**Supplementary Figure 1. Mechanism for signal-induced opening of the DNA box.**

**a**, An atomic model of the DNA box held closed by "locks" (orange and blue) that are double helices formed by two short strands protruding from the lid and the main box, respectively. Each "lock" has a small sticky-end where a "key" sequence signal can bind and open the "lock" by strand displacement. If both "locks" are opened the lid of the box is effectively opened (b). The reporter system for detecting the lid opening is a Cy3-Cy5 FRET system. In the closed state the two fluorophores are in close proximity resulting in FRET emission from Cy5 (red star) when Cy3 is excited. In the open state the two flurophores are far apart and excitation of Cy3 only results in emission from Cy3 (green star).
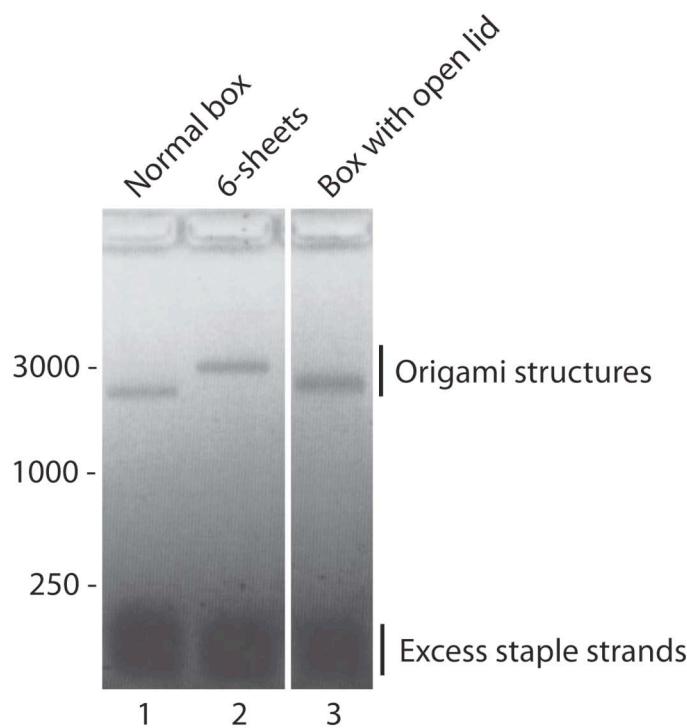
**Supplementary Figure 2. Native gel mobility assay.** The assembly reactions of the DNA box, 6-sheets, and box with one lid open were run on a native agarose gel containing 1 mM $MgCl_2$. The gel was ethidium bromide stained and imaged. Numbers on the left indicate a size marker for double-stranded DNA. The relative positions of the loading slot, the DNA origami structures, and the excess oligos are indicated on the right. Together, the migration pattern suggests an efficient formation of the DNA boxes: besides the band corresponding to the excess staple strands there is only a single sharp band in the DNA box sample migrating faster than both the 6-sheet particle and the open box.
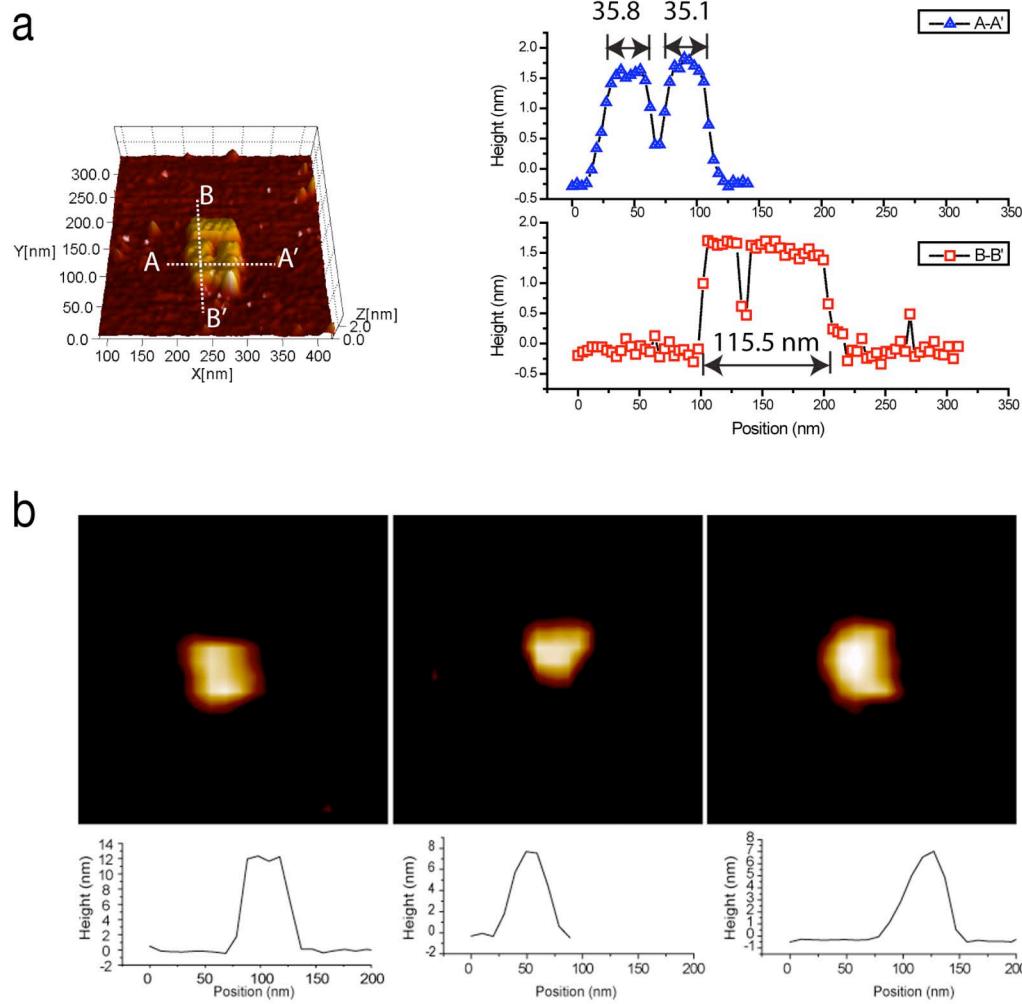
**Supplementary Figure 3. Height traces of the six sheets and the closed box. a**, 3D rendering of a single particle of the six faces without 3D links. Lines A-A' and B-B' show the height traces in the graphs to the right. Main dimensions are indicated with arrows. The measured dimensions are compatible with the expected sizes of the DNA sheets. The gap between the sheets measures approximately 14-16 nm, which is compatible with fully extended single-stranded linkers of 33 nucleotides. **b**, Three AFM images of DNA box particles with the corresponding height trace shown below.
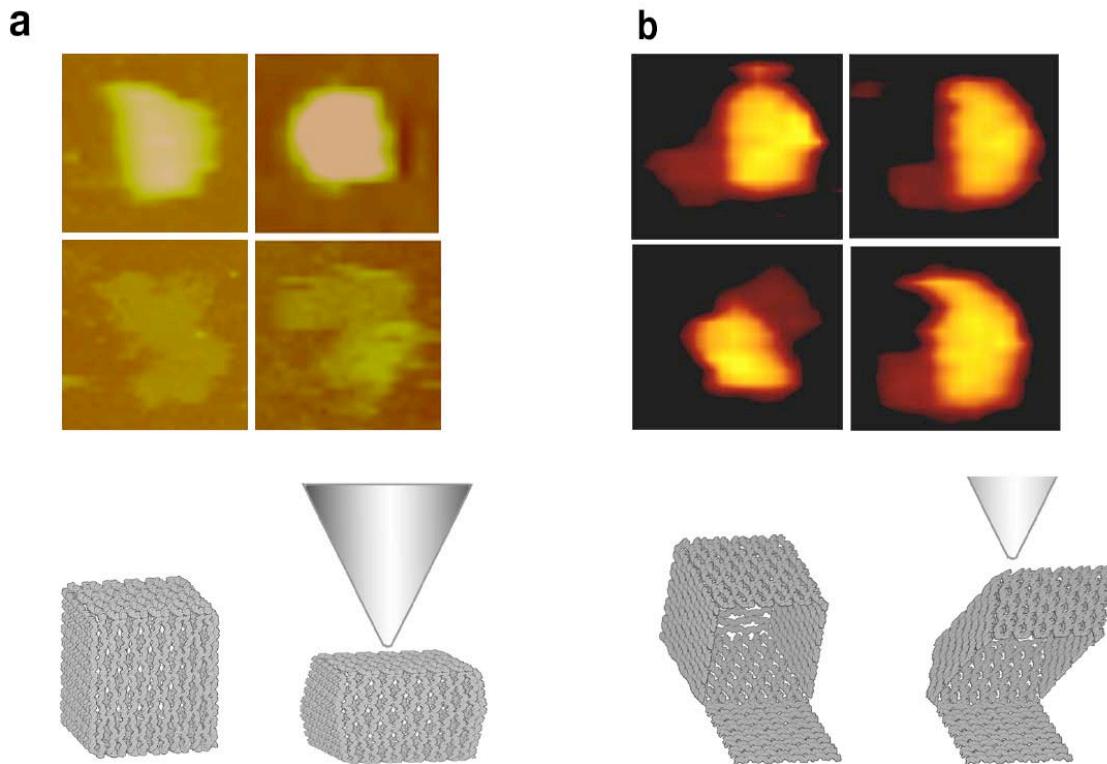
**Supplementary Figure 4. Collapse of box structure during AFM imaging. a**, AFM images of the DNA box. Two images on top show box-like dimensions. The two images below show particle-structures that upon AFM imaging changed shape into double- or single-layer DNA sheets. Cartoon below illustrate how the AFM tip might distort the soft and hollow DNA box. **b**, Four representative images of DNA box particles with an open lid. The cartoon below illustrates how the elongated main part of the particle is likely the result of a sideway collapse of main box, while the lid is laying flat on the surface.
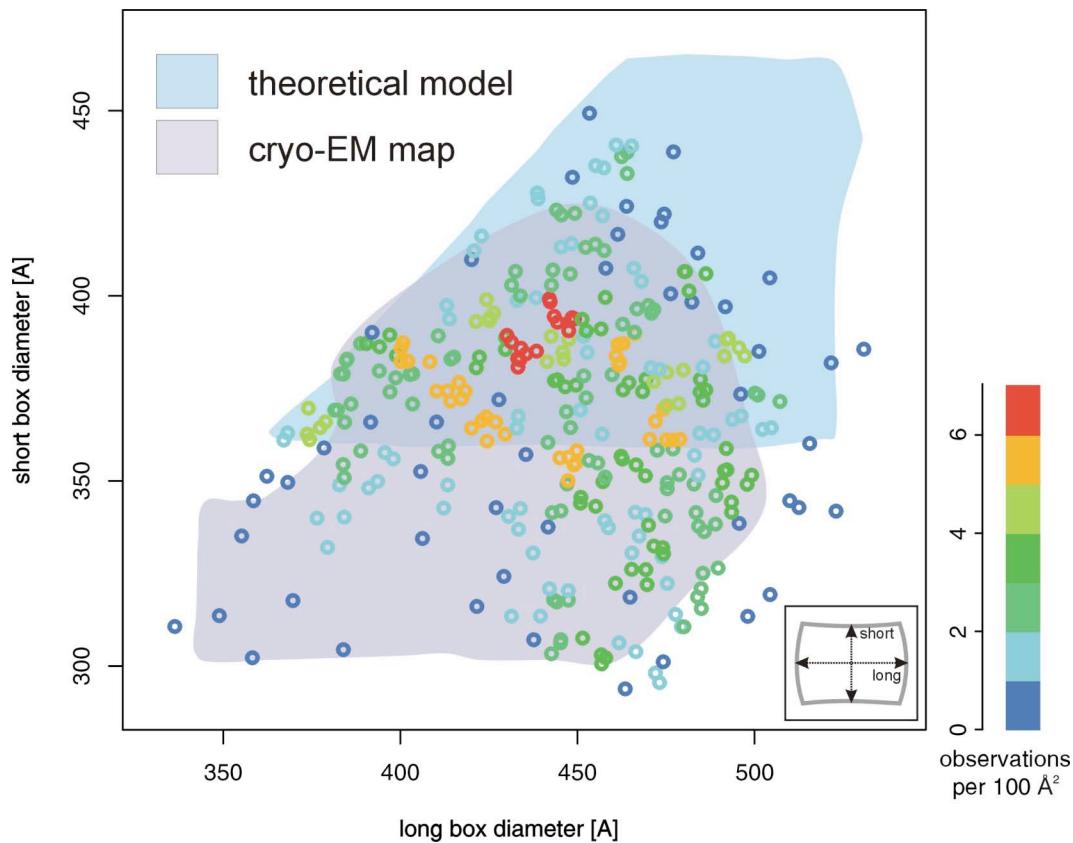
