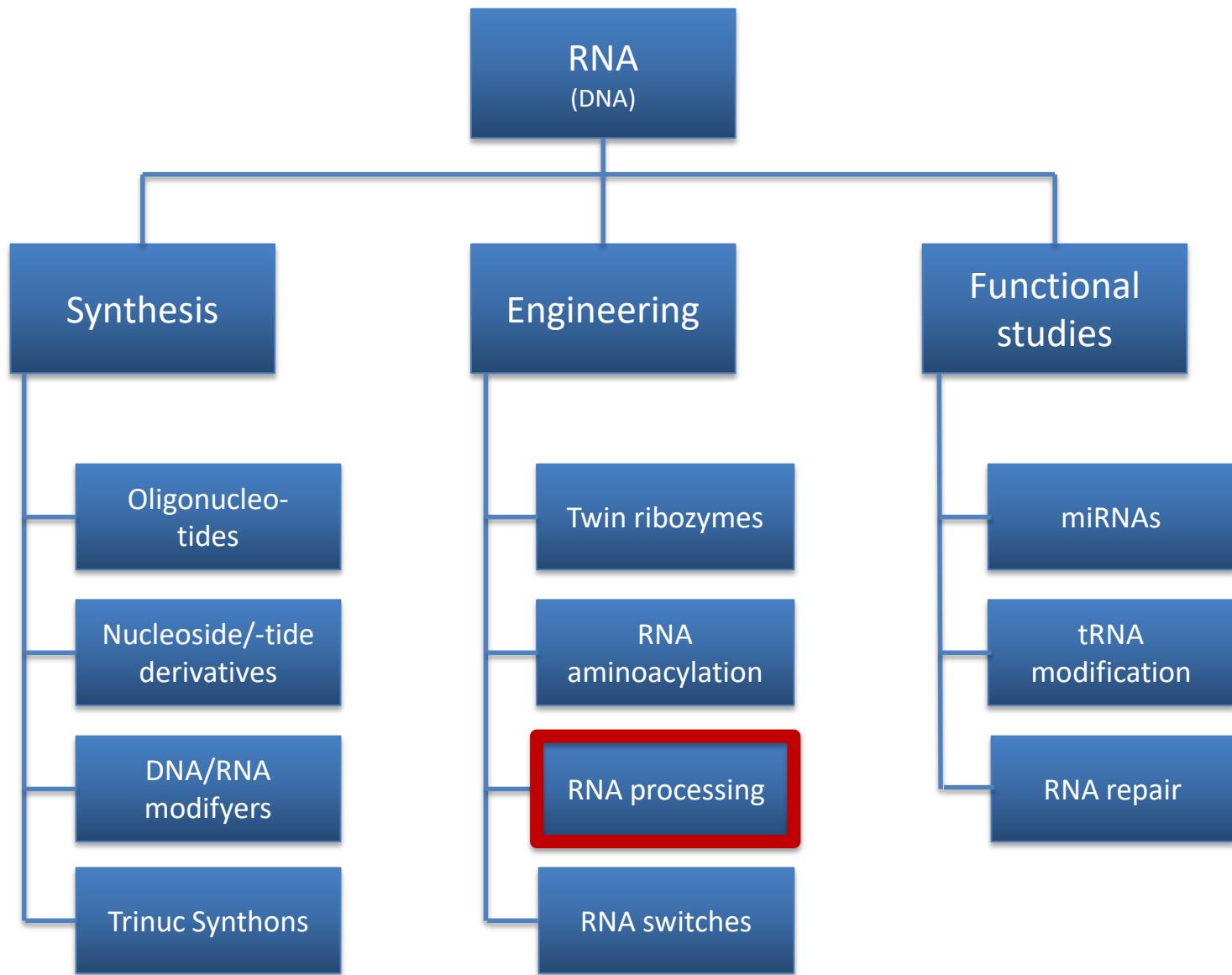


A small ribozyme as key mediator of diverse RNA processing pathways

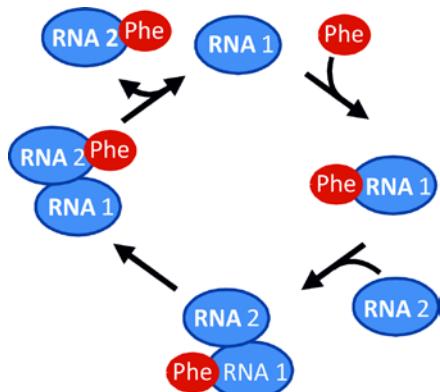
Sabine Müller, Darko Balke, Robert Hieronymus, Jikang Zhu
Institute for Biochemistry, University Greifswald





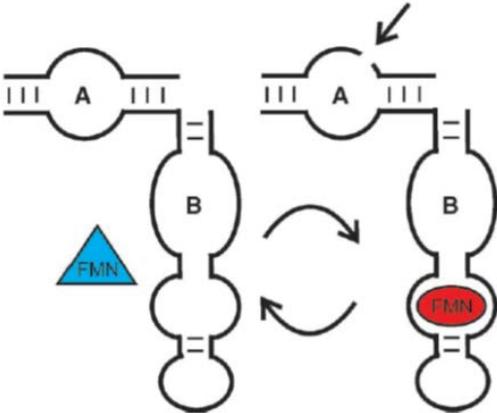
Engineering of RNA activity

Aminoacylation



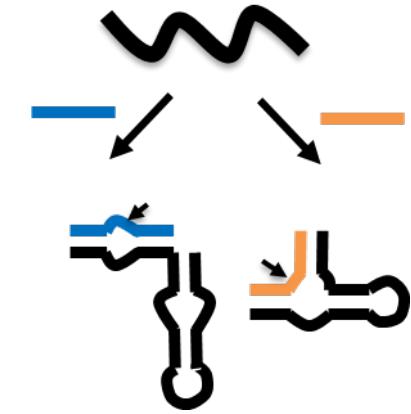
Xu et al., *ChemBioChem*, 2014

Regulation



Strohbach et al., *Angew. Chem. Int. Ed.*, 2006

Substrate promiscuity



Moeller & Balke, 2018

- Recombination
- Circularization (back splicing)
- Oligomerization
- Regular splicing

