Supporting Information

Effect of Temperature Ramp in Rapid Folding of 3D DNA Origami Structures

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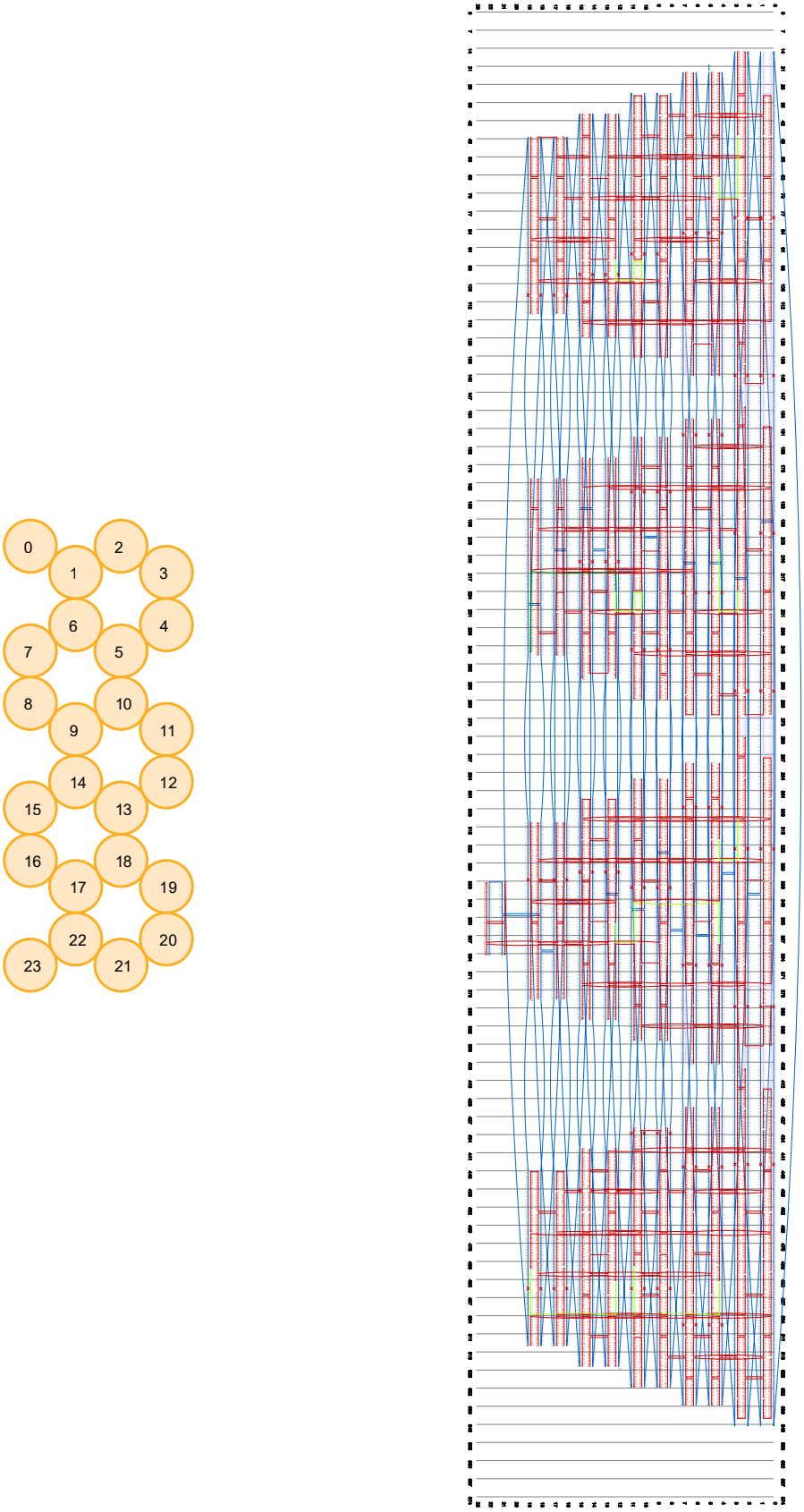

Figure S1. Strand routing diagram of the 20SB DNA origami structure.

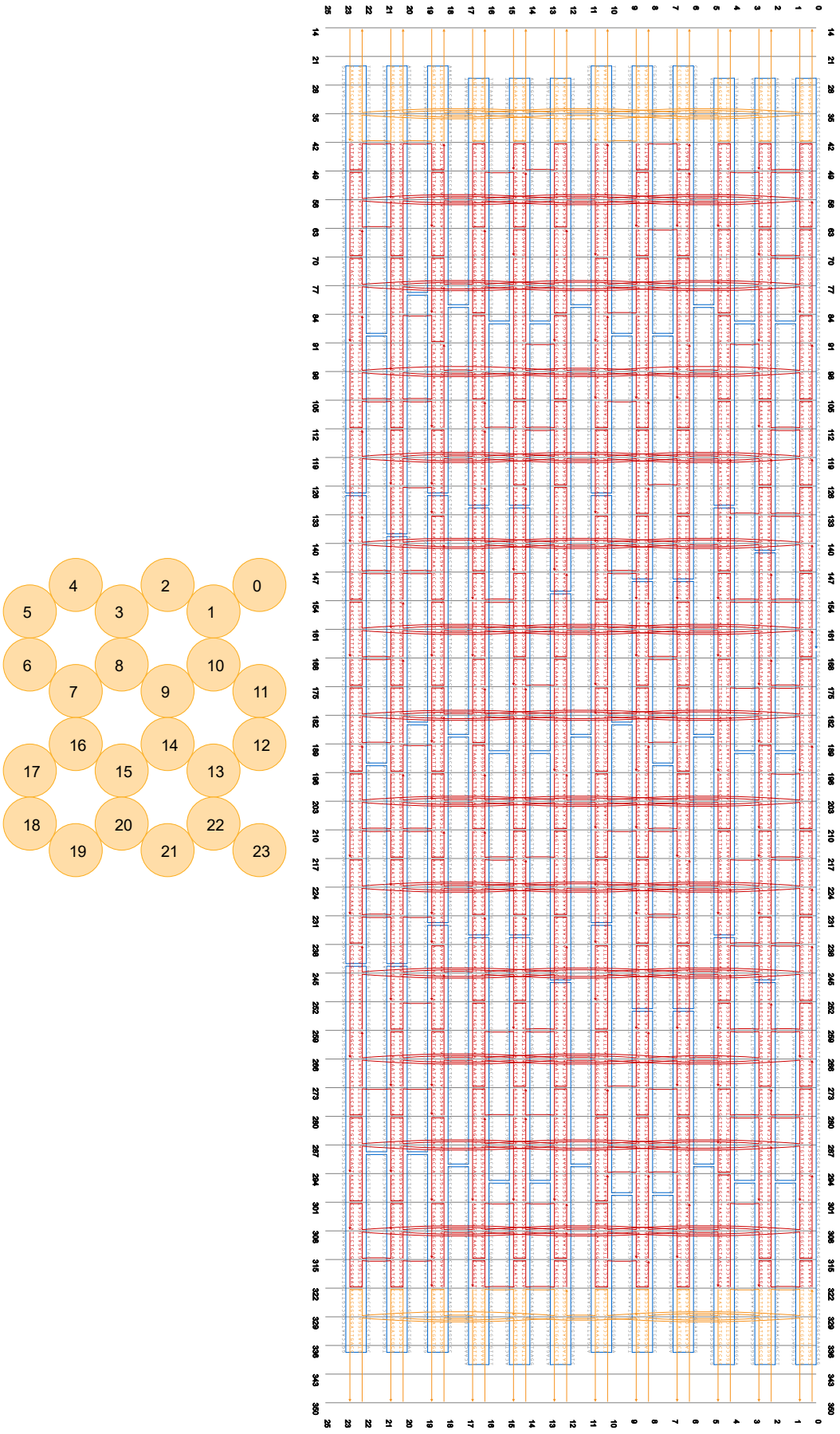

Figure S2. Strand routing diagram of the 24HB DNA origami structure.

