

Supplementary Materials

Table S1: Overview of the software covered in this review as well as their scope, main features and their website. "+" indicates an improvement, "-" a restriction of the software. The websites listed in this table have been last accessed on the 14th of April 2021.

CAD Software	Scope	Main Features	Website
Vienna RNA (1994, inactive) [1]	Secondary structures of RNA strands	Early tool to predict secondary RNA structures	-
Mfold (2003, inactive) [2]	Secondary structures of RNA & DNA strands	Early tool to predict secondary RNA/DNA structures	-
GIDEON (2006, inactive) [3]	<i>de novo</i> design of 3D DNA nanostructures	GUI for the design of DNA nanostructures Kink & Hairpin relaxation	-
SARSE (2008, inactive) [4]	Lattice-based DNA origami design	Semi-automated DNA origami design	sourceforge.net/projects/sarse (download, historical reference)
UNIQUMER3D (2009, inactive) [5]	<i>de novo</i> design of 3D DNA nanostructures	3D modelling tool for DNA constructs	-
Scaffold-based			
Cadnano 1.0 /2.0 (2009/2012) [6]	Lattice-based scaffolded DNA origami design	GUI allowing for design from scratch & manual manipulation of strands Lattice-based (honeycomb or square lattice) + 2.0 introduces undo button	cadnano.org/legacy cadnano.org (download)
Cadnano 2.5 (2018)			github.com/cadnano/cadnano2.5 (download)
scadnano (2020) [7]	Lattice-based scaffolded DNA origami design	Similar to Cadnano script based online tool	scadnano.org (direct use via browser)
Tiamat (2009) [8]	Lattice and scaffold free DNA nanostructure design	+ No geometrical constraints + corrects for: secondary structures, repetitions and GC-content - needs manual adjustment	yanlab.asu.edu/Resources.html (download)
vHelix (2015) [9]	Automated 3D wireframe DNA origami design	Automated 3D wireframe design + Scaffold automatically transverses through every edge evenly - Restricted to designs equivalent to a sphere	vhelix.net (download, req. Maya)
DAEDALUS (2016) [10]	Fully automated 3D wireframe origami design	Automated 3D wireframe origami design	daedalus-dna-origami.org (download, req. Maya)

		+ No geometrical restriction to a sphere + designs stable at low salt - no GUI	
PERDIX (2019) [11]	Fully automated 2D wireframe origami design	Automated 2D wireframe origami design + Arbitrary large 2D constructs - no GUI	perdix-dna-origami.org (download, req. Maya)
TALOS (2019) [12]	Fully automated 3D wireframe origami design (higher stability)	Automated 3D wireframe origami design + Increased mechanical stability due to six-helix edges - Material intensive, requires high salt conc.	talos-dna-origami.org (download, req. Maya)
METIS (2019) [13]	Fully automated 2D wireframe origami design (higher stability)	Automated 2D wireframe origami design + Increased mechanical stability due to six-helix edges - Material intensive, requires high salt conc. - no GUI	metis-dna-origami.org (download, req. Maya)
ATHENA (2020) [14]	Fully automated 2D & 3D wireframe origami design	Combines all features of DEADALUS, PERDIX, TALOS, METIS in an interactive GUI	github.com/lcbb/Athena (download, req. Maya)
Tile-based			
DNA Pen (2013) [15]	2D tile-based DNA designs	Free hand drawn or digitalized 2D design + Automatic inclusion of poly-T chains to prevent base stacking - only planar structures	guptalab.org/dnapen (download)
3DNA (2014) [16]	3D tile-based DNA designs	Digitalized 3D design + Allows for arbitrarily large structures + Accounts for GC content & Hamming distance	guptalab.org/3dna/index.html (download)
Hex-Tiles (2019) [17]	2D Triangulated Wireframe Structures using DNA Tiles	Triangulated 2D Wireframe Structures without a scaffold + Allows for arbitrarily large structures + Rolled up sheets resemble 3D hollow tubes + physiological salt conditions	github.com/tls-dna/hex-tiles(download)

