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Supporting information for article:

**Structure of an *Influenza A virus* N9 neuraminidase with a
tetrabrachion domain stalk**

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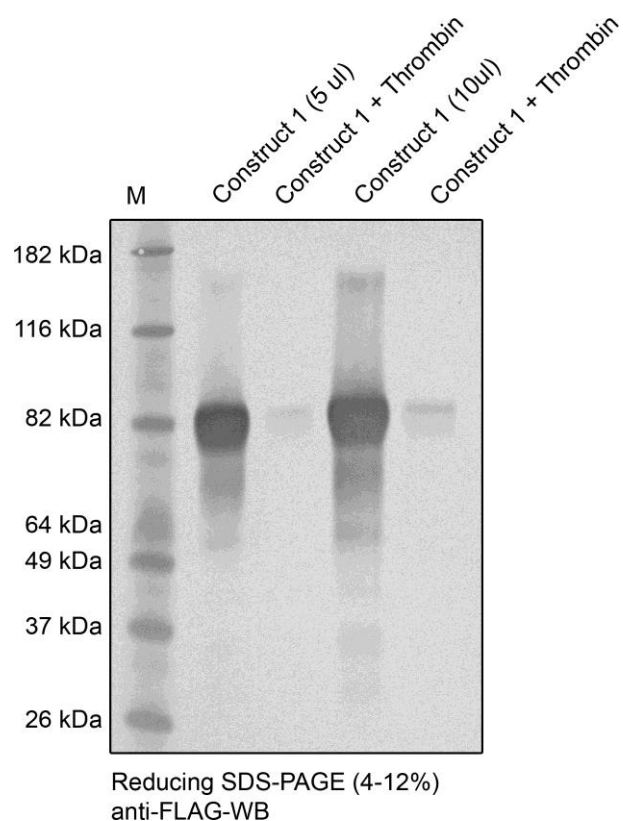


Figure S1 Approximately 3 mg of construct 1 NA was incubated with 130 U of thrombin as described in the Materials and Methods section. To verify the removal of the N-terminal FLAG-tag, 5 μ l and 10 μ l (\approx 12.5 μ g and 25 μ g) of the thrombin-cleaved construct 1 NA and the control sample were analyzed by anti-FLAG WB, respectively. Only a minor fraction of the NA showed anti-FLAG reactivity indicating the successful cleavage of the FLAG tag.

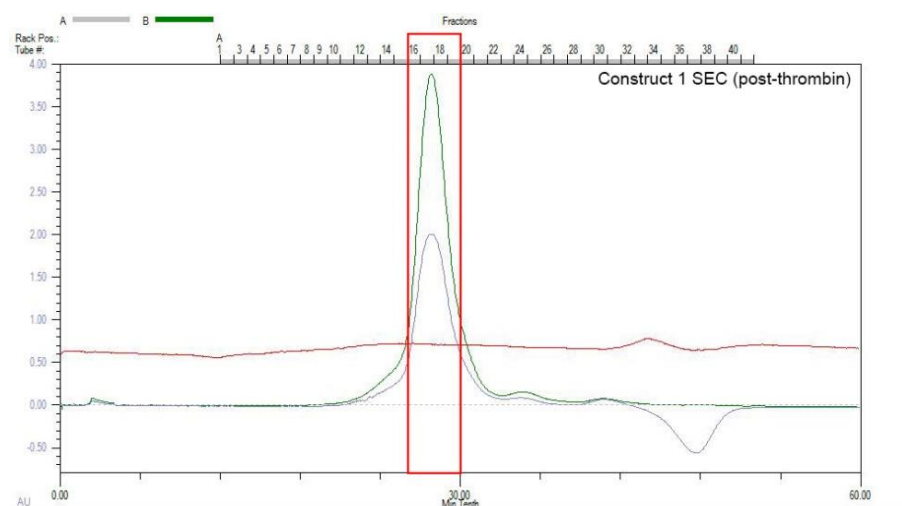


Figure S2 Approximately 3 mg of thrombin-cleaved construct 1 NA was purified by SEC as described in the Materials and Methods section, to remove excess thrombin, and the cleaved FLAG-tag from the NA. Fractions 16-19 of the SEC run were pooled based on their OD280 as well as their activity (data not shown). The pooled fractions were concentrated to 5 mg ml⁻¹ for subsequent crystallization as described in the manuscript.