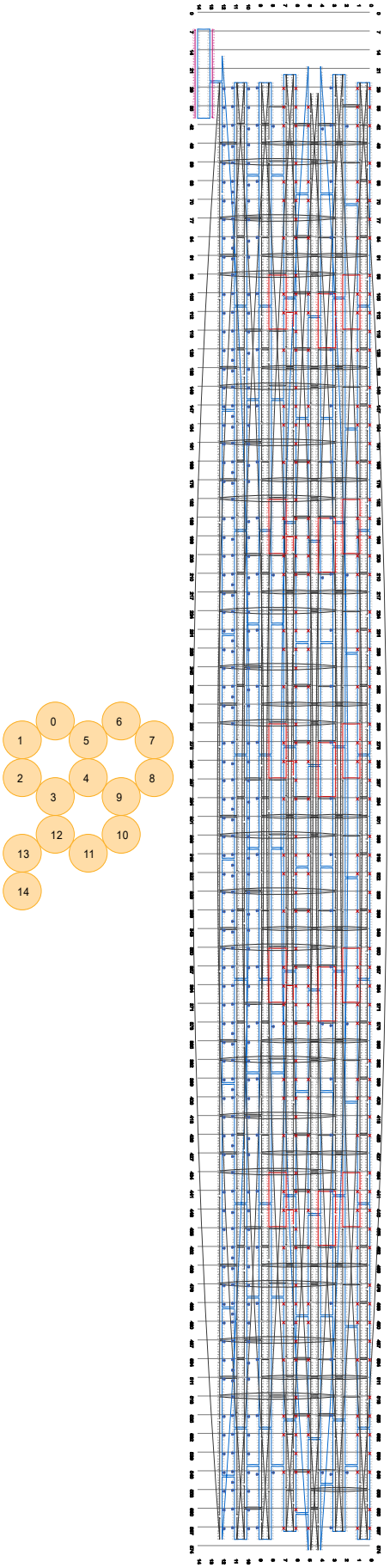

Figure S3. Strand routing diagram of the 13R DNA origami structure.

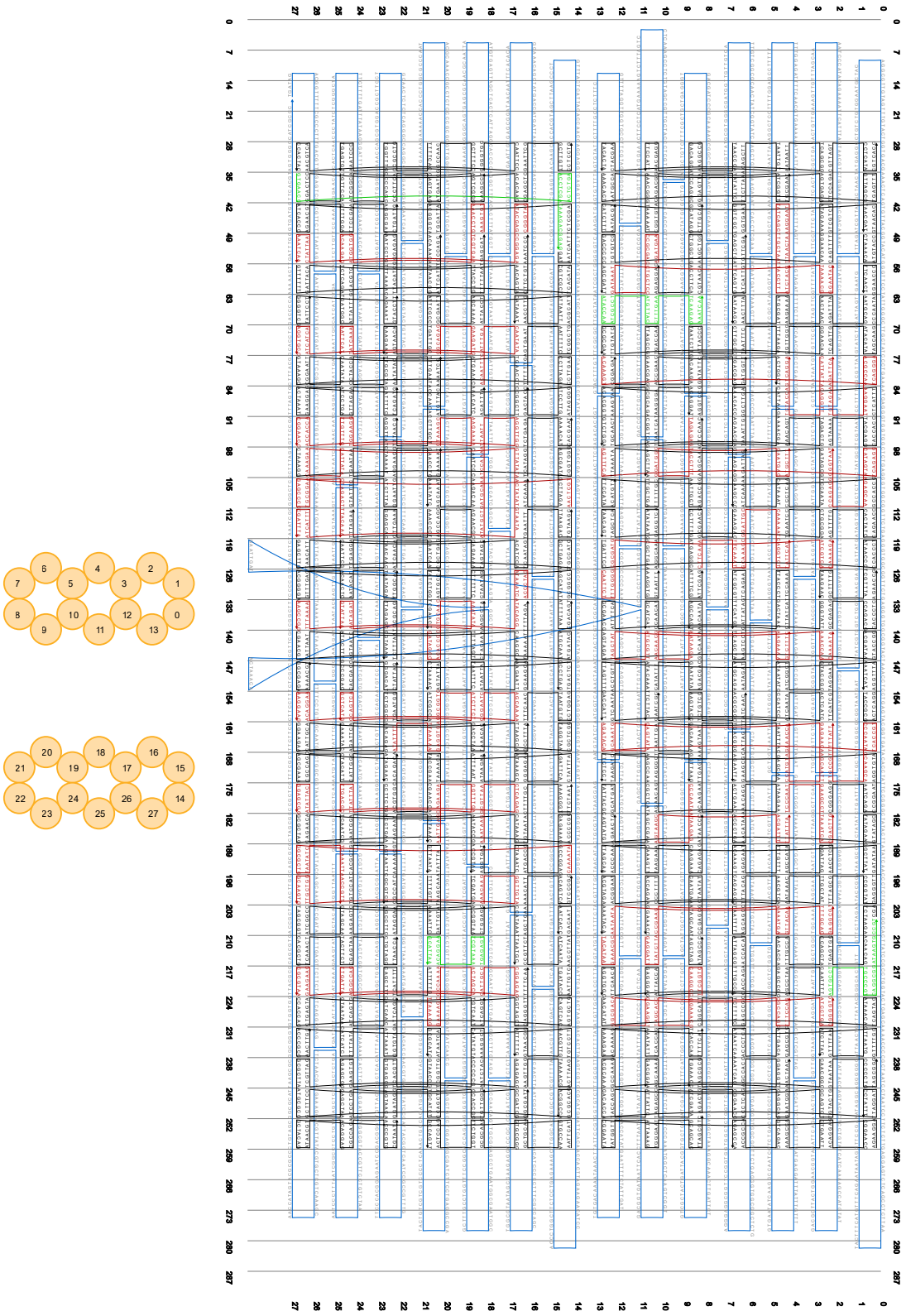

Figure S4. Strand routing diagram of the Cross DNA origami structure.

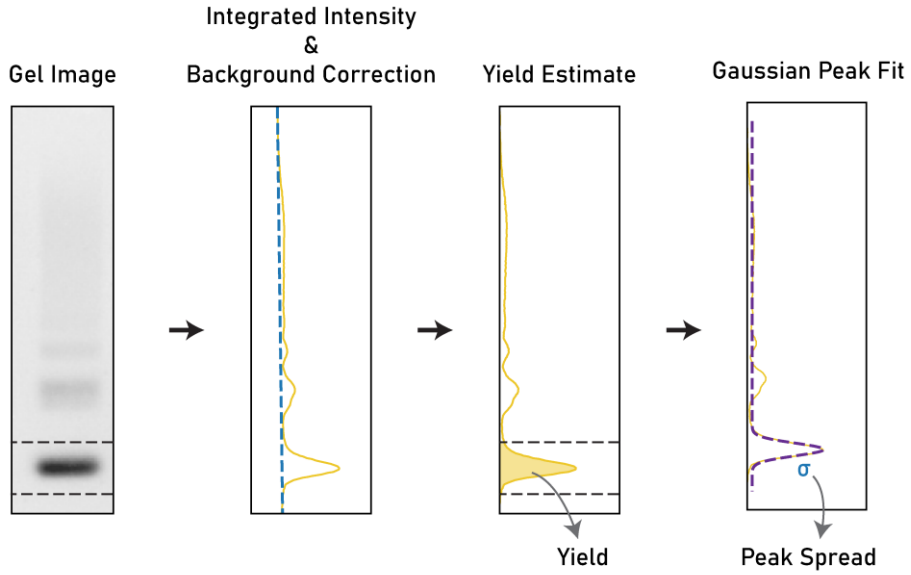

Figure S5. Gel Image Analysis. The raw gel images were cropped into individual bands, and a simple first-degree polynomial background correction was applied. To estimate yield, we defined a region encompassing the entire peak corresponding to the 30 h sample (as shown here). The area under the curve within this region was calculated, providing a measure of yield. Each peak was then fitted with a Gaussian function, and the standard deviation (σ) obtained from the fit was used to quantify peak spread, serving as a measure of structural non-uniformity.

