Supplementary Information

A modular DNA origami-based enzyme cascade nanoreactor

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1. Materials

All reagents are commercially available and applied without any further purification. In all procedures the water used was Milli-Q purified.

NeutrAvidin (NTV) protein, biotinylated horseradish peroxidase (B-HRP) (2.5 mg/ml) and 10x TAE buffer were purchased from Thermo Fisher Scientific. Biotinylated glucose oxidase (B-GOx) was purchased from VWR/Rockland Inc. (1 mg/ml). D-(+)-glucose, 3,3',5,5'-Tetramethylbenzidine (TMB), ethidium bromide (EthBr), hydrogen peroxide, agarose and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich.

2. DNA origami and enzymes

2.1. DNA origami annealing

DNA origamis were prepared by folding single stranded DNA from virus M13mp18 (New England Biolabs) with a set of short staple strands (Integrated DNA Technologies) (see Section 7 and 8 for the sequences and the design, respectively). The structures were prepared by mixing the following components:

- 10 µl scaffold (100 nM)
- 20 µl staples (500 nM) including biotinylated strands for NTV functionalization
- 5 µl 1x TAE
- 5 µl NaCl (50 mM)
- 10 µl MgAc (110 mM)

Origamis were annealed using Finnzymes Instruments Piko Thermal Cycler with the following procedure:

- 65 °C => 60 °C 15 min/°C
- 59 °C => 40 °C 90 min/0.5 °C
- after folding the origamis were stored at 4 °C

2.2. Purification of DNA origami

The excess amount of staples were removed in a non-destructive spin-filtering process. For filtering, we used either Millipore Amicon Ultra YM-100 filter columns with 100 kDa molecular weight cut-off. Filtration steps for 100 kDa filter are described below.

- 50 µl of DNA solution was mixed with 450 µl of folding buffer and injected into the filter.
- Solution was spun with 14,000 rcf, 3 min at room temperature.
- Flowthrough was discarded and 450 µl of folding buffer was added to the filter.
- Sample was spun in total 4 times repeating the procedure described above, expect for the last round the centrifugation time was set to 5 min.
- After the last spinning the filter was turned upside down in a fresh container and was spun 2 min at 1,000 rcf to collect the solution.

After filtration the volume of the solution was typically brought from 500 µl down to 17-20 µl.

2.3. DNA origami with avidin and enzymes

NeutrAvidin (NTV) was added to origamis in 200-fold excess (15 µl (1 mg/ml)) and then diluted to final volume of 100 µl by adding 65 µl 1x TAE + 20 mM Mg buffer.

Biotinylated horseradish peroxidase (HRP) was added to origamis in 20-fold excess (150 µl b-HRP (0.0022 mg/ml)) and incubated over night at room temperature.

Biotinylated glucose oxidase (GOx) was added to origamis in 200-fold excess (40 µl (0.1 mg/ml)) and incubated over night at room temperature.

After adding avidin, the excess amount of NTVs was removed using the same spin-filtering recipe as above (2.3). The excess amount of added HRP was also removed by the same method. For GOx-origami, other filtration steps were done as above, but the removal of the excess GOx was carried out using Pall Corporation Nanosep centrifugal device with Omega membrane and 300 kDa molecular weight cut-off with the following recipe:

- 50 µl of DNA solution was mixed with 450 µl of NaAc (5 mM, pH 5) and injected into the filter.
- Solution was spun with 5,000 rcf, 5 min at room temperature.
- Flowthrough was discarded and 450 µl of NaAc was added to the filter.
- Sample was spun in total 3 times repeating the procedure described above, expect for the last round the centrifugation time was set to 7 min.
- Finally 20 ul of NaAc was added to the membrane and the liquid was gently pipetted back and forth in order to collect origamis from the membrane.

2.4. Free enzyme activity (spin-filtering efficiency)

In order to ensure that above-mentioned filtering procedures work efficiently for both enzymes, initial rate of reactions for unfiltered and filtered (in 1x TAE + 20 mM Mg++) enzymes were tested. The concentration of the filtered enzyme was brought back to its initial value after the spin-filtering procedure. 3 µl of 50 nM HRP was mixed with 250 µl of substrate (pH ~5, 250 µM TMB, 2.5 mM sodium acetate, 20 mM D-glucose and 80 mM H_2O_2). Similarly, 3 µl of 0.6 µM GOx was mixed with 150 µl of substrate (pH ~5, 250 µM TMB, 2.5 mM sodium acetate, 20 mM D-glucose) and 2 µl of 50 nM HRP. The results shown in Fig. S1 indicate that practically all HRP can be removed when a sample is spin-filtererd as described above. In the case of GOx enzyme, the activity of filtered GOx is relatively high - nevertheless significantly lower than the activity of the unfiltered GOx – and that can be attributed to the TMB* formation of the added HRP indicator in the reaction buffer (HRP can catalyze the formation of TMB* to some extent without additional H_2O_2). Thus, the

observed activity of the dimer nanoreactor (with the excess amount of HRP completely removed) is due to the correctly bound enzymes inside the nanoreactor.