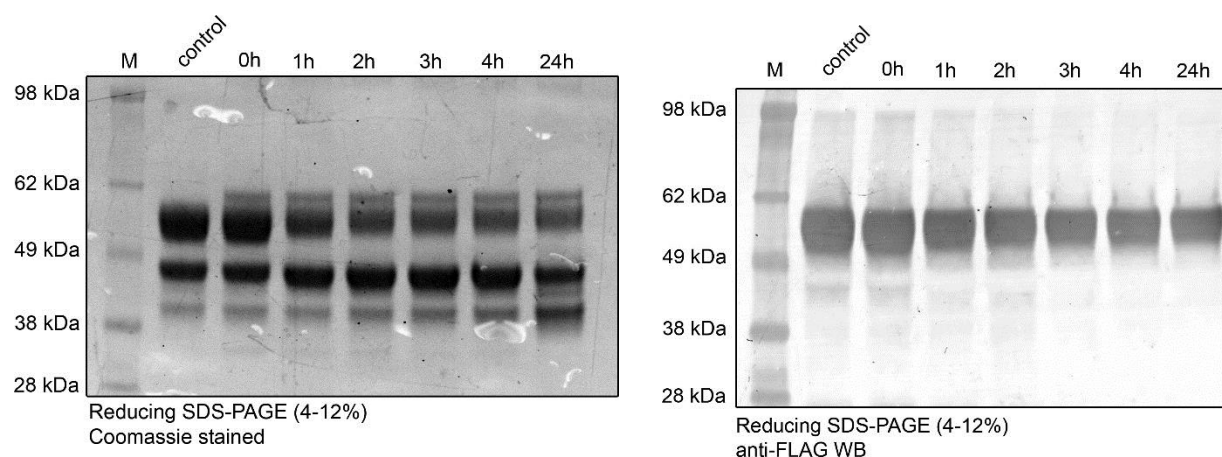


Figure S3 Kinetic analysis of thrombin cleavage of approximately 3 mg of construct 2 NA incubated with 100 U of thrombin for 24 h, as described in the Materials and Methods section. Only the upper band showed FLAG-reactivity in the corresponding WB indicating the presence of the N-terminal FLAG tag. The lower band which was already present in the control sample showed neither FLAG reactivity nor enzymatic activity (data now shown) suggesting this band might be a proteolytic fragment of the construct 2 NA head, generated during storage of the sample after its anti-FLAG affinity purification. The top band is an impurity from the commercially available thrombin preparation as it is only visible after immediate addition (0 h vs control) of thrombin to the construct 2 NA sample.

