Crystallization of Small Molecules in Lyotropic Liquid Crystals

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Single-crystal X-ray diffraction analysis emerges as the most dependable method for structural determination. It serves as the definitive method, particularly in challenging cases where other approaches fail to yield precise results. However, the generation of high-quality single crystals remains a frequently encountered obstacle. Here, we present a strategy employing an alignment medium to enhance the growth of single crystals for small organic compounds. Furthermore, a mathematical crystallization model based on the classical nucleation theory was conducted specifically designed for alignment media. The hypothesis suggests that alignment media effectively reduce solution entropy, thereby lowering the free energy barrier associated with crystal nucleus formation and facilitating the crystallization process. Employing an alignment medium derived from self-assembled oligopeptide nanotubes (AAKLVFF), we successfully cultivated single crystals of diverse organic compounds. Our discovery unveils an innovative strategy and an accessible approach for acquiring single crystals.

1. Introduction

Structural determination stands as a crucial cornerstone in contemporary chemical research. Among available techniques, single-crystal X-ray diffraction analysis is the most robust method for precisely depicting the three-dimensional structure of molecules at high resolution [1]. However, the acquisition of a suitable single crystal, which serves as the foundation of crystallography, remains a significant challenge [2]. To overcome this obstacle, several innovative and sophisticated strategies leveraging the absorption and coordination of guest molecules within porous complexes have been employed to facilitate molecular alignment. These approaches aim to overcome the limitations by ensuring compatibility in size and interactions between the guests and host frameworks [3–7]. Nonetheless, the successful application of these methods heavily relies on achieving an optimal match between the properties of the guests and the host framework.

Meanwhile, a repertoire of alternative approaches, such as co-crystallization [8], encapsulated nanodroplet methods [9], nanoscale confinement [10,11], shear flow [12], microfluidics [13,14], gel-induced crystallization [15,16], and lipidic mesophases [17,18], has been extensively advanced for crystallography. Particularly noteworthy is the adoption of lipidic cubic phases as foundational matrices, now established as standard and routine protocols for crystallizing membrane proteins [17-19].

We have recently pioneered the development of a series of dilute lyotropic liquid crystals as alignment media for measuring anisotropic NMR parameters which render usually 0.01–0.1% fraction according to solid state [20–25]. The molecules are oriented to impede random motion by the alignment media, introducing a degree of anisotropy wherein the strength of alignments represents the generalized degree of order (GDO) within the range of 10^{-3}–10^{-4} [26]. We envisioned that the lyotropic liquid crystalline combining the anisotropy of solid and the fluidity character of solution could employ as novel and promising matric for the crystallization. Due to the orientational nature of lyotropic crystalline, the alignment force has the potential to reduce the entropy of solute molecules, consequently, leading to the decrease of nucleation free energy barrier. Herein, we introduce a novel strategy for molecule crystallization induced by lyotropic liquid crystalline and present a mathematical model illustrating the proposed nucleation theory. Preliminary single X-ray diffraction data for certain organic molecules are also demonstrated.

Following an anisotropic NMR investigation of natural product curtachalasin D, an unexpected discovery emerged that a single crystal was unexpectedly discovered within the alignment

medium of oligopeptide AAKLVFF liquid crystal, despite numerous failed attempts at crystallizing the compound under varying conditions. The single X-ray diffraction data has been reported previously [27]. The unanticipated phenomenon spurred our investigation to illustrate a mathematic model of the proposal nucleation theory.

2. Results

2.1. The mathematic model of the proposal nucleation theory

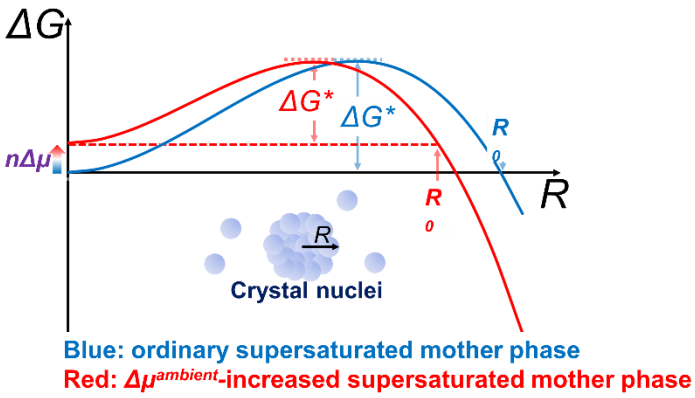

Figure 1. Nucleation free energy barrier and critical size of energy-stable crystal.

