

Optical anisotropy of crystallized vanillin thin film: the science behind the art

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SUMMARY

Vanillin, a common household flavoring agent, turns into mesmerizing artwork under a polarizing microscope. This artwork can be used to decipher crystal nucleation and growth mechanisms. The trick to create such artwork is to grow a crystalline vanillin thin film tens of micrometers thick to allow white light to be transmitted through a singular, instead of multiple overlapping, crystal grains. A series of experiments were carried out to determine the optimal conditions to grow such single crystal vanillin films. Vanillin crystal's chemical structure is anisotropic, specifically monoclinic. When a light wave propagates through its anisotropic crystallographic planes, it splits into two orthogonal beams, which in turn generates optical interference patterns unique to its molecular packing structure. We hypothesized that the resulting spectacular color pattern of vanillin micrographs was driven by its asymmetric crystal structure when vanilla macromolecules grew with covalent and or hydrogen bonding force during solidification. We employed a long-range molecular bonding model to simulate chemical interaction of multiple vanillin molecules. We interpreted the vanillin film growth mechanism as the radial propagation of single crystal phases in combination of amorphous phases to fill spacing between crystalline phases. The relationship between the optical interference pattern of recrystallized vanillin thin film and its microstructure was established through this study. The outcome of this study may shed light into engineering anisotropic crystals into micro to nanoscale optical devices with preferred optical reflectance.

INTRODUCTION

Crystalline materials refer to those exhibiting orderly arrangement of atoms, ions or molecules at a microscopic scale. The ordered microstructure is attributed to the intrinsic nature of the constituents to form repeating patterns in a three-dimensional space. The smallest group of the repeating pattern is called a unit cell, which represents the symmetry and structure of the entire crystal (1). There are a total of seven primitive crystal structures, namely cubic, triclinic, monoclinic, orthorhombic, tetragonal, trigonal and hexagonal (2). Some crystal structures are isotropic (e.g., cubic). One example of a cubic crystal is table salt (sodium chloride), where the Na⁺ cation and Cl⁻ anion are arranged with uniform spacing along three mutually perpendicular axes. Some are anisotropic such as monoclinic, one example is vanillin which has three axes of unequal lengths. The crystal structure plays

a critical role in determining many physical properties, such as electronic band structure and optical interference (2).

Vanillin (4-hydroxy-3-methoxybenzaldehyde) contains three oxygen atoms in different functional groups: alcohol, aldehyde, and ether (3). When crystallized, vanillin forms a monoclinic crystal structure with no sides of equal length at two edges that form 90° angles (a tilted parallelogram prism with rectangular bases) (4). In a vanillin crystal cell, four vanillin molecules form two pairs lying on adjacent parallel planes; each pair is linked by hydrogen bonds between the hydroxyl group and the oxygen atom of the aldehyde group, and between pairs. The same bonding structure extends in repeating three-dimensional cells and forms parallel linear polymer long range chains (5).

When light enters a non-equivalent axis of a vanillin monoclinic cell, it is refracted and split into two polarizing rays. When they emerge from the crystal, interference occurs and generates a spectrum of color. This phenomenon is called double refraction or birefringence (6). The optical path difference, D , between two splitting rays is a function of their respective refractive indices, n_1 and n_2 , and the specimen thickness, t , as shown in Equation 1 below:

$$D = (n_1 - n_2) \cdot t \quad (\text{Equation 1})$$

The Michel-Levy Birefringence Chart describes the relationship of a specimen's thickness as a function of birefringence ($n_1 - n_2$) and the resulting polarization colors at varying incident light wavelength and is commonly used to identify the optical index of a material if its thickness is known, or vice versa (6,7).

