

Supplementary Material

Polycistronic coexpression and nondenaturing purification of histone octamers

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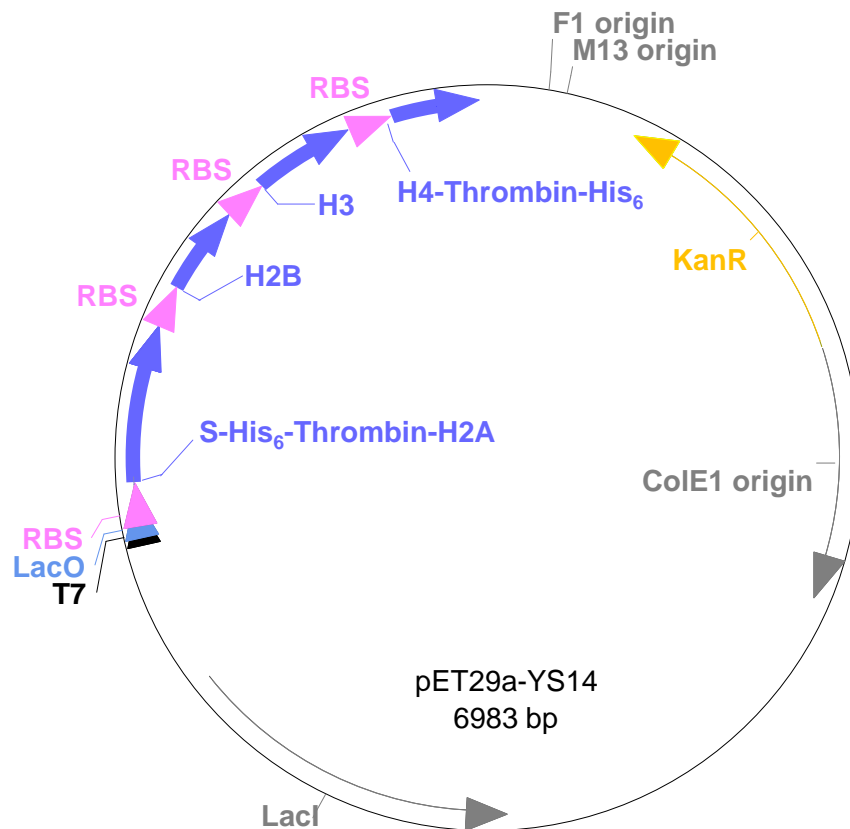
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1. Schematic map and DNA sequence of the histone-coexpressing polycistronic vector used in this study

Each histone gene is preceded by a ribosome-binding sequence (RBS). Both H2A and H4 contain His₆-tags which can be removed by thrombin digestion.



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AGGAATATATCCGAT (Total 6983 bp)

2. Methods

Polycistronic plasmid construction. Histone genes from *Xenopus laevis* were amplified by PCR from the plasmids originally made by the Luger laboratory [1]. To construct a polycistronic plasmid as depicted above (**Supplementary Material**, p. 2-3), we incorporated RBS along with a spacer sequence into the PCR primers for H2B, H3 and H4 (**Table S1**) [2]. An in-frame hexahistidine (His₆) tag was also inserted by PCR in between the pre-existing S-tag and thrombin sites in a pET29a vector so as to precede the N-terminus of H2A (**Table S1**). The stop codon in the C-terminus of H4 was deleted, and instead, an in-frame thrombin site was added between the C-terminus of H4 and a pre-existing His₆-tag in the pET29a vector, using PCR (**Table S1**). All PCR primers were purified using standard urea-PAGE protocols [3]. We assembled the histone genes into the engineered pET29a vector using standard cloning techniques using restriction enzymes as indicated in **Table S2**.

Histone expression. The resulting plasmid encoding all four core histones was transformed into BL21(DE3)pLysS cells and plated on to an LB agar plate containing kanamycin (50 µg/ml) and chloramphenicol (25 µg/ml). The plate was incubated overnight (~16 h) at 37 °C. For a 1 liter scale preparation, one colony was inoculated into 10 ml 2xTY media containing kanamycin (50 µg/ml) and chloramphenicol (25 µg/ml). This starter culture was shaken at 170 rpm for 4-5 h at 37 °C until slightly cloudy. The culture was then amplified to 1 liter of the same media and was grown for another 6-7 h at 37 °C. When the *OD*₆₀₀ reached ~0.4, histone coexpression was induced by adding 0.4 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). The culture was further shaken at 170 rpm at 37 °C for overnight (~20 h). Cells were harvested by centrifugation at 4,500 x g for 10 min at 4 °C. Cell pellets were processed immediately or stored at -80 °C for future purification.

