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变构药物设计方法学的系统创立以及在变构药物开发中的应用

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变构调节（allosteric regulation）是生物体内普遍存在的一种调控方式，是指小分子结合在空间上不同于活性位点的变构位点（allosteric site）扰动周围的残基并将信号传导至活性位点从而改变蛋白功能的现象。2009 年以来，上海交通大学医学院张健研究组开发了一系列变构药物设计方法，并将其应用于多个药物靶点，已获得多个原创性的候选药物。上述系列研究成果以“Allosteric methods and their applications: facilitating the discovery of allosteric drugs and the investigation of allosteric mechanisms”为题于 2019 年 1 月在线发表于国际著名学术期刊 *Acc Chem Res*，详细阐述了过去 10 年该研究组在变构机制和药物设计领域的工作。陆绍永副研究员是论文的第一作者，张健研究员是论文的通信作者。上海交通大学医学院细胞分化与凋亡教育部重点实验室是论文的通信单位。

近 20 年来，随着 X 射线晶体学、交换质谱、高通量筛选、电生理等实验技术的广泛应用，越来越多的变构蛋白和变构调节分子被鉴定出来。对变构分子结构的认识，包括蛋白的三维结构、变构调节剂的结构和变构蛋白－调节剂复合物结构等，均能促进基于结构的变构药物的开发。基于此，张健研究组于 2009 年起通过 10 年时间构建了变构知识数据库（AlloSteric database，ASD），对其中的变构位点、变构活性分子、变构调控网络、变构机制等进行系统的数据挖掘，创建了药物靶点变构组学（Allosterome），且已成为变构药物发现的起点。目前，ASD 已被 100 多个国家访问了 60 000 余次。

靶标中超过 90% 以上的都是蛋白质大分子，构象变化是决定蛋白功能发挥的动态开关。诱导蛋白构象变化，发现和改善靶标蛋白药效相关构象上的变构位点可为解决靶标难以利用这一问题提供契机。当前，变构位点的发现主要来自实验中的随机获得，缺乏对其本质上的理解和合理识别的方法，严重阻碍了其在创新药物研发中的应用。为解决上述瓶颈问题，张健等建立了用于发展和评价变构位点识别方法所需的标准体系（ASBench），为变构位点识别方法的建立和评价提供了高质量的数据基础和有益的方法指导。自 2015 年该标准体系对外公开以来，国内外新发展的变构位点识别方法中，有超过半数应用了 ASBench。

以 ASBench 为基础，张健等发展了变构位点比较组学的分析算法，阐明了变构位点空间和残基变化概率的分布，并在此基础上设计了普遍适用于蛋白质靶标的变构位点识别方法（Allosite）。Allosite 是国际上首个可以公开使用的变构位点识别方法，自 2013 年发布以来该方法的使用频率和识别性能均在同类方法中名列前茅。2017 年，张健等将动态微扰方法引入到 Allosite 中以提高其预测准确率，并利用该方法发现了 SIRT6 和 CK2 等新的变构位点。

根据对 ASD 中变构共晶结构的分析，张健等发现相比于活性位点的相互作用，变构位点的相互作用中疏水作用更多，导致现有的打分函数并不适用于评价变构分子和蛋白的结合模式。因此，2016 年张健等利用 ASD 中的变构共晶结构和亲和力数据，建立了变构结合模式打分函数（Alloscore）以预测变构小分子和蛋白的结合能力，为变构先导化合物发现和优化提供了专门的工具。

突变引起的变构调控失常往往与肿瘤的发生发展密切相关。张健等将 33 类肿瘤的 7 000 例临床样本中的突变映射到 ASD 中的变构蛋白结构上发现，这些突变中的有害突变主要富集在蛋白的变构位点和活性位点。基于上述结论，2017 年研究组开发了从临床样本直接识别肿瘤新靶标的方法（AlloDriver），即根据映射在变构位点的突变分布来识别针对不同肿瘤的靶标蛋白。利用 AlloDriver，张健等发现了非小细胞肺癌的新靶标 PDE10A，并且利用化学生物学的方法验证已上市的 PDE10A 的药物可以有效杀伤肺癌细胞株，为肺癌的治疗提供新的思路。

