Materials and Methods

BL21(DE3) Chemically Competent Cell and Trans5α Chemically Competent Cell were purchased from Beijing TransGen Biotech Co., Ltd. Difco™ LB Broth, Miller (Luria-Bertani medium) was bought from Becton, Dickinson and Company. Sangon Biotech (Shanghai) Co., Ltd. offered ampicillin, 1,4-dithiothreitol (DTT) and Ethylenediaminetetraacetic acid (EDTA). Tris(hydroxymethyl)methyl aminomethane was purchased from (Tris) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) Amresco Inc. Disodium hydrogen phosphate (Na₂HPO₄), guanidine hydrochloride (Gn·HCl) and other common reagent were bought from Sinopharm Chemical Reagent Co., Ltd. Sodium iodide and hydrazine hydrate were obtained from J&K SCIENTIFIC Ltd.

The synthesis of α-chloro acetamide 2

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\text{Tert-butyl 2-(2-bromoacetamido)ethylcarbamate (6): A solution of chloroacetyl chloride (2.74 g, 24.4 mmol) in DCM (30.00 mL) was added dropwise to a solution of tert-butyl-(2-aminoethyl)-carbamate (3.24 g, 20.40 mmol) and triethylamine (3.06 g, 30.00 mmol) in DCM (30.00 mL) at –20 ºC. The reaction was stirred overnight at room temperature. The crude product was washed with 1 M HCl, sat. NaHCO₃ and brine, dried over Na₂SO₄, concentrated in vacuo and purified by column chromatography on silica (5:1-1:1, petro ether/EtOAc) to afford the desired product 6 as a white solid (3.62 g, 75.30 %).}^{1} \text{H-NMR (300 MHz, CDCl₃): } δ 4.94 (s, 1H), 4.03 (s, 2H), 3.43-3.37 (m, 2H), 3.32-3.30 (m, 2H), 1.43 (s, 9H). \text{13C-NMR (300 MHz, CDCl₃): } δ 166.78, 156.76, 79.95, 42.51, 41.18, 39.79, 28.34. \text{HR-MS, m/z calcld for C₉H₁₇ClN₂O₃ 236.0928; found [M-H] - 235.0858, [M+Cl] - 271.0613, [M+COOH] - 281.0912.}

\text{N-(2-aminoethyl)-2-chloroacetamide hydrochloride (2): Compound 6 (3.00 g, 10.71 mmol) was dissolved in 4 M HCl/1,4-dioxane solution (20.00 mL) and stirred for 2 hours at room temperature. The reaction mixture was evaporated to afford 2 as a white solid (1.74 g, 94.80 %) and used for the next step without further purification.}^{1} \text{H-NMR (300 MHz, D₂O): } δ 4.03 (s, 2H), 3.44-3.41 (t, J = 6.0 Hz, 2H), 3.32-3.30 (m, 2H), 1.43 (s, 9H). \text{13C-NMR (300 MHz, D₂O): } δ 170.70, 42.14, 39.01, 37.15. \text{HR-MS, m/z calcld for C₄H₉ClN₂O 136.0403; found [M+H]⁺ 137.0482, [M+Cl]⁺ 271.0613, [M+COOH]⁺ 281.0912.}

Expression of Ub(1-75)- NHNH₂

Plasmid pTYB2-Ub(1–75) was transformed into E. coli BL21(DE3) Chemically
Competent Cell and monoclonal colony was selected to grow in 10 ml LB with 100 μg/ml ampicillin overnight at 37 °C under shaking at 220 rmp. The 10 ml LB with *E. coli* was transferred into 1 L LB with 100 μg/ml ampicillin and shaken at 37 °C until OD$_{600}$ reached 0.8. 0.2 mM IPTG was added to induce expression of protein. The cell was grown at 18 °C for another 18h and then could be harvest by centrifugation at 4500g for 10 min at 4 °C. After the cell resuspension and ultrasonification in lysis buffer (500 mM NaCl, 20 mM Hepes, and 1 mM EDTA, pH 7.5), the supernatant was separated from the pellet by centrifugation at 12000g for 30 min. Then it was loaded on about 10 ml chitin beads, which had been pre-equilibrated with lysis buffer. After about 1 h, the column was washed by lysis buffer for about 30 column volume. Finally, Ub(1-75)-NHNH$_2$ was cleaved from chitin beads by incubating the column in cleavage buffer (500 mM NaCl, 20 mM Hepes, 1 mM EDTA, 8% hydrazine hydrate, pH 7.5) for 3 h. After purified by HPLC and lyophilized, Ub (1-75)-NHNH$_2$ was obtained. The yield was about 3mg/L.

