Electronic Supplementary Material (ESI) for RSC Advances.

SUPPLEMENTARY INFORMATION

Imaging a specific mRNA in pollen with atomic force microscopy

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Inventory of Supplementary Information:

SUPPLEMENTAL TEXT

Model system III: The interaction between the DNA probe and the 1,515-nucleotide cRNA embedded in a polymer matrix

To measure the force value in a condition similar to the sectioned pollen surface, the RNA was dissolved in LR White resin, and the mixture was cast into a block. Sections of 150 nm thickness were prepared with an ultra AFM knife and placed on a glass slide. The DNA probe complementary to the sequence between nucleotides 944–977 of the *AtMAT1;4* mRNA was immobilized on the 27-acid dendron–modified AFM tip. The 1,515-nucleotide of the *AtAMT1;4* was synthesized *in vitro* from the cDNA template of *AtAMT1;4*.

First, low-resolution force maps of the sections were obtained (**Fig. S2B**). A flat and smooth area (10 μm × 10 μm) was selected for AFM topographic imaging, and then the force curves were recorded at an interval of 1.0 μm (a total of 100 pixels). The mean force value was obtained from 30 measurements per pixel. The interaction force values were categorized into five groups, and the same coloring system as that in the main text was used. The unbinding-force value for a curve with multiple unbinding events was calculated from the last rupture event, and was incorporated into the force histogram. The most probable unbinding-force observed was 45 ± 1 pN (n = 266/400; σ = 6). As the RNA concentration decreased, the number of pixels colored yellow and light yellow also decreased.

High-resolution force mapping was performed at 10 nm intervals (**Fig. S2C**). Again, the most probable unbinding-force value of 44 ± 1 pN (n = 182/400; σ = 4)

was obtained, and the number of specific pixels decreased at low concentrations. Even at 6×10^3 molecules/ml, relatively high number of RNA was detected in the area (100 nm × 100 nm). This result indicates that the RNA molecules may not be distributed evenly in the resin, and maps of multiple sites should be obtained to correlate with the concentration. Although the number of specific pixels was not linearly related to the RNA concentration in this system, the result supports that the 34-mer DNA probe was able to hybridize with the embedded *AtAMT1;4* mRNA. To confirm the specificity, the interaction was recorded for the antisense RNA in the resin (**Fig. S2D**). The most probable unbinding-force observed was 33 ± 1 pN (n = 34/400; σ = 4). Number of the specific pixels dramatically decreased in the force map, whereas the yellow colored pixels were still present. It is noteworthy that the number of yellow pixels is not correlated with the concentration. This observation is reminiscent of the background in the maps for the pollen sample.