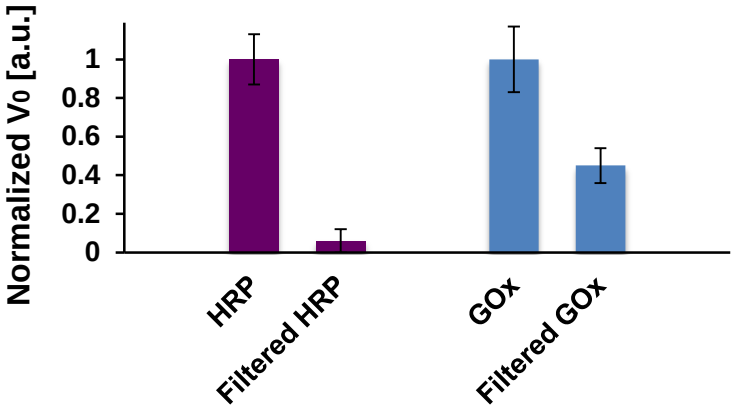

Figure S1. *Normalized initial rate of reactions in arbitrary units for unfiltered and filtered HRP and GOx samples (determined from triplicate samples).*

3. Concentration of DNA origami determined by UV/VIS spectroscopy

DNA origami concentration (c_{DNA}) was estimated using Beer-Lambert relation, $A_{260} = \varepsilon_{260} c_{DNA} l$, where A_{260} is absorbance at 260 nm wavelength, ε_{260} is the approximated extinction coefficient (0.9 * 10^8 M^{-1} cm^{-1}) [S1] and l is the length of the light path in centimeters). Final DNA concentration after filtration steps varied typically between 1 nM - 5 nM. The concentration was determined using BioTek Eon microplate spectrophotometer, Varian Cary 100 Bio UV-Visible spectrophotometer or Perkin-Elmer Lambda 950 UV/VIS Spectrometer.

4. Agarose gel electrophoresis

The quality of origami folding and dimerization of DNA origami units were verified by agarose gel electrophoresis using BIO-RAD Power Pac Basic equipment. 1-2 % agarose gels were prepared by dissolving 1-2 g of agarose into 100 ml of 1x TAE buffer (40 mM tris(hydroxymethyl)aminomethane, 19 mM acetic acid, 1 mM ethylenediaminetetraacetic acid) with 11 mM Mg++ stained with 30 µl of ethidium bromide (EthBr) solution (0.625 mg/l). 1x TAE + 11 mM Mg++ was used as a running buffer. Samples were stained with 6 X Blue Loading Dye (New England Biolabs). As a reference we used an M13mp18 scaffold strand. The gels were run with a constant voltage of 90 V for 45-90 minutes.

5. TEM imaging

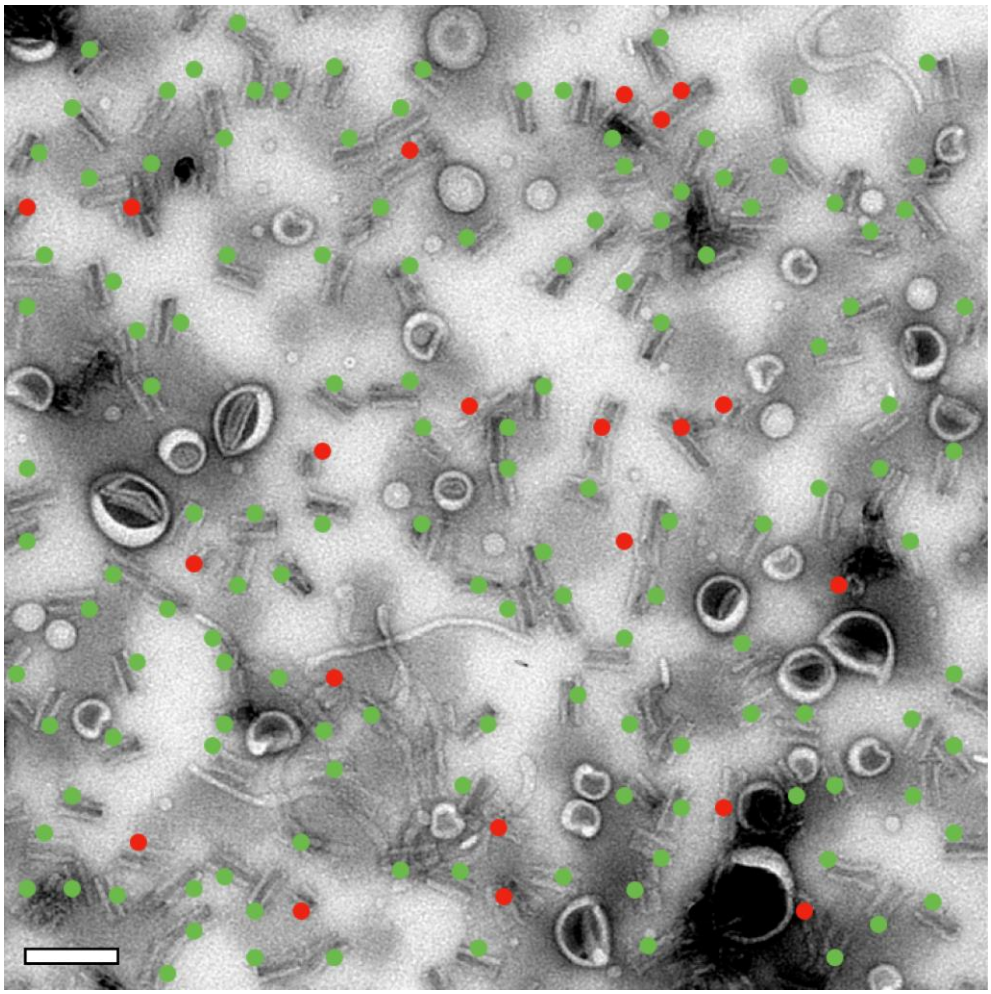

Figure S2. *Additional TEM image of origami dimers. In this typical image taken after 1-day incubation of monomers at room temperature, 86 % of all the observed objects are correctly formed dimers (marked with green dots) and only 14 % of the objects (marked with red dots) are either monomers or incorrectly assembled multimers. The scale bar is 100 nm.*

