MECHANISM OF THE APTAMER RECOGNITION BY SARS-COV-2 SPIKE PROTEIN REVEALED BY NANOPORE SEQUENCING AND MOLECULAR MODELING

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Nanopore sequencing (ONT)

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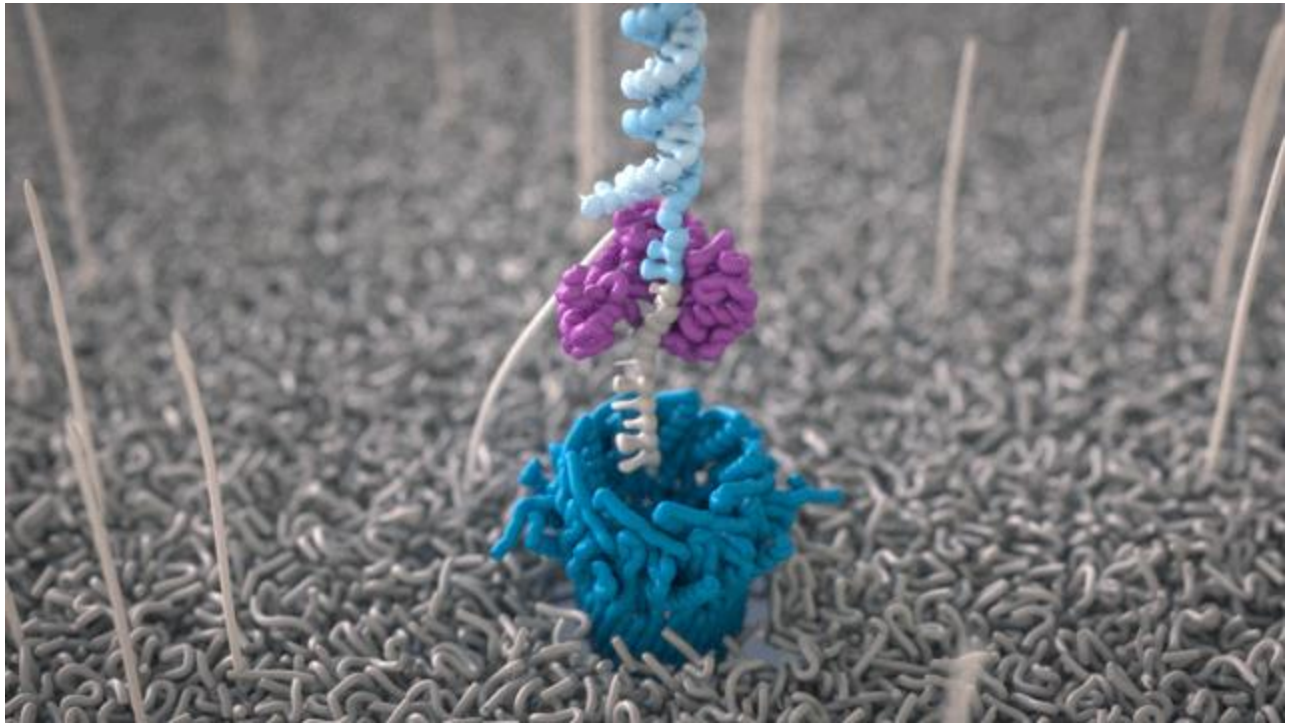
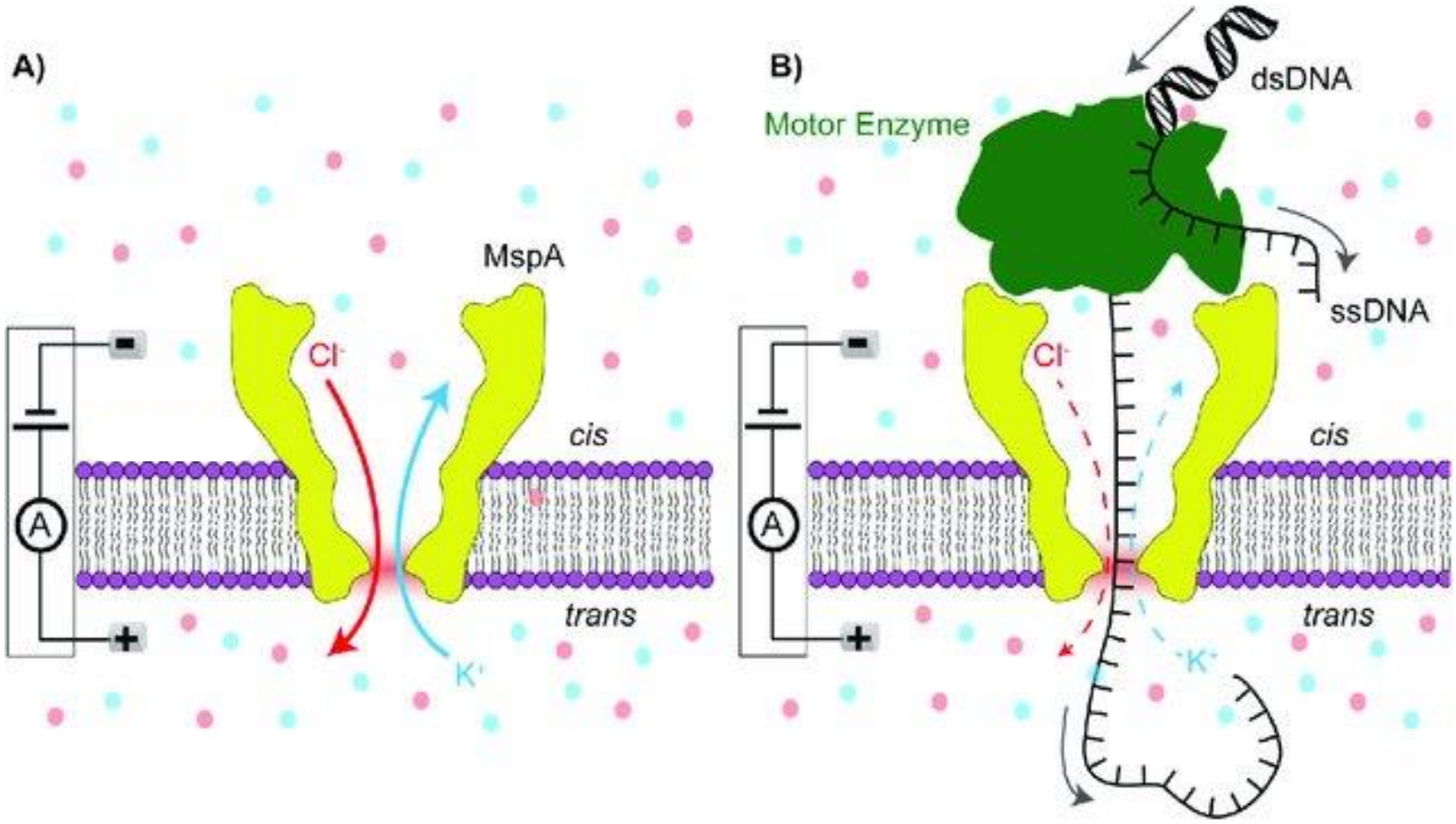


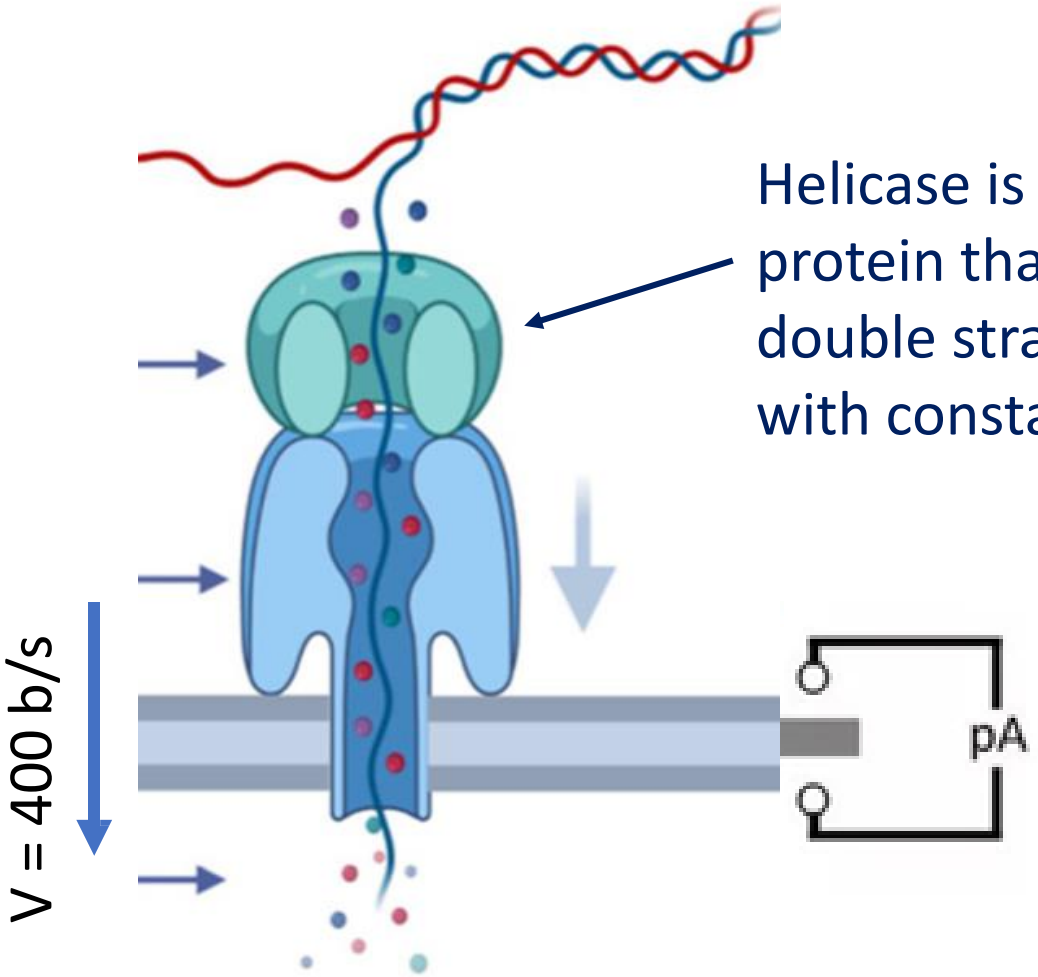
- Nanopores are transmembrane proteins incorporated into a synthetic membrane (usually SiN_x or SiO_2)

