

The Kinetics and Thermodynamics of Watson-Crick Base Pairing-Driven DNA Origami Dimerization

Supporting Information

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Supporting Note 1: Nanostructure specifics

DNA Origami Specifics

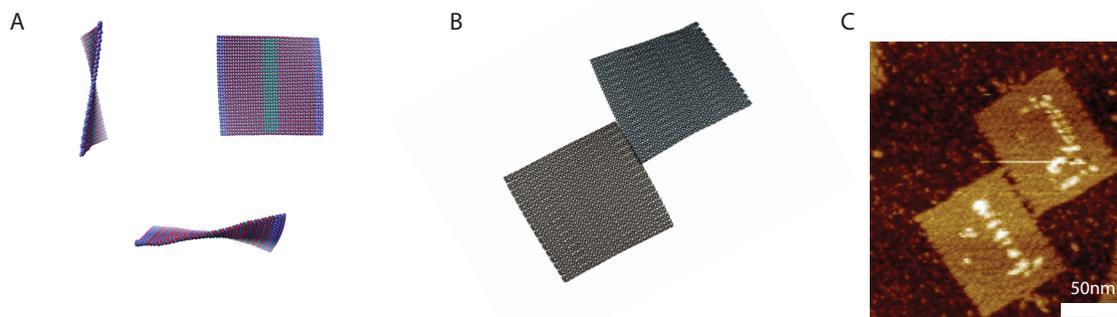


Figure S1: CanDO and CaDNAno models of DNA origami tiles used in this work. The tile components were designed in caDNAno¹ to be 32 helices tall with a helicity of 10.44 bases/turn, using 7008 bases of the ssDNA. T1 and T2 have the same origami design and staple sequences, except for the hairpins that distinguish the two tiles and the linker staples between the two tiles. For sequence specifics see Figure S33 and SI Note 7. (A) CanDO structure prediction of a single, tall, flat rectangle component². (B) Model of T1 and T2 binding generated from CanDO atomic model structure prediction without hairpins. However, CanDO structure prediction does not account for the curvature induced by hairpins on the origami surface, which has been shown to influence origami curvature³. The two tiles have many (>45) hairpins in order to ensure the differentiation of each origami component on the AFM, even if some hairpin staples did not properly incorporate into our system or were not distinguishable because of imaging artifacts: Due to the nature of the experiments described in this paper, it is important to be able to distinguish the identity of as many of the origami as possible. We rotated T2 relative to T1 by 180 degrees to facilitate the binding between homogeneous interfaces⁴. (C) AFM image of T1 and T2 binding with 4 linkers with 5bp SEs. Scale bar is 50nm. As shown in Figure 1, the distance the linkers span between T1 and T2 is 21bp, or about 2 full turns of DNA.

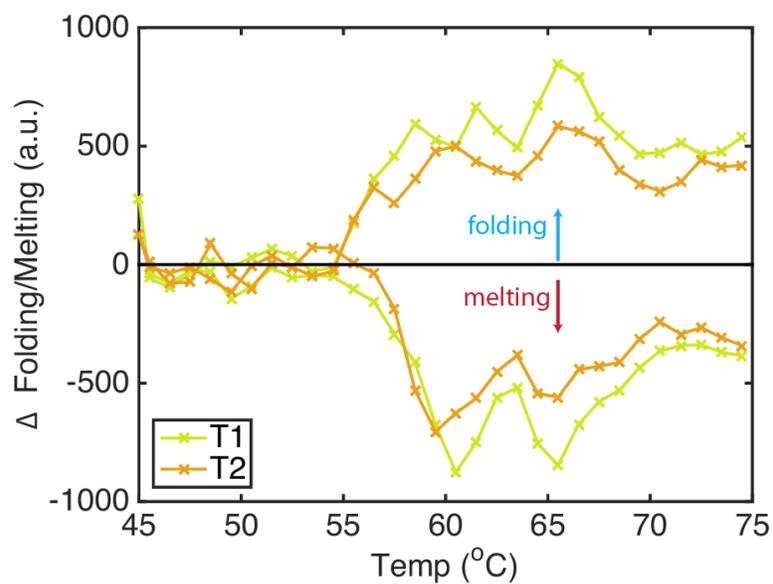


Figure S2: DNA origami components melt above 55°C. Tile component melting and folding temperature as determined by DNA intercalation dye SYBR Green, similar to the protocol previously described in Ref. ⁵. Rate of folding and melting of T1 and T2, as determined by the fluorescence derivative with respect to temperature of the full object reaction normalized by their respective staples-only reaction. The rate of cooling and heating is 1°C per hour. The melting of the DNA origami components takes place starting at 56°C, thus, all thermodynamic and kinetic protocols take place below this temperature.

Supporting Note 2: Fluorescence Specifics

Fluorescence Strategy

We chose to use a Texas Red®-X (NHS Ester) fluorophore, as its fluorescence has been shown to be relatively invariant to changes in temperature, pH, and nucleic acid sequence⁶. We used the Iowa Black® RQ dark quencher (IDT, Coralville, IA) to quench the Texas Red fluorophore. The fluorophore is located on a linker strand on Tile 1 (T1), on the end of a 3' sticky end (SE) and the quencher is on the 5' end of the Tile 2 (T2) linker strand (see Fig. 1 in main text). Contact quenching occurs when T1 and T2 bind via complementary linker SE sequences. Upon binding, the quencher contacts (within ~2 nm) the Texas Red fluorophore, which has been shown to produce high (> 95%) quenching efficiencies⁶. However, we found that the first anneal or melt cycle had noisy fluorescence values. Data was reproducible only after the first cycle, after the fluorophore / quencher pair had been interrogated multiple times (at least 20). We speculate that this could be a fast photobleaching process, some non-uniformity in the Texas Red or Iowa Black Quencher synthesis, or some initial reaction of the dyes to the DNA or buffer environment.

Post Processing Fluorescence Measurements

To account for the changes in Texas Red fluorescence with temperature, the fluorescence reading for T1 alone was used as a baseline. The fluorescence of T1 alone was subtracted from the fluorescence readings of all T1+T2 reactions. Additionally, as well-to-well background fluorescence varies in a real time thermal cycler, all fluorescence baseline-adjusted measurements in a given interface design were linearly shifted in order to maximize the overlap between samples, using a least squares fitting algorithm, and the average of the samples was then

linearly shifted to an initial, arbitrary starting value and then normalized to 1, in order to more easily and consistently interpret the change in fluorescence across all samples. The average and standard deviation of the fluorescence quenching were calculated using at least three replicates per linker architecture. Fluorescent data was smoothed *via* a moving average smoothing function built into MATLAB (*smooth* function).

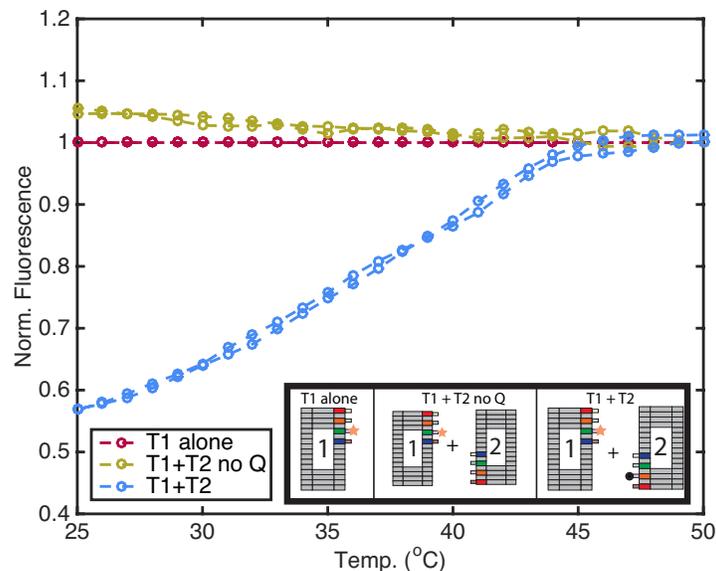


Figure S3: T1 – T2 binding does not reduce fluorophore activity when no quencher is present. Normalized fluorescence (to T1 alone) change versus temperature for T1 alone, T1 and T2 together without a quencher and T1 and T2 together with a quencher, as shown in the schematic in inset. Dimers decrease fluorescence with decreasing temperature when a quencher is attached to T2 but increase slightly fluorescence if a quencher is not present. A similar increase has been previously reported in Ref. ⁷ and is hypothesized to occur as a result of the fluorophore being “squeezed out” of interactions with DNA by the formation of dsDNA. In this hypothesis, the ability for the fluorophore to become excited could be enhanced upon dimerization because the fluorophore is no longer integrated into a stacking position within the ssDNA portion of the SE, allowing the dye to rotate more freely.

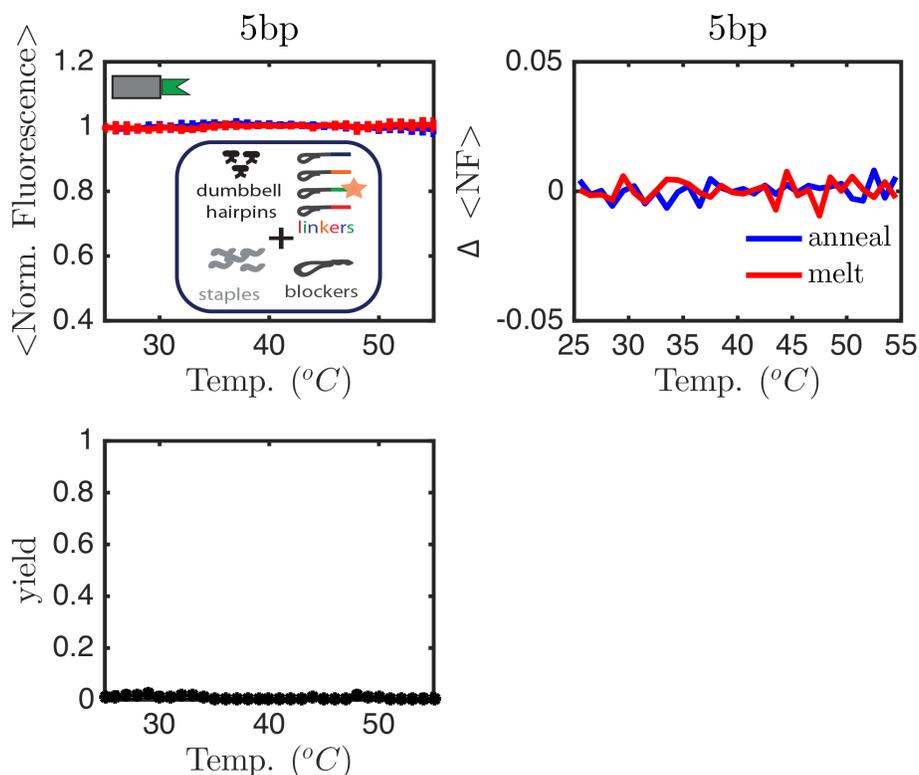


Figure S4: Free linkers in solution (unbound to origami) do not bind to their complementary linker for 5bp SEs. Reaction mixtures for T1 and T2 were prepared without the scaffold (see inset diagram in top left plot for components included in the reaction) for 4SE systems with 5bp SEs. Samples were normalized with the scaffold-free reaction mixture of T1 alone. The plot in the top left shows the mean normalized fluorescence as a function of temperature. Error bars represent one standard deviation of the reported quantity. The plot in the top right shows the change in mean normalized fluorescence values as a function of temperature. The 5bp linkers do not show any change in fluorescence with temperature in the range of temperatures tested, indicating that free linkers in solution do not bind to their complement, as shown in the plot in the bottom left. Yield calculations assume the same fluorescence to yield calibration measurements obtained in SI Figure S6.

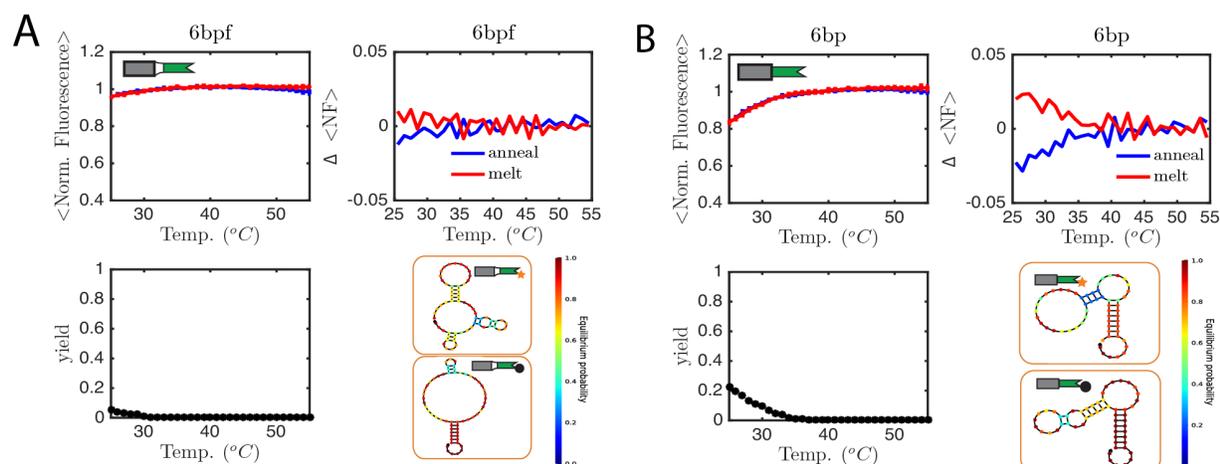


Figure S5: Free 6bp linkers in solution (unbound to origami) can bind to their complementary linker for linkers with 6bp SEs. Reaction mixtures for T1 and T2 were prepared without the scaffold for 4SE systems with (A) floppy linkers and (B) non-floppy linkers with 6bp SEs. Samples were normalized with the scaffold-free reaction mixture of T1 alone. The plots in the top left show the mean normalized fluorescence as a function of temperature. Error bars represent one standard deviation of the reported quantity. The plots in the top right show the change in mean normalized fluorescence values as a function of temperature. The plots in the bottom left show and the diagrams in the bottom right show the secondary structure of the fluorophore and the linker according to the NUPACK⁸ structure predictions at 35°C. For the system with non-floppy linkers, free linkers appear to bind to one another below ~35°C achieving ~20% yield at 25°C, which indicates that there is potentially competition between origami components and free linkers in binding to an origami interface. The floppy linkers, although their SE sequence is identical to the non-floppy linkers, do not show as much hybridization as the non-floppy linkers, yielding ~5% at 25°C, which is likely due to difference in the secondary structures between the floppy and non-floppy linkers. Specifically, the NUPACK prediction for the floppy linker of T1 (with the fluorophore) indicates secondary structure involving 3bp in its SE, most likely preventing or significantly slowing the reaction between the two free floppy linkers. Secondary structure is an important factor, along with SE length and sequence, in determining the extent to which free linkers bind in solution. Yield calculations assume the same fluorescence to yield calibration measurements obtained in SI Figure S6.

Supporting Note 3: Thermodynamic measurements

Fitting thermodynamic parameters

Finding thermodynamic parameters for the T1-T2 interaction was achieved using the definition for Gibbs free energy:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0, \quad (1)$$

and the van't Hoff Equation, assuming a two state transition between tiles:

$$\ln(K_{eq}) = -\frac{\Delta H^0}{RT} + \frac{S^0}{R}, \quad (2)$$

where ΔH^0 is the standard enthalpy change and ΔS^0 is the standard entropy change across a range of absolute temperatures, T and $R = 0.00198 \frac{kcal}{mol K}$ is the universal gas constant. K_{eq} is the equilibrium constant and defined as:

$$K_{eq} = \frac{yield_{eq}}{C_0 * (1 - yield_{eq})^2}, \quad (3)$$

where $yield_{eq}$ is defined as the yield of dimers at equilibrium and C_0 is the initial concentration of T1 and T2 (assuming negligible pipetting error so that the concentrations are the same). The $yield_{AFM}$, or the fraction of material in dimers as determined by a series of AFM scans, is determined by the following formula:

$$yield_{AFM} = \frac{2 * (N_D)}{(N_{T1}) + (N_{T2}) + 2 * (N_D)} \quad (4)$$

where N_D corresponds to the number of observed dimers, excluding those in the flipped conformation, (see Figure 2B), and N_{T1} and N_{T2} correspond to the counts of individual T1 and T2 components, respectively. Although flipped dimers would also result in a decrease in fluorescence, it was too difficult to determine with the AFM images if the flipped dimers were

bound by linkers or happened to land next to one another in a flipped confirmation. For this reason, we excluded the flipped dimers in the yield calculation altogether. We expect this would not have a significant impact on the results, as likely some of the flipped dimers would indeed be in a quenched state, and others would be in a fluorescent state. Overall, the flipped dimers accounted for less than ~10% of all of the structures for all AFM scans. For the majority of interface designs and temperatures tested, flipped dimers accounted for < 3% of all structures. The T_m , or the temperature at which the yield is 50% (see Figure 2F), was calculated using the yield curves (SI Figures S7-S14 bottom right plots).

