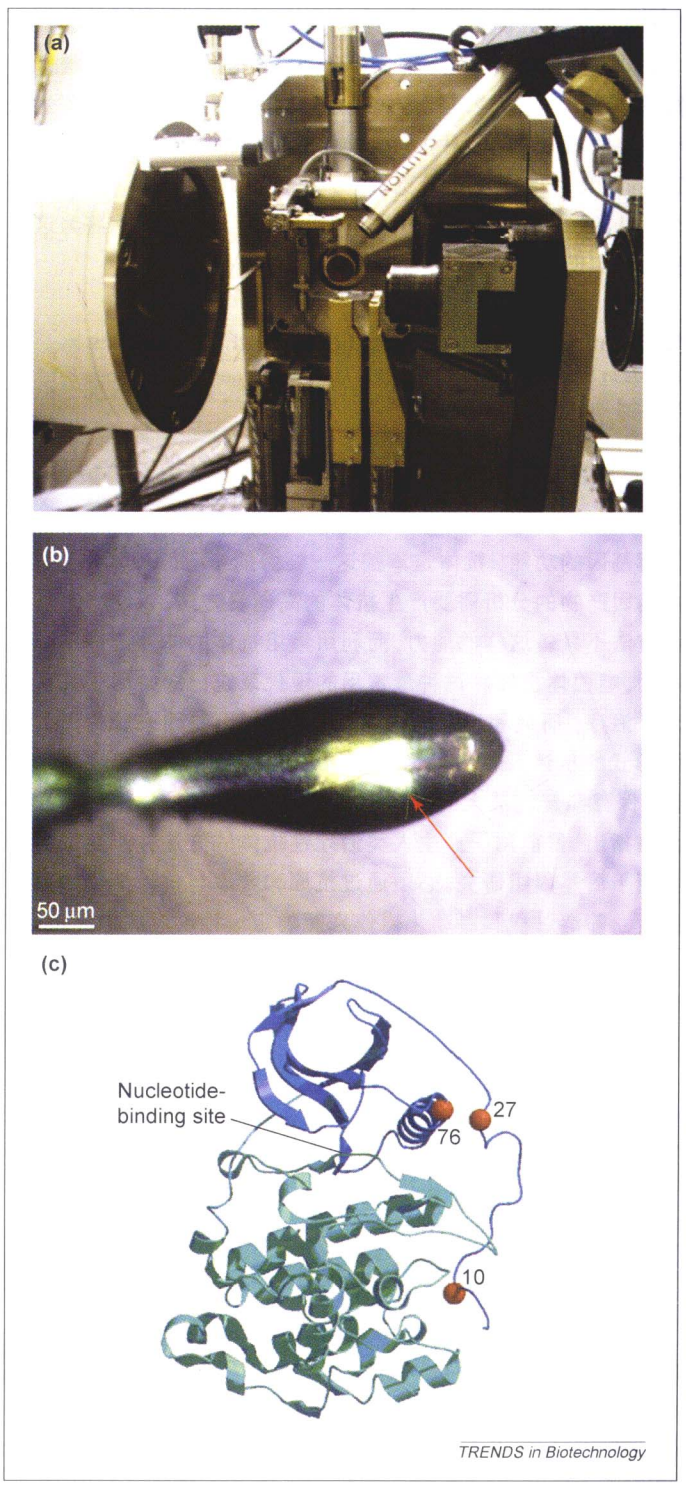
不间断地提供消耗性底物和去除反应产物的连续无细胞翻译系统，能使个别蛋白质在体外持续和大量的合成。无细胞反应混合物的改进，包括有效产生能量的新方法，对无细胞蛋白质合成工艺产生了更大的影响。基因组研究中对基因产物进行鉴定的需要，高通量结构蛋白质组学的发展，无细胞局限的蛋白质工程的需要（包括使用非天然的氨基酸），以及生产无毒性、低表达和不稳定蛋白质的需要都会引起分子生物学家、生物技术学家和药理学家对无细胞蛋白质合成技术的兴趣。

111 Protein nanocrystallography: a new approach to structural proteomics

Trends in Biotechnology, Volume 22, Issue 3, March 2004, Pages 117-122
Eugenia Pechkova and Claudio Nicolini

蛋白纳米结晶学：结构蛋白质组学的新方法

本文描述了一种结构蛋白质组学的新方法，该方法利用纳米技术产生并定性衍射的、稳定的和抗辐射的小尺度晶体。我们相信通过基于纳米技术的蛋白薄层模板结晶化而得到的蛋白质微晶，以及原子力显微镜、微重量测量、同步微聚焦等技术突破，已经使得纳米结晶技术成为一种能在原子水平上生成和定性稳定蛋白质微晶的独特技术。一条崭新的从艺术到科技的途径已经在蛋白结晶学中展开，它可能被用来揭开许多系统中许多尚未解决的谜团。



117 The proteomics bottleneck: strategies for preliminary validation of potential biomarkers and drug targets

Trends in Biotechnology, Volume 22, Issue 1, January 2004, Pages 4-7

Steven Bodovitz and Thomas Joos

蛋白质组学研究的瓶颈：初步确认潜在生物标志和药物靶点的策略

蛋白质组学技术已经被证明对于发现潜在的生物标志和药物靶点是非常有效的，然而初筛中，更多的应用了低效及低通量技术而非高通量技术，这产生了一个瓶颈。以下策略可以减缓这个瓶颈：第一，可以通过把蛋白质组分成一些更小的，更具有生物学意义的部分来限制潜在的生物标志和药物靶点的数量；第二，运用高产出和高通量的筛选技术来拓宽瓶颈；第三，将更多的初步确认的技术应用到发现过程中。新的和浮现中的技术将为这些策略的实现带来希望。

