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**LS-4-P-2882 Three-dimensional reconstruction of the S885A mutant of the human mitochondrial Lon protease**

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The Lon protein is a protease belonging to the superfamily of ATPases Associated with diverse cellular Activities (AAA+). Its main function is the control of protein quality and the maintenance of proteostasis by degrading misfolded and damaged proteins, which occur in response to numerous stress conditions [1]. Lon protease has been also shown to participate in regulation of levels of transcription factors that control pathogenesis, development and stress response, e.g. [2]. Furthermore, it seems to play an important role in aging [3], and it is supposed to be involved in mtDNA replication, translation, or repair [1, 4, 5]. We focus our interest on the structure of human mitochondrial Lon (hLon) protease whose altered expression levels are linked to some severe diseases, such as epilepsy, myopathy, or lateral sclerosis [5].

During the last decade, sub-structures of bacterial and human Lon have been resolved by X-ray scattering (e.g. [6-8]), and more recently a 3D structure of an E. Coli Lon dodecamer active at physiological protein concentrations was resolved with electron microscopy [9]. At the moment, it is assumed that Lon subunits assemble into oligomeric structures whose conformations are supposed to differ at ATP, ADP, and protein substrate binding [1]. However, neither the full 3D structure of the Lon holoenzyme nor the mechanism of Lon action is known [4, 5].

Here, we present the first 3D structure of an ADP-bound Lon S885A mutant obtained as a result of preliminary negative staining studies (Fig. 1). The S885A mutant has a point mutation on the proteolytic domain, which completely disables its proteolytic function. 2D classification of the collected dataset revealed classes with regular hexameric arrangement (Fig. 1B), 3D refinement with C6 symmetry applied revealed that the Lon mutant was formed as a hexameric ring of a 120 Å diameter having 90 Å in height. Its resolution was estimated at 19 Å by the FSC=0.5 criterion. We will also present an update of this mutant structure obtained by cryo-electron microscopy.

References


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Fig. 1: Preliminary results from negative stain. (A) Micrograph of negatively stained Lon particles. Scale bar: 50 nm. (B) Typical class averages. (1) top-view showing a hexameric arrangement of the Lon protease, (2)-(3) side-views. Scale bar: 5 nm. (C)