2024年第6期总7期

饲料用酶工程

本期导读

前沿资讯

学术文献

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前沿资讯

1. 中科院天津工业生物所发布REME平台推动非天然反应酶挖掘与评估

简介：近日，中国科学院天津工业生物技术研究所生物设计中心发布了首个集成的反应酶挖掘与评估平台——REME（https://reme.biodesign.ac.cn）。该平台结合底物原子到产物原子映射、原子类型变化识别和反应相似性计算，实现了相似反应的计算、快速排序和可视化。用户可以根据功能基团筛选相似反应，并进一步通过酶号或序列同源性筛选或扩展候选酶。REME平台结合多种人工智能方法对候选酶进行多角度评估（如kcat、Km、最佳温度和pH），帮助科研人员迅速识别潜在酶。REME平台的推出为非天然反应的酶挖掘和评估提供了新的解决方案。

来源：中科院天津工业生物所

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2. Bioengineered enzyme creates natural vanillin from plants in one step（利用生物工程酶一步式从植物中产生天然香兰素）

简介：Professor Toshiki Furuya from the Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science, and his graduate students Shizuka Fujimaki and Satsuki Sakamoto, successfully developed an enzyme that generates vanillin from plant-derived ferulic acid. “Ferulic acid, the raw material, is a compound that can be obtained in abundance from agricultural waste such as rice bran and wheat bran. Vanillin is generated simply by mixing ferulic acid with the developed enzyme at room temperature. So, the established technology can provide a simple and environmentally friendly method for producing flavor compounds,” explains Prof. Furuya. Their study was published on May 10, 2024 in Applied and Environmental Microbiology.The researchers used genetic engineering approaches to modify the molecular structure of an enzyme ‘Ado.’ Ado is originally an oxidase enzyme that adds an oxygen atom to the substrate isoeugenol. In its native state, it does not have the ability to convert ferulic acid into vanillin. Using structural modeling analysis, the researchers were able to predict amino acid changes in Ado which would enable its interaction with ferulic acid. On these lines, they conducted a series of experiments by replacing phenylalanine and valine amino acid residues at specific positions in the structure of Ado, with various other amino acids. They went on to examine the ferulic acid conversion ability of the various engineered mutant proteins.

来源：Eurek Alert

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3. University of Groningen chemists produce new-to-nature enzyme containing boron（格罗宁根大学科学家合成一种新的天然硼酸酶）

简介：Boronic acid has been used in organic chemistry for decades, even though it is not present in any organism. ‘It gives rise to different chemical reactions than those we find in nature,’ explains Gerard Roelfes, Professor of Biomolecular Chemistry & Catalysis at the University of Groningen. His group created an enzyme with boronic acid at its reactive centre and then used directed evolution to make it more selective and to improve its catalytic power. Furthermore, enzymatic reactions are more sustainable than classical chemical reactions, as they take place at low temperatures and without toxic solvents. The study was presented online in the journal Nature on 8

May.
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学术文献

1. Advances and prospects in microbial production of biotin(微生物生产生物素的研究进展与展望)

简介：Biotin, serving as a coenzyme in carboxylation reactions, is a vital nutrient crucial for the natural growth, development, and overall well-being of both humans and animals. Consequently, biotin is widely utilized in various industries, including feed, food, and pharmaceuticals. Despite its potential advantages, the chemical synthesis of biotin for commercial production encounters environmental and safety challenges. The burgeoning field of synthetic biology now allows for the creation of microbial cell factories producing bio-based products, offering a cost-effective alternative to chemical synthesis for biotin production. This review outlines the pathway and regulatory mechanism involved in biotin biosynthesis. Then, the strategies to enhance biotin production through both traditional chemical mutagenesis and advanced metabolic engineering are discussed. Finally, the article explores the limitations and future prospects of microbial biotin production. This comprehensive review not only discusses strategies for biotin enhancement but also provides in-depth insights into systematic metabolic engineering approaches aimed at boosting biotin production.
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2. Int&in: A machine learning-based web server for active split site identification in inteins(Int&in:用于对内含肽进行主动分割位点识别的基于机器学习的网页服务器)