Cell lysis and Nickel-affinity chromatography. Cell pellets were resuspended in 60 ml of lysis buffer (20 mM Tris-HCl pH 8.0, 2.0 M sodium chloride, 1 mM phenylmethanesulfonylfluoride

or Pefabloc SC (Centerchem) and 0.5 mM tris(2-carboxyethyl)phosphine. Resuspended cells were lysed by EmulsiFlex-C3 high pressure homogenizer (Avestin) and clarified by centrifugation at 38,500 x g at 4 °C for 1 h. The supernatant was collected and imidazole stock solution was added to adjust the concentration to 30 mM. The clarified lysate was then loaded onto a 5 ml HisTrap FF column (GE Healthcare) pre-equilibrated in the Ni-buffer A (20 mM Tris-HCl pH 8.0, 2.0 M sodium chloride, 0.5 mM tris(2-carboxyethyl)phosphine followed by a 10 column volumes (CV) wash with Ni-buffer A containing 30 mM imidazole and then another 10 CV wash with Ni-buffer A. The bound proteins were eluted by increasing the imidazole concentration from 30 mM to 500 mM linearly over 23.5 CV. Each fraction was analysed by 18% SDS-PAGE: 10 µl of sample was mixed with the same volume of 2x SDS loading buffer, incubated at 98 °C for 10 min and centrifuged at 17,000 x g for 10 min, after which all supernatant was loaded on the gel. Electrophoresis was run at 150 V for 80 min.

Thrombin digestion. Thrombin digestion was carried out by adding purified thrombin (Sigma) in 25:1 mass ratio and incubating the samples at 4 °C for 3 h. The digestion was confirmed by SDS-PAGE.

Size exclusion chromatography. Thrombin-digested histones were then concentrated up to 3 mg/ml with ultrafiltration using Amicon YM50 membrane (MWCO 50 kDa) at 4 °C (Millipore). The concentrated sample was then injected onto a Superdex 200 10/300GL column. The histone octamer peak was eluted at an elution volume of 12.8 ml. The peak fractions were pooled and concentrated up to 8 mg/ml, aliquotted and flash-frozen in the presence of 50% glycerol for long-term storage. The yield of pure histone octamers after the size-exclusion column was typically 3-5 mg per liter culture.

Nucleosome reconstitution. Nucleosome reconstitution was done essentially as described [4; 5]. The histones and DNA were mixed in the molar ratio of octamer:DNA at 1.1:1 and dialyzed

sequentially against TE buffers (10 mM Tris-HCl pH 8.0 and 1 mM EDTA) containing 1 M, 600 mM and 0 mM sodium chloride, each for at least one hour. The last dialysis was usually done overnight. The results were verified on 5% native polyacrylamide gels run in 0.25x TBE at 130 V for 1 h at room temperature.

FRET measurements. The DNA substrate was internally labeled at positions 6 and 81 in a 601 sequence with Cy3 and Cy5 respectively (Fig. S3A) [6]. The FRET by the dye pairs was measured as described to validate the formation of NCP by the histones [6].

3. Supplementary Tables S1 & S2

Table S1. PCR primers used in this study.

Name	Sequence
BglII_His ₆ _KpnI_F	C AGCCCAGATCTG CACCACCACCACCACCAC <u>GGTACC</u> CTGGTG
BglII_His ₆ _KpnI_R	CACCAG <u>GGTACC</u> GTGGTGGTGGTGGTGGTG <u>CAGATCT</u> GGGCT G
NcoI_xeH2A_F	acttga <u>CCATGG</u> gt ATGTCAGGAAGAGGCAAA
EcoRI_xeH2A_R	cacaca <u>GAATTC</u> TCACTTGCTCTTGGCCGA
EcoRI_RBS_xeH2B_F	acttga <u>GAATTC</u> AATAATTTTGTTTAACTT TAAG <u>AAGGAG</u> ATATACAT ATGGCCAAGTCCGCTCCA
SalI_xeH2B_R	cacaca <u>GTCGAC</u> TTACTTGGCGCTGGTGTA
SalI_RBS_xeH3_F	acttga <u>GTCGAC</u> AATAATTTTGTTTAACTT TAAG <u>AAGGAG</u> ATATACAT ATGGCCCGTACCAAGCAG
NotI_xeH3_R	cacaca <u>GCGGCCGC</u> CTAAGCCCTCTCGCCTCG
NotI_RBS_xeH4_F	acttga <u>GCGGCCGC</u> AATAATTTTGTTTAACTT TAAG <u>AAGGAG</u> ATATACAT ATGTCTGGTCGTGGTAAA
XhoI_xeH4 -Thrombin_R	cacaca <u>CTCGAG</u> GCTGCCGCGCGGCACCAGGCCGCTGCT ACCACCGAAACCGTACAG

*Restriction enzyme sites are underlined and RBS's are indicated in red.

