DNA Origami Failure Catalogue

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Definitions

D1.Anneal: When one heats and then reduces the temperature of a solution over a period of time to aid in the synthesis of a product, we call it annealing. Annealing can increase yields; however, new research and better understanding about the formation of DNA origami may eliminate this step [1]. Some researchers also refer to annealing as the process of bringing two strands together, similar to hybridizing.

D2.Antiparallel: When two helix bundles are parallel to each other, each helix bundle will have two single stranded DNA: one going from the 3' to the 5' end and one going from the 5' to the 3' end (in reverse). Thus a pair of the single stranded DNA between the two helix bundles in parallel goes in opposite directions (3' to 5' and 5' to 3'), and we call this antiparallel. Specifically, in DNA origami, the rastering of the scaffold orients it such that the scaffold is always antiparallel with itself in adjacent helix bundles connected by crossovers. See Chen et al. for an example of anti-parallel helices [2].

D3.Atomic Force Microscopy: An atomic force microscope uses a probe (also known as a tip or cantilever) to scan the topography of small molecules with 5nm resolution. The result of this operation is a height map corresponding to the height at which the needle interacted with the molecule of interest.

D4.Base Pair: When two single stranded DNA domains hybridize (come together) their individual nucleotides (bases) connect according to Watson and Crick's theory for pairs (A with T and C with G). One such

pair is called a base pair. Base pairs are also a measurement of length for double stranded DNA.

D5.Blunt Ends: When a segment of double stranded DNA ends, exposing one face of the hybridized base pair unstacked, we call this a blunt end.

D6.Blunt End Stacking: Between base pairs there exists a force that pulls the bases closer together. The hydrophobicity of the base pairs (their desire to avoid water molecules) causes them to "stack." Thus when blunt ends come together they will stack next to each other. With DNA origami this effect noticeably causes structures to align along their blunt ends, creating long chains of DNA origami.

D7.CaDNAno: Software that allows for the rapid prototyping of DNA origami [3].

D8.CanDo: Software that takes a file from CaDNAno and approximates the resulting origami's rigidity, global twisting, and conformation. See Kim et al. for an explanation of how the software works [4].

D9.Cation: A positively charged atom or molecule. See the definition for salt.

D10.Chirality: A molecule is chiral if it is unique from its mirror image. Conversely, an achiral molecule is not unique, e.g. in origami, an achiral structure looks the same when you flip it or orient it a different way.

D11.Coaxial Stacking: When two nonadjacent DNA domains hybridize with two adjacent domains along a single stranded DNA (ssDNA). In DNA origami, adjacent staples have coaxial stacking. See SantaLucia and Hicks for some examples [5].

D12.Complementary: A nucleotide, A, is complementary to another nucleotide, B, if the unordered pair (A,B) is equivalent to either (A,T) or (C, G).

D13.Codomain: A codomain, A, is a domain whose sequence of nucleotides are reversed and complementary to another domain, B.

D14.Crossover: When the backbone of one single stranded DNA jumps from one helix bundle to another, we call it a crossover (for use of the word "jumps" see Woo and Rothemund [6]). Crossovers can be made with either the scaffold or the staple strands.

D15.Domain: A domain of DNA is a specific, continuous sequence of single stranded DNA.

D16.DNA Origami: A method for creating nano structures by folding a long length of DNA called a scaffold. See Rothemund's original paper [7].

D17.Electron Microscopy: Using an electron beam, a small object is imaged either through the transmission of the electron beam or through the conversion of the beam into other forms of energy such as heat. In transmission electron microscopy, the molecules to be observed often need to be dehydrated. Single particle cryo-electron microscopy does not require dehydration, but it does require the solution to be frozen.

D18.Fluorescence Microscopy: Fluorescence microscopy is a way of analyzing DNA strands with light emitting fluorophores in such a way that the appearance or disappearance of visible light informs one on the state of an experiment. Techniques such as Förster resonance energy transfer (FRET), total internal reflection fluorescence (TIRF) and fluorescence quenching use fluorophores, and are all techniquess used in fluorescence microscopy.

D19.Gel Electrophoresis: A process where macromolecules (such as DNA) are put into a gel with an electric current. The result is the filtration of DNA molecules (including hybridized DNA) by their size in different parts of the gel as they migrate in presence of a voltage difference. One can use this to separate complete DNA origami from materials, the ratio of which represent the yield.

