Synchrotron-Based Protein Crystallography at NSRRC Provides Opportunities in Precision Medicine

Recent advances in X-ray crystallography, cryo-electron microscopy (cryo-EM) and related biology-system techniques speed uncovering the mystery based on developments in proteomics, genomics and glycomics. Synchrotron-based X-ray crystallography is a powerful technique to resolve crystal structures of biological molecules based on the X-ray diffraction of a single crystal of a biological sample in diagnosing cancer, chronic disease, autoimmune diseases and disorder, and for the purpose of drug design.

In year 2018, NSRRC users unfolded several crucial biological complicated structures related to disease, such as the echinomycin–DNA complex, open-stated topoisomerase II (Top2) DNA-gate, and three prime repair exonuclease 1 (TREX1). Synchrotron-based technology hence plays a crucial role to create opportunities for opening a window to future diagnostic medicine, medical therapy and drug design. And precision medicine, attracts persons concerned about the issues of health care, preventive medicine and disease therapy, which is an approach to set a therapy strategy for precision treatment of patients based on fully understanding the proteomic and genetic information of a disease. According to this fundamental biological knowledge, incorrect base pairing and mutations of the DNA molecule are known to be highly possibly induced by an alteration of the interaction between DNA bases and damaged DNA bases, sufficient to cause genetic disease and cancer. For eight non-Watson-Crick alternatives, throughout the replication processes, the mismatches sometimes occur in the DNA duplex, but potentially mutagenic and caused local structural deformations can make a mismatch-repair system rapidly reform the damaged DNA bases. Deficiencies in the mismatch-repair system enhance the possibility of mutation and consequently increase the risk of genetic disease and cancer. Given the intrinsic pleiotropy of carcinogenesis and the steady advance towards targeted therapy, an ability to pinpoint whether a particular cancer arises from a specific deficiency of the mismatch-repair system or from other causes could be highly applicable in diagnostic medicine for cancer diagnosis and therapy. Based on the structure-specific recognition of echinomycin-targeting mismatched base pairs in DNA, it would open a clinical possibility for cancer diagnosis and therapy. Echinomycin, a potent anticancer antibiotic, functions by intercalating into DNA at sites of cytosine and guanine separated by only one phosphate group (CpG). Pei-Ching Wu (National Chung Hsing University) and his collab-