Table S2. Restriction enzymes and affinity tags used for the histones.

Histone	(5') Restriction Enzyme	(3') Restriction Enzyme	Affinity tags
H2A	NcoI	EcoRI	An N-terminal S-tag followed by a His ₆ -tag and a thrombin cleavage site.
H2B	EcoRI	SalI	None
H3	SalI	NotI	None
H4	NotI	XhoI	A C-terminal His ₆ -tag preceded by a thrombin site.

4. Supplementary Figures S1-S3

Fig. S1. Coexpression and copurification of histone octamers. **(A)** All four histones were induced and overexpressed upon adding 0.4 mM IPTG. Induced and uninduced cells are indicated by (+) and (-) IPTG, respectively. M: molecular weight marker. **(B)** Most histones were recovered in the soluble fraction of the cell lysate. C: total cell lysate, S: cell supernatant, P: cell pellet fractions. All lanes represent 50 μ l of cell culture. **(C)** The affinity tags on H2A and H4 could be digested completely within 3 h of thrombin addition at 4 °C. Undigested and digested samples are indicated with (-) and (+) thrombin, respectively. Numbers above the lanes 3-5 indicate the number of hours after adding thrombin.

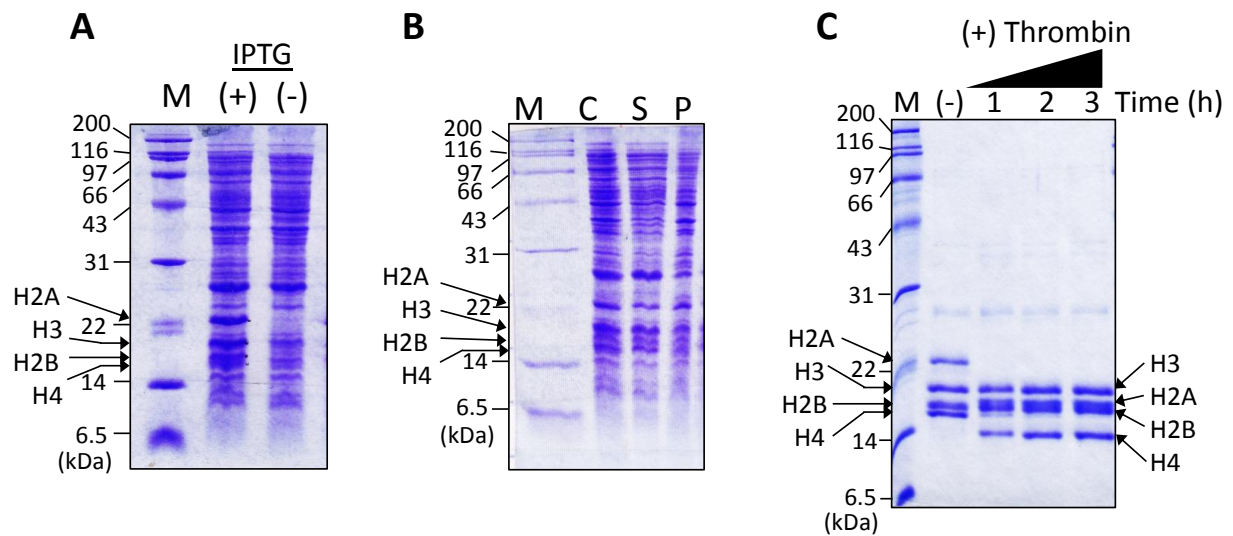


Fig. S2. Characterization of the copurified histones using size-exclusion chromatography. **(A)** Calibration curve for the Superdex 200 column used in this study. According to this curve, the molecular weight of the histone complexes eluting at 12.8 ml (Fig. 1D) is estimated to be 111 kDa, closely matching the expected molecular weight of the octamer (111 kDa). K_{av} and LogMW were calculated as described in the product manual for the size-exclusion column molecular weight calibration kit (GE Healthcare). **(B)** Histones purified as shown in Fig. 1D, were subject to a second size-exclusion chromatography. The chromatogram (top) and SDS-PAGE analysis of the fractions (bottom) show that the four histones again elute at 12.8 ml while maintaining the stoichiometric ratio. However, the void volume peak observed in Fig. 1D no longer exists. M: molecular weight marker, I: input, 7-14 indicate fraction numbers. **(C)** SDS-PAGE of histones prepared by a conventional method [6] (lane 1) and the histones copurified in this study (lane 2).