RNA synthesis at the origin of life



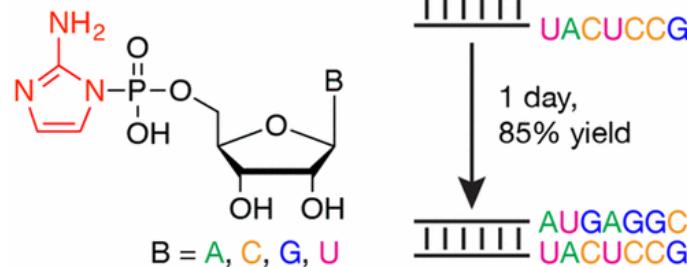
3C B=cytosine

3U B=uracil

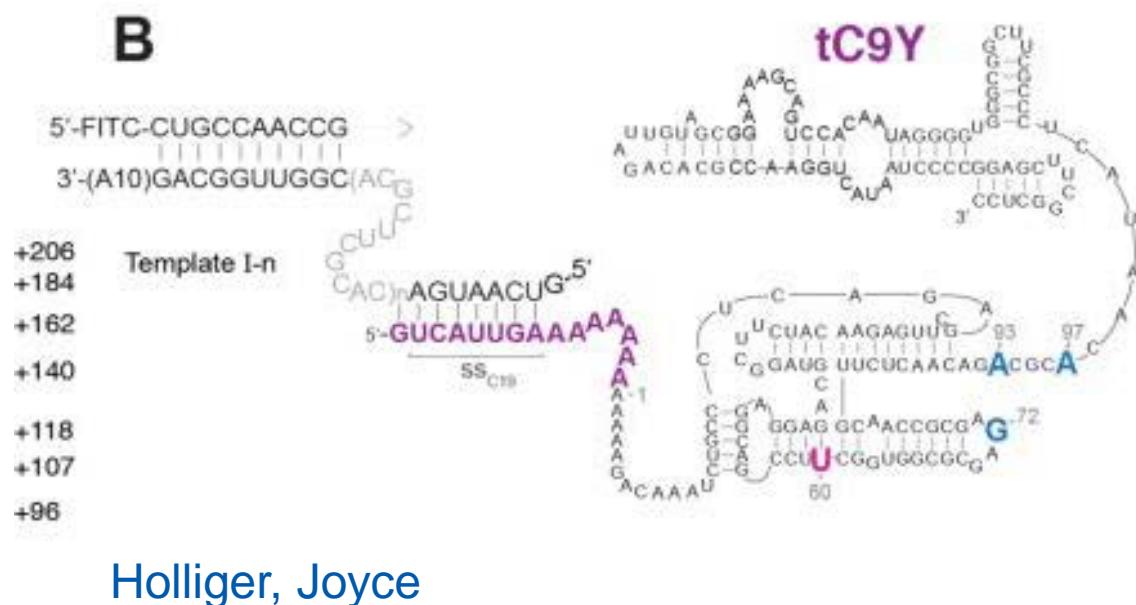
3OA B=8-oxo-adenine

3OI B=8-oxo-hypoxanthine

Sutherland, Powner,
Carell



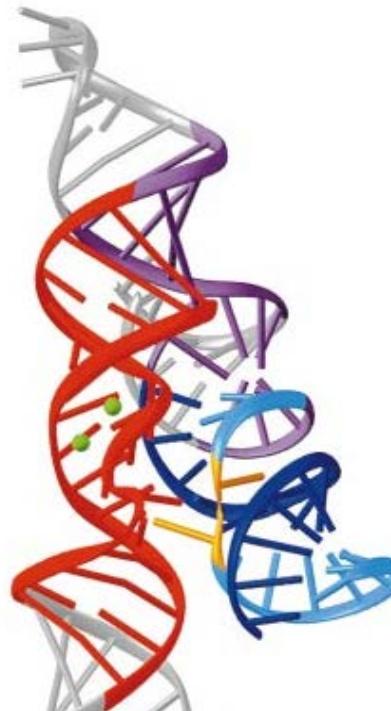
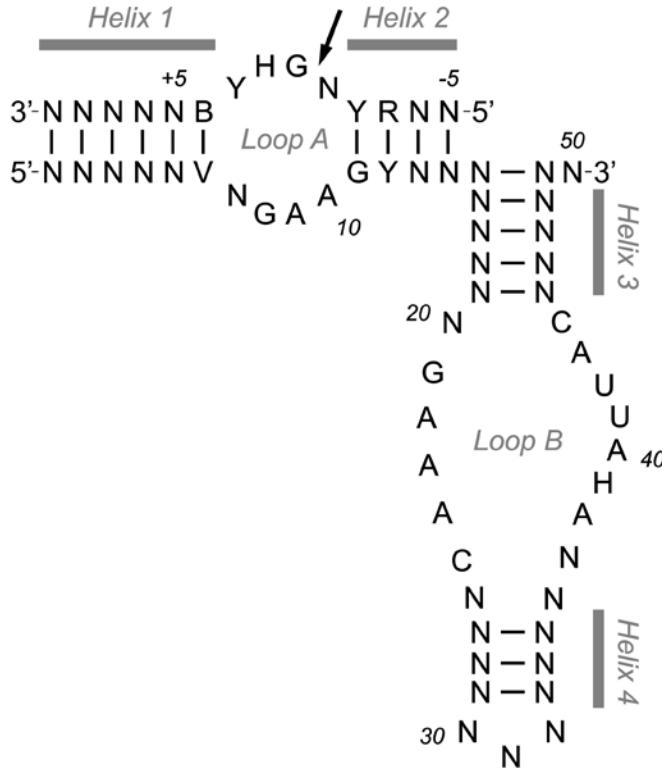
Szostak, Richert



