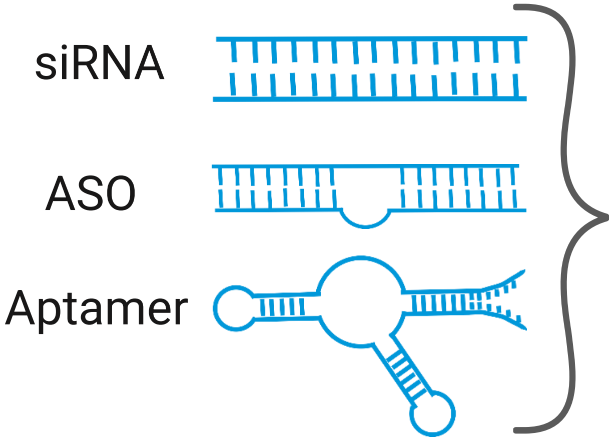
SCAMT

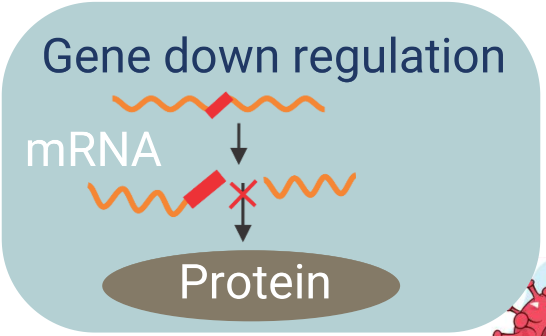
MULTIVALENT DEOXYRIBOZYME CONSTRUCTIONS FOR EFFICIENT CLEAVAGE OF TARGETED RNA

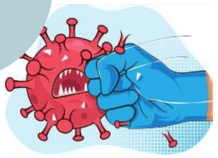
Completed by:
student of group # A42424
Batsa M.
Supervised by:
Professor PhD, Vladimir Vinogradov
Co-supervised by:
Professor PhD, Dmitry Kolpashchikov

Therapeutic nucleic acid for gene therapy



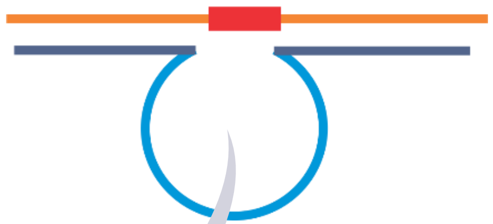
Therapeutic effect





Gene knockdown tools

RNA-cleaving DNAzymes (DZ)



Advantages of DZs
- high selectivity
- Independent of any additional protein interaction

Limitations of therapeutic nucleic acid approaches
- Off-target effects
- Low efficiency
- High cost of synthesis

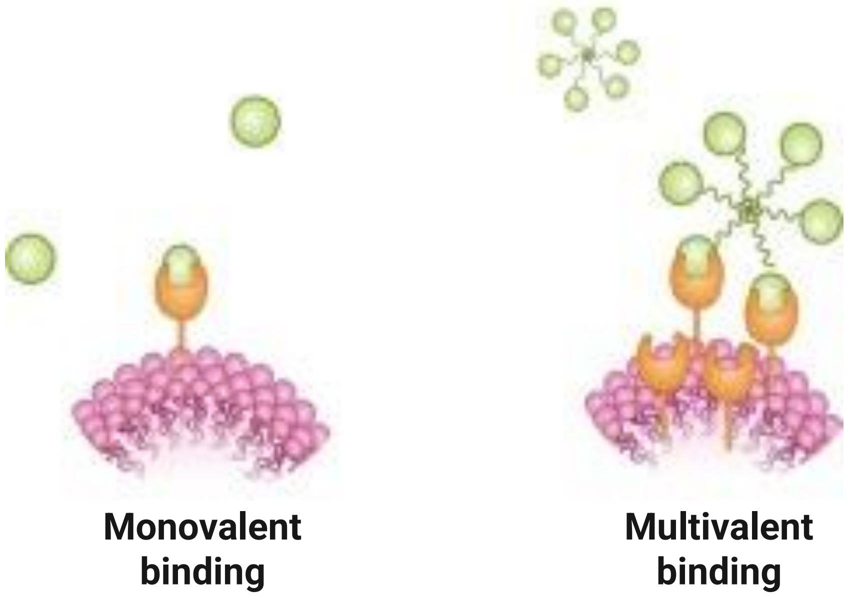
Levin, 2019; Xiong et al., 2021

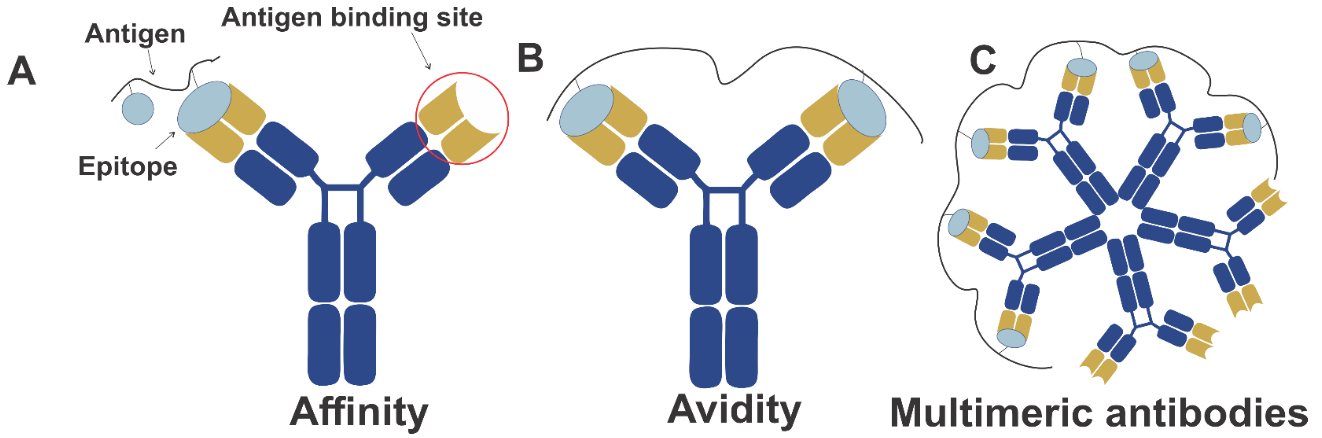
Limitations of DZs
- Weak affinity to folded targets
- Inefficient cleavage activity

How do we tackle the limitations of DZs?
1. Chemical modification and computational prediction
2. Multivalent based RNA-cleaving DNAzymes