2018 年，经过多年来变构数据和方法的积累，张健研究组在 Allosite、Alloscore、Allosterome 等方法的基础上，通过构象采样方法发展了变构小分子药物发现和设计平台（AlloFinder）。用户可以利用 AlloFinder 发现新型变构调节分子并评估其生物学功能。基于此，张健等发现了 STAT3 位于 CC 结构域（coiled-coiled domain）的全新变构位点及靶向该位点的新型变构抑制剂 K116。经突变实验及细胞功能实验的验证，K116 的确是通过结合在 STAT3 新的变构位点来产生抗肿瘤效应。

综上所述，张健研究组创建了 ASD 和 ASBench 的变构研究准则，并以此为基础开发了一系列变构药物设计方法如 Allosite、Alloscore、AlloDriver、AlloFinder 等，分别用于解决变构位点的探测、变构结合模式的评价、变构靶标的识别及变构药物的开发等创新药物研发中的科学问题。更为重要的是，张健等利用该系列方法在多个实例上已得到了验证，例如 SIRT6、CK2、PDE10A、STAT3 等。这些药物设计方法为国际各大药物开发机构的研究人员理解变构机制和开发变构药物提供了可行的方法和实施案例。

Allosteric methods and their applications: facilitating the discovery of allosteric drugs and the investigation of allosteric mechanisms

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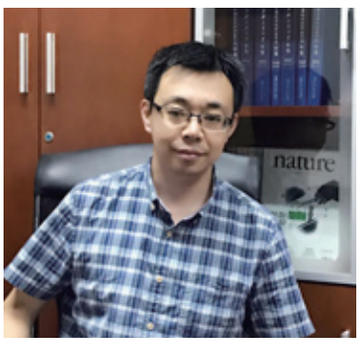
Abstract:

Allostery, or allosteric regulation, is the phenomenon in which protein functional activity is altered by the binding of an effector at an allosteric site that is topographically distinct from the orthosteric, active site. As one of the most direct and efficient ways to regulate protein function, allostery has played a fundamental role in innumerable biological processes of all living organisms, including enzyme catalysis, signal transduction, cell metabolism, and gene transcription. It is thus considered as “the second secret of life”. The abnormality of allosteric communication networks between allosteric and orthosteric sites is associated with the pathogenesis of human diseases. Allosteric modulators, by attaching to structurally diverse allosteric sites, offer the potential for differential selectivity and improved safety compared with orthosteric drugs that bind to conserved orthosteric sites. Harnessing allostery has thus been regarded as a novel strategy for drug discovery.

Despite much progress having been made in the repertoire of allostery since the turn of the millennium, the identification of allosteric drugs for therapeutic targets and the elucidation of allosteric mechanisms still present substantial challenges. These challenges are derived from the difficulties in the identification of allosteric sites and mutations, the assessment of allosteric protein-modulator interactions, the screening of allosteric modulators, and the elucidation of allosteric mechanisms in biological systems.

To address these issues, we have developed a panel of allosteric services for specific allosteric applications over the past decade, including (i) the creation of the Allosteric Database, with the aim of providing comprehensive allosteric information such as allosteric proteins, modulators, sites, pathways, etc., (ii) the construction of the ASBench benchmark of high-quality allosteric sites for the development of computational methods for predicting allosteric sites, (iii) the development of Allosite and AllositePro for the prediction of the location of allosteric sites in proteins, (iv) the development of the Alloscore scoring function for the evaluation of allosteric protein-modulator interactions, (v) the development of Allosterome for evolutionary analysis of query allosteric sites/modulators within the human proteome, (vi) the development of AlloDriver for the prediction of allosteric mutagenesis, and (vii) the development of AlloFinder for the virtual screening of allosteric modulators and the investigation of allosteric mechanisms. Importantly, we have validated computationally predicted allosteric sites, mutations, and modulators in the real cases of sirtuin 6, casein kinase 2α, phosphodiesterase 10A, and signal transduction and activation of transcription 3. Furthermore, our developed allosteric methods have been widely exploited by other users around the world for allosteric research. Therefore, these allosteric services are expected to expedite the discovery of allosteric drugs and the investigation of allosteric mechanisms.



