SUPPLEMENTARY INFORMATION

An RNA origami robot that traps and releases a fluorescent aptamer

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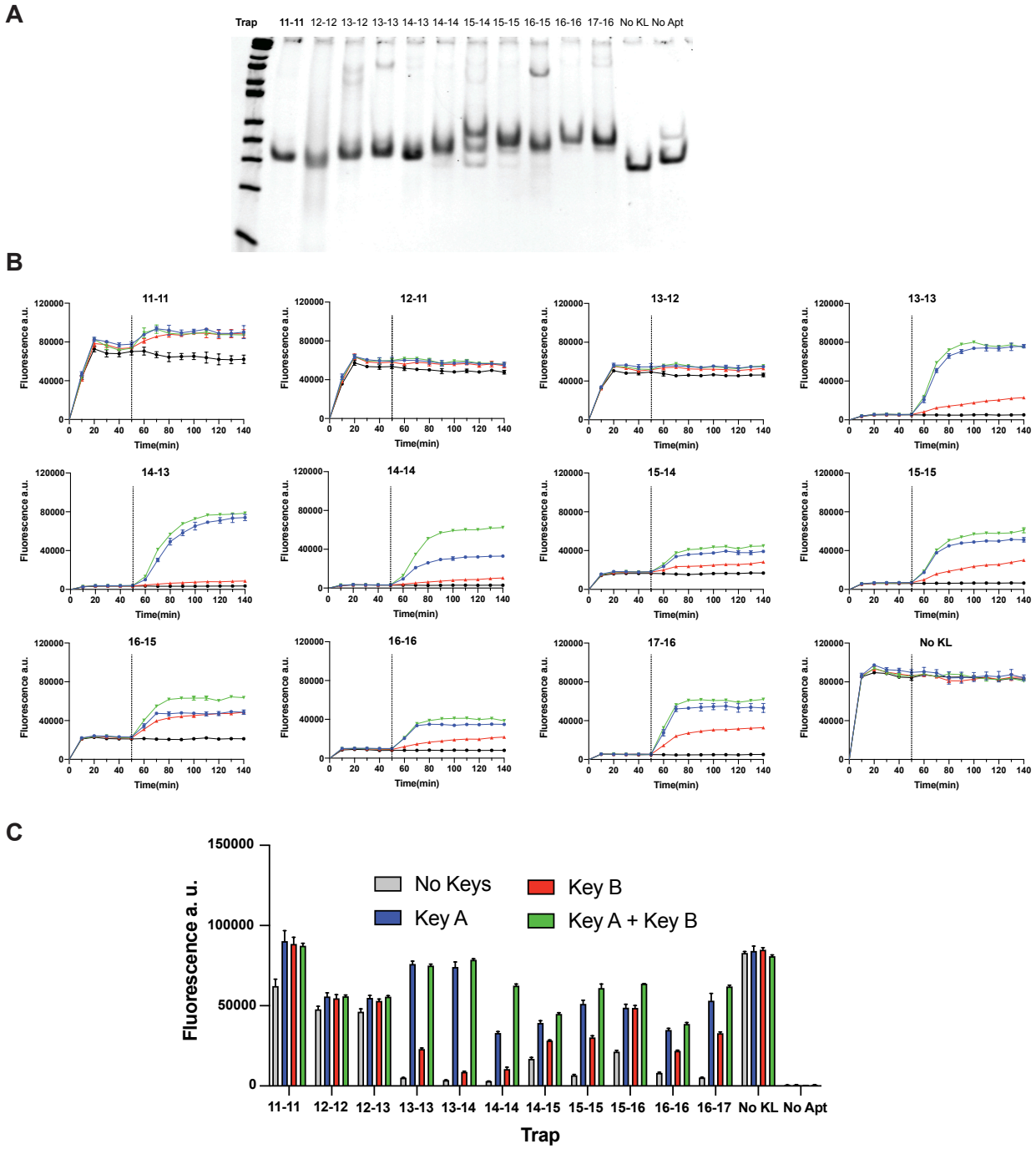

Figure S1. Design screening for a co-transcriptionally folded RNA mechanical trap.
A. 8% Native PAGE gel showing the state of the different designs after SEC purification. **B.** Design screening. Monitored fluorescence over time of the different designs. The different RNAs were produced and purified to a final concentration of 100 nM. The fluorophore DFHBI-1T (1 µM) was added after the first measurement and 5X (500 nM) single stranded RNA keys were added after 50 minutes (black dashed line). Data corresponds to 3 technical replicates, error bars represent mean ± SD. **C**. Fluorescence observed at 140 mins (85 minutes upon addition og the RNA keys) for the different designs.