Thermal Protocol for fluorescence quenching thermodynamics experiments

The annealing protocol is as follows, with fluorescence readings taken at the end of each temperature hold at integral degrees:

1. 25 to 55°C at +1°C per 15 minutes (melt 1)
2. 55 to 25°C at -1°C per 15 minutes (anneal)
3. 25 to 55°C at +1°C per 15 minutes (melt 2)

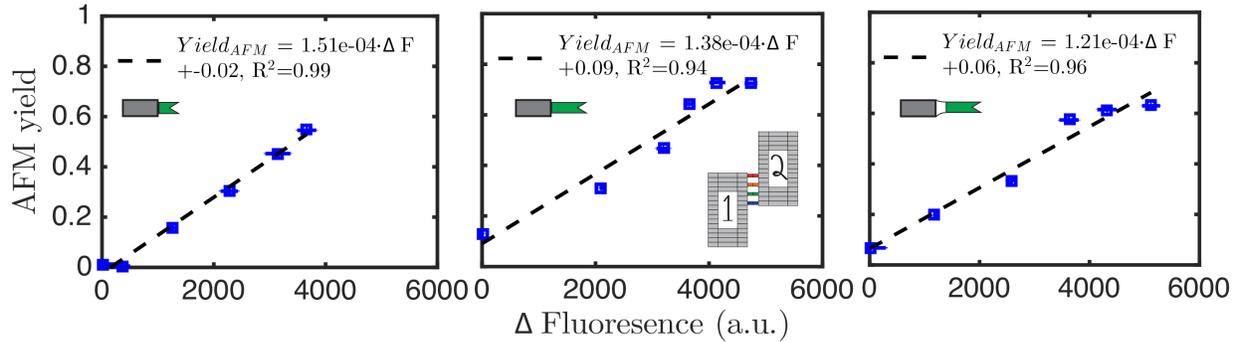


Figure S6: Fluorescence quenching data as a proxy for AFM yield. Change in fluorescence versus AFM yield for 5bp SE (top left), 6bp SE (top right), and 6bp SE floppy (bottom left) SE (bottom right). Samples were annealed from 55°C to 25°C at a rate of 1°C per 15 minutes for the 5bp and 6bp floppy SEs systems and a rate of 1°C per hour for the 6bp SEs systems (see Methods). All systems used in this calibration process have four. All AFM yield data points have, at minimum, 300 counted objects. All fluorescence measurements were performed in at least triplicate. Error bars for the change in fluorescence represent one standard deviation; error bars for AFM yield generated by bootstrapping and indicate one standard deviation and are too small to see.

Thermodynamic Data

5 base pair SE

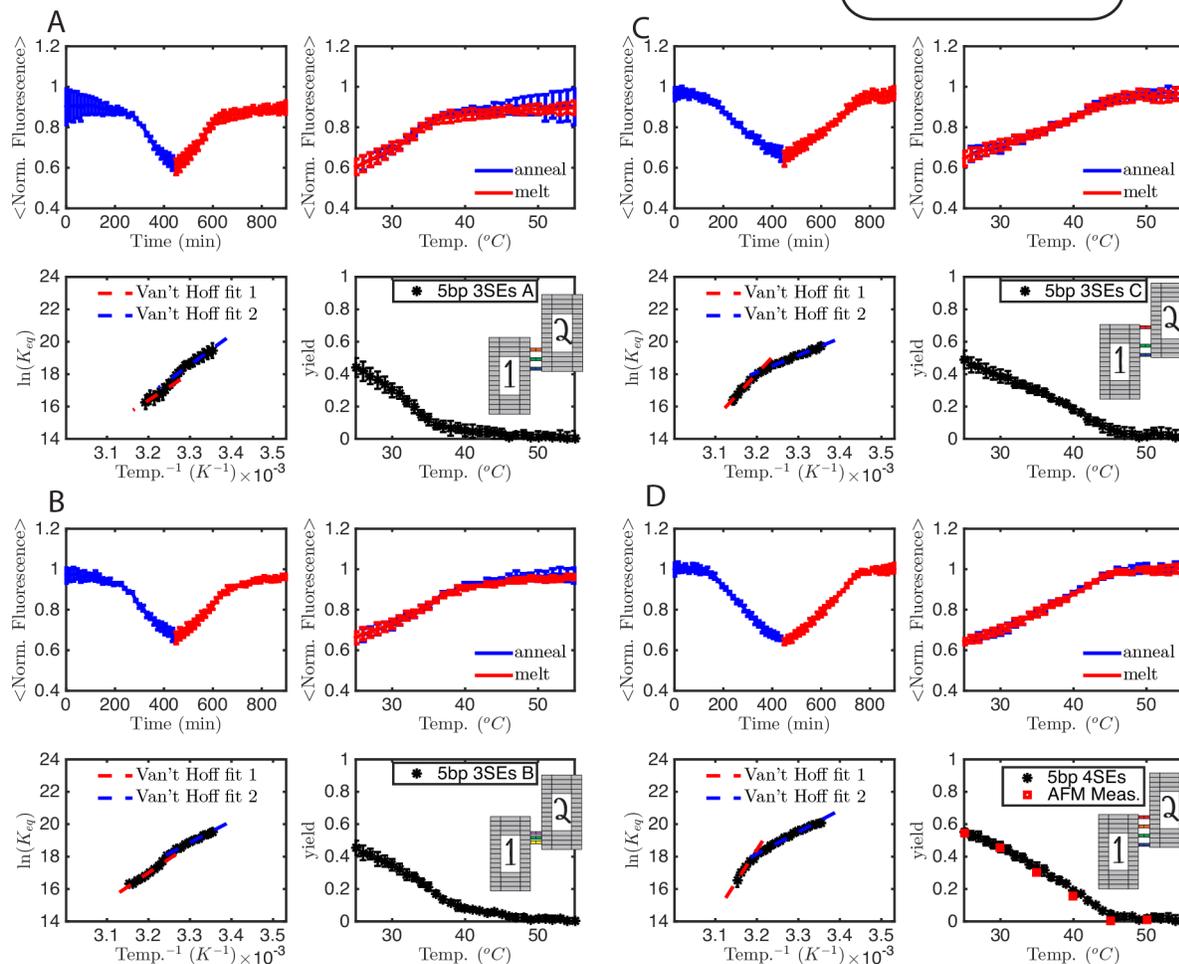


Figure S7: Thermodynamic measurements for interfaces with 5bp SE linkers. Interfaces are comprised of (A) 3 linkers in arrangement “A” (B) 3 linkers in arrangement “B”, (C) 3 linkers in arrangement “C”, (D) 4 linkers. Four plots per interface are (top left) fluorescence versus time, holding at 15 minutes per degree, (top right) fluorescence versus temperature, (bottom left) van’t Hoff plot and (bottom right) yield as a function of temperature. Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 5bp SE linker shown at the top of the figure. Error bars represent one standard deviation of the reported quantity.

5 base pair SE (Cont'd.)

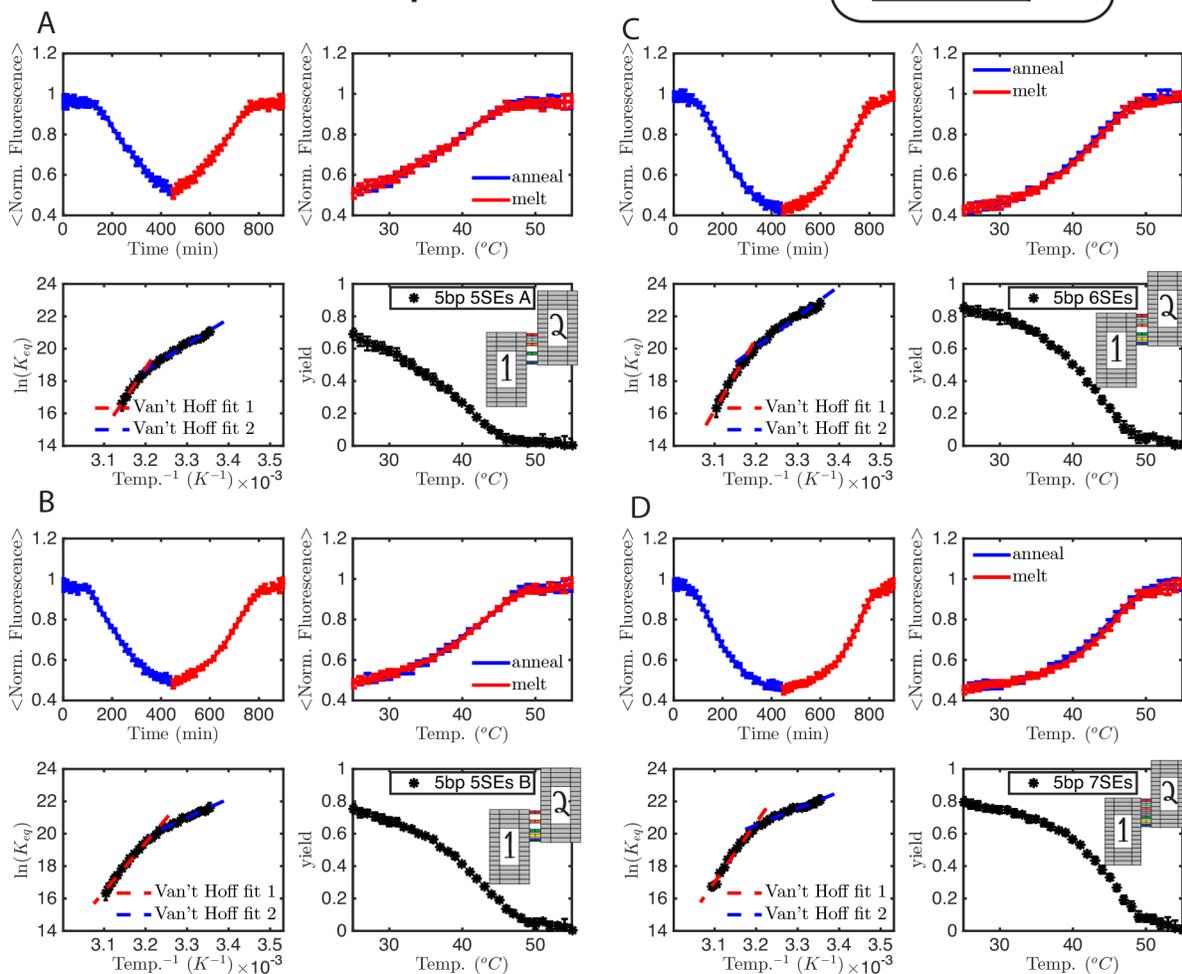


Figure S8: Thermodynamic measurements for interfaces with 5bp SE linkers (continued). Interfaces are comprised of (A) 5 linkers in arrangement “A” (B) 5 linkers in arrangement “B”, (C) 6 linkers, (D) 7 linkers. Four plots per interface are (top left) fluorescence versus time, holding at 15 minutes per degree, (top right) fluorescence versus temperature, (bottom left) van’t Hoff plot and (bottom right) yield as a function of temperature. Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 5bp SE linker shown at the top of the figure. Error bars represent one standard deviation of the reported quantity.

6 base pair SE

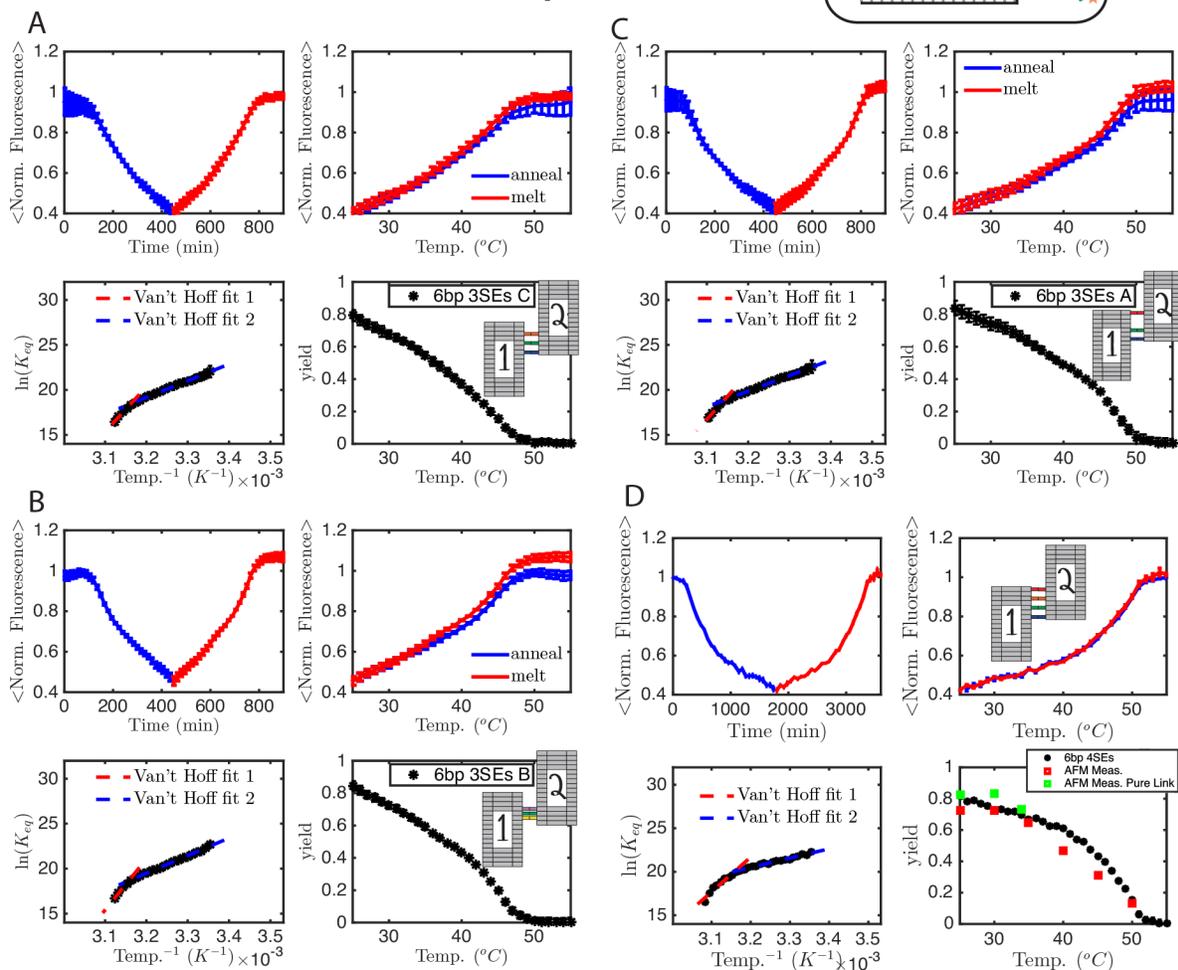


Figure S9: Thermodynamic measurements for interfaces with 6bp SE linkers. Interfaces are comprised of (A) 3 linkers in arrangement “A” (B) 3 linkers in arrangement “B”, (C) 3 linkers in arrangement “C”, (D) 4 linkers. Four plots per interface are (top left) fluorescence versus time, holding at 15 minutes per degree, (top right) fluorescence versus temperature, (bottom left) van’t Hoff plot and (bottom right) yield as a function of temperature. Note that (D) depicts yield as measured by AFM for linkers ordered PAGE purified (“AFM Meas. Pure Link”) and with standard desalting (“AFM Meas.”). Note the limits on the y-axis is larger than in the plots for other SE lengths for the van’t Hoff plots, reflecting the higher K_{eq} values. Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 6bp SE linker shown at the top of the figure. Error bars represent one standard deviation of the reported quantity.

6 base pair SE (Cont'd).

