Understanding solution processing of inorganic materials using cryo-EM

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Abstract: Cryo-electron microscopy (cryo-EM) single particle analysis (SPA) has revolutionized biology, revealing the hydrated structure of numerous macromolecules. Yet, the potential of SPA to study inorganic materials remains largely unexplored. An area that could see great impact is solution-processed device materials, where solution changes affect everything from crystal morphology for perovskite photovoltaics to stability of photoluminescent quantum dots. While with traditional microscopy, structures underlying these effects can only be analyzed after drying, cryo-EM allows characterization of in-solution structures, revealing how features arise during processing. A top candidate for such characterization is found in chalcogenide glasses (ChGs), which researchers in the 1980s proposed take on solvent-dependent solution nanostructures whose morphologies have yet to be confirmed. Here we show that cryo-EM can directly image ChGs in solution and combine with other techniques to connect solution structure to film characteristics. Our results bring closure to a long open question in optoelectronics and establish SPA as a tool for solution-processed materials.

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1. Introduction

Solution processing has wide-ranging applications from photonic crystals to solar cells [1,2], and similar to how the structure of a hydrated biological macromolecule controls function, the solvated structure of an inorganic or hybrid material controls properties [3,4]. Unfortunately, structural characterization of such materials has traditionally been limited by a lack of techniques that can directly probe solutions. Biologists have combated this issue very successfully with cryo-transmission electron microscopy (cryo-EM)—a powerful tool that has revealed the solution structure of key biological macromolecules [5,6], though its use in materials science has largely focused on beam sensitive solids like lithium [7,8] rather than on solutions. Two cryo-EM methods popular in biology for determining three-dimensional (3D) solution structures are single particle analysis (SPA) and tomography. The latter involves taking many micrographs of the same particle at different tilts and thus is limited by its electron dose to less volatile aqueous systems or organic systems with large particles visible at lower magnification [9]. SPA, however, is not subject to these limitations as it does not require repeat exposures; each area of the sample is imaged once, simultaneously capturing thousands of particles. By assuming the particles are structurally identical and imaged in random orientations, several softwares can average the micrographs into a single 3D structure [10,11]. SPA is thus a strong candidate for determining the in-solution structures of inorganic materials.
Arsenic (III) sulfide (As$_2$S$_3$) chalcogenide glass (ChG) is an attractive initial sample for SPA due to its technological relevance and the existing attempts to understand its solution structure. Falling in a class of materials of interest to the photonics community for their high optical nonlinearities, mid-infrared transmission, photoinduced phenomenon, and stability as glasses at room temperature, As$_2$S$_3$ has found applications in areas like information storage and sensing [12,13]. As with other solution-processed materials, many of these unique properties are known to depend on structure—losses in transmission, for instance, depend on porosity, and nonlinearity depends on bonding and microstructure [14–16]. In 1983, Chern and Lauks demonstrated that As$_2$S$_3$ can be solution-processed in amines and proposed it takes on a round, cluster-like structure in propylamine and in butylamine [17]. Five years later, Guiton and Pantano proposed a longer, chain-like structure for As$_2$S$_3$ in ethylenediamine [18]. Though widely cited by other works in the subsequent decades, neither theory has been verified by direct experimentation and questions remain about the specifics of the morphologies proposed—for instance, does propylamine gives platelets or spheres, and what are the length scales of structure in the systems? These are knowledge gaps cryo-EM is uniquely suited to fill.