121 Mating antibody phage display with proteomics

Trends in Biotechnology, Volume 22, Issue 1, January 2004, Pages 8-14

Michael Hust and Stefan Dübel

结合噬菌体抗体库的蛋白质组学

“后基因组”研究的下一个主要任务是通过分析蛋白质组了解基因的功能，抗体是掌控这一任务的中心。90000多个人类基因产物的分析需要产生抗体的高通量方法。以动物为基础的方法不具备这样的能力，而利用重组抗体的体外选择系统则不同。噬菌体抗体库这种最常用的现代系统已经给出了几百个用于治疗、研究和诊断的抗体。利用从不同基因来源构建的不同形式的抗体库，人们已经发展了许多克隆和突变策略来创建、保存和开发最大的抗体多样性。对噬菌体抗体库涵盖面的评估指出，目前用于产生人类治疗性抗体的方法并不能很容易地用于后基因组研究。必须通过抗原和文库与抗体形式的紧密整合、筛选过程和自动化操作来能确定一种改进的操作过程。只有通过优化生物学、生物信息学和技术三者的相互作用才能创建一个高通量的过程，以满足蛋白质组分析和微阵列技术的要求。

128 Comparison of network-based pathway analysis methods

Trends in Biotechnology, Volume 22, Issue 8, 1 August 2004, Pages 400-405

Jason A. Papin, Joerg Stelling, Nathan D. Price, Steffen Klamt, Stefan Schuster and Bernhard O. Palsson

以网络为基础的途径分析方法的比较

最近几年中出现了以网络为基础定义的生化途径。这些途径的定义强调对整个生化反应网络的平衡使用。基本模式和极端途径这样两个相关的定义，已经产生了新的有关生化网络功能的假设。通过比较和对比以前发表的人红细胞和人幽门螺旋菌代谢重建的基本模式和极端途径，可以说明这两个方法间的关系。在实际的代谢网络分析中，使用这两种方法得到网络特性的描述需要谨慎地加以解释。

134 Biomarker discovery and validation: technologies and integrative approaches

Trends in Biotechnology, Volume 22, Issue 8, 1 August 2004, Pages 411-416

Sergey E. Ilyin, Stanley M. Belkowski and Carlos R. Plata-Salamán

生物标志的发现和确证：技术和整合方法

生物学标志是一个新兴的领域，现已被用于疾病进展的诊断、病程阶段划分、预后和监测，以及对治疗干预和个性化治疗的进展所表现出临床反应的监测，以降低临床试验的损耗。而且，生物标志对卫生经济学也有积极的影响。“生物标志”这个词已经广泛地用于治疗领域和其他方面，对其性质的考虑应该联系临床、生理学、生物化学、发育学、形态学和分子测量等方面。在药物实验中，人们已建议将生物标志用于效果的测定和病人群体的分类、药代动力学和药效动力学关系的推断以及安全性的监测等方面。数据收集和分析的不同技术的连接和整合是生物标志物的鉴定、描述、确证和应用的关键。“整合功能信息学”代表了这一技术整合中的一个新方向。

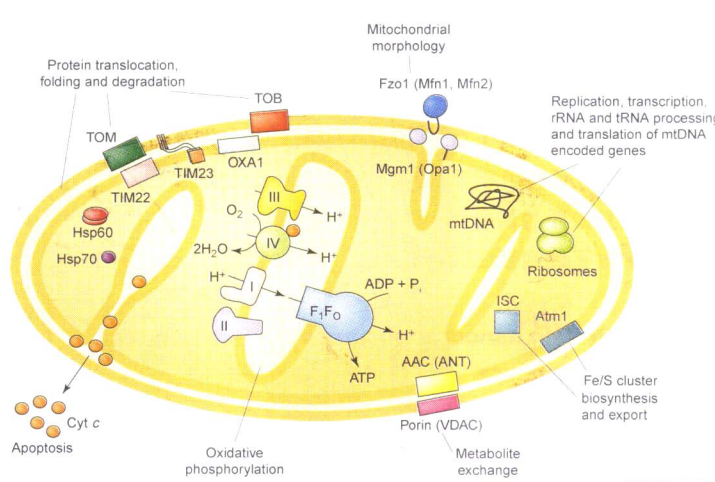
140 Mitochondriomics or what makes us breathe

Trends in Genetics, Volume 20, Issue 11, November 2004, Pages 555-562

Andreas S. Reichert and Walter Neupert

线粒体组学，是什么使得我们呼吸

在高等真核生物中，线粒体执行了一些基本的细胞过程，包括氧化磷酸化、铁硫簇的形成和凋亡等。这个细胞器的功能失常和人类的许多疾病有关。为更好地了解线粒体的功能，多种最新的蛋白质组学、遗传学、转录物组学和生物信息学方法已经开始用于测定酵母、植物和哺乳动物线粒体中的全套蛋白质。这里，我们综述了这些研究，并讨论了各种策略的进展和局限性。不同策略的整合使用被证明是具有高灵敏度和特异性的鉴定线粒体蛋白质组的成功而有用的方法。人们现在已得到了酿酒酵母最为完整的数据，在其线粒体中鉴定出了约700种不同的蛋白质。