Optical birefringence can be readily revealed between crossed polarizing plates when a thin layer of sample is placed under a transmitted light polarizing microscope. Usually, samples are prepared by melting or diluting them in a solvent on a transparent glass slide. When solidified, polymer chains may form a range of phases, from orderly crystalline to disorderly amorphous phases or a mixture of them, namely semi-crystalline phases. Entanglements of long polymeric chains are suspected to be the root cause of the amorphous phase (8,9). The resulting morphology strongly depends on supercooling conditions, chemical gradients ahead of solidification frontier and pre-existing defects. Nucleation refers to when a very tiny solid crystal grows out of its liquid solution, and typically the crystal will continue to grow (10). For example, spherulites are spherically symmetric semi-crystalline structures grown out of a slowly cooled molten polymer. Semi-crystalline describes a mixture of crystalline and amorphous phases, the latter refers to a disorderly atomic structure. The process of slow cooling allows its molecular chains to form an orderly crystalline configuration (11). The radial growth around a defective site is probably the most energetically favorable way to form the hydrogen bonds between atoms inside the crystal.

Although methods to obtain crystal interference images are well established, little is known about how these images are fundamentally related to their specific crystal structures and we are not aware of such research on vanillin crystal. In this study, we aimed at understanding the fundamental relationship of vanillin crystal's optical interference behavior and its crystalline microstructure, specifically how its structural anisotropy leads to its colorful patterns. We grew thin films of vanillin out of their diluted solution on glass slides and characterized their crystallization process through a polarizing optical microscope. We captured spectacular color patterns, i.e., birefringence, and used them to decipher crystal nucleation and growth mechanism. We developed a polymer crystallization-based physics model to understand the experimental data.

First, we studied birefringence color patterns to understand the fundamental material science of vanillin crystallization at a macroscopic level. We observed that vanillin crystals are composed of “matte” disorderly phases (likely amorphous or very small crystal clusters) and thin long plates. Our study also revealed that vanillin macromolecules present a linear long range polymer chain structure and thus preferentially one directional (instead of two directional) grown crystal morphology was expected when vanillin crystallized. Afterwards, we modeled a vanillin atomistic scale chemical bonding structure within a monolithic crystal unit cell to understand vanillin chemical bonding and the resulting long range chain morphology. Finally, we interpreted the color of vanillin crystalline phase using the Michel-Levy color interference chart. We concluded that the resulting mesmerizing color patterns of vanillin can be fundamentally understood through its atomic level polymer bonding structure for its crystalline phase, while that of the second phase presents a shade of several colors indicating either totally disordered or many small crystal cluster types of structures.

In a longer term, we believe that the possibility to uncover the driving force for vanillin molecules to self-assemble into artistic patterns opens up endless opportunities to engineer microscale structures with preferred functionality without relying on expensive microfabrication facilities (12–15).

RESULTS

We grew vanillin thin films grown out of their dilute solutions under a variety of conditions such as concentration of vanillin solutions. We then examined the films under a polarizing microscope, and selected the films which generate distinctive optical birefringence patterns for further study. After around 5 to 10 seconds of incubation, we observed that crystallites started to emerge at seemingly random locations, with more occurrences at the edge of the glass slide. We examined a total of about 50 samples. Approximately one third of the films failed to generate beautiful optical patterns because their thickness and/or grain size was not suitable for producing birefringence. We observed that nucleation of these crystallites prefers to be around pre existing particles (such as dust or undissolved vanillin particles). This indicates heterogeneity instead of homogeneous nucleation (16,17).

The crystallites were primarily faceted plates in the range of tens of micrometers initially. These crystallites propagated rapidly in a radial manner until adjacent crystal grains began to impinge. Afterwards, the growth stopped and began to form polygonal boundaries between adjacent crystal grains.

