A New Hope for Crystallization

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Recent work from Dan Li and co-workers, published in Chem, reported the application of a cyclic trinuclear Ag^I *pyrazolate complex* (Ag_3Pz_3) *as an adaptive crystallization agent for a series of difficult-to-crystallize organic molecules.* Ag_3Pz_3 *was also employed for the direct structural elucidation of mixed organic compounds in addition to natural products in crude extracts.*

The intrinsic physicochemical properties of a chemical substance, such as its reactivity, biological activity, or optical properties, are dictated by its molecular structure. Thus, molecular structure elucidation is key to understanding structure-property relationships, and the subsequent application of such knowledge is essential to design-driven materials engineering. In particular, assigning the absolute configurations of biologically active compounds such as peptides and drug molecules is of utmost importance. A lack of detailed understanding of the physiological effects of stereochemistry can result in catastrophe, as in the infamous case of the drug thalidomide.[1]

Chemists employ analytical techniques such as nuclear magnetic resonance (NMR) and mass spectroscopy (MS) to gain crucial structural insights. However, the potential for mis-assignment persists, especially in large or complex molecules that feature stereocenters. Single-crystal X-ray diffraction (SCXRD) provides a clear picture of the three-dimensional arrangements of atoms in molecules, but it requires high-quality single crystals, which are often obtained through classical crystallization methods such as solvent evaporation or anti-solvent diffusion. However, organic compounds can be difficult to crystallize, especially complex and flexible ones.[2,3]

In an attempt to circumvent these issues, new strategies have emerged to facilitate SCXRD analysis. Effective molecular packing depends on non-covalent intramolecular interactions; hence, crystallization can be facilitated by increasing the strengths of these interactions by introducing an agent that interacts well with the chosen target. The crystalline sponge method pioneered this strategy, where a crystalline porous network acts as a sponge that can absorb and align a desired substrate in its cavities through host-guest interactions. These non-covalent interactions result in the ordering of guest molecules throughout the material, allowing for subsequent analysis by SCXRD. A range of porous networks have been investigated as crystalline sponge materials, including metal-organic frameworks, metal-macrocycle frameworks, and porous organic materials.[4,5] The broader applicability of these materials may be restricted, however, by constraints imposed by the sizes and geometries of the cavities, which can limit the substrate scope.

The crystalline sponge method can be described as a post-crystallization strategy, whereas co-crystallization is based on the simultaneous crystallization of both the substrate and the crystallization agent. These co-crystals may form as a result of different non-covalent interactions, such as arene stacking or hydrogen bonding.[6,7] In contrast to the crystalline sponge method, the size and shape of the substrate are not restricted. Co-crystallization has until now lacked the generality of the crystalline sponge method, however.

In work recently published in *Chem*, researchers at Jinan University led by Dan Li investigated the applications of a cyclic trinuclear Ag^I pyrazolate complex (Ag_3Pz_3) as an adaptive crystallization agent for molecules that are otherwise difficult to crystallise.[8]

Cyclic trinuclear complexes (CTCs) such as Ag_3Pz_3 offer a tunable platform which can provide non-covalent interactions with a range of substrates.[9] Density functional theory (DFT) calculations on Ag_3Pz_3 highlighted its potential as a co-crystallization agent because of a positive electrostatic potential on the central Ag_3N_6 metallacycle. The authors postulate that the presence of this π-acidity may allow Ag_3Pz_3 to bind substrates bearing electron-rich functionalities in co-crystals. Additionally, the trifluoromethyl groups protruding from the exterior of the crystallization agent may facilitate C-H$\cdots$F interactions, further restricting the degrees of freedom in co-crystallized structures and in turn promoting the ordered molecular arrangement required for crystallization. The presence of the heavy silver atoms also aids structure elucidation as a result of stronger X-ray diffraction.[10]