Fokina et al., 2015

Monovalent versus multivalent binding





- Monovalent binds to a single ligand while multivalent binds to several ligands
- The number of ligands and the core structure (linear, circular, or radial) strongly influence the degeneracy coefficient, which is a measure of the energy states of the possible binding interactions

Böhmer et al., 2021

Aim and Objectives of Research

Hypothesis

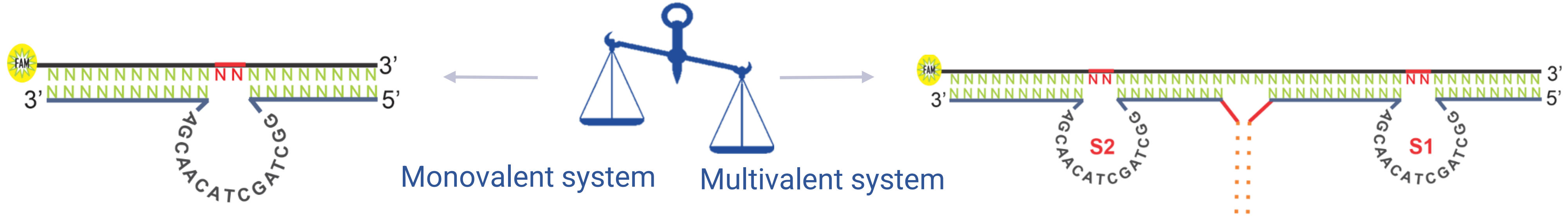
The weak affinity of RNA-cleaving DNAzymes near physiological conditions could be enhanced through multivalent DNA models

Aim

To design bivalent RNA-cleaving DNA-enzymes and compare their catalytic activities with monovalent 10-23 DNAzymes

Objectives

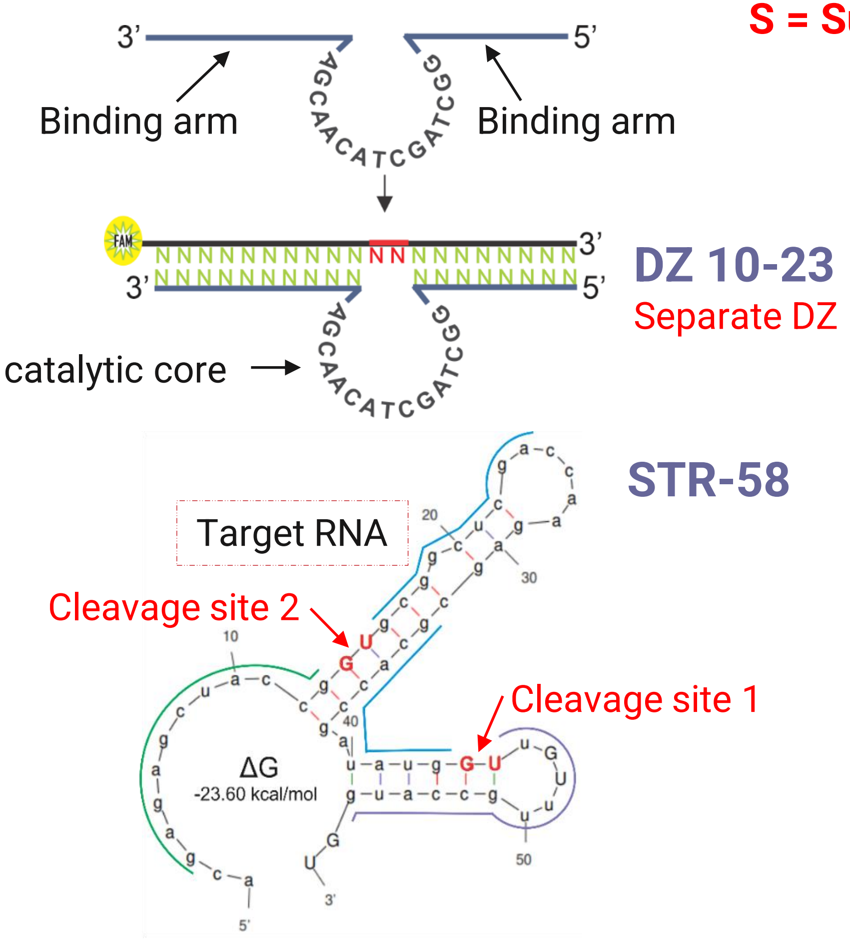
1. To demonstrate that multivalent systems cleave targeted RNA more efficiently than monovalent systems
2. To determine if two RNA-cleaving DZs covalently linked in a single nanostructure provide advantages in RNA cleaving activity in comparison to two separate DZs
3. To investigate the kinetics of the optimized RNA-cleaving DNAzymes



