

Supplementary material:

Direct observation and rational design of nucleation behavior in addressable self-assembly

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1 EXTENDED METHODS

If not otherwise noted, chemicals were purchased from Sigma Aldrich Chemie GmbH (Munich, Germany).

1.1 Annealing protocols

DNA sequences for the target cuboid structure are given in Sec. SI-5. For the all-BB system, sequences were taken from Ref. 1 (Table S8), and DNA strands were appropriately decoupled to split the relevant boundary bricks for the face-BB, edge-BB and no-BB systems. All sequences were purchased from Eurofins Genomics in 100 μM stocks in ddH_2O , and then pooled using a Tecan Genesis Workstation 150 liquid handling robot. We used a strand concentration of 153 nM in 1 \times assembly buffer, i.e., a solution of 15 mM MgCl_2 , 0.5 mM EDTA and 5 mM Tris, pH 8. The strand solution was denatured at 90 $^\circ\text{C}$ for 10 min and then gradually cooled. We used two linear cooling protocols: (i) in the 15.2 h protocol, the reciprocal cooling rate was 12 min K^{-1} , and (ii) in the 66 h protocol, it was 52 min K^{-1} . The annealed samples were stored at 4 $^\circ\text{C}$.

Prior to the reference DLS measurement (positive control), the all-BB sample assembled in the 66 h protocol was supplemented with 2.5 mM EDTA to reduce high-molecular-weight contamination. In the context of DLS experiments, we therefore refer to this sample as ‘purified’.

Prior to the reference AFM imaging (positive control), the all-BB sample assembled in the 66 h protocol was ultrafiltered using Amicon (UFC510024, Millipore, Merck KGaA, Darmstadt, Germany) filter units, with a molecular weight cut-off of 100 kDa, to reduce the fraction of small particle contamination and to improve the image quality. To this end, the assembled sample was mixed with pre-chilled 1 \times assembly buffer to the maximum admitted volume and centrifuged for 10 min at 14 000g at 4 $^\circ\text{C}$. Subsequently, the flowthrough was discarded, and the filter unit was loaded with buffer and centrifuged again. This process was repeated three times in total. Finally, the concentrated filtrate was eluted at 1000g for 2 min at 4 $^\circ\text{C}$.

1.2 Atomic force microscopy

Samples were prepared following the 66 h annealing protocol. A freshly cleaved mica disc was coated with 100 μL of 0.5 wt% poly-L-ornithine solution for 5 min and rinsed three times with 1 \times assembly buffer. In order to be able to image samples in liquid mode, an acrylic glass ring was glued by Thin Pour (Reprorubber) onto a slide to surround the mica disc and form

a fluid cell. For each sample, 1.5 pmol per brick was deposited on the coated mica for 10 min. Afterwards, the cell was filled with 1 \times assembly buffer and imaged using the JPK Nanowizard 3 atomic force microscope and a BioLever Mini cantilever in intermittent contact mode in liquid. Images were recorded with a target amplitude of 15 nm.

Quenching experiments, designed to stop the hybridization reaction at a given temperature during the annealing protocol, were done by immobilizing 5 μL of the reaction mixture on poly-L-ornithine coated and pre-equilibrated mica discs. As a negative control, the same procedure was performed using a random selection of ssDNA strands that did not contain complementary sequences.² For both the all-BB structure and the negative control, samples were quenched from 318 K, 314 K, 311 K, 308 K and 300 K during annealing protocol (i) and imaged by AFM, as described above, at ambient temperature.

1.3 Agarose gel electrophoresis

Assembly of DNA brick structures was confirmed by non-denaturing agarose gel electrophoresis. Samples (300 fmol per brick) were analyzed on a gel made from 2 wt% agarose in 0.5 \times TBE and 10 mM MgCl_2 . Electrophoresis was performed at 80 V and 4 $^\circ\text{C}$ for 2 h. The gel was post-stained with 0.5 $\mu\text{g mL}^{-1}$ ethidium bromide solution and scanned in using the Intas GDS gel set instrument for structure visualization. To estimate structure yield, the band intensity was approximated by fitting densitometry profiles with an SQP algorithm to Gaussian functions using the GelBandFitter software.³ The mass of the structure fractions was estimated via a 1 kb standard (GeneRuler, ThermoFischer Scientific) and related to the total mass loaded of 850 ng.

1.4 Static and dynamic light scattering

The same conditions as in the 15.2 h annealing protocol were used and the measurement was performed in the last 2 min of the 12 min cooling step. 20 μL samples were filled into ZEN2112 quartz cuvettes (Malvern), covered by molecular biology grade mineral oil, and sealed with a plastic lid that was further fixed with tape. Light scattering was measured using a Malvern Zetasizer NanoZSP apparatus at an angle of 173 $^\circ$. The viscosity of the samples was determined at five temperatures spanning the region of interest and fitted to $\eta/(10^{-5} \text{ Pa s}) = 1.78 \times \exp[617/(T/\text{K} - 138.5)]$. The refractive index was measured to be 1.331.

For dynamic light scattering, the intensity auto-correlation

function was computed from 12 measurements at 10 s intervals. We interpreted the DLS data in the dilute limit by assuming that all particles diffused independently of one another, since the total strand concentration (approximately $40 \mu\text{M}$) implies that single strands ($R_h \sim 2.7 \text{ nm}$) in solution occupied a volume fraction of approximately 0.2%. We further assumed that, after the initial equilibration period of 10 min, the distribution of cluster sizes remained nearly constant over the DLS measurement period at the end of each temperature step; this assumption is consistent with our observations of rate-limiting structure nucleation.

When analyzing DLS data for solutions comprising a range of particle sizes, the inverse Laplace transform used to obtain a particle size distribution from the intensity auto-correlation function is not uniquely determined,⁴ and the choice of fitting functions and parameters can affect the final result.⁵ We have therefore computed multiple fits to the distribution of hydrodynamic radii using several regularization methods, including a range of different smoothing exponents and a maximum entropy constraint (Fig. S3). Although the agreement is not perfect for the individual data points, the trends for the distributions and the fits to a linear combination of Gaussian functions are nearly the same regardless of the regularization procedure used, indicating that the conclusions drawn from the DLS data are robust with respect to the choice of regularization method. We chose to use the smoothness constraint regularization method recommended in Ref. 5 with a smoothing exponent of $m = 8/5$ (corresponding to Fig. S3b) for all data reported in the main text.

1.5 Fluorescence annealing

In the fluorescence annealing experiments, the same conditions as in the 15.2 h annealing protocol were used, except that 10 nM SYBR green I solution⁶ was added to the strand mixture. SYBR green I in buffer solution was analyzed as a negative control. Samples were placed on a MicroAmp Fast Plate 96-well tray and sealed with adhesive film. The plate was loaded onto the ABI Prism 7900HT-Fast Real Time PCR system, with dye excitation effected by an argon ion laser at 488 nm. The fluorescence signal was detected at 525 nm every 8.5 s and averaged over time at each temperature, and its derivative with respect to temperature was computed numerically. The data were smoothed via a Gaussian filter with a standard deviation of 1.5 K.

2 MONITORING STRAND HYBRIDIZATION

2.1 Fluorescence measurements

We monitored the progress of domain hybridization during the annealing protocol via fluorescence, using SYBR green I as a double-stranded DNA probe (see Sec. 1.5).⁷ We observed a dominant maximum in the fluorescence derivative between 335 K and 350 K for all structures with boundary bricks (Fig. S4a), indicating a significant amount of base-pairing at relatively high temperatures. However, as we discuss in the main text, no complete structures were assembled at these temperatures.

Comparison with theoretical annealing curves suggests that the assembly of boundary-brick structures is a two-step process. To demonstrate this, we show in Fig. S4b the temperature derivative of the equilibrium number of base pairs in a solution of monomers and dimers,^{8,9} assuming that stable misbonding

between non-complementary domains cannot occur (see below). The high-temperature transitions correspond to the hybridization between pairs of boundary bricks (where continuous 24-bp segments are hybridized) or between one scaffold strand and one boundary brick (with 16-bp hybridized segments). Consequently, the assembly of the full structure must occur in the presence of these pre-formed clusters. These calculations also indicate that the fluorescence-signal contributions from each domain length overlap significantly, since the domain melting temperatures vary widely according to their specific sequences, and each hybridization reaction tends to occur over a broad ($\gtrsim 10 \text{ K}$) range of temperatures. In particular, the theoretical annealing curves predict a broad maximum associated with the 8-bp domains near 295 K.

Analysis of fluorescence data has previously been used to distinguish between single- and multi-step assembly mechanisms for DNA tile systems with varying domain lengths. For example, a similar step-wise assembly process was seen in DX-tile structures comprising short (10- and 11-bp) and long (21-bp) hybridizations, and the presence of two distinct maxima in the fluorescence derivative was interpreted as evidence of hierarchical assembly.¹⁰ By contrast, fluorescence measurements of DNA-brick crystallization using equal-length domains exhibited no evidence of hierarchical self-assembly.¹¹ In our measurements, there appear to be multiple local maxima in the annealing curves at temperatures below the scaffold-strand $T_m \approx 315 \text{ K}$, the highest temperature at which our theoretical calculations predict that a lattice of scaffold strands can be thermodynamically stable. However, these signals are significantly weaker than the higher-temperature hybridizations which dominate the fluorescence signal. Interpreting the lower-temperature maxima is additionally hindered by several known sources of bias, including high background signals⁶ and the preferential binding of SYBR green I to GC-rich sequences.¹² Furthermore, the intercalating SYBR green I probes distort the double-helical structure of DNA molecules,¹³ which increases their melting temperatures¹⁴ and precludes a quantitative analysis.

2.2 Hybridization calculations

All hybridization calculations were carried out using the SantaLucia parameterization and the solution conditions described in the Methods section of the main text. In this section, we consider a two-state model (i.e. bonded or not bonded) for each domain and examine the simple case where pairs of strands hybridize to form dimers, but not larger multimers. We denote the hybridization free energy between complementary domains on a pair of strands i and j by ΔG_{ij} . The equilibrium probability that a strand i is correctly hybridized with its putative neighbor strand j is

$$p_{ij}(T) = \frac{\rho \exp(-\Delta G'_{ij}/k_B T)}{1 + \rho \exp(-\Delta G'_{ij}/k_B T)}, \quad (1)$$

where ρ is the dimensionless strand number density, k_B is the Boltzmann constant, T is the absolute temperature, and we assume that all species are present in equal concentrations. The hybridization free energies are written as $\Delta G'_{ij}$, to indicate that we use the longest complementary subsequence of strands i and j , which, due to the random sequence design, is occasionally longer than the intended domain length. To calculate the total change in base-pairing during an annealing protocol (Fig. S4b), we took the temperature derivative of the ensemble average of

correctly formed base pairs,

$$\text{Hybridization} = -\frac{d}{dT} \sum_{\substack{i < j \\ j \in \mathcal{E}(i)}} l_{ij} p_{ij}(T), \quad (2)$$

where l_{ij} is the length of each hybridizing domain.

3 CLUSTER POPULATION RATIOS

We assume that annealing is slow, so that nucleation is always rate-limiting. We can write the nucleation barrier height as

$$F^\ddagger = -n^\ddagger k_B T \ln \rho_m + \varepsilon(T) E^\ddagger + C, \quad (3)$$

where n^\ddagger is the number of independent subunits in the critical nucleus, E^\ddagger is the number of 8-bp bonds in the critical nucleus, and C is a constant that accounts for the (effective) number of parallel nucleation pathways, as well as the rotational entropy terms. The bond energy ε is a decreasing function of temperature, while the per-species monomer concentration ρ_m , indicating the number of monomers per unit volume, also decreases as the reaction progresses. Initially, we have ρ_T of each species. For simplicity, let us assume that, given this initial monomer concentration, the barrier is infinitely high above some critical temperature T_0 . Nucleation begins once $T \leq T_0$, where F^\ddagger is finite. (In reality, nucleation can begin as soon as the target structure, or any large cluster, becomes thermodynamically stable. However, the nucleation rate is proportional to $\exp(-F^\ddagger/k_B T)$, so the highest barrier that can be crossed depends on the cooling rate.)