**Supplementary Figure 5. Distribution of box dimensions as determined by cryo-EM.** Distribution of diameters of 337 class averages of the DNA boxes with well visible edge signal. The central long and the central short diameters are plotted using the number of dots per area for color-coding as shown on the right (blue, rare observations; red, frequent observations; for details of the measurements, see inset). The purple area shows the theoretical distribution of diameters of reprojections from the cryo-EM 3D reconstruction assuming even angular distribution. Correspondingly, the light blue area shows the theoretical distribution of diameters of reprojections from the theoretical model. Overall, 60.5% of the cryo-EM class averages exhibit dimensions compatible with the theoretical model. 81.6% of the cryo-EM class averages exhibit dimensions compatible with the experimental cryo-EM map.
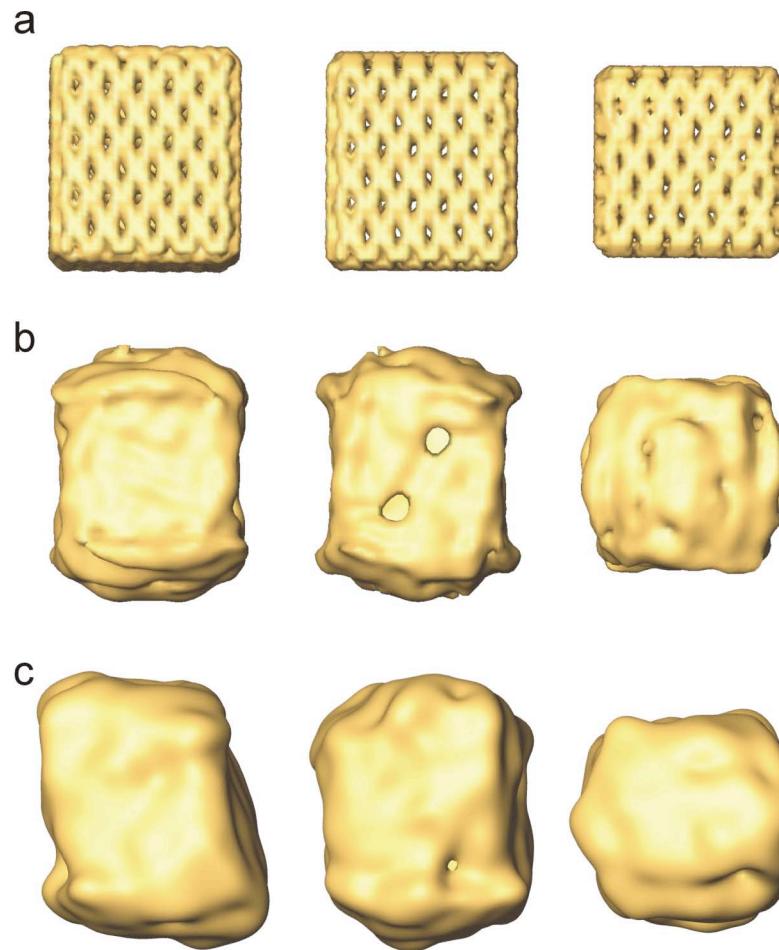
**Supplementary Figure 6. Single-particle cryo-EM reconstruction.** Surface view of the theoretical DNA box model (**a**) and the cryo-EM reconstruction applying D2 symmetry (**b**) and no symmetry (i.e. C1) (**c**) seen from the front (left), from the side (middle) and from the top (right).
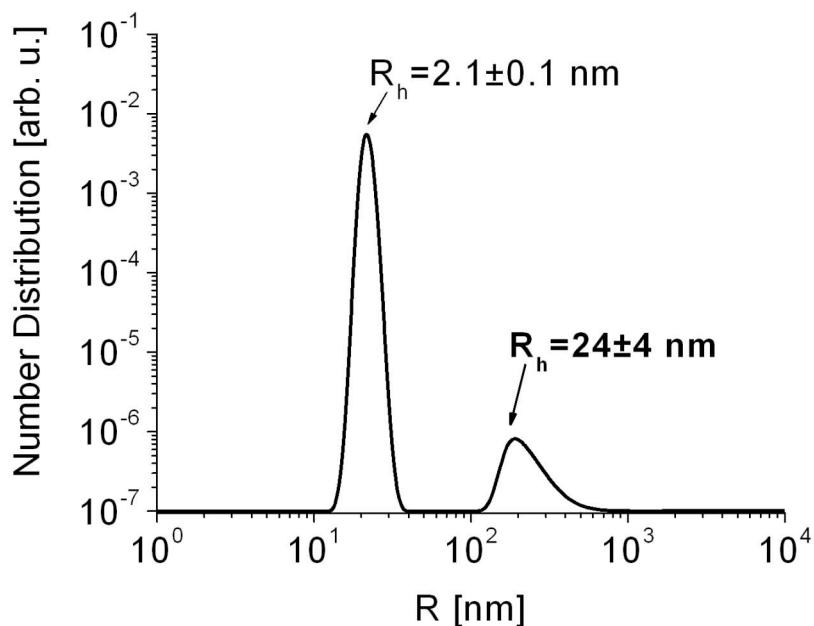
**Supplementary Figure 7. DLS measurements.** Results from dynamic light scattering (DLS) measurements. The obtained value of the hydrodynamic radius for the box ($24 \pm 4$ nm) is very similar to the one expected for the atomic based model (25.2 nm). However, the peak is broad, probably indicating the presence of deviating shapes of the DNA boxes. The DLS also reveals the presence of smaller structures corresponding to the excess oligos (2.1 nm).
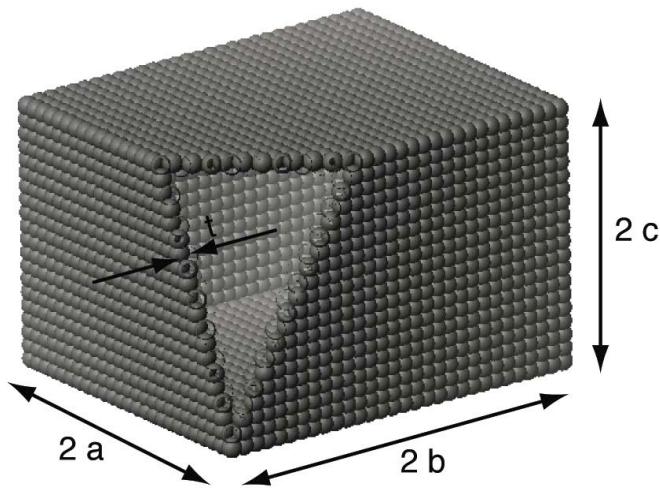
**Supplementary Figure 8. SAXS box model.** The SAXS box model with adjustable dimensions, $2a$, $2b$, $2c$, and wall thickness, $t$.
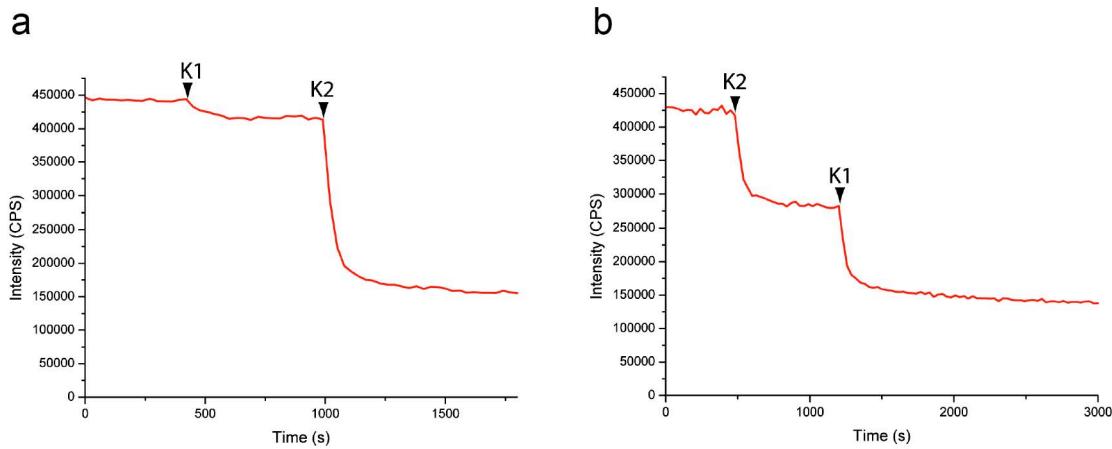
**Supplementary Figure 9. Kinetic study of the sequential addition of key oligos. a**, Key 1 was added after 500 s and key 2 after 1000s. **b**, Key 2 was added after 500 s and Key 1 after 1200 s. The time of addition is indicated with an arrow on the graph.