Design Concept

Monovalent DZ

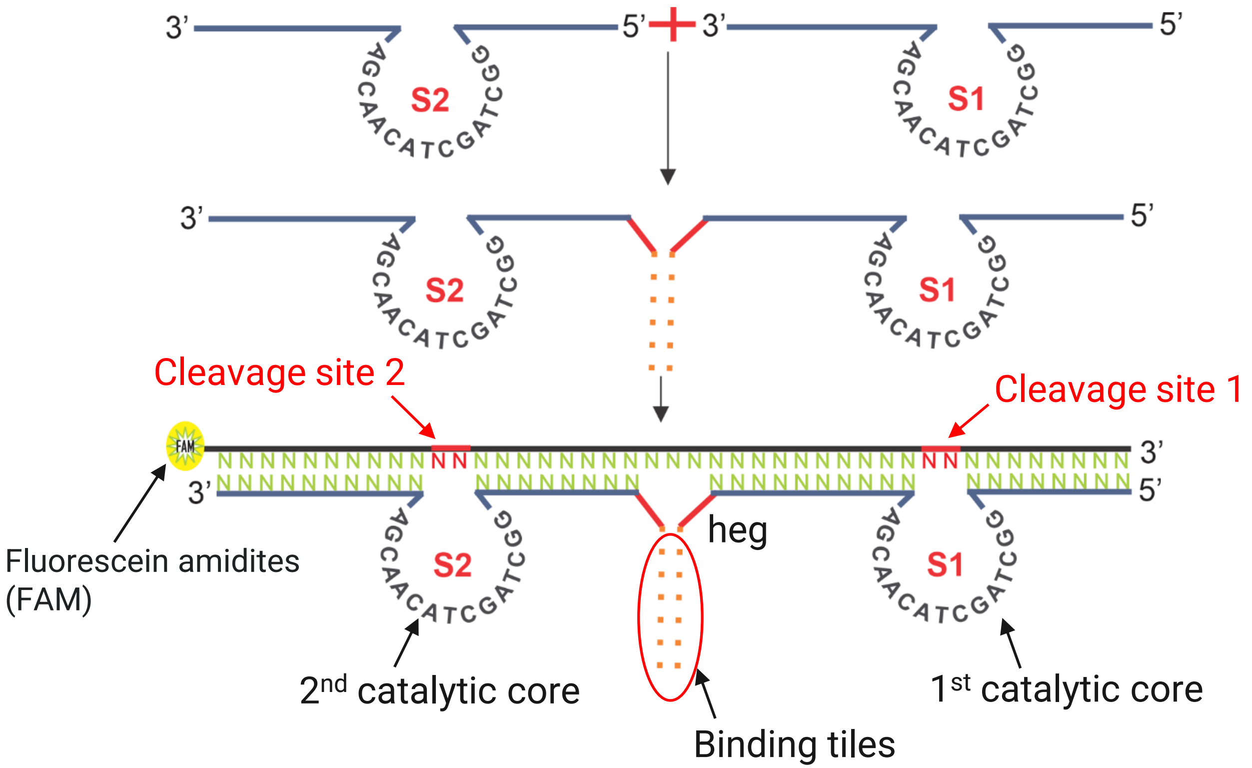
DZ = DNAzymes
S = Subunit



STR-58 is a fragment from streptomycin resistance cassette strA

Bivalent DZ

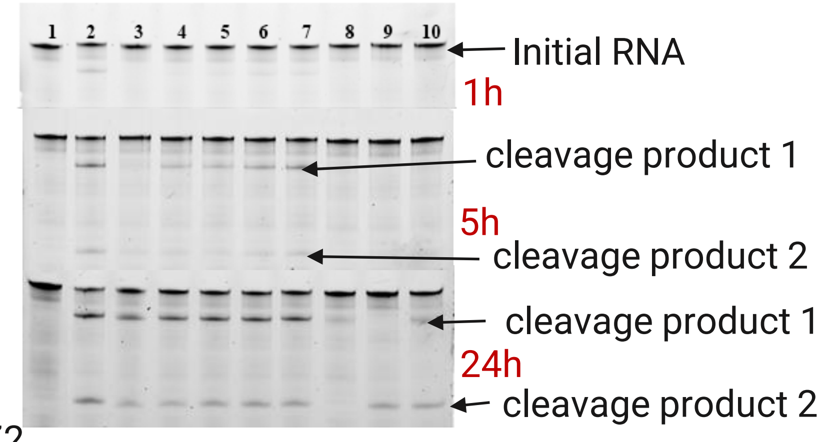
Conjugation of two oligonucleotides by HEG linker



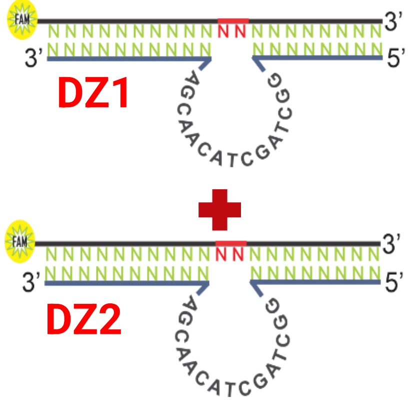
HEG (hexaethylene glycol modification) can be conjugated at the 5’ or 3’ end of oligonucleotides

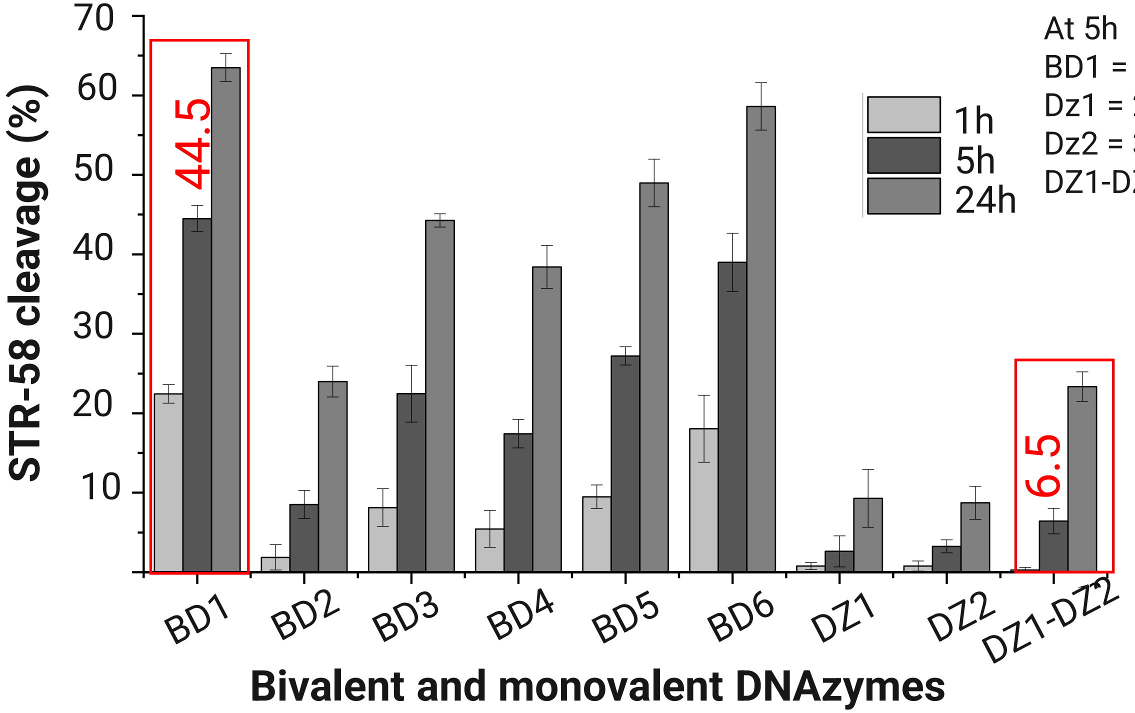
Bivalent and monovalent under multiple turnover conditions