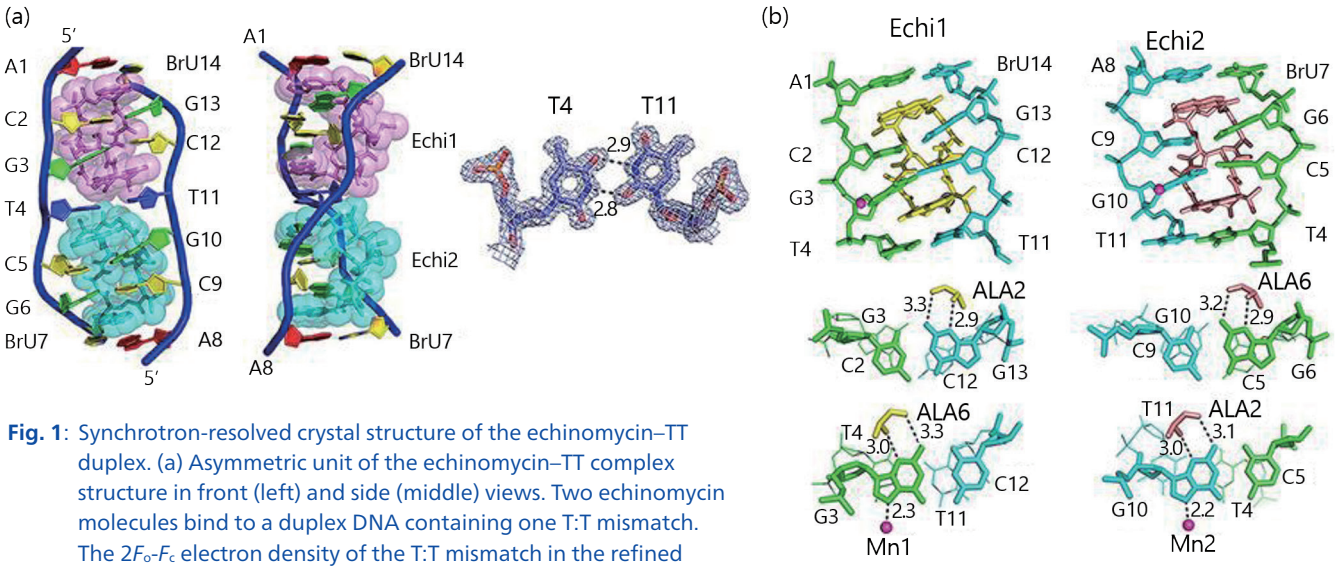

Fig. 1: Synchrotron-resolved crystal structure of the echinomycin–TT duplex. (a) Asymmetric unit of the echinomycin–TT complex structure in front (left) and side (middle) views. Two echinomycin molecules bind to a duplex DNA containing one T:T mismatch. The $2F_o$-F_c electron density of the T:T mismatch in the refined structure is shown at right, contoured at 1.0 σ. Hydrogen bonds are represented with dotted lines; numbers indicate the distance between the two contributing nuclei in Angstroms. (b) Skeletal models showing antibiotic binding sites in the echinomycin–TT complex. The diagrams illustrate the stacking interactions and hydrogen bonding at the CpG steps. Mn^{2+} ions are shown as magenta spheres. Dotted lines represent the direct interactions between nuclei; numbers show the distances. [Reproduced from Ref. 1]

