Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Direct visualization of the conformational change of FUS/TLS upon binding to promoter-associated non-coding RNA

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Supplementary Movies legends:

Movie S1.

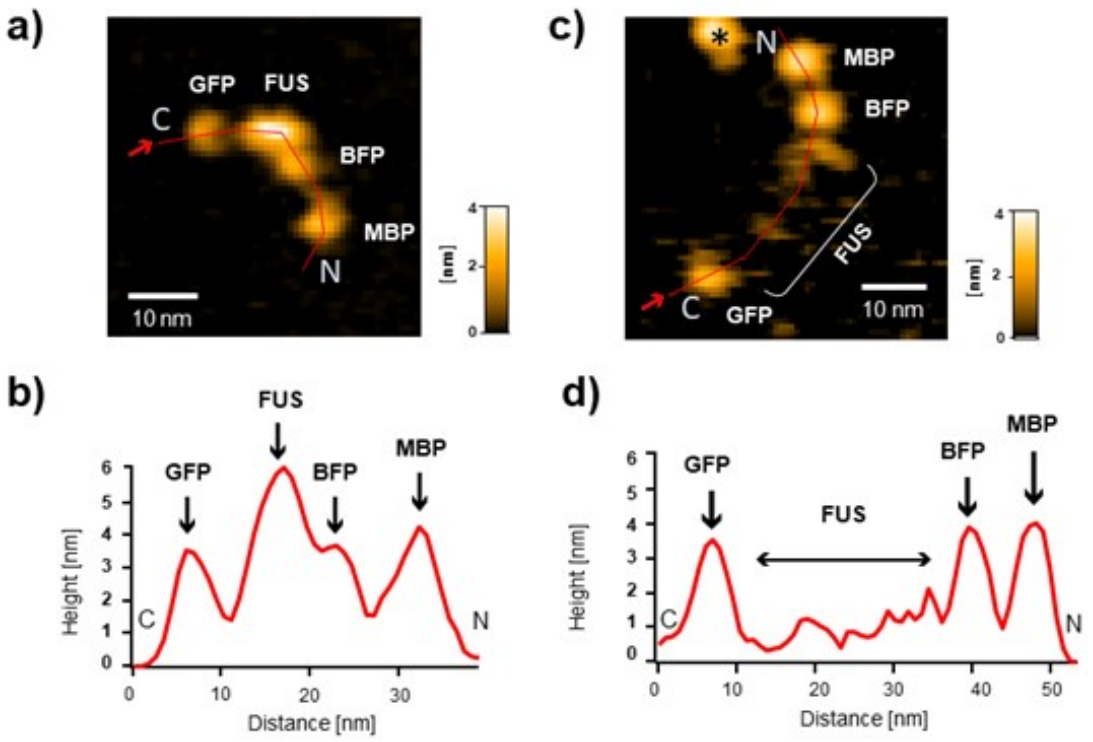
HS-AFM movie to show the compact-to-extended conformational transition of the FUS fusion protein upon the addition of pncRNA. A 2 μL droplet of the 5 nM fusion protein was placed on the APTES-mica surface. Fourteen-fold equimolar amount of pncRNA to the fusion protein was added to the solution in the chamber at time 0. Scanning area: 60 nm x 47 nm, pixel size: 72 pixels x 56 pixels, recording rate: 5 frame/s, playing rate: 10 frame/s. Blue and green dots are added to the movie using Igor Pro software to indicate BFP and GFP, respectively.

Movie S2.

HS-AFM movie of the FUS fusion protein in the absence of pncRNA. A 2 μL droplet of the 10 nM fusion protein was placed on the APTES-mica surface. Tracking was started from time 0. Scanning area: 58 nm x 47 nm, pixel size: 70 pixels x 50 pixels, recording rate: 5 frame/s, playing rate: 10 frame/s. Blue and green dots are added to the movie using Igor Pro software to indicate BFP and GFP, respectively.

Movie S3.

HS-AFM movie of the FUS fusion protein in the presence of pncRNA. A 2 μL droplet of the 10 nM fusion protein was placed on the APTES-mica surface. Equimolar amount of pncRNA to the fusion protein was added to the solution in the chamber. Tracking was started from time 0, while pncRNA was added earlier. Scanning area: 60 nm x 50 nm, pixel size: 72 pixels x 60 pixels, recording rate: 5 frame/s, playing rate: 10 frame/s. Blue and green dots are added to the movie using Igor Pro software to indicate BFP and GFP, respectively.