1- Control
2- BD1
3- BD2
4- BD3
5- BD4
6- BD5
7- BD6
8- DZ1
9- DZ2
10- DZ1-DZ2



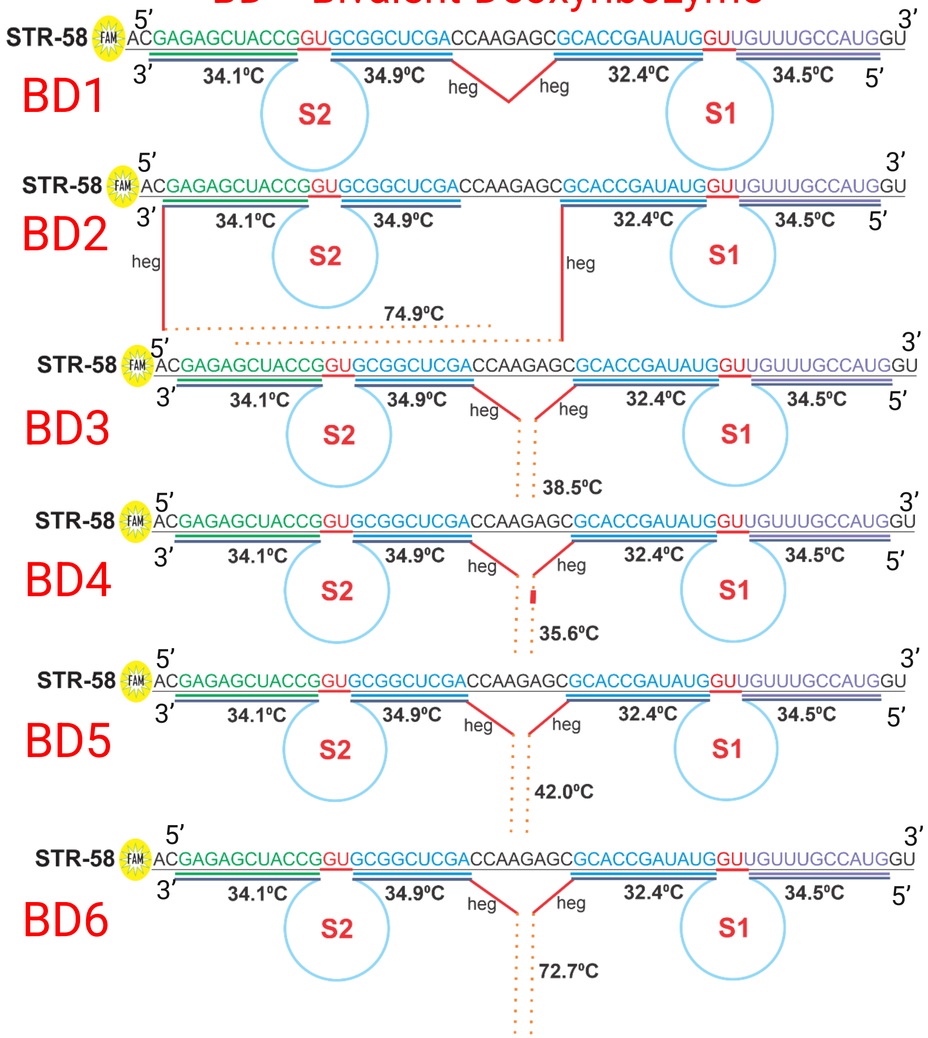
Monovalent DZ





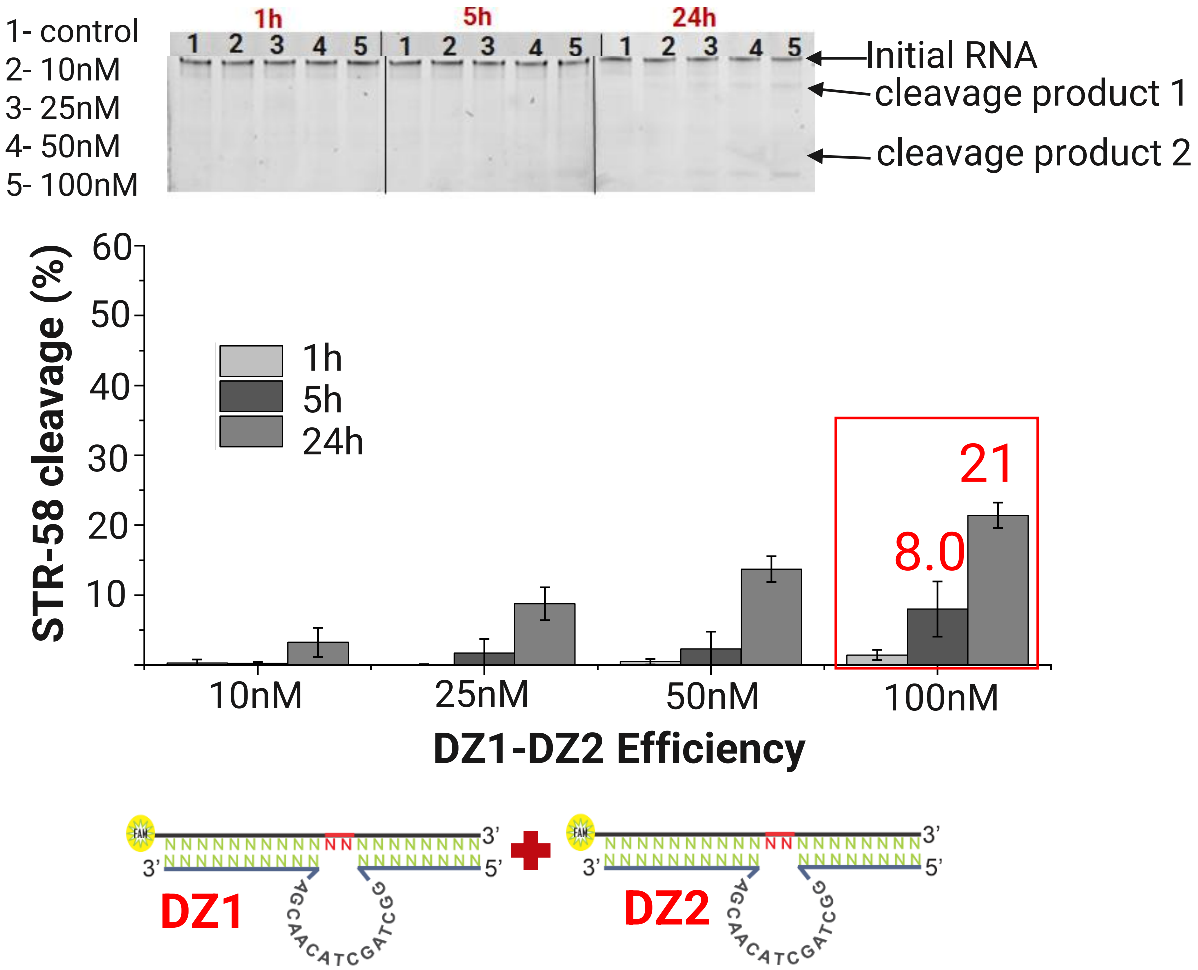
At 5h of incubation
BD1 = 44.5% (0.89 h^{-1})
Dz1 = 2.6% (0.05 h^{-1})
Dz2 = 3.3% (0.07 h^{-1})
DZ1-DZ2 = 6.5% (0.13 h^{-1})

