

Supporting Information for:

**Decoupling Geometric and Topological Contributions to Conformational Free-Energy Landscapes
in DNA Origami Nanosheets**

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caDNAno Schematics of Nanosheet Designs

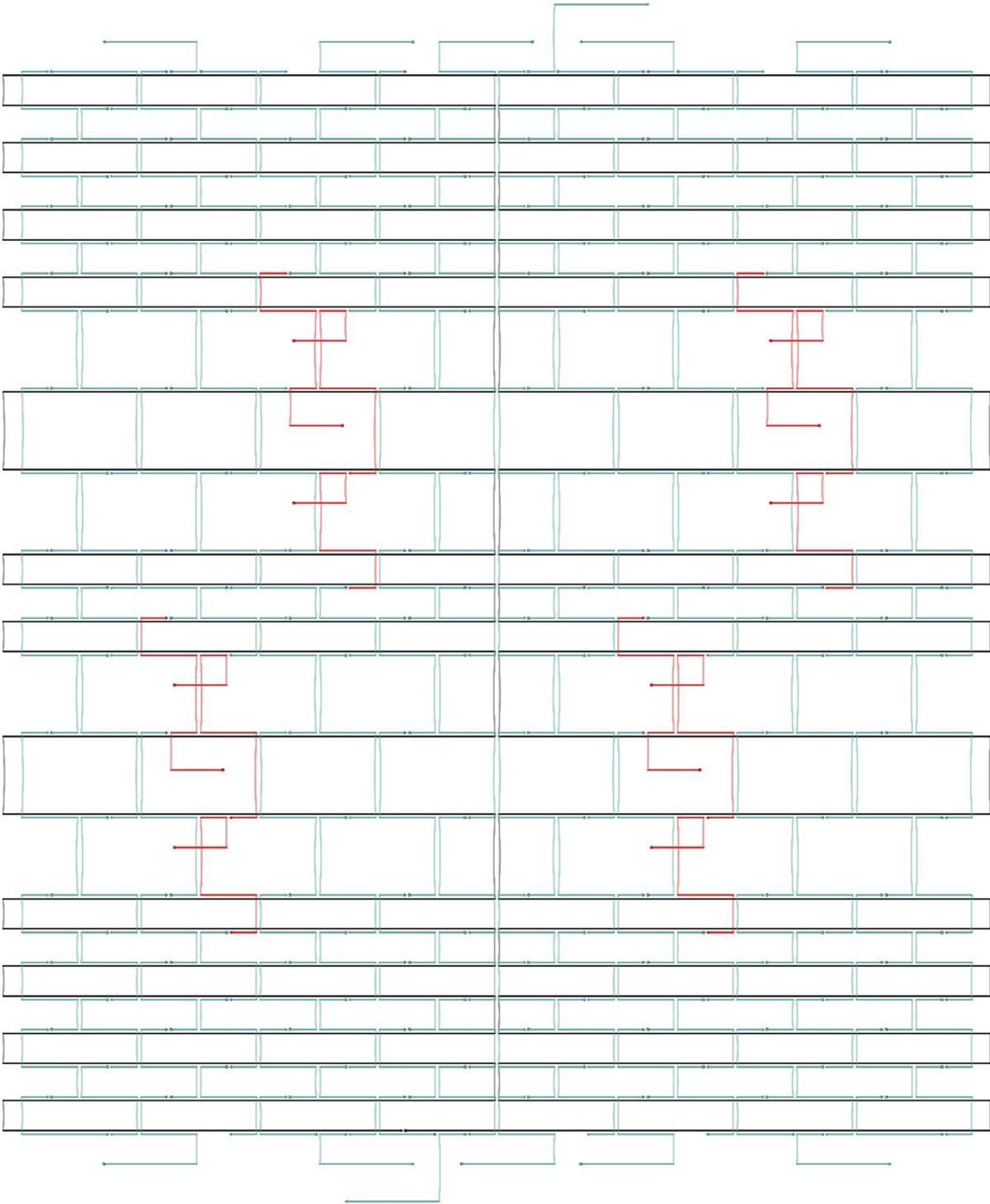


Figure S1. caDNAno schematic of the seamed main nanosheet design. Red staples indicate strands carrying polyA overhangs that serve as payload linkers.

