# Crowning Proteins: Modulating the Protein Properties Using Crown Ethers

*This report features the work of Cheng-Chung Lee, Manuel Maestre-Reyna, Andrew H.-J. Wang and their co-workers published in Angew. Chem. Int. Edit.* ***53****, 13054 (2014).*

Although protein X-ray crystallography is the main path to obtain structural information about proteins, protein crystallizability is still the main bottleneck in efforts on structural genomics. An optimization of crystallization is typically achieved on only a basis of case by case, trial and error. In contrast, on first identifying a ring-shaped binding mode for low-molecular weight polyethylene glycol (lmw PEG) in several protein crystal structures, Andrew H.-J. Wang and his co-workers established crown ether (18-crown-6; CR) as a reliable additive for crystallization and a powerful tool for crystallization. Their work shows that crown ethers can modify greatly the surface behavior of a protein by stabilizing either intra- or intermolecular interactions. Crown ethers can serve to modulate the diverse behavior of protein surfaces beyond crystallization, such as oligomerization, domain-domain interactions and stabilization in organic solvents.

As a basis to establish CR as surface modulator, they studied CR structural analogues, lmw PEG (MM < 600 g/mol). In protein co-crystal structures, these show two distinct conformational types—linear lmw PEG presenting an extended conformation and ring-shaped lmw PEG with a conformation similar to the CR structure. In the latter type, they typically make van der Waals (vdW) contacts with aromatic or aliphatic residues, or they coordinate primary amines (lysine) or guanidinium moieties (arginine) (Fig. 1(a)). They showed also that 68% of all deposited ring-shaped lmw PEG, but only 58% of linear PEG, mediated protein–protein contacts in the crystal. These observations indicate that CR, having physicochemical properties similar to those of lmw PEG, might be more constrained and hence better chelators and vdW partners for protein surfaces.

To investigate the effects of CR in protein crystallization, they performed sparse-matrix crystallization screening on several protein targets—Pin1R14A, DMP19, Rbma, SARS-CoV 3CL protease, lysozyme, myoglobin, and trypsin—based on common commercially available conditions. We found that CR affected crystal growth positively in most cases, with an exception of trypsin.

On solving the structures of all crystals obtained in the presence of CR, they observed the direct interactions with it in crystals of DMP19, Pin1R14A, hemoglobin and RbmA. In the latter, CR improved the crystal quality and resolution, making it possible to solve the complicated structure. In contrast, the DMP19, Pin1R14A and hemoglobin structures presented novel CR interactions with common characteristics (Fig. 2). Three distinct CR interaction modes comprise the K-crown, the C-crown and the KC-crown modes. In the first, it interacts with proteins in a similar fashion to ring-shaped lmw PEG on

