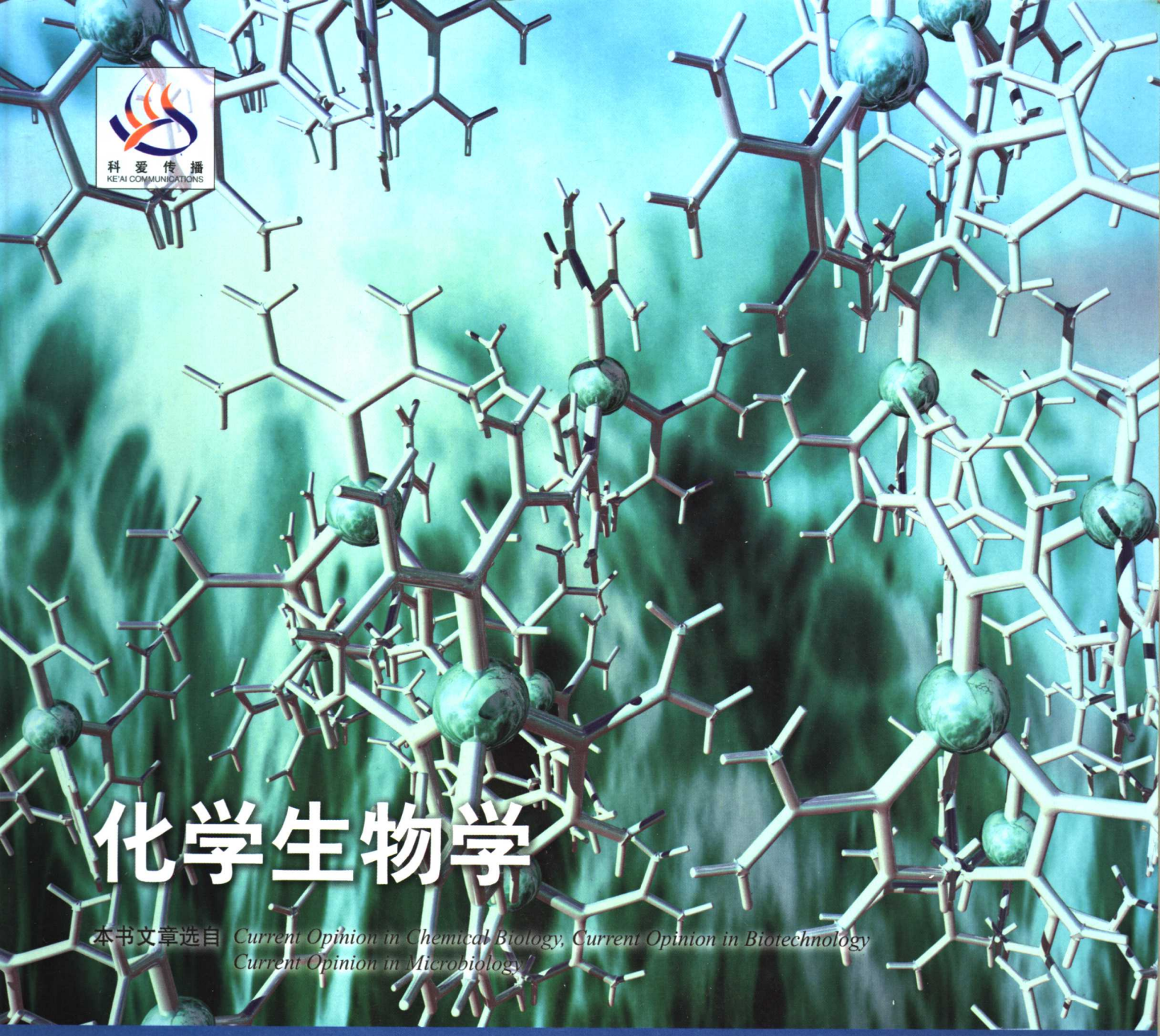

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KE'AI COMMUNICATIONS

化学生物学

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NEW FOCUS in Life Sciences

生命科学新视野 7

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5 Enzymatic tools for engineering natural product glycosylation

Current Opinion in Chemical Biology 2006, 10:263-271

Sophie Blanchard and Jon S Thorson

天然产物糖基化的工具酶

糖基化的天然产物为许多现有一线药物的进一步开发提供了可靠的发展平台。为了探索糖基在这些化合物中的作用，多个研究小组将注意力集中在发展化学和酶学手段上，以使天然产物糖基化具有多样性。在可利用的各种途径中，体内通路工程，也称作"组合生物合成"，正成为天然产物糖基化的新兴方法，该方法基于糖类生物合成的基因盒以及糖基转化酶在体内的共表达而实现天然产物糖基化。这篇综述对组合生物合成进行了最新总结，着重阐述了核苷－糖生物合成途径以及糖基转移酶的研究近况。

14 Nucleic acid aptamers and enzymes as sensors

Current Opinion in Chemical Biology 2006, 10:272-281

Naveen K Navani and Yingfu Li

作为传感器的核酸适配子和酶

核酸的功能是一个无穷无尽的产生新发现和发明的宝库，极大地促进了我们对 DNA 及 RNA 多面性的认识。众所周知，核酸可以作为酶（脱氧核酶和核酶），亦可作为受体（适配子），并且这些功能性核酸（FNAs）既可从自然界获得也可从随机核酸库中分离得到。天然或人工的功能性核酸的可利用性为这些"智能"分子在化学、生物领域中的应用开辟了一片广阔天地。这篇综述简要总结了近期生物分子检测、药物研发和纳米科技等领域中应用功能性核酸作为新型传感器的进展。

24 Catalytic antibodies and their applications

Current Opinion in Biotechnology 2005, 16:631-636

Carl Veith Hanson, Yasuhiro Nishiyama and Sudhir Paul

催化抗体及其应用

催化抗体（CAbs）在自然情况下存在于健康个体之中并可以作为其天然免疫系统的一部分，但是它们却更多地出现在患有自体免疫疾病的个体中。催化抗体也能通过人工工程或免疫系统诱导产生。它们的作用机制包括亲核催化、构象张力诱导、与金属离子配位以及稳定过渡态等。具有临床意义的催化抗体近期主要应用在：将可卡因转化为无精神兴奋作用的形式、降解尼古丁、靶向化学疗法中前药的活化、紫外线辐射的防护、HIV传染性的抑制以及破坏老年性痴呆症(Alzheimer's disease，AD)中 β-淀粉质的聚集。人工催化抗体将可能越来越多地应用于科研、临床医学、诊断和药物生产等领域。

30 The evolution of DNA polymerases with novel activities

Current Opinion in Biotechnology 2005, 16:370-377

Allison A Henry and Floyd E Romesberg

新活性 DNA 聚合酶的人工进化

DNA和RNA聚合酶在自然界中进化，并在特定环境下对特定底物发挥功能。因此，尽管这些酶的商品化已经使生物技术产业发生了革命性的进步，但其应用依然有限。具有非天然功能的聚合酶将有着更为巨大的应用前景。为实现这一目标，已发展出一些基于酶活性的筛选和精选技术。利用这些技术，一些合成各种不同聚合物的聚合酶被开发出来，包括将2'-O-甲基修饰的核苷或非天然碱基对掺入到核酸链之中。这些结果预示着在不久的将来，人工设计裁剪的聚合酶将能产生，并实现其各种特定用途。