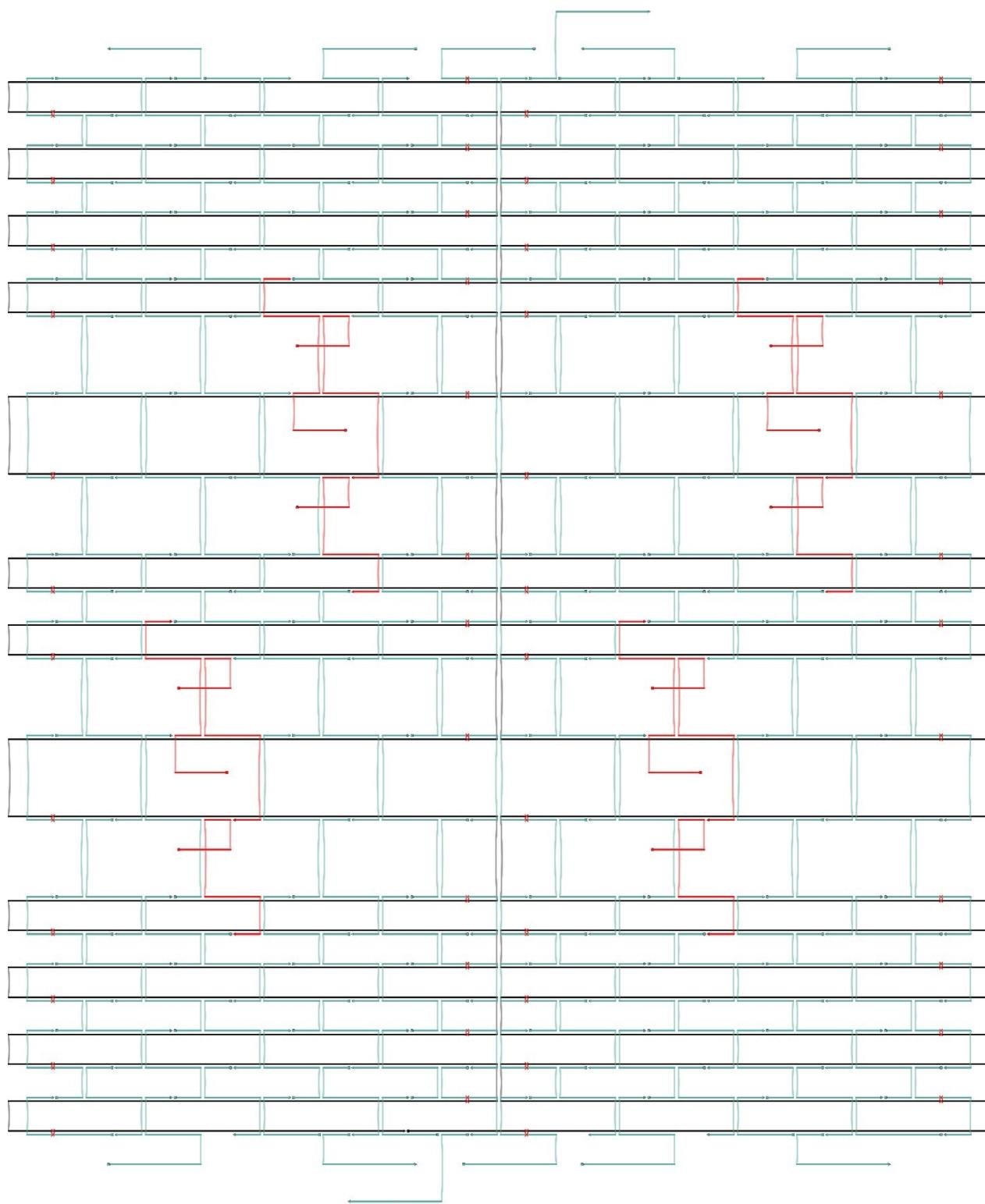


Figure S2. caDNA schematic of the seamed 2skip nanosheet design.