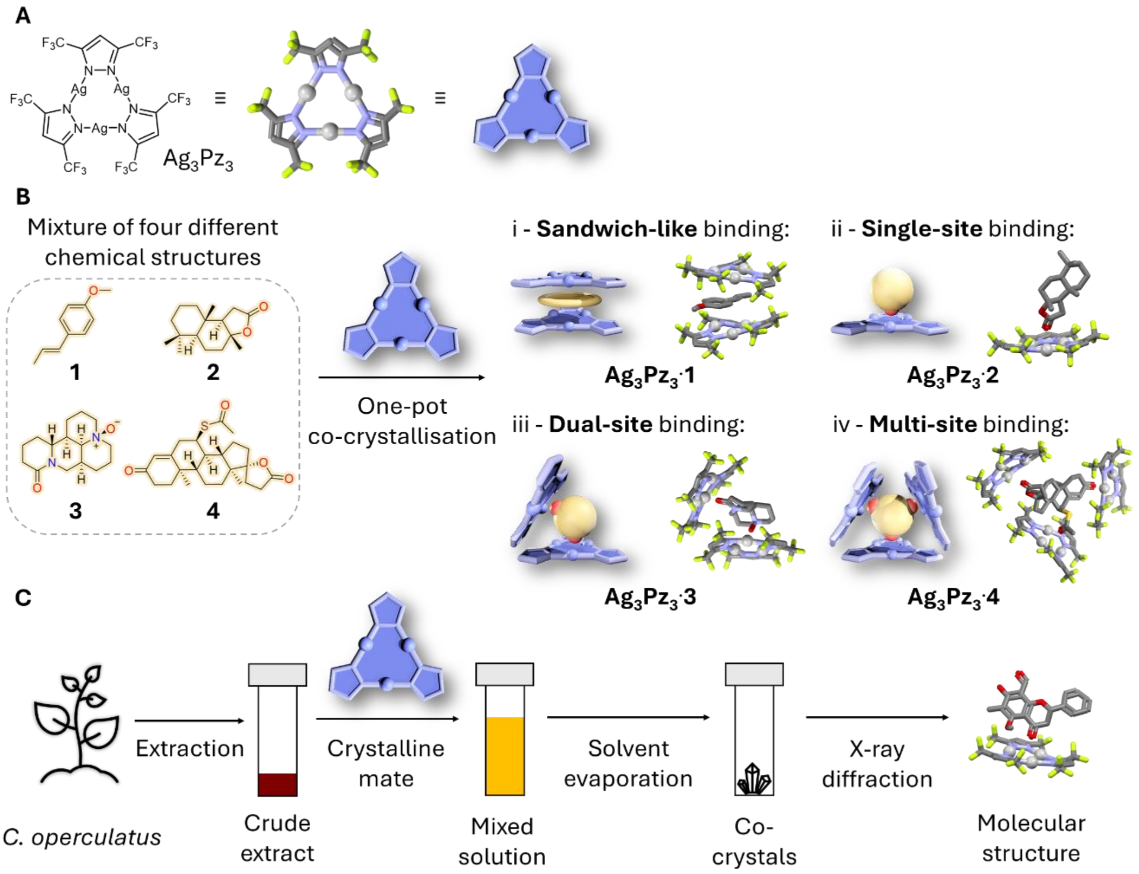

Figure 1. Ag_3Pz_3 as an adaptive crystallization agent developed by Li and co-workers

(A) Chemical and crystal structures of the cyclic trinuclear Ag^I complex Ag_3Pz_3.

(B) One-pot co-crystallization of a mixture of four compounds with distinct chemical functionalities.

(C) Workflow for the co-crystallization of a crude extract of *C. operculatus* with Ag_3Pz_3.

The authors obtained co-crystals suitable for SCXRD with Ag_3Pz_3 and a range of Lewis-basic oxygenated organic molecules. Although co-crystallization is not limited by the size or shape of the substrate, its geometry and number of Lewis-basic sites do impact the binding mode observed in the crystal structure. Figure 1B illustrates the four key binding modes that were observed between the organic molecules and Ag_3Pz_3, dependent on substrate shape and its electronic properties. For instance, planar molecules co-crystallized with Ag_3Pz_3 in a sandwich-like binding mode, with strong interactions indicated by substrate-crystallization agent distances that were shorter than the sum of their van der Waals (vdW) radii. When investigating the crystallization behavior of non-planar structures, they found that oxygen-containing molecules co-crystallized with Ag_3Pz_3 with either single-site, dual-site, or multi-site binding modes, depending on the number of distinct electron-rich sites and resultant interactions with the π-acidic Ag_3N_6 metallacycle. Furthermore, numerous C-H···F interactions were observed between the substrates and Ag_3Pz_3, leading to restricted freedom and more effective crystal packing.

Ag_3Pz_3 was observed to adopt subtly different structural conformations in order to maximize interaction with the functionalities of each compound. For instance, it adopts a concave shape in the trinuclear complex in Ag_3Pz_3·**2**, as shown in Figure 1B. The utility of Ag_3Pz_3 as a crystallization agent was further demonstrated through the successful growth of high-quality single co-crystals with twenty-five distinct oxygen-containing organic molecules, encompassing a range of molecular architectures and functionalities. For instance, Li *et al.* were able to decipher the full stereochemistry of the quassinoid eurycolactone E, which bears 10 contiguous stereocenters, for the first time.

They also demonstrated that Ag_3Pz_3 could be used for the simultaneous elucidation of the structures of multiple molecules, which crystallized individually with Ag_3Pz_3, from a mixture of organic compounds. Both mixtures of similar molecules that engage in the same binding mode and mixtures of molecules with different binding modes were investigated. Interestingly, similar single co-crystals to those obtained in homogenous mixtures were obtained in both cases. This proof-of-concept study may thus provide a useful strategy towards structure elucidation while avoiding the need for extensive and difficult separation processes. This method might prove especially useful for rapid structure elucidation in complex chemical mixtures, as only trace amounts of the substrate are required to obtain high-quality single co-crystals.

To further illustrate the potential of their crystallization technique, Li and co-workers applied the same methodology to a crude extract of *C. operculatus*, a plant with leaves and buds that have well documented health benefits ranging from anticancer to cardiotonic effects. After extracting the air-dried buds with methanol, the crude extract was taken up in a mixture of dichloromethane and methanol and Ag_3Pz_3 was added. The solution was left to evaporate at room temperature, producing four different single crystals. This method led to the identification of five flavonoids, including two new ones by SCXRD, while avoiding the need for time consuming separation processes.

This work demonstrates the highly adaptive nature of Ag_3Pz_3 as a crystallization agent for the structural elucidation of organic molecules that are difficult to crystallize on their own. Ag_3Pz_3 interacts with compounds in distinct binding modes in response to both their electronic and structural properties. This methodology is thus complementary to the crystalline sponge method: given that there are no shape or size restrictions this strategy may prove applicable to cases where the crystalline sponge method is not successful. Li and co-workers demonstrate that Ag_3Pz_3 can be applied directly for the structural elucidation of organic molecules in mixtures, in addition to natural products in crude extracts, thus potentially expediting the discovery of natural products. The facile modification of CTCs might also pave the way for further application, such as by introducing chiral groups to allow for stereoselectivity, or by exchanging Ag^I for Au^I to enhance interaction with more polarizable substrates. Li and co-workers have impressively demonstrated the broad applicability of their co-crystallization strategy, and the simple and cost-effective synthesis of Ag_3Pz_3 promises to make it a powerful and easily accessible crystallization agent for structure elucidation.

Declaration of interests

The authors declare no competing interests.

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