38 Understanding enzyme action on immobilised substrates

Current Opinion in Biotechnology 2005, 16:385-392

Peter J Halling, Rein V Ulijn and Sabine L Flitsch

酶对固定化底物的作用

随着人们对自动化合成与筛选方案的兴趣越来越浓厚，固相载体化学和生物化学成为了有吸引力的技术。对固定于表面的底物进行研究已用以分析酶的可接近性、动力学及热力学性质。为监测酶对固定在聚合物玻璃珠或金表面等固相物上的底物的作用，人们发展了一些新颖的方法，包括荧光测定、基质辅助激光解吸离子化飞行时间质谱(matrix-assisted laser desorption/ionization time of flight，MALDI-TOF）以及采用石英晶体微量天平检测直接固定于固相表面的底物分子的重量变化。此外还有人报道了在单个载体珠表面实现空间分辨的方法。这些方法更好的表征了酶进入玻璃珠内部的能力，并且促进了提高酶可接近性的新型载体材料的发展。相较于溶液中的反应，固相表面反应的平衡点移动较大，这点可以利用水解酶来开发应用。同时，固体物的反应动力学的修饰利用也引起了重视。

46 Mining genomes and 'metagenomes' for novel catalysts

Current Opinion in Biotechnology 2005, 16:588-593

Manuel Ferrer, Francisco Martnez-Abarca and Peter N Golyshin

从基因组和"宏基因组"中寻找新型催化剂

基因组学和宏基因组学领域的研究进展极大的改变了我们对微生物生物多样性及其在生物技术上应用前景的认识。鉴于环境中大约99%以上的微生物的发酵培养极难进行，人们对它们的基因组、基因及其编码酶的活性知之甚少。对环境 DNA（或称为宏基因组）的分离、存档和分析能帮助我们发掘微生物的多样性、获取它们的基因组、确定蛋白质编码序列甚至重建其生物化学途径、洞察这些生物体的性质和功能。建立和分析（宏）基因组文库将是一个收获和存档环境遗传资源的有力途径，使我们能够鉴别生物物种的存在、以及这些物种的作用，并且能告诉我们人类如何从其遗传信息中获益。

52 Ultra-high-throughput screening based on cell-surface display and fluorescence-activated cell sorting for the identification of novel biocatalysts

Current Opinion in Biotechnology 2004, 15:323-329

Stefan Becker, Hans-Ulrich Schmoldt, Thorsten Michael Adams, Susanne Wilhelm and Harald Kolmar

基于细胞表面展示及荧光活化细胞分类的超高通量筛选，寻找新型生物催化剂

微生物细胞表面或微珠表面的酶库可用荧光底物进行筛选，因为这些底物可以将反应产物与对应的酶连接起来。种类超过109的酶库可用流式细胞仪以30000细胞/秒的速度进行定量分析和筛选。基于荧光活化细胞分类筛选技术来观察酶的定向进化将很有前途，并且已经在近期取得进展。

59 Improved β-lactam acylases and their use as industrial biocatalysts

Current Opinion in Biotechnology 2004, 15:349-355

Charles F Sio and Wim J Quax

改良的β－内酰胺酰基转移酶及其作为工业生物催化剂的应用

尽管β-内酰胺酰基转移酶在传统上用于青霉素G和头孢菌素C的水解过程，新的突变的酰基转移酶已能用于对所选择的发酵产物进行水解以及参与半合成的β-内酰胺抗生素的合成。三维结构分析和定点突变研究增进了对这些酶催化机制的认识。工艺设计极大地提高了水解及合成的产率，这些工艺设计包括酶固相化及新型反应介质的应用。利用β-内酰胺酰基转移酶进行立体选择性酰基化或水解，达到外消旋混合物的拆分也已经取得重大进展。

66 Enzyme catalysed deracemisation and dynamic kinetic resolution reactions

Current Opinion in Chemical Biology 2004, 8:114-119

Nicholas J Turner

酶催化的去消旋化和动态动力学拆分

外消旋手性混合物的动态动力学拆分或手性拆分领域中，已经发展出新的催化剂和反应条件。一些特殊的官能团，包括手性二级醇、α-氨基酸、胺基和羧基，都能够很好的适用于这种拆分方法。这一过程中普遍采用的方法是将具有立体选择性的酶与特定的化学试剂结合使用。化学试剂的作用是将拆分反应过程中不参加酶反应的手性异构体外消旋化，或者使它生成能够实现外消旋化的中间体。在某些例子中，另一种酶（消旋酶）也引入参与反应底物之间对映体的互变，用于辅助立体选择性酶实现双酶的动态立体选择性拆分。

72 Peroxide-utilizing biocatalysts: structural and functional diversity of heme-containing enzymes

Current Opinion in Chemical Biology 2004, 8:127-132

Isamu Matsunaga and Yoshitsugu Shiro

利用过氧化物的生物催化剂：含有亚铁血红素的酶的结构与功能的多样性

含亚铁血红素的酶（如过氧化物酶、过氧化氢酶和过氧加氧酶P450等）在它们特定的反应中都要利用过氧化物。这些酶催化的各类反应中存在着共同的高反应活性的中间体——氧铁卟啉π-阳离子自由基（oxo-ferryl porphyrin π-cation radical），这个中间体由过氧化物同亚铁血红素的三价铁反应生成。亚铁血红素酶催化的反应主要是由底物接近反应活性位点的难易性决定的。此文描绘了含亚铁血红素酶，特别是亚铁血红素的末端分枝结构，通过共享通用的活性中间体对它们功能进行精确调控的机制。同时，根据过氧化物酶的分子机制和结构数据，作者提出通过修饰血红素蛋白的末端分枝元素，能实现此类酶的功能转换。

78 Hydrogenases: active site puzzles and progress

Current Opinion in Chemical Biology 2004, 8:133-140

Fraser A Armstrong

氢化酶：活性位点之谜与进展

关于氢化酶反应，目前的研究已经不仅仅是X-射线衍射晶体结构。利用先进的电子顺磁共振技术（EPR）和理论方法，"瞬态"氢原子在潜在的催化活性中间体的定位等主要难题正在逐步得到解决。该文对氢化酶活性位点不同状态的结构、活化机制以及氢循环的最新进展进行了讨论，并试图给出一个逐步统一的机制。

86 Enantioselective biocatalysis optimized by directed evolution

Current Opinion in Biotechnology 2004, 15:305-313

Karl-Erich Jaeger and Thorsren Eggert

通过定向进化优化对映选择性生物催化剂

定向进化方法现在被广泛应用于优化各种酶的性质，包括与生物技术相关的特征如稳定性、区域选择性，特别是对映选择性。原则上讲，优化对映选择性反应有三种不同的方法：通过生物体设计发展全细胞体系的生物催化剂；对已知的对映选择性的酶进行优化以适合酶学工艺过程；从无对映选择性的野生型酶进化出新型对映选择性生物催化剂。

95 Evolving haloalkane dehalogenases

Current Opinion in Chemical Biology 2004, 8:150-159

Dick B Janssen

卤烷烃去卤化酶的人工进化

近年来结构生物学、生物化学和计算化学的发展使科学家能够从深层次上理解碳-卤键消除的生物化学机制，洞察卤烷烃去卤化酶如何高效完成其催化活性。与水相中的非酶催化的反应相比，酶

