Missing gold atoms in lysozyme crystals used to grow gold nanoparticles

To the Editor — Wei *et al.* reported the growth of gold nanoparticles within protein single crystals of hen egg white lysozyme (*Nature Nanotech.* **6,** 93–97; 2011). Visible absorption spectra and transmission electron microscopy were used to prove the co-crystallization of gold nanoparticles within the crystals, and from an analysis of crystallographic data, the authors proposed a structural characterization of the process.

We tried to reproduce the experiments performed by Wei *et al.,* obtaining red, well-diffracting crystals of lysozyme after 1 month of soaking in the presence of the precursor $ClAuS(CH_2CH_2OH)_2$. However, when we refined our crystal structures we found no gold atoms.

This finding prompted us to analyse the models of Wei *et al.* deposited in the Protein Data Bank (codes 3P4Z, 3P64, 3P65, 3P66, 3P68), in which nine different gold atoms are present (four isolated gold atoms and a 5-atom cluster). Three out of these five structures show a gold ion bound to His15 and to a monoatomic ligand (Supplementary Fig. 1), which Wei *et al.* reasonably modelled as a chloride ion, considering that lysozyme crystals were grown in high NaCl concentrations. Five more gold ions are clustered together in the last two deposited crystal structures.

For eight out of the nine gold atoms found in the structures deposited by Wei *et al.*, we believe that the authors' interpretation is questionable. This is due to the following reasons: first, almost all gold atoms have been placed by Wei *et al.* where Cl^- or Na^+ ions are usually found in isomorphous crystals of lysozyme that are grown using NaCl as a precipitant and sodium acetate as a buffer solution; second, Au ions form their most stable complexes with 'soft' donor atoms such as P, S or N — in these crystal structures, the Au ions have been placed in atypical coordination (for example, close to Tyr23, Ser24, Thr43); third, an analysis of electron density maps shows large negative peaks at the position of Au atoms (Supplementary Figs 2–5). The negative peaks disappear when gold atoms are replaced with Cl^-, Na^+ ions or water molecules. Independent refinements, performed using structure factors of the deposited models, show that the replacement of gold atoms with Cl^- and Na^+ results in a significant decrease of both R-factor and R-free (that is, a better agreement between structural models and experimental crystallographic data) in all the five structures (Supplementary Table 1).

Ultimately, three out of five crystal structures solved by Wei *et al.* are likely to correspond to lysozyme with only one Au^+ ion bound to His15. The last two crystal structures are gold-free.

We consider the ability of gold nanoparticles to grow within protein single crystals a stimulating result that has interesting implications. Our findings weaken, but do not invalidate the hypothesis suggested by Wei *et al.* of a protein-mediated metal ion transfer preceding the nanoparticle formation, a result that has recently been supported by Baksi *et al.* (*Nanoscale* **5,** 2009–2016; 2013). However, the structural analysis by Wei *et al.* cannot be used to unveil protein–gold nanoparticle interactions because no gold atom is unambiguously found in the lysozyme structures reported, apart from one ion bound to His15 in the first three structures. This means that structural data on biomolecule-directed gold clusters is still lacking and that the molecular basis of protein–gold nanoparticle recognition requires further investigation. ❐

Additional information

Supplementary information is available in the online version of the paper.

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Pre-market testing of nanomaterials in food is both practical and necessary

To the Editor — Andrew Maynard's Thesis article[1] asks an important question about how we respond to new data suggesting health impacts associated with nanomaterials contained in food products that are already on the market. He uses the example of synthetic amorphous silica (SAS), a nanomaterial used in food. A study released in 2012 suggested that the SAS had a very different toxicity profile to its nano colloidal counterpart[2]. The colloidal silica, which had virtually the same particle size as the SAS, had no toxic effects, but the SAS was shown to lead to the production of hydroxyl and reactive oxygen species.

In another study published in 2014, rats that were fed SAS developed fibrosis of the liver and the nanostructured silica accumulated in the spleen[3]. Based on a history of no known impacts, do these studies provide sufficient evidence to justify some kind of regulatory intervention? Maynard answers no. He justifies this based on a history of no known impacts associated with SAS and because of the potential impacts on consumers