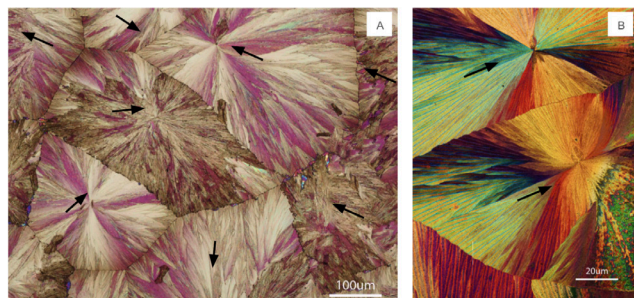


Figure 1: Images of crystallized vanillin thin film. (A) Vanillin grains imaged at a lower magnification with approximately six vanillin grains present, (B) Vanillin grains imaged at a higher magnification; two vanillin grains are present. Black arrows point to each individual grain.

Approximately six vanillin grains were solidified and grain boundaries were evident (Figure 1A). A higher magnification revealed detailed microstructures of two grains (Figure 1B).

Vanillin crystal is anisotropic and has three refractive indexes, with $\alpha = 1.551 \pm 0.002$, $\beta = 1.694 \pm 0.002$ and $\gamma = 1.80 \pm 0.005$, yielding three theoretical birefringence values of $n_1-n_2 = 0.143$, $n_1-n_3 = 0.249$, $n_2-n_3 = 0.106$. These theoretical birefringence values were imported, along with two major interference colors (violet red and violet gray) identified in Figure 1 of vanillin crystals, into an interactive Michel-Levy Birefringence chart (7). We estimated that $n_1-n_2 = 0.143$ and $n_2-n_3 = 0.106$ were primary refractive crystallographic orientations and the polymerized film thickness was 12–14 μm (Figure 2A, 2B).

We examined one single vanillin grain and observed that this grain grew from a central nucleus radially and formed colorful stripes along its propagation direction until it was impinged by a neighboring crystal grain at a “zig-zag”

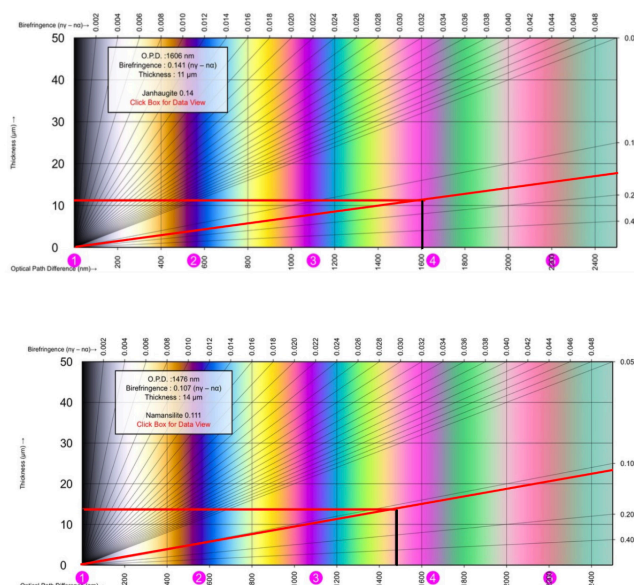


Figure 2: Michel-Levy Birefringence chart of the vanillin film. For the interference colors captured in Figure 1A, violet red in (A) and violet gray in (B), the birefringence value and the film thickness were estimated.