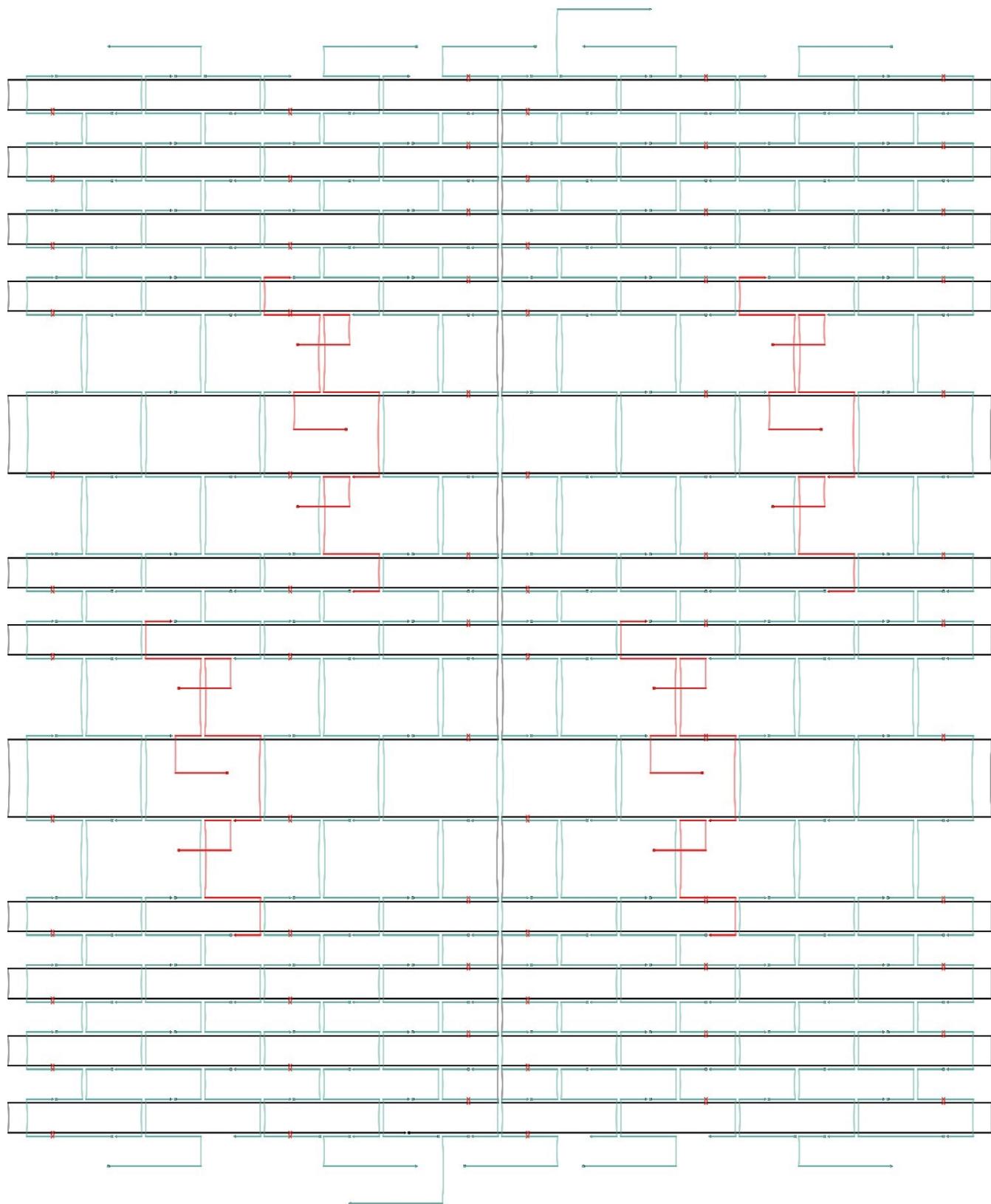


Figure S3. caDNAno schematic of the seamed 3skip nanosheet design.

