

Supplementary data for:

The impact of solubility and electrostatics on fibril formation by the H3 and H4 histones

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The supplementary material provides additional information on: 1) the hypothetical evolution of the histone fold by domain-swapping; 2) the conditions tested to promote fibril formation by the hMfB, hPyA1 and H2A-H2B histone dimers; 3) structural characterization of the H3 and H4 fibrils; 4) predictions of aggregation prone regions in H3 and H4; and 5) pH dependence of H3-H4 fibrillation kinetics.

Supplemental Table S1. Conditions surveyed for fibrillation of the histone dimers.

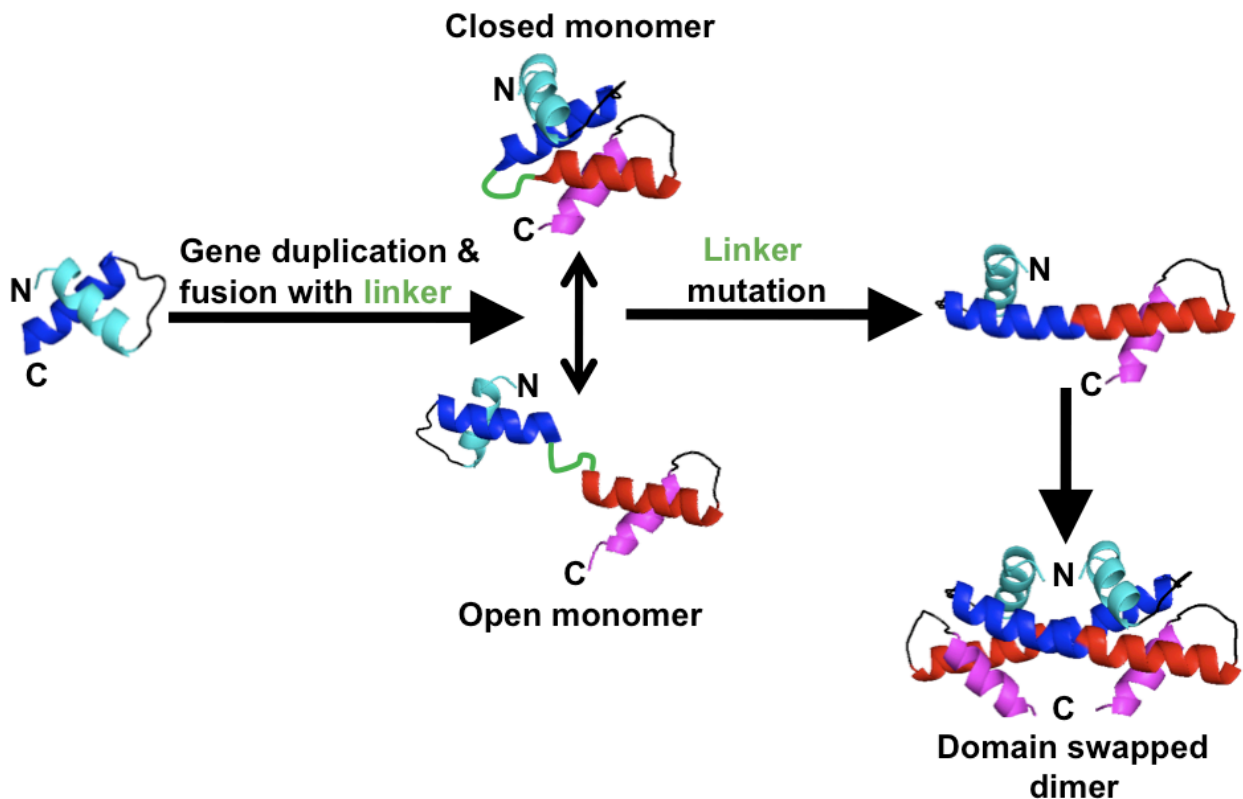
Conditions were assayed by ThT fluorescence, and the lack of fibrils was confirmed by the gel-based assay as described in the text. The samples were incubated for at least four days. X indicates that no fibrillation was detected under the specific condition listed on the left. Where concentrations were varied, the conditions used for each histone dimer is listed in the appropriate column. A dash means that these experimental conditions were not tested.