Nanopore sequencing (ONT)

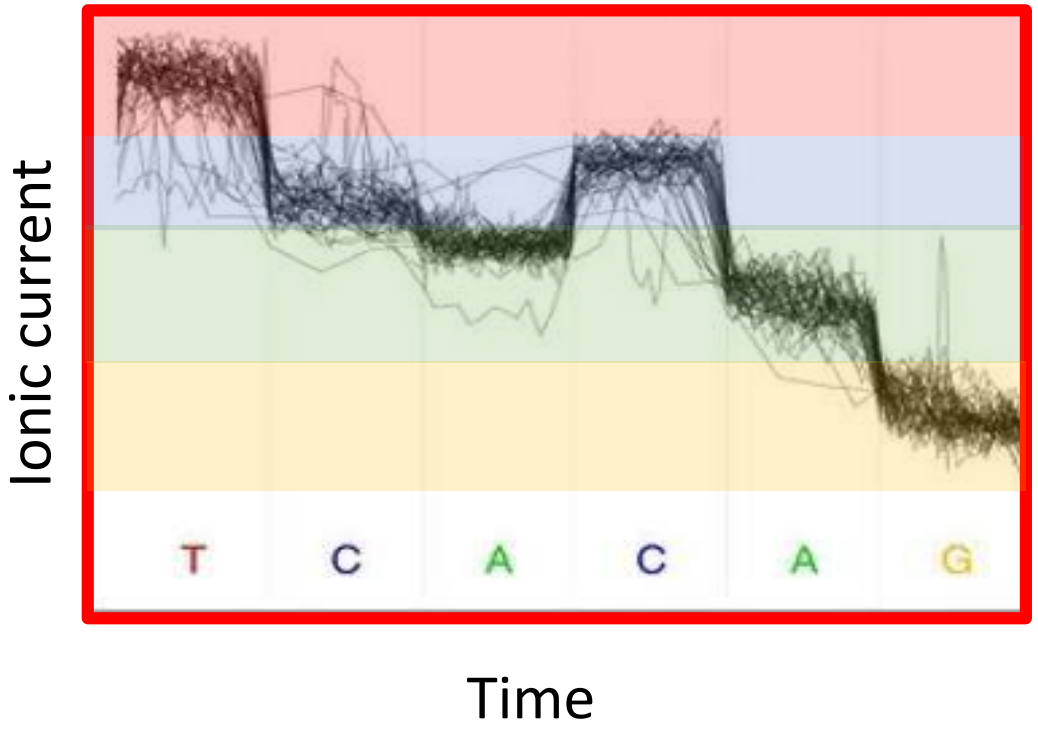


- KCl is utilized as electrolyte. Application of a constant potential difference leads to the ionic current

Nanopore sequencing (ONT)



Helicase is a motor protein that divides double strand DNA with constant rate



- Application of a constant potential difference to the silicon chip produces an electric field inside the membrane and charges the interfaces with opposite ions. This facilitates ion current and DNA/RNA

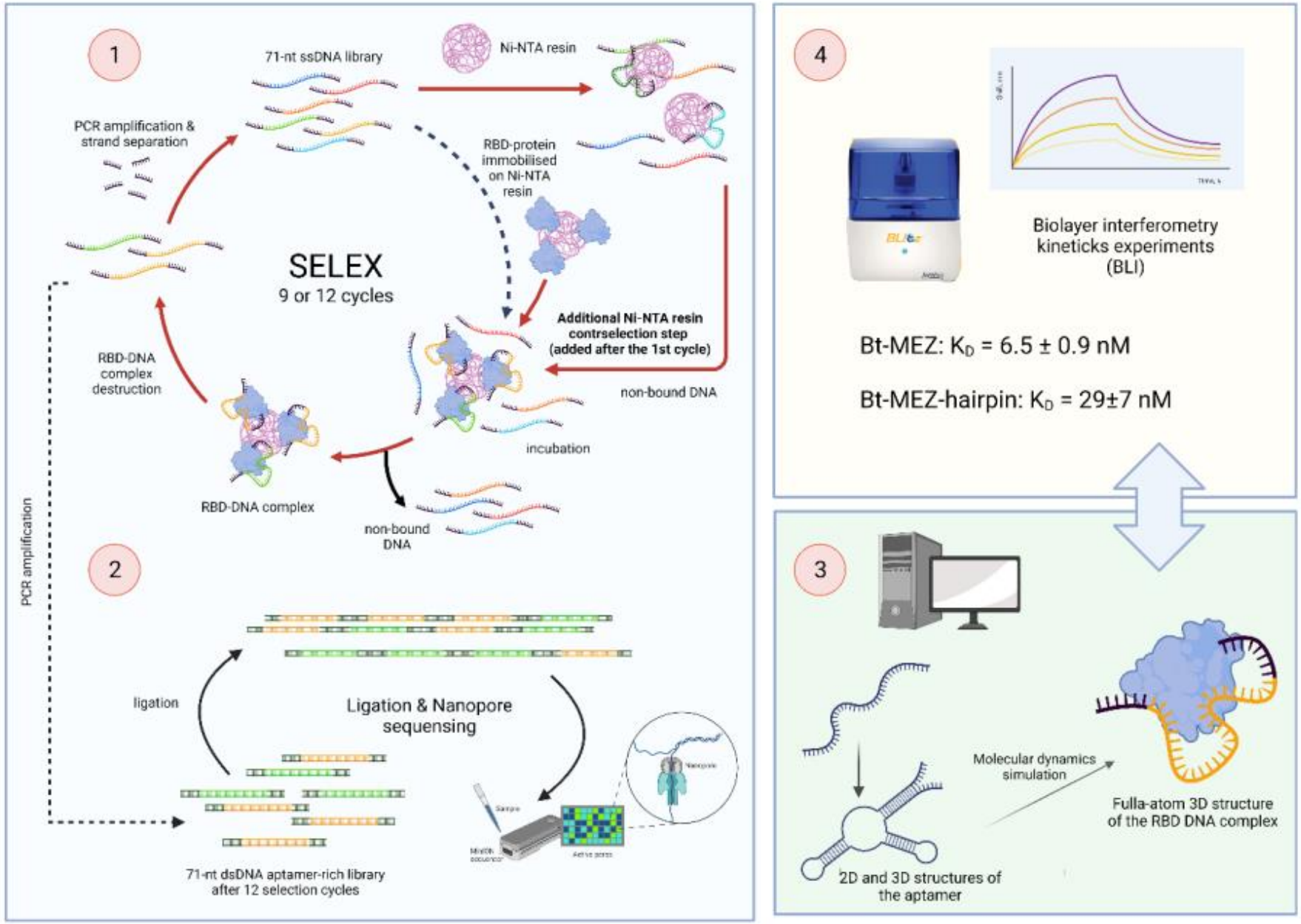
Some applications of nanopore sequencing

- *De novo* genome assembly
- Determination of the single nucleotide polymorphism and large rearrangements
- Determination of oligonucleotide sequences?