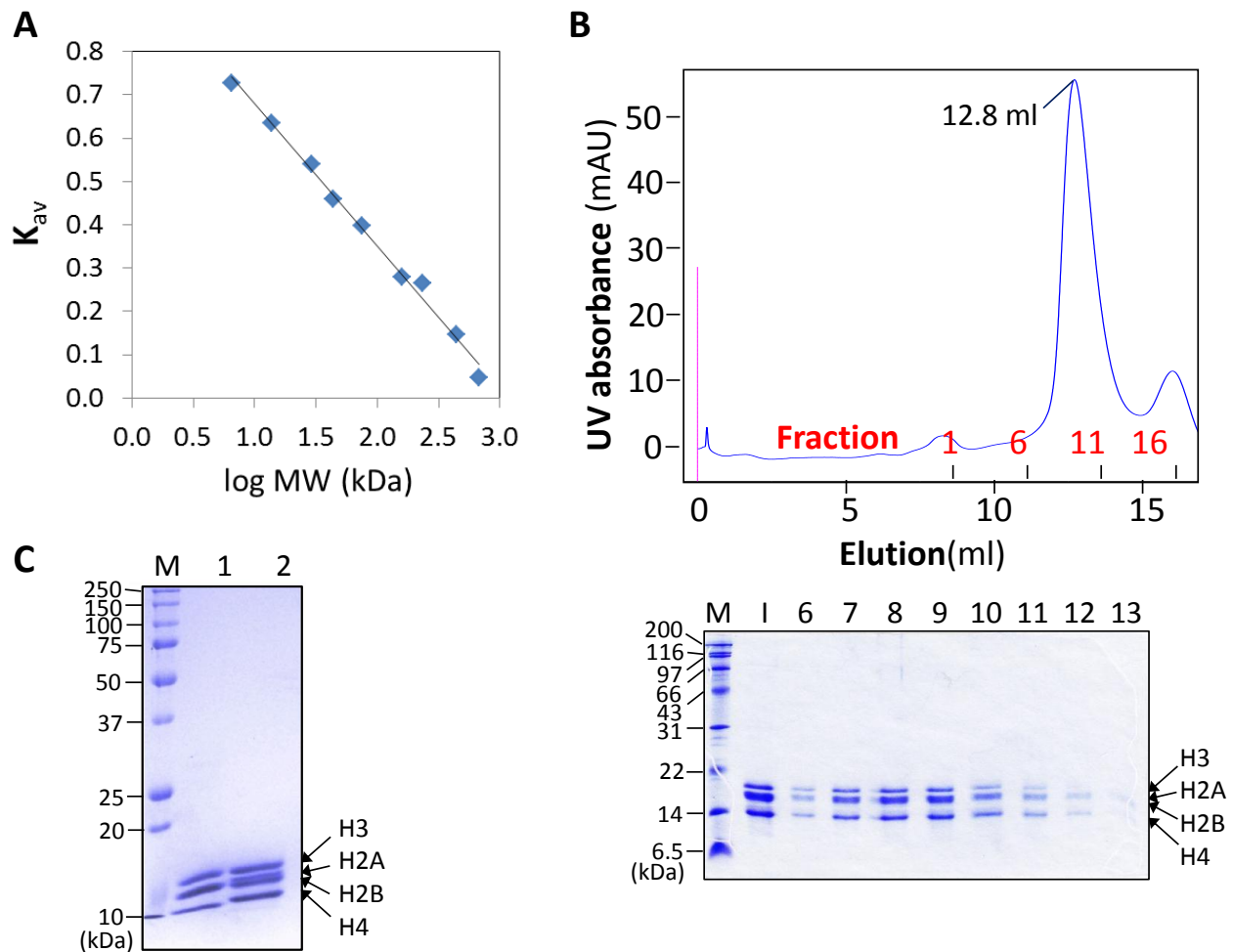
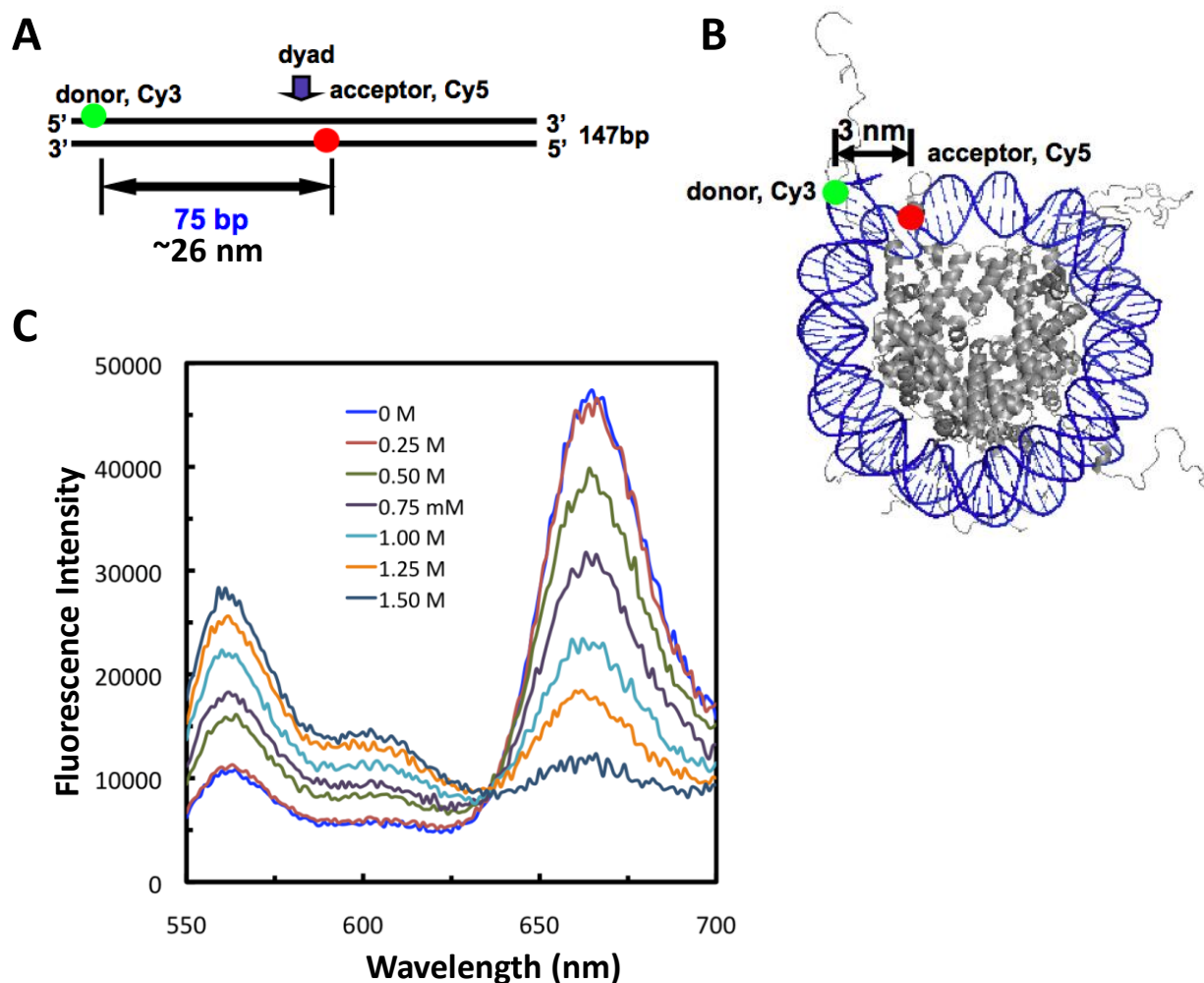


Fig. S3. Characterization of the copurified histones using FRET. **(A and B)** Schematic diagrams depicting the relative positions of the donor (Cy3) and acceptor (Cy5) fluorophores on a naked “601” DNA **(A)** and after the DNA forms NCP **(B)**. The formation of NCPs with the histones can be confirmed by the FRET peak of the acceptor at 670 nm as shown in Fig. 2B. **(C)** Fluorescence emission spectra of NCPs reconstituted with the copurified histones show that the NCPs dissociate as the salt concentration increases. This salt-induced dissociation profile is similar to that of NCPs with control histones (Fig. 2C)



5. References

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- [4] K. Luger, T.J. Rechsteiner, and T.J. Richmond, Preparation of nucleosome core particle from recombinant histones. *Methods Enzymol* 304 (1999) 3-19.
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