Supplementary Materials:

Role of nanoscale antigen organization on B-cell activation probed using DNA origami

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Supplementary Materials
Supplementary Figs. 1–26
Supplementary Tables 1–22
Supplementary Text 1
Supplementary References
Supplementary Fig. 1. All-atom model of the icosahedral scaffolded DNA origami NP. The all-atom model was generated using DAEDALUS\textsuperscript{1}. 
Supplementary Fig. 2. Sequence design and all-atom model of the 6 Helix Bundle scaffolded DNA origami nanoparticle. (Left) Secondary structure of the 6HB rendered using caDNAno. (Right) All-atom model of the 6HB DNA-NP rendered using CanDo^2,3.
Supplementary Fig. 3. Overhang placement on the edges of the DNA-nanoparticles. (a) Secondary structure of an edge of the DNA icosahedron with ssDNA overhangs. (b) Zoom-in of the secondary structure of the 6HB showing available sites for ssDNA overhangs.
Supplementary Table 1. Properties of the flexible linkers used for testing the role of scaffold rigidity in antigen presentation. Flory Radius was determined from Ma et al., and Radius-of-Gyration for the PEG polymer from Linegar et al. (Rg=0.0215xMw^{0.583}).

| Linker material | Linker name | MW [Da] | Number of units (Bases or PEG monomers) | Contour Length [nm] | Flory Radius [nm] | Radius-of-Gyration [nm] |
|-----------------|-------------|---------|----------------------------------------|---------------------|------------------|------------------------|
| ssDNA           | ssDNA5      | 8930.9  | 5                                      | 3.2                 | N/A              | N/A                    |
| ssDNA           | ssDNA12     | 11123.3 | 12                                     | 7.6                 | N/A              | N/A                    |
| ssDNA           | ssDNA24     | 14881.8 | 24                                     | 15                  | N/A              | N/A                    |
| ssDNA           | ssDNA35     | 18210.9 | 35                                     | 22                  | N/A              | N/A                    |
| ssDNA           | ssDNA47     | 21916.2 | 47                                     | 30                  | N/A              | N/A                    |
| ssDNA           | ssDNA83     | 30767.9 | 83                                     | 52                  | N/A              | N/A                    |
| PEG             | Bis-Mal-PEG-1 | 308.3  | 2                                      | 0.6                 | 0.5              | 0.61                   |
| PEG             | Bis-Mal-PEG-2 | 2000   | 45                                     | 12.6                | 2.8              | 1.8                    |
| PEG             | Bis-Mal-PEG-3 | 7500   | 170                                    | 47.7                | 6.1              | 3.9                    |
Supplementary Fig. 4. Transmission electron microscopy images of the icosahedral DNA-NP with 60 overhangs without eOD. The electron microscopy images are from 3 technical replicates [10 images were taken per replicates] with similar results.
Supplementary Fig. 5. TEM images of the 6HB DNA-NP with 5 overhangs without eOD. The electron microscopy images are from 3 technical replicates [10 images were taken per replicates] with similar results.
Supplementary Fig. 6. Agarose gel electrophoresis of the icosahedral DNA-nanoparticle with varying numbers of overhangs. (n=2 biological replicates for each gels with similar results [M. Marker]). The bands visible below 500 bp are the excess of staple strands visible before purification.
Supplementary Fig. 7. Agarose gel electrophoresis of the 6 Helix Bundle DNA-nanoparticle with varying numbers of overhangs. (n=1, M. Marker, Sc. Scaffold). The very bright bands visible below 500 bp are the excess of staple strands visible before purification.
Supplementary Fig. 8. Schematic of the peptide Nucleic Acid linker designed for antigen attachment to DNA-nanoparticles. The sequence is the following: (Maleimide)-GGK-cagtccagt-K-(CONH₂).
Supplementary Fig. 9. MALDI-TOF analysis of the synthesized PNA strand. Mass spectrometry analysis of maleimide modified PNA strand (expected: 2927.22 m/z, measured: 2957.29 m/z). Two distinct batches of PNA strand were synthesized and MALDI-TOF analysis was performed both times with similar results.
Supplementary Fig. 10. Absorption spectrum analysis of peptide nucleic acid strand conjugation to the eOD-GT8. UV-vis spectrum of the PNA strand alone (grey), eOD alone (black), the eOD-PNA conjugate (red), and the sum of absorbance from eOD alone and PNA alone (dotted pink). UV-VIS analysis was performed three times independently with similar results.
Supplementary Fig. 11. eOD-GT8 antigen conjugation to DNA-nanoparticles with single stranded DNA overhangs characterized with agarose gel electrophoresis. (a) eOD-GT8 conjugation to various 6HB constructs. (b) eOD-GT8 conjugation to various icosahedral constructs. All purified structures were run on a 1.5 % agarose gel pre-stained with EtBr and run for 2 h at 70 V. For (a) and (b) gels n=1. The bands visible below 500 bp in the bottom of the gels in (a) and (b) for 6HB-0x and Ico-0x are the excess of staple strands visible before purification.
Supplementary Fig. 12. Percentage of antigen modification of the DNA-NPs determined by fluorimetry of eOD-GT8-PNA modified with AF647. 

a. Fluorescent imaging of agarose gel acquired with Typhoon FLA 7000. 
b. Example of standard curve acquired with fluorescent eOD-GT8. This experiment has been repeated two times with similar results. 
c. Quantification of eOD-GT8 coverage on different DNA NPs from fluorescence spectroscopy. Error represents standard deviation of the mean (n=3 biological replicates/group for lco-1x, lco-5x, lco-30x, and 6HB-5x-7nm; n=2 biological replicates/group for lco-60x; n=1 sample/group for 6HB-1x, and 6HB-2x-7nm).
Supplementary Fig. 13. Tryptophan assay to determine percentage coverage of DNA-nanoparticles with unlabeled eOD-GT8-PNA. (n=1 sample/groups)
Supplementary Fig. 14: Representative distances between eOD-GT8 immunogens on the icosahedral DNA-NP. Distances were measured from the center of the antigen using UCSF Chimera and are listed in Supplementary Table 2.
Supplementary Table 2. Distances between eOD-GT8 antigens in a 1, 2, 3, 4, and 5-mer icosahedral DNA-NP. Distance were measured using UCSF Chimera⁶ as shown in Supplementary Fig. 14 from the center of the antigens (n=1). The error is determined using the estimated linker size (~3 nm).

| Antigen # | 1                  | 2                  | 3                  | 4                  | 5                  |
|-----------|--------------------|--------------------|--------------------|--------------------|--------------------|
| 1         | 56 ± 6 nm          | 43.3 ± 6 nm        | 39.4 ± 6 nm        | 36.5 ± 6 nm        |
| 2         | 56 ± 6 nm          | 40.3 ± 6 nm        | 40.9 ± 6 nm        | 44.5 ± 6 nm        |
| 3         | 43.3 ± 6 nm        | 40.3 ± 6 nm        | 33.9 ± 6 nm        | 27.6 ± 6 nm        |
| 4         | 39.4 ± 6 nm        | 40.9 ± 6 nm        | 33.9 ± 6 nm        | 49.2 ± 6 nm        |
| 5         | 36.5 ± 6 nm        | 44.5 ± 6 nm        | 27.6 ± 6 nm        | 49.2 ± 6 nm        |
Supplementary Fig. 15. B cell fluorescence response with high valency eOD-GT8 loading on the icosahedral DNA-nanoparticle. Icosahedral DNA-NPs modified with eOD-GT8 were added to B cells loaded with Fluo-4 DNA and normalized to baseline levels. Ico-0x with 0 copy number, Ico-10x, Ico-30x, and Ico-60x contained 10, 30, and 60 copies of eOD-GT8, respectively. eOD-GT8-60mer is the positive control protein NP containing 60 copies of eOD-GT8 per NP (representative individual calcium trace). DNA-NPs and eOD-GT8-60mer were used at a 2 nM equivalent eOD-GT8 concentration. (n=3 biological replicates with similar results).
Supplementary Fig. 16. Increasing valency of the low affinity antigen eOD-GT5 does not enhance B cell activation. (a) Raw fluorescence of cells loaded with Fluo-4 calcium probe (representative individual calcium trace). This experiment has been repeated three times independently. (b) Normalized Area Under the Curve [AUC] of calcium release from cells stimulated with the icosahedral DNA-NPs displaying different valencies of the low affinity immunogen eOD-GT5. Ico-0x and Ico-10x were used at 25 nM equivalent eOD-GT8 concentration and eOD-GT8-60mer control at 2 nM equivalent eOD-GT8 concentration. Error bars represent standard deviation of the mean (n=3 biological replicates/group).
Supplementary Fig. 17. Flow cytometry of labeled eOD-DNA-nanoparticles binding to B cells. Fluorescently labeled eOD-GT8-PNA attached to 6HB-2x-7nm, 6HB-2x-28nm, (Data as presented in Figure 3b) or the Ico-30x was incubated with gLVRC01 B cells for 30 minutes on ice at a fixed antigen concentration of 5 nM. (a) Representative flow cytometry plots of Ico-30x, 6HB-2x-7nm, and 6HB-2x-28nm binding to antigen-specific B cells. Two independent flow cytometry experiments were performed with similar results. (b) Quantitation of data from (a) (n = 3 biological replicates/group) where error bars represent standard deviations of the mean and the P-values are from a one-way ANOVA, followed by Tukey post hoc comparison test (***: p<0.01; ****: p<0.001; NS: Not statistically significant, p=0.9999. All P-values are available in the Supplementary Table 18).
Supplementary Fig. 18: Comparison of linear versus 2D clustering of five copies of antigens on Icosahedron and 6 Helix Bundle. (a) Calcium signaling of cells loaded with Fluo-4 calcium probe normalized by unstimulated levels with buffer-only control curves subtracted for 6HB structures with varying inter-antigen distances (representative individual calcium trace) at a fixed antigen concentration of 5 nM. Experiment was performed independently three times (biological replicates) with similar results. (b) Total calcium signaling from cells stimulated with 6HB 5-mer structures presenting eOD-GT8 antigen compared with Ico 5-mer structures presented in Fig. 4. Fluo-4 AUC is normalized as in Fig. 2 (n=3 biological replicates/group), where error bars represent standard deviations of the mean and P-values are from 2-way ANOVA, paired Student’s t-test (*: p<0.05; **: p<0.01; ***: p<0.001. All P-values are available in the Supplementary Table 19).
Supplementary Fig. 19: Comparison of distance between linear antigen presentation on a 6 Helix Bundle 5-mer rod (6HB-5x-11nm) and 2D presentation on an Icosahedron 5-mer (Ico-5x-22nm). Distance were determined in chimera. (n=1)
Supplementary figure 20: Comparison of B cell triggering by icosahedral versus pentagonal bipyramid geometries. (a) Ten copies of eOD-GT8 presented by the two opposing faces of an 84bp edge-length pentagonal bipyramid (PB) DNA-NP construct, with antigen attachment at the midpoints between vertices. Tryptophan fluorescence and agarose gel electrophoresis were used to characterize antigen coverage. (n=3 technical replicates/group for fluorescence/absorbance measurements with similar results and n=1 for the agarose gel experiment) (b) Comparison of inter-antigen distance distributions for the PB-10 and Ico-10 reveal only a small relative shift towards larger average and maximal distances for the Ico-10. Distances were binned in 3 nm steps corresponding to the distances between the O3’ at functionalized nick positions in the atomic model from DAEDALUS1. (n=1) (c) Raw fluorescence of Ramos cells loaded with Fluo-4 calcium probe after incubation with functionalized DNA-NPs (representative individual calcium trace). This experiment has been done 3 times (biological replicates) with similar results (d) Normalized Area Under the Curve [AUC] of calcium release from cells after stimulation with functionalized DNA-NPs. Total antigen concentration for all experiments was 2 nM. Error bars represent standard deviation of the mean (n=3 biological replicates/group).
**Supplementary figure 21: Comparison of B cell triggering by model peptide antigens on DNA-NPs versus LNPs.**

(a) 45 copies of two model peptide antigens, p5 or p31, were conjugated to the pentagonal bipyramid DNA-NP, with three outward facing attachment sites uniformly spaced along each edge. A ratiometric absorbance assay at 260 nm and 647 nm and two-color agarose gel electrophoresis were used to quantify coverage of the peptide antigens. (n=3 technical replicates/group for fluorescence/absorbance measurements with similar results and n=1 for the agarose gel experiment)  

(b) DLS of liposomes presenting the p5 peptide antigen revealed a mean Lipo-p5 particle size of 100 nm with a PDI of 0.071. (n=1)  

(c) Raw fluorescence of primary B cells loaded with Fluo-4 calcium probe after incubation with peptide-functionalized DNA-NPs versus Lipos (representative individual calcium traces). This experiment has been done 3 times (biological replicates) with similar results.  

