GlrR thus competes with DNA for the HTH motif of GlrA. These scientists used gel shift to show that GlrR can compete with DNA from GlrA at a concentration above 0.3 μM, but DNA cannot displace GlrR from a GlrR-GlrA complex. They showed also that the GlrR-GlrA complex regulates the ehx promoter positively and the fhhDC promoter negatively. Such a differential regulation allows pathogens to control precisely the gene expression involving its pathogenesis.

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Drug Discovery with Protein X-ray Crystallography

*This report features the work of Ya-Hui Chi, Hsing-Pang Hsieh, Su-Ying Wu and their co-workers published in Proc. Natl. Acad. Sci. USA **110**, E1779 (2013); of Su-Ying Wu, Hsing-Pang Hsieh and their co-workers published in J. Med. Chem. **56**, 3889 (2013); and of Pür Nordlund, E. Andreas Larsson and their co-workers published in J. Med. Chem. **56**, 4497 (2013).*

The objective of the huge investment in human genome projects and the subsequent structural genomics is to improve the quality of life. A direct result of this investment is the production of new and superior drugs to combat human diseases. Structurally based drug design is thus a main focus of beamlines for protein X-ray crystallography. Taiwan's government has supported our protein X-ray crystallography beamlines through National Research Program for Genomic Medicine in 2002-2010 and National Core Facility Program for Biotechnology since 2011. This year, three publications on the design of improved drugs are selected for this report.

One way to find suitable drug targets is to inspect proteins that are expressed abnormally in cells associated with certain diseases. Aurora kinases are the targets for several cancers. A few compounds for aurora kinases are currently in clinical trials. Aurora kinases are involved in chromosomal activities, especially during cell division and the separation of duplicated DNA. A group of scientists led by Ya-Hui Chi, Hsing-Pang Hsieh and Su-Ying Wu in National Health Research Institutes, Taiwan, characterized the inhibitors of aurora kinases, IBPR001 and IBPR002, and found efficacy better than MLN8237 and VX-680, the first-generation small molecules, to inhibit

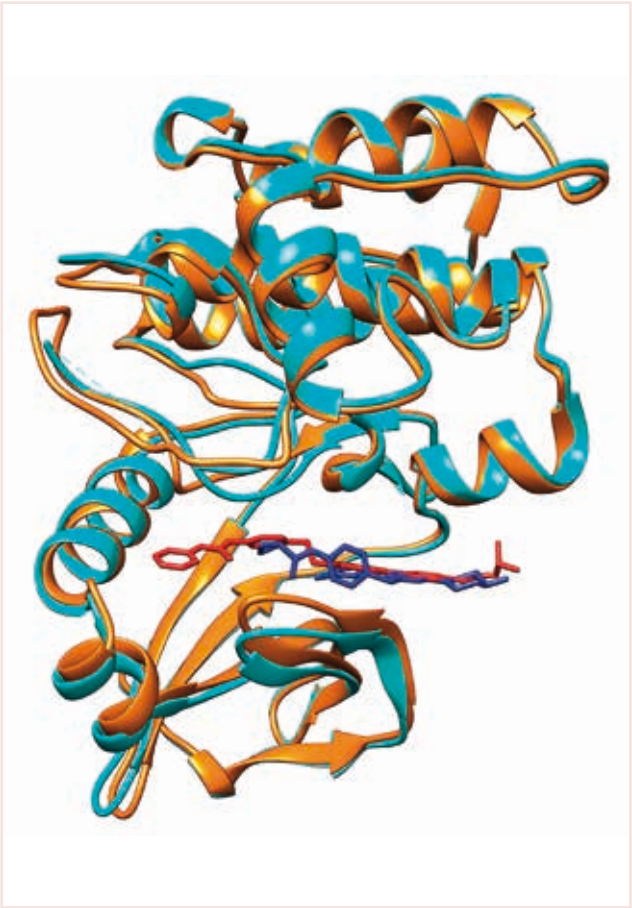

Fig. 1: Compare structures of Aurora A/IBPR001 and Aurora A/VW-680. Superimpose Aurora A/IBPR001 (Aurora A in orange and IBPR001 in red) and Aurora A/VX-680 (Aurora A in cyan and VX-680 in blue). IBPR001 clearly extends further into back pocket of Aurora A, thus, the binding affinity is increased.

aurora kinases.[1] They investigated the phosphorylation of human hepatoma up-regulated protein (HURP), a substrate of aurora kinases, affected by these inhibitors.