Transmission electron microscopy (TEM) images were taken with Tecnai 12 Bio Twin instrument. Samples were prepared on Formvar carbon coated or carbon only copper grids (Electron Microscopy Sciences) by placing a 3 µl drop of the sample solution on the grid. The sample drop was left on the grid for 1 min after which the excess solution was blotted away with a piece of filter paper. Samples were negatively stained by applying 3 µl of stain (0.5 % uranyl acetate in Milli-Q water) onto the grid and removing the excess stain with a piece of filter paper. Additional 3 µl drop of uranyl acetate was applied to the grid and excess liquid was blotted away after 20 s. Finally, the samples were dried under ambient conditions for at least 5 min before imaging.

6. Progress curves for HRP- and GOx-origamis

The product concentration (absorbance of TMB* at 650 nm) as a function of time for spin-filtered HRP- and GOx-origamis were monitored using BioTek Eon microplate spectrophotometer or Perkin-Elmer Lambda 950 UV/VIS Spectrometer (see Fig. S3). As a reference sample we used same amount of DNA origami fabricated and treated similarly but which did not contain NTV-modifications. The origamis were mixed in 1:100 – 1:10 ratio with the substrate (see the caption of Fig. S3) resulting in ~100 pM of origami concentration in each measurement. The results show that origamis with NTV-modifications have significantly higher enzymatic activity than the ones without NTVs, thus indicating proper binding of the enzymes to the NTV-sites of the origami. In addition, the results show that the spin-filtering efficiently removes the excess amount of enzymes and that the unspecific binding of the enzymes to origamis is insignificant.

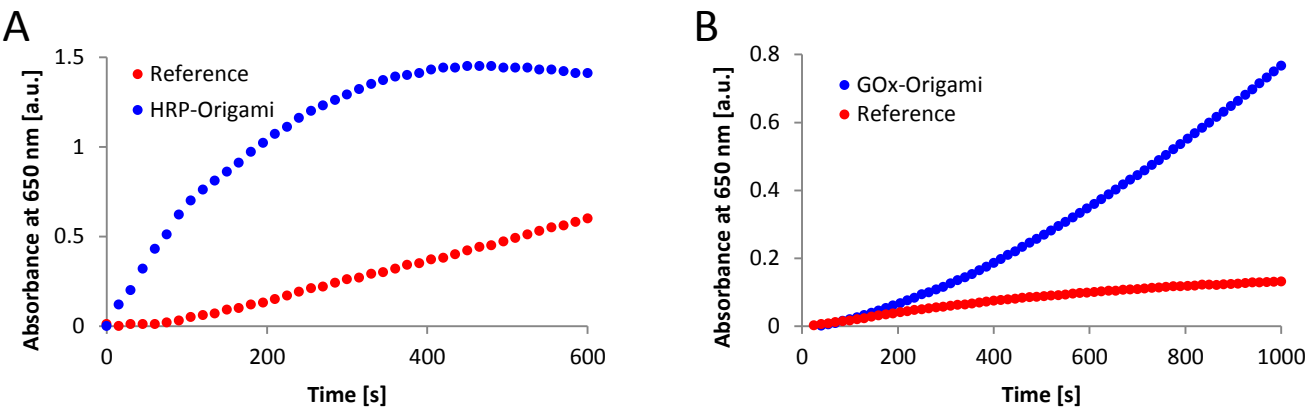

Figure S3. *(A) Progress curve (change in TMB* absorbance in arbitrary units) for DNA origami equipped with HRP (blue). The reference (red) is same amount of DNA origami fabricated and treated similarly but without NTV-modifications. The substrate (pH ~5) consists of 80 μM TMB, 4.5 mM sodium acetate and 4 mM H_2O_2. (B) Progress curve (change in TMB* absorbance in arbitrary units) for DNA origami equipped with GOx (blue). The reference (red) is same amount of DNA origami fabricated and treated similarly but without NTV-modifications. The substrate (pH ~5) consists of 150 μM TMB (in DMSO), 3.5 mM sodium acetate, 10 mM D-glucose and 0.3 nM B-HRP.*

In Fig. S4 the initial rate of reactions for origami units and the dimer nanoreactor are shown. The results are similar as the maximum rate of reactions shown in the main article (Fig. 4), since typically the initial rate of reaction was exactly same as the maximum rate of the reaction.

