

Researchers observe real-time homogeneous nucleation using cryo-TEM

Theories of homogeneous crystallization have been around for a long time, beginning with J.W. Gibbs, of Gibbs free energy fame, in 1876. In recent years, the classical nucleation theory of spontaneous ion-by-ion or molecule-by-molecule formation of crystal nuclei from a liquid or solution has been joined by a nonclassical theory. A number of nonclassical pathways have been observed, including a two-step mechanism in which an amorphous dense phase is formed first, before subsequently reorganizing to form crystal nuclei. While this bimodal, nonclassical mechanism has been confirmed before by various means, recently researchers at the Weizmann Institute of Science in Rehovot, Israel, have observed the crystallization process visually at real time and molecular scale using cryogenic transmission electron microscopy (cryo-TEM). They report their research in a recent issue of *Nature Chemistry* (doi:10.1038/NCHEM.2675).

Ronny Neumann of the Department of Organic Chemistry at the Weizmann Institute of Science has been working with polyoxometallates as catalysts since 1988. A certain manganese polyfluoroxometallate (MnPFOM) has a formula of $K_8[H_2F_6NaMn^{IV}(OH)W_{17}O_{55}]$. About a year and a half ago, he and his team observed something unusual when performing a reaction that liberates oxygen from this MnPFOM. “What we saw was something we didn’t expect—we saw pre-organization in this reaction under certain conditions,” Neumann says. “When there was pre-organization the reaction was faster than when there wasn’t. So that started it all. We thought if we could pre-organize or not pre-organize molecules at will, then maybe we could find a way to visualize both the classical and nonclassical nucleation process using cryogenic TEM.”

To test whether pre-organization at will was possible, Neumann and colleagues prepared two different MnPFOM solutions: one with NaCl and one with CsCl salt in the solution. They hypothesized that because the MnPFOM molecules are anionic, their solubility can be tuned due to the different solvation properties of cations leading to different phase formations. The NaCl solution was placed on a quantifoil TEM grid at 60°C then cooled rapidly to -180°C in ethane to vitrify it. The same process was performed with the CsCl solution. But the differences in crystallization appeared when the TEM beam began to slowly heat the supersaturated solutions, shifting them to equilibrium.

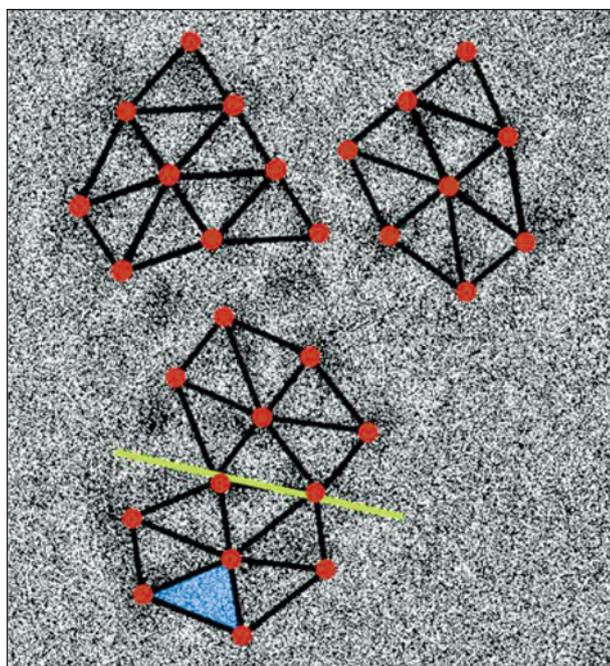
In the NaCl solution, which was initially homogeneous, the MnPFOM molecules attached one by one to form round aggregates of approximately 5–15

molecules. A TEM tomogram showed that the MnPFOM molecules nucleated and grew in a two-dimensional (100) plane aligned with the x, y directions of the TEM foil. In the CsCl solution, the Cs^+ cations induced a dense phase in which the MnPFOM molecules formed a non-ordered aggregate in which they occupied positions close to, but not commensurate with, their ideal crystal lattice positions; when heated, this aggregate collapsed into an ordered lattice. Crystalline structure was determined by comparison with an x-ray diffraction structure. “The important thing about these compounds is that we can really control the formation of different types of phases, and this control allowed us to do this research,” Neumann says. “With Cs cations you get amorphous aggregates and with Na cations you get purely homogeneous nucleation.”

The two-step aggregation-crystallization process of the Cs solution has a lower overall energy barrier than the one-step classical homogeneous spontaneous random aggregation of molecules in the Na solution, so the two-step process is faster, as predicted. Being able to observe this process with TEM could have implications down the road for a better understanding of protein crystallization and self-assembly of molecules, according to Neumann.

“In recent years, evidence of nonclassical nucleation pathways (often known as two-step nucleation models) has appeared in the literature of ionic systems, small molecule organic systems, and proteins and also has been supported by molecular modeling studies,” says Allan S. Myerson, professor of the practice of chemical engineering in the Department of Chemical Engineering at the Massachusetts Institute of Technology, who was not involved in this work. “In this new research, the authors experimentally observed nucleation using cryo-TEM. Their work demonstrates that both classical and nonclassical nucleation mechanisms can occur depending on the cation present. This observation demonstrates the complexity of nucleation and that nucleation pathways can depend on the nature of the substance involved.”

Tim Palucka



Relative position of molecules derived from the x-ray diffraction crystal structure (red circles) overlaid on the transmission electron microscope image. Credit: *Nature Chemistry*.