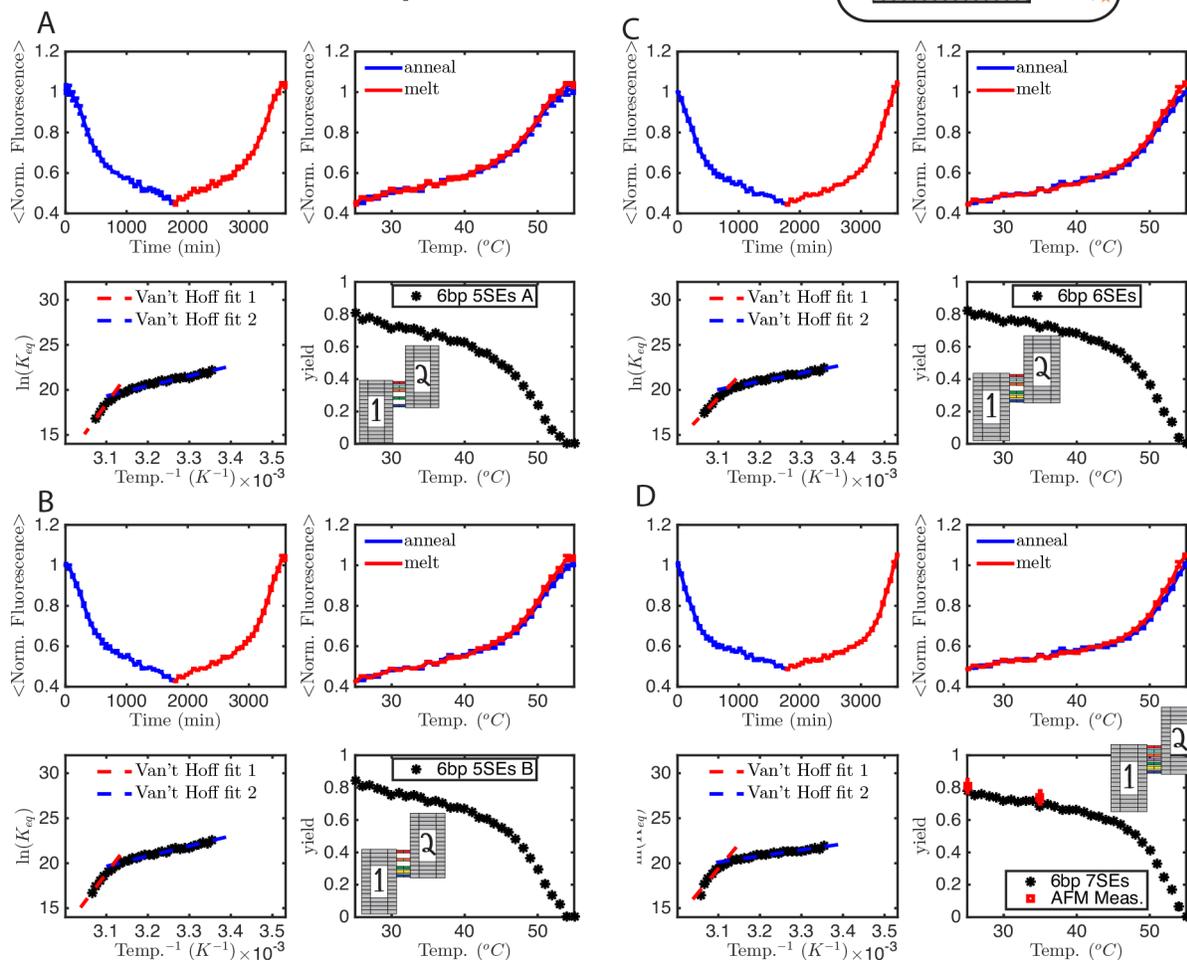


Figure S10: Thermodynamic measurements for interfaces with 6bp SE linkers (continued). Interfaces are comprised of (A) 5 linkers in arrangement “A” (B) 5 linkers in arrangement “B”, (C) 6 linkers, (D) 7 linkers. Four plots per interface are (top left) fluorescence versus time, holding at 15 minutes per degree, (top right) fluorescence versus temperature, (bottom left) van’t Hoff plot and (bottom right) yield as a function of temperature. Note the limits on the y-axis is larger than in the plots for other SE lengths for the van’t Hoff plots, reflecting the higher K_{eq} values. Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. To “spot check” other interface designs aside from the 4 linker, yield for 6bp 7SE at 25°C was verified on the AFM to be ~81% at 25°C, and ~74% at 35°C, suggesting the yield is indeed near 0 at 55°C. Cartoon schematic of an individual 6bp SE linker shown at the top of the figure. Error bars represent one standard deviation of the reported quantity.

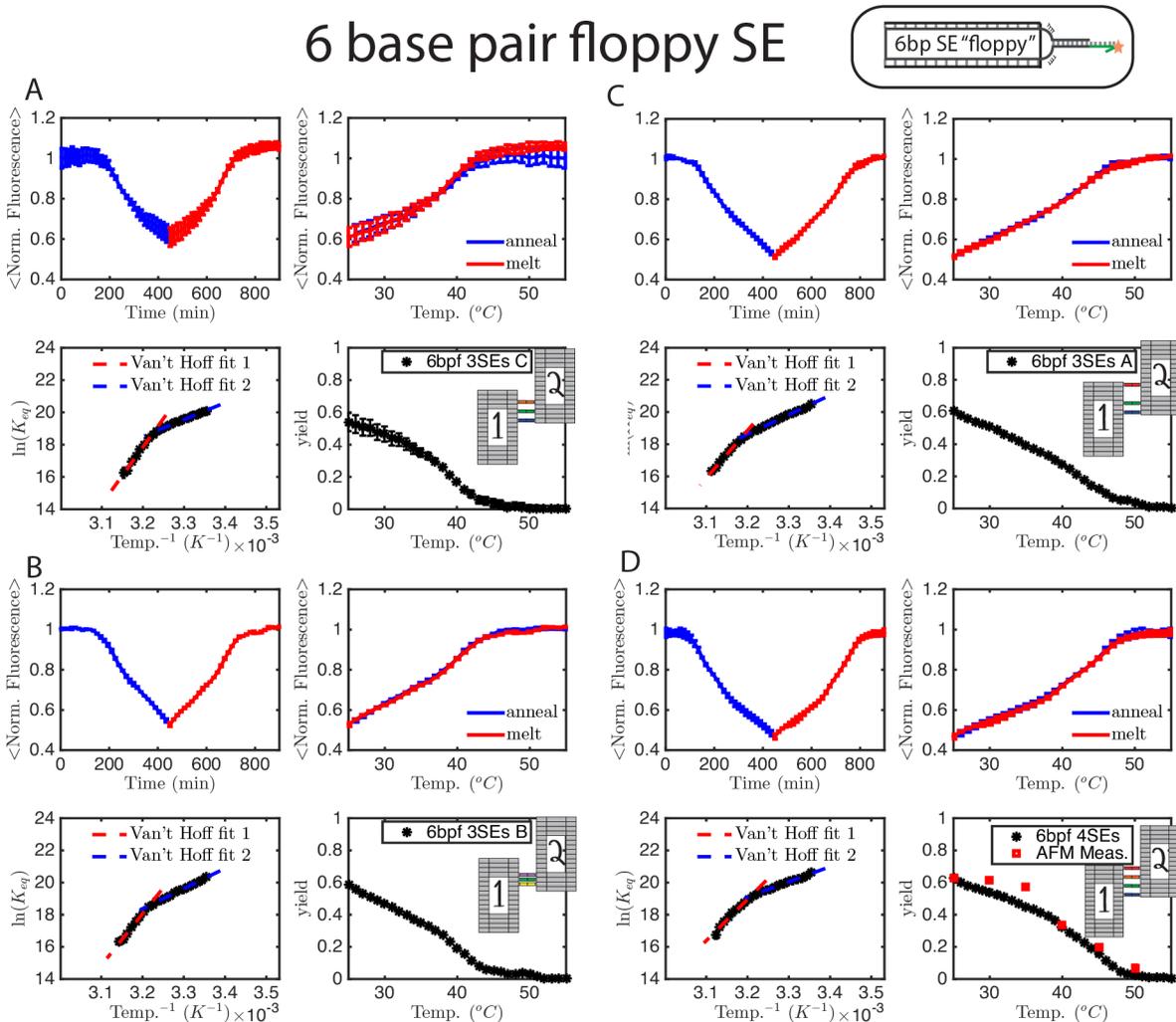


Figure S11: Thermodynamic measurements for interfaces with 6bp SE floppy linkers. Interfaces are comprised of (A) 3 linkers in arrangement “A” (B) 3 linkers in arrangement “B”, (C) 3 linkers in arrangement “C”, (D) 4 linkers. Four plots per interface are (top left) fluorescence versus time, holding at 15 minutes per degree, (top right) fluorescence versus temperature, (bottom left) van’t Hoff plot and (bottom right) yield as a function of temperature. Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 6bp SE floppy linker shown at the top of the figure. Error bars represent one standard deviation of the reported quantity.

6 base pair floppy SE (Cont'd.)

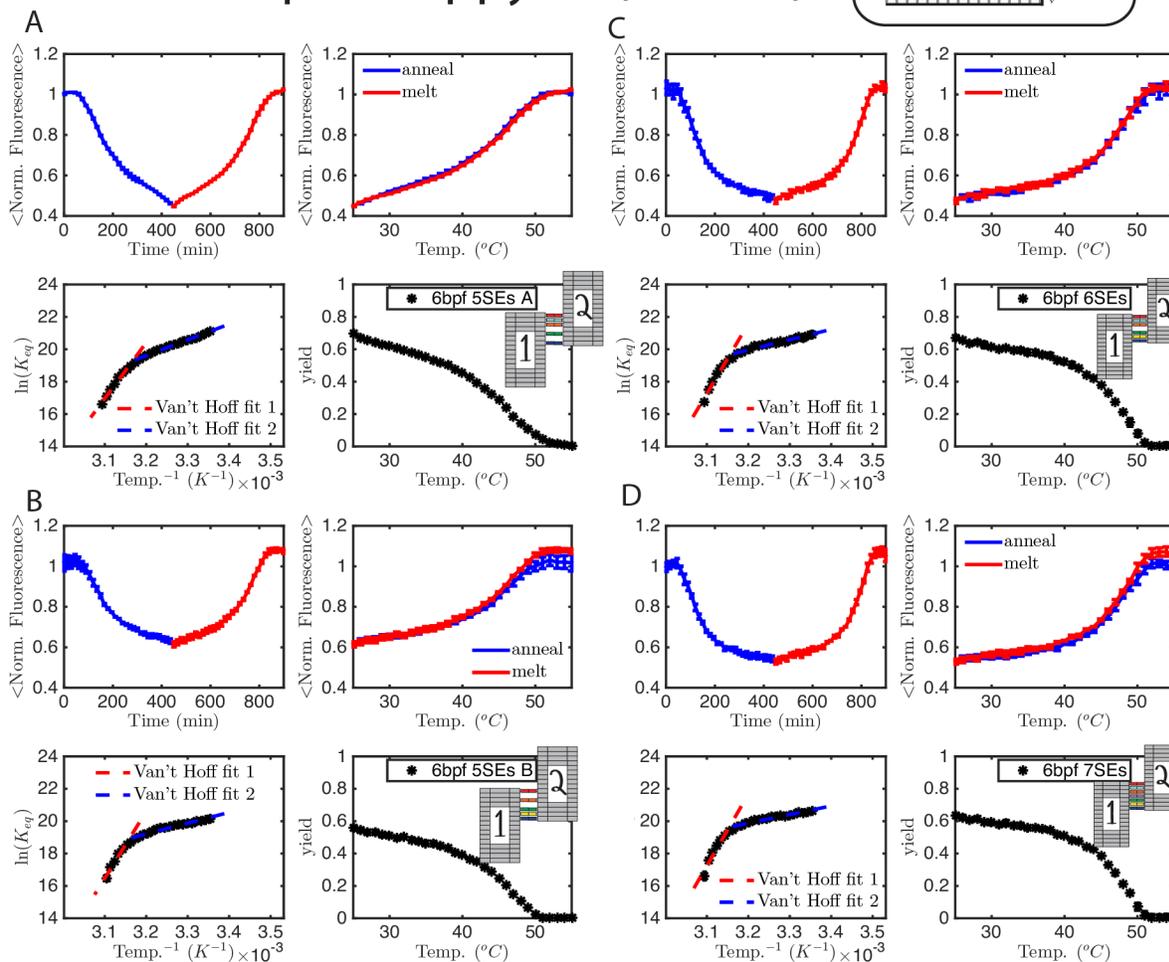
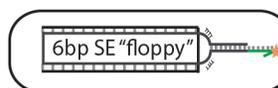


Figure S12: Thermodynamic measurements for interfaces with 6bp SE floppy linkers (continued). Interfaces are comprised of (A) 5 linkers in arrangement “A” (B) 5 linkers in arrangement “B”, (C) 6 linkers, (D) 7 linkers. Four plots per interface are (top left) fluorescence versus time, holding at 15 minutes per degree, (top right) fluorescence versus temperature, (bottom left) van’t Hoff plot and (bottom right) yield as a function of temperature. Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 6bp SE floppy linker shown at the top of the figure. Error bars represent one standard deviation of the reported quantity.

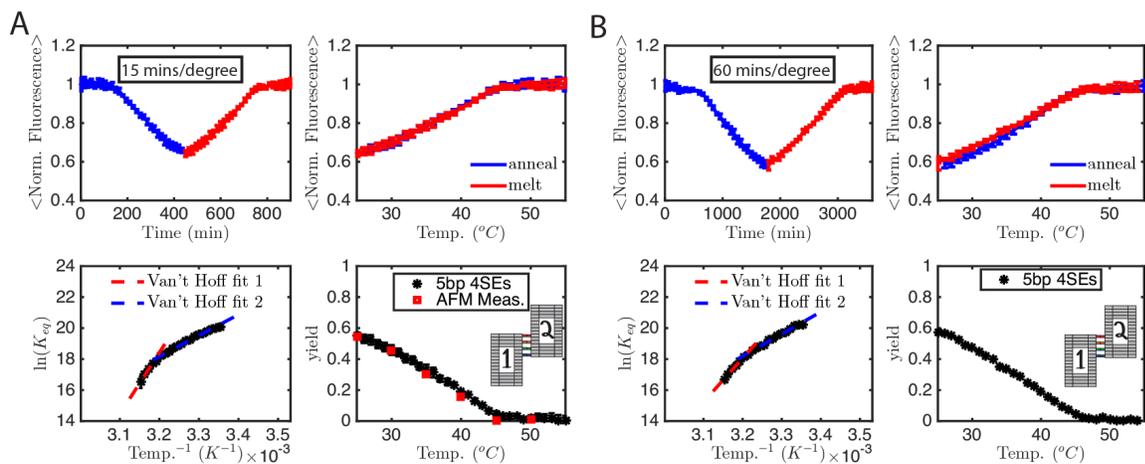


Figure S13: Dimerization achieves equilibrium in 15 minutes for 4 linkers with 5bp SEs. Anneal/melt curves for (A) thermal protocols with heating/cooling rates of 1°C per 15 minutes and (B) 1°C per 60 minutes. Neither thermal protocol shows hysteresis in an anneal/melt cycle and both achieve approximate yields of $\sim 60\%$ at 25°C . We therefore believe reactions achieve equilibrium quickly, within 15 minutes for 5bp linkers. Inset diagrams depict the linking schematic.

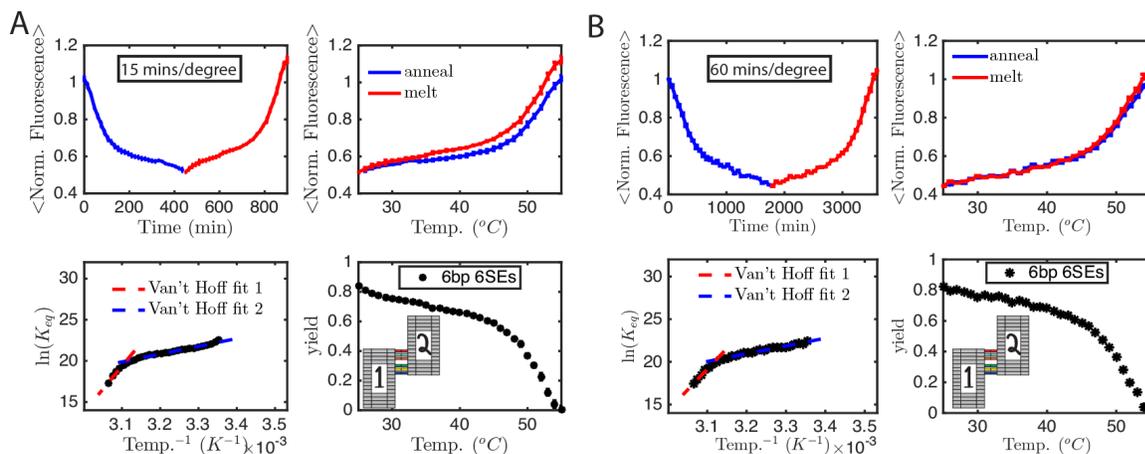


Figure S14: Dimerization achieves equilibrium in 60 minutes for 6 linkers with 6bp SEs. Anneal/melt curves for (A) thermal protocols with heating / cooling rates of 1°C per 15 minutes and (B) 1°C per 60 minutes. The faster heating / cooling thermal protocol shows hysteresis in an anneal/melt cycle while the slower protocol does not. This is also the case for interfaces with 4 – 7 linkers and 6bp SE. Inset diagrams depict the linking schematic.