Holliger, Joyce

RNA processing

Hairpin ribozyme



-derived from *Tobacco Ringspot Virus* Satellite RNA

-positively charged co-factors (Mg^{2+}) required for activity

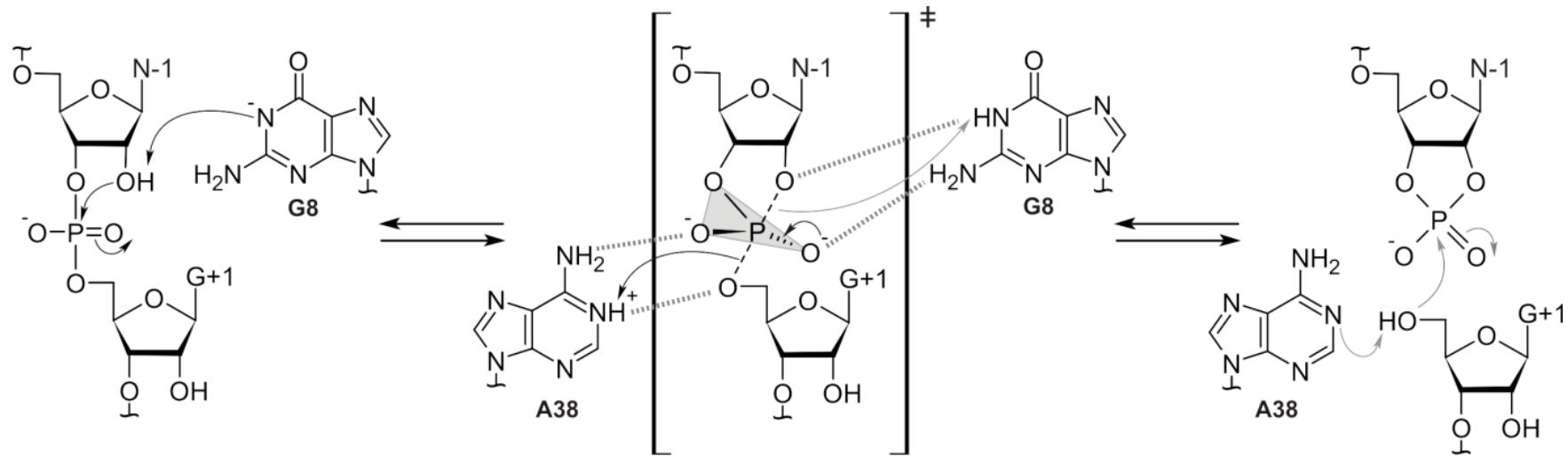
-bent active conformation with contacts between loop A and loop B