## Supplementary Note 1. Details of the design process

The DNA origami design software program with documentation and tutorials is available here: http://www.cdna.dk/origami/. A folding path of the circular M13 single-stranded DNA was devised through the six faces of a box. This is done as an interrupted DNA origami sheet for face A, B, C. The latter is linked to face D that by a central seam returns to the adjacent edge. Face E is folded similar to face A, B, and C, and extends into lid F that is folded as face D. The M13 backbone strand connects face F and A. These links are compatible with the folding of a 3D box.

First, bitmap files for the six faces are composed with dimensions as described previously[13] and the size was adjusted to use as many of the 7,249 nucleotides of the m13mp18 sequence as possible (1232 nts for face A, B, C, E and 1056 nts for face D and F, giving a total of 7040 nts). The design leaves 209 nts unpaired, or 34 nts for each of the 6 linkers between faces. The dimensions of face A, B, C, E are 38.08 x 32 $nm^2$ and the dimensions of face D and F are almost a square (32.6 x 32 $nm^2$).

Next the actual folding of the m13mp18 single stranded DNA is performed using the `DNA-origami-fold` script with the following parameters:

| Faces | file | -B | -S | -H | -P | -O |
|-------|------|-----|-----|-----|-----|-----|
| ACE | face.bmp | 15 | no | left | 5p | 7.5 |
| B | face.bmp | 15 | no | right | 5p | 7.5 |
| DF-left | lid-left.bmp | 15 | right | right | 3p | 23.5 |
| DF-right | lid-right.bmp | 15 | left | left | 5p | 23.5 |

Where option -B is the line from where to start folding. -S is the option to make a left or right or no seam. -H is the option to start folding from left or right. -P is the option to start the folding with a 5' or 3' end of the DNA. Option -O sets the origin of staple strand cross-overs in the plane of the DNA origami sheet. After the initial folding

the design was modified in the editor software to insert T-loops and extensions at the staple strands forming the edges.