D20.Helix Bundle: A helix bundle (hb) is a consecutive section/collumn of antiparallel double stranded DNA.

D21.Inter-helical Gap: Two adjacent helix bundles connected by crossovers will repel each other such that there exists a gap between the two helices.

We call this the inter-helical gap.

D22.Nucleotide: A nucleotide (nt) is one base, sugar and phosphate of a single stranded DNA. It can have one of four possible bases, A, T, C or G, which bind according to Watson Crick interactions.

D23.Overwound: When nucleotides are deleted between ideal crossovers (every ~ 10.4 nt), each nucleotide must extend its twist per nucleotide. This results in less than 10.4 nt per twist causing each nucleotide to be overwound. Mathematically, examine a full twist of 10.4 nt for a domain A versus a full twist of 9 nt for a domain B. Domain A's nucleotides have a $\frac{1}{10.4}$ twist whereas B's nucleotides have a $\frac{1}{9}$ twist. Thus B is overwound by $\frac{1}{9} - \frac{1}{10.4} \approx 0.15$.

D24.Polarity: Just as atoms have positive or negative charges, molecules will have polarity, a vector from negative to positive of a change in charge over a length of the molecule. Polarity largely affects hydrophobicity (retraction from water), as hydrophobic molecules are non-polar and hydrophilic molecules are polar.

D25.Salt: For creating DNA origami in solution, the solution must be composed of water and a concentration of counterions. Most commonly DNA nanotechnology will use NaCl, MgCl2, MgOAc or NICl2 to provide cations Na+ or Mg2+, but there are many other options as well. The salt will bind with the backbone of the DNA, reducing its polarity. With too much polarity (too little salt), DNA is unable to bind.

D26.Scaffold: The scaffold for DNA Origami consists of a long single stranded DNA usually > 7000 nt in length, which is folded to create a desired structure. Often, DNA nanotechnology uses the genome of the m13 bacteriophage; however, other long single stranded scaffolds could also work.

D27.Seam: In DNA origami, a seam is a contour of the origami for which the scaffold strand does not cross. Seams can be closed by crossing the contour with staple strands.

D28.Seed: A seed domain is a domain of a staple that binds with a codomain of the scaffold that is 14 nt in length, as presented by Ke

et al [8]. However, the length, 14nt, should not be considered as a necessary constraint for a seed domain, as any domain that binds with high specificity before other staple domains should theoretically work the same way as a seed.

D29.Single-Layer 3D Origami: 3D Origami that is comprised of flat polygons (made from anti parallel helix bundles), constructing the surface area of an object, e.g. a hollow cube. See Linko and Dietz for more examples [9].

D30.Solution: Solution is generaly a specific ratio of salt mixed with water.

D31.Staple: A staple is a single stranded DNA that binds two disparate parts of a scaffold strand. The two disparate domains are held in place by their domains and the backbone of the staple.

D32.Strain: The strain on a segment of DNA can be thought of as the potential energy created by deforming the DNA idealized as a spring from its lowest energy state. Think of strain on DNA as the squeezing, stretching, twisting, and bending of a double helix.

D33.Strand Displacement: When part of one dna strand binds to another single stranded domain, allowing it to "invade" adjacent hybridized domains. See Qian and Winfree for more information [10].

D34.Transmission Electron Microscopy: The dehydrated version of electron microscopy. See the definition for electron microscopy.

D35.Underwound: When nucleotides are inserted between ideal crossovers (every 10.4 nt), each nucleotide must shorten its twist per nucleotide. This results in more than 10.4 nt per twist causing each nucleotide to be underwound. See the definition for overwound for calculating under or over winding.

D36.Walker: Molecular motors, such as kinesin and dynein, transport molecular payloads in alive cells. Similar attempts have been made with DNA nanotechnology. Most move a strand of DNA using strand displacement such that the motor moves with a bias in one direction along

a track (also known as a substrate since the walker interacts with it).

D37.Yield: The yield is the fraction of correctly formed DNA origami over the total amount possible. This can be measured using gel electrophoresis. Researchers will some times rely on visual analysis via AFM to determine yield; however, this method introduces observer bias and sampling error.

