Last Update: April 10, 2023

DNA CRYSTAL SYNTHESIS

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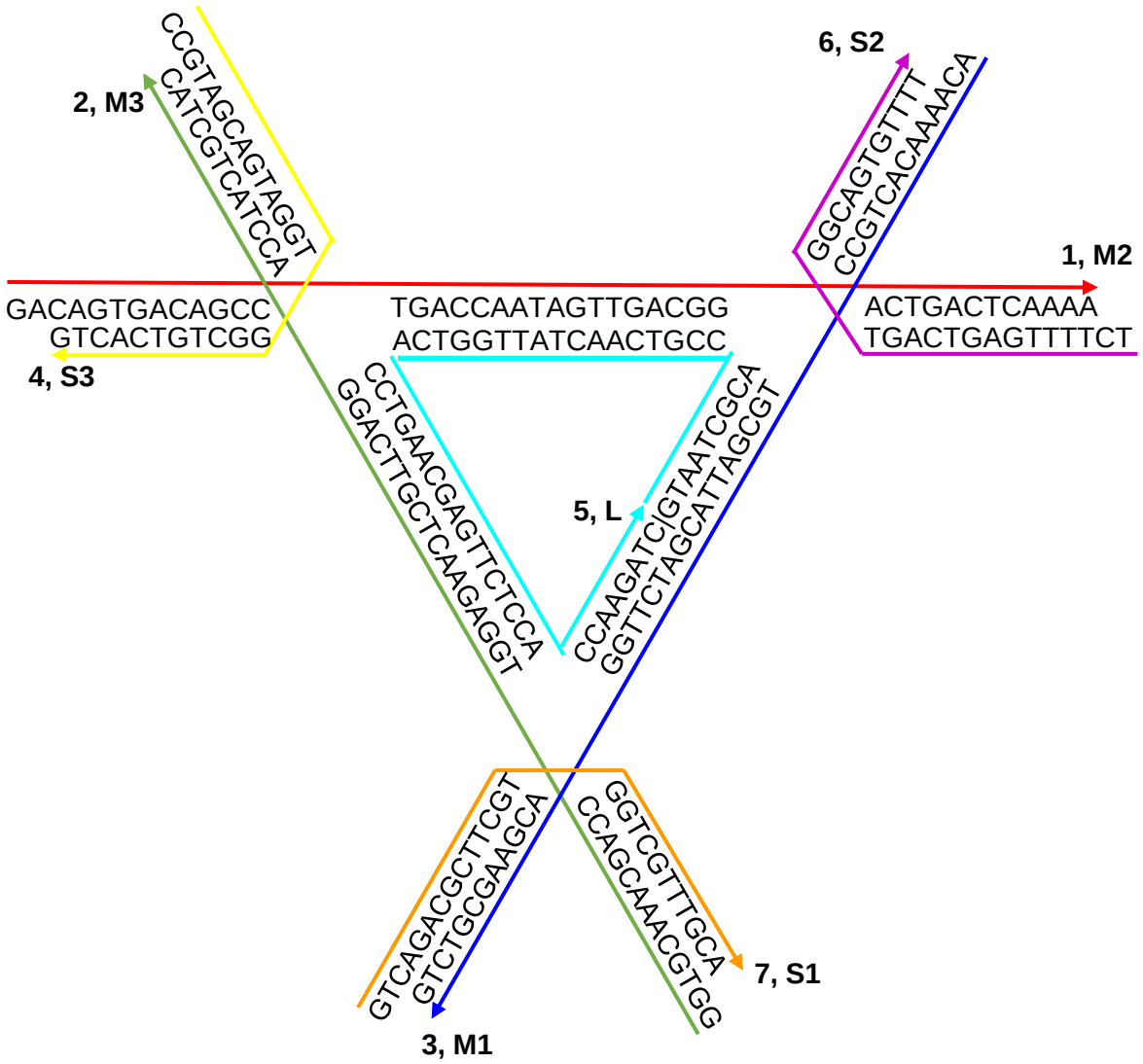
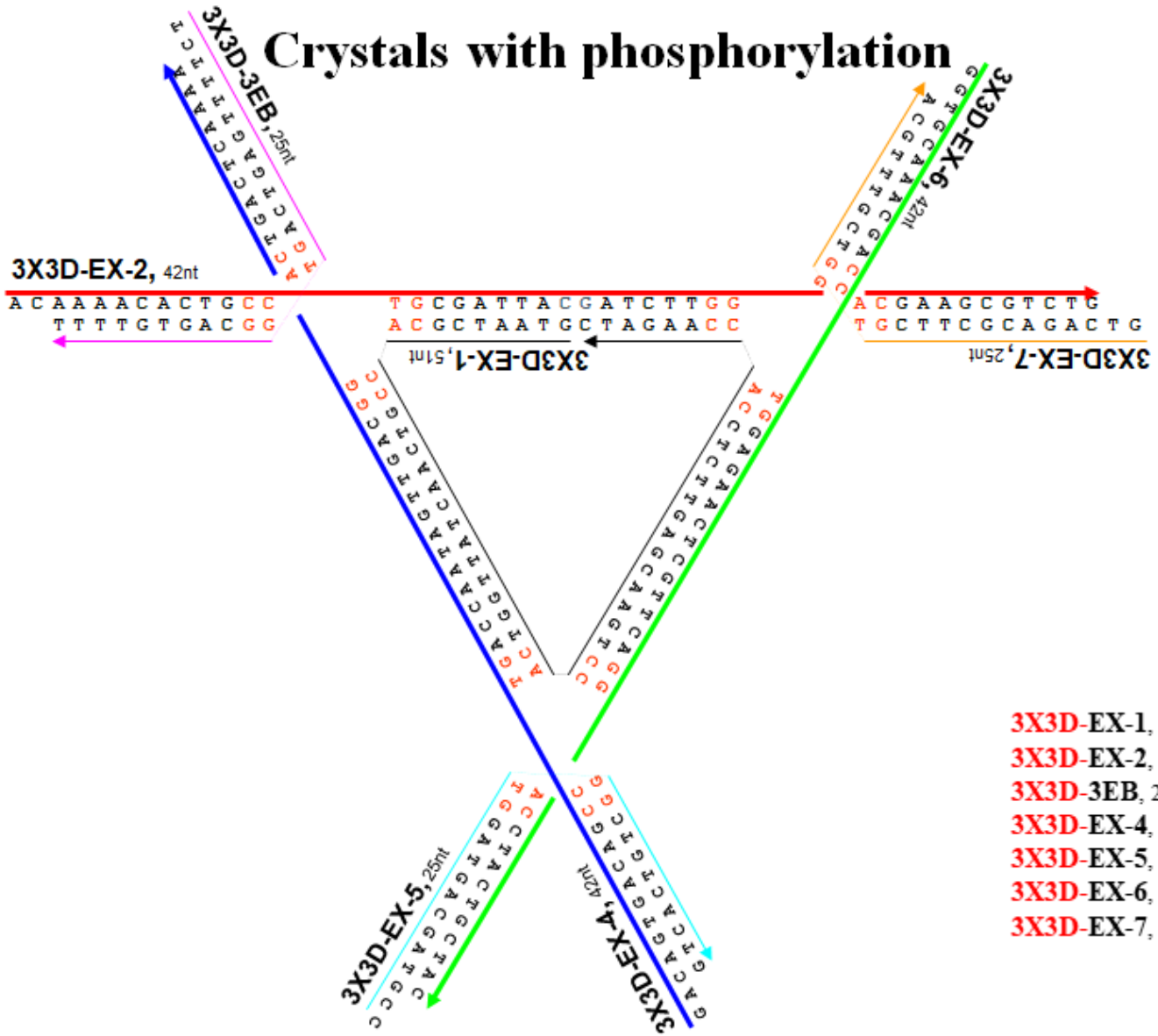