Measured Thermodynamic Parameters: ΔH^0 and ΔS^0

Table S1: Measured Thermodynamic Parameters for 5bp SE linkers (kcal/mol)*

Interface	$-\Delta H_{VH1}^0$	$-298\Delta S_{VH1}^0$	ΔG_{VH1}^0	$-\Delta H_{VH2}^0$	$-298\Delta S_{VH2}^0$	ΔG_{VH2}^0
5bp 3SE A**	32.6±0.3	21.4±0.2	-11.2±0.0	36.8±0.9	25.2±0.9	-11.6±0.0
5bp 3SE B	34.2±3.3	22.6±3.1	-11.6±0.3	26.3±1.4	14.7±1.2	-11.6±0.2
5bp 3SE C	54.4±3.2	41.2±3.2	-13.2±0.2	20.8±2.7	9.2±2.6	-11.7±0.3
5bp 4SE	78.5±5.8	64.0±5.7	-14.5±0.2	26.9±3.1	14.9±3.1	-12.0±0.2
5bp 5SE A	75.2±6.0	60.6±5.9	-14.6±0.1	31.4±5.1	19.0±4.7	-12.5±0.4
5bp 5SE B	59.8±6.0	45.6±5.5	-14.2±0.5	23.2±8.2	10.4±7.8	-12.8±0.4
5bp 6SE	88.0±27.7	71.8±25.9	-16.3±1.8	37.5±14.2	23.9±13.6	-13.6±0.6
5bp 7SE	72.6±13.2	57.0±12.3	-15.6±0.9	20.2±5.0	7.2±4.7	-13.1±0.4

*VH1 is the fit for higher temperatures (~55°C to ~45°C); VH2 for lower temperatures (~45°C to 25°C). Error indicates one standard deviation of reported quantity and was calculated based on best fits to individual reaction samples.

**Nomenclature of interfaces (e.g. 5bp 3SE A) in this table is in the same order throughout SI. Please refer to Figures S7-S8 for interface cartoon schematic.

Table S2: Measured Thermodynamic Parameters for 6bp SE linkers (kcal/mol)

Interface	$-\Delta H_{VH1}^0$	$-298\Delta S_{VH1}^0$	ΔG_{VH1}^0	$-\Delta H_{VH2}^0$	$-298\Delta S_{VH2}^0$	ΔG_{VH2}^0
6bp 3SE						
A	102.0±5.3	84.4±4.9	-17.6±0.4	34.3±7.4	21.0±6.8	-13.3±0.5
6bp 3SE						
B	112.7±9.1	95.0±8.6	-17.7±0.5	38.8±6.7	25.5±6.2	-13.3±0.4
6bp 3SE						
C	98.5±8.2	81.9±7.6	-16.6±0.6	36.7±3.5	23.7±3.3	-13.0±0.2
6bp 4SE	79.2±15.7	62.8±14.5	-16.5±1.2	21.2±2.6	8.1±2.5	-13.1±0.1

6bp 5SE						
A	126.9±3.2	106.3±3.0	-20.6±0.3	22.0±0.9	9.0±0.8	-13.1±0.1
6bp 5SE						
B	121.1±0.2	100.8±0.1	-20.4±0.0	22.3±1.1	9.0±1.0	-13.3±0.1
6bp 6SE	96.4±2.1	77.8±1.9	-18.6±0.2	18.0±0.3	4.8±0.3	-13.2±0.0
6bp 7SE	109.7±4.3	89.9±4.0	-19.8±0.3	13.4±0.3	0.5±0.2	-12.9±0.0

Table S3: Measured Thermodynamic Parameters for 6bp floppy SE linkers (kcal/mol)

Interface	$-\Delta H_{VH1}^0$	$-298\Delta S_{VH1}^0$	ΔG_{VH1}^0	$-\Delta H_{VH2}^0$	$-298\Delta S_{VH2}^0$	ΔG_{VH2}^0
6bpf 3SE A	58.6±8.9	44.8±8.4	13.8±0.5	24.0±1.7	11.9±1.7	-12.1±0.1
6bpf 3SE B	63.6±3.4	50.0±3.3	13.6±0.1	25.0±1.2	13.0±1.2	-12.0±0.0
6bpf 3SE C	70.0±5.9	56.2±5.6	13.8±0.4	20.7±3.1	8.8±2.8	-11.9±0.3
6bpf 4SE	58.5±5.5	44.5±5.1	14.0±0.4	19.4±0.6	7.1±0.5	-12.2±0.1
6bpf 5SE A	68.3±1.9	53.0±1.7	15.2±0.2	19.3±0.6	6.8±0.6	-12.5±0.0
6bpf 5SE B	81.7±7.2	65.8±6.6	15.9±0.6	13.2±1.4	1.3±1.3	-11.9±0.2
6bpf 6SE	85.2±0.6	68.6±0.7	16.7±0.0	12.6±0.7	0.3±0.6	-12.4±0.1
6bpf 7SE	87.6±11.8	70.8±11.0	16.9±0.8	10.6±0.5	-1.6±0.6	-12.2±0.1

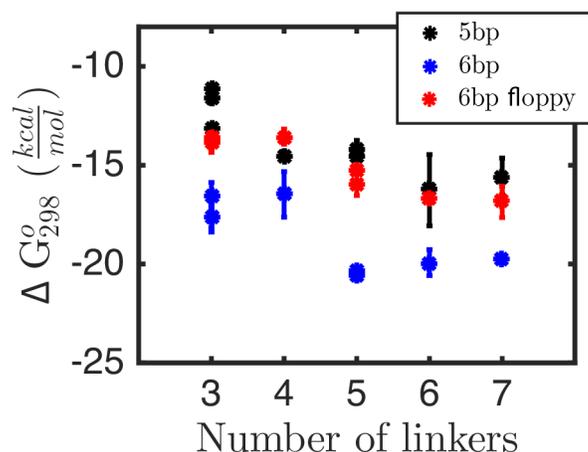


Figure S15: Measured values of standard Gibbs free energy at 298K, ΔG_{298}^0 , for all interface designs. All values plotted are from van't Hoff fit 1 (higher temperatures), as this fit indicates reflects the thermodynamic properties of the fully-formed interface (see Results and Discussion section of main text for further discussion). Specific values can be found in Tables S1-S3. Error bars indicate one standard deviation of the ΔG_{298}^0

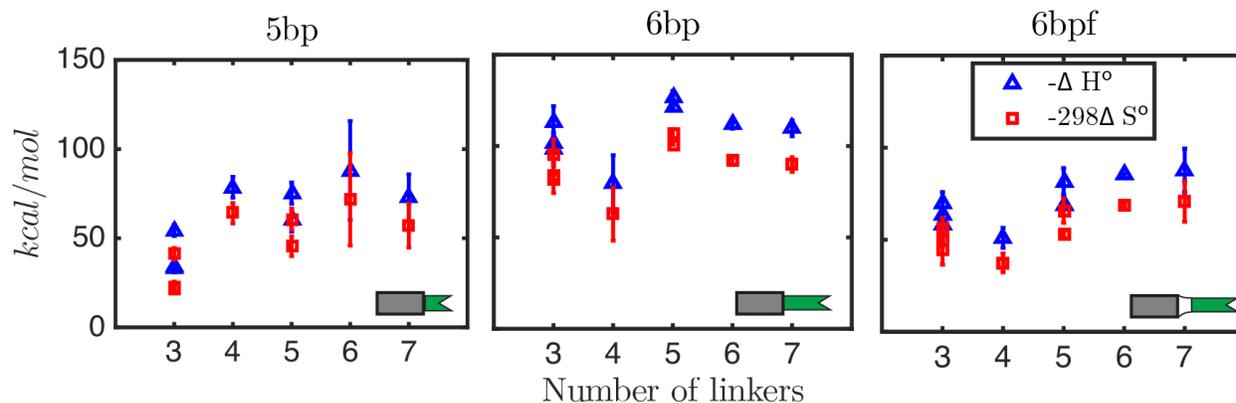


Figure S16: Measured values of standard enthalpy, $-\Delta H_{298}^0$, and entropy $-298 * \Delta S_{298}^0$ for all interfaces. Plots are shown as follows: (left) 5bp SE, (middle) 6bp SE, and (right) 6bp floppy SEs. All values plotted are from van't Hoff fit 1 (higher temperatures), as this fit indicates reflects the thermodynamic properties of the fully-formed interface (see Results of main text for further discussion). Specific values can be found in Tables S1-S4. Inset depicts cartoon schematic of linker design. Error bars indicate one standard deviation of the reported quantities.

Supporting Note 4: Kinetic measurements

The reaction between T1 and T2 produces the Tile 1-Tile 2 dimer, D according to the reaction:



where k_{on} is the the second order forward reaction rate constant and k_{off} is the first order reverse reaction rate constant. In our experiments, the initial concentration of components T1 and T2 were equal, at $C_0 = 5\text{nM}$. The yield of the dimer is defined as the fraction of total material of one of the component types in the complex, *i.e.* $yield = \frac{[D]}{C_0}$. Since we know the equilibrium concentrations for all of the species from our thermodynamics experiments, we know the equilibrium constant, $K_{eq} = \frac{k_{on}}{k_{off}} = \frac{[D]_{eq}}{[T1]_{eq}[T2]_{eq}}$ at all temperatures studied. With this information, we can fit a k_{on} for the dimerization reaction:

$$\frac{d[D]}{dt} = k_{on}[T1][T2] - k_{off}[D], \quad (6)$$

which expands to:

$$\frac{d(yield)}{dt} = k_{on} \left((1 - yield)^2 C_0 - \frac{(yield)}{K_{eq}} \right). \quad (7)$$

In the same fashion, we obtain an expansion for Eq. 6 to fit the k_{off} for the dimerization reaction:

$$\frac{d(yield)}{dt} = k_{off} (K_{eq}(1 - yield)^2 C_0 - yield). \quad (8)$$

We used least squares fittings from the fluorescence data to Equations 7 and 8 to obtain the reaction rate constants, k_{on} (SI Figures S17-S22) and k_{off} (SI Figures S23-S28), respectively. When fitting the reaction rate constants, we used the K_{eq} as obtained *via* the fluorescence measurements, where the yield was calibrated with the atomic force microscope (SI Note 3), in the thermodynamic protocol. Specifically, three sets of experiments are used to separately measure the three parameters we measured for each reaction: the equilibrium constant, the forward reaction rate constant, and the reverse reaction rate constant.

Thermal Protocol for fluorescence quenching kinetics experiments

Kinetic Measurements: Determining k_{on}

1. Hold 55°C for 15 minutes (take fluor. measurement every 5 minutes)
2. Hold 25°C for 15 minutes (take fluor. measurement every 6 seconds for 2 minutes, every 15 seconds for 8 minutes, every 30 seconds for 5 minutes)
3. Repeat steps 1 and 2, increasing the hold temperature of step 2 by 5°C every iteration until 50°C.

Kinetic Measurements: Determining k_{off}

1. Hold 25°C for 15 minutes (take fluor. measurement every 5 minutes)
2. Hold 55°C for 15 minutes (take fluor. measurement every 6 seconds for 2 minutes, every 15 seconds for 8 minutes, every 30 seconds for 5 minutes)
3. Repeat steps 1 and 2, decreasing the hold temperature of step 2 by 5°C every iteration until 30°C.

Kinetic Data: Forward Reaction Rate Constant 5 base pair SE

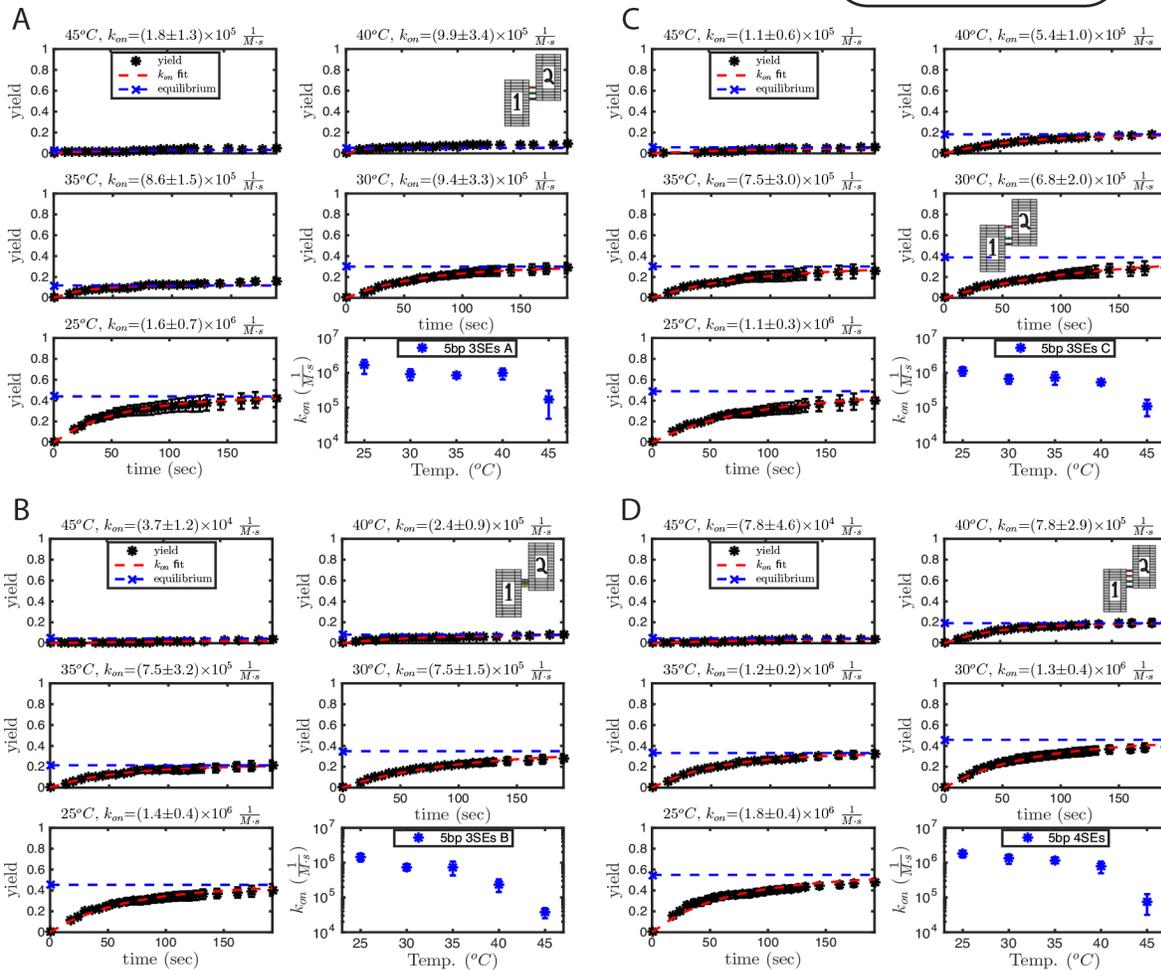
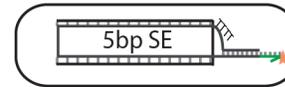


Figure S17: Kinetics measurements: forward reaction rate constants for interfaces with 5bp SE linkers. Interfaces are comprised of (A) 3 linkers in arrangement “A” (B) 3 linkers in arrangement “B”, (C) 3 linkers in arrangement “C”, (D) 4 linkers. Six plots per interface are fits for k_{on} for temperature jump experiments from 55°C to (top left) 45°C, (top right) 40°C, (middle left) 35°C, (middle right) 30°C, (bottom left) 25°C and (bottom right) k_{on} as a function of temperature. Values for k_{on} as a function of temperature are not reported when the error bar is equal to or greater than the value of k_{on} . Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 5bp SE linker shown at the top of the figure. Error bars indicate one standard deviation of the reported quantity.

5 base pair SE (Cont'd.)

