Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information:

Co-self-assembly of Multiple DNA Origami Nanostructures in a Single Pot

Joshua A. Johnson,[a] Vasiliki Kolliopoulos [b] and Carlos E. Castro*[a,c]

[a.] *Biophysics Graduate Program, The Ohio State University, 281 W Lane Ave, Columbus, OH 43210*
[b.] *Department of Chemical and Biomolecular Engineering, The Ohio State University, 281 W Lane Ave, Columbus, OH 43210*
[c.] *Department of Mechanical and Aerospace engineering, The Ohio State University, 281 W Lane Ave, Columbus, OH 43210*

Contents:

1.1 Initial isothermal folding gels

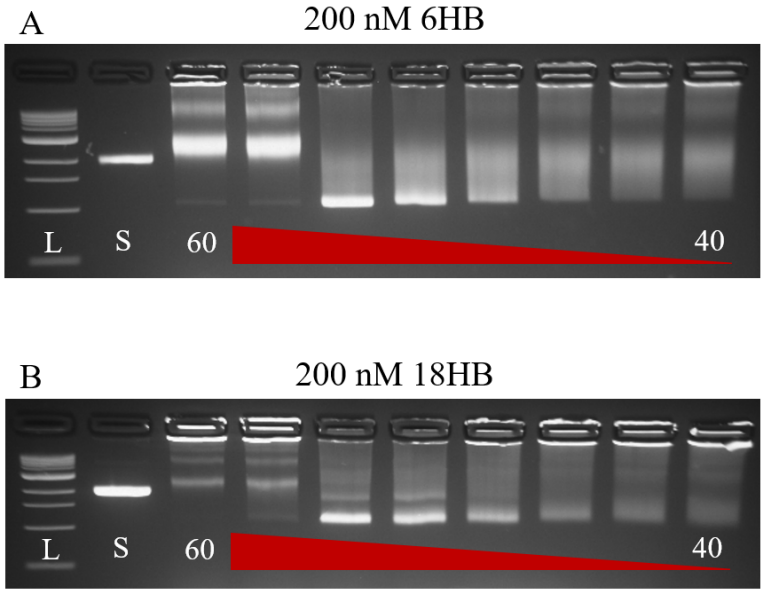

Figure S1. Isothermal anneal of 6HB (A) and 18HB (B) with 200 nM staples in a thermal gradient between 40°C and 60°C.

1.2 Kinetics of folding for individual nanostructures

We wanted to compare folding kinetics to help determine if the observed differences in yields were due to lower folding rates or simply an insufficient excess of staple strands. Using isothermal annealing at 53 °C we also explored the folding kinetics for each structure. Laser-scanned images of agarose gels revealed a clear concentration dependence on folding rates for the 6HB and the 18HB. We found that the 6HB tended to take longer than the 18HB to reach a maximum yield however traces of well folded structure tended to appear earlier than the 18HB. From most gel shift assays of folding kinetics, it was clear that a semi-folded intermediate form of the 6HB tended to emerge first in the lagging band of lanes corresponding to early time points. We found quantification of these lagging bands to be difficult due to the wide distribution of the lagging structure band and due to the emergence of a dimer band in 18HB kinetics assays. Additionally, manual quantification using programs such as ImageJ or even semi-automated methods using

previously developed MATLAB code would often yield results that were very sensitive to human input and also did not reflect the more apparent trends observed from simply looking at the gels. To make this process more consistent and higher throughput, we developed a fully automated process to quantify all kinetics gels. The program for processing kinetics automatically finds the optimal rotation for each gel to ensure the folded bands are aligned across all lanes, then finds each lane and extracts the lane profile which is then aligned and scaled with all other lane profiles. Thus, a single range is needed to specify which bands should be measured across all gels.

We chose to measure the peak heights of all folded bands and all lagging bands and attempted to fit them to a three-state model. In this model, we simplify the kinetics of DNA origami folding to an initial unfolded state, an intermediate partially folded state, and a completely folded state. We ignore possible pathways in which partially folded structures are kinetically trapped, preventing them from reaching a fully folded state. Additionally, we only consider the forward reaction rates and assume that the reverse reaction rates are negligible giving us only two rate parameters and a scaling factor to find a fit. This model is consistent with previously developed 3-phase models for fitting kinetics of binding individual staples which fit best when assuming one fast and one slow rate constant[1]. The chemical reaction is depicted below:

$$[U] \overset{k_1}{\Rightarrow} [P] \overset{k_2}{\Rightarrow} [F] \tag{1}$$

Where [U] is the concentration of unfolded structure, [P] is the concentration of structures in an intermediate folding state, and [F] is the concentration of folded structures. The system of differential equations corresponding to this reaction are listed below:

$$\frac{dU(t)}{dt} = -k_1 * U(t) \quad (2)$$

$$\frac{dP(t)}{dt} = k_1 * U(t) - k_2 * P(t) \quad (3)$$

$$\frac{dF(t)}{dt} = k_2 * P(t) \quad (4)$$

Where k_1 is the forward reaction rate of unfolded structure into an intermediate state, and k_2 is the forward reaction rate of structure in an intermediate state into the folded structure. The solutions to this system of differential equations which we used for fitting are listed below:

$$F(t) = \frac{k_1(1-e^{-k_2 t})+k_2(1-e^{-k_1 t})}{k_1-k_2} \quad (5)$$

$$P(t) = \frac{-k_1(e^{-k_1 t}-e^{-k_2 t})}{k_1-k_2} \quad (6)$$

It is important to note that for most gels, there is some degree of aggregation visible and it is unclear if any folded or partially folded structures can come from aggregates over long enough times. Additionally, DNA origami folding is certainly much more complicated than our simple 3-state model and while we can achieve reasonable fits for early time points, many fits fail to match the data for 24 hour or 60 hour time points especially for data associated with lagging bands. For individual kinetics assays, we know that over long enough timescales, a tighter band appears for 18HB structures which coincides with the lagging band associated with partially folded structures. From TEM images of this band at later time point, we know that this band corresponds to well-

formed 18HB structures that have become dimers (Figure S1). Thus, when fitting data, a weighting function is applied which allows the fitting function to prioritize fitting of earlier time points over later time points.

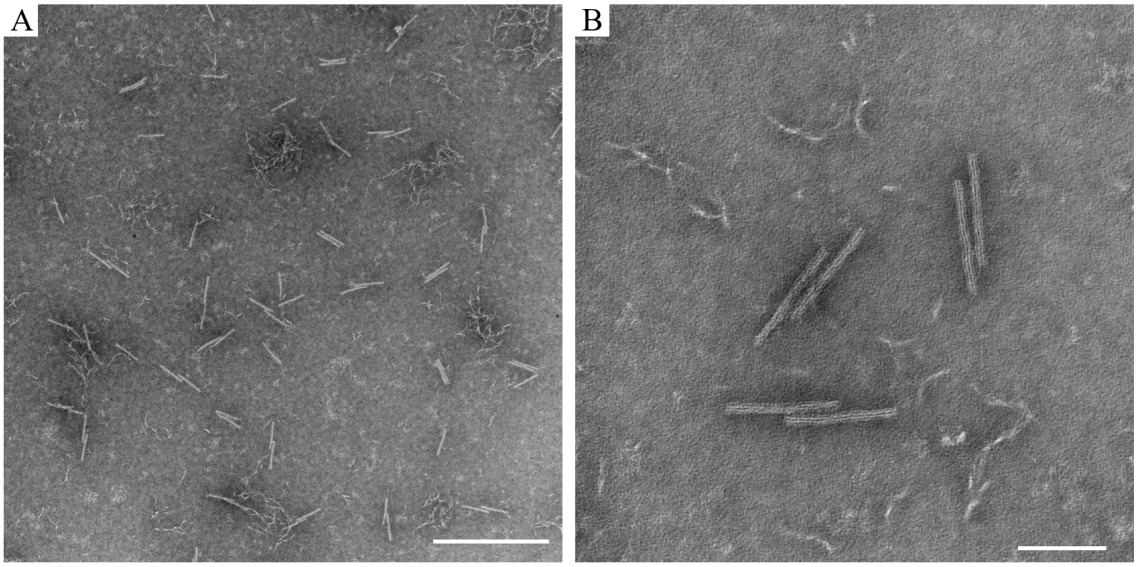

Figure S2. TEM images of 18HB dimer band. (A) Zoomed-out view shows that 18HB dimers comprise the majority of visible structures. Scale bar is 500 nm. (B) Zoomed-in view shows 18HB structures are not compromised by dimerization. Scale bar is 100 nm.

All gels for individual kinetics assays were repeated in triplicate and an example of each is shown in panel A of Figure S2 through Figure S10. In all cases for the 6HB, the yield of folded structure appears to level off between 8 and 24 hours, however, a substantial increase in yield after 60 hours makes fitting to simple exponentials more difficult. The 6HB fold notably fast at 30 nM (Figure S3) and 40 nM (Figure S4) with folded bands appearing after only 30 minutes. For kinetics assays of 18HB folded at lower staple concentrations (Figure S5 and Figure S6), there is an apparent lower yield of 18HB dimers which allows for fits to appear better for later time points. However, for staple concentration higher than 30 nM, a strong dimer band arises starting at the 2 hour time

point. Thus fits in Figure S7 through Figure S10 appear to only match early time points. For all measurements peak band intensities are normalized against the peak intensity of a 200nM control.

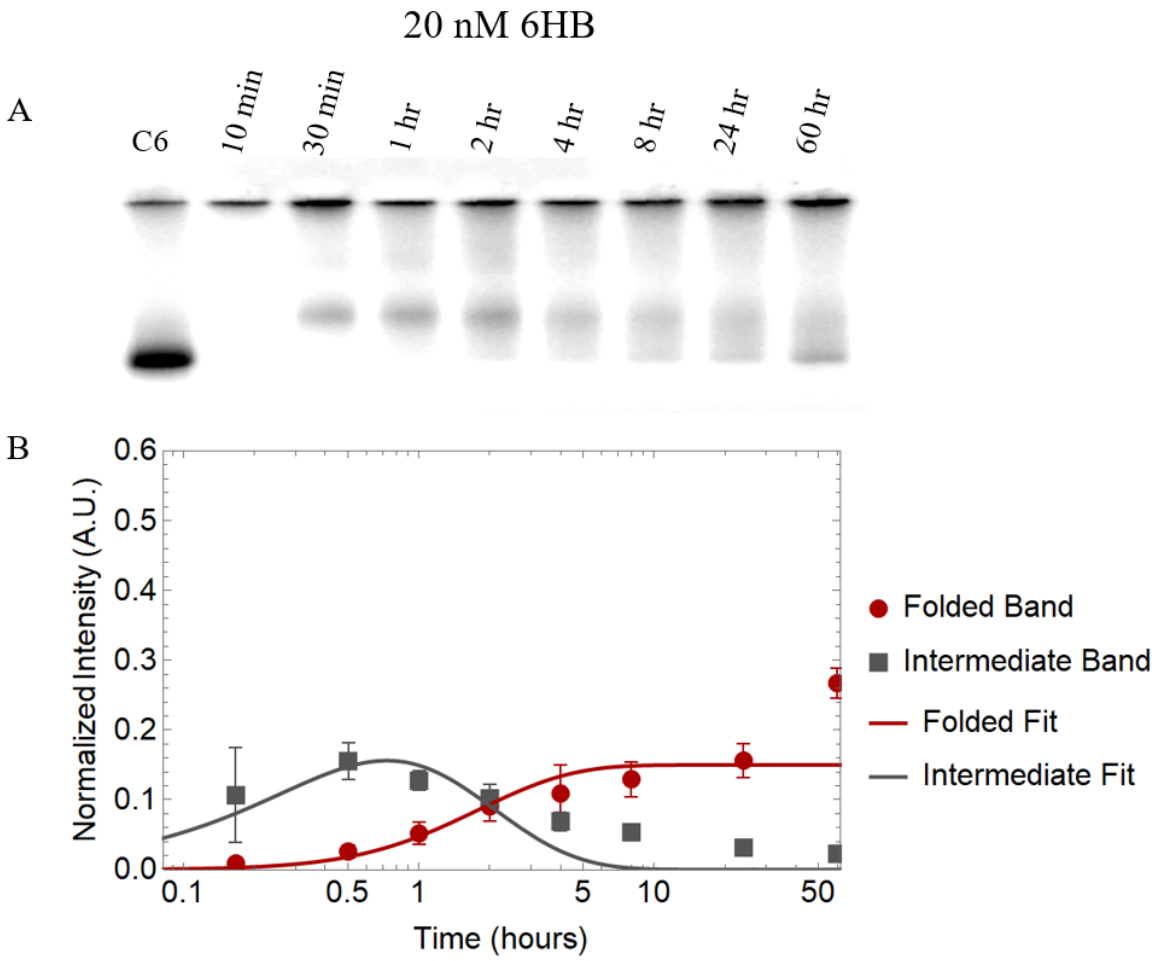

Figure S3. Individual kinetics of folding with 20 nM 6HB staples.(A) Example of laser-scanned agarose gel. First row is a control of 6HB folded at 200 nM staples in a 2.5 day anneal. Other lanes correspond to 20 nM 6HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively.

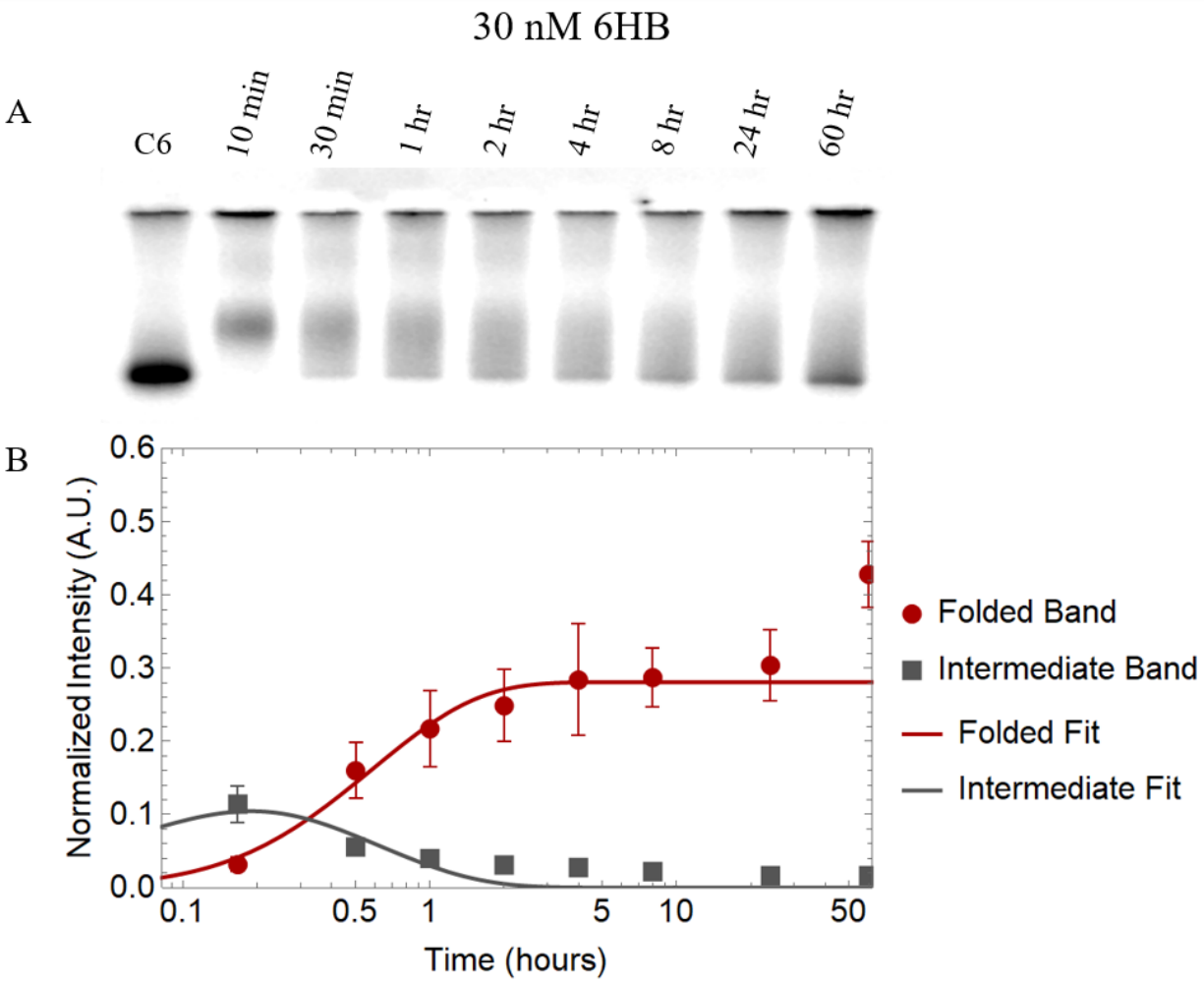

Figure S4. Individual kinetics of folding with 30 nM 6HB staples.(A) Example of laser-scanned agarose gel. First row is a control of 6HB folded at 200 nM staples in a 2.5 day anneal. Other lanes correspond to 30 nM 6HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively.

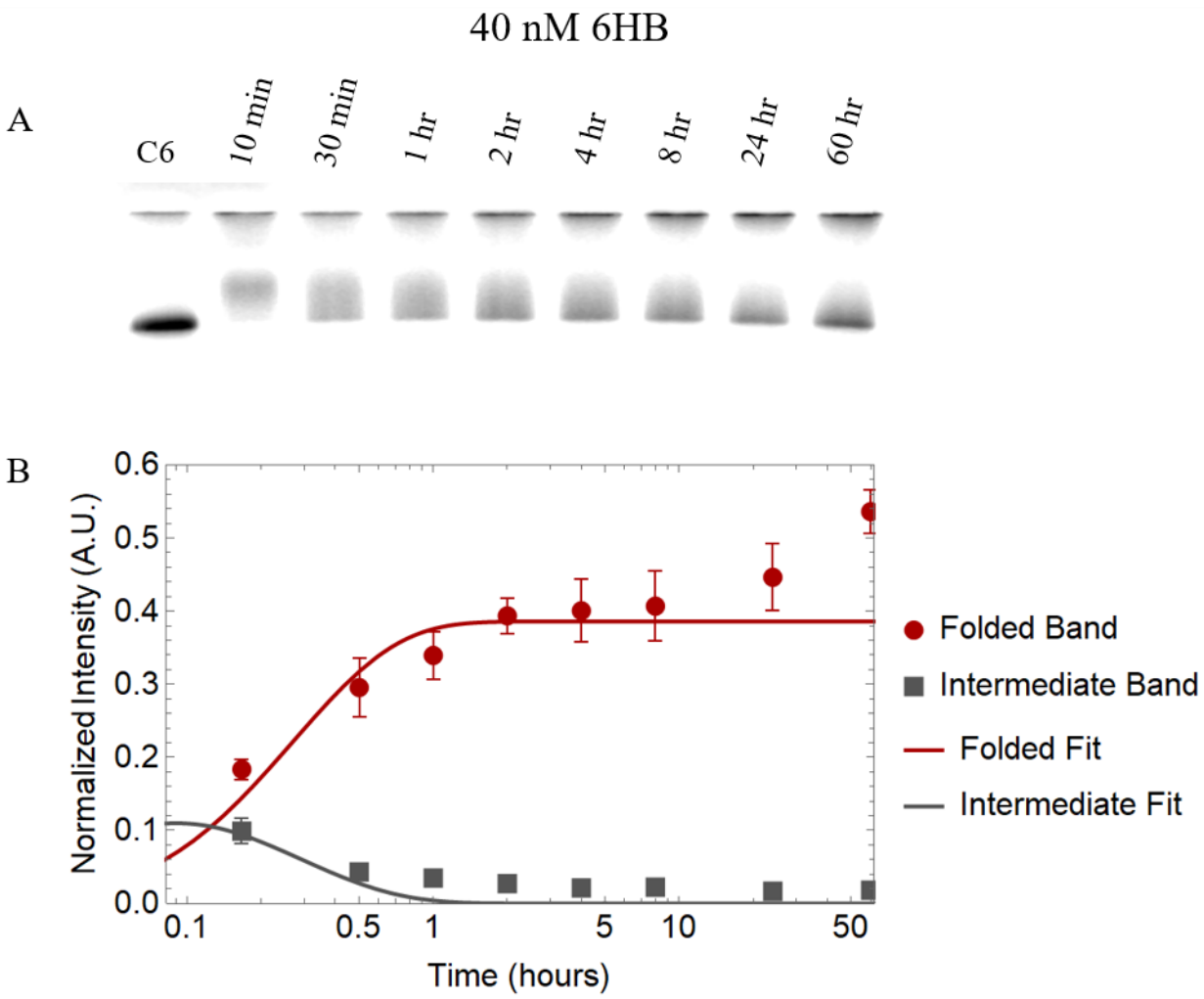

Figure S5. Individual kinetics of folding with 40 nM 6HB staples. (A) Example of laser-scanned agarose gel. First row is a control of 6HB folded at 200 nM staples in a 2.5 day anneal. Other lanes correspond to 40 nM 6HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively.

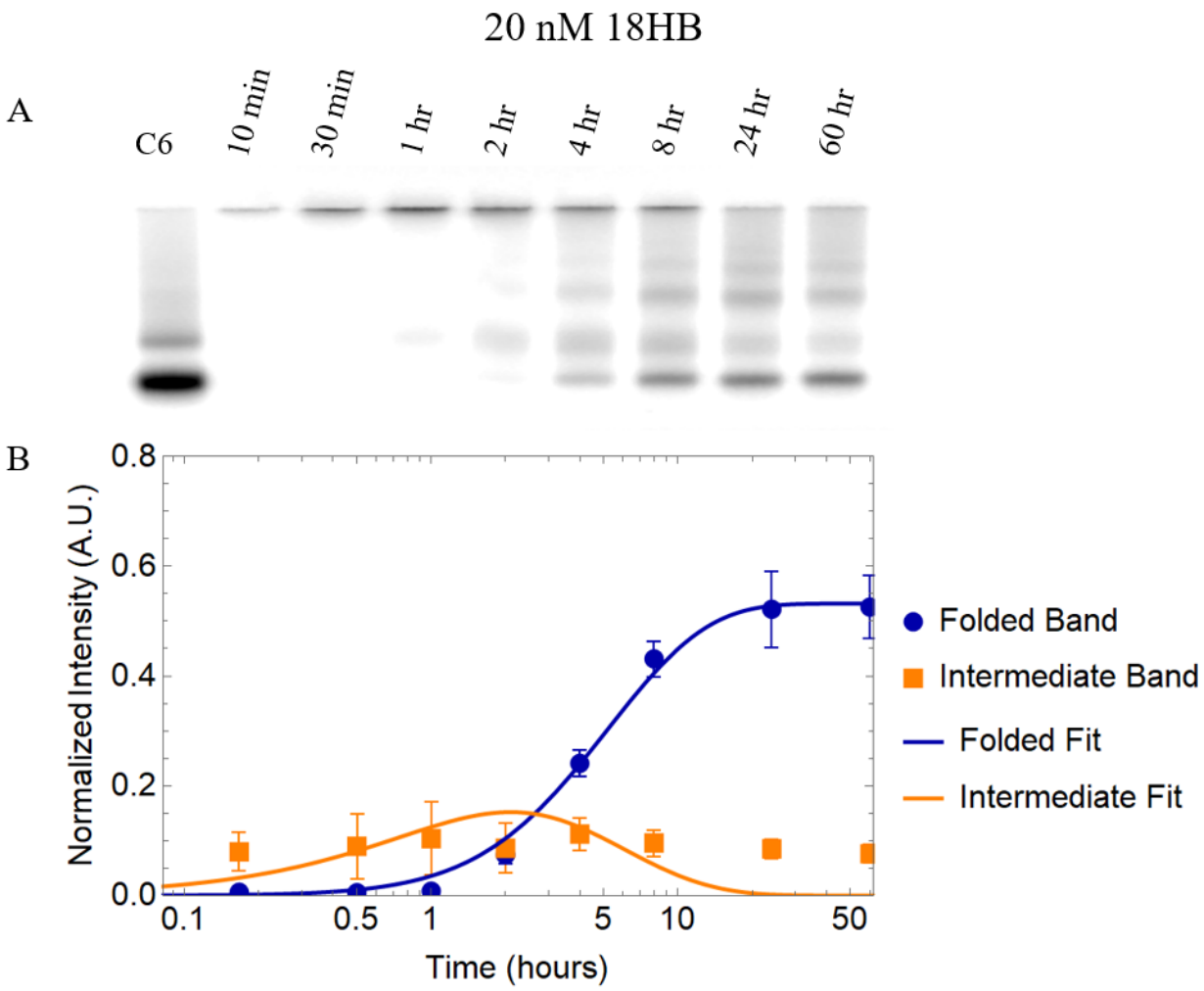

Figure S6. Individual kinetics of folding with 20 nM 18HB staples.(A) Example of laser-scanned agarose gel. First row is a control of 6HB folded at 200 nM staples in a 2.5 day anneal. Other lanes correspond to 20 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively.