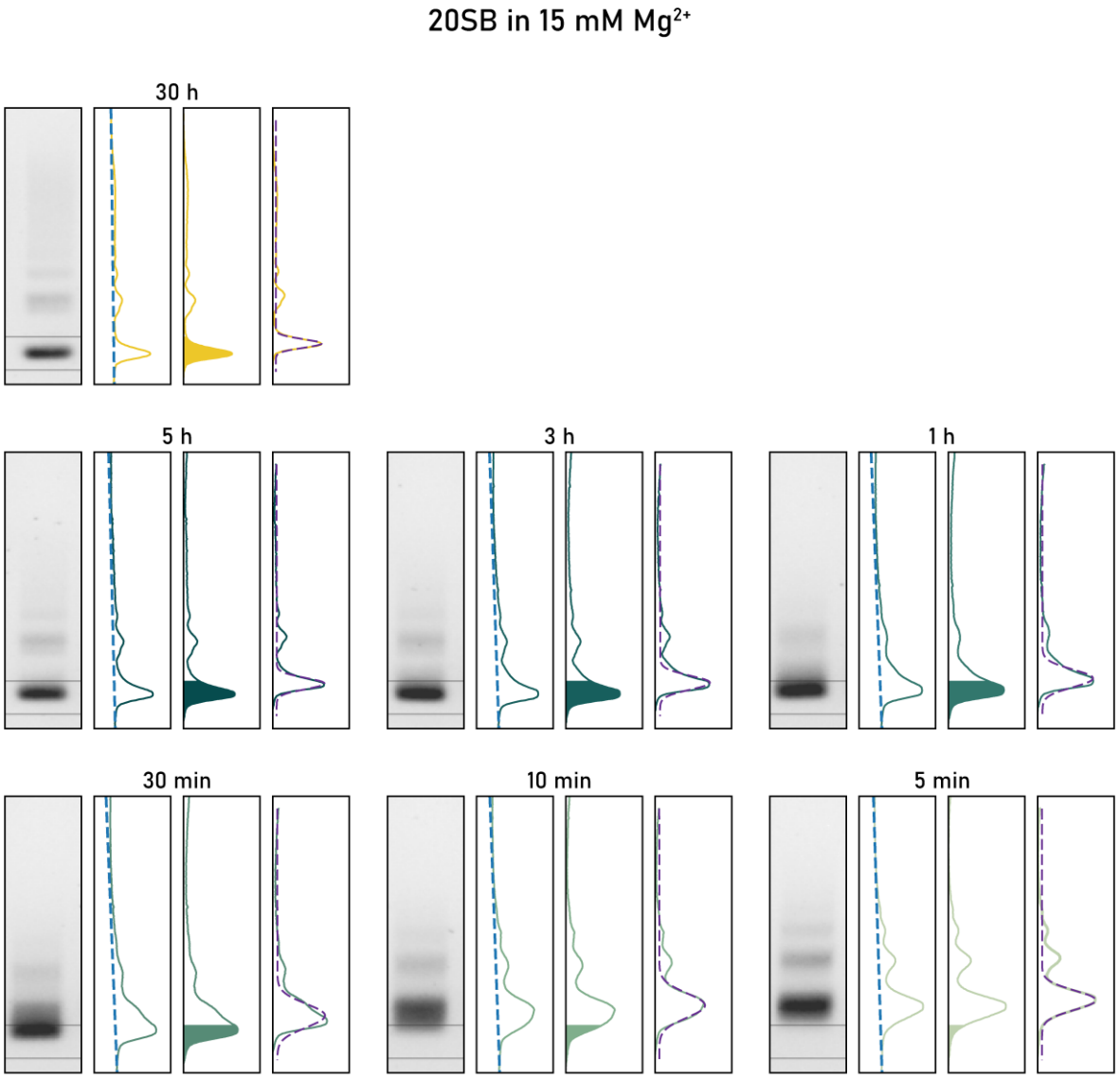

Figure S6. An example of the gel image analysis applied to all bands of a given structure. The 30 h sample region was used as a reference, and corresponding regions were defined for all other samples at the same vertical level. The area under the curve within this region was then calculated for each sample to estimate yield. A Gaussian peak fit was applied to all intensity curves, and the standard deviation (σ) extracted from the fit parameters was used to quantify peak spread, providing a measure of structural non-uniformity.

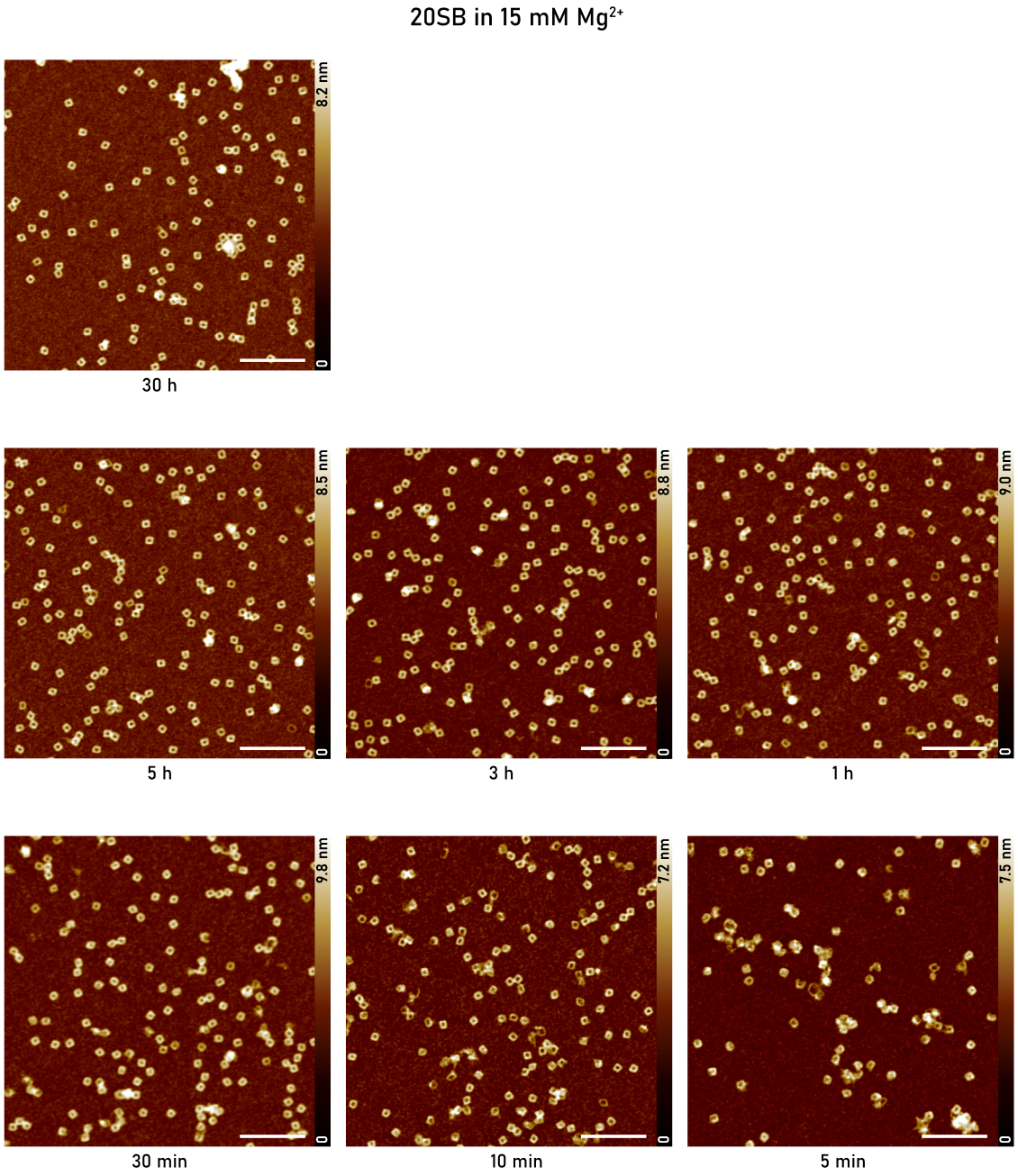

Figure S7. AFM images of the 20SB structure folded in 15 mM Mg^{2+} and 1×TE buffer. The 30 h sample was folded using the standard stepwise protocol, while all other samples were folded using the 60–40 protocol, with the total folding time indicated below each image. Even when samples were folded for 5 min, some fully formed structures were observed, along with the majority of DNA clusters of about the same size as the fully formed structures. The number of fully formed structures increases with the increase in folding time. Scale bars: 400 nm.