Nucleation will proceed at a given temperature until ρ_m decreases to a point where F^\ddagger is again insurmountable. Denoting this critical barrier height by F_0^\ddagger , we can relate the final monomer concentration ρ_m at any temperature to the initial concentration at the critical temperature,

$$F_0^\ddagger - C = -n^\ddagger k_B T \ln \rho_m(T) + \varepsilon(T) E^\ddagger \quad (4)$$

$$= -n^\ddagger k_B T_0 \ln \rho_T + \varepsilon(T_0) E^\ddagger, \quad (5)$$

so that

$$\frac{\rho_T}{\rho_m} = \exp\left\{-\frac{E^\ddagger}{n^\ddagger} \left[\frac{\varepsilon(T)}{k_B T} - \frac{\varepsilon(T_0)}{k_B T_0}\right]\right\}. \quad (6)$$

Assuming that the intensity of each peak is proportional to the concentration of unassembled strands (m) or assembled structures (c), respectively, the ratio of the scattering intensities is

$$\frac{I_c}{I_m} = \frac{R_{h,c}^6}{N R_{h,m}^6} \left(\frac{\rho_T - \rho_m}{\rho_m}\right) \quad (7)$$

$$= \frac{R_{h,c}^6}{N R_{h,m}^6} \left(\exp\left\{-\frac{E^\ddagger}{n^\ddagger} \left[\frac{\varepsilon(T)}{k_B T} - \frac{\varepsilon(T_0)}{k_B T_0}\right]\right\} - 1\right), \quad (8)$$

where N is the number of distinct subunits in the target structure. Furthermore, because $\varepsilon/k_B T$ is a nearly linear function of T in the range of interest (Fig. S9), we expect the intensity ratio to have a functional form

$$\frac{I_c}{I_m} = \text{const} \times \{\exp[-a(T - T_0)] - 1\}, \quad (9)$$

where $a = (E^\ddagger/n^\ddagger)(d\beta\varepsilon/dT)$ and $\beta = 1/k_B T$. Using a linear fit to the energies as a function of temperature at temperatures

of interest (Fig. S9), $d\beta\varepsilon/dT \approx 0.34 \text{ K}^{-1}$. From the theoretical free-energy profiles shown in the main text, we know that for edge BBs, $E^\ddagger/n^\ddagger = 7/6$, whilst for face BBs, the ratio is $6/5$. Hence we can estimate that $1/a \approx 2.5 \text{ K}$.

To calculate the intensity associated with each peak in the DLS data, we first fitted a sum of Gaussians to the distribution function, $f(R_h)$. We then numerically integrated the peak associated with the Gaussian function f_g , according to

$$I_{c/m} = \int_0^\infty \min[f_{g,c/m}(R_h), f(R_h)] d \ln R_h. \quad (10)$$

In reality, the appearance of aggregates at low temperatures, which tend to increase the mean R_h of the assembled population, means that the ratio of the scattering intensities is not exactly proportional to the ratio of the cluster concentrations. However, this effect is relatively small over the range of temperatures of interest (approximately 8 kelvin below T_0 ; see Fig. 4a). Instead, the exponential increase in the intensity ratio as a function of decreasing temperature shown in Fig. 5c is driven primarily by an exponential decrease in the scattering intensity of the unassembled population upon cooling below T_0 . Such behavior is consistent with the theoretically predicted evolution of the unassembled-strand population shown in Fig. 5b.

4 SUPPLEMENTAL FIGURES

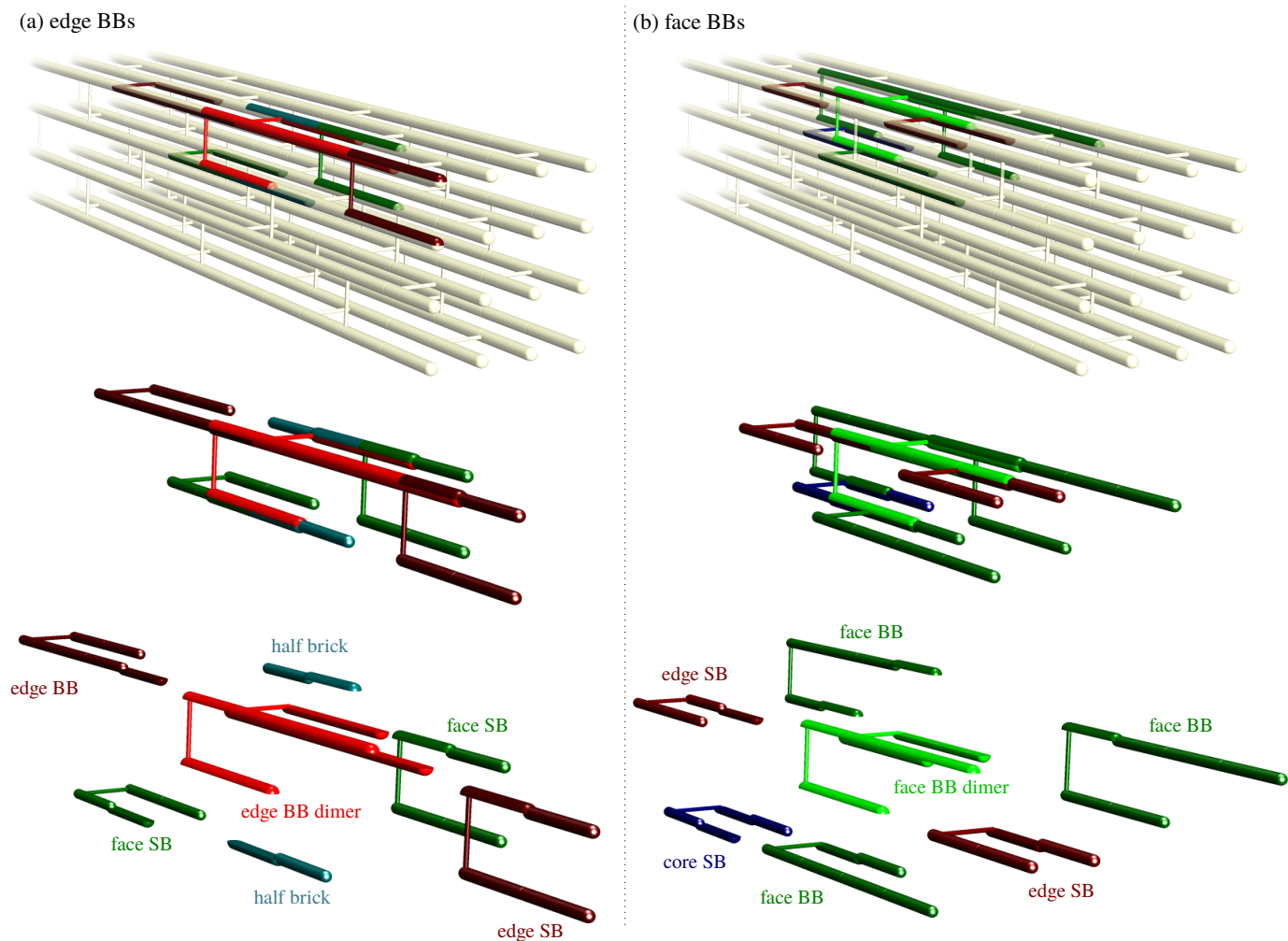


FIG. S1. Boundary brick dimers and their nearest neighbors in a schematic representation. In the top panel, the location within the target structure is shown. In the bottom panel, the neighbors are shown spread out and labeled to make their identification clearer. ‘SB’ stands for a 32-nt ‘scaffold brick’. The edge-BB system’s nucleation properties were also investigated by merging some bricks, as described in the main text. In particular, the ‘merged-A’ building block corresponds to the edge BB dimer shown in red. The ‘merged-B’ building block corresponds to the edge BB dimer and one of the face SBs shown in dark green. Either one of these face SBs could have been chosen, as both of them have direct connections to core strands. In our simulations, the face SB that is merged with the edge BB dimer is the one whose center of mass is nearer the cuboid’s principal axis in the target structure.

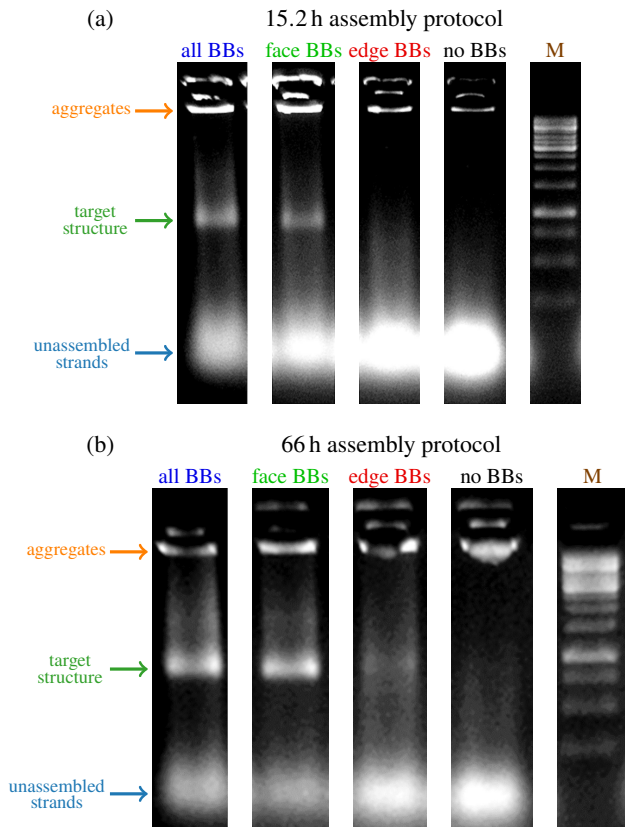


FIG. S2. Gel electrophoresis of samples at the end of (a) 15.2 hour or (b) 66 hour linear assembly protocols. Samples were assayed in 2% agarose gel. Lane M contains a GeneRuler 1 kb ladder that was used to reference the assembly yield. The bands corresponding to the target structures, unassembled strands and aggregates are indicated.

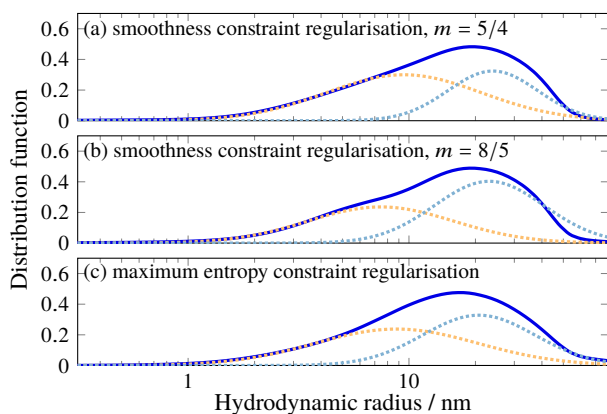


FIG. S3. Example size distribution functions (solid blue lines) for the all-BB system at 310 K determined using three regularization methods. We used a smoothness constraint functional⁴ with smoothing exponents⁵ of (a) $m = 5/4$ and (b) $m = 8/5$, as well as (c) a maximum entropy constraint with a Gaussian prior distribution.⁵ Dotted lines show the Gaussian functions determined by fitting a linear combination of Gaussians to each distribution function.

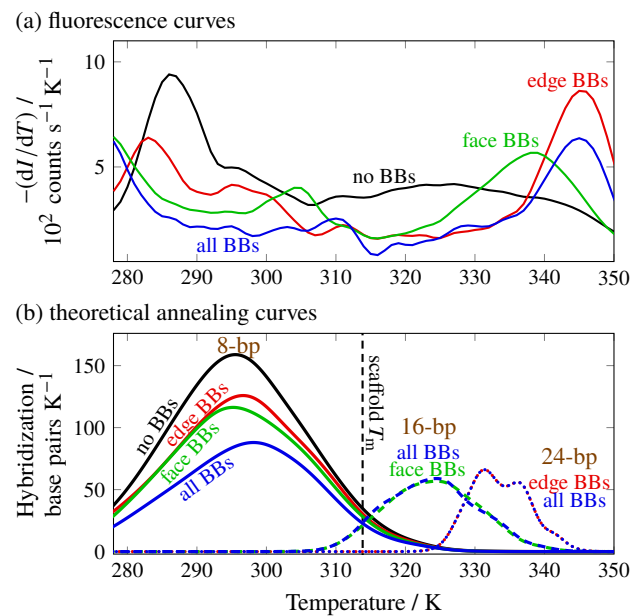


FIG. S4. (a) The derivative of the fluorescence signal I with respect to the temperature obtained from a 15.2 h annealing protocol. (b) The corresponding theoretical annealing curves (see Sec. 2.2). In agreement with the experimental fluorescence data, contributions from 16- (dashed lines) and 24-bp (dotted lines) hybridizations dominate at higher temperatures. The predicted scaffold-strand T_m is also shown.