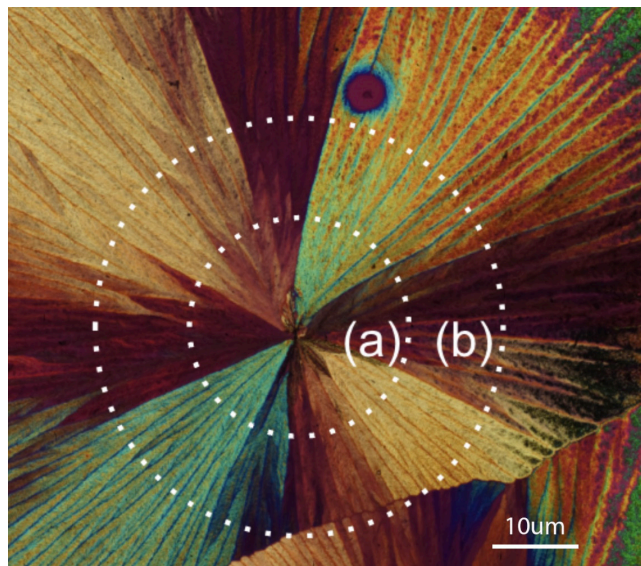


Figure 3. The color change of a vanillin grain circumferentially. Two circles were drawn surrounding central nucleate, to study color changes along each circle, inner circle (a) likely represents a thicker film than outer circle (b) as this grain grows radially.

shaped boundary (**Figure 3**). This morphology is classified as spherulite, or spherical in shape. As stated previously, vanillin crystal is monoclinic and has three distinct reflective indices, generating cyan, yellow, and red interference colors. A 530 nm retardation plate was inserted when taking these images, so a second-order birefringence spectrum of the Michel-Levy chart was used to interpret the polarizing image. Two circles are drawn for analyzing the interference color. Starting from cyan along one circle, the color changes to light orange to dark red and finishes with cyan. (**Figure 3**).

We used increased magnification to study the micrographs at the grain boundary region of several vanillin crystals (**Figure 4A**). Areas sharing the same color indicate the same crystallographic orientation, whereas distinctive colors represent different crystal orientations. The grain boundary is faceted. An intriguing phenomenon is the existence of strips traversing through the same grain, maintaining almost the same color and dimension longitudinally. This indicates that the strip features a single crystal growing in the preferred orientation (**Figure 4B**).

Images of two adjacent vanillin grains are presented in **Figure 5**. Morphology wise, transverse strips sandwiched

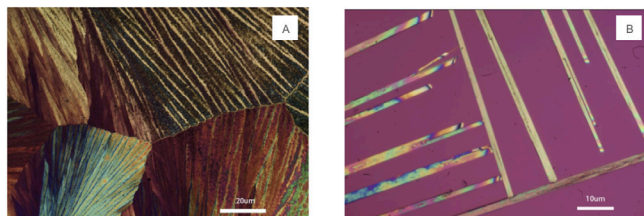


Figure 4. Vanillin grain boundaries and their formation. Multiple vanillin grains separated by a mutual grain boundary (A). Crystallite plate arrays impinged by another array of plates grown in a different orientation (B).

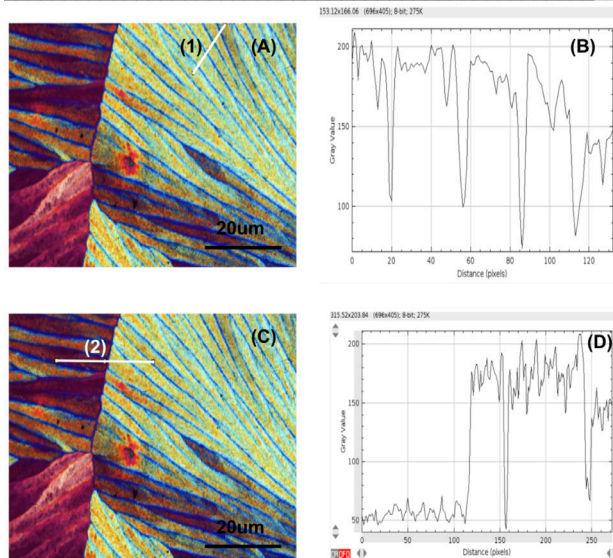


Figure 5. Two adjacent vanillin grains separated by a mutual boundary in (A) and (C), line scans and color intensity profiles were captured in (B) and (D) to study phase change. The periodic color intensity fluctuation in (1) shows alternating amorphous phases (yellow) to crystal phase (dark blue), and in (2) color intensity changes due to transition from one grain to its neighbor which likely have different orientation.