The faces were inserted on the m13mp18 sequence using the CLCbio workbench (http://www.clcbio.com). The thermodynamically stable DNA hairpin mentioned previously[2] was placed at the linker between face A and B, which left 33 bases for the remaining linkers. The sequence regions are then exported and used for primer design of staple strands. To `pdbgenerator` script was used to produce molecular models of the six faces, which were subsequently oriented to form the box using the PyMol program (http://pymol.sourceforge.net/). Finally, the `oligotrack` program is run with the sequence files exported from the CLCbio workbench and produces a representation within the SARSE editor and an oligo list for the DNA synthesis.

## Supplementary Note 2. Design of oligos for the six faces

The designed staple strands are listed below with the faces of the box color-coded. Face A (red), face B (green), face C (blue), face D (yellow), face E (purple), face F (orange). To facilitate the editing of the final design it is convenient to use color-coding of the staple strands. T-loops and T-extensions are colored black.

### 2D staple strands:

### Face A

```
A-13,118      ATACAGTAACAGTACCTTAACGTCAGATGAATTTTTTTGAGTAA
A-17,14       ATTACCTGTTTTGGATTATACTTCTGAAATAATCCT
A-17,46       CAAAATCGACCTACCATATCAAAATCCTGATT
A-17,78       GGATTCGCCAGAAATAAAGAAATTAACCACCA
A-23,31       GGGTTAGACGCAGAGGCGAATTATTCATTTCA
A-23,63       ACGTAAAACTGATTGCTTTGAATACCAAGTTA
A-23,95       TTTCAGGTTTTTACATCGGGAGAAACAATAAC
A-37,14       GATTGTTTTTTTGATTAGAGCCGTCAATGCACTAAC
A-37,46       ATCAGATGGATTTAGAAGTATTAGATTGAGGA
A-37,78       GAAGGAGCGACAACTCGTATTAAACTGGTCAG
A-37,110      CATTATCATATTAATTTTAAAAGTTTTTTGAACCTC
A-43,31       CATTTGAGATGGCAATTCATCAATTAATGGAA
A-43,63       AACAATTCGGAATTATCATCATATTTATTTGC
A-43,95       CCGAACGTTTTTGCGGAACAAAGAGCGTAGAT
A-57,14       AACTAATATTTTCGAACCACCAGCAGAACATTAAAA
A-57,46       AGGTTATCGGCGGTCAGTATTAACTAATGCGC
A-57,78       TTGGCAAACCACGCTGAGAGCCAGACAGACAA
A-57,110      AAATATCATAAAGCATCACCTTGCTTTTCCTTCTGA
A-63,31       AGAGGTGATAAAATATCTTTAGGAAGATAATA
A-63,63       CAACAGTGTCAACAGTTGAAAGGAACTTTACA
A-63,95       GAAAAATCAACCCTCAATCAATATTCCTTTGC
A-77,14       ATACCGAATTTTGAAATACCTACATTTTAGGAAAAA
A-77,46       GAACTGATAAATGGATTATTTACATCCAGAAC
A-77,78       TATTTTTGTCACACGACCAGTAATAAGAACTC
A-77,110      CCTGAAAGCCAACAGAGATAGAACTTTTTTGATTAG
A-83,31       ATCGTCTGAGCCCTAAAACATCGCGATAAAAC
A-83,63       TTCACCAGAATGGCTATTAGTCTTACCGCCTG
A-83,95       CATTCTGGCGTAAGAATACGTGGCCAGCAAAT
A-97,14       CGCTCATGTTTTTTTTAGACAGGAACGGAGGCCGATTAAAGGGATT
A-97,46       AATATTACGAAGTGTTTTTATAATCCTCGTTA
A-97,78       AAACTATCTAAAAGAGTCTGTCCAGGTTGCTT
A-97,110      TAATAACATTGTAGCAATACTTCTTTTTCGCTTAAT
A-103,31      AATCCTGACGCCAGCCATTGCAACGACGCTCA
A-103,63      CCACCGAGGGCCTTGCTGGTAATATTGGCAGA
A-103,95      ATTAACCGTCACTTGCCTGAGTAGAAAAGGGA
A-117,46      GAATCAGAGCGGGAGCTAAACAGGTACGCCAG
A-117,78      TGACGAGCACGTATAACGTGCTTTCAGTGAGG
A-117,110     GCGCCGCTACAGGGCGCGTACTATTCACGCAA
```

### Face B

```
B-13,31       GCCCCCGATTTAGAGCTTGACGGGGTGAACCA
B-13,63       GCGAACGTGGCGAGAAAGGAAGGGAAAAACCG
B-13,7        TTCACTAAATCGGAACCCTCGAGGTG
B-13,95       GAAAGGAGCGGGCGCTAGGGCGCTTTTTTTAAAGAA
```

```
B-27,14     CCGTAAAGTTTTGGCGAAAATCCTGTTTCTGGTTTG
B-27,46     TCACCCAATCGGCAAAATCCCTTAGGCCCTGA
B-27,78     TCTATCAGCCCGAGATAGGGTTGAGACGGGCA
B-27,110    CGTGGACTGAACAAGAGTCCACTATTTTGGCGCCAG
B-33,31     TTCCGAAAATCAAGTTTTTTGGGGTAAAGGGA
B-33,63     AAGAATAGGGCGATGGCCCACTACGAAAGCCG
B-33,95     CCAGTTTGCCAACGTCAAAGGGCGAAGAAAGC
B-47,14     CCCCAGCATTTTTAACTCACATTAATTGTGCCTAAT
B-47,46     GAGAGTTGCGCTTTCCAGTCGGGACGAGCCGG
B-47,78     ACAGCTGATGCATTAATGAATCGGATTGTTAT
B-47,110    GGTGGTTTGGCGGTTTGCGTATTGTTTTGTAATCAT
B-53,31     TCACTGCCCAGCAAGCGGTCCACGGATGGTGG
B-53,63     GTGCCAGCTTGCCCTTCACCGCCTTAAATCAA
B-53,95     CGGGGAGATTCTTTTCACCAGTGAGTGTTGTT
B-67,14     GAGTGAGCTTTTTTGGGTAACGCCAGGGCTGCAAGG
B-67,46     AAGCATAAGTTGTAAAACGACGGCGCTATTAC
B-67,78     CCGCTCACTGCCTGCAGGTCGACTTGTTGGGA
B-67,110    GGTCATAGTACCGAGCTCGAATTCTTTTCAAAGCGC
B-73,31     GTCACGACAGTGTAAAGCCTGGGGCGTTGCGC
B-73,63     AGCTTGCAAATTCCACACAACATAAACCTGTC
B-73,95     TCCCCGGGCTGTTTCCTGTGTGAACCAACGCG
B-87,14     CGATTAAGTTTTGGCGCATCGTAACCGTCACGTTGG
B-87,46     GCCAGCTGGGGGACGACGACAGTAACAAACGG
B-87,78     AGGGCGATCGCACTCCAGCCAGCTGCGAGTAA
B-87,110    CATTCGCCGGTGCCGGAAACCAGGTTTTCTGTAGCC
B-93,31     CAGTTTGAGCGAAAGGGGGATGTGTTTTCCCA
B-93,63     AGGAAGATCGGTGCGGGCCTCTTCCAGTGCCA
B-93,95     CCGCTTCTATTCAGGCTGCGCAACCTAGAGGA
B-107,14    TGTAGATGTTTTATTTAAATTGTAAACGTTAATATT
B-107,46    CGGATTGAATTCGCATTAAATTTTTGTTAAAT
B-107,78    CAACCCGTTTTTTAACCAATAGGAACGCCATC
B-107,110   AGCTTTCATTCGCGTCTGGCCTTCTT
B-113,31    TTGTTAAACCGTAATGGGATAGGTGCATCTGC
B-113,63    CAGCTCATCGGATTCTCCGTGGGATCGGCCTC
B-113,95    AAAAATAATCAACATTAAATGTGATTCCGGCA
```