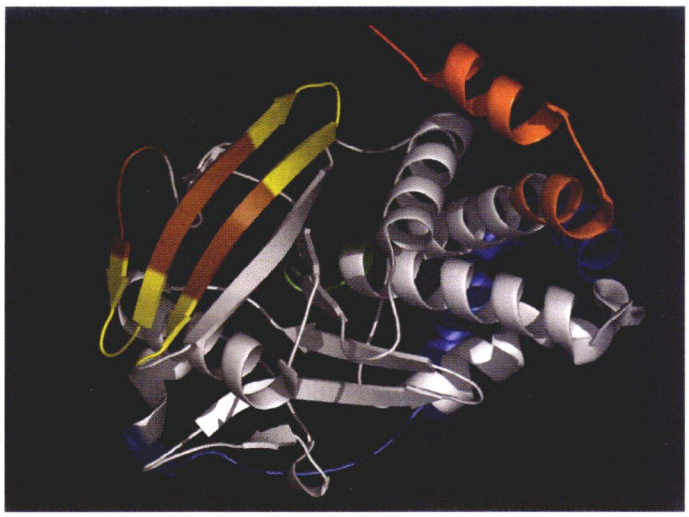
148 Do transposable elements really contribute to proteomes?

Trends in Genetics, Volume 22, Issue 5, May 2006, Pages 260-267

Valer Gotea and Wojciech Makałowski

转座子真的对蛋白质组有贡献吗?

最近的研究表明：起初把转座子（transposable elements, TEs）认为是DNA的一些无用的、自私的或者垃圾部分是不准确的，转座子有着复杂的调控功能，对许多基因的编码区域也有着很大的贡献。因为转座子的贡献在之前只是在转录水平上被记录，所以我们找寻了一些能够支撑转座子盒（TE cassettes）的翻译的证据。我们研究发现，尽管那些包含转座子编码片断的蛋白质的比例（大概0.1%）可能被低估了一些，但还是远远小于在转录水平上的与转座子相关的蛋白质的比例（大概4%）。所有情况下转座子盒都起源于古老的转座子，这与把转座子片断整合进入功能蛋白质需要很长的进化周期的想法是一致的。因此我们指出：功能蛋白质中不大可能包含新近生成转座子的转座子盒，后者的功能主要局限在调控上面。



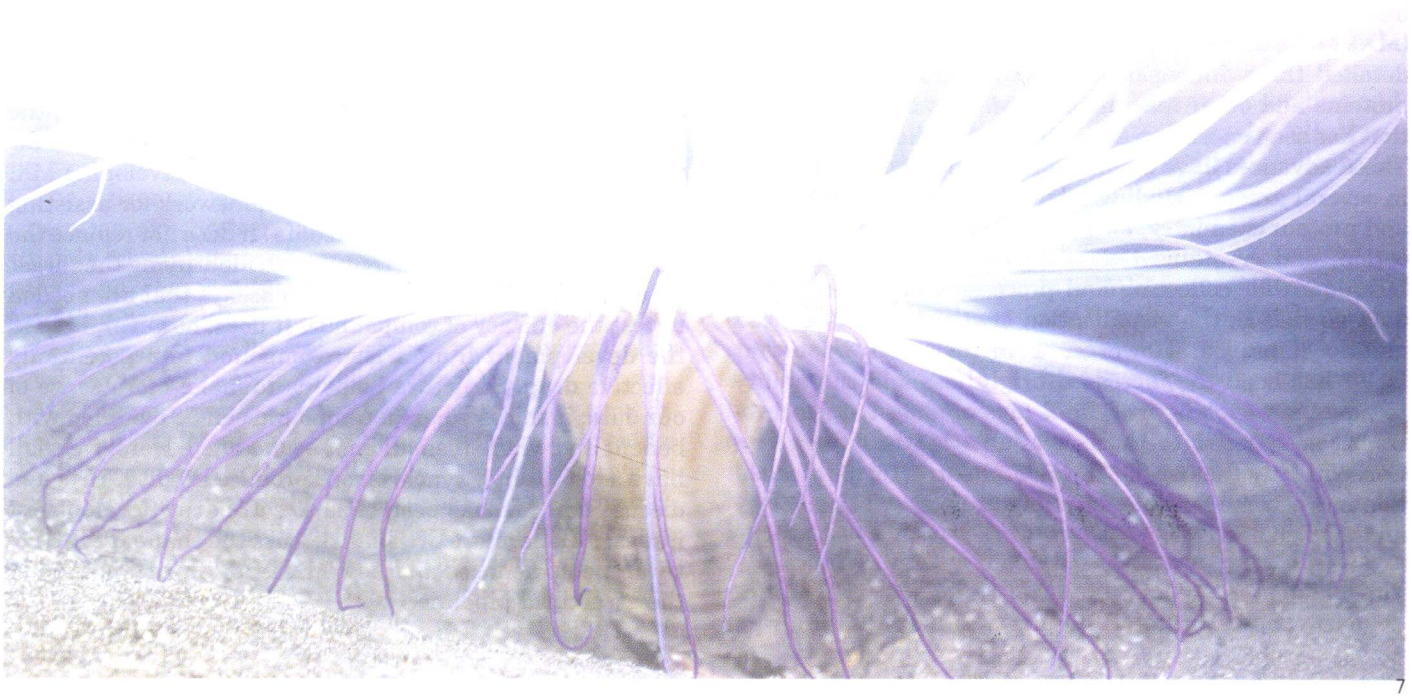
156 Single cell proteomics for personalised medicine