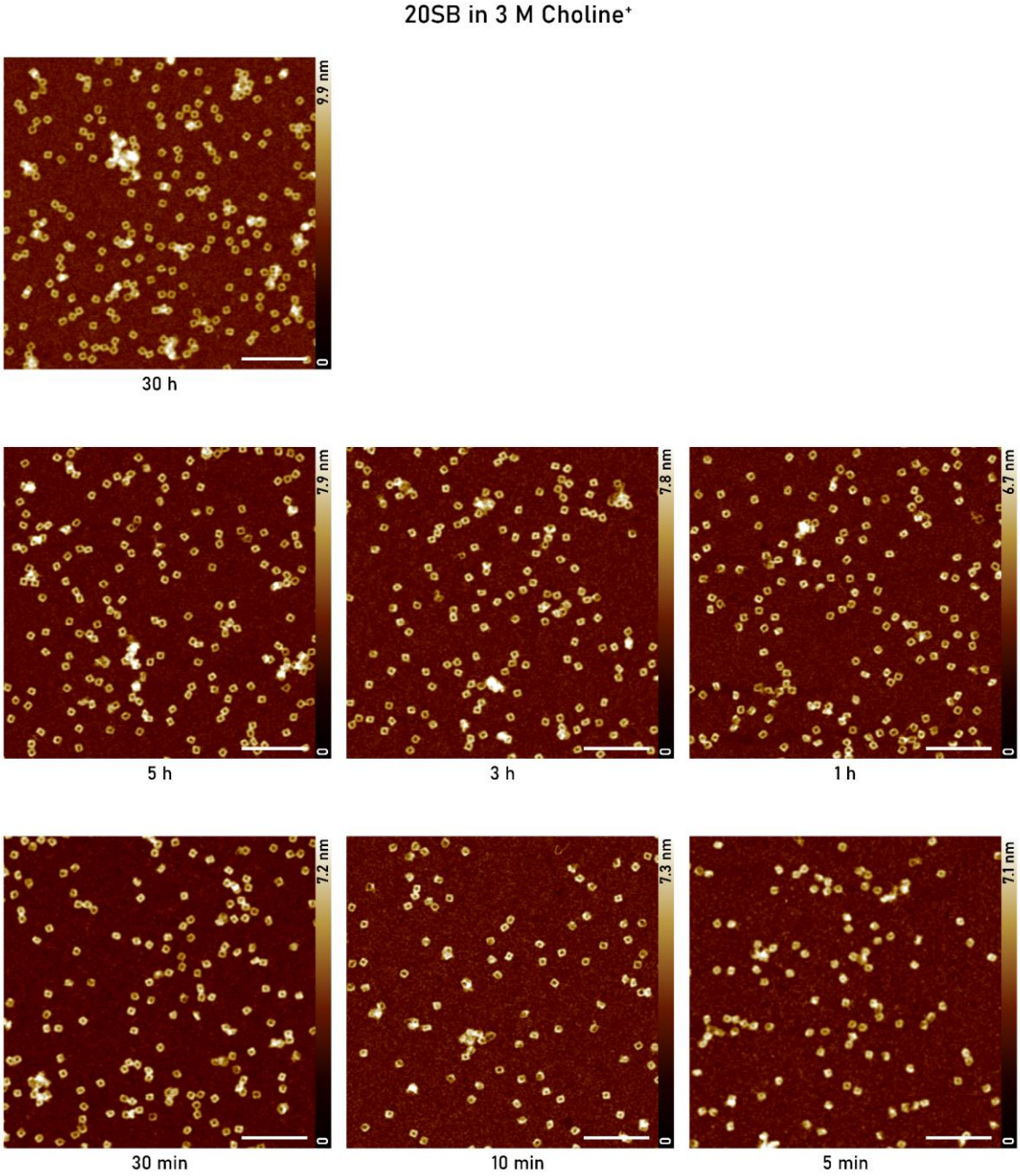

Figure S8. AFM images of the 20SB structure folded in 3 M Choline$^+$ and 1×TE buffer. The 30 h sample was folded using the standard stepwise protocol, while all other samples were folded using the 60–40 protocol, with the total folding time indicated below each image. Here, samples folded for 5 mins or 10 min have a higher number of fully formed structures compared to the 20SB structures folded in 15 mM Mg^{2+} (Figure S7). However, for longer folding times, the behavior is very similar in both conditions. The number of fully formed structures increases with the increase in folding time. Scale bars: 400 nm.

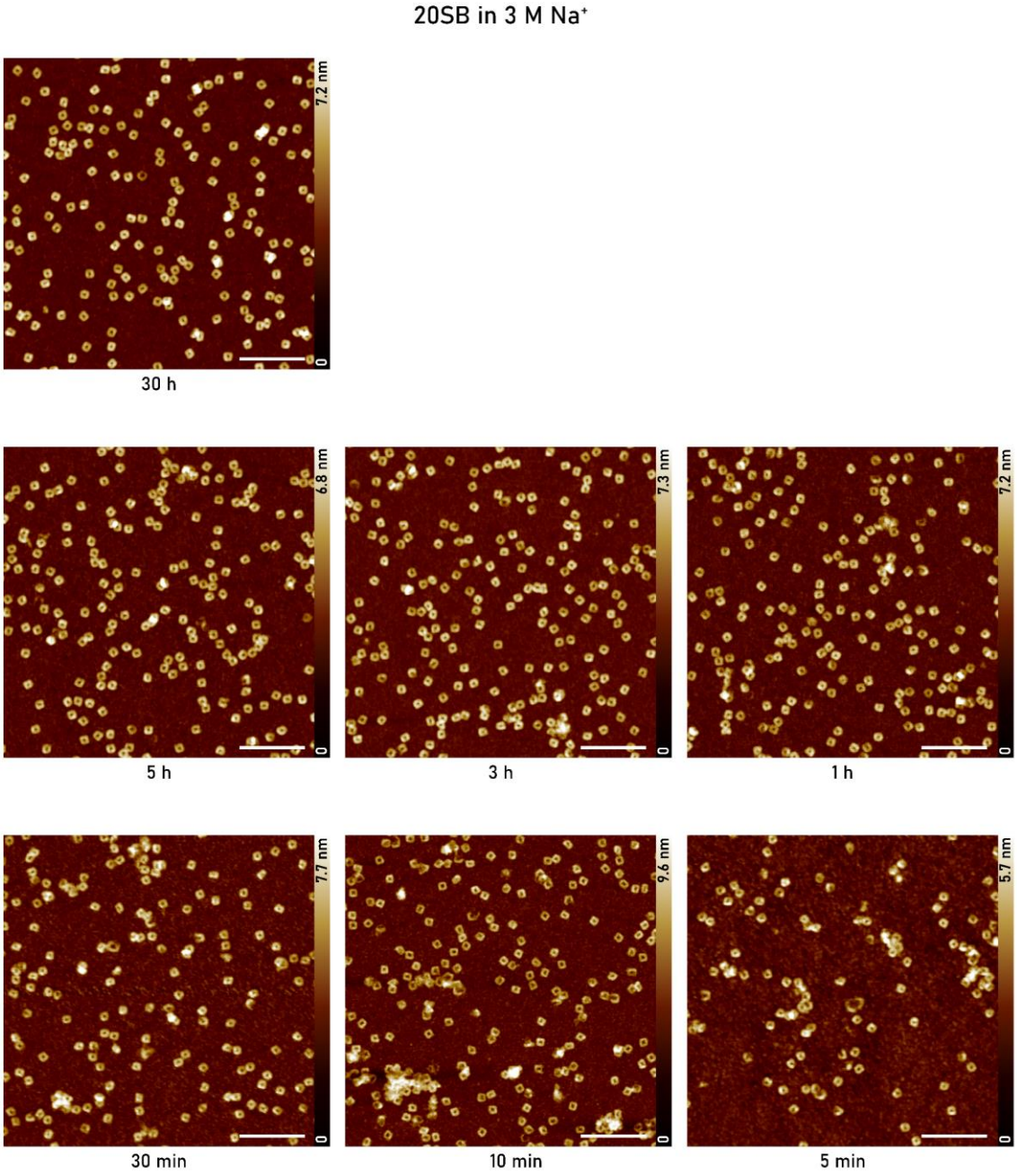

Figure S9. AFM images of the 20SB structure folded in 3 M Na^+ and 1×TE buffer. The 30 h sample was folded using the standard stepwise protocol, while all other samples were folded using the 60–40 protocol, with the total folding time indicated below each image. Similar to the results in Figure S8, samples folded for 5 min or 10 min also have a higher number of fully formed structures compared to the structures in Figure S7. There is no significant difference in the folding behavior for longer folding times. The number of fully formed structures increases with the increase in folding time. Scale bars: 400 nm