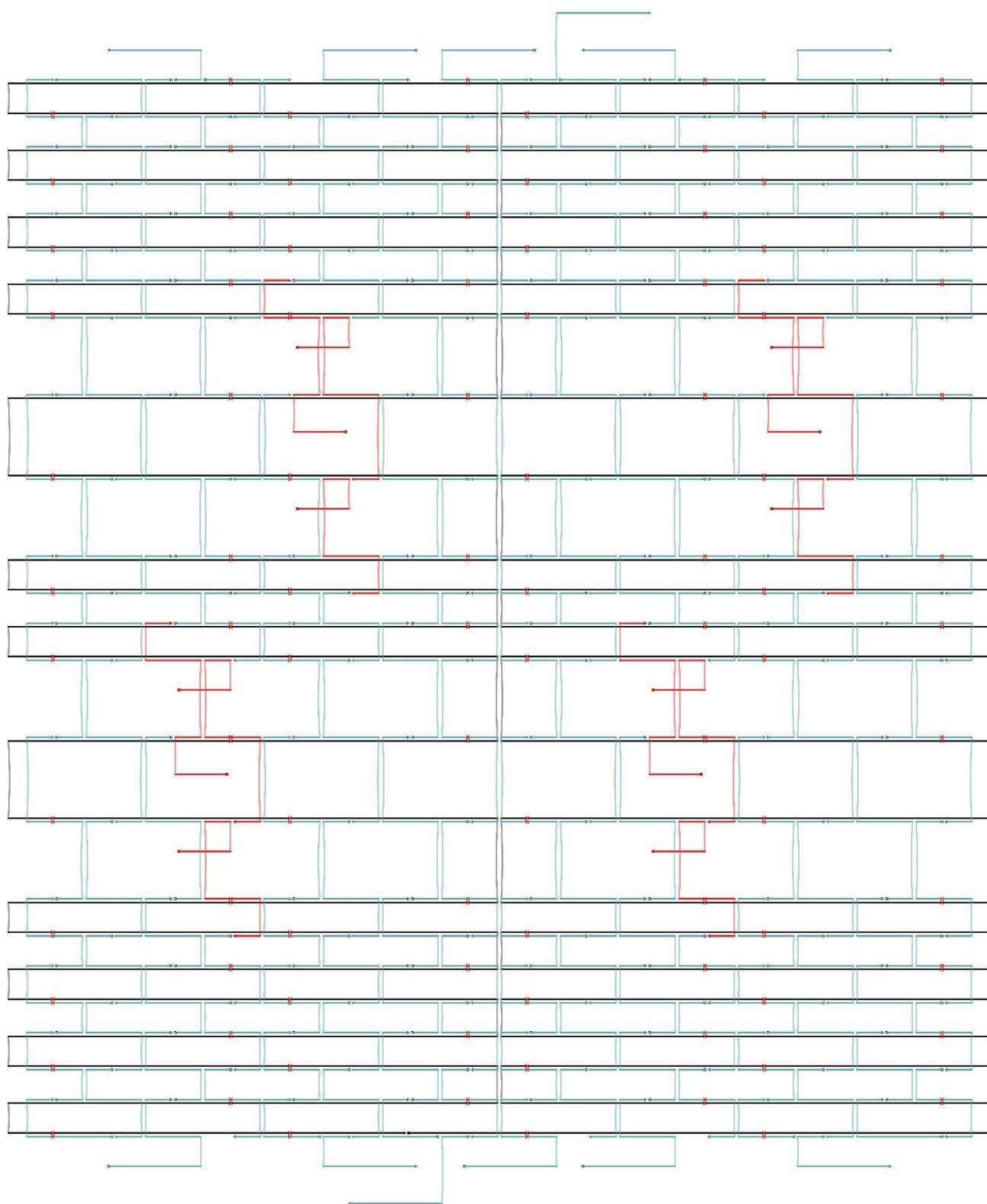


Figure S4. caDNAno schematic of the seamed 4skip nanosheet design.