Trends in Molecular Medicine, Volume 10, Issue 12, December 2004, Pages 574-577

Sander H. Diks and Maikel P. Peppelenbosch

单细胞蛋白质组学在个体化医疗中的应用

最近出现的多色流式细胞仪与磷酸基特异抗体结合的方法，可用于测定单个细胞的信号传导中间体的相对磷酸化水平。现已很清楚，在细胞因子的刺激下，个别白细胞在磷蛋白形式上表现出了明显的差异，这些形式与疾病相关。因此，在疾病的分子诊断上，单细胞磷酸化蛋白质组学技术可能较其它蛋白质组学方法有优势，也许将有助于个体化医疗的实现。



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New light for science: synchrotron radiation in structural medicine

Thomas L-M. Sorensen, Katherine E. McAuley, Ralf Flaig and Elizabeth M.H. Duke

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Macromolecular crystallography (MX) is a powerful method for obtaining detailed three-dimensional structural information about macromolecules. MX using synchrotron X-rays has contributed, significantly, to both fundamental and applied research, including the structure-based design of drugs to combat important diseases. New third-generation synchrotrons offer substantial improvements in terms of quality and brightness of the X-ray beams they produce. Important classes of macromolecules, such as membrane proteins (including many receptors) and macromolecular complexes, are difficult to obtain in quantity and to crystallise, which has hampered analysis by MX. Intensely bright X-rays from the latest synchrotrons will enable the use of extremely small crystals, and should usher in a period of rapid progress in resolving these previously refractory structures.

Introduction

Imagine searching for clues in a darkened building with a beam of torchlight, and then finding a light switch. The entire room is suddenly illuminated, and that which was previously hidden is visible. Light itself is a potent tool in many areas of research. Our visual sense is most highly developed, and seeing helps in discovery and understanding. This article focuses on a particularly bright and pure form of light, the light produced by a particle accelerator known as a synchrotron.

One of the main applications of synchrotron light in biology is in the field of macromolecular crystallography (MX). MX is one of the most powerful methods for obtaining detailed three-dimensional structural information about proteins and other macromolecules. It is a research field that has seen enormous advances during the past decades. These advances have stemmed from improved techniques in several areas, including advances in molecular biology and protein chemistry, for the cloning, expression, purification, and crystallisation of proteins. In addition, the techniques for collecting and processing crystallographic data as well as the visualisation of structures have been greatly improved. However, a further and important factor has been the increased brightness and the ability to tune the wavelength of the X-rays produced at synchrotrons, as opposed to those available at the laboratory. Consequently the use of MX to obtain structural information has become feasible for many projects within biology and medicine.

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MX comes with both a basic and applied 'flavour'. Within the past decade, MX has improved our basic understanding of many aspects of biology: examples include how cations are moved across biomembranes through channels and pumps [1,2], and how the ribosome deciphers the genetic code and translates it into polypeptide chains [3–6]. At the same time, MX has had considerable impact on many drug discovery programs and has had an important role in the delivery of marketed drugs against at least seven enzyme targets. MX has also been a key technology in the development of drugs using a much larger number of targets, some of which have entered clinical trials. A recent review [7] identified >60 compounds that are in the clinic and curing patients where structural information about the protein target is available. This highlights the opportunity of using MX to provide valuable information about drugs and their targets. Furthermore, some 25 of these are in the top 200 by drug sales. How important structural information has been for the drug development process is hard to assess from the outside. In a pharmaceutical company, MX is a complementary approach, contributing in concert with more traditional drug discovery. The development of HIV protease inhibitors in the 1990s is recognised as a classic example where the drug design was based on a structural understanding of the active site of the protease. In several cases, such as the anti-cancer drug Gleevec (imatinib) [8], the structure of the target molecule did not become available until late in the process, and instead of driving the design of the original drug, the information was used to address how to improve the drug.

Structure determination is not always straightforward, and structural biology still faces challenges; however, the insights we have gained from the efforts invested so far fully justify putting further resources into developing MX. Structural information provides a framework for assisting the interpretation of functional data. It does not replace the functional investigations that lead to identifying the target of interest nor the rapid functional assays that are needed to test the efficacy of compounds. It does, however, help in the development from a lead compound to a potentially effective drug. Structure helps us to make more sense of our data; it shows where a compound can be modified with benefit and where it should not be modified. Here, we shall have a look at how synchrotrons produce bright X-rays and discuss how MX has been used to solve drug development issues. Furthermore, we shall discuss the challenges structural medicine faces and how MX is an important step in the structural pipeline.

