Supporting Information for "Influence of a Heptad Repeat Stutter on the pH-Dependent Conformational Behavior of the Central Coiled-Coil from Influenza Hemagglutinin HA2"

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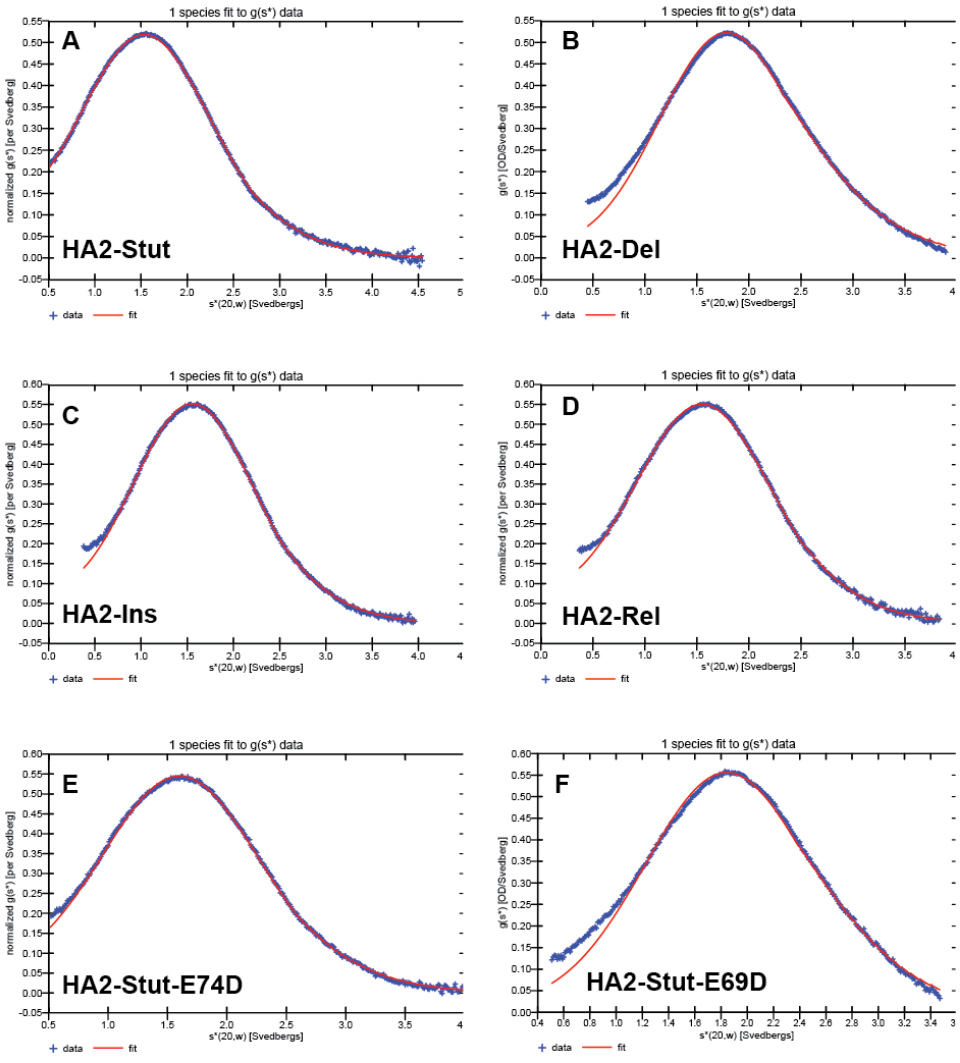

Figure S1. Velocity analytical ultracentrifugation of HA2-Stut (A), HA2-Del (B), HA2-Ins (C), HA2-Rel (D), HA2-Stut-E74D (E), and HA2-Stut-E69D (F). Molecular weight estimates are listed in the main text and in Table S1.

Table S1 – Results from sedimentation velocity analytical ultracentrifugation data analysis.