Solvent conditions at 60 μ M monomer	hMfB ^a	hPyA1	H2A+H2B
pH 2, macromolecular crowding 200 mg/ml PEG + 0.5 or 1 M NaCl	X	--	X
pH 5, macromolecular crowding 200 mg/ml sucrose, Ficoll or PEG + 0.5 M NaCl	X	X	X
pH 2, destabilizing cosolute Ethanol (%) + NaCl (M)	25% + 1 M		25% + 1 M
	10 & 25% + 0.5 M	10 & 25% + 0.5 M	
0.5, 1 and 2 M urea + NaCl (M)	0.5 & 1 M	0.5 M	1 M
Varied pH			
pH 2 + NaCl (M)	1 M	--	1 M
pH 5 + 0.5 & 1 M NaCl	X	X	X
pH 1 and 3 + NaCl (M)	1 M	0.5 M	1 M
pH 9 and 11 + 0.5 M NaCl	X	X	--
Varied salt			
pH 2 + Na ₂ SO ₄ (M)	0.1 & 0.2 M	0.1, 0.2 & 0.5 M	0.2 M

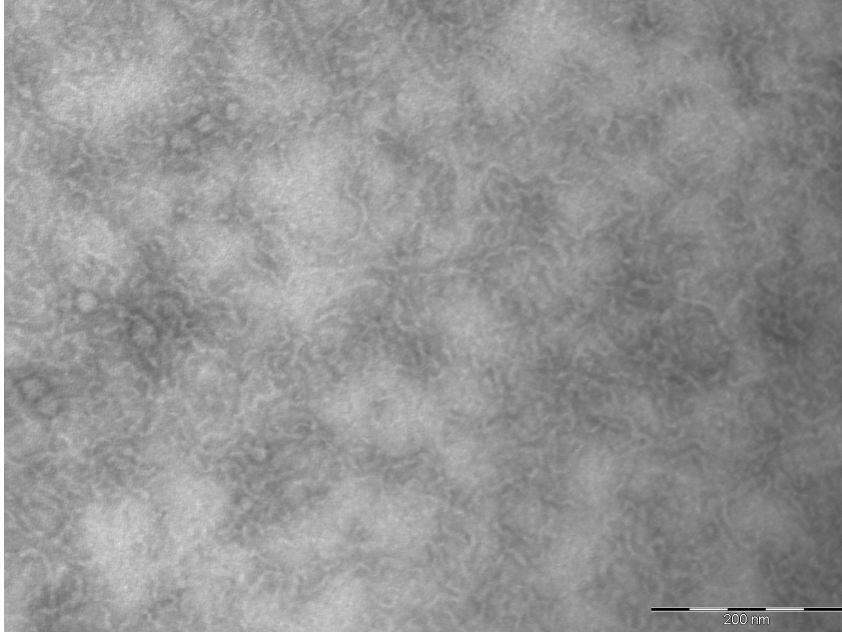
^aFor hMfB, these experiments utilized the M35Y mutant, which incorporates a unique Tyr residue for easier monitoring of protein concentration.¹

Supplemental Figure 1. Hypothetical evolution of a domain-swapped dimer from the duplication and fusion of a helix-strand-helix (hsh) motif.

Sequence and structure comparisons suggest an evolutionary relationship between the histone hsh motifs, the helical portion of the C-domains of AAA+ proteins (ATPases Associated with diverse cellular Activities) and the N-terminal domain of Clp/Hsp 100 proteins.² In these monomers, such as the RuvB C-domain,³ there is a linker/hinge between the two hsh motifs so that they interact to form a “closed” globular monomer, shown as an intermediate in this scheme. The structures in this figure are based on the canonical histone fold, derived from the coordinates of the archaeal hMfB histone (1BFM.pdb).⁴



Supplemental Figure 2. TEM of fibrils formed from H3-H4 oligomer initially folded at pH 7.2 and shifted to pH 5 fibrillation conditions as described in the legend of Figure 4 of the paper. The scale bar is 200 nm.



Supplemental Figure 3. SDS-PAGE of products from limited Proteinase K treatment of fibrils containing H3 and/or H4 histones.

Fibrillation conditions: 1 M NaCl, 50 mM sodium acetate, 50 mM sodium phosphate, pH 5, at 23 °C. Protein concentrations were 30 µM monomer for unfolded H3 and H4 (0.34 to 0.46 mg/ml) or 30 µM of each monomer for unfolded H3+H4 and folded (H3-H4)₂ (0.79 mg/ml). Initial unfolded conditions were 10 mM HCl. Initial conditions for the folded tetramer were 200 mM KCl, 20 mM potassium phosphate, pH 7.2.

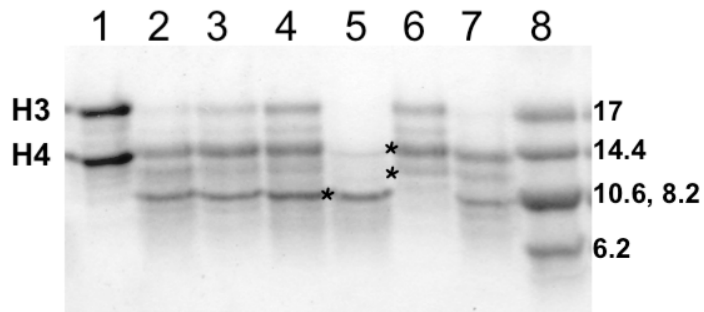
Lanes:

1. H3+H4 fibrils prior to proteolysis
- Lanes 2 to 7, samples after proteolysis
2. Fibrils from initially folded (H3-H4)₂ tetramer
3. Fibrils from initially unfolded H3+H4, sample 1
4. Fibrils from initially unfolded H3+H4, sample 2
5. Fibrils from H4 alone
6. Fibrils from H3 alone
7. Fibrils from initially unfolded H3+H4, sample 3
8. Low molecular weight markers

* indicates H3 bands and H4 band subjected to N-terminal sequencing.

