**Ligand transport pathways in proteins**

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**Abstract**

Analysis of tunnels in proteins started with a visual identification in single static structures. However, with the improvement of computational power and better algorithms, the number of conformations that can be obtained for a protein increased considerably, mainly by the usage of molecular dynamics simulations. This increase in data available for analysis, raised difficulties and limitations in the study of tunnels, primarily in the resources and time required to perform such analyses.

In my doctoral research, I have contributed to the development of tools and methodologies for the analysis of tunnel networks in proteins. Tunnels in proteins are relevant structural features that affect not only the catalytic processes but also the dynamics and selectivity. Nowadays, with the increase in computational capabilities, not only the structural data produced has grown immensely, but also the type of information available, mainly the tracking of ligand movement. The first paper included here allows for an integrative view of tunnel geometry description and the assignment of ligand transport to them, providing non-subjective results and reproducibility compared to visual analysis. The second publication grants the possibility of analyzing tunnels from large datasets quickly and cheaply, overcoming the requirements of specialized hardware to perform this task. Furthermore, this methodology also allows for the identification of previously disregarded narrow tunnels by employing a smaller probe.

In the second part of this thesis, I present two practical applications of the methodologies developed in the first part. The third publication focuses on the selective transport of the ABCG46 transporter of *Medicago truncatula*, where I showed how the changes to the tunnel block or allow the translocation of ligands through it. The fourth and final paper is a study about water transport on five hydrolase enzymes, where we showed how water can traverse tunnels of bottleneck radii lower than a single water molecule, highlighting the importance of narrow tunnels.