2. Results

2.1. Development of the freezing protocol

Sample preparation is the most significant obstacle to analyzing solutions of As$_2$S$_3$ and other inorganic materials though cryo-EM. A limiting factor even to biologists, existing methods are heavily sample dependent and have been developed with aqueous solutions in mind [19]. Organic solvents bring unique challenges to traditional freezing, and while some progress has been made in this area [9,20,21], attention must be paid to achieve samples uniform enough for SPA. Figs. 1(a)–1(h) show the results of adapting traditional freezing methods to the nontraditional ChG in amine system. While aqueous cryo-EM samples are plunge frozen in liquid ethane to achieve fast cooling [22], we find liquid ethane is detrimental when used with the amines—consistent with results for other solvents [20,21]—yielding grids with large empty patches that can be seen in Figs. 1(a) and 1(b). Glow discharging, a common cryo-EM pre-treatment used to make grids hydrophilic, also decreases quality, resulting in grids poorly wet by the solvents as shown in Fig. 1(c). It is thus determined that freezing in liquid nitrogen (LN$_2$) without grid pre-treatment, while nonviable for aqueous samples, produces the most uniform grid for the amines, as shown in Fig. 1(d).

Optimizing sample thickness further complicates the preparation; a thick layer of solution will take longer to freeze and block more of the electron beam, but thin layers are fragile and difficult to achieve. A typical approach to controlling thickness is by blotting excess solution off the grid with filter paper before plunging into the cryogen. This process has been well documented for biological samples using a Vitrobot [23], but needs adaptation to succeed with ChGs in organic solvents. Our solutions display less capillary action than water with the Vitrobot filter paper and evaporate quickly due to solvent volatility. Thus, while a traditional Vitrobot protocol may include several seconds of blot time and a zero or negative blot force, we find a quicker blot at a slightly positive force setting is most effective for the amine solutions. Figs. 1(d)-(f) show grids of propylamine alone (Fig. 1(e)) and As$_2$S$_3$ in propylamine (Figs. 1(d) and 1(f)) prepared with such parameters. Figs. 1(e) and 1(f) are imaged at tilts to measure the thickness of the frozen layer; both show an average hole-edge thickness around 100 nm (103.7 nm and 97.7 nm, respectively) plotted in Fig. 1(h). This thickness is similar to values achieved with biological specimens and enables imaging of the solute [24]. In contrast, Fig. 1(g) shows a grid of propylamine prepared with blot parameters typical of a biological sample, producing a frozen layer with thickness above 1 µm that cannot be properly imaged. Thus, our freezing protocol is unique in its production of consistent quality grids with the solutions used and in the future can be applied to the numerous other material systems that require organic solvents.
Fig. 1. Development of sample preparation. (a) and (b) Propylamine grids frozen in liquid ethane are nonuniform with many empty holes. (c) As$_2$S$_3$ in propylamine grid glow discharged prior to freezing shows solution has wet the grid poorly. (d) and (f) As$_2$S$_3$ in propylamine plunge frozen in LN$_2$ without glow discharging; (f) is imaged at a tilt of $\alpha = -50^\circ$ for thickness measurement. (e) Propylamine grid prepared in the same manner as (d) and (f), imaged at a tilt of $\alpha = -70^\circ$. Grids in (a)-(f) are all prepared with blotting parameters determined most suitable. (g) Propylamine grid prepared with blotting parameters in ranges used for biological materials is too thick for imaging. (h) Average thickness measurements from grids shown in (e)-(g). Measurements locations for (e) and (f) are marked by red lines, and error bars represent standard deviation of the three. Measurement of the grid in (g) was conducted by measuring a protruding feature at 0 and nonzero tilt and relating to thickness by trigonometry; error bar is the calculated thickness of a flat feature by the same technique. Scale bars: (a), (c), and (d) 10 $\mu$m; (b) 40 $\mu$m; (e) and (f) 500 nm; (g) 50 $\mu$m.