(d) Normalized Area Under the Curve [AUC] of calcium flux from cells integrated until 140 s versus 420 s after stimulation with functionalized DNA-NPs (PB-45) versus Lipos. Total antigen concentration for all experiments was 1 nM. Error bars represent standard deviation of the mean (n=3 distinct samples/group) and P-values are from 2-way ANOVA, paired Student’s t-test (left bar graph [after 140s]: for p5 vs PB-p5 p=0.0053; for p5 vs PB-p31 p=0.0166; for PB-p5 vs lipos-p5 p=0.0203; for PB-p31 vs lipos-p5 p=0.0471, and right bar graph [after 420s]: for p5 vs PB-p5 p=0.0478; for p5 vs PB-p31 p=0.0423; for p5 vs lipos-p5 p=0.0095)
Supplementary figure 22. Confocal fluorescence imaging of fixed Ramos cells with 30-mer icosahedral DNA-NP bearing fluorescent eOD-GT8. 30-mer fluorescent eOD-GT8 conjugated icosahedral DNA-NP were added to VRC01+ Ramos cells at a concentration of 5 nM and fixed in solution at various timepoints. (a) Images of Ramos cells with the Icosahedral 30-mer (Ico-30) bound, IgM (BCR) stained with an f(Ab) fragment conjugated to JF549 and also stained with phalloidin (Ph.) conjugated to Alexa Fluor 405. Heterogeneity in the surface expression of the B cell receptor between B cells is apparent from the image. The binding of the icosahedral DNA-NP with fluorescent eOD-GT8 is restricted to Ramos cells expressing VRC01 IgM. Experiment was repeated twice with similar results. The experiments in (a) were repeated twice (biological replicates) with similar results. (Scale bar: 20 μm). (b) The probability distribution of total eOD-GT8 intensity on cells illustrates that a proportion of Ramos cells bind little or no eOD-GT8 even at long times (30 minutes) following antigen addition. (c) Plots of individual cell total BCR intensity vs total eOD-GT8 intensity further illustrate the strong correlation between surface BCR expression and eOD-GT8 binding, as well as the increase in eOD-GT8 binding over time. (Number of cells per conditions; 1min: 119 cells; 5 min: 103 cells; 30 min: 30 cells, from the same sample).
Supplementary figure 23: Fluorescence quantification of confocal microscopy images. Fluorescent eOD-GT8 conjugated Ico-30x was added to VRC01+ Ramos cells at a concentration of 5 nM and fixed in solution at various timepoints. (a) Average total eOD-GT8 fluorescence per cell increases over time for all constructs. (b) The pixel-based Pearson correlation between eOD and BCR intensity also increases over time. Total fluorescence is calculated across all z-sections and average is computed across all cells. (c) The pixel-based Pearson correlation between eOD-GT8 and pTyr intensity varies between the antigen organizations tested. Total fluorescence is calculated across all z-sections and average is computed across all cells. For (a), (b), and (c) Error bars represent standard errors of the means across all cells per condition and P-values are from a two-way ANOVA, paired Student’s t-test (*: p<0.05; **: p<0.01; ***: p<0.001. All P-values are available in the Supplementary Tables 20-22). (Number of cells per conditions; Ico-30x: 1min: 14 cells, 5 min: 183 cells, 30 min: 15 cells; 6HB-2x-7nm: 1min: 26 cells, 5 min: 22 cells, 30 min: 19 cells; 6HB-2x-28nm: 1min: 15 cells, 5 min: 26 cells, 30 min: 23 cells; Control: 29 cells from the same sample)
Supplementary figure 24: Confocal microscopic imaging of DNA-NPs on Ramos B cells from Figure 5 separated by channels. (a) BCR channel, (b) eOD-GT8 channel, (c) Phalloidin channel. The experiments in (a), (b), and (c) were repeated twice (biological replicates) with similar results. (Scale bar: 5 μm).
Supplementary table 3. List of primers used for amplification of DNA-NP scaffolds.

| Structure      | 5’-primer (forward) | 3’-primer (reverse) |
|----------------|----------------------|---------------------|
| 6-HB           | CCCTTTAGGGTTCCGATTTA | GCTGAAAAAGGTGGCATCAAT |
| Icosahedron    | TCTTTGCCTTGCTGTATGA  | GCTAACGAGCGTCTTTCCA  |
Supplementary Text 1:
A membrane proximal BCR signaling model

We propose a membrane proximal BCR signaling model in silico to interpret the monotonically increasing BCR signaling that is observed with increasing distance between eOD-GT8 immunogens presented using DNA-NPs. The current model is adapted from a related, previously published BCR signaling model. The model is composed of membrane proximal signaling reactions that occur at early times following BCR binding and triggering, and diffusion of BCR and associated proteins. We note that this model is not designed to investigate various competing mechanisms proposed for BCR triggering, namely, breakdown of auto-inhibited BCR multimers into smaller active BCR clusters upon antigen binding, formation of BCR clusters due to conformational change in BCRs when BCRs bind to antigens, or actin-mediated restriction of BCR mobility limiting ITAM phosphorylation. Instead, the current model proposes one possible mechanism to explain the gradual increase in BCR activation observed from Calcium flux with increasing distance between eOD-GT8 immunogens displayed using rigid, DNA-NP origami scaffolds.

The model describes the following membrane proximal BCR signaling events as biochemical reactions. Upon antigen binding to BCRs, tyrosine residues in immunoreceptor tyrosine based activation motifs, or ITAMs, associated with the BCRs are initially phosphorylated by the SFKs that reside in the plasma membrane. Cytosolic Syk kinases bind to partially and fully tyrosine phosphorylated ITAMs with SH2 domains and, once they are recruited to the BCR-antigen complex, bound Syk molecules can further phosphorylate ITAMs associated with the same BCR or neighboring BCRs. These phosphorylated ITAMs in turn recruit more Syk molecules and generate a positive feedback in ITAM phosphorylation. Further details regarding these signaling reactions can be found in Mukherjee et al. The model also assumes that Syk molecules recruited by antigen-bound BCRs can phosphorylate ITAMs that come into physical contact with BCR-bound Syk molecules. These ITAMs can be associated with BCRs that are not bound to any antigen. Experiments reported in Kläser et al. support this assumption. To simplify our model, we do not model further downstream signaling reaction events (e.g., PI3K activation). In B cells, once ITAMs are fully phosphorylated, downstream signaling proteins are recruited that lead to activation events such as calcium signaling. Thus, in our model we used the total number of fully phosphorylated ITAMs as a measure of the strength of BCR signaling.

We assume that BCRs form clusters at the nanoscale when stimulated by eOD-GT8 immunogens. The assumption of the formation of such BCR clusters upon antigen stimulation has been found in many experiments, including super-resolution microscopy experiments. Our model does not describe any mechanism for the formation of these BCR clusters, but rather investigates the functional consequences of such cluster formation. BCRs in a single cluster are modeled as discrete particles, where each cluster is modeled by a circular region of radius 15 nm in the B cell plasma membrane where BCRs unbound by antigen can diffuse freely. However, unbound BCRs are restrained to remain within the cluster domain. If two BCR clusters overlap, then unbound BCRs can freely diffuse within the two overlapping cluster regions. Each BCR cluster can accommodate multiple BCRs, and we assume that the number of BCRs that can physically be accommodated (or the maximum BCR density) in a single cluster of radius 15 nm has an upper bound equal to 5 BCR molecules. Therefore, the total number of BCR molecules that can be accommodated in two overlapping BCR clusters is less than the total number of BCRs when these clusters do not
overlap, due to steric occlusion. As a result, the number of BCRs available for downstream signaling effectively increases as the region of overlap between any pair of clusters decreases. For example, increasing the distance ($d$) between the two antigens in an eOD-GT8 dimer decreases the overlap between two BCR clusters that would form in the vicinity of each antigen-BCR complex (Supplementary fig. 25).

Supplementary Fig. 25. Schematic diagram showing BCR molecules (red circles), BCR cluster regions (grey circle) and antigens (yellow stars) as incorporated in the model. BCR molecules not bound to antigens are allowed to freely diffuse in the BCR cluster regions. Three cases of overlaps, namely, (a) high, (b) medium, and (c) no overlap, generated by increasing values of the inter-antigen spacing $d$ in a eOD-GT8 dimer are shown schematically. The total number of BCR molecules increase in the BCR clusters as the overlap between the clusters decreases.

We also note that the above model implicitly assumes that the formation of BCR clusters and Syk mediated signaling reactions occur in the model when antigens separated up to a distance of 80nm are presented on a rod. B cells are not appreciably stimulated by monomers of the immunogens displayed in the eOD-GT8 dimers or when these immunogens are connected by flexible linkers. Application of this model in the above scenarios have to be accompanied with modified assumptions where the immunogens do not give rise to clustering of BCRs or Syk mediated phosphorylation of ITAMs associated with unengaged BCRs. There are similar limitations in using our model to predict B cell signaling when antigens in the rod are separated beyond 80nm. The model assumptions regarding BCR clustering and signaling for antigens separated by longer distances (e.g., $\geq$ 80nm) could pertain to “long range” molecular interactions facilitated by cytoskeletal forces between BCR molecules/clusters interacting with spatially separated antigens, and, studying these molecular mechanisms as $d$ increases is outside the scope of the current model but could be an exciting future direction.

Numerical Simulation of the processes described in the model. We implemented a spatially-resolved in silico numerical model described in Mukherjee et al.7 to investigate early timepoint BCR signaling events triggered by eOD-GT8 immunogens. The simulation region represented the
B cell plasma membrane containing BCRs, antigens, and a thin (thickness $l_0 = 0.5$ nm) cytosolic region beneath the plasma membrane. The simulation region composed of the BCR cluster regions (Supplementary fig. 25) is discretized using $N$ equal sized cubic chambers of dimension $l_0 \times l_0 \times l_0$. For the case of stimulation by eOD-GT8 dimers, BCR molecules in the model are distributed with a fixed density of 5 molecules/[(π $15^2$)×(nm)$^2$] within two clusters, each of radius 15 nm. We defined the fraction of overlap, $f$, as, $f = $ the total area of the overlapping BCR clusters/$(2 \times$ area of a single circular BCR cluster). A total number of $\text{int}[10(1-f)]$ BCR molecules are distributed uniformly in the cluster regions at the beginning of the simulation. The centers of the two BCR clusters are separated by a distance $d$, which is varied from 7 nm to 80 nm. This set-up is extended to 5 BCR clusters for studying stimulation by 5-mer eOD-GT8 using the same scheme as above. BCRs within a cluster do not diffuse outside of the region. In addition, Src family kinase (SFK) molecules and the kinase Syk are distributed uniformly and randomly within the simulation volume. The SFK and Syk molecules diffuse freely in 2D/3D space and phosphorylate tyrosine residues in the ITAMs following signaling reactions described in Supplementary Table 4. BCRs in each individual cluster can interact with a single antigen presented by the eOD-GT8 origami construct. For the dimer construct, as the inter-antigen distance $d$ between the eOD-GT8 antigens is increased from 7 nm to 80 nm (Supplementary Fig. 25), and for the 5-mer eOD-GT8 the minimum distance $d$ between the antigens is increased from 7 nm to 80 nm. Reaction and diffusion processes are explicitly simulated using the spatial Gillespie method implemented in the software package Stochastic Simulation Compiler\textsuperscript{17}.

Importantly, the preceding model is coarse-grained in nature, thus does not describe the different microscopic states of BCR-antigen binding that were used in a particle-based Monte Carlo model to interpret surface plasmon resonance measurements in a recent DNA origami study that characterized the impact of inter-antigen distance on antibody binding. There, the "spatial tolerance" of single antibodies was determined, whereby a sharp decrease in binding affinity was observed when the inter-antigen distance increased beyond 17nm, the maximum inter-antigenic distance that is tolerated by an individual, flexible IgG\textsuperscript{18}. Because that study was focused on the discrete nature of antibody binding to a discrete lattice of antigenic sites presented using DNA origami, such particle-based modeling was suitable. This is in contrast to the present work that sought to test the previously established positive feedback mechanism\textsuperscript{7}, which was hypothesized here to explain BCR activation induced by the spatial constraints imposed on clusters that form upon antigen binding. In this cellular membrane environment, dozens to hundreds of protein components may be involved, rendering single-particle-based models extremely computationally demanding. Notwithstanding, incorporating these additional structural features of BCRs and their kinetic on/off-rates would be interesting to explore in future work.

Results

BCRs in the simulation box composed of multiple BCR clusters were stimulated by antigens displayed on a single origami dimer or pentamer of eOD-GT8, where the minimum distance $d$ between the antigens was increased monotonically from 7 to 80nm. ITAM phosphorylation kinetics reached the steady state by approximately 200s. Immunogens were assumed to bind BCRs irreversibly due to their high affinity\textsuperscript{19}, whereby once the antigen binds to a BCR it does not unbind. The ITAMs of antigen-bound BCRs were initially phosphorylated by SFKs, and the phosphorylated ITAMs recruited Syk kinase to the antigen-BCR complex. The bound Syk kinase then phosphorylated ITAMs associated with other neighboring BCRs that were not bound to any antigen, which then react with bound Syk molecules in the simulation. The variation of the total
number of fully phosphorylated ITAMs time-averaged between 300s to 5000s with \(d\) for eOD-GT8 1D dimer shows larger increase in ITAM phosphorylation up to \(d = 21\) nm, subsequently saturating to a fixed-value for larger values of \(d\) (Supplementary Fig. 26a and c). ITAM phosphorylation increased with increasing \(d\) as the total number of BCRs present in the clusters increased with increasing \(d\). This was because as \(d\) increased the overlap between the clusters decreased (Supplementary Fig. 26b and c), thereby lowering the steric interaction between the BCR molecules that made it possible for larger numbers of BCR molecules to reside within the clusters. However, experiments with eOD-GT8 1D pentamer (Supplementary Fig. 18b) show apparent saturation of calcium flux at a smaller \(d\) (~11nm) compared to that predicted by the model (~28 nm, Supplementary Fig. 26b). This disagreement could be due to the mechanisms that are not included in the model, such as limitation in BCR signaling by cytoskeletal forces\(^\text{12-14}\), which could generate a saturation on BCR signaling at a lower value than that predicted in the model.