Nomenclature followed by: Ruixin / Harshith
Prof. Choi's Lab

Motif of Asymmetric 4-Turn Crystal

Nomenclature followed by: Mengxi / Cuizheng
Prof. Mao's Lab



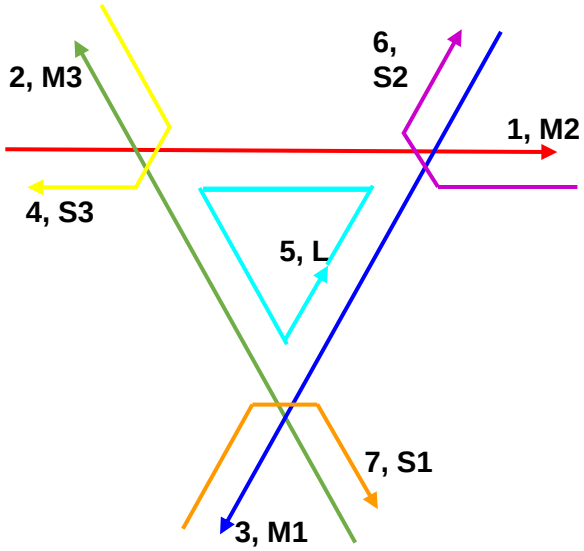
3X3D-EX-1, 51 nt: GTAATCGCA CCGTCAACTATTGGTCA CCTGAACGAGTTCTCCA CCAAGATC
3X3D-EX-2, 42 nt : ACAAAACACTGCC TGCGATTACGATCTTGG ACGAAGCGTCTG
3X3D-3EB, 25 nt : TCTTTTGAGTCAGT GGCAGTGTTTT
3X3D-EX-4, 42 nt : GACAGTGACAGCC TGACCAATAGTTGACGG ACTGACTCAAAA
3X3D-EX-5, 25 nt : CCGTAGCAGTAGGT GGCTGTCACTG
3X3D-EX-6, 42 nt : GGTGCAAACGACC TGGAGAACTCGTTCAGG ACCTACTGCTAC
3X3D-EX-7, 25 nt : GTCAGACGCTTCGT GGTCGTTTGCA

Nomenclature of Asymmetric motif sequences

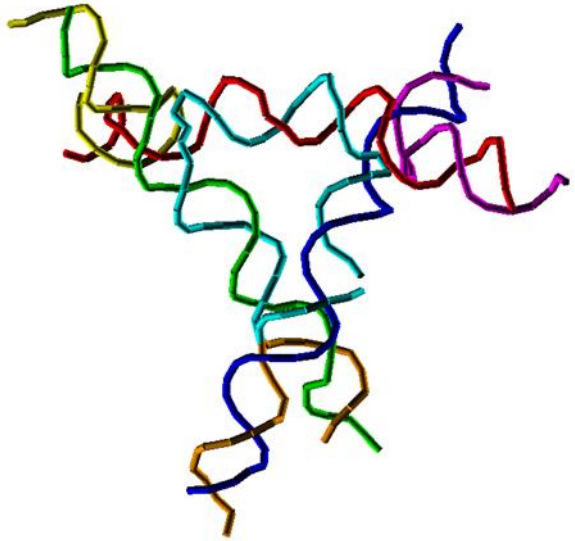
Mengxi / Cuizheng		Sequence	Ruixin / Harshith	
Name	**Colour**		**Name**	**Colour**
3X3D-EX-1	Black	GTAATCGCA CCGTCAACTATTGGTCA CCTGAACGAGTTCTCCA CCAAGATC	5,L	Cyan
3X3D-EX-2	Red	ACAAAACACTGCC TGCGATTACGATCTTGG ACGAAGCGTCTG	3,M1	Blue
3X3D-3EB	Pink	TCTTTTGAGTCAGT GGCAGTGTTTT	6,S2	Pink
3X3D-EX-4	Blue	GACAGTGACAGCC TGACCAATAGTTGACGG ACTGACTCAAAA	1,M2	Red
3X3D-EX-5	Cyan	CCGTAGCAGTAGGT GGCTGTCACTG	4,S3	Yellow
3X3D-EX-6	Green	GGTGCAAACGACC TGGAGAACTCGTTCAGG ACCTACTGCTAC	2,M3	Green
3X3D-EX-7	Orange	GTCAGACGCTTCGT GGTCGTTTGCA	7,S1	Orange

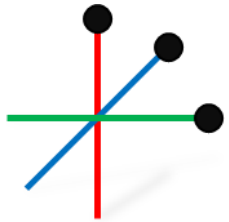
- Ruixin introduced the nomenclature, color for sequences to depict the red (1,M2) strand which has less energy than other directions and they form the strands which are in the thickness direction. Look into () paper to understand the directions for crystal simulations and mechanical deformations

