



## Electron cryomicroscopy structure of N-ethyl maleimide sensitive factor at 11 Å resolution

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Abstract	N-ethyl maleimide sensitive factor (NSF) belongs to the AAA family of ATPases and is involved in a number of cellular functions, including vesicle fusion and trafficking of membrane proteins. We present the three-dimensional structure of the hydrolysis mutant E329Q of NSF complexed with an ATP-ADP mixture at 11 Å resolution by electron cryomicroscopy and single-particle averaging of NSF.alpha-SNAP.SNARE complexes. The NSF domains D1 and D2 form hexameric rings that are arranged in a double-layered barrel. Our structure is more consistent with an antiparallel orientation of the two rings rather than a parallel one. The crystal structure of the D2 domain of NSF was docked into the EM density map and shows good agreement, including details at the secondary structural level. Six protrusions corresponding to the N domain of NSF (NSF-N) emerge from the sides of the D1 domain ring. The density corresponding to alpha-SNAP and SNAREs is located on the 6-fold axis of the structure, near the NSF-N domains. The density of the N domain is weak, suggesting conformational variability in this part of NSF.
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