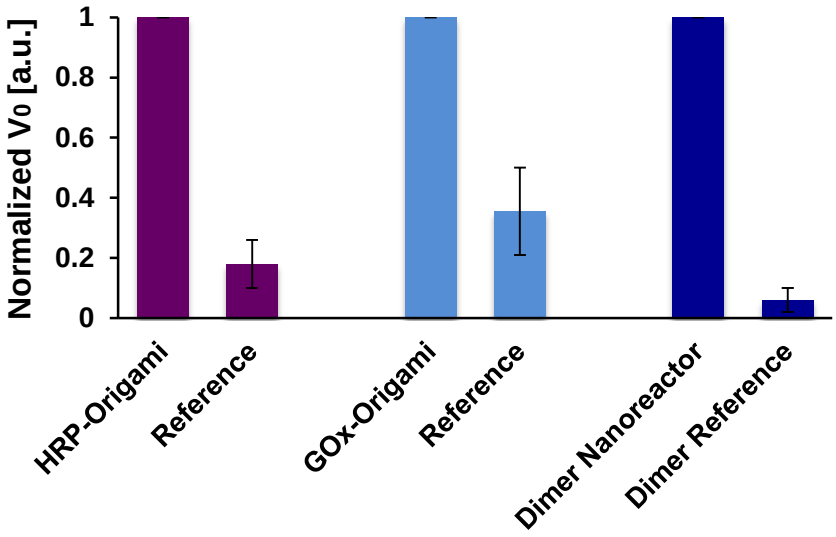

Figure S4. *Initial rate of reactions (V_0) for enzymes attached to DNA origami units and for the assembled dimer nanoreactor. Initial rate of reaction (formation of TMB*) for the sample is normalized to 1 in each case (for independent samples), and the performance of the sample is compared to a reference, which is fabricated and treated similarly but does not contain NTV binding sites.*

7. Strands for DNA origami units

Biotinylated strands for **both** units (3 strands):

Sequence (5′ -> 3′)	**Bases**
Biotin-AAACATTAAATTTTGCTCCAACACGTTG	28
Biotin-AGCTTTCAACATTAAATAGTGAATTTGCCAGAATGATTGAC	41
Biotin-ACGAGGCAATTCCAACGAAACGCAAAGACGTTCAGCTA	38

Core strands for **both** units (91 strands):

Sequence (5′ -> 3′)	**Bases**
CGTAATACATCAACATCTGGCC	22
AGGCAATGCAGCTGATTGCCTTAAACGGGCCTAAAAAGGCGTTGCTTATC	50
CAATCCAATTTATTTACTCATCCAACATATAAAAGAGCATGTAAAACCAA	50
TATATTTAGGATAAATGACCCAAGAATT	28
TTTCACCGCAGCAACCGCGAAAGAC	25
AATTCGGAAAAGCCCTATAGCCCGGAAAATATAATCAATTGATA	44
GAGCTGCTCAGAGAAAATACGTGAGGC	27
AATATGATACAAACTACAAGGTTTCAGGCCACCCTTCTAGGTGT	44

TAGTAAATTTCAACCCGAACCTCAA	25
AATTCACAGAGCCCTGACTATTATAATTATGTA	33
CGCGAGATCTTCTATAAGAACTGTTT	26
CAGCACCTTTTCATGGAAGGGCGCCAT	27
AATGCTTATAAATAAGTAAAATAACGGA	28
TTTCAGAAGATAAAACAGAGCGAACGAATATACGTGG	37
TCAATCACAGGTCAAGAACCGGATAGCA	28
TTGCCCTGACGATAATCATCTAAAGAA	27
CGATTAAGTTGGTGACCTTCAAAAGCTGGCGTTAAGACCTAA	42
ATCATTTTATCAGTTTGGATACGTAAATTTAACG	34
GAAATACGCATTTTCGAACCAGACAGCCAGGTTTGAGG	38
TGTTACTTGGGAACCTAGGCTGGCGTAACGCCAGGG	36
CTTGCTAAAAAAAAGTAGGATGGCTTAGA	29
CACAGACAATAGCCATTACATGGAA	25
TCACCCTCAGCAGAAATCGGCAACATTAGACG	32
TTGAAAACTCTGAGAAGGAGGTTGAAATCAAAATCATAGGATAGCGATAG	50
TTCCTGTAGTTACGAGGCATAAATAGCG	28
CGCCATTCGATCGGAAAGGGGACGTTGTGCAGGTCCGATTGACAAAGAC	49
GAAGCGTTGAGTTAAGCAATAGACGCTGGAGGGTGG	36
AGCCCAATCACCAGTATTCAAAAAGGGT	28
GGTCATTTTTGCGAACCCTCAGAGAAAGGCGGAGTGTCTTTCCAGACGT	49
ATCGGTTATAAAGCAAAAGGTTTAAAGGCCGCTGTTTAGCTATGGGGCGC	50
TGTGATGAAACCATAGCAAGCGCCATAGCATTTT	34
CTTAGATTGAGTGAATAATTTTCGTTGGGTCAATCG	36
GTACCGCTCATCGTAGGAATCCTATTATTTATCC	34
ATCACCGTACTCCACCCTCTTGCCTGGAGATCTACAAAGGCTGTCAGAAG	50
ACATGACATTCAACGACTCTAGAGGAAGACGGTCAATAAACA	42
AAGGTGGGAGAACACTTTCCAGAATCGG	28
GGCAGAGTTTAACAACGCCAAAGCACCAAGTCACGGATGTGCTGCAAGG	49
ACAGTCAAAGCGAAAAACAACTGAATTTTCTGTATGGGAAGG	42
TAATTGCTATAATGAAGTACGGTGTCTAAAGCTAAGCTTAATCATCAC	48
TACATTTGACGCCTGTAGCATTCCACAGTTTTGTC	35
AACGATTACCAGAAGCCAAAAGAACTGCAAGCCGTTATAAGA	42
TATCGGTGAATTACCAAATCTAGGCTTAGCCTTAGAATCC	40
TTCGCGTTAATGCCCCAGAGGAGAGGCTTTTGCAAAACATTAAATTT	47
CACCCTAGCATTGACGACTACCTTTTTCACCCTCCCGGAACGGTTT	46
TAAAGTGTAAACCTGTCGAAGAATACACTAACGCCGGAAGCA	42
CGAAAAACCGTTGGAAATACAACTGAACACCCCGTCAAAGGG	42
ACGCTCACTATCAAGCCATTGCTGACCT	28
TTTTTCTCCAACGCGTTTTTTGTTTAA	27