简介：Inteins are proteins that excise themselves out of host proteins and ligate the flanking polypeptides in an auto-catalytic process called protein splicing. In nature, inteins are either contiguous or split. In the case of split inteins, the two fragments must first form a complex for the splicing to occur. Contiguous inteins have previously been artificially split in two fragments because split inteins allow for distinct applications than contiguous ones. Even naturally split inteins have been split at unnatural split sites to obtain fragments with reduced affinity for one another, which are useful to create conditional inteins or to study protein-protein interactions. So far, split sites in inteins have been heuristically identified. We developed Int&in, a web server freely available for academic research () that runs a machine learning model using logistic regression to predict active and inactive split sites in inteins with high accuracy. The model was trained on a dataset of 126 split sites generated using the gp41-1, Npu DnaE and CL inteins and validated using 97 split sites extracted from the literature. Despite the limited data size, the model, which uses various protein structural features, as well as sequence conservation information, achieves an accuracy of 0.79 and 0.78 for the training and testing sets, respectively. We envision Int&in will facilitate the engineering of novel split inteins for applications in synthetic and cell biology.
来源：Protein Science

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3. Harnessing noncanonical crRNA for highly efficient genome editing(利用非规范 crRNA 实现高效基因组编辑)

简介：The CRISPR-Cas12a system is more advantageous than the widely used CRISPR-Cas9 system in terms of specificity and multiplexibility. However, its on-target editing efficiency is typically much lower than that of the CRISPR-Cas9 system. Here we improved its on-target editing efficiency by simply incorporating 2-aminoadenine (base Z, which alters canonical Watson-Crick base pairing) into the crRNA to increase the binding affinity between crRNA and its complementary DNA target. The resulting CRISPR-Cas12a (named zCRISPR-Cas12a thereafter) shows an on-target editing efficiency comparable to that of the CRISPR-Cas9 system but with much lower off-target effects than the CRISPR-Cas9 system in mammalian cells. In addition, zCRISPR-Cas12a can be used for precise gene knock-in and highly efficient multiplex genome editing. Overall, the zCRISPR-Cas12a system is superior to the CRISPR-Cas9 system, and our simple crRNA engineering strategy may be extended to other CRISPR-Cas family members as well as their derivatives.The inclusion of base Z has the potential to heighten the binding affinity between complementary nucleic acids. Here, the authors integrated base Z into CRISRP-Cas12a crRNA to augment the interaction between the crRNA and the target DNA, resulting in a significant enhancement of editing efficiency.
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4. A systematic analysis of regression models for protein engineering(蛋白质工程回归模型的系统分析)

简介：To optimize proteins for particular traits holds great promise for industrial and pharmaceutical purposes. Machine Learning is increasingly applied in this field to predict properties of proteins, thereby guiding the experimental optimization process. A natural question is: How much progress are we making with such predictions, and how important is the choice of regressor and representation? In this paper, we demonstrate that different assessment criteria for regressor performance can lead to dramatically different conclusions, depending on the choice of metric, and how one defines generalization. We highlight the fundamental issues of sample bias in typical regression scenarios and how this can lead to misleading conclusions about regressor performance. Finally, we make the case for the importance of calibrated uncertainty in this domain. Supervised machine learning is increasingly used to predict the function and properties of proteins. The performance obtained with these methods relies on a multitude of factors including how data is represented, how observations are distributed, how training is conducted, and how performance is measured. In this paper, we systematically assess the importance of these different components in a protein regression pipeline. We discuss the benefits of using representations extracted from protein language models, the impact of the choice of regression algorithm, and the role of uncertainty. Finally, to avoid misleading performance claims, we stress the need for carefully aligning the train/test setup to reflect the setting in which the prediction algorithm will ultimately be applied.
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5. Paternal microbiome perturbations impact offspring fitness(父系微生物组干扰影响后代的适应性)

简介：The gut microbiota operates at the interface of host-environment interactions to influence human homoeostasis and metabolic networks 1-4. Environmental factors that unbalance gut microbial ecosystems can therefore shape physiological and disease-associated responses across somatic tissues 5-9. However, the systemic impact of the gut microbiome on the germline-and consequently on the F1 offspring it gives rise to-is unexplored 10. Here we show that the gut microbiota act as a key interface between paternal preconception environment and intergenerational health in mice. Perturbations to the gut microbiota of prospective fathers increase the probability of their offspring presenting with low birth weight, severe growth restriction and premature mortality. Transmission of disease risk occurs via the germline and is provoked by pervasive gut microbiome perturbations, including non-absorbable antibiotics or osmotic laxatives, but is rescued by restoring the paternal microbiota before conception. This effect is linked with a dynamic response to induced dysbiosis in the male reproductive system, including impaired leptin signaling, altered testicular metabolite profiles and remapped small RNA payloads in sperm. As a result, dysbiotic fathers trigger an elevated risk of in utero placental insufficiency, revealing a placental origin of mammalian intergenerational effects. Our study defines a regulatory 'gut-germline axis' in males, which is sensitive to environmental exposures and programmes offspring fitness through impacting placenta function. Disturbances in the gut microbiota of male mice manifest as fitness defects in their offspring by affecting plancenta function, revealing a paternal gut-germline axis.