We also carried out a simulation where antigens were displayed on a planar two-dimensional pentamer of side \(d\) in order to investigate the effect of the geometry of displayed antigens on B cell signaling. The BCR signaling increased gradually with increasing \(d\) (Supplementary Fig. 26e) and saturated at larger values of \(d\). The behavior potentially captures the gradual increase in the calcium flux observed with increasing inter-antigen distances in Ico-5mer (Supplementary Fig. 18b). This behavior in the model can be mechanistically explained by the increase in the number of BCR molecules as the overlap between the BCR clusters decreased with increasing \(d\) (Supplementary Fig. 26f).

Furthermore, we investigated the effect of lower antigen affinity by performing a simulation of the model when B cells were stimulated by lower affinity antigens (\(k_{\text{off}}=0.1\) s\(^{-1}\)) displayed on eOD-GT8 1D dimer and eOD-GT8 2D pentamer. In this case, the BCR signaling increased monotonically with increasing \(d\) (Supplementary Figs. 26g and h) which is qualitatively similar to that observed for high affinity antigens (Supplementary Figs. 26a and e), however, the total number of fully phosphorylated ITAM decreased for the lower affinity antigens compared to its higher affinity counterparts (Supplementary Figs. 26g and h). The antigen-BCR complexes have shorter lifetimes compared to that formed by the high affinity antigens. Therefore, Syk-induced phosphorylation of ITAMs is less efficient in the case of stimulation with lower affinity antigens as once the antigen unbinds from the BCR, Syk molecules quickly dissociate from the BCR complex. This results in a lower number of fully phosphorylated ITAMs when weak affinity antigens stimulate BCRs. The mechanism could potentially underlie the lower amount of calcium flux observed for B cell stimulation by low affinity eOD-GT5 compared to its high affinity eOD-GT8-60mer stimulation (Supplementary Fig. 16)
Supplementary Fig. 26. Results from our *in silico* BCR signaling model. 

(a) Variation of the total number of fully phosphorylated ITAMs corresponding to antigen spacing, *d*, when BCRs were stimulated by a single eOD-GT8 1D dimer. The total number of ppITAMs was divided by its value.
A. Reactions and reaction rates

Supplementary Table 4. Parameters used in the model

| Reaction | $k_{on}$ (µM$^{-1}$s$^{-1}$) | $k_{off}$ (s$^{-1}$) | $k_{cat}$ (s$^{-1}$) | Notes |
|----------|-----------------------------|---------------------|-------------------|-------|
| $R + L \rightleftharpoons R_b$ | 0.02 | 0.0 | N.A. | The antigen has a very small unbinding rate ($k_{off} \sim 10^{-5}$ s$^{-1}$) (see main text), thus we set $k_{off}$ to zero for the simulation time scale. $k_{on}$ is estimated by requiring that the kinetics reaches the steady state in about 200s. |
$R_b + Ly \rightleftharpoons R_b - Ly$
$R_b - Ly \rightarrow R'_b - Ly$
$R'_b - Ly \rightarrow R''_b - Ly$  

0.04 | 6.0 | 3.5 | $k_{on}$ for Lyn binding is estimated, and $k_{off}$ and the catalytic rate ($k_{cat}$) are similar to that in Ref. (Mukherjee, 2013 #1).

$R + Ly \rightleftharpoons R - Ly$
$R - Ly \rightarrow R' - Ly$
$R' - Ly \rightarrow R'' - Ly$  

0.04 | 50 | 3.5 | Fast binding (complex lifetime ~1/50 s) of Lyn to unbound BCRs is assumed to generate a basal level of ITAM phosphorylation. Increasing or decreasing the $k_{off}$ value by 5 or 4 times, respectively, does not change the qualitative nature of the results (Supplementary Fig. 26i).

$R'_b + Sy \rightleftharpoons R'_b - Sy$  

0.03 | 10 | N.A. | $k_{on}$ for Syk binding is estimated, and $k_{off}$ is similar to that in Ref. (Mukherjee, 2013 #1).

$R' + Sy \rightleftharpoons R' - Sy$  

0.03 | 120 | N.A. | Fast binding (complex lifetime ~1/100 s) of Syk to unbound BCRs is assumed to generate a basal (tonic) level of ITAM phosphorylation.

$R'' + Sy \rightleftharpoons R'' - Sy$  

0.03 | 0.01 | N.A. | $k_{on}$ for Syk binding is estimated, and $k_{off}$ is similar to that in Ref. (Mukherjee, 2013 #1).

$R'' + Sy \rightleftharpoons R'' - Sy$  

0.03 | 10.0 | N.A. | $k_{on}$ and $k_{off}$ values for Syk binding are estimated.

$R'_b - Sy / R' - Sy$
$\rightarrow R''_b - Sy / R'' - Sy$  

N.A. | N.A. | 0.05 | Phosphorylation rate of ITAMs by Syk is estimated.

$-Sy + R / R_b \rightarrow -Sy + R' / R'_b$  

0.004 | N.A. | N.A. | Phosphorylation rate of neighboring ITAMs by ITAM bound Syk is estimated. This generates the positive feedback. Increasing or decreasing the rate by 5 or 4 times, respectively, does not change the qualitative nature of the results (Supplementary Fig. 26i).

$-Sy + R' / R'_b \rightarrow -Sy + R'' / R''_b$  

0.004 | N.A. | N.A. | Phosphorylation rate of neighboring ITAMs by ITAM bound Syk is estimated. This generates the positive feedback.
Increasing or decreasing the rate by 5 or 4 times, respectively, does not change the qualitative nature of the results (Supplementary fig. 26i).

| Reaction                                      | $k_1$ | $k_2$ | Notes                                      |
|-----------------------------------------------|-------|-------|--------------------------------------------|
| $R_b^- / R^- + Sh \rightleftharpoons Sh - R_b^- / Sh - R^-$ | 0.006 | 1.0   | N.A. Phosphatase SHP-1 binding/unbinding rates are estimated. |
| $R_b^{**} / R^{**} + Sh \rightleftharpoons Sh - R_b^{**} / Sh - R^{**}$ | 0.06  | 0.1   | N.A. Phosphatase SHP-1 binding/unbinding rates are estimated. SHP-1 with two SH-2 domains of SHP-1 binds to fully phosphorylated ITAMs at a higher rate compared to the partially phosphorylated ITAMs (Ref. {Mukherjee, 2013 #1}). |
| $R_b^- - Sh / R^- - Sh \rightarrow R_b / R + Sh$ | N.A.  | N.A.  | 0.3 Value taken from Ref. {Mukherjee, 2013 #1}. |
| $R_b^{**} - Sh / R^{**} - Sh \rightarrow R_b^* / R^- + Sh$ | N.A.  | N.A.  | 0.3 Value taken from Ref. {Mukherjee, 2013 #1}. |

R: free BCR, L: antigen, $R_b$: antigen-bound BCR, $R^*$: partially phosphorylated ITAM associated with R, $R^{**}$: fully phosphorylated ITAM associated with R, Ly: Lyn (SFK), Sy: Syk, Sh:SHP-1 (phosphatase), -Sy: pITAM or ppITAM bound Syk.

Diffusion constants for the plasma membrane bound and cytosolic molecules are set to 0.01 ($\mu m$)$^2$/s and 10.0 ($\mu m$)$^2$/s, respectively.
## B. Number of molecules inside the simulation box

| Species name | Number (eOD-GT8 1D dimer) | Number (eOD-GT8 1D or 2D 5-mer) | Notes |
|--------------|-----------------------------|----------------------------------|-------|
| Antigen      | 2                           | 5                                | Determined by ligands used in experiments. |
| BCR          | 10 (when there is no overlap) | 25 (when there is no overlap)    | The BCR numbers in the clusters are consistent with previous experiments and size of the BCRs (see [Supplementary text 1](#)). |
| Lyn          | 5                           | 12                               | Estimated. |
| Syk          | 10                          | 25                               | Estimated. Increasing or decreasing the value by 2 times does not change the qualitative nature of the results ([Supplementary fig. 26i](#)). |
| SHP-1        | 10                          | 25                               | Estimated. |
Supplementary Table 5. P-values for Fig. 2.

### Fig 2c

| Row   | PBS    | Ico-0x | Ico-1x | Ico-2x | Ico-3x | Ico-4x | Ico-5x | Ico-10x | eOD-GT8-60mer |
|-------|--------|--------|--------|--------|--------|--------|--------|---------|---------------|
| PBS   | 0.493436 | 0.89191 | 1.13E-05 | 0.00088 | 0.000346 | 5.27E-05 | 0.000834 | 0.00051624 |
| Ico-0x| 0.493436 | 0.345284 | 0.006204 | 0.008562 | 0.001881 | 0.003691 | 0.000346 |
| Ico-1x| 0.89191 | 0.345284 | 0.001098 | 0.003013 | 2.37E-05 | 0.000771 | 5.24E-05 | 0.001885169 |
| Ico-2x| 1.13E-05 | 0.006204 | 0.001098 | 0.030904 | 0.005501 | 0.000295 | 0.008987 | 0.0004083111 |
| Ico-3x| 0.00088 | 0.008562 | 0.001098 | 0.003013 | 0.030904 | 0.093347 | 0.009024 | 0.001885169 |
| Ico-4x| 0.000346 | 0.001881 | 2.37E-05 | 0.005501 | 0.000295 | 0.003691 | 5.24E-05 | 0.0004083111 |
| Ico-5x| 5.27E-05 | 0.003691 | 0.001881 | 0.003013 | 0.030904 | 0.093347 | 0.009024 | 0.001885169 |
| Ico-10x| 0.000834 | 0.001193 | 5.24E-05 | 0.008987 | 0.004083 | 0.000487 | 0.137352 | 0.141879698 |
| eOD-GT8-60mer| 0.000516 | 0.005257 | 0.001885 | 0.004083 | 0.000487 | 0.137352 | 0.141888 | 0.595305897 |

### Fig 2d

| Row   | PBS    | Ico-0x | Ico-1x | Ico-2x | Ico-3x | Ico-4x | Ico-5x | Ico-10x | eOD-GT8-60mer |
|-------|--------|--------|--------|--------|--------|--------|--------|---------|---------------|
| PBS   | 0.08999 | 0.389131 | 0.02394 | 0.016294 | 0.003536 | 0.01102 | 0.001067 | 0.005889142 |
| Ico-0x| 0.08999 | 0.977052 | 0.004753 | 0.008062 | 0.004916 | 0.001656 | 0.000369 | 0.00640514 |
| Ico-1x| 0.389131 | 0.977052 | 0.010846 | 0.000153 | 0.006472 | 0.020584 | 0.015051612 |
| Ico-2x| 0.02394 | 0.004753 | 0.010846 | 0.052715 | 0.002812 | 0.00911 | 0.001676 | 0.02058405 |
| Ico-3x| 0.016294 | 0.008062 | 0.000153 | 0.052715 | 0.052538 | 0.013243 | 0.003372 | 0.065446961 |
| Ico-4x| 0.003536 | 0.004916 | 0.006472 | 0.02812 | 0.052538 | 0.010054 | 0.035724 | 0.175582909 |
| Ico-5x| 0.00102 | 0.001656 | 0.002778 | 0.00911 | 0.013243 | 0.010054 | 0.127902 | 0.624728469 |
| Ico-10x| 0.001067 | 0.000369 | 0.000657 | 0.001676 | 0.003372 | 0.035724 | 0.127902 | 0.84904309 |
| eOD-GT8-60mer| 0.005889 | 0.006405 | 0.015052 | 0.020584 | 0.065446 | 0.175583 | 0.624728 | 0.849043 |
Supplementary Table 6. P-values for Fig. 3b.

| Fig 3b | Row   | 6HB-2x-7nm | 6HB-2x-28nm | PBS  |
|--------|--------|------------|-------------|------|
| NaN    | NaN    | 0.9999     | 0.0003      |
| NaN    | 0.9999 | NaN        | 0.0003      |
| NaN    | 0.0003 | 0.0003     | NaN         |
### Supplementary Table 7. P-values for Fig. 3c.

