InsPection of electron density maps supports wrongly modeled hexakisphosphate (InsP6) bound to African swine fever mRNA-decapping enzyme g5Rp

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InsPection of electron density maps supports wrongly modeled hexakisphosphate (InsP6) bound to African swine fever mRNA-decapping enzyme g5Rp

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In April 2022, a paper entitled "Structural Insight into Molecular Inhibitory Mechanism of $InsP_6$ on African Swine Fever Virus mRNA-Decapping Enzyme g5Rp" was published in the *Journal of Virology* (1). In this article, the authors focus on the g5Rp Nudix hydrolase from African swine fever virus (ASFV), the causative agent of swine infection. This enzyme is endowed with a diphosphoinositol polyphosphate (PP-InsP5) phosphohydrolase activity converting PP-InsP5 into inositol hexakisphosphate (InsP6) as well as with a low mRNA-decapping activity (2–4). They also claim to have solved the crystal structure of g5Rp bound to the InsP6 inhibitor at 2.25 Å resolution (PDB code: 7DNU). However, the 2Fo-Fc electron density map calculated from the experimental diffraction data does not match with InsP6 (Fig. 1, compare panels A and B). Indeed, several InsP6 atoms do not fit into the 2Fo-Fc electron density map and some phosphate groups fall within negative regions of the Fo-Fc difference map (Fig. 1A), indicating incorrect modeling of InsP6. Additional observations further support this statement: (i) the mean B factor (148.4 $Å^2$) for the modeled InsP6 is very high compared to the mean B factor of all the g5Rp atoms (46.6 $Å^2$); (ii) phosphate groups from symmetry-related InsP6 molecules are located less than 3 Å from each other (Fig. 1A), which is energetically highly unfavorable; (iii) the side chain of positively charged Lys residues (K8 and K133) are modeled so as to form strong electrostatic interactions with negatively charged InsP6 phosphate groups but are not defined by electron density, indicating that these side chains are in fact highly flexible, which should not be the case due to their assumed roles in InsP6 binding; and (iv) the Fo-Fc omit difference map calculated in the absence of InsP6 bound to g5Rp model also fails to confirm the presence of InsP6 in this region of g5R (Fig. 1C and D). This latter Fo-Fc difference omit map suggests the presence of a yet to be identified ligand, different from InsP6 and which could originate from the buffers used during protein purification (unfortunately not indicated in the Materials and Methods of the article) or which could have been co-purified with g5Rp as frequently observed in crystal structures (5–7). This incorrect structure does not call into question most of the experimental results obtained by biochemical methods. However, I believe it is important to alert the scientific community about this error, so that this structure is not used as a basis for future research aimed, for example, at trying to develop anti-ASFV drugs on the basis of this incorrect structural information. The authors of this crystal structure should also be encouraged to correct it and publish an erratum in the *Journal of Virology*, as suggested for other similar problems (8).

The great advantage of X-ray crystallographic structures over molecular docking models is that the former generate models based on experimental diffraction data. In recent years, more and more tools have been developed to identify ligands corresponding to Fo-Fc difference maps and thus help X-ray crystallographers identify molecules

