

Electronic Supplementary Information

**Non-destructive handling of individual chromatin fibers
isolated from single cells in a microfluidic device utilizing an
optically driven microtool[†]**

**Hidehiro Oana^{*§^a}, Kaori Nishikawa^{§^a}, Hirotada Matsuhara^{^b}, Ayumu Yamamoto^{^{b,c}},
Takaharu G. Yamamoto^{^d}, Tokuko Haraguchi^{^{d,e}}, Yasushi Hiraoka^{^{d,e}}, and Masao Washizu^{^a}**

^a Department of Mechanical Engineering, Graduate School of Engineering,
The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan

^b Graduate School of Science and Technology, Shizuoka University,
836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

^c Department of Chemistry, Shizuoka University,
836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

^d Advanced ICT Research Institute, National Institute of Information and Communications
Technology,
588-2, Iwaoka, Nishi-ku, Kobe 651-2492, Japan

^e Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamadaoka, Suita 565-0871,
Japan

[*] To whom correspondence should be addressed

Hidehiro Oana, Ph. D.

Department of Mechanical Engineering, Graduate School of Engineering

The University of Tokyo

7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan

E-mail: oana@mech.t.u-tokyo.ac.jp; Tel: +81-3-5841-6338

[†] Electronic Supplementary Information (ESI) available. See DOI:10.1039/x000000x.

[§] Both authors contributed equally to this work.

1. *Schizosaccharomyces pombe* strains used in this study

Table S1 Strain list

Strain	Genotype
SHM12-D3	h ⁻ ade6-210 leu1 lys1 ura4 hht2 ⁺ -GFP::ura4 ⁺ [1] hta1 ⁺ ::GFP-kan ^r [2]
SHMT1769p2	h ⁻ leu1-32 lys1-131 ura4 aur1::aur1 ^r -Phht1-hht1 ⁺ -mRFP ^[3] cnp1::nat1-Pcnp1-GFP-cnp1 ⁺ [4]

Source:

[1] a) H. Wang, X. Tang, J. Liu, S. Trautmann, D. Balasundaram, D. McCollum, M. K. Balasubramanian, *Mol. Biol. Cell*, 2002, **13**, 515–529. b) D.-Q. Ding, A. Yamamoto, T. Haraguchi, Y. Hiraoka, *Dev. Cell*, 2004, **6**, 329–341.

[2] A gift from Dr. Takashi Toda (Cancer Research UK, London Research Institute, UK).

[3] This study.

[4] Y. Takayama, H. Sato, S. Saitoh, Y. Ogiyama, F. Masuda, K. Takahashi, *Mol. Biol. Cell*, 2008, **19**, 682–690.

2. Preparation of spheroplasts from yeast cells

1. Grow 10 mL culture to an OD₆₀₀ of 0.3–0.7 in YES medium.
 2. Harvest cells (at 500 × g, 5 min), decant the supernatant, and resuspend the pellet in 10 mL of ultrapure water (produced by Milli-Q Gradient A10 system; Merck Millipore).
 3. Wash twice with 10 mL of ultrapure water (at 500 × g, 5 min).
 4. Harvest cells and resuspend the pellet in 1 mL of:
 - 10 mg/mL Lysing Enzymes (from *Trichoderma harzianum*)
 - 1.4 mg/mL Zymolyase 100T
 - 0.65 M KClIncubate at 37°C with rotation at *ca.* 3 rpm for 45–60 min until spheroplasts have formed.
 5. Harvest spheroplasts (at 3500 rpm, 3 min, 4°C) and resuspend the pellet in 1 mL of 1 M sorbitol.
 6. Wash twice (at 3500 rpm, 3 min, 4°C) with 1 mL of 1 M sorbitol.
- Then, obtained suspensions of spheroplasts are stored at 4°C and used within two days.

