Circularization and multimerization of synthetic ribozymes

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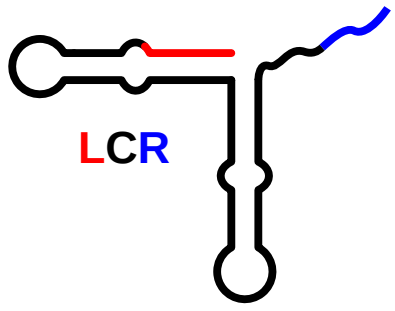
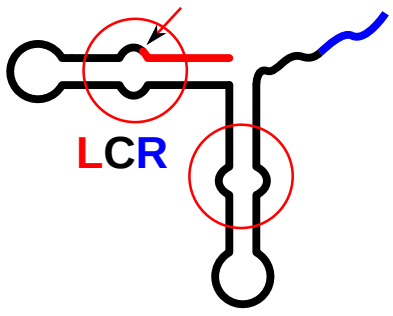
Outline

- **The topic**
  Design of self-polymerizing RNA
- **The strategy**
  Biochemistry, Physics and Computational Biology
- **The loop**
  {Design, Implementation, Evaluation}

The topic: Design of self-polymerizing RNA



The topic: Design of self-polymerizing RNA



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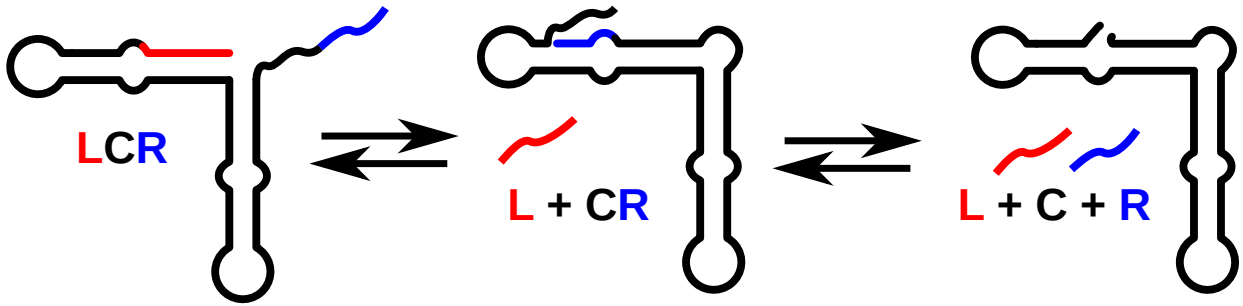
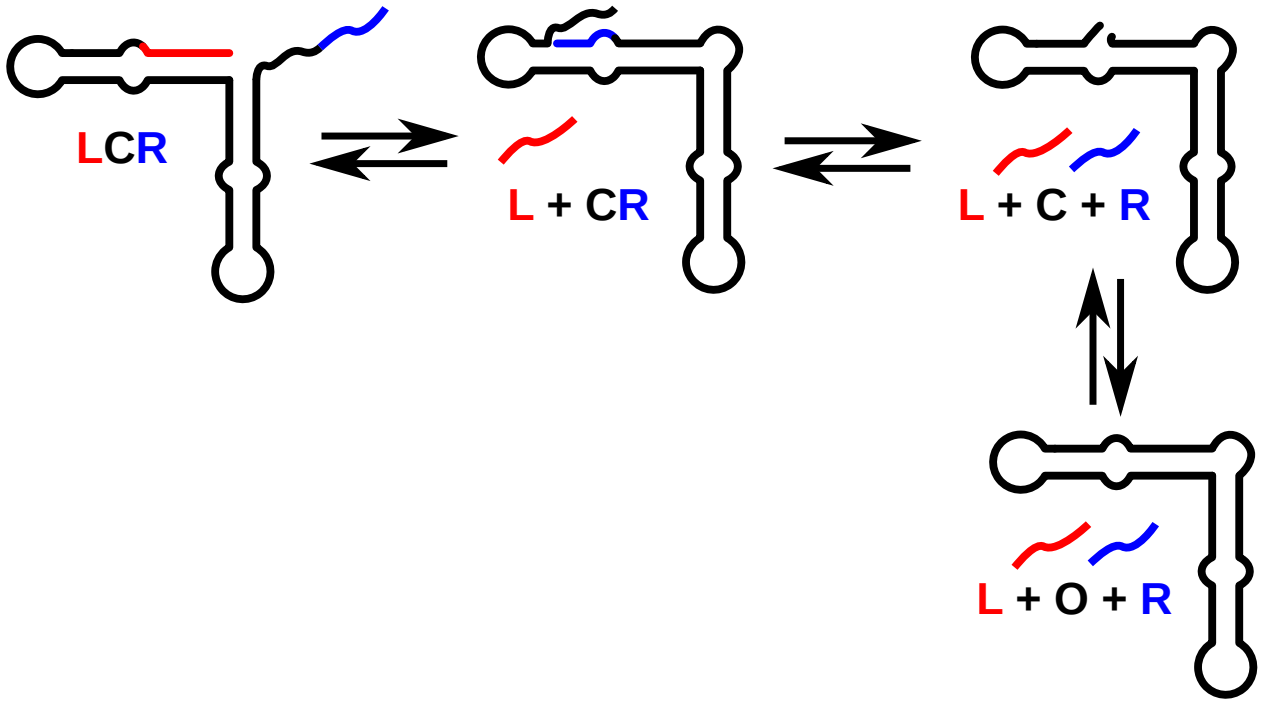
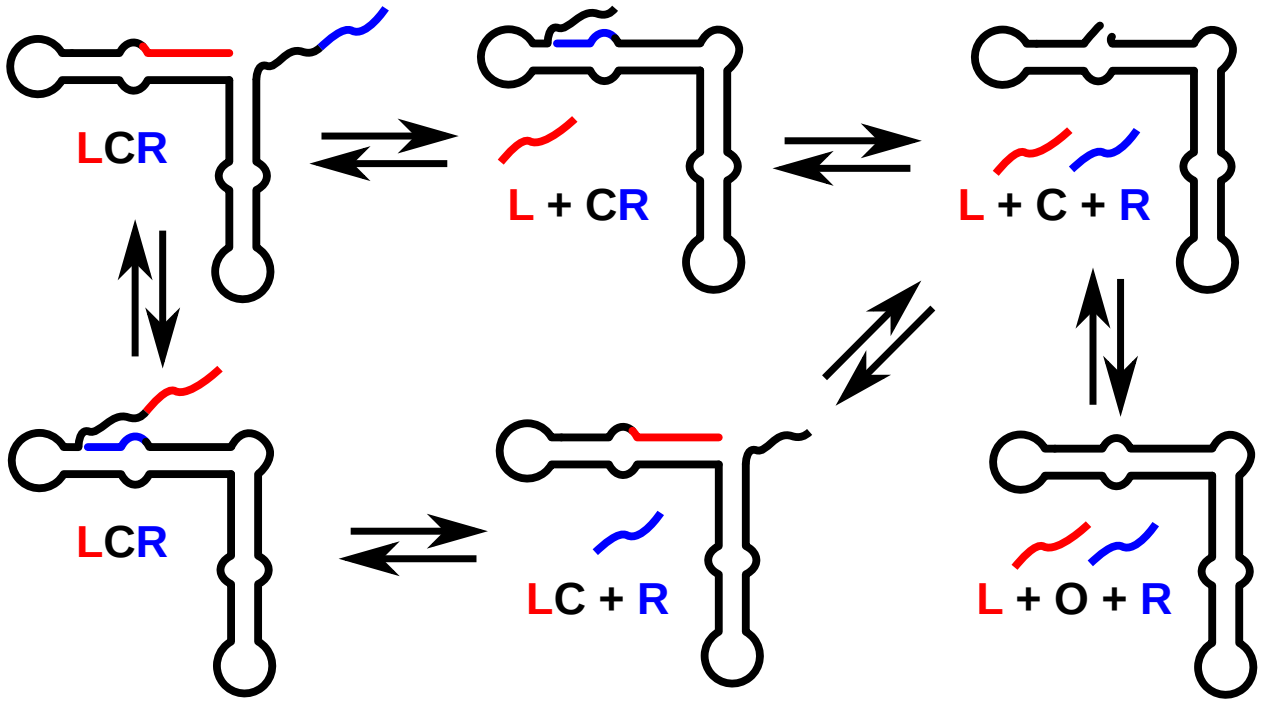