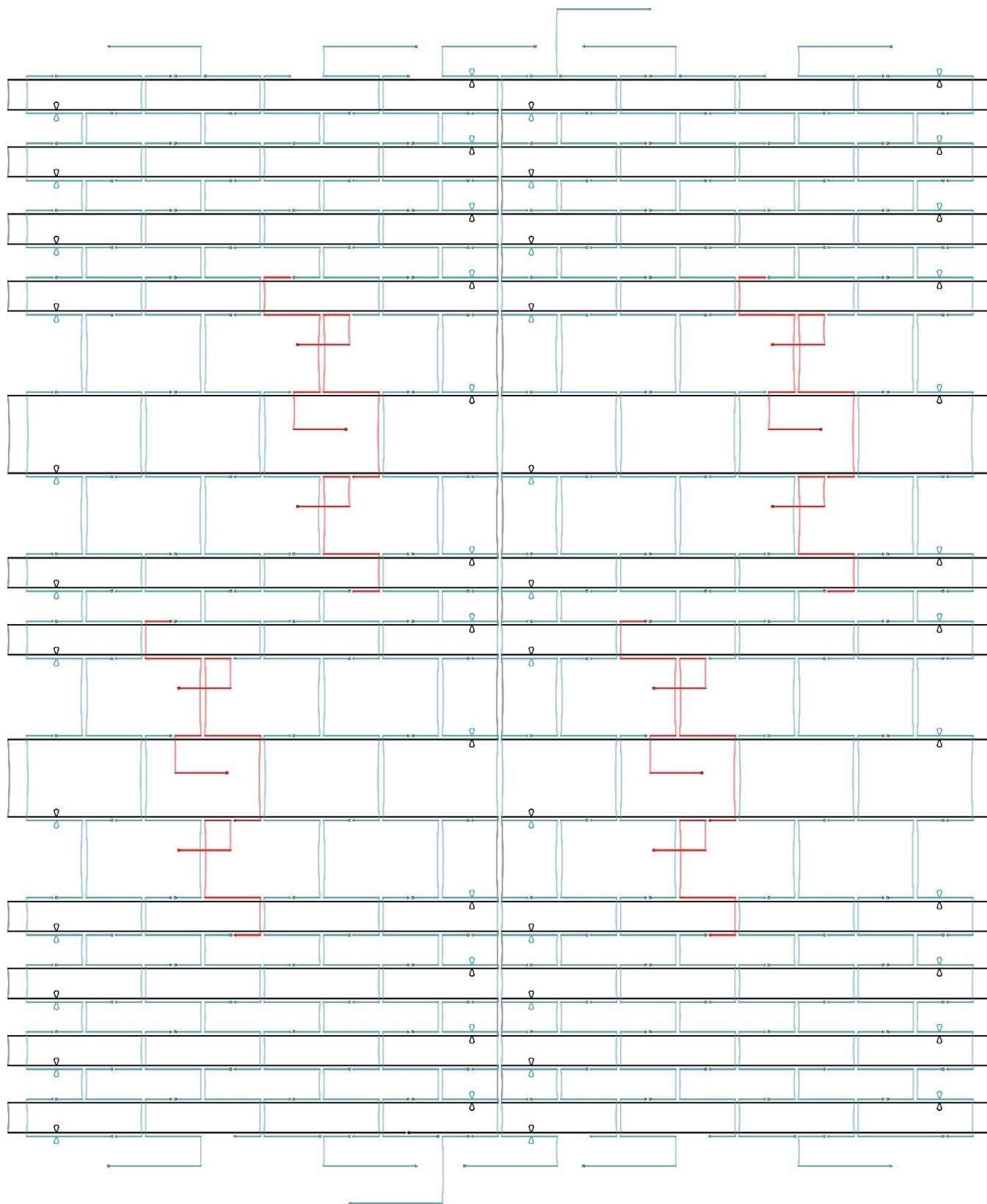


Figure S5. caDNAo schematic of the seamed 2insert nanosheet design.