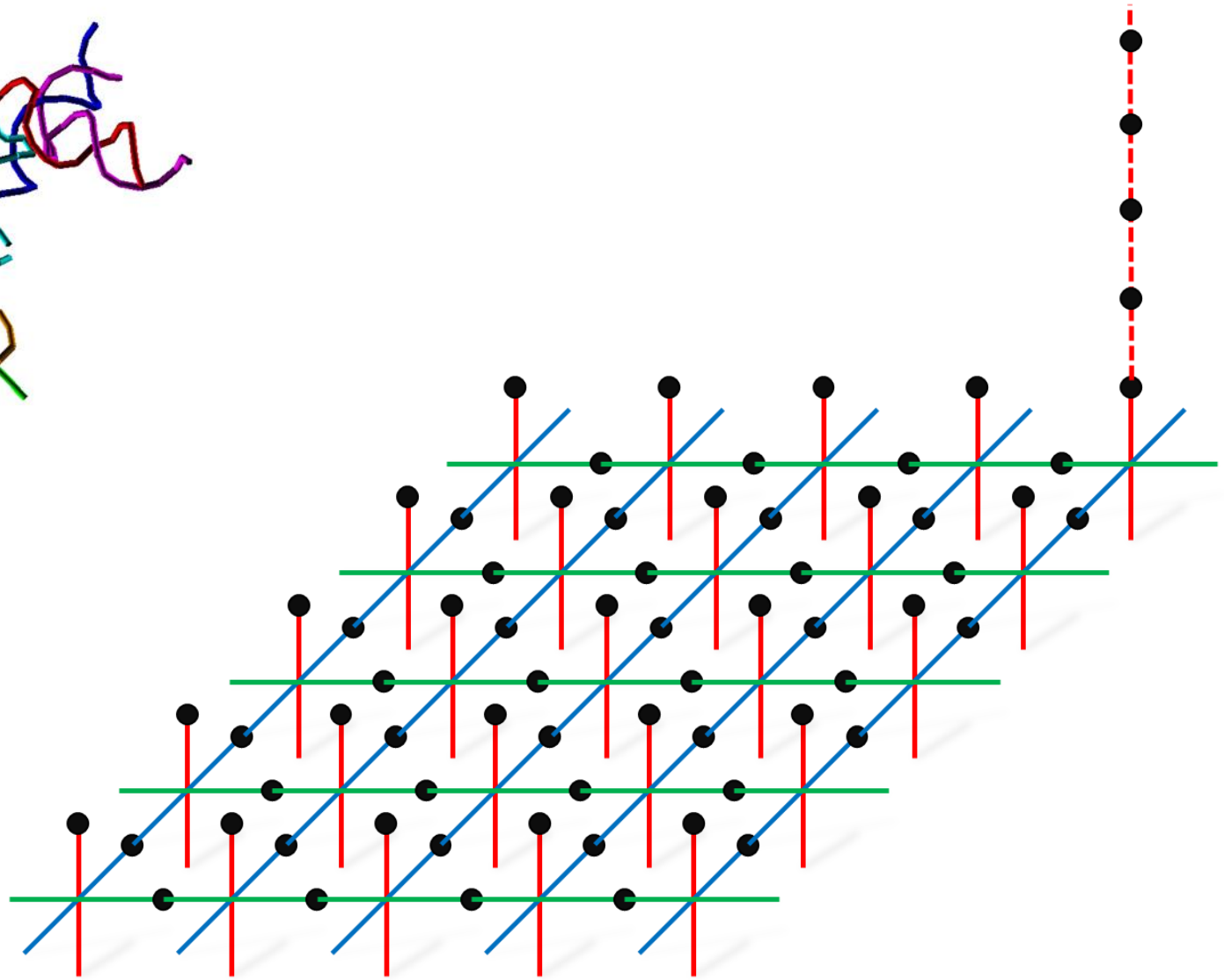
DNA Crystal Assembly Visualization



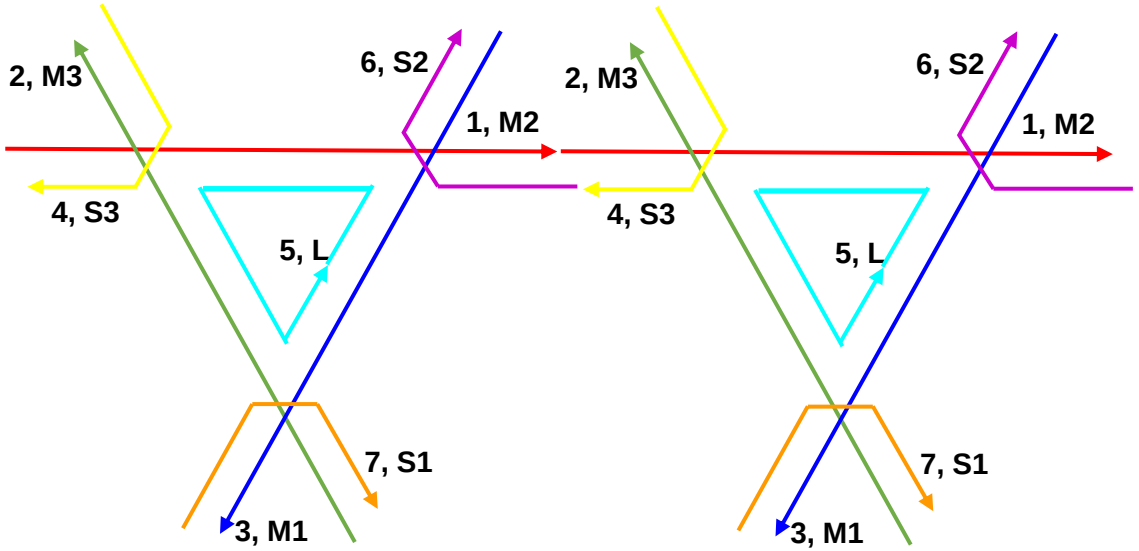
Asymmetric Motif

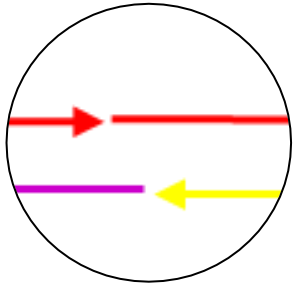






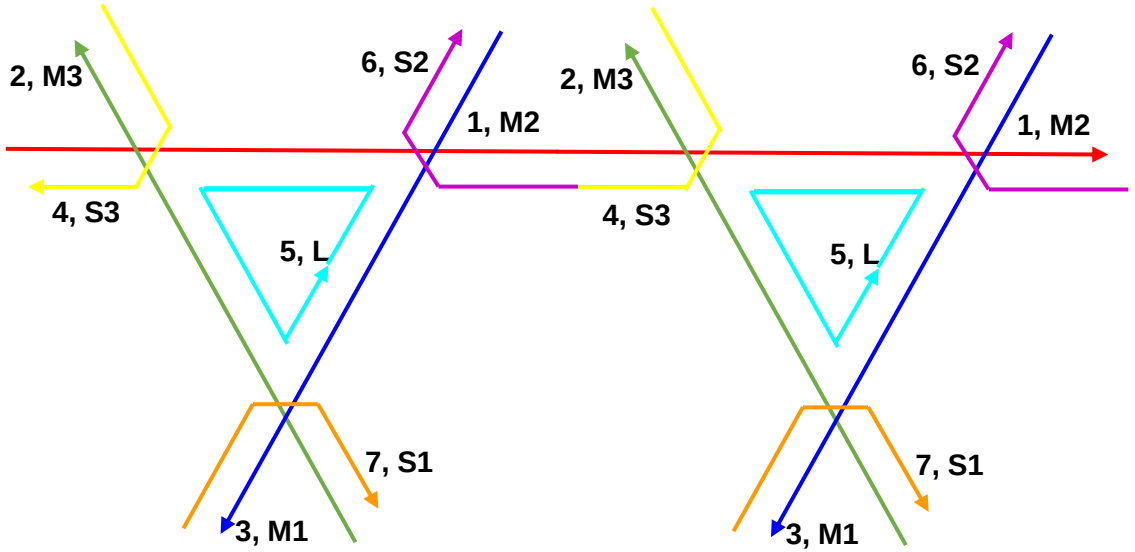
Native Crystal

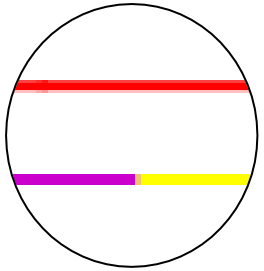




H-bond interaction

Ligated Crystal



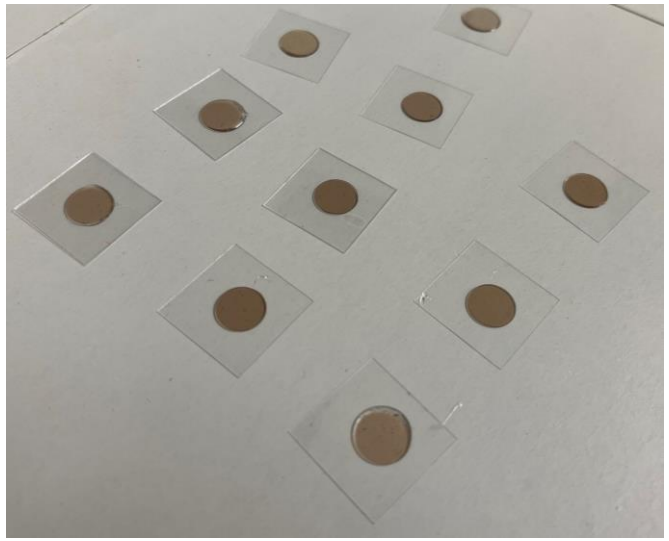


Ligation

DNA Crystal Synthesis

❖ Mica Surface Preparation

1. Crystal Synthesis is generally done on many samples (4 – 8). This is because of less probability of crystals of desired scale growing on the surface.
2. We use mica as the substrate to grow the crystals. Take 8 qt. of new mica discs and the corresponding number of glass coverslips.
3. Using the epoxy glue attach the mica on each of the glass coverslip and leave it overnight for strong adhesion.
4. Using scotch tape peel the mica top layer to make a fresh clean and flat surface.
5. Use the UV glue to make a circular boundary on the mica. Make sure to leave free space for crystal drop, not less, not more than desired area.