<i>Analytical tools</i>			
UNAFold (2008) [18]	DNA & RNA folding and hybridization prediction	Continuation of Mfold	www.unafold.org (download)
NUPACK (2010) [19]	DNA folding and hybridization prediction	Suitable for multiple strand analysis	nupack.org (download & direct use via browser)
ViennaRNA Package 2.0 (2011) n[20]	RNA secondary structure prediction	RNA secondary structure prediction	www.tbi.univie.ac.at/RNA/#download (download)
CanDo (2011) [21]	2D & 3D modeling of DNA nanostructures	Finite element modeling framework for DNA origami assemblies input: caDNAno or Tiamat	cando-dna-origami.org (submission via browser)
oxDNA/oxDNA2 (2015) [22] oxView (2020) [23]	2D & 3D Coarse-grained modelling of DNA & RNA assemblies	Coarse-grained modelling of DNA/RNA for DNA origami assemblies Includes Monte Carlo and Molecular Dynamics simulations Easy to visualize via browser-based oxView	dna.physics.ox.ac.uk/index.php (download) sulcgroup.github.io/oxdna-viewer (direct use via browser)
TacoxDNA (2019) [24]	Web-based interface for converting common formats of DNA structures	input: XYZ coordinate file, cadnano, Tiamat, CanDo, oxDNA, PDB output: oxDNA, PDB	tacoxdna.sissa.it (direct use via browser)
MrDNA (2019) [25]	Fast analysis of DNA nano-structures with high resolution	Faster prediction of low- & high-resolution models at Near-Atomic Resolution Predicts 3D shape & equilibrium properties input: cadnano, vHelix, DAEDALUS, CanDo, oxDNA, PDB	gitlab.engr.illinois.edu/tbgl/tools/mrdna (download)
Adenita (2020) [26]	Universal approach for the design and/or analysis of DNA nano-structures	Combines several previous approaches and also other molecular structures input: cadnano, vHelix, DAEDALUS	samson-connect.net/element/dda2a078-1ab6-96ba-0d14-ee1717632d7a.html (download, req. SAMSON)
SNUPI (2021) [27]	Rapid analysis of DNA Origami structures with high resolution	Rapid analysis due to a multiscale analysis framework Predicts 3D shape, equilibrium dynamic	github.com/SSDL-SNU/SNUPI (download)

		properties & mechanical rigidity input: cadnano	
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Table S2: List of used oligonucleotide sequences for AFM imaging shown in figure 9.