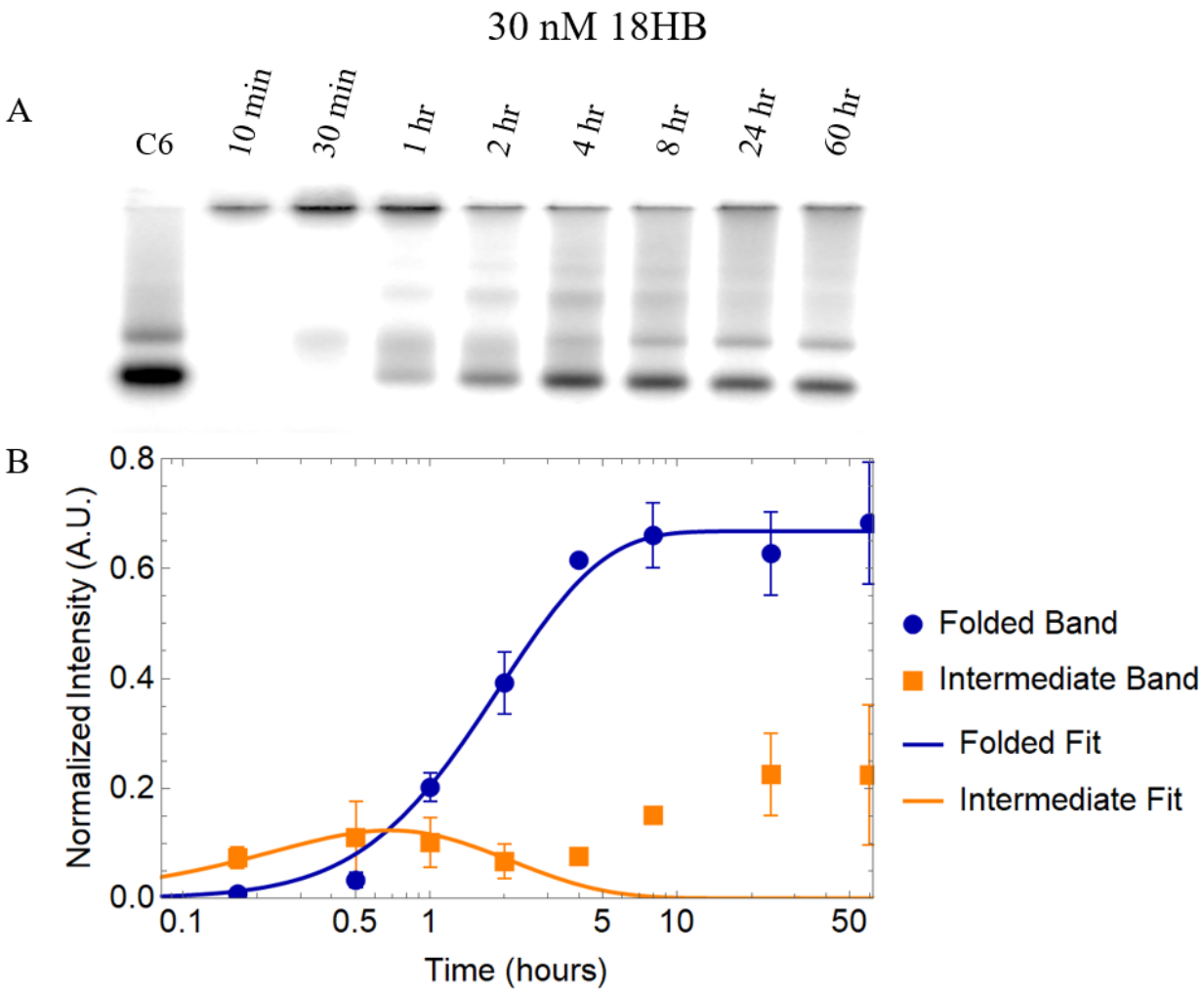

Figure S7. Individual kinetics of folding with 30 nM 18HB staples. (A) Example of laser-scanned agarose gel. First row is a control of 6HB folded at 200 nM staples in a 2.5 day anneal. Other lanes correspond to 30 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively.

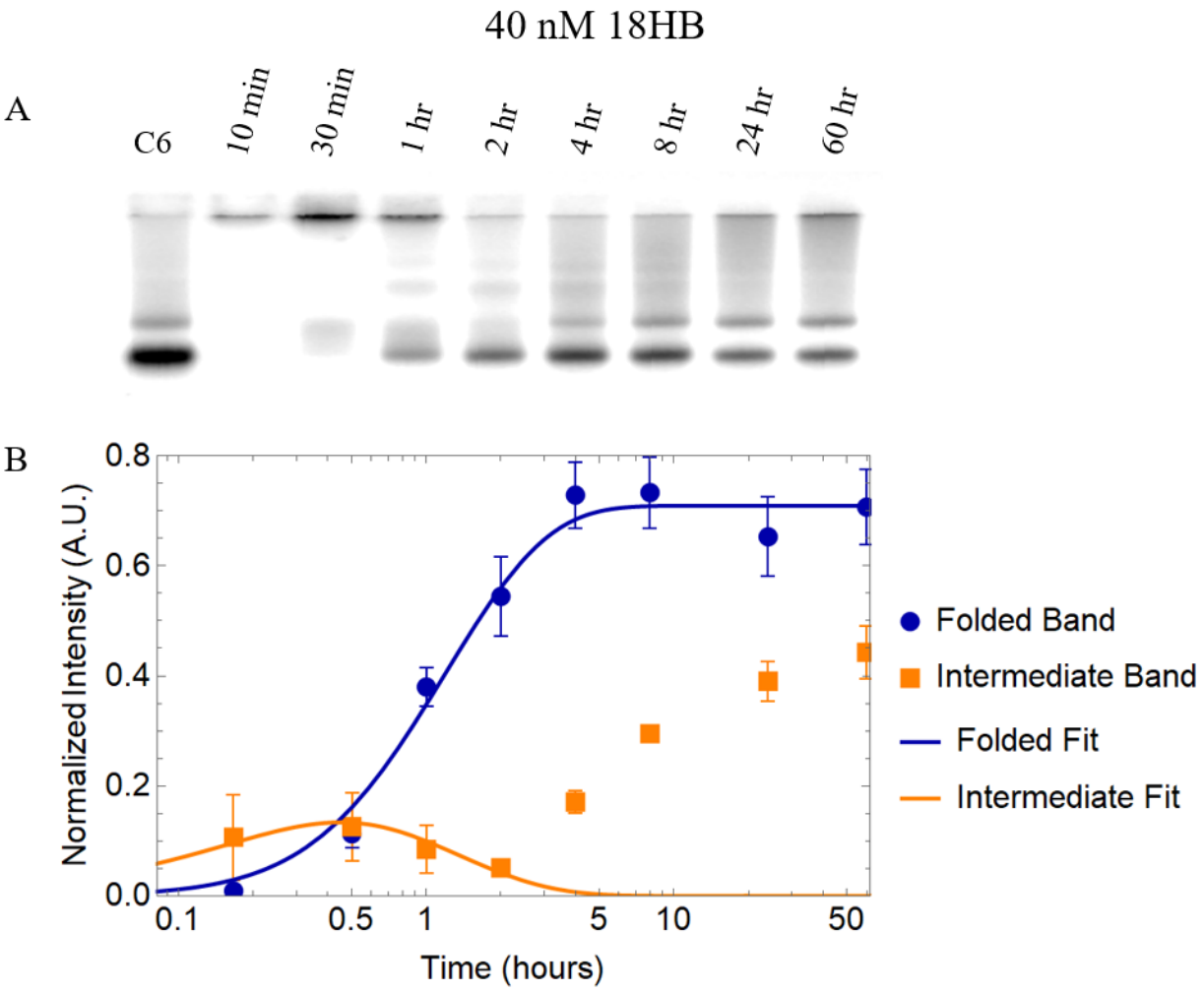

Figure S8. Individual kinetics of folding with 40 nM 18HB staples. (A) Example of laser-scanned agarose gel. First row is a control of 6HB folded at 200 nM staples in a 2.5 day anneal. Other lanes correspond to 40 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively.

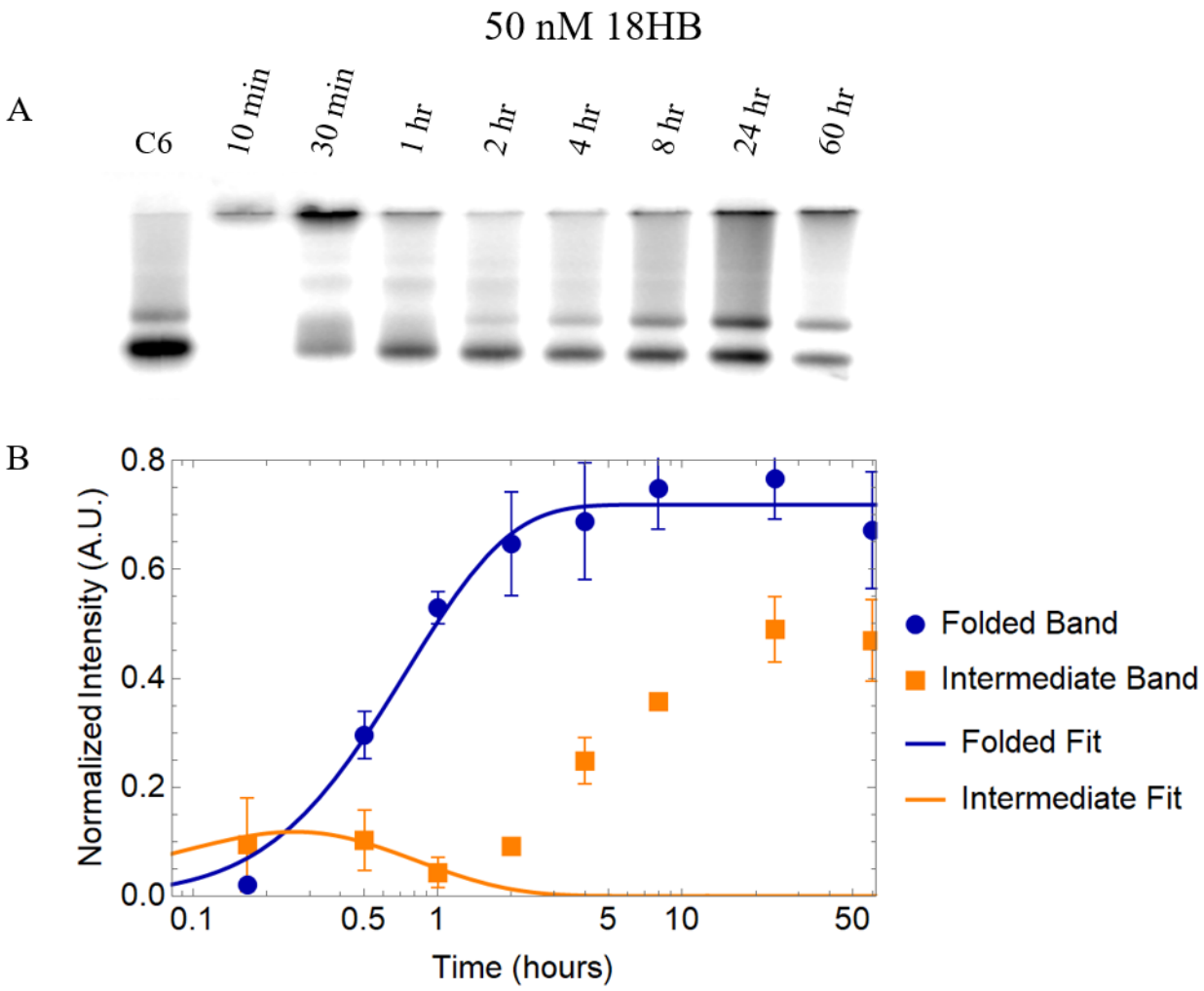

Figure S9. Individual kinetics of folding with 50 nM 18HB staples. (A) Example of laser-scanned agarose gel. First row is a control of 6HB folded at 200 nM staples in a 2.5 day anneal. Other lanes correspond to 50 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively.

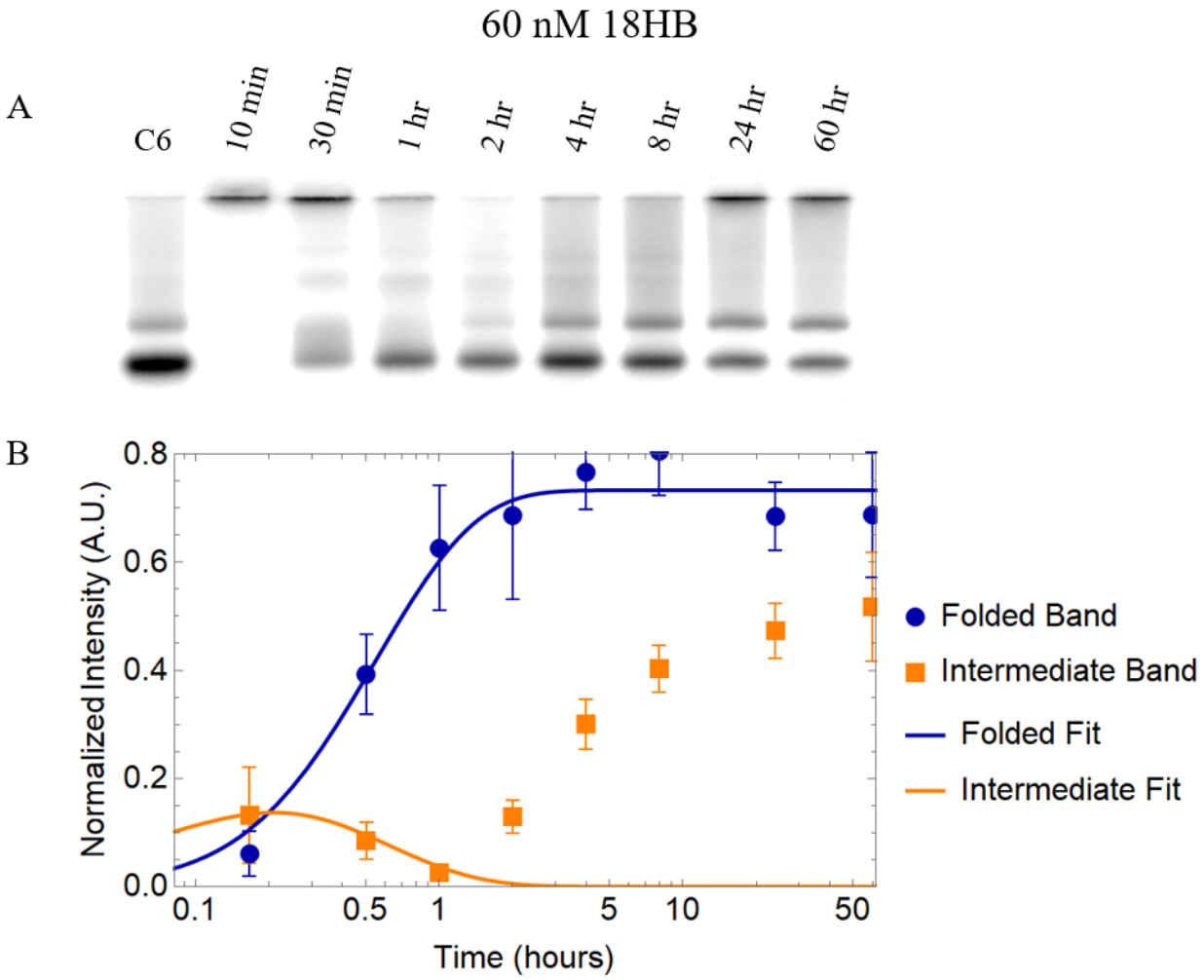

Figure S10. Individual kinetics of folding with 60 nM 18HB staples. (A) Example of laser-scanned agarose gel. First row is a control of 6HB folded at 200 nM staples in a 2.5 day anneal. Other lanes correspond to 60 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively.

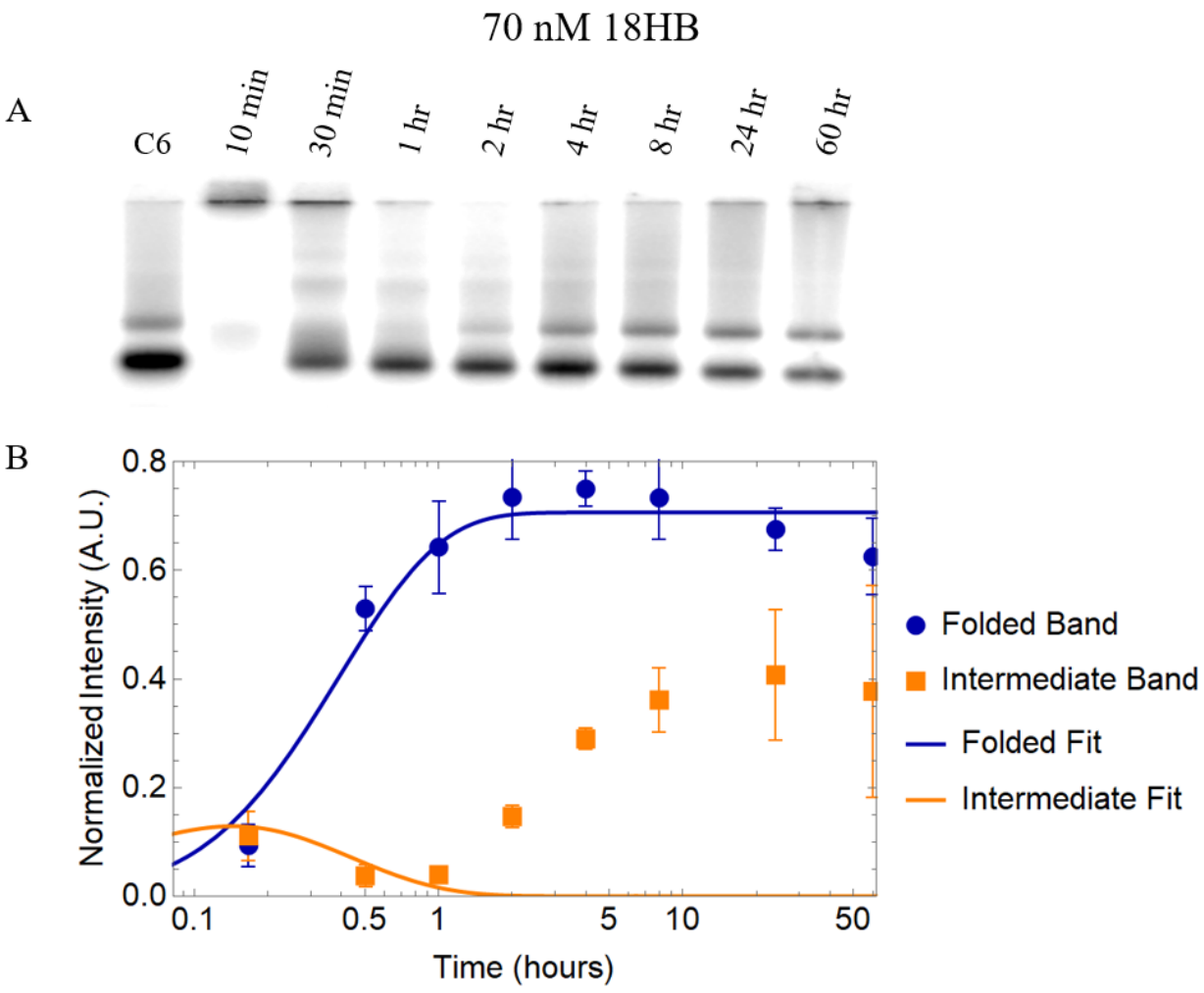

Figure S11. Individual kinetics of folding with 70 nM 18HB staples. (A) Example of laser-scanned agarose gel. First row is a control of 6HB folded at 200 nM staples in a 2.5 day anneal. Other lanes correspond to 70 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively.

Table S1 - Fitted folding rates for 6HB

Conc.	k_1	k_2
20	0.63 ± 0.07	2.54 ± 0.25
30	1.74 ± 0.22	12.0 ± 1.7
40	3.8 ± 0.4	25.0 ± 4.0

Table S2 - Fitted folding rates for 18HB

Conc.	k_1	k_2
20	0.213 ± 0.018	0.90 ± 0.16
30	0.55 ± 0.05	3.0 ± 0.6
40	0.89 ± 0.11	4.5 ± 1.0
50	1.39 ± 0.22	8.2 ± 2.6
60	1.96 ± 0.35	9.5 ± 3.2
70	2.7 ± 0.4	14 ± 4.0

1.3 Initial Co-self-assembly reactions

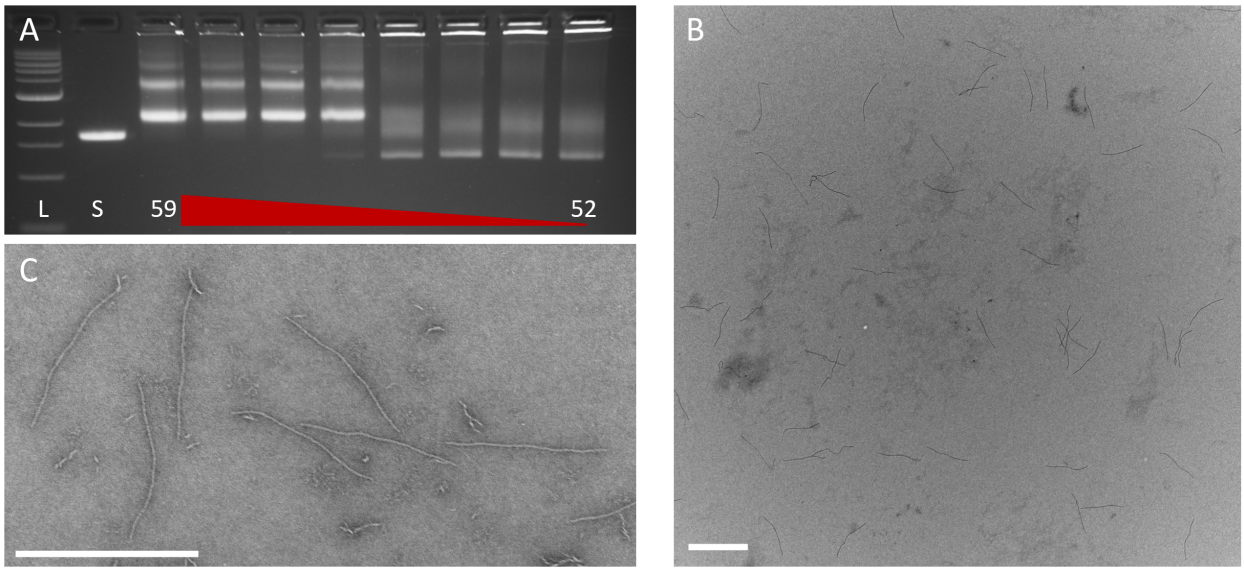

Figure S12. Combination fold of 200 nM 6HB staples and 200 nM 18HB staples. (A) Isothermal folding reactions between 59°C and 52°C run in an agarose gel. (B) Broad view and (C) close up view of TEM sample made from lead band corresponding to 54.2°C. Scale bars = 500 nm.