| Fig 3c | Row         | PBS    | ssDNA linker | ssDNA linker 7.6nm | ssDNA linker 30nm | ssDNA linker 52nm | PEG linker 0.6nm | PEG linker 1.8nm | PEG linker 3.9nm | 6HB-0x | 6HB-2x-28nm |
|--------|-------------|--------|--------------|--------------------|-------------------|-------------------|------------------|------------------|------------------|--------|-------------|
|        | PBS         | NaN    | 0.148663     | 0.037021           | 0.002094          | 0.024562          | 0.042811         | 0.034649         | 0.046538         | 0.733514 | 0.00185     |
|        | ssDNA linker| NaN    | 0.148663     | 0.039737           | 0.003992          | 0.028994          | 0.065672         | 0.052425         | 0.046739         | 0.308796 | 0.000185    |
|        | ssDNA linker 7.6nm | 0.037021 | 0.039737 | NaN               | 0.071463          | 0.026132          | 0.403086         | 0.185208         | 0.063565         | 0.027456 | 0.002904    |
|        | ssDNA linker 30nm | 0.002094 | 0.003992 | 0.071463 | NaN               | 0.743805          | 0.335171          | 0.808859         | 0.02302          | 0.000414 | 0.000436    |
|        | ssDNA linker 52nm | 0.024562 | 0.028994 | 0.026132 | 0.743805 | NaN               | 0.152415          | 0.962907         | 0.59734          | 0.017098 | 0.006259    |
|        | PEG linker 0.6nm | 0.042811 | 0.065672 | 0.403086 | 0.335171 | 0.152415 | NaN               | 0.040614         | 0.304591         | 0.029590 | 0.005721    |
|        | PEG linker 1.8nm | 0.034649 | 0.052425 | 0.185208 | 0.808859 | 0.929207 | 0.040614 | NaN               | 0.803306         | 0.025533 | 0.00897     |
|        | PEG linker 3.9nm | 0.046538 | 0.046739 | 0.063565 | 0.62302 | 0.59734 | 0.304591 | 0.803306 | NaN               | 0.040575 | 0.14995     |
|        | 6HB-0x       | 0.733514 | 0.308796 | 0.027456 | 0.000414 | 0.017098 | 0.029590 | 0.025533 | 0.040575 | NaN | 0.000119     |
|        | 6HB-2x-28nm  | 0 | 0.000185 | 0.002904 | 0.000456 | 0.006259 | 0.005721 | 0.00897 | 0.14995 | NaN |
Supplementary Table 8. P-values for Fig. 4.

| Fig 4b | Ico-5x-3nm | Ico-5x-11nm | Ico-5x-15nm | Ico-5x-22nm | Ico-0x |
|--------|------------|-------------|-------------|-------------|-------|
| Row    |            |             |             |             |       |
| Ico-5x-3nm | 0.076169193 | 0.0299739  | 0.003011634 | 0.001800419 |       |
| Ico-5x-11nm | 0.076169193 |             | 0.008038514 | 0.010558122 |       |
| Ico-5x-15nm | 0.0299739   | 0.033635109 |             | 0.007096408 |       |
| Ico-5x-22nm | 0.003011634 | 0.008038514 | 0.047160949 |             | 0.001332615 |
| Ico-0x   | 0.001800419 | 0.010558122 | 0.007096408 | 0.001332615 |       |
### Supplementary Table 9. P-values for Fig. 5.

#### Fig 5c

| Row           | Control       | 6HB-2x-7nm | 6HB-2x-28nm | Ico-30x | eOD-GT8-60mer |
|---------------|---------------|------------|------------|---------|---------------|
| Control       | 0.000727854   | 0.001599224| 0.00087076 | 0.006121495 |
| 6HB-2x-7nm    | 0.000727854   | 0.031436033| 0.011184711| 0.160429357 |
| 6HB-2x-28nm   | 0.001599224   | 0.031436033| 0.303764192| 0.415272711  |
| Ico-30x       | 0.00087076    | 0.011184711| 0.303764192| 0.477876625  |
| eOD-GT8-60mer | 0.006121495   | 0.160429357| 0.415272711| 0.477876625  |

#### Fig 5d

| Row           | 6HB-2x-7nm | 6HB-2x-28nm | Ico-30x |
|---------------|------------|------------|---------|
| 6HB-2x-7nm    | 0.555857459| 0.014166752|
| 6HB-2x-28nm   | 0.555857459| 0.007375736|
| Ico-30x       | 0.014166752| 0.007375736|
**Supplementary Table 10. Scaffold sequences.** (Red indicate the primers binding site)

| Name       | Sequence                                                                 |
|------------|--------------------------------------------------------------------------|
| Icosahedron| **C**                                                                    |
| 6-HB       | **TCCTTGCCTGCTGCTGCT**                                                   |
|            | **GTGCTTTCACTCTGTTCTCAGT**                                               |
|            | **AACCTGTTTATTTGGGAATATTGT**                                             |
|            | **ATATGGAATATTGT**                                                      |
|            | **ATATGGAATATTGT**                                                      |

*Red indicate the primers binding site*
## Supplementary Table 11. 6-Helix Bundle staple list.

| Staple name | Sequence |
|-------------|----------|
| 6HB_1       | TGCTGCATTCACCACATTAAT |
| 6HB_2-polyT | AATTCGGTGTATCTTTTGGTAATTCAGCTCATTTT |
| 6HB_3       | GCCGGCCCTGGCCCTTCATGCTC |
| 6HB_4       | AGATCAAGAATAAATGTGT |
| 6HB_5       | TGACCGGTCAATCTGAGAGA |
| 6HB_6       | CCATCAAAATTAATCGGTCA |
| 6HB_7       | TGTAACGTTAACAACGGACTG |
| 6HB_8       | GAGGCCGCCACAACTTGTAAATTCGCCAATAATTCGCTG |
| 6HB_9       | ATCAACAGAGTCTAGAGAATCGGATGAGATGGGCGCA |
| 6HB_10      | AGGTAAATTGGCTGCTTGTG |
| 6HB_11      | TTTTTAACCAATAAACGAC |
| 6HB_12      | CCAGCTGAGCTGATGGAAACC |
| 6HB_13      | TGGCCCTTTAATATTTACGAGC |
| 6HB_14      | CTGGTGGAACGGGTATAAATTT |
| 6HB_15      | TCCGTTGGTATAATTTGCGT |
| 6HB_16      | AGAAAGGACATAAAGGCTAAATCAGTATAATCAGGTGCAATCTG |
| 6HB_17-polyT| TTTTTTTTTTTTTTCA |
| 6HB_18      | AATTCGGTGTATCTTTTGGTAATTCAGCTCATTTT |
| 6HB_19      | TTGAGGCGGTATGACCC |
| 6HB_20      | TTGCTGGTCATAATGCGGAAAATCCGTGAGCCGCAAAGCGCCA |
| 6HB_21      | TGGCAATGTTCAACGAAACCTACCACAAAAACGTGGAAGAGG |
| 6HB_22      | AATGCGCCCTATTATTATATGGTTACAGCAGAAAAAGAGG |
| 6HB_23      | CTAACTCCCAAGTGCGTCCCTCCAAGCCCTCTCTCGCCGATTC |
| 6HB_24      | CCGAAGCCAACGGGGTGGTTTTTTTTGCTGCAATCCAGTT |
| 6HB_25      | GAGTAACAAACAGGGAGTGAG |
| 6HB_26      | GTGACGAGAGATCGAGCTCG |
| 6HB_27-polyT| TTTTTTTTTTTTTTTTCA |
| 6HB_28      | TCAATAAAAATATTGAAAGC |
| 6HB_29      | TGCGCCACGGCAAGGATATTC |
| 6HB_30      | TGCGCTTCAAGTGGAGAAG |
| 6HB_31      | CGAAACCTTGGAGCATGCC |
| 6HB_32      | GGTAACGGCGCCACGGGGA |
| 6HB_33-polyT| GTCAAGGGCGAAAACGGTCTATTTTTTTT |
| 6HB_34      | ACCGGTTAGGCTATCTGAAATTTACCTAGCATTAATGGGTGCCA |
| 6HB_35-polyT| TTTTTTTTTTTTTTTTTGCAAGAGAGTCCAGATTTTTTTTT |
| 6HB_36      | TCTAACAACAGTGCGCTGGCACTGTGCTTTGCTTTCAGAGATGTC |
| 6HB_37      | GAATCGGATAAAAGTGTAAATTTGAAACGCTTCTGAGTTAGTG |
| 6HB_38-polyT| GGGCGTGGCCAAATTTTTTT |
| 6HB_39      | GCGGTAGGAGAGGCGAGAGAGGTGACGGAGAGGAGGACGGATCGT |
| 6HB_40  | TCGAGGGGACGACGACAGTA |
| 6HB_41-polyT | TTTTTTTTTGCTTTGCATGCTGTTCAGTGAGCTTTTTTTTTTT |
| 6HB_42  | CCCCATATCTGAGTGGCAGGATAGGCTATTAATCCCAAC |
| 6HB_43  | ACGTGGCGGTAATCACGTCA |
| 6HB_44  | TTCCGGGCAAATAAGGGTG |
| 6HB_45  | TACCAACCGGAATGTTCCGAAATCGACCGTCTCTGCCCAG |
| 6HB_46  | CGCTTTTGTATTAACGAAAAAGCCCCAAAAACCCGTTATTCG |
| 6HB_47  | CGGGTCCAGCTGTTGAGACGGCAACGGAAGGGGATG |
| 6HB_48  | GTGAAATAATCATGGGAGGTCGACTCTAGTTTGTAAGGAAG |
| 6HB_49-polyT | TTTTTTTTTTTATCACCTTATAAATTTAAA |
| 6HB_50  | GTAATACGATTCAACCTTATATAATCAACTCCAGCCAGCT |
| 6HB_51  | TGTCGTTGGCCTATTATAGATTGTATAAGCCATTTAATGTGAGC |
### Supplementary Table 12. 6-Helix Bundle modified staple list.

#### 6 Helix Bundle 5 mer-7nm: Modified staples list

| Staple name | Sequence |
|-------------|----------|
| 6HB_47      | CTGGGTCGCCAGTGTGGAGACGGGCAACGGAAAGGGGATGTACCTGGAACTG |
| 6HB_46      | CGCGTCTATTTAGAGAGAAAGGCCCAGAAACCCGTTATTTACCTGGAACTG |
| 6HB_39      | GGCTAGGAGGCGAGAGAGATGTCAGGAAAGGGGATGTACCTGGAACTG |
| 6HB_34      | ACCGGTATTCTGTAATTTATGACGATATTCTGGTACCTGGAACTG |
| 6HB_20      | TGGCCTGTCATTGCCAGGAAAGTCTGGTCAGGAAAGGGGATGTACCTGGAACTG |

#### 6 Helix Bundle 5 mer-11nm: Modified staples list

| Staple name | Sequence |
|-------------|----------|
| 6HB_37      | GATCGTGATAAAGTTAATTGTGAAGCGCTGTAGTAAGTGTACCTGGAACTG |
| 6HB_39      | GCGGTAGGAGACGGGCAACGGAAAGGGGATGTACCTGGAACTG |
| 6HB_16      | AGAAAGGAACATTTAAAAGCAGCTAAATCGCGTGCATCTGGTGCTTACCTGGAACTG |
| 6HB_51      | TGCCGTTGGCCTTAATAAGATTGTATAATGCGCTATTTACCTGGAACTG |
| 6HB_36      | TCTCAACTAGCGCTGCTGTTTACCTGCCGACGATTACCTGGAACTG |

#### 6 Helix Bundle 5 mer-15nm: Modified staples list

| Staple name | Sequence |
|-------------|----------|
| 6HB_37      | GATCGTGATAAAGTTAATTGTGAAGCGCTGTAGTAAGTGTACCTGGAACTG |
| 6HB_46      | CGCGTCTATTTAGAGAGAAAGGCCCAGAAACCCGTTATTTACCTGGAACTG |
| 6HB_34      | ACCGGTATTCTGTAATTTATGACGATATTCTGGTACCTGGAACTG |
| 6HB_16      | AGAAAGGAACATTTAAAAGCAGCTAAATCGCGTGCATCTGGTGCTTACCTGGAACTG |
| 6HB_21      | TGCAATGTTCACACAGCCACATCACCACAAAAAGCAGGACGTTACCTGGAACTG |

#### 6 Helix Bundle 5 mer-17nm: Modified staples list

| Staple name | Sequence |
|-------------|----------|
| 6HB_11      | TTTTTAACCAATAAACGACTTACTGGACTG |
| 6HB_39      | GCGGTAGGAGACGGGCAACGGAAAGGGGATGTACCTGGAACTG |
| 6HB_21      | TGCAATGTTCACACAGCCACATCACCACAAAAAGCAGGACGTTACCTGGAACTG |
| 6HB_24      | CGGAGCAACGCCGCTAGTGTACCTGGAACTG |
| 6HB_9       | ATCCACCAAGAGTCTAGAGAAATGATGATGAGGTGGGCACATTACTGGAACTG |

#### 6 Helix Bundle 2 mer-7nm: Modified staples list

| Staple name | Sequence |
|-------------|----------|
| 6HB_39      | GCGGTAGGAGACGGGCAACGGAAAGGGGATGTACCTGGAACTG |
| 6HB_34      | ACCGGTATTCTGTAATTTATGACGATATTCTGGTACCTGGAACTG |