Synchrotron light

When electrons experience acceleration, they emit radiation with a range of wavelengths. In a synchrotron, electrons travelling at speeds that approach the speed of light are made to travel in near-circular paths by using powerful magnets, which causes them to emit so-called synchrotron radiation. The earliest synchrotrons were used as sources of high-energy particles for use in sub-atomic physics research, and the radiation they generated was regarded as an unwelcome by-product, representing an unavoidable loss of energy from the particles. These original synchrotron sources were termed first generation. However, when the nature of the radiation became better understood [9,10], its potential for application in its own right began to be appreciated. The first experiments were performed with a muscle sample [11] and immediately showed how exposure times could be dramatically shortened compared with the existing experimental technique using a conventional X-ray source. The next stage in the evolution of the synchrotron was the provision of sources dedicated solely to, and therefore optimised for, the production of synchrotron radiation. These were termed second-generation sources. These second-generation sources have now evolved into third-generation sources, where the fundamental difference is the types of magnets used to generate the synchrotron radiation. In a third-generation source, special additional magnets, termed insertion devices, are inserted into the existing near-circular electron-beam path to produce radiation with properties specific to the scientific questions being probed.

There are now in excess of 70 synchrotrons around the world, many of which are wholly or largely dedicated to the generation of synchrotron radiation for use in areas including materials science, semiconductor research, geology, inorganic crystallography, small-molecule crystallography and, increasingly, structural biology. Synchrotron technology has evolved over time, and synchrotrons differ in size and design depending on their purpose. New synchrotrons are often designed to provide optimum performance around a wavelength of 1 Å, making them ideal for meeting the requirements of structural biology. A parameter that is often used to rank the performance of new synchrotron facilities is the emittance of the machine: emittance is the product of the electron-beam source size and the solid angle of the emitted radiation. In general terms, the lower the emittance, the better the source.

The Diamond Light Source (Figure 1) is a third-generation synchrotron, located near Oxford in the UK, which is scheduled to become operational in early 2007. It belongs to the family of medium-energy synchrotrons operating around 3 GeV and has been designed to have as low an emittance for a synchrotron at this energy as is possible with existing technology (Box 1).

Most large particle-accelerators are funded by governments, but the Diamond Light Source is unusual in that a significant fraction of the funding (14%) has been provided by the Wellcome Trust, the largest medical research charity in the UK, highlighting the importance of synchrotron radiation for medical research. The Diamond synchrotron will become a hub for those in the research community who promote complementary techniques (e.g. in imaging and use of neutrons), where they can also take advantage of the co-localisation with other central experimental facilities such as the ISIS neutron source.

Figure 1. Diamond Light Source – the new UK synchrotron.

X-ray macromolecular crystallography

In a structural biology experiment, a crystal of the target molecular species is probed using a narrow beam of X-rays and the resulting diffraction pattern captured. Patterns from different incident beam angles, generated by rotating the crystal in the X-ray beam, can be combined and analysed to construct 3D electron-density maps. When combined with other information, such as the amino acid sequence of a protein, the 3D structure of the molecule can be determined. The short wavelength of X-rays (of the order of 1 Å) permits atomic-level resolution, if the sample quality permits it, and sophisticated software is now available to accelerate all steps of structure determination, from the analysis of diffraction pattern data, structure solution and refinement, to model building.

Structure-based drug design

Detailed knowledge of the structure of enzymes and other proteins has a crucial role in the design and discovery of drugs for treatment of important diseases. There are numerous examples in which structural biology has been important, including treatments for illnesses such as HIV/AIDS [12,13] and influenza [14] (Table 1). Here we discuss two examples of the development of an anticancer drug and steps towards a drug for tuberculosis. In all these examples, synchrotron radiation has a central and ongoing role.

Chronic myeloid leukaemia

Chronic myeloid leukaemia (CML) is a relatively rare cancer that is usually associated with a reciprocal chromosome translocation involving the long arms of chromosomes 9 and 22 [15]. This translocation produces a fusion of two genes, *bcr* (breakpoint cluster region) and *abl* (Abelson leukaemia virus). The *abl* gene encodes for an intracellular, non-receptor tyrosine kinase, c-Abl, which is closely related to the Src family of tyrosine kinases. In keeping with other tyrosine kinases, the activity of c-Abl is normally tightly regulated, but the Bcr–Abl fusion protein lacks a small N-terminal region that is important for autoinhibition, resulting in constitutive activation of

Box 1. Diamond machine operation and MX beamline features

- Within the linac, a stream of low-energy electrons is produced from an electron gun by thermionic emission from a heated cathode. The electrons are accelerated to an energy of ~100 MeV by a linear accelerator.
- A 'booster synchrotron' then receives the electrons and propels them around the 160 m circumference booster. The electrons are further accelerated by radio frequency energy sources.
- The electrons are then injected into a storage ring some 560 m in circumference, consisting of 24 straight sections alternating with 24 'bending' magnets, which deflect the electron beam around the bends.
- The storage ring accumulates electrons until the desired operating current is achieved. The electron beam in the storage ring is only a few tens of one μm in diameter. The electrons are traveling at 99.9% of the speed of light.

The main components are shown in Figure I in this box.