1.4 Detailed co-self-assembly characterizations

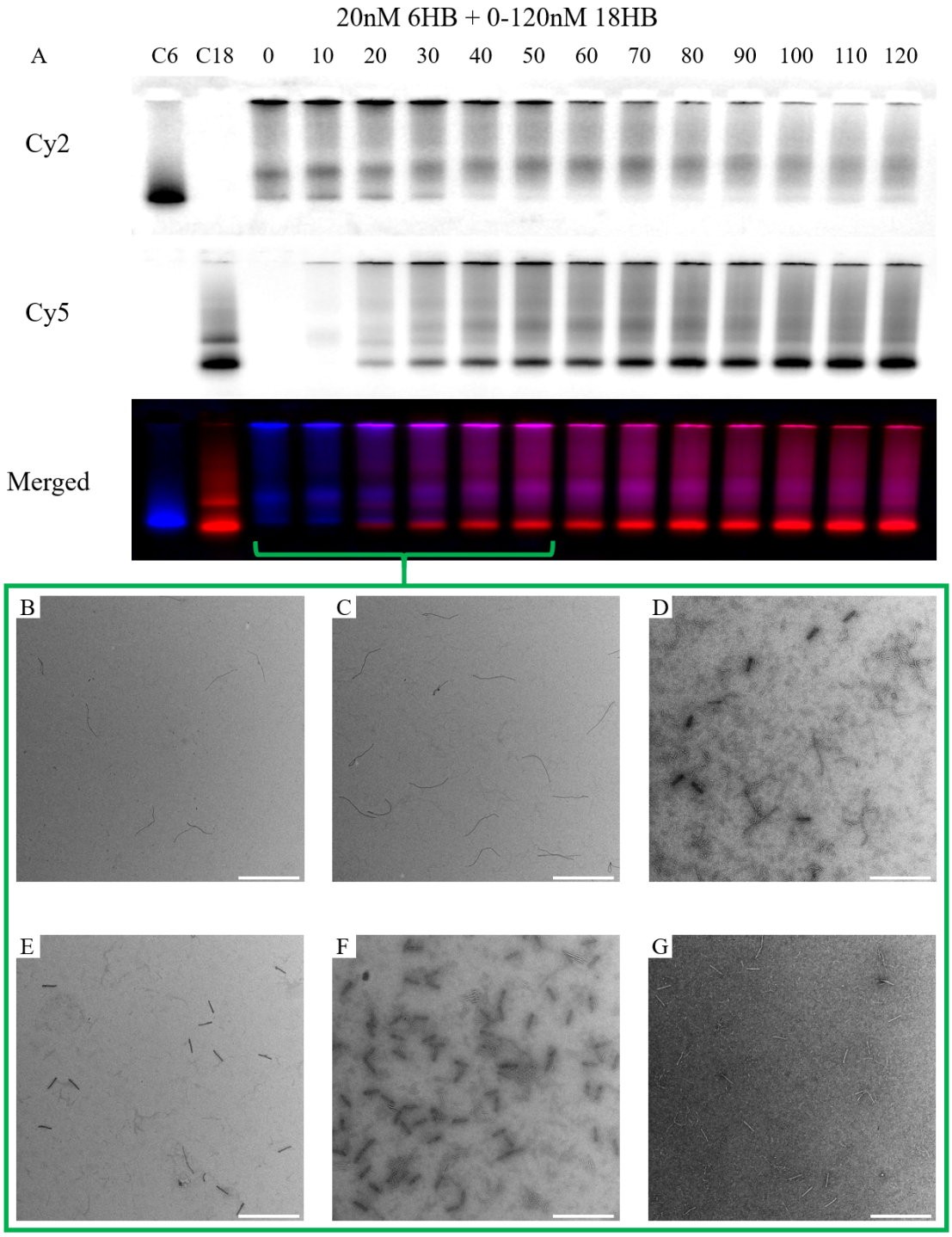

Figure S13. Co-self-assembly with 20 nM 6HB staples and 0-120 nM 18HB staples. (A) Laser scanned images of agarose gel with Cy2, Cy5, and both channels merged. B through G showing TEM images corresponding to the lead bands of 20 nM 6HB + 0 through 50 nM 18HB staples respectively. Scale bars = 500 nm.

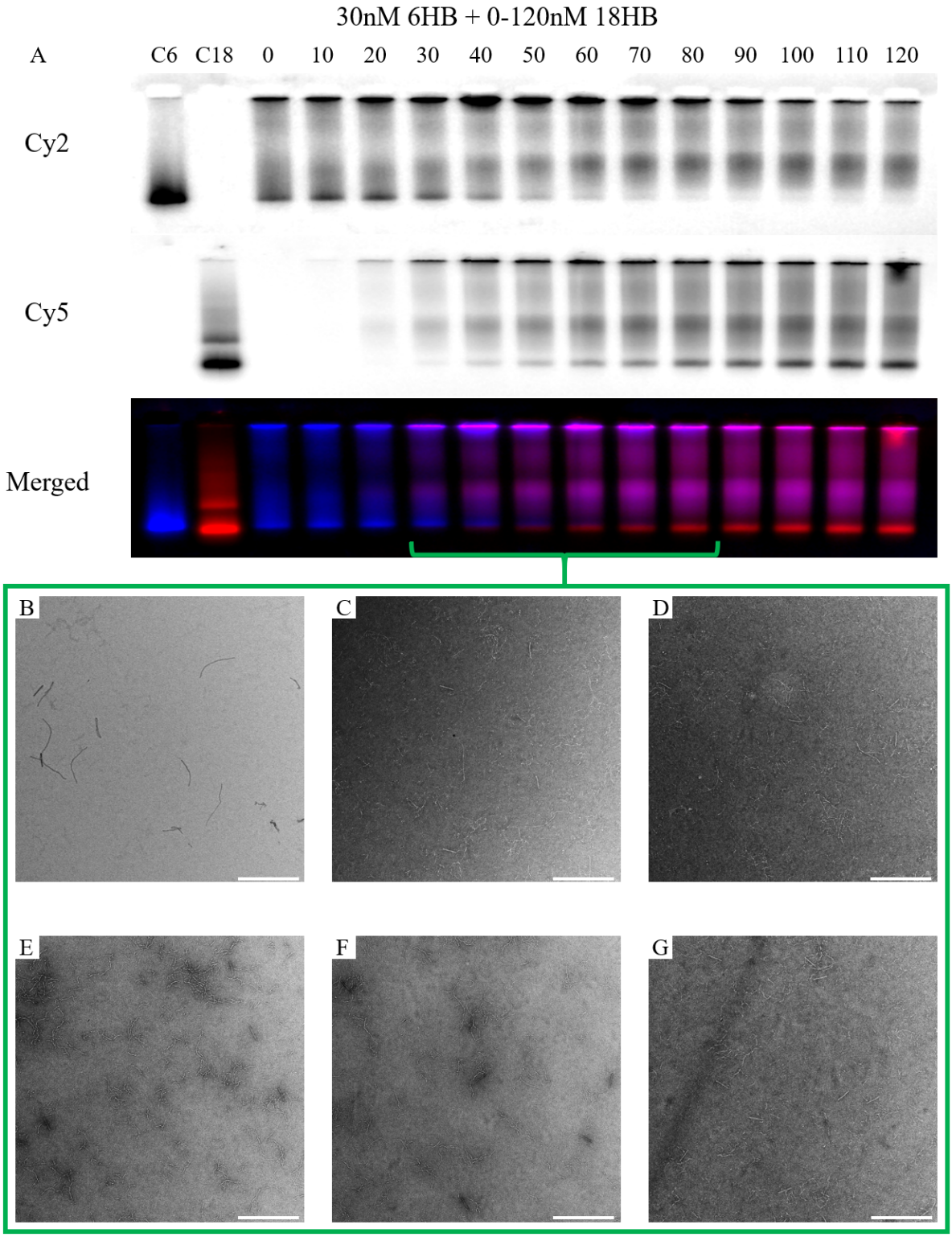

Figure S14. Co-self-assembly with 30 nM 6HB staples and 0-120 nM 18HB staples. (A) Laser scanned images of agarose gel with Cy2, Cy5, and both channels merged. B through G showing TEM images corresponding to the lead bands of 30 nM 6HB + 30 through 80 nM 18HB staples respectively. Scale bars = 500 nm

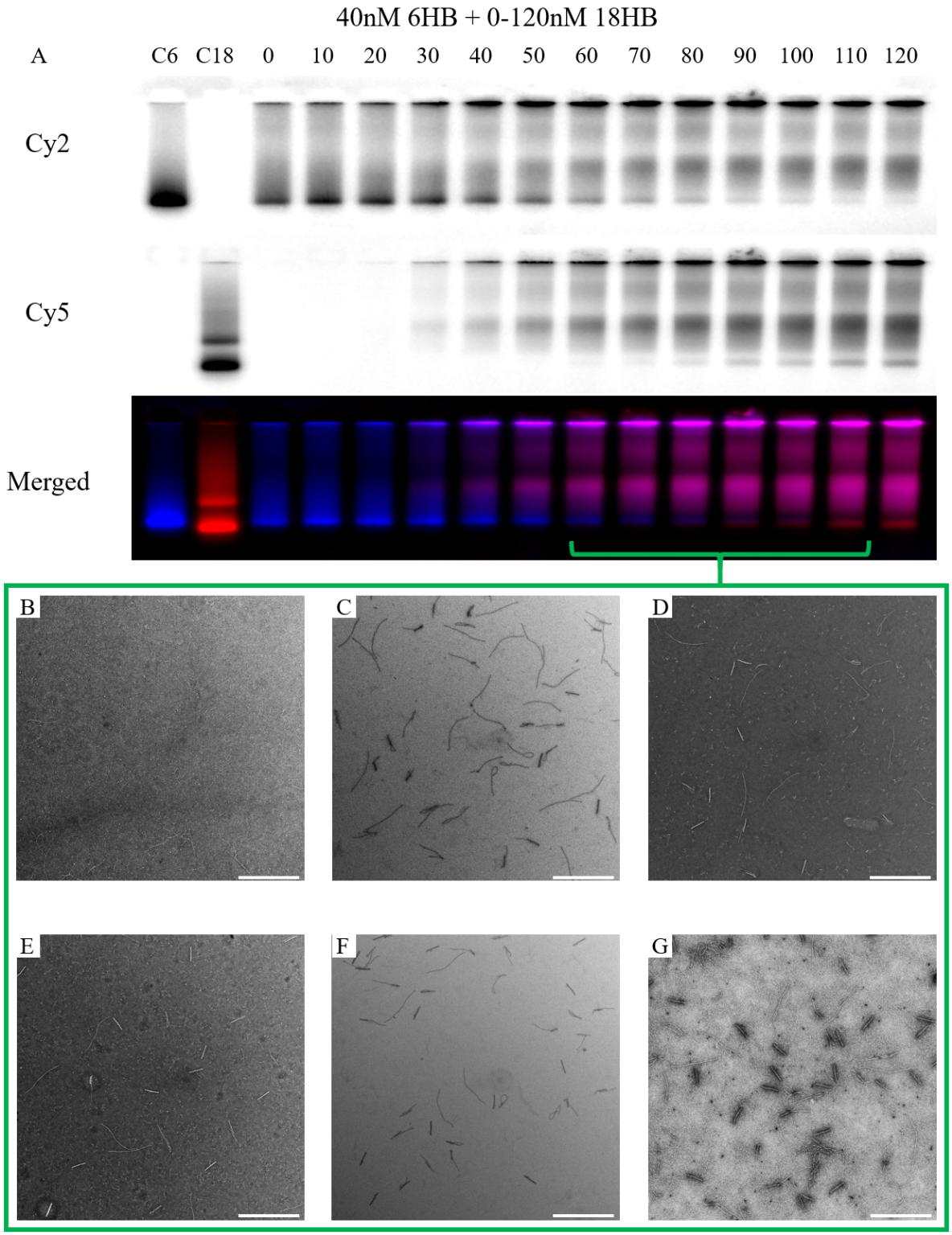

Figure S15. Co-self-assembly with 40 nM 6HB staples and 0-120 nM 18HB staples. (A) Laser scanned images of agarose gel with Cy2, Cy5, and both channels merged. B through G showing TEM images corresponding to the lead bands of 40 nM 6HB + 60 through 110 nM 18HB staples respectively. Scale bars = 500 nm

1.5 Co-self-assembly kinetics

To gain further insight into the co-self-assembly process, we compared the kinetics of folding both individually and in co-self-assembly reactions. Specifically, to gain insight into early folding pathways we examined the slower bands on the gel, which correspond to partially folded structures. We noticed that for all concentrations a broad, slow migrating band appears at early time points and then, more gradually, well-folded bands appear (Fig. S16A). From TEM images of structures excised from these lagging bands we see partly formed structures which reveal a variety of structural intermediates. After 10 minutes, structures excised from the lagging gel band each had one or more region that appeared to be a nucleating 6HB (Fig. S16D). After 30 minutes the lagging band appears to dominantly contain two types of structures (Fig. S16E). One type is a 6HB intermediate which appears to be kinked with some regions stuck to each other. The other types are structures which appear to be a chimera of a partially folded 6HB and partially folded 18HB. For later time points, the lagging band appears to diminish and the overall yield of structures increases (Fig. S16H). This result appears to confirm that early folding events are critical when multiple folding pathways are available. Even though folding conditions may favor 18HB when evaluated as a function of overall yield, smaller regions of the 6HB appear more likely to form at earlier times which is consistent with our predications based on individual kinetics data. Data pertaining to the kinetics of all other co-self-assembly reaction for 20 nM, 30 nM, and 40 nM 6HB staples each combined with 20 nM to 70 nM 18HB staples is provided in below (Figures S17-S35).

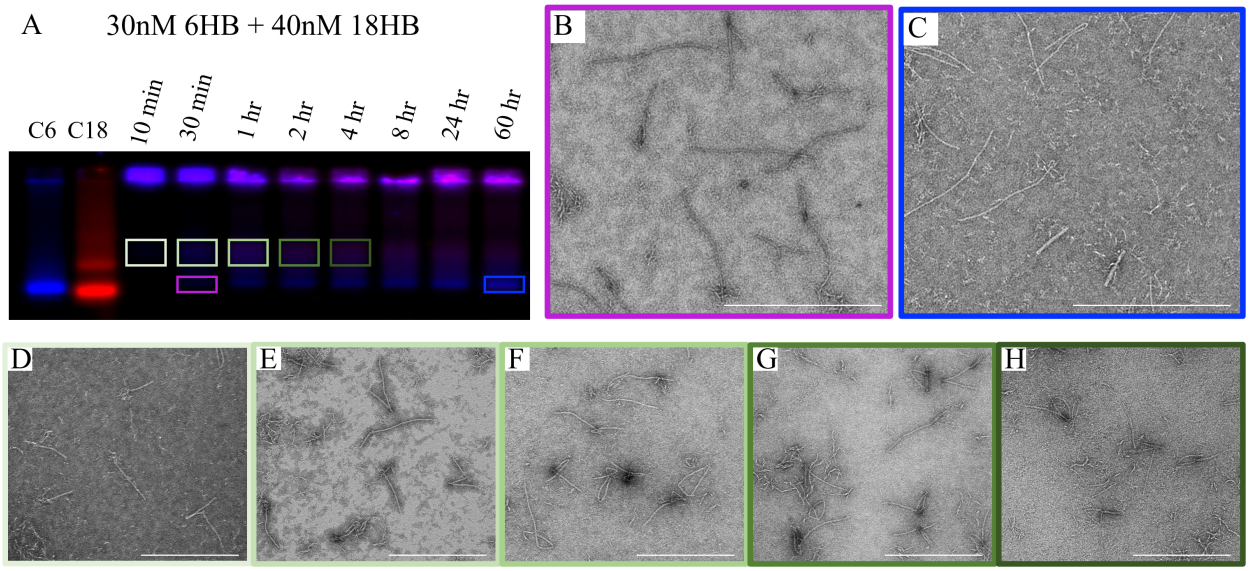

Figure S16. Kinetics of folding in a mixture containing 30 nM 6HB staples and 40 nM 18HB staples. TEM images showing partially folded structures purified from agarose gels corresponding to co-self-assembly reaction cryo-quenched after (A) 10 minutes, (B) 30 minutes, (C) 1 hour, (D) 2 hours, and € 4 hours. Laser scanned agarose gel images of a folding reactions which have been cryo-quenched at times ranging from 10 minutes to 60 hours. TEM images of well folded structures from cryo-quenched reaction after (G) 30 minutes and (H) 60 hours. Scale bars = 500 nm.

1.6 Modeling of co-self-assembly reaction kinetics

We quantified kinetics assays of co-self-assembly reactions for mixtures containing the following : 20 nM 6HB staples and 20 to 70 nM 18HB staples (Figure S13 through Figure S18); 30 nM 6HB staples and 20 to 70 nM 18HB staples (Figure S19 through Figure S24); 40 nM 6HB staples and 20 to 70 nM 18HB staples (Figure S25 through Figure S30). Similar to the analysis of individual folding kinetics, later time points for all fits were subject to reduced weights ensure the model fit earlier folding behavior better. Our model assumes there the transitions between partially folded and fully folded states are irreversible, the fitted functions for partially folded structures

must necessarily go to zero at longer times. This is clearly not true when examining the experimental data and it is likely that the remaining traces of structures within the regions corresponding to partially folded structures may have come from previously aggregated structures, similar to the appearance of 18HB dimers at later timepoints (Figure S2). For simplicity and for the sake of better describing early folding behaviors, preferential weighting was given to data from earlier time points. Band intensities for gels imaged in a Cy2 fluorescence channel were normalized relative to the 6HB control lane. Likewise, band intensities for gels imaged in the Cy5 fluorescence channel were normalized relative to the 18HB control lane. The data for each channel was fit separately to the same 3-state model as described in section 1.1. All assays corresponding to co-self-assembly reactions containing 20 nM and 40 nM 6HB staples were replicated in duplicate while assays corresponding to co-self-assembly reaction containing 30 nM 6HB staples were repeated in triplicate.

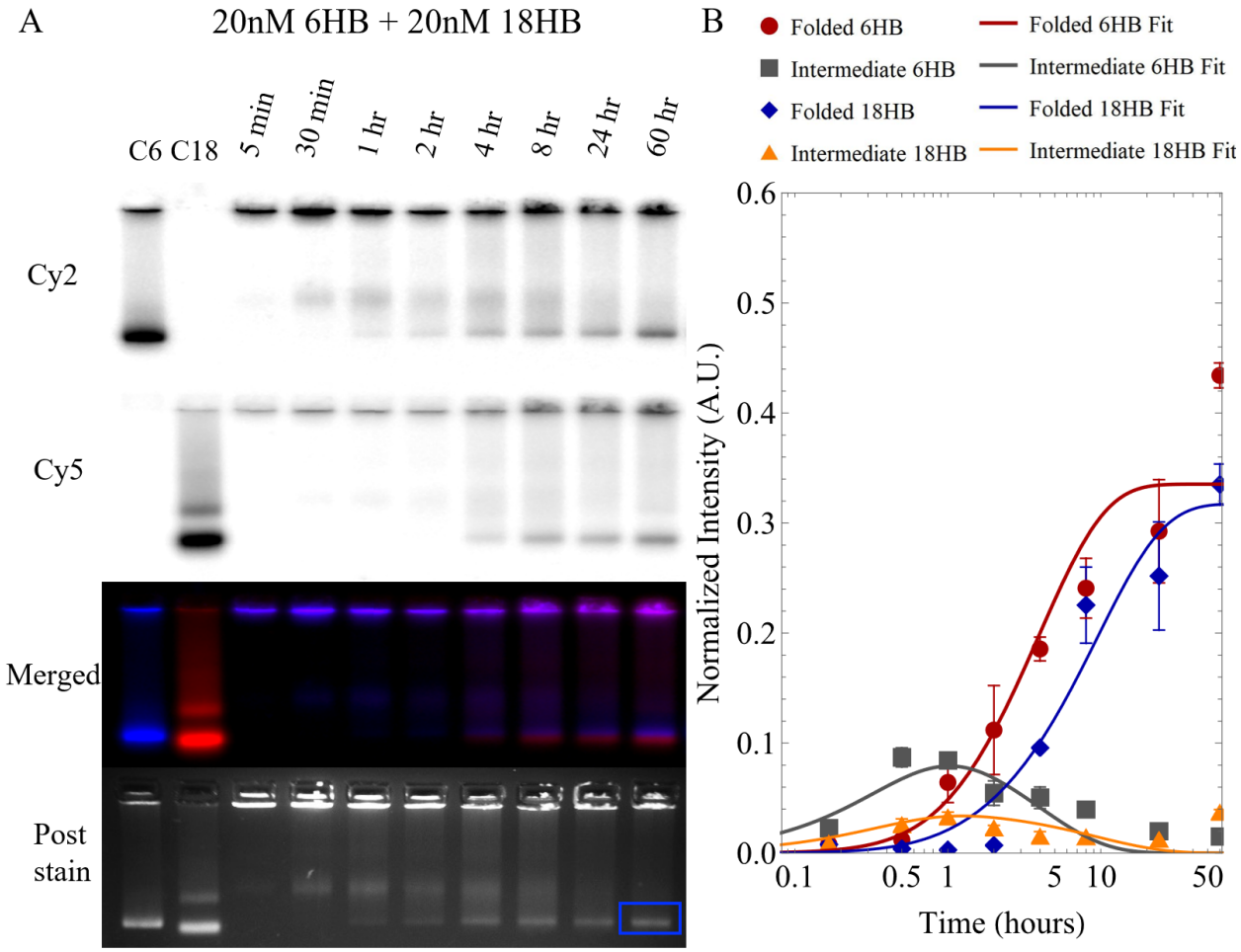

Figure S17. Kinetics of co-self-assembly with 20 nM 6HB staples and 20 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 20 nM 6HB and 20 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