The topic: Design of self-polymerizing RNA



The topic: Design of self-polymerizing RNA



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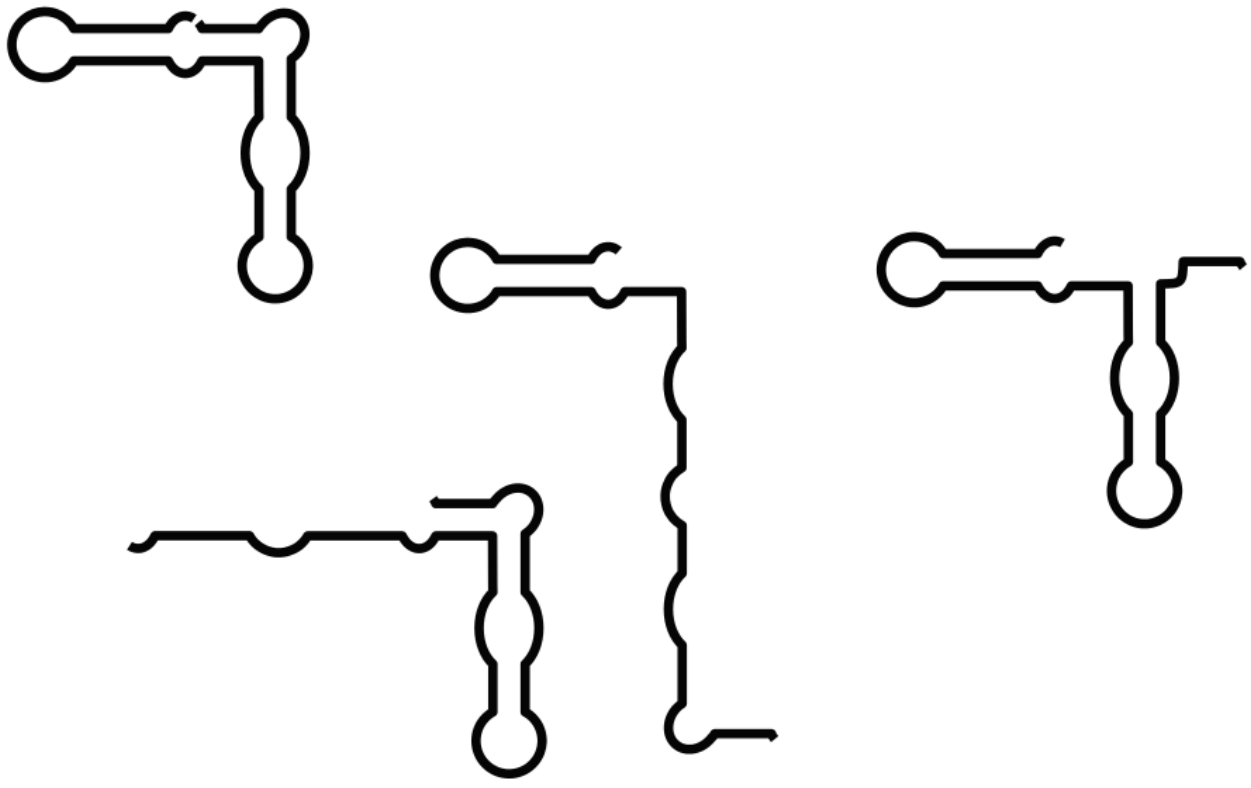
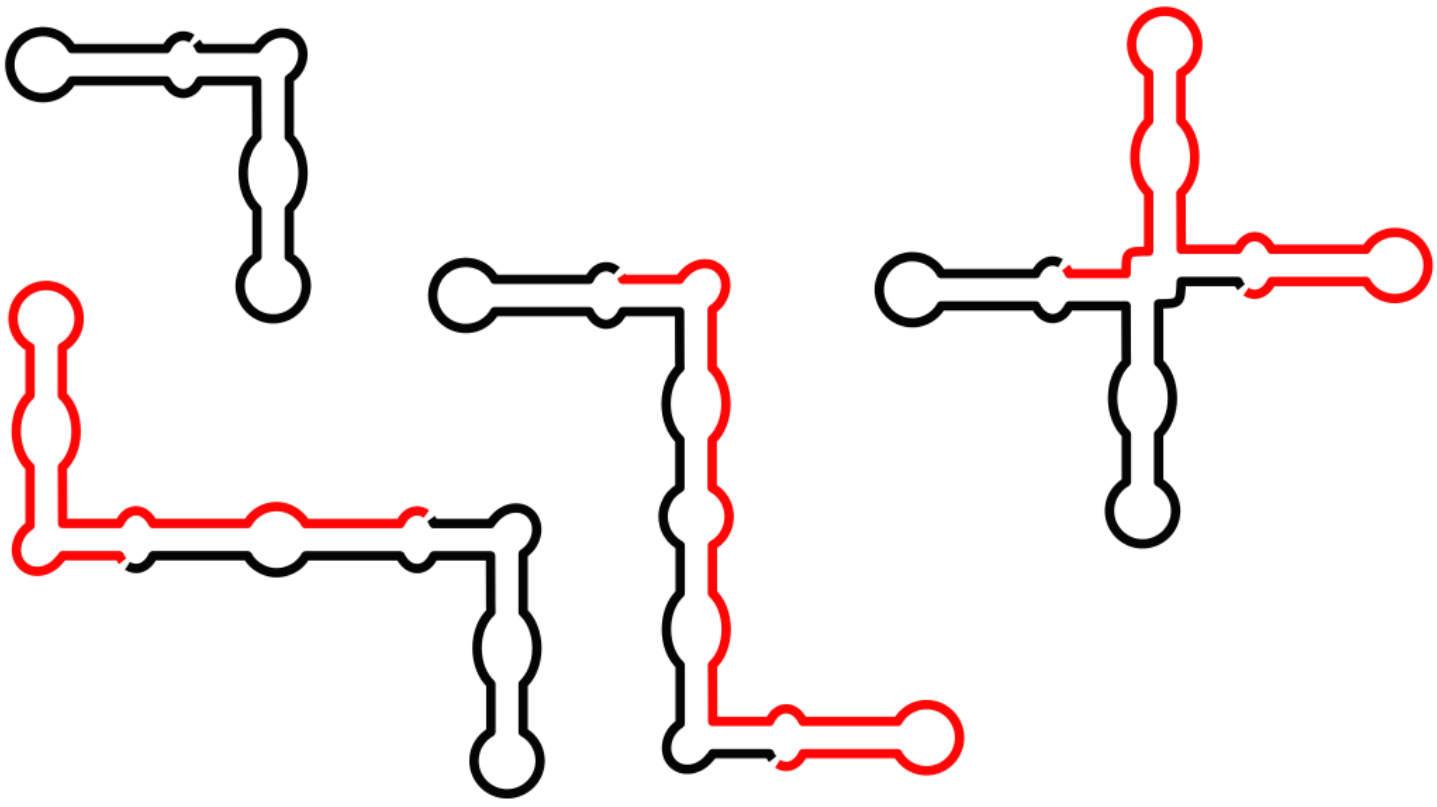
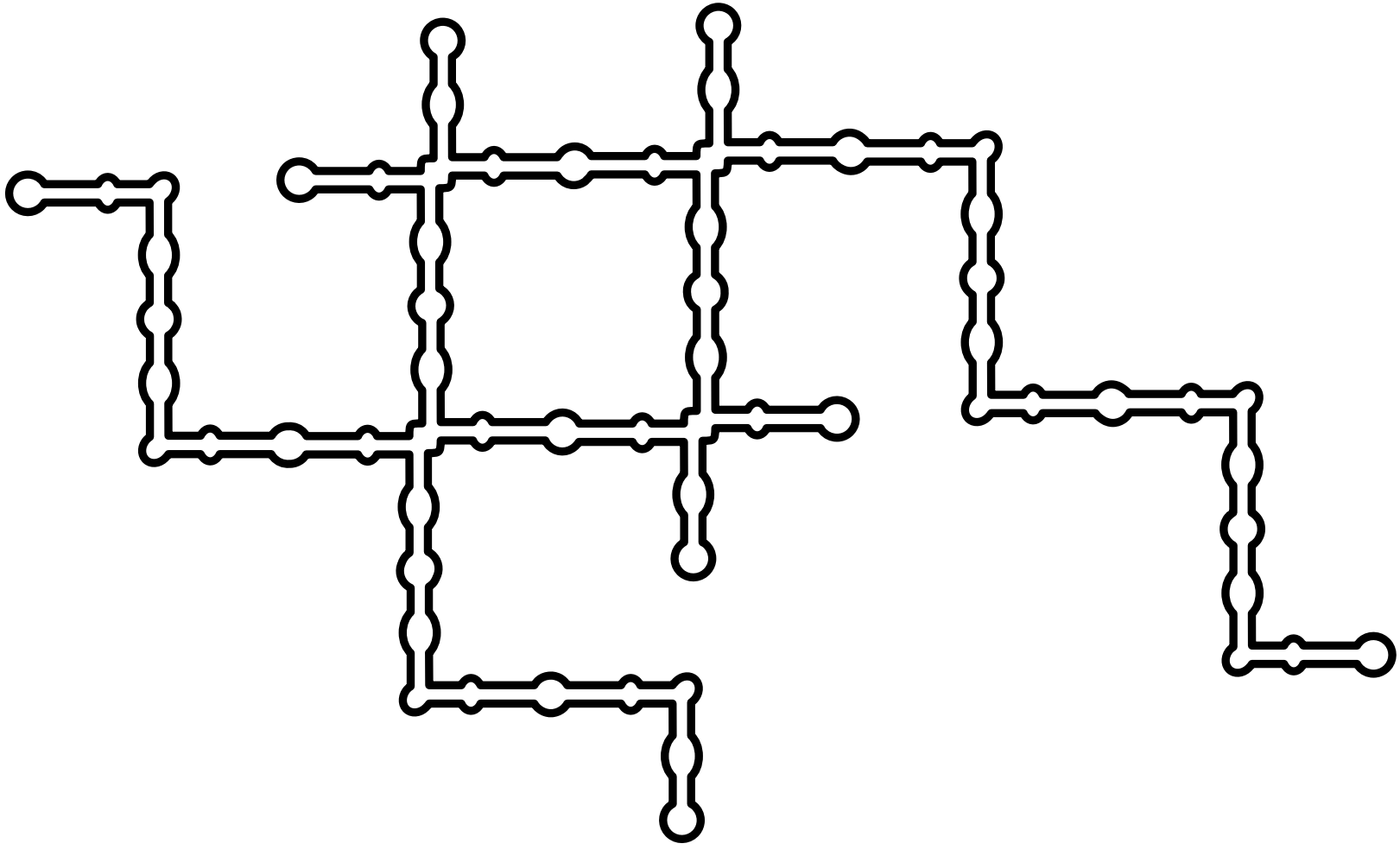