SUPPLEMENTAL FIGURES

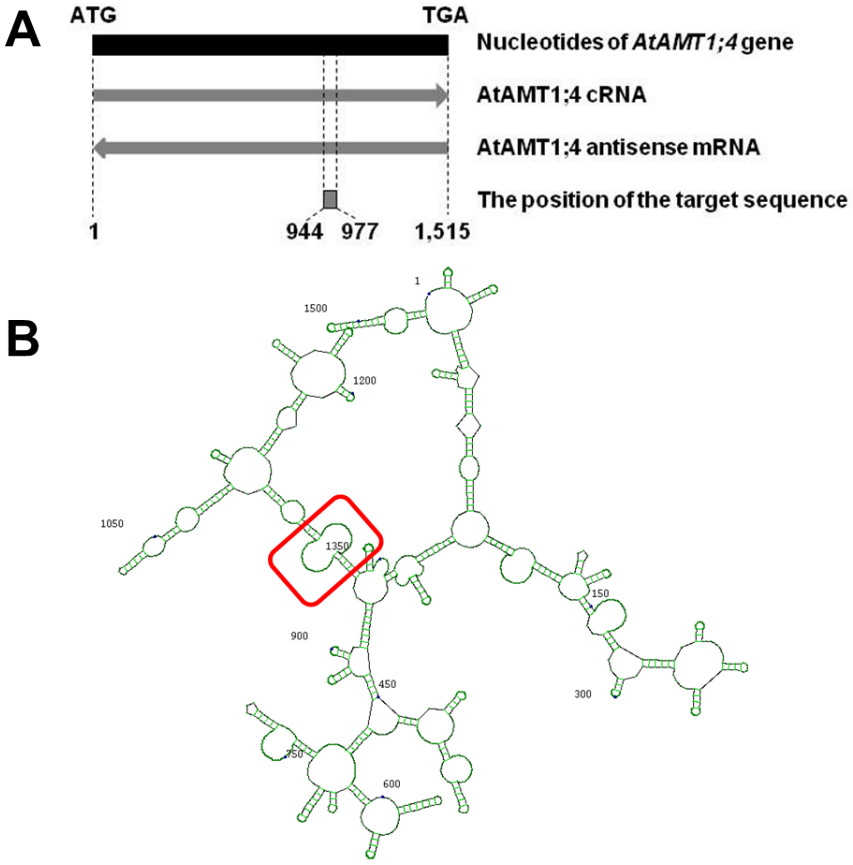

Fig. S1. The location of the DNA probe sequence in the predicted secondary structure of *AtAMT1;4* mRNA. **(A)** Position of the target region in the open reading frame of *AtAMT1;4* mRNA. **(B)** A possible secondary structure of *AtAMT1;4* mRNA. The red box shows the detection part of the *AtAMT1;4* mRNA (5′-AA AGA CUU AUU GAU GGG UAU UGG AAU GUA ACU GA-3′) with the 34-mer DNA (5′-TC AGT TAC ATT CCA ATA CCC ATC AAT AAG TCT TT-3′) probe.