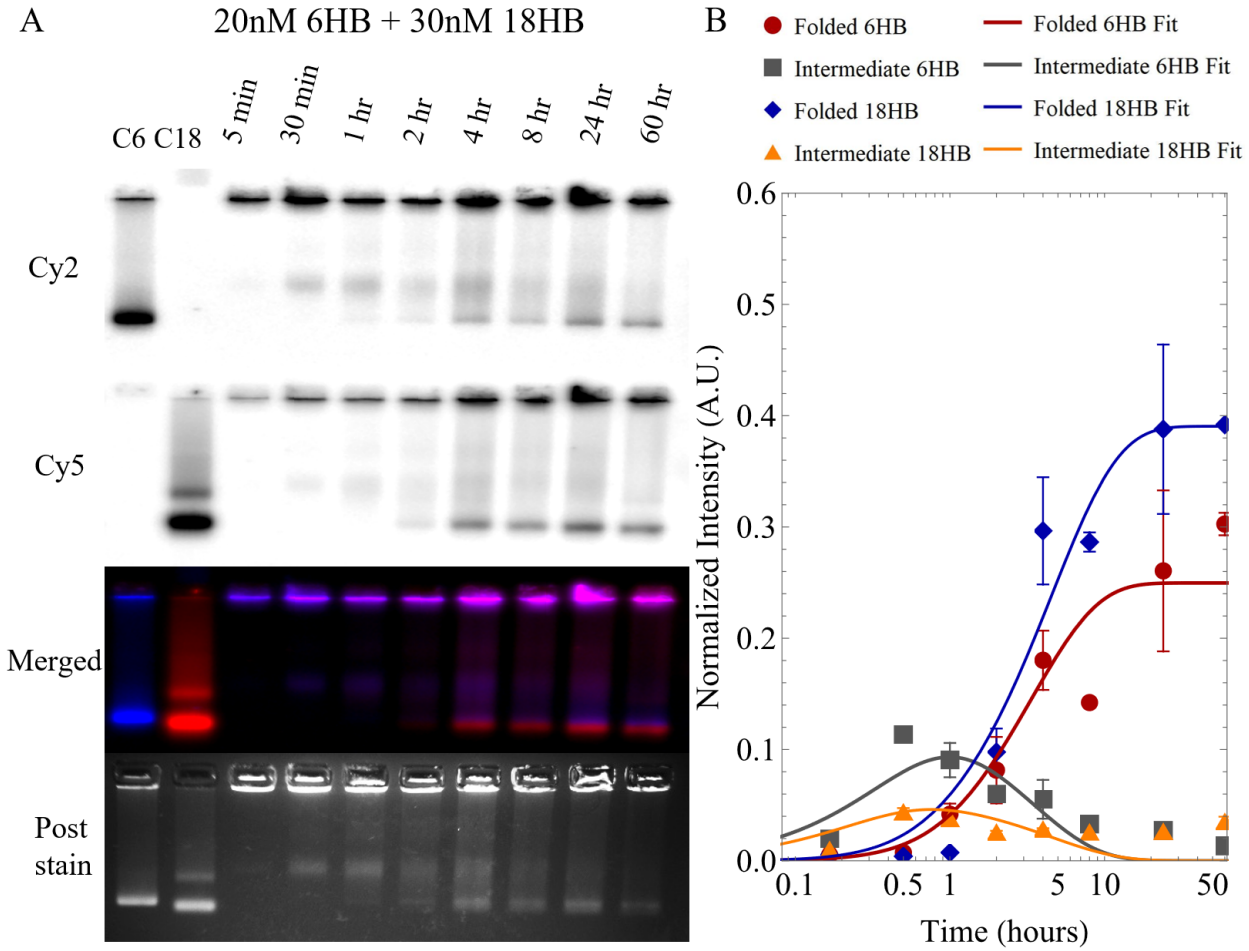

Figure S18. Kinetics of co-self-assembly with 20 nM 6HB staples and 30 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 20 nM 6HB and 30 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

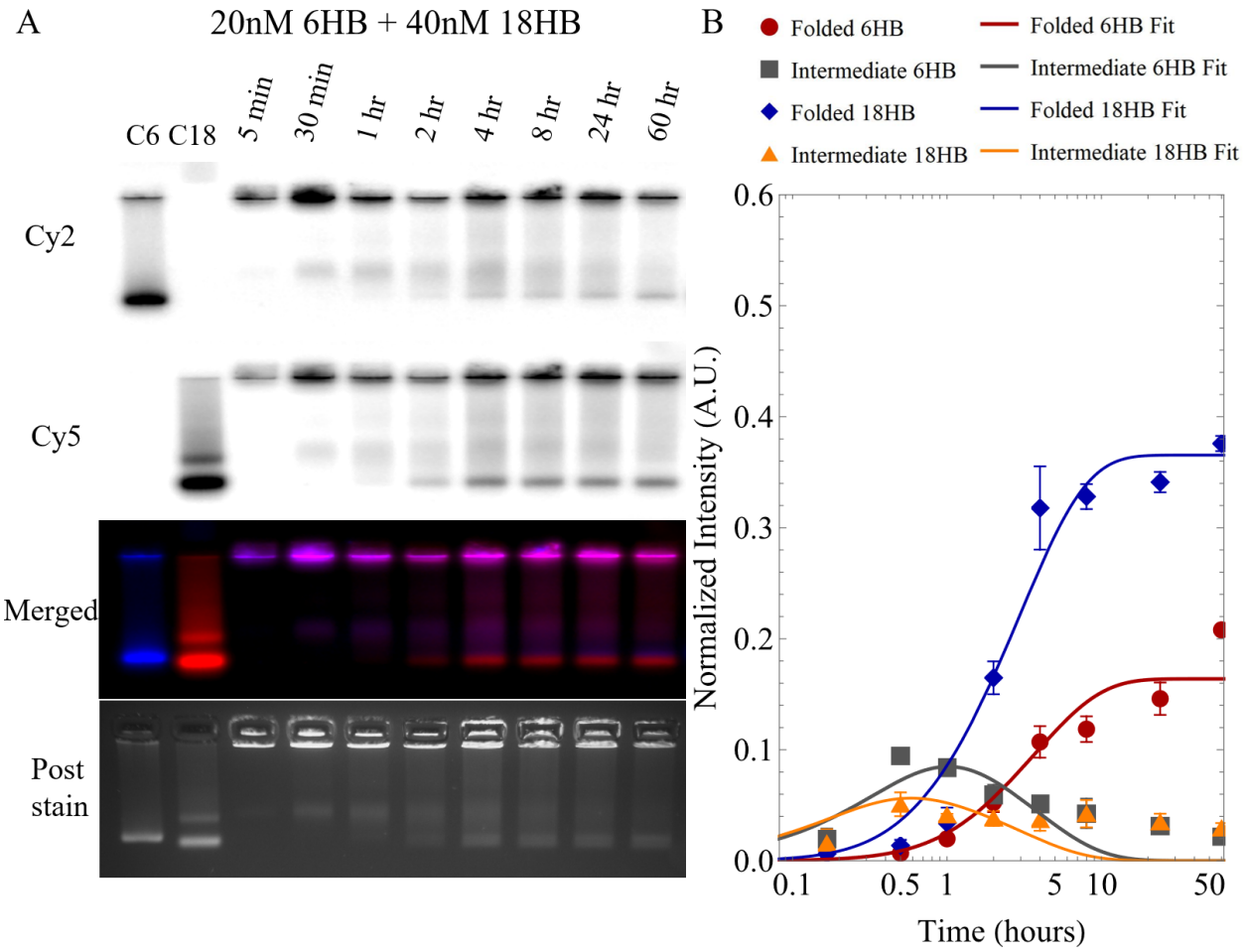

Figure S19. Kinetics of co-self-assembly with 20 nM 6HB staples and 40 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 20 nM 6HB and 40 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

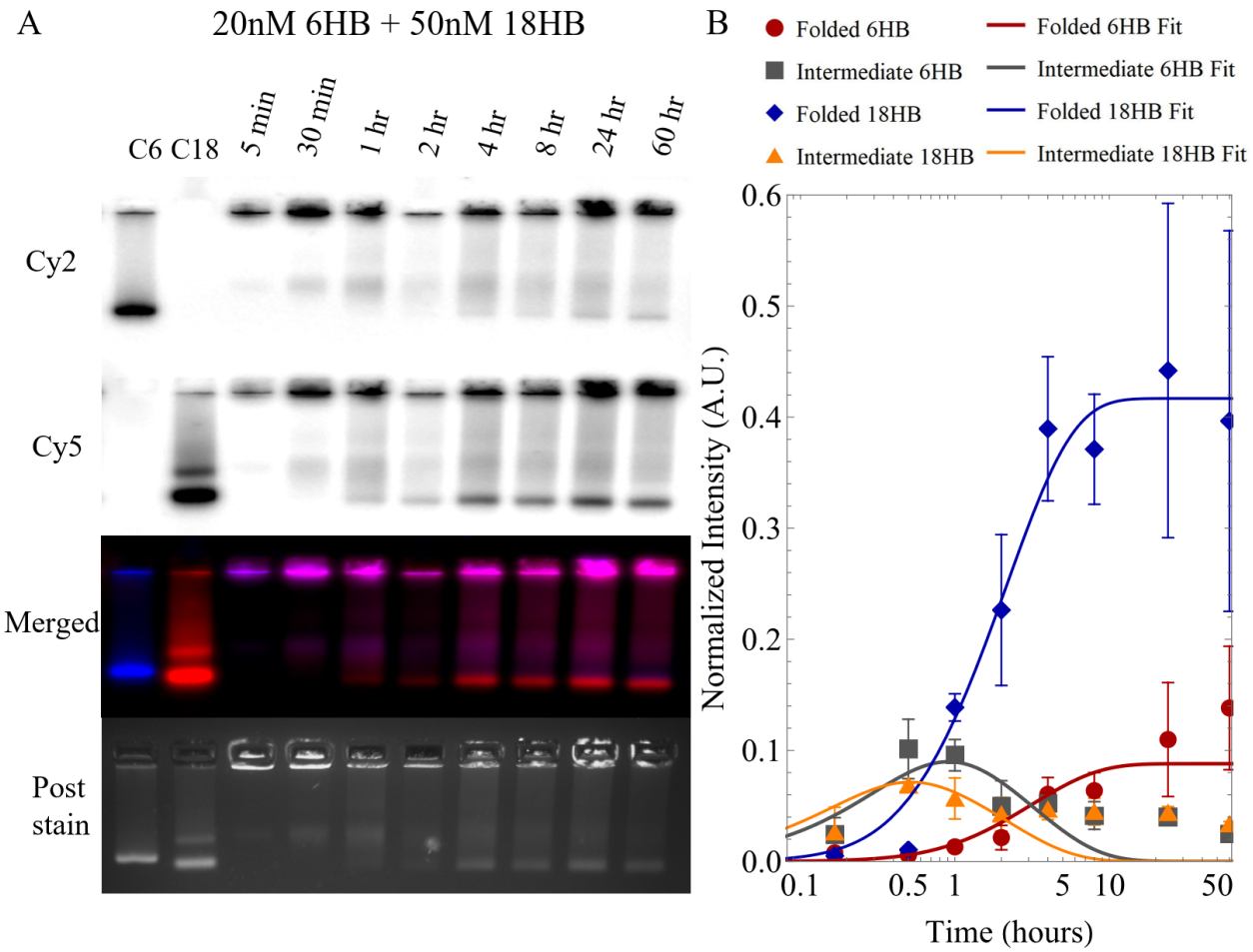

Figure S20. Kinetics of co-self-assembly with 20 nM 6HB staples and 50 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 20 nM 6HB and 50 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

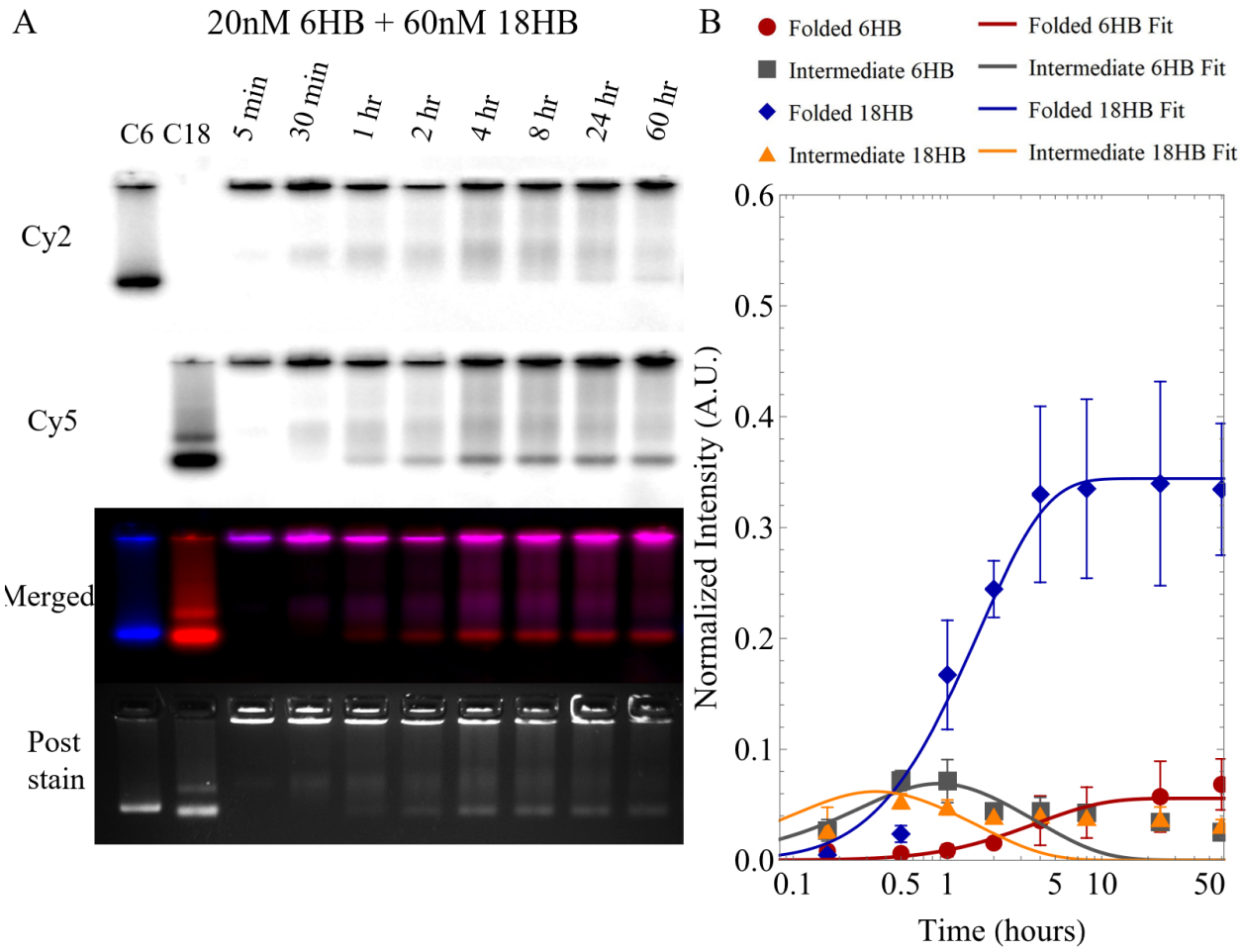

Figure S21. Kinetics of co-self-assembly with 20 nM 6HB staples and 60 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 20 nM 6HB and 60 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

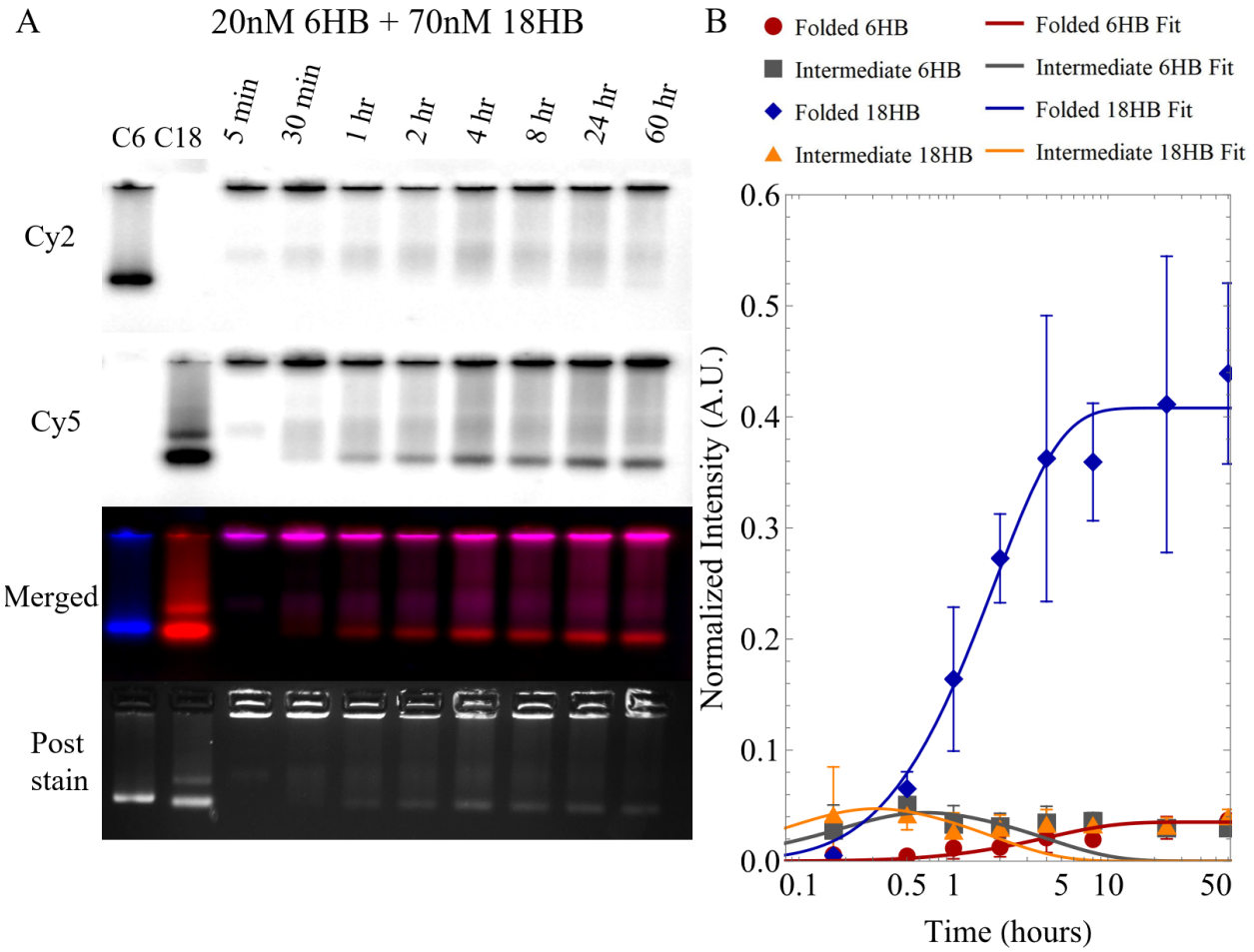

Figure S22. Kinetics of co-self-assembly with 20 nM 6HB staples and 70 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 20 nM 6HB and 70 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

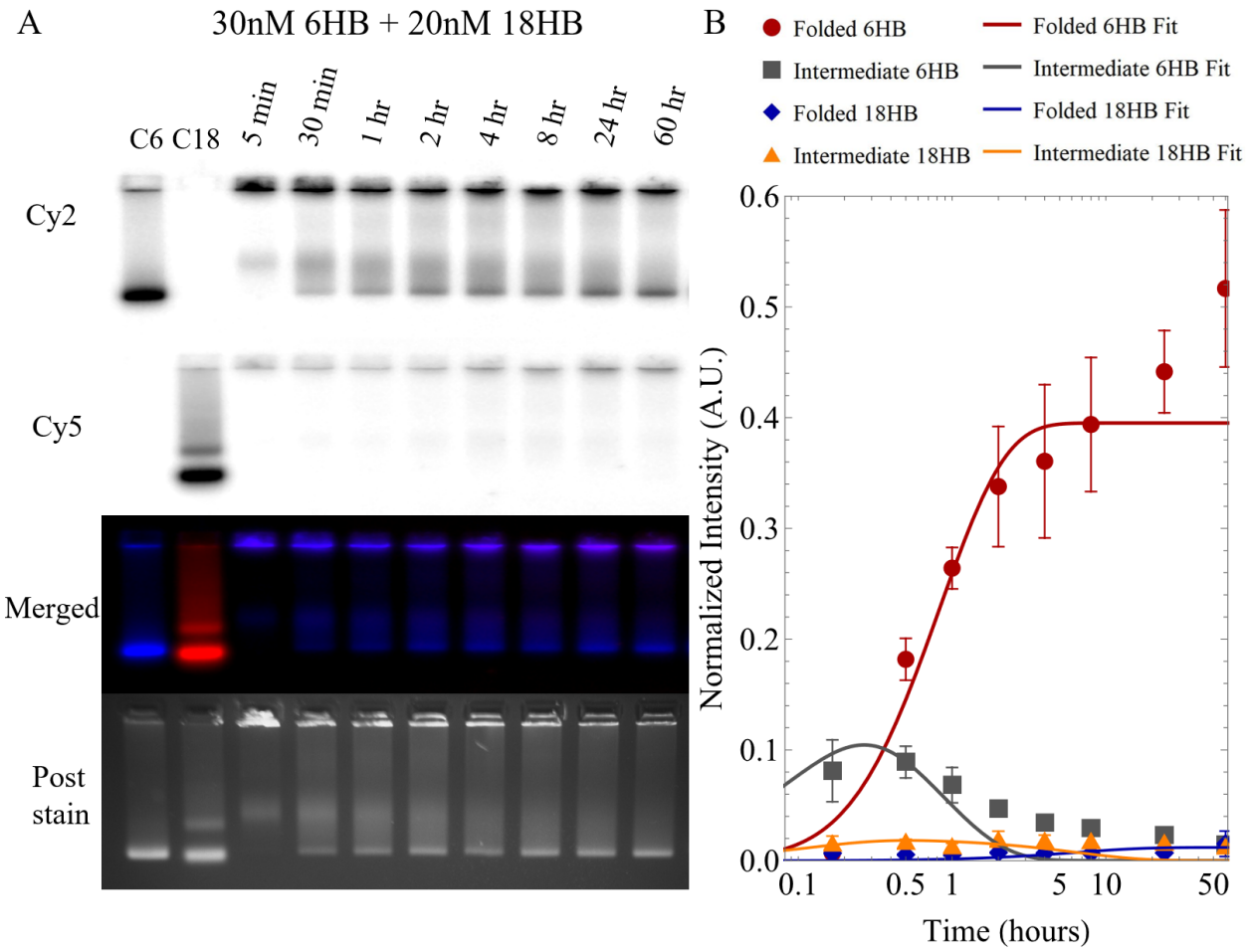

Figure S23. Kinetics of co-self-assembly with 30 nM 6HB staples and 20 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 30 nM 6HB and 20 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

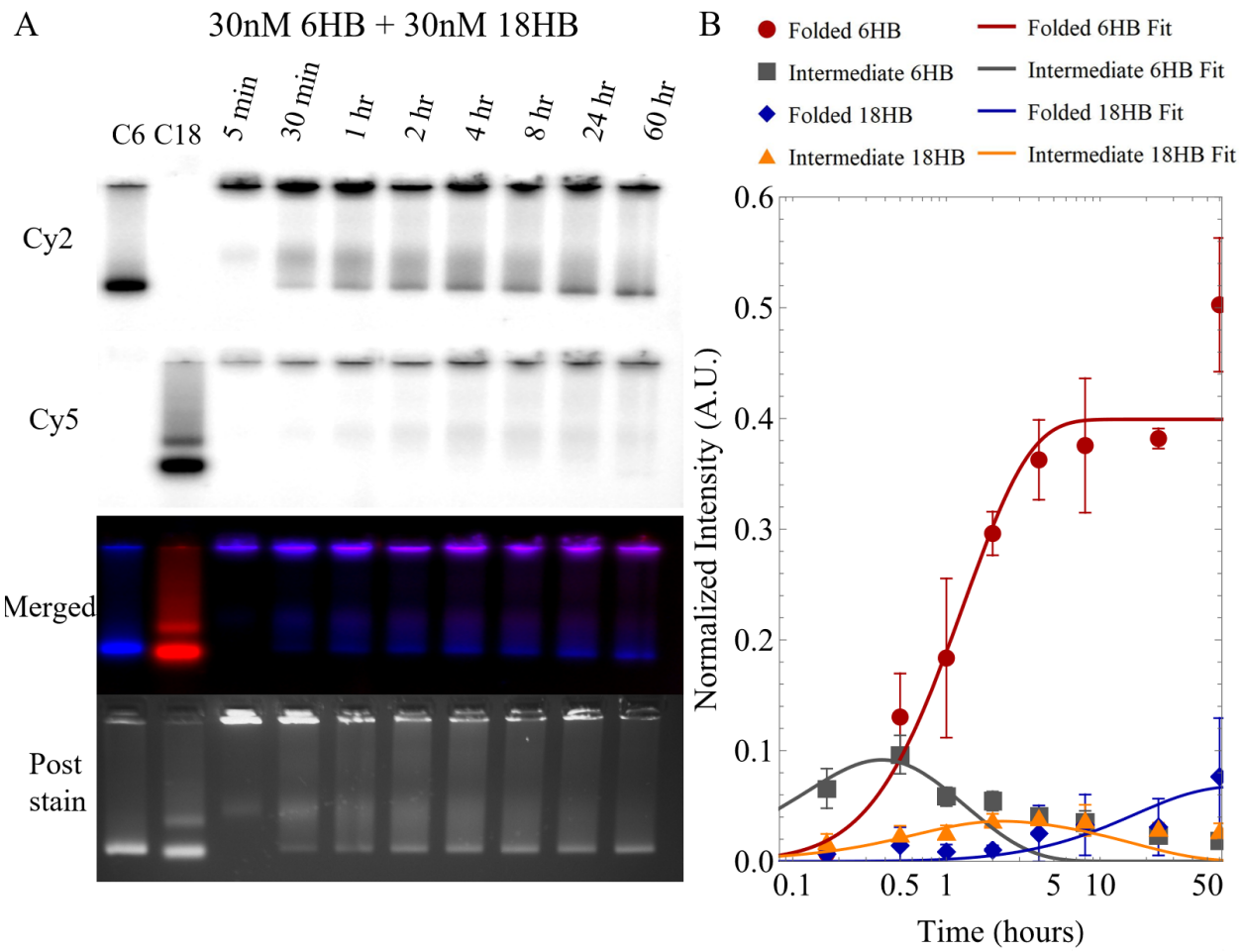

Figure S24. Kinetics of co-self-assembly with 30 nM 6HB staples and 30 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 30 nM 6HB and 30 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

