Producing Irreversible Topoisomerase-II-Mediated DNA Breaks

A coordination bond formed between a transition-metal ion and a reactive side chain in a protein might exhibit a conditional liability and alter the spatial arrangement of ligands, prospectively employing metal coordination chemistry in drug development.

Imbalances in enzyme activity are etiological factors for numerous diseases, including inflammation, metabolic disorders, cardiovascular irregularities and cancers. The modulation of an enzyme function with bioactive small molecules is hence a commonly employed therapeutic strategy; many successful drugs are enzyme inhibitors or poisons. Most drugs bind their targets *via* non-covalent forces, rendering the interactions reversible in nature. In contrast, irreversible inhibition has been achieved mainly via the formation of a covalent bond between an inhibitor and its target. Despite the superior potency *in vitro* displayed by these so-called covalent inhibitors, their broader clinical applications are generally limited by pronounced adverse effects due to off-target reactivity and potential immunogenicity arising from the resulting protein-inhibitor adducts. Knowing that the stability of coordination complexes is determined in part by the number and geometric distribution of metal-coordinating ligands, Nei-Li Chan and Tsai-Kun Li of National Taiwan University, Tun-Cheng Chien of National Taiwan Normal University and their coworkers envisaged that the coordination bond formed between a transition-metal ion incorporated in an organic scaffold and a reactive side-chain functional group(s) in a target protein might exhibit a conditional liability. Perturbing the conformational state of the target protein might alter the spatial arrangement of ligands, leading to a rupture of the coordination linkage.

In previous work, structural analyses revealed drug intercalation between the base pairs flanking the DNA cleavage site, which effectively stabilizes Top2cc by blocking religation of the cleaved DNA ends.[1] The specificity displayed by these drugs towards the site of Top2-induced DNA cleavage can be rationalized by their interactions with the surrounding protein residues. The presence of a methionine residue(s) in the drug-binding pocket in two human Top2 isoforms hTop2α and hTop2β indicates that site-specific incorporation of a Pt^{2+} reactive center into a drug might enable the formation of a Pt^{2+}-thioether bond with the methionine side chain and boost the drug's efficacy by strengthening its interaction with human Top2cc. The Top2-targeting anticancer drug etoposide is an ideal candidate to test this concept because of the well comprehended relation between structure and activity regarding its three constituting moieties. The tetracyclic aglycone core composed of rings A–D mediates DNA intercalation and the appended E-ring provides specific interactions with protein residues located on the DNA minor groove side; both moieties are required for optimal drug action and are sensitive to modifications.[2] Conversely, the pocket that houses the glycosidic group on the DNA major groove side not only is spacious enough to accommodate structurally distinct chemical groups but also harbors a potentially Pt^{2+}-reactive methionine residue(s). Replacing the glycosidic moiety with a Pt^{2+}-containing group might thus allow the formation of a Pt^{2+}-thioether bond between the drug and hTop2 isoforms. A diammine linker has already been used to introduce Pt^{2+} into podophyllotoxin. The modeling analysis by the authors indicated that adjusting the length of the reported linker could place the Pt^{2+} within a favorable distance to conjugate to a nearby methionine and to confer a potent Top2-poisoning activity on the resulting compounds. They proposed to name these compounds etoplatins, representing Pt^{2+}-conjugated etoposide derivatives (**Fig. 1**).

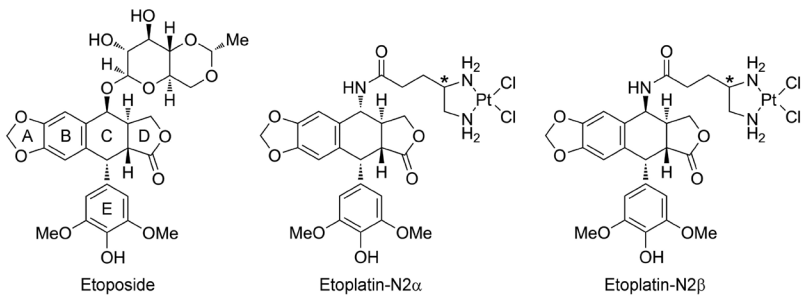

Fig. 1: Polycyclic aglycone rings A–D and pendant ring E of etoposide are labeled. A cis-dichlorodiammineplatinum(II) moiety was introduced *via* an amide linkage to the C4 position of the aglycone core in α and β configurations about ring E to produce etoplatin-N2α and N2β, respectively. [Reproduced from Ref.3]