I.2D Chirality

Assumed Properties: Symmetry, Chirality, Reference Marker

Affected System Goals: Analysis, Observation Bias

Failure Type: Compound

Description: Though 3D origami can also have issues with chirality, most notably asymmetrical 2D origami used for walkers may convince researchers of false confirmations depending on the the orientation of the origami (face up vs face down).

Origin: An origami dolphin first shows proof of concept that 2D origami can land with approximately equal probability on either of its two faces [11]. Wickham et al. used chiral origami for a DNA spider walker that followed a track from start to stop. They discovered that when the origami was face down on the mica, people were more likely to identify a spider in the stop position, even when there was no walker [12]. A walker that had to navigate a symmetrical set of tracks along a 2D origami was given a "reference marker" in the lower right hand corner to give the structure visible chirality [13]. Jungmann et al. found in an experiment resulting in digital numbers displayed by FRET based DNA paint strands that the digit "5" was showing sometimes when "2" was intended [14]. It is assumed that the "5" was actually a "2" flipped face down.

Cause: When depositing 2D Origami on a surface to be observed it can with approximately equal probability land on either side. This can lead

to false observations or unexpected behavior.

Detection: Origami that is absolutely not chiral will land on either side with equal probability, and it will not be possible to tell which side it is on. Also, if results of a chiral origami experiment seem to correlate with one side and not the other, then the orientation of the origami with respect to the observer likely matters.

Solution/Mitigation: For non-chiral origami, one should implement chirality in design. For example, reference markers let the observer (researcher) distinguish between which paths the walker was on [12]. Next, to distinguish between whether the topside (side facing the observer) or the bottom side are giving false observations, one can implement a double blind experiment [13] where the real outcome is known. Thus one can compare the expected outcomes from either orientation of the origami, comparing them both with the real outcome to get an estimate of the bias associated with each.

II.AFM Distortion of Origami

Assumed Properties: AFM (tip, cantilever), Single-Layer 3D Origami (Hollow 3D Origami), Higher Resolution (higher force on tip)

Affected System Goals: Analysis, Feedback

Failure Type: Component

Description: Atomic Force Microscopy returns either an altered structure, or it damages the structure.

Origin: Found when examining a DNA Origami dolphin. The tail of the dolphin was distorted when applying extra force on the AFM tip: "this tail distortion can clearly be assigned as an influence of the force exerted by the AFM tip" [11]. Furthermore, the direction of scanning affected the dolphin's tail [11]. In several 3D origami shapes with hollow cores [15][16][17], the AFM tip caused the shapes to either be shorter than expected [16] or caused the shapes to completely collapse [15][17].

Rothemund found that 26-helix squares were being stretched by the tip of the AFM creating hourglass shapes [7]. Furthermore, in labeled staple experiments (where the staple formed a hairpin), the AFM may have also damaged the labeled staples [7].

Cause: Atomic Force Microscopy works by scanning a 3 dimensional surface with a needle in order to create a height-map corresponding to the surface. (Note, this does not mean it only works for 3D origami. 2D origami also has a height). The force applied by the needle touching an origami can distort or destroy the intended structure of the origami.

Detection: Increasing the resolution for an AFM, which also increases the force, may create noticeable distortions [11]. Differences in design and AFM output, especially differences in height or in one direction, imply an unwanted interaction with the tip. Sequential imaging can show deformations that occur over time, which may suggest unwanted interactions with the needle [7].

Solution/Mitigation: Use a different imaging technique such as Electron Microscopy or DNA paint [14]. The technique chosen will depend greatly on the design and cost constraints of the experiment. If an AFM is the only choice, then lowering the force or resolution should help.

III.Compression Limit for Helix Bundles

Assumed Properties: Single Stranded Domain, Tension, Base Pair (Hybridization), Compression

Affected System Goals: Assembly, Task

Failure Type: Compound, Component

Description: Origami does not form due to buckling under compression forces. A helix bundle will fold/remain folded with a compression force less than the critical Euler Force F_c (also known as the buckling force).

$$F_c = pi^2 * P * k_b * T/L^2$$

T = Temperature
k_b = Boltzmann constant
pi = 3.14...
P = Persistence length of bundle
L = Length at which helix buckles

Origin: Experimentally verified in tensegrity structures that formed kites with struts composed of 12-helix-bundles (12-hb) [18].