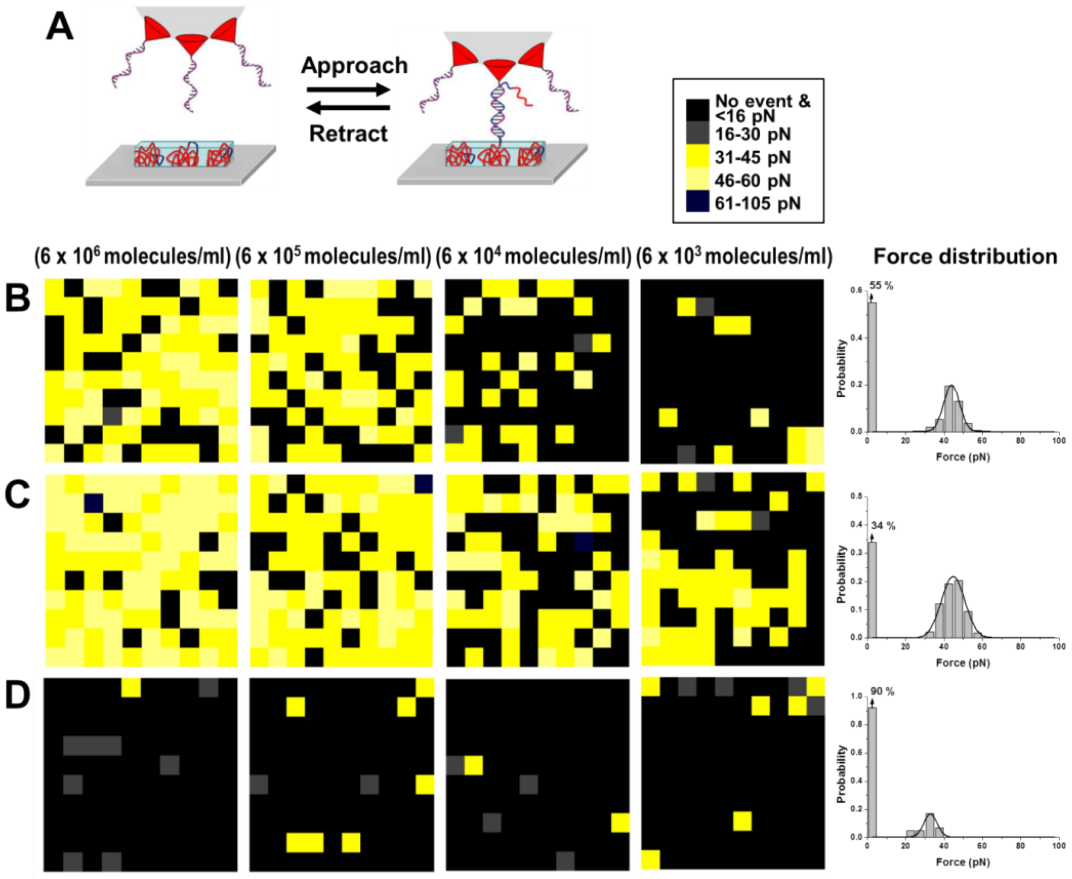

Fig. S2. Mapping the distribution of the *AtAMT1;4* cRNA in cRNA-embedded resin sections. **(A)** Schematic drawing of the experimental setup employed for measurement of the interaction between the 34-mer DNA probe and the 1,515-nucleotide cRNA embedded in LR White resin. **(B-D)** Force maps of *AtAMT1;4* cRNA at various concentrations. **(B)** Low-resolution maps (1.0 μm interval), **(C)** high-resolution maps (10 nm interval), and **(D)** low-resolution maps for the antisense RNA embedded in resin (1.0 μm interval).

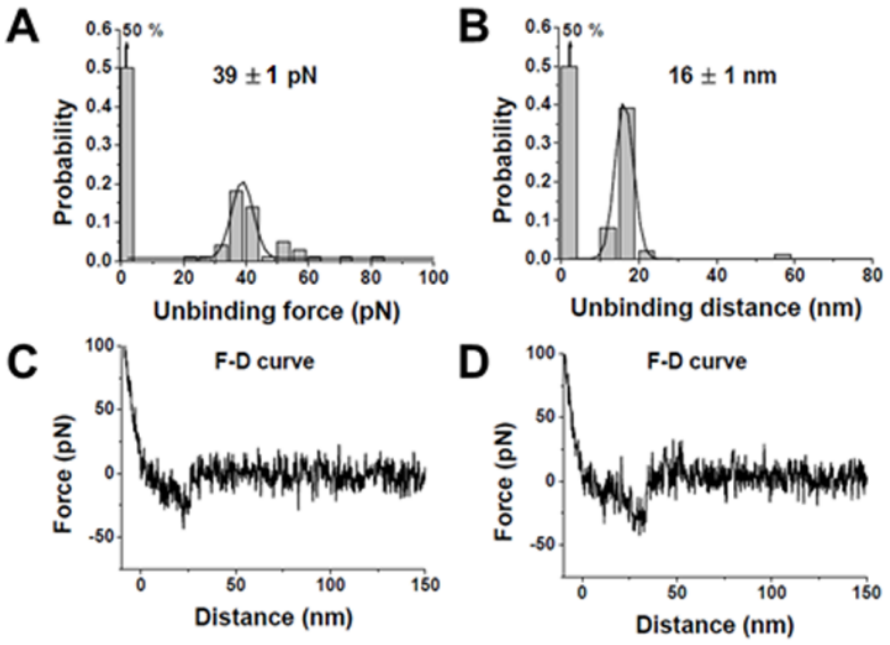

Fig. S3. Histograms for the specific interaction between the 1,515-nucleotide *AtAMT1;4* mRNA and the DNA probe on the sectioned pollen surface, and the representative force-distance curves. **(A)** The adhesion force histogram corresponding to the DNA-mRNA interaction on the sectioned pollen surface (the percentage value next to the *y*-axis represents the probability of no event). Gaussian fitting gave the most probable value of 39 ± 1 pN. **(B)** The stretching-distance histogram; Gaussian fitting gave the most probable value of 16 ± 1 nm. **(C** and **D)** The representative force-distance curves in this experiment.