中无水活性空穴和强氢键作用协同降低了过渡态的能量势垒。尽管所有已知的卤烷烃去卤化酶都属于α/β水解酶折叠家族，但突变酶的性质和瞬态动力学研究显示它们的机理和动力学细节还存在着一些有趣的不同点。为了改善环境中某些重要的难降解化合物的酶促降解过程，人们正在对这些酶进行定点突变和定向进化的研究。

105 Harnessing microbial activities for environmental cleanup

Current Opinion in Biotechnology 2006, 17:274-284

Frank E Löffler, Elizabeth A Edwards

利用微生物活性进行环境净化

人类活动将大量有毒的有机和无机化合物释放到环境中。含有毒废物的溪流威胁着日益减少的饮用水资源，对陆地、河口以及海洋生态系统产生了冲击。有毒废物的清除具有技术挑战性，而采用传统技术进行清除的费用也超过了最富有国家的经济能力。最近，发展用于污染物转化和消除的微生物并将之实际应用，取得了一定的成功。因此，利用天然存在细菌的活性，尤其是其在厌氧还原过程中的能力，是一种有前途的恢复被污染的地表环境、保护饮用水储备和维护生态系统健康的途径。

116 Bioethanol

Current Opinion in Chemical Biology 2006, 10:141-146

Kevin A Gray, Lishan Zhao and Mark Emptage

生物乙醇

为了缓解世界性的不可再生能源紧缺问题，寻找代替源自石油的燃料，将成为一种有效的途径。从玉米谷物（淀粉）和甘蔗（蔗糖）中提取得到的乙醇是当今最普遍的可再生能源。这些原料的来源有限，木质纤维素类生物质被认为将成为未来乙醇供给的有效来源。尽管如此，利用这些生物质的商品化过程，仍然存在着技术和经济上的诸多障碍。当前正在发展多种将生物质有效转化为燃料和化学品的技术。这些技术采用了低成本的热化学预处理、高效的纤维素酶和半纤维素酶，以及高效、高活力的发酵型微生物。在过去的几年中，各个方面的长足发展使其商业化成为了可能。

122 Chemical diversity through biotransformations

Current Opinion in Biotechnology 2004, 15:591-598

Michael Müller

生物转化带来的化学多样性

多样性是生物合成具有的内在特征。这一内在特征能够开发并成功地应用到有机合成之中。目前在很多领域取得了长足进展，其中包括多功能酶和混和催化的应用、从单一底物合成多种产物、通过不同的生物转化方式生产同一产品、以及体内生物转化的应用等等。

130 Metabolic engineering of *Escherichia coli* and *Corynebacterium glutamicum* for biotechnological production of organic acids and amino acids

Current Opinion in Microbiology 2006, 9:268-274

Volker F Wendisch, Michael Bott and Bernhard J Eikmanns

生产有机酸和氨基酸的大肠杆菌与谷氨酰胺棒状杆菌的代谢工程

工业微生物可以作为生物催化剂，发展新的或者优化已知化工过程，以从来自可再生植物生物质的化学物中得到生物技术产品。通过代谢工程设计适合的菌株是成功的关键。这取决于有效的基因操作技术和对代谢途径及其调控机制的深入了解。这篇综述总结了工业化的模型菌——大肠杆菌和谷氨酰胺棒状杆菌的代谢工程研究最新进展，它们可以产生有效的基因重组生物催化剂，从而生产出醋酸盐、丙酮酸盐、乙醇、D-和L-乳酸盐、琥珀酸盐、L-赖氨酸和L-丝氨酸。

137 Recent advances in oxygenase - catalyzed biotransformations

Current Opinion in Chemical Biology 2006, 10:156-161

Vlada B Urlacher and Rolf D Schmid

加氧酶催化的生物转化的最新进展

为了进行有机化合物的选择性生物氧化，加氧酶一直被广泛地研究着。蛋白质工程的发展促成了有着广泛改变的底物特异性的亚铁血红素和黄素单加氧酶的产生，这些酶所催化的放大反应也已见报道。辅助因子的再生仍然是这类反应应用的关键。蛋白质工程为理解双氧化酶的结构及功能提供了帮助。

143 The soil metagenome - a rich resource for the discovery of novel natural products

Current Opinion in Biotechnology 2004, 15:199-204

Rolf Daniel

土壤宏基因组－发现新型天然产物的宝库

土壤中的微生物是天然产物最有价值的来源，它提供了有生产价值的重要抗生素和生物催化剂。但是近年来，由于土壤中的大部分微生物不易培养，使用传统培养技术发现新型生物分子的效率非常低。新型培养依赖和分子培养非依赖方法的发展，带来了从土壤微生物中发现新产物的新时代。尤其是基于来自土壤宏基因组的复杂文库的构建与筛选，其为全面探索和开发土壤微生物的巨大的遗传与代谢多样性提供了诸多机会。采用这种策略已经分离得到了一系列新型生物催化剂和生物活性分子。

149 Biocatalysis in pharmaceutical preparation and alteration

Current Opinion in Chemical Biology 2006, 10:169-176

Barrie Wilkinson and Brian O Bachmann

药物制备与改造中的生物催化

越来越多的人使用"合成生物学"这个术语来描述小分子化合物的生物催化合成，这种合成或者通过逐步的生物转化或者通过人

工改造的生物合成途径进行。这种新创术语灵活地将两个传统的独立领域——天然产物的生物合成和代谢工程统一起来。这篇综述总结了生物活性前体及产物的发现和开发中，这两项技术的最新进展。

157 High-throughput screening of biocatalytic activity:applications in drug discovery

Current Opinion in Chemical Biology 2006, 10:162-168

R Anand Kumar and Douglas S Clark

生物催化活性的高通量筛选：在药物发现中的应用

酶能够催化多种多样的反应，推进了生命进程，因而被用作有价值的药物靶标。从庞大的化合物库中寻找药物候选物时，高通量的方法已经变得越来越重要，而且某些灵敏可靠的分析手段已经被用于高通量筛选方法对于催化活性的测量。高通量生物催化筛选平台使得快速筛选酶靶标成为可能，很有可能对药物发现的各阶段产生影响，包括先导化合物的确认和优化、ADME/Tox评价等等。这些进展使得高通量生物催化剂筛选技术已经成为不可缺少的制药工具。

164 High-throughput screens and selections of enzyme-encoding genes

Current Opinion in Chemical Biology 2005, 9:210-216

Amir Aharoni, Andrew D Griffiths and Dan S Tawfik

酶编码基因的高通量筛选与精选

天然源（基因组和cDNA文库）和人工源（基因文库）基因库的利用，要求发展和应用新型技术，用以从大量文库中筛选和精选各种酶活性。该文总结了利用定向进化和功能基因组学精选酶编码基因的新进展。该综述将集中讨论用相对简单的方法（如非机械手法）从大型文库（超过10^6基因变量）中挑选以及体外分区法进行的高通量筛选。

171 Predicting enzyme function from protein sequence

Current Opinion in Chemical Biology 2005, 9:202-209

Jeremy Minshull, Jon E Ness, Claes Gustafsson and Sridhar Govindarajan

从蛋白质序列预测酶功能

两个主要原因促成蛋白质序列预测酶功能的研究。一是为了鉴定生物体的组分，进而鉴定其功能，二是为了创造具有特殊性质的酶。基因组学、基因表达分析、蛋白质组学以及代谢组学很大程度上将注意力放在理解在一个生物体内信息如何从DNA序列流向蛋白质功能。这篇综述集中于讨论反方向的信息流，即从天然酶研究探讨改进催化剂设计的方法。