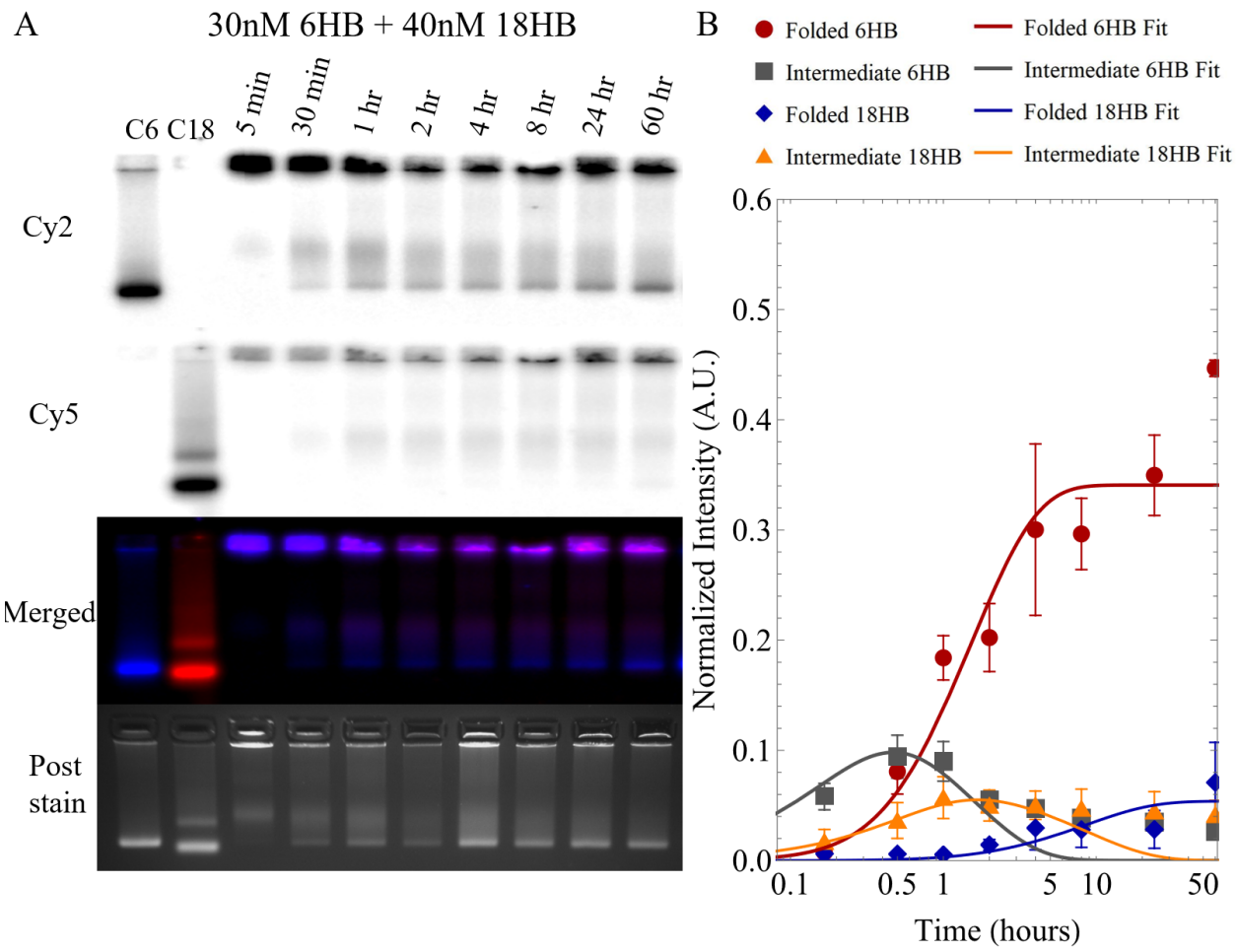

Figure S25. Kinetics of co-self-assembly with 30 nM 6HB staples and 40 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 30 nM 6HB and 40 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

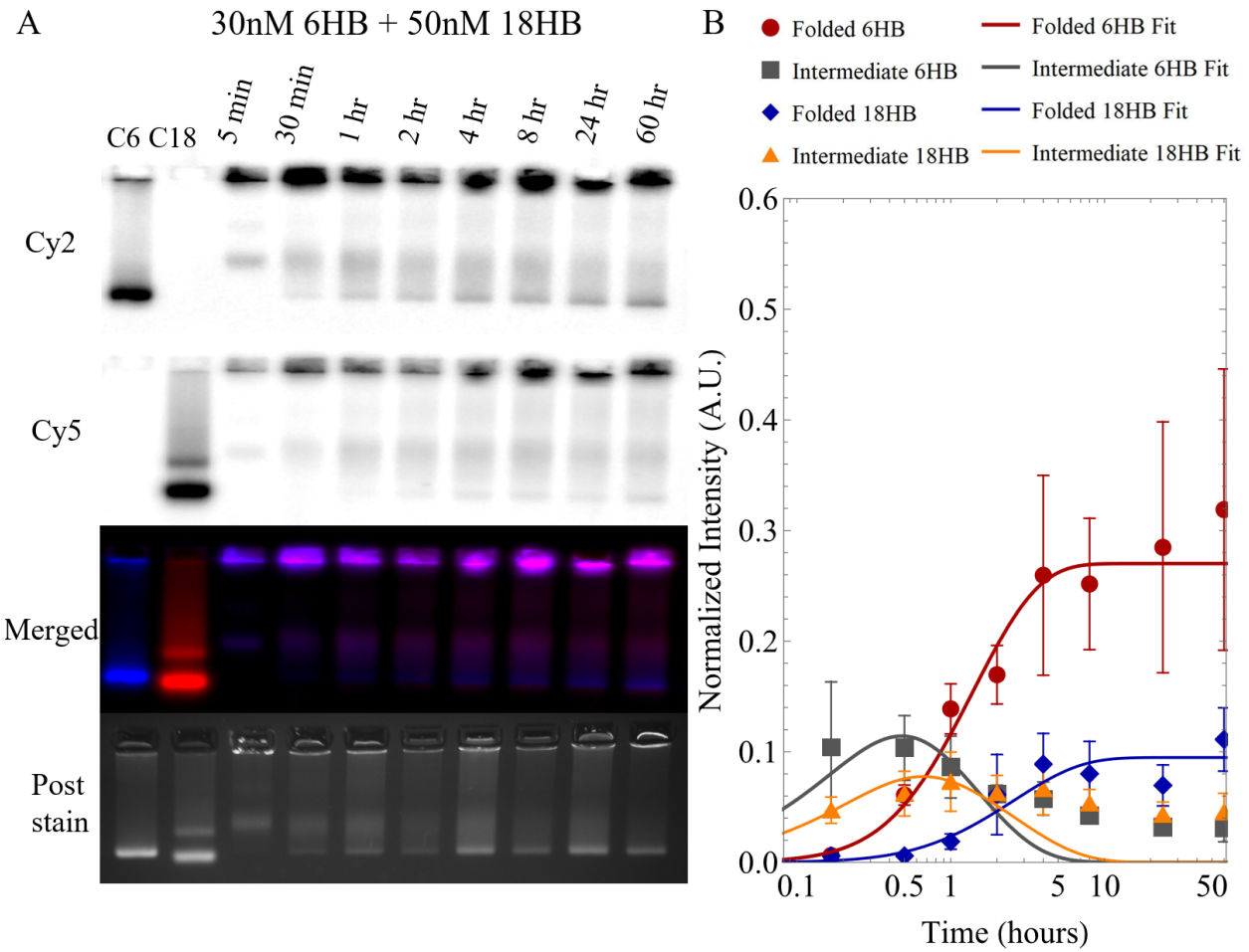

Figure S26. Kinetics of co-self-assembly with 30 nM 6HB staples and 50 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 30 nM 6HB and 50 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

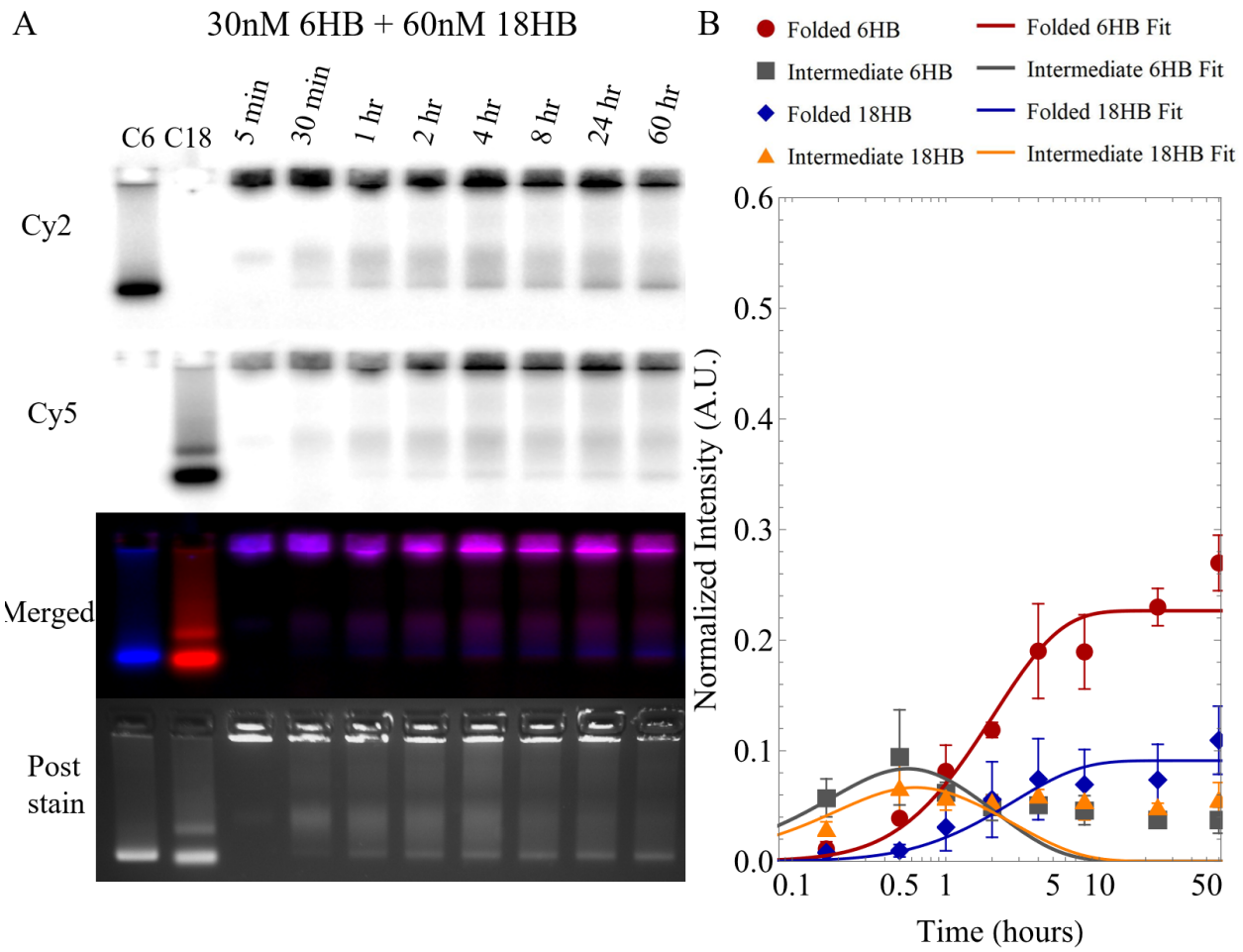

Figure S27. Kinetics of co-self-assembly with 30 nM 6HB staples and 60 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 30 nM 6HB and 60 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

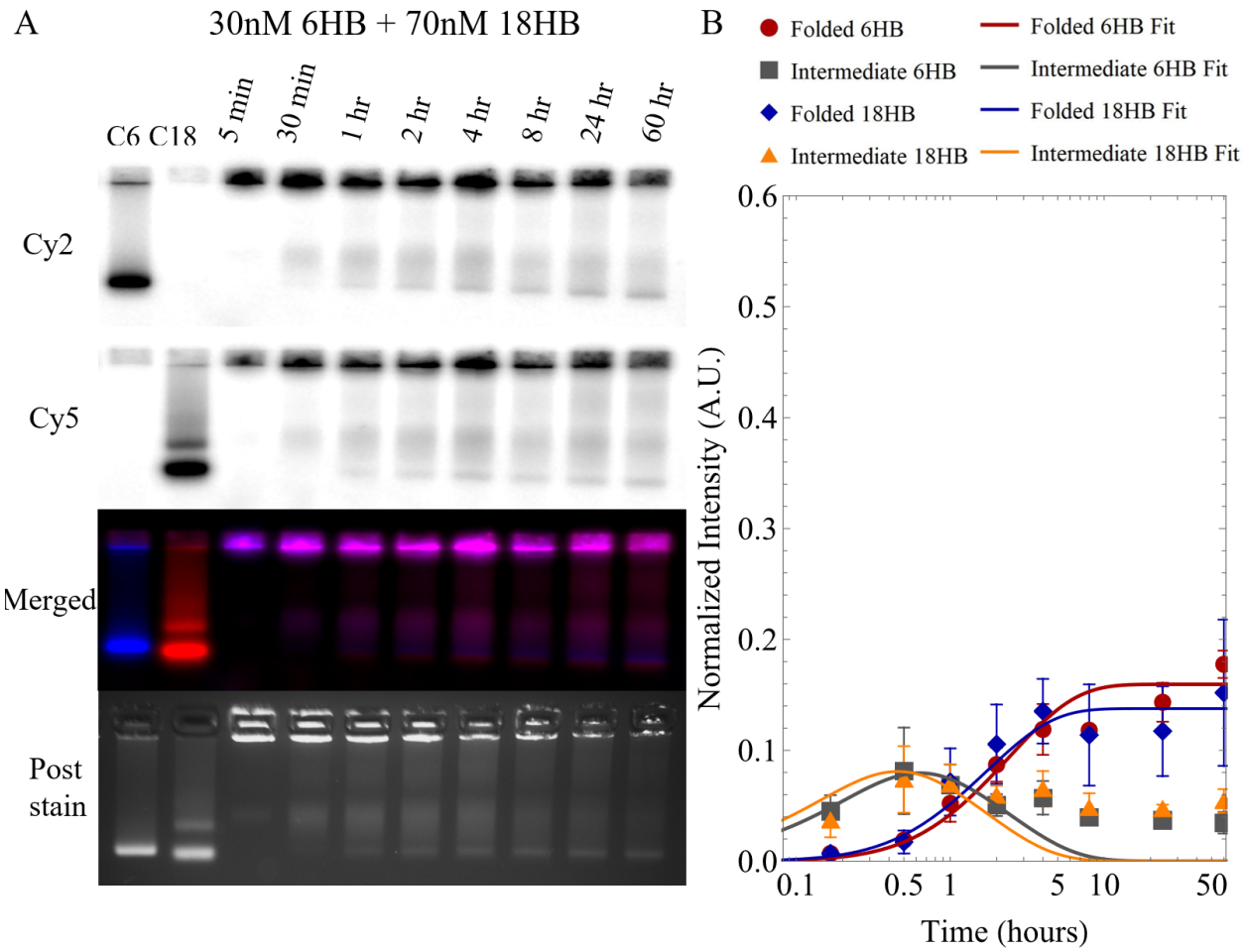

Figure S28. Kinetics of co-self-assembly with 30 nM 6HB staples and 70 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 30 nM 6HB and 70 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

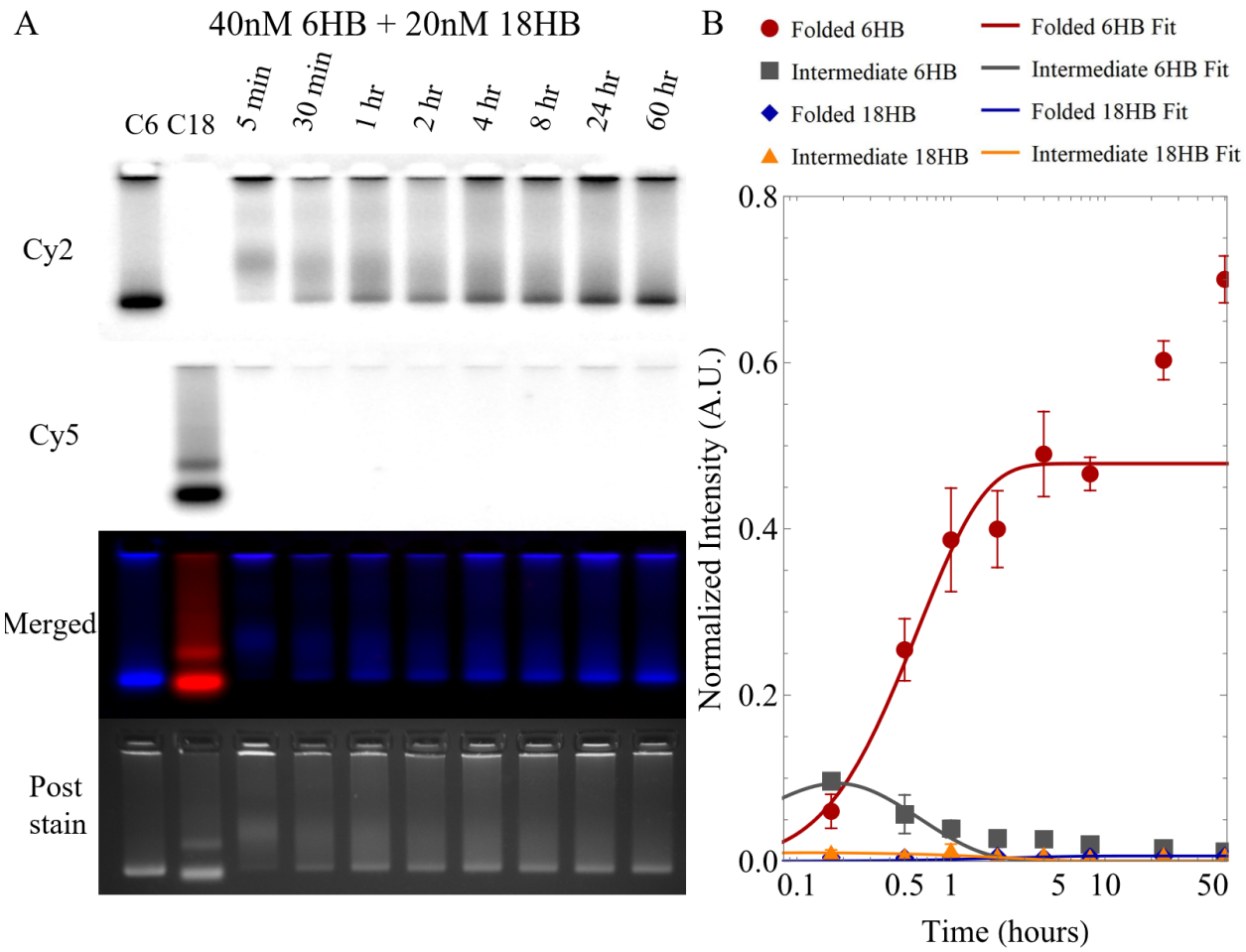

Figure S29. Kinetics of co-self-assembly with 40 nM 6HB staples and 20 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 40 nM 6HB and 20 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

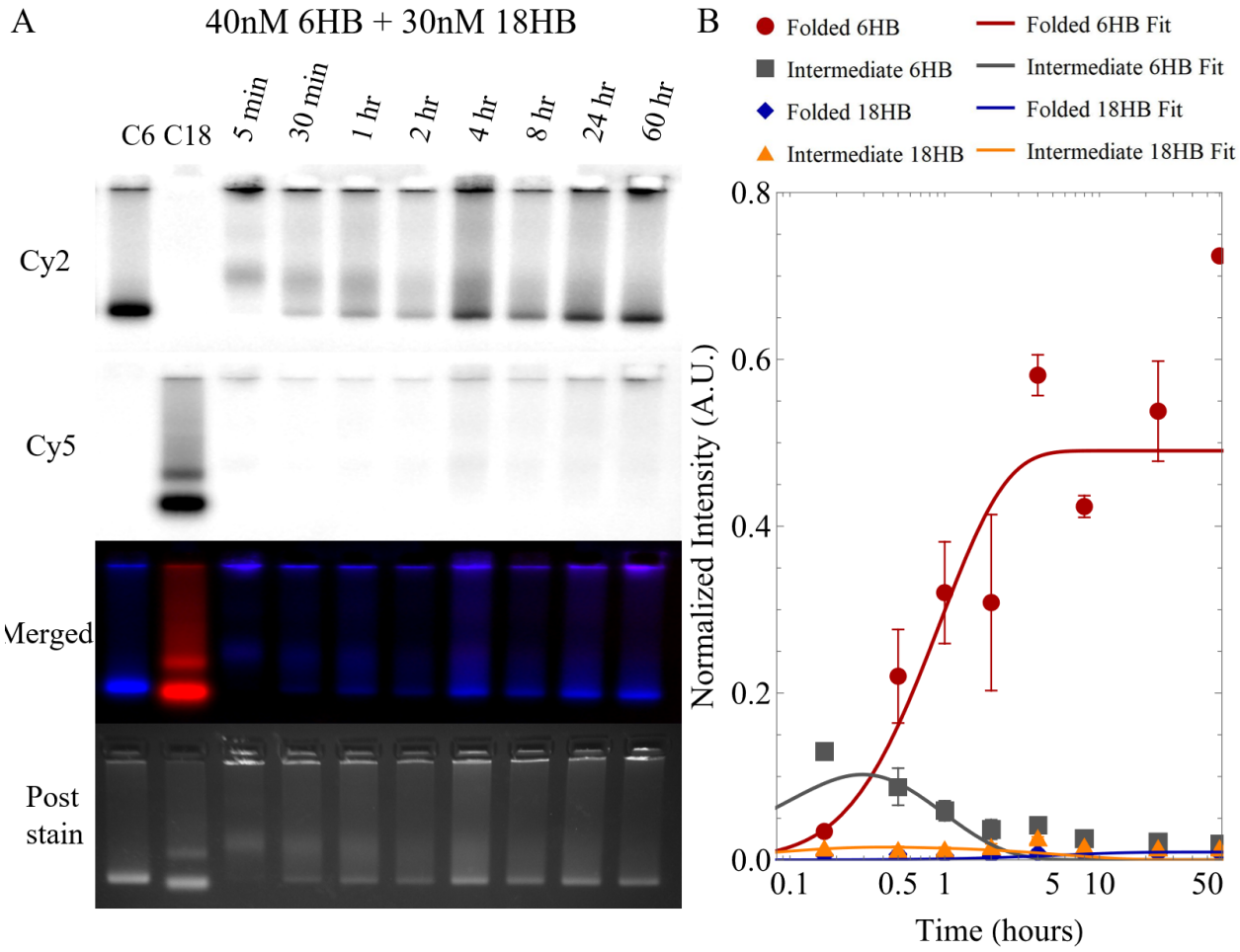

Figure S30. Kinetics of co-self-assembly with 40 nM 6HB staples and 30 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 40 nM 6HB and 30 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