oligo1	CAGGCAAAGCGCCATTCGCCATTCAGGCTGCGCAATCCAGCCA
oligo2	GTAACAACCCGTCGGATTCGGCCAGTGCCAAGCTTGCA
oligo3	AAAAGCCCCAAAAACAGGAGCACCGCTTCTGGTGCCGG
oligo4	ATCACCATCAATATGATATTCATCAACATTAAATGTGA
oligo5	CCTCAGAGCATAAAGCTAATATGTACCCCGTTGATAA
oligo6	TGCGAACGAGTAGATTTAGTGAGAAAGGCCGGAGACAG
oligo7	AGCAAACCTCCAACAGGTCAATTAGCAAAATTAAGCAAT
oligo8	TTGAATCCCCCTCAAATGCATATAACAGTTGATTCCCA
oligo9	ACATAACGCCAAAAGGAATGCTTCAAAGCGAACCAGAC
oligo10	GTTTAATTTCAACTTTAATTGCGGAATCGTCATAAATA
oligo11	TTGAAAGAGGACAGATGAATACCACATTCAACTAATGC
oligo12	GGCAAAAGAATACTACTAAAAGTAGTAAATTGGGCTTGA
oligo13	GCTTGCAGGGAGTTAAAGGTAAGGGAACCGAACTGACC
oligo14	CCAGTCACGACGTTGTAAAACGACTCCGTGGG
oligo15	GGCGATTAAAGTTGGGTAACGCCAGGGGATAGG
oligo16	ACGCCAGCTGGCGAAAAGGGGATGTCGTAACC
oligo17	AAGGGCGATCGGTGCGGGCCTCTTGACGACA
oligo18	AACAAACGTTCTGTAGCCAGCTTCAACCGT
oligo19	TCACGTTGAACGCCATCAAAAATGAGGGTAG
oligo20	GTGCATCTTTTGTTAAATCAGCTCGCTATCAG
oligo21	GTATCGGCGTTAATATTTTGTTAAACAAGA
oligo22	GCTTCCGAGATTGTATAAGCAAACTAGCAT
oligo23	GTCTGGCCGCGGATTGACCGTAATGGTTTTT
oligo24	ACCAATAGGGTGTAGATGGGCGCATGCTGCAA
oligo25	ATTAAATTGCCAGTTTGAGGGGACCGCTATT
oligo26	TTGTAAACCTCAGGAAGATCGCACCTGTTGGG
oligo27	TCTAGCTGTAAAGATTCAAAAGGGTTTGACCA
oligo28	CTATTTTTTTTAAATGCAATGCCATAACCTG
oligo29	GTCATTGCGCAAGGATAAAAAATTCGCGAGCT
oligo30	GAATCGATATACTTTTGCGGGAGAATAGTAG
oligo31	GTCAATCAATCGGTTGTACCAAAAACAGGCAA
oligo32	TGTGTAGGATAAATTAATGCCGAAATTCGC
oligo33	CTCATATATGAGAGATCTACAAAGATTTTTTA
oligo34	ATTTCAACCTGAGAGTCTGGAGCAAATTCGC
oligo35	ACCCTGTAGAACGGTAATCGTAAATATTTAAA
oligo36	TTAGATACCTGGAAGTTTCATTCTTTTAAACA
oligo37	TTTAGCTATGTTTTAAATATGCAAATCAAAA
oligo38	GAAAAGGTCTTAATTGCTGAATATATAGTCAG
oligo39	TAGCATTAATAAGAGGTCATTTTGTATTAAG
oligo40	GGCAAAGAGGATTAGAGAGTACCTTCGCGTTT
oligo41	TACGGTGTATTTTCGCAAATGGTCATGAGTAA
oligo42	AGCTCAACATATTTTCATTTGGGGTTAGAACC
oligo43	GCTTAGAGGGCATCAATTCTACTAAGCCTTT
oligo44	TCCTTTTGACATCCAATAAATCATACATTATG
oligo45	G TTCAGAAGGATAGCGTCCAATACCATTGTGA
oligo46	ATCAGGTCCAGAGGGGGTAATAGTGGTCAT
oligo47	AAGCAAAGAATAGCGAGAGGCTTTGAAAAATC
oligo48	AGGAAGCCCCCTCGTTTACCAGACAACAACA
oligo49	TAATTGATACGAGGCATAGTAAGAGTTGAGA
oligo50	TTTAGACTAACGAGAATGACCATAACTAAAG