5. Shine the portable UV light (High Wave) for 10-15 sec to initiate the polymerization of the glue.
6. Repeat steps 5 & 6 for all the prepared mica glass coverslip assembly.
7. Arrange all the mica in an order and make a combined UV exposure for 10 - 15 min.
8. Leave it overnight for the glue to polymerize completely.

❖ Motif Solution Preparation

9. 5 μL of motif solution is placed on each mica. Calculate the required amount of total solution to be made to accommodate all mica.
10. Since we are synthesizing Asymmetric motif, all the 7 strands are unique. Make the solution containing all strands with a concentration of 2 μM motif in 0.2X TAEM buffer. Use the following sheet to calculate required volume of strands from stock solution.
11. Thermal Anneal the motif solution using the protocol (95-65-50-37-22)

❖ Growing Crystals

12. Take the small petri dishes and fill them with glass coverslips to a particular height.
13. Introduce the mica – coverslip assembly in each one of the petri dish.
14. 1 mL of 1X TAEM Buffer should be introduced into each petri dish.
15. Drop 5 μL of annealed motif solution on the mica and seal the petri dish with paraffin.
16. Store all the petri dishes in a closed container to each uniform heat distribution and leave it undisturbed for 7 days.

❖ Ligation of Crystals

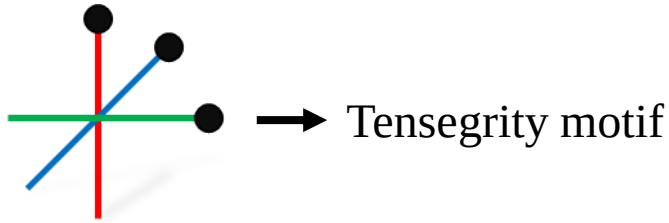
17. Look under microscope if crystals were grown on any of the mica.
18. Carefully using pipette wash the crystals (add and remove fluid) with 5 μL of 5X TAEM without disturbing the crystals.
19. Repeat the washing for 3-4 times.
20. Use the sheet to prepare the ligase solution.
21. Drop 5 μL of ligase solution on each mica and seal back with paraffin. Leave it overnight for the ligase to bond all the phosphorylated strands.
22. The surface of mica will become very sticky after the ligation. Wash the crystals again with 5X TAEM 4-5 times to reduce the stickiness.
23. Store the crystals hydrated in petri dishes till you are going for AFM measurements.

Ligation Schemes in DNA Crystals

Details on 5x5x5 crystal:

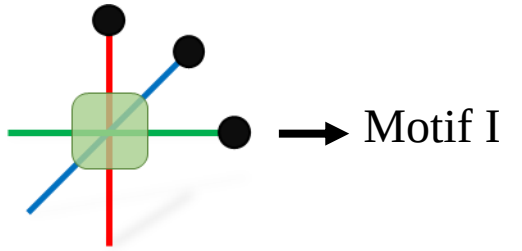
- No. of sticky end cohesions b/w motifs: 300
- Each sticky end cohesion = dsDNA duplex (2 DNA strands)
- No. of covalent bonds needed for complete ligation = 600
- To not ligate – Use unphosphorylated strand

Schemes of Ligation:

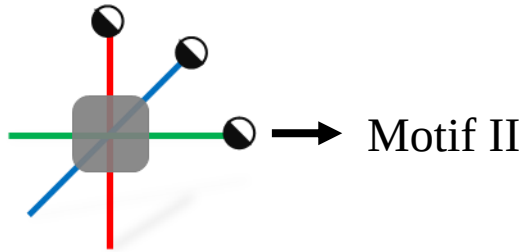


If phosphate is available b/w 2 sticky ends, ligase forms a covalent bond and joins the strand

1. 100% - ligate at all sticky ends
2. Phosphates avoided only at major strands – 50% uniform
3. Phosphates avoided at only one major strand (let red) – 83%
4. Phosphates avoided at only two major strands (let blue and green) – 66%
5. 2 different motifs forming a unit cell (alternate lig. and partial lig.) – 75%