“matte” textured phases are the characteristics of the vanillin crystals. Detailed color intensity profile using ImageJ software shows that alternating color intensity peak and valley across line scan (a) which corresponds to crystal strips (dark blue color) and amorphous like phase (yellow), and line scan (b) shows color intensity transition when across a grain boundary, which indicates very different grain orientation between these 2 neighboring grains.

We applied the Visualization for Electronic and Structural Analysis (VESTA) software to simulate vanillin chain structure formation when polymerized. VESTA is a 3D visualization program for structural models, volumetric data such as electron/nuclear densities, and crystal morphologies (18). As shown in **Figure 6A**, two molecules were covalently bonded along a plane parallel to the c axis, in the background is a unit cell of monoclinic with a, b, c axis vector denoted for both top down and side views. **Figure 6B–D** illustrate continuous growth of vanillin crystalline chains with more molecules joining molecular stacking through chemical bonding sequentially from 4, to 20, to more than 100 molecules. Clearly we can observe that vanillin crystals maintain linear instead of planar polymer chain topology as the macromolecule extends in three dimensions, characterized by O-H...O bonds within the linear chains as well as between the linear chains.

DISCUSSION

It is evident that vanillin has a spherulite-like morphology, featured by thin strips of crystals eradicating radially with second phases in between as a repeating unit. Distinctive colors manifest as different orientations of these crystalline phases or varying thickness along radial directions as a thin liquid layer spreads across a glass slide substrate. Multiple spherulites nucleate and grow until they impinge upon each

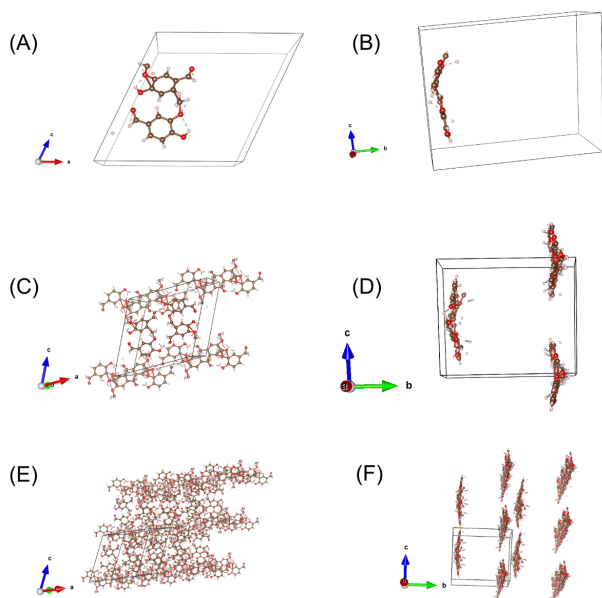


Figure 6. Vanillin molecular bonding configurations of top down (left) and side views (right). (A and B) 2 molecules, (C and D) 10 molecules, and (E and F) 100 molecules, all lying in one plane on the a and c axes, A is top down view and B is side view, similarly for C, D and E and F. Oxygen (red), carbon (gray) and hydrogen (light pink), with a monoclinic unit cell in the background.

other (Figure 1B).

Assuming that the film thickness around circle A (Figure 3) is comparable due to radial growth from the same nuclei, this color pattern tells us that at least two values of birefringence of vanillin crystal are present. While the radial strips remain the same color, the matter in between strips changes color from red to light orange to light yellow to light orange and then finishes with dark red. This indicates the change of film thickness in comparison to circle A (Figure 3).

When crystallized, vanillin forms a linear chain polymeric structure, linked by intermolecular hydrogen bonds; these elongated plates support that vanillin polymerization is dominated by one directional growth relatively well (5). It is possible that these polymer chains arrange themselves tangentially, causing the solidified regions to grow radially from nuclei. This hypothesis is also supported from a micrograph taken from a different vanillin glass slide, where elongated narrow crystal plates protrude out of a main reservoir of vanillin and propagate until they are arrested by crystal plates growing from other directions (Figure 4).