Cause: In the original experiment the compression was caused by single stranded DNA (ssDNA). The ssDNA was tightened until the struts (in this case 12-hb) failed (they no longer formed the intended structure as shown by lower yields when examined by gel electrophoresis).

Detection: Detection is done primarily through gel electrophoresis, though the effect of buckling may be observable with electron microscopy.

Solution/Mitigation: Calculate the compression force exerted on the helix bundle in question (or all helix bundles) and reduce the cause of the force or adjust the length of the helix bundle [18]. Information on the forces can also be found in an older study by Smith et al. [19]. Also, note that gel purification was found to lower persistence length which changes the calculation of the force [18].

IV.Denatured Base Pairs

Assumed Properties: Staple Domain (Hybridization), Base Pair (Hybridization)

Affected System Goals: Analysis

Failure Type: Cumulative

Description: For base pair by base pair (bp) conformations most imaging techniques are insufficient. While they can give an image of the general

shape of the origami, one cannot tell which segments of the origami are denatured (unattached), if any.

Origin: A solution to this problem was addressed by Wagenbauer et al. [20], whose technique can identify regions where 3 or more nucleotides are unbound.

Cause: Current techniques for showing whether or not intended domains of DNA strands have hybridized are based on imaging techniques such as electron microscopy (EM) or atomic force microscopy (AFM). Such techniques do not have bp resolution.

Detection: Denatured base pairs are hard to detect. Other than the method described in the solution, gel electrophoresis alone can show large amounts of denatured bps through bands corresponding to structures other than the origami. If a structure has near one hundred percent yield, this measure may not help.

Solution/Mitigation: To show how many base pairs are hybridized correctly, Wagenbauer et al. designed a circular de-Brujin sequence containing all domains of length 3. (In other words all permutations of A,C,T,G of length 3 were contained as a substring of the larger de Brujin sequence.) The de-Brujin sequence was then split from 64 nt domains into two smaller 47 nt and 27 nt domains to avoid secondary structures such as hairpins. These strands are the 'defect labels' [20]. In one's own experiments, Wagenbauer's method can be implemented in the following steps:

1. Attach a fluorophore to the defect labels.
2. Add the defect labels to the solution containing the origami in question.
3. Use gel electrophoresis to separate the denatured defect labels from the origami.
4. Compare the intensities of the fluorescence in each band to see how many of the defect labels have attached to the origami structure. See Wagenbauer et al. for a comparison of results [20].

Wagenbauer et al. used this information to fine tune the annealing ramp (cooling step) for 42 helix bundle (hb) 3D origami structure.

V.Electrostatic Repulsion

Assumed Properties: Crossovers, Underwinding, Overwinding, Inter-helix Gap, Crossover Spacing

Affected System Goals: Assembly

Failure Type: Component, Cumulative

Description: Having more nucleotides (nt) between crossovers results in a greater gap between helix bundles (hb) due to the repulsion. Underwinding can relieve strain created from the repulsion, while overwinding can decrease the distance between two strands, increasing the strain. The effect is similar to when one tries to push the negative ends of two magnets together.

Origin: Rothemund reported on this effect in his original paper on DNA origami [7]. Specifically, he reported an "inter-helix gap of 1 nm for 1.5-turn [16 bp] spacing and 1.5 nm for 2.5-turn [26 bp] spacing." In multilayer origami with 7bp spacing the inter-helix gap shrinks to 0.5 nm [21]. Finally Ke et al. found that underwinding staples to give 3D structures relief from electrostatic repulsion resulted in higher yields [8].

Cause: Electrostatic Repulsion is due to the polarity of the sugar backbone of DNA. The negatively charged backbone's hydrophilicity along with the nonpolar bases drives DNA to form; however, for two anti-parallel dsDNA strands the negative dipole moment of the sugars will also cause the strands to repel from each other.

Detection: For 2 dimensional origami the gap can be found by measuring the y-resolution of the origami (using AFM or EM). A similar technique can be used for 3D origami. For origami that has low yields, electrostatic repulsion could be part of the problem.

Solution/Mitigation: For origami that needs a specific inter-helical gap, reducing or increasing the space between crossovers will reduce or increase the gap. For 3D origami, targeted insertions which increase the base pairs between crossovers underwind the structure relieving tension

created by electrostatic repulsion. This also can be interpreted as increasing the inter-helix gap. Furthermore, an intercalator such as ethidium, combined with underwinding can increase yields. (One should note that this result is design dependent, and the underwinding should be done such that the interaction with the intercalator results in an "equilibrium twist" of 360 degrees [8].)