They solved the structure of Aurora A kinase with IBPR001 (PDB: 4JBO) and VX-680 (PDB: 4JBQ). Both compounds sit inside the ATP-binding pocket and form hydrogen bonds with Glu211 and Ala213 in the hinge region, but the diphenylurea moiety of IBPR001 reaches deeper into the back pocket whereas VX-680 cannot (Fig. 1). The DFG (Asp-Phe-Gly)-in conformation of the active loop adapted by Aurora A/IBPR001 structures shows that the conserved Glu181 forms two hydrogen bonds with the phenylurea group of IBPR001, whereas Phe275 of this motif points at the cyclopropyl group of VX-680. They also solved the structure of Aurora A kinase with IBPR002 (PDG: 4JBP). The complex structures of Aurora A-IBPR002 are superimposable with Aurora A-IBPR001 and an additional piperidinol group extended toward the solvent region. They further tested IBPR002 on mice and found that it works well, similarly as VX-680. The growth of xenograft colorectal cancer cells is significantly inhibited. They also found the underlying reason that the phosphorylation of HURP is inhibited. Its role in spindle formation and stability is thus affected. Both IBPR001 and IBPR002 have efficacy in inhibiting the HURP phosphorylation in cells better than VX-680 and MLN8237. This work used beamlines **BL13B1** and **BL13C1** at the TLS and Taiwan Beamline **BL12B2** at SPring-8.

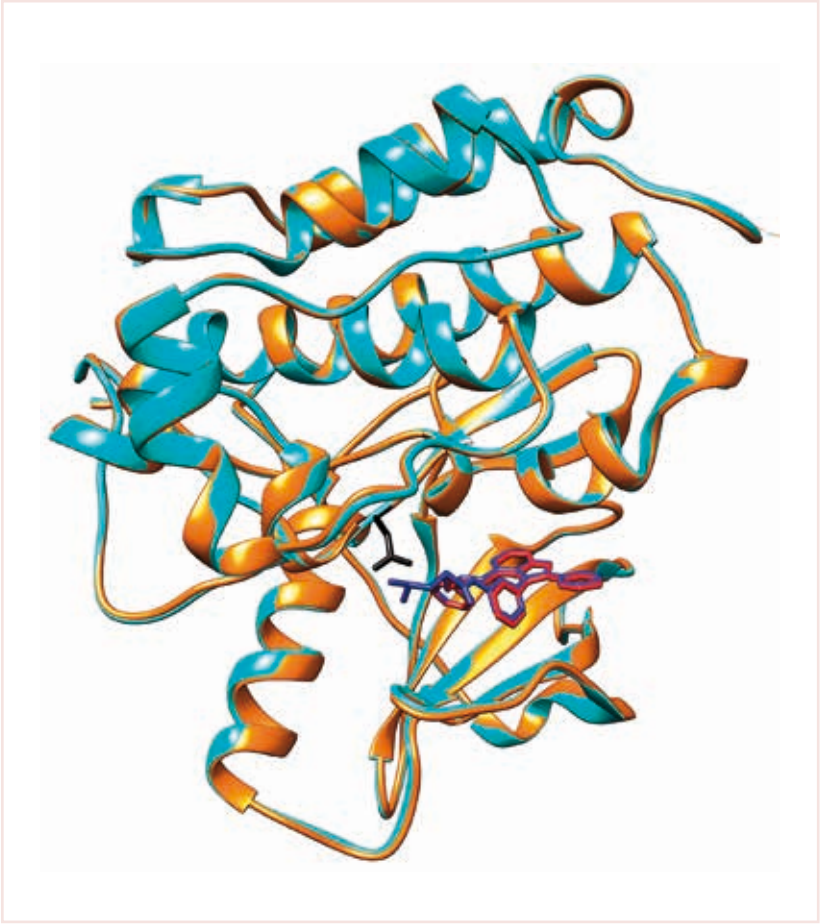

Fig. 2: Comparison of structures of EGFR/**2a** and EGFR/**4b**. Superimposed EGFR/**2a** (EGFR in orange and **2a** in red) and EGFR/**4b** (EGFR in cyan and **4b** in blue). The Asp831 (black) forms a salt bridge to **4b** but is too far from **2a**. **4b** is thus a 50-fold improvement in inhibiting EGFR.

Wu and Hsieh further investigated the active loop of kinase.[2] Kinase is an enzyme that transfers a phosphate group from high-energy molecules to proteins; this process is called phosphorylation. More than 500 kinases that are in human genome share a highly conserved catalytic domain. The kinase activity can be turned on and off with regulatory elements of which one is an activation loop, which undergoes a large conformational change from the closed to opened form upon phosphorylation to allow substrate binding. The highly conserved DFG motif is located at the beginning of the activation loop.

