Solid-phase assembly of micrometer-scale crisscross DNA origami structures

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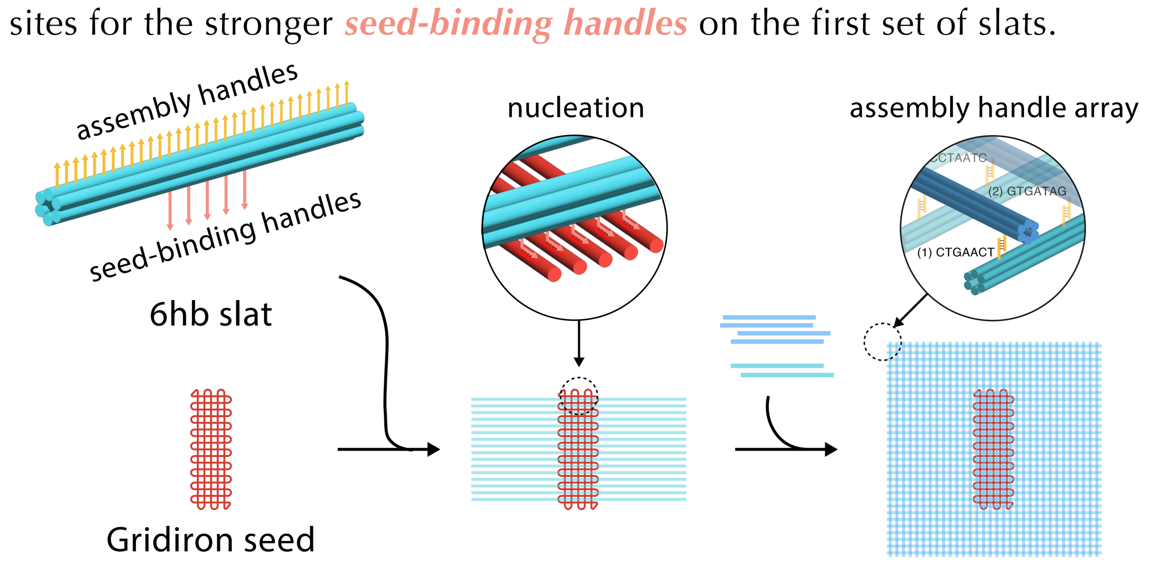
Hierarchical self-assembly

Hierarchical self-assembly of DNA origami structures enables the programmable construction of micrometer-scale architectures with nanoscale registration. However, the production of large, addressable structures remains challenging as the substantial number of unique components needed leads to slow growth kinetics and labor-intensive workflows. Additionally, typical origami purification methods cannot distinguish fully assembled structures from partially formed assemblies or remove unincorporated components. This limits the scalability and practicality of DNA origami megastructures for diverse applications ranging from molecular computing to synthetic cell construction to integrated electronic devices and beyond.

We introduce here an assembly approach based on the crisscross DNA origami polymerization paradigm[1], enabling **stepwise assembly on magnetic bead solid supports**. Rapid fluid exchange through magnetic pulldown allows control over growth conditions for multi-step processes. This technique enables the selective purification of fully formed crisscross structures from partial assemblies and excess origami components. Furthermore, **diverse architectures can be produced from a minimal set of origami building blocks** by varying their assembly sequence, similar to other solid-phase synthesis strategies. Finally, we demonstrate that enzymatic ligation can be used to modify standard staple sets for folding origami components, providing a **cost efficient alternative to liquid handling automation** for the preparation of unique origamis bearing desired cargo handles. Together, these advances provide a streamlined and scalable method for producing micron-scale hierarchical origami structures, expanding their accessibility and potential for diverse applications.