VI.Global Twisting

Assumed Properties: Crossovers, 3D origami, CanDo, CaDNAno, Overwinding, Underwinding, Square Lattice, DNA helix

Affected System Goals: Assembly

Failure Type: Component

Description: Origami has too much or unwanted torsion that affects structural functionality [22].

Origin: It is found in experiments on 3D origami comprised of 2 and 3 layers, but not found in origami of 6 or 8 layers [21].

Cause: Local underwinding of helices results in global relaxation [21][23]. In short, a global right-hand twist results from the strain of underwinding. Note that the CaDNAno software [3] designs square-lattice 3D origami with global twisting by default.

Detection: One can design the origami to form multimers such that the twist is elongated over multiple monomers. This makes the total twist easier to measure as a function of length [21]. Computational methods, such as CanDo, for detection also exist [4].

Solution/Mitigation: Change the initially imposed double helical twist density such that staples are overwound instead of underwound. One way to accomplish this is deletion of base pairs spaced evenly along the length of the scaffold (192-bp in the original paper). The experiment was carried out with 10.5 bp/turn (removing 3 bp to get (192 - n)/(24

* 0.75) = 10.5, n = 3), 10.44 bp/turn (removing 4, n = 4), and 10.39 bp/turn (removing 5, n = 5). No twist was observed for the 10.44 or 10.39 bp/turn designs [21]. Reasons for why the staples had to be overwound are speculative, but the authors offer the work by Mathew-Fenn et al. [22] as a possible explanation. Stein et al. also has a nice explanation of how to fix global twisting using CanDo [24].

VII.Helix Bundle Flexibility

Assumed Properties: CanDo, Coaxial stacking, Crossovers, Flexibility, 2D Origami

Affected System Goals: Task, Assembly

Failure Type: Component

Description: In solution a DNA helix bundle (hb) has a certain amount of flexibility that can either prohibit or enable the operation of a desired task.

Origin: Nanotechnologists involved in producing origami have been aware of, and have purposefully tried to utilize the flexibility of origami. A good example comes from Anderson et al. who tried to make a dolphin tail that wiggled [11].

Cause: Flexibility is an intrinsic property of DNA. Single stranded DNA has several degrees of freedom in its backbone. Double stranded DNA (dsDNA) reduces, but does not eliminate the aforementioned freedom through Watson-Crick base pairs (bp). In practice, double stranded DNA has a persistence length of 50nm, after which dsDNA acts like a flexible rod as opposed to a rigid beam.

Detection: Methods for detection in solution require examining the differences from different instances of the same origami design in solution using a method that can capture the physical formation of many DNA origami such as TEM. Also, the software utility CanDo [25][4] can predict a lower bound for the flexibility of a helix bundle caused by perturbations from

the heat of the solution. It creates a heat map which corresponds to the movement (in nm) of a helix bundle (hb) in solution.

Solution/Mitigation: To increase rigidity, one can either change the placement and quantity of crossovers (increasing for 3D origami), or if the current DNA origami is 2D, then one can make their design 3D by adding layers.

VIII.Inter-Origami Base Stacking

Assumed Properties: Stacking, Exposed Blunt Ends

Affected System Goals: Assembly, Task, Analysis, Origami Separation, Individual Origami, Coagulation

Failure Type: Interaction

Description: When the ends of staples, also known as blunt ends, on two Origami structures meet, they have a tendency to "stick" to each other. This can cause unexpectedly large chains of 2D origami or can be manipulated purposefully. See Douglas et al. [26] for an example of stacking with 3D origami.

Origin: Found by accident in the original paper on origami by Rothemund [7], but also investigated and manipulated by Woo and Rothemund [6]. A good example of how to use base stacking to make a large structure is the stack cross [26].

Cause: Each base of DNA, though non-polar, does have an electron cloud (pi-cloud) that encourages bases to stack on top of one another (pi-bond). This occurs even when nucleotides are not attached by a sugar-phosphate backbone.

Detection: Can be detected by atomic force microscopy (AFM) or potentially by fluorescence resonance energy transfer (FRET) if the sides of the origami are labeled. The edges of the origami are where the blunt

ends will adhere to the blunt ends on other origami causing blunt end stacking.