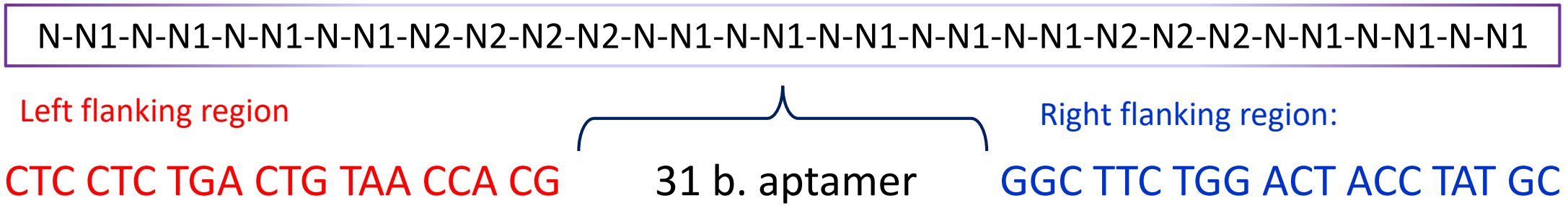
\+ Ability to read long reads (up to 100 000 b.)
– High errors in sequence determination

In vitro selection of an aptamer targeting SARS-CoV-2 spike protein with nanopore sequence

The overall workflow



Aptamer library



N = 45:05:45:05 A/C/G/T – enriched with purine bases

N1 = 05:45:05:45 A/C/G/T – enriched with pyrimidine bases

N2 = 25:25:25:25 A/C/G/T

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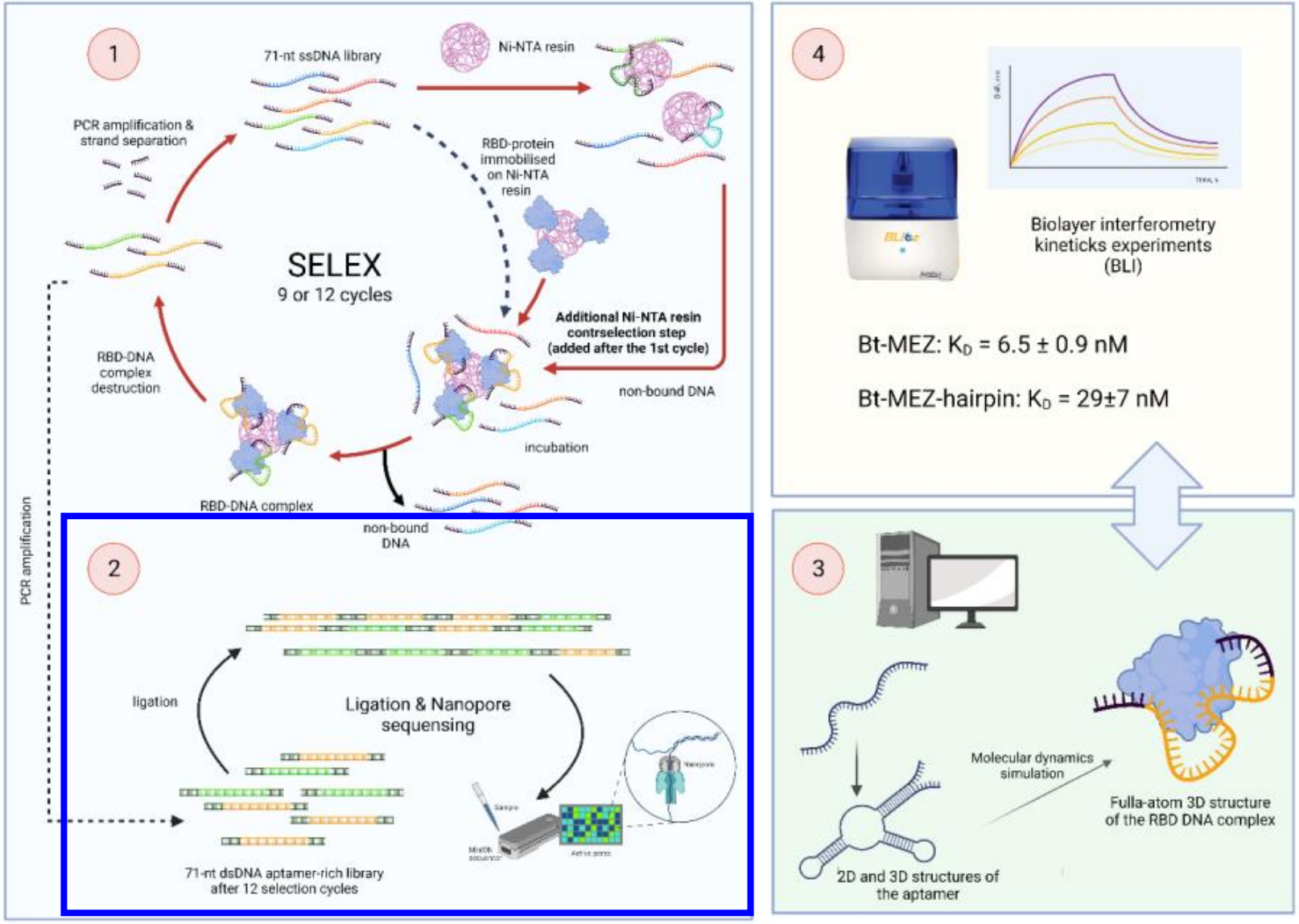
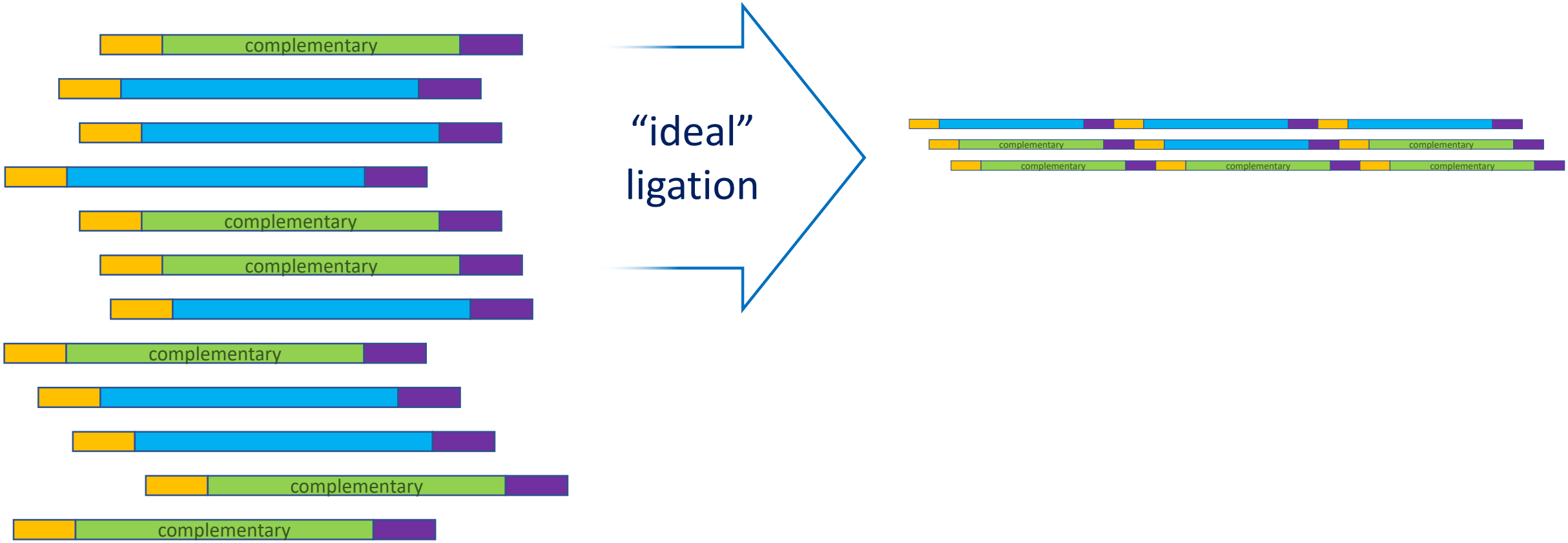
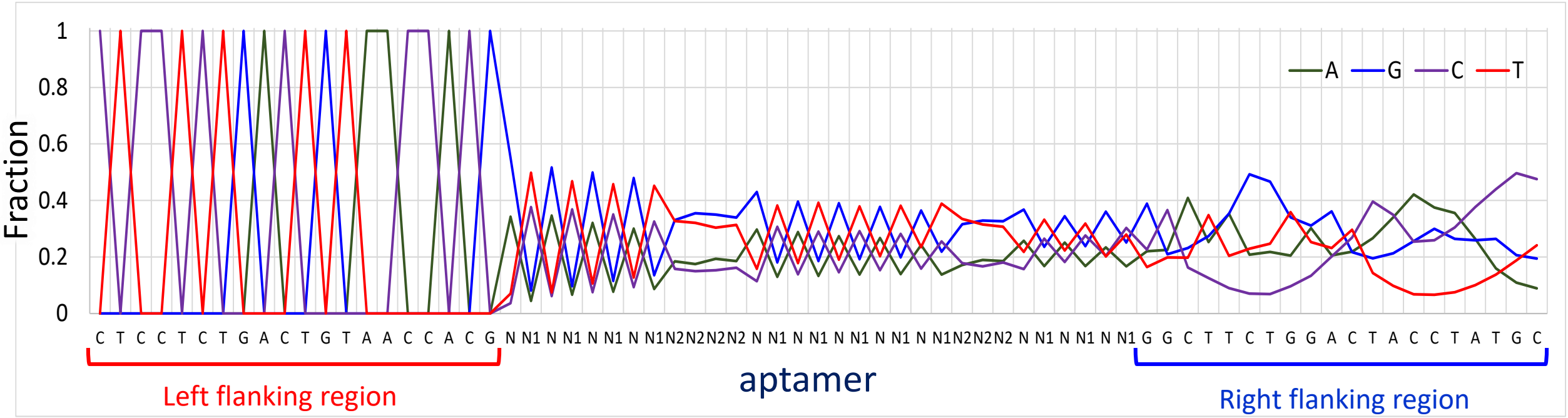