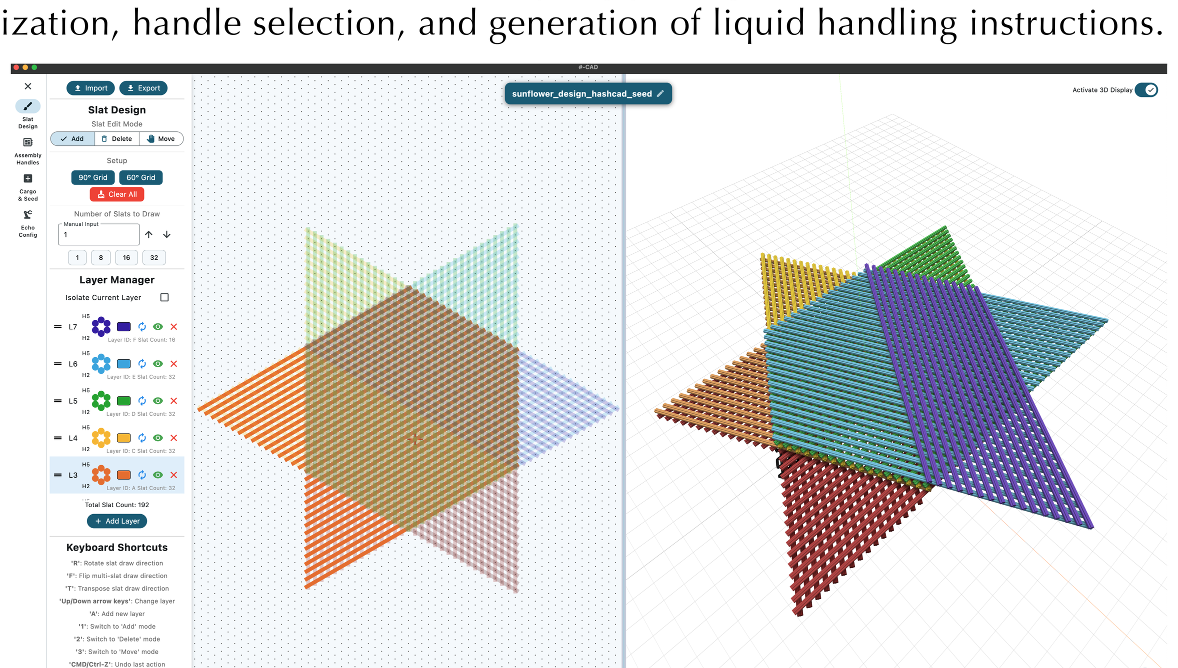
The crisscross assembly mechanism

involves the hybridization of a single *6-helix bundle origami slat* with many other origami slats. Growth is achieved through cooperative binding of *weak assembly handles*. To overcome the energetic barrier of initiation, a ***gridiron seed origami*** nucleates assembly by providing binding sites for the stronger *seed-binding handles* on the first set of slats.