- For <50% ligation
  - Partial surface ligation
  - Inner core native

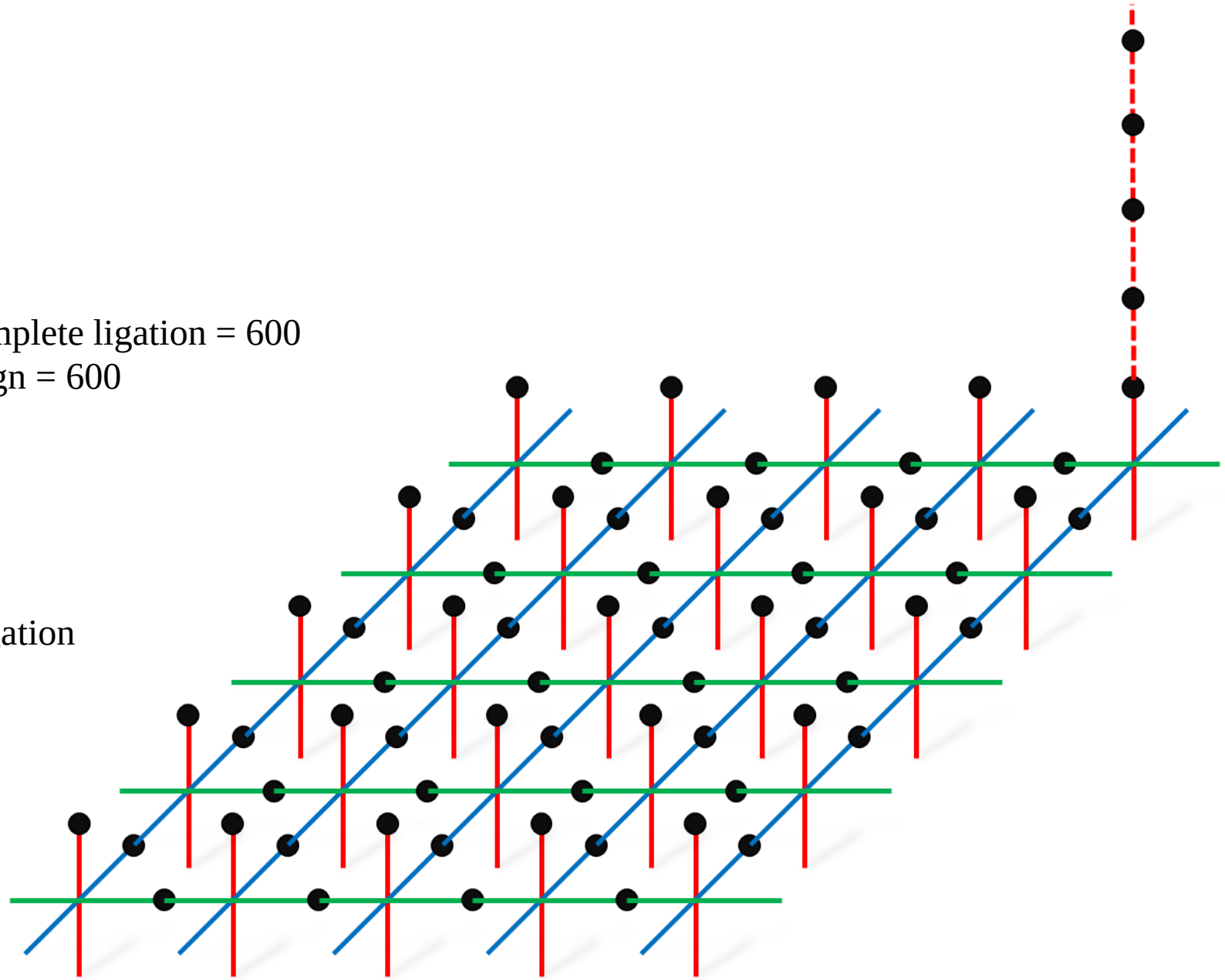


I. Fully Ligated Crystal

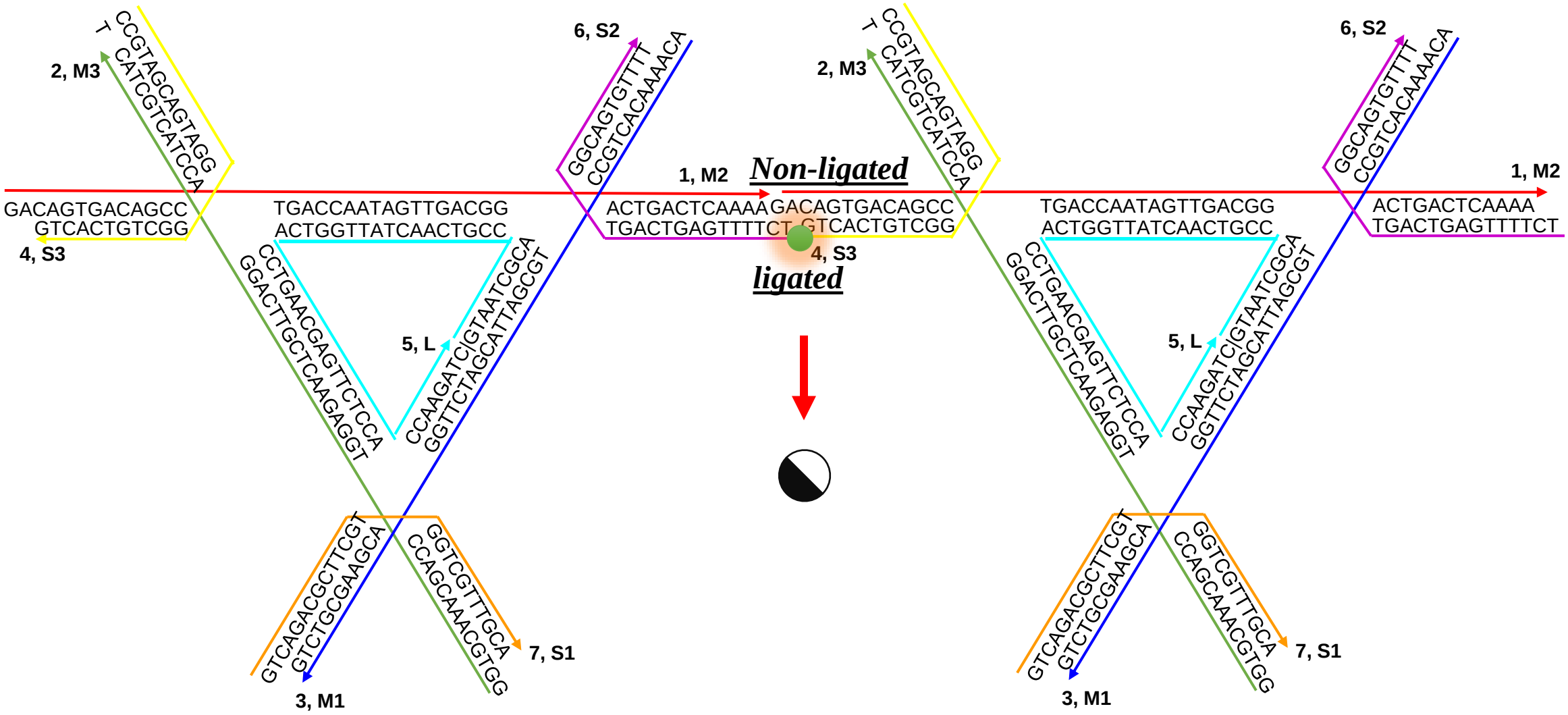
- No. of covalent bonds needed for complete ligation = 600
- No. of covalent bonds ligated in design = 600