179 Synthesis and modifications of carbohydrates, using biotransformations

Current Opinion in Chemical Biology 2004, 8:106-113

Alison M Daines, Beatrice A Maltman and Sabine L Flitsch

应用生物转化进行碳水化合物的合成和修饰

对于生产稀有的和非天然的单糖以及选择性地生成糖苷键而言，酶一直是重要的催化剂。多酶体系已经应用于多步反应以及辅助因子再生的“一锅煮”策略之中。通过活性位点突变而产生糖苷合成酶，能够显著地提高糖苷酶在糖基化反应中的效率。最近的报道表明，可以通过定向进化对糖苷合成酶的底物特异性进行扩展和优化。目前从基因组数据库中已经鉴定出新型糖基转移酶，并证明其能以高选择性和高产率糖基化复杂代谢物，如糖肽抗生素。一个新兴的领域就是将糖苷合成酶和糖基化转移酶应用到固相载体反应中，从而使其具备在微阵列中应用的潜力。

187 Whole organism biocatalysis

Current Opinion in Chemical Biology 2005, 8:174-180

Takeru Ishige, Kohsuke Honda and Sakayu Shimizu

全生物体生物催化

在化学品的工业合成中，使用生物催化剂作为一种环境友好的合成方法已经吸引了越来越多的注意。微生物细胞由于极具多样性而在“化学－酶学合成”中发挥着主导的作用。通过大批量筛选，人们已经发现了多种具有独特催化能力的微生物，并将其实用化。与此同时，高级分子生物技术已经成为开发更有效生物催化剂的有力工具。

Enzymatic tools for engineering natural product glycosylation

Sophie Blanchard and Jon S Thorson

Glycosylated natural products have served as reliable platforms for the development of many existing front-line drugs. In an effort to explore the contribution of the sugar constituents of these compounds, research groups have focused upon the development of chemical and enzymatic tools to diversify natural product glycosylation. Among the complementary routes available, *in vivo* pathway engineering, also referred to as 'combinatorial biosynthesis', is an emerging method that relies upon the co-expression of sugar biosynthetic gene cassettes and glycosyltransferases in a host organism to generate novel glycosylated natural products. An overview of recent progress in combinatorial biosynthesis is highlighted in this review, emphasizing the elucidation of nucleotide-sugar biosynthetic pathways and recent developments on glycosyltransferases.

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Introduction

Natural products are a reliable source of drug leads and often contain sugar attachments that can influence a compound's specificity and pharmacology [1,2]. Thus, there is an interest in developing chemical and enzymatic tools to diversify natural product glycosylation patterns. Because no single universal glycosylation tool exists, accomplishing natural product glycodiversification will continue to require an array of complementary tools. To date, the strategies generally employed to create libraries of glycosylated natural product variants include total synthesis or semisynthesis [3•,4,5], glycorandomization [6,7••] and *in vivo* pathway engineering (often also referred to as 'combinatorial biosynthesis') (Figure 1). Combinatorial biosynthesis relies upon co-expression of sugar biosynthetic gene cassettes and glycosyltransferases in a host organism containing an endogenous or exogenously delivered aglycon, to generate novel glycosylated natural products. While chemical and enzymatic strategies for glycorandomization have recently been reviewed [8,9••], this article focuses upon developments within the past two years relevant to combinatorial biosynthesis. Specifically, we emphasize the elucidation of nucleotide-sugar biosynthetic pathways — a critical first step in the design of sugar biosynthetic gene cassettes — and the discovery of novel promiscuous glycosyltransferases — the core catalysts for combinatorial glycosylation strategies.

Elucidation of nucleotide-sugar biosynthetic pathways

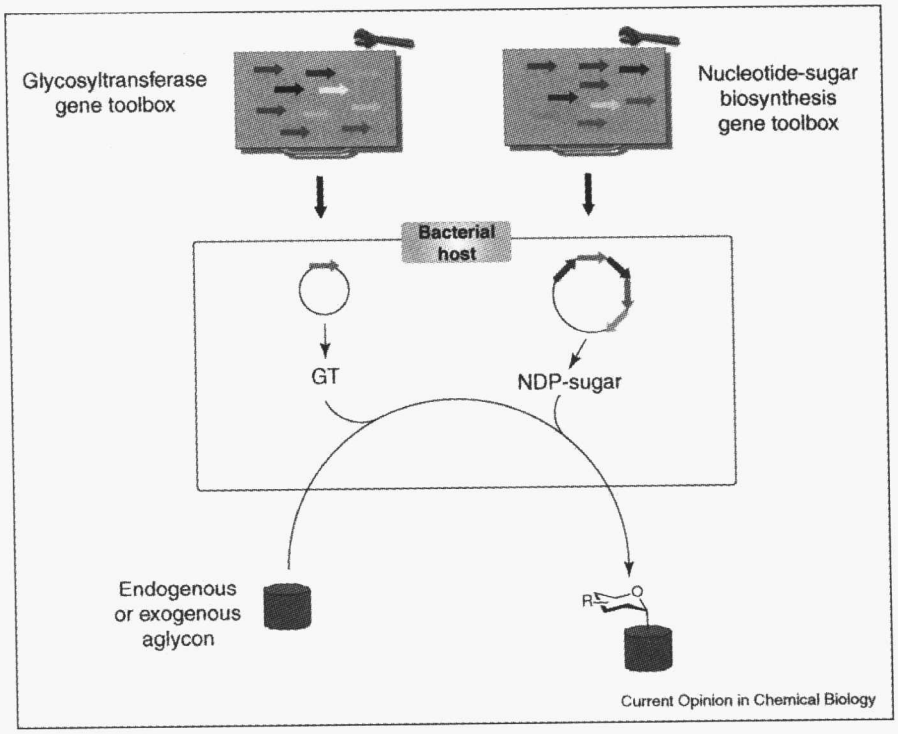
Glycosylated natural products generally contain sugars that are deoxygenated at C2, C3, C4 and/or C6. Because the chemical synthesis of deoxyhexoses is difficult [10•], understanding deoxyhexose biosynthesis is critical to the glycodiversification of natural products [11]. The first two steps in deoxyhexose biosynthesis are activation of a monosaccharide-1-phosphate by the tandem NDP-hexose nucleotidylyltransferase/NDP-hexose 4,6-dehydratase-mediated conversion to an NDP-4-keto-6-deoxyhexose (Figure 2a; boxed) — the immediate biosynthetic precursor of most deoxyhexoses [11]. This species can undergo further enzymatic modifications before being transferred by a glycosyltransferase to an aglycon, where the sugar can be subjected to a final array of tailoring enzymes [12,13]. These latter modifications provide even more diversification to natural product glycosylation.

The pool of available nucleotide-sugars is increasing as gene clusters of glycosylated natural products are discovered and as new sugar biosynthetic pathways are elucidated [14]. The past two years have seen significant advances in the elucidation of biosynthetic pathways for NDP-deoxyhexoses, NDP-deoxyaminohexoses, and NDP-pentoses, as described below. Also, the *in vitro* reconstitution of the entire TDP-L-mycarose biosynthetic pathway has been disclosed. Although the following sections highlight recent developments, there remain many novel nucleotide-sugar pathways to be elucidated — promising the discovery of rich new enzyme-catalyzed chemistry and a supply of exciting new combinatorial glycosylation tools.