-catalyzes the reversible cleavage of a specific phosphodiester bond

-internal equilibrium is shifted towards ligation

Rupert & Ferré-D'Amaré, *Nature*, 2001, 410, 780

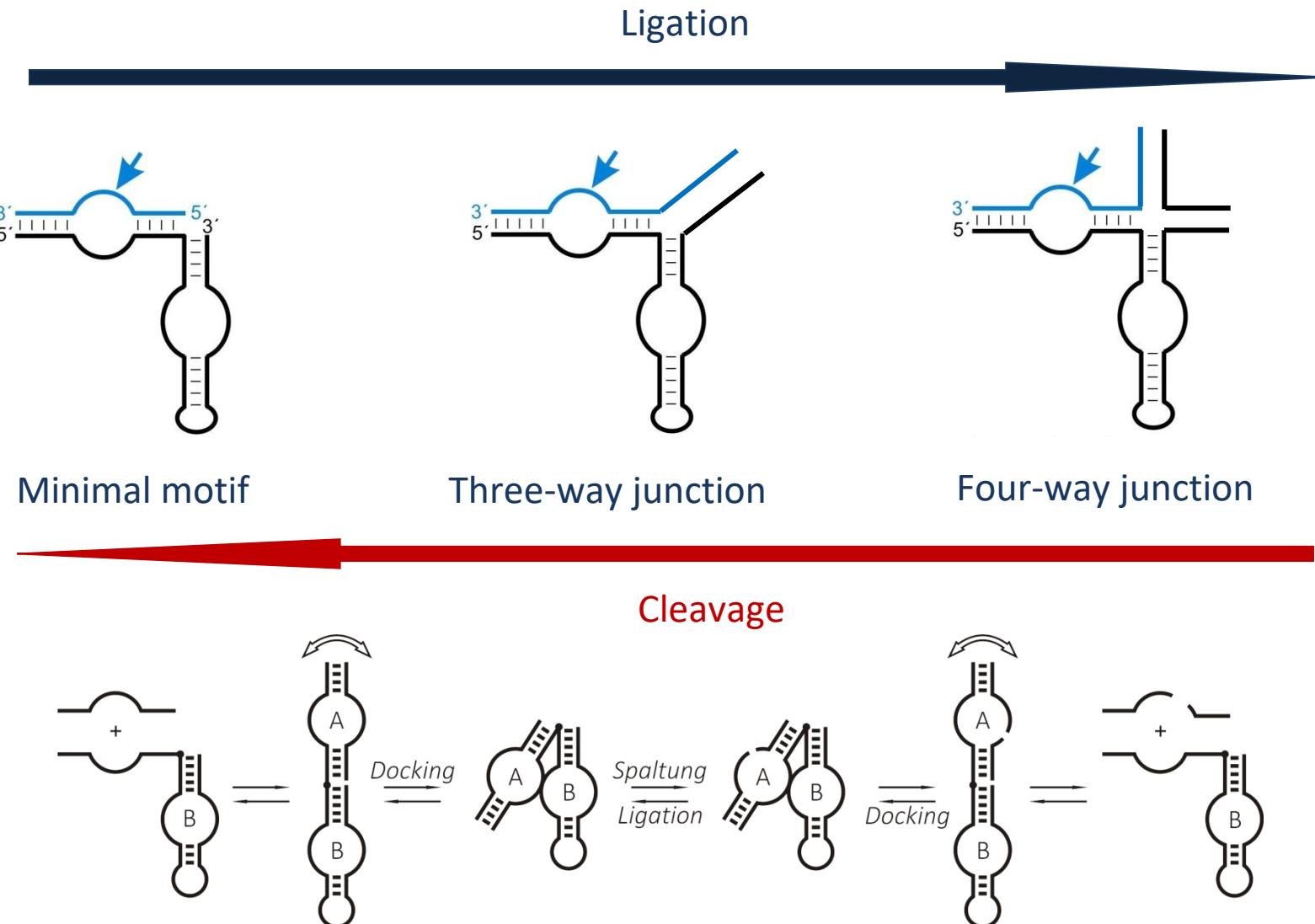
Hairpin ribozyme cleavage & ligation



- General acid base catalysis
- Stabilization of the charged transition state
- In line geometry

→ Ligation is enthalpically favored, cleavage is entropically favored

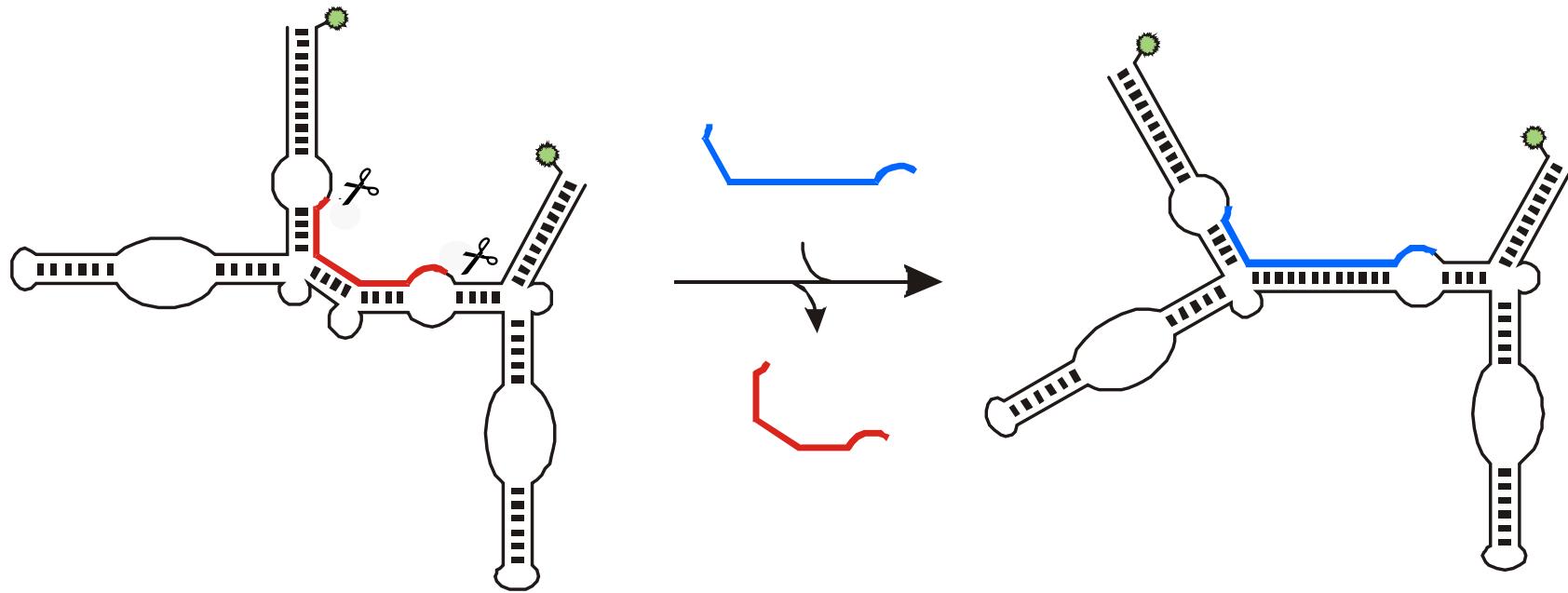
Ligation profits from structural stabilization



Zhuang et al., *Science* 2002, 296, 1473

Hairpin ribozymes with stable secondary (and tertiary) structure favor ligation, while hairpin ribozymes that are less stable, however stable enough to fold into a catalytically competent structure, favor cleavage.

