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# Structural Basis for the Recognition and Cleavage of Polysialic Acid by the Bacteriophage K1F Tailspike Protein EndoNF

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An α-2,8-linked polysialic acid (polySia) capsule confers immune tolerance to neuroinvasive, pathogenic prokaryotes such as *Escherichia coli* K1 and *Neisseria meningitidis* and supports host infection by means of molecular mimicry. Bacteriophages of the K1 family, infecting *E. coli* K1, specifically recognize and degrade this polySia capsule utilizing tailspike endosialidases. While the crystal structure for the catalytic domain of the endosialidase of bacteriophage K1F (endoNF) has been solved, there is yet no structural information on the mode of polySia binding and cleavage available. The crystal structure of activity deficient active-site mutants of the homotrimeric endoNF cocrystallized with oligomeric sialic acid identified three independent polySia binding sites in each endoNF monomer. The bound oligomeric sialic acid displays distinct conformations at each site. In the active site, a $Sia_3$ molecule is bound in an extended conformation representing the enzyme–product complex. Structural and biochemical data supported by molecular modeling enable to propose a reaction mechanism for polySia cleavage by endoNF.



*Keywords:* polysialic acid; endoNF; glycosidase; host recognition

## Introduction

On the surface of mammalian cells, polysialic acid (poly-α2,8-linked Neu5Ac; polySia) plays an important role in cellular motility and neuronal plasticity, and has implications in tumor metastasis.[1] This natural abundance triggers tolerance of the immune system to polySia.[2] Thus, a thick polySia capsule expressed by neuroinvasive bacteria such as *Escherichia coli* K1 and *Neisseria meningitidis* serogroup B conveys resistance to the host immune system and serves as a disguise mechanism during host invasion.[3] However, in order to gain access to the bacterial surface and to inject their genetic material, bacteriophages of the K1 family evolved the ability to specifically recognize and degrade the bacterial polySia capsule utilizing tailspike proteins with endosialidase activity.[4–10] In contrast to exosialidases (E.C. 3.2.1.18), which remove single, terminal sialic acid moieties from sialoglycoconjugates, endosialidases (E.C. 3.2.1.129) are highly specific for polySia and cleave within the polymer chain.

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Abbreviations used: polySia, polysialic acid; PDB, Protein Data Bank; TBA, thiobarbituric acid; MD, molecular dynamics; TMB, 3,3′,5,5′-tetramethylbenzidine; BSA, bovine serum albumin; PBS, phosphate-buffered saline.

The catalytic domains of viral, bacterial, and eukaryotic exosialidases share the common overall topology of a six-bladed β-propeller and have conserved binding properties. With the exception of some bacterial sialidases, an arginine triad binds the carboxyl function of sialic acid, while its glycerol function is hydrogen bonded by a glutamic acid residue. The substrate's *N*-acetyl function is typically buried in a hydrophobic pocket formed by tryptophan and isoleucine residues.[11–14] Like many glycosylhydrolases, exosialidases typically perform cleavage by two successive $S_N2$ reactions via a covalently bound sialyl-enzyme intermediate. It was shown that a conserved tyrosine, in close proximity to the anomeric carbon of sialic acid, acts as nucleophile and binds the intermediate product.[15,16]

The homotrimeric endosialidase of bacteriophage K1F endoNF (Fig. 1) specifically recognizes and processively degrades α-2,8-linked polySia, with $Sia_3$ molecules as the major cleavage product.[17,18]