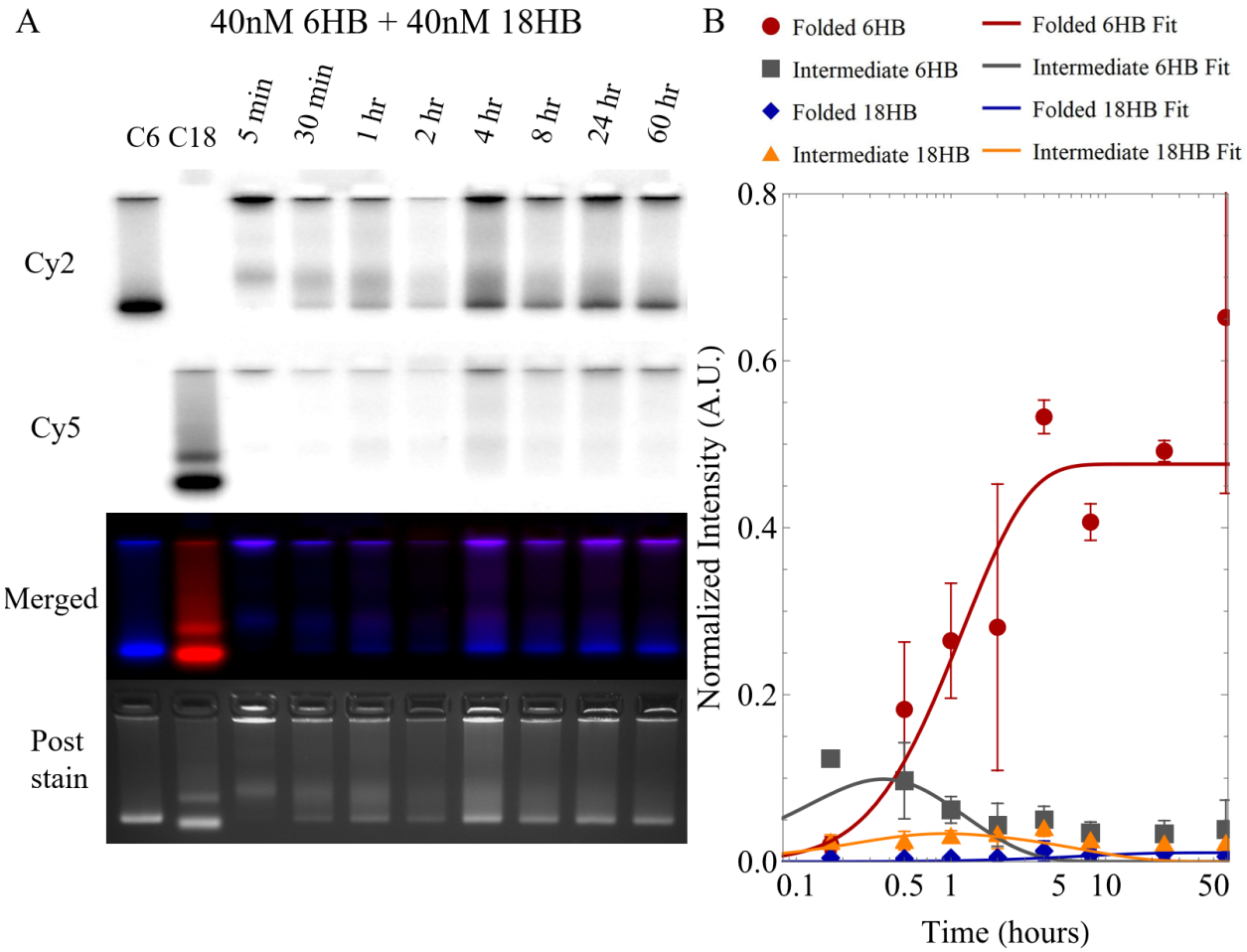

Figure S31. Kinetics of co-self-assembly with 40 nM 6HB staples and 40 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 40 nM 6HB and 40 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

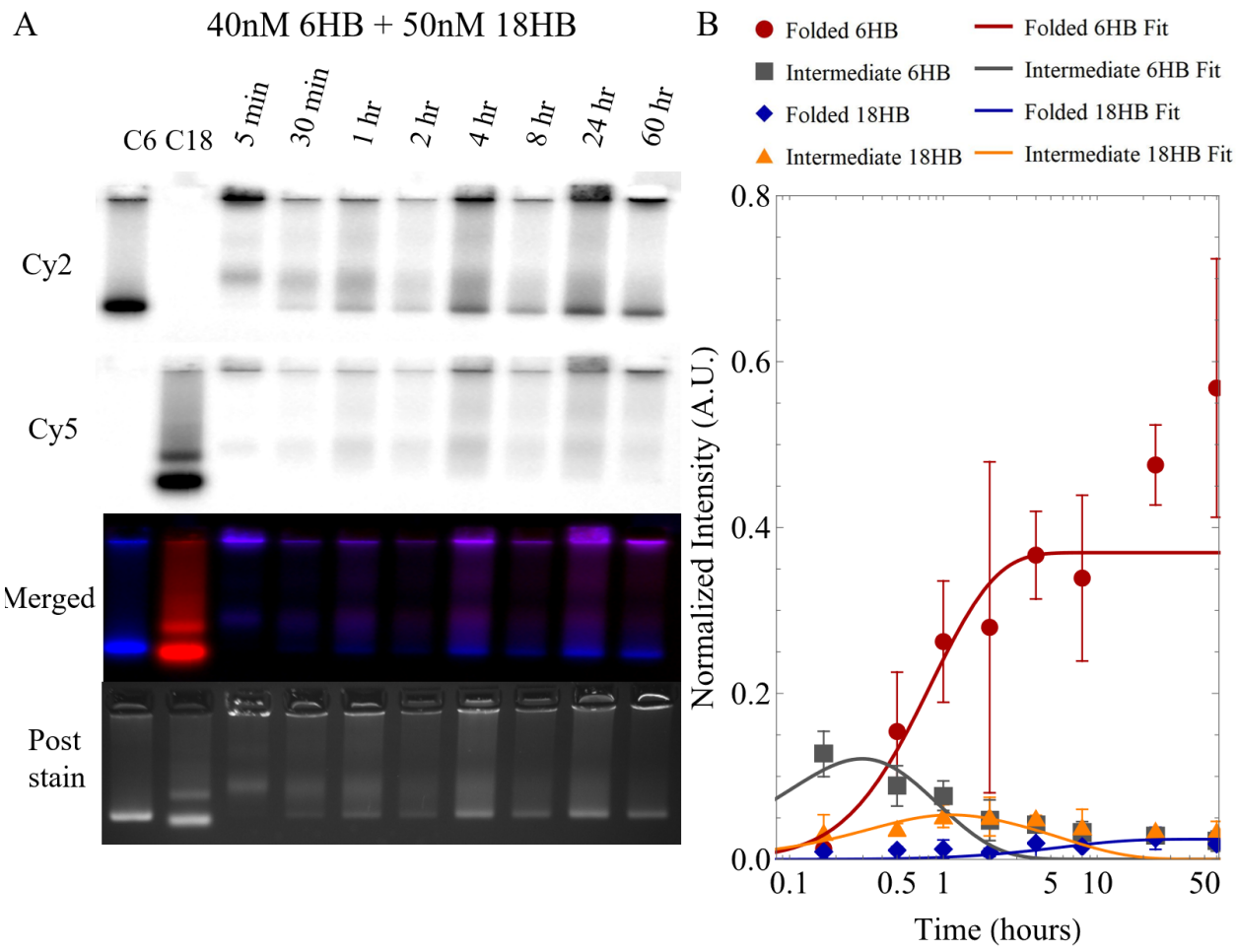

Figure S32. Kinetics of co-self-assembly with 40 nM 6HB staples and 50 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 40 nM 6HB and 50 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

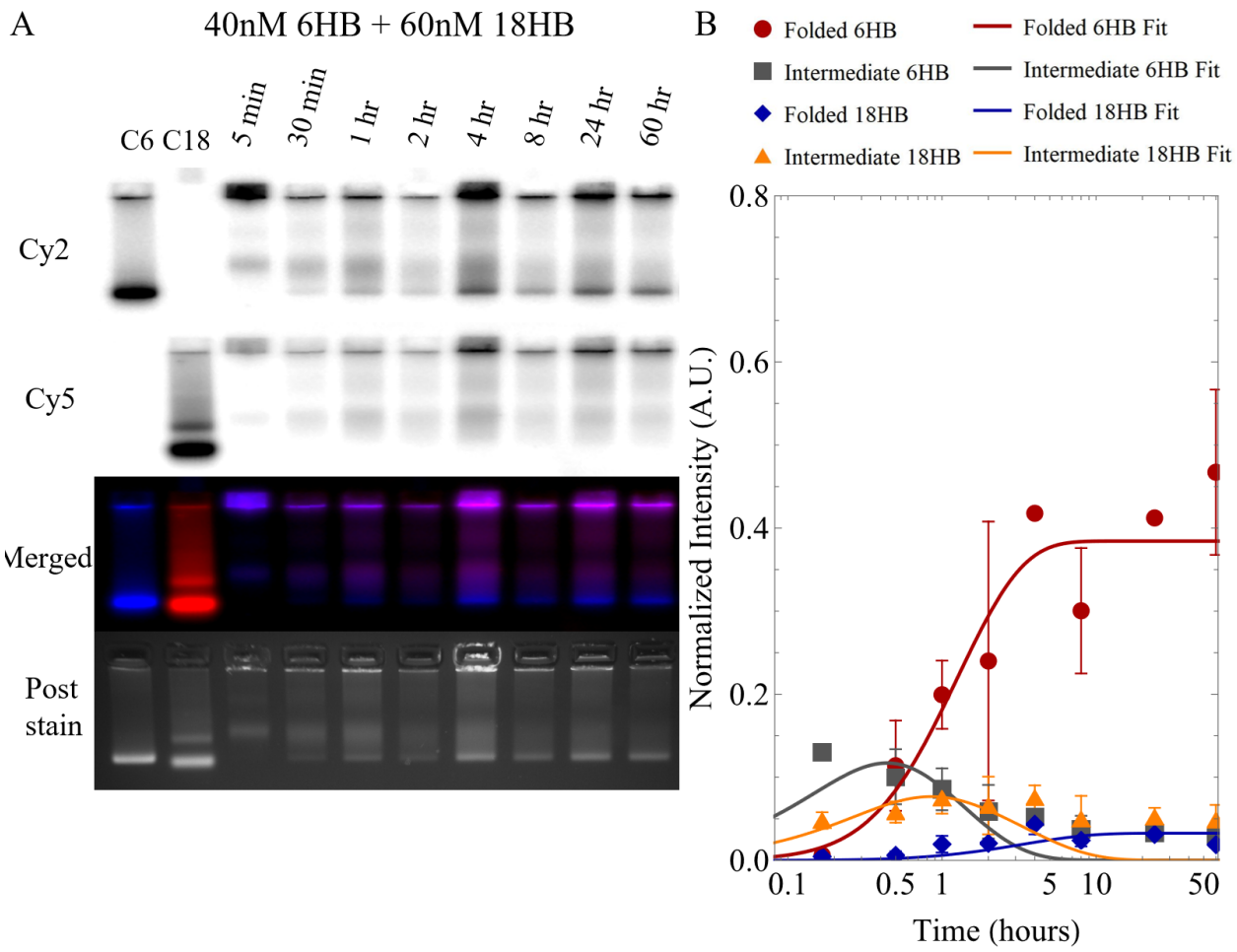

Figure S33. Kinetics of co-self-assembly with 40 nM 6HB staples and 60 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 40 nM 6HB and 60 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

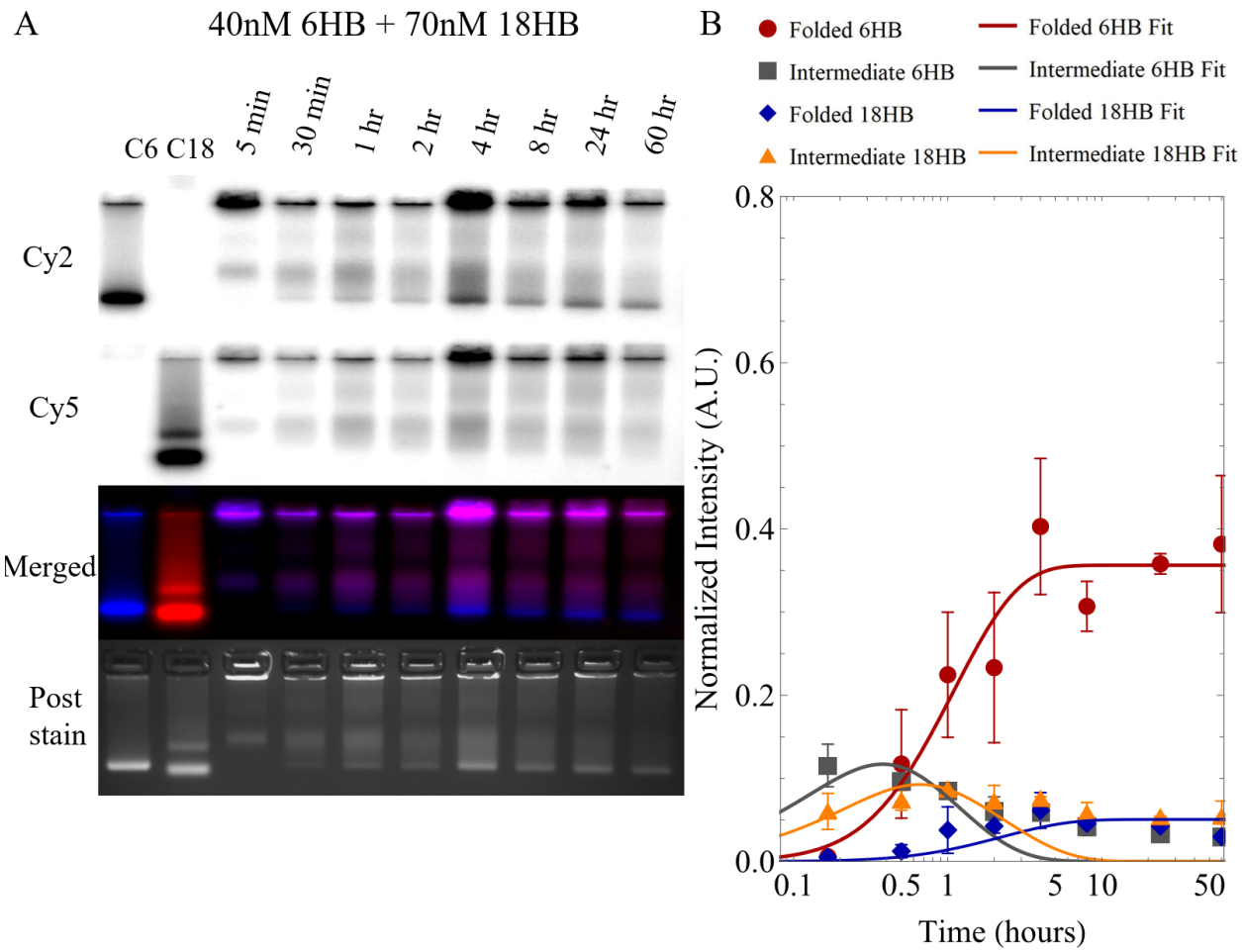

Figure S34. Kinetics of co-self-assembly with 40 nM 6HB staples and 70 nM 18HB staples. (A) Example of laser-scanned agarose gel using a Cy2 channel and Cy5 channel along with a merged imaged of both channels and a corresponding image of the gel with EtBr post-stain. First two rows are controls of 6HB and 18HB folded separately with 200 nM staples in a 2.5 day anneal. Other lanes correspond to mixtures with 40 nM 6HB and 70 nM 18HB folded at 53 °C for various lengths of time before quenching the folding reaction in liquid nitrogen. (B) Quantification of intensities for the leading and lagging band corresponding to folded and partially folded structures respectively and associated fitted curves.

From these results there are some conditions which are particularly noteworthy. The kinetics assay with 20 nM 6HB and 20 nM 18HB staple concentrations (Figure S13) have slow enough kinetics to more clearly see trends even at longer timescales. Additionally, traces of the lagging band more clearly diminish at later times making it a closer fit to our 3-state model. The extracted

rates k_1 and k_2 for transitioning to partially folded and fully folded state respectively appear to follow concentration dependent trends which are similar to those described in individual folding kinetics assays. Mainly, for assays containing 20 nM 6HB staples we see that both rates are greater for transitions to the partially and fully folded 18HB states in mixtures with 40 nM 18HB staples and above. However, in mixtures with 30 nM and below, we see that the rate of formation of partially folded 6HB becomes greater than the rate of formation of partially folded 18HB (Figure S31 A). Also, the rates of formation of fully folded 6HB and 18HB are roughly equivalent which could possibly explain why 6HB structures fold with higher yield in co-self-assembly reaction than would be expected from individual folding characterizations. Similarly, for co-self-assembly reactions with 30 nM 6HB staples we see the rates of partially and fully folded structure formation are greater 6HB structures when 18HB staple concentrations are about 60 nM or below (Figure S31 B). Finally, for co-self-assembly reactions with 40 nM 6HB staples we see that the rate of partially folded structure formation is greater for all conditions while the rate of folded structure formation is only greater for 18HB when staple concentrations are less then 20 nM. We estimate, however, that this particular fitted value for the transition to fully folded structure in mixtures containing 40 nM 6HB and 20 nM 18HB are very likely inaccurate due to the very low overall band intensity in these gels.

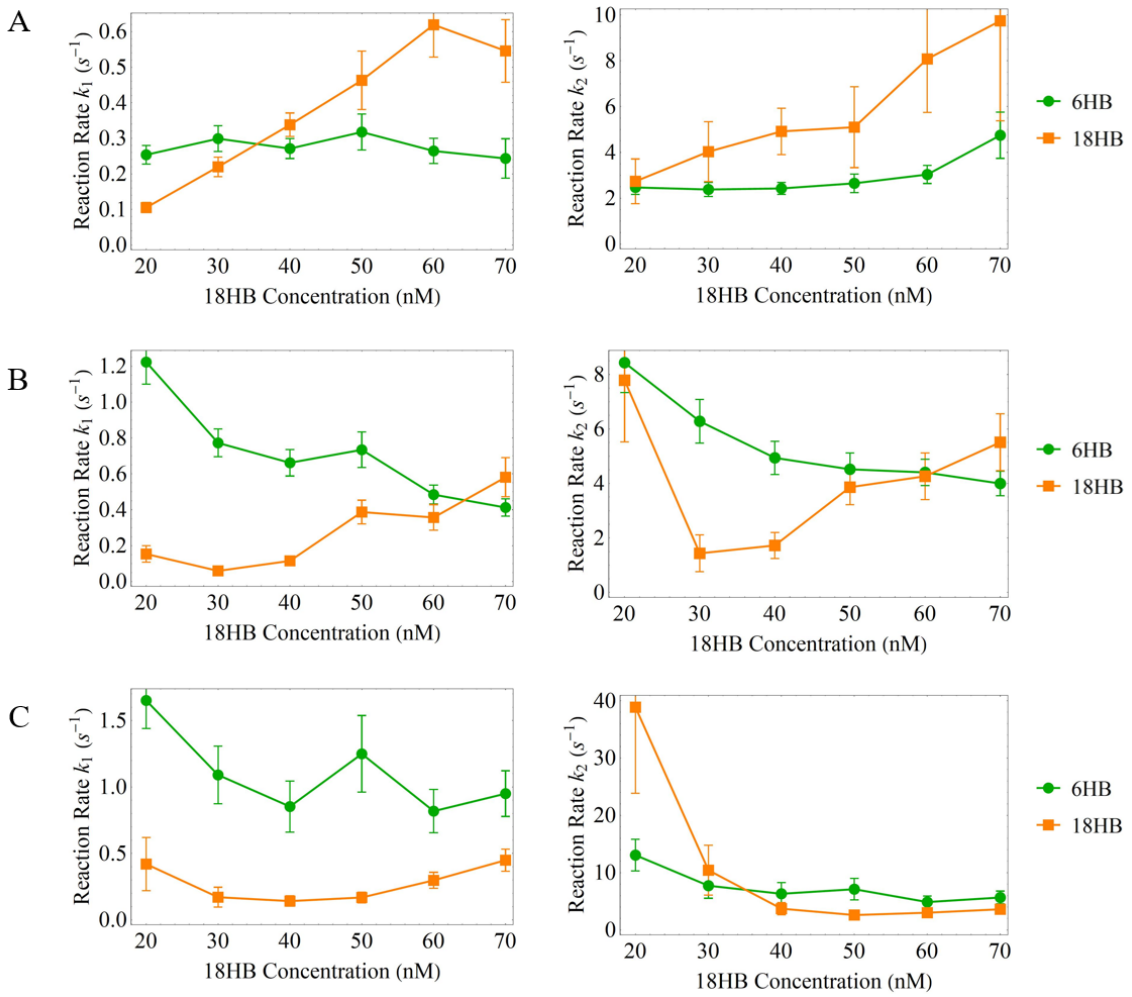

Figure S35. Extracted folding rates for co-self-assembly reactions. Summary of results for rate of formation of partially folded structures (left column) and fully folded structures (right column) for mixtures containing 20 nM (A), 30 nM (B), and 40 nM (C) 6HB staples each with 20 nM to 70 nM 18HB staples.

1.7 Staple and Origami Design Comparisons

Comparing the staples sets and overall design of each origami provides some insight into how one might design structures to have specific folding rates and yields in co-self-assembly reactions. The 6HB structure (Figure S36) is distinct in that all bundles only share 2 neighbors and has no internal core of helices with three neighbors. As a result, the frequency of crossovers is reduced in the 6HB and staples can have more segments which bind to longer portions of the

scaffold. The 18HB (Figure S37) has a core in which the central 6 bundles must all have 3 neighbors and therefore must have more frequent crossovers. In our design, many staples needed to have smaller binding segments to accommodate the standard staple lengths.

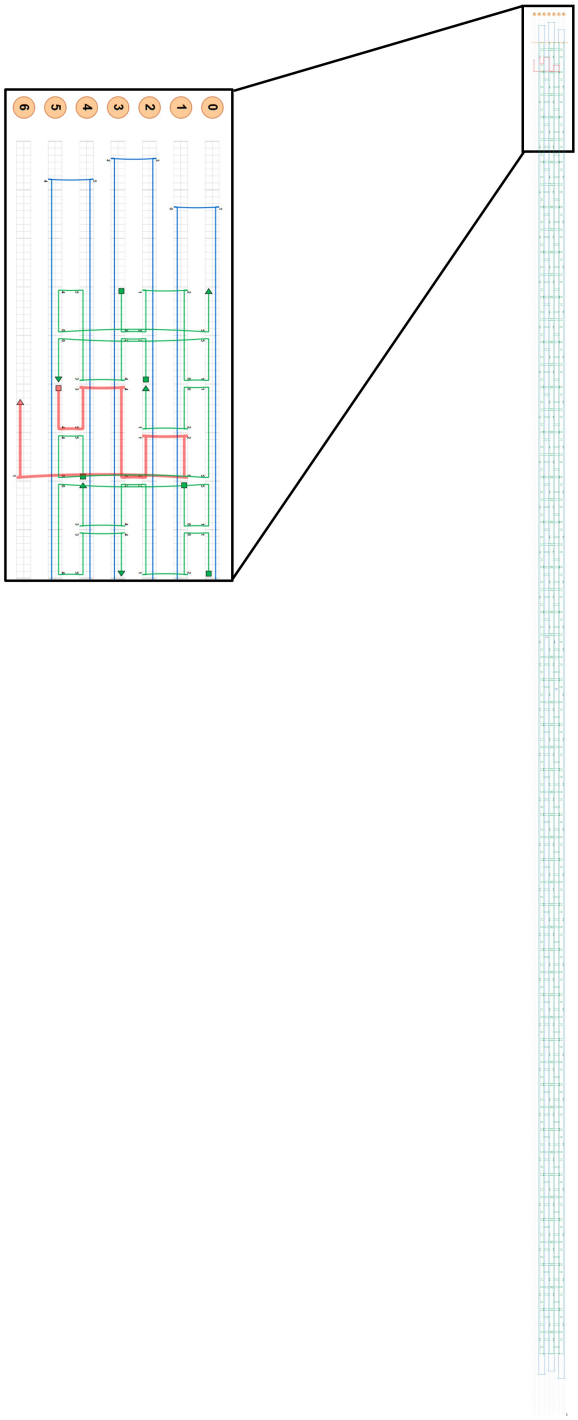

Figure S36. caDNAno schematic of 6HB DNA origami with an overhang to bind to a fluorescently labeled oligo.