To examine the effects of etoplatins on the catalytic functions of Top2, the authors first compared the potency of these platinum organometallic compounds in blocking the relaxation of Top2-mediated DNA with that of etoposide. Although etoplatin-N2α and etoposide displayed similar inhibitory activities, a concentration of etoplatin-N2β one twenty-fifth that of etoposide sufficed to produce a comparable effect, indicating that etoplatin-N2β is significantly more effective in inhibiting the relaxation activity of both hTop2α and hTop2β. Given that etoplatin-N2β is produced on replacing the glycosidic moiety of etoposide with a thioether-directed reactive center containing Pt^{2+}, and that both human Top2 isoforms exhibited increased sensitivity towards etoplatin-N2β, they reasoned that the Pt^{2+} center of etoplatin-N2β most likely forms a coordinate bond with the side-chain thioether moiety of Met766 in hTop2α and the spatially equivalent Met782 in hTop2β. This speculation that a Pt^{2+}-thioether coordinate bond is formed between etoplatin-N2β and Top2 predicted an enhanced stability of the resulting cleavage complex, presumably less reversible and thus more resistant to EDTA treatment. Whereas the DNA breaks induced by etoposide are readily reversible on pre-treating the cleavage complex with EDTA, as indicated by the disappearance of the smeared DNA fragments and restoration of the full-length linear substrate DNA, the breakage resulting from etoplatin-N2β-mediated hTop2 poisoning cannot be resealed.

roplatinum(II) moiety in the general vicinity of the methionine residue(s) located in helix α4 of the Top2 winged-helix domain. For etoplatin-N2β, one chloride ion is replaced by the methionyl S^{δ} of M782 in hTop2α (M766 in hTop2β) with electron density clearly connecting S^{δ} and Pt^{2+}, indicating that a coordinate bond with a refined bond length 2.3 Å has been formed (**Fig. 2**). Because of the structural constraints imposed by the alternative stereochemistry at the C4 chiral center, the dichloroplatinum(II) moiety of etoplatin-N2α is directed nearer the +1/+4 base pair, which places the Pt^{2+} more distant (~4.3 Å) from the methionyl S^{δ} (**Fig. 2**). No coordinate bond formation was observed and both Pt^{2+}-ligating chloride ions are retained in etoplatin-N2α. Together, the results obtained from crystallographic and modeling analyses provide convincing evidence that etoplatin-N2β acts as a potent and irreversible poison of human Top2 isoforms through its capability of forming a coordinate bond.

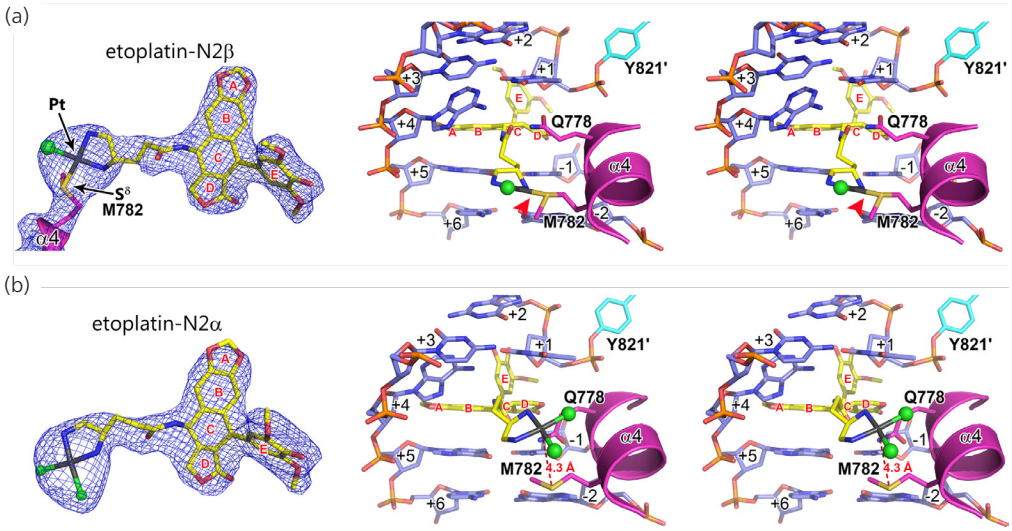

Fig. 2: Detailed view of the etoplatin binding site. Both etoplatin-N2β and -N2α bind to the DNA cleavage sites in the hTop2βcc crystal structure as etoposide, but only etoplatin -N2β forms an irreversible Pt^{2+}-thioether coordinate bond. [Reproduced from Ref. 3]

To confirm the proposed mechanisms of the action of etoplatins, this team performed X-ray crystallographic analysis on the etoplatin-stabilized cleavage complexes of hTop2β using beamlines **TLS 13B1** and **TLS 15A1.**[3] Similar to etoposide, both etoplatins trap Top2cc on targeting the enzyme-mediated DNA breaks, with the aglycone core intercalating between the base pairs flanking the cleavage site and ring E protruding towards the DNA minor groove to interact with the surrounding residues (**Fig. 2**). As expected, the diammine linker extends towards the side with the DNA major groove and places the reactive dichlo-

