Mechanism of fully-reversible, pH-sensitive inhibition of human glutamine synthetase by tyrosine nitration.

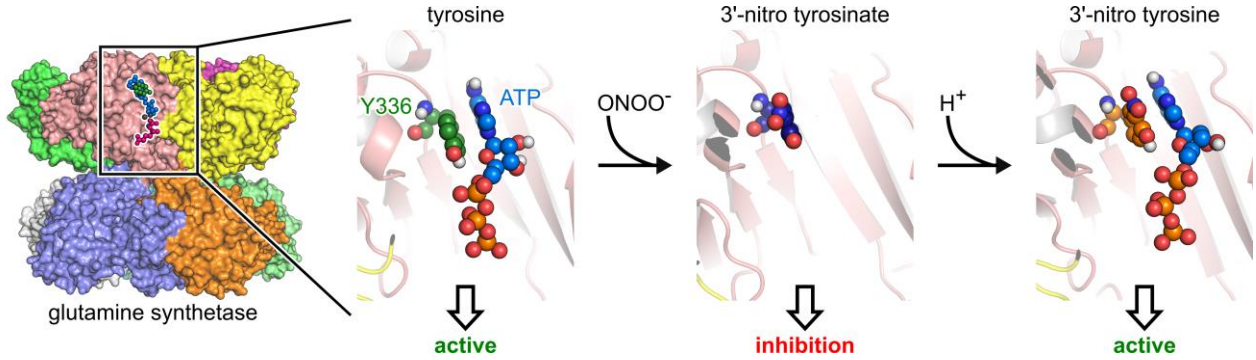
Benedikt Frieg[1,2], Boris Görg[3], Natalia Qvartskhava[3], Mohanraj Gopalswamy[1], Thomas Jeitner[4], Nadine Homeyer[1], Dieter Häussinger[3*] and Holger Gohlke[1,2*]

[1] *Institute for Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Düsseldorf, Germany*

[2] *John von Neumann Institute for Computing (NIC), Jülich Supercomputing Centre (JSC), and Institute for Complex Systems - Structural Biochemistry (ICS-6), Forschungszentrum Jülich GmbH, Jülich, Germany*

[3] *Clinic for Gastroenterology, Hepatology, and Infectious Diseases, Heinrich Heine University Düsseldorf, Düsseldorf, Germany*

[4] *Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, NY, USA*



Glutamine synthetase (GS) catalyzes the ATP-dependent ligation of toxic ammonia and glutamate to glutamine, the most substantial free amino acid. This reaction – and therefore GS – are indispensable for the human nitrogen metabolism [1, 2] and changes in GS catalytic activity have been linked to a broad range of neurological diseases [3, 4]. In particular, GS catalytic activity is highly sensitivity to tyrosine nitration, a post-translation modification under "nitroxidative stress" conditions, which causes GS inhibition [5, 6]. As to human GS, nitration of tyrosine 336 (Y336) inhibits GS activity [5]. Although Y336 nitration modifies key properties of the amino acid, the molecular mechanism by which Y336 nitration inhibits GS, however, is not understood.

Here, we show by means of unbiased MD simulations, binding and configurational free energy computations that Y336 nitration hampers substrate (ATP) binding, but only in the deprotonated and negatively-charged state of residue 336. By contrast, for the protonated and neutral state, our computations indicate an increased binding affinity for ATP. pK_a computations of nitrated Y336 within GS predict a pK_a of ~4. Thus, at physiological pH nitrated Y336 exists almost exclusively in the deprotonated and negatively-charged state. *In vitro* experiments confirm these predictions, in that, the catalytic activity of nitrated GS is decreased at pH 7 and pH 6, but not at pH 4. These results indicate a novel, fully reversible, pH-sensitive mechanism for the regulation of GS activity.

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