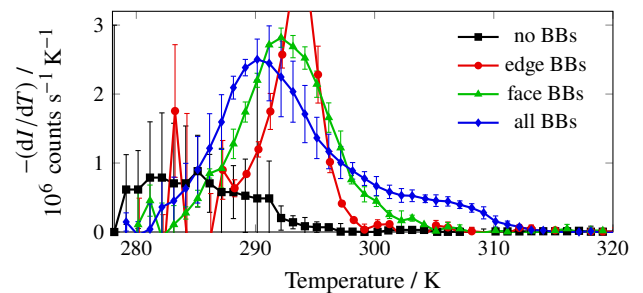


FIG. S5. The derivative of the static light scattering intensity I with respect to temperature for self-assembly following the 15.2 h annealing protocol.

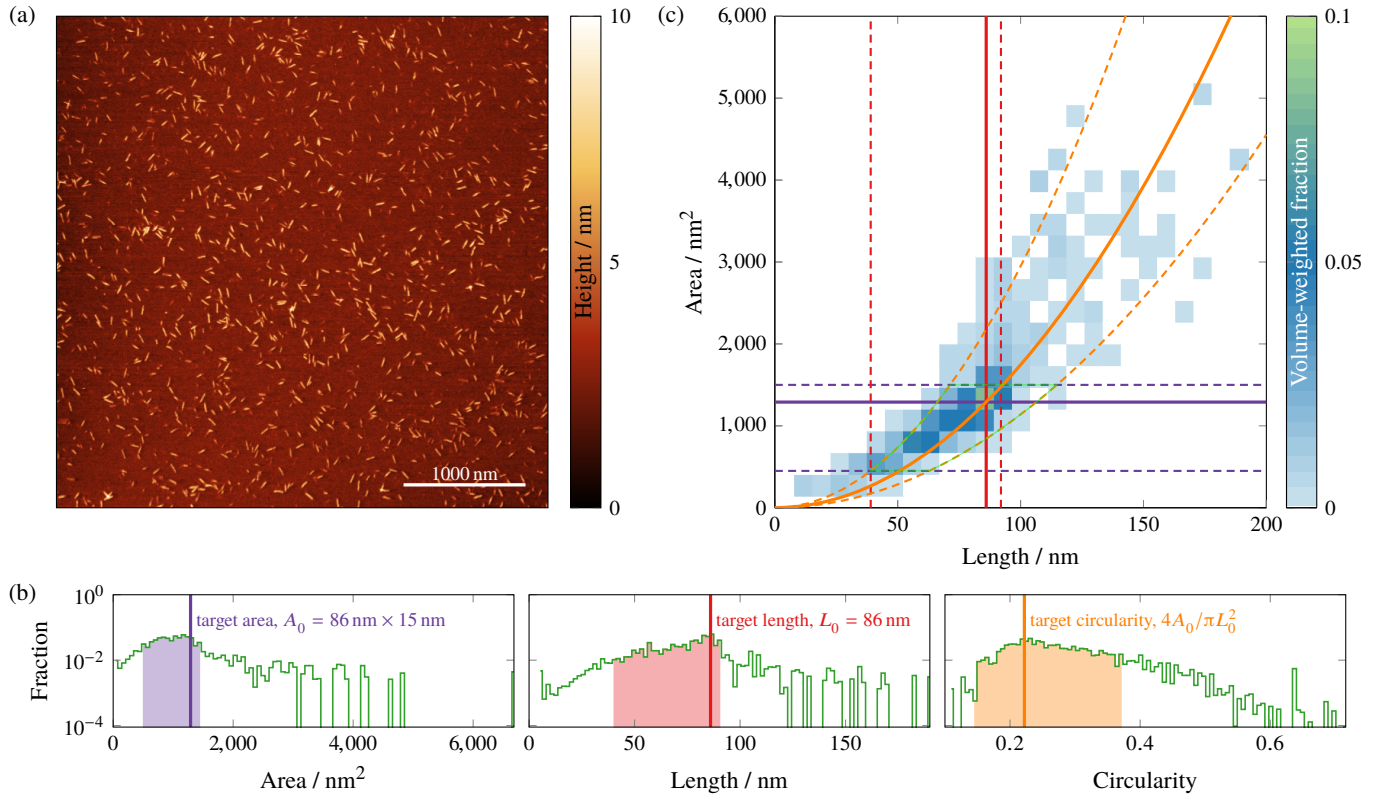


FIG. S6. Criteria for identifying target structures via AFM. (a) AFM image of the positive-control all-BB structures (see Sec. 1.1). (b) Area, length and circularity distributions of the particles identified in this image (see Methods). All distributions are weighted by the particle volume to prevent tiny particles from skewing the distributions. The solid vertical lines show the expected values for an ideal target structure when treating the cuboid as a cylinder with diameter $d \sim 15$ nm. (c) Two-dimensional volume-weighted distribution of imaged particle areas and lengths. The solid lines indicate the expected values for an ideal target structure, while the dashed lines correspond to the boundaries of the shaded regions in panel (b); the parabolic curves show the circularity, $4A/\pi L^2$. Notably, the peak of this distribution (green square) coincides with the expected area, length and circularity. Based on this distribution, we chose to use the area and circularity criteria ($450 \text{ nm}^2 \leq A \leq 1500 \text{ nm}^2$ and $0.145 \leq 4A/\pi L^2 \leq 0.375$, indicated by the translucent green lines) to identify particles as correctly assembled structures. Using these criteria, the AFM-determined yield of the positive control is 53 %.

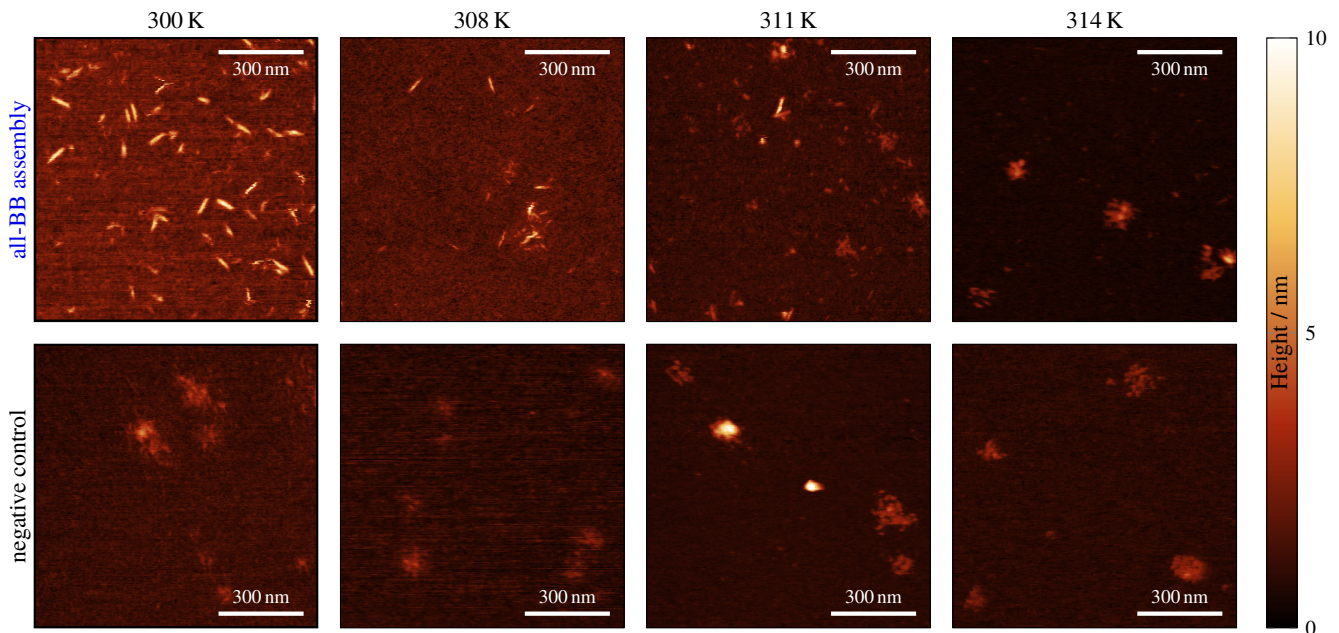


FIG. S7. AFM images for all-BB structures (top row) and negative controls (bottom row). Samples were prepared by rapidly quenching the system from the indicated temperature (column labels) during the annealing protocol (see Sec. 1.2). The negative control consists of a collection of similar-length oligonucleotides that were not designed to have complementary sequences. The bright amorphous clusters seen in the negative control and the all-BB structures quenched from high temperatures indicate large aggregates of oligonucleotides that form during quenching.

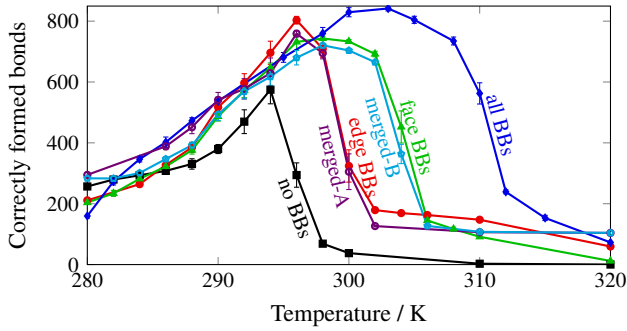


FIG. S8. The number of correctly formed bonds in the system as a function of temperature from Monte Carlo simulations. Each data point corresponds to an average over ten independent simulations in the long time limit once nucleation has occurred. Error bars give the standard deviation in each case.

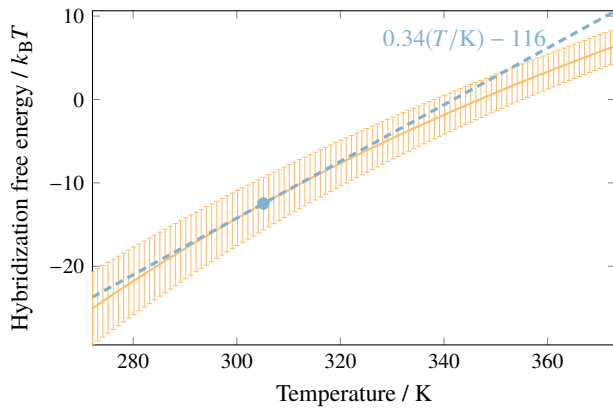


FIG. S9. The mean hybridization free energies of 8-bp interactions as a function of temperature for the no-BB structure, computed via the SantaLucia thermodynamic model,⁸ with error bars reflecting the standard deviation. The tangent to the curve at 305 K is also shown, demonstrating that the hybridization free energy is well described by a linear function over the region of interest (295 K to 315 K).

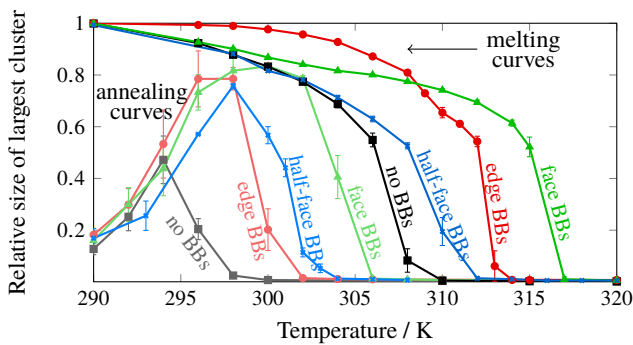


FIG. S10. Melting and annealing curves for the half-face BB structure in Monte Carlo simulations (cf. Fig. 4c and Fig. 6b).