Preparation to the sequencing



Initial aptamer library



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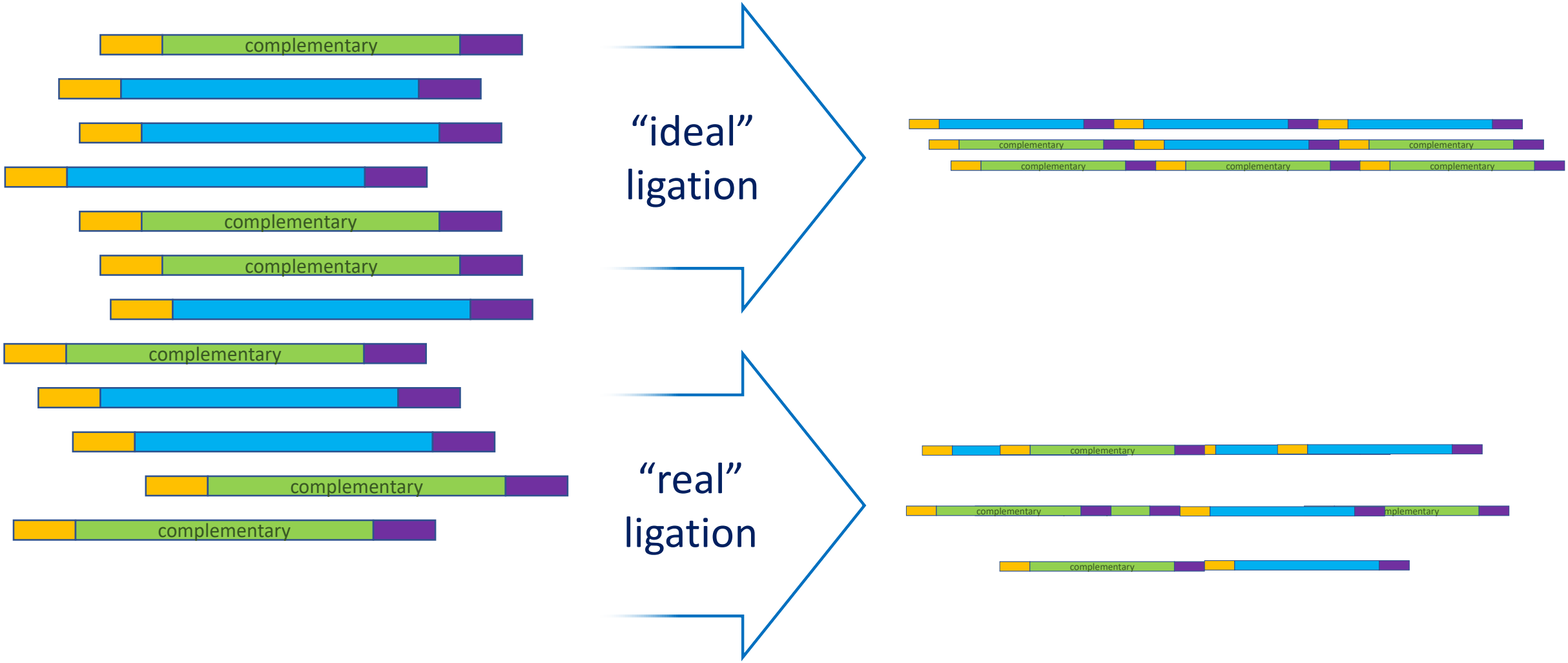
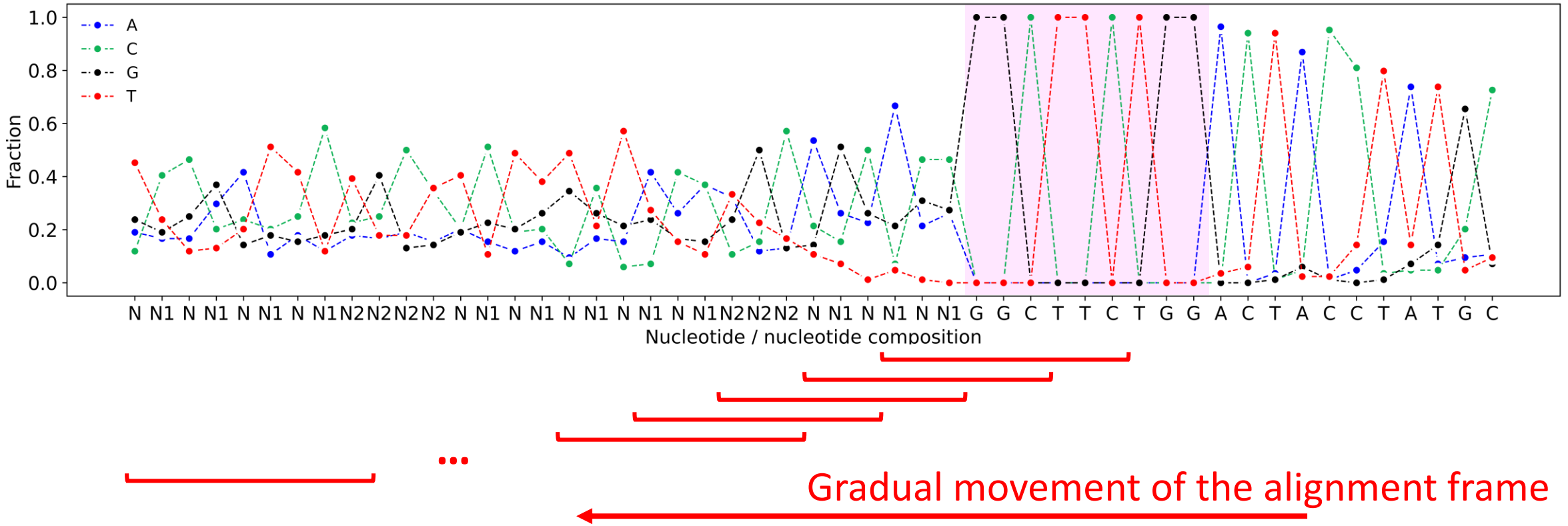


Data analysis



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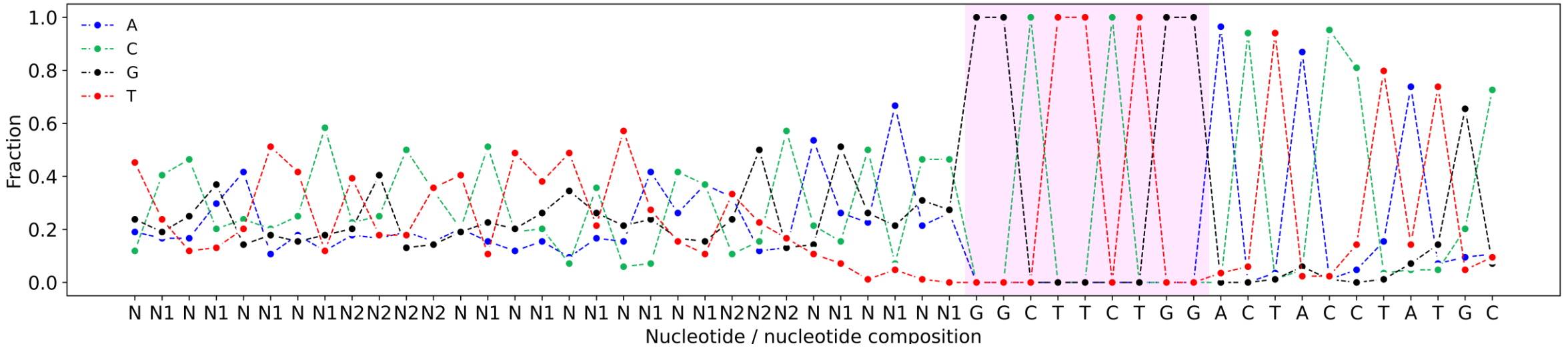