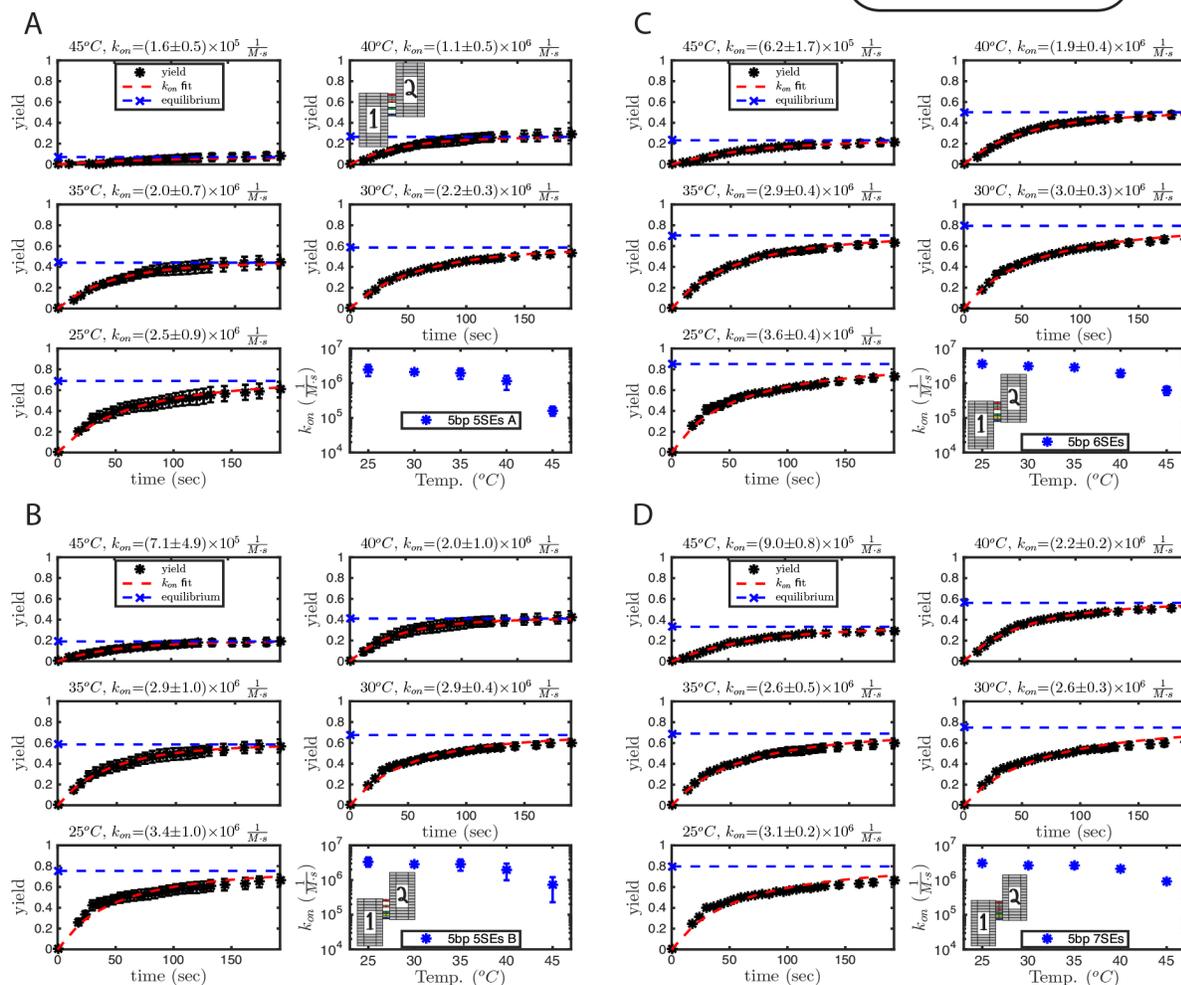


Figure S18: Kinetics measurements: forward reaction rate constants for interfaces with 5bp SE linkers (continued). Interfaces are comprised of (A) 5 linkers in arrangement “A” (B) 5 linkers in arrangement “B”, (C) 6 linkers, (D) 7 linkers. Six plots per interface are fits for k_{on} for temperature jump experiments from $55^{\circ}C$ to (top left) $45^{\circ}C$, (top right) $40^{\circ}C$, (middle left) $35^{\circ}C$, (middle right) $30^{\circ}C$, (bottom left) $25^{\circ}C$ and (bottom right) k_{on} as a function of temperature. Values for k_{on} as a function of temperature are not reported when the error bar is equal to or greater than the value of k_{on} . Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 5bp SE linker shown at the top of the figure. Error bars indicate one standard deviation of the reported quantity.

6 base pair SE

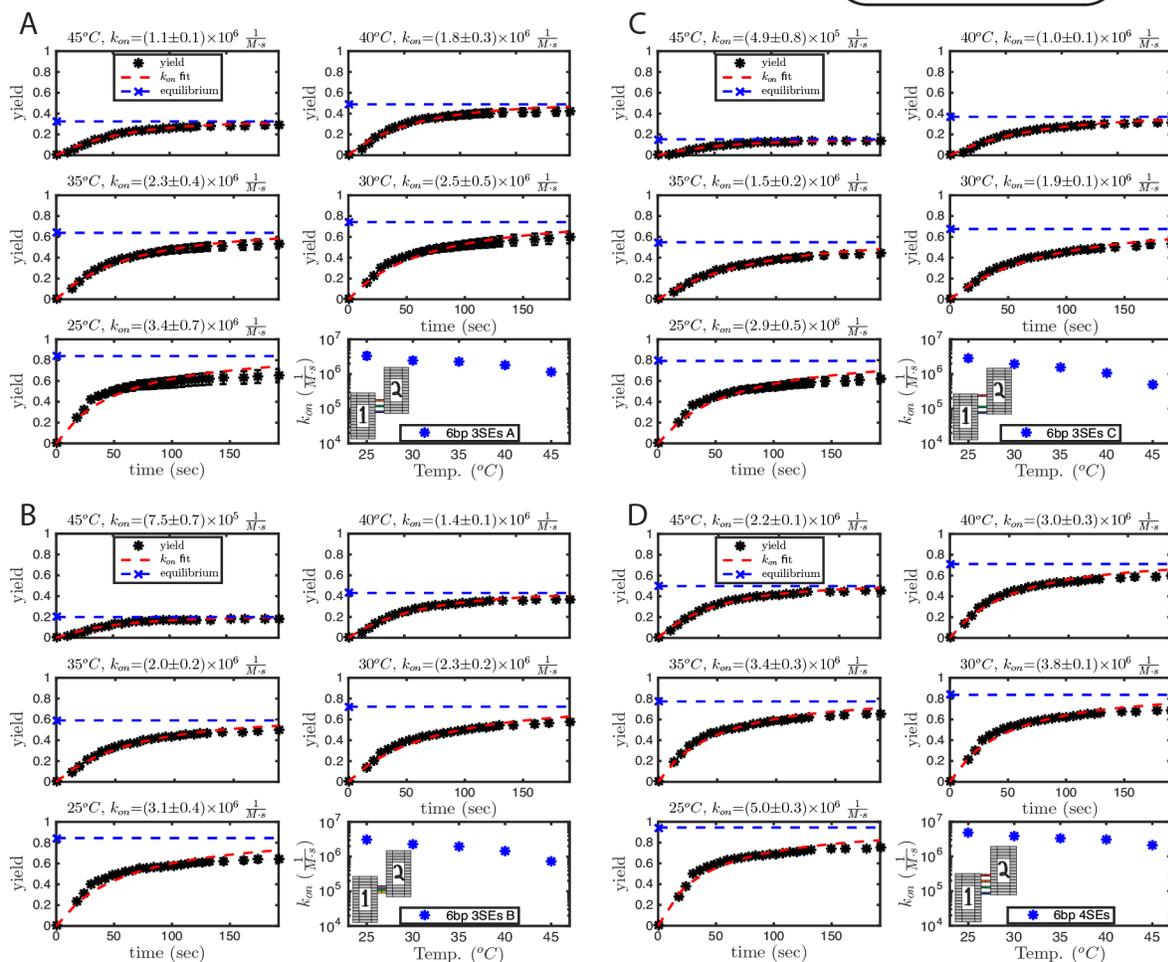
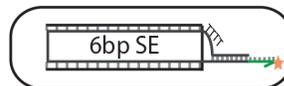


Figure S19: Kinetics measurements: forward reaction rate constants for interfaces with 6bp SE linkers. Interfaces are comprised of (A) 3 linkers in arrangement “A” (B) 3 linkers in arrangement “B”, (C) 3 linkers in arrangement “C”, (D) 4 linkers. Six plots per interface are fits for k_{on} for temperature jump experiments from 55°C to (top left) 45°C, (top right) 40°C, (middle left) 35°C, (middle right) 30°C, (bottom left) 25°C and (bottom right) k_{on} as a function of temperature. Values for k_{on} as a function of temperature are not reported when the error bar is equal to or greater than the value of k_{on} . Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 6bp SE linker shown at the top of the figure. Error bars indicate one standard deviation of the reported quantity.

6 base pair SE (Cont'd.)

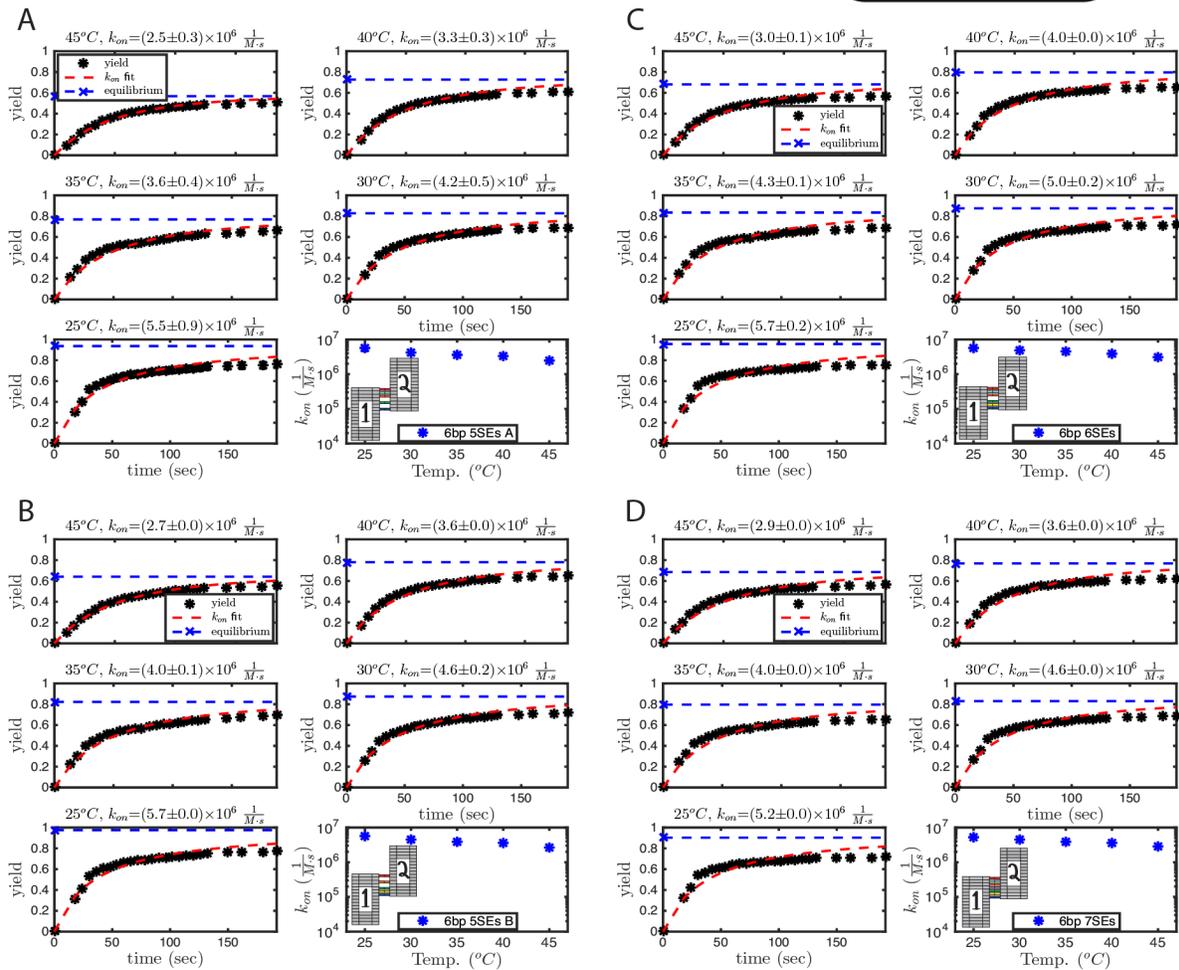


Figure S20: Kinetics measurements: forward reaction rate constants for interfaces with 6bp SE linkers. Interfaces are comprised of (A) 5 linkers in arrangement “A” (B) 5 linkers in arrangement “B”, (C) 6 linkers, (D) 7 linkers. Six plots per interface are fits for k_{on} for temperature jump experiments from 55°C to (top left) 45°C, (top right) 40°C, (middle left) 35°C, (middle right) 30°C, (bottom left) 25°C and (bottom right) k_{on} as a function of temperature. Values for k_{on} as a function of temperature are not reported when the error bar is equal to or greater than the value of k_{on} . Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 6bp SE linker shown at the top of the figure. Error bars indicate one standard deviation of the reported quantity.

6 base pair floppy SE

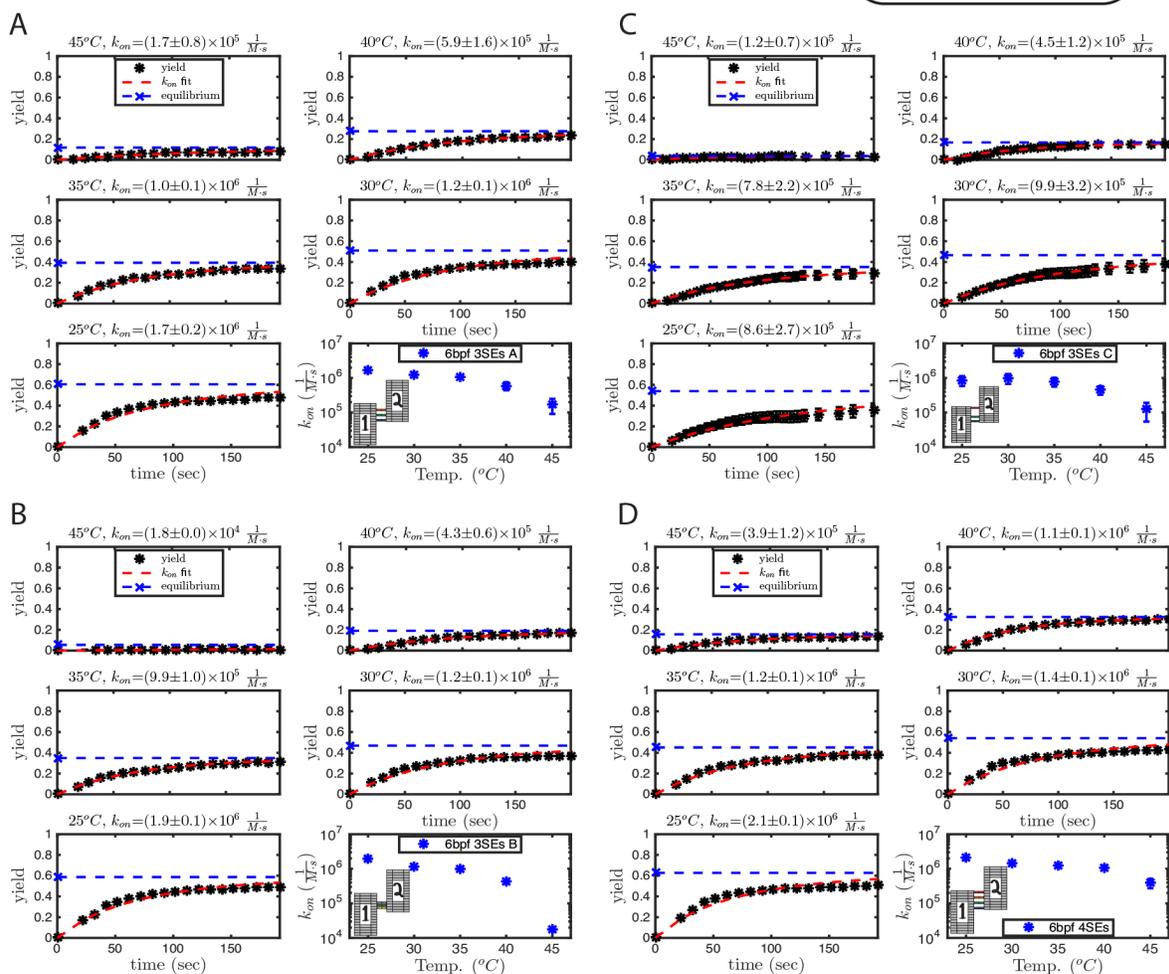
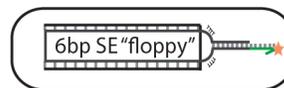


Figure S21: Kinetics measurements: forward reaction rate constants for interfaces with 6bp SE floppy linkers. Interfaces are comprised of (A) 3 linkers in arrangement “A” (B) 3 linkers in arrangement “B”, (C) 3 linkers in arrangement “C”, (D) 4 linkers. Six plots per interface are fits for k_{on} for temperature jump experiments from 55°C to (top left) 45°C, (top right) 40°C, (middle left) 35°C, (middle right) 30°C, (bottom left) 25°C and (bottom right) k_{on} as a function of temperature. Values for k_{on} as a function of temperature are not reported when the error bar is equal to or greater than the value of k_{on} . Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 6bp SE floppy linker shown at the top of the figure. Error bars indicate one standard deviation of the reported quantity.

6 base pair floppy SE (Cont'd.)

