The crystal structures of post-reactive states of 2'-5'-oligoadenylate synthetase provide new insights into the mechanism of the innate immune signaling

Pavel Kats[1], Xiaoyi Zhou[1], Ole Zeymer[1], Jan Lohöfener[1], Nicola Steinke[1], Petra Baruch[1,2], Rune Hartmann[3], Dietmar J. Manstein[1,2] and Roman Fedorov[1,2]

[1] Institute for Biophysical Chemistry, [2] Research Division for Structural Biochemistry, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Kats.Pavel@mh-hannover.de, Germany; [3] Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark.

The innate immune sensors activate interferon-driven antiviral responses upon recognition of PAMPs and serve as a rheostat for the metabolic activity of the microbiota and its exposure to diet, xenobiotics, and infections. The ability to modulate innate immune sensors opens new ways to novel antiviral and anti-inflammatory drugs and therapies against cancer and many aging-associated metabolic, neoplastic, autoimmune, or autoinflammatory disorders. The innate immune sensor 2'-5'-oligoadenylate synthetase (OAS) is among the most promising targets for the development of new antivirals. In light of the recent discoveries of the crucial role of OAS in protection against the COVID-19 infection [1], the activation of OAS by small-molecule agents is of particular interest for anti covid therapies. OAS belongs to the large and diverse superfamily of nucleotide triphosphate transferases (NTPTs) which catalyze key cellular processes in all kingdoms of life. A similar mechanism of catalysis in NTPTs is ensured via a highly conserved structure of the active site. Targeting active sites of NTPTs may interfere with other important biological processes through unspecific inhibition (cross-reactivity). At the same time, allosteric mechanisms facilitating the regulation of activity and exchange of reaction components are often specific for each enzyme. Allosteric effects thus can be used to avoid cross-reactivity issues and develop specific activity modulators of the innate immune sensors. However, the identification of allosteric networks requires a comprehensive structural and mechanistic description of the enzymatic cycle.

Towards this end, we established earlier the detailed mechanisms of nucleic acid-induced activation of OAS1, the individual roles of nucleic acid and substrates, and the sources of 2'-specificity of product formation [2]. In our recent structural studies, we observed the series of metastable intermediate states associated with the exchange of reaction components of OAS1. These intermediate steps provide new insights into the product release mechanism, which determines the initial concentration of signaling molecules 2'-5'-oligoadenylates that activate the RNase-L immune pathway. Here we report the results of our kinetic crystallography investigations of these states together with the mutagenesis and computational chemistry studies, which together provide important insights for both the fundamental understanding of the rate-limiting steps of the innate immune signaling and the development of allosteric activity modulators targeting these mechanisms.

[1] Zhou *et al*. A Neanderthal OAS1 isoform protects individuals of European ancestry against COVID-19 susceptibility and severity. Nat. Medicine. 27:659-667 (2021).

[2] Lohöfener *et al*. The Activation Mechanism of 2'-5'-Oligoadenylate Synthetase Gives New Insights Into OAS/cGAS Triggers of Innate Immunity. CELL Structure. 23(5):851-862 (2015).