#### 6 Helix Bundle 2 mer-14nm: Modified staples list
| Staple name | Sequence |
|-------------|----------|
| 6HB_46      | CCGCTTTACATTAAAGAAGCCCTCACAACCGTTATTACGTTACTGGACTG |
| 6HB_34      | AACCCTTTACATCAGCTCGTTAAATCTAGCCATTAAATGGGTGCAGCGAATTACTGGACTG |

**6 Helix Bundle 2 mer-21nm: Modified staples list**

| Staple name | Sequence |
|-------------|----------|
| 6HB_46      | CCGCTTTACATTAAAGAAGCCCTCACAACCGTTATTACGTTACTGGACTG |
| 6HB_20      | TTGCCCTGTCAATATGGCGAAAATCCTGTCAGGCAAAGCGCCATTACTGGACTG |

**6 Helix Bundle 2 mer-28nm: Modified staples list**

| Staple name | Sequence |
|-------------|----------|
| 6HB_37      | GAATCGGATAAAAGTAAATGAAACGCTCTGTATAGTAAATTGTACTGGACTG |
| 6HB_20      | TTGCCCTGTCAATATGGCGAAAATCCTGTCAGGCAAAGCGCCATTACTGGACTG |

**6 Helix Bundle 2 mer-80nm: Modified staples list**

| Staple name | Sequence |
|-------------|----------|
| 6HB_11      | TTTTTAACCAATAAGCTACTTACTGGACTG |
| 6HB_42      | CTTTATCCCTGAGTGGCCCAGATAGGGTTATTAACACTCCACTTACTGGACTG |
**Supplementary Table 13. Icosahedron staple list.**

| Staple name | Sequence |
|-------------|----------|
| Ico_52_1    | ACGGGAGAATTTTTTAACTGAAACATATCGAGAGTTTTTTAATACCACATCATCGGCATTTTTTTTCGGTCATA |
| Ico_52_2    | GAAAAGTACTATCTTTACGAGGGCATCCCA |
| Ico_52_3    | ATTCTGCCGATATACAGTTGCTTTTTAA |
| Ico_52_4    | GCGGTTTAAAATAGGTATTAAGCCCAAATAAT |
| Ico_52_5    | AAGAGCAATTTGCGCTATGAGCTAGAGTAGATTTTTTTAGTTGAC |
| Ico_52_6    | GAAAACGAGTAATTTTTTCTATGAAACAGACAGGAGGTTTATGATATATTTGATATA |
| Ico_52_7    | CCATTAGCAAGTTTTGCGCCAAAGACGATCCGATTTTTACGAGATCG |
| Ico_52_8    | ATTGCTGAAATTTTTTAAAATGCTGATAGCGTTGCTTTTTGAGTTTCAATAAGAGATAGATTTTTTAGTTGAC |
| Ico_52_9    | CCTTCTAGATTCTTTTTATTTTTTTCTCCTTCTTCTTTTATA |
| Ico_52_10   | GCTAATAACATTAGATACATTCTTTGCAGTTTTAA |
| Ico_52_11   | GAGGCTCTGAATCAAACTGACAGGAAATAG |
| Ico_52_12   | GAGATCTTGGGCGCGAGCTGTTTAACGT |
| Ico_52_13   | CAAAATCCAACCTCAAGGGTTCAGGATTA |
| Ico_52_14   | CAAGGCGACCTTTTTAGACCGAGAAAGAAATAGCCAGCAGTCCTTTTTCTTTACAGAGGAGCGG |
| Ico_52_15   | GCCACCAAGAATACATATATAAACACGGGAA |
| lco_52_81 | GAAACGAAACATAAAGGGAGGCAACCATAAAT |
|-----------|---------------------------------|
| lco_52_82 | AAGACACCACGTTTTTGAAATAAGTTTTTTACCCAGCGCTTTTTCAAAGACAAAAGGTTAAATATTGTTTTTGGGAAATT |
| lco_52_83 | ACGGAATACCCCTTTTTAAAAGAAACTGAGCAAAAGTATTTTTTGGAAATACAT |
| lco_52_84 | TAATCGAAAATCTTTTTACCGGAACCACCGCCACCCCTCAGTTTTTAAACGACCCACC |
### Supplementary Table 14. Icosahedron modified staple list.

| Staple name  | Sequence                                                                                       |
|--------------|-----------------------------------------------------------------------------------------------|
| Ico_52_VM_1  | ACGGGAGAAATTTTTTAATGACACATATCAAGAGTTTTTAACCCACACATCATCGGCCATTTTTTTTTTTCGGTGATA               |
| Ico_52_VM_2  | GAAAGATCACTATCTCTCCAGAAGCCATCCCA                                                           |
| Ico_52_VM_3  | ATTCTGCTCCATATAACAGTTGCTTTTTAA                                                            |
| Ico_52_VM_4  | GCCGGTTGAAGATGTTAAAGCCCAATACGAATTCGCCCATCAGAATTTT                                           |
| Ico_52_VM_5  | AGAGCAATTTGCCCTTTAGGCTCAGACTCTGA                                                          |
| Ico_52_VM_6  | CAGAACCAAGAAGAACATAAGG                                                                          |
| Ico_52_VM_7  | ATAGCAATAGGACAGATACC                                                                         |
| Ico_52_VM_8  | TTTGCTGAAATATTTTTTAACAGCTGTATACGGGTCTGCTTGAAGTTCTAATCAGAGTAGATTTTTTTTTTTTTTTGA               |
| Ico_52_VM_9  | CCTGTTAGCCTTTTTATATTTTTCACTTTTTAATTGCTTTTTCTTTTTTTGATA                                      |
| Ico_52_VM_10 | GTCATAAAGTATTAGATCAGATTTCGGGTAAATT                                                          |
| Ico_52_VM_11 | GAGGCCTACCTGCAAACAGTCAGACAATATTG                                                           |
| Ico_52_VM_12 | GAGATCTTGGGCAGCGCTGCTTTAAGCTGAAGGYES                                                        |
| Ico_52_VM_13 | CAAAATGGCAATCTCAGACCGCTGACAGTTA                                                            |
| Ico_52_VM_14 | CAAAGGCCAACTTTTTTAGCAGGGAAGAAAAATAGCAGCTTTTTCTTTTACAGAGGAACACACCCCACTTTTTAGAGGCGGA             |
| Ico_52_VM_15 | GCCACCAAGAATACACATAAACACACGAGGAGG                                                           |
| Ico_52_VM_16 | CGCATTAGCCAGAAGCCACCACCCACCTACAGGACC                                                          |
| Ico_52_VM_17 | AATCAGGCTAATTTTTTCAAGACGTCTACTACATATATTTTTACCTCAGTTT                                        |
| Ico_52_VM_18 | ATTACCCACCAGCATGAGACAGTGGGTTAGGG                                                             |
| Ico_52_VM_19 | CAGTCGACATTTGAGAAGACGGGATATTC                                                             |
| Ico_52_VM_20 | GGCCTGACCTTCTTCTTTATCAAGAGTAACGATGCTTGCCTTTTTGTATTCAC                                      |
| Ico_52_VM_21 | GSAAXGCGCAATTTCTTCTCTGAATAAAGGCACTCAGCTTTTTTTTGGAGGGAACAGGGCAACTTTTTTGAGGGCGAAGGAGG         |
| Ico_52_VM_22 | GCCGAATAAACAATATATATATCTCAGCTCC                                                           |
| Ico_52_VM_23 | TCAAGACCGAGCCACCCAGACCTTCAGTTAAAA                                                          |
| Ico_52_VM_24 | AGCAGCGATACCTTACGCAGTAAAGCTCATAC                                                          |
| Ico_52_VM_25 | ATGCGCTTTCTCTTGGCGGTAGCCTCACCCTTC                                                       |
| Ico_52_VM_26 | GCTTGCAAGGGATTTTTTGTAAAGGCTGATGATACGTCAACTGATGACTGAGATGCTGACCTTTTTTGTATACAG                   |
| Ico_52_VM_27 | GGTGGTGAATATAAGGGTTTTAAACCGGTTGACA                                                          |
| Ico_52_VM_28 | TCTTTTCACCCCCCTATTAGGCGGGTGCTAGT                                                        |
| Ico_52_VM_29 | TATTTCTGAAATCTATTTTATAGAAAGTATACGACATAATGACATATTTTTTACACATTCC                             |
| Ico_52_VM_30 | GAACCTATTTAAGGCCCTTGCCCGATAGGCA                                                           |
| Ico_52_VM_31 | GCACGCTATACCAATGAAACACATTATTTCG                                                            |
| Ico_52_VM_32 | AGTGGCGCTAAGGCTGAGACTCTCAAGA                                                              |
| Ico_52_VM_33 | GAAGGATTTCTTTAAACAGCTGTTAGACCCGT                                                      |
| Ico_52_VM_34 | TCAGGTGCGCCACACGAAAGGTGTAATAAGG                                                         |
| Ico_52_VM_35 | TTAAGCCCATCCATAAGCGGTTAAATTTGCG                                                            |
| Ico_52_VM_36 | CAGACGTAAGCAGACCGCTACAGAGGCCTTTG                                                          |
| Ico_52_VM_37 | AGGACTAACAGACGGCTCATATAAGGGAAC                                                         |
| lco_52_VM_38         | CATAGGCTTGAACGGTGTACAGATTAATTTC |
|---------------------|---------------------------------|
| lco_52_VM_39         | AACTTTAATGGGCTTGAATGTTGCGGCG    |
| lco_52_VM_40         | TAAGAGCAACATTCAATTTGAGTCTGTGCC  |
| lco_52_VM_41         | TGACGAGACCAAAAGGAAATTAGGAGGCTAG |
| lco_52_VM_42         | TCGAGCTTTACAGACGACGCTAATAAACCAAA |
| lco_52_VM_43         | ATAGGAGACTTCAAATATCGCGTTTTAAT   |
| lco_52_VM_44         | AGAGCTTTAAGAGCTTACATTGCGGATTT   |
| lco_52_VM_45         | CATCAAAATAGTCAGAAGCAAAGGATGCTT  |
| lco_52_VM_46         | AAATAGGGCTCAACATGTTTTATATTATCG  |
| lco_52_VM_47         | CGTAGATGCTCAGATAGAAGTACCTCAATAGC |
| lco_52_VM_48         | GAACAAAGTTACTTGAGCCCAT          |
| lco_52_VM_49         | TTGGGAATATTCATTTAGCAAG          |
| lco_52_VM_50         | CGTCACCAGCCAGAAGAAGAACCAGAAGACG |
| lco_52_VM_51         | CATATAAATGCTTAAAGGTAATATACAC   |
| lco_52_VM_52         | CACCATTAGCGACAGAAAAATCAGTGCCGTC |
| lco_52_VM_53         | GAGAGGGTAGTACGTACGCCTGATAACAGTAG |
| lco_52_VM_54         | TGATATAAGTATTTTTTTAGCCCGGAACCGCCACCTCTCTTTTAAAGCACTCCTCTATTTTTTATAGGAGGAAATTTGTGAGACG |
| lco_52_VM_55         | CAGCTGTGCTTTTTTTTCGAGTGATAGATTAGCGGTCTTGGTTTTGTCCTG |
| lco_52_VM_56         | GTTTAGTATAGGTGTACATTACCGTGATTTG |
| lco_52_VM_57         | GGGAGGAAAGGGGACATTCGACATTCCAGAG |
| lco_52_VM_58         | TTCAGGTCTCCCTCAAGACCGCCACCTCCAGA |
| lco_52_VM_59         | GCCACCACAGGAGATGGGAATATATATTTTT |
| lco_52_VM_60         | TGAATTTTCTGTGTTTTTTATGGGATTTTGAAGAAATTGTGTTTGGGAACCCCACTACCTCTATTGTGTTTGGGAAAAATCATTATTAGGTTTT |
| lco_52_VM_61         | GAGTTTCTGCTATTTTTCCAGATATTAGTAGGCTTGGTTTTGTAACGATCT |
| lco_52_VM_62         | GAAAACGTACGTTAATAAAAACGTCACAGGCG |
| lco_52_VM_63         | TCAAACGCTTACACGCTGGTACATCAATAAT |
| lco_52_VM_64         | GACAGCCCTTACACGCTGGTACATCAATAAT |
| lco_52_VM_65         | AACCCCCGAATGATCTTAAATAATTCACACA |
| lco_52_VM_66         | TTAGTTAAAAAGTGTGTCGTTGCTTTAAGTAAAC |
| lco_52_VM_67         | GGAAACACTAGTTAATAAAGCAGCAGGAGCAG |
| lco_52_VM_68         | AGCGGAGTGCTTAAACAAAATTTCTGAAAAACAG |
| lco_52_VM_69         | TACAACGTTTACAAAGCGGCAAGAGTTTCC |
| lco_52_VM_70         | CGTTTATTCCTCTAAAGGGCCTCTTTGGAATAACA |
| lco_52_VM_71         | CTAAACACGGAAGAGGCAAAAAATTGTAT |
| lco_52_VM_72         | CTAGGAGGACTTTTTTTCCAAAGTACCTCTTTGCTTATTTTTCCACCAGCCA |
| lco_52_VM_73         | AGTTTATTGTATTTTTTATGGGCTGTTATACGTAGGCTGTTTTGGAACGAGGCCAGACCTTATTCTTTTCAGGTAGGTT |
| lco_52_VM_74         | GCTCCATGAAATTGTGCTGGAATCCTCATTATA |
| lco_52_VM_75         | TACCACTGCTATTATAAGAAGCTGCCGACCT |
| lco_52_VM_76         | CATCTGGATTATTTTTTATGCTTACGTAGGCTGTTTTGGAACGAGGCCAGACCTTATTCTTTTCAGGTAGGTT |
| lco_52_VM_77         | AAATAGGGCTTAAAAACGAGACGAACTCTTTTGGAGTAAAAAAATCACTATTACAGGGTTT |
| lco_52_VM_78         | CCACATTCCATCTAGTGGAGATTTAGTAAAT |
| Staple modified name | Sequence |
|----------------------|----------|
| Ico_52_VM_79         | GTTTAGACTGCCAGGAGGGTAATAGGAAATA |
| Ico_52_VM_80         | TGGATAGCGTCTTTTTCAATACTGCGTCAAATGCTTTTTTTAAACAGTTCAAGGTCTTTACCTTTT |
| Ico_52_VM_81         | TCTGACTATTA |
| Ico_52_VM_82         | AGATTAAGAGGTTTTTAAGCCCGAAAATGACATATAAAA |
| Ico_52_VM_83         | CAACCCCAACCTAAAGGCTTTGCTTTTTAAACAGTTCAAGGTCTTTACCTTTT |
| Ico_52_VM_84         | TGGATAGCGTCTTTTTCAATACTGCGTCAAATGCTTTTTTTAAACAGTTCAAGGTCTTTACCTTTT |
| Ico_52_VM_85         | ACGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| Ico_52_VM_86         | GCCGGAAGGCTAGTGCGA |
| Ico_52_VM_87         | TAATCCAAATCTTTTTACCAGAAACCCCAACCTCAGTTTTTTAACGCCACC |
|                      |           |
| Icosahedron 60 mer eOD-GT8: modified staples list | |

