NUCLEATION

What atoms do when they get together

How atoms organize during the earliest stages of nucleation has been a subject of speculation for over a century. Using atomically resolved electron microscopy, the formation and ordering of metal clusters from individual atoms has now been observed in carbon nanotubes that serve as 'test tubes'.

J. J. De Yoreo and B. A. Legg

Classical Nucleation Theory (CNT)[1], the physical model of crystal nucleation that was first proposed by Gibbs to explain the formation of rain drops, was dominant throughout the twentieth century but has since been challenged by experiments, theory and simulations for a wide range of materials. CNT contains three essential features. First, as a cluster grows, competition between decreasing chemical potential terms and increasing surface energy terms leads to a maximum in the work of formation and, therefore, a free-energy barrier and associated critical cluster size. Second, this barrier is overcome by thermally driven fluctuations that generate a steady population of unstable clusters that grow and shrink through reversible addition of monomers — these may be atoms, ions, molecules or colloids, depending on the crystallizing system. Third, an ancillary unnecessary assumption in nearly all formulations of CNT is that the clusters exhibit the same structure as the final crystal.

Conflicts between these features of CNT and the observed behaviour of nucleating systems inspired hypotheses of alternative mechanisms, often referred to as non-classical[2]. Many involve two-step pathways in which amorphous particles or dense liquid droplets appear first and eventually give rise to the stable form[3]. Whether these intermediates are metastable — or in fact represent the stable form at a very small size — has remained largely unresolved, as has the formation mechanism of the precursors themselves, whether by the atomistic dynamics envisioned in CNT or a fundamentally different physical process[4].

Writing in *Nature Chemistry*, Ute Kaiser, Andrei Khlobystov and co-workers now provide some clear answers to these questions by documenting[5] the temporal evolution of both the number of atoms and degree of crystallinity in individual clusters of Fe, Au and Re. An atomically accurate description of the nucleation of metal nanocrystals was obtained by applying in situ low-voltage aberration-corrected transmission electron microscopy (TEM) to growing metal clusters confined within single-walled carbon nanotubes (SWCNT), which served as electron-transparent test tubes. To create the metal clusters, SWCNTs were annealed in a vapour of an appropriate organometallic complex and the organic ligands were then eliminated by thermal dissociation. Delivery of atoms to the clusters occurred through electron-beam-driven diffusion of amorphous carbon, which the metal atoms were complexed to. Attraction of the metal atoms to the cluster led to the atoms' detachment from the amorphous carbon and attachment to the growing cluster.

For all three metals, amorphous clusters first formed, then transformed to crystalline phases after they exceeded a certain size (Fig. 1a,b). In the case of Fe, the nucleation process started with formation of a diatomic seed that atoms were repeatedly delivered to, resulting in a structurally dynamic amorphous cluster that crystallized into the γ-Fe phase after sufficient incubation time. Analysis of numerous clusters showed that, while the diatomic seeds can dissociate, clusters containing about 10 atoms neither dissociate nor crystallize, while those containing about 17 atoms or more always crystallize. Thus, under the conditions of the experiment, there is a size range where the amorphous phase is stable and another where the crystalline phase is stable. Each exhibits a critical size for nucleation and the amorphous-to-crystalline transformation occurs via fluctuations in the individual atomic positions of the constituent atoms.

The atomic details of crystallization in the amorphous cluster were also captured in the case of Au. During exposure of an ~100-atom cluster to the electron beam, a sub-nm ordered region formed, dissociated, reformed and fluctuated in size until it exceeded about 1 nm, after which, crystallinity spread through the rest of the cluster to form a single crystal.

Investigations on Re clusters show that cluster coalescence events can enhance the probability of crystallization. Collision of two stable, amorphous and ~10-atom clusters led to the formation of a larger cluster whose size favoured crystallization, and atomic reorganization into a single crystal was observed.