oligo51	AAGTTTGGCTTTACCCTGACTATTAATGCTGT
oligo52	AAAACCAACGGATTGCATCAAAAAGCGGATG
oligo53	TATCATAACGAAAGACTTCAAATATTAATTGC
oligo54	ATTACCTTGAGAAACACCAGAACGACACTCAT
oligo55	TATACCATGCTCATTCACTGAATAGCGCGAA
oligo56	TACGTTAAGATATTTCATTACCCAATCATCGCC
oligo57	TTATTACACCTTCATCAAGAGTAAACCTGCT
oligo58	TTTAGGAACGGTGTACAGACCAGGCGCAGACG
oligo59	GCCCTGACATGCGATTTTAAGAACTAAAATG
oligo60	AACAAAGCGTCAGGACGTTGGGAATGCAAAAG
oligo61	AAGAACCGTAAAACGAACTAACGGGACGATA
oligo62	CTGGCTGAGGTAGAAAGATTCATCAGCAACAC
oligo63	GTAAAAATACCCAGCGATTATACCAAAGGCTT
oligo64	ACTTTTTCACAAACGGAGATTGTGAATCAACGT
oligo65	AGCAACGGTGTGTGCGAAATCCGCGTCTTGAC
oligo66	CAGCAGCGCTTAGCCGGAACGAGGCGCATAGG
oligo67	AGTTTCGTCACCAGTACAAAACCGATATATTCGGTCGC
oligo68	CAGGCGGATAAGTGCCGTCAATAGAAAGGAACAATA
oligo69	TTTGATGATACAGGAGTGTGGAACCCATGTACCGTAAC
oligo70	CTCAGAGCCACCACCCTCAATTAGCGGGTTTTGCTCA
oligo71	CACCGTAATCAGTAGCGACTCCAGTAAGCGTCATACAT
oligo72	ACAAAAGGGCGACATTCAACCGCCACCCTCAGAACCGC
oligo73	GCAATAATAACGGAATACCACCAATGAAACCATCGATA
oligo74	ACACCCTGAACAAAGTCAGCATATGGTTTACCAGCGCC
oligo75	TTACCAACGCTAACGAGCGTACCAGAAGGAAACCGAGG
oligo76	GTTTTTATTTTCATCGTAGCATTAGACGGGAGAAATTA
oligo77	CAACATGTTTCAGCTAATGCCAGCTACAATTTTATCCTG
oligo78	TCATATGCGTTATACAAATCACTCATCGAGAACAAGCA
oligo79	AAAATCTCGTTTCAGCGGAGTGAGGAGAGGGT
oligo80	CTTTAATTGTATGGGATTTTGCTGTGTATCA
oligo81	CGAGGTGATCGTCTTTCCAGACGTGCCACCCT
oligo82	ATAGTTGCCTCATAGTTAGCGTAACACCCTC
oligo83	CCACGCATACTACAACGCCTGTAGGATAGCAA
oligo84	TGATATAACAAGAGAAGGATTAGGGAGCCGCC
oligo85	CCGTACTAACATGAAAGTATTAAGCCAGCAT
oligo86	CAGAACCGCCCCCTGCCTATTTCGGACGATTG
oligo87	AGAGCCACTGAGTAACAGTGCCCGATCCTCA
oligo88	GCCCAATAACTGGTAATAAGTTTTTCTCTGAA
oligo89	AGACTCCTGTATAGCCCGGAATAGAAACAAC
oligo90	TATTCTGACAGGAGTTTAGTACCTAGTAAAT
oligo91	AGTTAATGCCACCCTCAGAACCGCCGATCTA
oligo92	CAGTGCCTCACCTCATTTTCAGGCATTCCAC
oligo93	ACCAGAACACCGCCTCCCTCAGAGCCGATTGA
oligo94	TGACAGGCAAAATCACCGGAACCAAAATTATT
oligo95	GCCTTGATCCCCTTATTAGCGTTTCACCGACT
oligo96	TTAAAGCCGCGCGTTTTTCATCGGCGCAAAAT
oligo97	TTTACCGTAGAATCAAGTTTGCCTCAAGGCCG
oligo98	CCACCGGACACCACCAGAGCCGCCGAGGCTG
oligo99	TTCATAATAGGTTGAGGCAGGTCAGAACCTAT
oligo100	GTCATAGCATTACAAAACAAATAATATAAAC
oligo101	AGACTGTAAGAATGGAAAGCGCAGAACGGGGT
oligo102	GGGAGGGACAATCAATAGAAAATTAGGGTAAT
oligo103	CATTAATAAAGACACCACGGAATCCCACAAG
oligo104	TGAGCCATTACATAAAGGTGGCAAGAGCAAGA
oligo105	CACCAGTAACGCAGTATGTTAGCATTACCGA
oligo106	GAAACGTCCAAAAGAACTGGCATGGATAGCCG

