**The x-ray structure of the viral protein Npro reveals its proteolytic activity and immune modulatory function.**

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The bi-functional protein Npro is a key effector of pestiviruses such as classical swine fever virus (CSFV) to suppress host cell anti-viral defense mechanisms. Synthesized as the N-terminal part of the single viral polyprotein, Npro releases itself via an auto-proteolytic cleavage event. Mutational studies suggested an atypical catalytic triad consisting of Glu22, His49 and Cys69. These observations were the basis to assign Npro to a new subfamily of cysteine proteases, C53. Processed, proteolytically inactive Npro was reported to exert an additional function as it interfered with transcription factor Interferon Regulatory Factor 3 (IRF3)-signalling and targeted it to the proteasome. Three conserved cysteine residues which form a TRASH-motif were suggested to coordinate zinc and were proposed to be essential for this interaction. As a result, Npro suppresses the production of IFN-α/β - typical mediators for anti-viral immune responses - thus enabling the virus to evade this host cell defense mechanism.

Here we present the crystal structure of Npro at 1.25 Å resolution. The structure analysis revealed a two domain architecture comprising an all-β fold together with random coil elements. Crystal structures in substrate- and product-bound conformations revealed the relevance of the putative catalytic residues and further explained the enigmatic latency of the protease. The structure-deduced catalytic mechanism explains the observed one-time cleavage *in cis*. The interaction domain harbors the TRASH-motif as well as distinct electrostatic binding sites. Their geometric arrangement relate to Npro's function in transcription factor targeting.