TTGAGTCACCCTCATATTTAGATTCAAATCACCATC	36
ATAACCGATACCACCAGCTTAAACAGCTTGCATCGCCCACGC	42
TGAAACAAACATCTGAGTAACTATTTCGGAAGGATTAGGATGCGTAG	47
AGTATCGTCACCAATAAATAAGCTCATTC	29
GGACGAACTAACGGAGGGATAGGTCACTCTGCCACTTTCCG	41
GGTCAGTTCTAAAGTGCTGAATCCTTTTGATAAGA	35
AGTTGAGGGAAGAATTATGCGTCAACTTGAAACACACGTAAC	42
GAAAGCGTAAGAATTCGGTCGCAGGGAGGGCATCA	35
TCAGATGGAAACAATGTTTAGACGATAA	28
TAATTTTCAAACAAATATCGCGGAAGCA	28
CGAGCCAGACGACAATCATAAAGCCGGA	28
GGAGAATTCTACATTTTAACGAGCGTATAAAAACAGG	37
TTTTCCCTTACCATTCGATAG	21
ATTCTACAGCAAAATTAAGCAGTACCAA	28
CGGGCAACCAGCTGATAAACAGCCATAAGAACGCGCGAAAG	41
CGGTATTATTACCGGGGTATTGAAACCATCCCATC	35
CAAGACCAGAGCCGCAACCTCCCGTTAATTAGAAAGCGCCAAAAGGAACC	50
AGTGACAACTGTTGCGCGACCG	22
TCAAGAGAGGCGCAGAACTGAAATTCTGTATCAACAATAGA	41
GCCTGTTTCCAGACGTAATAAGCTTAAT	28
GTTTTGCTCAGATATAAGCAAAAACTAGCATG	32
ATTTTCAGGACAGAAATAAAGAAATTTAGCGGG	33
GCCTAAATCAAGATCACTTCACCGCCTGCGAGGGTCTTTTGCGGGATCG	49
GGTGGTTGCGGTCCCTTTTACAGAGAGAATAACCTTTCCAGA	42
AAAAGGGCACCACGTGTTATCGGGTGCC	28
ATTTGACAATATATGTAAGACGCTGAGACATTTAGCAAAAGCACTGATTG	50
CTTTGAATACCATTTCAATCAACACTATGCAGATACATAAATTCATC	47
GAGAATAATTTTTTAAGGAGCGAGGTGAA	29
CGTCAAAACATTAATGTCGGGAAAGCCTGCGCTCACGCTCCCCGGGTACC	50
ATATCAAACCTTTTGCTCCAGACCGTTTTAAGCATCAAATCAGGT	45
TTGAGGACTCAATCTGAAAAA	21
CCCCGGTCCCCCTCACTTTACCACAACATT	30
CAAAATTAATTAAGAGTCTTACAGGAAAACGACGACAG	38
CAAAGCGGATTTTCGAGCAGTATTATAGATAA	32
AGCATCGGAAGCCCTGAGAGAGTTAGTGAGA	31
TCAGGCTGCGCACTCGCCACCAAGAACCGC	30
ATCAGAAGTTTTGCCCTGCCAGTGCCCGTATAAAAAGATGA	41
GGGTAATTTCATTGCTGATTGATGATGGC	29
GAGCTCGGTGAAATGAATAAGATACATA	28
AAACCAAGTAAGAGTACCTGAACAATTTC	29

ATGCAGAACGCCTAATTTCACAAC	24
ATTTAATCGCCTCCTGCCTCAGGAAGATCGATAAGGC	37
AAAATCTAGTTTCAGCCGGAG	21

Core strands for **GOx**-unit (23 strands):

Sequence (5′ -> 3′)	**Bases**
CTGCGGCTGAATACATCATA	20
ATTAACAAAACATCTTTTTGAAACCCTTCAACACGACCAGT	41
AAGAAATATCATCCGAAACA	20
TATTTGCTATACTTAATCGTCTAAACAGTTCAGAAAACGA	40
AAAAGCTAAACACCAAATCACAGAACGAGTAGTAAA	36
TGCGGGAGGTTTTGAAGCCTTAATTTGCCAGTTACAAAATGAAAATA	47
ACTAACAACTAATAGATTAGAGCCGTCAAGACTTTAAAAAGT	42
GTTTCATCGTCATTTATTTAGAAATGGTTGAAATGG	36
CAAATCTATATAAGACGTTGATTTAGGAA	29
GACTTCAATTCGACAACT	18
ATCGGGAAATATACTCAAAAT	21
AAGTACAAAACACTCAAGAACCGCCCAATAGCAA	34
GAATGACCATAAACAAAGAACGTTAT	26
TACAATTTTATCCTGAATCTTACCAACGCTGACGCTC	37
ATACGAGGAGATTTGAATAATAATCAATAATCGGCTGT	38
TTCACCAGTCACAGGAAAAAATCGTCCAATAACAGCAACG	40
TGAGAATGCCGGAAACATATGCGTTATACAGTAGGGAGAATATAAAGT	48
ATTAATTAACCTTGCATAAATATTACCT	28
TAAAGTCCAACTTGCGACCTGCTCCA	26
CAACTACTGTAGCCAGCAAAAATATC	26
AGACTTTTTCATGAGGAGGCTTTGAGGACTAA	32
CGGTGTACAGACCTAATCTTGACAAGAACCGG	32
TTCTGGCCAACAGAGATAGATGGCTATTAGTCTTT	35

