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Supporting Information

DNA Origami Seesaws as Comparative Binding Assay

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DNA Origami Design

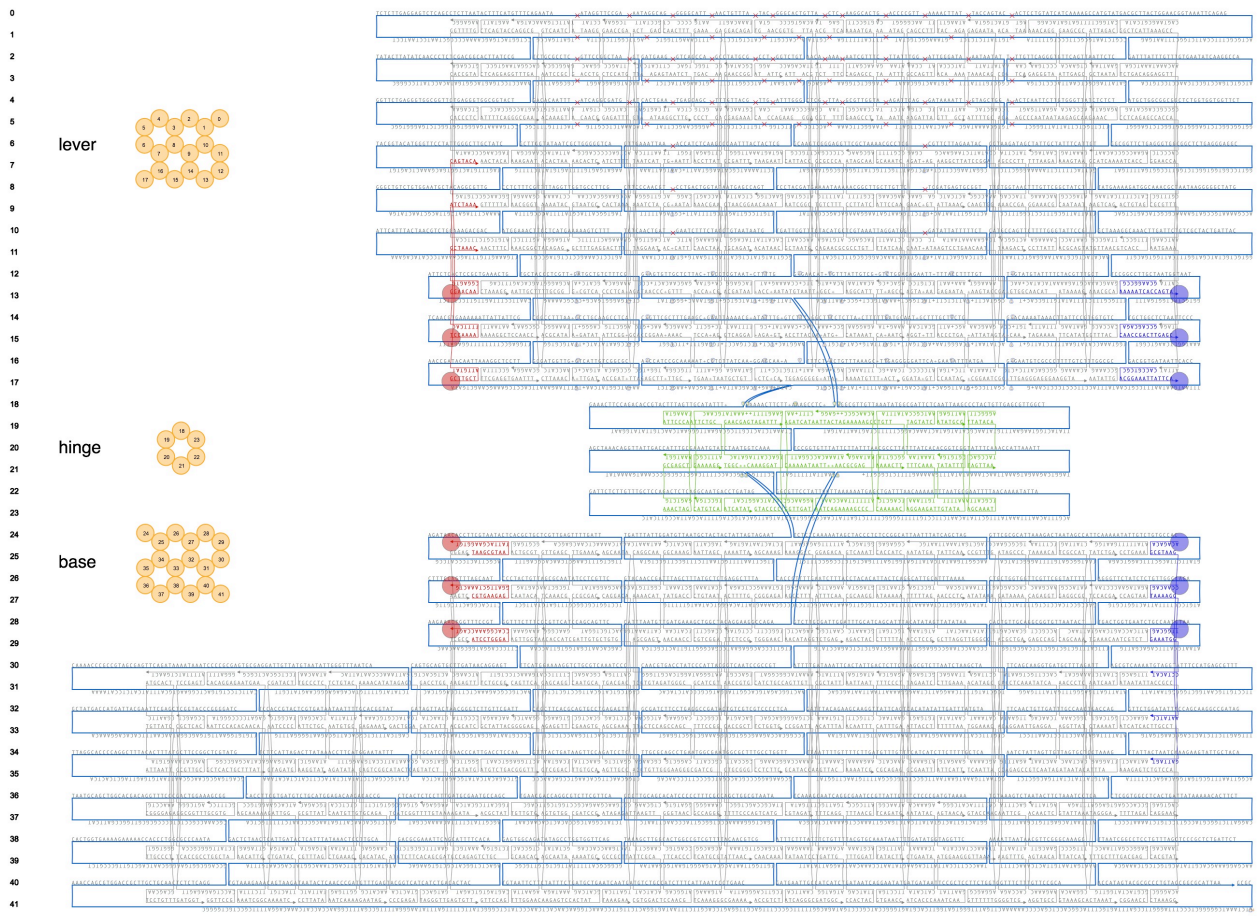


Figure S1: Design schematics of the seesaw structure. The extension positions are highlighted by red (left) and blue (right) circles. The staples forming the hinge are coloured green. Base deletions are marked by red crosses and base insertions are marked by blue loops.

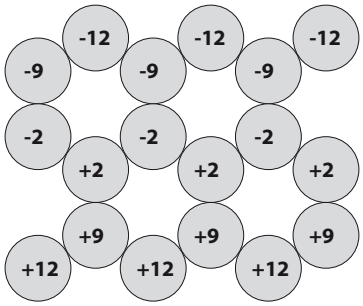


Figure S2: Number of deleted and inserted bases in a region of 98 base pairs to achieve the 60° bending of the lever.