We proposed a model to interpret the spherulite-like morphology of vanillin crystal based on the constructed linear polymer chain structure (Figure 8). We elaborated the detailed phases in vanillin spherulite structure as the following: phase (a) refers to crystalline phase, which is composed of a highly ordered long linear polymer chain of vanillin radiating from the central nucleus. It was reported in literature that a long linear polymer chain folds into lamellae at a nanometer scale (19), which is possible; however, this current study will not be able to verify the previous statement due to lack of experimental equipment to collect relevant data; phase (b) refers to the amorphous phase, where residual vanillin

molecules solidify to fill spaces between long crystals; A high magnification examination shows microscale roughness and shades of varying colors, which indicate a possible disordering amorphous microstructure. Phase (c) refers to the nucleus, commonly foreign particles or defects on glass slides, which facilitate an easier heterogeneous nucleation at a lower thermodynamic energy barrier (17). The presence of linear crystalline phase (a) and disordering amorphous phase (b) can be supported where alternating of crystalline strips and “matte” amorphous-like phases coexist. Figure 5 further supports the strip morphology most likely represents crystalline phase when arrays of long plates grow almost parallel out of solution in one dominant direction, which is typical of orderly crystal structure (Figure 5). Similarly molecular modeling results also support a linear polymer chain structure of vanillin crystal (Figure 7). When multiple spherulites nucleate and propagate next to each other, eventually they meet each other, forming a mutual boundary to separate each individual spherulite, and then growth stops, as shown multiple spherulites reside on a glass slide (Figure 1).

This study is limited by access to advanced lab equipment, such as temperature and humidity-controlled chambers, where vanillin crystal growth kinetics can be explored. Another limitation of the study is the lack of access to a lab with elemental analysis and x-ray scattering capabilities so the vanillin crystal phases presented in this study cannot be quantitatively characterized to elucidate some of the

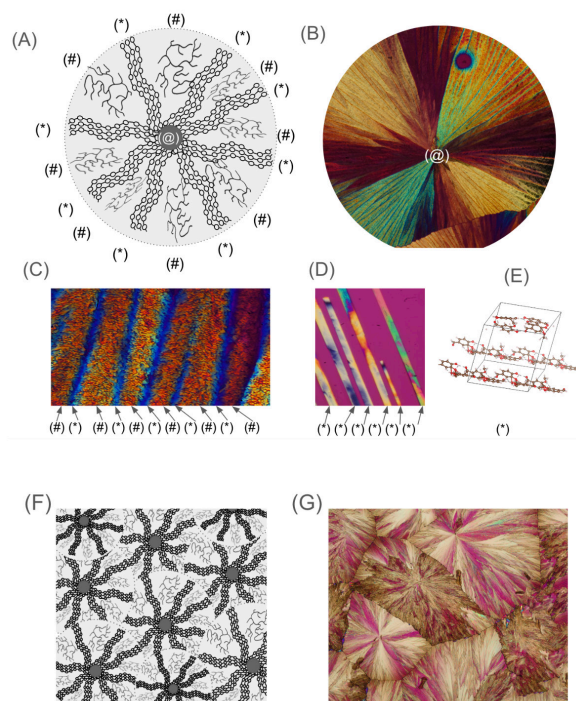


Figure 7. Single spherulite molecular model illustration (A), showing single crystal platelets (*), amorphous phases (#) filling in between, and (@) nucleation sites. Image (B) is used to verify the model. Vanillin spherulite has semi-crystalline phases, as shown in C, D and E. F illustrates multiple spherulite models when several grains share mutual boundaries, G refers to the image taken of multiple grains to verify the model.

hypotheses presented. Access to a microbeam X-ray diffraction technology could have supported verifying whether chain folding indeed occurs within each of the crystal plates. It is also possible that the relationship between vanillin crystal structure and its colorful appearance could be interpreted in alternative theories, especially when more quantitative measurement results could be obtained with advanced lab equipment. In the long term, this series of studies aim at understanding chemical and physical aspects of anisotropic organic chemical crystallization, and therefore hopefully establishing a methodology to engineer the self-assembly process of grain orientations for micro or nanoscale optical devices applications.