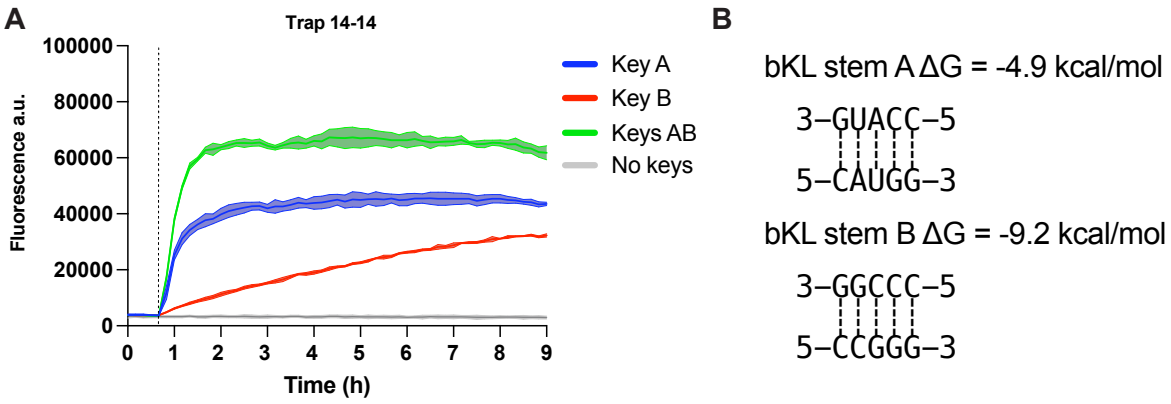

Figure S2. Opening of Trap-14-14 and stabilities over extended time period.
A. Monitored fluorescence over 9 hours of trap 14-14 (100 nM) in solution with DFHBI-1T (500 nM) after addition of RNA keys (500 nm) at RT (dashed line indicates key addition). Data represents 3 technical replicates ± SD. **B.** Free energies of the bKL stems calculated with Nupack.