- Ligation: 100%

● ⟶ Full Ligation



50% ligated crystal - Design

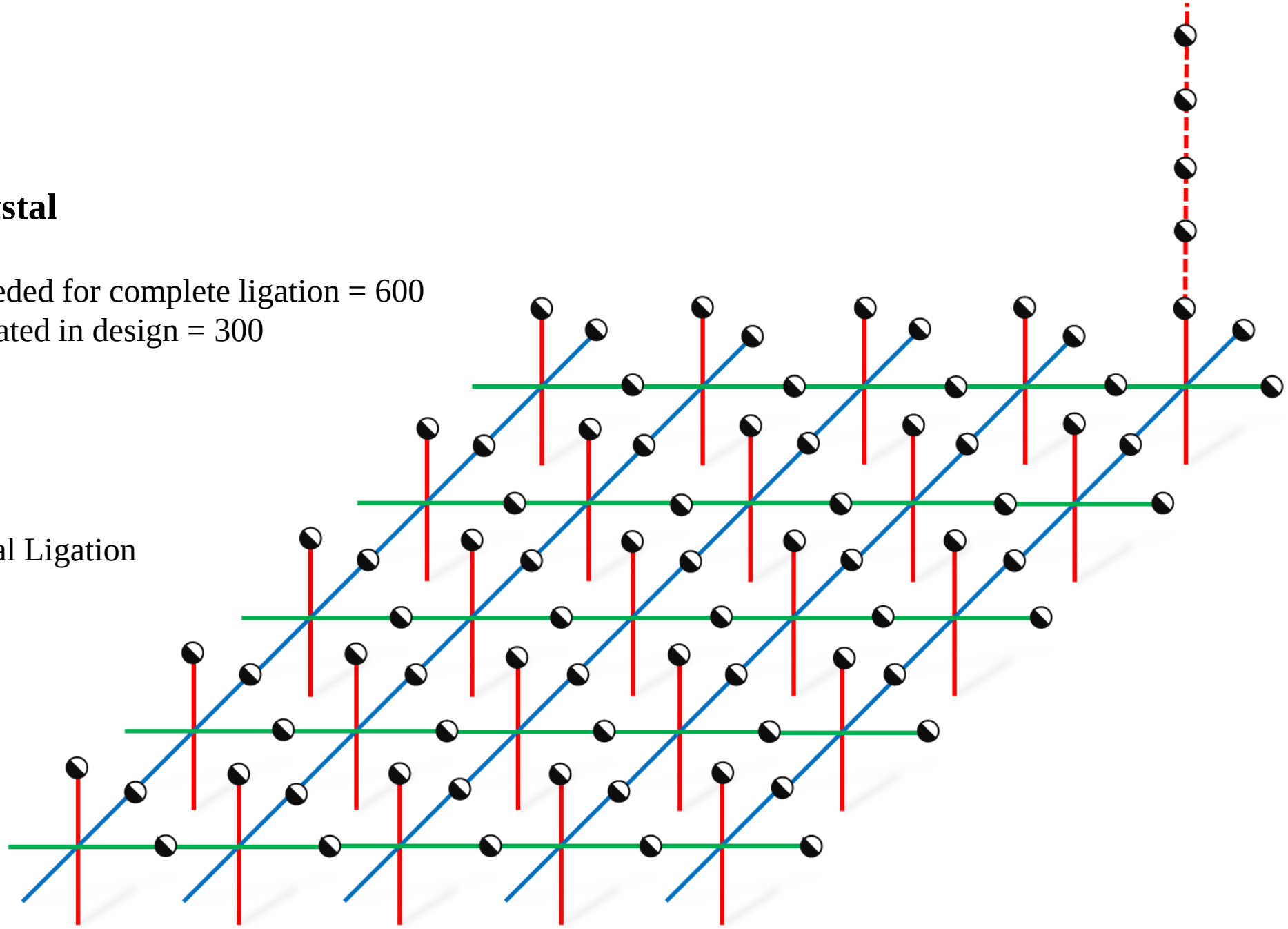


II. Partially Ligated Crystal

- No. of covalent bonds needed for complete ligation = 600
- No. of covalent bonds ligated in design = 300

- Ligation: 50%

◔ → Partial Ligation

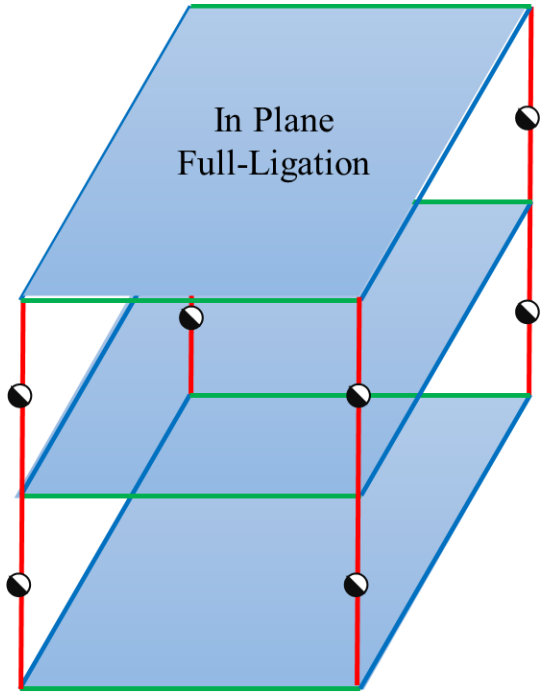


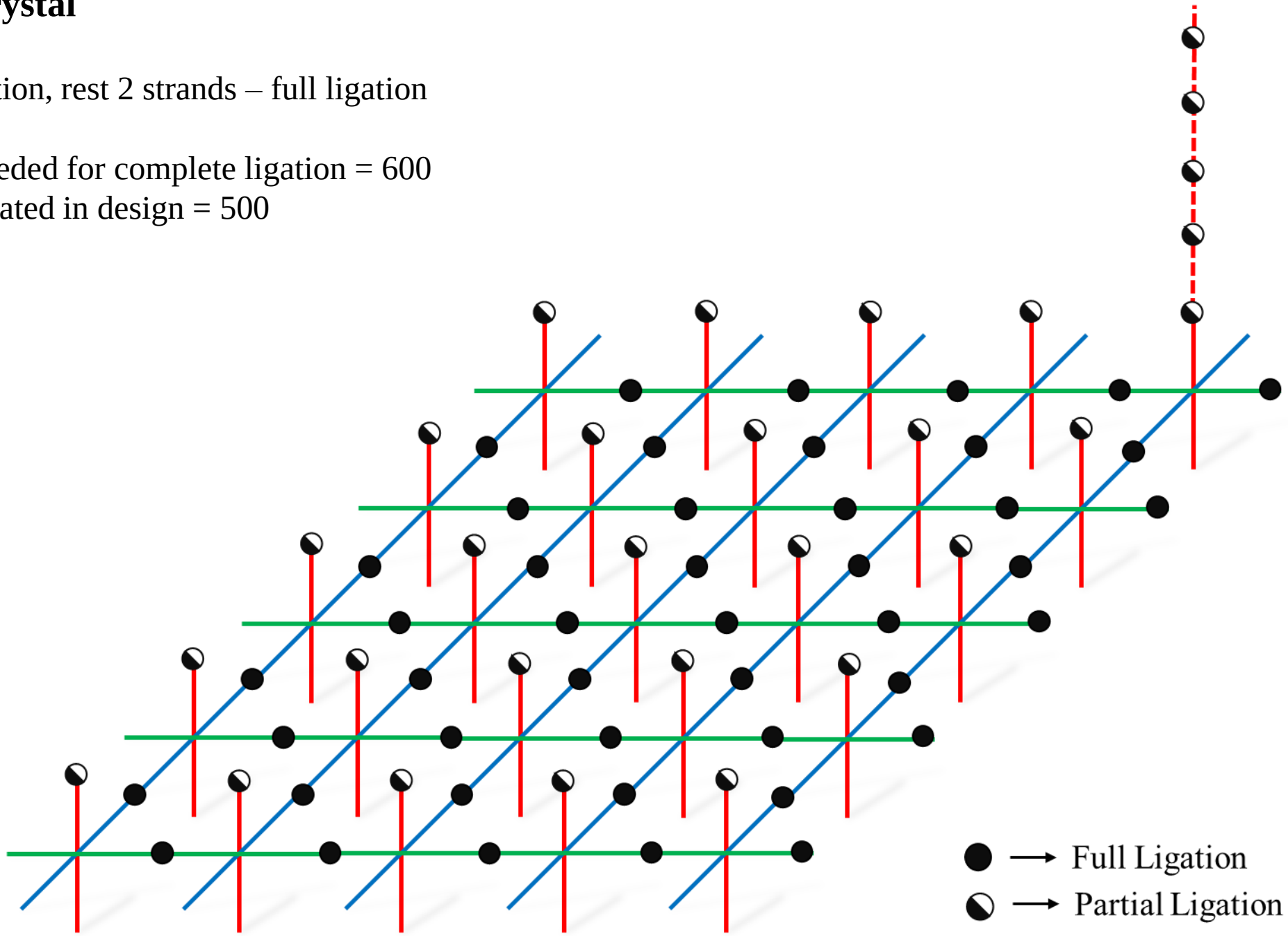
III. Partially Ligated Crystal

- Red strand – partial Ligation, rest 2 strands – full ligation

- No. of covalent bonds needed for complete ligation = 600
- No. of covalent bonds ligated in design = 500