Even with the well frozen solvent layer, there are still a variety of issues and artifacts that can arise and lead to a lack of well-preserved solute particles suspended within [19,23]. We therefore must validate our sample and the presence of imageable As$_2$S$_3$ particles. Figs. 2(a) and 2(b) show example raw cryo-EM micrographs for propylamine alone and As$_2$S$_3$ in propylamine. The dark features in Fig. 2(b) represent electron-dense As$_2$S$_3$ structures, made more clear by the contrast-enhanced version of the micrograph in Fig. 2(c). Figs. 2(d)–2(f) show the equivalent example micrographs for ethylenediamine. The lack of features in Figs. 2(a) and 2(d) confirms that the solvents are vitrified during freezing, thus preserving the in-solution structure of the ChG. While resolution is limited by the $<5e^{-}/\AA^2$ dose needed to preserve the sample at this magnification, comparing Fig. 2(b) with Fig. 2(e) makes apparent that As$_2$S$_3$ takes on different structures in the two solvents—a rounder structure in propylamine and a longer, thinner structure in ethylenediamine—consistent with the predictions from literature [17,18]. To further validate the identity of these particles, we employ two additional methods independent of cryo-EM: liquid cell transmission electron microscopy (TEM) and cryo-energy dispersive X-ray spectroscopy (cryo-EDS). Fig. 3(a) shows an example liquid cell TEM micrograph of As$_2$S$_3$ in propylamine. The presence of round features similar to those in Fig. 2(b) confirms that the structures imaged by cryo-EM are present in the liquid solution and are not an artifact of freezing. Fig. 3(c) shows the results of cryo-EDS performed on the same solution, comparing a point spectrum taken from a feature to a spectrum taken from an empty grid. The spectra clearly show the presence of As and S in the sample.
Fig. 2. Example cryo-EM micrographs. Raw cryo-EM micrographs of propylamine (a) and ethylenediamine (d) show no features, as expected for vitrified solvents. Raw cryo-EM micrographs of 0.4 mol L\(^{-1}\) As\(_2\)S\(_3\) in propylamine (b) and ethylenediamine (e) show As\(_2\)S\(_3\) structures as dark features with solvent-dependent shapes. Panels (c) and (f) are contrast-enhanced versions of (b) and (e). Scale bar is 40 nm for all panels.

Fig. 3. Independent validation of cryo-EM method. (a) Liquid cell TEM micrograph of As\(_2\)S\(_3\) in propylamine shows similar features to cryo-EM micrograph in Fig. 2(b), confirming these are not an artifact of freezing. Scale bar is 10 nm. (b) Cryo-EDS point spectrum of a feature on an As\(_2\)S\(_3\) in propylamine grid compared to spectrum of an empty grid confirm As and S are present in the sample.

2.2. Single particle analysis

Now confident in our sample preparation, we can proceed with the cryo-EM analysis, employing SPA methodology to obtain 3D averages from the two-dimensional micrographs. Atomic resolution SPA requires that the particles imaged are atomically identical, as is typically the case for biological macromolecules but not for ChGs due to their glassy nature. This places a fundamental limit on the resolution of our analysis, echoed by similar work on glasses in aqueous solution [25]: 3D reconstructions from SPA will represent the average nanoscale shape of the structures imaged. Fig. 4(a) shows the 3D nanostructure of As\(_2\)S\(_3\) in propylamine, reconstructed using EMAN2 [10]. The structure is round, in agreement with Chern and Lauks’s pioneering work from the 1980s; notably, it is round in all directions, while Chern and Lauks predicted “platelets or clusters” [17]. Our results allow us to narrow the scope of this decades old theory by concluding that As\(_2\)S\(_3\) forms clusters, not platelets, in propylamine. It is a different story for As\(_2\)S\(_3\) in ethylenediamine. Fig. 4(b) shows the 3D reconstruction for this system from three different viewing angles; it is much longer and more anisotropic than the structure in propylamine. This is consistent with Guiton and Pantano’s work from the 1980s, in which they proposed that...
ethylenediamine chelates the $\text{As}_2\text{S}_3$ and forms solution structures consisting of “branched $\text{As}_4\text{S}_6$ chains” [18].

**Fig. 4.** 3D reconstructions of $\text{As}_2\text{S}_3$ solution structure. Structure of $\text{As}_2\text{S}_3$ in propylamine (a) and in ethylenediamine (b) as determined by cryo-EM SPA, each shown from three different viewing angles. Scale bars are 3 nm.