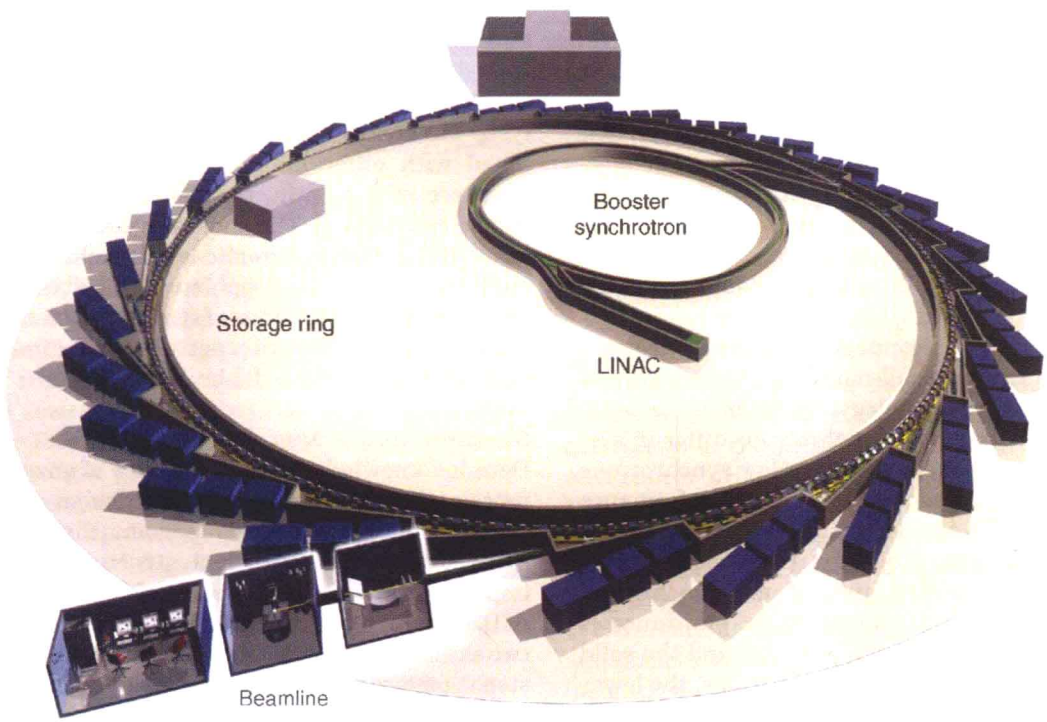
Where the electron beam in the storage ring is curved, either by the bending magnets or by special insertion device magnets, synchrotron radiation is emitted in a narrow cone in the forward direction but at a tangent to the path of the electron beam. This radiation is drawn off in a series of tangential 'beamlines' located around the storage ring. The spectrum of radiation produced is influenced by the magnetic-field strength used; therefore, insertion devices can be tuned to produce synchrotron radiation of different types. Each beamline represents a source of radiation dedicated to a particular technique or techniques.

Ultimately, Diamond will host up to 40 beamlines. The first phase of construction will include three beamlines dedicated to MX, which will become available to users in early 2007. These beamlines will have the following features:

- they will be tuneable over the wavelength range 0.5–2.5 Å;
- they will contain fluorescence detectors to enable accurate wavelength setting for MAD;
- they will include robotic sample changing and crystal centring systems, and automated data collection;
- they will provide an on-axis optical viewing system;
- samples can be cryo-cooled to 100 K.

One beamline will be equipped with biological containment to category 3 level for pathogenic samples.

Laboratory facilities and accommodation for users will be available at the Diamond complex, and an adjacent research centre will be set up to act as a focus and centre of excellence for synchrotron-related research, including aspects of sample preparation.



TRENDS in Biotechnology

Figure I. The key elements of a synchrotron.

the Abl kinase. Bcr–Abl can transform haematopoietic cells, leading to increased proliferation and a reduced dependence on growth factors, producing a population of cells with a modest survival and proliferative advantage compared with normal cells [16]. The resulting gradual expansion of the number of granulocyte and/or macrophage progenitor cells characterises the chronic phase of CML. Bcr–Abl-transformed cells also show an enhanced mutation rate, and accumulation of further mutations is thought to be responsible for the eventual disease progression to a terminal blast crisis, characterised by the accumulation of either myeloid or lymphoid blast cells [17,18].

Understanding the central role of enhanced Bcr–Abl kinase activity led to a search for inhibitors of the enzyme, culminating in the development of a highly specific inhibitor, imatinib (Gleevec; Novartis), which is now approved for first-line treatment of CML in the USA and Europe [8]. Virtually all patients treated with imatinib during the chronic phase of CML undergo complete and lasting remission [19], and the drug is generally well tolerated. However, if treatment is initiated during

Table 1. Examples of marketed drugs for which structural biology has provided information about the target protein

Commercial drug	Active compound	Supplier	Disease target	Protein target	PDB entry
Gleevec	Imatinib	Novartis	Chronic myeloid leukaemia, Gastrointestinal stroma tumours	Abl tyrosine kinase C-Kit Platelet-derived growth factor tyrosine kinase (PDGF TK)	1XBB
Herceptin	Trastuzumab	Genentech	Breast cancer	HER2 receptor	1N8Z
Lipitor	Atorvastatin	Pfizer	High cholesterol levels	HMG–CoA (3-hydroxy-3-methylglutaryl–coenzyme A) reductase	1HWK
Avandia	Rosiglitazone	GlaxoSmithKline	Type 2 diabetes mellitus	Peroxisome proliferator-activated receptor-gamma (PPARγ)	2PRG
Actonel	Risedronate	Procter and Gamble Pharmaceuticals	Osteoporosis	Farnesyl diphosphate synthase	1YV5
Evista	Raloxifene	Eli Lilly	Osteoporosis	Oestrogen receptor	1ERR
Casodex	Bicalutamide	AstraZeneca	Prostate cancer	Androgen receptor	1E3G
Norvir	Ritonavir	Abbott	HIV	HIV protease	1HXW
Ciprobay	Azithromycin	Pfizer	Bacterial infections	Type-I inosine monophosphate dehydrogenase	1AJ6
Relenza	Zanamivir	GlaxoSmithKline	Influenza virus	Influenza neuraminidase	1A4G