Parameter	HA2-Stut	HA2-Del	HA2-Ins	HA2-Rel
C_o (OD)	0.9085 (0.9055-0.9116)	0.7886 (0.7858-0.7912)	0.8921 (0.8900-0.8941)	0.8834 (0.8808-0.8860)
$s_{20,w}$ (S)	1.648 (1.645-1.650)	1.852 (1.850-1.854)	1.647 (1.646-1.649)	1.645 (1.643-1.647)
MW_{app} (s/D; kDa)	14.34 (14.21-14.47)	17.42 (17.25-17.60)	14.79 (14.70-14.88)	14.38 (14.26-14.49)

Parameter	HA2-Stut-E74D	HA2-Stut-E69D
C_o (OD)	0.8908 (0.8884-0.8930)	0.7846 (0.7814-0.7876)
$s_{20,w}$ (S)	1.695 (1.693-1.697)	1.907 (1.905-1.910)
MW_{app} (s/D; kDa)	15.18 (15.08-15.29)	17.44 (17.26-17.64)

[a]Values in parentheses indicate 68% confidence intervals from data fitting

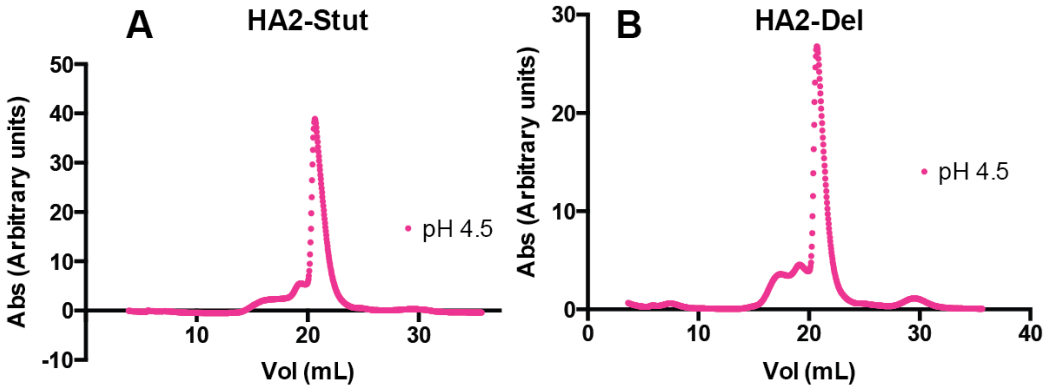

Figure S2. Gel filtration analysis of HA2-Stut (A) and HA2-Del (B).

