TITLE: Critical roles of Human SIRT3 modulators, from nicotinamide to iso-nicotinamide

AUTHORS: Xiangying Guan1✝, Eric Knoll1✝, Raj Chakrabarti2\*

1 PMC Advanced Technology, LLC, NJ, USA

2 Department of Chemical Engineering, College of Engineering, Carnegie Mellon University, PA, USA

✝ Both authors contributed equally to the results of this work.

\* To whom correspondence should be addressed:

Raj Chakrabarti, Ph.D.

Associate Professor of Chemical Engineering and Center for Advanced Process Decision-Making

Department of Chemical Engineering

College of Engineering

Carnegie Mellon University

Doherty Hall 3122

5000 Forbes Avenue

Pittsburgh, PA 15213

Phone: (412) 268-5615

Email: rajc@andrew.cmu.edu

ABSTRACT *(150 words limit)*

Human sirtuin type 3 (hSIRT3), a major mitochondrial protein, has an NAD+ - dependent deacetylase activity regulating the global lysine acetylation. hSIRT3 is associated with human pathologies, such as metabolic syndrome, cancer, and geriatric diseases. The kinetics and mechanism of inhibition of hSIRT3, as well as that of Sir2, were investigated *in vitro* and computationally. Physiological concentrations of nicotinamide competitively inhibit human recombinant hSIRT3 versus NAD+. The critical roles of nicotinamide and its analogue (iso-nicotinamide) as inhibitor/activator of hSIRT3 were discussed as well. Induced fit protein-ligand docking along with a subsequent binding affinity estimation using molecular mechanics /generalized born surface area (MM/GBSA) calculations show that nicotinamide binds approximately equally well to the two alternate binding sites of Sir2, known as the AB or AC pockets, and that nicotinamide preferentially binds to the AC pockets of hSIRT3. These results provide important insights for the development of SIRT3-specific modulators.