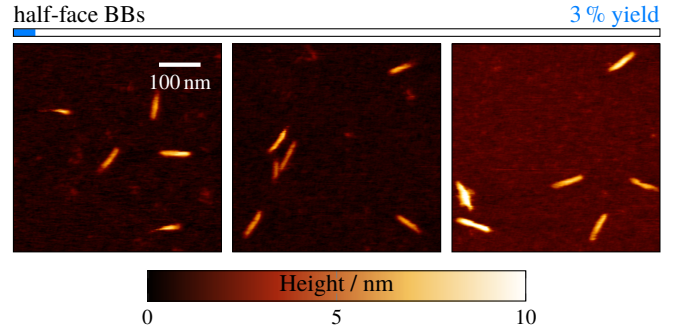


FIG. S11. AFM images and the yield, as determined by gel electrophoresis, for the half-face BB structure.

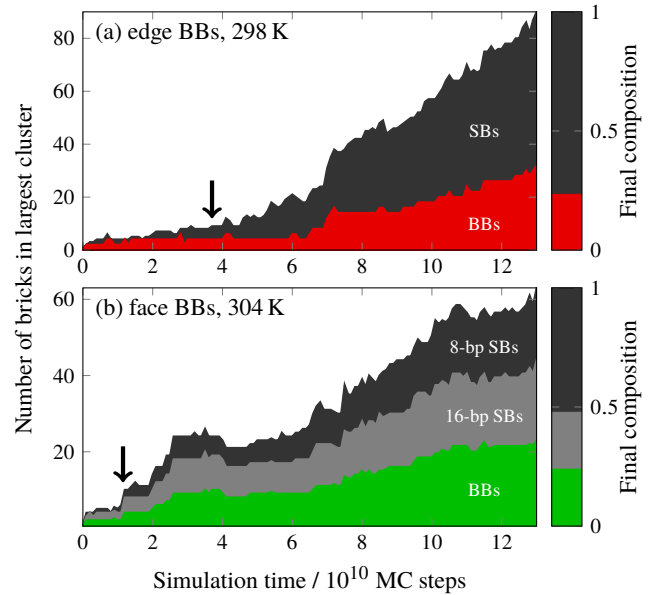


FIG. S12. The size of the largest cluster as a function of time in representative Monte Carlo trajectories for the (a) edge-BB and (b) face-BB systems. The composition of the largest cluster is shown in terms of boundary bricks (BBs) and scaffold bricks (SBs). In the face-BB system, the scaffold bricks are further divided into those that have only 8-bp hybridizations with neighboring bricks and those which form 16-bp hybridizations with face BBs. The relative proportions of each type of brick in the target structure are shown in the right-hand panel in each case. Both trajectories start at roughly the point where a fluctuation leads to nucleation and subsequent growth. Arrows indicate approximately where the theoretically predicted post-critical nucleus (see Fig. 7) first appears in each trajectory. In (a), this cluster has two BB dimers (four BBs) and four SBs. In (b), this cluster has three BB-SB dimers (three BBs and three 16-bp SBs) and two 8-bp SBs. In both cases, the cluster size increases rapidly after this nucleation event. BBs dominate in the early part of the growth phase shown here, where they comprise a greater proportion of the post-critical clusters than they do in the final assembled structure.

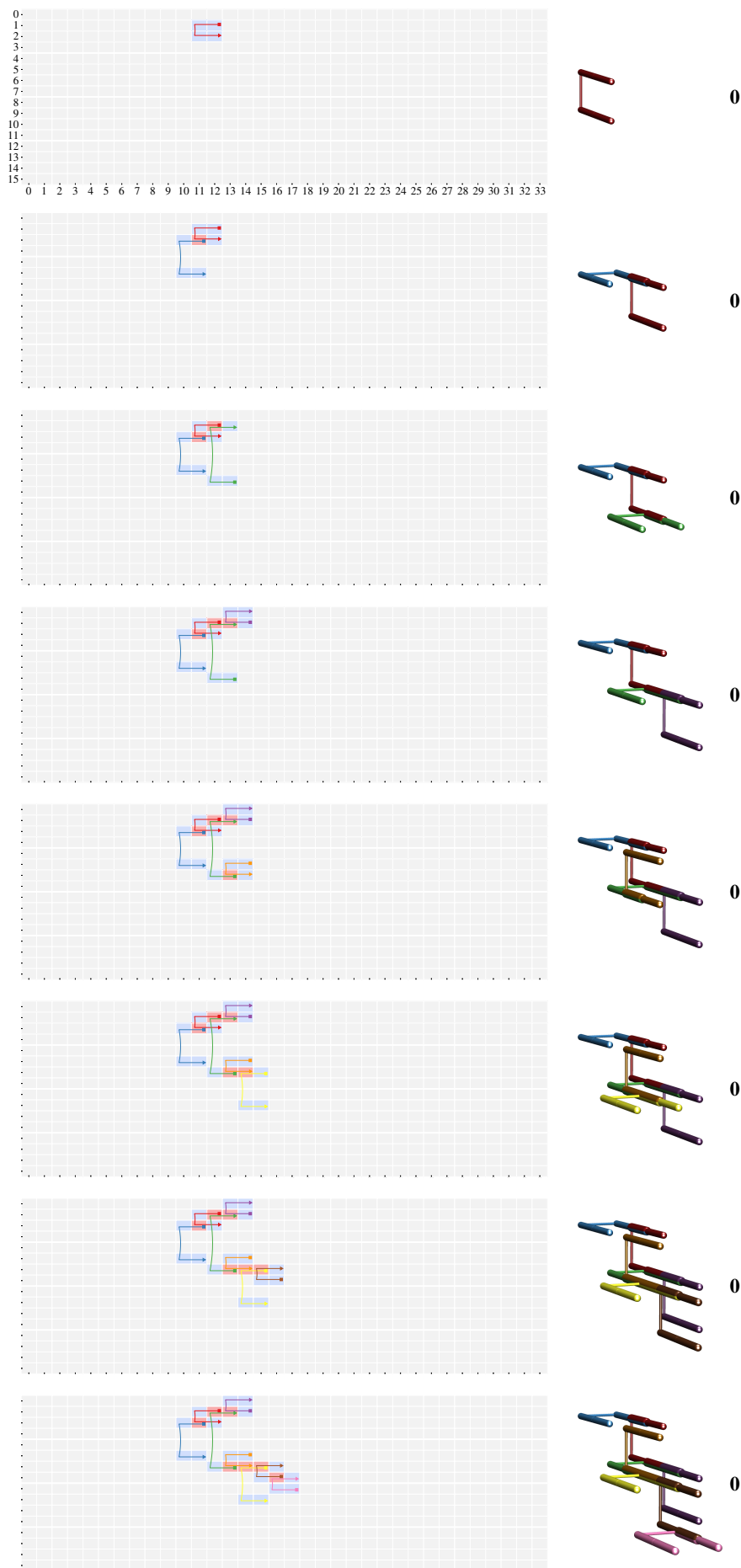


FIG. S13. Example low free-energy nucleation pathway for the no-BB system in two representations: a Cadnano-style connectivity diagram and a three-dimensional rendering. In the latter, DNA brick domains are represented by cylinders. Non-bonded domains are represented by smaller cylinders, while where two DNA bricks are bonded, larger multicolored cylinders are used. Each new monomer or multimer added to the cluster along the nucleation pathway is colored in a different hue. The bold number to the right of each structure indicates the number of multimers in the structure.

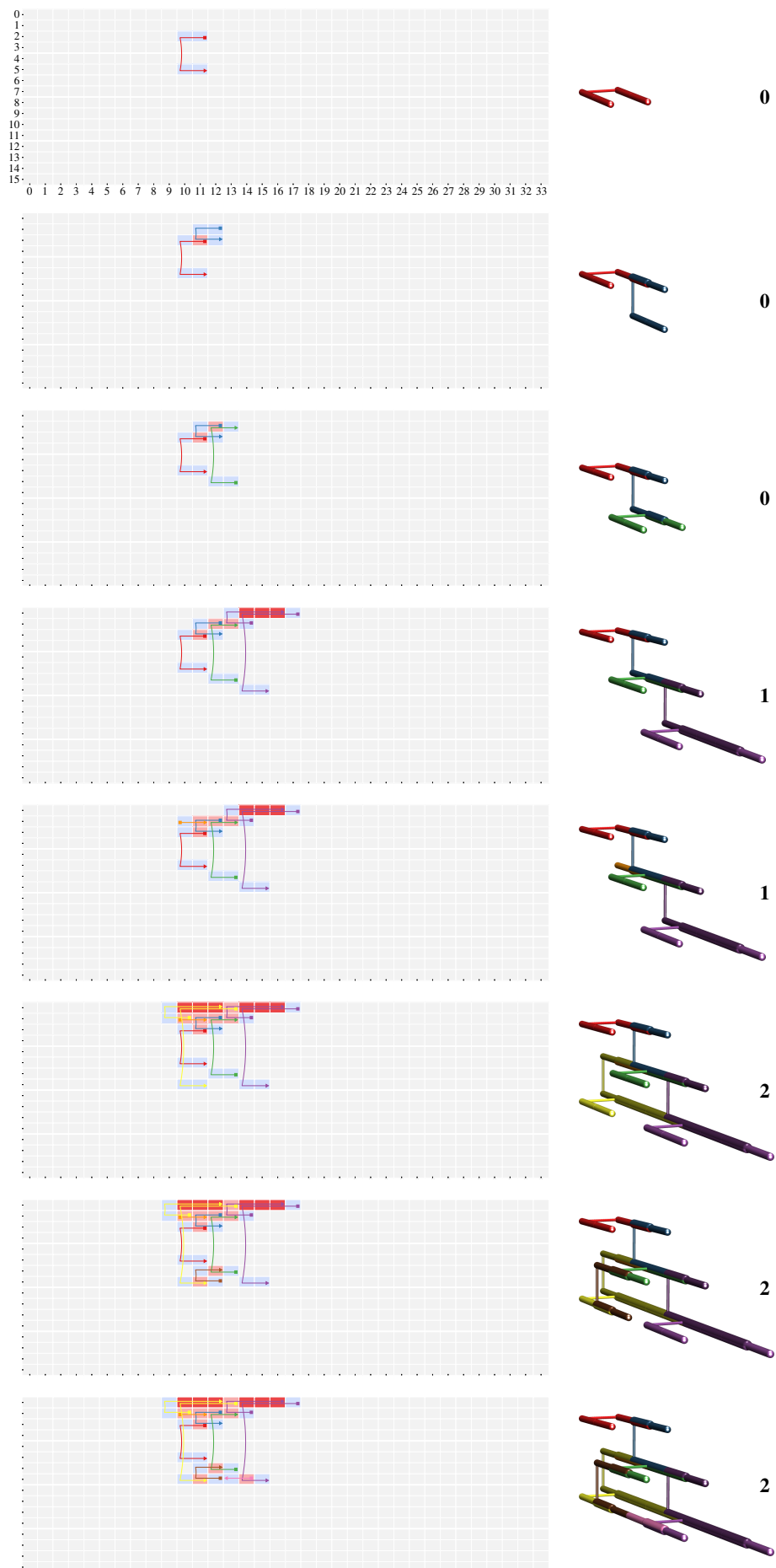


FIG. S14. Example low free-energy nucleation pathway for the edge-BB system in two representations, as in Fig. S13.