Hairpin derived twin ribozyme

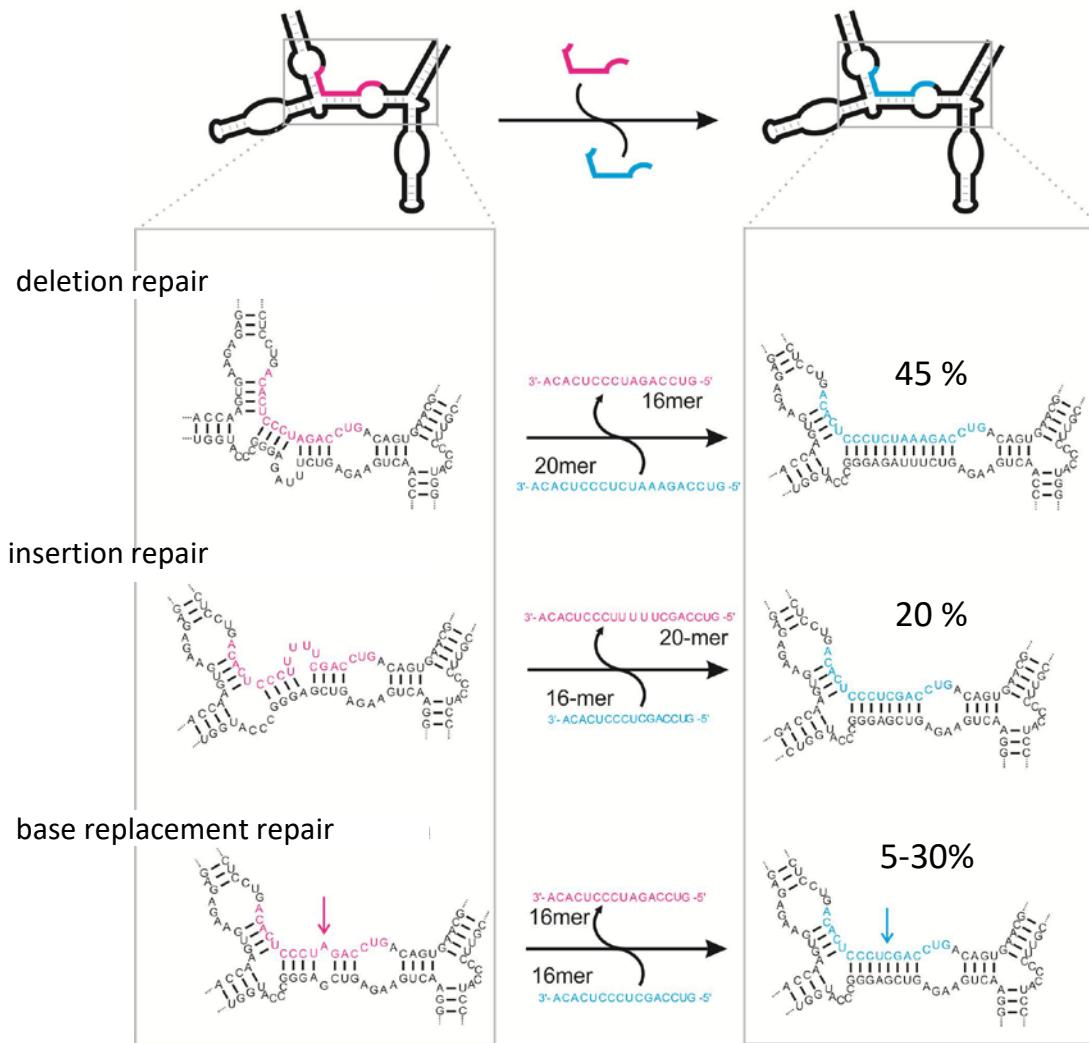


Two RNA cleavage reactions and two ligations

Activity control by stability modulation of the twin ribozyme-substrate/product complexes

→ Facile dissociation of the red segment versus stable binding of the blue segment