## Face C

```
C-13,118    TTTACAAAGGCTATCAGGCTATTTTTGAGAGATCTTTTACATTATG
C-17,14     ATCAGAAATTTTAAGATTCAAAAGGGTGGAGTAATG
C-17,46     TAGCATGTGTCAAATCACCATCAATAGAACCC
C-17,78     ACAAGAGATCTAGCTGATAAATTAAGCCTTTA
C-17,110    CTATCAGGCTATTTTTGAGAGATCTTTTACATTATG
C-23,31     CGGAGACACAATCATATGTACCCCGGTTGATA
C-23,63     TCAACCGTATCGATGAACGGTAATCGTAAAAC
C-23,95     GAGGGTAGTCATTGCCTGAGAGTCTGGAGCAA
C-37,14     TGTAGGTATTTTTCTACTAATAGTAGTAAAAAGGTG
C-37,46     TCATATATAATCATACAGGCAAGGTTTAGCTA
C-37,78     TTTCAACGTTAAGCAATAAAGCCTTTAGATAC
C-37,110    ACCCTGTAATCGGTTGTACCAAAATTTT-AATTCTGC
C-43,31     ATCCAATATTTAAATGCAATGCCTAGAAAGGC
C-43,63     TAGCAAAACAAGGATAAAAATTTTTATGATAT
C-43,95     AAAGCTAAATACTTTTGCGGGAGAATGCCGGA
C-57,14     GCATCAATTTTTGCGGATGGCTTAGAGCATAAGAGG
C-57,46     TATTTTCAATGCTGTAGCTCAACAGGATTAGA
C-57,78     ATTTCGCACTAAAGTACGGTGTCTGAACCAGA
C-57,110    GAACGAGTATAACAGTTGATTCCCTTTTCAAATATC
C-63,31     TGAATATATTTGGGGCGCGAGCTGGCATTAAC
C-63,63     ATATGCAAAATGGTCAATAACCTGCAAAGAAT
C-63,95     CATTCCATAGATTTAGTTTGACCACAGAGCAT
C-77,14     TCATTTTTTTTTGTTCAGAAAACGAGAATCAAATGC
C-77,46     GAGTACCTATCAGGTCTTTACCCTCGGAATCG
C-77,78     CCGGAAGCAGCAAAGCGGATTGCAAAAATGTT
C-77,110    GCGTTTTAGAAGCCCGAAAGACTTTTTTGCAAAAGA
C-83,31     AATCAAAATTAATTGCTCCTTTTGTTAATTGC
C-83,63     TAGTCAGAAAACTCCAACAGGTCATGTTTTAA
C-83,95     ATTAAGAGATTCGAGCTTCAAAGCGGAAGTTT
```

```
C-97,14      TTTAAACATTTTAACTAATGCAGATACATAGGAATACCACATTC
C-97,46      TCATAAATTACGAGGCATAGTAAGATTACAGG
C-97,78      TAGACTGGCCCTCGTTTACCAGACACGTTAAT
C-97,110     AGTTTTGCATAGCGAGAGGCTTTTTTTTTATACCAG
C-103,31     AAAGGAATATTCATTGAATCCCCCTGACCATA
C-103,63     TATCATAAATAGCGTCCAATACTGGACTATTA
C-103,95     AAACCAAACAGAGGGGGTAATAGTTCAAAAG
C-117,46     TAGAAAGATTCATCAGTTGAGATTTAACGCCA
C-117,78     AAAACGAACTAACGGAACAACATTAGCAACAC
C-117,110    TCAGGACGTTGGGAAGAAAAATCTGACGATAA
```

## Face D

```
D-13,102     TTGCAGGGAGTTAAAGATTCGGTCGCTGAGGCTTTTCGAGGTGA
D-17,30      AGCAACGGATGAGGAAGTTTCCATGGCACCAA
D-17,62      TCAGCAGCGAAAGACAGCATCGGAACGAGGGT
D-23,15      ACTTTTTCCTACAGAGGCTTTGAG
D-23,47      TAAAATACGACAACAACCATCGCCTAGTTGCG
D-23,79      ACCGATATGCCGCTTTTGCGGGATCGTCACCC
D-37,30      CCTAAAACTCTTTGACCCCCAGCGGATTTGTA
D-37,62      CCGACAATGTAATGCCACTACGAATAAACGGG
D-37,94      ATTTCTTATTTATCAGCTTGCTTTTTTTAAAGGAAT
D-43,15      AACACTCAGAAAGAGGCAAAAGAATTTTGACTAAAG
D-43,47      AAGCGCGAAAGGCTCCAAAAGGAGGAAAATCT
D-43,79      TGTATCGGAACAGCTTGATACCGACACGCATA
D-57,30      TCATCGCCCATGTTACTTAGCCGGCGAACTGA
D-57,62      CCAAAAAAAACAAAGTACAACGGAATTATACC
D-57,94      TGCGAATAATAGAAAGGAACAACTTTTTTCGTCTTT
D-63,15      ACCTGCTCTGATAAATTGTGTCGATTTTTACACTAA
D-63,47      GCAGACGGAAACAACTTTCAACAGTGTATGGG
D-63,79      GAGTGAGAATAATTTTTTCACGTTCCTTTAAT
D-77,30      CCAACTTTGCGCATAGGCTGGCTGGATATTCA
D-77,62      ATTTTGCTTCAATCATAAGGGAACAACGAGGC
D-77,94      CCAGACGTACGATCTAAAGTTTTGTTTTGTACCGTA
D-83,15      CAGACCAGGAAAGAGGACAGATGATTTTAATCCGCG
D-83,47      CAAGAGTAGCATTCCACAGACAGCAACTACAA
D-83,79      TTAGCGTATAGTAAATGAATTTTCTTTCAGCG
D-97,30      TTACCCAAAAGGCTTGCCCTGACGGAGATGGT
D-97,62      CGCCTGTAATCTTGACAAGAACCGACCTTCAT
D-97,94      ACACTGAGCCCAATAGGAACCCATTTTTTTAGTACC
D-103,15     CAGTGAATATCAACGTAACAAAGCTTTTACGGTGTA
D-103,47     CAGAACGAGAGCCACCACCCTCATAGAACCGC
D-103,79     ATAGCAAGTTTCGTCACCAGTACACCTCATAG
D-117,30     TTAATTTCAACTTTAATCATTGTGTTTTTGCTCATT
D-117,62     CACCCTCAGTAGTAAATTGGGCTTAGAAACAC
D-117,94     GCCACCCTCAGAACCGCCACCCTCTTTCAGGG
```

