

Supporting Information

Polyamines Mediate Folding of Primordial Hyperacidic Helical Proteins

Dragana Despotović^{1,‡,*}, Liam M. Longo^{1,2,3,‡}, Einav Aharon¹, Amit Kahana^{1,4}, Tali Scherf⁵, Ita Gruic-Sovulj⁶ and Dan S. Tawfik^{1,*}

¹Department of Biomolecular Sciences, Weizmann Institute of Science, 7610001

Rehovot, Israel

²Earth-Life Science Institute, Tokyo Institute of Technology, 1528550 Tokyo, Japan

³Blue Marble Space Institute of Science, Seattle, 98154 Washington, USA

⁴Department of Molecular Genetics, Weizmann Institute of Science, 7610001 Rehovot, Israel

⁵Department of Chemical Research Support, Weizmann Institute of Science, 7610001 Rehovot, Israel

⁶Department of Chemistry, Faculty of Science, University of Zagreb, 10000 Zagreb, Croatia

[‡]These authors contributed equally to this work.

*Corresponding authors: E-mail addresses: dragana.despotovic@weizmann.ac.il; dan.tawfik@weizmann.ac.il

Supporting Figures

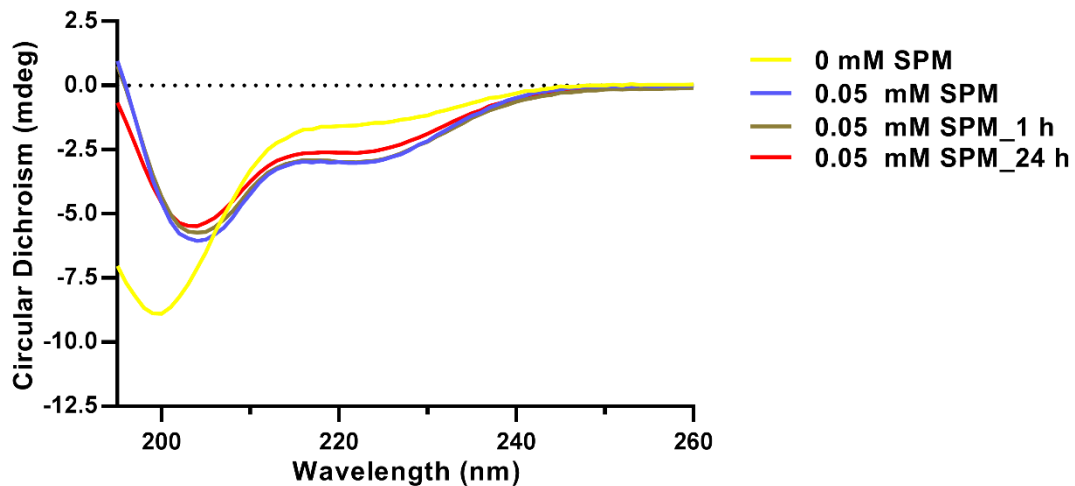
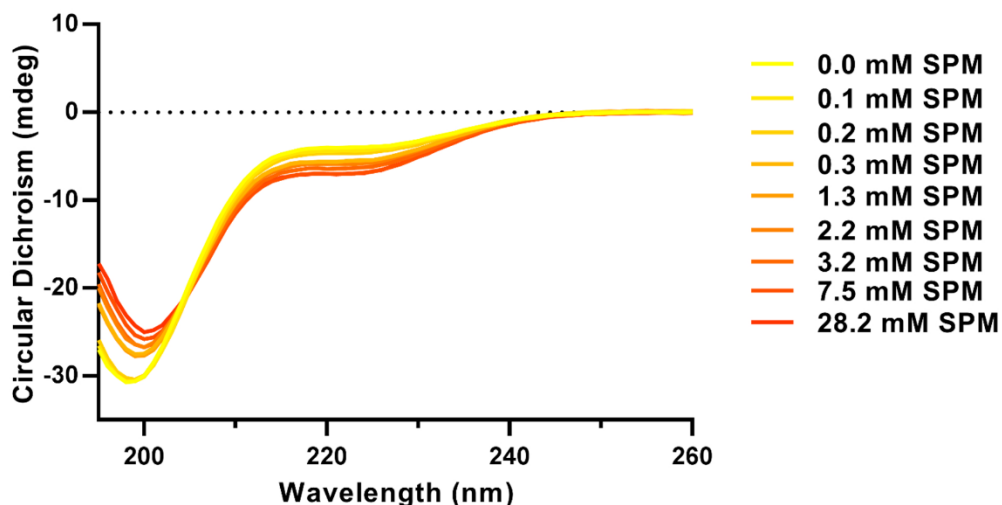


Figure S1. Circular dichroism spectra of Acidic-(HhH)₂ upon incubation with spermine (SPM). Spectra were taken immediately after addition of 0.05 mM spermine and again after 1 h and 24 h of incubation. The resulting spectra are very similar, demonstrating that for Acidic-(HhH)₂ polyamine-induced structure formation is very fast.