3. Partial unfolding of the entangled chromatin fibers by fast flow

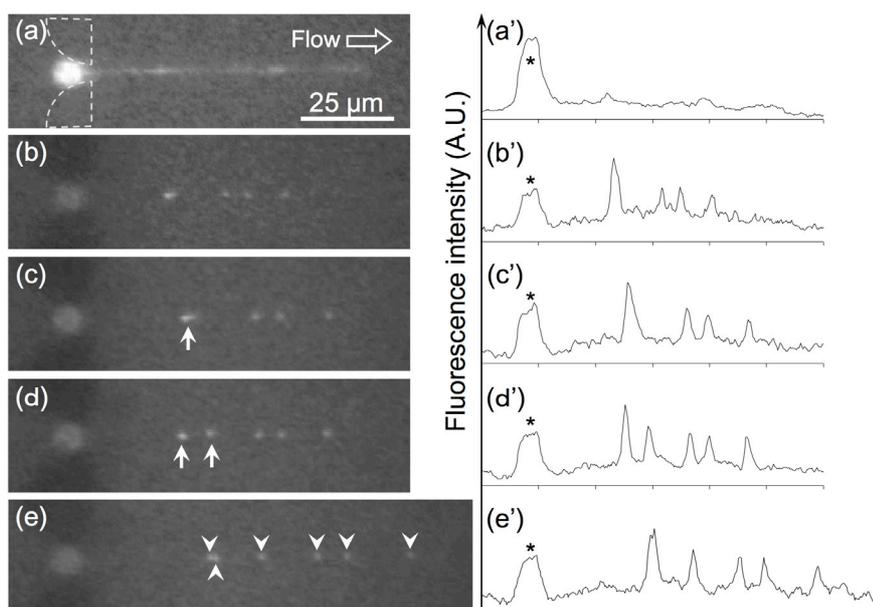


Fig. S1 Left: representative image of immunostained chromatin fibers under the flow of the bursting solution (0 mM NaCl), which were immobilized at the micropillars after immunofluorescence staining in the micropocket. (a) Fluorescence image of bundle of the tethered chromatin fibers visualized by RFP tagged to histone H3 under a flow rate of *ca.* 110 μm/s. (b) Fluorescence image of Cnp1-GFP enhanced by the Alexa488-conjugated anti-GFP antibody under a flow rate of *ca.* 110 μm/s. (c) Image of the fluorescent spots of the Alexa488 just before the split of the far left fluorescent spot (indicated by a white arrow) under a flow rate of *ca.* 130 μm/s. (d) Image of fluorescent spots of Alexa488 just after the separation of the fluorescent spot under a flow rate of *ca.* 130 μm/s. Separated fluorescent spots are indicated by white arrows. (e) Image of fluorescent spots of Alexa488 under a flow rate *ca.* 300 μm/s. Fluorescent spots are indicated by white arrowheads. Right: profiles of fluorescence intensities along the chromatin fibers. Each profile (a' to e') corresponds to the respective fluorescence image on the left (a to e). Far left trapezoidal shapes of fluorescence profiles (indicated by '*') were derived from auto-fluorescence of the antibody-conjugated microsphere. A movie file is available in the Electronic Supplementary Information (Movie 3).

Fig. S1 shows a representative image of a bundle of immobilized chromatin fibers after immunofluorescence staining in the micropocket. When the immobilized chromatin fibers were visualized by RFP tagged to histone H3, a *ca.* 75-μm-long bundle of chromatin fibers was observed (Fig. S1a). When green fluorescence of the Alexa488-conjugated anti-GFP antibody for the immunostained

GFP-tagged Cnp1 was observed, there were four fluorescent spots, and the far left spot was brighter than the others (Fig. S1b). The integrated fluorescence intensity of the fluorescent spot on the far left of Fig. S1b' seems to be comparable to the sum of the integrated fluorescence intensities of the other three fluorescent spots. This indicates a piling up of the positions of the centromeres. When the flow rate was slightly increased, the immobilized chromatin fibers were slightly elongated (Fig. S1c), and suddenly, the brightest fluorescent spot was split into two spots (Fig. S1d). Comparing Fig. S1c and Fig. S1d shows that the positions of the three downstream fluorescent spots were not shifted during the split. This indicates that chromatin fibers loading downstream of the three fluorescent spots were not on the same chromatin fiber loading upstream at the fluorescent spot that was three times brighter. Furthermore, the integral fluorescence intensity of one of the fluorescent spots generated by the split is comparable to that of each of the three downstream fluorescent spots, and the integral fluorescence intensity of the other is roughly twice that of each of the three downstream fluorescent spots (Fig. S1d'). This split of the far left fluorescent spot indicates that entangled chromatin fibers were unfolded partially and one of the three piled up centromere regions became detached by the flow while the two regions of centromere remained piled up. Finally, when the flow rate was increased further, the far left fluorescent spot split in two, which resulted in six fluorescent spots along the bundle of partially unfolded chromatin fibers (Fig. S1e, e').

4. List of Movies

Movie 1:

Immobilization of the chromatin fibers to the pair of micropillars by placing the microsphere in the gap between the micropillars (played at 2× speed) corresponding to Fig. 4a.

Movie 2:

Immobilization of the chromatin fibers to the isolated triangle micropillar by winding (played at 2× speed) corresponding to Fig. 4b.

Movie 3:

Split of the green fluorescent spot on the tethered chromatin fibers under flow (in real-time) corresponding to Fig. S1.