These scientists used epidermal growth factor receptor (EGFR, also called human ErbB receptor tyrosine kinase), a drug target in oncology, as a model. Overexpression of these kinases commonly results in cancers. Several drugs that inhibit EGFR have been approved by FDA: cetuximab, a monocolnal antibody, is for colorectal cancer and gefitinib, erlotinib and lapatinib are small molecules. The former two

are for non-small-cell lung cancer and the latter for combination therapy of HER2-positive breast cancer. Inhibitors to EGFR have, however, various adverse effects because these inhibitors affect other kinases in the human body. All these small molecules and second-generation ones bind to the hinge region of the EGFR kinase and either extend toward the solvent-exposed region or expand to the ATP binding site, which disrupts an important salt bridge. Based on their previous drug, **2a**, discovered with high-throughput drug screening, the group of Wu and Hsieh used structure-based drug design to develop a new one, **4b**, which can interact with the conserved DFG motif; the resulting compound has 50-fold improvement in EGFR inhibition. The N,N-dimethyl-amino tail forms a salt bridge with the side chain of Asp831. They first solved the structure of the EGFR kinase domain in complex with compound **2a** (PDB: 4JQ7). The structure is at an active conformation, and the salt bridge of Lys721 and Glu738 exists. **2a** is located in the ATP binding cleft, and the furanopyr-imidine core aligns in the hinge region. This structure shows a distance 5-7 Å between a perpendicular phenyl group (ring 5) and the side chain of Asp831.

A series of compounds were synthesized to diminish this gap; indeed **4b**, containing a two car-bon-chain linker, was the most potent one with 49-fold improvement. The structure of EGFR in complex with **4b** was then solved, and EGFR still adapted an active conformation with a salt bridge between Lys721 and Glu738 (PDB: 4JQ8). A special and strong hydrogen bond and charge network in the ATP-bind-ing pocket are formed with **4b**. In short, **4b** acts as a clamp that wraps around Asp831 to block the ATP-binding, thus to prevent phosphate transfer to a sub-strate. On comparison of the structures of EGFR/**2a** and EGFR/**4b**, the additional N,N-dimethylamino tails of **4b** provide an additional salt bridge with the kinase. This tail also provides extra intramolecular hydrogen bonds. All these enhancements explain the superior inhibition of **4b**. An interaction of inhibitors with the DFG motif has been observed in many kin-ase structures. It is worth noting that even **4b** targeted the conserved DFG motif; it exhibited great selectiv-ity in a small fraction of the protein kinases. The ex-planation is that the DFG motif might adapt to varied conformations in other kinases. Many other important residues are affected by the binding of this chemical compound. This work used beamlines **BL13B1** and **BL13C1** at the TLS and Taiwan Beamline **BL12B2** at SPring-8.

The group of Pür Nordlund and E.Andreas Larsson in Singapore and Sweden works on drugs targeting tankyrases, which is involved in cancers and myelin-degrading diseases.[3] They used a fragment-based ligand design to discover novel inhibitors. Tankyrase (TNKS) is in the family of poly-ADP-ribosylating enzymes (PARP) that transfer ADP-ribose moieties from NAD^+ to various substrates and typical-ly decrease activity of these protein substrates. TNKS1 and TNKS2 are tankyrases that have recently targeted in colon cancer. This group targeted TNKS2 and useed a combination of biophysical methods to dis-cover new inhibitors. A thermal shift assay was used to find and to optimize fragments that bind tightly to TNKS2. Protein X-ray crystallography was used to expand these fragments, and their properties were characterized by various techniques. An initial library of 500 fragments is used to bind with TNKS2; melting temperatures are measured over a range of fragment concentration. The idea is that a tightly bound frag-ment increases the melting temperature of TNKS2. While analysis of the data was not straightforward for TNKS2 and much analysis was done by manual inspection of raw data, this group nevertheless found two compounds – 4-methyl-1,2-dihydroquinolin-2-one (**2**) and 4-chloro-1,2-dihydrophthalazin-1-one (**3**), which stabilize TNKS2 by 1.1 and 1.3 °C at 1 mM. Further experiments confirm the validation. On solving the structures of TNKS2 with compounds **2** and **3** (PDB: 3W51 and 4J1Z, respectively), they con-cluded that the 7-position of **2**, pointing toward the extended pocket responsible for adenosine binding, is an interesting position for expansion. Compounds in a series are being synthesized based on this strat-egy, and compound **11**, bearing an *o*-fluoro substitu-ent, invoked a large shift of melting temperature even at small ligand concentrations. The structure of