A

EIEEASVEELTEVPGIGPELAEEILEELAS/E
 EIEEASVEELTEVPGIGPELAEEILEEL

**B**

EIEEASVEELTEVPGIGPELAEEILERLAS/E
 EIEEASVEELTEVPGIGPELAEEILERL

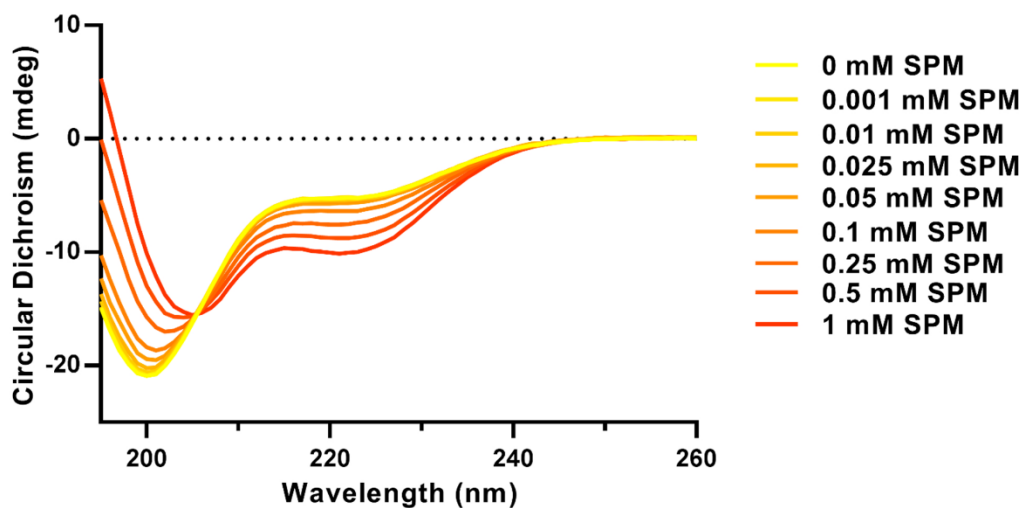


Figure S2. Spermine titration of acidified Primordial-(HhH)₂. Shown are titrations of 5 μ M protein with spermine (SPM); the protein sequences are shown above the respective titrations graphs. **A.** Complete acidification of Primordial-(HhH)₂ (replacement of all Arg residues to Glu) resulted in an unfolded protein that is only weakly responsive to spermine addition. **B.** Based on previous experience with the (HhH)₂ fold (see *Main Text*) we reverted two positions to arginine (colored blue in the sequence), which resulted in acquisition of an α -helical structure upon addition of spermine.