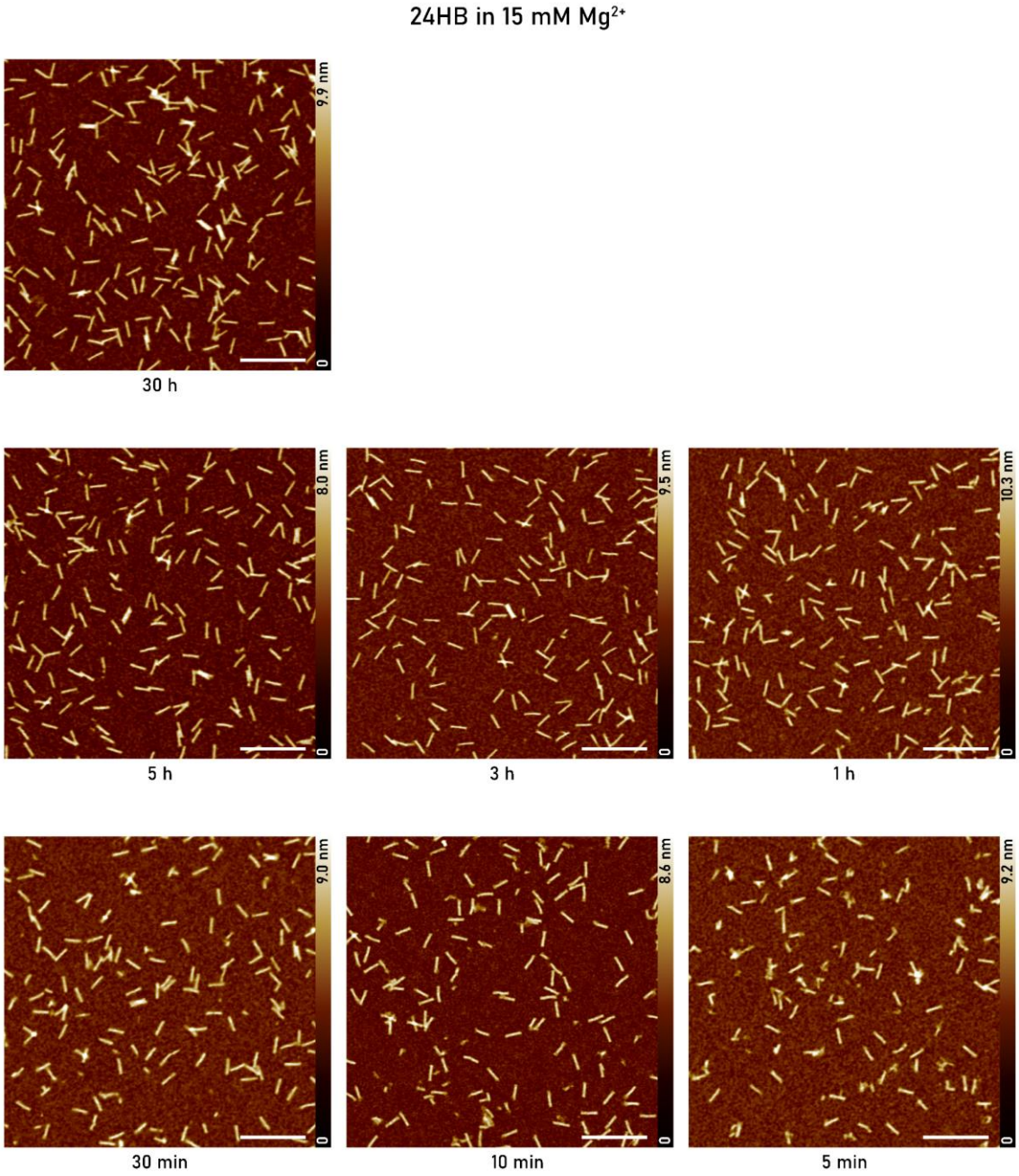

Figure S10. AFM images of the 24HB structure folded in 15 mM Mg^{2+} and 1×TE buffer. The 30 h sample was folded using the standard stepwise protocol, while all other samples were folded using the 60–40 protocol, with the total folding time indicated below each image. In this case, the structural defects are not easily noticeable (the 10-minute sample looks very close to the 30-hour sample in terms of proper folding). However, the 10 min sample has broken/partially folded origami. The number of fully formed structures increases with the increase in folding time. Scale bars: 400 nm.

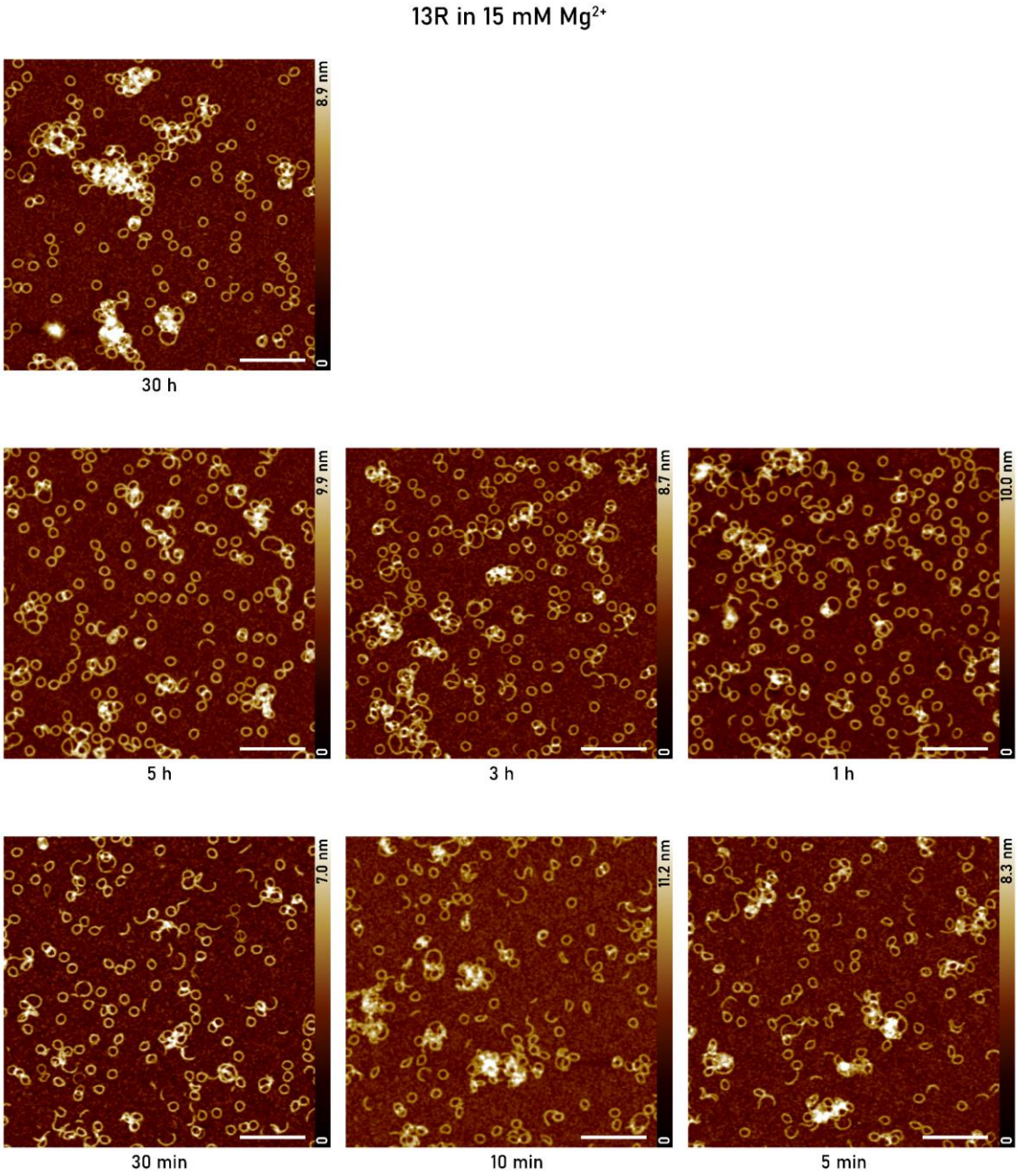

Figure S11. AFM images of the 13R structure folded in 15 mM Mg^{2+} and 1×TE buffer. The 30 h sample was folded using the standard stepwise protocol, while all other samples were folded using the 60–40 protocol, with the total folding time indicated below each image. Here, it can be clearly seen that higher folding times result in more multimers and aggregates. The shorter folding time samples exhibit a high number of partially folded, broken ring structures, but they tend to form fewer aggregates. Scale bars: 400 nm.