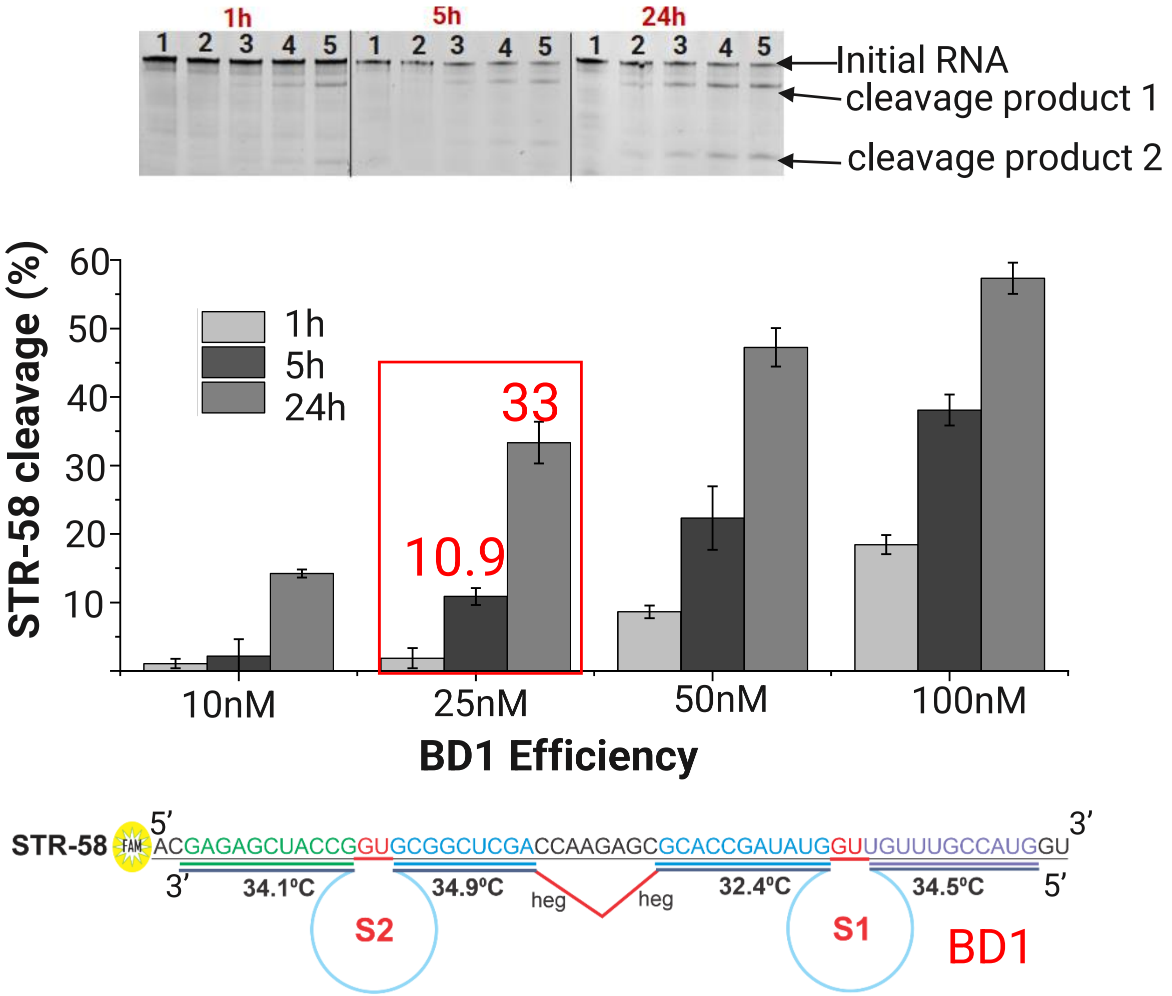
BD = Bivalent Deoxyribozyme



DZs conjugated with linker increased catalytic activity

Catalytic efficiency of DZ1-DZ2 and BD1

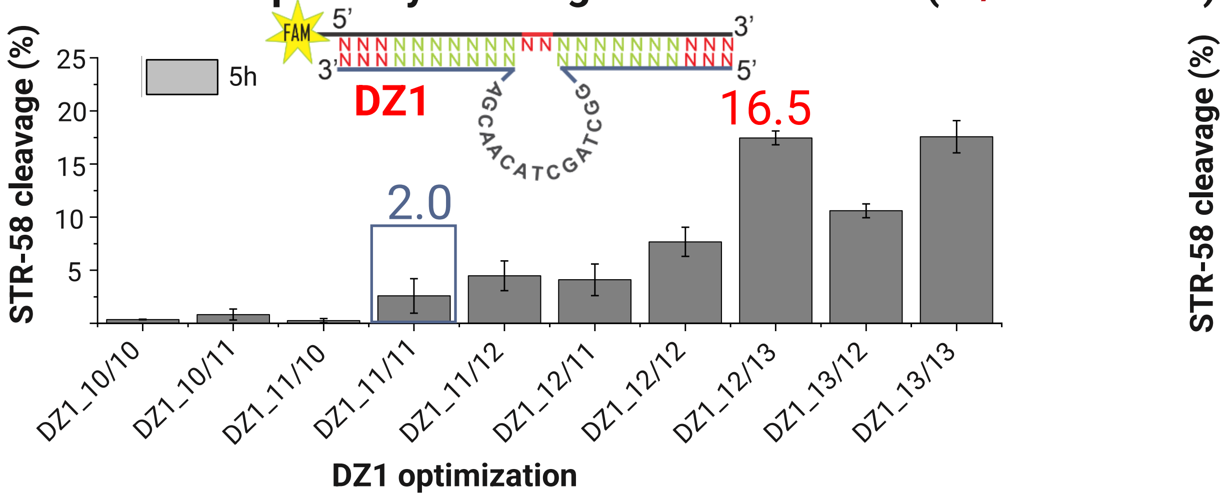




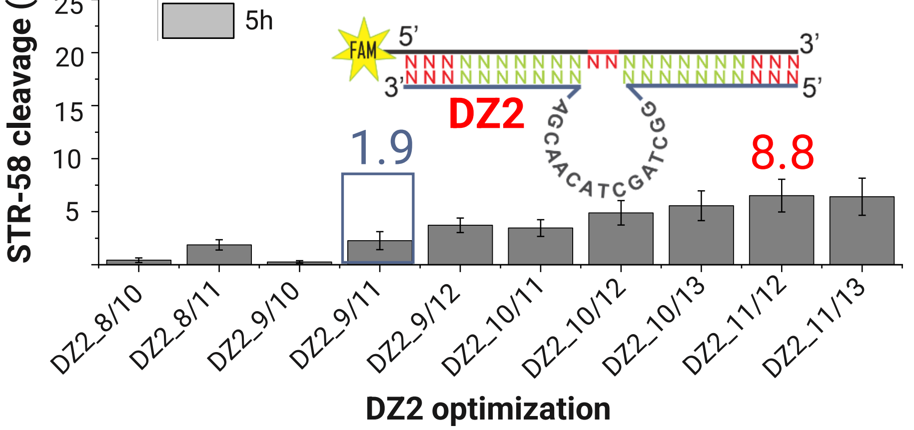
BD1 demonstrated higher catalytic efficiency at 25 nM as compared to DZ1-DZ2 at 100 nM after 5 h of incubation