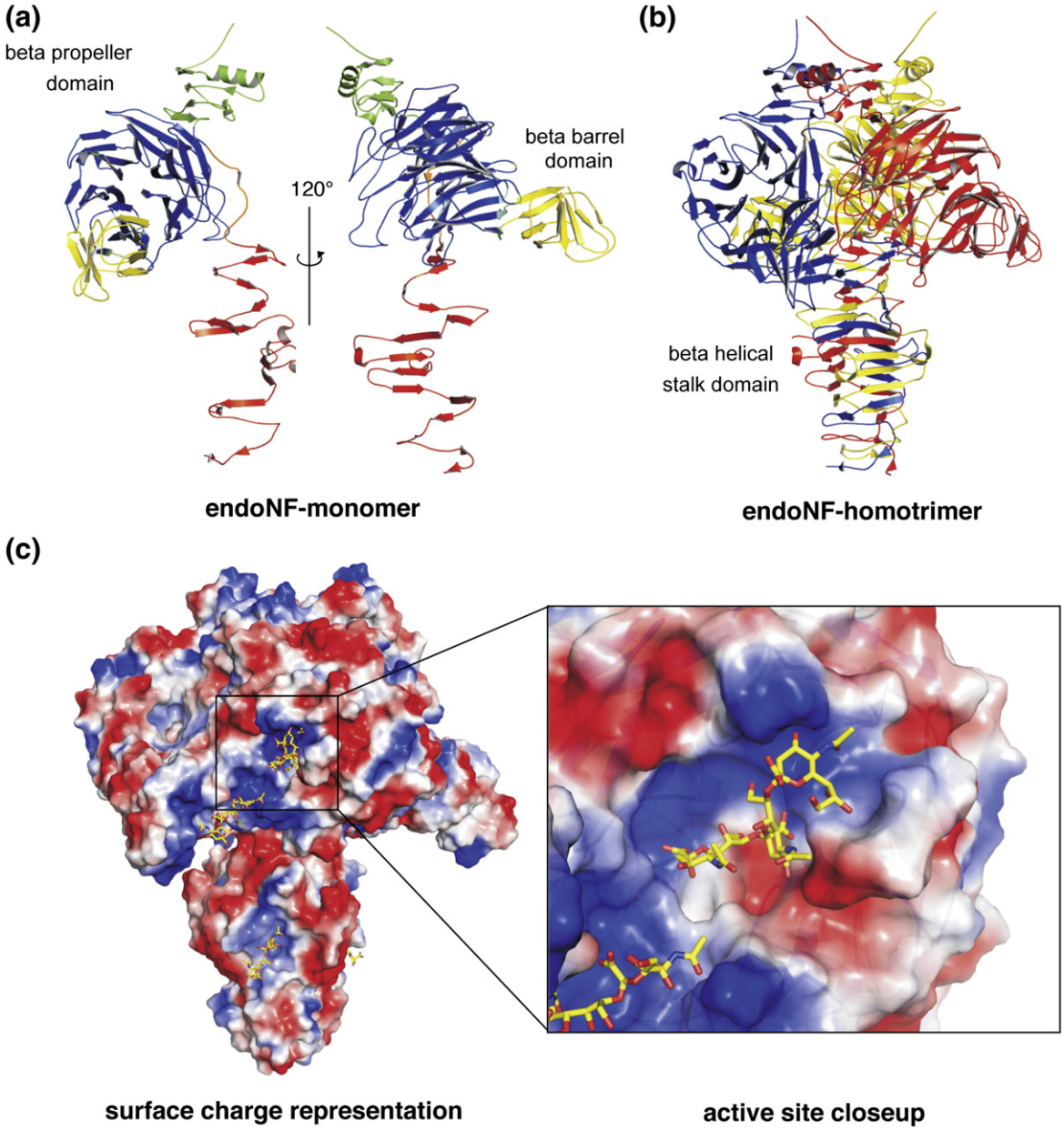


**Fig. 1.** Overall topology of endoNF. (a) The monomeric polypeptide chains of endoNF. Each monomer consists of a small helical N-terminal domain, followed by the neuraminidase β-propeller, which is intermitted by a β-barrel domain. The six-bladed β-propeller domain comprises the active site. However, only trimeric endoNF is fully functional. (b) The functional homotrimeric form of endoNF. The general topology of endoNF resembles a mushroom-like shape with a head region and a stalk domain. The stalk domain is composed of the β-strand, which forms a triple β-helix in the native trimer. (c) The distribution of positive charges (blue) and negative charges (red) on the surface of endoNF-H350A is displayed on a qualitative basis. The negatively charged polySia residues (yellow sticks) bind to the positively charged areas on endoNF, indicating the path that an extended polySia chain would take into the active site. The magnification highlights the active site of endoNF-H350A with bound $Sia_3$.