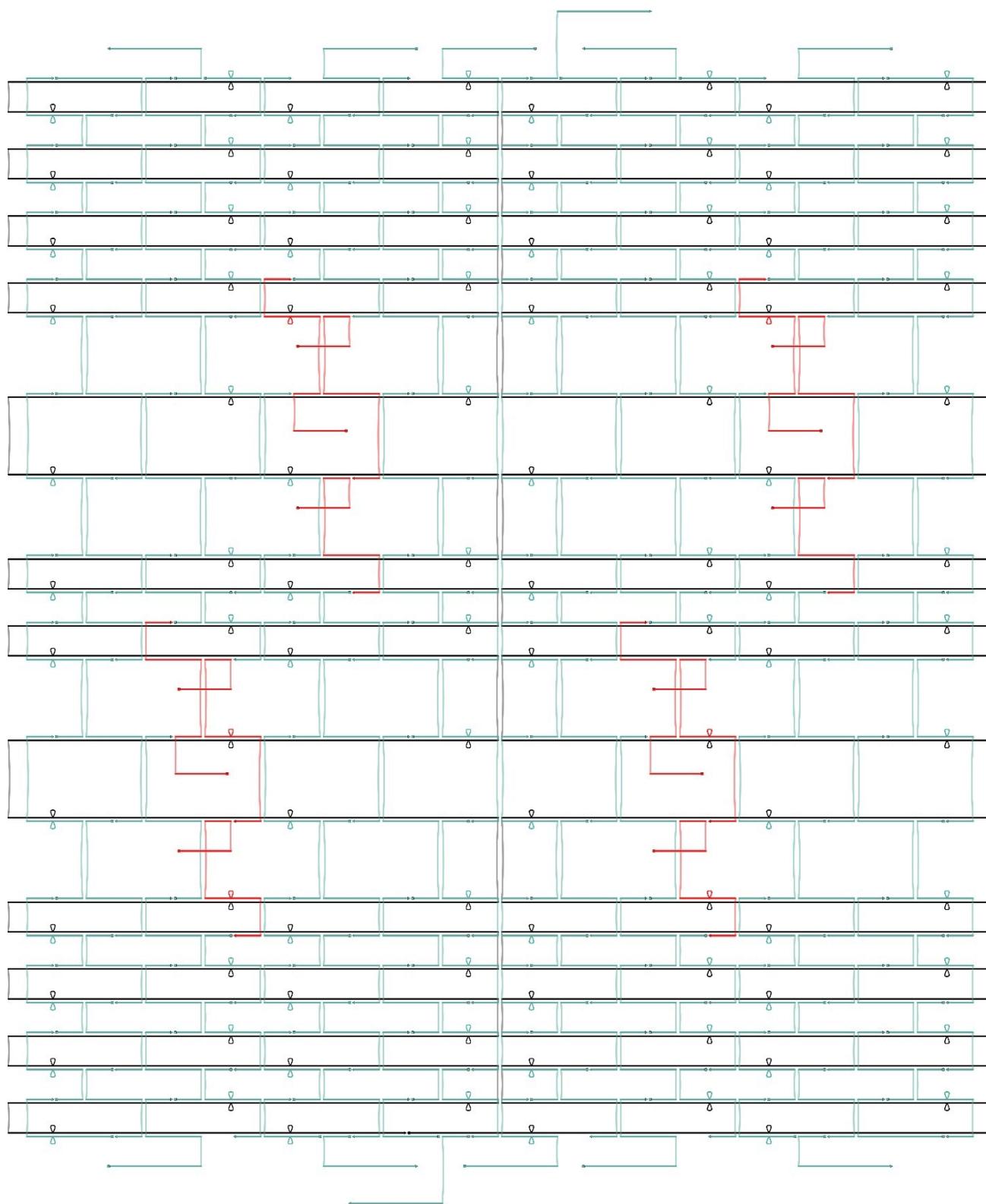


Figure S6. caDNAo schematic of the seamed 4insert nanosheet design.

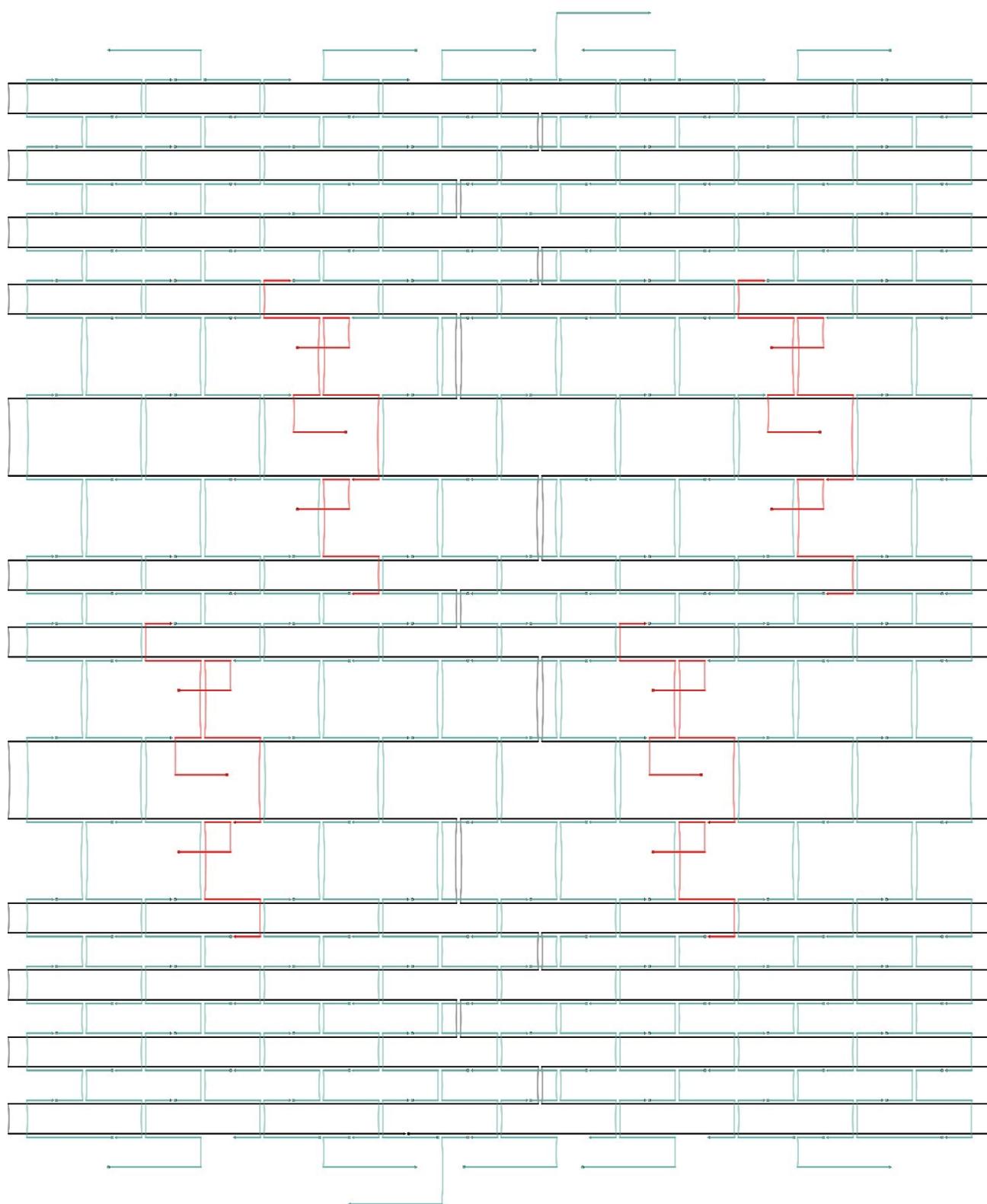


Figure S7. caDNAo schematic of the seamless main nanosheet design.