### 2.3. Atomic structural analysis

To demonstrate how cryo-EM betters our understanding of the solution process as a whole, we combine SPA with X-ray pair distribution function (PDF) analysis—a more traditional materials science technique capable of probing local atomic structure in the corresponding dried systems. Previous work has suggested different drying mechanisms for each solvent as a result of the solution structure—cluster growth and aggregation for $\text{As}_2\text{S}_3$ in propylamine resulting in films that retain cluster-like features on a longer length scale [26,27] and a process similar to gelation for $\text{As}_2\text{S}_3$ in ethylenediamine [18]. Fig. 5(a) compares the X-ray PDFs of samples prepared by drying the solutions without annealing. The amorphous nature of the samples is clearly revealed by the absence of obvious structural order above $\sim 6\text{ Å}$. Nevertheless, local structural orders ($\sim 2-4\text{ Å}$) are evident for both samples, consistent with previous PDF studies on similar ChGs [28,29], and show a solvent dependency. Figs. 5(b) and 5(c) show the first atom-atom correlation intensity deconvoluted into a central peak at $\sim 2.24\text{ Å}$ and a right side shoulder at $\sim 2.5\text{ Å}$, each represented by a Gaussian. While 2.24 Å corresponds to the expected heteropolar As-S bond length, the 2.5 Å shoulder has been seen in previous studies and is thought to represent homopolar As-As “wrong bonds” in the amorphous structure [28,30]. The relative amounts of homo- and heteropolar bonds in ChGs has been shown to affect their nonlinear refractive index, a key property for device applications [15,16]. Comparing the relative areas of the shoulder and main peaks in our PDF data shows that the 2.5 Å correlation is more prominent in the $\text{As}_2\text{S}_3$ in propylamine, where it has a relative area of $\sim 11\%$, than in ethylenediamine, where it has a relative area of $\sim 2\%$.

This difference in local structure is consistent with both solution structures we have observed with cryo-EM. The cluster structure we determined in propylamine was proposed by Chern and Lauks to consist of bulk $\text{As}_2\text{S}_3$ within an amine salt shell [17]. The presence of this salt would mean there are molecules in the solution whose As:S ratios differ from that of $\text{As}_2\text{S}_3$, consistent with the “wrong bonding” observed by PDF. The chain-like structure observed in ethylenediamine, meanwhile, does not require the formation of nonstoichiometric As-S components, consistent with the smaller amount of “wrong bonding” observed for this system. Thus, by combining
cryo-EM SPA with PDF analysis, we are able to further corroborate the long held theories on \( \text{As}_2\text{S}_3 \) solution structure and to conclude that nanostructural differences between the solutions correspond with atomic structural differences between the dried materials. This suggests not only that these local atomic structures arise in solution but that they propagate—at least in part—to the dried state.

2.4. Optical characterization

Connections between the solvated and dried material are further observed with an investigation of linear optical properties. As shown in Fig. 5(d), the solution of \( \text{As}_2\text{S}_3 \) in propylamine shows a higher indirect band gap than the solution of \( \text{As}_2\text{S}_3 \) in ethylenediamine—2.66 eV compared to 2.54 eV—a trend that should be expected based on the size differences of the cryo-EM determined structures. This trend propagates to the as-cast films, which show band gaps of 2.44 eV for the propylamine system and 2.33 eV for the ethylenediamine system. While the lower band gaps of the films compared to the solutions suggest that elements of the in-solution structure are removed during conventional spin-coating and drying, differences between the films can still be correlated to differences between the solutions and solution structures. This demonstrates how understanding the in-solution structures through cryo-EM may unlock additional avenues for tuning ChG properties.