来源：Nature

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6. Engineering Peroxygenase Activity into Cytochrome P450 Monooxygenases through Modification of the Oxygen Binding Region(通过修改氧结合区域将过氧化酶活性引入细胞色素 P450 单加氧酶中)

简介：Cytochrome P450 enzymes (CYPs) are biocatalysts for the generation of fine chemicals including natural products, drug metabolites, and flavor and fragrance compounds. However, both the high cost of the required nicotinamide cofactors and their need for additional electron transfer proteins limit their use. Here, we investigate whether CYPs can be converted into more efficient peroxygenases through protein engineering of the enzyme's oxygen activation machinery. We improve the peroxygenase activity by modifying selected residues within the I-helix to more closely resemble those of a natural peroxygenase. We produced mutants containing two, four, and six mutations, within this region of the I-helix. In our model CYP system, the double mutant in which glutamine and glutamate residues replaced aspartate and threonine, respectively, was found to have significantly higher peroxygenase activity for the O-demethylation of 4-methoxybenzoic acid than a single glutamate mutant prototype. Importantly, it functioned better at lower H2O2 concentrations and could convert all the added substrate to product. All the mutants maintained the stereoselectivity of the CYP enzyme for the epoxidation of 4-vinylbenzoic acid. The X-ray crystal structures of these enzymes showed significant structural changes at the oxygen-binding groove in the I-helix. In crystallo reactions with 4-methylbenzoic acid exhibit electron density corresponding to the 4-(hydroxymethyl) benzoic acid metabolite. We extended this mutagenesis strategy to a bacterial steroid-hydroxylating CYP and an uncharacterized CYP from a thermophilic bacterium. In these instances, we generate peroxygenases, which catalyze the regio- and stereoselective hydroxylation of progesterone and the hydroxylation of fatty acids at low hydrogen peroxide concentrations.

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7. Practical Machine Learning-Assisted Design Protocol for Protein Engineering: Transaminase Engineering for the Conversion of Bulky Substrates(蛋白质工程实用机器学习辅助设计规程：用于大分子底物转化的转氨酶工程)

简介：Protein engineering is essential for improving the catalytic performance of enzymes for applications in biocatalysis, in which machine learning provides an emerging approach for variant design. Transaminases are powerful biocatalysts for the stereoselective synthesis of chiral amines but one major challenge is their limited substrate scope. We present a general and practical variant design protocol for protein engineering to combine the advantages of three strategies, including directed evolution, rational design, and machine learning, and demonstrate the application of the protocol in the protein engineering of transaminases with higher activity toward bulky substrates. A high-quality data set was obtained by rational design of selected key positions, which was then applied to create a machine learning model for transaminase activity. This model was applied for the data-assisted design of optimized variants, which showed improved activity (up to 3-fold over parent) for three bulky substrates, maintaining enantioselectivity of the starting enzyme scaffold as well as improving the enantiomeric excess (up to >99%ee).
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8. CRISPR–dCas12a-mediated genetic circuit cascades for multiplexed pathway optimization(CRISPR–dCas12a介导的遗传回路级联用于多路通路优化)

简介：The production efficiency of microbial cell factories is sometimes limited by the lack of effective methods to regulate multiple targets in a coordinated manner. Here taking the biosynthesis of glucosamine-6-phosphate (GlcN6P) in Bacillus subtilis as an example, a 'design-build-test-learn' framework was proposed to achieve efficient multiplexed optimization of metabolic pathways. A platform strain was built to carry biosensor signal-amplifying circuits and two genetic regulation circuits. Then, a synthetic CRISPR RNA array blend for boosting and leading (ScrABBLE) device was integrated into the platform strain, which generated 5,184 combinatorial assemblies targeting three genes. The best GlcN6P producer was screened and engineered for the synthesis of valuable pharmaceuticals N-acetylglucosamine and N-acetylmannosamine. The N-acetylglucosamine titer reached 183.9 g liter (-1) in a 15-liter bioreactor. In addition, the potential generic application of the ScrABBLE device was also verified using three fluorescent proteins as a case study.
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