to genome-integrated origin-terminator constructs, in which the direct conjunction of the terminator and origin sequence is present in the synthesized circular DNA, but not in the genomic template. This mechanism for expression of DNA circles from a genomic template is similar to the mechanism of induction used by integrating ("temperate") filamentous phages[542], such as CTXϕ and its satellite phages. In the present scenario, it would need to be adapted to mammalian chromosomes and mammalian rolling circle viruses such as the porcine circovirus[192]. Partial homology between origin and terminator sequences might also need to be mutated away to completely prevent cutting of the genome-embedded sequences.

It would also likely be necessary to optimize the absolute and relative expression of Cas1/Cas2, and perhaps further optimize the system, via directed evolution of these proteins, in order to ensure sufficiently high spacer acquisition rates to achieve time-resolved recording on timescales of practical interest.

7.3 Autonomously stretching and directly visualizing a single DNA double helix: a route to versatile 1D nano-arrays?

Using structural DNA nanotechnology[525], we (joint with Mingjie Dai and Ralf Jungmann) have designed and experimentally validated a system to stretch out a single DNA double-helix or ssDNA strand of arbitrary user-programmable sequence within a defined "frame" structure. This could be used to display the strand for high-resolution microscopy studies (e.g., electron microscopy or atomic force microscopy), or to harness it as a completely addressable scaffold – down to the 0.34 nm linear precision of the DNA base pair – for arranging nanoscale components along a 1D array.

The DNA double helix represents a versatile substrate for constructing one-dimensional nanostructures: arbitrary DNA sequence motifs can be arranged in any order along its length, allowing sequence-specific DNA binding moieties to be placed in close proximity. To capture the potential

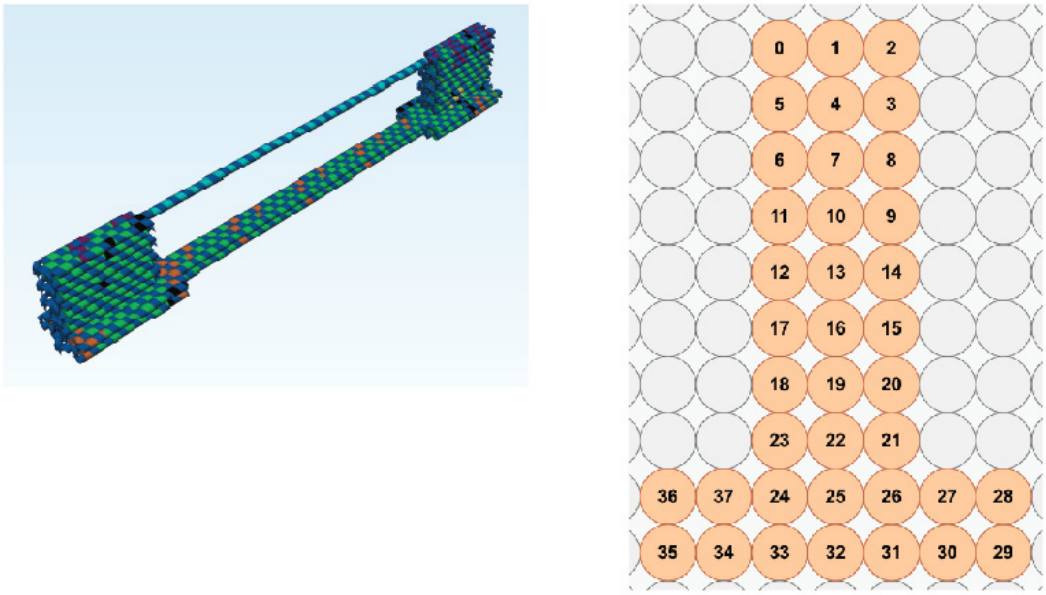

Figure 7.7: Design of the DNA tightrope in caDNAno2. The three-dimensional view and square-lattice [341] cross-section of the scaffolded DNA origami nanostructure are shown. Each cylinder or circle represents a single DNA double helix, linked with its neighbors by single-stranded crossovers, in a pattern reminiscent of basket weaving.