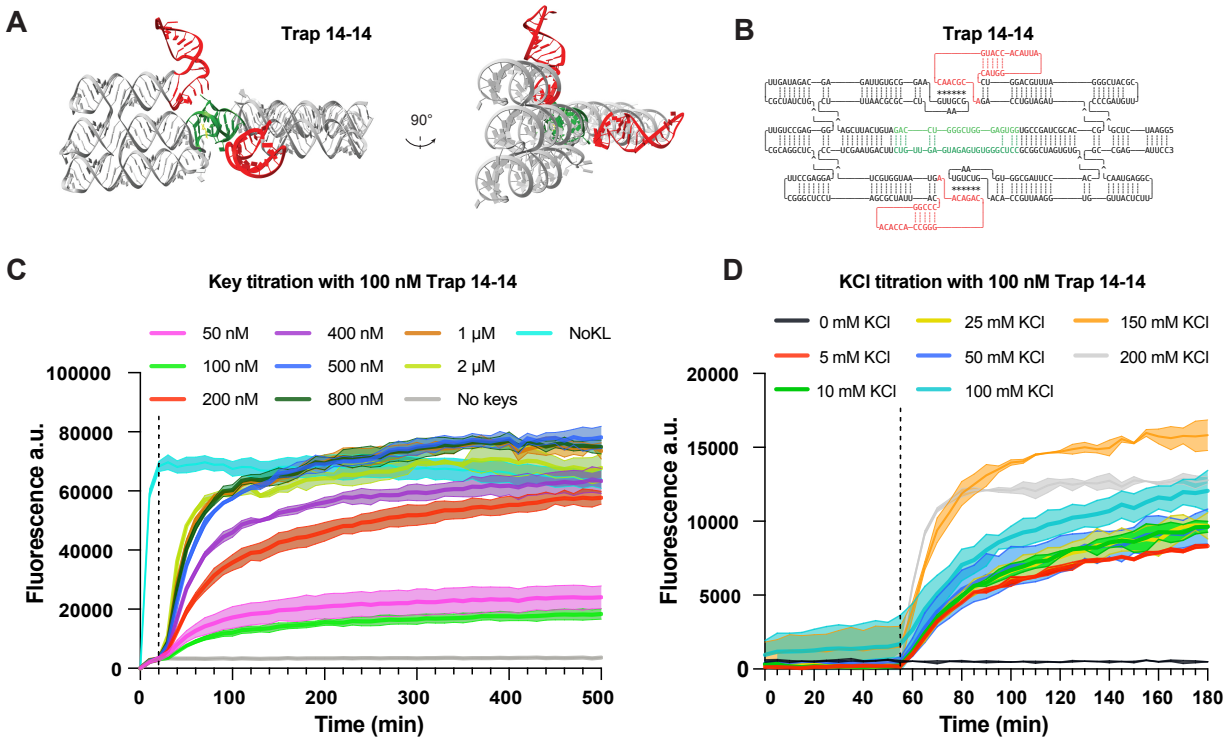

Figure S3. Model and properties of the Trap-14-14 design.
A. Computer-generated 3D model with RNAbuild [1]. The iSpinach aptamer is depicted in green, bKLs in red and DFHBI-1T ligand in yellow. **B.** RNA sequence text blueprint. The iSpinach motif is highlighted in green and the bKLs in red. **C.** ssRNA key titration. Monitored fluorescence over time of Trap-14-14 RNA (100 nM) in complex with DFHBI-1T (1 µM), different concentrations of ssRNAs were added after the first fluorescence measurement. The No kissing loops (NoKL) positive control is shown in cyan blue. Data corresponds to 3 technical replicates, error bars represent mean ± SD. **D.** KCl titration. Monitored fluorescence over time of Trap-14-14 (100 nM) in buffers with different KCl concentrations. Data corresponds to 3 technical replicates, error bars represent mean ± SD.

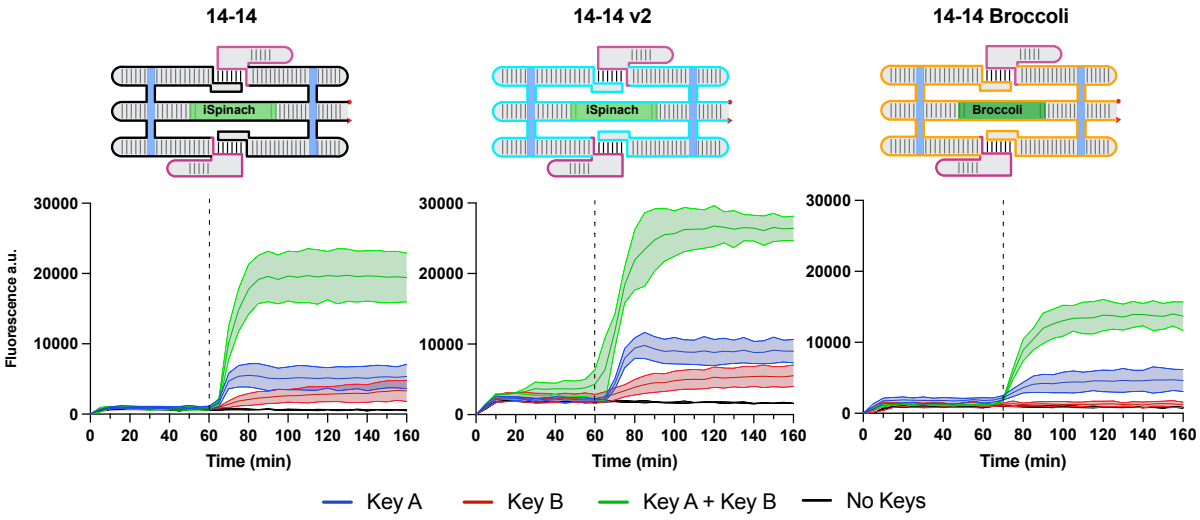

Figure S4. Function of Trap 14-14 with different sequence designs and Broccoli aptamer.

Observed fluorescence for the 14-14 design variants over time. 14-14 v2 has a different scaffold sequence (blue line). 14-14 Broccoli has the Broccoli aptamer and a different scaffold sequence (orange line). DFHBI-1T (500 nM) keys (500 nM) were added to 100 nM RNA after 60 minutes (dashed line). Data corresponds to 3 technical replicates; error bars represent mean ± SD.

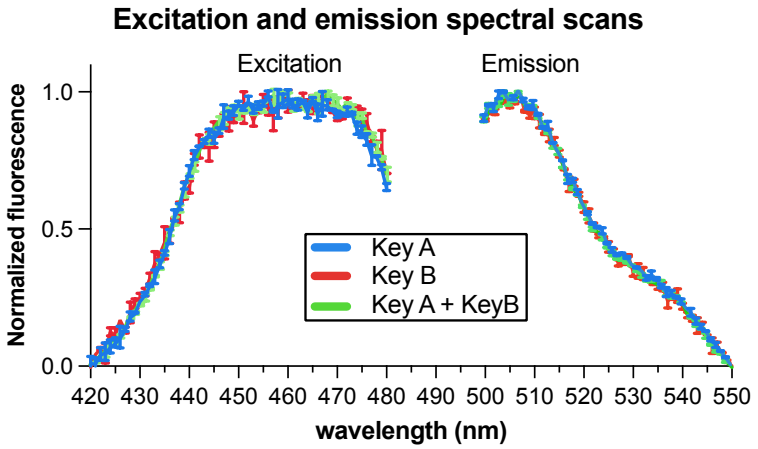

Figure S5. No effect of key addition on excitation and emission spectra.
Excitation and emission spectra of DFHBI-1T (500 nM) in complex with the Trap 14-14 (100 nM) at different key conditions (500 nM).