## Face E

```
E-13,118     AAACATGAAAGTATTAGGAACCTATTATTCTGTTTTGGCAGGTC
E-17,14      AAGTATAGTTTTTTGATGATACAGGAGTGCGTCATA
E-17,46      AGGCGGATTTAACGGGGTCAGTGCAAGCGCAG
E-17,78      GGATTAGGCGTATAAACAGTTAATAACAAATA
E-23,31      AATAAGTTAAGTGCCGTCGAGAGGGTTGATAT
E-23,63      ACAGTGCCATTAGCGGGGTTTTGCTCAGTACC
E-23,95      CCTATTTCAGAGGCTGAGACTCCTCAAGAGAA
E-37,14      CATGGCTTTTTTACCGCCTCCCTCAGAGCAGAGCCA
E-37,46      TCTCTGAACGCCACCCTCAGAGCCTGCCATCT
E-37,78      AATCCTCACCACCAGAACCACCACGCATTTTC
E-37,110     AGACGATTATTGACAGGAGGTTGATTTTCTTTAGCG
E-43,31      CTCAGAACTTTACCGTTCCAGTAAGTACTGGT
E-43,63      CAGAGCCGTTAAAGCCAGAATGGACTTGAGTA
E-43,95      CCGCCAGCGGCCTTGATATTCACAGCCCCCTG
E-57,14      CCACCGGATTTTTTAGAGCCAGCAAAATTTGAGCCA
E-57,46      TTTCATAAACCATTAGCAAGGCCGTTCATTAA
E-57,78      GGTCATAGAACCATCGATAGCAGCGAGGGAGG
```

```
E-57,110      TCAGACTGACAGAATCAAGTTTGCTTTTGCCAAAGA
E-63,31       GCACCATTTCAAAATCACCGGAACCCGCCACC
E-63,63       ACCAATGACCCCCTTATTAGCGTTACCACCCT
E-63,95       CAGTAGCGTAGCGCGTTTTCATCGCAGAGCCG
E-77,14       TTTGGGAATTTTCAAACGTAGAAAATACTTACGCAG
E-77,46       AGGTGAATACATATAAAAGAAACGCCCAAAAG
E-77,78       GAAGGTAATAAGTTTATTTTGTCAGGAAACCG
E-77,110      CAAAAGGGCATATGGTTTACCAGCTTTTAGTAAGCA
E-83,31       AGGTGGCATATCACCGTCACCGACCACCAGTA
E-83,63       CCACGGAAATATTGACGGAAATTAGAAACGTC
E-83,95       AGAAAATTCGACATTCAACCGATTACCGTAAT
E-97,14       TATGTTAGTTTTATTGAGCGCTAATATCACAAAGTCAGAGGGTATT
E-97,46       AACTGGCAAGAATTGAGTTAAGCCCATTAGAC
E-97,78       AGGAAACGAAACAATGAAATAGCATACAGAGA
E-97,110      GATAGCCGAGCCCTTTTTAAGAAATTTTTGTTTAAC
E-103,31      AACCCACATGATTAAGACTCCTTAATACATAA
E-103,63      AGAGCAAGCAATAATAACGGAATACAAAGACA
E-103,95      CTTACCGAAACAAAGTTACCAGAACAATCAAT
E-117,46      GGGAGAATTAACTGAACACCCTGAAGAGAGAT
E-117,78      GAATAACATAAAAACAGGGAAGCGCAATAATA
E-117,110     GTCAAAAATGAAAATAGCAGCCTTATAGCTAT
```

## Face F

```
F-13,102      TTGCTCAACAGTAGGGCTCCAGTATAAAGCCAACTTTTGCGTTAAA
F-17,30       TCGAGCCATAAAGTAATTCTGTCCTGCAGAAC
F-17,62       AACGCCAACATGTAATTTAGGCAGAGGCATTT
F-23,15       ACAAAAGGGTAATAAGAGAATATATT
F-23,47       ACAATAAAGTTTAGTATCATATGCTTACTAGA
F-23,79       AATTCTTATAATTGAGAATCGCCATATTTAAC
F-37,30       GCGCCTGTAATAATATCCCATCCTCGGCTGTC
F-37,62       AAAAGCCTCAACATGTTCAGCTAAAGACGACG
F-37,94       TAAGAATACCGTGTGATAAATAAGTTTTAAAGAACG
F-43,15       ACAAGAAATTATCAACAATAGATATTTTAAGTACCG
F-43,47       AGCATGTATCTGACCTAAATTTAATTAGTTAA
F-43,79       AATACCGAAACACCGGAATCATAAGTTATACA
F-57,30       TTTCCTTAGCACTCATCGAGAACAATTACCGC
F-57,62       TTTCATCTGAAACCAATCAATAATAATTTACG
F-57,94       CGAGAAAAAATCCAATCGCAAGACTTTTCAAAATCA
F-63,15       CAAGTACCTCATTCCAAGAACGGGTTTTAGTCCTGA
F-63,47       GTTTTTATGGGTTATATAACTATAACCTCCGG
F-63,79       CTGATGCACTTTTTCAAATATATTTGGTTTGA
F-77,30       GCCCAATATATTCTAAGAACGCGAGTTTTGAA
F-77,62       CTTAGGTTTTTCATCGTAGGAATCAGCAAGCC
F-77,94       TAGGTCTGTCAATAGTGAATTTATTTTTGTAAATCG
F-83,15       TTATCCGGGCAAGCAAATCAGATATTTTTATTAAAC
F-83,47       AGCGAACCAGCGATAGCTTAGATTGAATCCTT
F-83,79       GAGAAGAGAGAGACTACCTTTTTATGTAAATG
F-97,30       GCCTTAAAAATTTTATCCTGAATCTAATTTGC
F-97,62       GAAAACATTCCCGACTTGCGGGAGGGCGTTTT
F-97,94       TCGCTATTGAATAACCTTGCTTCTTTTTAACAAAT
F-103,15      CCAGCTACTCAAGATTAGTTGCTATTTTTAGAAGGC
F-103,47      GCTAACGATGGAAACAGTACATAAGAATTACC
F-103,79      ATGTGAGTAATTAATTTTCCCTTAAAGACGCT
F-117,30      CAGTTACAAAATAAACAGCCATATTTTTTTTTGCAC
F-117,62      TTTTTTAAGCGTCTTTCCAGAGCCTTACCAAC
F-117,94      TAATTACATTTAACAATTTCATTTATCAATAT
```

## Supplementary Note 3. Design of 3D staple strands

Here we describe the design of the staple strands connecting the edges of the box. Most of these staple strands are double crossovers and the oligos involved are listed next to each other. The same color code applies as for the 2D staple strands. The oligo design supports hierarchical assembly: The oligo should have one region of 16 base pairs and several smaller, which will make the oligo anneal at one position during heat-annealing and secondary annealing will thus happen by proximity related mechanisms. In a few oligos we have designed two regions of 16 basepairs, which is only done when these regions are in close proximity on the linear sequence, which should also ensure that only one oligo anneals at this position.

First we construct the main box composed of face A, B, C, and E. By aligning the edges different options for crossover of staple strands are found. In the following figure the faces are viewed from the top with helix ends pointing upwards (crossovers are shown in red):
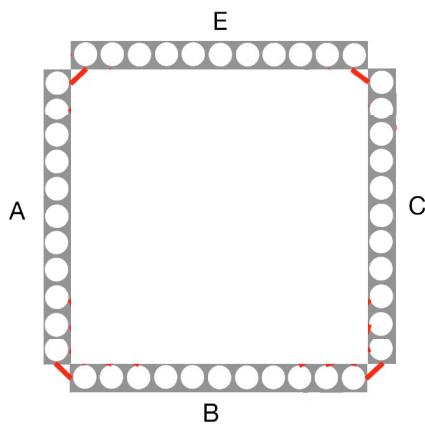