- Ligation: 83 %

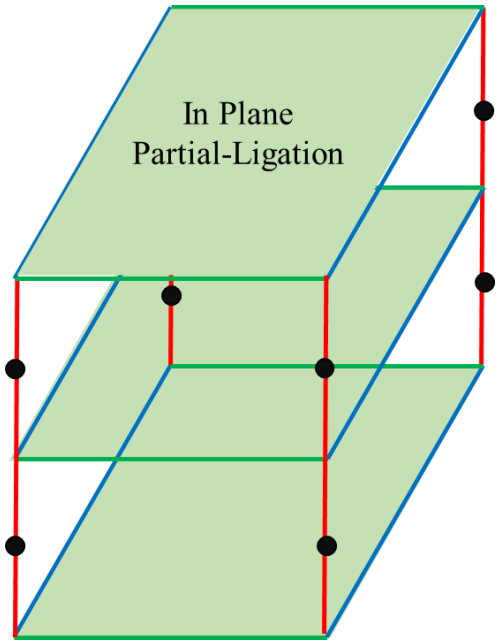


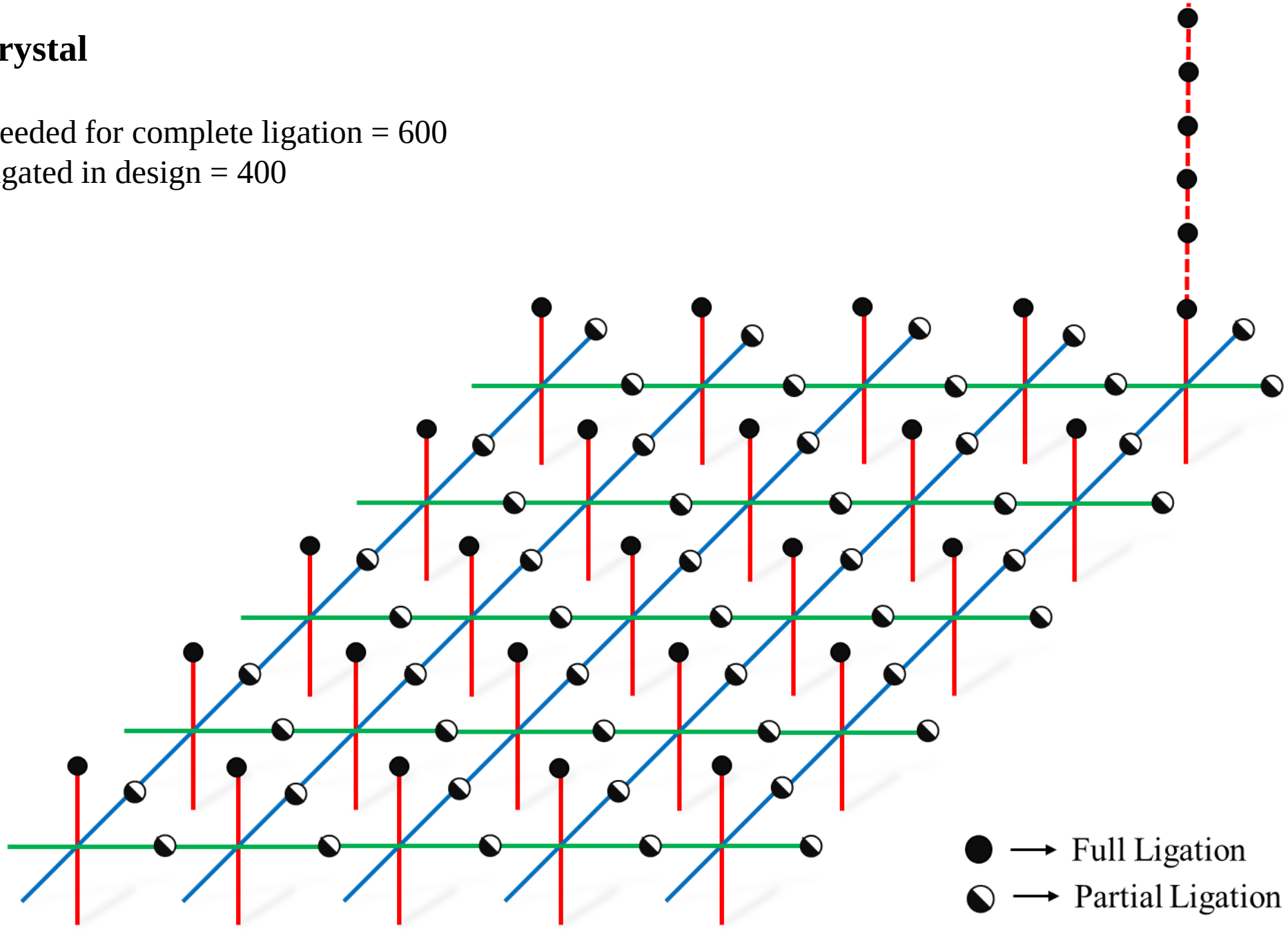


IV. Partially Ligated Crystal

- No. of covalent bonds needed for complete ligation = 600
- No. of covalent bonds ligated in design = 400