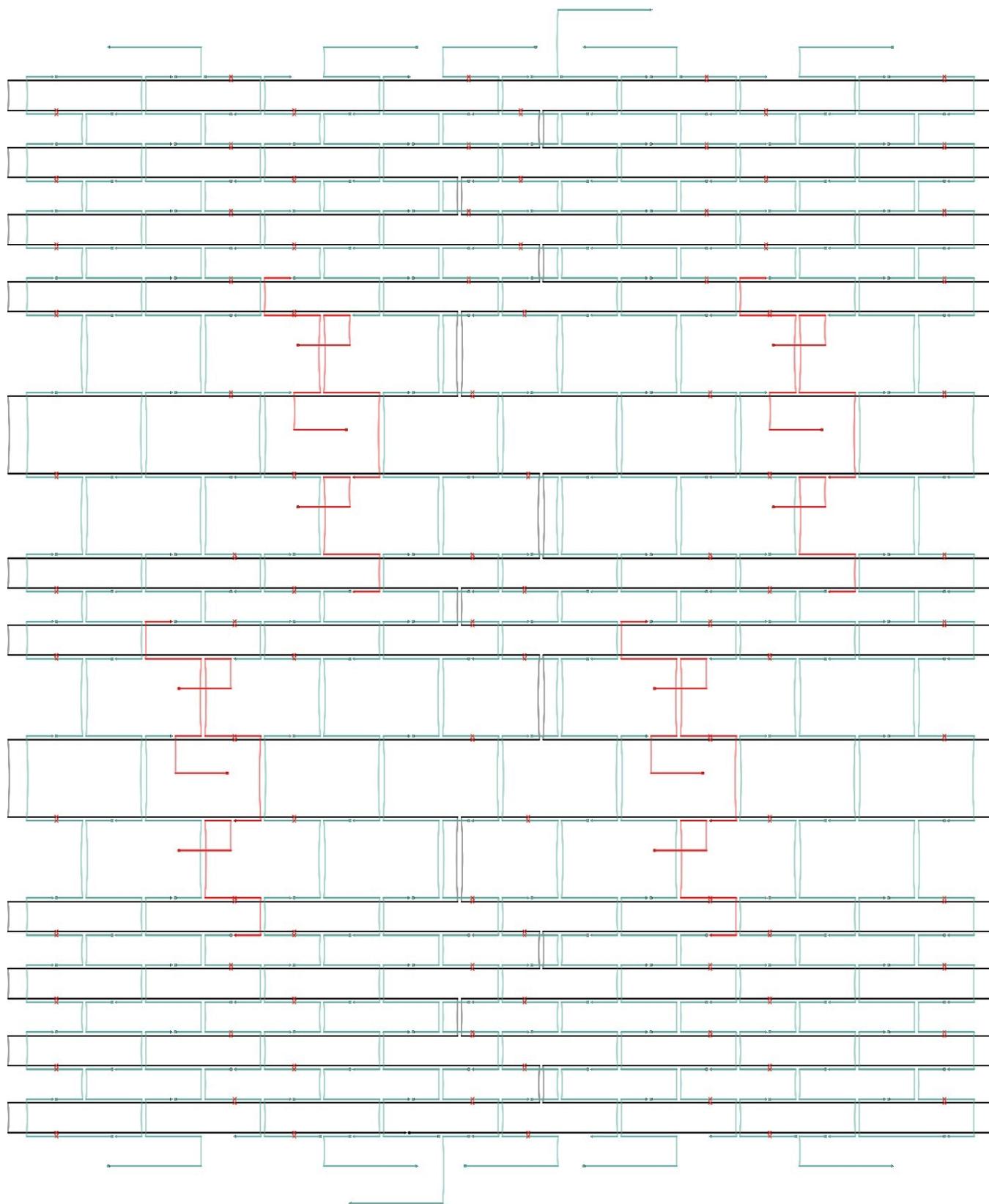


Figure S8. caDNAo schematic of the seamless 4skip nanosheet design.