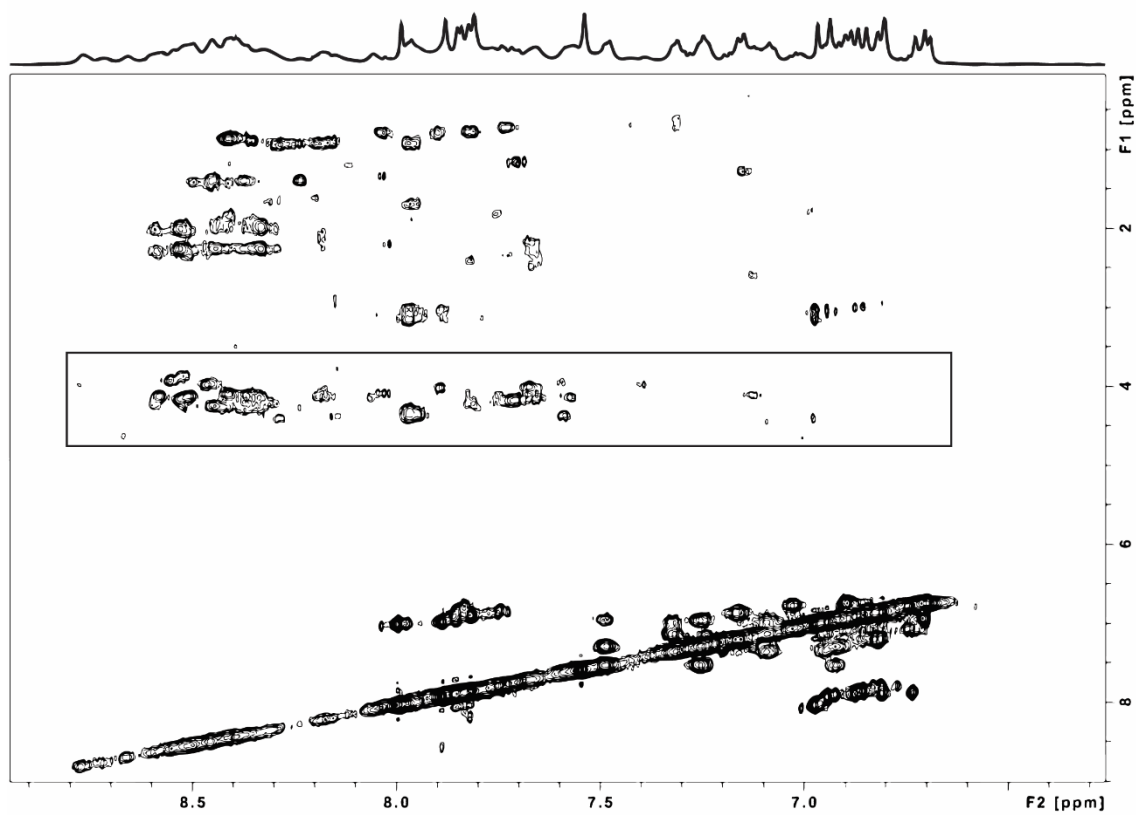


Figure S3. A 2D ^1H NMR TOCSY spectrum of Acidic-(HhH)₂ in the presence of 250 fold excess spermine at 293 K. The spectrum was acquired on a 600 MHz NMR spectrometer using 120-ms mixing time and reports on intra-residue interactions of amide groups with aliphatic groups as well as interactions within aromatic sidechains. The fingerprints region of the spectrum, revealing intra-residue ^1HN - $^1\text{H}^\alpha$ correlations, is marked with a box (see the main text).

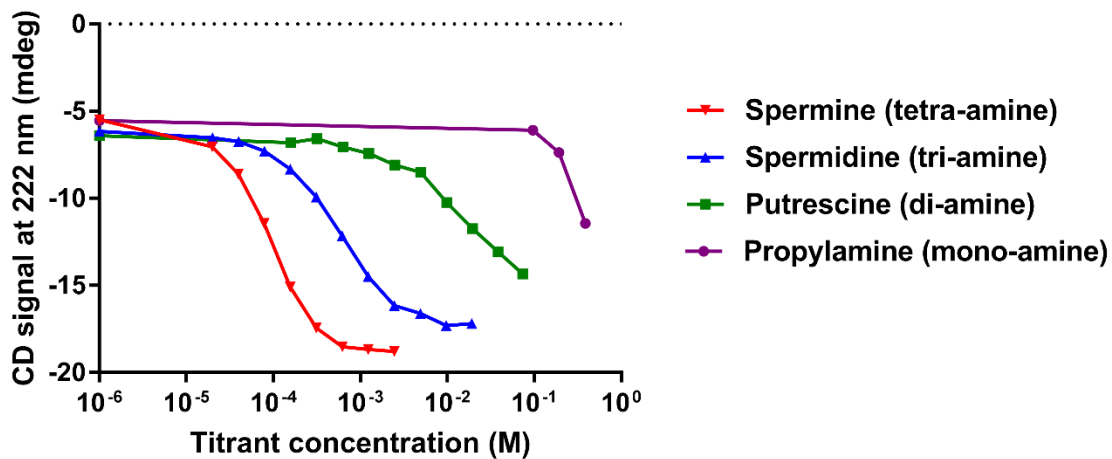


Figure S4: Titration of Acidic-(HhH)₂ with various polyamines. Circular dichroism spectra of 10 μ M Ni-NTA-purified Acidic-(HhH)₂ were collected upon addition of various concentrations of polyamines. Plotted here is the CD signal at 222 nm, a reporter of α -helical structure, after buffer subtraction and dilution correction. Estimated midpoint concentrations are: spermine = 0.09 mM, spermidine = 0.6 mM, putrescine = 23 mM; these values and the midpoint concentrations from the independent titration in **Figure 5** are within $\pm 20\%$ (the midpoint for propylamine could not be reliably estimated from this dataset, but the plot is qualitatively very similar to the titration in **Figure 5**). Midpoint values were estimated from a linear interpolation between points and assuming a saturated folded signal of -19 mdeg at 222 nm.