Scheme S2 The HPLC traces and ESI of recombinant Ub(1-75)-NHNH$_2$.

Expression of histones

Plasmids of H2A, H2BK34C, H3 and H4 were transformed into E. coli BL21(DE3) Chemically Competent Cell and monoclonal colony was picked to grow in 10 ml LB (Luria-Bertani medium) with 100 μg/ml ampicillin overnight at 37 °C under shaking at 220 rmp. The 10 ml LB with *E. coli* was transferred into 1 L LB with 100 μg/ml ampicillin and shaken at 37 °C. When OD$_{600}$ was about 0.8, 0.4 mM IPTG was added. The cell was grown at 37°C for another 5 h and then could be harvest by centrifugation at 4500g for 10 min at 4 °C. After the cell resuspension and ultrasonification in the lysis buffer (150 mM NaCl, 20 mM Tris, pH 7.5), the inclusion body was separated from the supernatant by centrifugation at 12000g for 30 min and then washed by wash buffer (100 mM NaCl, 50 mM Tris, 1 mM EDTA, 1 mM benzamidine, pH 7.5) for twice. It was then resolved by unfolding buffer (6 M GnHCl, 20 mM Tris, 10 mM DTT, pH 7.5) and centrifuged to remove the pellet. The supernatant was purified by semi-preparative HPLC to obtain each histone. The yield was about 10 mg/L.
Scheme S3 The HPLC traces and ESI-MS of recombinant H2BK34C.

**Synthesis of α-chloroacetamide Ub derivative**

10 mg recombinant Ub(1-75)-NHNH₂ was resolved into 1 ml acidic PBS buffer (6 M GnHCl, 0.2M Na₂HPO₄, pH 3.0). At – 20 °C, 59 μl of 0.2 M NaNO₂ in acidic PBS buffer was added to oxidize for 20 min. Then 100 eq. α-chloroacetamide was added and the pH was adjusted to 6.8. The reaction was carried out at room temperature for 2 h. When the reaction was completed, it was purified by semi-preparative HPLC.

**Synthesis of H2BK34sUbd**

1.86 mg α-chloroacetamide Ub derivative, 4.65 mg H2BK34C (1.6 equiv.) and 3.23 mg NaI were resolved in 80 μl PBS (6 M GnHCl, 0.2M Na₂HPO₄, pH 7). The pH was adjusted to 8 and the reaction was carried out at room temperature overnight. When the reaction was completed, it was purified by semi-preparative HPLC.

**Reconstruction of octamer**

The octamer was reconstructed according to standard procedures. Equal ration of four histones were resolved into unfolding buffer to 2 mg/ml separately. Mix them and adjust the protein concentration to 1 mg/ml. The mixture was dialyzed against 600 mL refolding buffer (2 M NaCl, 20 mM Tris, 1 mM EDTA, 1 mM DTT, pH 7.5) for 12 h for three times at 4 °C. Finally, it can be purified by size-exclusion chromatography (SEC 200).

**Reconstruction of nucleosome**

0.25 μg 601 DNA was adjusted to 2 M NaCl, 10 mM Tris, 1 mM EDTA and 1 mM DTT, and then 1.1 equivalent purified octamer was added. The mixture was transferred into a dialysis bag and put into 400 mL refolding buffer under stirring at 4 °C. The refolding buffer was diluted slowly by adding buffer of 10 mM Tris, 1 mM EDTA, 1 Mm DTT through a peristaltic pump. When the NaCl concentration was reduced to 0.15 mM, the refolding was completed. Native page gel was used to analyze the reconstructed nucleosome.

**Thermal stability essay**
5000X SYPRO Orange was diluted to 50X with nucleosome reconstruction buffer. The nucleosome solution was centrifuged to remove the precipitate, and 27 μl supernate was mixed with 3 μl 50X SYPRO Orange. Bio-Rad CFX96 Touch Real-Time PCR was used to measure the fluorescence intensity. The temperature of the sample raised from 25 °C to 95 °C by 1 °C, each temperature maintained for 1 min, and the fluorescence intensity was measured at each temperature to form the fluorescence intensity-temperature curve.

References
NMR SPECTRA

$^1$H-NMR spectrum of compound 6 in CDCl$_3$

$^{13}$C-NMR spectrum of compound 6 in CDCl$_3$

HR-ESI-MS spectrum of compound 6
$^1$H-NMR spectrum of compound 2 in D$_2$O

$^{13}$C-NMR spectrum of compound 2 in D$_2$O
HR-ESI-MS spectrum of compound 2

+ESI Scan (0.755 min) Frag=120.0V QH-2.d

Counts vs. Mass-to-Charge (m/z)

137.0482
(M+H)+

139.0454
(M+H)+

x10^5