The process of crystallization in a solvent can be comprehensively conceptualized as a thermodynamic phenomenon, with the driving force denoted by the difference ($\Delta\mu$) in the chemical potential of solute between the supersaturated mother phase and the crystal phase, as described in the formula (1) [10, 11, 28]. The pivotal initial step in this process is nucleation, which not only determines the feasibility of crystal formation but also significantly impacts the quality of the resulting crystals [10, 11, 28]. According to classical nucleation theory, nucleation is controlled by the interplay between the reduction in bulk free energy and the increase in surface free energy (Fig. 1) [10, 11, 28]. For spherical nuclei with a radius R, the overall free energy change of solute can be described as

$$\Delta\mu = \mu^{ambient} - \mu^{crystal} \quad (1)$$

$$\Delta G = -\frac{4\pi}{3}R^3\rho_c\Delta\mu + 4\pi R^2\gamma \quad (2)$$

Here, ρ_c represents the particle number density of nuclei, and γ denotes the surface free energy area density [10, 11, 28]. When the crystal starts growing and the radius of nuclei is very small, the predominant contribution to the overall free energy change arises from the positive surface free energy which hinders nucleation. Its maximum value, namely ΔG^*, is known as a nucleation-free energy barrier, which can be described as [10, 11, 28]

$$\Delta G^* = \frac{16\pi\gamma^3}{3(\rho_c\Delta\mu)^2} \quad (3)$$

Besides, the radius of energy-stable crystal needs to be more than a critical value R_0, which can be described as

$$R_0 = \frac{3\gamma}{\rho_c\Delta\mu} \quad (4)$$

In formula (1), when the architecture of crystal is certain, $\mu^{crystal}$ can roughly be considered as a constant, and $\mu^{ambient}$ is the only variable parameter. When the number of solute molecules (n) is certain, the value of $\mu^{ambient}$ has a relationship with enthalpy (H), thermodynamic temperature (T), and entropy (S), expressed as

$$G^{ambient} = n\mu^{ambient} = H - TS \quad (5)$$

When H and T values are certain as well, obviously, the lower S is, the higher $\mu^{ambient}$ and $\Delta\mu$ are. In alignment media, a part of solute molecules can be oriented along the direction of microscopic structures, such as liquid crystalline phases. (Fig. 2) [20–25]. The ordered solute molecules have lower entropy than those in ordinary isotropic solutions, as the proof showed below. Entropy (S) and microstates (W) have a relationship as

$$S=klnW \quad (6)$$

We separate the entropy of solute molecules into orientation entropy (S_{ori}) and other type of entropy (S_{others}). Besides, we assume that the influence of alignment media on solute molecules is the same as that of solvent molecules, except for the spatial anisotropic effects. Therefore, the S_{others} values in alignment media and isotropic solution are the same, and we only need to consider the difference of W_{ori}.

$$S = S_{ori} + S_{others} = klnW_{ori} + klnW_{others} \quad (7)$$

We set the number of solute molecules as M, and the total number of orientation quantum states is n, and the numbers of molecules distributing at quantum states 1, 2, 3, 4,…, n are a_1, a_2, a_3, a_4, …, a_n, respectively. Therefore, orientation microstates W_{ori} can be expressed as

$$W_{ori} = \frac{M!}{\prod_{i=1}^{n} a_i!} \qquad M = \sum_{i=1}^{n} a_i \quad (8)$$

It is easy to prove that W_{ori} value is maximum when all the solute molecules distribute at all orientation quantum states as evenly as possible, namely in isotropic conditions. In alignment media, by contrast, solute molecules are partially unevenly oriented, and then the

orientation microstates are always fewer. Given that q is the quotient of M and n, with a remainder of r, the conclusion can be described as

$$W_{ori} \leq \frac{M!}{(q+1)!^{r} \times q!^{n-r}} \tag{9}$$

Therefore, we can eventually conclude that the $\Delta\mu$ value of solute molecules in alignment media is higher than that in isotropic conditions. Intuitively, on the other hand, considering crystal as a fully oriented state and isotropic solution as a fully disordered state, the aligned state is an intermediate between them, which could be a low free energy bypass from solution to crystal.