| Staple modified name | Sequence |
|----------------------|----------|
| lco_52_60-mer-eOD-GT8-DS_2 | GAAAAGTACTATCTTACCGAAGCCATTCCCATTACTGGACTG |
| lco_52_60-mer-eOD-GT8-DS_3 | ATCTTGGCTCATATACAGTTGTTTTTAATATTCTGGACTG |
| lco_52_60-mer-eOD-GT8-DS_4 | GCGGGTGTAGTAATGGAACCTGATTACATTTCTGGTAAATTCTGGACTG |
| lco_52_60-mer-eOD-GT8-DS_5 | TTACTGGACTGAAGAGCAATTTGCCTTTAGCGTCAGACTGTA |
| lco_52_60-mer-eOD-GT8-DS_10 | GCCAAGAGATGCTATATTTCTGGACTG |
| lco_52_60-mer-eOD-GT8-DS_11 | AGGGGCTTACGCGGAAAGATCTGACATTTCTGGACTG |
| lco_52_60-mer-eOD-GT8-DS_12 | GAGGGTACTGCGGACGTCGCAATTTACTGGACTG |
| lco_52_60-mer-eOD-GT8-DS_13 | AAAAATGAGATGCTATATTTCTGGACTG |
| lco_52_60-mer-eOD-GT8-DS_15 | GCCCAAAGAATAACATACAAACAGGGGAGTTACTGGACTG |
| lco_52_60-mer-eOD-GT8-DS_16 | GCCGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| lco_52_60-mer-eOD-GT8-DS_18 | GAAAAGTACTATCTTACCGAAGCCATTCCCATTACTGGACTG |
| lco_52_60-mer-eOD-GT8-DS_19 | CAGGTAGATTATTTTGAATAGAGGCTTTATCTGGACTG |
| lco_52_60-mer-eOD-GT8-DS_22 | GCCAGAAAATAACAAATAATTTCTGGCCTTTTATTCTGGACTG |
| lco_52_60-mer-eOD-GT8-DS_23 | GCGGGTGTAGTAATGGAACCTGATTACATTTCTGGTAAATTCTGGACTG |
| lco_52_60-mer-eOD-GT8-DS_24 | AGGGGCTTACGCGGAAAGATCTGACATTTCTGGACTG |
| lco_52_60-mer-eOD-GT8-DS_25 | TTACTGGACTGAAGAGCAATTTGCCTTTAGCGTCAGACTGTA |
| lco_52_60-mer-eOD-GT8-DS_27 | GCCGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| lco_52_60-mer-eOD-GT8-DS_28 | GCCGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| lco_52_60-mer-eOD-GT8-DS_30 | GCCGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| lco_52_60-mer-eOD-GT8-DS_31 | GCCGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| lco_52_60-mer-eOD-GT8-DS_32 | GCCGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| lco_52_60-mer-eOD-GT8-DS_33 | GCCGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| lco_52_60-mer-eOD-GT8-DS_34 | GCCGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| lco_52_60-mer-eOD-GT8-DS_35 | GCCGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| lco_52_60-mer-eOD-GT8-DS_36 | GCCGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| lco_52_60-mer-eOD-GT8-DS_37 | GCCGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| lco_52_60-mer-eOD-GT8-DS_38 | GCCGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| lco_52_60-mer-eOD-GT8-DS_39 | GCCGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| lco_52_60-mer-eOD-GT8-DS_40 | GCCGGAATACCCCTTTTTAAAAAGACTGTAGCAACGTATTTTTGAAATACAT |
| Staple modified name | Sequence               |
|----------------------|------------------------|
| Ico_52_60-mer-eoD-GT8-DS_41 | TGACGAGACCAAAAGGAATTACGAGGCAATAGGTTACTGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_42 | TCGAGCTTACCAAGAGCAGATAAAACCAATTACTGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_43 | ATACGGAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_44 | AGAGCTTAAAGGCTATTTTTTGCGGAGATTGTACTGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_45 | CATCAAATATCGAGAGCAGATAAAACCAATTACTGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_46 | AAATAAGGTCATCAATGTTTTTATTATAGTATTGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_47 | CAGTATGTGGATATAGAAGCTTCCAAATATGCTTTACTGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_48 | GTATCAGGAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_49 | CAATAATATCATTAAGGATTATTACACTTACTGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_50 | CACCATAGGACAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_51 | GAGAGGTTAGTACCAAGAGGCTAACCAGTTACTGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_52 | GTATCAGGAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_53 | GAGAGGTTAGTACCAAGAGGCTAACCAGTTACTGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_54 | TACCCCTGATGGTTGATTAAAGCAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_55 | GGGGAGGAAAGCGCAGATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_56 | TACCCCTGATGGTTGATTAAAGCAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_57 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_58 | GTATCAGGAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_59 | AATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_60 | TTACCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_61 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_62 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_63 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_64 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_65 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_66 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_67 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_68 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_69 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_70 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_71 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_72 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_73 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_74 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_75 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_76 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_77 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_78 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_79 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_80 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |
| Ico_52_60-mer-eoD-GT8-DS_81 | GCCACCAAGAATCTCAATCTGCTTATATATGGACTG |

**Icosahedron 30 mer eOD-GT8: modified staples list**
### Icosahedron 10 mer eOD-GT8: modified staples list

| Staple modified name | Sequence                                      |
|----------------------|------------------------------------------------|
| lco_52_10-mer-eOD-GT8_DS_3 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| lco_52_10-mer-eOD-GT8_DS_12 | GAGAGTGTTTTGAGGACTTTGTTAGGTTGTTAGGACTTG       |
| lco_52_10-mer-eOD-GT8_DS_24 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| lco_52_10-mer-eOD-GT8_DS_30 | GAGAGTGTTTTGAGGACTTTGTTAGGTTGTTAGGACTTG       |
| lco_52_10-mer-eOD-GT8_DS_36 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| lco_52_10-mer-eOD-GT8_DS_40 | GAGAGTGTTTTGAGGACTTTGTTAGGTTGTTAGGACTTG       |
| lco_52_10-mer-eOD-GT8_DS_42 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| lco_52_10-mer-eOD-GT8_DS_44 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| lco_52_10-mer-eOD-GT8_DS_46 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| lco_52_10-mer-eOD-GT8_DS_48 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| lco_52_10-mer-eOD-GT8_DS_50 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| lco_52_10-mer-eOD-GT8_DS_54 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| lco_52_10-mer-eOD-GT8_DS_56 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| lco_52_10-mer-eOD-GT8_DS_60 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| lco_52_10-mer-eOD-GT8_DS_62 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| lco_52_10-mer-eOD-GT8_DS_64 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| lco_52_10-mer-eOD-GT8_DS_66 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |

### Icosahedron 5 mer eOD-GT8: modified staples list

| Staple modified name | Sequence                                      |
|----------------------|------------------------------------------------|
| lco_52_5-mer-eOD-GT8_DS_12 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| lco_52_5-mer-eOD-GT8_DS_24 | AGCTCTGGGTCATATACGATTTTGTTAAGTTGTTAGGACTTG     |
| Staple modified name | Sequence |
|----------------------|----------|
| Ico_52_5-mer-eOD-GT8-DS_40 | TAAGAGCGACATCTGAGGATATGCTTATGGCTTACTGGGACTG |
| Ico_52_5-mer-eOD-GT8-DS_66 | AGCGGAGTTGCTAAACAACTTTCAAACACATTGACTGGGACTG |
| Ico_52_5-mer-eOD-GT8-DS_80 | CAAAATCGAAAACGAGAATGACATATAAAATTACTGGGACTG |

**Icosahedron 4 mer eOD-GT8: modified staples list**

| Staple modified name | Sequence |
|----------------------|----------|
| Ico_52_4-mer-eOD-GT8-DS_24 | AGCAGCGATACCGTCCAGTAAGGCTTATGGCTTACTGGGACTG |
| Ico_52_4-mer-eOD-GT8-DS_40 | TAAGAGCGACATCTGAGGATATGCTTATGGCTTACTGGGACTG |
| Ico_52_4-mer-eOD-GT8-DS_66 | AGCGGAGTTGCTAAACAACTTTCAAACACATTGACTGGGACTG |
| Ico_52_4-mer-eOD-GT8-DS_80 | CAAAATCGAAAACGAGAATGACATATAAAATTACTGGGACTG |

**Icosahedron 3 mer eOD-GT8: modified staples list**

| Staple modified name | Sequence |
|----------------------|----------|
| Ico_52_3-mer-eOD-GT8-DS_24 | AGCAGCGATACCGTCCAGTAAGGCTTATGGCTTACTGGGACTG |
| Ico_52_3-mer-eOD-GT8-DS_40 | TAAGAGCGACATCTGAGGATATGCTTATGGCTTACTGGGACTG |
| Ico_52_3-mer-eOD-GT8-DS_80 | CAAAATCGAAAACGAGAATGACATATAAAATTACTGGGACTG |

**Icosahedron 2 mer eOD-GT8: modified staples list**

| Staple modified name | Sequence |
|----------------------|----------|
| Ico_52_2-mer-eOD-GT8-DS_24 | AGCAGCGATACCGTCCAGTAAGGCTTATGGCTTACTGGGACTG |
| Ico_52_2-mer-eOD-GT8-DS_80 | CAAAATCGAAAACGAGAATGACATATAAAATTACTGGGACTG |

**Icosahedron 1 mer eOD-GT8: modified staples list**

| Staple modified name | Sequence |
|----------------------|----------|
| Ico_52_1-mer-eOD-GT8-DS_80 | CAAAATCGAAAACGAGAATGACATATAAAATTACTGGGACTG |

**Icosahedron 5 mer-3nm eOD-GT8 vertex modified: modified staples list**

| Staple name | Sequence |
|-------------|----------|
| Ico_52_VM_6 | CAGAATCAAGGAAACAATGAATTACTGGGACTG |
| Ico_52_VM_7 | ATAGCAATAGAGCAGATAGCCTTACTGGGACTG |
| Ico_52_VM_48 | GAACAAAGTTACTGGGACTG |
| Ico_52_VM_49 | TTGGGAATTACCTAGCAAGTTACTGGGACTG |
| Ico_52_VM_86 | GCCGGAACGATCGATCGGACTG |