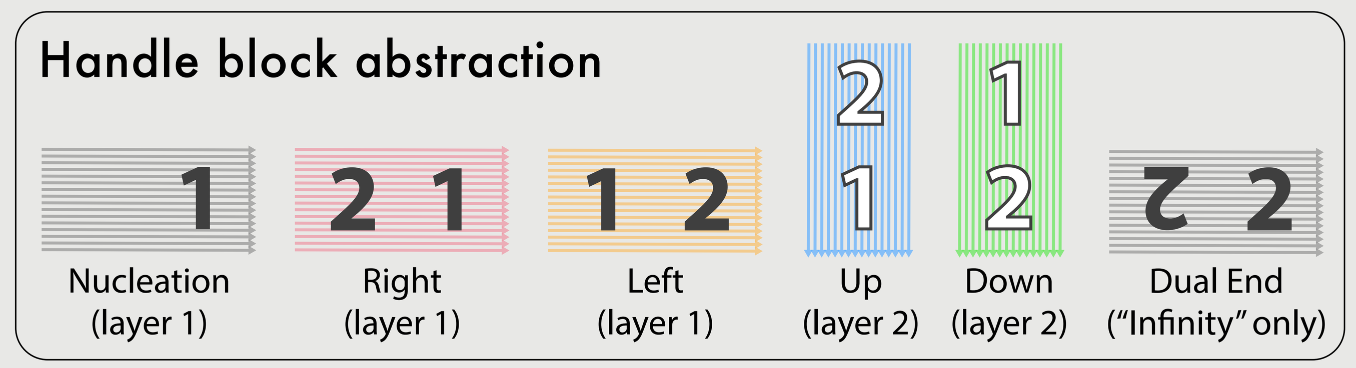
The design of crisscross origami

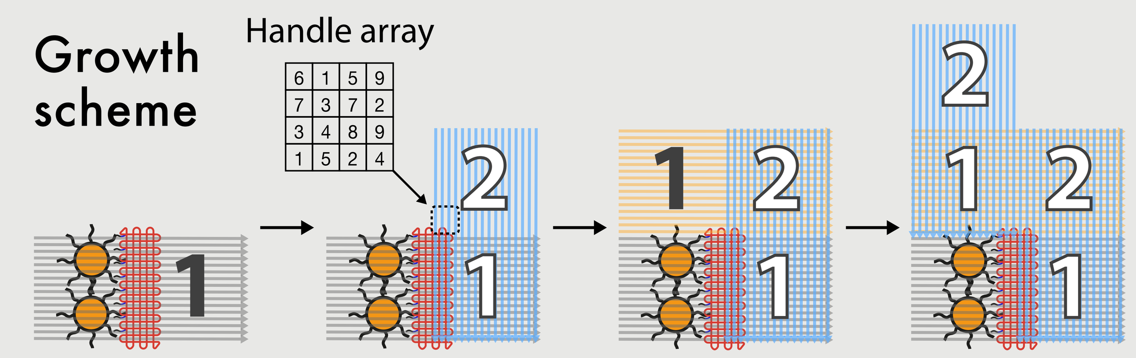
can now be achieved with the #-CAD software suite[2], which provides a GUI-based CAD environment as well as scripting packages for megastructure visualization, handle selection, and generation of liquid handling instructions.