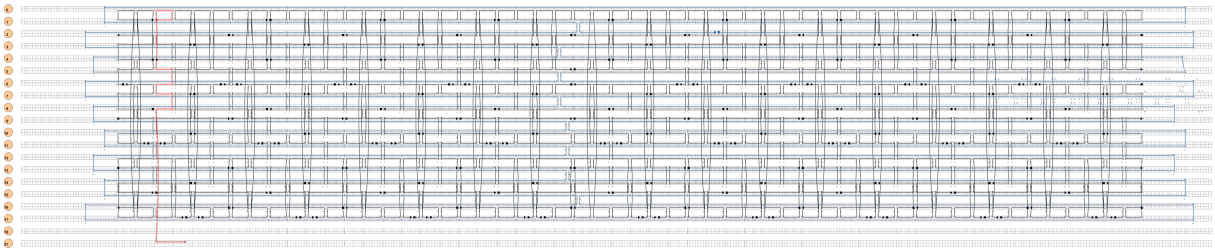

Figure S37. caDNAno schematic of 18HB DNA origami with an overhang to bind to a fluorescently labeled oligo.

Looking at the lengths of staple segments (Table S2) between crossovers we can see the 18HB has a large number of staple segments that are only 3 or 4 bases while the 6HB has a larger number of staple segments that are 14 bases long. Additionally, we calculated the melting temperature of both the staple as a whole and each of its segments between crossovers using the Santa Lucia nearest neighbor model.[2] It should be noted that these melting temperatures are simply calculated based on their sequence and doesn't take into account base stacking between neighboring staple segments. Regardless, we can see in Figure S38 that the histograms comparing each structures staple and staple segment melting temperatures are fairly similar with the exception of a large number or the 18HB 3 and 4 base long segments being calculated as effectively 0°C. Given that the cross section of the 6HB is smaller it would take fewer staples to nucleate a part of the structure. To see if there were any sections that might behave this way, we plotted a heat map of the structures where the strands with lower melting temperatures are more blue and strands with higher melting temperature are more red. In the heat map of the 6HB (Figure S39), there are some portion of the structure which stand out as being likely nucleation sites due to the strands in those areas having higher overall melting temperatures. Since all strands in the 6HB structure are 42 bases long, this is mostly reflective of local GC content. However, since we cannot distinguish which end is which or what orientation our structures are in TEM images, it is difficult to conclusively say whether the regions with higher GC content seen in our heat maps correspond with actual nucleation sites.

Table S2. Comparison of staple segments

Staple Segments

Length	3	4	7	14	21
6HB	0	0	689	176	3
18HB	51	51	667	118	6

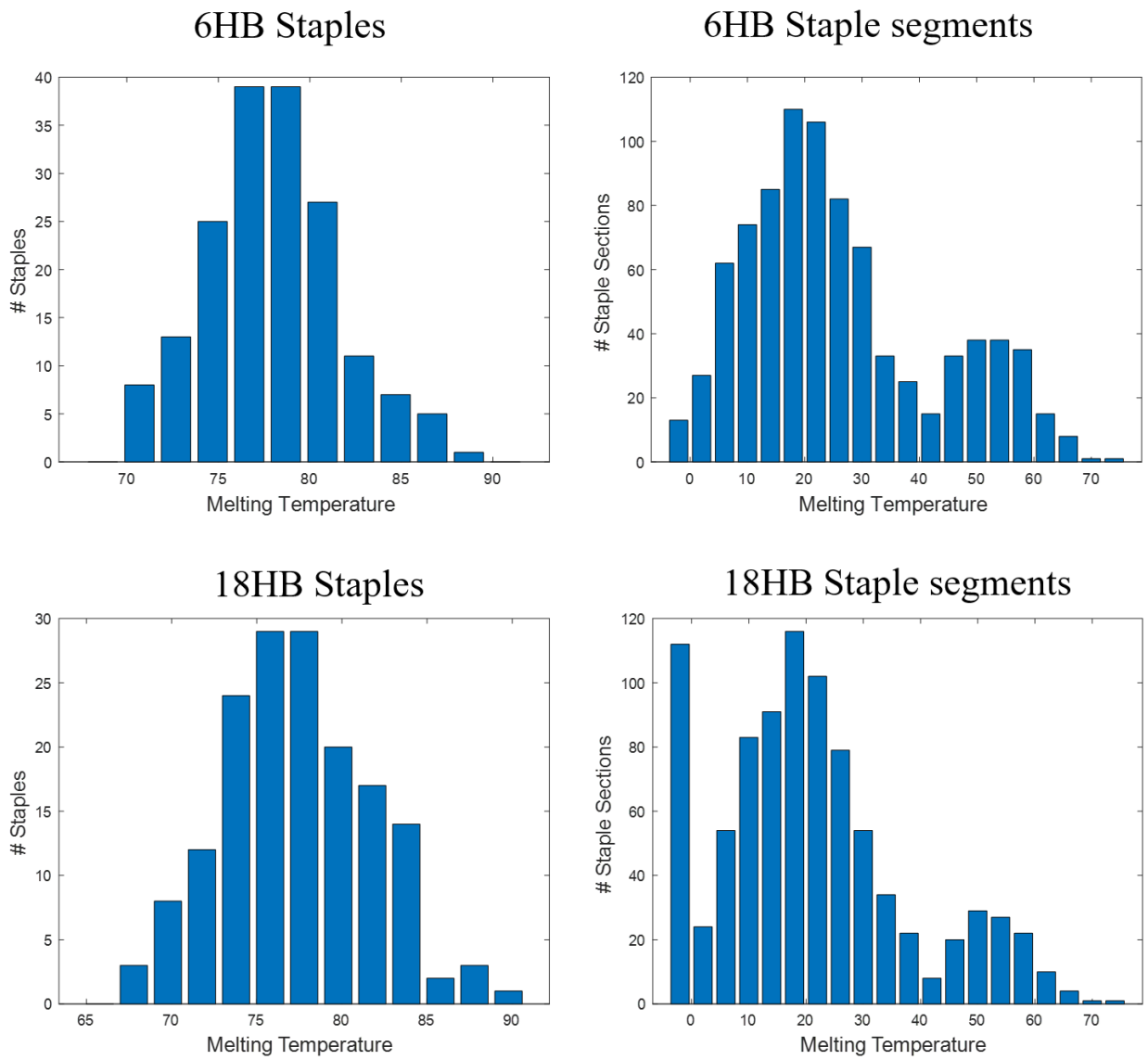

Figure S38. Comparison of melting temperatures for 6HB and 18HB staples and each segment of each staple between crossovers.

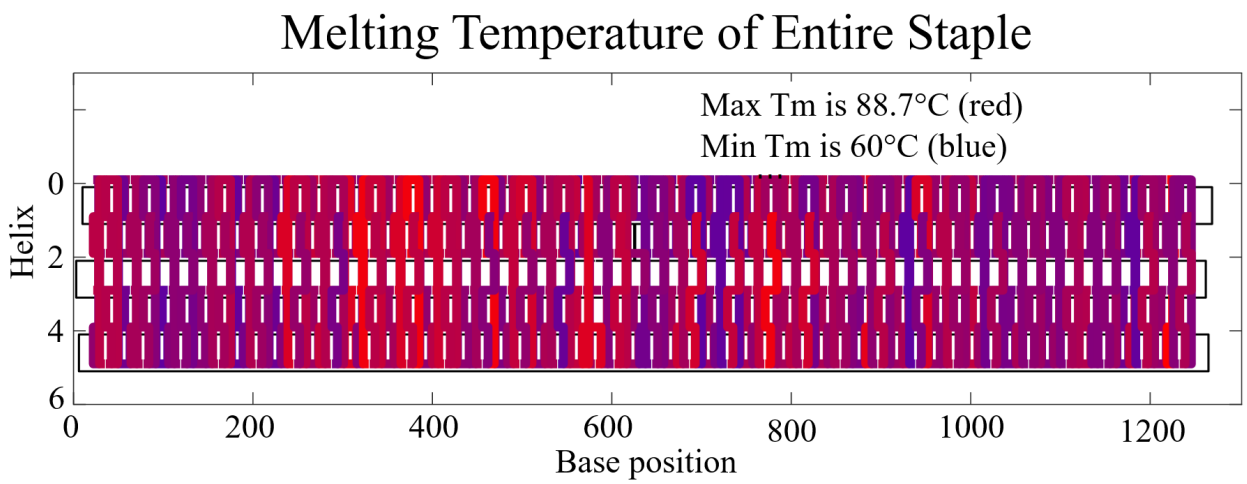

Figure S39. Heat map of 6HB structure in which strands with the highest melting temperatures are more red and strands with lower melting temperatures are more blue.

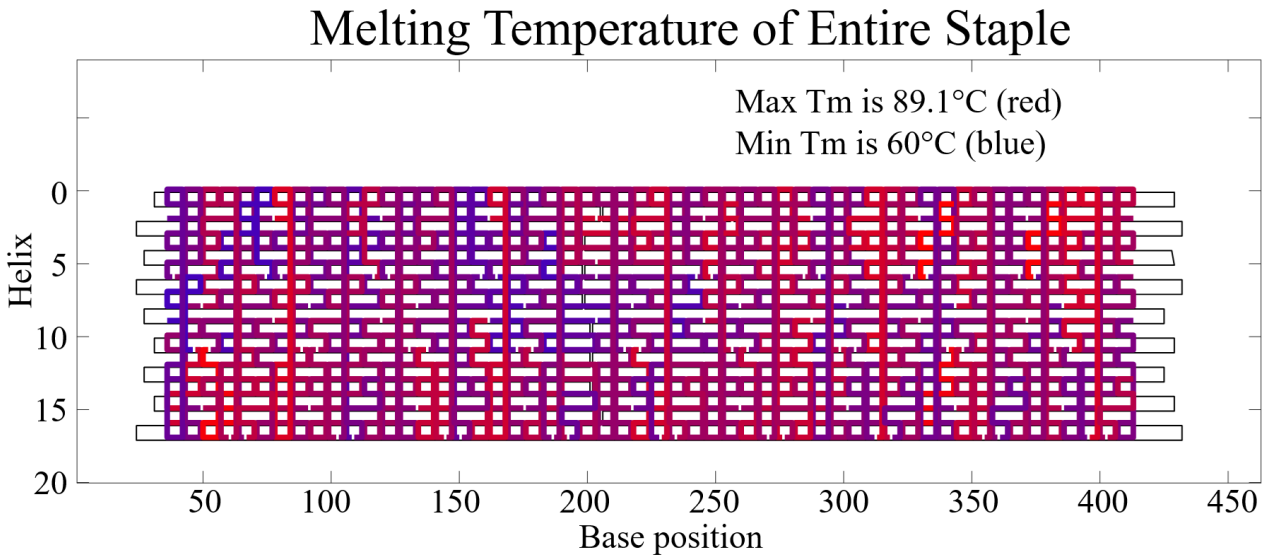

Figure S40. Heat map of 18HB structure in which strands with the highest melting temperatures are more red and strands with lower melting temperatures are more blue.

1.8 Methods and Materials

1.8.1 Individual folding reactions

Characterization for folding each structure individually follow the same protocol as described in other publications [3, 4]. Folding reactions were all prepared using 20 nM 7560 scaffold DNA, 1 mM EDTA, 5 mM NaCl, 5 mM Tris, and 18 mM $MgCl_2$ with a variable concentration of staples.

Additionally, a 5-fold excess of fluorophore labelled oligos relative to staple concentration was added since these oligos bind to overhangs on their respective structure instead of integrating directly as part of the structure. Folding mixtures were aliquoted into 8-tube strips at 50 µL per tube and placed in a thermocycler with the following protocol: 65 °C for 15 minutes, a temperature gradient between 52 °C and 58 °C for 4 hours, 4 °C indefinitely.

1.8.2 Co-self-assembly folding reactions

Characterization for folding reactions with a combination of multiple staple sets use the same protocol as specified in section 1.8.1 with the exception that a variable amount of both staple sets are added and a 5-fold excess of both corresponding fluorophore labelled oligos relative their respective staple concentration. All co-self-assembly reactions were made in smaller 50 µL batches since the concentration of each condition were different. Typically, a specific amount of 6HB staples was added to 13 tubes while a variable amount of 18HB staples were added (0-120 nM at 10 nm intervals). For gel characterization, no ethidium bromide was used and instead gels were laser-scanned in a Typhoon fluorescence imager using Cy2 and Cy5 channels sequentially. Once scanned, gels were post-stained in an ethidium bath containing a high excess of ethidium bromide for approximately 2 hours and then imaged on UV illumination table. Once sufficiently stained, bands of interested where excised and further processed for TEM characterization.

1.8.3 Kinetics assays

To characterize folding kinetics, we first made a standard folding reaction containing a variable concentration of 6HB and 18HB as described in section 1.8.1 or section 1.8.2 depending on whether the experiment was for individual or co-self-assembly kinetics. Folding reaction were incubated at 65 °C for 15 minutes, then at 53 °C for a range of times between 10 minutes to 60

hours. To quench the folding reactions, samples were quickly transferred into liquid nitrogen and kept frozen until just before loading them into an agarose gel.

1.8.4 Agarose gel characterization

Samples were then mixed with orange loading dye (containing no EDTA) and run in a 2% agarose gel (Invitrogen, Carlsbad, CA) in which the gel and running buffer contained 1x TE (1 mM EDTA, 10 mM Tris-Cl) with 11 mM $MgCl_2$ (Sigma-Aldrich, St. Louis, MO). For each gel a control structure was included. Control structures were folded at standard staples concentrations (200 nM) and slowly annealed from 65 °C to 4 °C over the course of 2.5 days. Gels were run at 100 V for approximately 1 hour while cooled in an ice water bath. For these individual folding reactions gels also contained 480 mg/mL of ethidium bromide. Gels were imaged on a UV illumination table. To verify which bands corresponded with well-folded structures, selected bands were excised and extracted using Freeze'N Squeeze™ gel extraction spin columns (Bio-Rad, Hercules, CA). Spin columns were spun at 10000x g for 10 minutes. The resulting effluent was subsequently used for preparation of TEM grids and further characterization.

1.8.5 Gel image quantification

Gels were processed using a Mathematica program processes all gels in a particular set simultaneously. The program finds an optimal rotational correction for each gel image, then finds all relevant lanes. The program detects the staple excess band and gel wells and crops the image to include to remove the staple excess bands. The program then adjusts the image pixel intensities with the folded bands of control structures for each respective image channel normalized to 1. The program then finds the lane profiles by summing all pixel values horizontally and performs a background subtraction using a background estimator function. The resulting background

subtracted profiles are then scaled to ensure that the location of folded bands are the same for all profiles. The peak intensities within the expected range of the folded bands and the partially folded bands are then measured at the same location for all profiles. The peak intensity values are then paired with their respective time, concentrations, and band locations which comprises the full set of kinetics data. For each concentration condition, a NonlinearFindFit function is applied to both the folded band data and partially folded band data in which the model being fit is the solution to our 3-state model. A range of starting conditions were tests to ensure the fidelity of the fits by finding ranges in which the model converged on reasonable values (i.e. values less then 10^6).

1.8.6 TEM grid preparation

TEM grids of samples were prepared as has been described in prior literature [3]. Briefly, 4 µL of gel purified samples were deposited on formvar copper mesh grids (Electron Microscopy Sciences, Hatfield, PA) for 4 minutes, rinsed with a 10 µL droplet of 2% Uranyl Formate, then incubated with 20 µL droplet for 40 seconds. Stained grids were imaged on a FEI Tecnai G2 Spirit TEM at 80 kV.

1.8.7 Staple sequences

Table S3. Staple sequences for 6HB origami.

TTAGCAATTTAAGATGACCCTGTAATACAGGTCAGCCAAGCG
AAACAAAACAGTAGCGCCAGGGTTTTCCGAAGCATGGGATTT
GAATAGGACCTTATTAAGAGCAACACTATGTATAATTGCTAT
GAACCAGATAAAAAGTTCTAGCTGATAATCAGAAAAGGCGCA
TGGTTGCTCAAATAGAATACCAAGTTACATTTTCGCCTCTTC
AATTTCTGGCACCATTAATTGCTGAATACAAAGAAATACCCA
ACGCGCCGCGTAGACACTAACAACTAATAAAGGAGTCACCAG
CTGAGAGGCGGGAGCTAATGAGTGAGCTTGTGCTGATTTAAC
GTCAGAAGTGAGAAACAAAGTCAGAGGGTTTCGGTGAATTTT
TAGGGCGTTAGGAGTTTTCAGGTTTAACTCAGCTAGCACCGC
CAAAAGGGCAAAGCGTCAATCATAAGGGAGTAAATCATAGCC

TTGCGGGCGTTCCATCGAGAGGGTTGATTGGCTCAAAAAGGA
AACCTACTCCCATCGCCAGTTTGAGGGGACCGCCTCGGCGAA
GTAACAAAATCAAATACGTGAACCATCATCATTTTTTATCAT
GCACCGTAAGAAACCAAGGATAAAAATTATTCGAGTGATAAA
GTCCAGAAGTACCTTTGAAAGGAATTGACTGCGCGGAGAGGC
TGGGAATGAAACCGGCCTCAGAGCATAAGAGGTCAAGAATAC
TGCAAATAGGTCTGATTCACCAGTCACACATTGCAGACGCATGTCGAGAGCAGTAGAC
TAGGCTGATTCCACACCGGAACCGCCTCAGCCTTTCCGGAGA
TTGTGTCATAGAAAAGCGACAGAATCAAGTTAAGCCCCTCAT
CGGTATTTGTACTGGGATTAGCGGGGTTGTTAATAATTTAGG
TATTATTAAAACCGGAGTGTTGTTCCAGTGTAGCCAATCATT
TGAGTAACCAATAGGCGTCTGGCCTTCCTTTGGAAACGTCAA
AATAGATAGAAAGGGCTGATTGCCCTTCACGACGACAAGAAA
TAAGAGCAATCAGTGGAACAACTAAAGGCATCGCCCTTCAAA
CCTGATTGGCTGTCAGGTCACGTTGGTGGTTTGCCACCCTAA
AGTCAGGAGTGCCGGTAAGCGTCATACAGCGAACCGCATTAA
TCAGACTGAGATAAATGCCTGAGTAATGAGATTAAAGCCGGA
AGGGACACAGAACATTCTCCGAACTCTGTTTACGCACTTTTT
GAGTACCAACATTAAAAGTAAGCAGATATAGCACCCCAAAAG
GCGGATAACGTTGGCTAATGCAGATACAAAAATTCTCCCGAC
CTGGATAGAGTCTGTTATCCCAATCCAACACCCTCACCCATG
AATAATACATATCATTGAGGATTTAGAAGGAAAGCGGCCCTG
GGTTGAGGTCTTTCATGTCAATCATATGCGAGAGGACGAGTA
CCACTATATAATTCCAAGCAAATCAGATCGGGGTCGACTCCT
ATTAAAAGAGGCCAGCTGTTTCCTGTGTCAGGAGACCTGTTT
ACGCTCACATCAAGTATTAACACCGCCTGATTAAAAAAGTGT
AATATCTCTGGCAAGCGCCAGGGTGGTTCTTTCCGATGCAGA
AATAAATCCAGCTAAAAAACAGGAAGATTCATAACAATCATT
AGTATCAAATTACCACGAACCACCAGCAAGAAGTGTTATCCG
ACAGGAGCTAAGAAAGCTCATTTTTTAAAGTTGAGAAACGAA
GGTGAGGGAACGGTCAACATACGAGCCGCAGTCACAAAGCCA
AGCAAACATTGAGGCCACGCATAACCGAACTAAAGTGGAAGT
ACTTTGATTTGTCGTGCCATCTTTTCATAGACGGGCCATCAA
AGGGAGCTCGACAATTATACTTCTGAATTGTAGAAGCGCATC
ATTTTTGCATTCAAGAAGAAAAATCTACTTGCTCATGATGAT
GTAACCGTGCAGCATTAGAGCTTGACGGGTATTAGGGGTTAG
ATTTTTTAAGTACAAAGCAAACTCCAACTTTTGCGTTACCGA
GCGTTGCCGCCAGCATTTAGGCAGAGGCAAAATCGCACCTTG
TTAATTAAAATACCGCTGAATTGTCAACTGGTGCTATAACAT
TACCAGAAAGCCCCCAATTTTATCCTGAGATATTCCCGCCAC
AGAAATTTGTTTATCGCACTCCAGCCAGTTTCTTTCGGGCGC
TAGACAGCGGTCAGAAAACAAAATTAATCCAGTATGACGTTG
TAATATCTTCTGGCCTGAGAAGAGTCAACGAGAAATCGCCCT

GCGAACCTTCAACGAATGAAATAGCAATAACCATCAATAATA
CTAACGGAGGATTAGTAATAAGTTTTAAATAGAAGGAACGCC
AAACGCTTTGGCAGAGAGACTACCTTTTTATGTAACATCTGT
CGTGGCGAATACATAAATTATTTGCACGTCCTGAACAGTATC
AAGAAACATGGTTTGTTAAAGGCCGCTTAGCATCGAACGAGT
AAATCATCCAATCGATACCGACAGTGCGATCGGCTACAGGAA
AATAATCGTTTGGACTCGTATTAAATCCAATCGGACCAGCAG
CATGAGGCCATCGCGAGGGAAGGTAAATTTACGCAGTAGCAT
TGGTTTGATTTTCCGTGGCACAGACAATATTAGTATGTTACC
CCACCAGATATTATGAGCAAACAAGAGAGTAAAATCTCATTC
TGCCAGCAACTGTTAAGTACCGACAAAAAACAATAGGTCAGT
AATACCATTAAATCCGCGAGGCGTTTTATGGCTTTGTACCAG
AAACCAACTGATTATTTGAGTAACATTACCCAAATCAAAATC
TGAGACGGGAAGATCAACAATAGATAAGTAAAACAAGCCGTC
CTGAACCTTTGACGGCCCGCTTTCCAGTGTGCGGGAGCCAGT
AAACAATCCCCGATAGCGGTCCACGCTGTAGATGGACCAATC
AACGCCAATTCATTCAAATGAAAAATCTGCTTTCCATTAATT
AATAAGATTGCTTTTCAAACCCTCAATCCAGGGCGCCTGTCG
GAGGCTTACGGCTACAGTTGATTCCCAATTCATTTCATATAA
GGTAGCAGCAGGGAACCAGCGCCAAAGAGTGGCAAGGGGCGC
TACCGTACATTACCATACTGCGGAATCGGGCTATCACGATTT
AAAGCCTTGGGTAAGGCTTAATTGAGAAAAAAGAATGCCACG
AGAATAATGAGTGACGCGAACTGATAGCCTGTCCAGAATTCG
CCCTCAGAACGTCAGAGAGATCTACAAATCATAAAAAGAACC
GTAAATTACCGCCAAGACGATTGGCCTTATCTTACGTTGATA
ATTAAGTGGGGTGCCTAAACAGGAGGCCGCAACAGGATGATG
CCCGAAAAAATGCACCCACAAGAATTGAGTTTGCCTCAGCGG
CAAATATTAAGACGCAACAGAGATAGAAATCGGCCGGTTGGT
ATCAACATAGGGTTTCTATCAGGGCGATCCTATTTCGGAACC
TAATCATTTCTTCTAATCATAATTACTACAGTACACATCGCC
AGAGAGTTGCATCTCTAATTTACGAGCAAATGGAAACTTTAC
TTCTGGTGTATTGGGTGTAGCGGTCACGGGAAGGTGAATATA
CAAGAGAAACAACATTATTAAGAACGTGAGGCTGAAGTGCCT
ATTTTCAATGCCCCCTGCCACCAGAAGGAGCAAGCTGAGCGA
GTGAATTTGTATCAAAAGCCAGAATGGAAGATTAGGCAAATA
CTTAGATATTTTAGCCGCTTCTAATCTAACCTCCTTTGCTGG
GTGGATGGGTCATACCGAGTAAAAGAGTCCTAAAATAAATCA
CTGTATGGCAGACGGGATTGCATCAAAATGTAGGTGCGCTAA
ACTGAACTAGCGTTTCTTTCCAGACGTTAACCGAATATTATA
GAGCCTTCACTCATGCTCCTTTTGATAAAGCTAAAAAAGTTA
GGAGTGAAAATCATATATTACCGCCAGCCGACCAGTTTATCA
AACGAGCGCAGGTCCCCTCAGAACCGCCCACCAGACTTTTGC
AAAAGAAAACTAGCCAGAGCCTAATTTGCAGCATTGAGCCAC