Twin ribozyme mediated fragment exchange



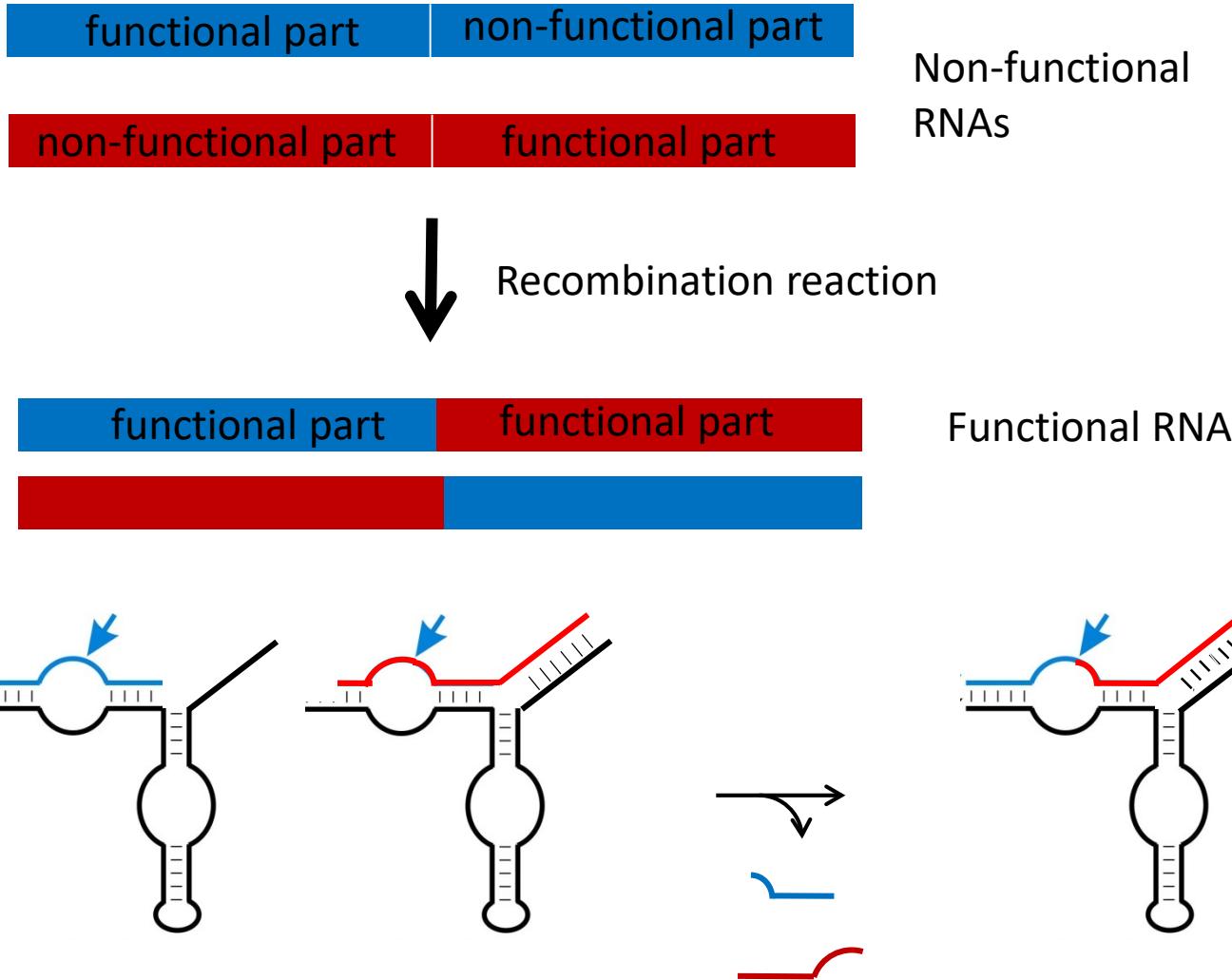
Welz et al., *Angew. Chem. Int. Ed.*
2003; Ivanov et al., *FEBS J.*, 2005;
Balke et al., *ChemMedChem*, 2014,
Balke et al., *Org. Biomol. Chem.*, 2016