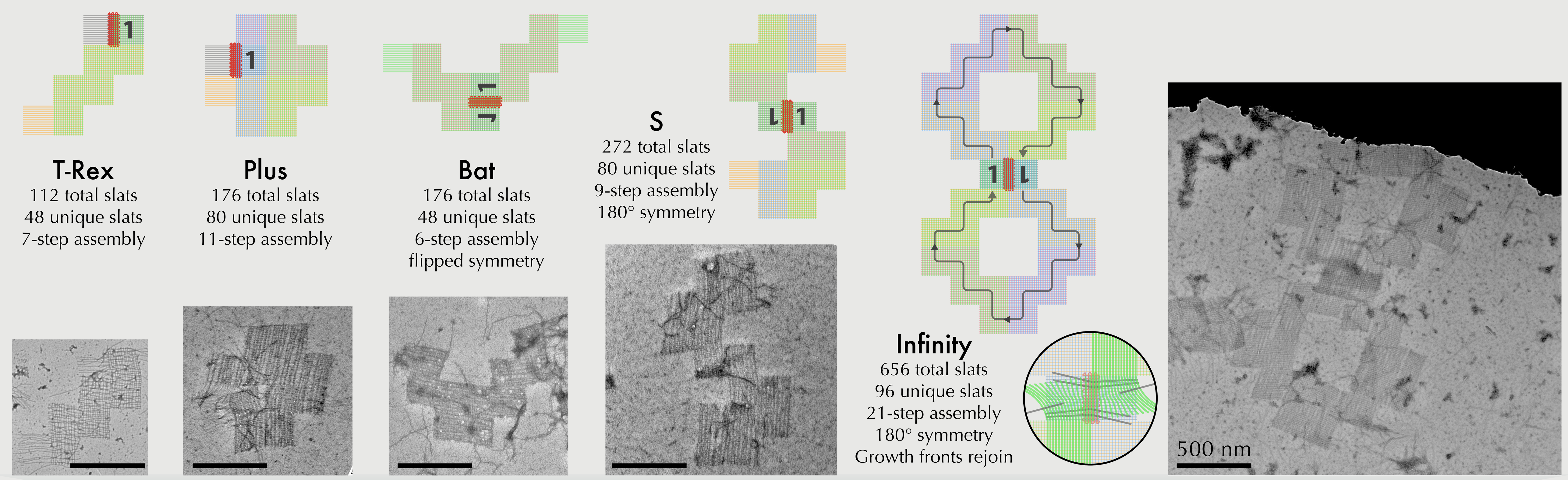


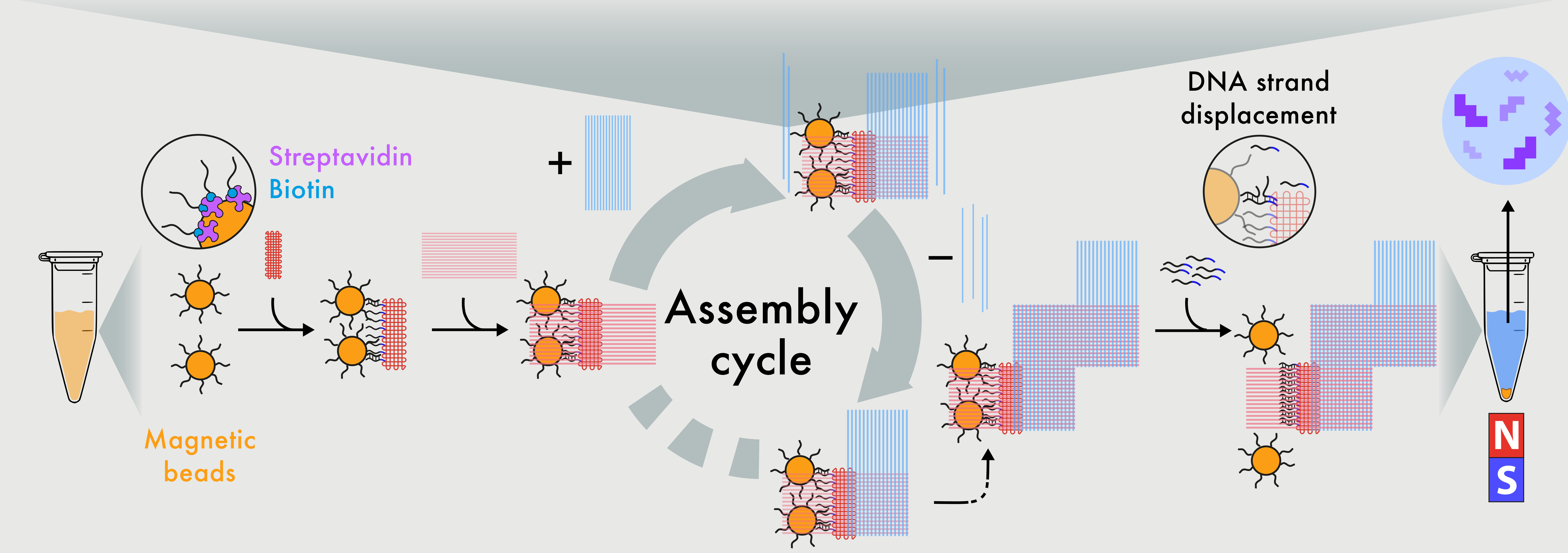
Arbitrary shapes can be stepwise assembled from a finite set of subunits

on magnetic beads, thanks to rapid buffer exchange cycles. Sets of 16 slats form growth blocks, which present one of two pre-selected handle sequence arrays in a specified orientation and complementarity that determines assembly direction. Introducing symmetry to the initial (nucleation) slats allows structures to assemble through two parallel growth fronts.