**Icosahedron 5 mer-11nm eOD-GT8: modified staples list**

| Staple modified name | Sequence |
|----------------------|----------|
| Ico_52_5-mer-11nm-eOD-GT8-DS_3 | ATTCTGCGTCCATATAACAGTTACTGGGACTG |
| Ico_52_5-mer-11nm-eOD-GT8-DS_4 | GCCGTTTTAGAATGGTTAAGCCCAATAATTACTGGGACTG |
| Ico_52_5-mer-11nm-eOD-GT8-DS_30 | GAACCTATTGAGGCGCGTTACTGGGACTG |
| Ico_52_5-mer-11nm-eOD-GT8-DS_49 | CAATAATCAATATTGAGGCGCGTTACTGGGACTG |
| Staple modified name | Sequence                        |
|----------------------|---------------------------------|
| Ico_52_5-mer-11nm-eOD-GT8-DS_51 | GAGAGGTTAGTACCCAGGGATAACCATGTTACTGGACTG |
| **Icosahedron 5 mer-15nm eOD-GT8: modified staples list** | |
| **Staple modified name** | **Sequence**                        |
| Ico_52_5-mer-11nm-eOD-GT8-DS_2 | GAAAAGTACTATCTTACCGAAGCCATTCATCCATTTACTGGACTG |
| Ico_52_5-mer-11nm-eOD-GT8-DS_5  | TTACTGGACTGAAGCAAATTTGCTTACTGGACTG |
| Ico_52_5-mer-11nm-eOD-GT8-DS_31 | GCACCGTATCCCAATGAAACCATTATTTCGTTACTGGACTG |
| Ico_52_5-mer-11nm-eOD-GT8-DS_48 | GCACCGTATCCCAATGAAACCATTATTTCGTTACTGGACTG |
| Ico_52_5-mer-11nm-eOD-GT8-DS_50 | CACCATTAGACCGAGCAATGCTGCTTACTGGACTG |
| **Icosahedron 5 mer-22nm eOD-GT8: modified staples list** | |
| **Staple modified name** | **Sequence**                        |
| Ico_52_5-mer-11nm-eOD-GT8-DS_10 | GTCAATAACATTAGATACATTTCGGGTAATTCTACTGGACTG |
| Ico_52_5-mer-11nm-eOD-GT8-DS_28 | TCTTTTCAGCCCCCTTATTTAGCGGGTCTGTTACTGGACTG |
| Ico_52_5-mer-11nm-eOD-GT8-DS_32 | AGTTGCGCTAAAGGCTGAGACTCCTCAAGATTACTGGACTG |
| Ico_52_5-mer-11nm-eOD-GT8-DS_47 | CAGTAGATTAGGTGATCACCAGTGATTACTGGACTG |
| Ico_52_5-mer-11nm-eOD-GT8-DS_54 | GTTTAGTTAGGTATACCCAGTGATTACTGGACTG |
| Staple name | Sequence |
|------------|----------|
| PB-84-2    | ATAAAGTTTTTGCCTTTCCTGCAGCATGAGATGGAGGCGG |
| PB-84-3    | TGGCGTCGCCGCTACAGGGCGCACTTAACGAGCAACCTTT |
| PB-84-4    | GCTGTGTTACCCGCGCCGCTAAATGCGGCCTTCCGGCTG |
| PB-84-5    | GTCAAGCTGCGTTTTTTCGTAACCCACCATTGCTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-6    | GTGTAGCGAGCCGGCGAACGTGGCGAGAAAGGGCGCTGGCAA |
| PB-84-7    | CGGGCGCTAGGAAGGGAAGAAAGCAGCTATAT |
| PB-84-8    | CTCTGTAAATCGCCATGTTGTCAGGCAACCTAAACGCTATTTAAAAAC |
| PB-84-9    | TCTAGTCTAGCTCTACATTTTACCTATTTAAAAAGGAAGGACCCG |
| PB-84-10   | GTGTAGCGAGCCGGCGAACGTGGCGAGAAAGGGCGCTGGCAA |
| PB-84-11   | CGGGCGCTAGGAAGGGAAGAAAGCAGCTATAT |
| PB-84-12   | TCTAGTCTAGCTCTACATTTTACCTATTTAAAAAGGAAGGACCCG |
| PB-84-13   | GTGTAGCGAGCCGGCGAACGTGGCGAGAAAGGGCGCTGGCAA |
| PB-84-14   | CGGTAGCGAGCCGGCGAACGTGGCGAGAAAGGGCGCTGGCAA |
| PB-84-15   | GCGAAAAAGAACGTGGACTCCAACAATCAGTA |
| PB-84-16   | CTCTGTAAATCGCCATGTTGTCAGGCAACCTAAACGCTATTTAAAAAC |
| PB-84-17   | TCTAGTCTAGCTCTACATTTTACCTATTTAAAAAGGAAGGACCCG |
| PB-84-18   | GTGTAGCGAGCCGGCGAACGTGGCGAGAAAGGGCGCTGGCAA |
| PB-84-19   | AAAAGTCATAGGAAGCAGCTATTTAAAAAGGAAGGACCCG |
| PB-84-20   | ATAAAGTTTTTGCCTTTCCTGCAGCATGAGATGGAGGCGG |
| PB-84-21   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-22   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-23   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-24   | ACTGATTTAAGTATGGATGAA |
| PB-84-25   | AATGTGCGGGCGAACTACTTACTCTCACTTTTACGGAAGGGAGGGGAGG |
| PB-84-26   | TCTGTGTTACCCGCGCCGCTAAATGCGGCCTTCCGGCTG |
| PB-84-27   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-28   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-29   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-30   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-31   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-32   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-33   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-34   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-35   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-36   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-37   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-38   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
| PB-84-39   | GGGTGCAAAGATTTTTCTTAACGTGATAATTATAATCTGGAGCCACGAACGGGGATTTTTGTCAGGCAAC |
CTCGGTCGCCGTTTTTCATACACTATATGCAGTGCTGTTTTTCCATAACCAT
AGAGAATTTCAGAATGACTTGTTGAGTAGCATGAGCAGTA
GATGCTGAAGATTTTTTCAATGATGATTGTTGCACTTTTAA
GAACGTGGGACAGTTGGTTACATCGAATTTTTCCCGGAA
CTTGAGAGCTGGATCTCAACAGCAGCTGAATCTG
TCTATACAACGAAACCTTGCACTCGTAAGATC
GAGTATTCAAACCCTGATAA
AAGAGTATATGCTTCAATAATATTACATGCGA
TGTGACCTAGAGAATAAGTAAATTGAAAAAGG
TTATCGGACCATTTTTTCGTGATTACGTTTTCTTAGATTTTCGTCAGGTGG
CTTCCACCGGCGCCGGTGGCTCAACAATCAAACGAAATGCC
AACTAAGAGAGTACAGCGA
TGCGCAGCAAGCTTTGCTAGGACAGGATTTTGGGACGACCTAGCA
GCTAACGCAACTCCCGGGTTGGTTCCAAGATGAGAAGTG
GATCGAAGGATTTTTTATGTGACGTAAGCAAGATATTTTTTTAATACCGT
TTCACGACGACAGTCTGGCAATACTAGTACAAGTAATATC
CTGGTTACTAGTTTTTGTACATGTTAATAGTTATGTGTTTTTTTGCAAGGTAG
TAACCTCAGGGAAGTTAGAA
AATTATTGAGTAGTACATAATT
### Pentagonal Bipyramid 45 mer eOD-GT8: modified staples list

| Staple name | Sequence |
|-------------|----------|
| PB-84-2-Over | ATAAAGTGGTTGGGCCTCTGGACATGGAGGTGGGCTTCAGGACTTG |
| PB-84-3-Over | TGGGCTGGGTCCTACAGGGAGAATTTGAAATGCGCCGGTACCTGGACTG |
| PB-84-4-Over | GCCGGCTGGGTCCTACAGGGAGAATTTGAAATGCGCCGGTACCTGGACTG |
| PB-84-6-Over | GTTGGTGGGTCCTACAGGGAGAATTTGAAATGCGCCGGTACCTGGACTG |
| PB-84-7-Over | CCGGGCTGGGTCCTACAGGGAGAATTTGAAATGCGCCGGTACCTGGACTG |
| PB-84-8-Over | TCTGACATATGGAGGCTGGGTCCTACAGGGAGAATTTGAAATGCGCCGGTACCTGGACTG |
| PB-84-10-Over | TACCTTGAAGGAACACACGAGGCCGGTACCTGGACTG |
| PB-84-11-Over | TTCAATTGATGGAGGCTGGGTCCTACAGGGAGAATTTGAAATGCGCCGGTACCTGGACTG |
| PB-84-12-Over | TCGGGCTGGGTCCTACAGGGAGAATTTGAAATGCGCCGGTACCTGGACTG |
| PB-84-13-Over | CGATGGGTCCTACAGGGAGAATTTGAAATGCGCCGGTACCTGGACTG |
| PB-84-14-Over | GCGAAAAAGAACGTGGACTCCAACAATCAGTATTACTGGACTG |
| PB-84-15-Over | CTTTCTTATATGGAGGCTGGGTCCTACAGGGAGAATTTGAAATGCGCCGGTACCTGGACTG |
| PB-84-16-Over | ECBCTTATATGGAGGCTGGGTCCTACAGGGAGAATTTGAAATGCGCCGGTACCTGGACTG |
| PB-84-17-Over | GAGATAGGATCAGCTCATTTTTTAACCAAAAAAGAATAGACCTTACTGGACTG |
| PB-84-18-Over | TATAAATCTAGGCCGAAATCGGCATGTTGTAGTTACTGGACTG |
| PB-84-19-Over | AAAGTCAATAGGAAGCCGATAAGAAATCCCTTTACTGGACTG |
| PB-84-20-Over | GGTTGTAAATGACCAAAATCCCTTAACGTTCATATGTACCCCTTACTGGACTG |
| PB-84-21-Over | GACCCCAAGAGTTTTCGTTCCACTCCCGTATTTTACTGGACTG |
| PB-84-22-Over | GACCGGGGTGTGGCGCGGTATTATGAGCGTCATTACTGGACTG |
| PB-84-23-Over | GGTGCCTCCGAAATAGACAGATCGAAAAGCATTTACTGGACTG |
| PB-84-24-Over | CTTACGGATACTACCACTCACGAGCTGAGATTTACTGGACTG |
| PB-84-25-Over | GTGCGCTTCCGAAATAGACAGATCGAAAAGCATTTACTGGACTG |
| PB-84-26-Over | CTATATGCTTCCGAAATAGACAGATCGAAAAGCATTTACTGGACTG |
| PB-84-27-Over | GGGTGTCCTTCCGAAATAGACAGATCGAAAAGCATTTACTGGACTG |
| PB-84-28-Over | GGTTGTAAATGACCAAAATCCCTTAACGTTCATATGTACCCCTTACTGGACTG |
| PB-84-29-Over | GACCCCAAGAGTTTTCGTTCCACTCCCGTATTTTACTGGACTG |
| PB-84-30-Over | GACCGGGGTGTGGCGCGGTATTATGAGCGTCATTACTGGACTG |
| PB-84-31-Over | GGTGCCTCCGAAATAGACAGATCGAAAAGCATTTACTGGACTG |
| PB-84-32-Over | CTTACGGATACTACCACTCACGAGCTGAGATTTACTGGACTG |
| PB-84-33-Over | GGTTGTAAATGACCAAAATCCCTTAACGTTCATATGTACCCCTTACTGGACTG |
| PB-84-34-Over | GGTTGTAAATGACCAAAATCCCTTAACGTTCATATGTACCCCTTACTGGACTG |
| PB-84-35-Over | GACCGGGGTGTGGCGCGGTATTATGAGCGTCATTACTGGACTG |
| PB-84-36-Over | GACCGGGGTGTGGCGCGGTATTATGAGCGTCATTACTGGACTG |
| PB-84-37-Over | GACCGGGGTGTGGCGCGGTATTATGAGCGTCATTACTGGACTG |
| PB-84-38-Over | GACCGGGGTGTGGCGCGGTATTATGAGCGTCATTACTGGACTG |
| PB-84-39-Over | GACCGGGGTGTGGCGCGGTATTATGAGCGTCATTACTGGACTG |
| PB-84-40-Over | GACCGGGGTGTGGCGCGGTATTATGAGCGTCATTACTGGACTG |
| PB-84-41-Over | GACCGGGGTGTGGCGCGGTATTATGAGCGTCATTACTGGACTG |
| PB-84-42-Over | GACCGGGGTGTGGCGCGGTATTATGAGCGTCATTACTGGACTG |
| PB-84-43-Over | GACCGGGGTGTGGCGCGGTATTATGAGCGTCATTACTGGACTG |
{ suppressed }
### Pentagonal Bipyramid 10 mer eOD-GT8: modified staples list

| Staple name | Sequence |
|-------------|----------|
| PB-84-3-Over | TGCGCTCGCGCTACAGGGGCACATTAAGCAGGACCACTTATTACTGGACTG |
| PB-84-8-Over | TCTGACATGTCCGGTCCCAGGAAAGGAGTTACTGGACTG |
| PB-84-12-Over | TCTAGGTGAATCAAGTTTTTTGGGGTCGATAATTTAAAAGGATTACTGGACTG |
| PB-84-19-Over | AAAGTCAATAGGAAGCCGCATAAGAAATCCCTTTACTGGACTG |
| PB-84-25-Over | GGTGCCCTCCGAATAGACAGATCAGAAAAACATTTACTGGACTG |
| PB-84-29-Over | CTGATGACCGCCCTTTGCGATGTGGGGGGCCAGATTTACTGGACTG |
| PB-84-46-Over | TCTATACACGGAAACCTTGCACCTCAGATCCTTACTGGACTG |
| PB-84-48-Over | AAGAGATATGCTGGTGTAATAATATAGATGCCGATTACTGGACTG |
| PB-84-52-Over | TTGGTCGTCTGATACCCCTATTTTTTATAAAAAGAAAAAGGGTTACTGGACTG |
| PB-84-60-Over | CTATGCTCATAGAAGGCGTGTATTACCCCTTTACTGGACTG |
## Supplementary Table 17. ssDNA linker scaffold sequences.