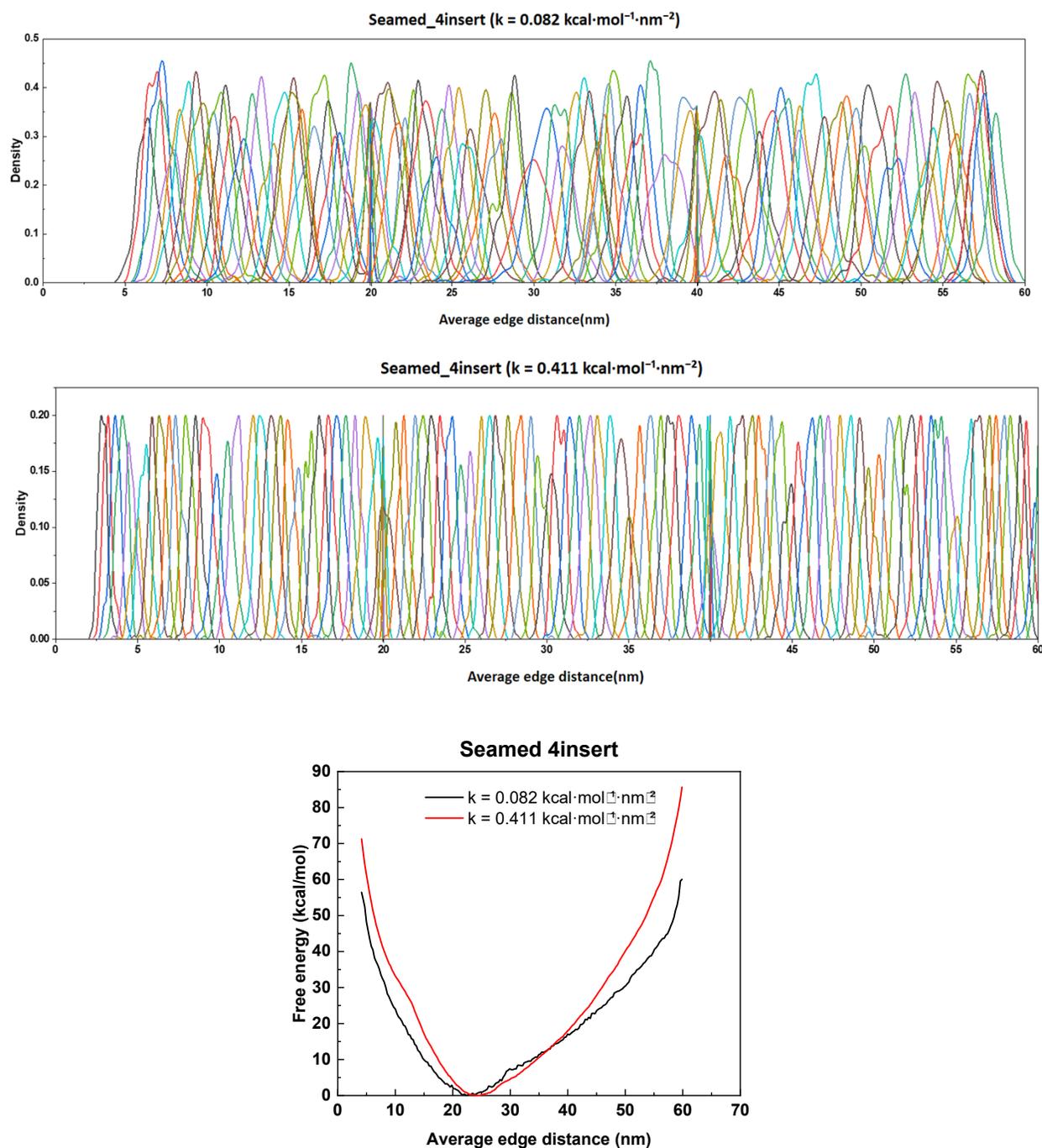


Figure S9. Stiffness sensitivity analysis for the seamed 4insert nanosheet.

Order-parameter histograms and reconstructed free-energy profiles obtained using different harmonic restraint stiffness values. While increased stiffness reduces sampling efficiency at large inter-edge separations and leads to higher apparent free energies in the open regime, the location of the free-energy minimum and the transition region remain unchanged. These results confirm that a stiffness of $0.082 \text{ kcal}\cdot\text{mol}^{-1}\cdot\text{nm}^{-2}$ yields unbiased free-energy landscapes even for highly strained designs.