NDP-deoxyhexoses

The common NDP-4-keto-6-deoxyhexose (Figure 2a; boxed) can undergo deoxygenation at C2, C3, and/or C4. Surprisingly, the enzymatic deoxygenation at each of these three positions proceeds via drastically different

Figure 1



A glycosyltransferase gene toolbox and a nucleotide-sugar biosynthesis gene toolbox provide the basis for a general *in vivo* pathway engineering strategy to generate novel glycosylated natural products. The aglycon can be fed to the host, or produced by the host strain itself. A variation of this strategy incorporates organisms that already possess some of the genes necessary for the preparation of the nucleotide-sugar and/or its transfer to the aglycon. GT, glycosyltransferase; NDP, nucleotide diphosphate.

mechanisms [15]. Whereas deoxygenation at C2 occurs via β-elimination followed by hydride reduction, C3 deoxygenation occurs via an unprecedented pyridoxal 5′-phosphate (PLP)-dependent radical-based mechanism [11,15]. In contrast, Liu and co-workers recently revealed C4 deoxygenation to proceed via an amino sugar intermediate (Figure 2b). Specifically, amino sugar **2**, formed via DesI-mediated transamidation of **1**, was converted by a newly characterized *S*-adenosylmethionine (SAM)-dependent deaminase DesII [16•], to form the corresponding 4,6-dideoxyhexose (**3**). Hexose **3** was ultimately converted to the C4-deoxygenated nucleotide-sugar TDP-desosamine.

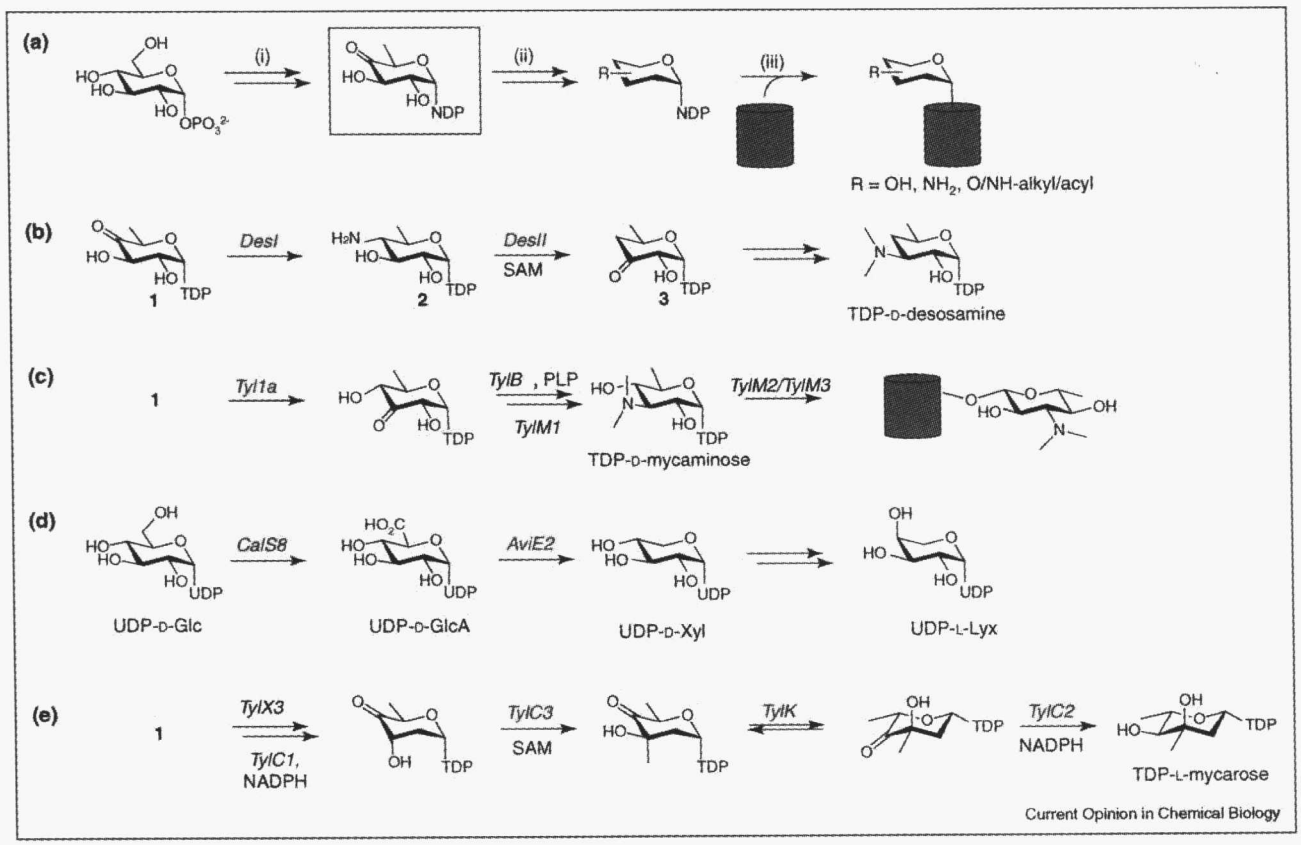
NDP-deoxyaminohexoses

Deoxyaminohexoses are found in many biologically active natural products. These sugars are generally formed from keto sugars via standard aminotransferase-catalyzed transamination reactions [17•]. Liu and co-workers recently characterized SpnR as the aminotransferase in the D-forosamine biosynthetic pathway [18], a pathway that is particularly promising for combinatorial biosynthesis given the promiscuity of downstream tailoring enzymes. In another NDP-deoxyaminohexose biosynthetic study, Liu and co-workers found that Tyl1a, not the previously postulated TylM3, was required for the isomerization of the canonical TDP-4-keto-6-deoxyhexose intermediate (Figure 2c; **1**) in the synthesis of TDP-D-mycaminose [19]. TylM3 was subsequently shown to assist the glycosyltransferase TylM2 in catalyzing the transfer of TDP-mycaminose to the aglycon (see below).

NDP-pentoses

Recent work from Bechthold and co-workers provided additional insight into the genesis of the unusual pentoses appended to a variety of secondary metabolites. In their study, AviE2 from the avilamycin A biosynthetic pathway was found to form UDP-D-xylose from UDP-D-glucuronic acid via decarboxylation (Figure 2d) [20]. Such decarboxylation reactions are common in pentose primary metabolism; however, this was the first biochemical characterization of a sugar decarboxylase from secondary metabolism. A similar biosynthetic pathway for the deoxypentose moieties found in enediyne antitumor antibiotics was previously proposed based upon the biochemical characterization of the UDP-glucose dehydrogenase CalS8 [21]. Genes encoding homologs for these enzymes have also recently been discovered in the indolocarbazole *Actinomadura melliaura* AT2433 gene cluster (Q Gao, JS Thorson, unpublished data).