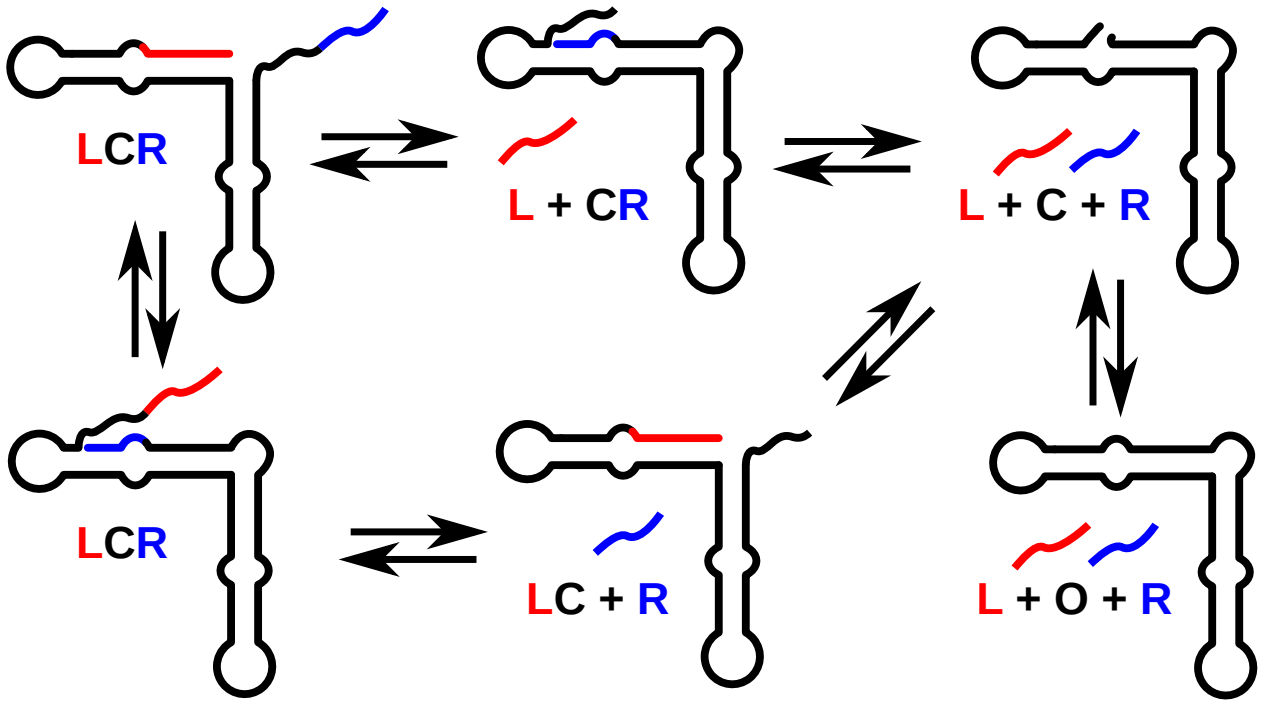
The topic: Design of self-polymerizing RNA



The topic: Design of self-polymerizing RNA

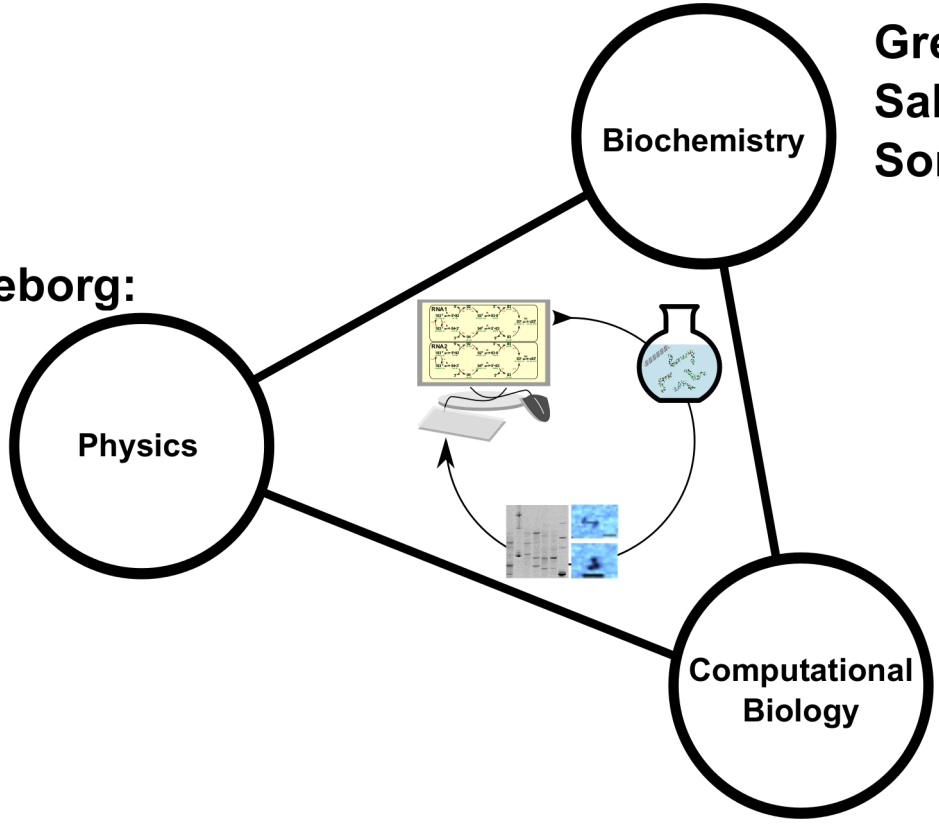


The topic: Design of self-polymerizing RNA



- Does this system exist in nature?
- What is the biological relevance?

The strategy: Biochem., Physics and Comp.Bio.