Coord	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	N	N1	N	N1	N	N1	N	N1	N2	N2	N2	N2	N	N1	N	N1	N	N1	N	N1	N	N1	N2	N2	N2	N	N1	N	N1	N	N1
	T	A	G	G	G	A	A	A	C	A	C	G	A	T	A	G	A	A	T	C	C	G	A	A	C	A	G	C	A	C	C

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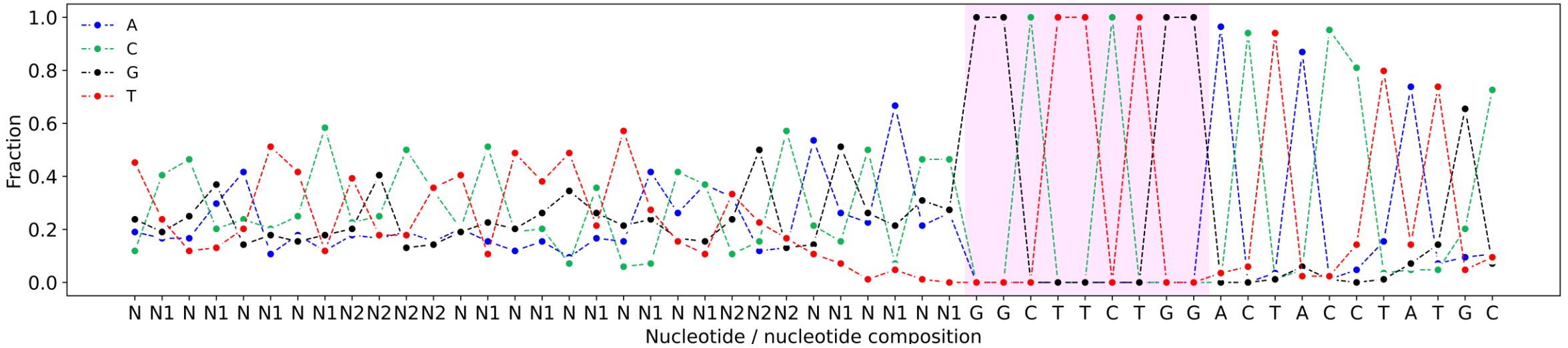

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	T	A	G	G	G	A	A	A	C	A	C	G	A	T	A	G	A	A	T	C	C	G	A	A	C	A	G	C	A	C	C

Determination of the aptamer sequence from the left side leads to worse results as the enrichment at these coordinates is less pronounced. The 3’-end is more important for binding.

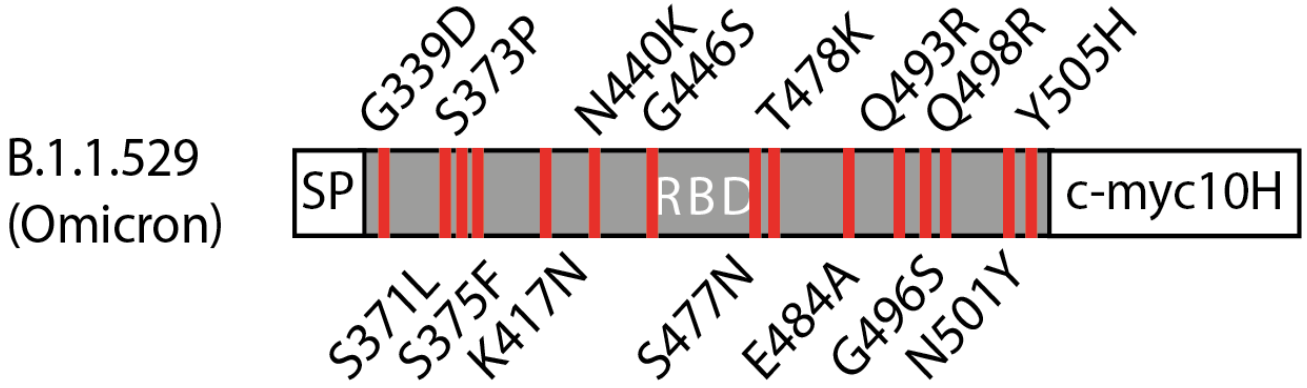
RBD – aptamer complex

RBD from Wuhan strain:

K_d = 6.5 ± 0.9 nM

RBD from Omicron strain:

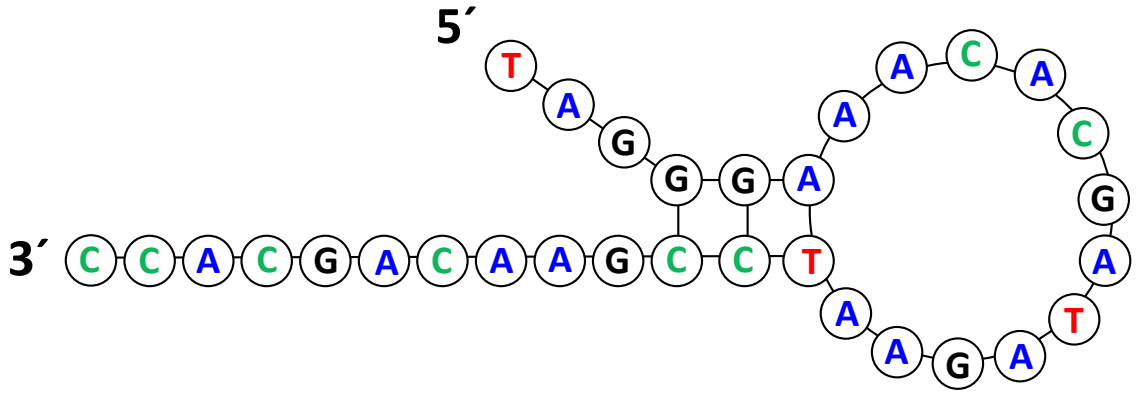
No binding

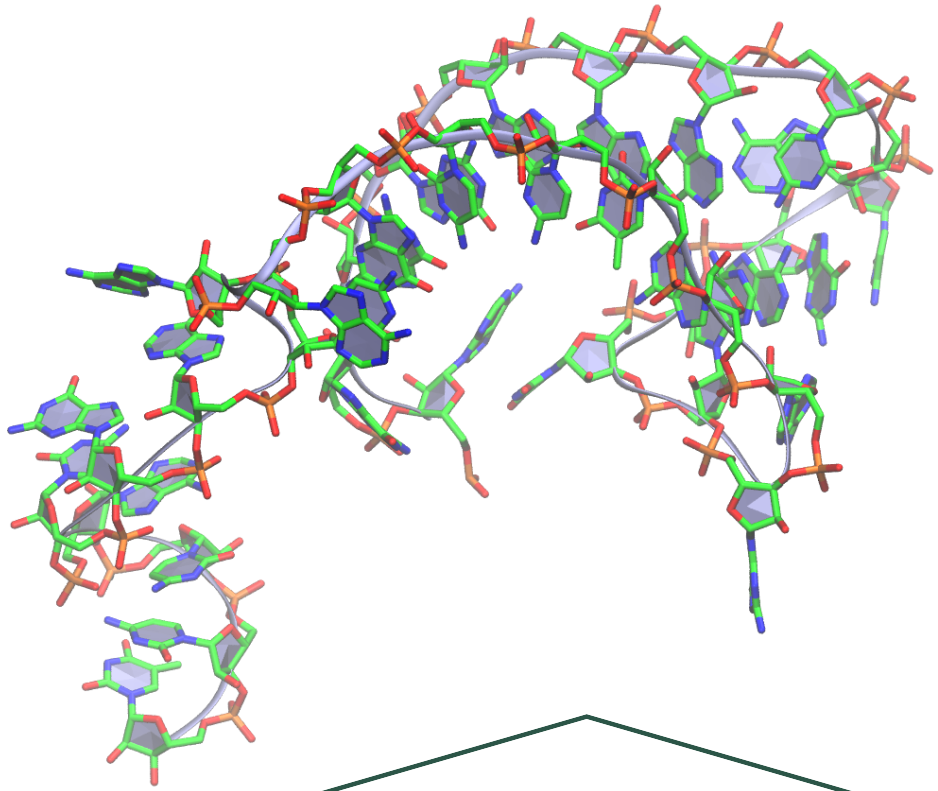


Molecular modeling: preparation of the 3D structure

AGGGAAACACGATAGAATCCGAACAGCACCT

RNAstructure
Web Servers for RNA Secondary Structure Prediction

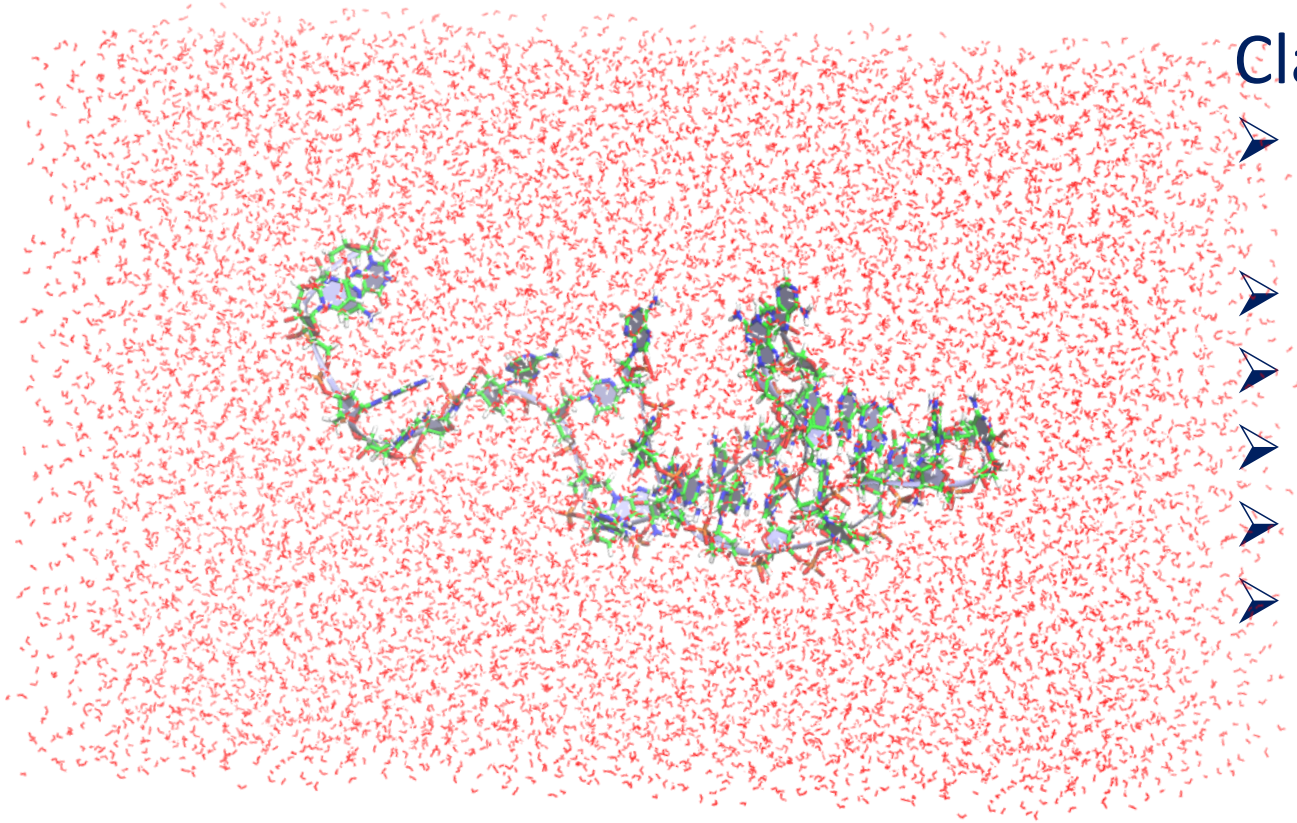




RNAComposer
Automated RNA Structure 3D Modeling Server

AGGGAAACACGATAGAATCCGAACAGCACCT
..(((.....)))..........

MD simulations of the aptamer in water



Classic MD

- CHARMM36 force field for oligonucleotide and TIP3P for water molecules;
- 500 ns trajectory with 1 fs time step;
- NPT, p = 1 atm, T = 300 K ;
- System neutralization with Na^+;
- 36 500 atoms in system;
- Software: NAMD.

Modeling of RBD – aptamer complex

Molecular docking: HADDOCK server

1

13 structure with the highest interaction energy

2

50-200 ns molecular dynamics simulations to determine stability of complexes predicted by docking

Only **ONE** complex turned out to be stable in MD simulations

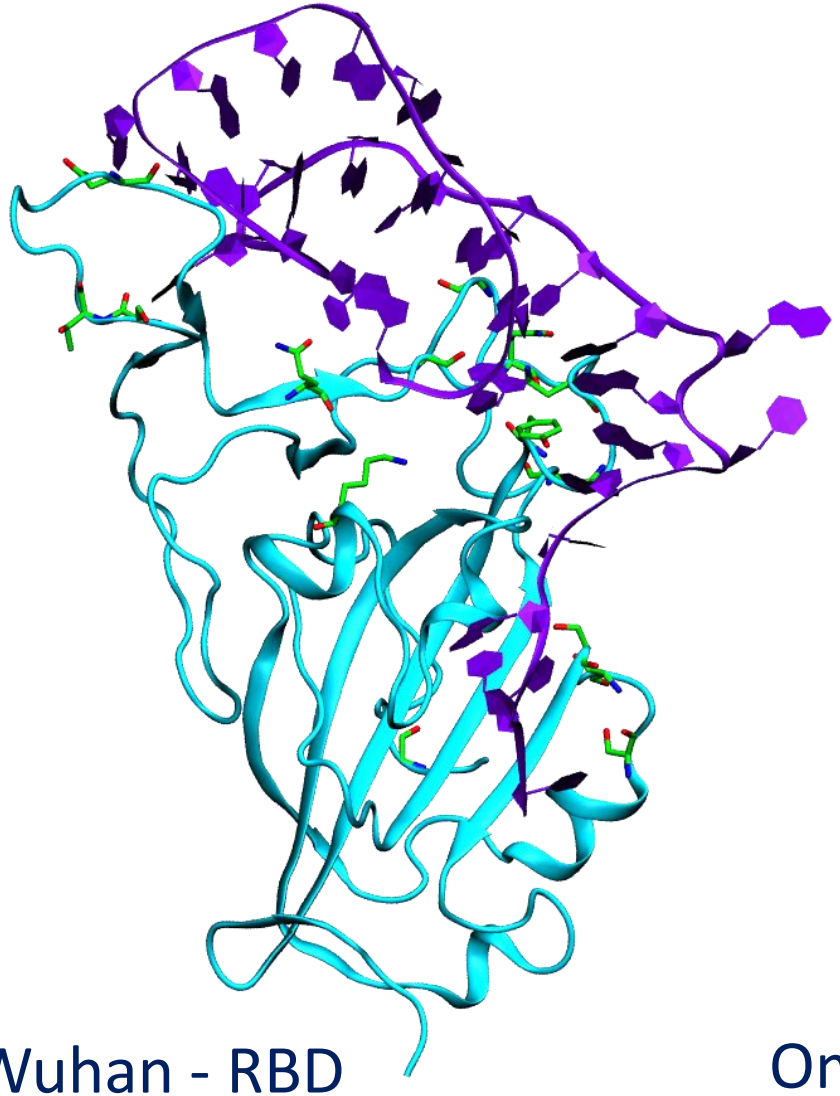
3

3D structure of the Omicron-RBD-aptamer complex by motifs of the complex with the Wuhan-RBD