Connecting strands for **GOx**-unit (14 strands):

Sequence (5′ -> 3′)	**Bases**
AATCAACAGTTGAAAGGAATTATCTAAAATATCTTTAGGCGT	40
AGTTCCTTATCATTCCATCTTTGCACCAATAAAATACGTAA	41
TTTAGTTAATTTCAAAACTTTTTCAAATACAT	32
GGACACATTCAACTAATCATAACCAGACGACTGGATAGCGTTTAAAT	47

GGCGAATTATTCAAGTTACAAAATCGCGCATA	32
TTGCACTACGAAGGACCCCCAGCGAAAT	27
TGCGGCTTGAGATGGTTTAATTATTTTAAGAACTGGCTCAT	40
CAAATTAGATACATTTCGCTAGATTTAGTTTG	32
AGAGTACCTTAACTCCAACAGGTCAGATGTGT	31
TACCAAGCGGCCTGATGAAATCCTGAAAGAGGACACCAAGC	37
GCTACAGAAGTTTCTAGTTGCTATTTTGCACCCAAG	36
TTCCTGATTGCGGAATCAAAAAAAGATTAAGAGGAAGCCCCCGG	44
CGCGCGCGAACTGATAGCCCTACCGCCTGCAACAGTGCCAC	40
ATTCTGCGAATCCATATAACAGTTGAAAATCA	31

Side strands for **GOx**-unit (56 strands):

Sequence (5′ -> 3′)
TTTTTTTTAATTGCGTTGCGCTCATGAGCTAACTCACATTTTTTTTTTTT
AATATTTTTATTCTGAAACATGAAAGTTTTTTTTT
GGGAGGGAAGGTAAATATTGACGTTTTTTTTTT
AGTTTGGAACAAGAGTCCATTTTTTTTTT
TTTTTTTTAAGTAAGCAGATAGCCAAGCCCTTTTTAAGAATTTTTTTTT
TTTTTTTTTTCTATTAAAGAACGTGGACTCCAA
TTTTTTTTTATATCAGAGAGATAACCCACAAGAAAATCCCTTATAAATCA
GCAGCCATCTTACCGGAACAAAGCGGGGAGAGGCGGTTTGCTTTTTTTTT
TTTTTTTTGCATCGTAACCGTGCAGTTGGTGTAGATGGGCTTTTTTTTTT
TTTTTTTTATGTTAGCAAACGTAGCTCCTTATTACGCAGTTTTTTTTTT
TTTTTTTTCAATAGGAACGCCATCCAGCTCATTTTTTAACTTTTTTTTTT
GTAACAACCCGTCGGATTTTTTTTTT
TTTTTTTTGTAACACTGAGTTTCGTAGGAACCCATGTACCTTTTTTTTTT
TTGTTAAATAAAAATAATACAGGAGTGTACTGGTATTTTTTTTTT
TAATGAGCTGCCCGAGGCATGATTAAGA
TTTTTTTTTTAAGCCTTTATTTCAACGCATAAATGCAATGCCTGAGTATT
TTTTTTTTTTCCAGAACCACCACCAGAGCCGCCGCCCAGAGCCTCACCGG
TTTTTTTTTTATGTGTAGGTAAAGATTCACCGTTCT
TTTTTTTTTTGTATTGGGCGCCTTTGCCCCAGCATTTTTTTTTT
TTTTTTTTAAGAAACAATGAAATAGCCCAATAATAAGAGCTTTTTTTTTT
TTTTTTTTTTTATTAAGAGGCTGAGACTCCTCAAGAGAACCTATGTTAA
TTTTTTTTTTAAATCATACAGGCAAGGCATGTAATACTTTTGCGGGAGTT
TTTTTTTTTTTTCTCCGTGGGAACAA
TTTTTTTTATTACGCCAGCTGGCGTGCGGGCCTCTTCGCTTTTTTTTTTT
TGAGCGAACGGCGGGAAAGCGATAAATC

TTTTTTTTCGACAATGACAACAACATACCGATAGTTGCGCTTTTTTTTTT
TTTTTTTTTTGGCGAAAATCCTGTTTGAT
TTTTTTTTCCACCCTCAGAACCGCAGGAGGTTTAGTACCGTTTTTTTTTT
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TTTTTTTTTTAAATTCATATGGTTTACCAGCGC
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TTTTTTTTAATAATAACGGAATACGAAACCGAGGAAACGCTTTTTTTTTT
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TTTTTTTTTTATTCACAAACAACAGTCTCTGAATTTTTTTTTT
TTTTTTTTTTTTACCGTTCCAGTAAGCGTCATACATGGCAAGAAAA
TTTTTTTTTTATAAGTTTTAACGGGGT
TTTTTTTTCATAGCTGTTTCCTGTAATTCGTAATCATGGTTTTTTTTTT
TTTGCCTTTAGCGTCAGACTGCCCCCTTATTAGCGTTTGCTTTTTTTTT
TTTTTTTTTTGAAATTATTCATTAAAGGTGAATTATCATGTAATTTA
TTTTTTTTGCCGTCGAGAGGGTTGTACCAGGCGGATAAGTTTTTTTTTT
TTTTTGAAGAGTCTGGAGCAAACAAGAGAATTTTTTTTT
TTTTTTTTGGAATTGCGAATAATAGAAAGGAACAACTAAATTTTTTTTT
TTTTTTTTTTATCAGCTTGCTTTCCTTTAATTGTATCGGTTTTTTTTTT
CTCATTAAGGCAGGTCAGACGATTGGCCTTGATTTTTTTTTT
CAGTGCCTTTTTGATGATTCGCGTTAAATG
TTTTTTTTCGGAAACCAGGCAAAGGCACCGCTTCTGGTGCTTTTTTTTTT
TTTTTTTTTTTCGATGAACGGTAATCGTAAAACAGGAAGA
GTCATAGTAGCGCGGTAATCAGTAGCGACAGAATCAAGTTTTTTTTTTT
TTTTTTTTCATCTTTTCATAATCAAAAACCACCCTCAGAGCCGCCATTTT