Sequences of ssDNA Extensions

name	left	right
20 nt: lever 20 nt: base	5′-TACGAGTCCATGGGATCTGA-staple-3′ 3′-ATGCTCAGGTACCCTAGACT-staple-5′	5′-staple-AGTCTAGGGTACCTGAGCAT-3′ 3′-staple-TCAGATCCCATGGACTCGTA-5′
15vs30: lever 15vs30: base input:	5′-GTCCATGGGATCTGA-staple-3′ 3′-CAGGTACCCTAGACT-staple-5′	5′-staple-GACCTCTAGCTCCTTTACAAAGTACAGGTT-3′ 3′-staple-CTGGAGATCGAGGAAATGTTTCATGTCCAACCTGATAG-5′ 5′-GACCTCTAGCTCCTTTACAAAGTACAGGTTGGACTATC-3′

Theoretical prediction of expected distribution

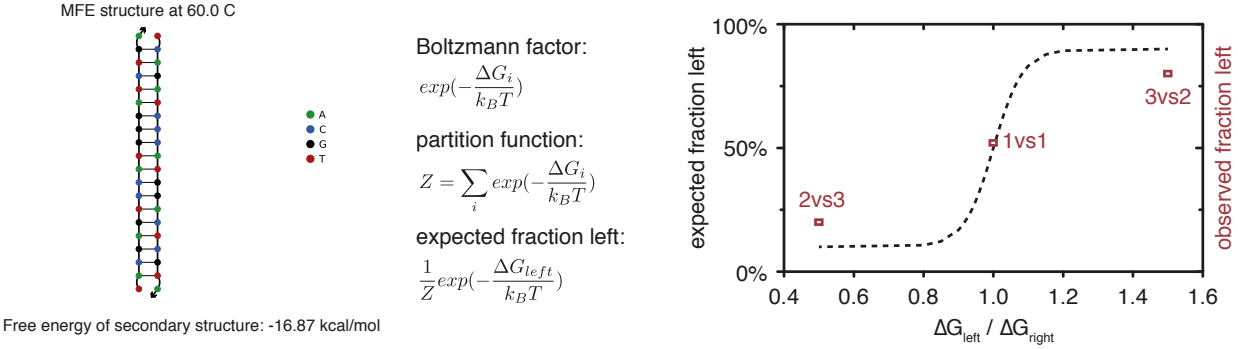


Figure S3: The Gibbs free energy ΔG for the 20 basepair duplex formed by the ssDNA extensions was calculated for 60°C (calculated T_M) using the NUPACK web server. These values were used to assign a Boltzmann factor for the states *left* and *right.* The expected fraction of structures in the *left* state was calculated for ratios of $\Delta G_{left}/\Delta G_{right}$ ranging between 0.5 (corresponds to the 2vs3 sample) and 1.5 (corresponds to the 3vs2 sample). The calculated expected fraction was then normalized to the observed values for the minimum (0vs3 sample) and maximum (3vs0 sample) concentration of *left* structures and plotted (dashed line).

Thermal Annealing Protocol

temperature [°C]	time [s]	time [min]
65	120	2
64	180	3
63	180	3
62	180	3
61	180	3
60	900	15
59	900	15
58	1800	30
57	2700	45
56	3600	60
55	4500	75
54	5400	90
53	5400	90
52	5400	90
51	5400	90
50	5400	90
49	5400	90
48	5400	90
47	5400	90
46	5400	90
45	5400	90
44	4500	75
43	3600	60
42	2700	45
41	1800	30
40	1800	30
39	1800	30
38	900	15
37	900	15
36	480	8
35	480	8
34	480	8
33	480	8
32	480	8
31	480	8
30	480	8
29	120	2
28	120	2
27	120	2
26	120	2
25	120	2