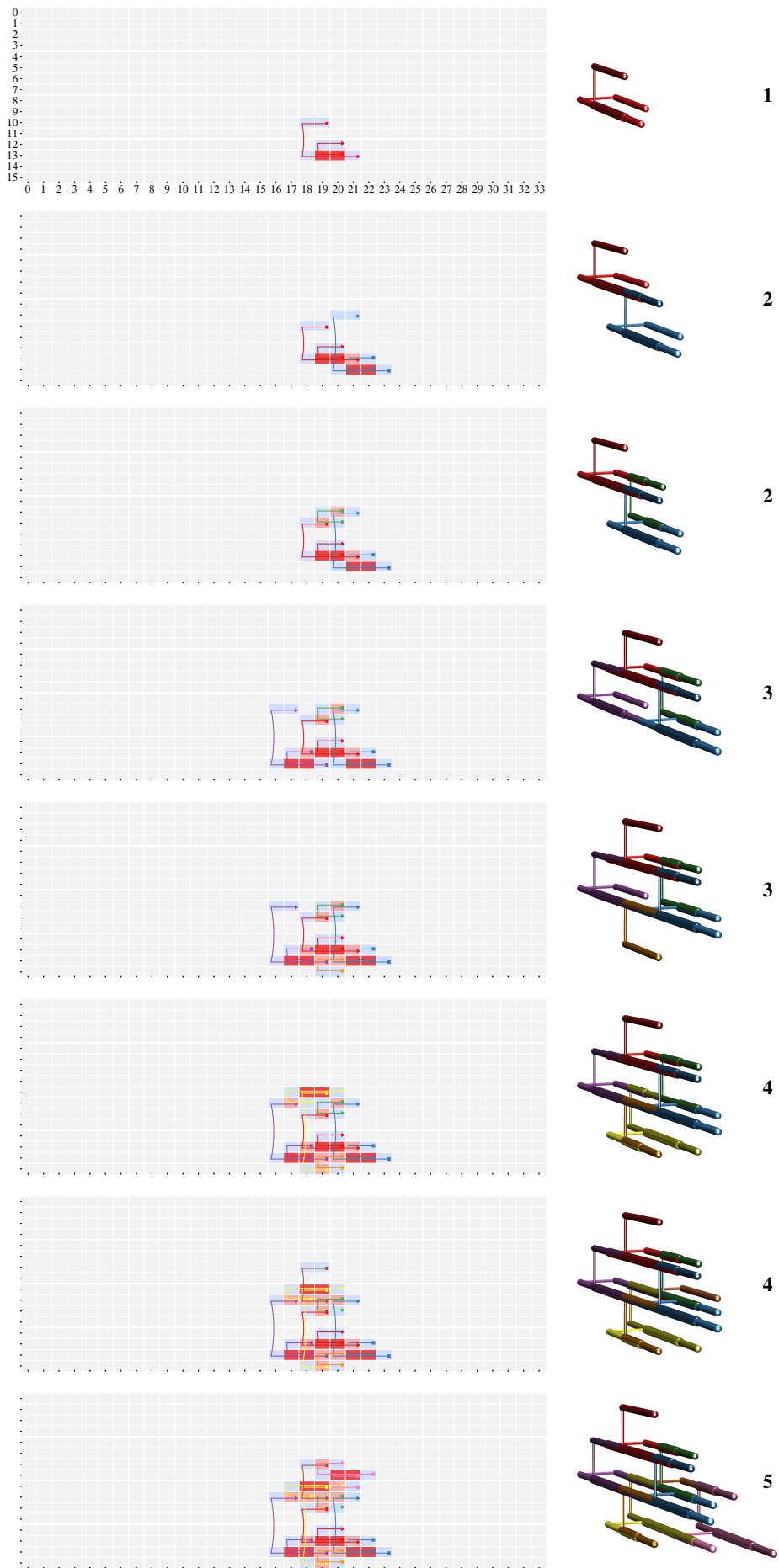


FIG. S15. Example low free-energy nucleation pathway for the face-BB system in two representations, as in Fig. S13.

5 DNA BRICK SEQUENCES

The following sequences comprise our library of DNA bricks.

ID	Sequence
1	TTCTTAAA TTTTTTTT
2	TGGCTGA TTTTTTTT
3	TTTTTTTT CCGCGTAA
4	TTTTTTTT CCAACAGG
5	TATGGTGA TTTTTTTT TTTTTTTT TGGCACCC
6	ATCCGAAG TTTTTTTT TTTTTTTT CGCGGACA
7	CGCTGATC TTTTTTTT TTTTTTTT GGGGATGA ATCCTTCC AACATCTC
8	TCGATAT TTTTTTTT TTTTTTTT GCACTGCC CCTACTCT CACCTTTC
9	ATATCAGA GAGCACAG
10	TCCGGGAT GGGTGCCA
11	AGAGTCTG GAGATGAT
12	GCTTCGGC CTTGTTGG
13	TAATATAA TGTCCGGG GATCAGCG AATTTCGAC
14	AGTTAGCA TTACGCGG GTACCTCG GACCGCTA
15	GGAAGGAT TCATCCCC TTTAAGAA TGATCGCA CCAGGTTA AGTGGCTC
16	TACATCTC GCTCGTCC TCACCATA ATCCACCA CCCATGCA GAAAGATA
17	GCGCGCTT CTGTGCTC ATCCCTTG CCCCAGAA
18	TCCCTAAA GTCGAATT GAGATGTA AGTTTCAC
19	ACCTTTTA ATCATCTC CAGACTCT TTTTTTTT TTTTTTTT GAGCCGAT
20	GGGAGATT TAGCGGTC CAGGGTAC TTTTTTTT TTTTTTTT GGACGAGC
21	CAGTCTTT GAGATGTT GAAACATCT TTTGGTTG
22	AAGGTAAT GTGAAACT CAATGATA TGACGGAT
23	ACATCTCG GAAAGGTT AGAGTAGG GGCAGTGC TCAGCCAA ACAGTGGG
24	CTTACACT TTCCGGAG CAAGGGAT ATCGGCTC CTTCGGAT TCTCTGGC
25	TATCATTT GCCAGAGA TGCTAATC GTATGACG GACCAGGT CTCCTAGG
26	TGCATGGG TGGTGGAT CGCGAAGC TGCTGTGA ATGATCGG TCATATGT
27	ACAATGGT ATCTTTTG AAGCGCAC GTAACCCG TAATTCGG AGCCCGGC
28	ACCTGGTC CGTCATAC TAAAAGTT AGAAGTAA GTAGGGTA CCGCTGGG
29	GCTCTTAA TATCTTTC GGTTCGGG GAGTAAGG
30	GTTTAGTG ATCCGTC AACTATTG TATTGGCC
31	CACAGGAA GAGCCACT TAACTTGG TGGCATCA ATCCCGGA CACGTA
32	CCACAAAG TCTTGTAT AAAGACTG TGCAGAGT CTTTACGC GCTTGAAC
33	TTCCTTTT CAACAAA AGATGTTT CCCCATGT TTATATTA CAAAAGAT
34	CCGAATTA CGGTTTAC CGAGATGT TTTACTTG ACTTGGGA TTAGGATC
35	CTTCCCTG GGCCAATA CTTTGGTG TCCACTAT
36	CAGTCCGA CCTAGGAG CGGTCTGG CTGTGAGG
37	GCGTAAAG ATCTCGCA TTTCTGGT CCAAAACT AATCATA TCACGGTT
38	TTCAAGCAG CCTTACTC CCGCAACT TTTACGTT TTTAGGGA ATACAAGA
39	GTGCTCTT GAGCGGTT TAAAGAGC CTCGCTTC AAGTCCGT GTCGGTAG
40	AAAACCTC ACATATGA CCGATCAT TCACGACA AATCTCCC GCTAGATG
41	ATGGAGCTG GCCGGGCT ATTAATCT CACGATCA
42	GTTGTTCC ATAGTGA AAGAGCAC GTTAACTC
43	GTAGTTTA CCCAGCGG TACCCTAC TTTACTCT AGTGTAAG CAACCCAT
44	GCACTCAA CCTCACAG CCGAGCCG CATCTAGT ATTACTTT CACCGCTC
45	GCCGACTC GTTCAAGC ACTATTAT CTGGCTAT
46	ACTGAGGC GAGTTAAT TTTATGTT TAACCTGC
47	TCATGTGG GATCCTAA TCCCAACT GCAAGTAA AAAAGGAA TTATGAGA
48	GATCACAA ATATGGCA AAAGTAAT ATGGGTTG CACTAAAC TATAGCAA
49	GAACATAA TTGTATA TCGGACTG TGGGCGTA CCTACACC ACAGATGC
50	AGCGACTT GAAGCGAG GAGGTTTT GCGACTCA TTGCTGAG TTCGAGTC
51	ATATAGGT CTGACACC CAGCTCCC AGGTAAGT GTATTATC GCGTCAAT
52	GGTGTAGG TACGCCCA TAAACTAC TTTTCCAC GTTGCCCT CGACCGAT
53	GGAGGGTT CTACCGC ATAT1TCT TTTGCCCT
54	TGTTGAGG GCAGGTTA ACCTATAT AGCCATTT
55	AGCTTGGG AACCGTGA GTATGAT AGTTTGG CTGCTGAA TGTCCGTG
56	GCACCATT TGACACC GGTTCGCG CAGCAGA CATCTTTT GGGTTCGT
57	AGCCTACG ATAGCCAG ATAATAGT TCTCATA CAGGCAAG GGTGTACG
58	GATAATAC ACTTACTC CCACATGA AGGTCTAC CAGAAGCT ATGTACCC
59	CGGAGAG AAATGGCT AATGTTCG ACAAATATG
60	CGTCCAGC GCATCTGT GATCCAGC TCAGATTT
61	AAAAGATG TCTGTAC CCAAGCTT TAGCATAA AACTGTG TGTCTTAT
62	TGCCAAGT AAGGGCAA AGCAATAT CACGGACA GGAACAAC GGGTGTCA
63	GCGTATGC TGAATGGA AACCTCCC TACGTTTT GCGATGTT GATGGGAA
64	TTTAGTTT GCGTCGAA CTCAGCAA TGAGTCCG TTGAGTCG GTGGATAA
65	ATCGCTCA ATTGACGC GACTTTGT GTAATCTC
66	CATACACA CATATTGT GCATACGC CCTGTGTT
67	CTGAGCGT ATCCGTCG AGGGCAAC GTGAAAA TTGTGATC CCCTCCCA
68	AATAGGCT ATTCAAGA GCTGAGC TGGCGGAG GCACGTAT GTCTTGAG
69	TAGGGCTA AAATCTGA CGTGGATC TTATCCAC GCCTCACT CCATTTCA
70	ACCATCGC AAAACGTA AAACATAA TGACGTTT GAGGTGAT ACGTAAAG
71	GCGGCTGG ACGACCCC GCCGTGGC ACGACTGA
72	GTAATGCG AAAACAGG AGCCTATT CATCAAGG
73	GATATCCA CGGTACAT AGTTTCTG GTAGACCT CGTAGGCT AGAGCGGT
74	TATTAAGT TTTCCGCG TGAGCGAT CAATGACC TCATATGG ACACACCA
75	GATCTCGA GAGATTAC ACAAGTCT TGGGAGGG CCTCAACA TCTTGAAT
76	ATACGTGC CTCGCGCA ACGCTCAG CCGTCTCG TAGGGTGA CGTATTAG
77	CAAATCCC TTCCCATC AAATACGA CTAAGCCG
78	CGTTGCAC CCTGTGAT ACTTAATA TTAAGGTT
79	GTTTGTCT ATAGGACA CAACAGTT TTATGCTA ACTTGGAC CGGGCATC
80	GCAGGGTG AAAGTGAG CCGAGCCG GCGCAGGG CTCGACGG TTAATTTG
81	ACTCTCGT TCAGTCTG CGCACGGC ACCGCTCT CTCTCCGC CGGCGAAA
82	CCATATGA GGTCAATT TGGATATC TCCGTGCA GTAAACGA TAGGTTTC

Table continues ...