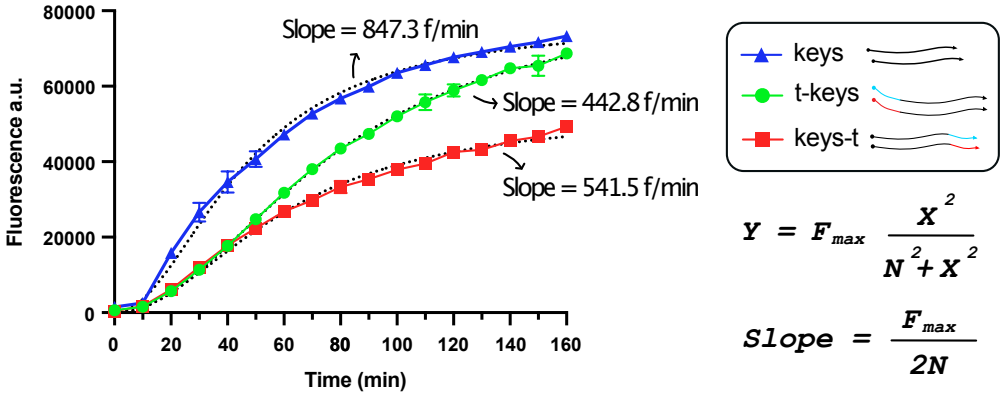

Figure S6. Effect of adding toeholds the ends of RNA keys.
Fluorescence activation of 100 nM Trap 14-14 after addition of keys (500 nM) with no toehold or toehold placed at 5'- (t-keys) or 3'- (keys-t) ends (500 nM). Data represents 3 technical replicates ± SD. F_{max} represents the maximum fluorescence and *N* represents the inflection point, i.e., time point (x) at which fluorescence has its maximum increase. Model fit $R^2 = 0.993$ for keys, $R^2 = 0.998$ for t-keys and $R^2 = 0.993$ for keys-t.

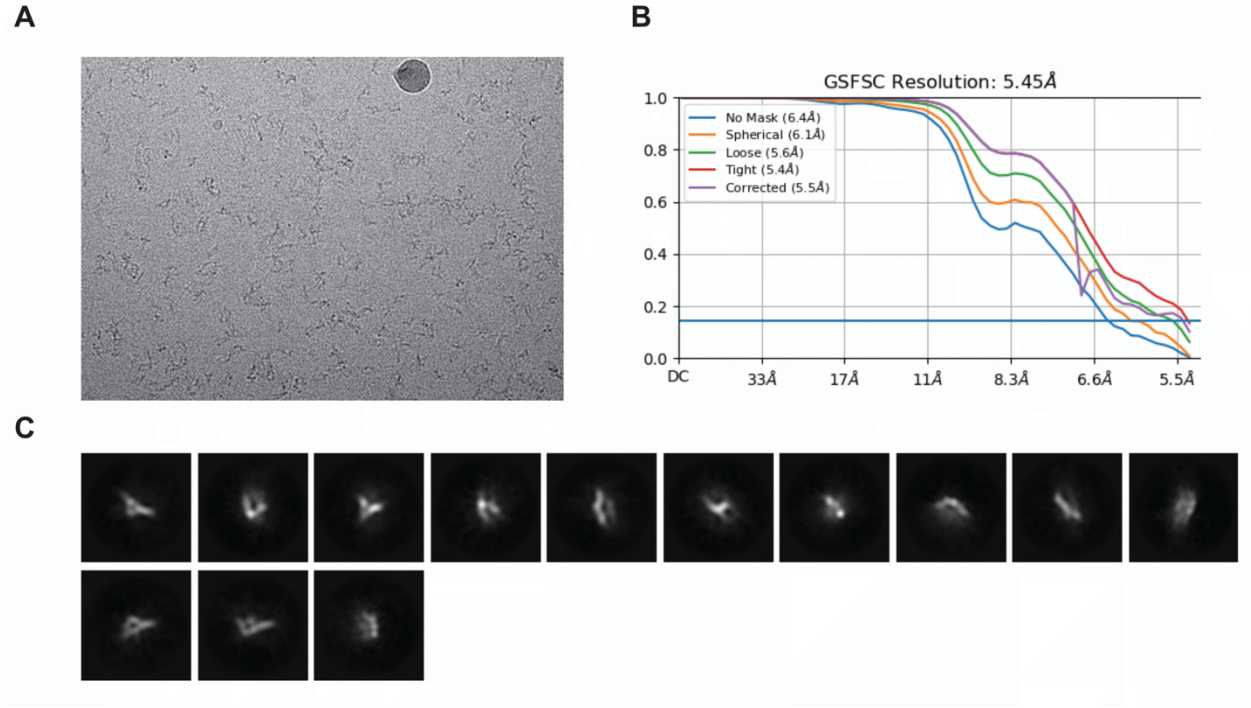

Figure S7. Cryo-EM analysis of Trap-14-14 with no keys.
A. Example cryo-EM micrograph from the locked Trap-14-14 dataset. **B.** Gold-standard Fourier Shell Correlation for the locked state of Trap-14-14. **C.** 2D Class. Averages from the final particle stack of the locked Trap-14-14 dataset.