Agarose gel analysis

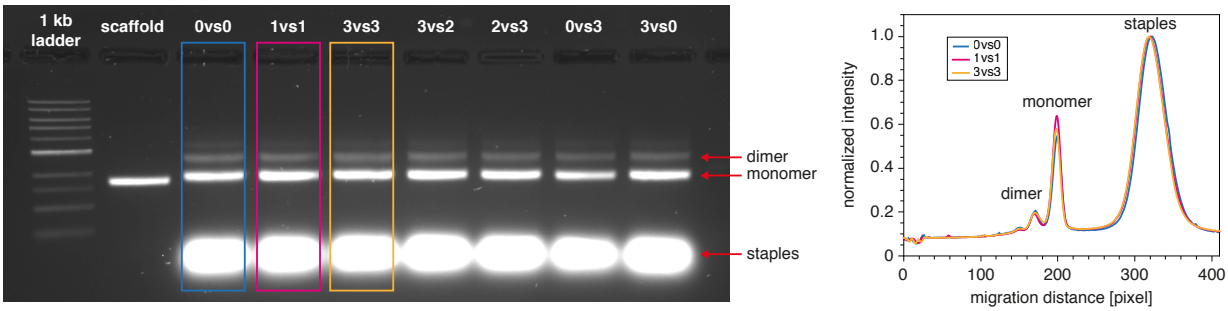
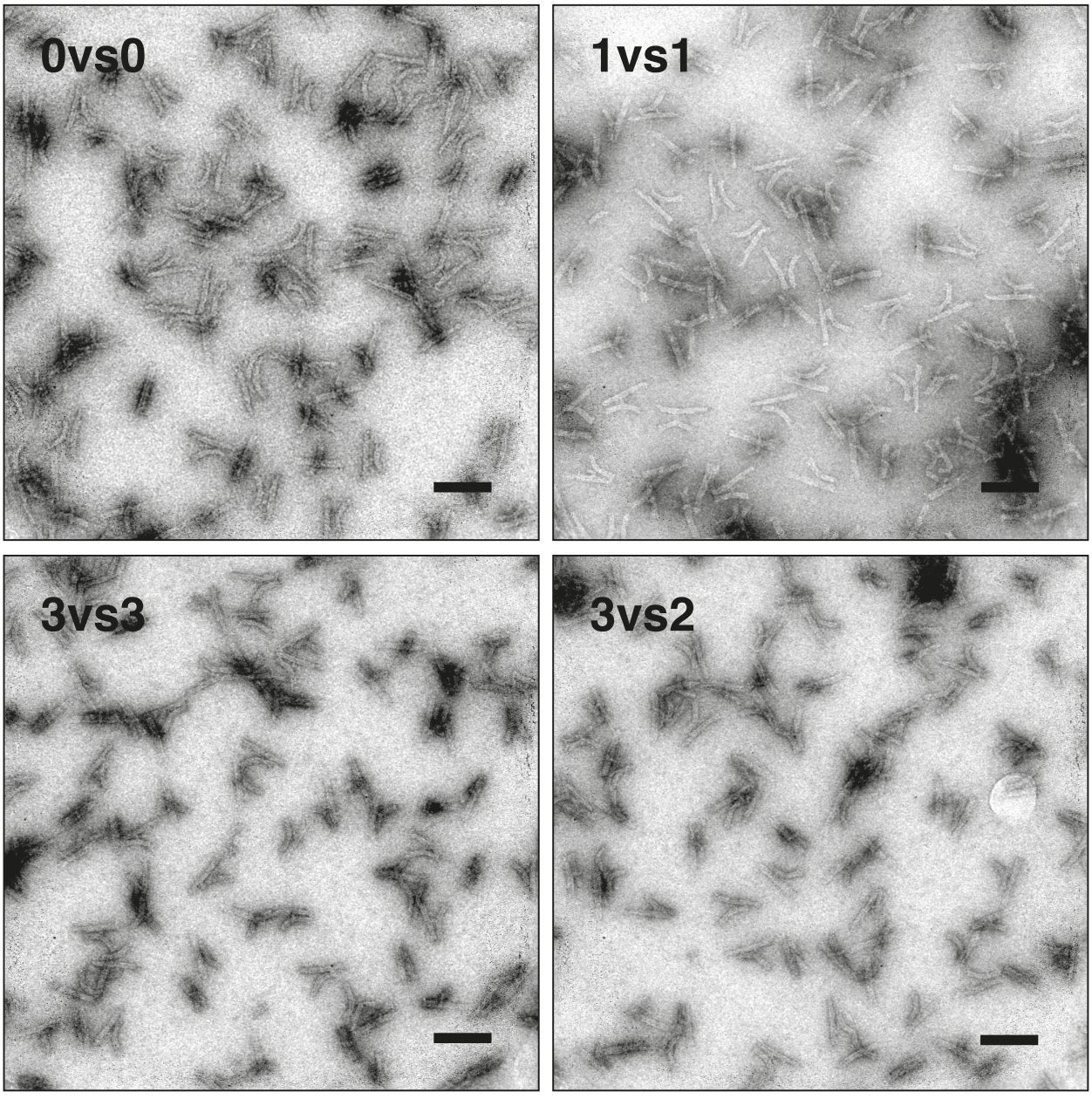