Figure 2



In vivo nucleotide-sugar biosynthesis. **(a)** A general biosynthetic route initiated by NDP-sugar formation, followed by multienzyme functionalization and glycosyltransferase-catalyzed glycosylation of a natural product aglycon. The first stage (i) is catalyzed by a nucleotidylyltransferase and a 4,6-dehydratase; the second stage (ii) requires multiple enzymes, which may lead to deoxygenation, oxidation, epimerization, transamination and ketoreduction (although not explicitly illustrated, alkylation can occur also at this stage); the third stage (iii) is glycosyltransferase-catalyzed transfer to the natural product aglycon; the sugar may be further modified after stage (iii) by additional enzymatic steps. **(b)** Proposed route for C4 deoxygenation in the biosynthesis of TDP-D-desosamine. **(c)** The biosynthesis of TDP-D-mycaminose. **(d)** The hypothetical biosynthetic pathway of UDP-L-lyxose is initiated by a UDP-glucose dehydrogenase (the biochemically characterized CalS8 from calicheamicin biosynthesis is shown) followed by a UDP-glucuronic acid decarboxylase (the biochemically characterized AviE2 from avilamycin A biosynthesis is illustrated) to give UDP-D-xylose. **(e)** Complete biosynthetic pathway of TDP-L-mycarose from tylosin producer *Streptomyces fradiae*. Glc, glucose; GlcA, glucuronic acid; Lyx, lyxose; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NDP, nucleotide diphosphate; PLP, pyridoxal 5′-phosphate; SAM, *S*-adenosylmethionine; TDP, thymidine diphosphate; Xyl, xylose.

Reconstitution of NDP-sugar pathways

An understanding of the complexity of multistep biosynthesis *in vivo* and *in vitro* is essential to harness the biosynthetic machinery necessary to produce 'tailor-made' molecules and to extend structural diversity. Such understanding was demonstrated when the entire biosynthetic pathway of TDP-L-mycarose, one of the three sugar components of the macrolide antibiotic tylosin, was reconstituted *in vitro* by Liu and co-workers [22]. Their experiments provided a critical basis from which to assign roles for the key enzymes (TylC3, TylK and TylC2) involved in TDP-L-mycarose biosynthesis (Figure 2e). Although TylK and TylC2 demonstrated moderate substrate flexibility, these two enzymes act downstream from the highly specific enzyme TylC3, the intolerance of which was postulated to limit the diversity of products available via the tylosin biosynthetic pathway.

Expanding the glycosyltransferase tool box

Generating glycosylated metabolites via combinatorial biosynthesis relies upon access to an array of glycosyltransferase genes. Most glycosyltransferases are single polypeptides; however, it was recently discovered that the macrolide glycosyltransferase DesVII required an auxiliary protein, Des VIII, for *in vitro* and *in vivo* activity [23••,24]. Since this seminal discovery, other pairs of glycosyltransferases and activating proteins have been discovered, such as AknS/AknT (aclacinomycin) [25], TylM2/TylM3 (tylosin) [26], and MycB/MydC (mycinamycin) [26]. Walsh and co-workers reported the activity of the glycosyltransferase EryCIII to also be greatly

enhanced by EryCII or AknT activation *in vitro*; however, once EryCIII was fully 'activated' it no longer required its protein partner [27]. Although the mechanistic role of activating proteins remains unclear [24], regardless of whether they require an activating protein, secondary metabolite-associated glycosyltransferases often exhibit inherent substrate promiscuity toward acceptor substrates and/or (deoxy)sugar donor substrates. As described below, this inherent flexibility can be exploited to generate novel glycosylated natural products.

Flexibility toward aglycons

Glycosyltransferases can display significant flexibility toward aglycons. One such glycosyltransferase is VinC from *Streptomyces halstedii* HC-34, which accepts the surprisingly diverse array of hydrophobic aglycons shown in Figure 3a [28•]. The macrolide glycosyltransferases OleD, OleI and MGT — which normally glucosylate macrolide glycoside acceptors as a macrolide inactivation mechanism — were also recently suggested to attach glucose to a structurally diverse set of aglycons including aromatics, coumarins, flavanols and macrolides, on the basis of a mass spectrometry assay [29]. Among the other glycosyltransferases capable of using natural product saccharides as acceptors, LanGT4 was used for the first time, to elongate the polysaccharide chain of a natural product beyond the wild-type length [30]. LanGT4 and LanGT1 were also recently found to attach two sugars within the hexasaccharide side-chain of landomycin A [31•].

Several enzymes that catalyze *N*-glycosylation [32••] and *C*-glycosylation [33••,34,35] have been discovered (Figure 3b). IroB, the first *C*-glycosyltransferase to be characterized *in vitro*, was reported last year [35]. Interestingly, UrdGT2, the *C*-glycosyltransferase involved in the biosynthesis of urdamycin A, was also able to form an *O*-glycosidic linkage, demonstrating unique catalytic flexibility and providing mechanistic insights [33••]. For *N*-glycosides, the two genes responsible for attaching α-L-ristosamine to the staurosporine aglycon — to provide a unique bridged *N*-glycosidic structure — were recently characterized *in vivo* [32••]. Specifically, the *N*-glycosyltransferase StaG displayed flexibility with respect to sugar donors, whereas the P450 oxygenase StaN only formed the second C–N linkage at the C(5)′ position with L-sugars. In a related study, the rebeccamycin *N*-glucosyltransferase (RebG) and downstream glucose-*O*-methyltransferase (RebM) were used *in vitro* and *in vivo* to modify unnatural indolocarbazoles (C Zhang, JS Thorson, unpublished data; see Update).