the blast crisis stage, the response rate is lower and almost all patients relapse [20,21]. Most relapsed patients have mutations in the Bcr–Abl kinase that reduce imatinib binding and confer varying degrees of resistance to the drug [22–24]. Understanding the mechanisms of resistance and the development of further drugs or treatment strategies to reduce the potential for resistance are now urgent priorities.

X-ray crystallographic studies have elucidated the structure of Bcr–Abl kinase (**Figure 2**) and provided some clues to its inhibition by imatinib and the effect of many mutations [25]. As with most kinases, normal Abl tyrosine kinase exists in either an 'open', active conformation or a 'closed', inactive (autoinhibited) state, in which an activation loop occludes the kinase domain. Imatinib binds to the ATP-binding site of Abl only in the inactive state [26–28], which contributes to the high specificity of the drug but might limit its effectiveness in at least two ways. First, it might limit the binding of the drug because, in CML, the fusion protein Bcr–Abl is predominantly in the active conformation. Second, it means that mutations that affect intramolecular regulatory interactions can reduce drug binding, as can mutations that affect the actual contact points between the kinase domain and the drug [27], thereby increasing the potential for resistance. Further structural studies have shown that other kinase inhibitors bind to Bcr–Abl in the active conformation (e.g. PD-173955 [29] and VX-680 [30]) or in either the active or inactive conformations (e.g. dasatinib; BMS-354825 [31,32]). It would be expected that these and other inhibitors with less stringent conformational requirements for binding could retain activity in the face of many of the mutations that confer resistance to imatinib [31,33], although with reduced specificity [34]. Protein kinases are involved in many forms of cancer and other diseases, and it is probable that the structural principles established from the molecular study of this relatively uncommon disease will inform the development of treatments in other areas [35].

Tuberculosis

Tuberculosis (TB) is a major health problem in many parts of the world. An epidemiological snapshot in 1997 indicated that 1.9 million people died of TB in that year and nearly one in three people worldwide was infected with the causative bacterium *Mycobacterium tuberculosis* [36]. The global burden of TB is increasing [37], partly because of the development of multiple drug resistance in *M. tuberculosis* and partly related to the current HIV pandemic [38]. An important feature of *M. tuberculosis* infection is that the bacteria can enter a persistent slow-growing or non-growing state, during which many current anti-TB drugs are ineffective. Enzymes involved in this persistent state are, therefore, important targets for new drug development. Malate synthase is an enzyme in the glyoxylate shunt pathway that is thought to be essential for the survival of *M. tuberculosis* in the persistent state [39]. This pathway is absent in mammals, making it a particularly attractive target for blockade. The 3D structure of malate synthase (to 2.1 Å resolution when the enzyme is complexed with its substrate glyoxalate and to 2.7 Å when complexed with its products, malate and coenzyme A) has been obtained by X-ray crystallography using synchrotron radiation [40,41]. Comparison of the active sites in the substrate- and product-bound forms has clarified the catalytic mechanism of the enzyme, and malate synthase is now regarded as a promising target for structure-based drug design. In obtaining the 3D structures, the 'tuneability' of synchrotron-generated X-ray radiation was exploited using the technique of multiple wavelength anomalous dispersion (MAD) [42]. Datasets were obtained from the same crystal at four different X-ray wavelengths, and differences in the scattering pattern between wavelengths provided enough information to solve the structure.

The cell wall of *M. tuberculosis* contains long-chain fatty acids, known as mycolic acids, that are also thought to be important in the persistence of infection. Some classes of mycolic acids in *M. tuberculosis* are modified by the incorporation of cyclopropane rings at specific sites along the long hydrophobic chain. The 3D structures of three mycolic acid cyclopropane synthase enzymes, each specific for cyclopropanation at different sites or in different configurations on mycolic acids, have been determined using synchrotron radiation and the MAD technique [43]. It emerges that the active sites of these