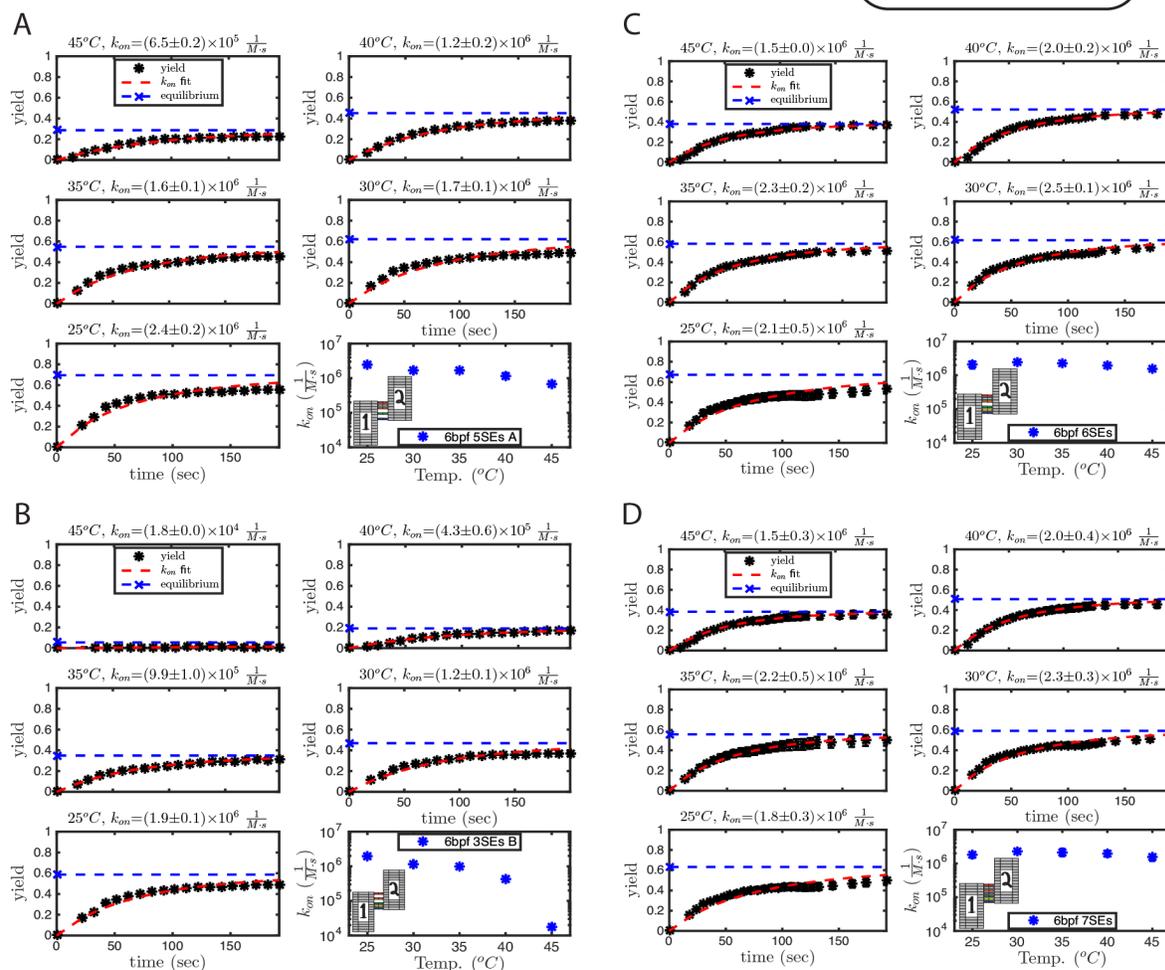
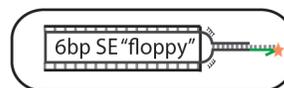


Figure S22: Kinetics measurements: forward reaction rate constants for interfaces with 6bp SE floppy linkers (continued). Interfaces are comprised of (A) 5 linkers in arrangement “A” (B) 5 linkers in arrangement “B”, (C) 6 linkers, (D) 7 linkers. Six plots per interface are fits for k_{on} for temperature jump experiments from 55°C to (top left) 45°C, (top right) 40°C, (middle left) 35°C, (middle right) 30°C, (bottom left) 25°C and (bottom right) k_{on} as a function of temperature. Values for k_{on} as a function of temperature are not reported when the error bar is equal to or greater than the value of k_{on} . Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 6bp SE floppy linker shown at the top of the figure. Error bars indicate one standard deviation of the reported quantity.

Kinetic Data: Reverse Reaction Rate Constant

5 base pair SE

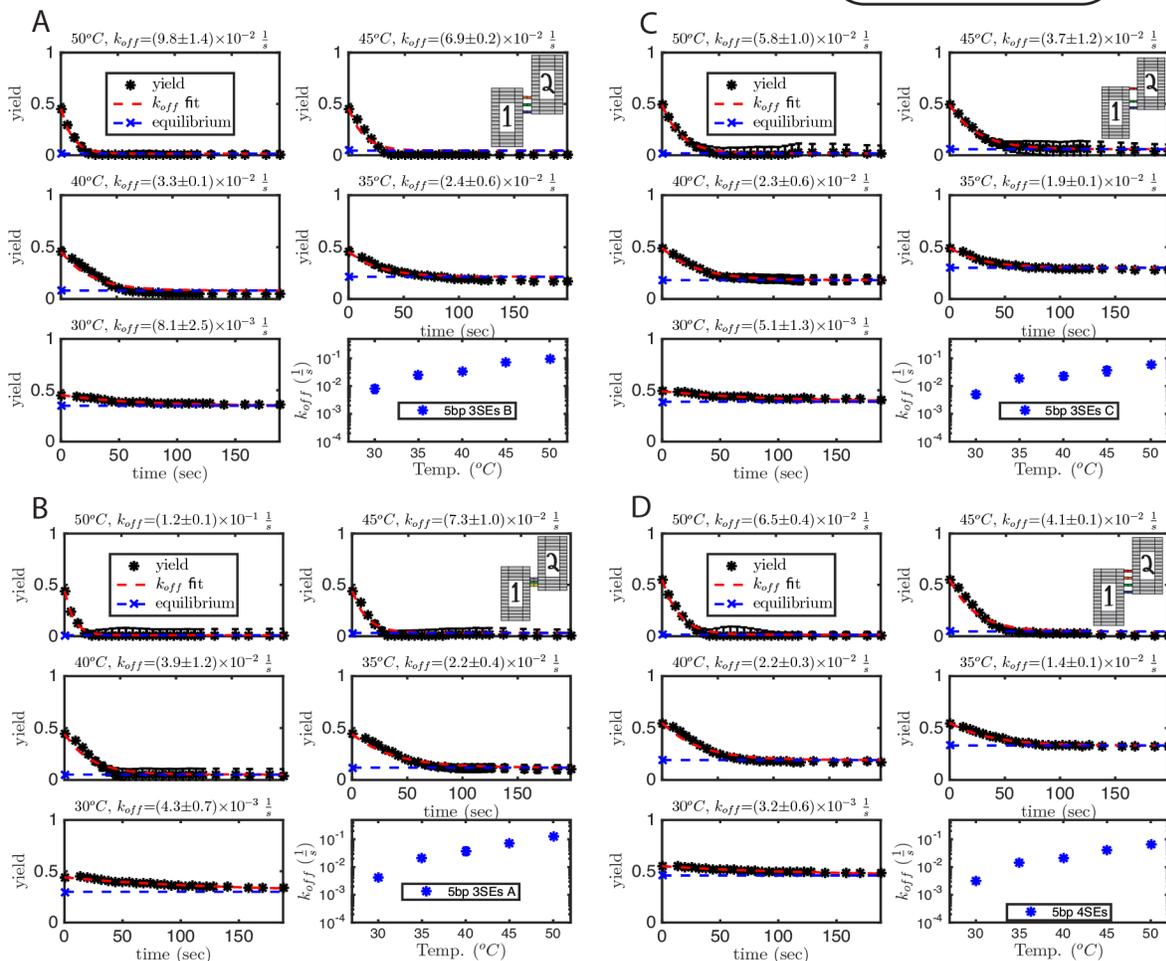


Figure S23: Kinetics measurements: reverse reaction rate constants for interfaces with 5bp SE linkers. Interfaces are comprised of (A) 3 linkers in arrangement “A” (B) 3 linkers in arrangement “B”, (C) 3 linkers in arrangement “C”, (D) 4 linkers, (E) 5 linkers in arrangement “A”, (F) 5 linkers in arrangement “B”, (G) 6 linkers and (H) 7 linkers. Six plots per interface are fits for k_{off} for temperature jump experiments from 25°C to (top left) 50°C, (top right) 45°C, (middle left) 40°C, (middle right) 35°C, (bottom left) 30°C and (bottom right) k_{on} as a function of temperature. Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 5bp SE linker shown at the top of the figure.

5 base pair SE (Cont'd.)

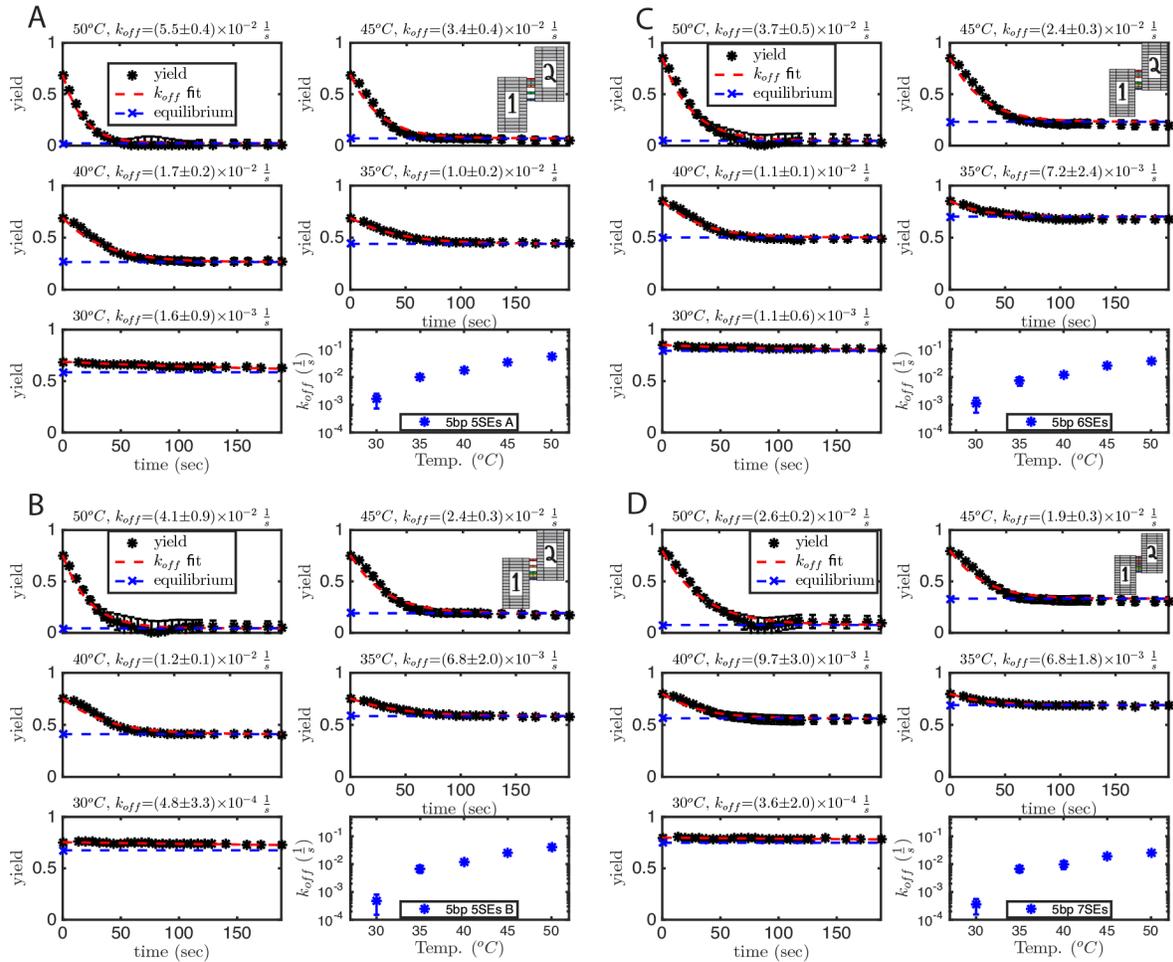


Figure S24: Kinetics measurements: reverse reaction rate constants for interfaces with 5bp SE linkers (continued). Interfaces are comprised of (A) 5 linkers in arrangement “A” (B) 5 linkers in arrangement “B”, (C) 6 linkers, (D) 7 linkers. Six plots per interface are fits for k_{off} for temperature jump experiments from $25^{\circ}C$ to (top left) $50^{\circ}C$, (top right) $45^{\circ}C$, (middle left) $40^{\circ}C$, (middle right) $35^{\circ}C$, (bottom left) $30^{\circ}C$ and (bottom right) k_{on} as a function of temperature. Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 5bp SE linker shown at the top of the figure.

6 base pair SE

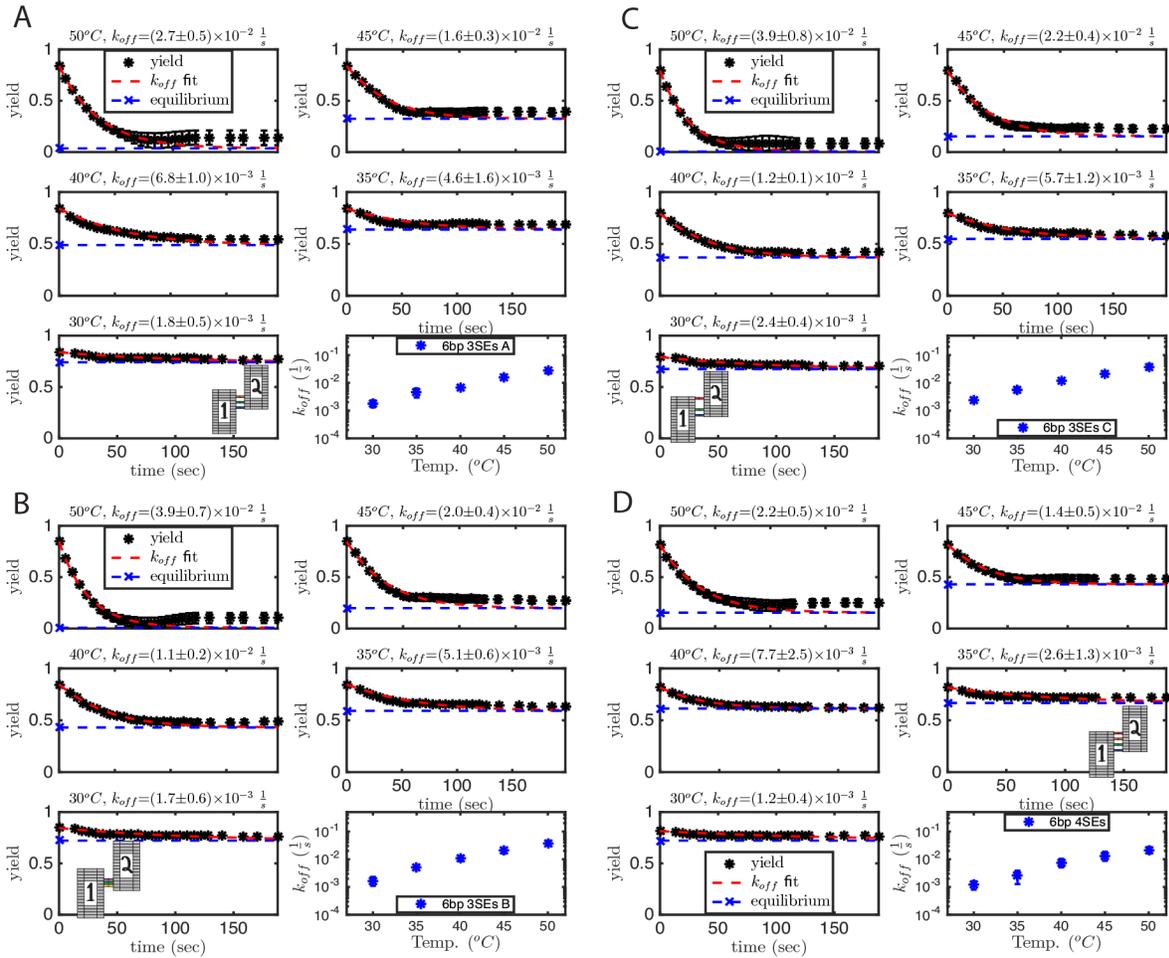


Figure S25: Kinetics measurements: reverse reaction rate constants for interfaces with 6bp SE linkers. Interfaces are comprised of (A) 3 linkers in arrangement “A” (B) 3 linkers in arrangement “B”, (C) 3 linkers in arrangement “C”, (D) 4 linkers. Six plots per interface are fits for k_{off} for temperature jump experiments from 25°C to (top left) 50°C, (top right) 45°C, (middle left) 40°C, (middle right) 35°C, (bottom left) 30°C and (bottom right) k_{on} as a function of temperature. Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 6bp SE linker shown at the top of the figure.