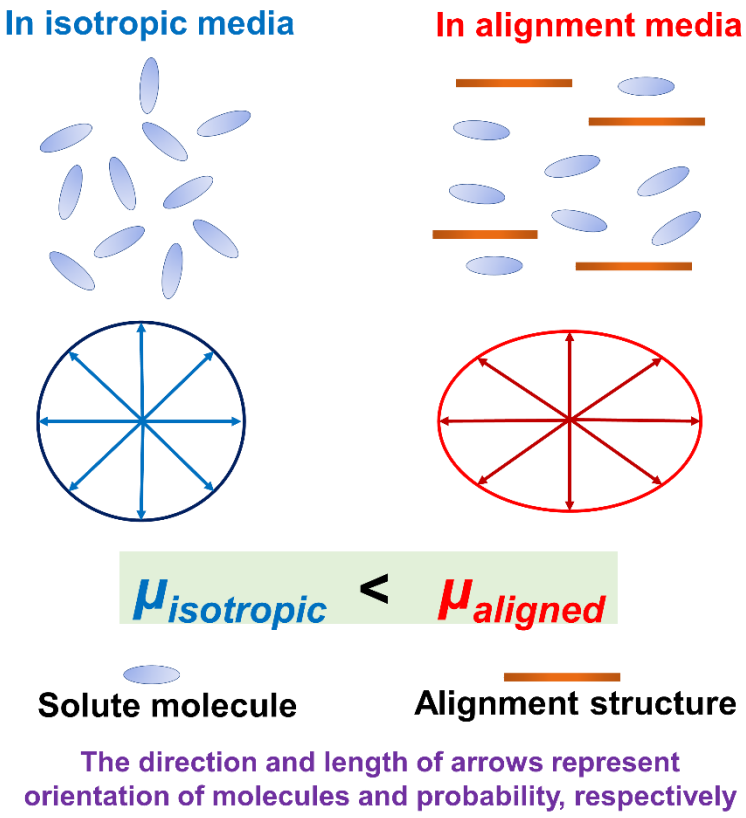

Figure 2. Comparison of the ambient chemical potential of solute molecules in isotropic solution and alignment media.

2.2. Preparation of crystals in lyotropic liquid crystalline phase

Subsequently, we selected the oligopeptide AAKLVFF liquid crystal as the alignment medium for implementing the method of crystallization due to its optimal combination of low critical concentrations, adequate fluidity, and straightforward preparation, rendering it an exemplary alignment medium for numerous applications in anisotropic NMR [22, 29, 30]. The peptide is capable of forming a stable lyotropic liquid crystal in methanol within a weight concentration range of 1–4%. At this concentration, the liquid crystalline phase exhibits sufficient liquidity and significant anisotropy. Notably, the well-ordered and highly aligned fiber structure of the peptide's beta-sheet imparts a unique ability to orient molecules along the fiber.

As anticipated, a diverse range of molecules, including organic synthesis agents (LCN079, **1**, and 10,10'-bianthrone, **2**), insecticides (santonin, **3**, and rotenone, **4**), natural products (aristolochic acid A, **5**, paecilin A, **6**, paxilline, **7** and columbin **8**), as well as approved drugs (piroxicam, **9**), were successfully crystallized within the AAKVLFF alignment medium. These compounds collectively represent a wide array of properties, functionalities, complexities, and diversities.