The proposed molecular model aims at understanding the phenomenon of vanillin crystallization in conjunction with a color spectrum captured by a polarizing microscope. Varying colors in solidified vanillin semi-crystals reveal the asymmetric crystal structure and resulting optical anisotropic behavior of thin films of vanillin deposited out of a solution. The relationship among its birefringence, intermolecular bonding characteristics in repeating monolithic unit cells, and existence of orderly crystal phase and disorderly amorphous phase post solidification is established preliminarily. When this underlying mechanism of vanillin spherulite formation is elucidated by experimental data and theoretical modeling, we could gain fundamental access to precisely manipulate its structure and morphology for scientific and or artistic merits. As one example, we could fabricate a low-cost nanoscale optical device by using vanillin's self-assembly behavior governed by its molecular bonding physics. As another example, we could apply similar analysis to other promising anisotropic crystals to generate distinctive optical interference and explore its application in the real world.

Studies like this on fundamental polymer chemical-physics could help to engineer preferred crystal orientations as arrays of microscopic optical devices in the future. Fabrication of nanoscale optical devices *via* molecular level self-assembly presents a unique advantage compared to highly expensive and technologically complex fabrication processes (12–15). The spontaneous organization of molecules governed by their chemical and physical law enables the development of ready-to-use devices at a much lower cost. One such application in life science is lab-on-fiber optrode, which can be easily integrated in hypodermic needles and catheters for *in vivo* theranostics and point-of-care diagnostics (13).

MATERIALS AND METHODS

99% high purity vanillin was purchased from ChemCenter for this study. The vanillin solution was prepared at room temperature. The vanillin powder was continuously added to a 1:1 mixture of water and 91% isopropyl alcohol and aggressively stirred until no more vanillin could be dissolved. The supersaturated vanillin solution was ready when slight vanillin particles become noticeable, approximately 10 mg/mL. Afterwards, we deposited a few droplets onto a microscope glass slide and observed the slide under a transmitted light microscope (Olympus BH-2) equipped with polarizing functionality. This microscope includes a polarizer, an analyzer, and a 530 nm retardation plate. A 16-megapixel digital camera was attached to the microscope to take images at 4x, 10x, and 40x magnifications. Tiny crystals began to form, and gradually the surrounding liquid depleted

and transformed into solid semi-translucent film. All of this occurred on a glass slide and was observed and captured by the digital camera.

A total of 50 samples of vanillin solution on glass slides were prepared by varying amount of vanillin dissolved (from 1–10 mg/mL), solution temperature (room temperature ~80° F, ~80° F degree outdoor, and ~40° F degree in refrigerator), and liquid droplets location on glass slides (at edge or at center of a slide). We discovered that a supersaturated solution prepared at room temperature yields the most satisfactory results in terms of nucleation and growth of crystals, and this condition was eventually used to collect data in this study.

Adobe Photoshop software was used to process the digital images so that multiple high magnification photos could be merged into one. ImageJ software was used to count the color intensity of vanillin images when crossing multiple grains and phases. The VESTA software was applied to simulate the vanillin polymerization chain structure formation. The input parameters including crystal system and lattice parameters for building vanillin monoclinic crystal were pulled from crystallography open database (20). Firstly, one molecule was constructed to visualize oxygen, hydrogen and carbon atomic bonding structure in one unit cell, then a second molecule was included to visualize these two intermolecular bonding configurations, and the multiple molecule bonding structure continues to expand to add 5, 10 and up to 100 molecules so as to present the formation of vanillin macromolecules.

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