3. Conclusion

Thus, by performing cryo-EM SPA for the first time on an inorganic material in organic solvents, we have shown that cryo-EM solution characterization can work in tandem with traditional film characterization to build a clearer picture of the solution process as a whole. We see cryo-EM SPA as a valuable tool for filling in gaps in understanding structure and property evolution during the solution process and have used it to fill a long open gap in the understanding of \( \text{As}_2\text{S}_3 \). We envision that future SPA studies of inorganic materials and non-aqueous solutions may span...
as vast a scope as solution processing itself. The structures of As$_2$S$_3$ explored mirror those of semiconductor quantum dots in both size and shape, offering a host of additional materials likely suitable for SPA. Organic solvents, too, find use not just with ChGs but also with perovskites and various low-dimensional materials, all of which are likely to succeed as cryo-EM samples using a freezing protocol similar to that which we have developed. This technique will only improve as it is more broadly adopted throughout materials science, giving us the ability to visualize and characterize device materials during processing, as opposed to after the fact.

4. Methods

4.1. Materials

As received As$_2$S$_3$ powder (Alfa Aesar 99.999%), confirmed amorphous by X-ray diffraction, was mixed with propylamine (Sigma-Aldrich 99.9%) or ethylenediamine (Fluka 99.5%) at 0.4 moles per liter solvent (mol L$^{-1}$ solv) and left for a week to dissolve at 22°C without stirring. Changes to parameters like concentration and mixing temperature have been shown to affect structure size in As$_2$S$_3$ solutions [26,27]; these factors can also be analyzed using cryo-EM but in this case were kept constant to isolate the effects of solvent choice. The 0.4 mol L$^{-1}$ solv concentration was chosen because it combined a relatively low viscosity with a relatively large As$_2$S$_3$ structure size in propylamine, ideal for cryo-EM sample preparation and imaging [27]. A 0.3 mol L$^{-1}$ solv solution of As$_2$S$_3$ in propylamine was also made for cryo-EDS only, as an even lower viscosity was needed. Solutions were prepared and stored in a nitrogen atmosphere glovebox to limit exposure to oxygen and moisture.

4.2. Electron microscopy

Cryo-EM samples were prepared with a Thermo Fisher Scientific Vitrobot Mark IV. Vitrobot chambers are normally brought to 100% relative humidity to prevent drying in aqueous samples, but since water vapor is a contaminant for non-aqueous samples, the Vitrobot was operated at ambient humidity. The temperature was also left at 20°C, rather than the 4°C commonly recommended for biological samples, as our previous work suggests the As$_2$S$_3$ in propylamine structure is temperature sensitive [27]. Samples for imaging were prepared on 200 mesh copper grids with Quantifoil substrates (R 1.2/1.3). For As$_2$S$_3$ in propylamine, 5 µL of 0.4 mol L$^{-1}$ solv solution were deposited on the as-received grid and blotted once at force setting 5 for 1s before being plunged into LN$_2$. For As$_2$S$_3$ in ethylenediamine, the same blot parameters and concentration were used, but with 4 µL of solution. Samples were imaged using a Thermo Scientific Titan Krios G3 with Volta Phase Plate, operated at 300 kV. Automated data acquisition was performed with EPU; micrographs were acquired at 165 kx magnification for 6 s through a Gatan BioQuantum Imaging Filter and collected by a Gatan K2 Summit Direct Electron Detection Camera operated in counting mode with a 20 eV slit inserted. The defocus ranged from $-0.5$ to $-2.0\mu m$, and the dose per acquisition was $4.7232$ e$^-$/Å$^2$.