H3 sequence: AXKSAPA (the same sequence was obtained for both bands)

H4 sequence: GITKPAI



Molecular weights determined by MALDI mass spectrometry:

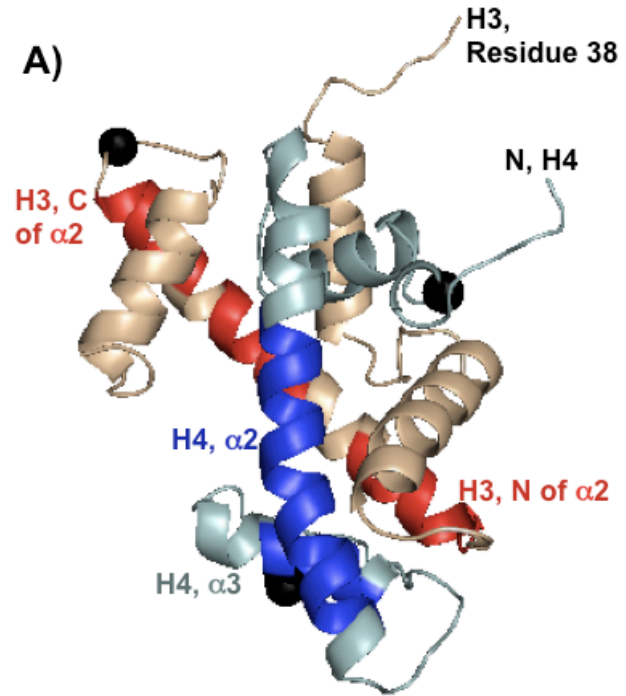
H3: 12,537, 11,804 and 10,423 daltons, corresponding to:

Residues 25 to 134 (C-terminus), 128 (α 3 of histone fold) and 115 (loop 2 of histone fold)

H4: 6647 and 6628 daltons, corresponding to:

Residues 28 to 84 and 86 (α 3 of histone fold)

Supplemental Figure 4. A) Ribbon diagram of the H3-H4 heterodimer indicating the regions protected by proteolysis in the fibril and predicted to be prone to aggregation and/or fibrillation. H3 and H4 are shown in shades of red and blue, respectively. The darker shades indicate segments that were identified by at least two prediction methods to be aggregation/fibrillation prone. Black spheres indicate the C α atoms of the terminal residues of the shortest polypeptide protected from proteolysis in the fibrils. For H3, the N-terminus of the proteolysis product—residue 25 in the N-terminal tail—was not resolved in this structure. The figure was generated from the H3-H4 structure in 1A01.pdb⁵ using MacPyMol (DeLano Scientific LLC, Portland, OR). B) Sequence of the *Xenopus* H3 and H4 histones. The N-terminal tails are represented by small letters, and helical regions are indicated above the sequences. The beginning and end of the segments protected from proteolysis in the fibril are indicated by bold and underline; the predicted aggregation/fibrillation prone sequences are colored as in Panel A. The H3 residues that differ between *Xenopus* and human histones are highlighted in green (S96C and A102G from *Xenopus* to human).



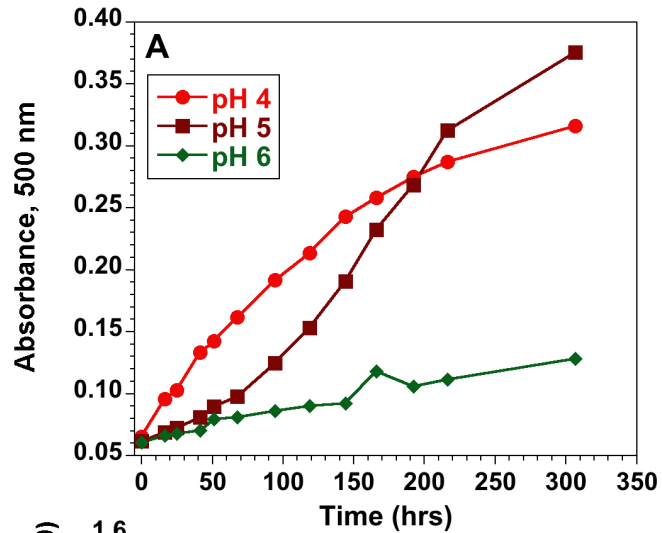
B)