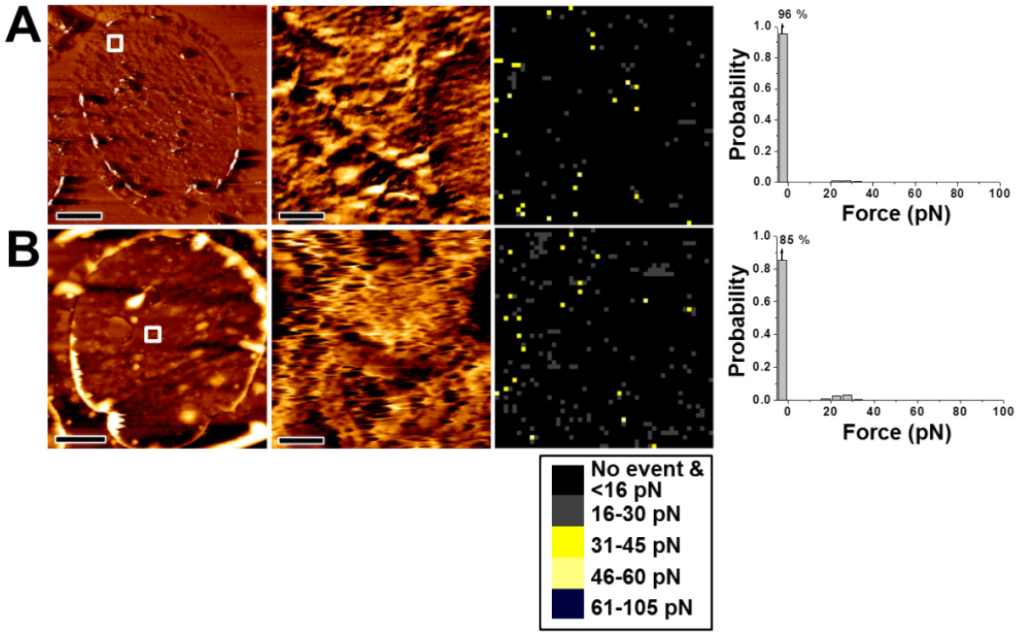

Fig. S4. Force maps and force distribution of *AtAMT1;4* mRNA from the control experiments. Images in the first column are low-resolution topographic images of *Arabidopsis* pollen sections (scale bars, 5.0 μm). White boxes represent the scanned area for the force mapping (20 nm interval) and the enhanced topographic images (scale bars, 200 nm). **(A-B)** After blocking with the antisense RNA **(A)** and after treating with RNase **(B)**.

SUPPLEMENTAL METHODS

Cleaning the substrates and AFM probes

Silicon substrates and fused silica (for dendron surface coverage analysis; data not shown) were cleaned in a Piranha solution by sonication [concentrated H_2SO_4: 30% H_2O_2, 7:3 (v/v)] for 4 h (*Piranha solution is very strongly oxidizing and should be handled with extreme caution*). The substrates were then washed thoroughly with deionized water. And these substrates were immersed in an aqueous cleaning solution; a mixture of deionized water, a concentrated ammonia solution, and 30 % hydrogen peroxide [5:1:1 (v/v/v)] in a Teflon beaker. The beaker was heated at 80 °C for 10 min. The substrates were taken out of the cleaning solution and rinsed carefully with deionized water. Silicon nitride AFM probes were dipped in the nitric acid solution [nitric acid:deionized water, 5:1 (v/v)] and then heated at 80°C during 20 min. The AFM probes were taken out from the solution and washed carefully with deionized water. The cleaned silicon substrates and AFM probes were put in a vacuum chamber (30–40 mTorr) for approximately 20 min and used immediately for the silylation.

Deprotection of carboanthrylmethoxy group

The dendron-modified substrates and AFM probes were stirred for 2 h in a methylene chloride solution dissolving trifluoroacetic acid (TFA) (1.0 M). After the deprotection reaction, they were immersed in a methylene chloride solution with 20% (v/v) diisopropylethylamine (DIPEA) for 10 min. The substrates were cleaned by sonication in methylene chloride and methanol each for 3 min sequentially. The AFM probes were rinsed carefully with methylene chloride and methanol for 3 min sequentially. Finally, the substrates and AFM probes were placed in a vacuum (30–40 mTorr).

Preparing NHS-modified substrates

The above deprotected substrates and AFM probes were dipped in an

acetonitrile solution containing di(N-succinimidyl) carbonate (DSC) (25 mM) and DIPEA (1.0 mM) for 4 h at nitrogen atmosphere. After the reaction, the substrates and AFM probes were placed in DMF with stirring for 30 min and rinsed with methanol. Finally, the substrates and AFM probes were placed in a vacuum (30–40 mTorr).