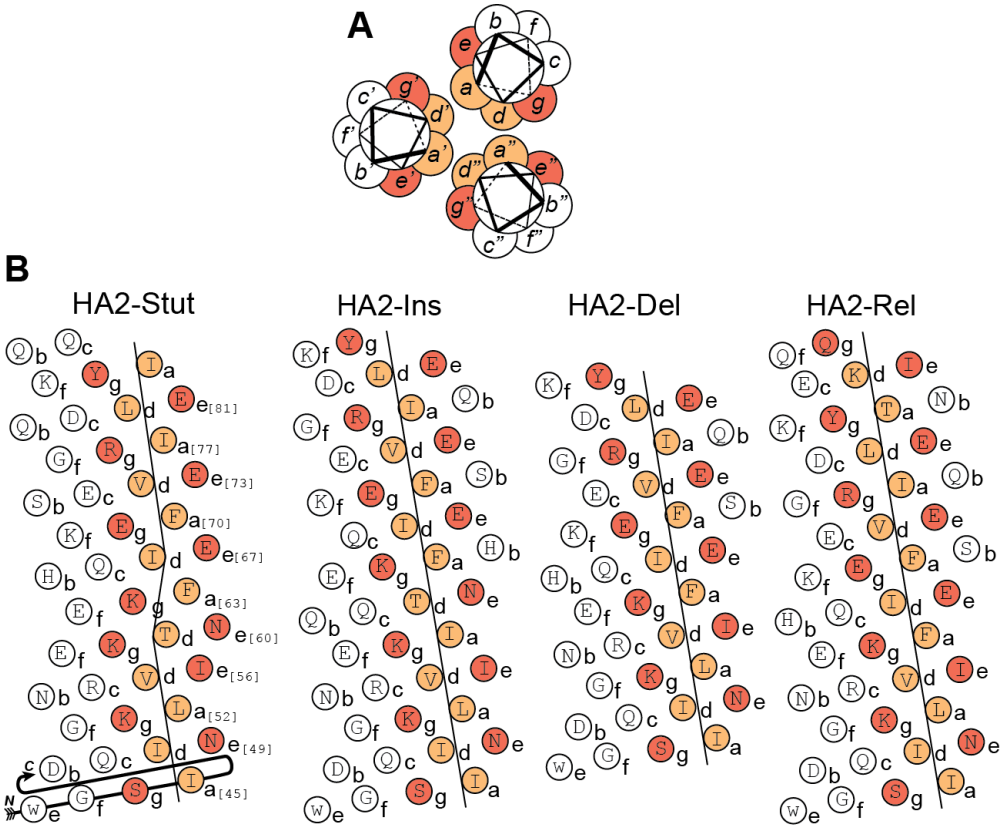

Figure S3. (A) Helical wheel diagram showing core (*a*/*d*) and flanking (*e*/*g*) interhelical interactions in a coiled-coil trimer. (B) Helical net analysis of HA2-Stut, HA2-Ins, HA2-Del, and HA2-Rel. Core *a*/*d* and flanking *e*/*g* residues colored as in panel A. Directionality of the polypeptide (N→C), and HA2 numbering shown on HA2-Stut.

Materials and Methods:

Gel filtration analysis

Analysis was performed on a Sephadex S75 column (10/300) at 4°C in 10 m*M* NaOAc pH 4.5 containing 100 m*M* NaCl. Protein samples were loaded at concentrations of 50–80 μ*M* and elution was monitored by absorbance at 280 nm. Both HA2-Stut and HA2-Del eluted as a single peak.

Analytical Ultracentrifugation

Sedimentation velocity analysis was performed on a Beckman XL-1 analytical ultracentrifuge with a Ti60 rotor. Samples of StutExt and DelExt were loaded into double sectors cells at a protein concentration of 180 μM, blanked against the sample buffer (10 mM sodium acetate, 100 mM NaCl, pH 4.5) in the reference sector. Two hundred scans were acquired at 58,000 rpm and 20 °C with sedimentation boundaries monitored by absorption at a wavelength of 280 nm. The sedimentation boundaries were directly fit as the derivative dc/dt using DCDT+ v2.4.0 to determine the sedimentation and diffusion coefficents, S and D, respectively from which was calculated the apparent molecular weight, $M_{w,app}$ (refs. S1,S2). Sixty to 80 absorbance scans were globally analyzed for each experiment. The observed values were normalized to standard conditions of 20°C and water ($s_{20,w}$ and $D_{20,w}$) by correcting for buffer density and viscosity. Buffer density, viscosity, and vbar were calculated using Sednterp (ref. S3).

S1. Philo, J S. (2000). A method for directly fitting the time derivative of sedimentation velocity data and an alternative algorithm for calculating sedimentation coefficient distribution functions. *Analytical biochemistry*, *279*(2), 151–63. doi:10.1006/abio.2000.4480

S2. Philo, John S. (2006). Improved methods for fitting sedimentation coefficient distributions derived by time-derivative techniques. *Analytical biochemistry*, *354*(2), 238–46. doi:10.1016/j.ab.2006.04.053

S3. Hurton, T., Wright, A., Deubler, G., & Bashir, B. (2012). SEDNTERP. Biomolecular Interactions Technology Center (BITC), University of New Hampshire. Retrieved from http://sednterp.unh.edu/