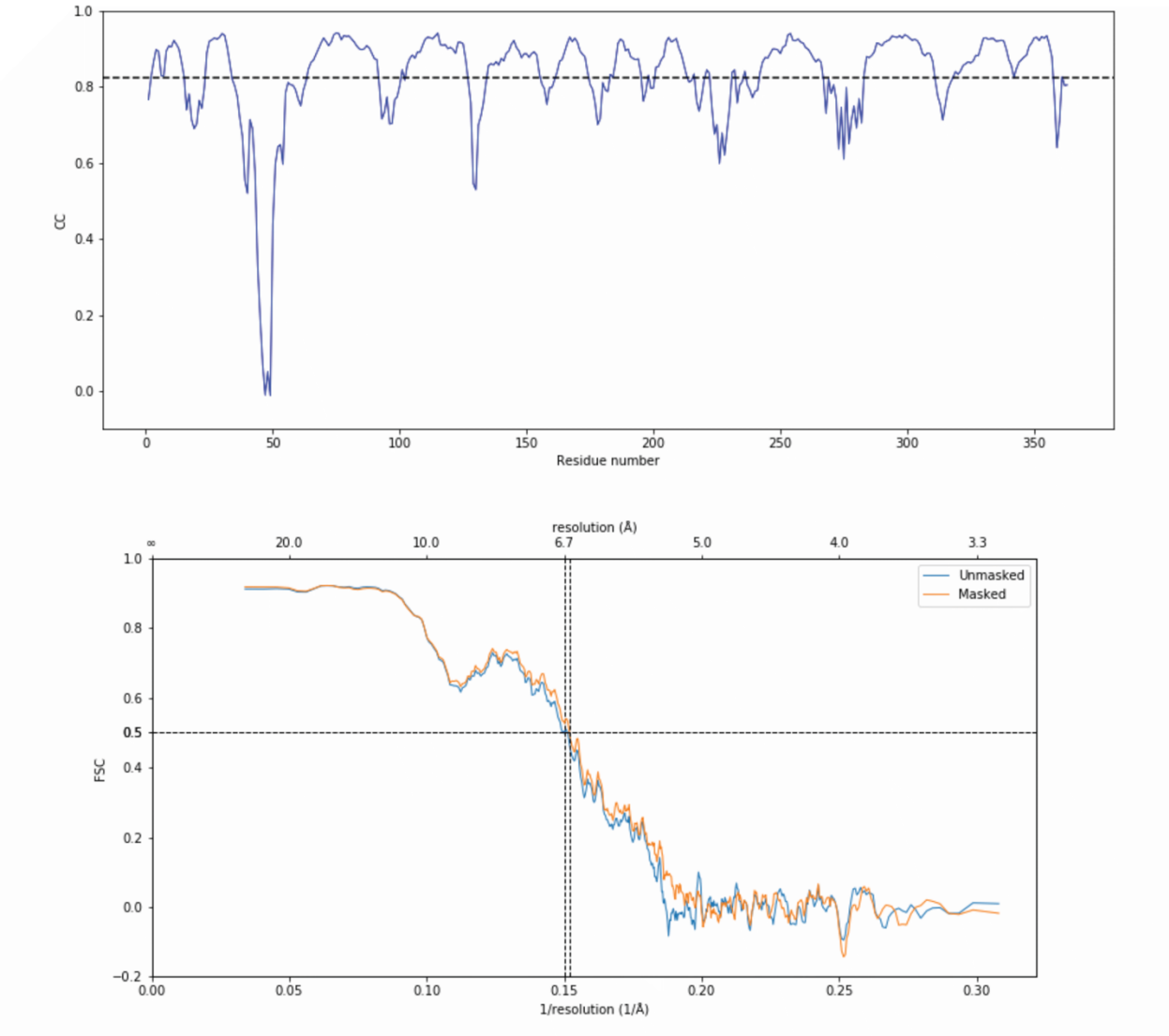

Figure S8. Cross Correlation and Fourier Shell Correlation.
Per residue Cross Correlation (top) and Fourier Shell Correlation (bottom) between atomic model and cryo-EM map for the trap 14-14 with no keys after phenix RSR.