TACCAAATTTAATTCTTTGACCCCCAGCAAAGGCTATTACCA
CTACGAATAAACAGATTATCACCGTCACTAACGGATTAGCAA
TAAAACGTTCCACAACGCCAGAATCCTGGAAGATAAACAATT
CCTCAGAGGGCTTGAAAAACCAAAATAGTACCCCGCAACGCT
TATGATAAAATCAACAGATGAACGGTGTGTTAGCGATCACCG
TTTGCACCCTCATTCCGTACTCAGGAGGCAACTTTCCTCGTT
TTTGTTTAACCGCCGTTTCGTCACCAGTTCTTGACTATTCAT
TGACCATTTCAACCCAGGGAAGCGCATTAATCAAATAACGAT
GCTGCGCTGCATTATTAATGCGCCGCTAAATATCTACGGATT
GTAATGAAATAACCTTAATTTCATCTTCAAACATAGACCTGA
AATCAGTATACCGATTTTTTAATGGAAAGAAAAAGAGCCAGG
TATCAGAGTAGCGCGCTAAACAACTTTCGTTACTTGAGGAAG
CTCACAAACGGCCATATACAAATTCTTATACATTTAAACAGA
ATCAGAACGACGATAGATGGTTTAATTTTTTAGTAACAAACA
ATTAGTCCGTTGTAGGATCCCCGGGTACTCATGCGTTAAATA
CAGTAACCGACGACACCAGGCAAAGCGCGCGCGGGTAACCAC
GCGAATTACATGTATGGCGAAAGGGGGAAACTCACTCGTTAG
AATTAAGTTAGAGCACCTAAAACGAAAGTTGCTTTAGCCATT
CACTTGCAGAATACCTTAGAATCCTTGATGACCTACAATGTC
CTGTAGCGCTGACCATGCTTTAAACAGTATTAATGACAGAGA
CCTTTATAGACCGGACGGAGATTTGTATAATTGCGGATAGCA
GAGCTGACATATAACAGAGGCTTTGAGGTATATTCGCGACAT
TAAGTGTAGACGGAGCAATACTTCTTTGATTTTTGGTCGCTA
ATATTTTGACTTCAGCGACCTGCTCCATAACAGTTTTTAGCG
TCATTTGTATGCGTGTGCCAAGCTTTCTGAAATTGTTTTTAT
AAAGAACAAGGTGACTTGATACCGATAGAATACGTGTAGCTC
AGTGAGAGAAATCCAATATCGCGTTTTATTTAGAACCAATAA
ACAACAAAAGTTTCATGCAACTAAAGTAAATAGTAGTATGTT
TCGATAACCTTAGTGACCGTGTGATAAAGTAAATCAATGGCT
AATTAACTTTAATGATAACCTTGCTTCTTAAGGCGCACGACT
ACTAAAATAATTGTAGCAAAATCACCAGGCCGAACTCGGTTG
CTAAAGTAAGAGGAAAATCAGGTCTTTACAAATCAAGAATTA
TGCCTGAGCGTCCACAAATCAACGTAACAATAGGAAGAGCCG
AGCCCTTGGCCGGAAAAATCTCCAAAAAGATTATAGATTAGA
AATTTACAGGTTTTGTTAATATTTTGTTTAACGCCTTATACC
ATTACGATGTAAACGAAGCCTTAAATCAAAGCGCATAGCCCG
ATATATGACACCGGAAGTGGTTGTGAATCGAGCTCTCACGCA
GGATGGCCAATAAAAGGAAACGCAATAACGACTTGCGAGGTG
TTTAAATGGCATAGGCGATTTTAAGAACATAAGTAGTCTCTG
CACACCCTCAACAGTTTACATCGGGAGAGGTAAAGCATTCAG
AGTGAATATTTTCAACCAGAGCCGCCGCCCAGTTAAACGGTA
ATTTACACATGGAATATAGGGGCCTTGAGCCCTGCATGCTGA
AATTCATGCAAAGATGTTTAGCTATATTTTCTGCGGAACGAG

ACCGCGCCAGTGCCATGAAAGTATTAAGGACTCCACAAGAGT
GGCCTCAGGCAACAAAGGGAAGAAAGCGAGATTAGGAAATAA
TCAACCGGTAGAAAGCATCAATTCTACTCGGTGTCACTTTTT
AACATGTCAATAAAATTAAGACTCCTTAATTGACGGACAATG
CCTTATACCCGTCGACTCATCGAGAACAAGCGGAAGCGGAAC
CGCCTGAGAATATAGGGAAGGGCGATCGCGGGAAACGTACTA
AAGCGTACTGAGTAGGGCTTAAGCTACGCTTATGAAATTTAA
AACAGCCAACCACCGGGATAGCAAGCCCAAAGCTGGTTTAGA
GACCGTATCCTGTTGCCGTAAAGCACTATTTGCCCATATAAT
TGGCAAAGCCGCGCATGAATCGGCCAACCATTCGCTAATTCT
TTTGGGGTTAAAAGTCAGATGATGGCAACAAGAACGCGGATT
GGGTAGCCCCTCAATTCATCAAGAGTAAACAAACTGCCGCCA
CCCTTATACCCTGAAGGCCGGAGACAGTCCCTGACCTGACCA
TGAATCCTATTTTTAAAATGAAAATAGCCCTCAGAACAACGC
AGGGCGACTGAAACCGTATAAACAGTTATCGTAGGAGCTTTC
GAATAACAGCCACCAGACAGCCCTCATAACAGACCACGAGAA
AATCAGACCAGCAGTCAATTACCTGAGCTCGCCATCAAGGCG
CACCCTCAAGGCTTCAGAGGGGGTAATAATCGATGCAAAATA
GGATATTACACTGAACCCTCAGAGCCACATAAGAAAGGTCAT
ACGAGGCGGATTTTGTTTTCATCGGCATTAATTGAAAAGATT
CCGCCAAGTAAACAGAAGAACTCAAACTCCCTTCTGCGATAG
TTCACATCTCTATGCAAGACAAAGAACGTAGTGAATAATAAA
CGAAACACACGTTGAACGTCACCAATGAAGCTATCGGAGAAG
CATATTCGTACCGCGATTCTCCGTGGGAAAATCGGCAAGTTT
GCTATTAGCTCACTAGCACGTATAACGTAAAGCATCGCAGAG
TTCATTCAAAGGTGATACATACATAAAGCAAAAGGGGTCGCT
ATCGTAAGTTTTGCGCCCTGACGAGAAAACCCTCAGACAGGA
TTCATTATGGCATGTCATACAGGCAAGGTAATGCTAATGCCA
CCAGAAGTAGAGCCATCGGTTTATCAGCAGGCAAATTTTTGC
GGTTTGCGCCGGAAAATAAACAACATGTGTCAGATTATCTAA
AAAGAAAGGCCCACAGAATAGCCCGAGATTAAATGCGTTTTT
TAACATCTTTAAATCATTAAACGGGTAATTGCGCCGAAATTA
GCGAAAAATGGGATTTTCCTTATCATTCTTCATCAGAACGTT
ATTAATTTCGAGGTTGATGGTGGTTCCGACAAACGGGGTATT
ATCAAAATAACAGGTAGAAAGATTCATCCCAATAGGCTTATC
/5Alex488N/TTTGTCTACTGCTCTCGAC

Table S4. Staple sequences for 18HB origami.

AGTAATTAATATAACTATATGTAAATGCCTACCTTTCAA
ATAAGAGCTGTCCACCCGACTTGCGGGACCTTAAATTTA
ATCTTACAAGAAACAGACGATATAGAACTGAAAGCGCCGTCA

CCTTTTATGCTTTGAACAGAGTGGCCTTGAGCCGCAGCCATT
ACCGCCTGATCATCGGGGAAGTATTAGACTTGATTAGAGTAAGAA
GATTAAGAGCCGAAGCCTTAAAGGTGAATTACGACTTGCGCCAGC
TGGGAATTTATTCACTTTTTAAGAAAAGGTTAAGCACAA
TTGCCGAACCTGACGACGACAATAATTTTCGAAATCCAA
GTTTTAGTATTTTGATAGGTCAGTTACAATACAGTAATTCGA
ACAGGGACATCACAGACACTAACAACTAATATACAAACAACAGTA
ACGGAAATAGAGCCCAGAACCACCACCAGATATTCCCAATAATAG
AAATCCGCCACAGCAAAATCACCAGGAAGGTAATAGCCG
TTATACAAAGATTTAGGCAGAGGCAACAACATGCGAGGC
CTCAGAGCCTCATTCCCACAACTACAATTAAGAACGTTCAGC
TCGCAAGCAAAATCCACCCAGGAATTGATAAGCAGAATATTG
ATATCTTTATTAAAAACGTCAGATGAATAAATCGCAATAAAAAAT
CAACTCGTAGGAGCAATATTTTTGAATGGACCAGTGCAGAGGAAT
ACGCCAAGAACGCGCTTATCCGGTATTCTTTATCCAATA
TAATGCACATGTAAACGCGAGAAAACTTAAGAGTCTGAA
AACAAAGGAGATAAAAAGCCAGTCACACGCTATTAATCTAAA
AGATTTTTTCATTTTTCACCAGAATGGATCAGAGCATTACCA
GTGCGAATTACAGGTTTTCCTTTGCCCGAACGAAGGTTGTCTTTA
TCTTATCAGATTACCAGCCGATTGAGGGAGGTAGCACCCACCACC
CAACTAGAAGGCCTGTTTATCAACACGCCATATATATTT
TTAGCAACATTCAAAAGGAAACCGAGGATAATTGAGTCT
ATGCGCGTGGCAGACAATTACCGCTGAGTTTCAAATTTAACA
TCAGATAGCTAACGATTAAGACTGAGCATAAAGAAATTTTAA
AGGGCGAGGCCGGACAGAACCGCCACCCAAGCGCAGCGCTAATAC
CTGTTTACATAACTGATTGAAAGGAATTGAGGTTATTAATTGCGT
CGATTTTCATCGCTTAATTGAGAATATAGATAAAGCAAA
TTACCCACCCTAACGTCACCAATGAGCGCCAATAATAAC
AGAGCCGCGTTCCAACAAAGTTTCCAGACAATAGCAGTCCTG
TAGTTAAAGCTTAGAGCGTCTCAGAGGGAACGCAAAGACAAA
AAGTTTGCAACAGTAGCCCTAAAACATCTCTGAAAATGATGATAG
GGCAAATAGTAACAACGTAAAACAGAAAAAAGAAGTGGATTAAAT
AACAAGACAGTAGGTTCTGACCTAAATTAATCCTTTTGC
CGCTCAAAAAATAAATCATTACCGCGCCGCCTAATGAAA
GGAATACACCCTGAGTAAGCGTCAATCGGCCATTAGTCAGTT
TCAAAATATCAAGATTGACGCTCATACAGAACCGCGATAGCA
ACAAACAAACTATTTGCTTATCATTTTGCGGATATCTGAAAATAC
CAGACTGAACCCAAAAGCATATGGTTTACCAAACCATCCTCCCTC
AAACGTAGGATATCCCATCCTAATCAGTATATTGAAAT
GCACCGTGAAAATTAACTGGCATGATTAAGACGGGTGAT
CGAACGACTACATTAAACAAACCCTTAGTAATGGTAAGCCAA
TTTTCATATAAACATAATTTTATTAATTTAGAACCAAACCAC
GATGAAATACACCACCACAAACCCTCAATCAAACAAAGTACCATA

ATCAATAAATCAGTCAGAGCCACCACCGTGGCTTTAGAATTATTAC
GGAGCCGGAACAGCGACAGAATCAATTTATTTTTATTAC
TCGCGTGTGATATACAAATTCTTACTTACGAGGTTTTTA
AAAATCATGTACTGCAGGGAATATTTATGCAAGCCCATGTAG
ACCGACCTATTAATGCCATATGCGCATTAGACTCCTGTCACA
CAGAAGGCAAATATGCAGAAGATAAAACGAAAAACACAATTTTCG
TGAACCTAGCGGAAAATAATGGAAGGGTACATTTAGCTCATGACA
AAACCAAATGCGTTAAATAAGGCGTTAACTTGCTTCAAA
GTATCATTCAATAACTCATCGAGAACAACCCAATCCTGT
GCAGTATATAAAAAGTAATAAGCAACAGAGAGGTGACCTTGC
GGATTATATTACCTAGCCATTGTTTTAAATCTTTTTTTAGCG
AAACATTTGAACTTCTGTTATCATCATATTCAAGCATCAGGCGGT
TAAGAATAACGTTAGCACACCACGGAATAAGGTTTGCCCATAATC
ACGATACCGCATCGGCTGTCTTTCCAAAAAGCTAAACAC
TCAGACTGCAAAGAAACGTAGAAAATACAGCCTTTAGTG
AACCAAGTTTTTTGTGTGAGTTGGAAACAATCCTGTCAGATG
CAGTATTTACCGCCTTTTTAAGAATAACATAAGAACTGTTTA
AAGAAACGTAGCGCTATTAGCGTTTGCCCGGGGTCACAGAGAGAA
CCTACAATATAACACCGAAATGAAAAATCTACTGATTAATTGTTT
AACGGATATATGCAATGCCTGAGTAATGTCTCCGTATCA
GTAAGCCCCCTGTTTTCATCGGCATAGGGCAACATATAA
AGCCGGAAATGGGAACTATCGACAGTTAAACAGTTTCAGCTC
ATGGCAACAGCAGCCCTGCAACAGTGCCTGGTAATAACGGAT
CGAAACATTACAGGCGTATAAGCCTTGCACGAGGTGCCGTAA
CGGAATCCAATATATTTAACGTCAAAGATTGAACGGGTATTA
GAACCCTTTTTGATATTAGAGAGTACCTAAGATTCGGGAACA
GGTCATACAGTGCCTAAATGAAAATAGCATACATAAAGGTCG
TGAGAGCTTAAAGCCTGGGGTGCCTAATTTTTTTGGGGTCGC
TTGCTCCCATATATTTTAAAATTACTAGTTATCATTCCAAAA
GTTAACATTAAAGTACACAGCGATTATACCATTGGAGTGAGAATA
TGACCGTAGCATAAAGTGTCATCAATATAGTACATATCCAGATGA
ATTTGGTCAGGAAGAGGTCATTTTTGATAAAAAAAGATT
GAAAGGATGACCCCACGGAGATTTGTATCGAACTACTGC
AGCACTAAACTCAATAGGTCACGTTGGTCCACACACTAACTC
TCCAACAAGGAATAACAACCCGTCGGATTGTAGGTATTTTTA
CTAGTAGAAGAATCGGATCACCCAAATCAAGGAGTGAGACATACG
TCATCTTACAACTAGCTAAACAACTTTCATGCCCCACGGAACGAG
CGGAGGATTTTAAGGAATTGCGAATATACACTTGATAAA
GTGAGTGAGAATATTTCAACGCAAGGCGGATGAGCAAAC
CAAAAGGGCGAGTACCACATTCAACTAAGACCGGAGCTTAGA
CTGTATGACCTATTCTACGTTAATAAAACATCGCCAAAACAC
ACATTAATGAACCAACCCTAAAGGGAGCCATCACTGGCGCATAAT
CACTACGTTGCGTTATCCGCTCACAATTGTAGATGTGCCTGATTT

GCGGGAGTGCTGAATTCAAAGCGAACCATGCAGATAACA
GCTTAATAAGCCTTAGGCCGGAGACAGTTTTCATCACAT
TGTGTGAGTGCATCTTGATTAAACATGAAGTAAATTTTTCAC
TTGTGTCGAAAAATATTCTGAGTAATAACCCCGATGATGGCC
AACTTGGGAAGAAATCCAAAGAGGCAAAAGAAATAATTGAATTTT
TTACGTAACCAATTGTTGCGCTCACTGCCCGTCAGGGCTTAGAGC
AAAATTCGAGCTATAATGCTGTAGCCCTGTAACCATCAA
GTTGAAATAAAACGGCGACCTGCTCCATTACCAGTAAGA
GTTTTAAGGAATTATTCCTGTTTGAGGGTCATAGCGTCGGGA
TTGACGGTACTTCTTGCCAGTAGCCAGCCAAATCATACTTTT
ACCAACCATCTCCATCTTTCCAGACGTTAAGTATTCAGGACGGCC
GGCGTAGCAAGGAAAGCCGAAAAACCGTCTACTTTCCATGTTTCC
CGCGTTCAACCAAAAACATTATGACTCAACATATATCGC
GACTTTTGTCGAAAAAAAGGCTCCAGCCACTAAGCCGGA
CTAAAGTCCTCAAGGAACTGGTAGTAAGACTTCAAGTTTTAA
TATGATATCTGGCCCGAGGCACTCATTAGTTACTTCGAAGGC
AACCTGTCAAAGGGCGGCGAACGTGGCGGCAAATTACAGTATATT
CCAACGTCGTGCCAATTCGTAATCATGGGACGACGAACCGTTTGA
AAATCGGACTAAAGAGGAAGCCCGAAAGAGCAACAAAAA
ATATGCATTGTACCGTTCTAGCTGATAACGCCATCCTAT
ACGAGGCATTTTAAAGAAGGACCATCACAGAAAGGGTGGACT
GGTACCGAGGAAGAAGTCTGTTTAGGATGTTAGCGCCTTTAA
ATACGGCCTCAGCTCGAGCTGCATTAATGAAAAAGAACAAGGGAA
CATTTATGCGGCAGACGGTAAAATACGTAATAAAGGAGTAACGAT
CCTCGATTAAGTACGGTGTCTGGAACAGAGCACCGGAGA
TTGTATCTAAACGGGTCAATCATAAGGGCATTGTGGTTT
GAAAGCGGTAAAAGTCGCACTATAGGAAATTAATGTAAAGCT
TCAAAAAGTTTACCTTAACCACCAGCCACTAGAGGACGCGCG
TGCCCACCGAAAAGGAGAAGAGTCCACTATTTCGGCCAATCCCCG
TTTCCATGGTTTATAGACAGCCCTCATATAGCGGGAATTACCAAC
CAGCTATTTTTAAGCAATAAAGCCTGTTTCATGATTGCA
GTACATTCCACCAGCTTGCTTTCGATTTTCATCTGACCA
CTGTAGCCAGGCGGATTTCAAGATAAAACAAAGCGTCCATAT
GGGTAGCTCATTTTAGACGACCTTTAATAACCGAAGAGGAAG
GGGAGAGTTGGAACCGGGCGCTAGGGCGTATAATCGGCACCGAAT
TTCCAGTGCGGTTTCCTGCAGGTCGACTGCTTTCCAGTGAGGTCA
AACAGTTCAAAATTGAGAGATCTACAAATTAAATTTAGC
GAATTAGGATTCCCATTATAGTCAGAAGACCAAAATTTG
ACTTTGATGGTTTAATAAGTGGTGTTTTCTGGCAAAGTGTTG
GCCAAGCTGCCGGACTGAGAACCGTCGAACAAACTTTCTTAA
GAGCTTGAGAAAGAGGAGAGGACTAAAGACTGGTGAATACAACGC
TTACTTCTGGTTGCATGGCGTATTGGGCGCCAGGGTTGGTGTAGC
ACAGCTTAGGCTTTCAGATGAACGGTGTAGTAGTAGATA

CTTTCCTGACTAATTCTGCGAACGAACAGGCAAGGTCAT
GGTCACGCAGAATCAACCAGGATTCGCAGGCTATCAGGCAAA
TCTTTACTGCAAAATGTTAAACAAAGCGAAACGACTTTTTCT
GCTACAGGATACCGGTTTCGTCACCAGTGAGGGTTAATTGGGAGG
TAAGGTACGCCTGCGCGGAATAGCCCGAGATAGGGTGGGGCCAGT
CGTTGAGTCTGATCCAATAAATCATGTAGATTAATCAGG
TAGCACACTGAATAGTTGCGCCGACCGAGGGTAGGCGCA
TACCGTACCGGAATGAAACACTGCCAGAAATCAAATAGTTTG
TGCCTGAAATATTTGAAGTTTCAGAACGACAGACCAGCAACG
CTTATAAAGTGAGAGTCACGACGTTGTACCATTCGCAGGAACGTA
TTTCACCATCAAAATAACCACCACACCCTTTTAGACCATTCAAAA
ACCATTACATTAACGAGCAAACAAGAGAAATATTTATAG
GTAGTAGGATACATCGAGAATGACCATAGGGGGTAAAAT
CCAGGGTCAACTGTGCCGATTTCACCGTAATAGGAACAACCA
TAGGCTGCTGACGAAGGTGTAAAAGGGAGCCGCGCAAAATCC
TAAGCTTGCCGCTGACCAGACAGCATCGGAAAATGACAACCCATG
TGTGGCTGCGTTTCCCACGGGCAACAGCTGAAATCGGCTTAATGC
TCGCCCACAGCGAATTCATCAAGAGTAAATTCAGTAGG
GTTTCAGAAAATTCGCAAATGGTCACAATTCTAACGGTA
AACAGTTAGACTGGAGATTGTGGCGATCTAAGTTGTCACCGC
GCCGCTAACAGGAGTGGGAAGATAAGCAATCGATGACTAATA
CCCTCAGCGCATAAGGGATAGCAAGCCCACTCAGGGAATAAGAAT
TTTAGAGCTAACAGGGCGGATGGTGGTTCCGATTGCCCTGGTAACG
CCGCATTTTCACCGATATATTCGGTTTGCGGGAAGAACC
CACCCTCCACCCTCTAACAAACCAATACCCTCAAAGTTTAGC
CCCCAAAAACTAGCTGAAAAGGTGGCATATAACCTTGCTTTA
CTGGCCCCCTGTTTCGTACTATGGTTGCTAGAATCGCCTCTT
CGAAAATTGAGAGATGCTGCAAGGCGATGGTGCGGAGAGCGGGTA
TACGTGGTCTGGCCAATACCATGAGAGATGATGCAGCCAGTATGCACAGTGAGACATC
ATCGTAAAACAGGAATAGCGTGCTGCTCTCTTGACATCGTCA
/5Alex647N/TTTGATGTCTCACTGTGCA

1.9 References

1. F. Schneider, N. Moritz and H. Dietz, *Sci Adv*, 2019, **5**, eaaw1412.
2. J. SantaLucia, Jr., *Proc Natl Acad Sci U S A*, 1998, **95**, 1460-1465.
3. C. E. Castro, F. Kilchherr, D. N. Kim, E. L. Shiao, T. Wauer, P. Wortmann, M. Bathe and H. Dietz, *Nat Methods*, 2011, **8**, 221-229.
4. J.-P. J. Sobczak, T. G. Martin, T. Gerling and H. Dietz, *Science*, 2012, **338**, 1458-1461.