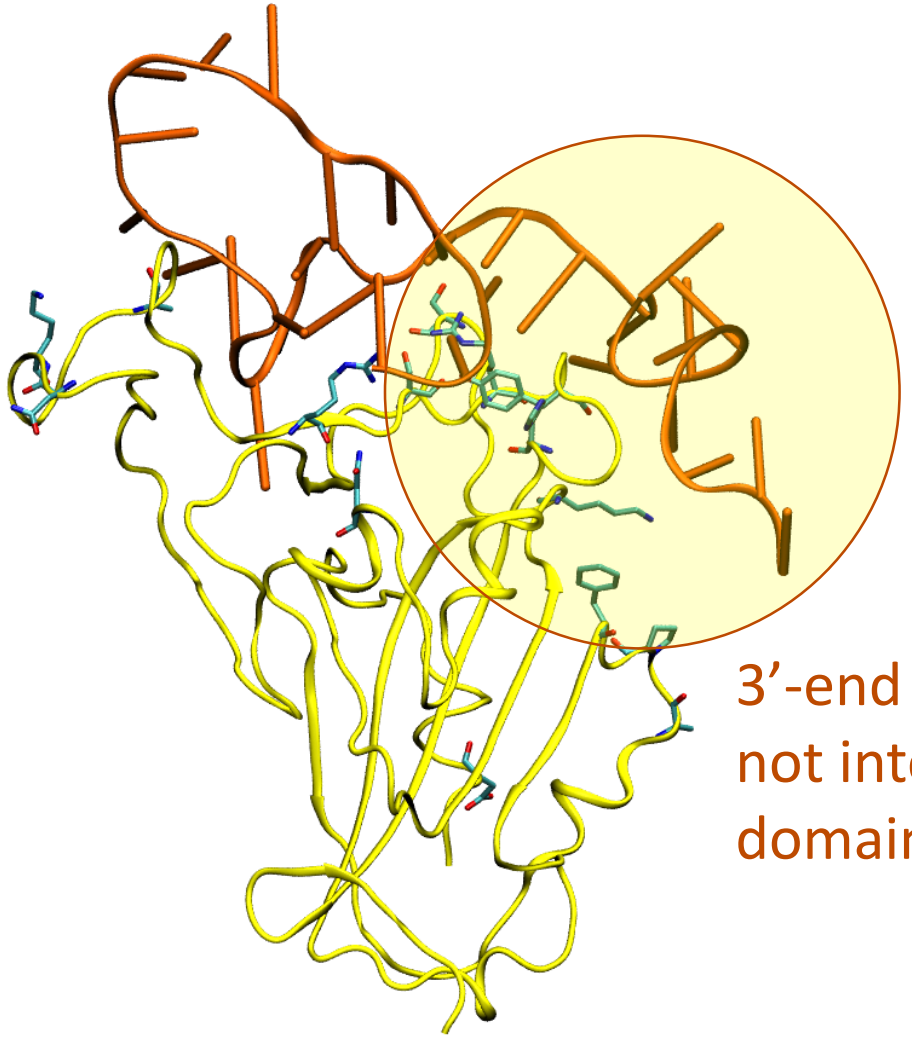
4

Sets of 200-300 ns MD trajectories

RBD – aptamer complex



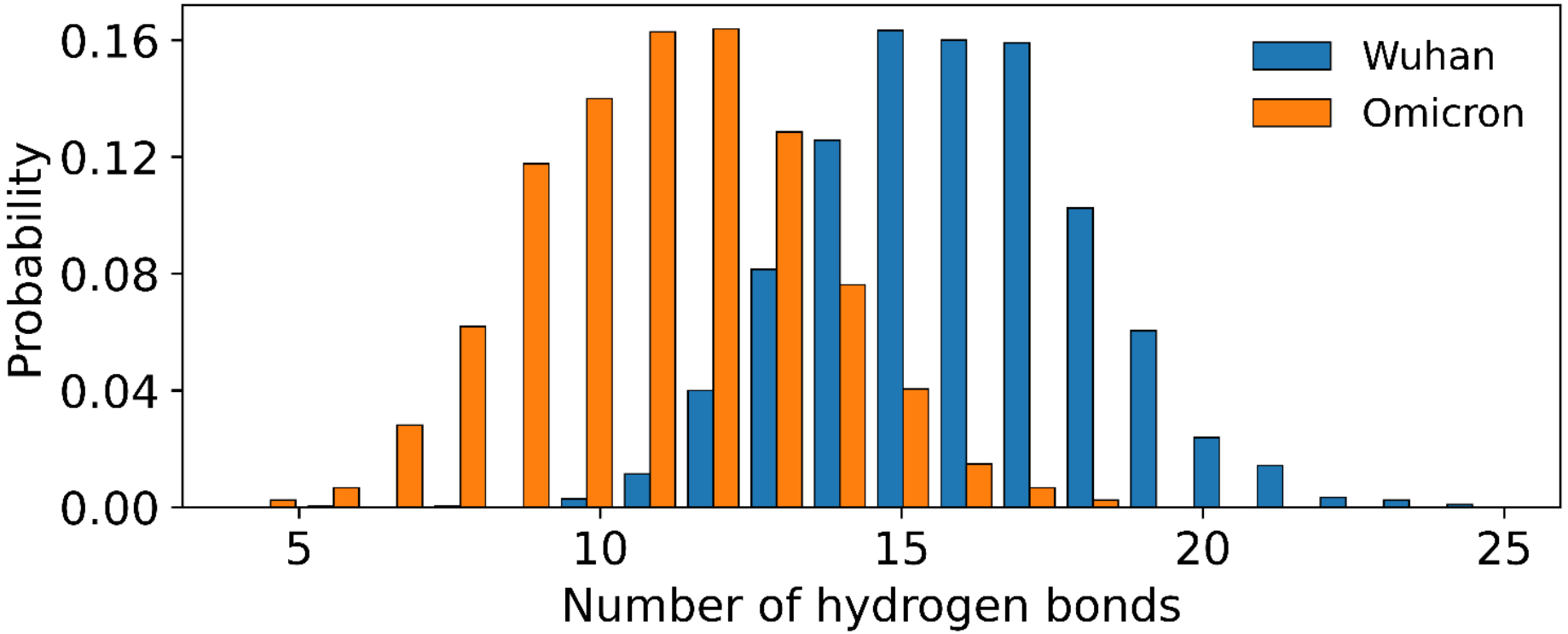
Wuhan - RBD



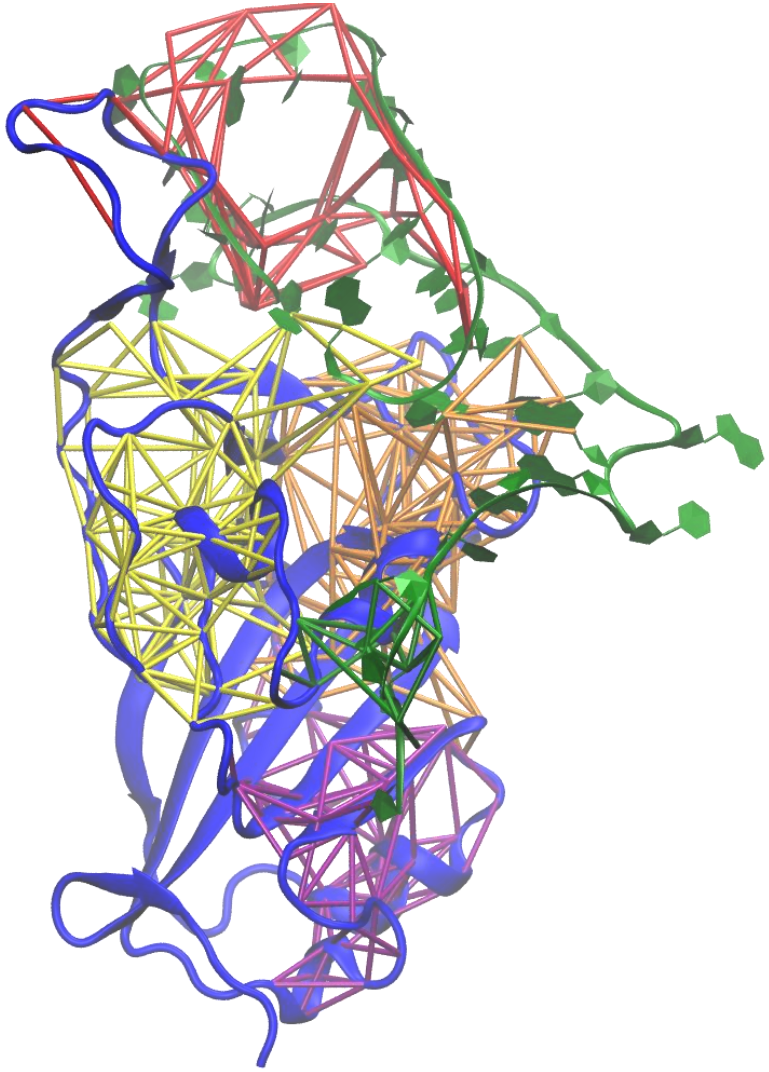
Omicron - RBD

3’-end of the aptamer does not interact with the RBD domain of the Omicron

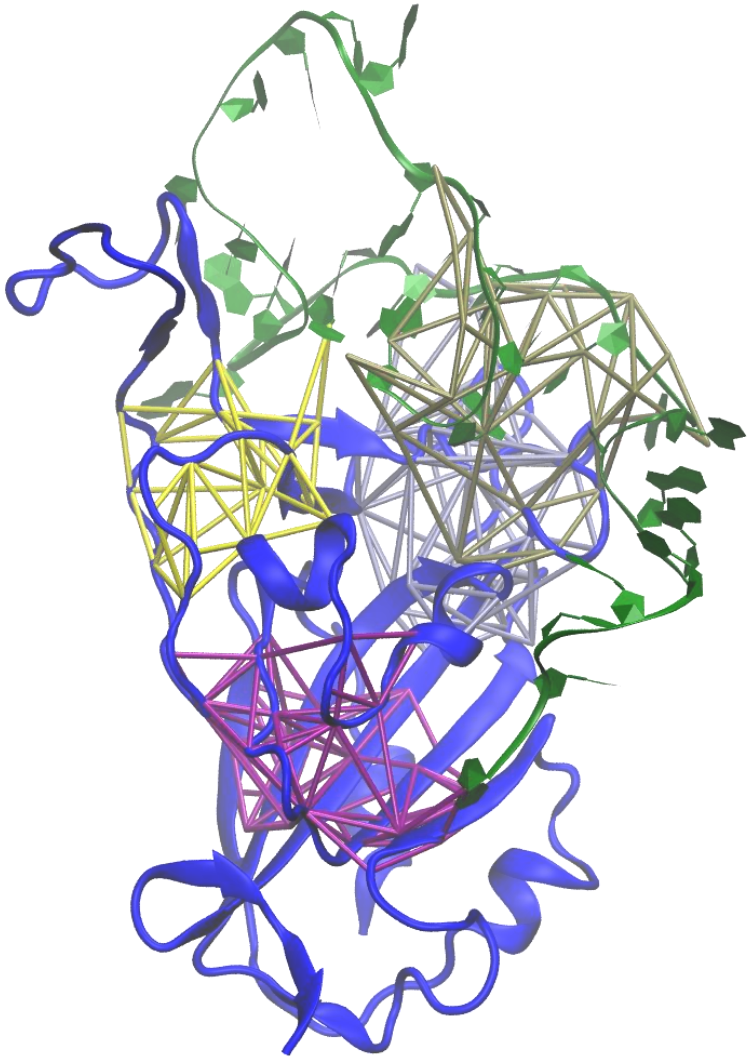
RBD – aptamer complex: hydrogen bond interactions