Optimization of monovalent DZs

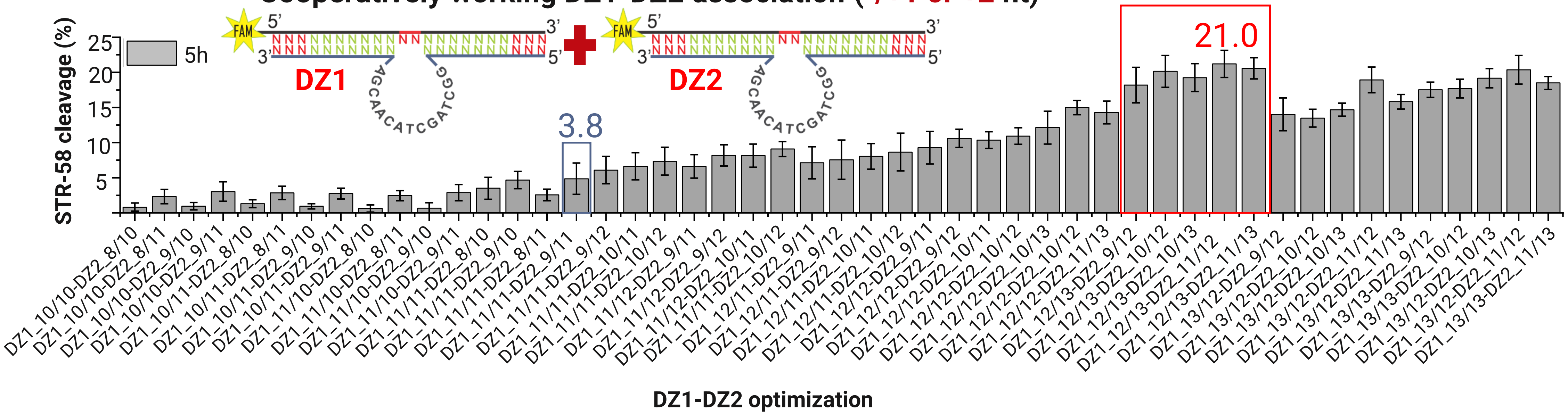
Separately working monovalent DZs (-1/+1 or +2 nt)



Increasing the length of the binding arms, increased cleavage activity



Cooperatively working DZ1-DZ2 association (-/+1 or +2 nt)