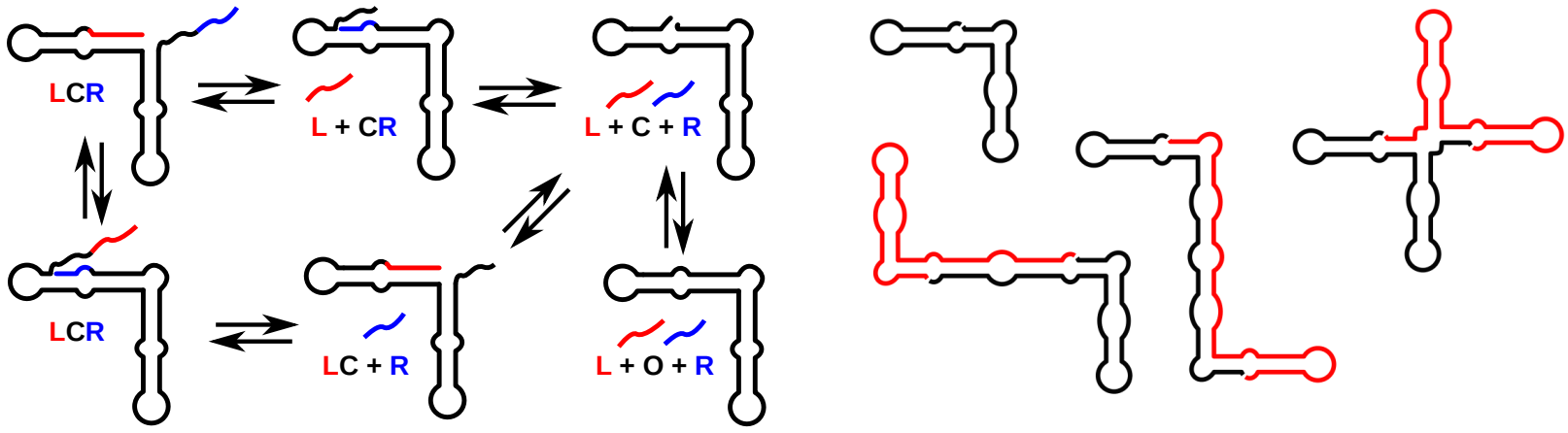


Greifswald:
Sabine Müller
Sonja Petkovic

Greifswald/Göteborg:
Mihaela Delcea
Stephan Block

Wien:
Ivo Hofacker
Christoph Flamm
Stefan Badelt

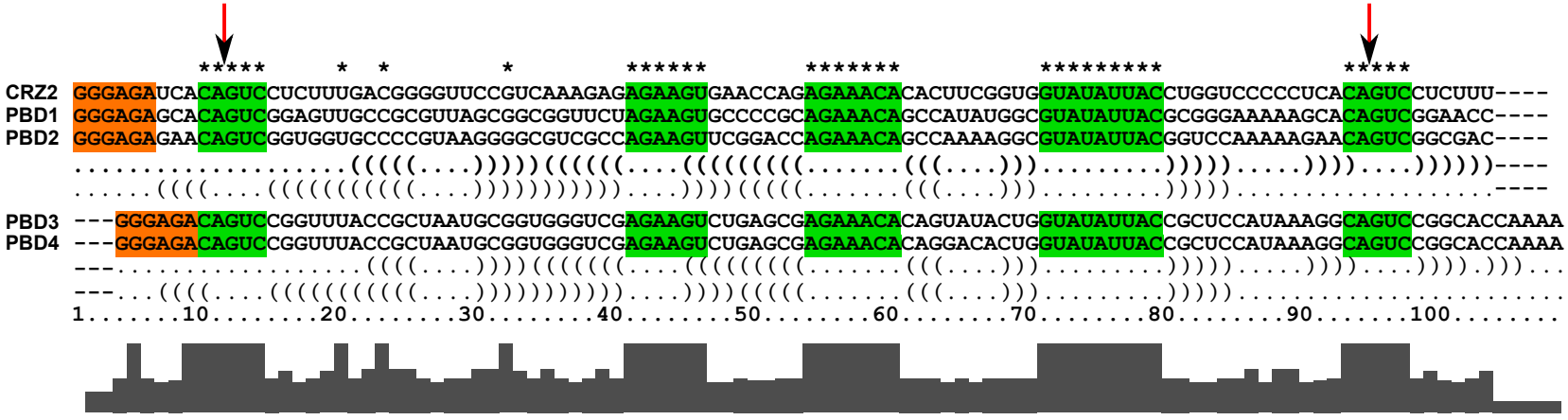
The loop: **Design**, Implementation, Evaluation



⇒ compute a set of candidate molecules (switch.pl)

⇒ maximize probabilities to form reactive conformations

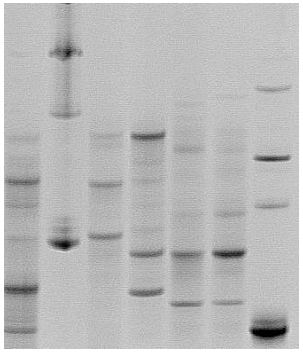
⇒ differ between probabilites to form active monomers and dimers



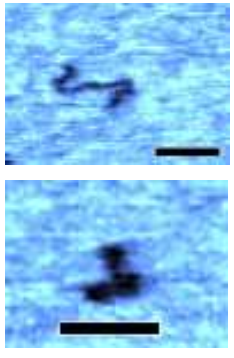
The loop: Design, **Implementation**, Evaluation

- Sample Preparation – Sonja Petkovic
  1. dsDNA synthesis
  2. *in vitro* Transcription (T7 RNA polymerase)
  3. full-length ribozyme extraction
  4. cleavage/ligation/denaturating conditions
- 1D and 2D Gel electrophoresis – Sonja Petkovic
- Atomic Force Microscopy – Stephan Block

Gel Electrophoresis



AFM



The loop: Design, Implementation, **Evaluation**

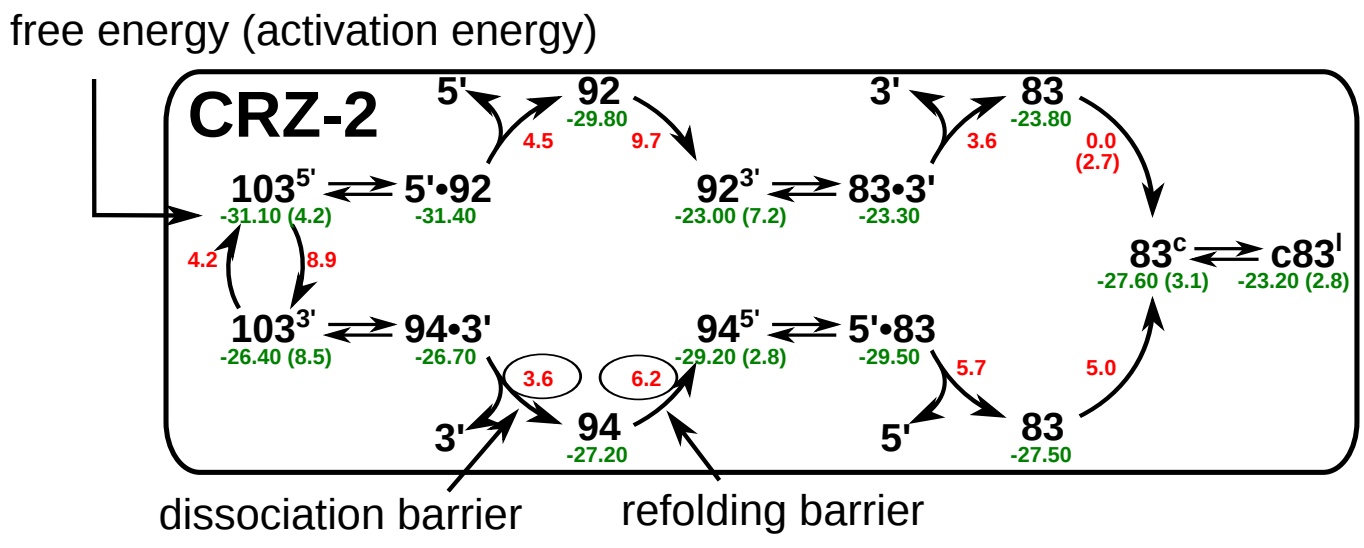
- Matching Results to Design Objective
- Identify deficiencies of Design Functions
- Include results into the second round of sequence design

