A Non-Canonical Tricarboxylic Acid Cycle Underlying Cellular Identity: An *In Silico* Perspective

Lihao Lin[1], Fabiola Maria Andrea Saputo[1] , Francesco Lapi[1], Isabella Cecilia Rizzo[1], Bruno Giovanni Galuzzi[2], Marco Ercole Vanoni[1], Chiara Damiani[1]

E-mail: l.lin6@campus.unimib.it

[1]Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy

[2] CNR Centro Nazionale delle Ricerche, Segrate (Milan), Italy

Keywords: Metabolic Modeling; Non-canonical Krebs cycle; ENGRO2 computational model;

Abstract: This study focuses on the exploration of a potential non-canonical pathway of the Krebs cycle, described in the article by Arnold, P.K., Jackson, B.T., Paras, K.I. et al. (A non-canonical tricarboxylic acid cycle underlies cellular identity. *Nature* 603, 477–481, 2022). In this article, the authors identified and described an alternative Krebs cycle, a metabolic pathway crucial for oxidizing nutrients to produce energy (in the form of ATP) and generating essential metabolites for various biosynthetic processes. The reactions involved in the alternative pathway differ from the traditional Krebs cycle, and the related genes are specifically expressed at different stages of cellular differentiation. Using the ENGRO2 computational model, we confirmed some of the results from Arnold et al.'s article, and new research insights emerged. The work highlighted that, while useful, the ENGRO2 network is insufficient to fully describe the behavior of the non-canonical cycle, requiring the integration of new pathways, such as ACSS2 and CrAT, for a more comprehensive analysis of fatty acid synthesis and cellular metabolism. These findings emphasize the importance of the combined approach between mathematical models and experimental evidence to investigate the mechanisms underlying these "new" metabolic pathways, paving the way for future research.