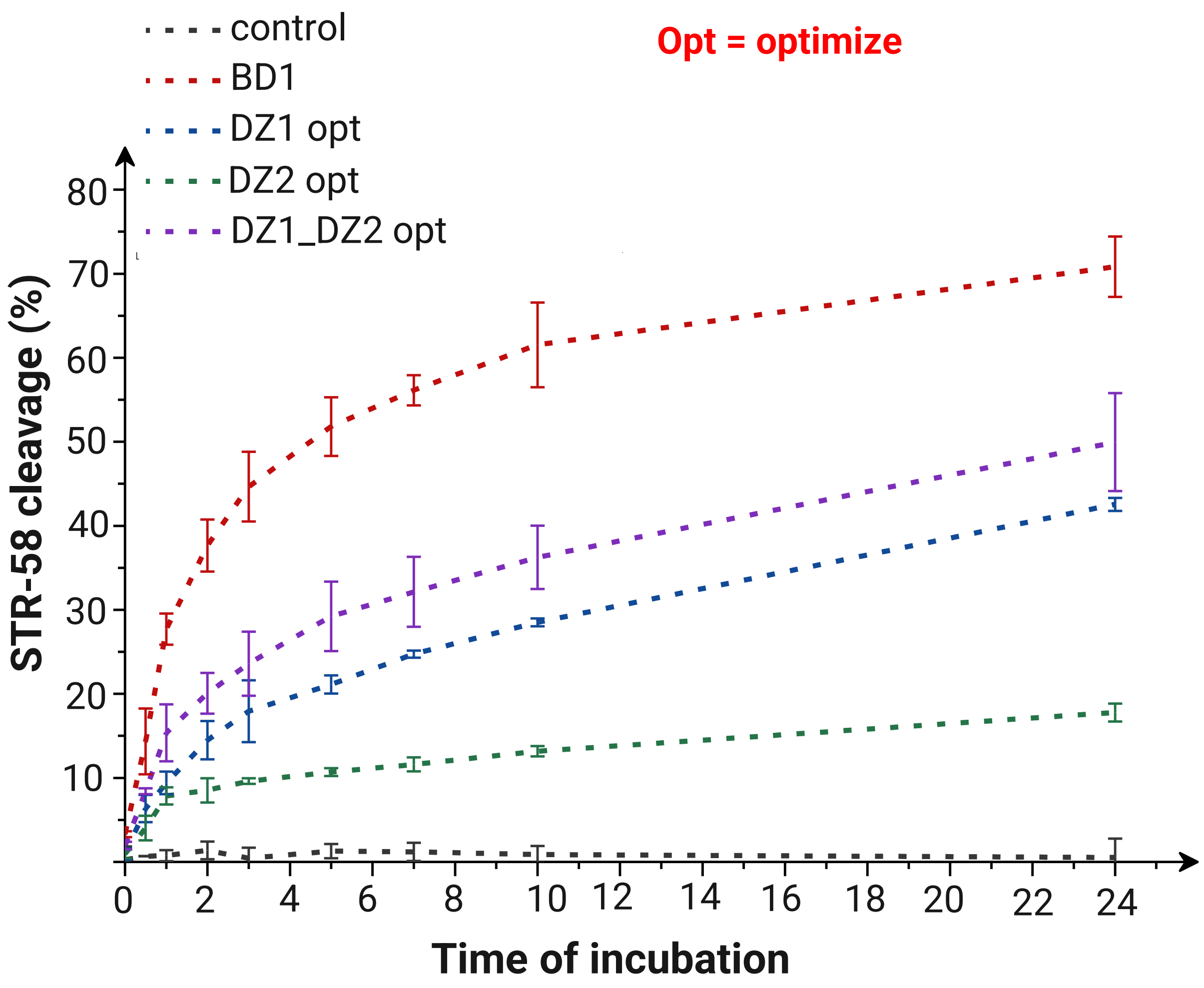


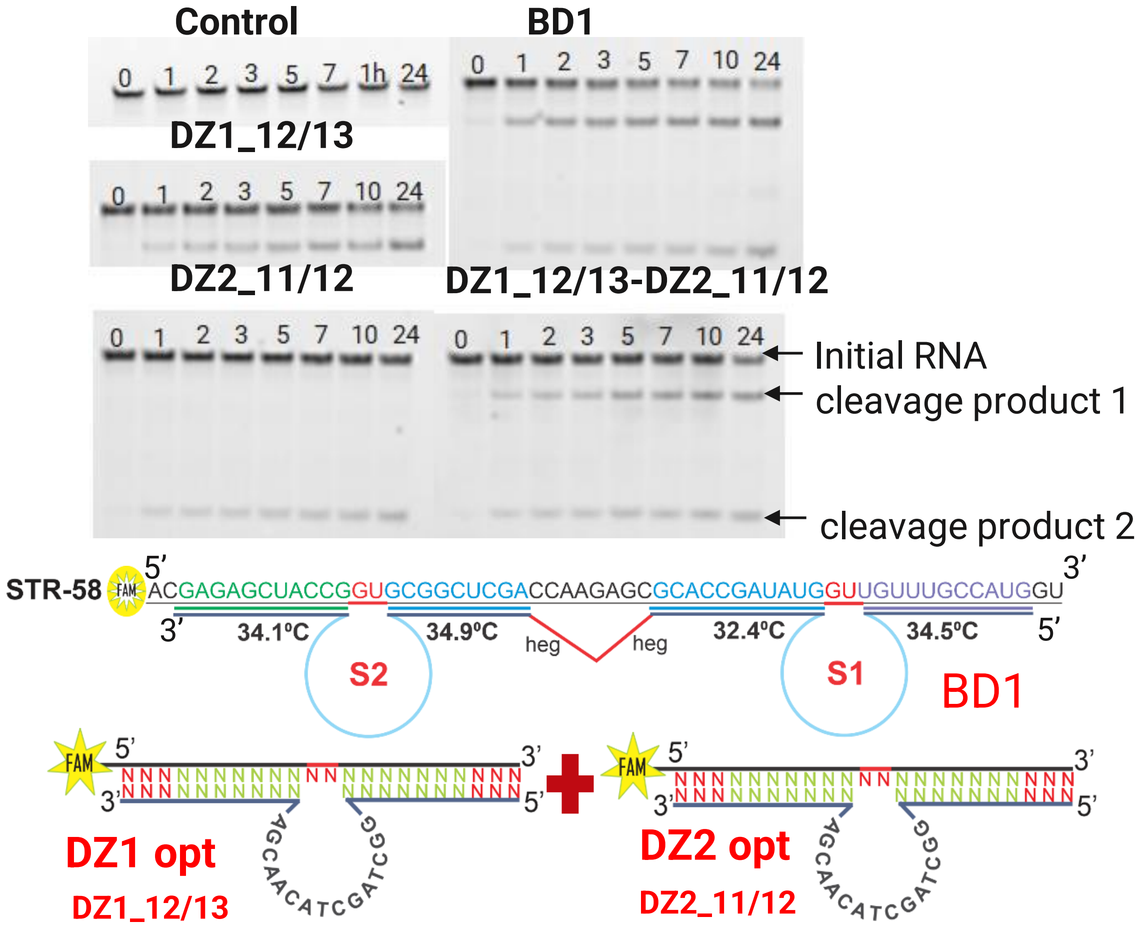
Kinetics of optimized DZs and BD1

At 5 h of incubation

BD1 = 51.8% DZ2 opt = 10.7%
DZ1 opt = 21.1% DZ1-DZ2 opt = 29.2%

Opt = optimize





Covalently linked BD1in a single nanostructure increased hybridization of the cleavage agent to the substrate

Conclusion

Multivalent system

BDs have demonstrated higher cleavage activity in cleaving STR-58 than monovalent constructions

RNA cleavage efficiency

Two DZs covalently linked in a single nanostructure increased DZ hybridization and affinity to substrates, resulting in higher catalytic activity at low concentrations than monovalent DZs at high concentrations

Optimization

Although increasing the length of the binding arms increased the efficiency of monovalent constructions, covalently linked DZs in single nanostructures are the most efficient constructions for RNA cleavage activity

Our multivalent DZ models demonstrated efficient catalytic cleavage activity, indicating a promising path for future DZ research