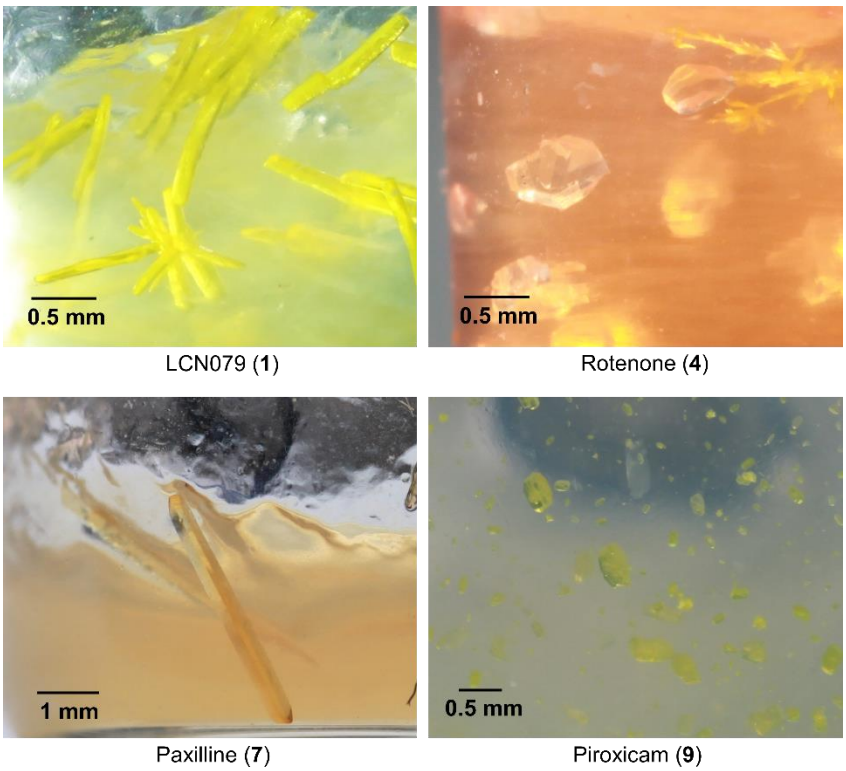

Figure 3. Representative single crystals of LCN079 (**1**), rotenone (**4**), paxilline (**7**), and piroxicam (**9**) grew in alignment medium of self-assemble oligopeptide AAKLVFF.

Specifically, crystallization in the presence of liquid crystals was observed to yield crystals suspended in the alignment medium, discernible by the naked eye. (Fig. S1.). The phenomenon was similar to the nucleation in colloidal suspensions and the mechanism has been extensively investigated [31–33]. Interactions between solutes and the surface of the beta-sheet peptide led to heightened solute concentrations near the surface. Molecular recognition at the interface of the surface and solute induces orientational alignment within the enriched solute layers, and these factors have the potential to facilitate nucleus formation [10, 11]. Additionally, the alignment medium heightened solute orientational order through liquid crystal anisotropy, aiding molecular alignment during the nucleation process. This phenomenon suggests that crystallization in lyotropic liquid crystals has the potential to promote nucleation and control crystal size. As expected, well-defined large single crystals were obtained as shown in Fig. 3 and Fig. S2. Several crystals reached dimensions of hundreds of μm³ in length, height, and width—ideal for standard in-house X-ray crystallography measurements. Notably, the crystal of paxilline exhibited a regular rectangular prism shape with 200 μm in width and over 3000 μm in length, significantly larger than those obtained under typical conditions (Fig. S3).