These findings demonstrate that, for all three metals, nucleation follows a two-step mechanism. In the first step, individual atoms add to clusters that are unstable until they become large enough to overcome the free-energy barrier for formation of stable amorphous clusters. In the second step, continued addition of atoms renders the amorphous cluster metastable as it exceeds the critical size for nucleation of the crystalline phase. Individual atomic additions and rearrangements enable both steps.

Some details of the transformation step remain unclear. For example, an observed gold cluster was amorphous prior to imaging and during initial exposure to the electron beam, under which it eventually crystallized. The researchers proposed that the transformation occurs because energy from the electron beam decreases the critical size for crystallization. Although this is certainly possible, nucleation probabilities also depend on kinetic factors[6] — such as bond breaking to enable reorganization — that are unknown and difficult to separate from that of free-energy considerations.

Investigations of nucleation processes that proceed via two or more steps date back to Ostwald[6] and a number of fundamentally different mechanisms have been invoked to explain their existence. In the simplest rationalizations based on CNT, particles of both a metastable and a stable phase are assumed to be generated by direct nucleation pathways, independently of each other. The inverse relationship between surface energy and phase stability is thought to enable faster formation of the metastable phase as long as the supersaturation is high enough[7], but the rapidly generated metastable particles will later dissolve away in favour of the more stable final phase. In a second class of pathways, developed to explain the