Figure S4: Agarose gel analysis of the folded samples from figure 2 (1% agarose gel, 1xTAE buffer + 11mM $MgCl_2$, run for 2 hours at 5.5 V/cm, cooled in ice water bath, stained with SybrSafe). A small fraction of the folded structures form dimers. This dimer formation is most likely unspecific and a result from base stacking. There is no significant increase in dimerization from specific intermolecular binding after closing of the structure via intramolecular binding. A comparison of the intensity profile of the 0vs0 (blue) sample (no specific intermolecular dimerization) with the intensity profile of the 1vs1 (magenta) and 3vs3 (orange) samples shows that there is no difference in the relative percentage of monomers and dimers. If specific dimerization via the complementary extensions occurs, it should be most pronounced in the 3vs3 sample.

Zoom-out TEM images



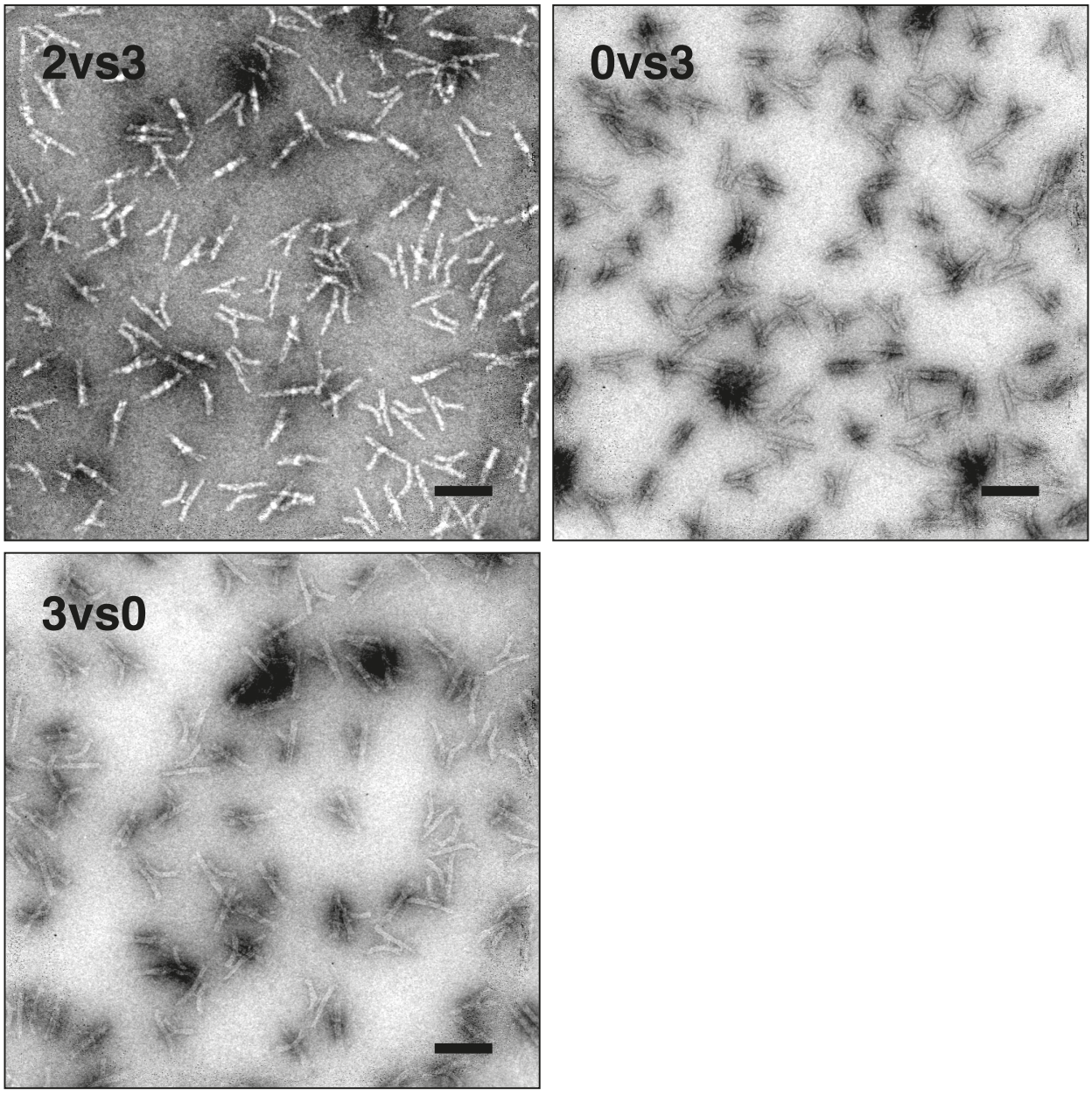


Figure S5: Exemplary zoom out TEM images (imaged at 12.000 x magnification) of all samples shown in figure 2. Only structures lying on their side were included in the analysis. Scale bars are 100nm.