ID	Sequence
83	CCAGTAAT AGCCTGAA CACCCTGC ATCGACGG
84	CTAGCAT CTCACAGC CTGGGAAG TAGTAAAG
85	CCGTGAG CCCTGCGC GACAAACG CATATCCT GTTTACTT AGGGATGG
86	TCTGGCGC CGGCTTAG TCGTATTT GATGCCCG TGTGTATG CTCACCTT
87	AAAAGATC ATAACGTT GGGATTTG TTCCCGCT ATAGGTAA CTGGGTTT
88	GTCAATTT CTTTACGT ATCACCTC AAACGCTA TAGCCCTA CGGATCAA
89	GAATTATA TGGTGTGT CCATATCA ACAAAACC
90	GCGCGGAA CCGTCGAT GATCTTTC GGACCGAG
91	GGCTTGCA CTAATACG TCACCCTA CAGACCGG TCAGGATC ATTTTGGG
92	TTTGTAAA CTTACCTA ATTCACAG TTGATCCG CGCATTAC AACGTTAT
93	CGGCGCGC CAAAATTA TAGTCGAT CATGCTTC
94	AGCTCATT CTCGGTCC AATTCGAG CATGTCCC
95	AAGCAAGA GAAACCTA TCGTITAC TGCAACGA ACGAGAGT GCTGGCAT
96	TGGGCCCG GGGTTTGT TGATATGG TCCAAAAT GTGCAACG GGAACACG
97	CTCGAATT GTGTTTCC ATGCTAGC GCCACAGC ACTACTTA AGACTTAG
98	TTACTTAT AGGCGGAA AAATTGAC ATTTCTAC AATCGTGA CGGTTTCT
99	CTTCTACC TCTTAATG TATAATCT ATCCTGTG GATTACGG GCGGTTAG
100	TAAGTAGT CGTGTGGC TGCAAGCC CTCACCTG ACCTCTTA GCACAATA
101	CCTAACAT AAACCCAG CGTGACGG TGAATCTT
102	GTGTAAGA GGGACATG GTGAGAAG GAACTCTG
103	ATTACCGT CCATCCCT AAGTAAAC AGGATATG GCGCCAGA CCACAATG
104	GCGGATGG AAAACGAC GCGGGCCG GGAAGGAC CTTTAGCC CCAAGGAG
105	TCCCAACG GAAGCGTA ATCGACTA ATCGACAG CATTACCG CATTAAAG
106	CCGTAATC CACAGGAT TCTTGCTT ATCTTAGT CCCCAGT GCTACATG
107	CATGGAAG CAGAGTTC CCATCGCG AAAATTGA
108	AACCCGCG CTAAGTCT TTAGAATC ACCTTAGG
109	GCCTAAAG GTCCTTCC ACGGTAAT CCGGGGTG TACGAACT CAACGCAC
110	CTCAGTTT AAGATTCA CCGTCACG CATTGTGG TTCGCCCG GTCGTTTT
111	GACCCCTC AGATCCCA ATGTTTAGC ATGTTAGC GTGCAACG GAAACACG
112	TCTTCAA AGAAAACG TCACGATT GTAGAAAT TTTACAAA GACGGACA
113	AATGTTCT CTAAGCCG AGTAAACA ATCAAGGA
114	GAGGAGGT TCAATTTT AGAGCTCG AGCGGAGT
115	GTAGGCTC TATTGTGC TAAGAGGT CAGGTGAG CCGGGCCA ATTGTACC
116	ACGGAAAC TCCTACCC GCGCGGTT GATGTCCG TCGCAAAA GCAAGCCG
117	GTCGCGCA CTAAGTCT GATTCTAA TGTCCCTG AATGAGCT TGGGATTC
118	GAAATATC TGTAGCT TTTGAAGA AGGCTCCG TCATTCCG TCCCCCAA
119	ACTCCGGC CTCTTGG TATATTAG CTTACCCA
120	GACCCGAC ACTCGCTT GTTCCCTT
121	GCGATTCC CATGTAGC ACTTGGGG ACTAAGAT CGTTGCGA ATTTTCCG
122	GTTACCCG TATAATTA AGAACATT CCGGTTCC GCGAATTG AGTACAAG
123	GCGCAGTT TCCTTGAT TGTTACTT GGTACAAT CTTTACAG GCGGTAGG
124	TTTTCGCA GGCACATC GAGCTTAC CCGTCTAT AGCAGAAAT GGTATAAA
125	CACCTACC GTGTCCTT CGTCTGCT CCGGTCCC
126	ATATTACA AAGAACGC GCGGTAAC ATCACATA
127	CGAGTCGT GTGCGTTG AGTTCGTA CACCCCGG AAAGTACG GAGAACAT
128	CCTAGGCG TAAGACCT GCGCGGAG AGACGTGC CACGCGAG CTATGTAG
129	CGCTGTCT TGGGTAAG CTAATATA GCGAAATG CATTACAG GGTATATA
130	CAATCCCG CGAACCCG CGAATCGC AGGACGAT CTCGTTCC GGTGCTAC
131	ACGTGGTC TATGTGAT CGCTAGG CTAACCTC
132	GCGACACA CGGCTTGC TGGACACA ATTTTAT
133	CTCGGTG GCACGTCT ACGACTCG TCGAACGA GCTCCGGT AGTCTCAA
134	ATTTTGGC GGAACGGG ACAGGACG ATGTTTCT ACCTTTC AGGTCTTA
135	CACGAGCT TTTACGCG GGTAGGTT TAAGACCT GCGCTTCA GCGGTAAC
136	TAGCCGCC TTGGGGGA CGGAATGA CCGAGCCT TCCGCCAC CCCTGACA
137	GAGTCCAC CTTGTACT CTTATCTC TCACCAGG
138	GCGGATGC GAGGTTAG AGTCTGGC AAGCGGTT
139	TCCCAATC TTTATACC ATTCTGCT ATAGACGG AACTGCGC GTCACCTC
140	AGGGATTG AGTACCCA TGTGTCCG AGATGCGA TATGACAG GTGAGCAC
141	TTCCGCGC ATAAAACT TGGTCGCA TGTCAAGG GTCGATAA GGGTAAAG
142	TCAGGCGC AGGTCTTA GCGGCGTA CGTCTATT TAACGTGC CTGATCAC
143	GGACTGTT CTACATAG TGACTTTG ACGAGGTT
144	TCAGCGCC AACCCGTT CAATCCCT AGCCGTTT
145	GCATGCCG GTAGCACC CGAACGAG ATGCTCTC AGACGAGC GATCTCTC
146	ATGACTCC AGGAGAGG GTGAGCTC CTACGTGG TGACGAAG AATACCTG
147	ATAACCAT CCTGGTGA AGGATAAG GCGAGATC TGTAAAT TGGTACT
148	CTGTATA TGCCATCT GATTGGGA GAGTCCAG GCAATAAA GAAACTGC
149	TTCATTAC GTTCTACG CGATGCTT TTGCCACA
150	CCTACGCC GAACGGCT GGAGTCAT CTCGGCAT
151	AGTTTCTT TTGAGACT ACCCGAGC TCGTTCGA GCCAAAAT CAAGGACG
152	AGTTAGAT CCATGCGA AACAGTCC GTATAGCG CCGACTG CCTCGACA
153	GCGTAAA AACCTCGT CCAAGTCA AGGAGATC GACCAAGT GCTCTCTC
154	CTTGTGTA CCACGTAG CCGCATGC AGCACCGA TATTGTAG GTCTTGGC
155	AAGTGTTA ATGCCGAG ATCTAATC TGATTTTT
156	CCGACCCT GTGCTCAC GCACGAGA GCGCTTCC
157	TCACCTGG CGTATAC AGGAAACT CAGGGGGC GAAGATAG AGAGCATA
158	TAAAGATG TGTGGCAA AAGCATCG CGTCTTGT GCATCCCG TCGCATGG
159	AAAGATGG TCTACATC AGGATAAG GCGAGTGG AAGCAGAG CATTTTGA
160	TACAGCGG GTGATCAG GCACGTTA AATAGACG GCGCGGAA GACCTCCG
161	AAGAGCAA ACGGTATT ATCCGTTT CACAGCGC
162	ACATGAGG AAAAAATCA CCATTTCT GTGCCAGT
163	TTACCTCT GCAGTTTC TTTATTGC CTGGACTC ATGGTTAT CCCTTCCC
164	TTACCAGC GTGCGGCC AGGTCCGG ACGAGCTT ACCCATGG CGCACCAC
165	TTTTCCT GGAAGCCT TCTCTGCT CCGCGTGC GCGCTGTA GATGTAGA
166	CTCTGCTT ATCTATCC CCGCTGAA AGGATTAT TCAATAAT GCACCTGC
167	TCCCTGTC TGTGAGC CCAGCGAC CACTTCTG
168	TCTAGCC ACTGGCAC CTTGGTAA TCTGGGCT
169	CAGCTTGA CGCAAGAC GTCAATA TGCCTGCT TTTAACCG TTTAGACC
170	ATTCAGAA TCGAGACC TTGCTCTT GGAGCTCT CCATCAGA TGGAGCTG

Table continues ...