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张　健（1978—），上海交通大学医学院研究员、课题组长，医药生物信息中心主任。2007 年获中国科学院上海药物研究所药学博士学位。2007—2009 年于美国密歇根大学从事博士后研究工作。2009 年回国受聘于上海交通大学医学院。

主要从事药物设计、药物化学和化学生物学研究，尤其专注于精准靶标识别和 first-in-class 原创药物先导发现等方向。以通信作者在 *Nat Chem Biol*、*Chem Rev*、*Chem Soc Rev*、*Am J Hum Genet*、*Nucleic Acids Res*、*J Med Chem* 等国际学术期刊发表 SCI 论文 70 余篇。近 5 年代表性工作包括：①发现肝癌靶标 SIRT6 原创激动剂 MDL-800（*Nat Chem Biol*，2018）。②发现结肠癌恶性转移靶标 APC-Asef 相互作用原创抑制剂 MAIT-203（*Nat Chem Biol*，2017）。③开发临床样本来源的全新靶标识别方法 AlloDriver（*Am J Hum Genet*，2017）。④开发变构药物设计方法以发现多种全新变构活性小分子（*Nucleic Acids Res*，2018、2016、2014、2011；*Bioinformatics*，2016、2015、2013）。由于“精准靶标识别和 first-in-class 药物发现”方面的贡献，先后获得美国化学会优秀导师奖、中国药学会生物医药创新奖、药明康德生命化学研究奖以及 2017 年中国十大科技新锐人物称号等。于 2013 年获国家自然科学基金优秀青年基金，2015 年入选中组部万人计划青年拔尖人才计划，2017 年获教育部青年长江学者荣誉称号，是国家自然科学基金创新群体成员。

ZHANG Jian born in 1978, professor, principal investigator, and director of Medicinal Bioinformatics Center of Shanghai Jiao Tong University School of Medicine. He got his Ph.D of pharmacy from Shanghai Institute of Materia Medica, Chinese Academy of Sciences in 2007. As a research fellow, he worked at University of Michigan from 2007 to 2009. Then he was employed by Shanghai Jiao Tong University School of Medicine since 2009.

Prof. ZHANG′s research interests mainly focus on drug design, medicinal chemistry, and chemical biology. Remarkably, he has made a series of cutting-edge breakthroughs in the identification of new anti-cancer drug targets and the discovery of first-in-class lead compounds. As the corresponding author, he has published over 70 SCI-indexed papers, such as *Nat Chem Biol, Chem Rev, Chem Soc Rev, Am J Hum Genet, Nucleic Acids Res, J Med Chem* and so on. The representative works over the past five years include: ① the discovery of first-in-class allosteric activators (MDL-800) for liver cancer drug target SIRT6 (*Nat Chem Biol*, 2018). ② the discovery of first-in-class inhibitors (MAIT-203) for metastatic colorectal cancer drug target APC (*Nat Chem Biol*, 2017). ③ the development of AlloDriver to precisely identify new anti-cancer drug targets from clinical samples (*Am J Hum Genet*, 2017). ④ the development of allosteric methods to discover allosteric modulators (*Nucleic Acids Res*, 2018, 2016, 2014, 2011; *Bioinformatics*, 2016, 2015, 2013). Owing to significant contributions in the field of precise identification of drug targets and first-in-class drug discovery, Prof. ZHANG has earned several awards, including Excellent Research Advisor of American Chemical Society, Biomedical Innovation Awards of Chinese Pharmaceutical Association, Wuxi AppTec Life Science and Chemistry Awards, Top Ten Cutting-Edge Scientists of China in 2017. He also won Excellent Youth Fund of National Natural Science Foundation of China in 2013, Outstanding Young Scholars of Ten Thousand Talent Program of Organization Department of Central Committee of CPC in 2015, and Changjiang Young Scholars of Ministry of Education of China in 2017. Currently, he is the member of Creative Research Groups of National Natural Science Foundation of China.

药物设计研究组

张健研究组一直致力于全新靶标识别和原创药物先导化合物的发现工作。利用药物设计、药物化学、生物信息学和化学生物学等方法，课题组构建了原创药物研发技术体系，针对肿瘤进行了系统的研究工作。通过发展变构药物设计系列方法，从临床样本中直接发现肿瘤的全新靶标，并克服靶标的位点缺陷发现原创抗肿瘤先导化合物。

Prof. ZHANG′s group works on the identification of novel targets and the discovery of first-in-class leads. By the integration of methods and techniques in drug design, medicinal chemistry, bioinformatics, and chemical biology, ZHANG′s group has set up a platform for drug discovery in Shanghai Jiao Tong University School of Medicine and made a systematic research against cancer. ZHANG′s group developed a set of allosteric drug design methods in the last ten years. Using these allosteric methods, they have found several novel targets directly from clinical cancer samples and then developed first-in-class anti-cancer leads against cancer by overcoming site deficiency.

5 篇代表性论文：

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