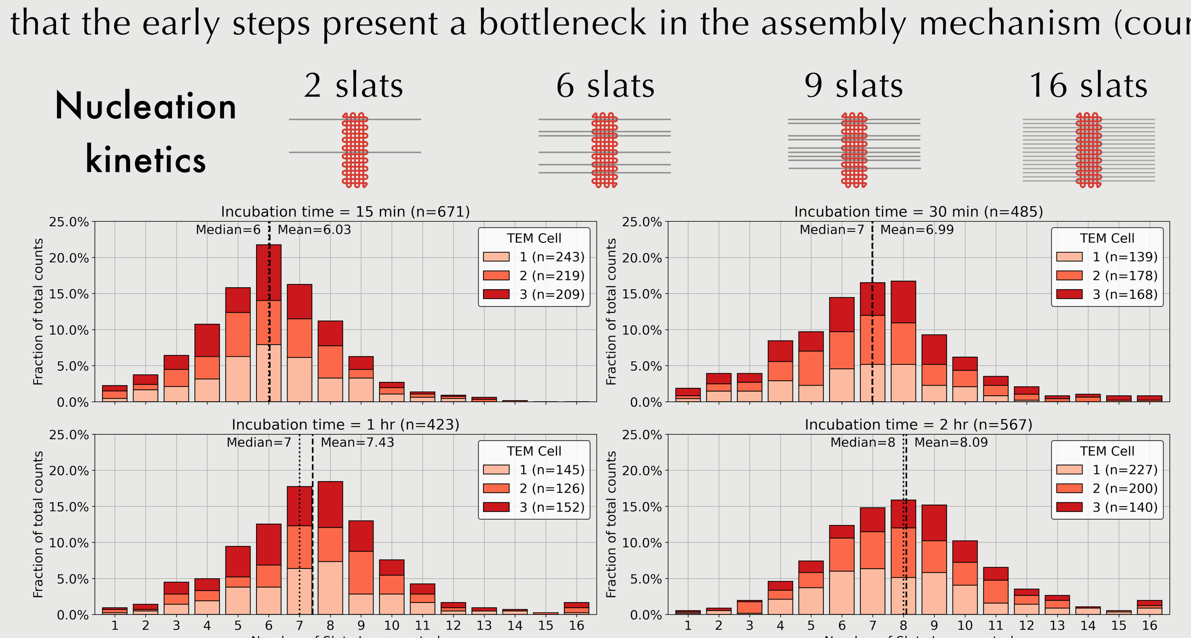


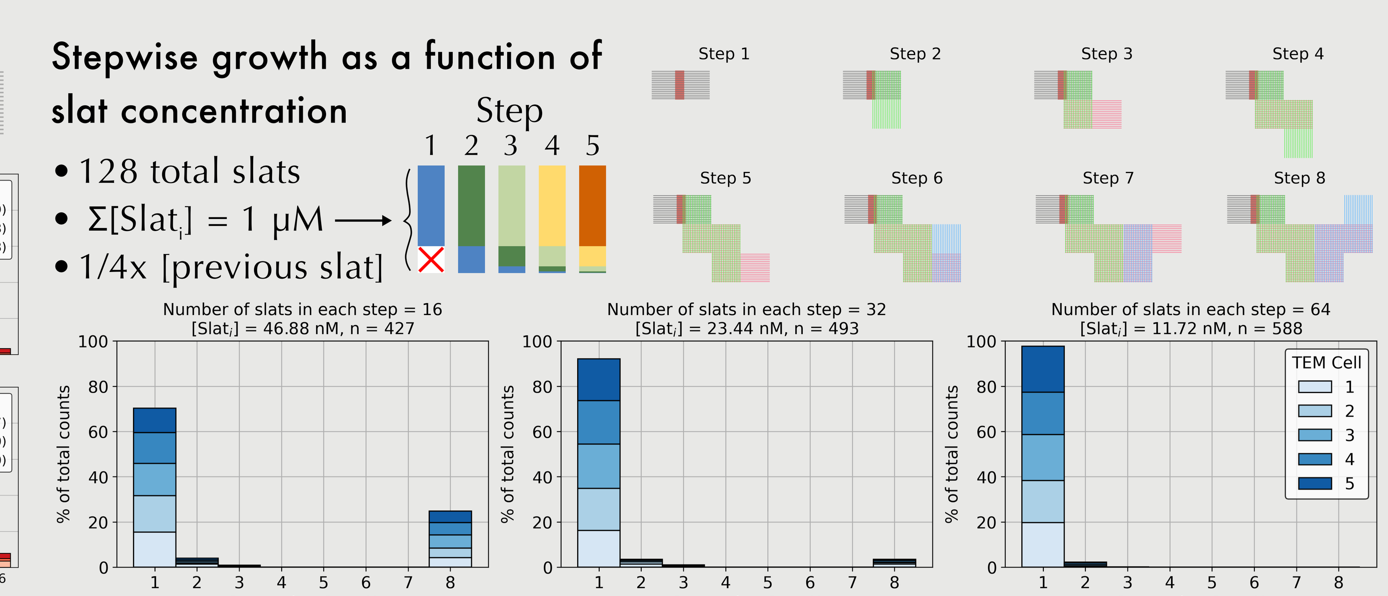




Nucleation is initially fast and stepwise assembly shows all-or-nothing behavior

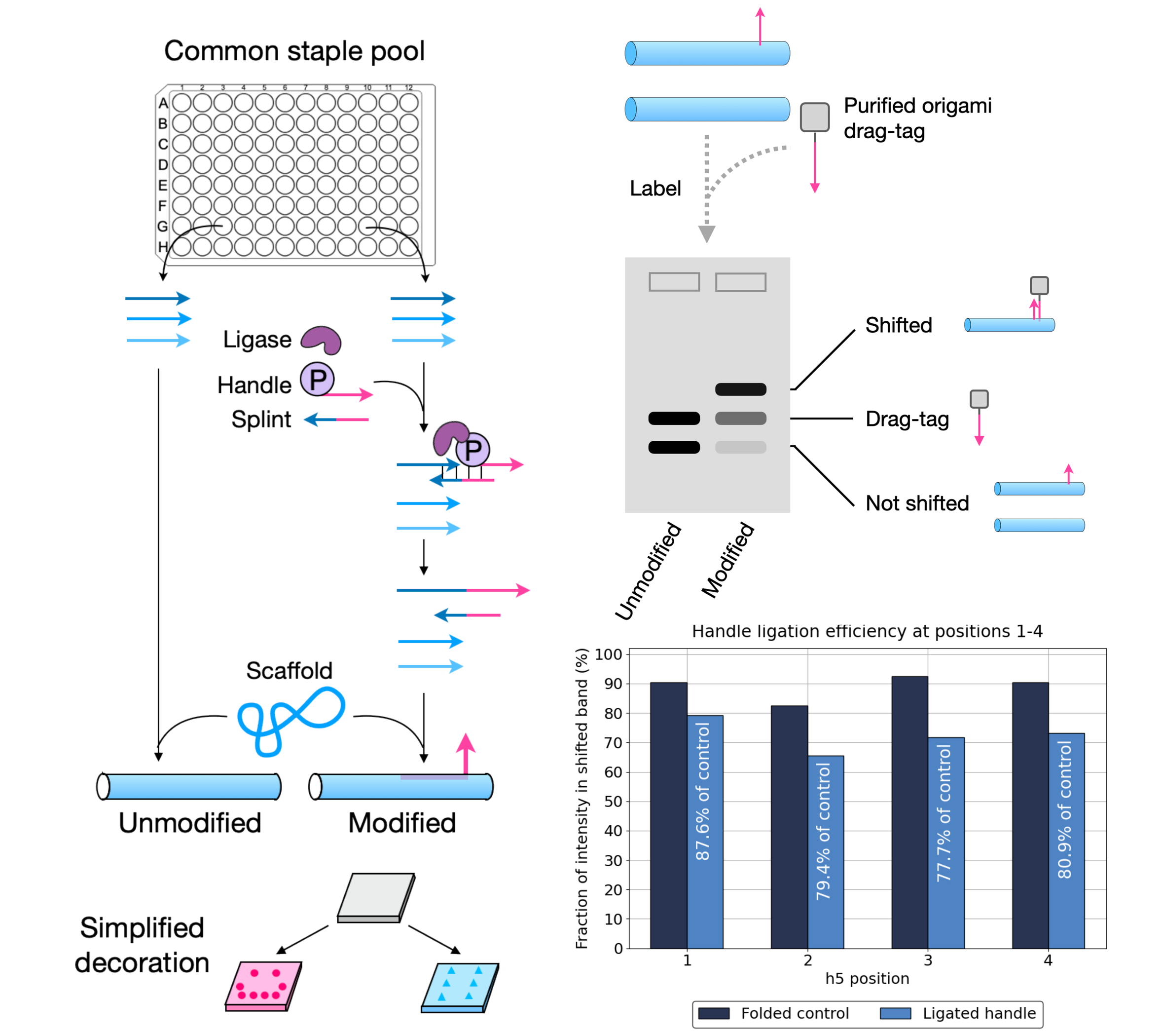
once ideal growth conditions are met for solid-phase crisscross assembly. An average of 6 nucleation slats attach to the seed within 15 minutes, but subsequent attachments occur more slowly (aggregated counts from TEM micrographs, **left**). When stepwise assembly is performed at 1 hour incubation steps with varying numbers of slats (while maintaining a constant overall slat concentration), crisscross structures are either terminated at Steps 1 - 3 or fully assembled, suggesting that the early steps present a bottleneck in the assembly mechanism (counts from TEM, **right**).