Samples for cryo-EDS were prepared on 300 mesh copper grids with PELCO single layer graphene on lacey carbon to provide more support for the sample under the stronger beam required to obtain a measurable EDS signal. To maintain thinness of the sample on the different grid, 0.3 mol L$^{-1}$ solv As$_2$S$_3$ in propylamine was used, with 4 µL blotted in the same manner as described above. Cryo-EDS spectra were acquired using a Thermo Scientific Talos F200X scanning/transmission electron microscope (S/TEM) in STEM mode and a Gatan 626 cryo-transfer holder. Liquid cell TEM micrographs were also acquired on the Talos F200X, this time in TEM mode with 0.4 mol L$^{-1}$ solv As$_2$S$_3$ in propylamine in a Protochips Poseidon Select in situ holder.
4.3. Thickness measurement

Thicknesses of cryo-EM grids were measured in ImageJ [31]. For grids thin enough to image through, a crack was found and imaged at some known tilt $\alpha$. The perceived thickness $h_{\alpha}$ was then measured and related to the actual thickness $h_0$ through $h_0 = \frac{h_{\alpha}}{\sin|\alpha|}$. For grids too thick to image through (Fig. 1g, which was blotted once for 2 s at force setting −20 to represent parameters common for biological samples), a protrusion of frozen solvent was imaged at no tilt and again at a known tilt $\alpha$. The width of the feature (in the direction perpendicular to the tilt axis) was measured in both images ($w_0$ and $w_{\alpha}$) and related to the thickness $h_0$ through $h_0 = \frac{w_{\alpha} - w_0 \cos|\alpha|}{\sin|\alpha|}$.

4.4. X-ray PDF analysis

The X-ray PDF analysis was conducted using the same methodology as in our previous work [29]. The 0.4 mol L$^{-1}$ solutions were dried in a ceramic mortar in the nitrogen atmosphere glovebox, then crushed into powder. The powder was packed into polyimide capillaries and sealed with epoxy before removal from the glovebox and measured on a Bruker D8 Advance X-ray diffractometer using Ag K$_\alpha$ radiation. The data were collected from a $2\theta$ of 3° to 130°, with a step size of 0.05° and a count time of 30 s per step. The PDF, $G(r)$, was then calculated by taking a sine Fourier transform of the measured total scattering function $S(Q)$, where $Q$ is the momentum transfer, as outline by Egami and Billinge [32]. The X-ray PDF data were obtained using PDFgetX2 [33] and standard data reduction procedures with a $Q_{\text{max}}$ of $\sim$20 Å$^{-1}$. Before calculating the PDF, the total scattering function $S(Q)$ was multiplied by a Lorch window function [34] to improve the signal/noise, similar to our previous work [29]. For peak analysis, a linear baseline was fitted to the first atom-atom correlation peak, which was then fitted to the sum of four Gaussian functions. The mean of the right hand side shoulder was fixed at 2.5 Å to enable comparison of its relative area in each system.

4.5. Band gap determination

Indirect band gaps were determined through absorption measurements and use of the Tauc relation [35]. Liquid samples were prepared by dropping 10 µL of solution (0.4 mol L$^{-1}$ for both propylamine and ethylenediamine) onto a glass slide, placing a cover slip, and sealing on all edges with Parafilm. As-cast film samples were prepared by spin-coating 50 µL of solution on a glass cover slip and allowing to dry. All samples were prepared in a nitrogen glove box and removed immediately prior to measurement. Absorbance measurements were carried out in a Cary 5000 UV-Vis-NIR spectrometer with a glass slide reference. Thickness of liquid samples was calculated using the known volume of solution and surface area occupied on the slide. Thickness of films was measured with an Olympus LEXT OLS4000 confocal microscope.

4.6. Reconstructions

3D reconstructions of the As$_2$S$_3$ solution structures were prepared using EMAN2 [10]. The structure in propylamine was reconstructed to 16.3 Å resolution using images of 1,374 particles, and the structure in ethylenediamine to 25.2 Å resolution using 1,127 particles. This is far fewer particles than is used for single particle reconstruction in biology—the amorphous and self-organized nature of the As$_2$S$_3$ structures implies they are not atomically identical and cannot be used to generate a true high-resolution single particle reconstruction, but rather to determine a low-resolution average structure shape. Thus, larger data sets would have increased the data acquisition and computing loads without significantly improving the analysis. Our resolution is consistent with what has been reported in the limited existing work on SPA with glassy materials [25].
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Disclosures
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