RBD – aptamer complex: dynamical network analysis

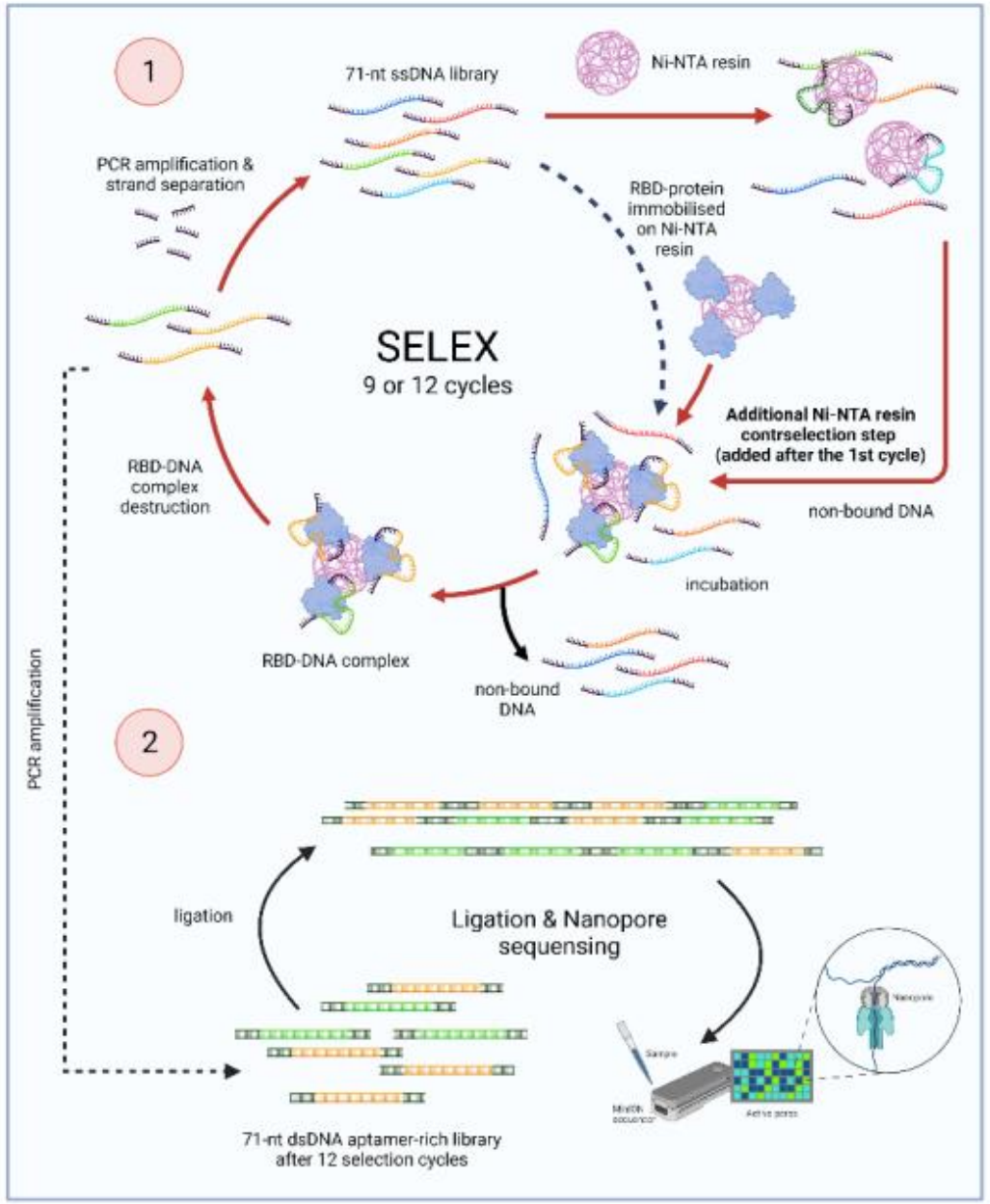


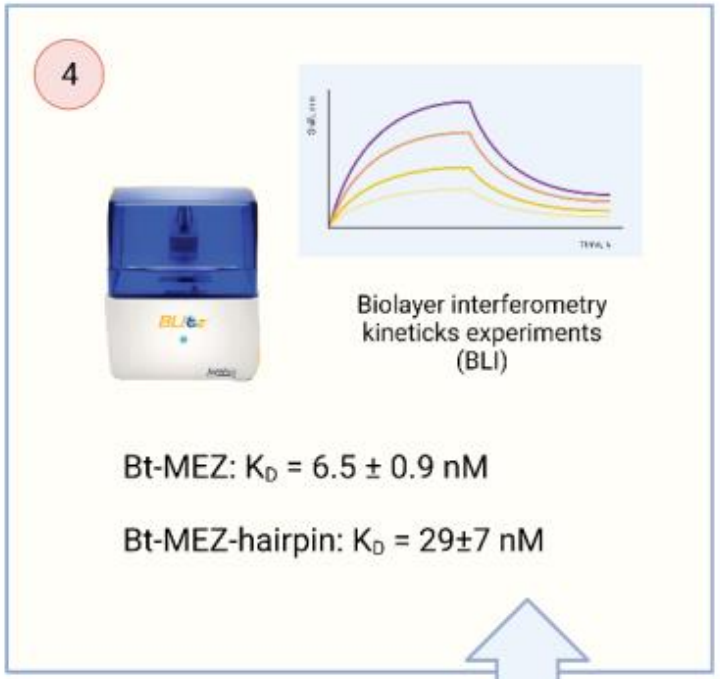
Wuhan - RBD

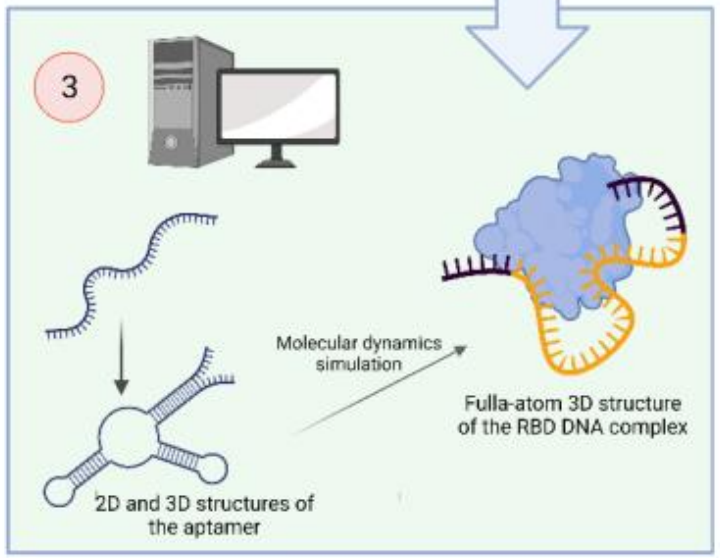


Omicron - RBD

Conclusion


1
71-nt ssDNA library
Ni-NTA resin
PCR amplification & strand separation
RBD-protein immobilised on Ni-NTA resin
SELEX
9 or 12 cycles
Additional Ni-NTA resin contrselection step (added after the 1st cycle)
RBD-DNA complex destruction
non-bound DNA
incubation
RBD-DNA complex
non-bound DNA
PCR amplification
2
ligation
Ligation & Nanopore sequensing
71-nt dsDNA aptamer-rich library after 12 selection cycles


4
Biolayer interferometry kineticks experiments (BLI)
Bt-MEZ: K_D = 6.5 ± 0.9 nM
Bt-MEZ-hairpin: K_D = 29±7 nM


3
Molecular dynamics simulation
Fulla-atom 3D structure of the RBD DNA complex
2D and 3D structures of the aptamer

Main contributors

Chemistry Department of the Lomonosov Moscow State University:

Zvereva M.

Grabovenko F.