Dr. Gonzalo A. Jaña
Associate Professor
Departamento de Ciencias Químicas
Facultad de Ciencias Exactas
Universidad Andres Bello
Autopista Concepción-Talcahuano 7100, Talcahuano
Chile
gonzalo.jana@unab.cl

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Dear Editor-in-Chief Professor Kenneth M. Merz Jr.,

Please find enclosed the manuscript entitled: "***Elucidation of the reaction mechanism of Cavia porcellus L-Asparaginase (CpA): A QM/MM study***", to be considered for publication in the *Journal of Chemical Information and Modeling.*

L-Asparaginase (L-ASNase) enzymes are used as part of first-line treatment for acute lymphoblastic leukemia (ALL). The L-ASNases currently used for ALL disease (EcA and EwA) are derived from bacterial organisms, triggering serious side effects in patients. Interestingly, several studies have indicated that L-ASNases of mammalian origin could be as effective as EcA or EwA, without their side effects. Therefore, understanding the catalytic mechanism of these mammalian enzymes is an essential step towards improving future ALL treatments.

In this work, the catalytic mechanism of L-ASNase from the mammalian *Cavia porcellus* (CpA) has been studied for the first time using high-level computational methods. Our findings contribute to elucidate the controversial mechanistic discussion regarding a traditional hydrolysis reaction versus the formation of a covalent intermediate between L-Asn and a Thr residue of the enzyme.

We have found that the mechanism involves the formation of the covalent intermediate, adding a step to the traditional chemical mechanism. Notably, we have also revealed that the Thr residue must be activated by a Tyr through a network of water molecules, this further step being essential to begin the reaction. After this critical step, hydrolysis is initiated by nucleophilic attack of the Thr residue, which corresponds to the rate-limiting step of the reaction (ΔE = 18.9 kcal·mol^{-1}). This finding is in agreement with previously reported experimental data. The subsequent steps studied involve the reorganization and reprotonation of the active site residues, thus promoting a new catalytic cycle.

In summary, this is the first QM/MM study addressing the enzymatic mechanism catalyzed by a mammal L-ASNase. Based on the results obtained, we propose a new step in the catalytic mechanism, which has not been previously reported for this type of enzyme. Furthermore, we identified significant electrostatic interactions and observed the formation of catalysis-relevant species, such as stabilization through an oxyanionic hole and a covalent intermediate, which turned out to be crucial for the CpA enzyme catalysis.

I am submitting the article to the Journal of Chemical Information and Modeling, because it is a leading journal in its field, and I believe that the manuscript should be of interest to an audience interested in computational biochemistry, especially in the area of enzyme catalysis.

Sincerely yours,

Dr. Gonzalo A. Jaña