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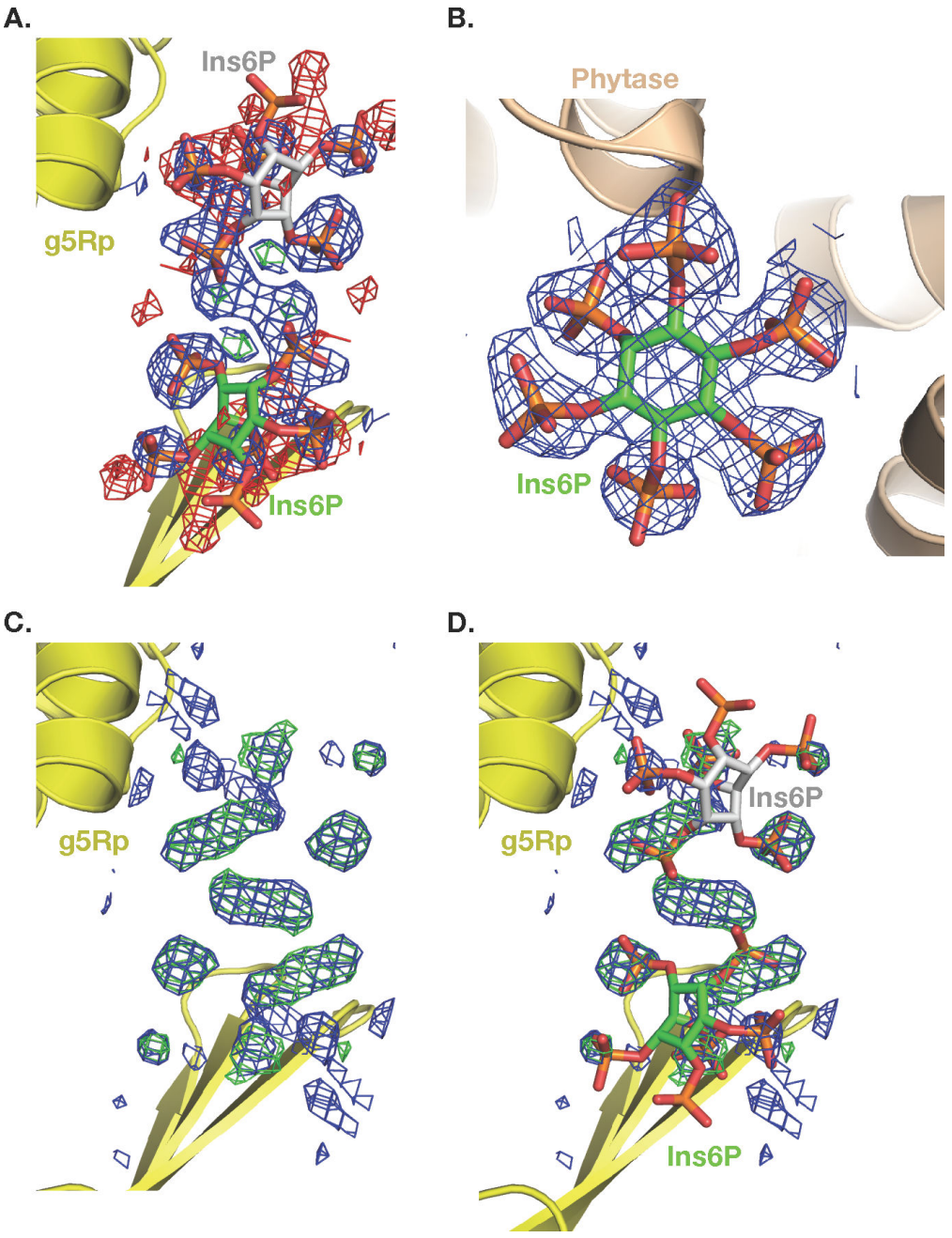

FIG 1 Electron density maps illustrating the incorrect modeling of InsP6. (A) 2Fo-Fc (contoured at 1 σ; blue) and Fo-Fc [contoured at −3 σ (red) or 3 σ (green)] electron density maps available from the Protein Data Bank [http://www.rcsb.org/; (9)] for the PDB entry 7DNU (1). The g5RP protein is colored in yellow. The carbon atoms of InsP6 bound to g5Rp and of its symmetrical mate are colored in green and gray, respectively. Phosphorus and oxygen atoms are colored orange and red, respectively. All panels of this figure were generated using the Pymol software (10). This color code and the map contouring levels are used in all panels. (B) Illustration of the 2Fo-Fc electron density map of InsP6 bound to *Escherichia coli* phytase [wheat; (11)], which was determined at a similar resolution (2.28 Å vs 2.25 Å for PDB entry 7DNU). The coordinates and electron density map were downloaded from the Protein Data Bank [http://www.rcsb.org/; (9)]. (C) 2Fo-Fc and Fo-Fc electron density maps calculated by the PHENIX.REFINE software in the absence of InsP6 bound to g5Rp (12). (D) Same as C, except that the InsP6 modeled by the authors are shown as sticks, showing a complete discrepancy between the shape of the electron density omit maps and the structure of InsP6.

bound to crystallized proteins (13, 14). These tools complement human expertise in electron density map analysis to help scientists avoid over-interpreting electron density maps. X-ray crystallographers need to be particularly attentive to this type of situation, as students or scientists with no expertise in structural biology may simply consider that since these results have been published in peer-reviewed journals, they are correct.

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