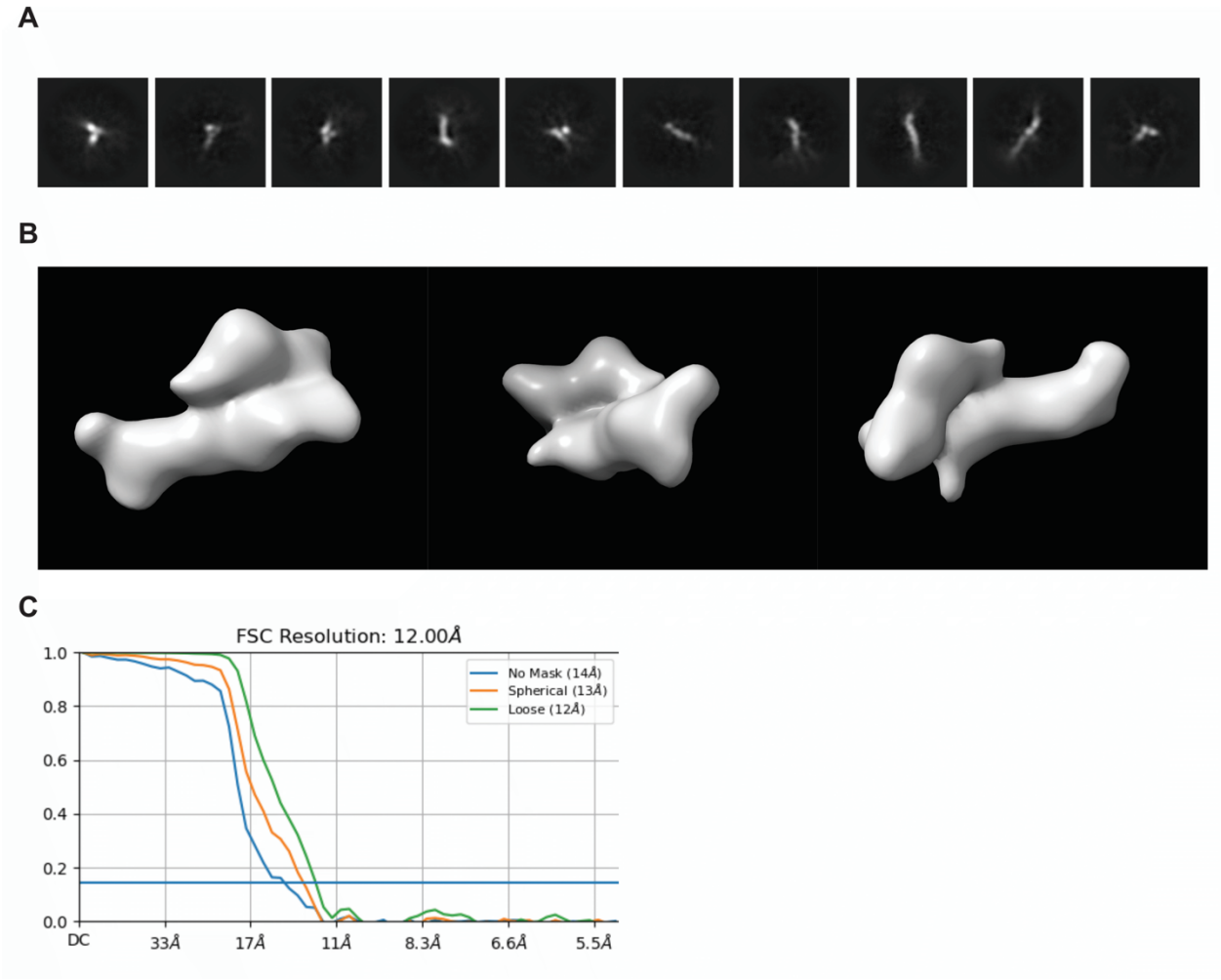

Figure S9. Cryo-EM analysis of Trap-14-14 upon addition of keys.
A. 2D Class averages from the. Final particle stack of the open Trap-14-14. dataset. **B.** Three views of the Trap-14-14 device with keys reconstruction. **C.** Gold-Standard Fourier Shell Correlation for the open state of Trap-14-14.