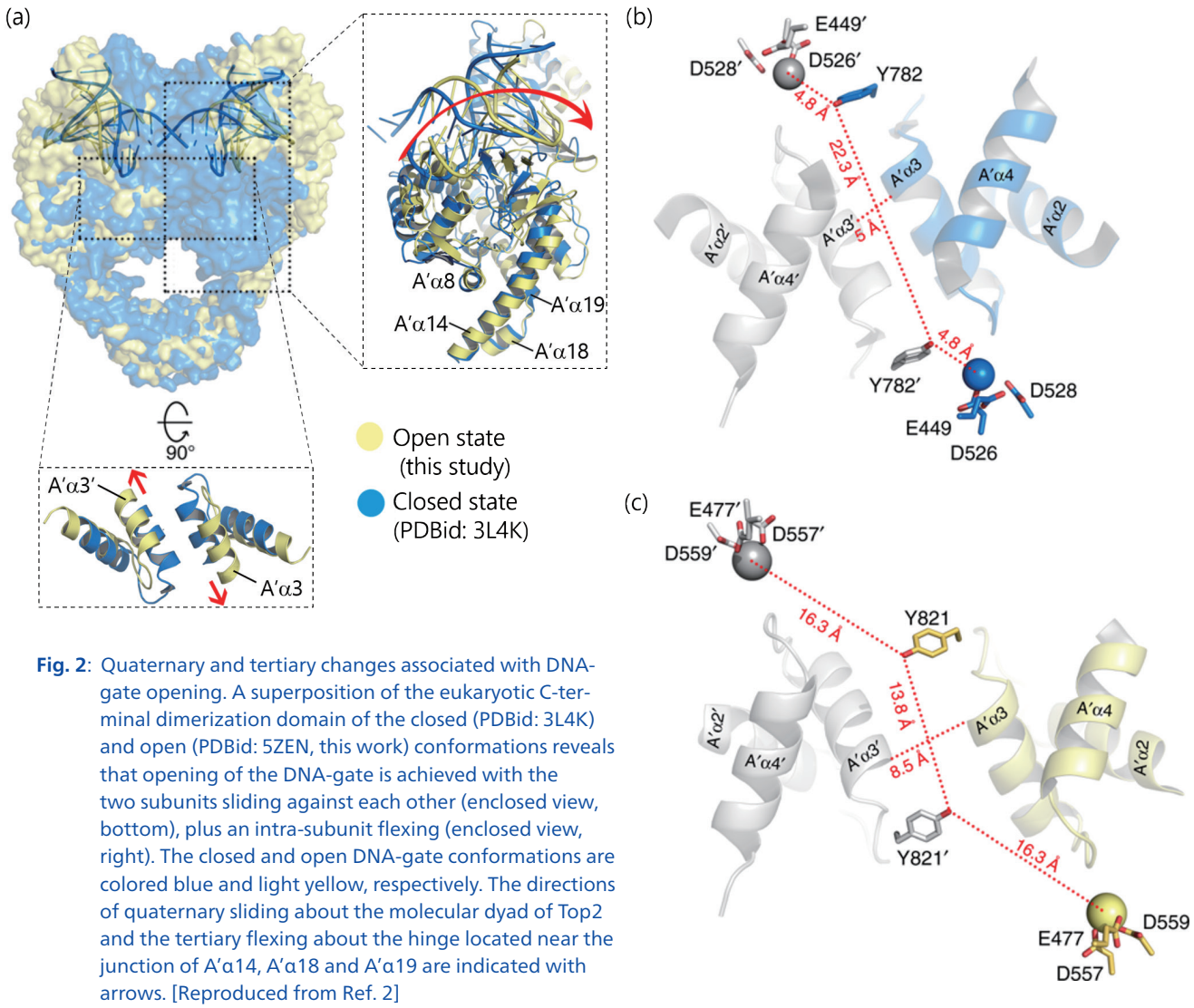

Fig. 2: Quaternary and tertiary changes associated with DNA-gate opening. A superposition of the eukaryotic C-terminal dimerization domain of the closed (PDBid: 3L4K) and open (PDBid: 5ZEN, this work) conformations reveals that opening of the DNA-gate is achieved with the two subunits sliding against each other (enclosed view, bottom), plus an intra-subunit flexing (enclosed view, right). The closed and open DNA-gate conformations are colored blue and light yellow, respectively. The directions of quaternary sliding about the molecular dyad of Top2 and the tertiary flexing about the hinge located near the junction of A′α14, A′α18 and A′α19 are indicated with arrows. [Reproduced from Ref. 2]

orators found that echinomycin binds specifically to consecutive CpG steps separated by a single T:T mismatch[1] as shown in **Fig. 1**. The conformation specificity appears to result from an enhanced cooperativity associated with another echinomycin insertion. The structure of the echinomycin–TT duplex resolved with synchrotron X-ray crystallography revealed that this preference originates from the staggered quinoxaline rings of the two neighboring molecules of echinomycin surrounding the T:T mismatch to form continuous stacking interactions within the DNA duplex. Unfortunately, echinomycin is an inhibitor of topoisomerase II (Top2), DNA helicase and DNA methyltransferase 1 partially in vitro, and is also highly cytotoxic and failed in most pre-clinical trials. Nevertheless, the authors propose that the enhanced cytotoxicity of echinomycin can be utilized against mismatch-repair-deficient (MMR-D) cell lines, which might raise the possibility of detection and treatment of MMR-D cancers in future.

Echinomycin also plays a role as an inhibitor of type-IIA topoisomerases (Top2s) known as an enzyme to relax supercoiled DNA and to introduce supercoils of both positive and negative contortions, which are found ubiquitously in eukaryotes and bacteria as essential DNA-manipulating enzymes. These ATP-driven enzymes direct the crossing of two DNA duplexes on exploiting protein conformational changes for binding and hydrolysis, which leads to a topological inversion of DNA molecule crossing. The temporary creation of a DNA double-strand break (DSB) on one DNA segment (G-segment) is necessary for a topological transformation of the Top2- mediated DNA to transport the duplex strand (T-segment) through. Fully understanding the biological response and function caused by varied conformations of DNA topoisomerases is hence expected to facilitate the developing targets of novel therapeutic agents in the treatment of infectious diseases. Top2s has one role to manipulate the handedness of DNA crossings and to introduce the DNA-gate, of which the molecular opening allows transport through another DNA segment to alter the DNA topology. As far as we know, all reported structures of Tops-

DNA for the DNA-gate are in the closed state. The research team led by Nei-Li Chan (National Taiwan University) is the first to report the high-resolution structure of Top2 DNA-gate in the open form,[2] as illustrated in **Fig. 2**, using protein crystallography facilities at **TLS 15A1** and **TLS 13C1** as well as at **SP12B2**, which directly mediates the resolution of topological strand crossings. This open structure unfolds the formation and molecular geometries of the T-segment-conducting path and even uncovers, unexpectedly and functionally relevant, the 3D conformational changes from closed form to open form of geometry. Semi-empirical theoretical calculation was utilized with steered molecular-dynamic (SMD) simulations to predict the reaction passage of the T-segment through the DNA-gate so as to provide further molecular insight into this central, yet elusive, step in Top2 catalysis.

In addition to the issue of incidence of cancer disease, a large human population suffers from inflammatory disease, which is an immune response due to white blood cells and cytokines produced to fight infection with foreign organisms, such as bacteria and viruses. The autoimmune disease is, however, a system of ourselves, introducing a chronic or acute inflammation response, and the causes of autoimmune diseases are generally unclear. Organ failure typically occurs in a late stage of disease caused by persistent fibrosis of the tissue. Chronic inflammation would also stimulate various cancers through DNA damage associated with persistent inflammation, possibly leading to cancer in turn.[3] Fortunately, the possibility of curing autoimmune diseases is increasing because of continuous development and improvement of the techniques of synchrotron-based crystallography, cryo-EM and the assistance of artificial intelligence in precision medicine. Kuan-Wei Huang (National Chiao Tung University) and his team reported the high-resolution structure of TREX1 resolved at the synchrotron-based crystallography facility in TPS;[4] one resolved structure is shown in **Fig. 3**. Malfunctioning of TREX1 is one factor that leads to inflammation and autoimmune disease such as inflammatory cardiomyopathy associated with an inflammatory heart muscle related to impaired function of the myocardium, systemic lupus erythematosus inducing inflammation in connective tissues, Aicardi-Goutières syndrome that is an inflammatory disorder most typically affecting the brain and the skin, retinal vasculopathy with cerebral leukodystrophy caused by progres-