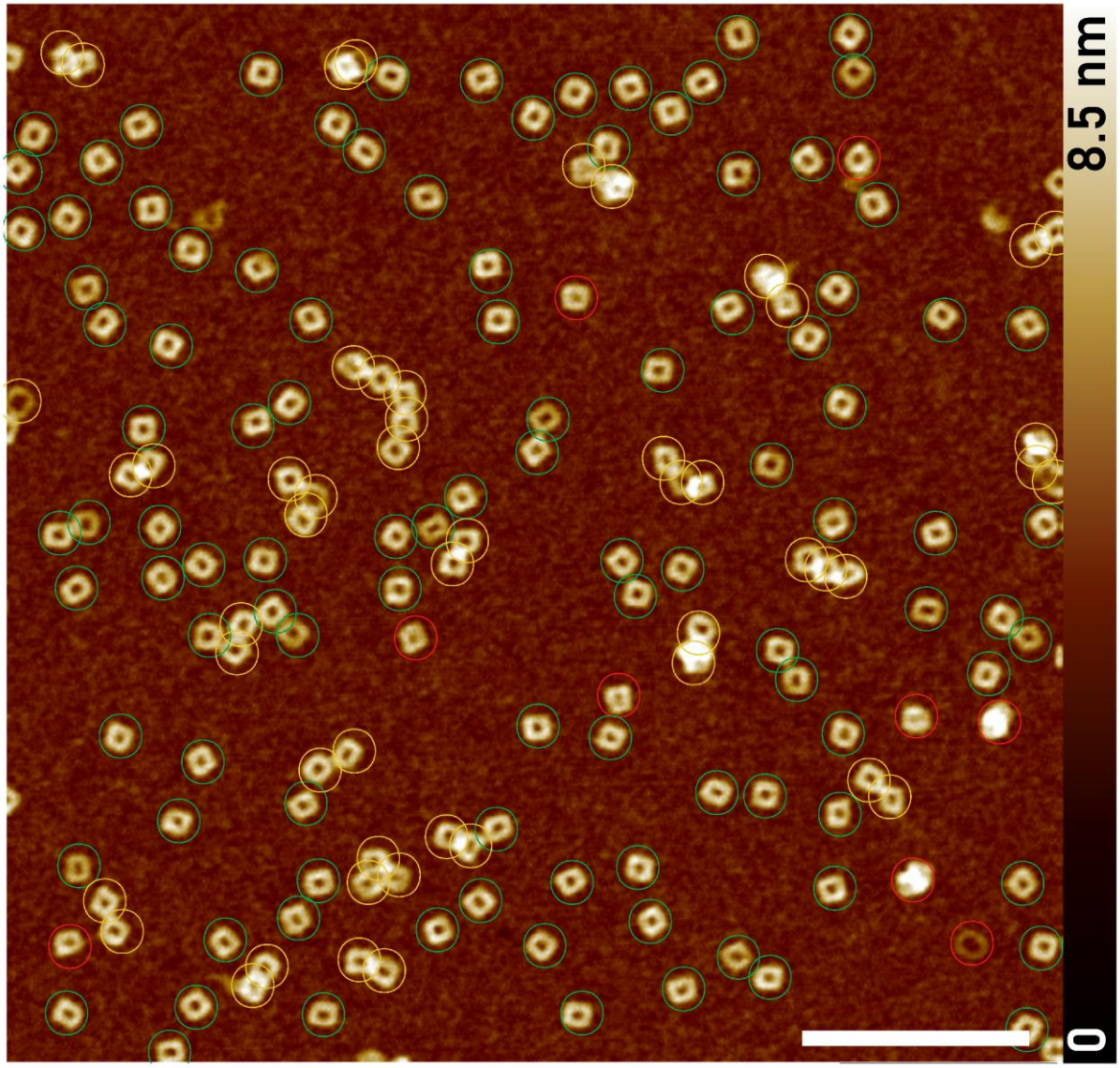

Figure S12. Calculation of the folding performance for the 20SB structure. The structures marked with green are considered fully folded, and the structures marked with red are considered misfolded. The structures that are either overlapping or on the edges, where it is difficult to categorise them as either folded or misfolded, are marked in yellow and are not considered in the calculations. Scale bars: 400 nm.

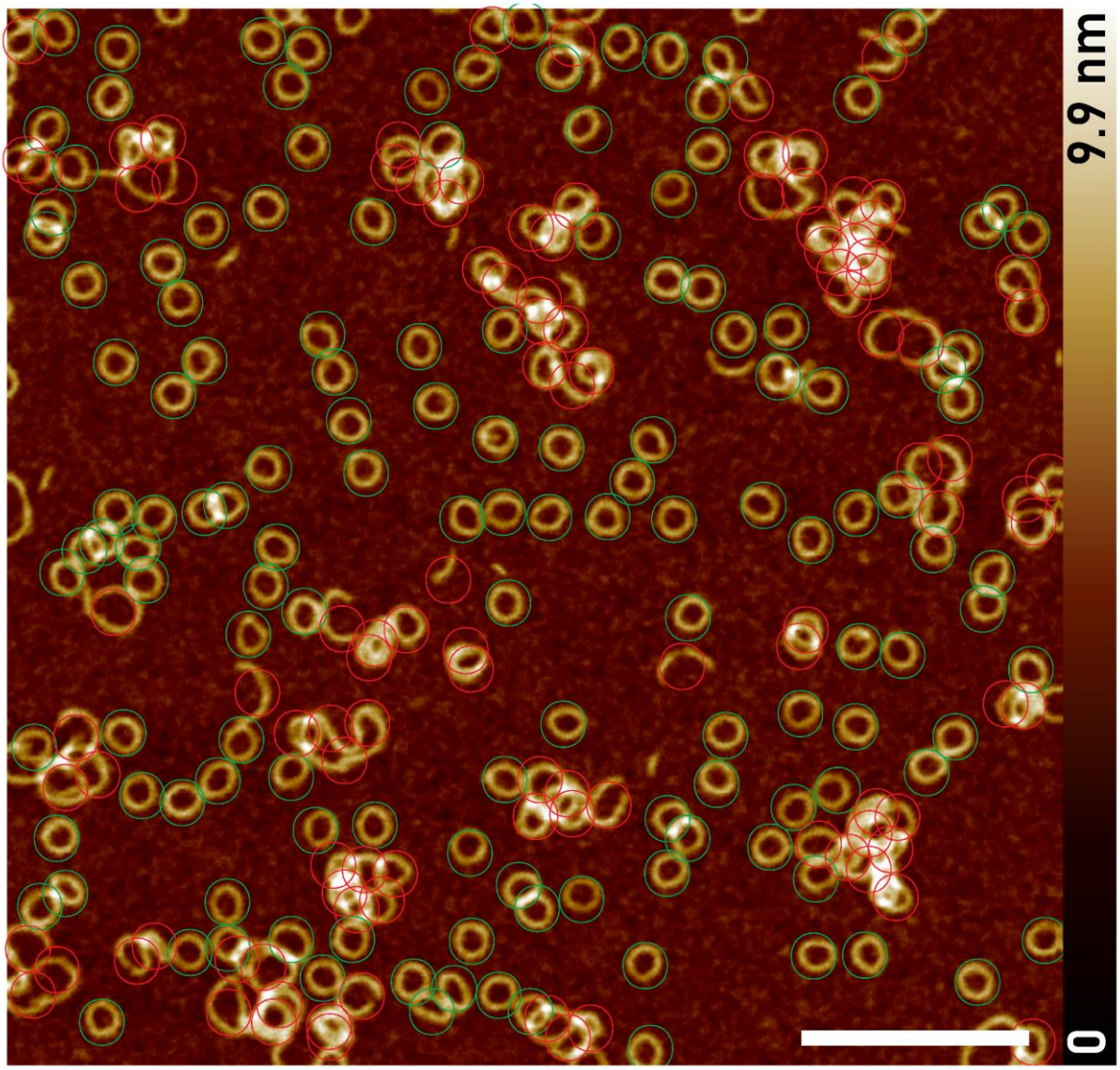

Figure S13. Calculation of the folding performance for the 13R structure. This structure tends to form numerous multimers and aggregates. Hence, only the intended monomeric structures are marked with green and are considered as fully folded. All other structures, including dimers, multimers, aggregates, partially folded, broken moon-like structures, are marked in red and considered misfolded. The structures on the edges, where it is difficult to categorise them as either folded or misfolded, are not considered in the calculations. Scale bar: 400 nm.