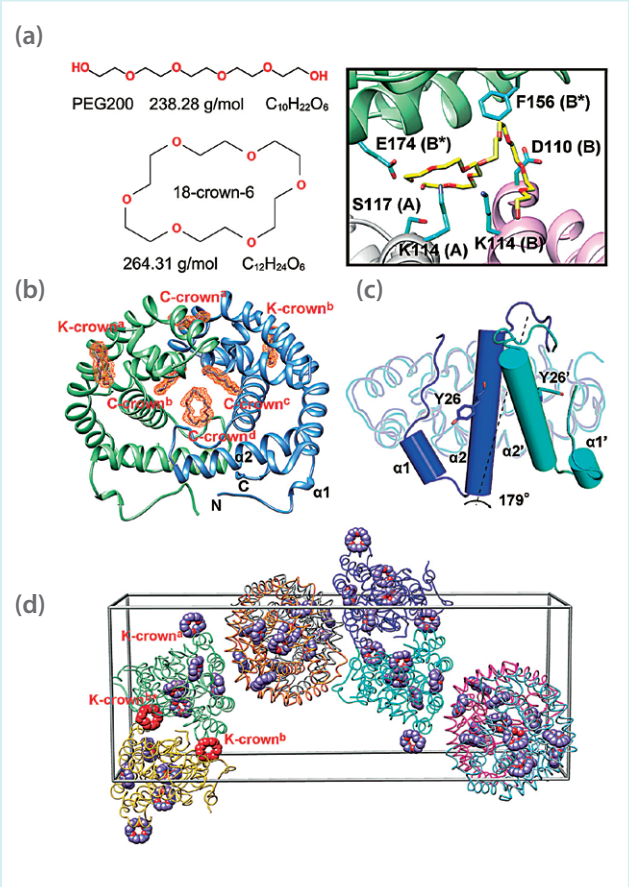


Fig. 1: PEG and 18-Crown-6 binding modes in a protein. (a) Top: PEG 200 in its linear conformation. Bottom: 18-crown-6 ether as a circular molecule. Right: Examples of lmw PEG adopting a ring-shaped conformation in various deposited crystal structures. dTMP kinase (2PLR) containing ring-shaped lmw PEG. (b) Structure of the DMP19•CR dimeric complex. Four C-crowns (C-crown$^{a-d}$) and two K-crowns (K-crown$^a$ and K-crown$^b$), in purple, bind each DMP19 dimer (green and blue). (c) Comparison of monomers of the published DMP19 structure (3VJZ, in blue) and the DMP19•CR complex (cyan). The region between the N-terminus and α-helix 2 alters greatly, rotating by 179°. (d) Crystal packing of DMP19 and CR (purple sphere). The two CR molecules interacting across separate unit cells, K-crown$^b$ and K-crown$^{b*}$, are shown in red.

coordinating the positive charge of a lysine axially (Fig. 2(a)). In the second, it stacks laterally either with aromatic and hydrophobic amino acids, or, via π-stacking, with carboxylic and guanidinium groups, which can clamp the CR (Fig 2(b)). The third binding has a mixed form, sharing characteristics of both the K- and C-crowns (Fig. 2(c)). As shown in Fig. 1(c), DMP19 revealed a new dimeric form containing in total six CR molecules, which caused α-helices 1 and 2 to rotate by 179° relative to the apo-structure. As the native dimeric state of DMP19

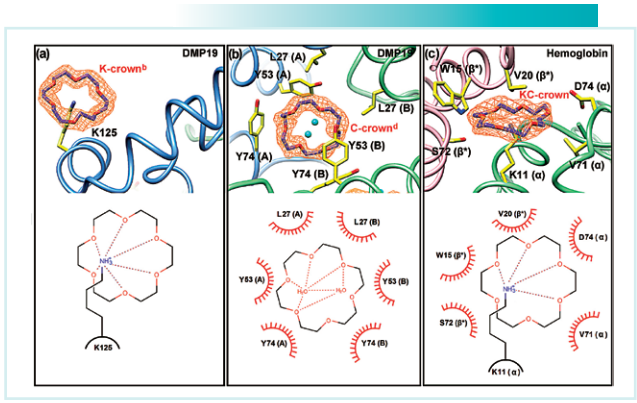


Fig. 2: 18-Crown-6 binding modes. The upper part of each panel illustrates the structure of the molecule; the lower part is a representation in which dashed lines represent hydrogen bonds and red semi-circles are hydrophobic contacts. (a) In the K-crown binding mode, a single lysine binds the CR axially. (b) Hydrophobic and p-orbital-containing side chains interact laterally with CR. No residue interacts with the central region of CR, which commonly, but not invariably, coordinates two water molecules. Letters in parentheses indicate the chain ID. (c) In the mixed KC-crown binding mode, the CR is coordinated axially with a lysine; hydrophobic and p-orbital-containing side-chains interact with it laterally. Letters in parentheses indicate the chain ID, with an asterisk indicating symmetry equivalents.

is the active conformation, they suggest that CR might interfere with the DMP19 function on altering its tertiary and quaternary structure.

In summary, the authors found that, on producing complexes, CR modified the properties of a protein surface, which resulted in alternative tertiary and quaternary structures. CR also increased the protein rigidity and, by CR–CR stacking, mediated direct interactions between hydrophobic patches and charged amino acids. They hence propose that CR, through its ability to modify protein surfaces, can serve in protein crystallography as a powerful additive, molecular probe to search for potential binding pockets, and reporter of protein conformational changes.

## References

1. C.-C. Lee, M. Maestre-Reyna, K.-C. Hsu, H.-C. Wang, C.-I. Liu, W.-Y. Jeng, L.-L. Lin, R. Wood, C.-C. Chou, J.-M. Yang, and A. H.-J. Wang, Angew. Chem. Int. Edit. **53**, 13054 (2014).
2. M. Maestre-Reyna, W.-J. Wu, and A. H.-J. Wang, PLoS ONE **8**, e82458 (2013).
3. H.-C. Wang, T.-P. Ko, M.-L. Wu, S.-C. Ku, H.-J. Wu, and A. H.-J. Wang, Nucleic Acids Res. **40**, 5718 (2012).