The loop: Design, Implementation, **Evaluation**

Gel Electrophoresis (Lane Intensity, Nr. of lanes):

RNA	103nt	91-97nt	83nt	circ83nt	> 83nt
CRZ2	-	+++	++	-	6×
PBD1	-	-	-	+++	2×
PBD2	+	+	-	++	4×
PBD3	+	+	-	+	2×
PBD4	++	+	-	+	2×

Computational Analysis:

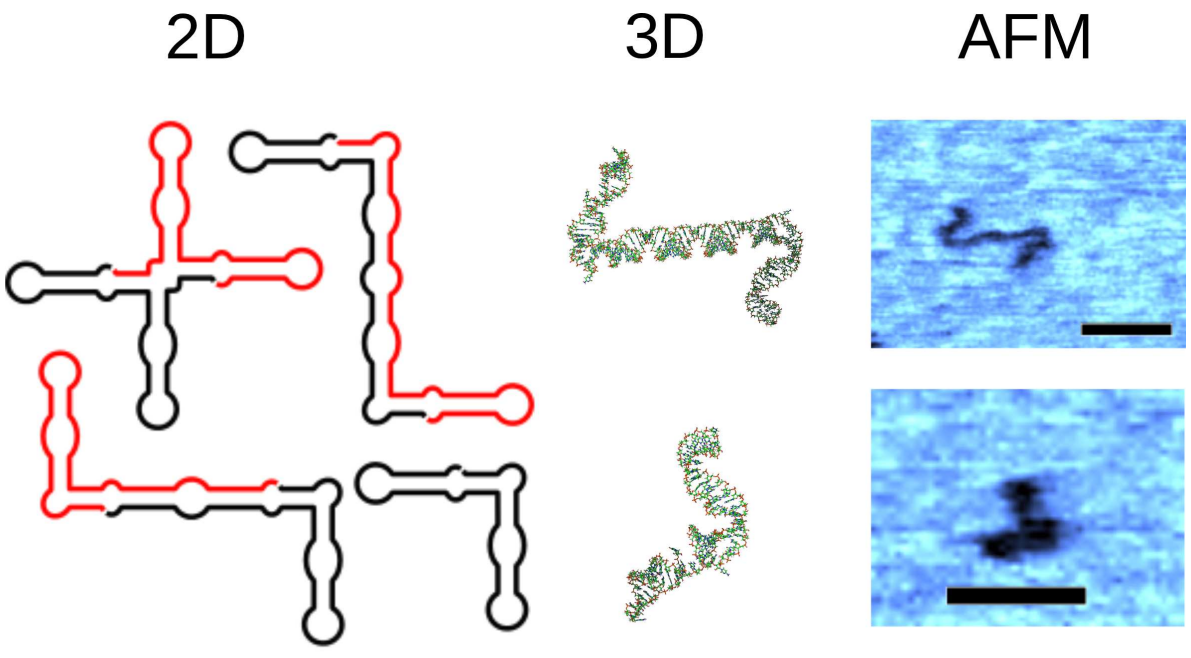


The loop: Design, Implementation, **Evaluation**

Preliminary Results – Atomic Force Microscopy:

RNA	Ratio (Monomers : Dimers : Trimers)
CRZ2	(7 :1:0)
PBD1	(10:3:1)
PBD4	(5 :1:1)

Computational Analysis:



Conclusion

Results:

- Design approach is sufficient to yield circular monomers
- Dissociation barriers do have an impact on efficiency
- Diversity of dimers has to be considered for optimization
- Optimizing for dimer-ligation does not lead to multimerization

Remaining Questions:

- Do molecules tend to cleave first and then multimerize (need of time resolved results)
- Will we be able to fit tertiary structure predictions to AFM images?

thanks to

	Supervisor	PhD committee	Experimenter
Ivo Hofacker	x	x	
Christoph Flamm	x		
Sabine Müller		x	x
Sonja Petkovic			x
Stephan Block			x
Peter Stadler		x	
and the whole TBI group for everything else			

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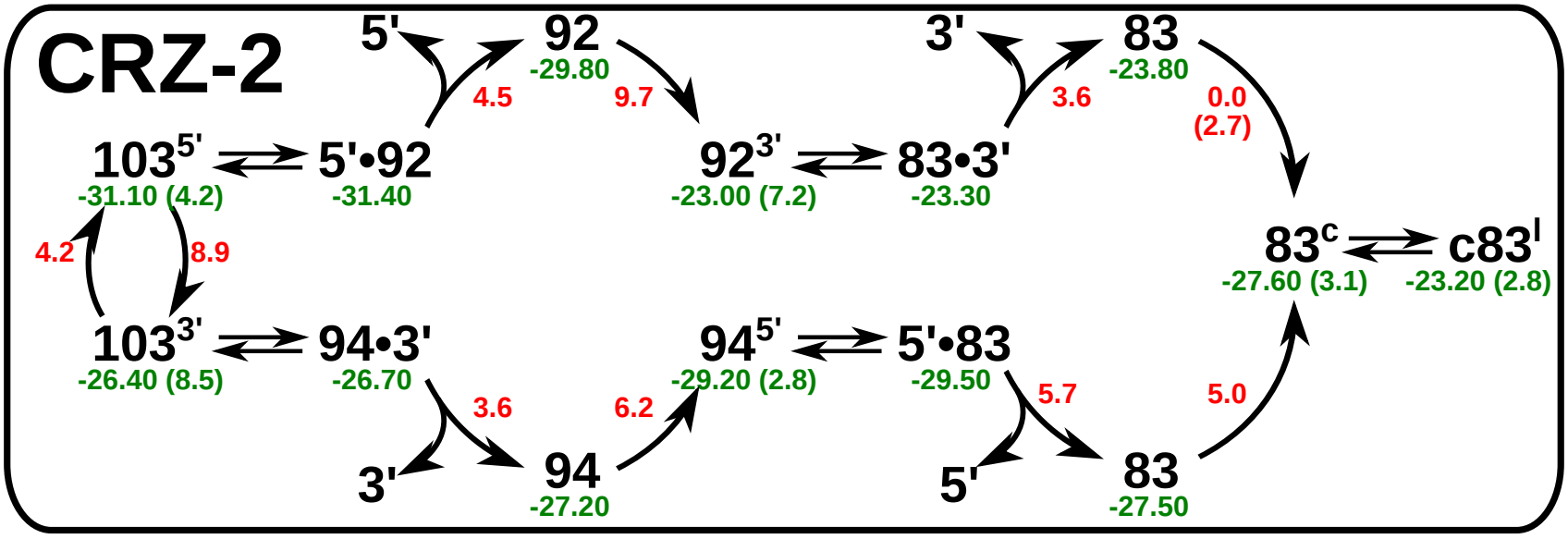
Fitness Functions