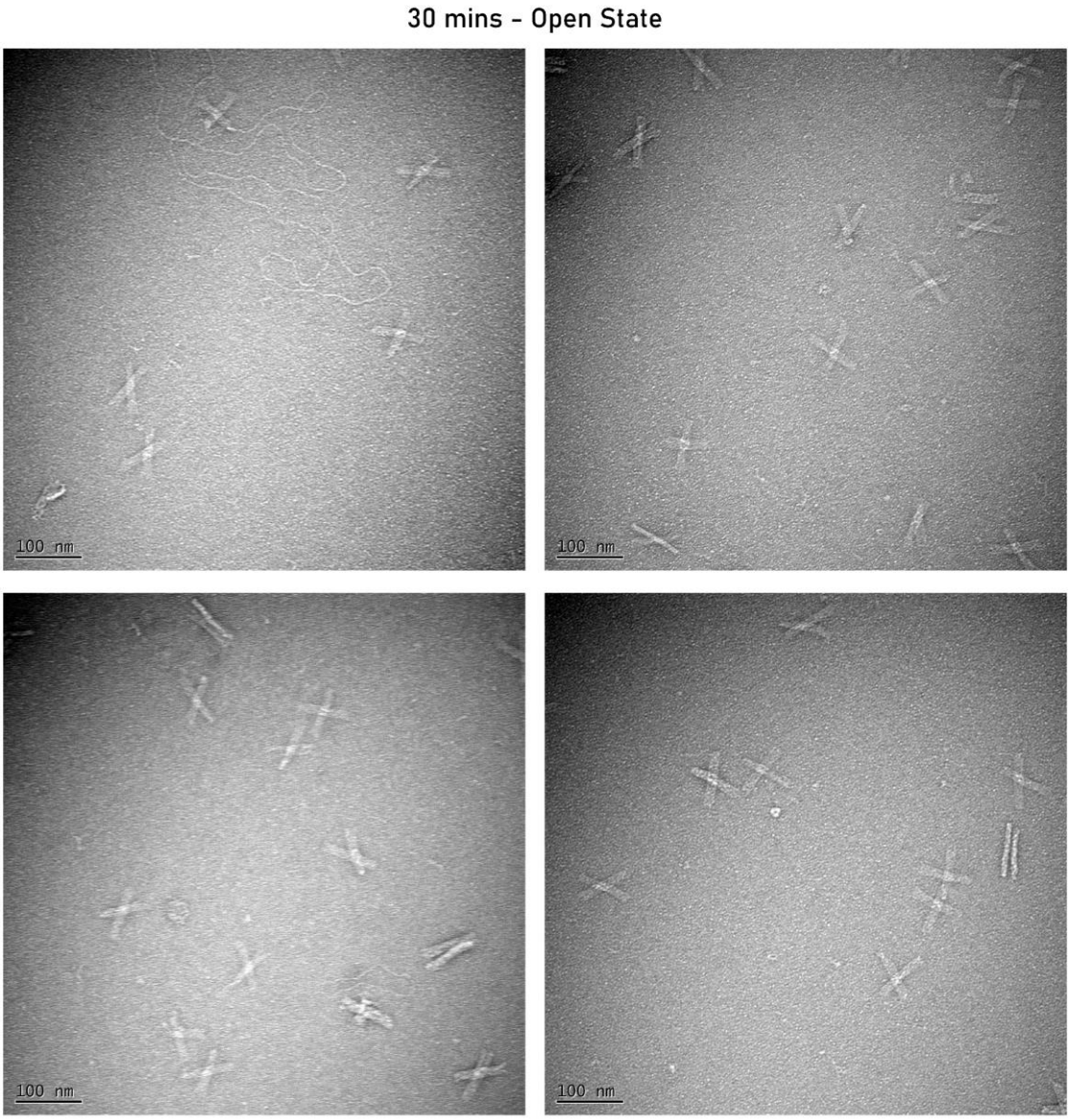

Figure S14. TEM images of the spin-purified Cross structure folded for 30 min using the 60-40 protocol in open state (i.e., before addition of Neutravidin).

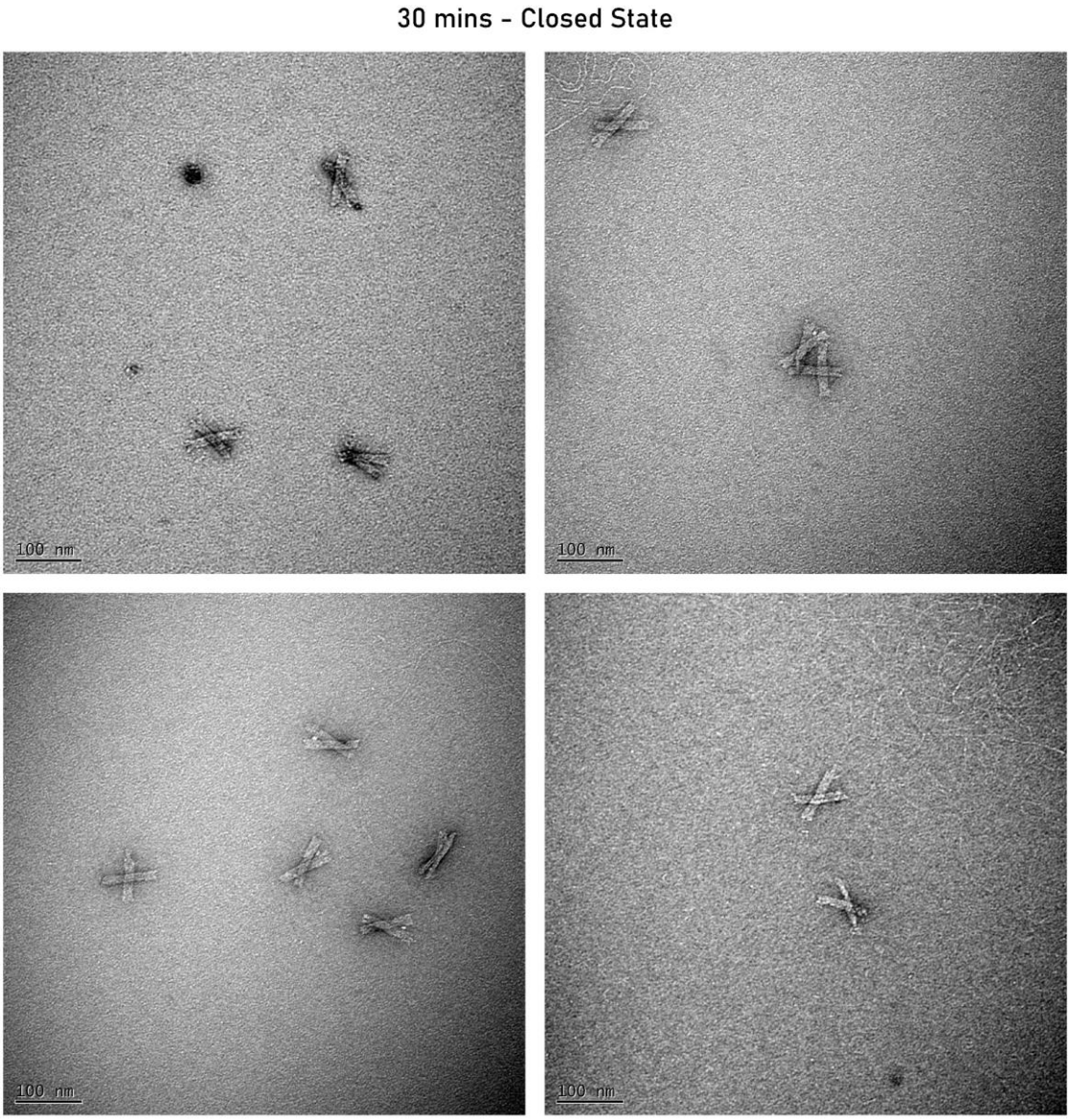

Figure S15. TEM images of the spin-purified Cross structure folded for 30 min using the 60-40 protocol in the closed state. The structures were incubated with Neutravidin for 1 h at room temperature to induce switching to the closed state before imaging.