H3 --- α N---
artkqtarks tggkaprkql atkaarksap atggvkkp hr yrpgTVALRE
----- - α 1----- - α 2---
IRRYQKSTEL LIRKLPFQRL VREIAQDFKT DLRFQSSAVM ALQEASEAYL
----- -- --- α 3----- -
VALFEDTNLC AIHAKRVTIM PKDIQLARRI RGERA

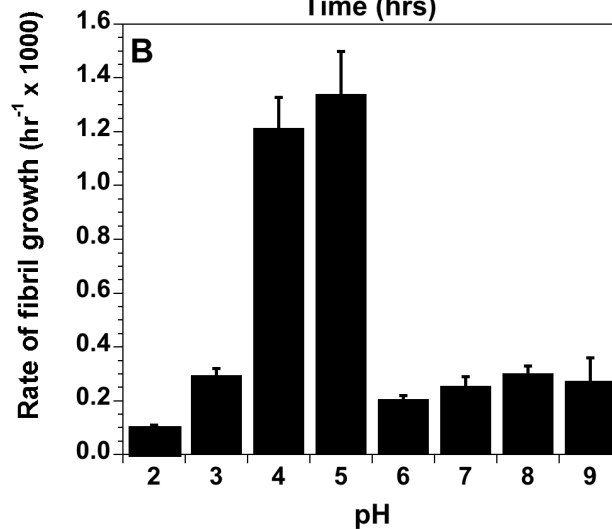
H4 --- α 1----- --
sgrgk $ggkgl$ gkgg $akrhrk$ vlr $dnigg$ it KPAIRRLARR GGVKRISGLI
----- α 2--- ----- --- α 3--- ---
YEETRGVLKV FLENVIRDAV TYTEHAKRKT VTAMDVVYAL KRQGRTLYGF GG

Supplemental Figure 5. The pH-dependence of fibril formation kinetics by H3-H4 (*i.e.* fibrillation initiated from folded $(\text{H3-H4})_2$ tetramer at pH 7). The formation of fibrils was monitored by turbidity (absorbance at 500 nm) and the gel-based assay; similar kinetics were observed for both methods (see inset of Figure 3).

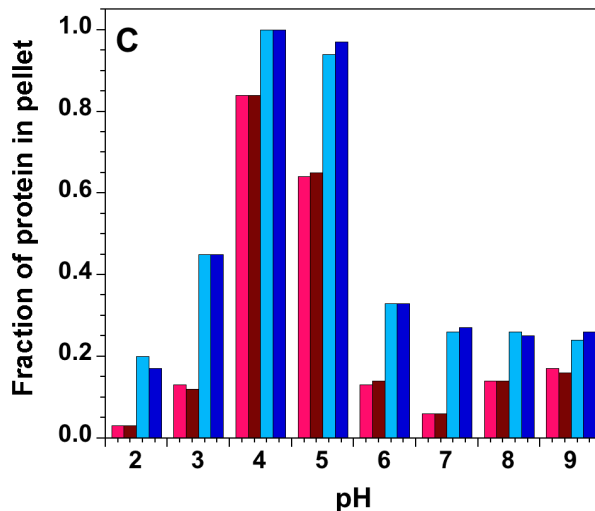
A) Representative time-dependent increases in turbidity. The lines are drawn to guide the eye and do not represent fits of the data.



B) Rate of fibril growth as a function of pH. The values were determined as described in the legend of Figure 5.



C) The fraction of H3 (light shades) and H4 (dark shades) in the pellet at two time points: 7.2 days (red) and 14 days (blue).



Conditions: 1 M NaCl, 50 mM acetic acid/sodium acetate and 50 mM phosphoric acid/ NaH_2PO_4 , 21 °C and 30 μM each of H3 and H4 monomers.

References

1. Stump, MR & Gloss, LM. (2008) Unique fluorophores in the dimeric archaeal histones hMfB and hPyA1 reveal the impact of nonnative structure in a monomeric kinetic intermediate. *Protein Sci* **17**: 322-332.
2. Alva, V, Ammelburg, M, Soding, J & Lupas, AN. (2007) On the origin of the histone fold. *BMC Struct Biol* **7**: 17.
3. Putnam, CD, Clancy, SB, Tsuruta, H, Gonzalez, S, Wetmur, JG & Tainer, JA. (2001) Structure and mechanism of the RuvB Holliday junction branch migration motor. *J Mol Biol* **311**: 297-310.
4. Starich, MR, Sandman, K, Reeve, JN & Summers, MF. (1996) NMR structure of HMfB from the hyperthermophile, *Methanothermus fervidus*, confirms that this archaeal protein is a histone. *J. Mol. Biol.* **255**: 187-203.
5. Luger, K, Mader, AW, Richmond, RK, Sargent, DF & Richmond, TJ. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**: 251-260.