ID	Sequence
171	TGGACCAC GCGCTGTG AACGGGAT GGGAAAGG GCGGTAGG GGCCGCAC
172	CCATGGGT ACTGCTGT GAGGGTAA CACGTTGG ACTCCTAC TGCCCCGCC
173	TTCAACAA TCAAAATG ATATTGTA GCGAGATA
174	CTACATTT AGCCCGA TTCTGAA CTGGGGCT
175	AACCTACTG TATGCTCT CTATCTTC GCCCCTCG CATCTTAA ATATAACT
176	CTAGTAAC CGACCGTT GACAGGGA CCACCCTG TCTTTCTC GTATATCA
177	CTAGGACC CAGAAGTG GTGCTGGG GGTCTAAA TAACACTT GGTCTCGA
178	TCTGATGG AGACGTCC TCAAGCTG CTGCCACG TGATTAGC GCTTAGGG
179	CATTCTAT AGCCCGCG GTTACTAG GCGCCTTG
180	GATGATCT GTGGTGGC CACAATTT GCTGGGAA
181	GAGAAAGA TGCGGTGG CAGTAGTT GTGATTAG AATTCGCT AAACCGGA
182	TATCCGCC TATCTCGC TACAATAT AGTTATAT CCTCATGT AACGGTCG
183	ATTAAGCT GTGGAATG TTGTTGAA CATACTCC TCATTAGG GTCCACCG
184	TTCCCTAAC GCAGGTGC ATTATTGA ATAATCCT AGGAAATA CCACTGCC
185	AGGGTTCA CAGCTCCA AAGCCTGT CGAGGCGT
186	GACTCTCT CAAGCGCC AGCTTAAT TAGTAAGG
187	TGTTTTAA GCGCGGCA GTAGGAGT CCAACGTG GTGGTCCA CGGCGACG
188	CTTTTCTG CTTCAGC AAATTTGT GCGAGTGG GGCTAGAA CATTCCAC
189	CTTGGGGG TGATATA CTCACTAA ACTCCTTC
190	CCTCCTAC CCTTACTA GCGGTACA CACCAAGA
191	AACCTACC CCTAAGC GCTAATCA CTGGGCG GGTCTAG GTTGAGA
192	TTACAGCG ACGCTCGC ACAGGCTT CGTCGCCG AAATGTAG CCATGAAT
193	TGTACCCG ATTCATGG AGATCATC CATTGACG TCGTITAGG TTTTTTTT
194	CCTAATGA GGAGTAGT GTTAGGAA TACCTGCT CCTGAATG TTTTTTTT
195	CTCCTACG CTCATAGT TGAACCTT TACTATCG
196	AGAGTTCT ACCAGCCA AGAGAGTC GCAGCCCT
197	CGTAACGA CGTCAATG TAAACACA ACAACCGA
198	CATTACAG AGCAGGTA CAGAAAAA GAACCGAC
199	TTTTTTTT CGATAGTA GGTAGGTT TTTTTTTT
200	TTTTTTTT AGGGCTGC CCCCAGAG TTTTTTTT
201	TTTTTTTT CGGTGGAC AGAATCTT TTTTTTTT
202	TTTTTTTT TCTTGGTG CGTAGGAG TTTTTTTT
203	TTTTTTTT TCGGTTGT CGCTGTAA TTTTTTTT
204	TTTTTTTT GTCCGTTT GATAGGAC TTTTTTTT
205	TTTTTTTT TCCGGTTT AGCGAATT CTAAATCA GCGCGATA TGGCTGGT
206	TTTTTTTT GAAGGAGT TTAGTGAG TCTGCAAC ATAGAATG CTATTGAG
207	CAGACTCT TTTTTTTT TTTTTTTT GAGCCGAT
208	CAGGGTAC TTTTTTTT TTTTTTTT GGACGAGC
209	TCTGATAT TTTTTTTT TTTTTTTT GCACTGCC
210	CGCTGATC TTTTTTTT TTTTTTTT GGGGATGA
211	CAAGGGAT ATCGGCTC CTTCCGTT TCTCTGGC
212	TACATCTC GCTCGTCC TCACCATA ATCCACCA
213	AGAGTAGG GGCAGTGC TCAGCCAA ACAGTGGG
214	GGAAAGAT TCATCCCC TTTAAGAA TGATCGCA
215	TATCATTG GCCAGAGA TGCTAATC GTATGACG
216	TGCATGGG TGGTGGAT CGCGAAGC TGTCGTGA
217	AGATGTTT CCCACTGT TTATATTA CAAAAGAT
218	TAACCTGG TGCGATCA ATCCCGGA CAGCTAAA
219	ACCTGGTC CGTCATAC TAAAAGGT AGAAGTAA
220	CCGATCAT TCACGACA AATCTCCC GCTAGATG
221	ACAATGGT ATCTTTTG AAGCGCAC GTAACCCG
222	CCGCAACC TTTACGTG TTTAGGGA ATACAAGA
223	TACCCTAC TTACTTCT AGTGTAAG CAACCCAT
224	CAAGCCG CATCTAGC ATTACCTT CACCCGTC
225	CCGAATTA CGGTTTAC CGAGATGT TTTACTGC
226	CCACAAAG TCTTGTAT AAAGACTG TGCGAGAT
227	AAAGTAAT ATGGGTGG CACTAACC TATAGCAA
228	GTGCTCTT GAGCGGTG TTAAGAGC CTCGCTTC
229	TCCCAAGT GCAAGTAA AAAAGGAA TTAAGAGA
230	CGGTAAG ATCTCGCA TTCTGGTG CAAAACCT
231	GAACATAA TTGCTATA TCGGACTG TGGGCGTA
232	ACGGACTT GAAGCGAG GAAAGTTT GCGACTCA
233	ATAATAGT TCTATAA CAGGCAAG GGTGCTAG
234	GTATGATT AGTTTTGG CTGCTGAA TGTCCTGG
235	GGTGTAGG TACGCCCA TAAACTAC TTTTCCAC
236	CTCAGCAA TGAGTCGC TTGAGTGC GTGGATAA
237	ATATAGGT CTGACACC CAGCTCCC AGGTAAGT
238	AGCAATAT CACGGACA GGAACAAC GGGTGTCA
239	AGGGCAAC GTGGAAAA TTGTGATC CCTCCCA
240	CGTGGATC TTATCCAC GCCTCAGT CCATTTCA
241	GATAATAC ACTTACCT CCACATGA AGGTCTAC
242	CGACCATT TGACACCC GAGTCGGC GTGACAGA
243	ACAAAGTC TGGGAGGG CCTCAACA TCTTGAAT
244	GCGTATGC TGAATGAG AACCTCTC TACGTTTT
245	AGTTTCTG GTAGACCT CGTAGGCT AGAGCGGT
246	AAAAAGAT TCTGTGAC CCCAAGCT TAGCATAA
247	AATAGGCT ATTCAAGA GCTGGAGC TGCCGCGAG
248	ACCATCGC AAAACGTA AAACCTAAA TGACGTTT
249	CGCACGGC ACCGCTCT CTCTCCGC CGCGGAAA
250	CAACAGTT TTATGCTA ACTTGGAC CGGGCATC
251	ATACGTGC CTCGCGCA ACGCTCAG CCGGCTCG
252	ATCACCTC AAACGTCA TAGCCCTA CGGATCAA
253	TATTAAGT TTTCCCGC TGAGCGAT CAATGACC
254	TCGTATTT GATGCCCG TGTGTATG CTCACTTT
255	TCACCCTA CAGACCCG TCAGGATC ATTTTGGG
256	ATTCCAG TTGATCCG CGCATTAC AACGTTAT
257	CCATATGA GGTCAATG TGGATATC TCCGTGCA
258	CGAGGGTG AAAGTGAG CCAGCCGC GCGCAGGG

Table continues ...

ID	Sequence
259	TGATATGG TCCAAAAT GTGCAACG GAAAACAC
260	GAAAGATC ATAAAGCT GGGATTTG TTCCGCCT
261	TCGTTTAC TGCACGGA ACGAGAGT GCTGGCAT
262	CCGTCGAG CCCTCGCG GACAAACG CATATCCT
263	CTCGAATT GTGTTTCC ATGCTAGC GCCACACG
264	TTACCTAT AGGCGGAA AAATTGAC ATTTCTAC
265	ATCGACTA ATGCCAGC ATTACTGG CATTAAAG
266	AAGTAAAC AGGATATG GCGCCAGA CCACAATG
267	TAAGTAGT CGTGTGGC TGCAAGCC CTCACCTG
268	TCACGATT GTAGAAAT TTTACAAA GACGGACA
269	CTTCTACC TCTAATG TATAATTC ATCCTGTG
270	CCGTCACG CATTGTGG TTCGCCGC GTCGTTTT
271	TAAGAGGT CAGGTGAG CGGGCCCA ATGTACC
272	GATTCTAA TGTCGGTC AATGAGT TGGGATCT
273	CCGTAATC CACAGGAT TCTTGCTT ATCTTAGT
274	GCCGATGG AAAACGAC CGGGCCGC GGAAGGAC
275	TGTTTACT GTTACAAT TCTTACAC GGGTAGGA
276	CGACTCT AGATCCCA ATGTTAGG AGCTAACA
277	ACTTGGGG ACTAAGAT CGTTGCGA ATTTTCCG
278	GCCTAAAG CACTTCC ACGGTAAT CGGGGGTG
279	ACGGAAAC TCCTACC GCGCGGTT GATGTGCC
280	GAAATATC TGTAGCT TTTGAAAG AGGCTCCG
281	CTAATATA GCGAAATC CTTCCATG TAATTATA
282	AGTTCGTA CACCCCGG AAACCTGAG GAGAATAT
283	TTTTCGCA GGCACATC GAGCCTAC CCGTCTAT
284	CGGAATGA CGGAGCCT TCCGCCAC CCGGCACA
285	GTTACCGC TATAATTA AGAACATT CGGGTTCG
286	ACAGGACG ATGTCTC ACCTCTTC AGGCTTAA
287	ATTCGCT ATAGACGG AACTGCGC GTCACCTG
288	TGGTCGCA TGTCAGGG GTCCGGTC GCCGTAAA
289	CAATTCCC CGAACCCG CGAATCGC AGGACGAT
290	CCTAGGCG TAAGACCT GCCGCTGC AGACCTGC
291	AGGATAAG GCAGTGAC TGTAATAT TGGTACT
292	GCCAGACT TTTACGGC GGTAGGTT TAAGACCT
293	CGAACGAG ATCGTCT AGACGACG GCTACTCT
294	CTCGGTG GCACGTCT ACGACTCG TCGAACGA
295	AGGGATTG AGTACCCA TGTGTCCG AGATGGCA
296	TCAGGGCG AGGTCTTA GCGGGTAA GCGTATT
297	CAAAGTCA AGGAGATC GACCACGT CCTCTCT
298	ACCCGAGC TCGTTCGA GCCAAAAT CAAGGACG
299	CTGTGATA TGCCATCT GATTGGGA GCGCTCAG
300	GCACGTTA AATAGACG GCGCGGAA GACCTCCG
301	ATGACTCC AGGAGAGG GTGAGCTC CTACGTGG
302	AAGCATCG CGTCCTTG GCATCCCG TACGTATG
303	TTTATTGC CTGGACTC ATGGTTAT CCCTTCCC
304	TCTCGTGC CCGAGGTC GCCGCTGA GATGTAGA
305	CTTCGCTA CCACGTAG CGGCATGC AGACGCA
306	AGTTAGAT CCATGCGA AACAGTCC GTATAGCC
307	AACGGGAT GGGAAAGG GCGCTAGG GGCCGCAC
308	AAGAATGG TCTACATC GTAATGAA GGATAGAT
309	GTCAAATA TGCGTCT TTTAACGC TTTAGACC
310	TCACTGGG CGCTATAC AGGAAACT CGAGGGGG
311	TTACCAGC GTGCCGCG AGGTCCGG ACAGCAGT
312	CTCTGCTT ATCTATCC CCGCTGAA AGGATTAT
313	GTCGCTGG GGTCTAAA TAAACTTT GGTCTCGA
314	CTATCTTC CCCCCTCG CATCTTAA ATATAACT
315	CCATGGGT ACTGTGTT GAGGGTAA CAGTGGG
316	ATTATTGA ATAACTCT AGGAAATA CCACTGCC
317	ATTCAGAA TCGAGACC TTGCTCTT GAGCGTCT
318	TACAATAT AGTTATAT CCTCATGT AACGGTGC
319	GTAGGAGT CCAACGTG GTGGTCCA CCGCCACG
320	AAATTTGT GGCAGTGG GGCTAGAA CATTCCAC
321	TCTGATGG AGACGTCC TCAAGTGC CTGCCAG
322	CTAGTAAC CGACCGTT GACAGGGA CCGCCGCA
323	ACAGGCTT CGTCGCCG AAATGTAG CCATGAAT
324	ATTAAGCT GTGGAATG TTGTTGAA CATACTCC
325	GCTAATCA CTGGGCAG GTCCTAG GTTGACGA
326	GAGAAAGA TGCCGTGG CAGTAGTT GTGATTAG
327	TGTACCGC ATTCATGG AGATCATC CATTGACG
328	CCTAATGA GGAGTATG GTTAGGAA GTTAGCT
329	TTAGTGAG TCTGCAAC ATAGAATG CTATTGAG
330	AGCGAATT CTAATCAC GCGCGATA TGGCTGGT
331	ACTTTTTA ATCATCTC
332	GGGAGATT TAGCGGTC
333	CCTACTCT CACTTTTC
334	ATCCTTCG AACATCTC
335	CTTACACT TTCGGGAG
336	CCCATGCA GAAAGATA
337	ACATCTCG GAAAGGTG
338	CCAGGTTA AGTGGCTC
339	GACCAGGT CTCTTAGG
340	ATGATCGG TCATATGI
341	TTCTTTT CAACCAAA
342	CACCAGAA GAGCCACT
343	TAGGGTGA CCGCTGGG
344	AAAACCTC ACATATGA
345	TAATTCGG AGCCCGCC
346	TTCAGCAG CCTTACTC

Table continues ...