Drude et al., *Biochem. Biophys. Res. Comm.*, 2007,

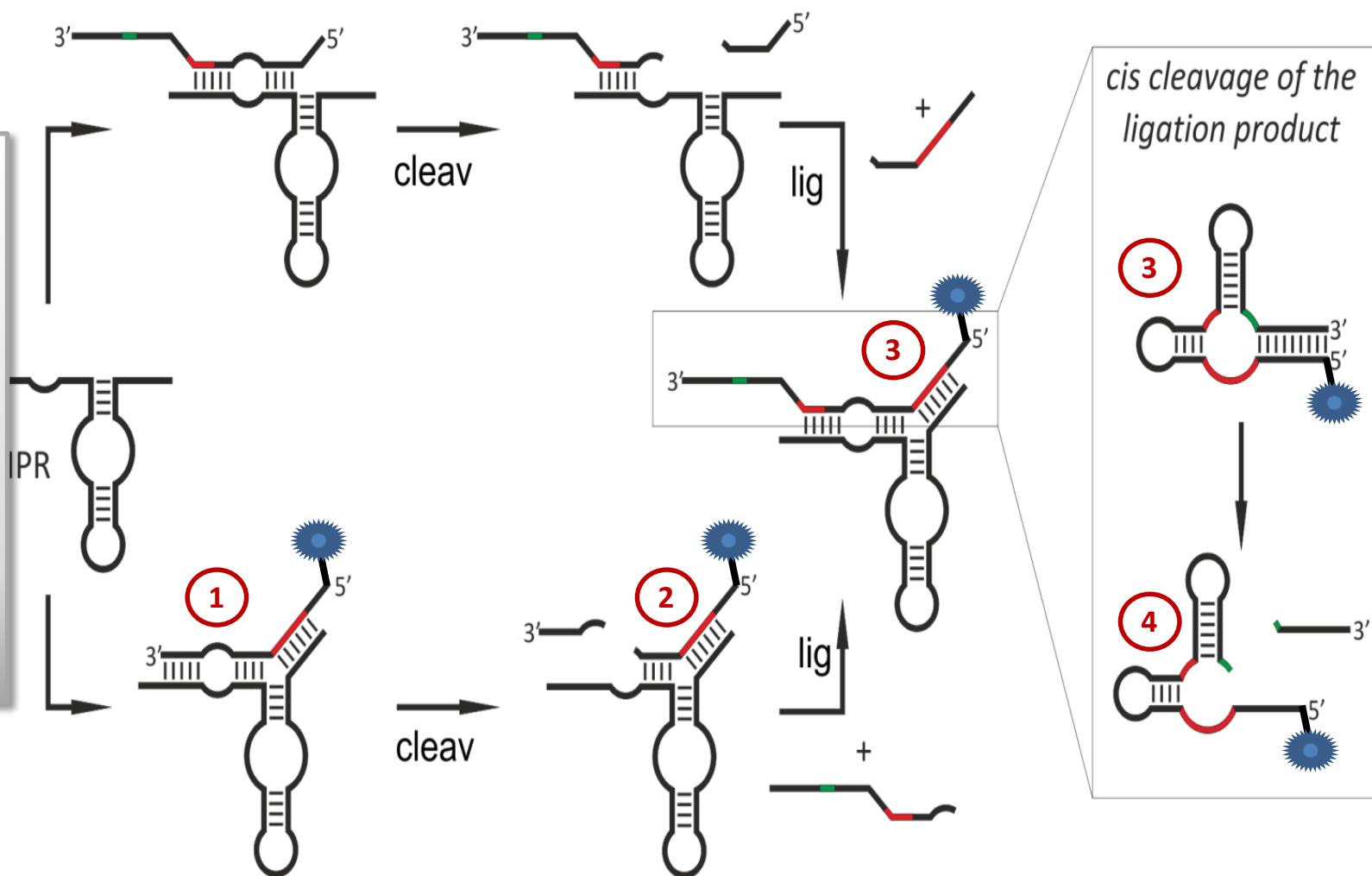
Vauléon et al., *ChemBioChem*
2005

Can we make simpler systems?

Simpler than twin ribozymes?

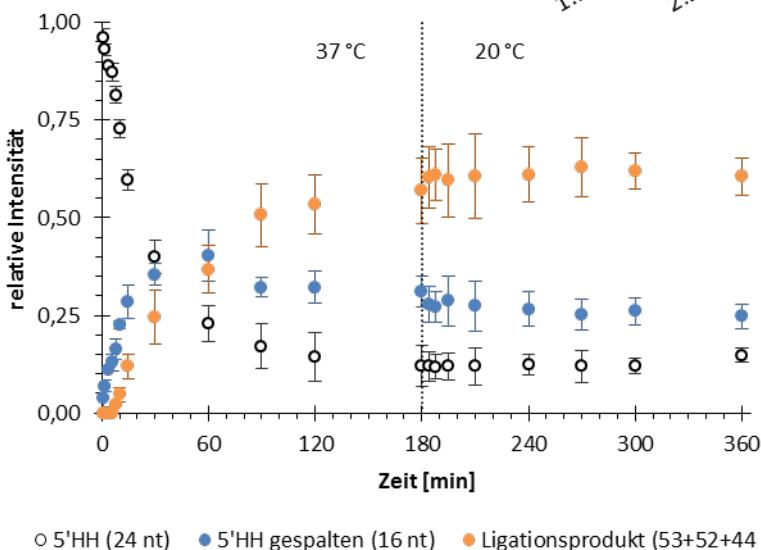
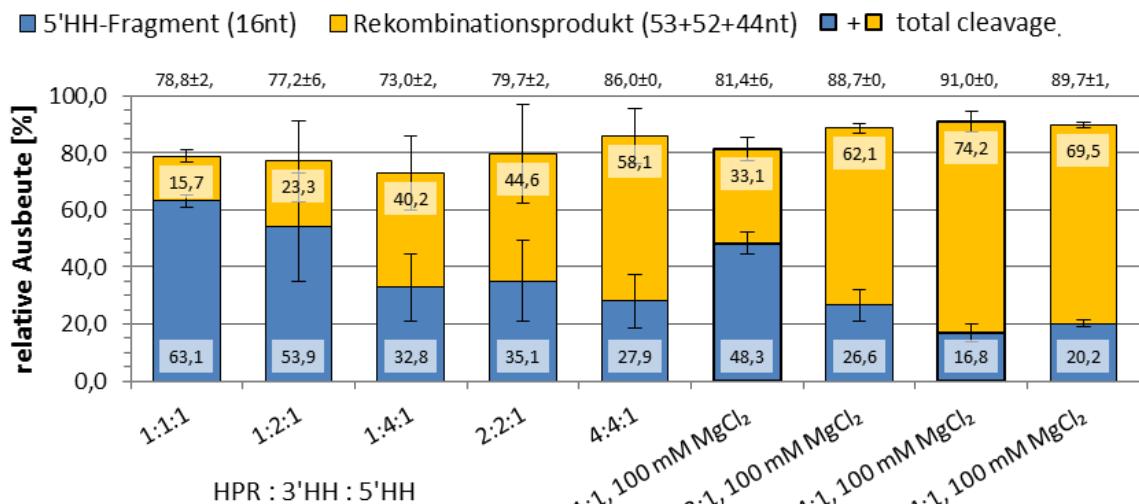
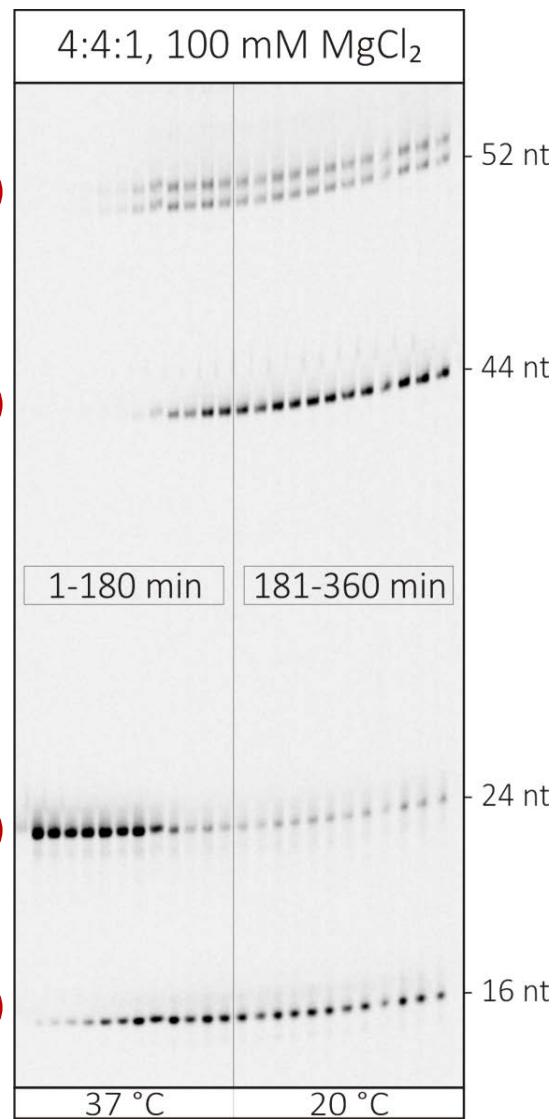