In summary, the authors performed a structure-based development of an etoposide derivative containing a dichloroplatinum(II) moiety to show that highly efficient enzyme-targeting is achievable on employing Pt^{2+} coordination chemistry.[3] Their work demonstrates a potential benefit of employing metal coordination chemistry in drug development. (Reported by Chun-Jung Chen)

This report features the work of Nei-Li Chan, Tsai-Kun Li and their co-workers published in Nucleic Acids Res. ***45****, 10861 (2017).*

TLS 13B1 SW60 – Protein Crystallography
TLS 15A1 Biopharmaceuticals Protein Crystallography

- Protein Crystallography
- Biological Macromolecule, Protein-DNA Structure, Life Science

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Parity-Dependent Slippage of DNA Hairpins for a Disease-Associated Repeat Expansion

The structure of the repeat hairpin provides a clue to understand the initial expansion of repetitive DNA sequences associated with neurological diseases.

Repetitive DNA sequences play a vital role in the maintenance of normal function and pathology. The expansion of DNA repeats, even in non-coding regions of the genome, might disrupt cellular replication, repair and recombination and ultimately lead to altered gene expression. DNA repeat expansions of many types are associated with neurological diseases that wreak devastating consequences.[1,2] To complicate matters further, pathological DNA expansions might occur spontaneously, so there is a great interest in understanding their mechanism.

It is generally acknowledged that hairpin loops (**Fig. 1(a)**) are critical for the expansion of repetitive DNA sequences, but the relation between the hairpin structure and the initiation of expansion remains unclear. A collaborative team led by Ming-Hon Hou (National Chung Hsing University) and I-Ren Lee (National Taiwan Normal University) combined X-ray crystallography with various biophysical methods to provide clues to this initiation.[3] They studied the behavior of a pentanucleotide TGGAA repeat hairpin, which is associated with a spinocerebellar ataxia type 31; using single-molecule fluorescence resonance-energy transfer (smFRET), they found that the hairpin was able to interconvert dynamically (slip) be-

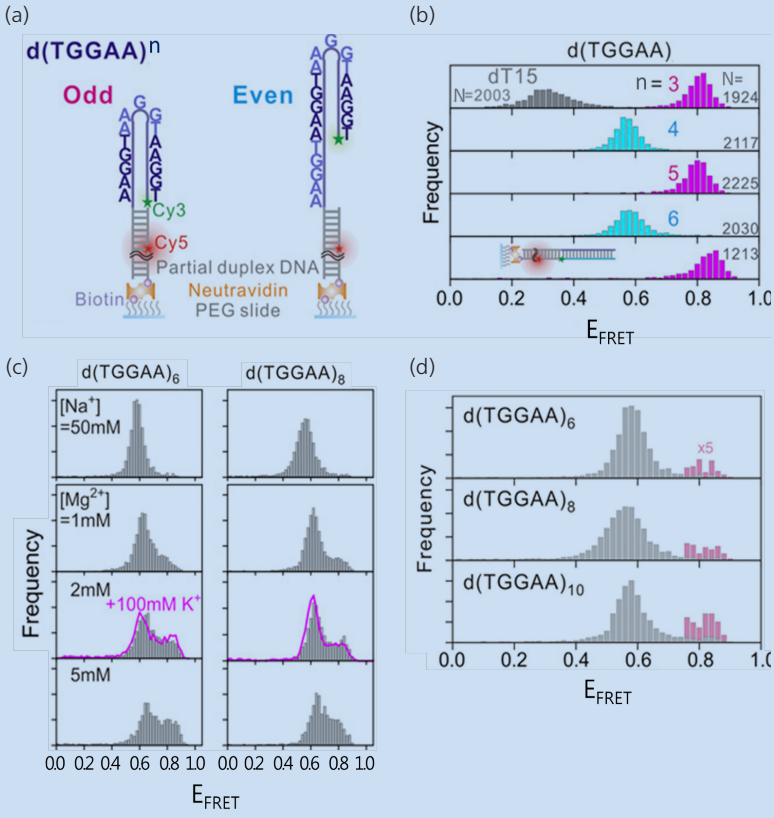

Fig. 1: Structural characterization of $d(TGGAA)_n$ using single-molecule FRET. (a) Illustrations of the single-molecule assay used in this experiment. (b) E_{FRET} histograms of $d(TGGAA)_{3-6}$ (colored) and the assay used as a caliper of the end-to-end alignment (cartoon at bottom). (c) E_{FRET} histogram of $d(TGGAA)_{6,8}$ under various salt conditions. The fractions of E_{FRET} > 0.8 increase with increasing concentrations of Mg^{2+}. (d) E_{FRET} histograms of $d(TGGAA)_n$, with n = 6, 8 and 10. [Reproduced from Ref. 1]