oligo107	TTTTGTCAAGGTAAATATTGACGGAGAGCCA
oligo108	AGAAACGCGGTGAATTATCACCGTGCCATCTT
oligo109	AAAATACATTGGGAATTAGAGCCAATTTTCG
oligo110	TCCTTATTGCACCATTACCATTAGTTAGCGTC
oligo111	TGAGCGCTTAAAAACAGGGAAGCGGAATCATT
oligo112	AATTGAGGAAAAATAGCAGCCTTTAGATATAG
oligo113	AACAATGATAAGAAACGATTTTTTACGCGAGG
oligo114	AGCCCTTTTAAACAGCCATATTATCGGGAGG
oligo115	AACAAAGTTCTTTCCAGAGCCTAAGTTGCTAT
oligo116	GAATAACAAATATCAGAGAGATAAAAGTTTA
oligo117	TCAAAAATTTAAGCCCAATAATAACATATAAA
oligo118	AATCCAAAAATAGCAATAGCTATCAACGTAG
oligo119	TTACAAAATTAAGAAAAGTAAGCAATTAAGAC
oligo120	ACCGCGCCATTAAACCAAGTACCGTCTTACCA
oligo121	AAGGCTTGCTGTCTTTCCTTATCGGGCTTAA
oligo122	CGTTTTAGTTTACGAGCATGTAGACGCCAACA
oligo123	TTTTGAAGCCTGAACAAGAAAAATGAGCCAG
oligo124	TTTGCACCAGAACGCGCCTGTTACAAAAGGT
oligo125	GAACGGGTCAATAGCAAGCAAAATCACAGAGA
oligo126	AATAATCGATCCGGTATTCTAAGAGTTTAACG
oligo127	CATCCTAACGAACCTCCCGACTTGTTATCCC
oligo128	AGATAAGTCCTTAAATCAAGATTATTTGCCAG
oligo129	ATGTGAGTGAATAACCTTGTAAGAAAAAGCCTGTTT
oligo130	CAATAACGGATTTCGCCTGATTAGGTTGGGTTATATAAC
oligo131	TCAGATGATGGCAATTCATGGAAACAGTACATAAAATCA
oligo132	ATTAGAGCCGTCAATAGATAGTACCTTTTACATCGGGA
oligo133	TATTAACACCGCCTGCAACAATTATCATCATATTCCTG
oligo134	AAAGGGACATTCTGGCCAATTAGGAGCACTAACAATA
oligo135	AATAACATCACTTGCCTGATAAAACAGAGGTGAGGCGG
oligo136	CGTATAACGTGCTTTCCTCCACCAGTCACACGACCAGT
oligo137	GGAGCCCCGATTTAGAGCGTAGCAATACTTCTTTGAT
oligo138	TAGCCCCGAGATAGGGTTGATACTATGGTTGCTTTGACG
oligo139	TTTGCGTATTGGGCGCCAGAGCACTAAATCGGAACCCCT
oligo140	TTCTTTTCGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAG
oligo141	GAAATTGTTATCCGCTCACAAATCCCTTATAAATCAAA
oligo142	CTTTGACCCGTAATGCCACTACGATCACGTTG
oligo143	ACAAAGTATGAGGAAGTTCCATAAAGGAGC
oligo144	TGATAAATCTACAGAGGCTTTGAGCTTGCTTT
oligo145	CCATGTTAAAAGACAGCATCGGAAGATACCG
oligo146	GTCAATCACCGCTTTTGCGGGATCACCATCGC
oligo147	ATTGCGAATAATAATTTTTAGGCACCAACCTAAAACGA
oligo148	TTTCAACACAAAAAAAAGGCTCCATAAACGG
oligo149	GAATTTTCTGTATCGGTTTATCAGGACTAAAG
oligo150	AAGTTTTGATTCTTAAACAGCTTCGAGGGT
oligo151	AGACAGCCGCCGACAATGACAACAGTCACCCCT
oligo162	AATCGTCGAATTACCTTTTTTAATCAATATAA
oligo163	TCCTTGACAAAATTAATTACATTTGAATAAT
oligo164	GACGCTGAAAAAGAAGATGATGAACAAAATTA
oligo165	AAAATCATGAGGCGAATTATTCATAAATTGC
oligo166	CCTCCGGCTTGCTTTGAATACCAAATGAATAT
oligo167	TTCAATTTGCTATTAATTAATTTTCGTTAAAT
oligo168	CAAGAAAAAAACATAGCGATAGCTGTTTGAAA
oligo169	ACCTGAGCGAAGAGTCAATAGTGAGTTAATT
oligo170	ATCGCGCAAGGTCTGAGAGACTACGAACGCGA
oligo171	TCCTGATTCCACCAGAAGGAGCGGAGTGCCAC
oligo172	GGAAGGGAGTAACATTATCATTTAATCTAAA