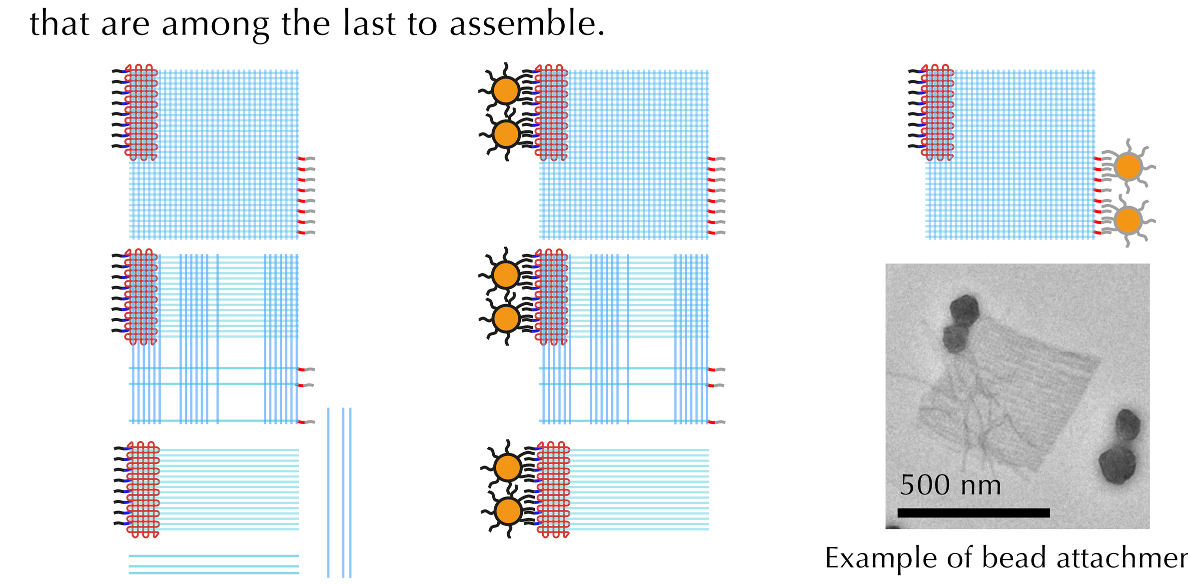
Enzymatic ligation of cargo handles

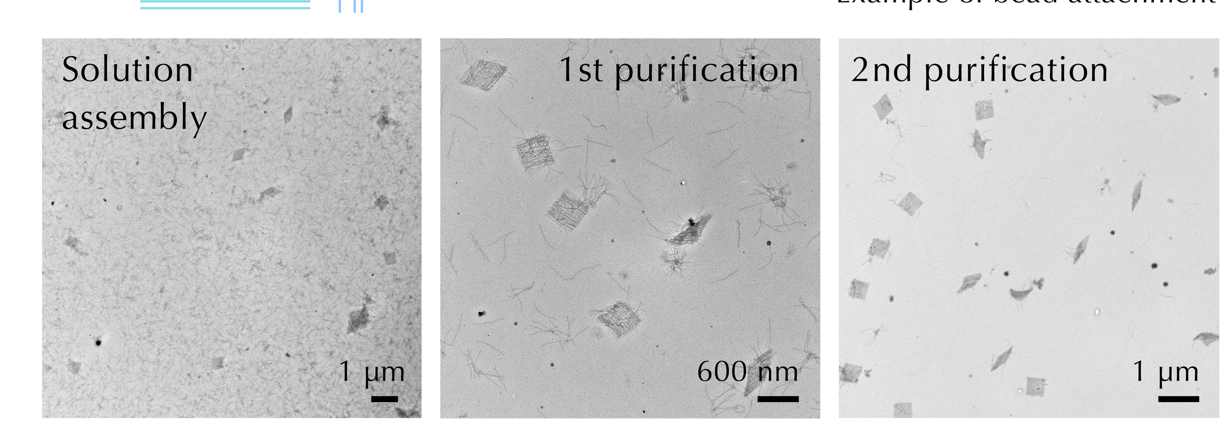
enables manual encoding of desired patterns on crisscross megastructures, without depending on liquid handling automation to repool staples.



Bead-based purification of structures

removes excess slats and partially assembled structures to yield completed structures at target concentrations. The first round of purification selects structures by bead attachment handles on the seed origami, while the second round selects by a different set of attachment handles on slats that are among the last to assemble.





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2. M. Aquilina, Hash-CAD, (2025), GitHub repository, https://github.com/mattaq31/Hash-CAD

#-CAD