Future Plans

Looking for PhD in

Molecular Biology,
Biotechnology,
and related fields

Research Interest

1. Genetic engineering
2. Genomics
3. Molecular mechanisms
4. Bioinformatics

Contact Details

kmichaelbatsa@gmail.com

+79117580878

SCAMT ITMO UNIVERSITY

Thank you for your attention

kmichaelbatsa@gmail.com

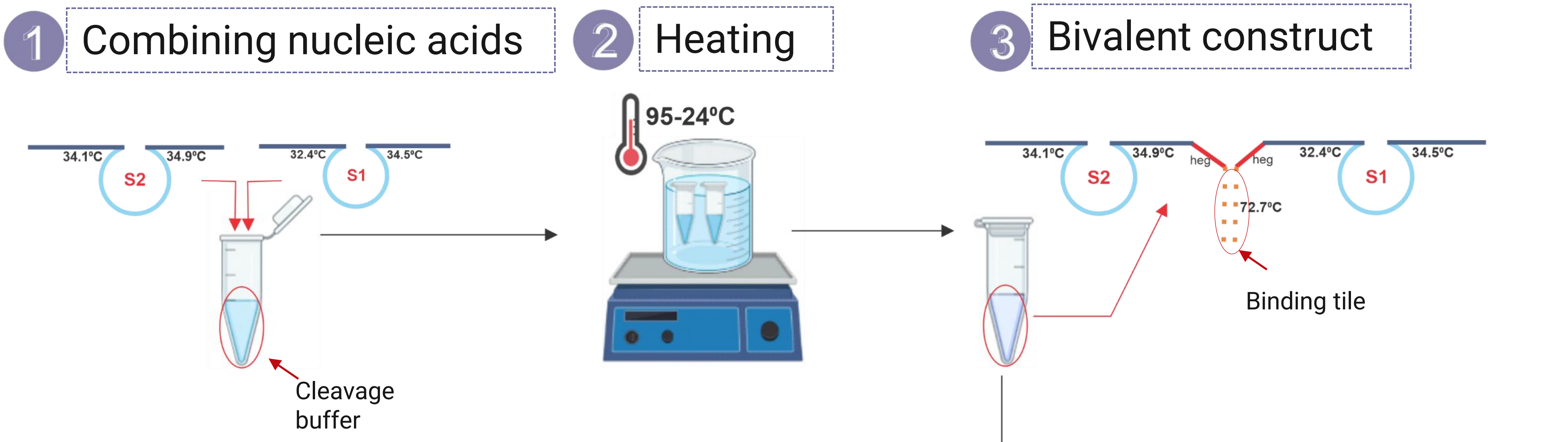
Supervisor
Ph.D. Dmitry Kolpashchikov

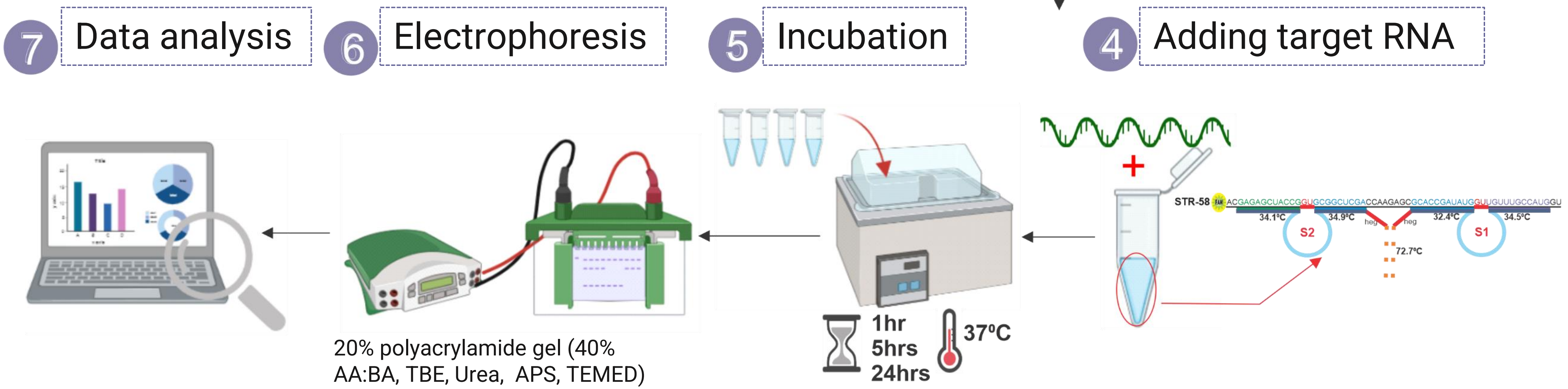
Assisted by
Mikhail Dubovichenko

Prepared by
Michael Batsa

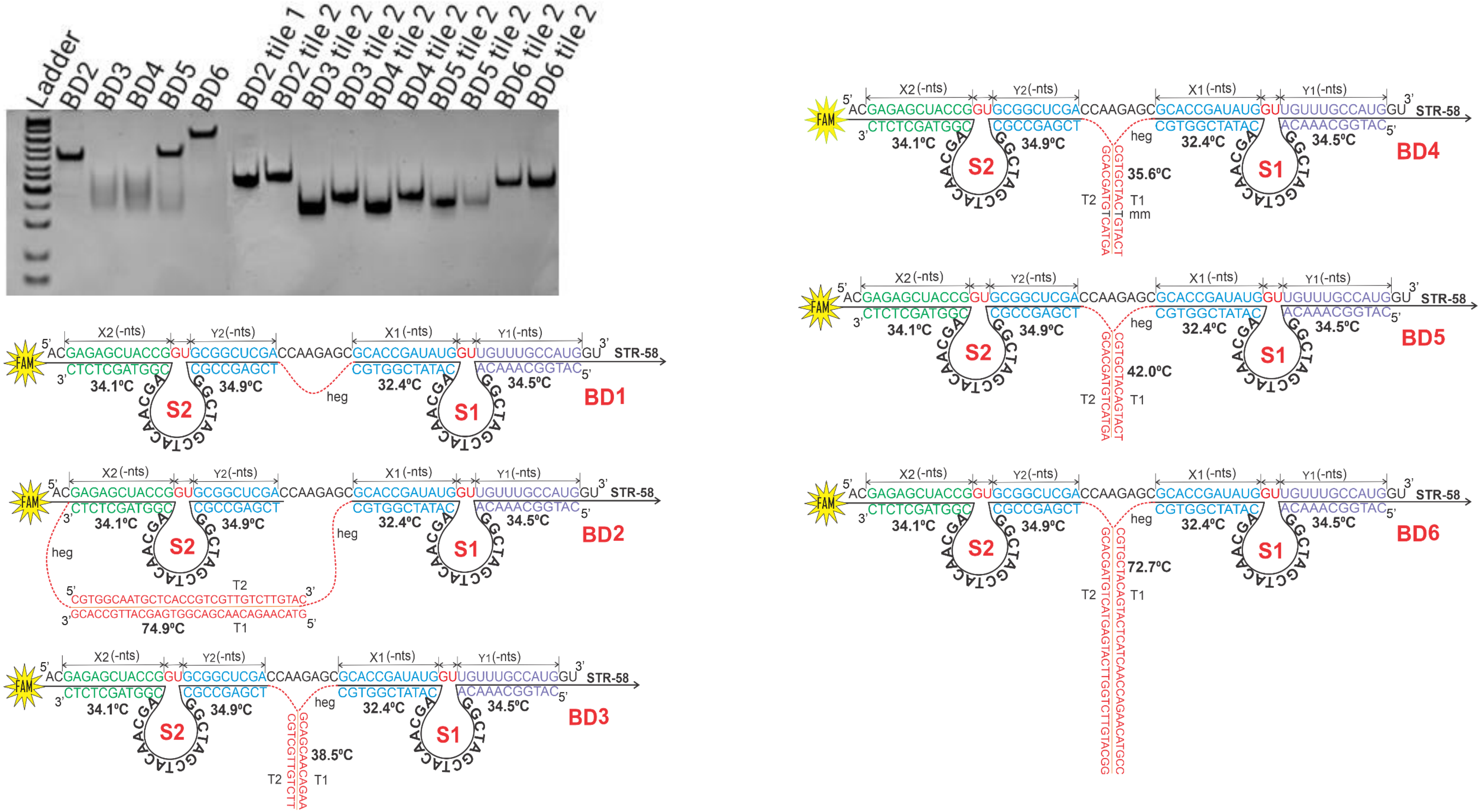
Near physiological conditions

KCl = 150 mM,
NaCl = 15 mM,
MgCl2 = 2 mM,
HEPES = 50 mM,
pH = 7.5 + 37°C)





APPENDIX 2 - Assembled BDs



APPENDIX 3 – BDs Efficiency