Supplementary Information

A Versatile DNA Origami Based Plasmonic Nanoantenna for Label-Free Single-Molecule Surface-Enhanced Raman Spectroscopy (SERS)

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1. DNA origami fork design

The DNA origami fork design with the nanoparticle capture strands is shown in Figure S1. The DNA origami nanofork is designed such that the scaffold DNA strand is routed through both DNA bridge helices. There are two times 5'-A$_{24}$ and two times 3'-(AAC)$_{8}$ strands extending from each arm on each side. In addition, there are two times 5'-A$_{24}$ and two times 3'-(AAC)$_{8}$ extending from the side of the bridge. Each particle capture sequence binds with the coating sequence in a zipper configuration, to ensure a close position of the particles to the bridge. One side of the arms is left open on purpose to enable free diffusion of external molecules into the hot spot if desired.

Figure S1. a schematic view of the DNA origami nanofork structures. (A), a side view of the nanofork with two different metal nanoparticle capture strands (blue and orange) protruding from each side of the fork from the arms and the bridge. (B) a side view of the same nanofork.
2. TEM images of Au DONAs

The TEM images of the Au DONAs do not show the actual DNA origami structure although negative staining was used (as in the TEM images of the nanofork structures alone), because the size of the DNA origami is too small and the material contrast too weak compared to the 60 nm Au particles.

Figure S2. (A)-(F) TEM images of shorter gap size DONA-AuNP dimers. The size of each image is 150 nm x 150 nm.
3. AFM images of Au DONA dimers

Figure S3. AFM image of Au DONA on a silicon substrate. The height profile of the dimer in the inset is shown on the right.
4. Single NP to DONA dimer ratio calculation

Figure S4. Example SEM image of Au DONA sample used the single NP to DONA dimer ratio calculation. The single NPs and dimers are highlighted by red and green circles, respectively.
5. AFM image of Au DONA monomers

Figure S5. AFM images of 40 nm AuNP monomer DONAs. (A) monomer DONAs with only poly(T) coated particles. (B) monomer DONAs with only (GTT)$_4$T$_4$ coated particles.
6. AFM images of different DNA origami nanoforks

Figure S6. AFM image of the short bridge Forks. The lines and the numbering correspond to the height data.
Figure S7. AFM image of the long bridge nanoforks. The lines and the numbering correspond to the height data.
7. The Correlated Raman-SEM maps of dye Ag and Au DONAs

The experimental parameters and the description of the measurements are discussed in the Methods section and the Raman measurement section below. Typically, the SEM images covered a smaller area than the Raman maps, so several SEM maps were stitched together to form an overall SEM map of the corresponding Raman map. The scale bars were used to fix the sizes of the overall SEM and Raman maps to the same size in Corel Draw. Then the maps were superimposed and the positions of the dimers were identified. The small step size was employed in order to achieve good enough overlap between adjacent points in the Raman map. Also, we excluded any dimers from the analysis that had larger aggregates, trimers or tetramers close by, since the assignment of the signal is not clear in these cases.

We observed that the typical TAMRA bands appear at around 1651 cm\(^{-1}\) (\(\nu(C=O)/\nu(C=C)\)), 1532 cm\(^{-1}\), 1509 cm\(^{-1}\) (both \(\nu(CC)\)) and 1360 cm\(^{-1}\) (\(\delta(COH)/\nu(CC)\)),\(^1\) which is accompanied by contributions from DNA coating strands \(\text{e.g.}\) the band at 1608 cm\(^{-1}\)). For Cy3.5, the characteristic peaks at around 1600 cm\(^{-1}\) (\(\nu(C=O)/\nu(C=C)\)), 1450 cm\(^{-1}\) and 1285 cm\(^{-1}\). For Cy5, the characteristic bands are at 1592 cm\(^{-1}\) (\(\nu(C=O)/\nu(C=C)\)), 1468 cm\(^{-1}\) (\(\nu(CC)\)), 1368 cm\(^{-1}\) (\(\delta(COH)/\nu(CC)\)) and 1232 cm\(^{-1}\) (\(\nu(CN)\)).
Figure S8. correlated SEM and Raman maps of Tamra modified DONA-AuNP sample. The colors and the number indicate the dimers areas and the corresponding spectra.
Figure S9. Correlated SEM and Raman maps of Cy5 modified DONA-AuNP sample. The colors and the number indicate the dimers areas and the corresponding spectra.
Figure S10. correlated SEM and Raman maps of Cy3.5 modified DONA-AuNP sample. The colors and the number indicate the dimers areas and the corresponding spectra.
Figure S11. Correlated SEM and Raman maps of Cy3.5 modified DONA-AgNP sample. The colors and the number indicate the dimers areas and the corresponding spectra.
8. The Raman reference spectra for the dye and the poly(T)

The dye-nanoparticle reference samples were prepared using the same coating protocol described in the Methods section. Each reference oligo sequence has the form of T_{28} – X – SH, where X is either Tamra, Cy5 or Cy3.5 in the case of dyes or empty for the pure poly(T) DNA reference. The dyes were always positioned close to the particle surface to maximize the plasmonic enhancement of the NP. The dye coated particles were deposited on silicon surface similarly as the DONAs. For gold, the Tamra and the poly(T) references were measured using 10s integration time and 10 accumulations and the power density was 602 W/cm² for 633 nm laser. For gold and Cy3.5, the same parameters were 4s, 10 accumulations and 6155 W/cm². For gold and Cy5, the parameters were 4 s, 10 accumulations and 4303 W/cm² for 633 nm laser. For silver and Tamra or Cy3.5, 1 s and 10 accumulations were used while for poly(T) 4s and 5 accumulations were used. All had a power density of 3070 W/cm² for the 532 nm laser. The Figure S12 and Figure S13 show the reference spectra for gold and silver, respectively.

Figure S12. Raman spectra of dye and poly(T) reference samples. (A) Tamra-oligo coated AuNP spectra measured using 633 nm laser. (B) T_{28}-oligo coated AuNP spectra measured using 633 nm laser. (C) Cy3.5-oligo coated AuNP spectra measured using 532 nm laser. (D) Cy5-oligo coated AuNP spectra measured using 633 nm laser.
Figure S13. Raman spectra of dye and poly(T) reference samples. (A) Tamra-oligo coated AgNP spectra measured using 532 nm laser. (B) T28-oligo coated AgNP spectra measured using 532 nm laser. (C) Cy3.5-oligo coated AgNP spectra measured using 532 nm laser. (D) Cy5-oligo coated AgNP spectra measured using 633 nm laser.

In DONAs, all dyes were measured using 633 nm laser with 3227 W/cm² power density. For Cy3.5 and Cy5, the integration time was 7s and for Tamra 4s. The pure DNA bridge Au DONAs were measured using 5s and 4635 W/cm² power density for 532 nm and 7s and 3240 W/cm² for 633 nm laser. In Ag DONAs, Tamra and Cy3.5 sample were acquired using 532nm laser with 4605 W/cm² power density and 10 and 8 s integration times, respectively. We did not observe any SM SERS signals at 532 nm using Au DONAs, which is in accordance with the FDTD simulations. For the pure DNA bridge case, the integration time was 8 s for both 532 nm and 633 nm laser and the power densities were 3070 and 2152 W/cm², respectively. Due to the raster scanning feature, the accumulation was 1.
9. UV-vis absorption spectra of Tamra, Cy5 and Cy3.5 oligos

Figure S14. normalized UV-vis absorption spectra of Tamra-, Cy5 and Cy3.5-oligos used in the DONA experiments. The absorption spectrum of TAMRA shows a main absorption band peaking at 561 nm and a shoulder at 530 nm. The main band and the shoulder for Cy3.5 appears at 597 and 560 nm, respectively. Cy5 has the same bands at 646 nm and 606 nm.
10. The average SERS spectra of DNA and dye Au DONAs

Figure S15. average spectra of multiple, individual-dimer spectra of (A) pure DNA bridge Au DONAs, (B) Tamra Au DONAs, (C) Cy3.5 Au DONAs and (D) Cy5 Au DONAs.
11. The correlated Raman-SEM maps of pure DNA bridge (control) Ag and Au DONAs

Figure S16. correlated SEM and Raman maps of Au DONA sample, where the bridge contains only DNA. The wavelength of the excitation laser is 633 nm. The colors and the number indicate the dimers areas and the corresponding spectra. The size of the inset images on the right are 1 µm × 1 µm.
Figure S17. correlated SEM and Raman maps of Ag DONA sample, where the bridge contains only DNA. The wavelength of the excitation laser is 532 nm. The colors and the numbers indicate the dimer areas and the corresponding spectra. The size of the inset images on the right are 1 µm × 1 µm.
12. Dark field scattering spectra of Au DONAs

Figure S18. Polarization dependent dark field (DF) scattering spectra from single dimer. (A) DF scattering spectra corresponding to the directions highlight in the inset. By turning the polarization 180°, the same initial spectral shape can be achieved. Differences in the height of the peaks are most probably due to slightly different focus. (B) DF scattering spectra showing all the measured orientation. (C) DF scattering spectra showing only the selected orientation near the gap axis and the transverse axis. (D) The spectrum of the halogen lamp used in the excitation.
Figure S19. Dark field scattering spectra of different DONAs. (A) – (F) DF scattering spectra showing the spectra along the gap axis (blue curve) and perpendicular to it (orange curve). The solid lines show an average fit whereas the real data is shown in the background. The arrows and the values highlight the position of the LSPR peaks.
13. Time series SERS measurements in dark field mode

The time series data is measured from DONAs containing single Tamra molecule in the middle of the bridge. Typical Raman measurements were usually carried out by performing the Raman mapping first and then imaging the sample using SEM or AFM. However, since the dark field imaging directly visualizes the nanoparticles, we used it and AFM images to identify the dimers from the dark field view (see Figure S20). This was done by taking first a dark field image close to an external scratch marker, placing the same sample to AFM and imaging areas next to the same scratch marker. By using the same particle patterns in dark field and AFM images, maps were correlated (see the green rectangles in Figure 20A and Figure 20B for example). The sample was placed back to Raman microscope and the view was moved to the previously imaged area. Then we moved our internal laser reference point on top of any identified dimer (the red boxes in Figure 20 for example), turned the imaging mode back to bright field and acquired the Raman spectra. The power density of the used 633 nm laser was 5380 W/cm² and the acquisition time was 3 s.

Noise in the dark field images was reduced by creating a Fourier transformed image of the original dark field image, removing the center pixels (circular mask, 2-pixel radius) from the transformed image and doing the reverse Fourier transformation. For any image processing, ImageJ software was employed. The Figure S20A and Figure S20B show the dimer d₁ from the Figure 4 (red boxes in all of the images). Two other dimer time series spectra (dimers d₂ and d₃) are shown in Figure S21C and Figure S21D and the correlated dark field and AFM maps in Figure S21A and Figure S21B. Here, we can also observe fluctuations in the main Tamra bands of 1654, 1537, 1509, 1360 and 1216 (bands highlighted by the vertical, dashed lines).
Figure S21. Correlated dark field and AFM images of the dimer \( d_2 \) and \( d_3 \) area and the time series spectra of both dimers. (A)-(B) correlated dark field and AFM maps of the measurement areas. Red and orange boxes are used as reference to locate the dimers. The dimers \( d_2 \) and \( d_3 \) are highlighted by the green and violet boxes in all of the images, respectively. The size of the both SEM images is 300 nm \( \times \) 300 nm. (C)-(D) time vs Raman shift contour plot for dimer \( d_2 \) and \( d_3 \) containing single Tamra molecule. The dotted lines indicate the main TAMRA peaks (1654, 1537, 1509, 1360 and 1216).

The intensities of the Tamra bands and fluorescence background decreased overall during the 600 s runtime (see Figure S22). This could be due to the measurement system going off focus during the relatively long runtime. However, for the dimer \( d_1 \), we plotted the time series of the main silicon peak at 520 cm\(^{-1}\) and observed no significant change in the peak intensity during the measurements, meaning that the initial focus on the surface was maintained during the measurements and the drop in the Tamra intensity might be due to changes in the morphology of the DONA samples: for example, it has been shown that laser power can be used to weld nanoparticles together.\(^2\) These kind of effects might lead into...
changes in the gap composition and the placement of the dye thus resulting in a drop in the Tamra peaks. Similar trends were observed in the case of dimers d$_2$ and d$_3$ (data not shown here).

Figure S22. the dimer d$_1$ time series data of the silicon band at 520 cm$^{-1}$, Tamra 1650 cm$^{-1}$ band and arbitrary 1050 cm$^{-1}$ band.
14. Protein coupling schemes

Figure S23. Scheme of the non-covalent and covalent coupling schemes to bind cyt c and HRP to the DNA bridge of the nanofork. Cyt c is coupled non-covalently to pyridine, which is connected to the DNA bridge, while HRP is cross-linked to a DNA staple strand, which is part of the DNA bridge. The cyt c and HRP models were adapted from [10.2210/pdb2B4Z/pdb] and [10.2210/pdb1HCH/pdb], respectively.
15. The correlated Raman-SEM maps of protein Au DONAs
Figure S24. correlated SEM and Raman maps of Cytochrome C modified DONA-AuNP sample.
Figure S25. Correlated SEM and Raman maps of HRP modified Nano-Fork-AuNP sample. The colors and the number indicate the dimer areas (laser power: 200 µw, Integration time: 12 s).
16. SERS spectra of DONAs with unspecifically bound proteins

Figure S26. correlated SEM and Raman maps of control DONA-AuNP sample without Cytochrome capture strand.
Figure S27. Correlated SEM and Raman maps of control DONA-AuNP sample without HRP capture strand.
17. Example of Au and Ag DONA gels

Figure S28. (A) and (B) depict the Au and Ag DONA samples after gel run, respectively. The blue rectangles highlight the dimer bands that are extracted from the gel.
18. Extra TEM images of DNA origami nanoforks

Figure S29. (A)-(D) extra TEM images of nanoforks on TEM grids.
19. Evaluation of the DNA coating layer thickness

Figure S30. the height data of non-coated 60nm AuNPs (152 particles). The heights were acquired from AFM images.

Figure S31. the height data of DNA coated 60nm AuNPs (359 particles). The height was acquired from AFM images.
The average DNA coating layer thickness was calculated from the average heights of the non-coated and coated 60nm AuNPs. Arithmetic averages were calculated (the vertical lines in Figure S30 and Figure S31) and the DNA layer thickness was calculated as the difference of the two average values divided by two, since the height value contains the DNA layer twice. This resulted in an average DNA coating layer thickness of 1.12 nm.

20. Plasmonic nanoparticle dimer simulations

The finite-difference time domain (FDTD) simulations were carried out using Lumerical FDTD Solutions software v8.19.1584. The model is shown in Figure S32. The gold and silver nanoparticles were defined as spheres with 30 nm radius and the refractive index (R.I.) of Ag/Au taken from Johnson and Christy. DNA coating layers were placed on top of the particles surfaces, where the DNA layers were set 1.12 nm thick according to the average layer thickness in the previous section and the R.I. was defined as 1.7. The substrate was Si (R.I. from 5) with 3 nm SiO$_2$ layer (R.I. = 1.44) on top of it. The direction of the incident light was perpendicular to the surface and the polarization axis of the field was set either along the central axis of the gap (labelled gap mode, parallel to the blue source arrows in Figure S32) and perpendicular to this axis (labelled off gap mode). The simulation range was from either 350 - 650 (only off-gap Ag dimer simulation) or 400 - 850 nm and the step size was 1 nm. Overall mesh of 1 nm was defined throughout the simulated volume, except in the gap area a finer mesh was used: the mesh size was set to that between particles surfaces there were at least 10 elements (e.g. 2 nm gap equals 0.2 nm mesh). The finer mesh included the gap and a small part of the surface of each particle. Monitors were placed in the gap and outside to visualize and plot the E-field in respect to the position and the wavelength. The reference field $E_0$ was calculated by removing the particles and the coating layers.

![Figure S32](image-url)

Figure S32. the model of DNA coated nanoparticle dimer on silicon surface used in the FDTD simulations. (A) the side view of the model. The dimer composed of two nanoparticles separated by distance d (np surface to np surface), where both have 1.12 nm DNA coating layer on top. The substrate is composed of 3 nm SiO$_2$ layer on top Si. The medium is defined as air. The polarization of the incident light (along the gap in the figure) is set either along the axis of the gap or perpendicular to it. (B) the isometric view of the same model. The x, y- and z-axes are defined in b.

We varied the particle surface to particle surface distance based on the gap distribution in the Figure 1 (from 3.5 nm to 1.2 nm). Since the Lumerical model does not take into account quantum mechanical
effects, we restricted the gap distance to higher values than 1 nm to avoid effects like e.g. tunneling. We also defined water in the gap, when the distance between DNA layers equal or smaller than 0.75 nm, since it is less probable that there would exist a pure air gap between the DNA layers and rather the ambient moisture could form a water layer between the DNA layers in these distances. This means that gap size of 2.5 nm and 3 nm included a water layer (R.I. = 1.33) in-between the DNA layers. In general, the gold dimer had the predicted FE of 315 at 637 nm for 2.5 nm gap (1.0x10^{10}), 396 at 710 nm and 1.5 nm gap (2.5x10^{10}) and 601 at 736 nm and 1.2 nm gap (1.31 x10^{11}). For the Ag dimers, the predicted FE is 183 (1.1x10^{9}) for 532 nm excitation and a 2.5 nm gap, 473 (5.0 x10^{10}) for 633 nm excitation and a 1.5 nm gap, while the highest FE (621; 1.4x10^{11}) is predicted to be at 664 nm for a 1.2 nm gap.

Figure S33 and Figure S34 show the field enhancement maps of the dimer gap region, when the distance between the nanoparticle surfaces is 1.2 nm, 2.5 nm or 3.5 nm and the excitation is at or close to one of the peaks in the FE curves in Figure 3. The highest field is always localized in the gap region with little or no visible coupling to the surface.
The off-gap FEs for both AuNP and AgNP were also solved (see Figure S35, Figure S36 and Figure S37). The AgNP dimer off-gap spectra had the LSPR at 380.6 nm for 3.5 nm gap and redshifted slightly to 384 nm when the gap was reduced to 1.2 nm. For gold nanoparticles, the same wavelengths are 529 nm to 525 nm, respectively. This corresponds to the plasmonic excitation of single AgNP and AuNP. Notably, for the case of 1.2 nm gap and AgNP dimer, we observed an additional peak corresponding to coupling between silicon substrate and the NP at 366 nm wavelength (see Figure S36C). In the case of gold, no such additional modes were observed (see Figure S37B). However, these modes are not localized within the
gap region hence the FE in the gap is 1.95 – 2.77 for silver and 0.59 – 0.66 for gold dimer and we would not expect to detect single molecule spectra in these cases.

Figure S35. the field enhancement curve of Au and Ag dimers in respect to the gap distance when the polarization of the incident light is perpendicular to the gap (the parallel excitation is shown in Figure 1). (A) the field enhancement of Ag dimer, when the distance is varied from 1.2 nm to 3.5 nm. The peaks are around 380 nm, which corresponds to the LSPR of single coated AgNP. (B) the field enhancement of Au dimer, when the distance is varied from 1.2 nm to 3.5 nm. The peaks are around 530 nm, which corresponds to the LSPR of single coated AuNP. The field values are extracted from the middle of the gap region in all of the cases.
Figure S36. The field enhancement maps of the Ag dimer at different excitation wavelengths, when the gap size is 2.5 nm and the polarization is perpendicular to the gap axis. (A)-(B) the top view FE maps when the excitation is at 366 nm and 383 nm, respectively. (C)-(D) the side view FE maps when the excitation is at 366 nm and 383 nm, respectively. The silicon substrate is at the bottom of the C and D figures. The excitation at 383 nm corresponds to LSPR of the single coated AgNP, whereas the 366 nm excitation seems to include coupling to silicon substrate as well as the dipole excitation.

Figure S37. The field enhancement maps of the Au dimer at 532 nm excitation, when the gap size is 2.5 nm and the polarization is perpendicular to the gap axis. (A) the top view FE map when the excitation is at 532 nm. (B) the side view FE map when the excitation is at 532 nm. The silicon substrate is at the bottom of the B figure. The excitation corresponds to LSPR of the single coated AuNP with some coupling to the silicon substrate.
21. The calculation of the hot spot volumes

Figure S38. The field enhancement maps of the hot spot of the Au dimer at 633 nm excitation, when the polarization is along the gap axis. The hot spot is defined as a volume, where \( \frac{E}{E_0} \) is roughly \( 10^8 \) - \( 10^9 \) or \( \frac{E}{E_0} \) is 100-178. (A)-(C) the top and the two side views of the hot spot around the gap area, when the gap size is 3 nm. The white arrows indicate the border of the hot spot volume. The hot spot volume resembles roughly a concave lens with the radius of 3.75 nm, the width at the edges of 3.8 nm and the width at the narrowest region of 3 nm. The total volume is then 159 nm\(^3\). (D)-(F) the top and the two side views of the hot spot around the gap area, when the gap size is 1.2 nm. The white arrows indicate the border of the hot spot volume. The hot spot volume is approximately a cylinder with an ellipsoidal cross section, where the length of the cylinder is 1.45 nm and the diameters are 2.8 nm and 3.02 nm. The total volume is then 38.6 nm\(^3\).
### 22. List of modified DNA origami nanofork strands

Table S1 – The list of the modified nanofork strands

| Strand   | Strand# in Table S1 | Sequence                                                                 | 5' end Mod. | 3' end Mod. | Supplier  |
|----------|---------------------|--------------------------------------------------------------------------|-------------|-------------|-----------|
| S173-NP cap. | 173                 | AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA GGG AGC CCC CGA TTT AGA GCT TAA ACC TGT CGT GCC AGC TGC ATT AAA ACA ACA ACA ACA ACA ACA AC |             |             | Metabion  |
| S129-NP cap. | 129                 | AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA TTT TGG TCG AGG TGC CGT AGA GGC GGT TTG CGT AAT GGG AAC AAC AAC AAC AAC AAC AAC AAC AAC AAC AAC |             |             | Metabion  |
| S161-Cy5    | 161                 | Cy5 - TTT TAA AGC ACT AAA TCG GAA CCC TTG AAT CGG CCA ACG CGC CCG GGG G | Cy5         |             | Metabion  |
| S161-Cy3.5  | 161                 | Cy3.5 - AA AGC ACT AAA TCG GAA CCC TTG AAT CGG CCA ACG CGC CCG GGG G | Cy3.5       |             | Sigma Aldrich |
| S161-Tamra  | 161                 | Tamra - TTT TAA AGC ACT AAA TCG GAA CCC TTG AAT CGG CCA ACG CGC CCG GGG G | Tamra       |             | Metabion  |
| S161-4-P-AA | 161                 | 4-Pyridine acetic acid - 4-P – TTT AAA GCA CTA AAT CGG AAC CCT TGA ATC GGC CAA CGC GCG GGG | 4-Pyridine acetic acid - 4-P |         | Biomers   |
| S161-thiol  | 161                 | AAA GCA CTA AAT CGG AAC CCT TGA ATC GGC CAA CGC GCG GGG TT - C3 - Thiol |             | C3-thiol    | Metabion  |

### 23. List of DNA origami nanofork staple strands

Table S2 – The list of the general DNA origami nanofork strands

| Strand# | Start | End | Sequence                                                                 | Length |
|---------|-------|-----|--------------------------------------------------------------------------|--------|
| 1       | 41[147] | 35[150] | TTTGACCAGCGGATAATCAAAGTTTCAAGGTTATAGTGCAA AAAACTCG                      | 46     |
| 2       | 8[233] | 8[215] | TATAAGTATAGCCGCTGT                                                       | 19     |
| 3       | 47[54] | 40[56] | AAATTTTATACAAAACCCACTACAGTGACGGGAAGCAAACCTCC                             | 44     |
| 4       | 60[173] | 65[178] | CACGACGAATTCGTTATTTATATTTAATCGATTACATTTTTGACG                           | 45     |
| 5       | 82[233] | 49[233] | GTGCCACGTAGACCGCGTGA                                                    | 20     |
| 6       | 27[172] | 22[168] | CGGGACAGATTTAATTTATAGCCGCTCAGTGAGTTGCAAAATGACG                        | 38     |
| 7       | 23[151] | 63[139] | AGACGTGAAAATCTCCAAAAATTTAATCCAAAACAGGGAGACG                             | 45     |
| 8       | 42[181] | 47[173] | AGCCTAATCTCCGAAACGGGGTATTAAATGCGTGCAGTTCAGCTGACCGTG                  | 48     |
|    | 9  | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  | 19  | 20  | 21  | 22  | 23  | 24  | 25  | 26  | 27  | 28  | 29  | 30  | 31  | 32  | 33  | 34  | 35  | 36  | 37  | 38  | 39  | 40  | 41  | 42  | 43  | 44  | 45  | 46  | 47  | 48  |
|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    |    | 35[138] | 38[138] | ATCAATAGGATTGTATAAGCAAAAAAGCCCCAAGAGGGGAAAACGAGA | 46  |
| 10 | 40[124] | 45[118] | AGCCCGAAGAGCCTCCAGGCAAGACAGCAGATTAGT | 34  |
| 11 | 26[160] | 59[160] | GTACAGAAGTAATCAACGACGATTAAGT | 28  |
| 12 | 22[209] | 63[202] | AGCCGAGTGAAGATACATGTATTAGATATCATT | 35  |
| 13 | 40[41] | 40[17] | AGAGAGTACCTTTAATTGCTTTTTTTT | 27  |
| 14 | 51[174] | 37[187] | GTTAAATCTAGGTGACTACATGACTG | 28  |
| 15 | 2[233] | 2[215] | CACCCCTCAGACGTGTACC | 19  |
| 16 | 56[233] | 11[233] | AGATGATGAATTATCACAGGGCGATTTGCTCAGT | 34  |
| 17 | 41[23] | 43[41] | TTTTTTTTCTTTGATAATTTTGCTTTTTTTTTCAC | 55  |
| 18 | 42[141] | 45[137] | GAAATCGGCGGCTTATCAAGCCGTTTTTATTAAATTCTGTCC | 42  |
| 19 | 44[48] | 47[53] | TGACCATTAGCTATGACCCCTACGCAAGGTTAA | 57  |
| 20 | 21[215] | 21[235] | ATGGGAATTTTGCTACGGGGTC | 21  |
| 21 | 40[139] | 48[131] | AAAAGATAGGCCAAAAAGGTAAAGTCAGAAAGGCGG | 37  |
| 22 | 38[202] | 47[202] | ATAGCTAATCAGAGCATAATTTATACAA | 28  |
| 23 | 91[25] | 90[25] | CGTGAACCATCACCCAAATCAAGTCGCCAGGGTGGTTTTTCTTTTCAC | 48  |
| 24 | 49[182] | 40[182] | GTAATCGGAAACCTAATTGGAACACCC | 28  |
| 25 | 43[17] | 44[17] | TACATCATGGCCATTGCGACATTGGCATTAAGTGA | 36  |
| 26 | 30[163] | 55[160] | TGGTTTAATATTTAAGTATCAGGGCGCAT | 31  |
| 27 | 39[138] | 41[146] | ATCTACAAACGGGAGACGTCAAATACGCAATCTG | 37  |
| 28 | 63[140] | 26[138] | TATAACGATACGAGTGTCGATAGGGCTG | 30  |
| 29 | 29[138] | 32[138] | CCCAGCTGGGCTTTCCGCAAGTGAACTGGAATTTACCCAGGTAAGA | 46  |
| 30 | 22[83] | 63[76] | AGGTTGAAATTTCTTAAGGCGGCTTTTTATAAGTAAACA AAC AAC AAC AAC AAC AAC AAC AAC | 59  |
| 31 | 37[138] | 40[140] | ATCACATGCGGCAATTAAAACTGAGAGCCAGATGTA | 36  |
| 32 | 28[160] | 57[160] | GCTCATGGAACCCCGCTGCTG | 28  |
| 33 | 43[42] | 42[15] | CTGAAATGTCAGAAGCTACAAACGGGTACATTTTGCGTTTTTTT | 41  |
| 34 | 32[160] | 35[166] | GAAACACATTTAGGTTGAAGCCACGCTTTCTGTAGTTTTTTGACGAT | 48  |
| 35 | 42[97] | 40[104] | AGGGTTGCGGTTGTCGGAACCGCCTAATGAGTACGCAATAT | 50  |
| 36 | 36[181] | 49[181] | AAAAGAACTGGTCAAATAGTAAGAAC | 28  |
| 37 | 65[91] | 24[98] | TATCGGCTTCTGACGAGCTACGGACGTACAGAG | 35  |
| 38 | 44[195] | 49[199] | TCCCTATGCAACAATGTAATTTTGCCGTACTAGATATATCTGAC | 46  |
| 39 | 51[221] | 81[241] | ATCACAATGGGCTTAAAAACTGAGAGCCAGATGTA | 35  |
| 40 | 46[233] | 1[233] | GGCTTAAAATGGCAGAGACTCACAACCCCATCAT | 34  |
| 41 | 50[212] | 55[213] | AAATGCCTGATAATCATAAGCTTACGTACTATAAA | 35  |
| 42 | 30[181] | 59[173] | CCATTACTAAGCTGTTTGAGGGGACAAAGGCAAGGCGGCCGCA | 48  |
| 43 | 65[116] | 22[126] | AACAATATTATTTAAAGGACATACAAACAAAGGCTCC | 38  |
| 44 | 22[125] | 63[118] | AAAAGGAGCTTTTACAGCAGGTTTTTAGGCTACT | 35  |
| 45 | 38[160] | 47[160] | TTTAAACAACTCAGGGGCTAGCCAATATG | 28  |
| 46 | 20[233] | 20[215] | TGCCCTGACTTATTTCTGT | 19  |
| 47 | 51[203] | 38[203] | ATGTAATAATATTCTCCTTTTTATAAGCAA | 28  |
| 48 | 45[138] | 42[142] | AGACGACGAGAACAAGCCCGTGATCTAAAGAAGTGGGTTCC | 42  |
CAGAGCCTCAATCACAATTCATCAATTGGTAACATCTTTACATGAAATG
AGTTTGATTTTCTGGGAGGGCGCAACCTTTGAAAGAGATATTCATACCCAGT
ATTGTCAATTATTTGGGATAGCAGCCTTTAGCGCGTTTGGAGCCA
AAAGGAAGCTGGCAAGAGTCTCTTCTTT
GTAACACCCAAACAGGAGCCTTTAGAATCGAAATAATATCCCATCCT
AAAGGAAGCTGGCAAGAGTCTCTTCTTT
GTAACACCCAAACAGGAGCCTTTAGAATCGAAATAATATCCCATCCT
AAA AAA AAA AAA AAA AAA AAA AAA AACGGGTGAATACATGGCGAG
TGGGTAAATCGGTGAGTAGTAAATTGTTAACGTAAGAAC
CGAGCTCGTTGTAATTGACAACAAAGCT
AAAAACGGGCCAAAACATTCAAAAAACGACTCATTACAGGCTTGAGA
TGGGTAAATCGGTGAGTAGTAAATTGTTAACGTAAGAAC
CCATTCACAATTTCCAGGACGTTGGGTAAATCACCCGTCACCCGTTTT
AGTTAATGCCCCGTTAGTA
AGGATTAGCGGGGATAGTTA
GGCAAAAAAATACTCGTCGGCGCGCAACAT
GACCAAACACCCTGCACTTTTTTTTT
CATTTAAAACTCATAAGACTCATAACCC
TGAACAAATAAGAAAAAGCATTAAACGCGCCTGTATTTACATCCACTTGGC
AATCCCTTCTGCAATGAAAGAAAGGAA
CCACCGAGGAAACCGTAGCCCCAACAGAAGTCAGAT
TTTTAGTAAGTTGAGATTATTATTGCGTCAA
GGAAGGTTAAAATGGAAGGG
ACCGTACTCAGGAGTACAA
CCTCGTGCCCGAACGCCGCTCACTCCATGGAACGGT
TGGCTTCGCTCAAGGCGGCCAGTGGGATCCCACACAACTGCTTT
CGGTCAAACTGACCAGACGGGCCACCAAAGCGTCAGAAAGGAACA
ATCTCAAGAATATTCTAGTGTGCA
TACATCCTTTAGGAGTGCAAGAGGAGGAGATGTA
TACATCTCTTTAGGAGTCAGAAGGAGCGGAGATGTA
AGCCCTCGTCATTCAAACCAGCGAAATCGTATTA
CGCGTTTTAATTCTGAGAGACAATAAATACTAAT
ACCCTCCTGCTATTAAAAACCGGAAAGTTTGATGTA
TACATCCTTTAGGAGTGCAAGAGGAGGAGATGTA
AGGCTTTTTAATCTGAGAGGAGCAAATGTTTAGTGA
TACATCCTTTAGGAGTGCAAGAGGAGGAGATGTA
AGGCCCTGCTATTAAAAACCGGAAAGTTTGATGTA
TACATCCTTTAGGAGTGCAAGAGGAGGAGATGTA
AGGCCCTGCTATTAAAAACCGGAAAGTTTGATGTA
TACATCCTTTAGGAGTGCAAGAGGAGGAGATGTA
TTTTTTACCGTTGTAGCAATAGTCCATCGATATATGTAATGCCACTACGAAGGCTTTTT
ACGAAGGCTTTTT
TTTTTTACCGTTGTAGCAATAGTCCATCGATATATGTAATGCCACTACGAAGGCTTTTT
ACGAAGGCTTTTT
TTTTTTACCGTTGTAGCAATAGTCCATCGATATATGTAATGCCACTACGAAGGCTTTTT
ACGAAGGCTTTTT
TTTTTTACCGTTGTAGCAATAGTCCATCGATATATGTAATGCCACTACGAAGGCTTTTT
ACGAAGGCTTTTT
45
| Line | Column 1 | Column 2 | Letter | Description |
|------|----------|----------|--------|-------------|
| 171  | 7[215]   | 49[220]  | ACTACAACCCAGAAGTAAGCAAAAACCTAAAT | 34 |
| 172  | 22[235]  | 63[220]  | GTTTTAAAACACTTTCAAGGGAGTGATACATTAACC | 37 |
| 173  | 90[91]   | 91[91]   | AAAAGGAGCCCCCCGATTTTAGCTTTAACCCTGTCGTCAGCTGCAATTAA | 50 |
| 174  | 84[233]  | 47[233]  | TATTAACACAAGCGCTCAACA | 20 |
| 175  | 72[233]  | 59[233]  | CAACAGTTGCTTACATCAGG | 20 |
| 176  | 65[179]  | 24[182]  | CTCAGTCAACAACTTTCCAGTGAACCAC | 31 |
| 177  | 57[221]  | 75[233]  | ATGGACAATATTITGGAATACCCTCAA | 27 |
| 178  | 58[233]  | 13[233]  | AACAAATACGTCATAGCGCCTTGCTAAGAGAAG | 34 |
| 179  | 0[233]   | 0[215]   | AGGGATAGGAAGCCCAAATA | 19 |
| 180  | 9[215]   | 51[220]  | AGCATTCCCATATAAGAATAATATCAAGCAGA | 34 |
| 181  | 40[76]   | 43[83]   | AAAAAAAAAAAAAAAAAAAAAAAAAAAGACCTGCCCCCTAGC | 73 |
| 182  | 63[203]  | 22[210]  | TTTGCGGAATGGATAGGTAAGACCGCTCCAGCAATGATACGATTTC | 49 |
| 183  | 30[202]  | 55[202]  | CCAGCAATTGCAGGTTAAATATAGGAA | 28 |
| 184  | 4[241]   | 4[215]   | TACATCCACCTCAGAACCCTGAAGTTTT | 27 |
| 185  | 65[217]  | 65[235]  | CACCAGTACACTTAGACG | 19 |
| 186  | 23[17]   | 25[41]   | TTTTTTTTTCCACGCATAACCACCAAGCGGGCCTTA | 55 |
| 187  | 43[168]  | 40[162]  | AGCGAATTTGCGCAATCCAAAGCTTATATAGTC | 34 |
| 188  | 31[138]  | 34[138]  | TGCACTCCCATAGTGCTACGTTGGTGGATATTCACTCAGCAACACT | 46 |
| 189  | 74[233]  | 57[233]  | TATTCGGAACCTCTGCAAC | 20 |
| 190  | 50[149]  | 57[150]  | AAATATTTAAGCTCATTTTCGGCAACAGGCGTATACTGGCTCAGGCTTC | 49 |
| 191  | 24[97]   | 65[90]   | GCTTTGCAAGGGCCGCTAAACTCAGATTACCGCCTGGCTGACGTCAAC | 49 |
| 192  | 38[182]  | 51[173]  | TCAATTGAAAAGGGGTGTGAATAAAAATGAGAATCGCATATGTTATTTT | 49 |
| 193  | 24[76]   | 62[70]   | AAAAAAAAAAAAAAAAAAAAAATCTCATGATTGGACCTCGGCTC | 45 |
| 194  | 62[241]  | 17[233]  | TACATCATTATCATCATACGTCACCAATCACCAGGCTATTCCGGAGATGTA | 49 |
| 195  | 52[174]  | 55[173]  | CATTAAACCATTTGGCTGAGGTGAC | 27 |
| 196  | 17[215]  | 59[220]  | TCCAGACCTGGAACCGACCATCATCTATAAAAAAGTA | 34 |
| 197  | 47[174]  | 42[182]  | CTAGCTTTTATATCATAGCGACGAGATTITTTCGGCT | 36 |
| 198  | 22[167]  | 65[157]  | TAATATTGTGTAAGAATCTCAGAGCAAAACGCTCAT | 46 |
| 199  | 24[181]  | 27[189]  | CACCAGACCCGAATGTGAATAATACGAGCAGCGTTCTCATTTC | 50 |
| 200  | 53[221]  | 79[233]  | AGCAGAATGAGCGCCTATGACATGCTCAGT | 27 |
| 201  | 62[69]   | 65[69]   | AGTCGGGGCTGCCTACATGACATTTC | 28 |
24. The caDNAno design of the DNA origami nanofork
25. References

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