Alternative glycosyltransferase sugar donors

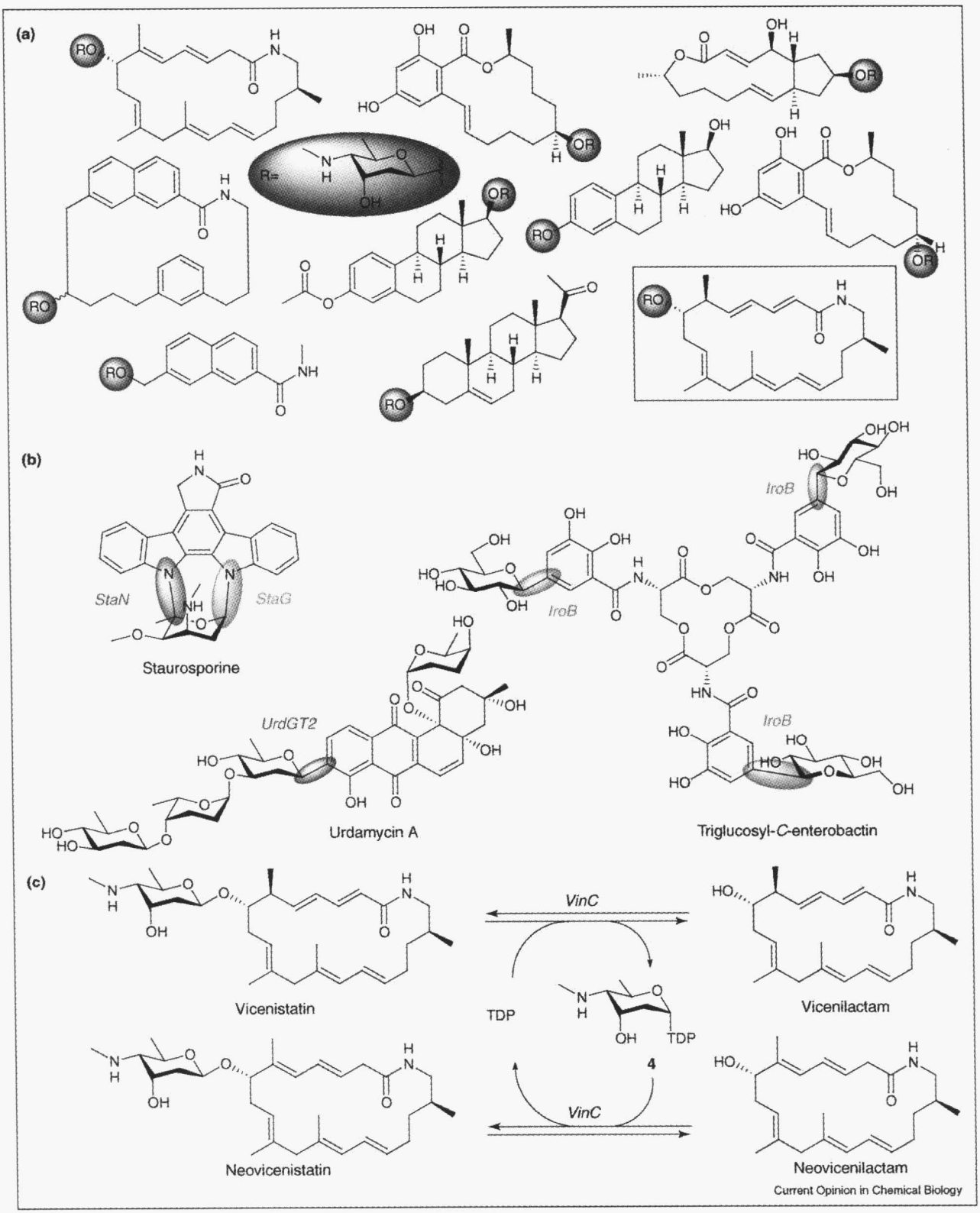
Glycosyltransferases are often promiscuous toward nucleotide-sugar donors, as exemplified by vancomycin glycosyltransferase GtfE [36•]. Other examples of glycosyltransferases that accept an array of nucleotide-sugar donors have been reported [14,37], and promiscuous glycosyltransferases have also been used to glycosylate avermectins, indolocarbazoles and enediynes (C Zhang, JS Thorson, unpublished data). In an interesting twist, a recent report disclosed a glycosyltransferase capable of using a glycosylated natural product as a sugar donor source [38•]. Specifically, VinC was demonstrated to transfer vicenisamine from vicenistatin to several aglycons (Figure 3c), a process postulated to proceed via the intermediacy of TDP-vicenisamine **4**. In contrast, there also exist highly specific glycosyltransferases, such as LanGT2 from *Streptomyces cyanogenus* [39] and NovM from *Streptomyces spheroids* NCIMB11891 [40]. Cumulatively, while glycosyltransferases have proven to be fairly versatile catalysts, glycosyltransferase directed-evolution and/or glycosyltransferase structural elucidation (for a recent example see [41•]) will continue to provide advancements for engineering of glycosyltransferases with enhanced properties [37].

In vivo diversification: pathway engineering or combinatorial biosynthesis

Early natural product glycosylation engineering was derived from gene-deletion or disruption experiments designed to elucidate sugar biosynthetic genes by generating glycosyl-modified shunt metabolites [14]. Solenberg and co-workers first described the use of heterologous glycosyltransferase genes to produce hydrid glycopeptide antibiotics [42]. Shortly thereafter, Hutchinson and co-workers advanced these experiments to the pathway engineering level when they replaced the native daunosamine 4-ketosugar reductase gene with an inverting 4-ketosugar reductase gene to ultimately convert the daunorubicin producer into an epirubicin-producing host [43]. These landmark experiments paved the way for the contemporary combinatorial glycosylation applications highlighted below.

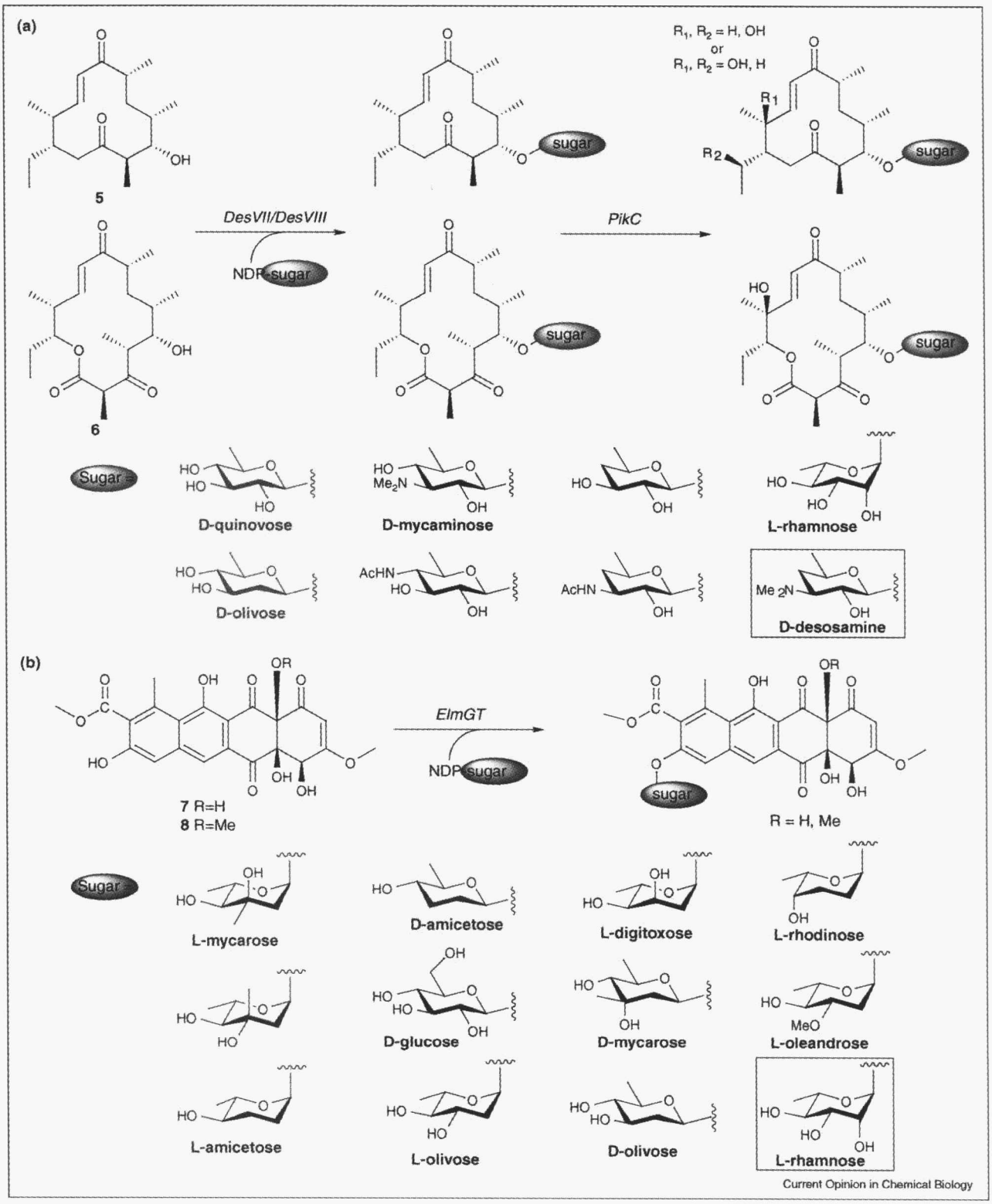
Salas and co-workers recently used combinatorial biosynthesis to dissect and reconstitute the entire rebbecamycin pathway [44••]. By elegantly combining these genes with those from staurosporine biosynthesis in a heterologous host (*Streptomyces albus*), over 30 indolocarbazole derivatives were generated, some glycosylated with natural and unnatural sugars [32••,44••]. Expanding upon the extensive macrolide-glycoside engineering work accomplished by Liu and co-workers (for their earliest example see [45]), Hong and co-workers recently engineered deoxysugar pathways for the generation of novel hybrid macrolide antibiotics [46]. Specifically, all of the TDP-D-desosamine biosynthesis genes within *Streptomyces venezuelae* were deleted and replaced with genes for the biosynthesis of TDP-4-keto-6-deoxy-D-glucose and TDP-D-olivose from the oleandomycin and urdamycin deoxysugar pathways. The promiscuous glycosyltransferase DesVII was able to attach both of these sugars to the macrolactones 10-deoxymethylnolide and narbonolide (Figure 4a). Several new derivatives of the antitumor compound elloramycin were