Supplementary Fig. S1: Length and height of the FUS fusion protein in the absence and presence of pncRNA. (a) Magnified HS-AFM image of the fusion protein in the absence of pncRNA. Frame rate: 0.2 s/frame and 55 × 55 pixels^2. (b) Height profile along the red line in the HS-AFM image in (a). (c) Magnified HS-AFM image of the fusion protein in the presence of equimolar amount of pncRNA. Frame rate: 0.2 s/frame and 60 × 60 pixels^2. *: another fusion protein. (d) Height profile along the red line in the HS-AFM image of (c).

Experimental Section

Protein preparation

The MBP-BFP-FUS-GFP-6xHis protein (fusion protein) was prepared as described in detail previously.[1] The essence of the preparation is followings. The fusion protein was expressed in BL21 (DE3) Gold Escherichia coli cells. The protein was induced by the addition of 0.1 mM isopropylthio-β-D-galactopyranoside (IPTG) for 20 hours at 20 °C. Cell pellets were sonicated in lysis buffer comprising 50 mM Tris-HCl, pH 7.6, 25 mM glucose, 1% CHAPS, 10 mM benzamidine, 5 U/mL DNase I, 1 mg/L RNase, and 0.2 g/L lysozyme. The supernatants were

purified by nickel-affinity column chromatography using Ni-sepharose beads (GE Healthcare Bio-Sciences) followed by size exclusion chromatography (SEC) using a HiloadTM 16/60 SuperdexTM 200 prep grade column (GE Life Sciences). Protein was stored at 4°C. 5 mM fresh dithiothreitol (DTT) was added to the purified fusion protein solution on the same day as the experiment.

Sample preparation for HS-AFM

Fusion protein was immobilized on a mica surface modified with 0.05% (3-aminopropyl) triethoxysilane (APTES) (Shin-Etsu Silicone, Tokyo, Japan). The mica surface was cleaved so as to have a clean and smooth surface in each experiment. After treatment with APTES, a droplet of the fusion protein was placed on the APTES-mica surface and incubated for 3 min. The remaining fusion protein molecules were removed by rinsing with measurement buffer (10 mM phosphate buffer, pH 6.8). After removing residual molecules, the sample stage was immersed in a chamber containing 70 μL of measurement buffer. For the observation in the presence of pncRNA, one- to fifteen-fold equimolar amount of pncRNA to the fusion protein placed on the APTES-mica surface was added to the solution in the chamber.

HS-AFM imaging

All HS-AFM experiments were performed using a laboratory-built high-speed atomic force microscopy (HS-AFM) [2–8] at room temperature. For HS-AFM measurement, small cantilevers (BL-AC7DS: Olympus, Tokyo, Japan) with a resonance frequency of approximately 0.8 MHz, a quality factor of ~2 and a spring constant of ~0.2 were used in the tapping mode. All properties of the small cantilevers were determined in liquid. To reduce the tip-sample loading force, the amplitude of cantilever oscillation was adjusted to 1-2 nm. Additionally, the set-point of amplitude for feedback control was set at ~90% of the free amplitude. Tracking as shown in Fig. 3a was performed for ten fusion molecules in the absence of pncRNA. Tracking as shown in Fig. 2a and 3c was performed for ten fusion molecules in the presence of pncRNA.

Image analysis

Image analysis were carried out using a laboratory-built software based on Igor Pro 8 (Wavemetrics, Inc., Lake Oswego, OR). All HS-AFM images were first processed by a Gaussian-filter smoothing and the background-tilt compensation using the first-order plane fitting. For the distance analysis between the BFP and GFP, the center position of BFP or GFP was independently determined by a tracking algorithm using two-dimensional correlation coefficient [2] and then the distance between the center posistions were calclated for each frame. To calculate the radius of gyration (R_g), we manually set the threshold to remove background and identify the regions of globular domains as shown by yellow-filed region. The center of mass to three globular domains was calculated based on the height distribution within each domain and the center position of each domain. Finally, the R_g was estimated using the distance between the center position of each domain and the center of mass for all domains.

All histograms are fitted with a normal Gaussian curve described as $f(x)=A\exp\{-(x-x_0)^2/2\sigma^2\}$, where x, x_0, σ^2 and A correspond to the measured distance, average, dispersion and a constant, respectively. The fitting was carried out using regression analysis built into IgorPro.

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