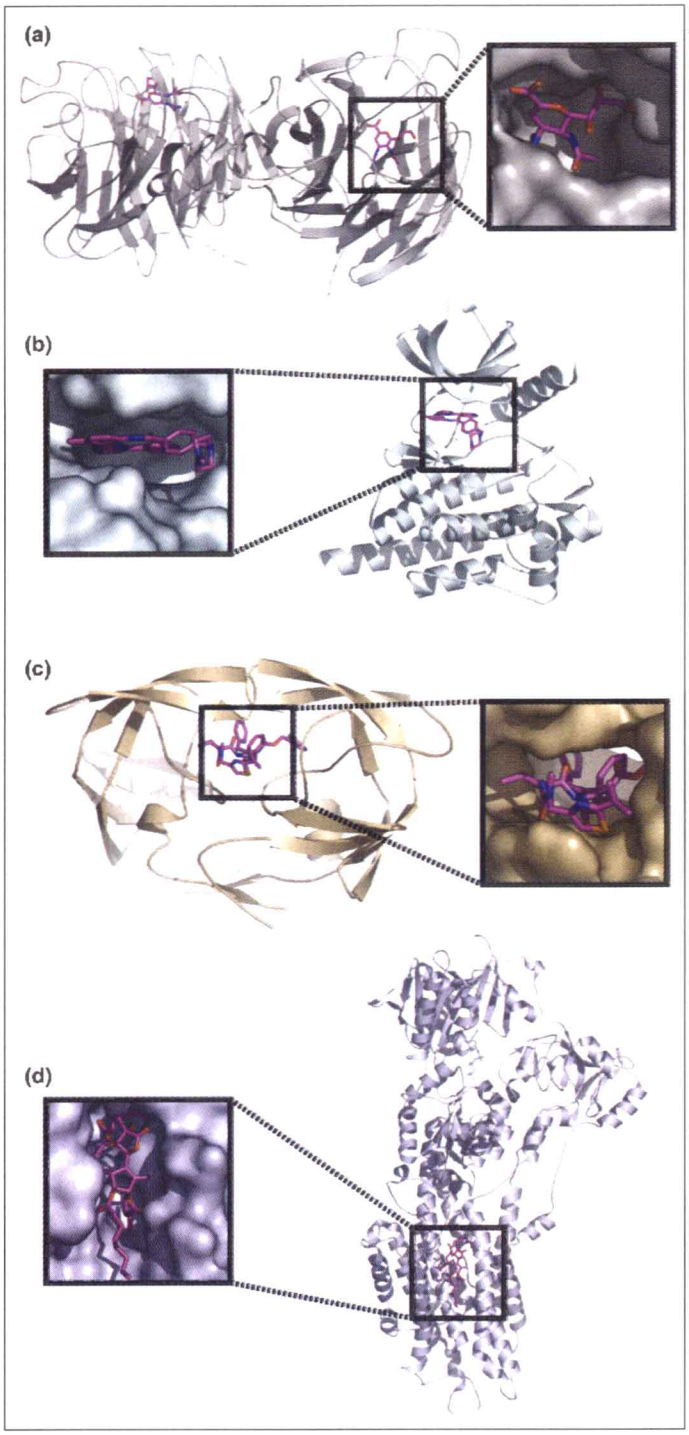


Figure 2. Examples of high-resolution structures of enzymes and inhibitors. **(a)** Influenza virus neuraminidase with the anti-flu drug Relenza (zanamivir) [66], PDB: 1A4G. **(b)** Spleen tyrosine kinase with the anti-cancer drug Gleevec (imatinib) [67] PDB: 1XBB. **(c)** Human immunodeficiency virus protease with the protease inhibitor Norvir (ritonavir) [68] PDB: 1HXW. **(d)** Ca^{2+}–ATPase with the inhibitor BOC-12ADT [69] PDB: 2BY4.

three enzymes are similar, raising the possibility that a single inhibitor might be found to be effective against all three, which should reduce the potential for the development of drug resistance. Furthermore, cyclopropanated mycolic acids are not known in mammals, making this pathway a potentially fruitful target for the development of new antibiotics [41,44].

The future for synchrotron radiation in structural biology and medicine

The above examples illustrate how basic structural biological research using synchrotron radiation is informing and driving, in a direct and immediate way, the search for new and refined treatments that will overcome some of the limitations of current drugs for important diseases. The third-generation synchrotron light sources that are now available or are under construction in several countries around the world (**Figure 3**) will accelerate and extend this process in several ways.

Greater access

Rapid progress in molecular biology, including large-scale projects such as the human genome project, has resulted in the availability of the amino acid sequences (primary structures) for a large and increasing number of proteins. 3D structures are currently available for only a fraction of these and represent a necessary further step in exploiting sequence information. Increased access to synchrotron light is, therefore, important. The building of additional synchrotron sources is one means to achieve this; another is to increase the productivity of each synchrotron beamline. Techniques for sample preparation and crystallographic data collection are continually being refined to optimise the use of available beamline time [45,46]. Another important area is the automation of the sample mounting, the alignment and the dismounting processes by using robotics. This will speed up the collection of diffraction datasets [47–49]. Synchrotrons are large and expensive instruments and researchers using them often have to travel long distances; however, advances in automation, networking and computing have made remote use a realistic alternative. It is increasingly possible for researchers to send crystal samples in by mail, screen large numbers of crystals, and collect diffraction data from the best of them without physically attending the synchrotron facility.

Greater brightness and quality

The new third-generation synchrotron sources produce radiation that is more intense and collimated than older sources. For example, the bending magnets of the new Diamond Light Source produce an increase of greater than three orders of magnitude in photon brightness (a measure of the number of photons per second in a given solid angle) compared with those of the current second-generation source in the UK, the Synchrotron Radiation Source at the Daresbury Laboratory, which is 25 years old. The availability of high-quality, intense radiation enables high-resolution crystallographic data to be collected quickly. This not only increases the throughput of samples but also enables time-resolved crystallography, using brief pulses of X-rays, to investigate processes such as ligand binding. For example, structural changes in the myoglobin molecule during the dissociation of bound carbon monoxide have been determined with a temporal resolution of ~150 picoseconds [50,51].

An additional and perhaps more important benefit of bright synchrotron light is that it enables crystallographic data to be collected from extremely small crystals: