Single Molecule DNA Origami Nanoarrays with Controlled Protein Orientation

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SUPPLEMENTARY MATERIAL

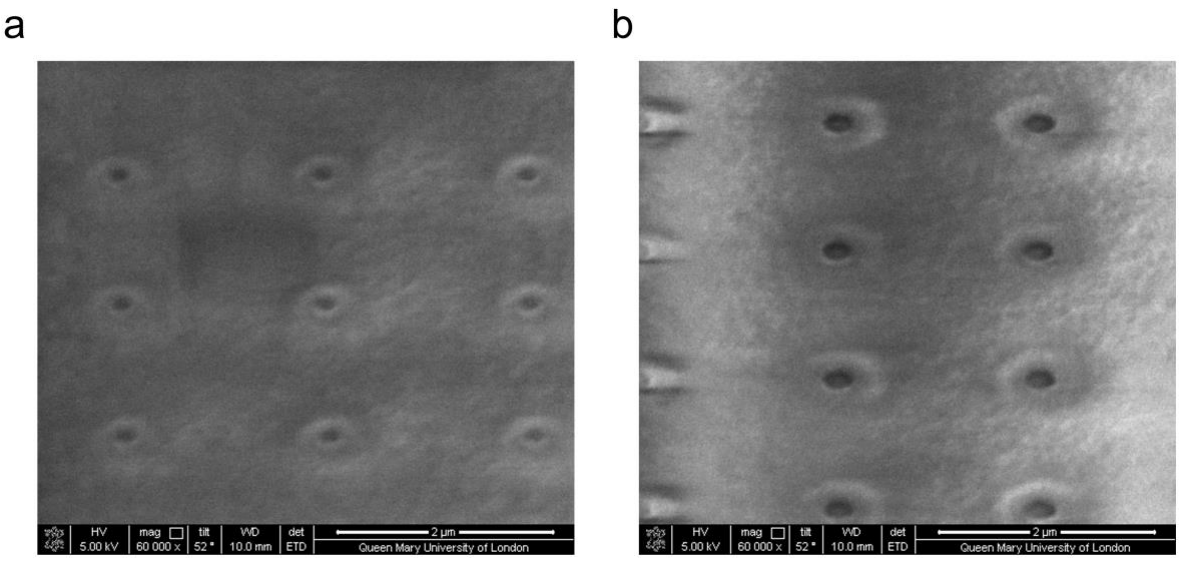

Figure SM1. Representative SEM images of the patterned nanoarrays with 2 µm period. (a) 150 nm nanoapertures. (b) 250 nm nanoapertures.

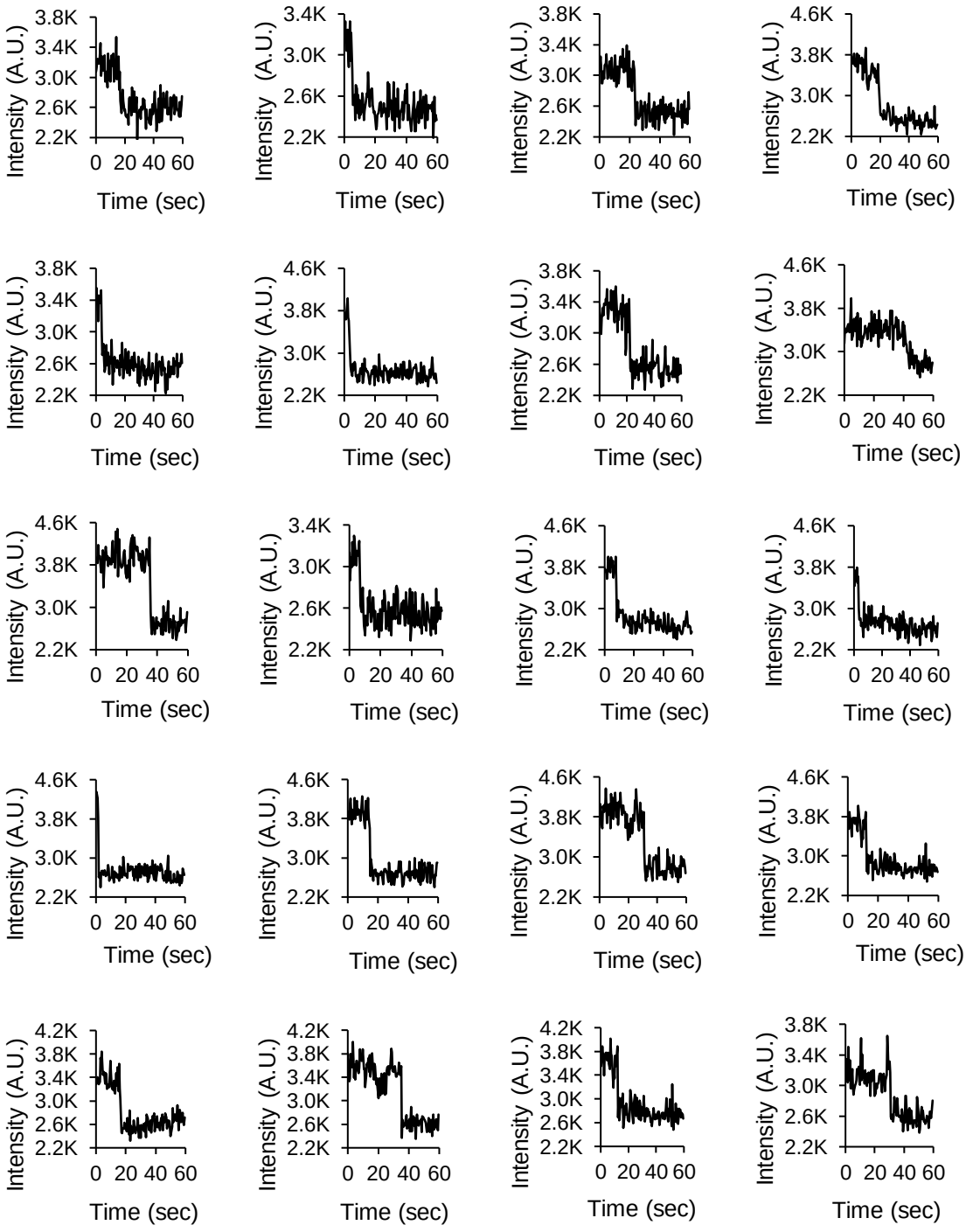

Figure SM2. Additional photobleaching events of a single ATTO 488 tethered to a DNA origami in the nanoarray in Figure 2 (main text).

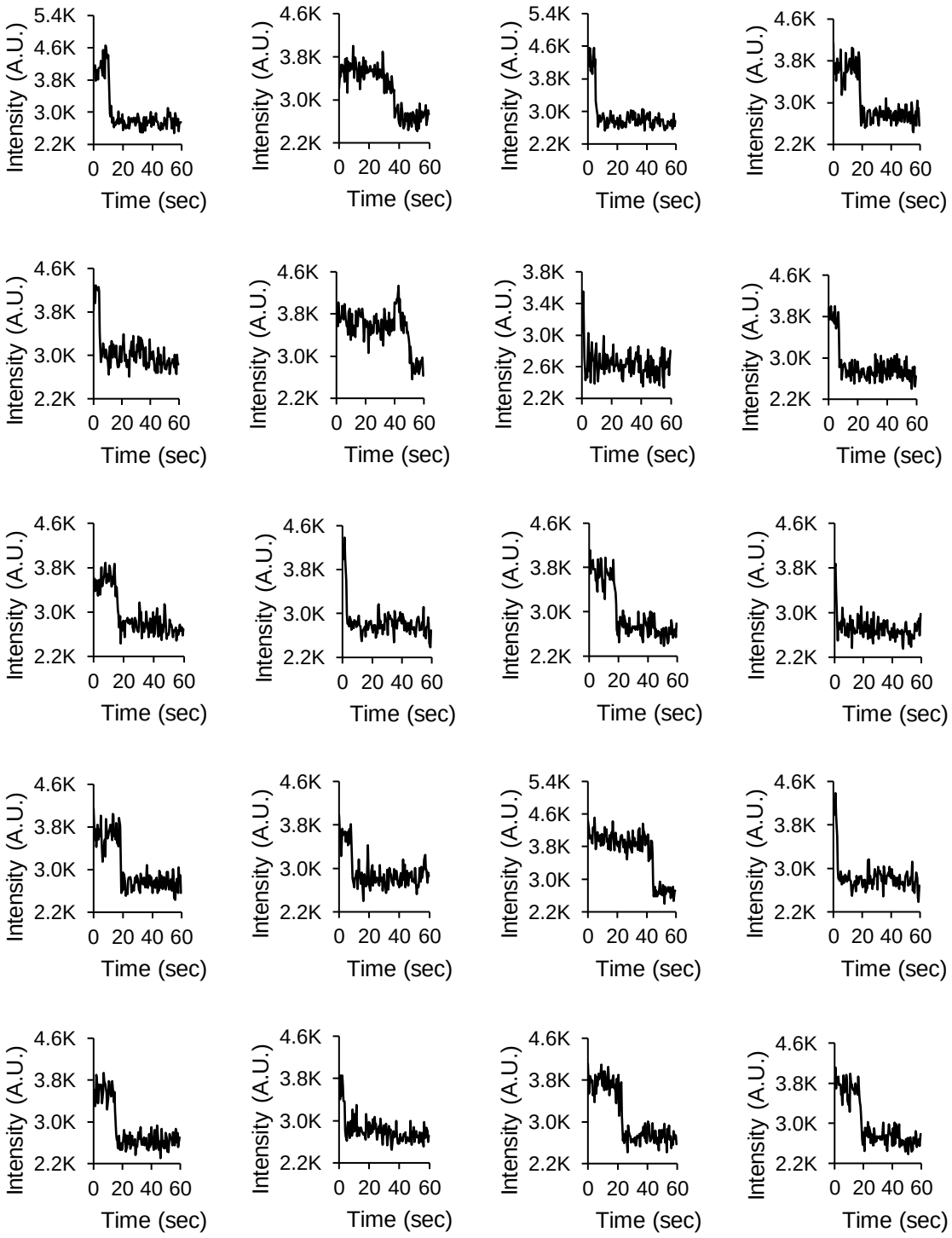

Figure SM2. (Continuation).

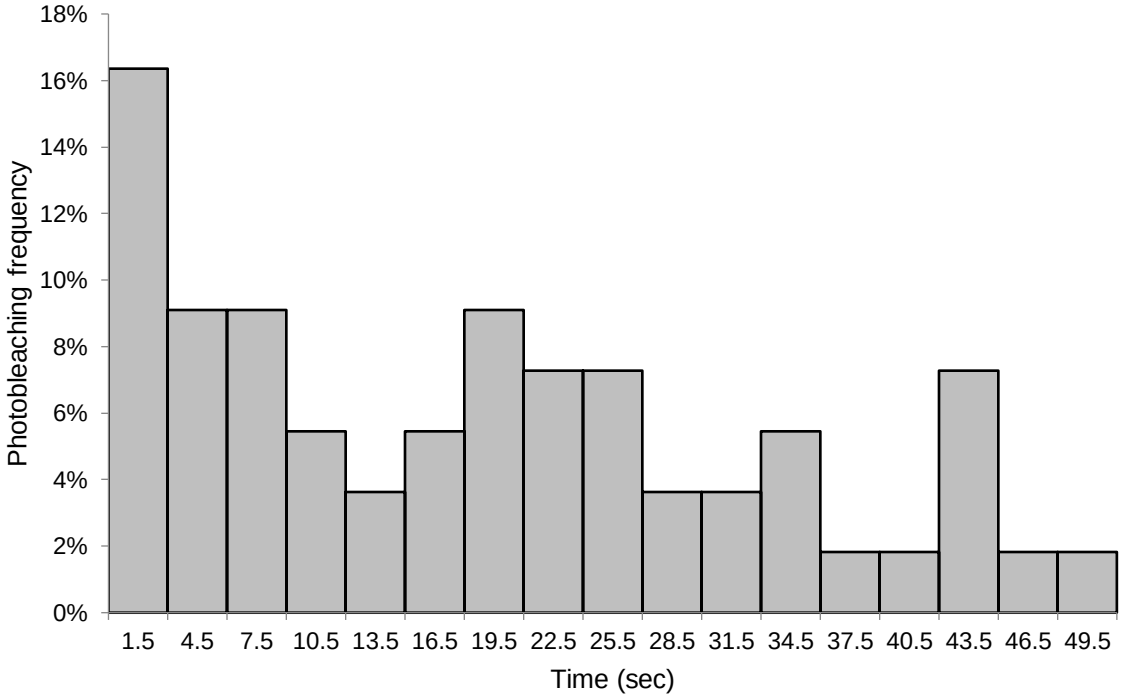

Figure SM3. Histogram of the single step photobleaching events (on to off transitions) of the nanoarray in Figure 2 in the main text.

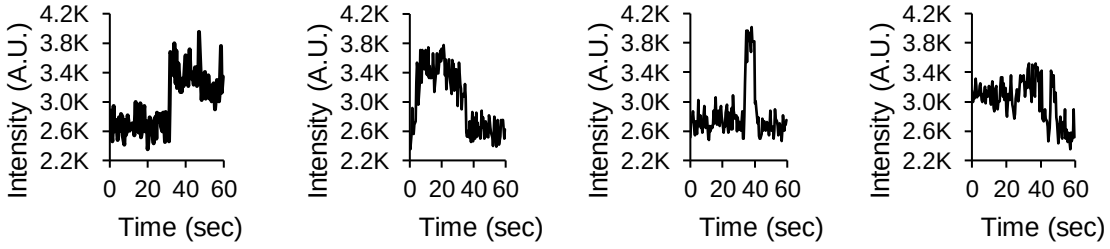

Figure SM4. Representative traces of the diverse single-step transition events. These are classified from left to right as: Low-High, Low-High-Low, Low-High-Low, and High-Low-High-Low.

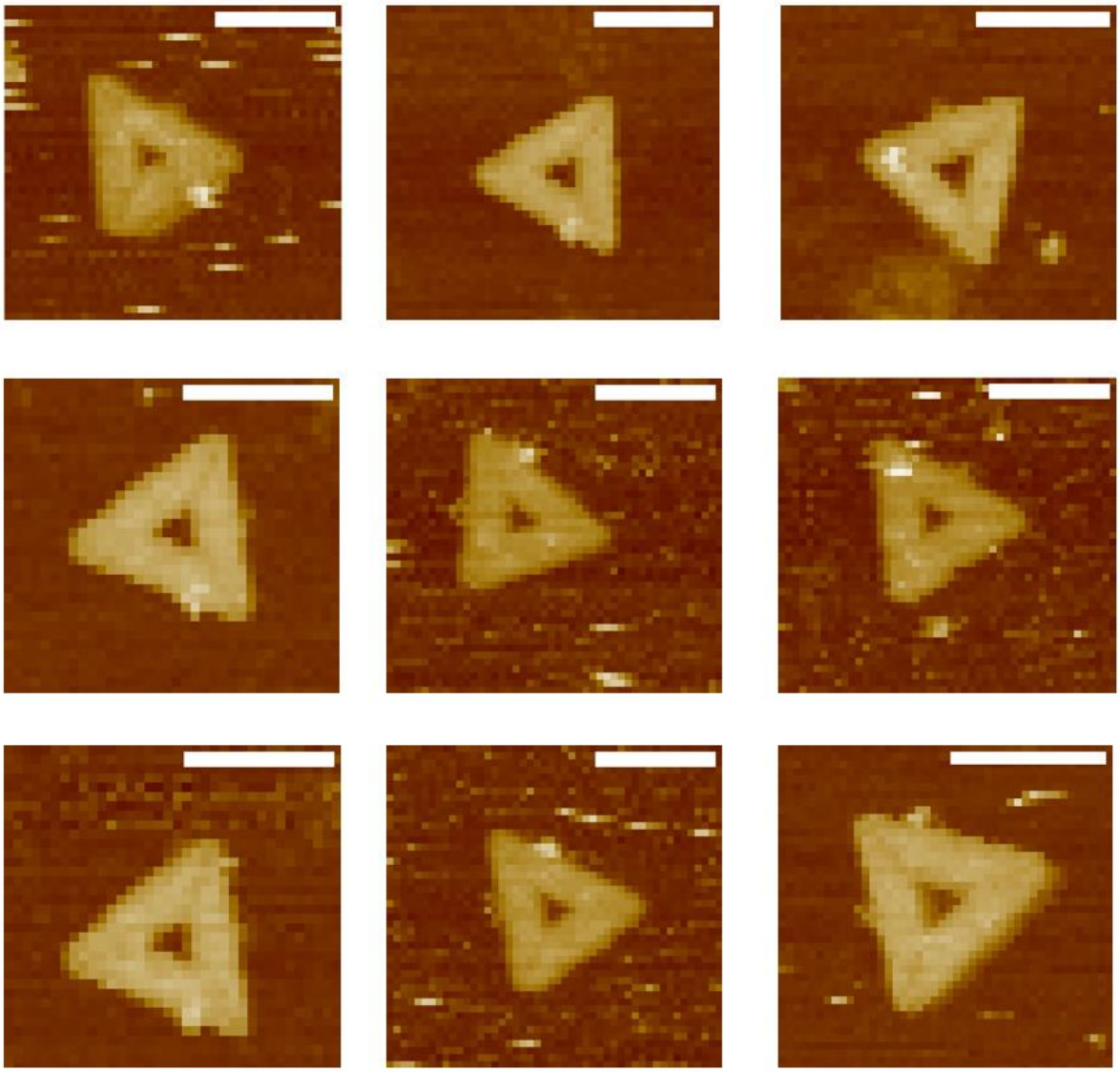

Figure SM5. Additional ‘AFM in fluid’ images of the protein in the edge of the DNA origami (see Figure 4b). Scale bar = 100 nm.

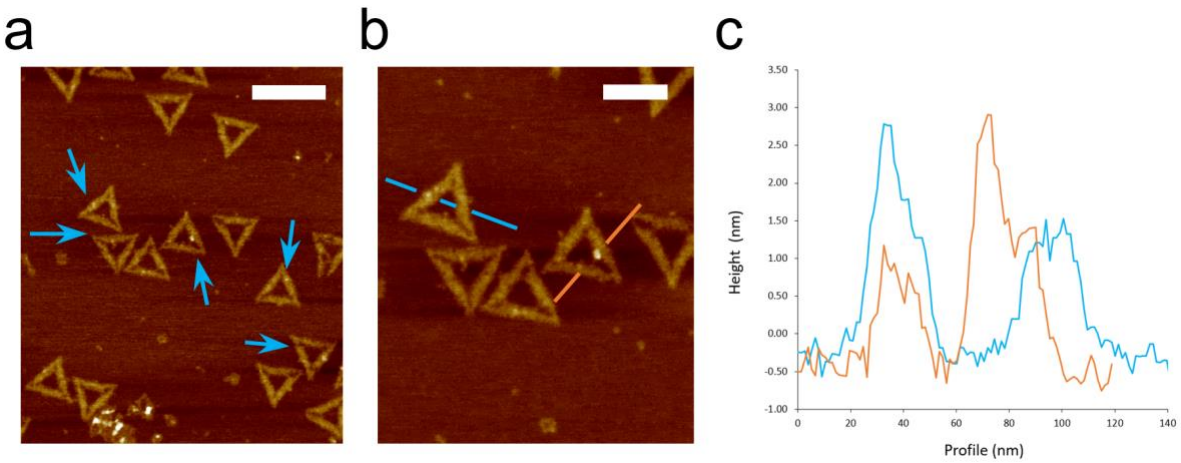

Figure SM6. Additional 'AFM in air' images of the protein on the face of the DNA origami (see Figure 4C). (a) Representative image of DNA origamis. Blue arrows indicate the position of the protein in the origami. (b) Zoom in while scanning. Blue and orange lines indicate the profile section used in (c). (c) Height profile of the blue and orange lines. Scale bar = 200 nm.

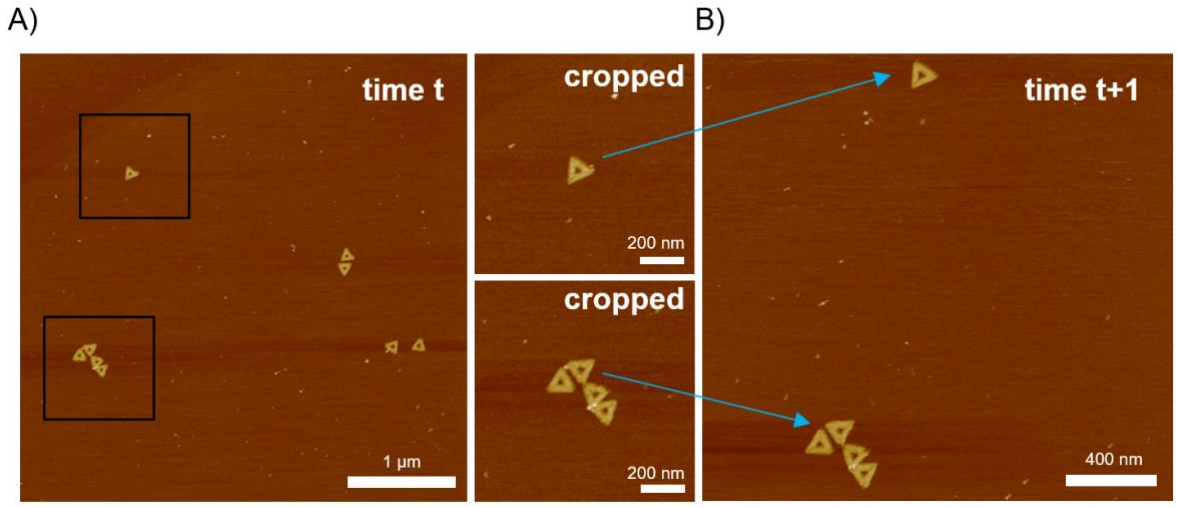

Figure SM7. Representative 'AFM in liquid' of GFP protein placed in the DNA origami's face. (a) Large area scan. (b) Cropped images taken from the frame in (a). Cropped images depict attached proteins. (c) Frame following the frame in (a) but scanning with zoom in. Buffer imaging was 1x TAE buffer/12.5 mM Mg^{2+}. Scale bars in (a-c) are 1 µm, 200 nm and 400 nm, respectively.

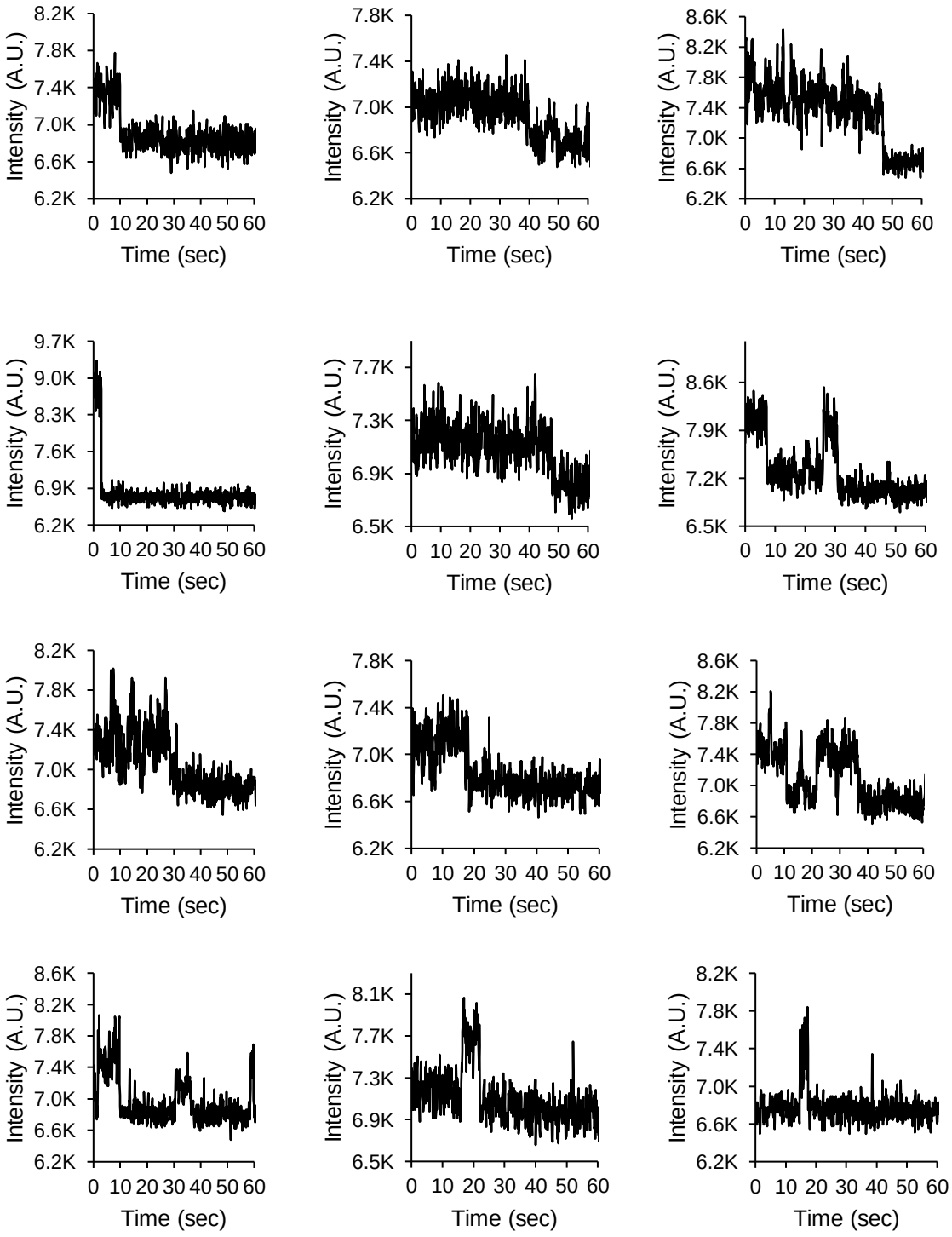

Figure SM8. Additional single molecule trajectory traces of GFP tethered to DNA origami.

Sequences for the Cy5 strand and amine strand:

Amine-strand: /5AmMC6/GGTCATTAGGTCATCGTAGC

Cy5-strand: /5Cy5/ ACAGAGCTGGAGTGGT GCTACGATGACCTAATGACC

Modified staple for the protein anchor and protein strand:

C31-EDGE-PROT: GGTACGCTCAACATTC CTGACACTGACTACTAACGG TTTT GCCACCGAGTAAAAGAACATCACTTGCCTGAGCGCCATTAAAA

A39-FACE-PROT: GGTACGCTCAACATTC CTGACACTGACTACTAACGG TTTT TTATCAAACCGGCTTAGGTTGGGTAAGCCTGT

DNA-PROT: CCGTTAGTAGTCAGTGTCAG GAATGTTGAGCGTACC /3AmMO/

Modified staple for the dye anchor and dye strand:

The ATTO 488 was attached to a DNA aptamer relevant to cortisol sensing (called dye strand).[1] As the sequences of the dye strand tended to self-dimerize, the capturing strand in the DNA origami was designed to hybridize to the dye strand in such a way that a single dye strand was placed on the face of the origami.

dye strand: /5ATTO488N/**AAGGAATGGAT** **CCACATCCATGG** ATGGGCAATGCGGGGTGGAGAATGGTTGCCGCACTTCGGCTTCACTGCAGACTTGA CGAAGCTT

A40-staple: GACTCTTGAA **CCATGGATGTGG** TT **ATCCATTCC** AAA TTAGTATCGCCAACGCTCAACAGTCGGCTGTC

Modified staples for the biotin anchor and biotin strand:

A52-staple: CCCATCCTCGCCAACATGTAATTTAATAAGGC CTGATGATTGATACCG

A44-staple: TCAATAATAGGGCTTAATTGAGAATCATAATT CTGATGATTGATACCG

A07-staple: AAAGACAACATTTTCGGTCATAGCCAAAATCA CTGATGATTGATACCG

A15-staple: GGAGGGAATTTAGCGTCAGACTGTCCGCCTCC CTGATGATTGATACCG

B52-staple: GTACAACGAGCAACGGCTACAGAGGATACCGA CTGATGATTGATACCG

B44-staple: ATTGTGTCTCAGCAGCGAAAGACACCATCGCC CTGATGATTGATACCG

B07-staple: AACCAGACGTTTAGCTATATTTTCTTCTACTA CTGATGATTGATACCG

B15-staple: GATTAGAGATTAGATACATTTCGCAAATCATA CTGATGATTGATACCG

C52-staple: CGCGCGGGCCTGTGTGAAATTGTTGGCGATTA CTGATGATTGATACCG

C44-staple: CCAGGGTGGCTCGAATTCGTAATCCAGTCACG CTGATGATTGATACCG

C07-staple: GGACATTCACCTCAAATATCAAACACAGTTGA CTGATGATTGATACCG

C15-staple: TGACCTGACAAATGAAAAATCTAAAATATCTT CTGATGATTGATACCG

Biotin strand: /5Biosg/CGGTATCAATCATCAG

Staple strands for the triangular DNA origami:

A01: CGGGGTTTCCTCAAGAGAAGGATTTTGAATTA

A02: AGCGTCATGTCTCTGAATTTACCGACTACCTT

A03: TTCATAATCCCCTTATTAGCGTTTTTCTTACC

A04: ATGGTTTATGTCACAATCAATAGATATTAAAC

A05: TTTGATGATTAAGAGGCTGAGACTTGCTCAGTACCAGGCG

A06: CCGGAACCCAGAATGGAAAGCGCAACATGGCT

A07: AAAGACAACATTTTCGGTCATAGCCAAAATCA

A08: GACGGGAGAATTAACTCGGAATAAGTTTATTTCCAGCGCC

A09: GATAAGTGCCGTCGAGCTGAAACATGAAAGTATACAGGAG

A10: TGTACTGGAAATCCTCATTAAAGCAGAGCCAC

A11: CACCGGAAAGCGCGTTTTCATCGGAAGGGCGA

A12: CATTCAACAAACGCAAAGACACCAGAACACCCTGAACAAA

A13: TTTAACGGTTCGGAACCTATTATTAGGGTTGATATAAGTA

A14: CTCAGAGCATATTCACAAACAAATTAATAAGT

A15: GGAGGGAATTTAGCGTCAGACTGTCCGCCTCC

A16: GTCAGAGGGTAATTGATGGCAACATATAAAAGCGATTGAG

A17: TAGCCCGGAATAGGTGAATGCCCCCTGCCTATGGTCAGTG

A18: CCTTGAGTCAGACGATTGGCCTTGCGCCACCC

A19: TCAGAACCCAGAATCAAGTTTGCCGGTAAATA

A20: TTGACGGAAATACATACATAAAGGGCGCTAATATCAGAGA

A21: CAGAGCCAGGAGGTTGAGGCAGGTAACAGTGCCCG

A22: ATTAAAGGCCGTAATCAGTAGCGAGCCACCCT

A23: GATAACCCACAAGAATGTTAGCAAACGTAGAAAATTATTC

A24: GCCGCCAGCATTGACACCACCCTC

A25: AGAGCCGCACCATCGATAGCAGCATGAATTAT

A26: CACCGTCACCTTATTACGCAGTATTGAGTTAAGCCCAATA

A27: AGCCATTTAAACGTCACCAATGAACACCAGAACCA

A28: ATAAGAGCAAGAAACATGGCATGATTAAGACTCCGACTTG

A29: CCATTAGCAAGGCCGGGGGAATTA

A30: GAGCCAGCGAATACCCAAAAGAACATGAAATAGCAATAGC

A31: TATCTTACCGAAGCCCAAACGCAATAATAACGAAAATCACCAG

A32: CAGAAGGAAACCGAGGTTTTTAAGAAAAGTAAGCAGATAGCCG

A33: CCTTTTTTCATTTAACAATTTCATAGGATTAG

A34: TTTAACCTATCATAGGTCTGAGAGTTCCAGTA

A35: AGTATAAAATATGCGTTATACAAAGCCATCTT

A36: CAAGTACCTCATTCCAAGAACGGGAAATTCAT

A37: AGAGAATAACATAAAAACAGGGAAGCGCATTA

A38: AAAACAAAATTAATTAAATGGAAACAGTACATTAGTGAAT

A39: TTATCAAACCGGCTTAGGTTGGGTAAGCCTGT

A40: TTAGTATCGCCAACGCTCAACAGTCGGCTGTC

A41: TTTCCTTAGCACTCATCGAGAACAATAGCAGCCTTTACAG

A42: AGAGTCAAAAATCAATATATGTGATGAAACAAACATCAAG

A43: ACTAGAAATATATAACTATATGTACGCTGAGA

A44: TCAATAATAGGGCTTAATTGAGAATCATAATT

A45: AACGTCAAAAATGAAAAGCAAGCCGTTTTTATGAAACCAA

A46: GAGCAAAAGAAGATGAGTGAATAACCTTGCTTATAGCTTA

A47: GATTAAGAAATGCTGATGCAAATCAGAATAAA

A48: CACCGGAATCGCCATATTTAACAAAATTTACG

A49: AGCATGTATTTCATCGTAGGAATCAAACGATTTTTTGTTT

A50: ACATAGCGCTGTAAATCGTCGCTATTCATTTCAATTACCT

A51: GTTAAATACAATCGCAAGACAAAGCCTTGAAA

A52: CCCATCCTCGCCAACATGTAATTTAATAAGGC

A53: TCCCAATCCAAATAAGATTACCGCGCCCAATAAATAATAT

A54: TCCCTTAGAATAACGCGAGAAAACTTTTACCGACC

A55: GTGTGATAAGGCAGAGGCATTTTCAGTCCTGA

A56: ACAAGAAAGCAAGCAAATCAGATAACAGCCATATTATTTA

A57: GTTTGAAATTCAAATATATTTTAG

A58: AATAGATAGAGCCAGTAATAAGAGATTTAATG

A59: GCCAGTTACAAAATAATAGAAGGCTTATCCGGTTATCAAC

A60: TTCTGACCTAAAATATAAAGTACCGACTGCAGAAC

A61: GCGCCTGTTATTCTAAGAACGCGATTCCAGAGCCTAATTT

A62: TCAGCTAAAAAAGGTAAAGTAATT

A63: ACGCTAACGAGCGTCTGGCGTTTTAGCGAACCCAACATGT

A64: ACGACAATAAATCCCGACTTGCGGGAGATCCTGAATCTTACCA

A65: TGCTATTTTGCACCCAGCTACAATTTTGTTTTGAAGCCTTAAA

B01: TCATATGTGTAATCGTAAAACTAGTCATTTTC

B02: GTGAGAAAATGTGTAGGTAAAGATACAACTTT

B03: GGCATCAAATTTGGGGCGCGAGCTAGTTAAAG

B04: TTCGAGCTAAGACTTCAAATATCGGGAACGAG

B05: ACAGTCAAAGAGAATCGATGAACGACCCCGGTTGATAATC

B06: ATAGTAGTATGCAATGCCTGAGTAGGCCGGAG

B07: AACCAGACGTTTAGCTATATTTTCTTCTACTA

B08: GAATACCACATTCAACTTAAGAGGAAGCCCGATCAAAGCG

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B12: AACTCCAAGATTGCATCAAAAAGATAATGCAGATACATAA

B13: CGTTCTAGTCAGGTCATTGCCTGACAGGAAGATTGTATAA

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B33: AGGGATAGCTCAGAGCCACCACCCCATGTCAA

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B36: GCGCAGACTCCATGTTACTTAGCCCGTTTTAA

B37: ACAGGTAGAAAGATTCATCAGTTGAGATTTAG

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B39: ATTTTCTGTCAGCGGAGTGAGAATACCGATAT

B40: ATTCGGTCTGCGGGATCGTCACCCGAAATCCG

B41: CGACCTGCGGTCAATCATAAGGGAACGGAACAACATTATT

B42: AGACGTTACCATGTACCGTAACACCCCTCAGAACCGCCAC

B43: CACGCATAAGAAAGGAACAACTAAGTCTTTCC

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B50: AGCGTAACTACAAACTACAACGCCTATCACCGTACTCAGG

B51: TAGTTGCGAATTTTTTCACGTTGATCATAGTT

B52: GTACAACGAGCAACGGCTACAGAGGATACCGA

B53: ACCAGTCAGGACGTTGGAACGGTGTACAGACCGAAACAAA

B54: ACAGACAGCCCAAATCTCCAAAAAAAAATTTCTTA

B55: AACAGCTTGCTTTGAGGACTAAAGCGATTATA

B56: CCAAGCGCAGGCGCATAGGCTGGCAGAACTGGCTCATTAT

B57: CGAGGTGAGGCTCCAAAAGGAGCC

B58: ACCCCCAGACTTTTTCATGAGGAACTTGCTTT

B59: ACCTTATGCGATTTTATGACCTTCATCAAGAGCATCTTTG

B60: CGGTTTATCAGGTTTCCATTAAACGGGAATACACT

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C06: AAGGAATTACAAAGAAACCACCAGTCAGATGA

C07: GGACATTCACCTCAAATATCAAACACAGTTGA

C08: TTGACGAGCACGTATACTGAAATGGATTATTTAATAAAAG

C09: CCTGATTGCTTTGAATTGCGTAGATTTTCAGGCATCAATA

C10: TAATCCTGATTATCATTTTGCGGAGAGGAAGG

C11: TTATCTAAAGCATCACCTTGCTGATGGCCAAC

C12: AGAGATAGTTTGACGCTCAATCGTACGTGCTTTCCTCGTT

C13: GATTATACACAGAAATAAAGAAATACCAAGTTACAAAATC

C14: TAGGAGCATAAAAGTTTGAGTAACATTGTTTG

C15: TGACCTGACAAATGAAAAATCTAAAATATCTT

C16: AGAATCAGAGCGGGAGATGGAAATACCTACATAACCCTTC

C17: GCGCAGAGGCGAATTAATTATTTGCACGTAAATTCTGAAT

C18: AATGGAAGCGAACGTTATTAATTTCTAACAAC

C19: TAATAGATCGCTGAGAGCCAGCAGAAGCGTAA

C20: GAATACGTAACAGGAAAAACGCTCCTAAACAGGAGGCCGA

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C23: TTAAAGGGATTTTAGATACCGCCAGCCATTGCGGCACAGA

C24: ACAATTCGACAACTCGTAATACAT

C25: TTGAGGATGGTCAGTATTAACACCTTGAATGG

C26: CTATTAGTATATCCAGAACAATATCAGGAACGGTACGCCA

C27: CGCGAACTAAAACAGAGGTGAGGCTTAGAAGTATT

C28: GAATCCTGAGAAGTGTATCGGCCTTGCTGGTACTTTAATG

C29: ACCACCAGCAGAAGATGATAGCCC

C30: TAAAACATTAGAAGAACTCAAACTTTTTATAATCAGTGAG

C31: GCCACCGAGTAAAAGAACATCACTTGCCTGAGCGCCATTAAAA

C32: TCTTTGATTAGTAATAGTCTGTCCATCACGCAAATTAACCGTT

C33: CGCGTCTGATAGGAACGCCATCAACTTTTACA

C34: AGGAAGATGGGGACGACGACAGTAATCATATT

C35: CTCTAGAGCAAGCTTGCATGCCTGGTCAGTTG

C36: CCTTCACCGTGAGACGGGCAACAGCAGTCACA

C37: CGAGAAAGGAAGGGAAGCGTACTATGGTTGCT

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C41: TTTCACCAGCCTGGCCCTGAGAGAAAGCCGGCGAACGTGG

C42: GTAACCGTCTTTCATCAACATTAAAATTTTTGTTAAATCA

C43: ACGTTGTATTCCGGCACCGCTTCTGGCGCATC

C44: CCAGGGTGGCTCGAATTCGTAATCCAGTCACG

C45: TAGAGCTTGACGGGGAGTTGCAGCAAGCGGTCATTGGGCG

C46: GTTAAAATTCGCATTAATGTGAGCGAGTAACACACGTTGG

C47: TGTAGATGGGTGCCGGAAACCAGGAACGCCAG

C48: GGTTTTCCATGGTCATAGCTGTTTGAGAGGCG

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C50: GGATAGGTACCCGTCGGATTCTCCTAAACGTTAATATTTT

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C53: CTAAATCGGAACCCTAAGCAGGCGAAAATCCTTCGGCCAA

C54: CGGCGGATTGAATTCAGGCTGCGCAACGGGGGATG

C55: TGCTGCAAATCCGCTCACAATTCCCAGCTGCA

C56: TTAATGAAGTTTGATGGTGGTTCCGAGGTGCCGTAAAGCA

C57: TGGCGAAATGTTGGGAAGGGCGAT

C58: TGTCGTGCACACAACATACGAGCCACGCCAGC

C59: CAAGTTTTTTGGGGTCGAAATCGGCAAAATCCGGGAAACC

C60: TCTTCGCTATTGGAAGCATAAAGTGTATGCCCGCT

C61: TTCCAGTCCTTATAAATCAAAAGAGAACCATCACCCAAAT

C62: GCGCTCACAAGCCTGGGGTGCCTA

C63: CGATGGCCCACTACGTATAGCCCGAGATAGGGATTGCGTT

C64: AACTCACATTATTGAGTGTTGTTCCAGAAACCGTCTATCAGGG

C65: ACGTGGACTCCAACGTCAAAGGGCGAATTTGGAACAAGAGTCC

Link-A1C: TTAATTAATTTTTTACCATATCAAA

Link-A2C: TTAATTTCATCTTAGACTTTACAA

Link-A3C: CTGTCCAGACGTATACCGAACGA

Link-A4C: TCAAGATTAGTGTAGCAATACT

Link-B1A: TGTAGCATTCCTTTTATAAACAGTT

Link-B2A: TTTAATTGTATTTCCACCAGAGCC

Link-B3A: ACTACGAAGGCTTAGCACCATTA

Link-B4A: ATAAGGCTTGCAACAAAGTTAC

Link-C1B: GTGGGAACAAATTTCTATTTTTGAG

Link-C2B: CGGTGCGGGCCTTCCAAAAACATT

Link-C3B: ATGAGTGAGCTTTTAAATATGCA

Link-C4B: ACTATTAAAGAGGATAGCGTCC

Loop: GCGCTTAATGCGCCGCTACAGGGC

REFERENCES

1. Martin, J. A. *et al.* Tunable stringency aptamer selection and gold nanoparticle assay for detection of cortisol. *Anal. Bioanal. Chem. 2014 40619* **406**, 4637–4647 (2014).