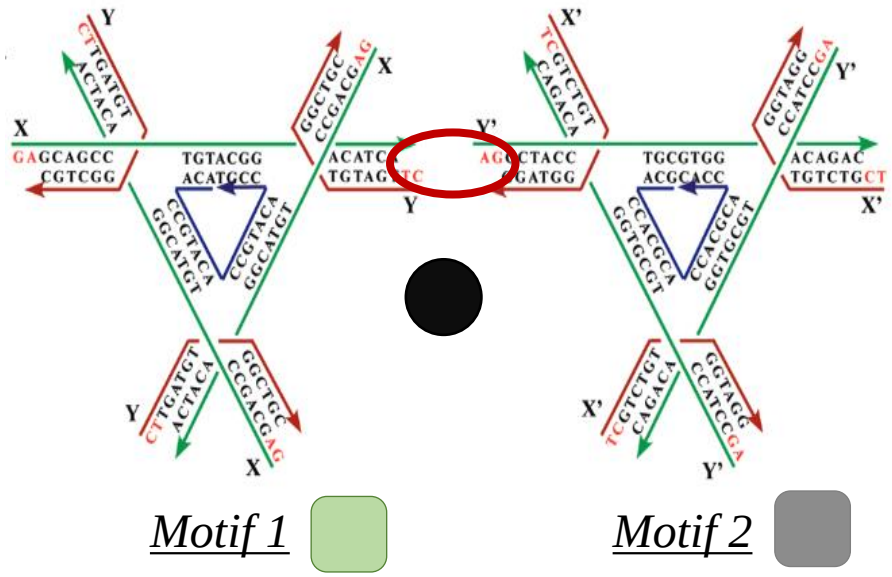
- Ligation: 66 %

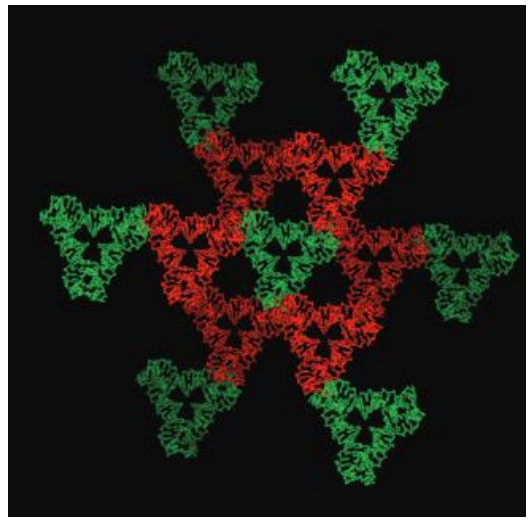


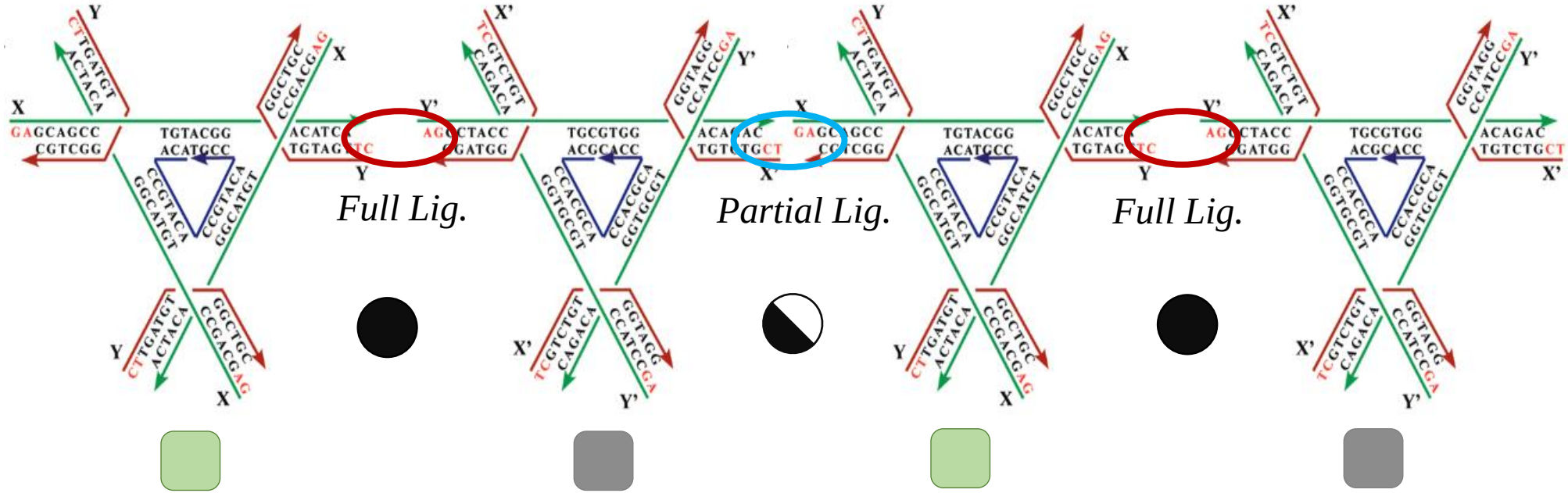


A DNA Crystal Designed to Contain Two Molecules per Asymmetric Unit, *Chengde Mao and Nadrian C. Seeman*

https://doi.org/10.1021/ja104833t



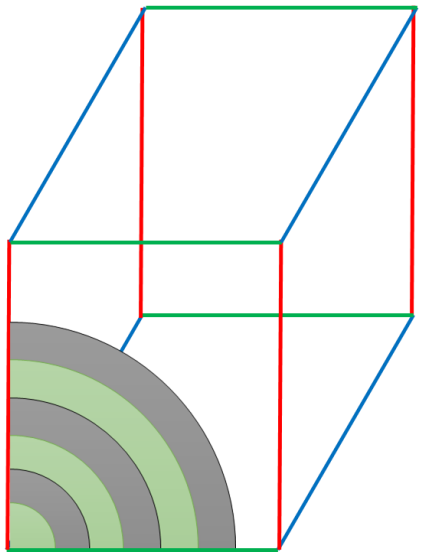


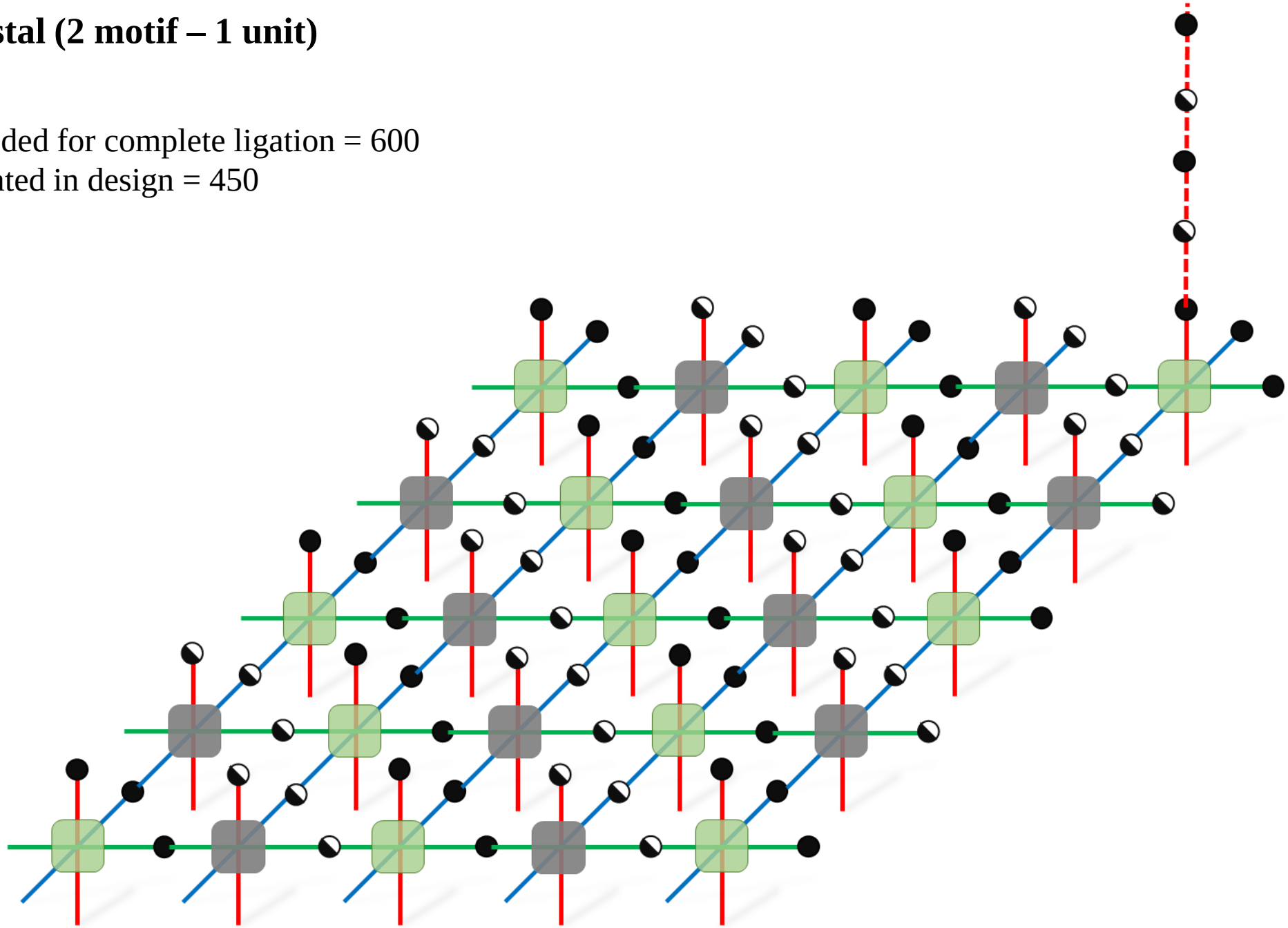


V. Partially Ligated Crystal (2 motif – 1 unit)

- No. of covalent bonds needed for complete ligation = 600
- No. of covalent bonds ligated in design = 450

- Ligation: 75 %





Notes

1. Make sure to make a clean flat surface of mica while peeling using scotch tape.
2. The central area left for the motif drop should be near to desired area (not too large or too small)
3. Ensure optimal number of glass coverslips are placed in petri dish. Too small height may cause the 1X TAEM solution to flow over the crystals. Very large height may cause the mica to fly and stick up to the cap of petri dish due to electrostatic attraction.