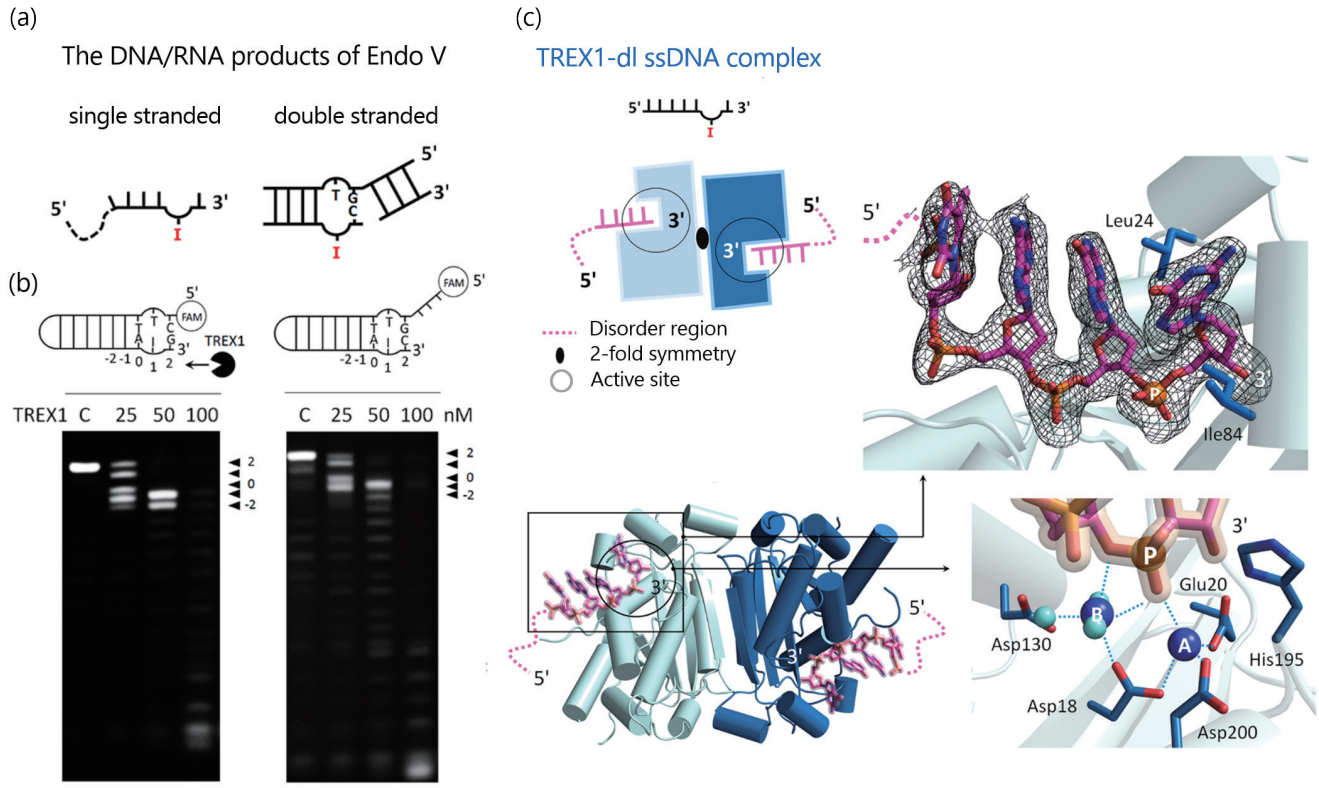

Fig. 3: TREX1-dI-ssDNA structure. (a) Schematic representation of DNA/RNA products generated by Endo V. (b) Nuclease activities of TREX1 in digesting bubbled DNA containing a hypoxanthine base (also named dI), including dI-bubbled DNA and dI-bubbled DNA with 50-overhang. The concentration of all substrates was 0.5 μM. (c) Overview of the TREX1-dI-ssDNA structure. The upper panel shows the dI-ssDNA molecule in the TREX1-dI-ssDNA structure. The omitted electron density map (black) is contoured at 2.0 σ. Scissile phosphate, Mg^{2+} and water molecules are shown in orange, blue and light blue balls, respectively. The hydrogen bonds between DNA, TREX1, water and Mg^{2+} are marked with dotted blue lines. Abbreviations are dI, deoxyinosine; Endo V, endonuclease V; Mg^{2+}, magnesium ion; ssDNA, single-stranded DNA; TREX1, three prime repair exonuclease 1. [Reproduced from Ref. 3]