Core strands for **HRP**-unit (22 strands):

Sequence (5′ -> 3′)	**Bases**
TCGATGAACGGTAATCGTAAAACAGGAAGAAATATTTTTATTCT	44
TAATAGTGTTGAGTGTGCTTGCTGAGTGTTCCAGTTTGGAACAAG	45
AGGTAAAGATTCACCGTTCTTTTTTGAAGAGTCTGGAGCAAACAAGAGAA	50
CGTTCCAGTAAGCGTCATACATGGCAAGAAAA	32

TGAGCGAACGGCGGGAAAGCGATAAATC	28
TAATGAGCTGCCCGAGGCATGATTAAGAAAAATACTTTATTTTGT	45
AGTTTTAACGGGGTCAGTGCCTTTTTGATGATTCGCGTTAAATG	44
CTCATTAAGGCAGGTCAGACGATT	24
GTCATAGTAGCGCGGTAATCACACCAGTCACCGTCACCGACTTGA	45
TAACCGTGCAGTTGGTGCCAGAGCCGCCGCCCAGAGCCTCACCGG	45
TTACCAGCGCGGGAGGGGGTGAATTATCATGTAATTTA	38
CTGAGTTTCGTAGGAACGAACCGCAGGAGGTAGGGTTG	38
GCAGCCATCTTACCGGAACAAAGCGGGGAGAGGCGGT	37
AAGAACGTGGACTCCAATGAACAAAGTCAGAGGGTAATTGAGCGCTA	47
TACCAGGGAGACTCCTCAAGAGAACCTATGTTAA	34
GGAATTGCGAATAATAGAAAGGAACAACTAAA	32
GGCGAAAATCCTGTTTGAT	19
CAATAGGAACGCCATCCAGCTCATTTTTTAAC	32
CTTTTCATAATCAAAAACCACCCTCAGAGCCGCCA	35
CATAGCTGTTTCCTGTAATTCGTAATCATGGT	32
TACAGGCAAGGCATGTAATACTTTTGCGGGAG	32
ATATCAGAGAGATAACCCACAAGAAAATCCCTTATAAAT	39

Connecting strands for **HRP**-unit (15 strands):

Sequence (5′ -> 3′)	**Bases**
AGAGGGTAGCTAAGCTGATAAATTAATGGAAA	32
TGTAAACGTTTTGTATAAGCAAATATCCAATA	32
ACCAAGAATAGCCCGAGATAGGAGTAGCATTAACATCCAATTTCCCA	47
TACTTCTCCGTGGGAACAAGTAACAACCCGTC	32
AACGATCTAAAGACAGCCCTCATAGTTAGAGC	31
TTGTTAAATAAAAATAATACAGGAGTGTACTGGTAAGA	38
TTGCATGCCTAAAACGACGGCCAGTGGATGAA	32
TTGCGGAAACCAGGCAAAGGCACCGCTTCTGG	32
TGCCGTATTGGGCGCCTTTGCCCCAGCA	28
CTTATGTTAGCAAACGTAGCTCCTTATTACGC	32
GCTTGCTTTCCTTTAATCAACGCATAAATGCAATGCCTGAGTAGATTAG	49
GCGTTGCGCTCATGAGCTAACTCACATTTTA	31
AAACAATGAAATAGCCCAATAATAAGAGCAGC	32
AATCGACAATGACAACAACATACCGATAGTTG	32
CCAGCTGGCGTGCGGGCCAGACTGCCCCCTTATTAGCGTTTGCTAT	46

Side strands for **HRP**-unit (52 strands):