Figure 3



Recent glycosyltransferase developments. **(a)** Alternative hydrophobic substrates for glycosyltransferase VinC from *S. halstedii* HC-34. The aglycon naturally transferred by glycosyltransferase VinC is boxed. **(b)** Examples of natural products bearing *C*- or *N*-glycosidic bonds. The bonds formed are circled, color-coded and labeled with the enzyme involved in their creation. **(c)** VinC-catalyzed transfer of TDP-vicenisamine **4** from vicenistatin to neovicenistatin. TDP, thymidine diphosphate.

Figure 4



Examples of combinatorial biosynthesis glycosylation applications. **(a)** Structures of glycosylated derivatives of 10-deoxymethylnolide (**5**) and narbonolide (**6**) (sugars referred to in text are colored in red). The sugar naturally transferred by DesVII/DesVIII is boxed. Subsequent hydroxylation by a P450 enzyme (PikC) generates the final products produced by *S. venezuelae*. **(b)** Structures of glycosylated derivatives of 8-demethyl-tetracenomycin C (**7**) and elloramycinone (**8**) (sugars referred to in text are colored in blue). The sugar naturally transferred by ElmGT is boxed. NDP, nucleotide diphosphate.

also recently generated via combinatorial biosynthesis using ElmGT [47,48], a flexible glycosyltransferase used extensively in past years with other aglycons (Figure 4b). Landomycin E derivatives were also created via engineering of biosynthetic pathways involving the glycosyltransferase LndGT4 [49].

Concluding remarks

The impact of sugar attachments on biological activity justifies the development of tools that diversify natural product glycosylation patterns [7••]. Each individual diversification tool has inherent advantages and disadvantages, but together these complementary methods provide access to an incredible array of novel glycosylated natural products. Combinatorial biosynthesis requires a genetically amendable host and remains limited to the combinations of existing naturally occurring enzymatic conversions to produce 'tailor-made' or random libraries of natural product glycosides. Thus, continued elucidation of new and exotic nucleotide-sugar pathways are critical to extending the diversity accessible by pathway engineering [10•,11,15,50•]. As a scalable fermentation-based approach, combinatorial biosynthesis may ultimately also provide scalable production of unique metabolites. Recent attempts to combine the use of 'unnatural' sugars in glycorandomization with the *in vivo* advantages of combinatorial biosynthesis have been successful [51•], and may ultimately lead to a single universal glycorandomization strain able to further complement the level of scalable diversity accessible via *in vivo* strategies (J Yang, JS Thorson, unpublished data). The discovery, characterization, and engineering of flexible glycosyltransferases will also remain critical to all enzyme-based glycodiversification strategies. Finally, pathway engineering can also be used to diversify natural product aglycons themselves, a facet of this strategy that, while not discussed here, has the potential to dramatically increase the number of available glycosylated natural product derivatives.

Update

The work referred to in the text as (C Zhang, JS Thorson, unpublished data) is now in press [52].

Acknowledgements

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Nucleic acid aptamers and enzymes as sensors

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The function of nucleic acids has been an endless source of discovery and invention that has drastically enhanced our appreciation of DNA and RNA as multifaceted polymers. It is now widely known that nucleic acids can act as enzymes (deoxyribozymes and ribozymes) and as receptors (aptamers), and that these functional nucleic acids (FNAs) can either be found in nature or isolated from pools of random nucleic acids. The availability of many natural and artificial FNAs has opened a new horizon for the development of 'smart' molecules for a variety of chemical and biological applications. This review provides a snapshot of recent progress in the application of FNAs as novel sensors for biomolecular detection, drug discovery and nanotechnology.

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Introduction

Our appreciation of the functional versatility of nucleic acids has grown over the past two decades. A quick perusal of the literature reveals that many functional RNAs, including ribozymes, microRNAs and riboswitches, exist in nature [1–3]. Furthermore, many man-made aptamers, ribozymes, deoxyribozymes and allosteric nucleic acid enzymes have been generated via '*in vitro* selection' or 'SELEX' (systemic evolution of ligands by exponential enrichment) techniques [4,5]. The realization that nucleic acid aptamers and enzymes are highly useful molecular tools has made the study of functional nucleic acids a very important part of chemical biology. The flexibility offered by nucleic acids (stability, ease of immobilization and susceptibility to various chemical modifications and labeling) has motivated molecular scientists worldwide to seek innovative applications for these species. This review discusses some new advances that harness the potential of nucleic acid aptamers and enzymes as sensor molecules. For simplicity, we will refer to nucleic acid aptamers and enzymes collectively as 'functional nucleic acids' or simply 'FNAs'. Although a broad definition of FNAs would also include hybridization-based probes such as standard molecular beacons and antisense oligonucleotides, we have chosen to focus on aptamers and nucleic acid enzymes in the strict sense.

Functional nucleic acids as sensors: general considerations

FNAs are attractive options for sensing applications. They can be generated by SELEX to bind diverse targets including those for which antibodies are difficult to obtain (such as toxins). Moreover, *in vitro* selection can be used to evolve FNAs under any pre-defined conditions, making it possible to design sensors for tasks that cannot be met with protein receptors. Nucleic acids can also be easily immobilized to provide a custom-made surface for specific applications. They have the added advantage of being able to refold to their native conformation following one round of sensing, and therefore represent reusable devices. The change in shape of FNAs upon binding to their target can be conveniently coupled with various signaling mechanisms for easy monitoring of molecular recognition events.

A biosensor is generally defined as an analytical device consisting of a biologically relevant molecular recognition element (MRE) integrated to a signal transduction element (Figure 1a). In this review, we will first examine the use of FNAs for easy detection of proteins, nucleic acids and metabolites, followed by a discussion on some of the latest activities involving FNAs as tools in drug discovery and nanotechnology.

Functional nucleic acids as sensors for protein detection

Many protein-binding aptamers have been isolated, a number of which have been exploited for proof-of-concept biosensing applications using various signal transduction mechanisms.

Optical sensing

Optical detection by fluorescence spectroscopy is a popular method, due largely to the ease with which FNAs can be fluorescently labelled, the availability of many different fluorophores and quenchers, and the inherent capability for real-time multiplex detection. Several strategies have been described for converting an existing aptamer into a fluorescent probe (recently reviewed in [6]). Two frequently adopted methods are the molecular beacon approach (Figure 1b) [7] and the duplex-to-complex switching approach (Figure 1c) [8]. The former