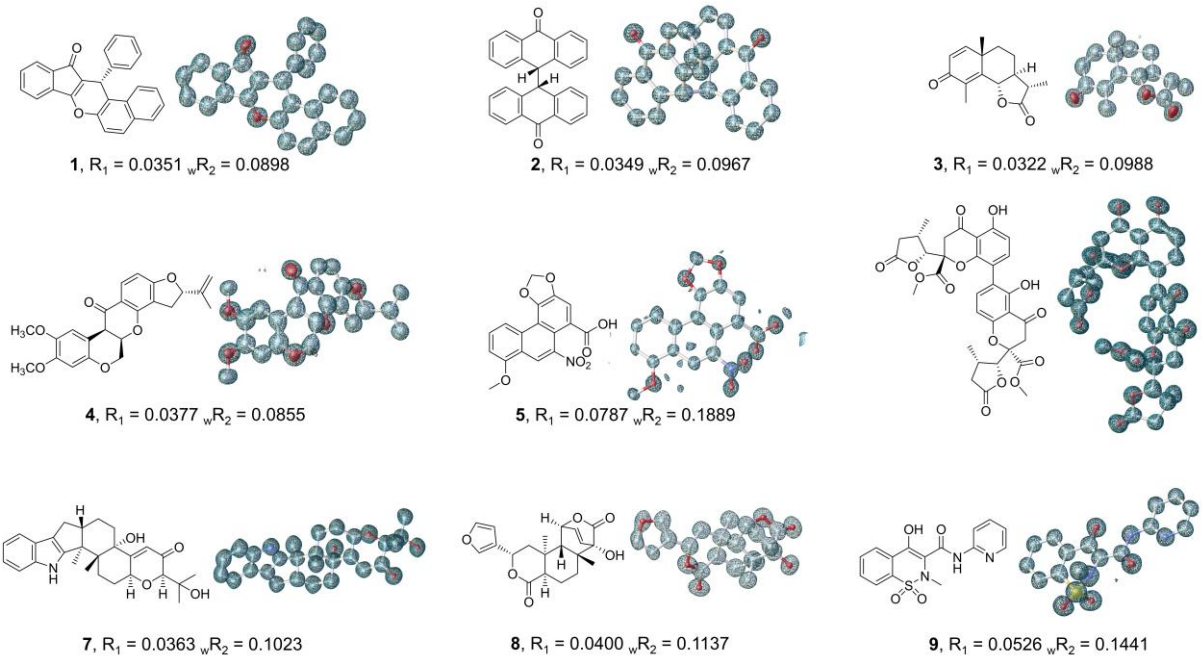

Figure 4. Organic compounds with single crystal cultivated in alignment medium of self-assemble oligopeptide AAKLVFF.

To assess the crystal quality comprehensively, X-ray diffraction analyses were conducted to elucidate the structures of the aforementioned compounds (Fig. 4). The data were collected using BRUKER D8 QUEST and Rigarku Xta-LAB Synergy Custom diffractometers,

employing either Cu Ka X-radiation (1.54184Å/1.54178Å) or Mo Ka X-radiation (0.71073Å). The R1(I>2σ) values for all nine datasets were below 8%, while the wR2 values were below 20%. Remarkably, the refined R and wR values proved highly satisfactory even without the imposition of any restraints. For columbin, paxilline, rotenone, and santonin, the absolute chemical structural assignments were verified through anomalous dispersion, resulting in Flack parameters of 0.07(7), −0.07(11), 0.02(2), and 0.03(5), respectively. These findings validate the suggested nucleation theory, indicating that the use of an alignment medium enables the crystallization of organic molecules without undermining the precision of structural determination.

3. Conclusion

In summary, we have introduced a strategy employing an alignment medium to enhance the growth of single crystals for small organic compounds. The mathematical model elucidated that the alignment force has the capacity to diminish the entropy of solute molecules, consequently decreasing the nucleation-free energy barrier. We believe that this concept, coupled with successful implementations in various cases, offers a novel perspective for advancing methods in single crystal cultivation. Furthermore, this advancement holds promise for application in generating larger crystals for functional materials, such as organic frameworks [34, 35], paving the way for future developments.

4. Materials and Methods

The suitable single crystals of each of the nine compounds were mounted, and analyzed by SCXRD. In all cases, high-quality data were recorded to a minimum completeness of 98% at a minimum resolution of 0.85Å on BRUKER D8 QUEST and Rigaku XtaLAB Synergy Custom diffractometers using Cu Kα X-radiation (1.54178Å/1.54184Å) or Mo Kα X-radiation (0.71073Å). Molecular structures were then obtained through structure solution and refinement using the Bruker Apex3 and OLEX2 [36] interface to the SHELX [37] suite of programs. The positions of the H-atoms were calculated geometrically with riding models. Furthermore, the absolute stereochemical assignments were confirmed by successful refinement of the Flack parameter derived from anomalous dispersion measurements for columbin, paxilline,rotenone and santonin. The R1(I>2σ) value of all those nine data is below 8% and wR2 value is below 20%. The graphics of crystal structures were generated with the programs Olex2.

[CCDC 2324606 (for **1**), 2324663 (for **2**), 2324615 (for **3**), 2324614 (for **4**), 2324608 (for **5**), 2155091 (for **6**), 2324610 (for **7**), 2324609 (for **8**), and 2324613 (for **9**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.]

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

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Alignment medium assists crystallization of small molecules by reducing the nucleation free energy barrier, which could be a novel tool to obtain single crystal.