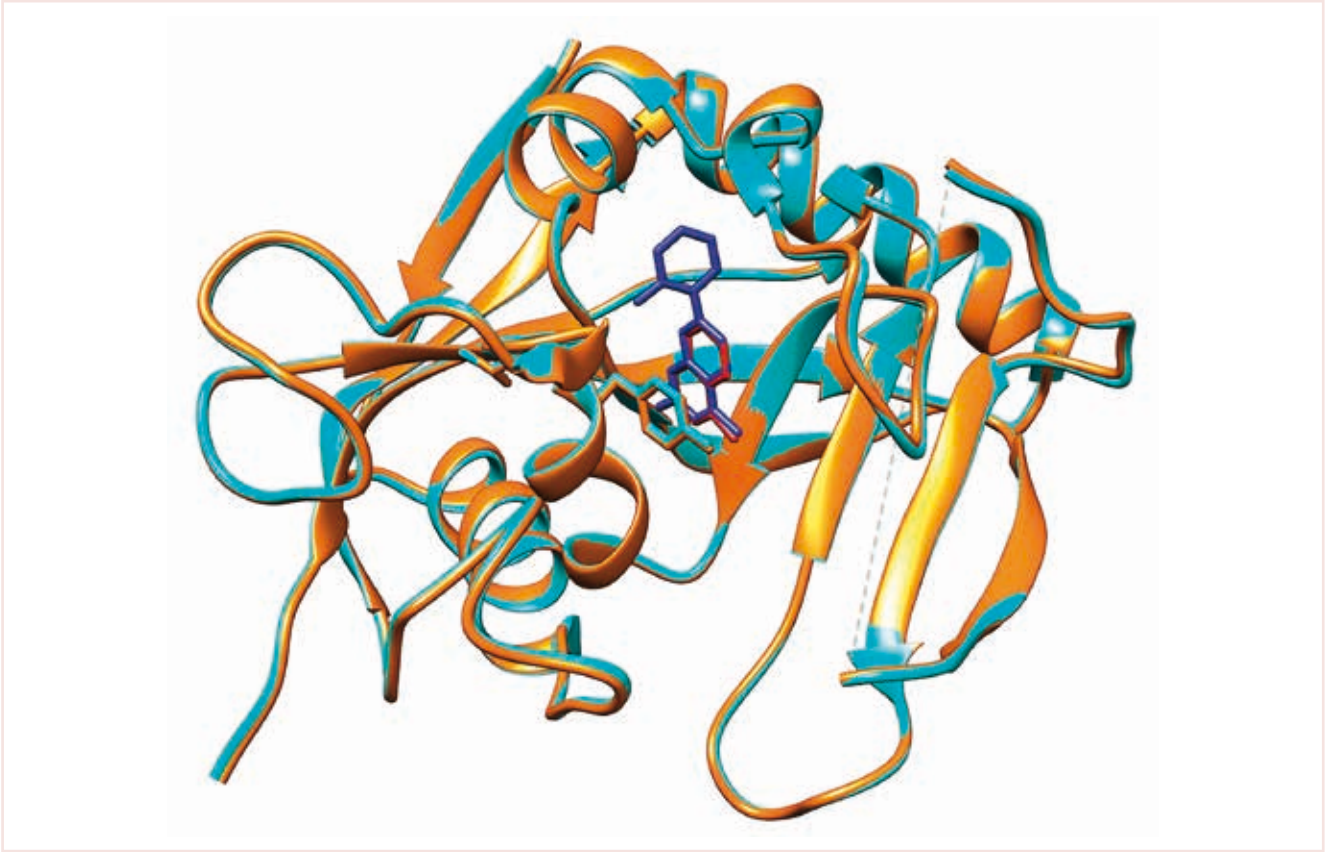

Fig. 3: Compare structure of TNKS2/**2** and TNKS2/**11**. Superimpose TNKS2/**2** (TNKS in orange and **2** in red) and TNKS2/**11** (TNKS2 in cyan and **11** in blue). The fluorine atom in compound **11** displaces a bonded water and interacts with carbonyl oxygen of Tyr1071.

TNKS2 with compound **11** was subsequently solved (PDB: 4IUE); this fluorophenyl moiety displaces a water in the structure of **2** and forms van der Waals interactions and nonpolar contacts with several residues (Ile1075, Phe1035, Tyr1050 and Pro1034). The analysis led to larger ortho- and para-substituents to maximize the interactions with Tyr1071. Several more compounds were synthesized and the structures were solved in complex with TNKS2. Some compounds eventually have the best binding affinity and a property to inhibit TNKS2 in their work. The selectivity is also improved during the process. This work used beamline **BL13B1** at the TLS and MX1 at Australia Synchrotron.

The fragment-based strategy led cleverly to a rapid development of high-affinity ligands for TNKS. By iterating through several biophysical techniques, including thermal shift assay and protein X-ray crystallography, less than 40 compounds were synthesized in this work; the efficiency is much greater than in traditional drug screening.

We can report only briefly on these discoveries. Each story deserves a detailed look at the original papers. Their efforts might one day become products for whoever needs them. That investment would never be too great.

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