ID	Sequence
347	GTAGTTA CCCAGCGG
348	CRACTCAA CCTCACAG
349	ACTTGGGA TTAGGATC
350	CTTTACGC GCTTGAAC
351	GATCACAA ATATGGCA
352	AAGTCCGT GTCGGTAG
353	TCATGTGG GATCCATA
354	AATCATA TCACGGTT
355	CCTACACC ACAGATGC
356	TTGCTGAG TTCGACGC
357	AGCCTACG ATAGCCAG
358	AGCTTGGG AACCGTGA
359	GTTGCCT CGACCGAT
360	TTTAGTTT GCGTCGAA
361	GTATTATC GCGTCAAT
362	GTCCAAGT AAGGCGAA
363	CTGAGCGT ATCGGTCG
364	TAGGGCTA AAATCTGA
365	CAGAAACT ATGTACCG
366	CATCTTTT GGGGTCGT
367	GATCCTGA GAGATTAC
368	GCGATGGT GATGGGAA
369	GATATCCA CGGTACAT
370	AACTGTTG TGCCTAT
371	GCACGTAT GCTTTGAG
372	GAGGTGAT ACGTAAAG
373	ACTCTCGT TCAGTCGT
374	CGTTTGTC ATAGGACA
375	TAGGGTGA CGTATTAG
376	GTCAATTT CTTTACGT
377	TCATATGG ACACACCA
378	TCTGGCGC CGGCTTAG
379	GGCTTGCA CTAATACG
380	TTGTAAA CTTACCTA
381	GTAACGTA TAGGTTTC
382	CTGACGG TTAATTTG
383	TGGGCCCG GGGTTTGT
384	ATAGGTAA CTGGGTTT
385	AAGCAAGA GAAACCTA
386	GTTTACTT AGGGATGG
387	ACTACTTA AGACTTAG
388	AATCGTGA CGGTTTCT
389	TCGCAACG GAAGCGTA
390	ATTACCGT CCATCCCT
391	ACCTCTTA GCACAATA
392	TCTTCAAA AGAAACCG
393	GATTACGG CGGCTTAG
394	CTCAGTTT AAGATTCA
395	GTAGGCTC TATTGTGC
396	GTGGCGGA CCTAAGGT
397	CCCAAGT GCTACATG
398	CTTTAGGC CCAAGGAG
399	GCGCAGTT TCCTTGAT
400	GATATTTT AAGGACAC
401	GCGATTCC CATGTAGC
402	TACGAACT CAACGCAC
403	TGCGAAAA GCAAGCCG
404	TCATTCCG TCCCCCAA
405	CGTCGTCT TGGGTAAG
406	CGAGTCGT GTGCGTTG
407	AGCAGAAT GGTATAAA
408	TAGCCGCC TTGGGGGA
409	GGGAATTG AGTACAAG
410	ATTTTGGC GGAACGGG
411	TCCCAATC TTTATACC
412	TTCCGCGC ATAAAACT
413	CTCGTTCC GGTGCTAC
414	CACGCGAG CTATGTAG
415	ATAACCAT CTGGTGA
416	CGCCCTGA CGTAGAAC
417	GCAATGCC GTAGCACC
418	GCTCGGGT AGTCTCAA
419	TATGACAG GTGAGCAC
420	TAACTGTC CTGATCAC
421	GCGTAAAA AACCTCGT
422	AGTTTCTT TTGAGACT
423	GCAATAAA GAAACTGC
424	TTACGCGG GTGATCAG
425	TGACCAAG AATACCGT
426	TTAAGATG TGTGGCAA
427	TTACCTCT CGAGTTTC
428	TATTTCTT GGAAGCCT
429	TATTTGAC GTCTTGCG
430	CCCAGTGA GCTCGACA
431	TGGACCAC GCGCTGTG
432	AAGCAGAG CATTTTGA
433	CAGCTTGA CGCAAGAC
434	GAAGATAG AGAGCATA

Table continues ...

ID	Sequence
435	ACCCATGG CGCACCAC
436	TCAATAAT GCACCTGC
437	CTAGGACC CAGAAGTG
438	AACTACTG TATGCTCT
439	ACTCTTAC TGCCCGCC
440	TTCCTAAC GCAGGTGC
441	CCATCAGA TGGAGCTG
442	TATCCGCC TATCTCGC
443	TGTTTTAA GCGGGGCA
444	CTTTTCTG CTTCACGC
445	TGATTAGC GCTTAGGG
446	TCTTCTC GTATATCA
447	TTACAGCG ACGCCTCG
448	TCATTAGG GTCCACCG
449	AACCTACC CCCTAAGC
450	AATTCGCT AAACCGGA
451	TCGTTACG TTTTTTTT
452	CCTGAATG TTTTTTTT
453	TTTTTTTT GAAGGAGT
454	TTTTTTTT TCCGGTTT

The following bricks were used in all structures investigated:

1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, 14, 17, 18, 21, 22, 29, 30, 35, 36, 41, 42, 45, 46, 53, 54, 59, 60, 65, 66, 71, 72, 77, 78, 83, 84, 89, 90, 93, 94, 101, 102, 107, 108, 113, 114, 119, 120, 125, 126, 131, 132, 137, 138, 143, 144, 149, 150, 155, 156, 161, 162, 167, 168, 173, 174, 179, 180, 185, 186, 189, 190, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204

In addition to the bricks common to all structures, the following bricks were used for each class of structure studied.

The **no-BB** system (330 bricks in total):

207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454

The **edge-BB** system (268 bricks in total):

8, 15, 19, 23, 26, 28, 31, 34, 37, 40, 43, 47, 50, 52, 55, 58, 61, 64, 67, 70, 73, 76, 79, 82, 85, 88, 91, 95, 98, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127, 130, 133, 136, 139, 142, 145, 148, 151, 154, 157, 160, 163, 166, 169, 172, 175, 178, 181, 184, 187, 191, 194, 205, 208, 210, 211, 212, 215, 217, 221, 222, 224, 226, 227, 228, 231, 233, 237, 238, 240, 242, 243, 244, 247, 249, 253, 254, 256, 258, 259, 260, 263, 265, 269, 270, 272, 274, 275, 276, 279, 281, 285, 286, 288, 290, 291, 292, 295, 297, 301, 302, 304, 306, 307, 308, 311, 313, 317, 318, 320, 322, 323, 324, 327, 329, 332, 334, 335, 336, 339, 341, 345, 346, 348, 350, 351, 352, 355, 357, 361, 362, 364, 366, 367, 368, 371, 373, 377, 378, 380, 382, 383, 384, 387, 389, 393, 394, 396, 398, 399, 400, 403, 405, 409, 410, 412, 414, 415, 416, 419, 421, 425, 426, 428, 430, 431, 432, 435, 437, 441, 442, 444, 446, 447, 448, 451, 453

The **face-BB** system (268 bricks in total):

7, 16, 20, 24, 25, 27, 32, 33, 38, 39, 44, 48, 49, 51, 56, 57, 62, 63, 68, 69, 74, 75, 80, 81, 86, 87, 92, 96, 97, 99, 104, 105, 110, 111, 116, 117, 122, 123, 128, 129, 134, 135, 140, 141, 146, 147, 152, 153, 158, 159, 164, 165, 170, 171, 176, 177, 182, 183, 188, 192, 193, 206, 207, 209, 213, 214, 216, 218, 219, 220, 223, 225, 229, 230, 232, 234, 235, 236, 239, 241, 245, 246, 248, 250, 251, 252, 255, 257, 261, 262, 264, 266, 267, 268, 271, 273, 277, 278, 280, 282, 283, 284, 287, 289, 293, 294, 296, 298, 299, 300, 303, 305, 309, 310, 312, 314, 315, 316, 319, 321, 325, 326, 328, 330, 331, 333, 337, 338, 340, 342, 343, 344, 347, 349, 353, 354, 356, 358, 359, 360, 363, 365, 369, 370, 372, 374, 375, 376, 379, 381, 385, 386, 388, 390, 391, 392, 395, 397, 401, 402, 404, 406, 407, 408, 411, 413, 417, 418, 420, 422, 423, 424, 427, 429, 433, 434, 436, 438, 439, 440, 443, 445, 449, 450, 452, 454

The **half-face-BB** system (299 bricks in total):

7, 16, 20, 32, 38, 39, 44, 56, 62, 63, 69, 80, 86, 87, 92, 104, 110, 111, 117, 128, 134, 135, 141, 152, 158, 159, 165, 176, 182, 183, 188, 207, 209, 211, 213, 214, 215, 216, 217, 218, 219, 220, 221, 223, 225, 227, 229, 230, 231, 232, 233, 234, 235, 236, 237, 239, 241, 243, 245, 246, 247, 248, 249, 250, 251, 252, 253, 255, 257, 259, 261, 262, 263, 264, 265, 266, 267, 268, 269, 271, 273, 275, 277, 278, 279, 280, 281, 282, 283, 284, 285, 287, 289, 291, 293, 294, 295, 296, 297, 298, 299, 300, 301, 303, 305, 307, 309, 310, 311, 312, 313, 314, 315, 316, 317, 319, 321, 323, 325, 326, 327, 328, 329, 330, 331, 333, 335, 337, 338, 339, 340, 341, 342, 343, 344, 345, 347, 349, 351, 353, 354, 355, 356, 357, 358, 359, 360, 361, 363, 365, 367, 369, 370, 371, 372, 373, 374, 375, 376, 377, 379, 381, 383, 385, 386, 387, 388, 389, 390, 391, 392, 393, 395, 397, 399, 401, 402, 403, 404, 405, 406, 407, 408, 409, 411, 413, 415, 417, 418, 419, 420, 421, 422, 423, 424, 425, 427, 429, 431, 433, 434, 435, 436, 437, 438, 439, 440, 441, 443, 445, 447, 449, 450, 451, 452, 453, 454

The **all-BB** system (206 bricks in total):

7, 8, 15, 16, 19, 20, 23, 24, 25, 26, 27, 28, 31, 32, 33, 34, 37, 38, 39, 40, 43, 44, 47, 48, 49, 50, 51, 52, 55, 56, 57, 58, 61, 62, 63, 64, 67, 68, 69, 70, 73, 74, 75, 76, 79, 80, 81, 82, 85, 86, 87, 88, 91, 92, 95, 96, 97, 98, 99, 100, 103, 104, 105, 106, 109, 110, 111, 112, 115, 116, 117, 118, 121, 122, 123, 124, 127, 128, 129, 130, 133, 134, 135, 136, 139, 140, 141, 142, 145, 146, 147, 148, 151, 152, 153, 154, 157, 158, 159, 160, 163, 164, 165, 166, 169, 170, 171, 172, 175, 176, 177, 178, 181, 182, 183, 184, 187, 188, 191, 192, 193, 194, 205, 206

6 REFERENCES

- ¹Y. Ke, L. L. Ong, W. M. Shih, and P. Yin, "Three-dimensional structures self-assembled from DNA bricks," *Science* **338**, 1177 (2012).
- ²M. Sajfudinow, K. Uhlig, A. Prager, C. Schneider, B. Abel, and D. M. Smith, "Nanoscale patterning of self-assembled monolayer (SAM)-functionalised substrates with single molecule contact printing," *Nanoscale* **9**, 15098 (2017).
- ³M. I. Mitov, M. L. Greaser, and K. S. Campbell, "GelBandFitter – A computer program for analysis of closely spaced electrophoretic and immunoblotted bands," *Electrophoresis* **30**, 848 (2009).
- ⁴S. W. Provencher, "A constrained regularization method for inverting data represented by linear algebraic or integral equations," *Comput. Phys. Commun.* **27**, 213 (1982).
- ⁵S. Hansen, "DLSanalysis.org: a web interface for analysis of dynamic light scattering data," *Eur. Biophys. J.* **47**, 179 (2018).
- ⁶H. Zipper, H. Brunner, J. Bernhagen, and F. Vitzthum, "Investigations on DNA intercalation and surface binding by SYBR Green I, its structure determination and methodological implications," *Nucleic Acids Res.* **32**, e103 (2004).
- ⁷J.-P. J. Sobczak, T. G. Martin, T. Gerling, and H. Dietz, "Rapid folding of DNA into nanoscale shapes at constant temperature," *Science* **338**, 1458 (2012).
- ⁸J. SantaLucia Jr and D. Hicks, "The thermodynamics of DNA structural motifs," *Annu. Rev. Biophys. Biomol. Struct.* **33**, 415 (2004).
- ⁹R. T. Koehler and N. Peyret, "Thermodynamic properties of DNA sequences: Characteristic values for the human genome," *Bioinformatics* **21**, 3333 (2005).
- ¹⁰W. Wang, T. Lin, S. Zhang, T. Bai, Y. Mi, and B. Wei, "Self-assembly of fully addressable DNA nanostructures from double crossover tiles," *Nucleic Acids Res.* **44**, 7989 (2016).
- ¹¹Y. Ke, L. L. Ong, W. Sun, J. Song, M. Dong, W. M. Shih, and P. Yin, "DNA brick crystals with prescribed depths," *Nat. Chem.* **6**, 994 (2014).
- ¹²S. Giglio, P. T. Monis, and C. P. Saint, "Demonstration of preferential binding of SYBR Green I to specific DNA fragments in real-time multiplex PCR," *Nucleic Acids Res.* **31**, e136 (2003).
- ¹³D. Suh and J. B. Chaires, "Criteria for the mode of binding of DNA binding agents," *Bioorg. Med. Chem.* **3**, 723 (1995).
- ¹⁴K. M. Ririe, R. P. Rasmussen, and C. T. Wittwer, "Product differentiation by analysis of DNA melting curves during the polymerase chain reaction," *Anal. Biochem.* **245**, 154 (1997).