Table S1. RNA blueprints and sequences

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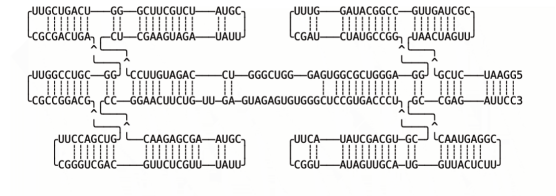
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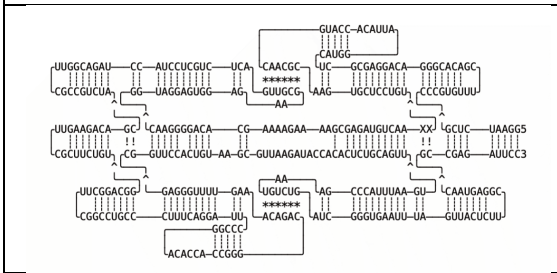
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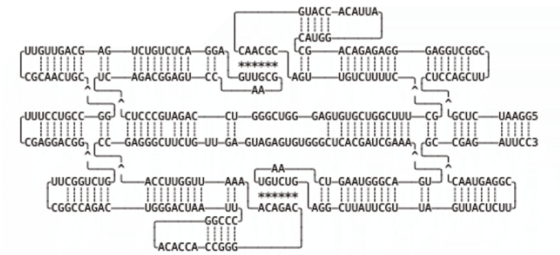
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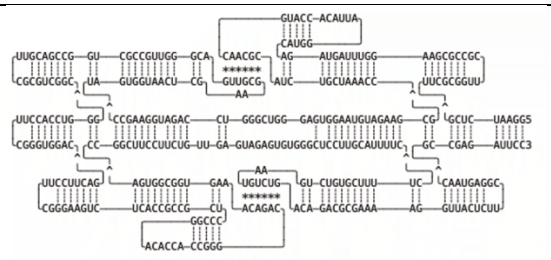

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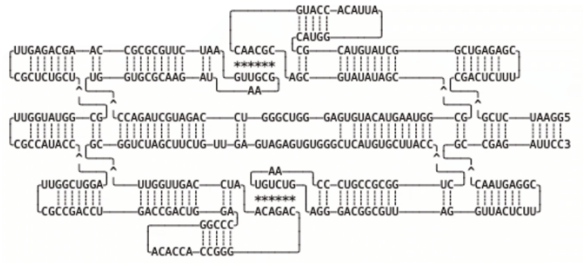

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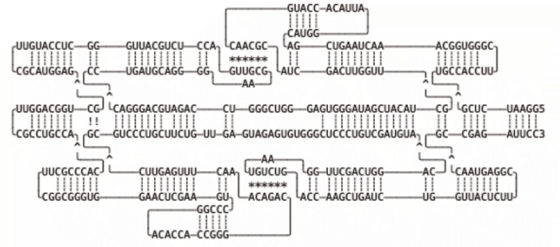

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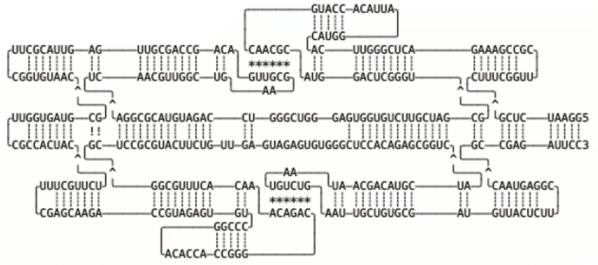

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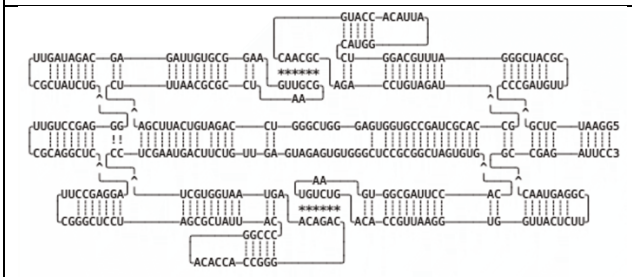

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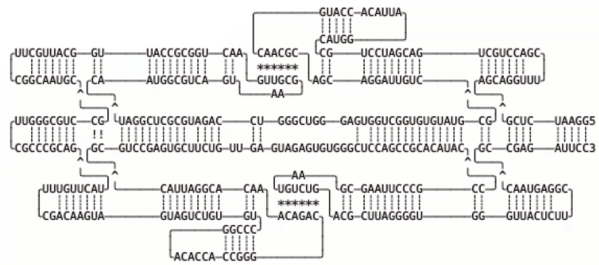

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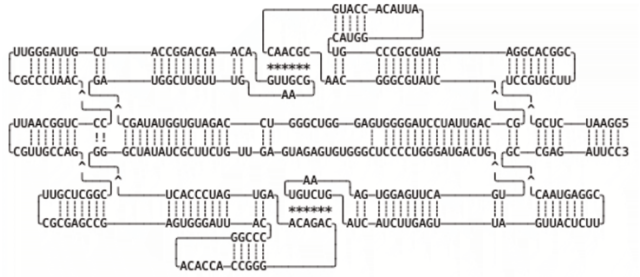