sive loss of capillaries caused by genetic disorder ultimately resulting in visual deterioration and a series of mini-strokes in the brain, and familiar chilblain lupus, which is a monogenic form of cutaneous lupus erythematosus in TREX1-deficient human beings. TREX1 is hence considered to take part in various cellular events such as DNA repair, immune regulation and viral infection. In addition to autoimmune-related diseases, this exonuclease might serve as a protein target for anticancer or antiviral therapies. A key for such broad attendance of TREX1 is the activity of precise trimming of the 30-overhang in a double-stranded DNA (dsDNA) and breaking of the terminal base pairing of the DNA duplex. The work of Huang established an integrated structural view of the versatile exonuclease functions of TREX1 and illuminated the molecular origin of unique catalytic properties of TREX1 in processing various DNA intermediates in the repair of DNA and in regulation of cytosolic immunity.

Overall, these collections of recent studies provide unprecedented knowledge at the molecular level of the echinomycin–DNA complex, the conformational change of the topoisomerase II (Top2) DNA-gate, enzymatic substrate processing involved in targeting cancer therapy or diagnosis, prevention of immune activation and responses to genotoxic stresses. All data of crystal structures were acquired at **TLS 13C1, TLS 13B1, TLS 15A1, TPS 05A** at NSRRC, and **SP12B2** at SPring-8. (Reported by Yao-Chang Lee)

This report features the work of: (1) Pei-Ching Wu and his co-workers published in Nucleic Acid Res. ***46****, 7396 (2018); (2) Nei-Li Chan and his co-workers published in Nature Commun.* ***9****, 3085 (2018); (3) Kuan-Wei Huang and his co-workers published in PLoS Biol.* ***16****, 2005653 (2018).*

TPS 05A1 Protein Microcrystallography
TLS 13B1 SW60 – Protein Crystallography
TLS 13C1 SW60 – Protein Crystallography
TLS 15A1 Biopharmaceutical Protein Crystallography
SP12B2 BM – Protein X-ray Crystallography
- Protein Crystallography
- Biological Macromolecule, Protein-DNA Structure, Life Science

References

1. P.-C. Wu, S.-L. Tzeng, C.-K. Chang, Y.-F. Kao, M.-J. Waring, M.-H. Hou, Nucleic Acid Res. **46**, 7396 (2018).
2. S.-F. Chen, N.-L. Huang, J.-H. Lin, C.-C. Wu, Y.-R. Wang, Y.-J. Yu, M. K. Gilson, N.-L. Chan, Nature Commun. **9**, 3085 (2018).
3. S. I. Grivennikov, F. R. Greten, M. Karin, Cell, **140**(6), 883 (2010).
4. K.-W. Huang, T.-C. Liu, R.-Y. Liang, L.-Y. Chu, H.-L. Cheng, J.-W. Chu, Y.-Y. Hsiao, PLoS Biol. **16**, 2005653 (2018).

Electron and Proton Transports in quinol:fumarate Reductase Provide Energy for Bacteria

The quinol:fumarate reductase (QFR), a membrane protein involved in anaerobic respiration with fumarate as the terminal electron acceptor, is utilized to produce usable chemical energy in bacteria. The electron and the coupled proton-transfer paths of QFR were elusive. Based on the structure of QFR from Desulfovibrio gigas, an anaerobe, the electron and proton paths of QFR are delineated.

Electron transport chains, comprising redox reactors (reduction and oxidation simultaneously occurring *via* redox reactors) in a series, are utilized to harvest energy in living organisms. The electrons flow from electron donors to acceptors, and are coupled with a proton transfer to compensate the excess charge in the cell membrane. Taking photosynthesis for example, plants utilize the electron-transport chain to harvest energy *via* redox reactions in a series from sunlight. The membrane-embedded quinol:-fumarate reductase (QFR), isolated from anaerobic bacteria *Desulfovibrio gigas* (*D. gigas*), is the target