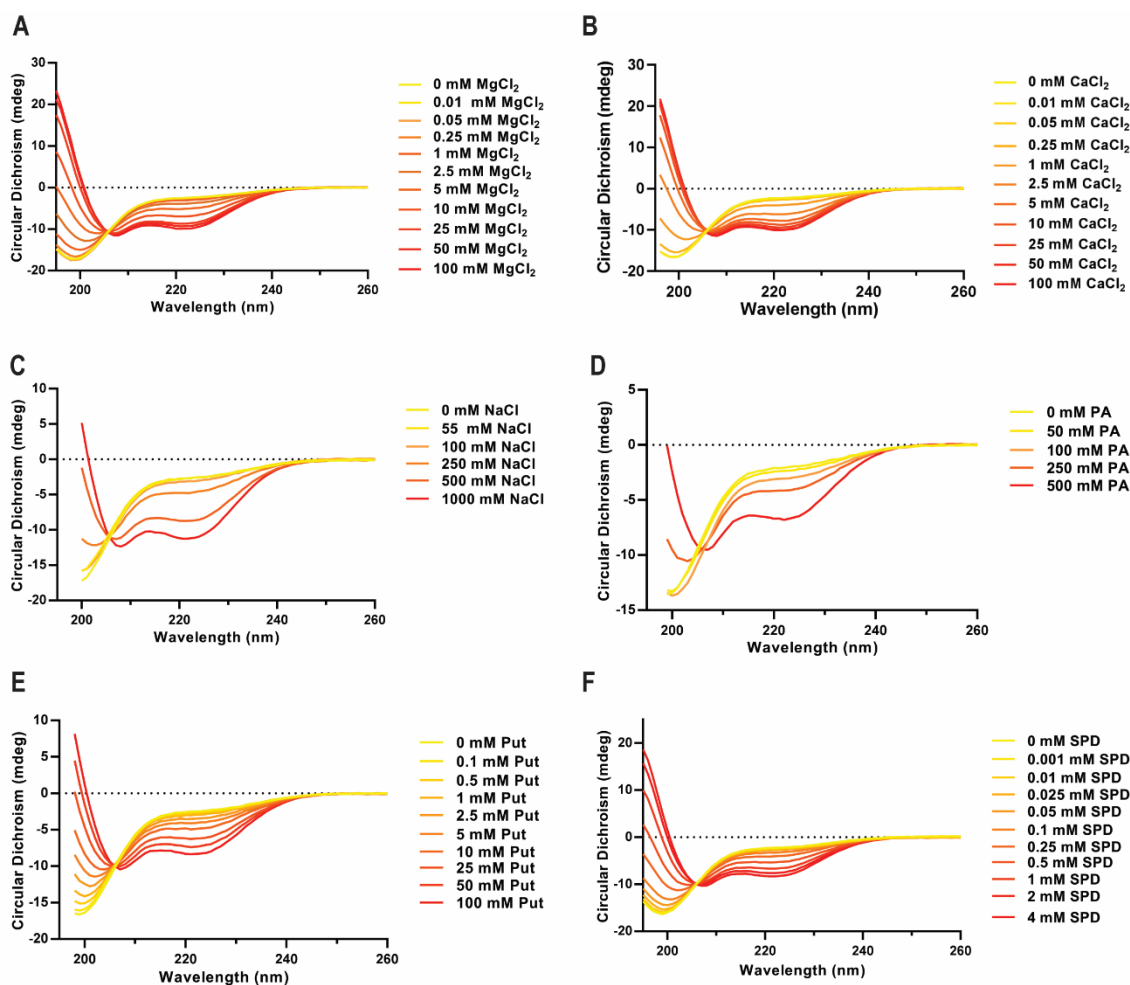


Figure S5. Circular dichroism spectra of Acidic-(HhH)₂ titrated with salts and polyamines. Circular dichroism spectra of 5 μ M Acidic-(HhH)₂ were monitored upon addition of: **A**, MgCl₂; **B**, CaCl₂; **C**, NaCl; **D**, propylamine (PA); **E**, putrescine (Put); **F**, spermidine (SPD). Spectra from the titration with spermine are presented in **Figure 3**. Each curve represents the average of two spectra after buffer subtraction and correction for dilution due to titration. Data points exceeding 700 V of applied voltage to the photomultiplier tube (PMT) were discarded. These data were used to generate the titration curves presented in **Figure 5**.