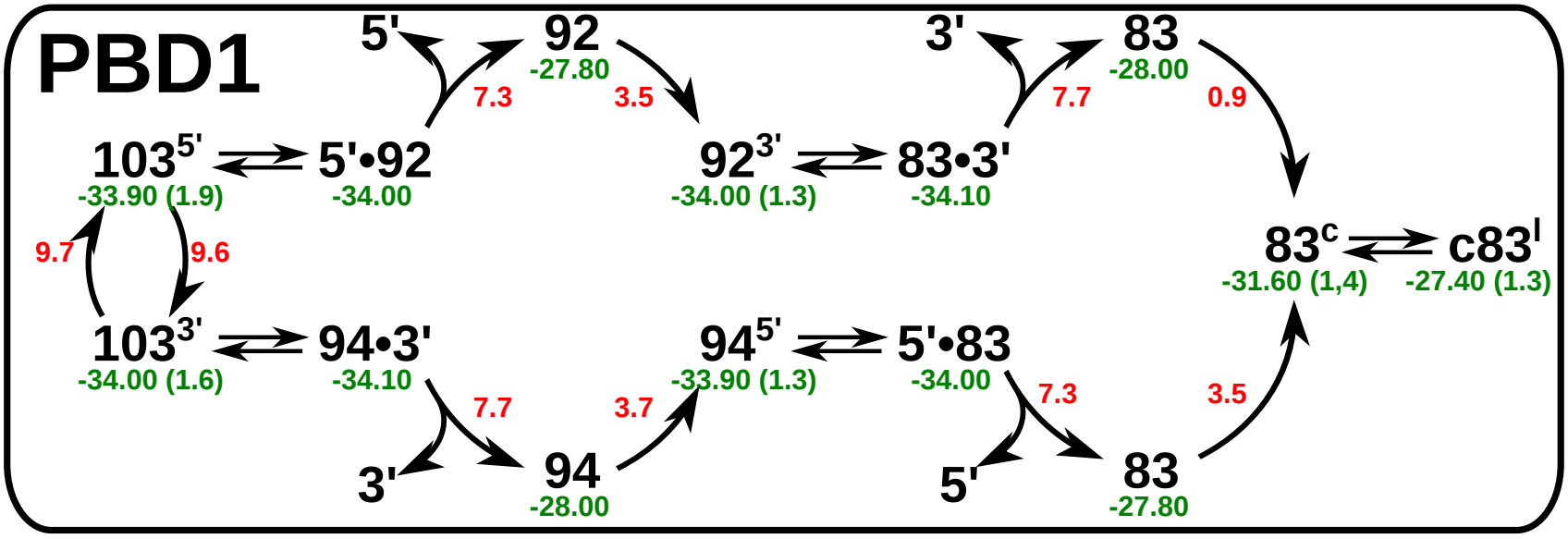
$$\kappa_1 = \left(\min \begin{cases} P(LCR^L), \\ P(CR^R) \end{cases} + \min \begin{cases} P(LCR^R), \\ P(LC^L) \end{cases} \right) \times e^{\frac{E(C^O) - E(O^C)}{kT}} \tag{1}$$

$$\kappa_2 = \frac{[LCR_{\text{dimer}}]_\theta}{[LCR_{\text{monomer}}]_\theta} \times \left(\min \begin{cases} P(LCR^L_{\text{dimer}}), \\ P(CR^R_{\text{dimer}}) \end{cases} + \min \begin{cases} P(LCR^R_{\text{dimer}}), \\ P(LC^L_{\text{dimer}}) \end{cases} \right) \times e^{\frac{E(C^{O_2}_{\text{dimer}}) - E(O_2^{C_{\text{dimer}}})}{kT}} \tag{2}$$