6 base pair SE (Cont'd.)

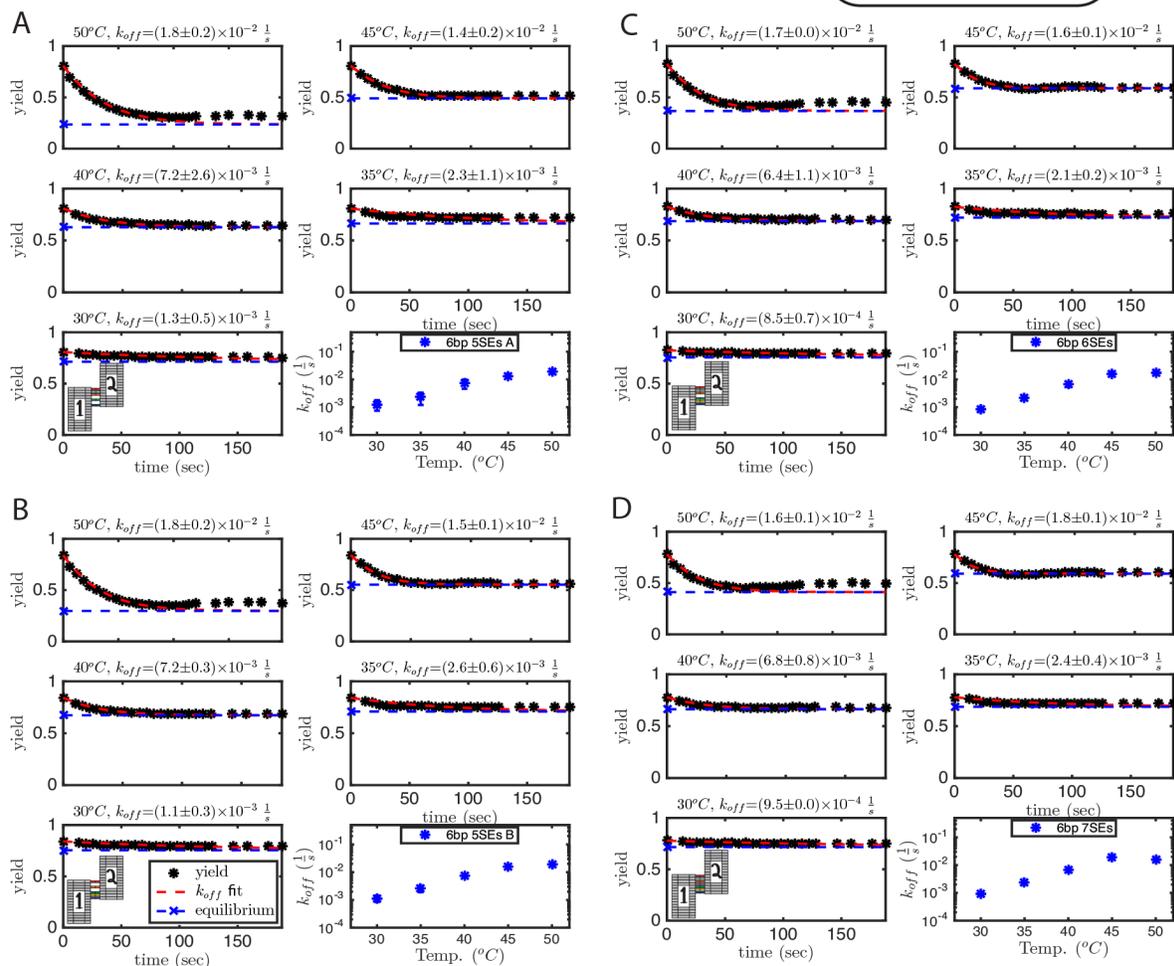
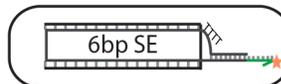


Figure S26: Kinetics measurements: reverse reaction rate constants for interfaces with 6bp SE linkers (continued). Interfaces are comprised of (A) 5 linkers in arrangement “A” (B) 5 linkers in arrangement “B”, (C) 6 linkers, (D) 7 linkers. Six plots per interface are fits for k_{off} for temperature jump experiments from $25^{\circ}C$ to (top left) $50^{\circ}C$, (top right) $45^{\circ}C$, (middle left) $40^{\circ}C$, (middle right) $35^{\circ}C$, (bottom left) $30^{\circ}C$ and (bottom right) k_{on} as a function of temperature. Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 6bp SE linker shown at the top of the figure.

6 base pair floppy SE

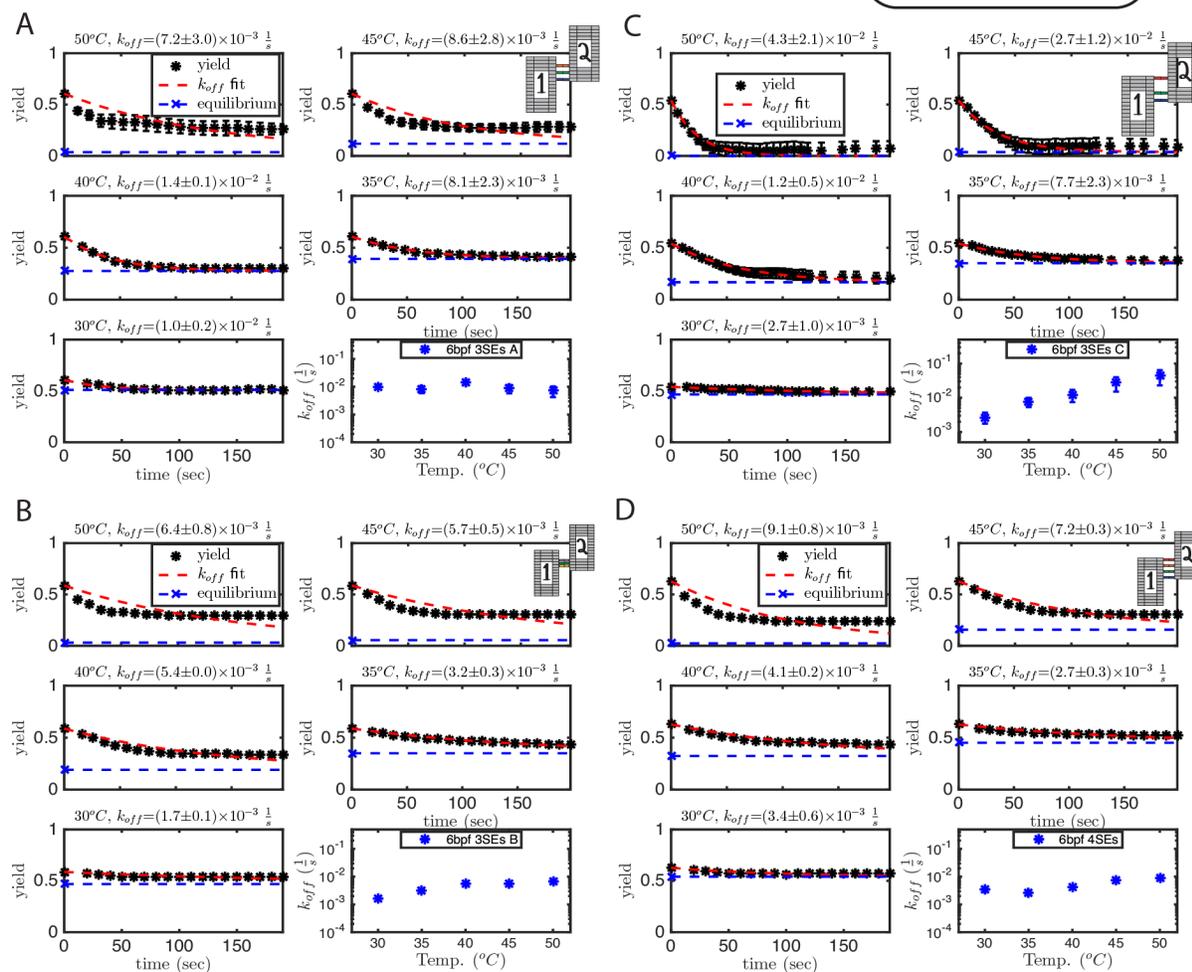
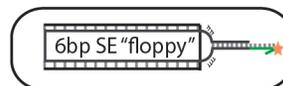


Figure S27: Kinetics measurements: reverse reaction rate constants for interfaces with 6bp SE floppy linkers. Interfaces are comprised of (A) 3 linkers in arrangement “A” (B) 3 linkers in arrangement “B”, (C) 3 linkers in arrangement “C”, (D) 4 linkers. Six plots per interface are fits for k_{off} for temperature jump experiments from 25°C to (top left) 50°C, (top right) 45°C, (middle left) 40°C, (middle right) 35°C, (bottom left) 30°C and (bottom right) k_{on} as a function of temperature. Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 6bp SE floppy linker shown at the top of the figure.

6 base pair floppy SE (Cont'd.)

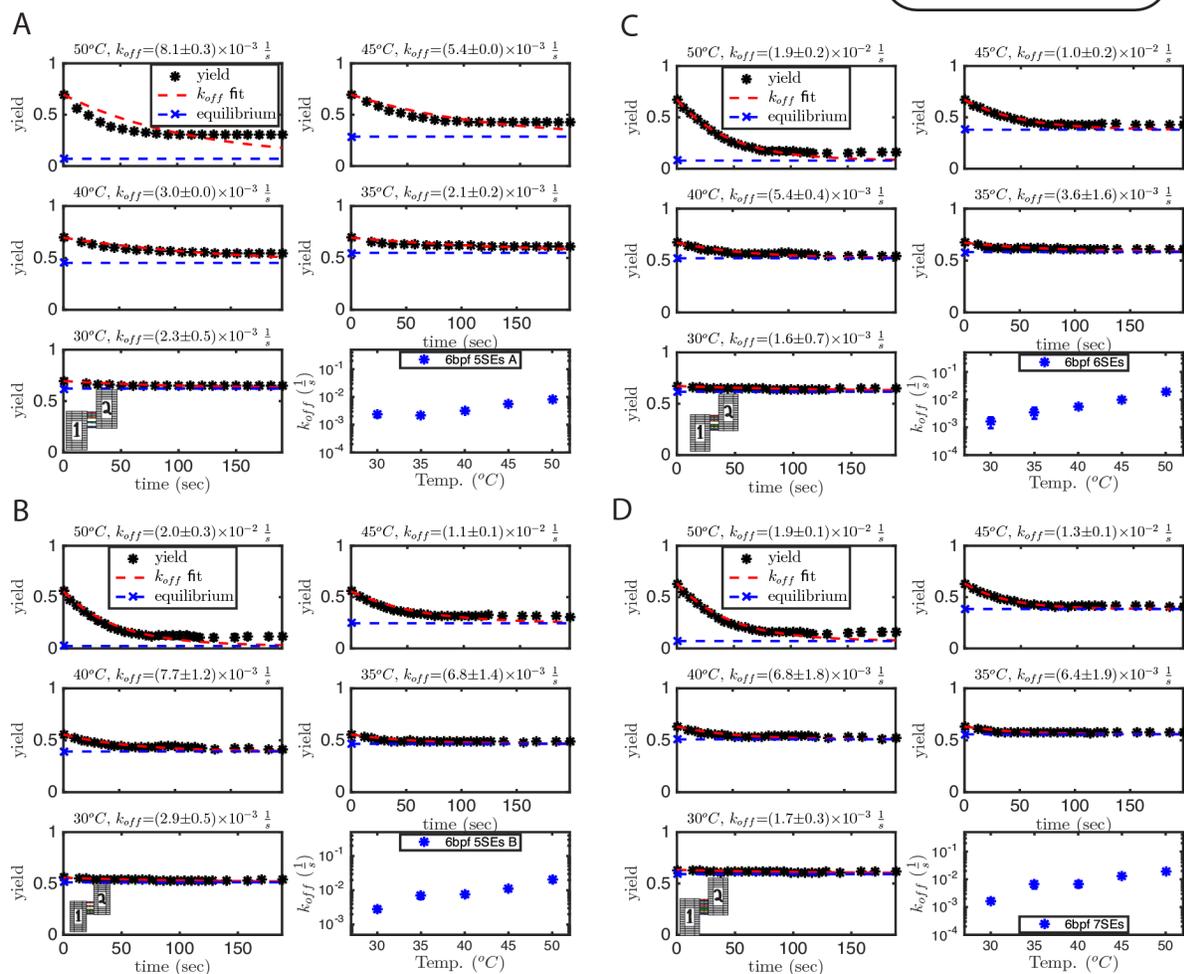
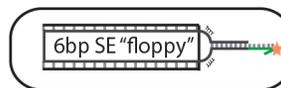


Figure S28: Kinetics measurements: reverse reaction rate constants for interfaces with 6bp SE floppy linkers (continued). Interfaces are comprised of (A) 5 linkers in arrangement “A” (B) 5 linkers in arrangement “B”, (C) 6 linkers, (D) 7 linkers. Six plots per interface are fits for k_{off} for temperature jump experiments from $25^{\circ}C$ to (top left) $50^{\circ}C$, (top right) $45^{\circ}C$, (middle left) $40^{\circ}C$, (middle right) $35^{\circ}C$, (bottom left) $30^{\circ}C$ and (bottom right) k_{on} as a function of temperature. Cartoon schematic of T1 and T2 to the left of plots depicts interface arrangement. Cartoon schematic of an individual 6bp SE floppy linker shown at the top of the figure.

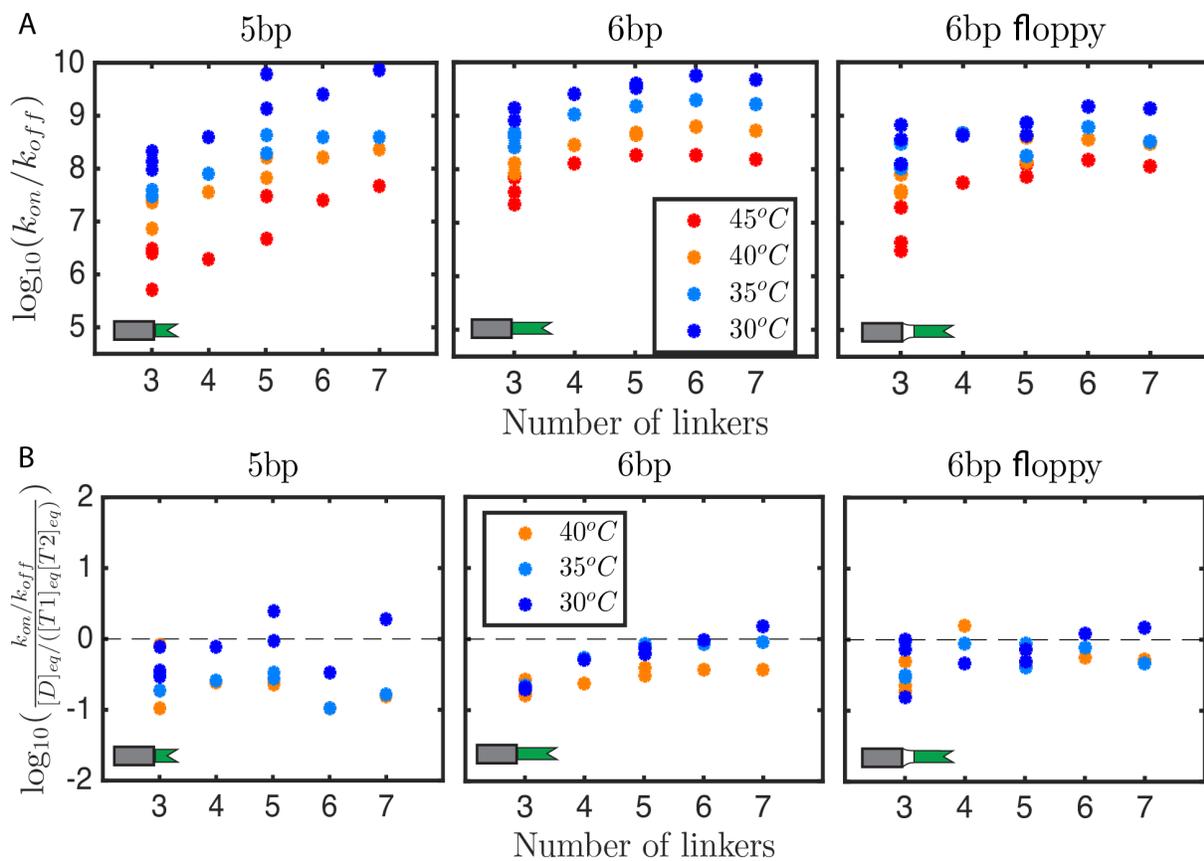


Figure S29: Equilibrium constants (K_{eq}) of dimerization. (A) K_{eq} values calculated from the fit k_{on} and k_{off} reaction rate constants and as a function of temperature and interface design. As expected, higher temperatures generally give lower values of K_{eq} across all interface designs. (B) Ratio of K_{eq} values found from fit reaction rate constants and yields at equilibrium and used as a measure of error in our fits. Ideally, the fit kinetic parameters would exactly reflect the equilibrium yield and thus the ratio of the two would be unity. However, these measurements are not truly independent, as equilibrium yields are used to fit reaction rate constants so we expect some degree of dependence between the two calculations. (Left) 5bp SE linker design, (middle) and (right) 6bp SE floppy linker designs. Black dashed lines serve as guide for the eye, indicating equal values for the equilibrium constant for both methods.