Solution/Mitigation: To get rid of the effects of inter-origami base stacking, single stranded segments of the scaffold along the edges can prevent the effect. There are two main ways to accomplish this:

1. Add staples which cause T loops (single stranded domains of only T nucleotides connected to an edge of the scaffold, such that it forms a loop). A's cannot form loops due to their single stranded persistence length. G's cannot form loops due to the formation of quadruplexes.

2. Leave single stranded sections of the scaffold unbound on the edges, preventing the formation of blunt edges.

Woo and Rothemund [6] encouraged blunt ends to form by:

1. Making all blunt end pairs "GC" instead of "AT", since "GC" blunt ends form strong pi bonds.

2. Correcting global twist. ("Chains of twisted origami [broke] with a characteristic offset" [6])

3. Preserve the B-form twist of the DNA, such that the major/minor grooves are aligned using "relaxed edges". Relaxed edges have the blunt ends of the staple on the edge of the origami lattice vs. the middle or inside of the staple on the edge.

IX.Remainder Strand Displacement

Assumed Properties: DNA Domain, Displacement, Multiple Unique Origami, Remainder DNA

Affected System Goals: Assembly, Yield

Failure Type: Interaction, Cumulative

Description: When multiple DNA origami with different underlying designs are mixed in the same solution, unbound remainder strands from

one solution can invade and displace strands in a differently designed origami originally mixed in a separate solution. When a DNA origami design uses less than all of the nucleotides in a scaffold, the rest of the scaffold is sometimes filled with remainder strands to prevent unpredictable binding with the remainder single stranded domain.

Origin: In Woo and Rothemund's work involving multiple origami with different designs, they found that staples left floating in solution from one Origami (remainder staples) would invade and unfold origami with a different design by attaching to single stranded loops [6, Supporting Info.]. Specifically, the remainder staples caused "large structural disruptions."

Cause: The different remainder strands from the different designs correspond to different domains along a scaffold. A domain that might be bound in one origami design will be unbound in another. Thus freely floating remainder strands will bind with these open domains when different origami designs are mixed. As Woo and Rothemund state, this process can continuously open up domains, and the newly formed double stranded DNA may be a more energetically favorable continuous duplex due to coaxial stacking [6, Supporting Info.]. They did not notice a similar error with just staple strands.

Detection: Potential unwanted interactions can be pre screened by examining the staple sequences. In solution, the structural disruptions depending on severity should show up using an AFM.

Solution/Mitigation: Woo and Rothemund solved this problem by aligning the remainder loops such that the remainder domains and strands were identical. They note that it is also possible to not add the remainder strands altogether [6, Supporting Info.]. One could also purify the different DNA origami with a gel or with size exclusion filtration columns before mixing them together to get rid of excess remainder strands.

X.Salt Buffers in Origami Patterning

Assumed Properties: Salt (Cation) Concentration, Thiol Gold Linkages

Affected System Goals: Task, Origami Patterning, Gold Patterning, Electrostatic Binding

Failure Type: Interaction

Description: Electrostatic binding approaches for the patterning of DNA origami require salt buffers with magnesium concentrations "incompatible with biochemical studies." Trying to reduce the salt buffers results in dissociation of DNA origami structures. [27]

Origin: This problem motivated the invention of a protocol for patterning DNA origami without using electrostatic binding to avoid the salt buffer problem mentioned.

Cause: Salt concentrations are necessary in solution in order for DNA to hybridize. Other studies on electrostatic arrays have done more intense inquiries of this aspect [28]. Salts that bind with DNA often contain Magnesium2+ and Sodium+. The binding affects the polarity of the DNA's backbone, making salt an important factor in experiments. This effect has also been studied [29].

Detection: Evident from the design of the experiment.

Solution/Mitigation: Origami can be attached using gold (which causes thiol-gold linkages with the origami) on passivated glass substrates that are etched using e-beam lithography. Samples were then measured using DNA-paint [14] and TIRFM.

XI.Side Products and Thermal Degradation

Assumed Properties: Temperature, Annealing, Thermal ramp

Affected System Goals: Assembly, Yield, Time

Failure Type: Compound, Cumulative

Description: Origami (especially 3D) takes long to fold or forms along

with many "molten-globule" side products when using a slow thermal ramping protocol[1].