Sequence (5′ -> 3′)
AAATGGTTGAAATGGATTATTTACATTTTTTTTTTT
TTTTTTTTTTTAAATATGCAACTACTGTAGCTCAACATGTTTTTTTTTTT
TTTTTTTTTTAGCTACAATTTTATCCTGAATCTTACCAACGCTGACGCTC
TTTTTTTTCTGGCCAACAGAGATAGATGGCTATTAGTCTTTAATTTTTTT
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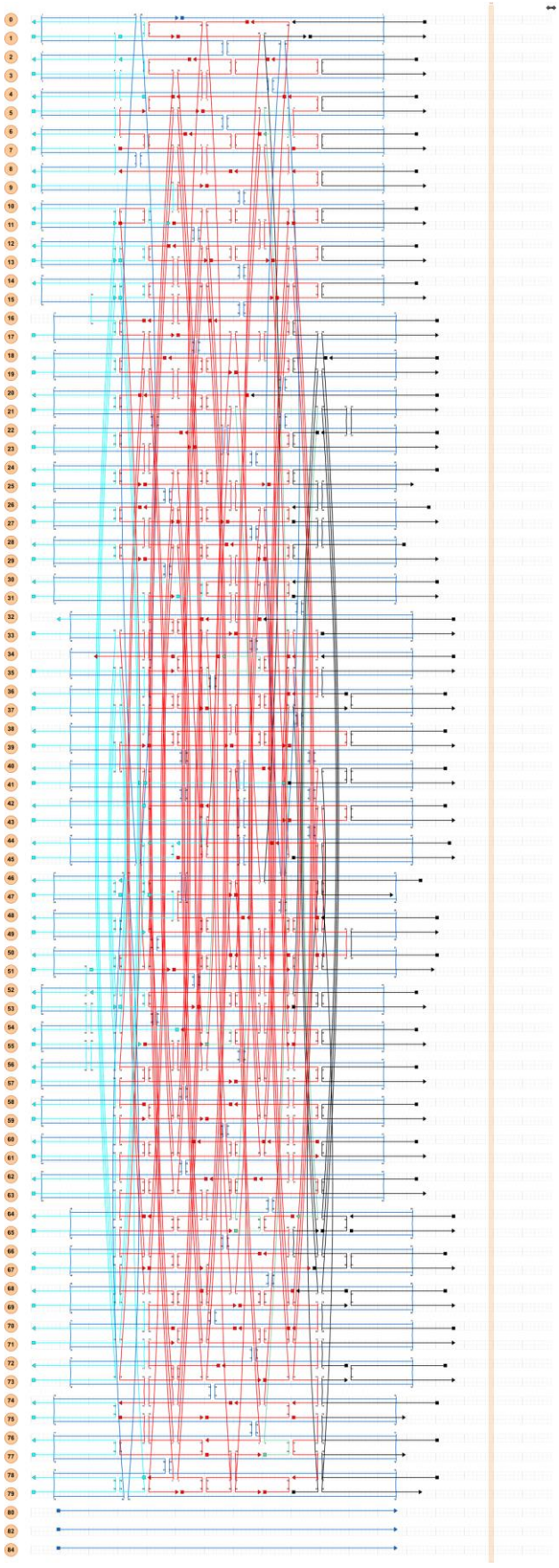
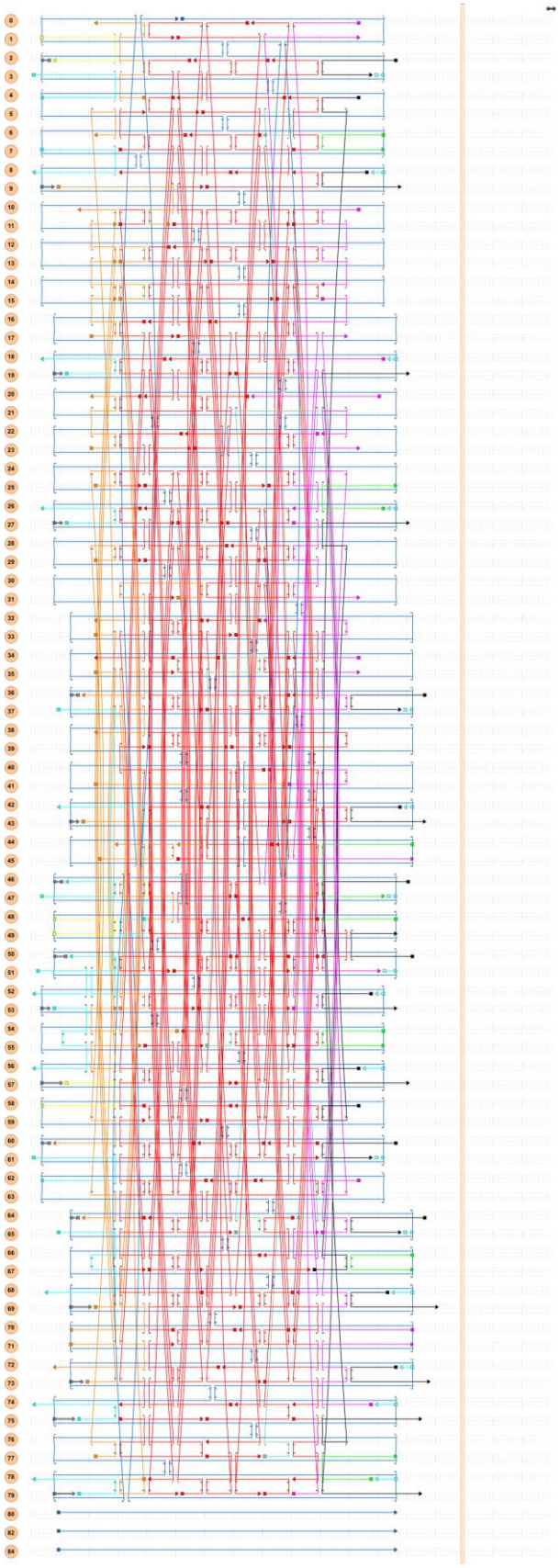
8. caDNAno designs

Left design: Core strands and right & left poly-T passivation strands (biotinylated strands in green)



Right design: Core strands and right & left connecting strands.



9. Supporting information references

[S1] A. M. Hung, C. M. Micheel, L. D. Bozano, L. W. Osterbur, G. M. Wallraff and J. N. Cha, *Nat. Nanotechnol.* 2010, **5**, 121-126.