| Name     | Sequence                                                                 |
|----------|--------------------------------------------------------------------------|
| Linker 5 | TTACTGGACTGTA AAAA ATTACTGGACTG                                         |
| Linker 12| TTACTGGACTGTTAAAAAAAAAAAA ATTACTGGACTG                                   |
| Linker 24| TTACTGGACTGTTAAAAAAAAAAAAA ATTACTGGACTG                                  |
| Linker 35| TTACTGGACTGTTAAAAAAAAAAAAA CCTGCAGGCATGCAAGCTTG GCAAAAAA ATTACTGGACTG  |
| Linker 47| TTACTGGACTGTTAAAAAAAAACCTGCAGGCATGCAAGCTTG GCACTGGCCGTCGTTAAAA ATTACTGG |
| Linker 83| TTACTGGACTGTTAAAAAAACCTGCAGGCATGCAAGCTTG GCACTGGCCGTCGTTTTTACAACCTGCGT |
|          | ACTCCCTGGCGTTACCCAAAAAATTACTGGACTG                                     |
Supplementary Table 18. P-values for Supplementary Fig. 17.

| Supplementary Fig 17b | 6HB-2x-7nm | 6HB-2x-28nm | Ico-30x | PBS |
|-----------------------|------------|-------------|--------|-----|
| Row                   |            |             |        |     |
| 6HB-2x-7nm            | NaN        | 0.9999      | 0.0062 | 0.0003 |
| 6HB-2x-28nm           | 0.9999     | NaN         | 0.0067 | 0.0003 |
| Ico-30x               | 0.0062     | 0.0067      | NaN    | <0.0001 |
| PBS                   | 0.0003     | 0.0003      | <0.0001 | NaN   |
# Supplementary Table 19. P-values for Supplementary Fig. 18.

| Supplementary Fig. 18b | Row | Ico-5x-3nm | Ico-5x-11nm | Ico-5x-15nm | Ico-5x-22nm | 6HB-5x-7nm | 6HB-5x-11nm | 6HB-5x-14nm | 6HB-5x-17nm |
|------------------------|-----|------------|-------------|-------------|-------------|------------|------------|------------|------------|
| Ico-5x-3nm             | 0.076169193 | 0.031459828 | 0.003011634 | 0.0060934  | 0.001818299 | 0.00699654  | 0.003652631 | 0.039674562 | 0.19798803  |
| Ico-5x-11nm            | 0.03365109  | 0.0086987514 | 0.22702024 | 0.464351908 | 0.48692314 | 0.014636992 | 0.014636992 | 0.049869231 | 0.032400632 |
| Ico-5x-15nm            | 0.029739739 | 0.0080018514 | 0.471609449 | 0.047160949 | 0.03965109  | 0.030624621 | 0.030624621 | 0.008038514 | 0.02869176  |
| Ico-5x-22nm            | 0.00311634  | 0.0080018514 | 0.047160949 | 0.03965109  | 0.48692314 | 0.030624621 | 0.030624621 | 0.008038514 | 0.02869176  |
| 6HB-5x-7nm             | 0.031459828 | 0.22702024 | 0.464351908 | 0.047160949 | 0.03965109  | 0.030624621 | 0.030624621 | 0.008038514 | 0.02869176  |
| 6HB-5x-11nm            | 0.00311634  | 0.0080018514 | 0.22702024 | 0.464351908 | 0.48692314 | 0.030624621 | 0.030624621 | 0.008038514 | 0.02869176  |
| 6HB-5x-14nm            | 0.000181289 | 0.0086987514 | 0.030624621 | 0.19798803  | 0.030624621 | 0.030624621 | 0.030624621 | 0.030624621 | 0.030624621 |
| 6HB-5x-17nm            | 0.000181289 | 0.0086987514 | 0.030624621 | 0.19798803  | 0.030624621 | 0.030624621 | 0.030624621 | 0.030624621 | 0.030624621 |
| Ico-0x                 | 0.001800419 | 0.000181289 | 0.010591222 | 0.007096498 | 0.00133615  | 0.000510378 | 0.000510378 | 0.000510378 | 0.000510378 |
Supplementary Table 20. P-values for Supplementary Fig. 23a.

| Row | 6HB-2x-7nm control | 6HB-2x-28nm control | ico-30x control | 6HB-2x-7nm 1min | 6HB-2x-28nm 1min | ico-30x 1min | 6HB-2x-7nm 5min | 6HB-2x-28nm 5min | ico-30x 5min | 6HB-2x-7nm 30min | 6HB-2x-28nm 30min | ico-30x 30min |
|-----|---------------------|---------------------|----------------|----------------|----------------|-------------|----------------|----------------|-------------|----------------|----------------|-------------|
| 1   | 1                   | 1                   | 0.713198       | 0.713198       | 0.713198       | 0.713198    | 0.713198       | 0.713198       | 0.713198    | 0.713198       | 0.713198       | 0.713198    |
| 2   | 0.002158            | 0.002158            | 0.000011       | 0.000002       | 0.000011       | 0.000011    | 0.000002       | 0.000011       | 0.000011    | 0.000002       | 0.000002       | 0.000002    |
| 3   | 0.187171            | 0.187171            | 0.240858       | 0.240858       | 0.240858       | 0.240858    | 0.240858       | 0.240858       | 0.240858    | 0.240858       | 0.240858       | 0.240858    |
| 4   | 0.063638            | 0.063638            | 0.140313       | 0.140313       | 0.140313       | 0.140313    | 0.140313       | 0.140313       | 0.140313    | 0.140313       | 0.140313       | 0.140313    |
| 5   | 0.010611            | 0.010611            | 0.082365       | 0.082365       | 0.082365       | 0.082365    | 0.082365       | 0.082365       | 0.082365    | 0.082365       | 0.082365       | 0.082365    |
| 6   | 0.007832            | 0.007832            | 0.06433        | 0.06433        | 0.06433        | 0.06433     | 0.06433        | 0.06433        | 0.06433    | 0.06433        | 0.06433        | 0.06433    |
| 7   | 0.004596            | 0.004596            | 0.002741       | 0.002741       | 0.002741       | 0.002741    | 0.002741       | 0.002741       | 0.002741    | 0.002741       | 0.002741       | 0.002741    |
| 8   | 0.000006            | 0.000006            | 0.000006       | 0.000006       | 0.000006       | 0.000006    | 0.000006       | 0.000006       | 0.000006    | 0.000006       | 0.000006       | 0.000006    |
Supplementary Table 21. P-values for Supplementary Fig. 23b.

| Row          | 6HB-2x-7nm control | 6HB-2x-28nm control | co-30x control | 6HB-2x-7nm 1min | 6HB-2x-28nm 1min | co-30x 1min | 6HB-2x-7nm 5min | 6HB-2x-28nm 5min | co-30x 5min | 6HB-2x-7nm 30min | 6HB-2x-28nm 30min | co-30x 30min |
|--------------|--------------------|--------------------|---------------|----------------|----------------|-------------|----------------|----------------|-------------|----------------|----------------|-------------|
| 6HB-2x-7nm control | 1                  | 1                  | 0.003383      | 0.000204      | 0.000006      | 0           | 0              | 0              | 0           | 0              | 0              | 0           |
| 6HB-2x-28nm control | 0.003383 | 0.003383 | 1              | 0.003383      | 0.00295       | 0.00009     | 0              | 0              | 0           | 0              | 0              | 0           |
| co-30x control | 0.003383 | 0.003383 | 0.00204        | 0.000006      | 1              | 0.00011     | 0              | 0              | 0           | 0              | 0              | 0           |
| 6HB-2x-7nm 1min | 0.000204 | 0.000204 | 0.001941       | 0.265912      | 0.069615      | 0.001196    | 0              | 0.000006      | 0           | 0              | 0              | 0           |
| 6HB-2x-28nm 1min | 0.000006 | 0.000006 | 0.000011       | 0.000006      | 0.617796      | 0.886269    | 0              | 0.14963        | 0.01779     | 0              | 0              | 0           |
| co-30x 1min    | 0.089615 | 0.089615 | 0.141963       | 0.866596      | 0.141963      | 0.352378    | 0              | 0.01779        | 0.000011    | 0              | 0              | 0           |
| 6HB-2x-7nm 5min | 0.000118 | 0.000118 | 0.000003       | 0.000003      | 0.000003      | 0.000003    | 0              | 0.000003      | 0           | 0              | 0              | 0           |
| 6HB-2x-28nm 5min | 0.000118 | 0.000118 | 0.000003       | 0.000003      | 0.000003      | 0.000003    | 0              | 0.000003      | 0           | 0              | 0              | 0           |
| co-30x 5min    | 0.000118 | 0.000118 | 0.000003       | 0.000003      | 0.000003      | 0.000003    | 0              | 0.000003      | 0           | 0              | 0              | 0           |
| 6HB-2x-7nm 30min | 0.000118 | 0.000118 | 0.000003       | 0.000003      | 0.000003      | 0.000003    | 0              | 0.000003      | 0           | 0              | 0              | 0           |
| 6HB-2x-28nm 30min | 0.000118 | 0.000118 | 0.000003       | 0.000003      | 0.000003      | 0.000003    | 0              | 0.000003      | 0           | 0              | 0              | 0           |
| co-30x 30min   | 0.000118 | 0.000118 | 0.000003       | 0.000003      | 0.000003      | 0.000003    | 0              | 0.000003      | 0           | 0              | 0              | 0           |
Supplementary Table 22. P-values for Supplementary Fig. 23c.

| Row            | 6HB-2x-7nm control | 6HB-2x-28nm control | ico-30x control | 6HB-2x-7nm 1min | 6HB-2x-28nm 1min | ico-30x 1min | 6HB-2x-7nm 5min | 6HB-2x-28nm 5min | ico-30x 5min | 6HB-2x-7nm 30min | 6HB-2x-28nm 30min | ico-30x 30min |
|----------------|--------------------|---------------------|----------------|----------------|----------------|-------------|----------------|----------------|-------------|----------------|----------------|-------------|
| 6HB-2x-7nm control | 1                  | 1                   | 0.005992       | 0.761281       | 0.042868       | 0.007829    | 0.12123       | 0.036727       | 0.002334    | 0.107816       | 0.287676       | 0.289732    |
| 6HB-2x-28nm control | 1                  | 1                   | 0.005992       | 0.761281       | 0.042868       | 0.007829    | 0.12123       | 0.036727       | 0.002334    | 0.107816       | 0.287676       | 0.289732    |
| ico-30x control   | 0.005992           | 0.005992            | 1              | 0.11614        | 0.644966       | 0.657305    | 0.264006      | 0.321065       | 0.70714     | 0.361906       | 0.076631       | 0.000305    |
| 6HB-2x-7nm 1min   | 0.761281           | 0.761281            | 0.011614       | 1              | 0.065448       | 0.012398    | 0.194184      | 0.067892       | 0.003777    | 0.168454       | 0.432416       | 0.157563    |
| 6HB-2x-28nm 1min  | 0.042868           | 0.042868            | 0.644966       | 0.005448       | 1              | 0.429847    | 0.563496      | 0.690172       | 0.423666    | 0.677585       | 0.244891       | 0.001632    |
| ico-30x 1min      | 0.007829           | 0.007829            | 0.657305       | 0.012398       | 0.429847       | 1           | 0.185128     | 0.191114       | 0.812695    | 0.255171       | 0.058407       | 0.000567    |
| 6HB-2x-7nm 5min   | 0.12123            | 0.12123             | 0.264006       | 0.104184       | 0.563496       | 0.185128    | 1             | 0.763754       | 0.152046    | 0.888485       | 0.574041       | 0.074056    |
| 6HB-2x-28nm 5min  | 0.036727           | 0.036727            | 0.321065       | 0.067892       | 0.690172       | 0.191114    | 0.763754      | 1              | 0.83684     | 0.903574       | 0.327207       | 0.001158    |
| ico-30x 5min      | 0.002334           | 0.002334            | 0.770714       | 0.003777       | 0.429847       | 0.812695    | 0.152046     | 0.163664       | 1           | 0.224367       | 0.003465       | 0.000007    |
| 6HB-2x-7nm 30min  | 0.107816           | 0.107816            | 0.361906       | 0.186454       | 0.677585       | 0.255171    | 0.888485      | 0.903574       | 0.24367    | 1              | 0.495438       | 0.013154    |
| 6HB-2x-28nm 30min | 0.287679           | 0.287679            | 0.076631       | 0.432416       | 0.244891       | 0.058407    | 0.574041      | 0.327207       | 0.00465    | 0.465438       | 0.010365       | 0.000007    |
| ico-30x 30min     | 0.289732           | 0.289732            | 0.903039       | 0.157605       | 0.001632       | 0.000567    | 0.014056      | 0.001158       | 0.000007    | 0.013154       | 0.000007       | 1           |
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