Supplementary information

Engineering ssRNA tile filaments for (dis)assembly and membrane binding

Nicola De Franceschi¹, Baukje Hoogenberg¹, Allard Katan¹ and Cees Dekker^{1,*}

¹ Department of Bionanoscience, Kavli Institute of Nanoscience Delft, Delft University of Technology, Delft,

The Netherlands

*Corresponding author: c.dekker@tudelft.nl

Table of contents

Supplementary Figure 1: Tiles T1 assembly into filaments
Supplementary Figure 2: Images used for filament length quantification
Supplementary Figure 3: tile binding to membrane via cholesterol or aptamer anchors
Supplementary Figure 4: T-cap design and Helicase binding
Supplementary Figure 5: isothermal tile refolding



Supplementary Figure 1: Tiles T1 assembly into filaments (**a**) Quantification of filament height by AFM line scan analysis. A Gaussian fit is indicated by the black line. (**b**) Tiles T1 run on agarose gel in the presence of 10 mM MgCl₂. Tiles T1 assemble in filaments that are too large to enter the gel and remain stuck in the loading well. (**c**) Tiles T1 run on agarose gel in the absence of MgCl₂.and in presence of 5 mM EDTA. Tiles T1 remain monomeric. This gel also shows the purity of the product obtained by *in vitro* transcription.



Supplementary Figure 2: Images used for filament length quantification. These images are representative AFM scans of (a) Filaments T1, (b) Filaments T2 and (c) Filaments T3. The automatic segmentation is shown as yellow traces on top of the filaments.



Supplementary Figure 3: tile binding to membrane via cholesterol or aptamer anchors. (a) Example of imaging of tiles attached to the membrane via cholesterol-oligo hybridized to the tiles. Tiles are detached by the mechanical action of the AFM tip. (b) Example of imaging of tiles attached to biotinylated lipids via biotin-aptamers that are part of the tile sequence itself.

4



а

Supplementary Figure 4: T-cap design and Helicase binding. (a) Design of T-cap. (b) Helicase Hel308 binding to tile filaments. White blobs correspond to individual helicase molecules bound to the filaments. The panel on the right shows a higher magnification. The height profile across the blue line is shown in the bottom panel.



Supplementary Figure 5: isothermal tile refolding. 500nM of T2 tiles were incubated for 5 minutes in buffer supplemented with 12.5mM MgCl₂ (left panel) or 5mM EDTA (right panel) and subsequently deposited on mica for AFM imaging. Spontaneous isothermal tile refolding, resulting in formation of filaments, is visible in the presence of MgCl₂. In the presence of EDTA the tiles remained unfolded due to self-repulsion of the ssRNA strand, resulting in the absence of filaments.