THE UNIVERSITY OF
WARWICK

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Dear Editor,

Please find enclosed the manuscript entitled **'Cryo-EM of multiple cage geometries reveals a universal mode of clathrin self assembly'** for consideration for publication in Nature.

Clathrin drives and coordinates transport vesicle formation in clathrin-mediated endocytosis and intracellular trafficking while also playing a pivotal role in mitosis by stabilising the mitotic spindle during cell division. Clathrin mediated endocytosis underpins multiple cellular functions including nutrient uptake, synaptic vesicle recycling, signaling, maintenance of cell polarity and development. In addition, the endocytic apparatus is used by some viruses (e.g. HIV, influenza) and bacteria to gain entry into cells, and some forms of cancer and neurodegenerative disease have been attributed to its malfunction. Thus clathrin and the processes it supports, are of fundamental importance to the health of eukaryotic organisms.

A central element of clathrin function lies in its ability to self-assemble into cage lattices and interact with multiple binding partners, yet the current structural information on clathrin in its assembled state is lacking. In 2004, a 7.8 Å cryo-EM map by Fotin *et al*, published in Nature, provided an alpha carbon model of assembled clathrin which was a landmark in the field and of enormous and sustained value. It has remained the highest resolution structure of clathrin assembled into a cage. Now, 14 years later, we present a much higher resolution cryo-EM map and molecular model of assembled clathrin at 4.0 Å together with lower resolution maps of five different clathrin cage types. This new structural information provides a significant advance in our understanding of clathrin assembly that complements recent progress in understanding endocytic dynamics in cells (Avinoam *et al*, Science 2015 and Scott *et al*., Nature Comms 2018).

Our analysis reveals the origin of the diverse lattice structures that clathrin makes and shows clathrin subunit interactions for the first time, including how the unusually stable trimerisation domain is formed. We conclude that clathrin can form multiple lattice assemblies by flexing of its subunits to maintain universal sites of contact. Because our manuscript explains how this single, fundamentally important protein is able to form diverse and flexible cellular structures in performing its function, we believe our findings will appeal to the wider readership of Nature.

This work is of central importance to multiple scientific communities because of the fundamental role clathrin plays in cellular processes including endocytosis, vesicle trafficking and mitosis, and in key areas of eukaryotic biology such as signaling, neuronal function and development.

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Despite the importance of clathrin-mediated endocytosis for many branches of biology, and the maturity of this field, structural data on the large scale macromolecular assemblies that clathrin forms as it performs its cellular role is lacking. This is due to the immense technical difficulty that must still be overcome to obtain such structural information.

The work we present is a first step in providing a molecular level understanding of the way in which clathrin engages with its partners in achieving coated vesicle formation and is a substantial technical and methodological advance for cryoEM structure determination. It is therefore of wide-reaching and fundamental relevance to all those with an interest in eukaryotic biology.

Yours sincerely,

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