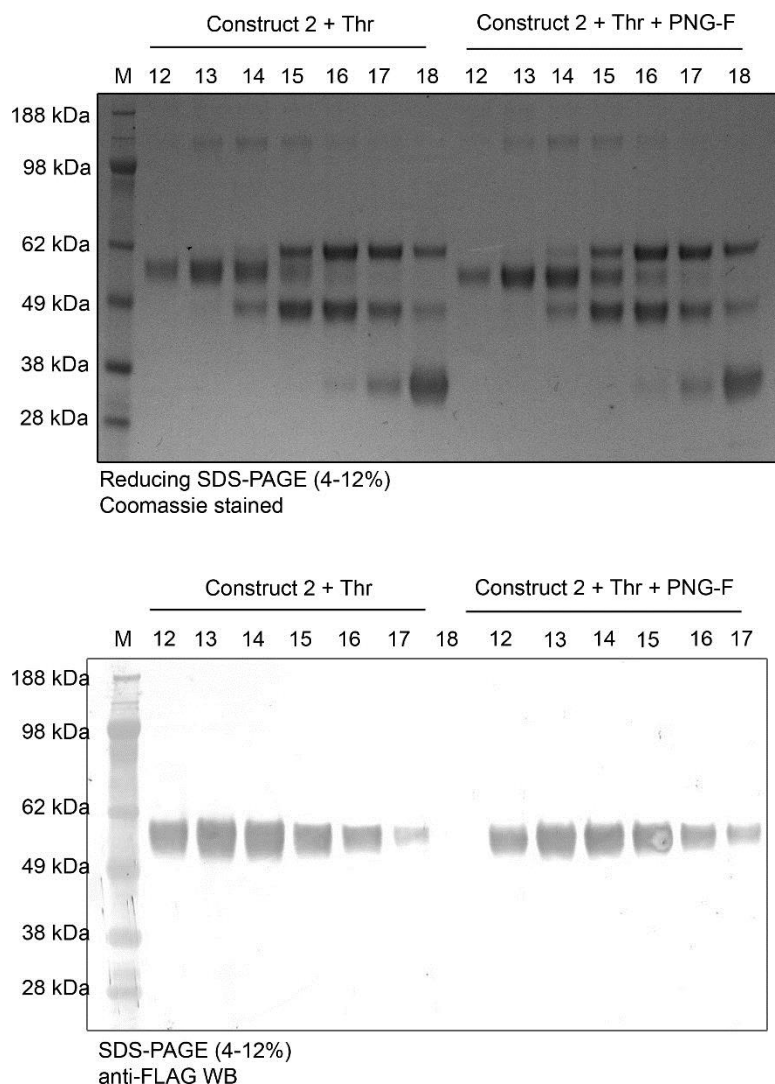


Figure S4 The thrombin-treated construct 2 NA was split after 48 h of incubation into 2 halves. The first half was incubated for an additional 24 h at RT, whereas the second half was incubated for an additional 24 h with 50 U PNGaseF, as described in the Materials and Methods section. Both samples were subjected to SEC and the fractions were analyzed by SDS-PAGE and anti-FLAG WB. Fractions 12-14 of both samples contained mainly the uncleaved NA as shown by its FLAG reactivity. The slightly lower band with a maximum at fractions 15 - 16 showed enzymatic reactivity (data not shown) but no FLAG reactivity suggesting the presence of functional construct 2 NA head with its TB-stalk removed. Fraction 15 + 16 of the thrombin and PNGaseF treated construct 2 NA were pooled and remaining FLAG-tagged NA was removed by incubating the sample with anti-FLAG affinity matrix. The flow through was concentrated down to 70 μ l with a concentration of 1.3 mg ml⁻¹ and used for subsequent crystallization, as described in the Materials and Methods section.

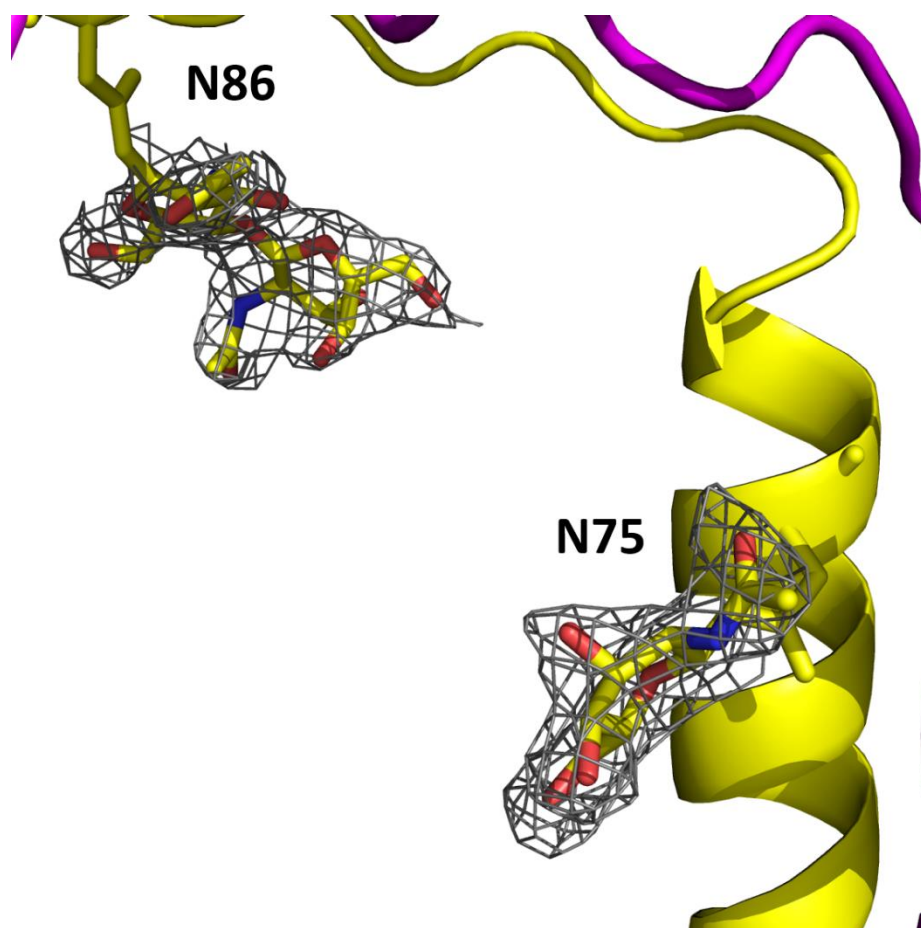


Figure S5 Glycosylation chains at residues N75(TB-stalk) and N86 (N9) of chain A overlaid with the omit (Winn et al, 2011) electron density map shown as a grey mesh contoured at 1σ within 1.6 Å of ligands. The figure was produced using PyMol (Schrodinger, 2010).

References

- Schrodinger, L. The PyMOL Molecular Graphics System, Version 1.3r1., 2010.
- Winn, M. D.; Ballard, C. C.; Cowtan, K. D.; Dodson, E. J.; Emsley, P.; Evans, P. R.; Keegan, R. M.; Krissinel, E. B.; Leslie, A. G. W.; McCoy, A.; McNicholas, S. J.; Murshudov, G. N.; Pannu, N. S.; Potterton, E. A.; Powell, H. R.; Read, R. J.; Vagin, A.; Wilson, K. S. Overview of the CCP4 suite and current developments. *Acta Crystallogr D* **2011**, 67, 235-242.