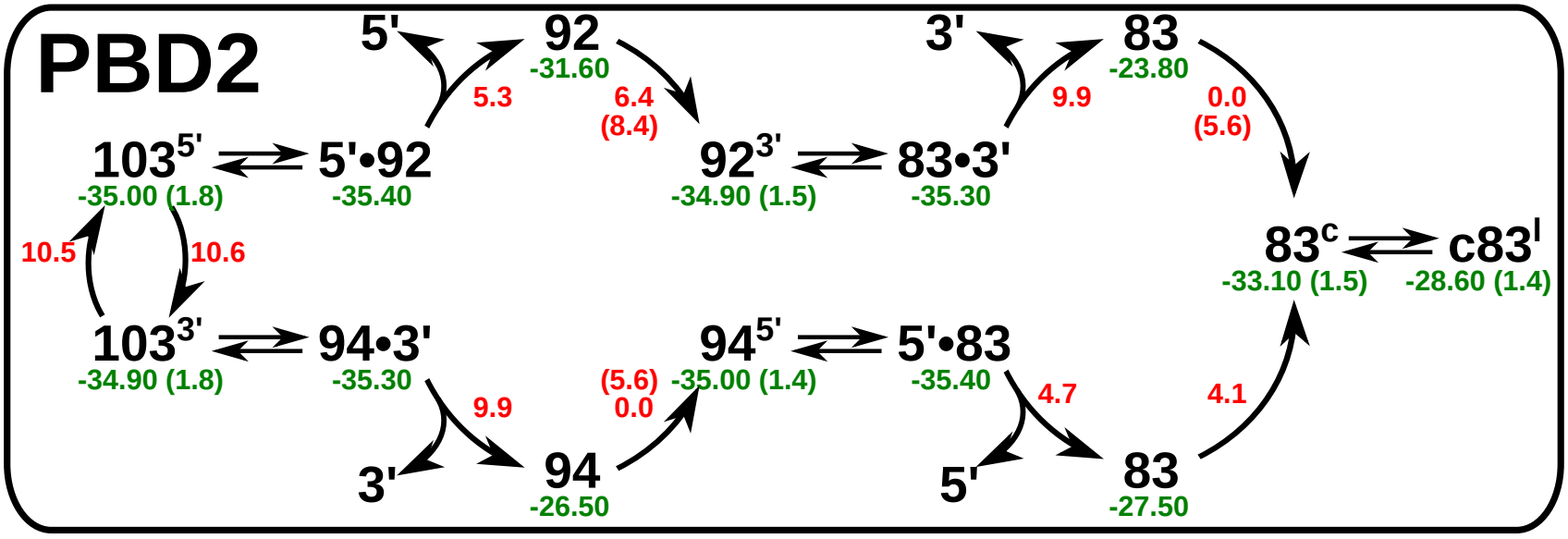
Cascades


CRZ-2
5'
92
-29.80
4.5
9.7
3'
83
-23.80
3.6
0.0
(2.7)
103^5'
-31.10 (4.2)
5'•92
-31.40
92^3'
-23.00 (7.2)
83•3'
-23.30
4.2
8.9
83^c
-27.60 (3.1)
c83^l
-23.20 (2.8)
103^3'
-26.40 (8.5)
94•3'
-26.70
94^5'
-29.20 (2.8)
5'•83
-29.50
3.6
6.2
5.7
5.0
3'
94
-27.20
5'
83
-27.50

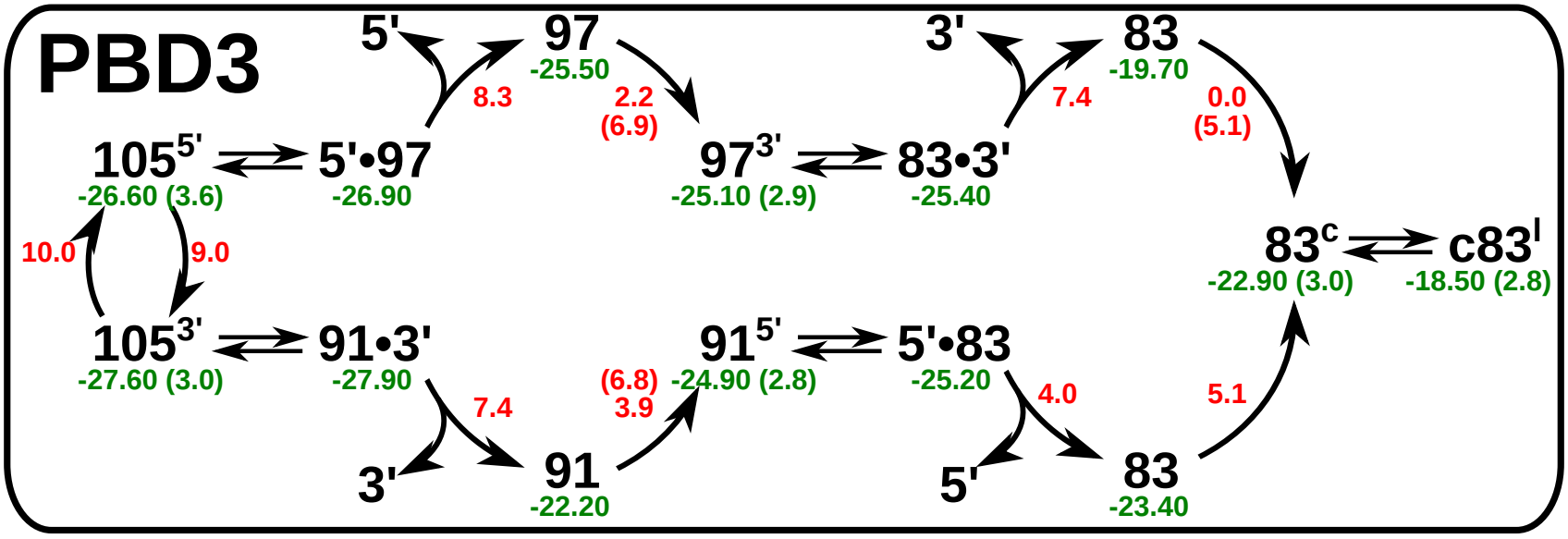
Cascades


PBD1
5'
92
-27.80
7.3
3.5
3'
83
-28.00
7.7
0.9
103^5'
-33.90 (1.9)
5'•92
-34.00
92^3'
-34.00 (1.3)
83•3'
-34.10
9.7
9.6
83^c
-31.60 (1,4)
c83^l
-27.40 (1.3)
103^3'
-34.00 (1.6)
94•3'
-34.10
94^5'
-33.90 (1.3)
5'•83
-34.00
7.7
3.7
7.3
3.5
3'
94
-28.00
5'
83
-27.80

Cascades


PBD2
5'
92
-31.60
5.3
6.4
(8.4)
3'
83
-23.80
9.9
0.0
(5.6)
103^5'
-35.00 (1.8)
5'•92
-35.40
92^3'
-34.90 (1.5)
83•3'
-35.30
10.5
10.6
83^c
-33.10 (1.5)
c83^l
-28.60 (1.4)
103^3'
-34.90 (1.8)
94•3'
-35.30
94^5'
-35.00 (1.4)
5'•83
-35.40
(5.6)
0.0
9.9
4.7
4.1
3'
94
-26.50
5'
83
-27.50

Cascades


PBD3
5'
97
-25.50
8.3
2.2
(6.9)
3'
83
-19.70
7.4
0.0
(5.1)
105^5'
-26.60 (3.6)
5'•97
-26.90
97^3'
-25.10 (2.9)
83•3'
-25.40
10.0
9.0
83^c
-22.90 (3.0)
c83^l
-18.50 (2.8)
105^3'
-27.60 (3.0)
91•3'
-27.90
91^5'
-24.90 (2.8)
5'•83
-25.20
(6.8)
3.9
7.4
4.0
5.1
3'
91
-22.20
5'
83
-23.40