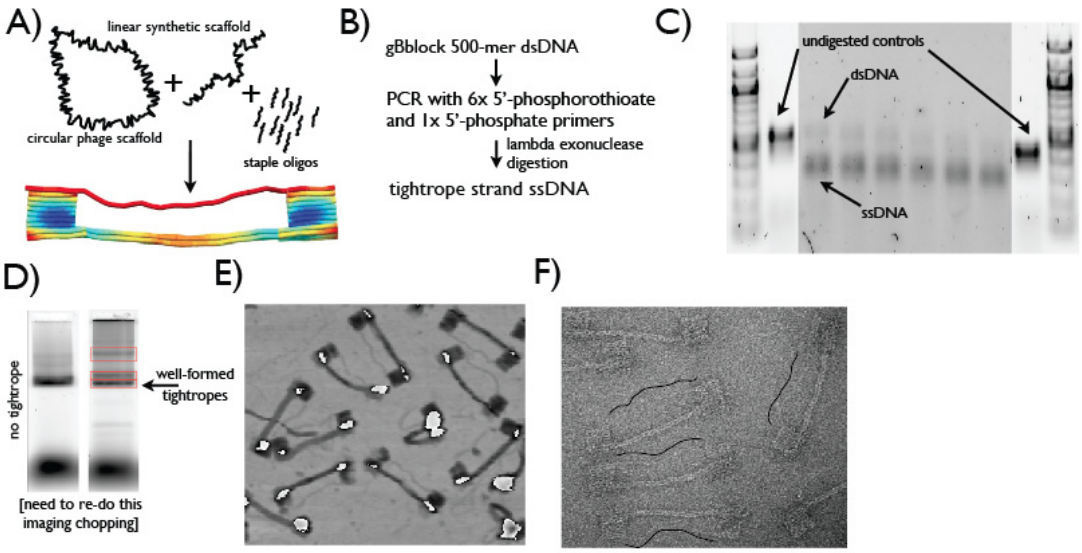

Figure 7.8: Construction and imaging of the DNA tightrope. A) The structure is assembled by combining circular m13mp18 phage ssDNA with DNA origami staple oligonucleotides and with a user-designed linear ssDNA strand generated from a synthetic dsDNA by exonuclease digestion. 3D model produced by CanDo[347]. B) Scheme for amplification and exonuclease digestion of the linear strand. C) Agarose gel illustrating the near-complete conversion of dsDNA to ssDNA by exonuclease digestion, for various lengths of starting dsDNA. D) Agarose gel confirming folding of the scaffolded DNA origami structure. E) AFM image showing visible tightropes. F) Negative stain TEM image showing visible DNA tightropes; black lines are drawn in, offset from the DNA tightropes, to highlight the paths of the DNA tightropes.

of the DNA helix for nanotechnology, it would be useful to constrain DNA to adopt a straightened linear conformation, rather than a random coil. While we have elsewhere assisted in demonstrating a DNA elongation and deposition method that relies on macroscale instrumentation[516], here we develop a simple and customizable DNA origami platform that stretches an arbitrary double-stranded DNA segment like a tightrope across two rigid pillars with a defined pillar-to-pillar spacing. In addition to straightening the DNA, the platform is easy to identify by imaging and can be functionalized with organic or inorganic groups at many addressable sites. The tightrope can be placed in tension by modulating its length relative to that of the supporting structure. This system should facilitate the rapid prototyping of one-dimensional nano-systems.

The support for the track was designed using caDNAno[167] and is assembled using standard 3D

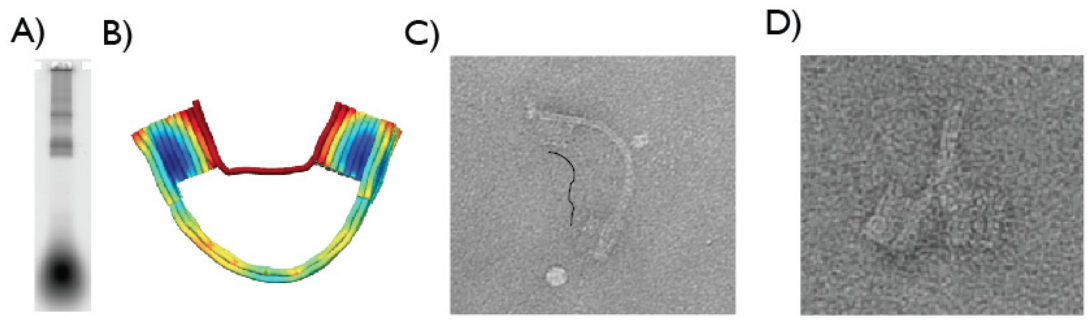

Figure 7.9: Effects of shortening the tightrope. A) Agarose gel showing successful folding of tightrope structures with shortened linear gBlock strands. B) CanDo [347] analysis showing the expected bent structure backbone resulting from mechanical strain. C) TEM image confirming the bent configuration; the tightrope is visible and an offset black line is drawn in to highlight its position. D) Another configuration observed in TEM, probably representing mechanical buckling of the structure backbone under strain.

scaffolded DNA origami techniques[166,108], whereby the m13mp18 genome is folded via 175 short staple strands. The tightrope strand begins as a 300 to 500-mer dsDNA "gBlock" available commercially from Integrated DNA Technologies. This gene block is then amplified by PCR with a phosphorothioate forward primer and a phosphate reverse primer, and the amplicons are subsequently exposed to lambda-exonuclease. The phosphate-primed strands are digested by the exonuclease, while the phophorothioate-modified strands remain intact, and can then be integrated with the support to form the ssDNA tightrope.

7.4 NM2CM: STRATEGIES FOR INTEGRATING TOP-DOWN AND BOTTOM-UP NANOTECHNOLOGY, TO CONSTRUCT FULLY PROGRAMMABLE BIO-CHIPS

No integrated architecture has yet been proposed which fully specifies the steps necessary to produce structures with a) overall sizes on the scale of today's computer chips (centimeters), b) addressable features on the 10 nm scale, and c) the ability to attach a wide range of discrete components at customizable locations. We have performed initial theoretical and experimental investigations into a scheme for nanometer-to-centimeter fabrication integration via top-down organization of DNA nanorods using