Origin: Sobczak et al. found that origami folds with higher yields at a set temperature that differs with each origami [1]. They cite side products and thermal degradation as possible causes for low yields under a slow annealing protocol. The thermal ramp they used reduced the temperature by 1° C per every 3 hours.

Cause: A slow annealing protocol (also known as a thermal ramping protocol), created "molten globule" precursors that remained without turning into the intended product origami [1].

Detection: Gel Electrophoresis can be used to determine if unintended side products are created due to the heating protocol. Sobczak et al. note that the precursors had greater "electrophoretic mobility", meaning that they can be identified by a distinct band on the gel [1].

Solution/Mitigation: Find the peak temperature at which the rate of folding is highest as described by Sobczak et al. Then set the temperature of the solution to the low boundary of the temperature peak. The solution is then heated to 65° C for 15 min (referred to as heat shock), then cooled immediately to the desired temperature. Yields were found to approach 100%, and folded within minutes instead of days [1].

XII.Unclear Structural Conformation

Assumed Properties: TEM, AFM, High Resolution, Cryo-TEM, Chirality, Symmetry, Static Structure

Affected System Goals: Analysis, Invasiveness

Failure Type: Component

Description: For detailed views of origami conformations the methods most popular have been atomic force microscopy, and transmission electron microscopy(TEM). Atomic Force Microscopy can alter the image,

and has a lower resolution. All non-cryo TEM, though better, can also cause a structure to conform to a state that it would not in solution. Each method comes with some drawbacks, and neither arguably is state of the art.

Origin: A technique using cryo-electron microscopy, an extension of TEM, along with software based class averages was able to determine the shape of 3D origami with an overall resolution of 11.5 Angstroms (115 nm) [30].

Cause: Viewing atoms at the scale of angstroms (1 A $= 1 * 10^{-10}$ meters) is challenging, albeit necessary for confirming the correct conformation of a device. Furthermore, alternative methods can further obscure a structural conformation by changing the structure of an origami. This method [30] offers one of the best ways to visually confirm the structure of origami while viewing it in solution.

Detection: When other viewing methods fail, this method should provide the most accuracy. However, it only works on chiral (asymmetrical), static (non-moving) structures.

Solution/Mitigation: Perform single particle cryo-electron microscopy as described by Bai et al [30]. This will work best on chiral origami, as chirality is necessary for identifying the orientation of the scaffold. Bai et al. use liquid ethane to freeze the origami in solution before performing electron microscopy. The microscope can then scan for all of the origami. The images of origami can then be collated into 2D class averages and 3D reconstructions using software.

XIII.Unoptimized Staple Configuration

Assumed Properties: Seed Segment, Staple Domain, 3D origami

Affected System Goals: Assembly, Yield

Failure Type: Component, Cumulative

Description: Low yields of 3D origami are sometimes caused by inefficient staple placement. Specifically, staples lack a seed segment described below.

Origin: Ke et al. reported certain staple strategies that were unable to fold a 24 helix bundle (24-hb) rod. They then through experimentation found a method of stapling that increased the yield from effectively 0% to greater than 20% without changing the length of the staples [8].

Cause: Ke et al. hypothesize that for staple strands that lack a 14 nt seed the origami gets stuck kinetically in a misfolded intermediate stage. However, it is also possible that the problem is due to thermodynamics. (14 nt domains on average bind earlier and with more favorability than domains of lesser length [31].) If it is due to kinetics, it is possible that the order in which staples bind to origami can be changed by altering the lengths of their domains binding with the scaffold.

Detection: If yield of origami is low (as shown by gel electrophoresis) or the shape is incorrect (as shown by electron microscopy of atomic force microscopy), the cause could be incorrect staple placement. As yields are higher for 2D origami, the staple configuration does not have as much of an effect.

Solution/Mitigation: Design staples such that they have a 14 nt seed domain. A seed domain is an uninterrupted stretch (no crossovers) of nt forming double stranded DNA with the scaffold. The seed domain is the part of the staple longer than the other domains, such that the seed domain binds first, hence it "seeds" the hybridization of the rest of the staple. It is also hypothesized that one can fine tune the times at which staples bind through these seed domains, leading to correct kinetic folding of the origami.

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