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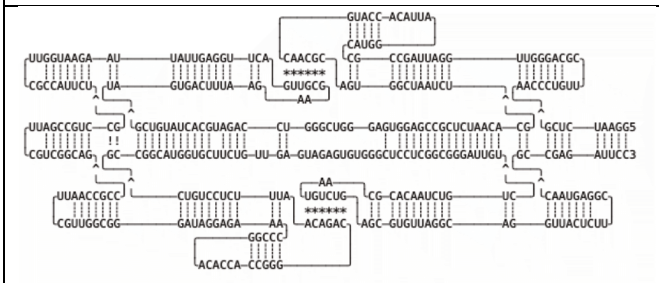

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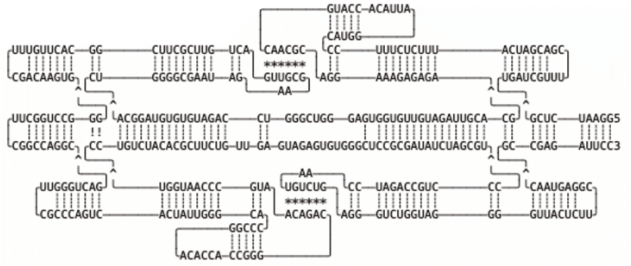

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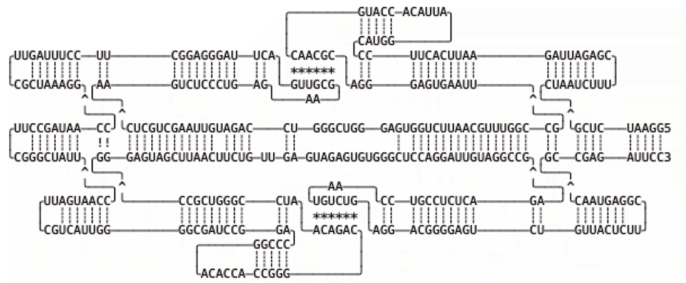

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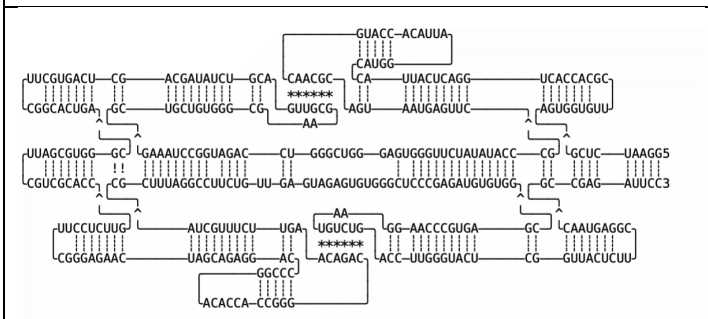

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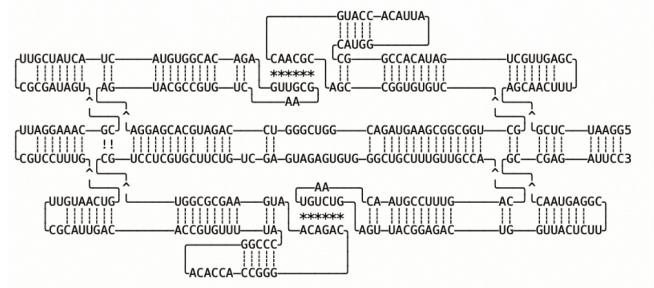

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Table S2. PCR primers and RNA keys.

PCR Primers (5’ to 3’)

Forward
CAGACTTCTTACGTGATCCATCCG
Reverse
GGAATCTCGGCGTTACTCCG

RNA keys (5’ to 3’)

Key A
GCGUUGCAUGGUGUAAU
Key B
UGUCUGCCCGGUGGUGU
Key C
AACCUAACUCAAUCUCU
Key D
UAACAAAACAGACAAAG
Key A with 3’ toehold
GCGUUGCAUGGUGUAAU-ACGUCG
Key B with 3’ toehold
UGUCUGCCCGGUGGUGU-UGAAGC
Anti-key t-A*
CGACGUAUUACACCAUGCAACGC
Anti-key t-B*
GCUUCAACACCACCGGGCAGACA
Key A with 5’ toehold
GUCCAC-GCGUUGCAUGGUGUAAU
Key B with 5’ toehold
CGAAGU-UGUCUGCCCGGUGGUGU
Negative control invader E
CUGCACAGAACGGGAUUCUUUCA
Negative control invader F
GGGAAGGUGUUUGCUGGGAGUGA

References

1. Geary, C., et al., *RNA origami design tools enable cotranscriptional folding of kilobase-sized nanoscaffolds.* Nature Chemistry, 2021. **13**(6): p. 549-558.