**Edge AB:** This edge is a simple continuation of the helix sheet. 3T spacers are added to allow the formation of a 90 degrees angle. To link the two faces together replace the staple strands A-97,14, A-117,46, A-117,78, A-117,110, B-13,7, B-13,31 , B-13,63, B-13,95 with the following staple strands:

```
AB-117,14    TAAAGGGATTTCACTAAATCGGAACCCTCGAGGTG
BA-13,31     GCCCCCGATTTGCGGGAGCTAAACAGGTACGCCAG
```

```
AB-117,46      GAATCAGATTTTTTTAGAGCTTGACGGGGTGAACCA
BA-13,63       GCGAACGTTTTTACGTATAACGTGCTTTCAGTGAGG
AB-117,78      TGACGAGCTTTTGGCGAGAAAGGAAGGGAAAAACCG
BA-13,95       GAAAGGAGTTTTACAGGGCGCGTACTATTCACGCAA
AB-117,110     GCGCCGCTTTTTCGGGCGCTAGGGCGCTTTTTTTAAAGAA
R-A-97,14      CGCTCATGTTTTTTTTAGACAGGAACGGAGGCCGAT
```

**Edge BC:** Edge BC is a continuous sheet like AB so the same rationale applies. To link face B and C together replace B-107,14, B-107,46, B-107,78, B-107,110, C-13,118, C-23,31, C-23,63, C-23,95 with:

```
BC-107,14      TGTAGATGTTTTATTTAAATTGTAAACGTTTGGTTGATA
CB-23,31       CGGAGACACAATCATATGTACCCCTTTTTAATATT
BC-107,46      CGGATTGAATTCGCATTAAATTTTTTTCGTAAAAC
CB-23,63       TCAACCGTATCGATGAACGGTAATTTTTGTTAAAT
BC-107,78      CAACCCGTTTTTTAACCAATAGGATTTTGGAGCAA
CB-23,95       GAGGGTAGTCATTGCCTGAGAGTCTTTACGCCATC
BC-107,110     AGCTTTCATTCGCGTCTGGCCTTCTTTTACAAAGG
R-C-13,118     CTATCAGGCTATTTTTGAGAGATCTTTACATTATG
```

**Edge CE:** Edge CE has the same characteristics as edge AE, so the same rationale applies. To link face C and E together replace C-117,46, C-117,78, C-117,110, E-117,46, E-117,78, E-117,110 with:

```
CE-117,46      TAGAAAGATTCATCAGTTTGCAGCCTTATAGCTAT
EC-117,110     GTCAAAATGAAAATATTTTTTGAGATTTAACGCCA
CE-117,78      AAAACGAACTAACGGATTTGGGAAGCGCAATAATA
EC-117,78      GAATAACATAAAAACATTTACAACATTAGCAACAC
CE-117,110     TCAGGACGTTGGGAAGTTTCACCCTGAAGAGAGAT
EC-117,46      GGGAGAATTAACTGAATTTAAAAATCTGACGATAA
```

**Edge AE:** Face A and E are oriented differently and have the characteristic that staple strands points down at coinciding positions. This is advantageous since this will enforce a rectangular bend using 3T linkers. Thus, it is possible to have double crossovers. To link face A and E together replace A-23,31, A-23,63, A-23,95, E-23,31, E-23,63, E-23,95 with the following 3D staple strands:

```
AE-23,31       GGGTTAGACGCAGAGGTTTAGACTCCTCAAGAGAA
EA-23,95       CCTATTTCAGAGGCTGTTTCGAATTATTCATTTCA
AE-23,63       ACGTAAAACTGATTGCTTTGGTTTTGCTCAGTACC
EA-23,63       ACAGTGCCATTAGCGGTTTTTTGAATACCAAGTTA
AE-23,95       TTTCAGGTTTTTACATTTTTCGAGAGGGTTGATAT
EA-23,31       AATAAGTTAAGTGCCGTTTCGGGAGAAACAATAAC
```

Now that the main box is formed we can attach the lids, D and F. Investigating the alignment of the edges a set of crossover types are chosen, that are schematized here (crossovers are shown in red):
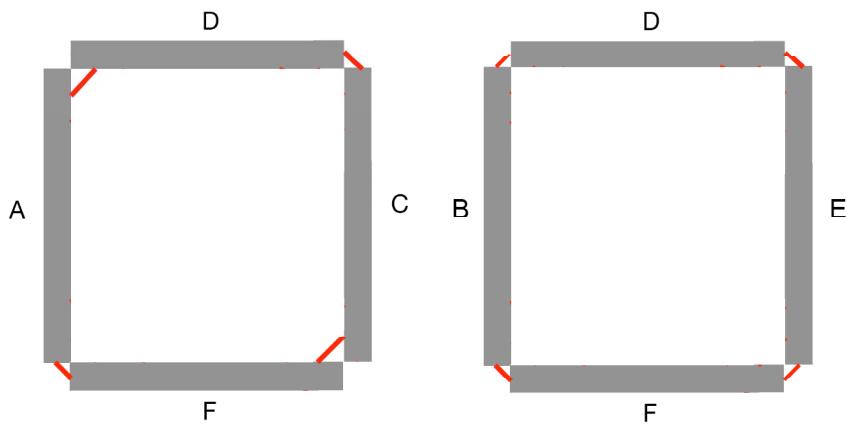


**Edge DE (hinge):** At edge DE the helix flank of face D and 11 helix ends of face E are aligned. Two positions are found along the edge where a staple strand of face D is close to a staple strand crossover of face E. These positions are used for 3D staple strand crossovers with a T4 spacer. To link face A and E together replace D-97,94, D-103,79, D-117,30, D-117,94, E-37,14, E-97,14 with:

```
DE-117,30    TTAATTTCTTTTTATTGAGCGCTAATATCACAAAGTCAGAGGGTA
ED-97,14     TATGTTAGTTTTTAACTTTAATCATTGTGTTTTTGCTCATT
DE-117,86    CAGAACCGTTTTTACCGCCTCCCTCAGAGCAGAGCCA
ED-37,14     CATGGCTTTTTTTCCACCCTCTTTCAGGGATAGCAAG
R-D-97,94    ACACTGAGCCCAATAGGAACCCATTT
R-D-97,102   TTTCGTCACCAGTACACCTCATAG
R-D-117,102  TTTTAGTACCGCCACCCT
```

**Edge AD (lid side):** The staple strand crossovers of face A and D does not meet. To crossover in register from helix to helix we make linkers inside the box. The staple strand points into the box on both faces 8 nucleotides from the edge. Thus the linker has to reach 3.8 nm for which a T8 spacer is used. To link face A and D together replace A-13,118, A-37,110, A-57,110, A-77,110, D-13,102, D-37,94, D-57,94, D-77,94 with:

```
AD-37,110    TTTTGAGTAACATTATCATATTAATTTTTTTTTTTAAGTTTTGTTTTGTACCGTA
DA-77,94     TTTCGTCTTTCCAGACGTACGATCTATTTTTTTTTTTAAAAGTTTTTTGAACCTC
AD-77,110    TTCCTTCTGACCTGAAAGCCAACAGATTTTTTTTTCTTGCTTTTTTTAAAGGAAT
DA-37,94     TTCGAGGTGAATTTCTTATTTATCAGTTTTTTTTTGATAGAACTTTTTTGATTAG
R-A-13,118   ATACAGTAACAGTACCTTAACGTCAGATGAATTT
R-D-57,94    TGCGAATAATAGAAAGGAACAACTTT
R-A-57,110   AAATATCATAAAGCATCACCTTGCTT
```

```
R-D-13,102        TTGCAGGGAGTTAAAGATTCGGTCGCTGAGGCTT
```

**Edge CD (lid side):** Edge CD is a normal seam, but we add T3 spacers to allow bend.

To link face C and D together replace D-63,15, D-103,15, C-37,110, C-77,110 with:

```
DC-63,15          ACCTGCTCTGATAAATTGTGTCGATTTAATTCTGC
CD-37,110         ACCCTGTAATCGGTTGTACCAAAATTTTACACTAA
DC-103,15         CAGTGAATATCAACGTAACAAAGCTTTGCAAAGA
CD-77,110         GCGTTTTAGAAGCCCGAAAGACTTTTTACGGTGTA
```