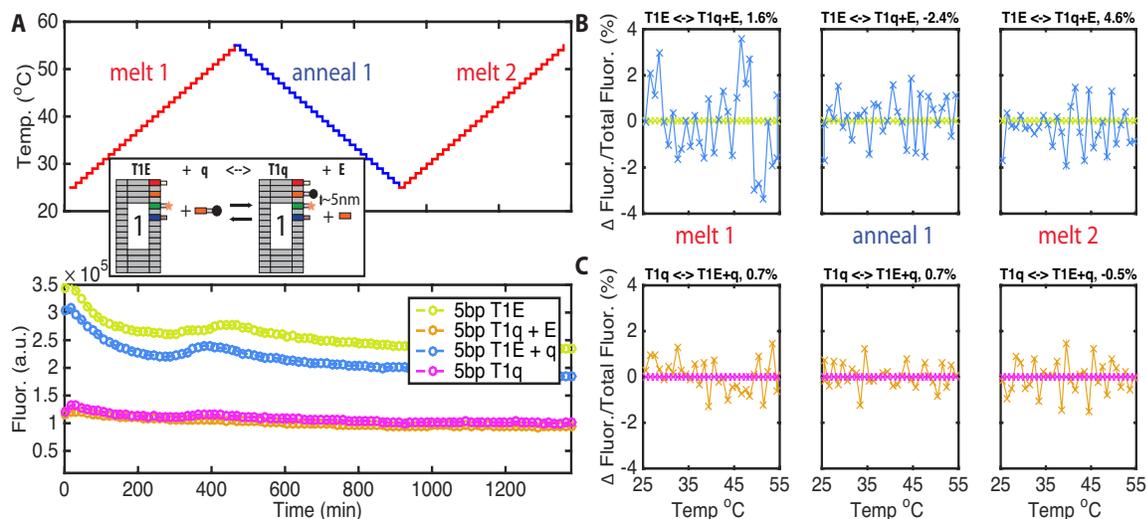


Figure S30: Less than ~5% of the edge linkers dissociate from origami, even at high temperatures. We wanted to know whether linkers were being swapped in between origami components (*i.e.*, a given T2 linker has the same scaffold-complimentary region as the T1 linker) and thus contributing to low yields. To test this, we mixed T1 with a 5bp 3SE interface (labeled “T1E”) with the corresponding T2 quencher (“q”) linker in solution and tracked the fluorescence over a (A) typical annealing protocol (top). Fluorescence would decrease if T1 with the quencher (“T1q”) formed from T1E + q. Tile schematic shown in inset. (Bottom) Raw fluorescence data over time shown in the lower part of (A). (B) Fluorescence of T1E + q was normalized by T1E to show how the quencher influences fluorescence for the first melt (left), anneal (middle) and second melt (right). To obtain an estimate for how much linker exchange occurs in solution on a given section of a thermal protocol, we calculated the integral of the normalized difference in fluorescence of the sample divided by the fluorescence difference between “T1E” and “T1q” (*i.e.*, the fluorescence difference between fully fluorescent and fully quenched states). Estimates for the percentages of linkers swapped are listed above the plots. Negative values indicate the reverse reaction occurs. (C) The same system in (B), but now comparing the swapping of “q” with “E” and normalized by T1q.

Supporting Note 5: Two-dimensional Lattice with 6bp Floppy Linkers

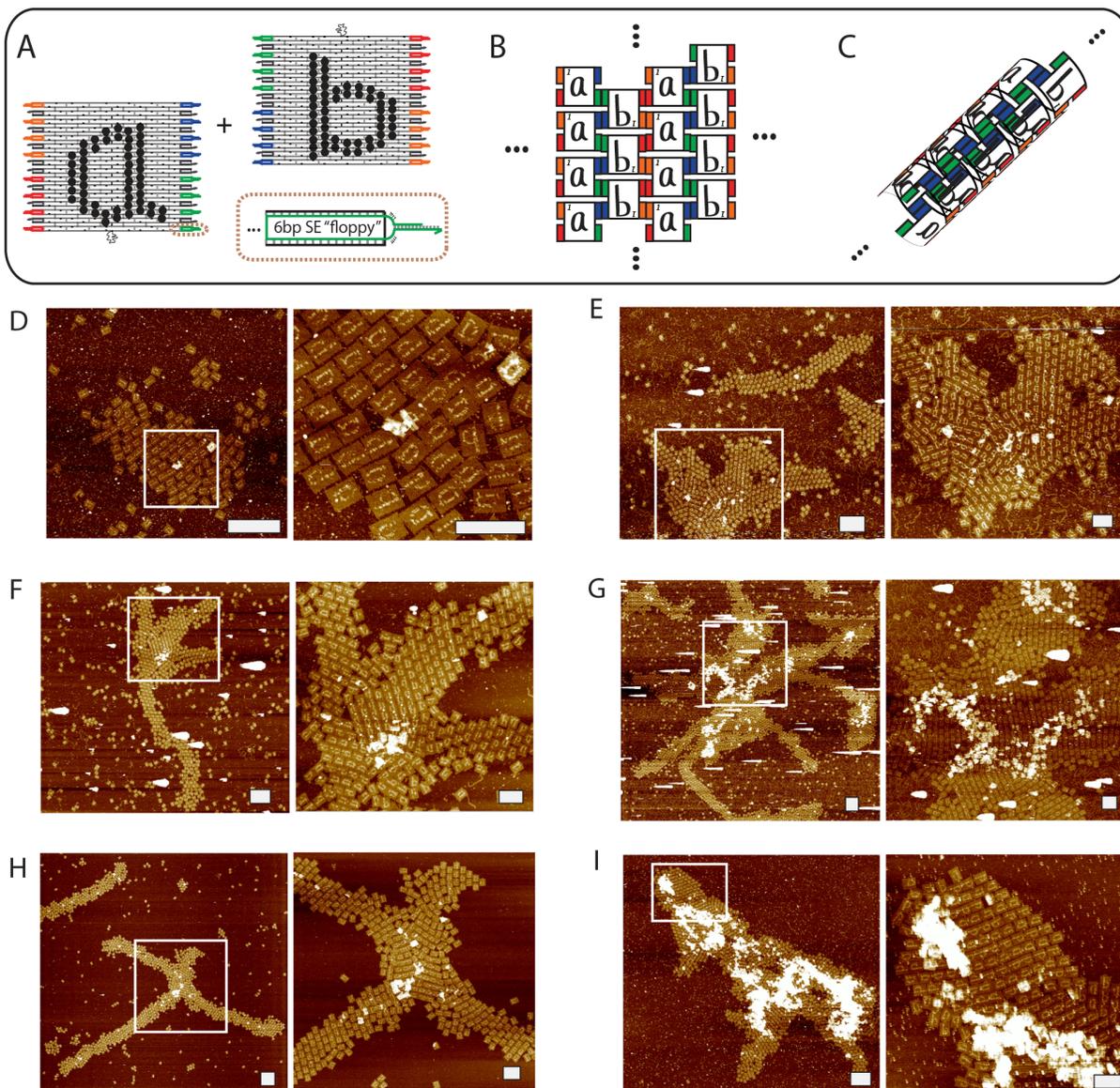


Figure S31: Two-dimensional origami tile lattice with two tiles labeled “a” and “b” designed with four 6bp floppy SEs per interface. (A) Schematic of tiles “a” and “b” that form a lattice. These tiles are structurally identical to T1 and T2, except for the hairpin staples and linking schematic. For simplicity, linker edges are shown in a uniform color, although each sticky end sequence and scaffold-linker complementary region has unique sequences. All linkers were 6bp floppy linkers, with 4SE on each interaction interface. (B) Schematic of 2D lattice (intended) and (C) tube (unintended) as possible confirmations of the “a”-“b” lattice. (D-I) AFM images of origami lattices annealed with various protocols (see Table S5). White rectangles on the top images indicate zoomed-in region. Scale bars on the zoomed-out (top) images are 500nm and on the zoomed-in (bottom) are 200nm.

Table S4: Concentration and Annealing Protocol for Infinite Lattice*

Image	$[A_0] = [B_0]$	Annealing Protocol
(D)	2nM	$55 \rightarrow 38^\circ C @ -\frac{1^\circ C}{hr}$
(E)	2nM	$55 \rightarrow 36^\circ C @ -\frac{0.33^\circ C}{hr}$ then hold at 38 for 48hrs
(F)	2nM	$55 \rightarrow 36^\circ C @ -\frac{0.33^\circ C}{hr}$, then hold at 42 for 48hrs
(G)	2nM	$55 \rightarrow 36^\circ C @ -\frac{0.33^\circ C}{hr}$, then hold at 38 for 3weeks
(H)	5nM	$55 \rightarrow 40^\circ C @ -\frac{0.5^\circ C}{hr}$, then hold at 40 for 6hrs
(I)	5nM	$55 \rightarrow 25^\circ C @ -\frac{0.5^\circ C}{hr}$, plated @25

*All solutions were diluted to 1nM before imaging and plated on the mica puck at 40C, unless otherwise noted

Supporting Note 6: Design of Origami Edge Staples and Linker Sequences

Linker sticky ends and intra-linker complementary regions were designed using custom MATLAB software written to minimize spurious interactions. The script minimizes crosstalk between non-complementary SEs and intra-linker regions while keeping all desired SE interactions at approximately equal binding strength $\pm 20\%$ (ΔG^0), according to the nearest neighbor model^{9 10} (Figure S32). Intra-linker complementary regions were also unique, and their crosstalk minimized with one another and the SEs. The core MATLAB code we modified was used previously in Refs. ¹¹ and modified as needed for this work. The software is available upon request.

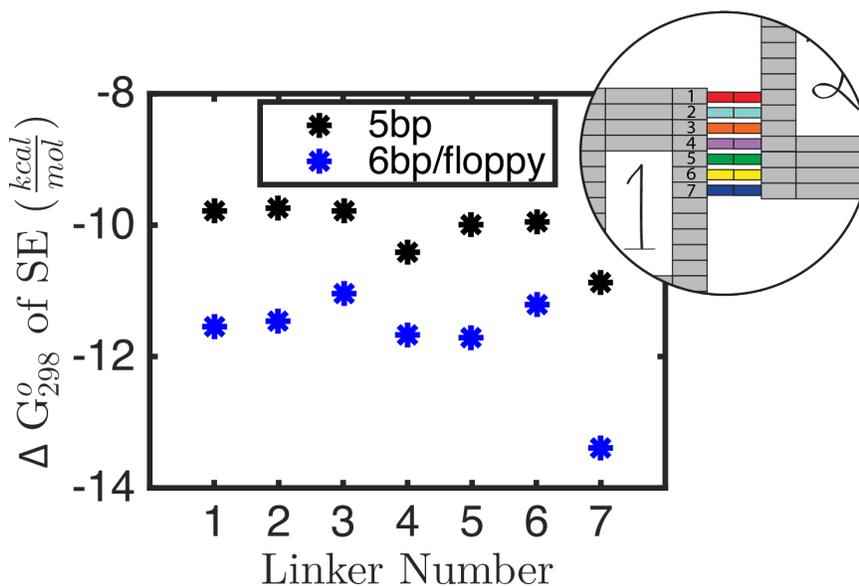


Figure S32: Designed strength of SE, ΔG_{298}^0 , as calculated by the nearest neighbor model⁹. The crosstalk between all SE strands was minimized and their relative strength was constrained to be within $\pm 15\%$. Linker number 1 corresponds to the top most linker on T1 while linker number 7 is the bottom most linker on T1, as depicted in inset. Note the 6bp SEs and 6bp floppy SEs have same sequence and thus same predicted free energy in our simple model.

Supporting Note 7: Extra Material

Extra supporting files include:

1. DNA sequences in excel file titled “sequences for obr.xlsx”

Table S 5: Nomenclature of Excel Spreadsheet with Sequence Specifics*

Tab Name	How sequences are named
Body staples	According to Cadnano file; hairpin sequence is the same as in ¹² .
Seam and body staples	
Body hairpin	
Seam and body hairpin	
Blockers	See SI Fig. S34
5bp linkers	
6bp linkers	
6bp floppy linkers	

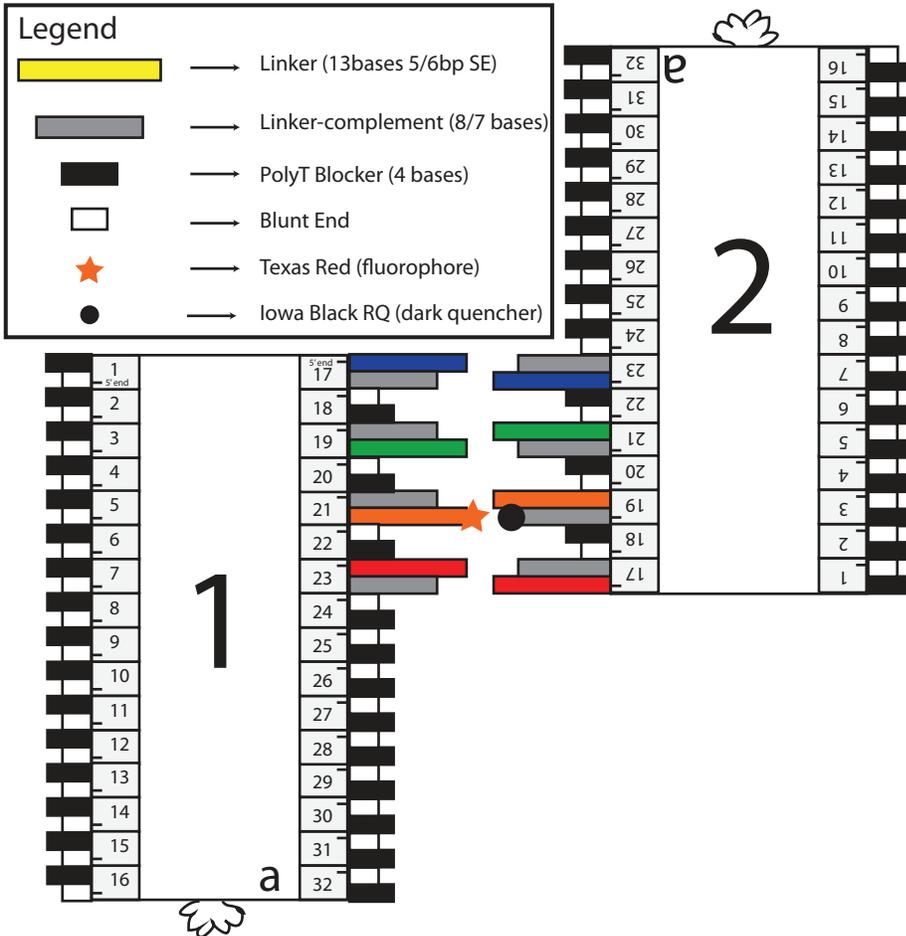


Figure S33: A diagram to understand the nomenclature of blockers and linkers in “sequences for obr.xlsx”. In this diagram, each edge sequence (blocker/linker) is labeled with a number. That number is indicative of the scaffold sequence of that edge. For example, the blocker “1” in T1 has the same sequence as the blocker “1” in T2 (i.e., same core staple-scaffold sequences concatenated with a poly T on the 3’ end). The 5’ end of the sequence is labeled with a dash (“-”). For example, in the excel file “tile_1_17_link” has sticky end complementarity to “tile_2_23_link” and both sticky ends occur on the 5’ end. Linker colors indicate sticky end complementarity. Every gray intra-linker complimentary region has a unique sequence. For all systems, the Iowa black quencher is covalently attached to the 5’ end of the T2 #19 linker whereas the fluorophore is always covalently linked to the 3’ end of the T1 #21 linker. The inset “a” in the origami schematic shows the relative orientation of T1 to T2.

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