Exemplary Set of Particles (0vs3 sample)

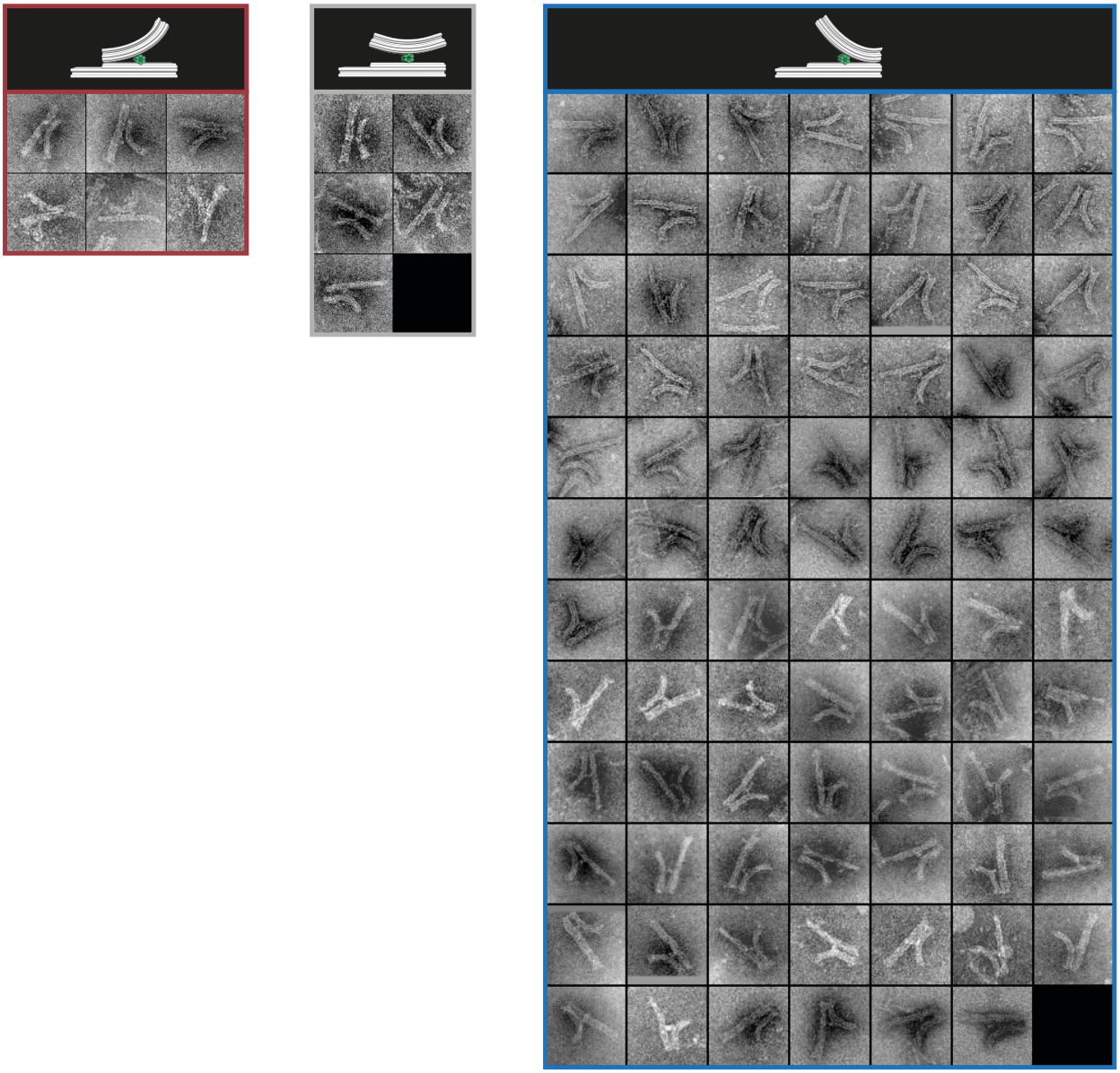


Figure S6: Three groups (*left*, *open*, and *right*) of TEM images of in total 94 particles of the 0vs3 sample. Particles were identified and boxed (300x300 pixel) from TEM micrographs taken at 30.000x magnification.