**Edge BD (lid tip):** At edge BD a helix flank of face D meets the helix ends of face B. The 3D staple strand crossovers are chosen to be distant from already existing crossovers and by the proximity of staple stand helical twist of face D to face B. At one position a face D staple strand points down at a face B staple strand crossover. Here no spacer is needed but we use a T2 linker to be on the safe side. The second possible crossover is less optimal and needs to reach almost a half or a full turn, so we use a T4 spacer here. To link face B and D together replace B-27,110, B-47,110, B-67,110, D-17,62, D-23,79 with:

```
BD-27,110         CGTGGACTGAACAAGAGTCCACTATTTGCGGGAT
DB-23,79          ACCGATATGCCGCTTTTTGGCGCCAGGGTGGTTT
BD-67,110         GGTCATAGTACCGAGCTCGAATTCTTTTACGAGGGT
DB-13,70          CGTCACCCTCAGCAGCGAAAGACAGCATCGGATTTTCAAAGCGC
R-B-47,110        GGCGGTTTGCGTATTGTTTTGTAATCAT
```

**Edge FE:** Edge FE is a similar situation to edge DE so the same design is used. To link face F and E together replace E-13,118, E-37,110, E-77,110, F-97,30, F-117,30, F-117,94 with:

```
EF-23,111         TTATTCTGTTTTTTTTAACAATTTCATTTATCAATAT
FE-117,94         TAATTACATTTTGGCAGGTCAGACGATTATTGACAGGAGGTTGATTTTCTTTAGCG
EF-77,110         CAAAAGGGCATATGGTTTACCAGCTTTTCAGTTACA
FE-97,30          GCCTTAAAAATTTTATCCTGAATCTAATTTGCTTTTAGTAAGCA
R-E-13,118        AAACATGAAAGTATTAGGAACCTA
R-F-117,22        AAATAAACAGCCATATTTTTTTTTGCAC
```

**Edge FC:** Crossover inside box with T8 spacer similar to edge AD, but F has different orientation. To link face F and C together we replace C-37,14, C-77,14, F-43,15, F-63,15, F-83,15, F-103,15 with:

```
C-37,14           TGTAGGTATTTTTCTACTAATTTTTTTTTACAAGAAATTATCAACAATAGATATTTTAAGTACCG
F-63,15           CAAGTACCTCATTCCAAGAACGGGTTTTAGTCCTGATTTTTTTTTTAGTAGTAAAAAGGTG
CF-77,14          TCATTTTTTTTTGTTCAGAATTTTTTTTTTTATCCGGGCAAGCAAATCAGATATTTTTATTAAAC
FC-103,15         CCAGCTACTCAAGATTAGTTGCTATTTTTAGAAGGCTTTTTTTTTAACGAGAATCAAATGC
```

**Edge FA:** At this edge the T4 loops meet (like edge CD), but with different directionalities. If a double crossover is made one oligo gets an 8 region on each face and the other oligo gets a 16 region on each face, which breaks with the hierarchical assembly design principle. Therefore, single crossovers are chosen on this edge, with a 16 region on face F and an 8 base region on face A. To do this it is necessary to break one of the crossovers of the double crossovers in face A and F. To link face A and F together replace A-37,14, A-57,14, A-77,14, F-37,94, F-57,94, F-77,94, F-97,94 with:

```
AF-37,14        GATTGTTTTTTTGTAAATCGTCGCTATT
AF-57,14        AACTAATATTTCAAAATCATAGGTCTG
AF-77,14        ATACCGAATTTAAAGAACGCGAGAAAA
R-A-43,7        TTGATTAGAGCCGTCAATGCACTAAC
R-A-63,7        TTCGAACCACCAGCAGAACATTAAAA
R-A-83,7        TTGAAATACCTACATTTTAGGAAAAA
R-F-37,94       TAAGAATACCGTGTGATAAATAAGTT
R-F-57,94       AATCCAATCGCAAGACTT
R-F-97,94       GAATAACCTTGCTTCTTTTTAACAAAAT
R-F-77,94       TCAATAGTGAATTTATTT
```

**Edge FB:** To link face F and B together replace B-47,14, B-87,14, B-67,14, F-23,79, F-17,62, F-17,30 with:

```
BF-47,14        CCCCAGCATTTTTTAACAACGCCAACATGTAATTT
FB-23,79        AATTCTTATAATTGAGAATCGCCATATTTTTTAACTCAC
BF-87,14        CGATTAAGTTTTGCATTTTCGAGCCA
FB-13,44        AGGCAGAGTTTTGGCGCATCGTAACCGTCACGTTGG
R-B-57,13       ATTAATTGTGCCTAATGAGTGAGCTT
R-B-73,7        TTTTGGGTAACGCCAGGGCTGCAAGG
R-F-23,23       TAAAGTAATTCTGTCCTGCAGAAC
```

To open the lid D we use staple strands for edge AB, BC, AE, CE, DE, FE, FC, FA, FB. To open both lids use staple strands for edge AB, BC, AE, CE, DE, FE.

## Supplementary Note 4. Adding locks to the lid of the box

Locks are added to actively open the lids of the box depending on external signals. We
make the following design with two different keys in lid BD. The sequence is
"randomly" generated.

```
B-Lock1          5'-BOX-GGCAGCTCGACTGATG-3'
D-Lock1          3'-BOX-CCGTCGAGCTGACTACGCTGACGT-5'

B-Lock2          5'-BOX-TTCTAGGCATCGTAAG-3'
D-Lock2          3'-BOX-AAGATCCGTAGCATTCCATCATGG-5'
```

The key oligos to open the lock and the unrelated oligo used as control are listed here:

```
Key1             5'-GGCAGCTCGACTGATGCGACTGCA-3'
Key2             5'-TTCTAGGCATCGTAAGGTAGTACC-3'
Jack              GGTCCGATAGATAGCTAAGCAGCGC
```

To insert lock 1 and 2 on edge BD replace B-27,110, B-47,110, B-67,110, B-87,110, D-
17,30, D-17,62, D-23,79 with:

```
D-Lock1          CGTCACCCTCAGCAGCGAAAGACAGCATCGGA-T-GGCAGCTCGACTGATG
B-Lock1          TGCAGTCGCATCAGTCGAGCTGCC-T-CAAAGCGCCATTCGCC
D-Lock2          ACCGATAT-GCCGCTTTTGCGGGAT-T-TTCTAGGCATCGTAAG
B-Lock2          GGTACTACCTTACGATGCCTAGAA-T-GGCGCCAGGGTGGTTT


R1-B-67,110      GGTCATAG-TACCGAGCTCGAATTC-TT
R1-B-93,103      GGTGCCGGAAACCAGG-TTTT-CTGTAGCC
R1-D-17,38       ACGAGGGTAGCAACGG

R2-B-27,110      CGTGGACT-GAACAAGAGTCCACTA-TT
R2-B-53,103      GGCGGTTTGCGTATTG-TTTT-GTAATCAT
R2-D-23,23       ATGAGGAAGTTTCCAT-GGCACCAA
```

A FRET system was designed to measure the opening and closing of the DNA box. A
Cy5 was placed on lid D and a Cy3 on face B, so that they get very close together when
the box is closed.

To place the Cy5 and Cy3 groups in the box replace B-47,110 and B-67,110 with:

```
Lock1-D-23,79    TGCAGTACCATCAGTCGAGCTGCC TGCGGGATCGTCACCCTCAGC
Lock2-D-13,70    (Cy5)AGCGAAAGACAGCATCGGA TTCTAGGCATCGTAAG
R1-B-47,110      GGCGCCAGGGTGGTTTGGCGGTTTGCGTATTG TT
R2-B-67,110      (Cy3)GTAATCATGGTCATAGTACCGAGCTCGAATTC
```