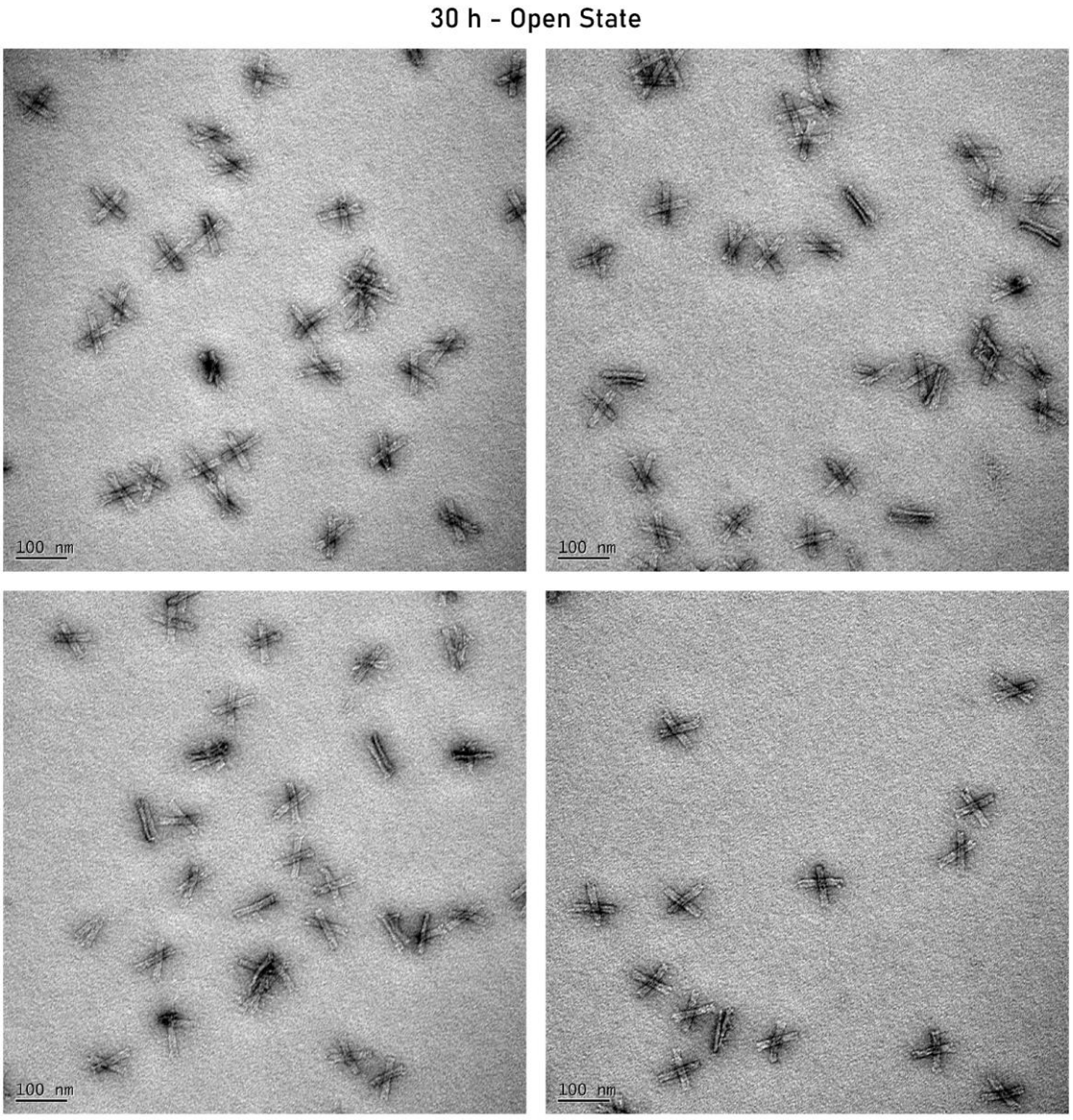

Figure S16. TEM images of the spin-purified Cross structure folded for 30 h using the standard protocol in the open state (i.e., before addition of Neutravidin).

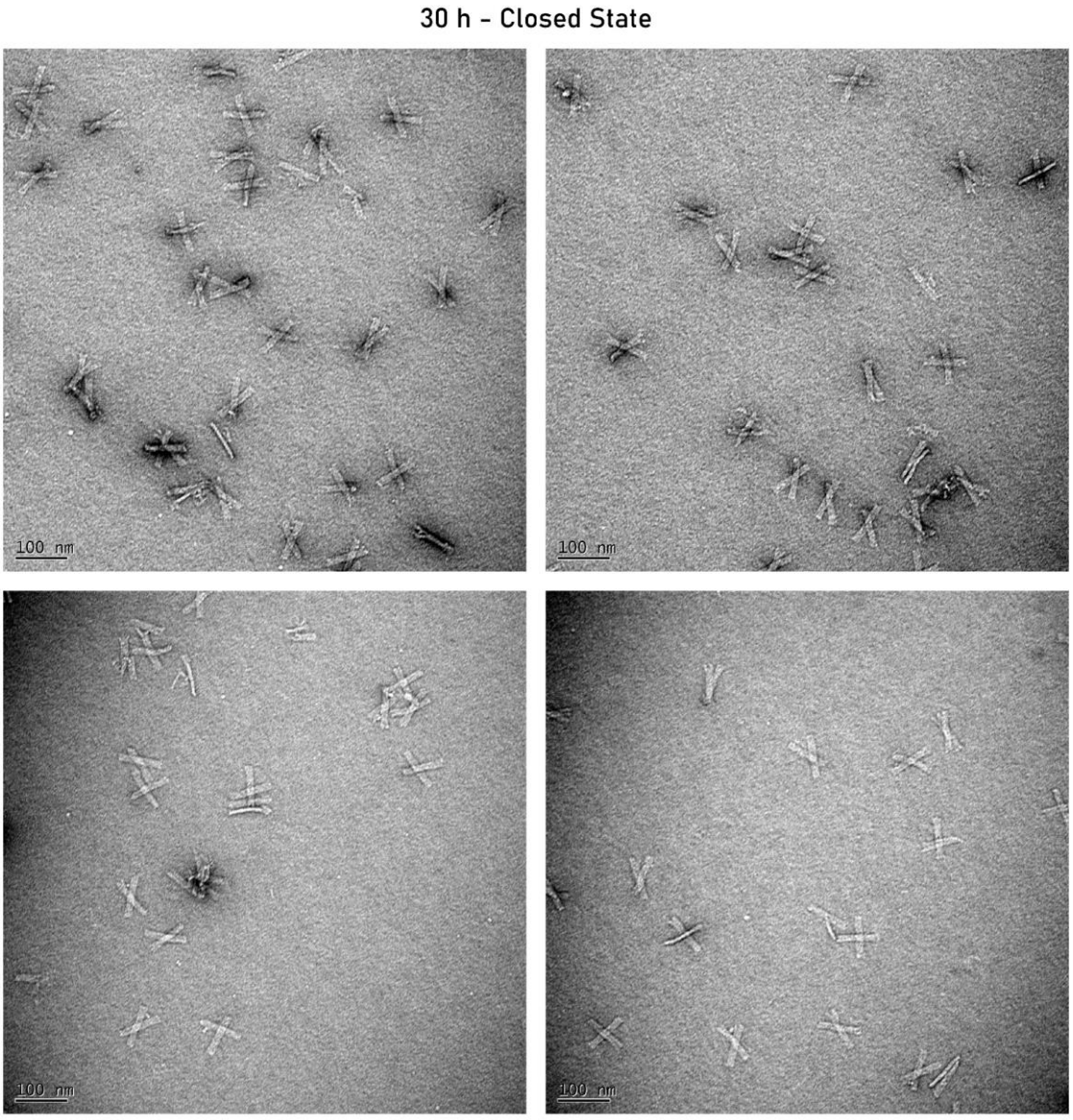

Figure S17. TEM images of the spin-purified Cross structure folded for 30 h using the standard protocol in the closed state. The structures were incubated with Neutravidin for 1 h at room temperature to induce switching to the closed state before imaging.

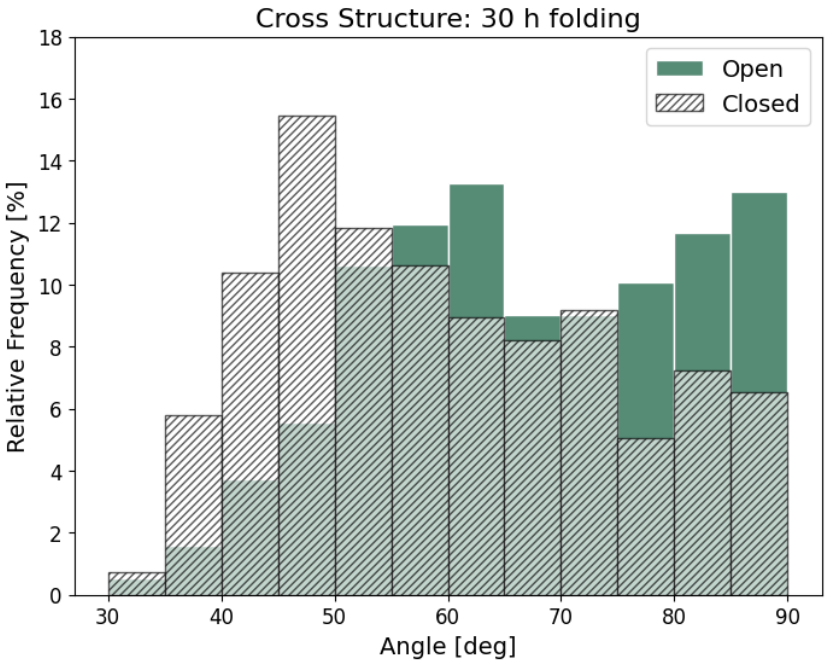

Figure S18. Histogram of the angles of the Cross structure folded for 30 h in the open state and closed state. (Total structures analyzed - Open: 377, Closed: 414)