oligo173	TTTGCACGTTTGGCCGAACGTTATTATCAAAC
oligo174	GTAGATTTTACAAACAATTCGACAGCAAATC
oligo175	ACAGTAACAATACATTTGAGGATTGTTATCTA
oligo176	CAAAGAAAAGTTTGGATTATACTTCTAACAAT
oligo177	AAAGTTTGTTAGAACCTACCATATACAAACAT
oligo178	TTAAATCCTAAAACAGAAATAAAGTTCAATT
oligo179	TTAGACTTTCAGGTTTAACGTCAGGTTACAAA
oligo180	GCTGAGAGAACCACCAGCAGAAGAGTAGAAGA
oligo181	GCATCACCTTAAAACATCGCCATAATATCCA
oligo182	CCTCAATCGGCTATTAGTCTTTAAGCAACAGG
oligo183	AACAGTTGAGAATACGTGGCACAGATTTTGA
oligo184	AAATATCTCAGAGATAGAACCCTTTTACATT
oligo185	ACCGAACGCCAGCAGCAAATGAAATGCGGAA
oligo186	CTGATAGCCTTGCTGAACCTCAAATAATTTTA
oligo187	TTTTGAATAATATCTGGTCAGTTGACTCGTA
oligo188	AAAGCGTAAAAGGAATTGAGGAAGTAGAAGTA
oligo189	ACTCAAACACGCAAATTAACCGTTTGTACGGG
oligo190	GAACAATTGAGGCCACCGAGTAAAGGAAGGG
oligo191	AAAAACGCGCCAGAATCCTGAGAATAGGGCGC
oligo192	CGCTCAATCGATTAAAGGGATTTTGCGTAAC
oligo193	GGCAGATTGTTAGAATCAGAGCGGGCCGCTAC
oligo194	TGTCCATCTATCGGCCTTGCTGGTTAAAAAT
oligo195	ATAATCAGATTACCGCCAGCCATTGCGCGAA
oligo196	AACGGTACTCATGGAAATACCTACACAATAT
oligo197	CAGGAGGCCGTCTGAAATGGATTACTGACCTG
oligo198	GAAAGCCGGGTCGAGGTGCCGTAAGGTGGTTT
oligo199	AAGAAAGCGTGAAACCATCACCCAACAGCTGA
oligo200	TGGCAAGTGAAAAACCGTCTATCAAGAGAGTT
oligo201	CACCACACTTAAAGAACGTGGACTGCCCCAG
oligo202	AGGGCGCGGTGTTGTTCCAGTTTGGGTTCGA
oligo203	TTTTTTGGGCGAACGTGGCGAGAAAAGAGTC
oligo204	GCCCCACTACGAAAGGAGCGGGCGCGTGTTTT
oligo205	CAAAGGGCGTAGCGGTACGCTGCAGACAGG
oligo206	GTCCACTACCGCCGCGCTTAATGCGAGCTAAA
oligo207	TTGCCCTCCGCTTTCAGTCGGGAAACCTG
oligo208	GCAGCAAGCTAACTCACATTAATTGCGTTGCG
oligo209	CAGGCGAAAGTGTAAGCCTGGGGTGCTTAA
oligo210	AATCGGCAAATTCACACAACATACGAGCCGG
oligo211	TCGTGCCAACCAGTGAGACGGGCAAATCAAG
oligo212	CTCACTGCTCACCGCCTGGCCCTGGGGCGATG
oligo213	TGAGTGAGCGGTCCACGCTGGTTTCCAACGT
oligo214	AAGCATAAAATCCTGTTTGATGGTGAACAAGA
oligo215	GTATAAAGACACCGGAATCATAATCTTCTGTA
oligo216	TTGAGAAGTGTGATAAATAAGGCCCTTAGAA
oligo217	TGTAATTTTGACCTAAATTTAATGTAGATTAA
oligo218	TAATAAGATTTCAAATATATTTTAATTTATC
oligo219	AAAGTAATCCAATCGCAAGACAAACTTTTAA
oligo220	AAGAATAACCAACGCTCAACAGTAATTCCAA
oligo221	TACCGACCTCGCCATATTTAACAAAACCAATC
oligo222	TCATCTTCAGGCAGAGGCATTTCAATATCC
oligo223	GAAAACTTGAATATAAAGTACCGATCAACAAT
oligo224	TGTAAATGCTGATGCAAATTCTGTCCAGACGACGACAA

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