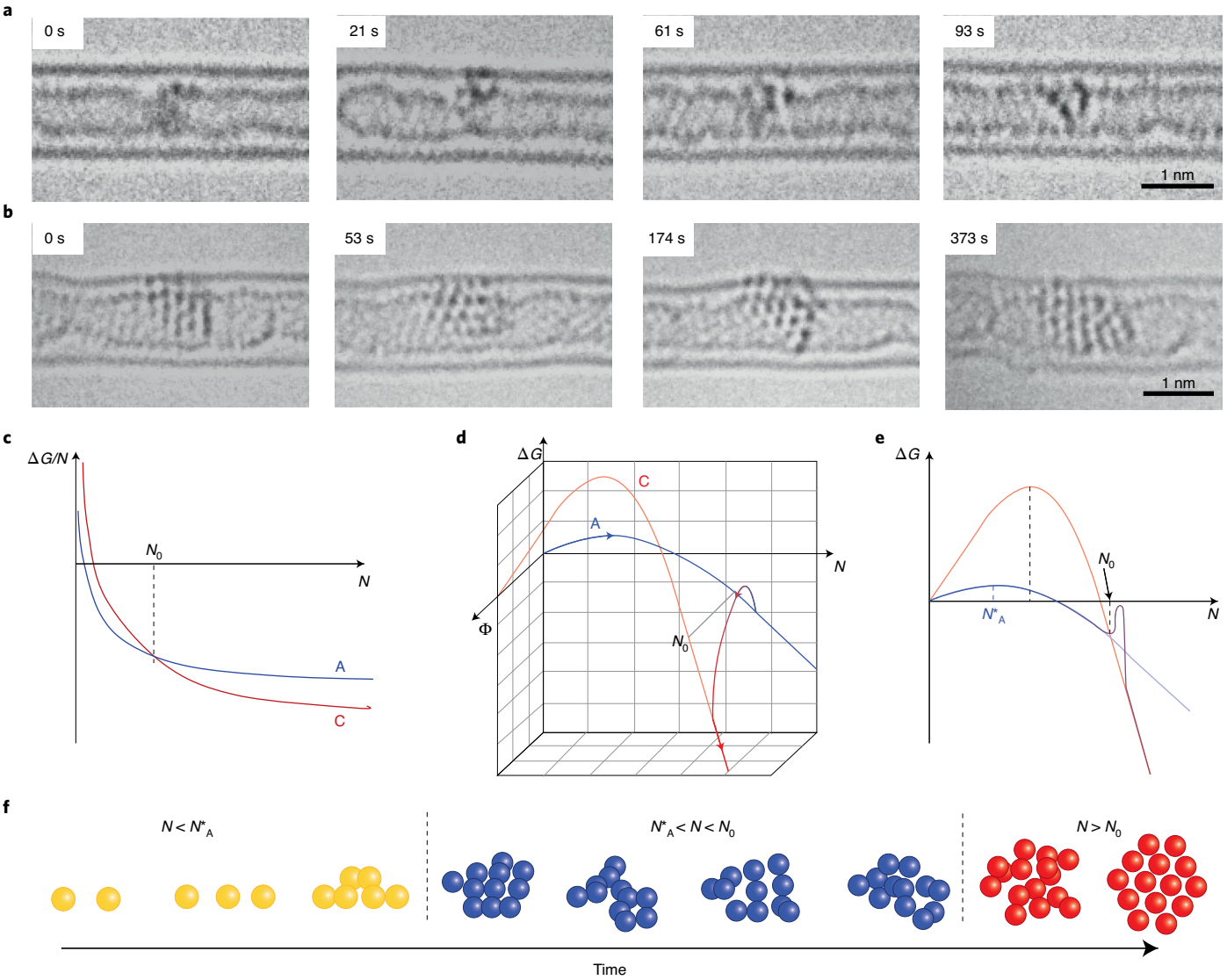

Fig. 1 | Two-step nucleation due to inversion in the stability of phases with particle size *N*. a,**b**, Fe clusters of about 10 atoms remain amorphous for the duration of the experiment (**a**), while γ-Fe clusters of 100 atoms are always crystalline (**b**). **c**, Free energy of formation ΔG versus size for crystalline (C) and amorphous (A) phases exhibit a crossover at N_0. **d**, The curves of ΔG for the two forms, showing that the direct barrier is larger for C than for A, but may be bypassed by transitioning from A to C at a larger size. Here Φ represents the degree of atomic order. **e**, Simplified curve of ΔG projected onto the ΔG–N plane for cases where fluctuations are large enough to ensure the low-energy phase will dominate. A nucleates first by overcoming the barrier at N^*_A and remains stable until it reaches the size N_0, where the relative stabilities revert back to the bulk relationship, then A converts to C by overcoming a second free-energy barrier. **f**, Schematic showing the nucleation process for Fe, Au or Re, where atoms aggregate to form an amorphous cluster (yellow) that is stable once it reaches N^*_A (blue) and then converts to the crystalline form once it exceeds N_0 (red). Panels **a** and **b** adapted with permission from ref. [5], Springer Nature Ltd.

behaviour of proteins, crystal nucleation occurs within unstable protein-rich liquid droplets that form through fluctuations within the region of crystal–solution coexistence[3,8]. The third mechanism (Fig. 1c–f) arises from the inversion of relative phase stabilities that can commonly occur in nanoparticle systems — for example, bulk rutile TiO_2 is more stable than anatase TiO_2, but nanoparticles of anatase are more stable than nanoparticles of rutile due to differences in surface energy[9,10]. In such cases, nucleation favours anatase particles, which are expected to transform into rutile as they grow. The results of the present study[5] provide the first atomic details for this third mechanism and show that it even applies to systems that only adopt one bulk crystalline phase, if the amorphous phase is also considered.

These findings[5] highlight the limitations and strengths of CNT. On the one hand, size dependence in both the stability of phases and the corresponding surface free energies is not considered in the theory, despite widespread acceptance of both. Consequently, a rich set of nucleation phenomena associated with transient phases is missed by CNT. Nonetheless, the simple notion that clusters must grow to a certain size before they are stable — and that they do so through inherent thermal fluctuations, manifest by random attachment and detachment of atoms — was soundly validated by the results. In short, while pathways to the crystalline state exhibit diversity because free-energy landscapes are complex, crossing those landscapes requires

uphill climbs, made possible through the statistics of fluctuating systems.

The conflict between CNT and recent observations of crystal nucleation parallels a similar crisis that occurred in the middle of the twentieth century when accepted theories of crystal growth clashed with observations. At the time, the growth of new crystal layers was assumed to be limited by the nucleation of new two-dimensional islands on existing crystal faces according to CNT, but careful measurements of growth rates were orders of magnitude larger than predicted values. This crisis was resolved by the realization of Frank, that dislocations, a ubiquitous feature of crystals, open up a barrier-free mechanism of growth[11]. The continuous supply of steps at the dislocation eliminates the need to nucleate a new island. Nonetheless, CNT remained a key component of crystal growth theory because it was needed to explain the rate at which new steps were generated at dislocations. While the pathway to growth deviated from that envisioned classically, the underlying physical chemical principle held steady. Moreover, electron microscopy played a central role in resolving the crisis by demonstrating the presence of dislocation steps on crystal surfaces. With the ability to now resolve individual atoms in a forming cluster, Kaiser, Khlobystov and colleagues have once again brought electron microscopy to the fore by providing proof that the lowest energy pathway to nucleation of metal particles bypasses the high barrier associated with directly forming the ordered state and instead takes an easier route via the amorphous state. Nonetheless, the mechanism by which amorphous nuclei form and transform holds true to the picture of atomic fluctuations envisioned by Gibbs nearly 150 years ago. ❐

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Competing interests

The authors declare no competing interests.

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CHEMICAL PROBES

Protein targeting with SAF(er) electrophiles

Electrophilic groups that undergo sulfur-exchange chemistry with protein nucleophiles can serve as the functional basis of chemical proteomic probes. A new addition to this class, sulfuramidimidoyl fluoride (SAF), which can be included in an array of covalent small molecule probes, exhibits a unique reactivity profile with proteins.

Thomas E. Speltz and Raymond E. Moellering

Chemical proteomics — the study of endogenous protein structure and function through the inclusion of small molecule probes in proteomic pipelines — often requires probes that covalently label target proteins. A number of such probes developed recently are based on sulfur-centred, electrophilic functional groups. These electrophilic 'warheads' are particularly useful as they balance chemical stability in aqueous environments with, in many cases, relatively broad reactivity with properly positioned protein nucleophiles[1]. Now, writing in *Nature Chemistry*, Jeffery Kelly, K. Barry Sharpless and co-workers describe the protein reactivity of small molecules functionalized with sulfuramidimidoyl fluoride[2] (SAF).

The most-studied functional groups capable of undergoing sulfur-exchange (SuFEx) chemistry are sulfonyl fluorides[3] and fluorosulfates[4], which react with a range of side chains in proteins such as amine and phenolate nucleophiles in protein lysines and tyrosines, respectively. This reactivity has been harnessed for the development of kinase-directed chemical probes, and has been more recently expanded to other enzyme and protein families[5]. Building on the utility of SuFEx chemistry, multiple sulfur-centred groups have been developed and deployed in the construction of electrophilic probes. These include the sulfonyl triazoles[6], used to profile tyrosines in lysates and live cells, as well as heteroaryl sulfones and sulfoxides that undergo S_NAr reactions with protein cysteines and likely other nucleophilic groups[7,8].

One overarching limitation of these sulfur-based electrophiles has been their synthetic inclusion in probes while safeguarding ensuing reactivity, which often demands that the electrophiles are incorporated as separate units near the end of a synthetic route. Additionally, there are limited opportunities to modify the electrophilic scaffold itself to tune reactivity and influence molecular recognition, for example with groups like sulfonyl fluorides, which are necessarily terminal. The development of new electrophilic groups that can address some, or all, of these limitations would be of great utility in chemical proteomic probe development.

Kelly, Sharpless and colleagues explored the proteome-wide reactivity of sulfuramidimidoyl fluoride (SAF) functionalized small molecules by applying an inverse drug discovery strategy — reacting a collection of small molecules with the proteome to identify protein(s) targeted (conversely, drug candidates are generally discovered by screening a large number of small molecules for their ability to modify the function of a target protein)[2]. They reasoned that sulfur–fluoride exchange by nucleophilic attack would only be possible in conditions where the SAF group is properly oriented near a nucleophilic