HPR mediated RNA recombination



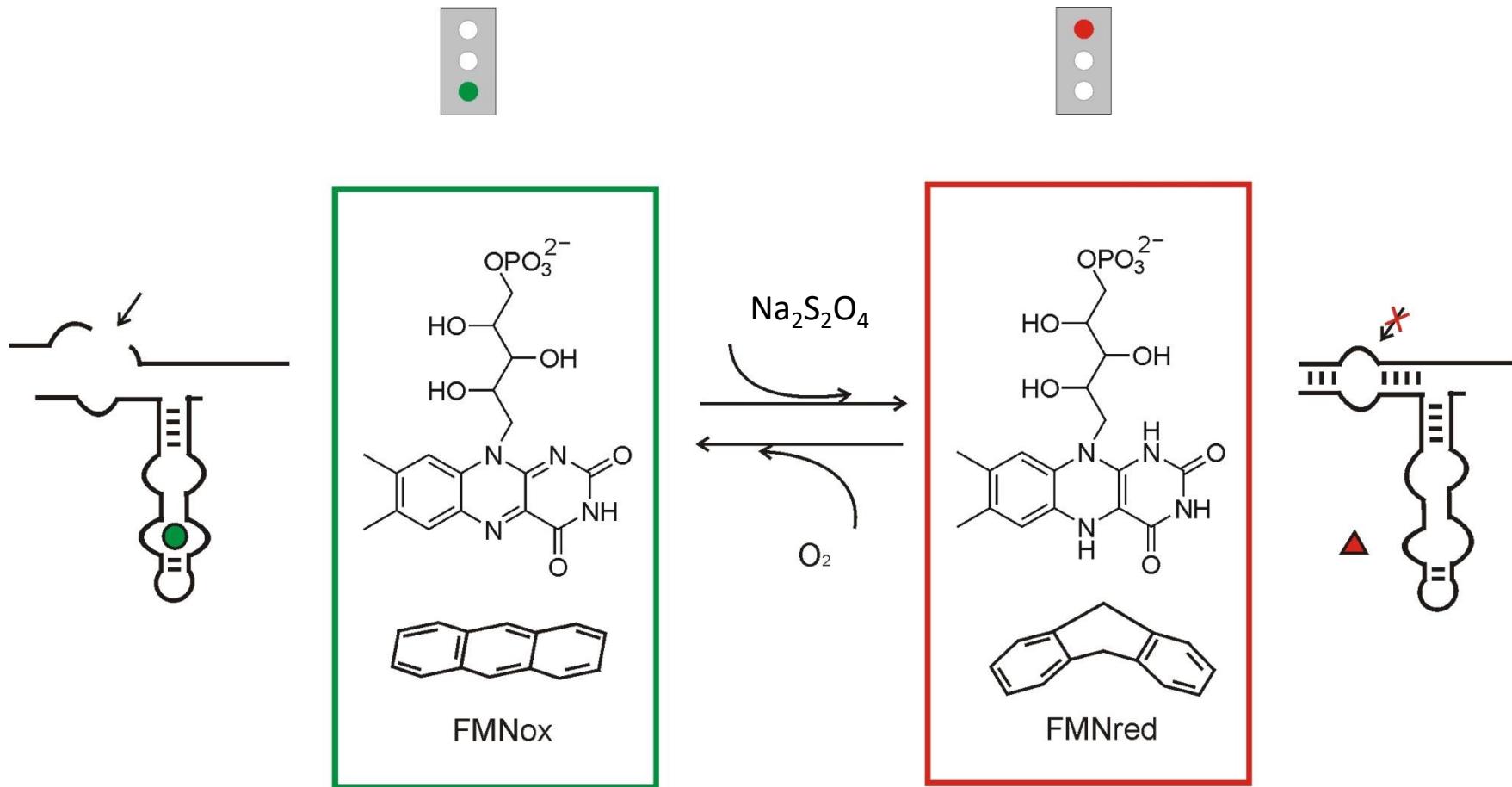
Hieronymus R, Godehard SP, Balke D, Müller S., *Chem Commun (Camb)*. 2016, 52: 4365-8.

HPR mediated recombination



Hieronymus R, Godehard SP, Balke D, Müller S., *Chem Commun (Camb)*. 2016, 52: 4365-8.

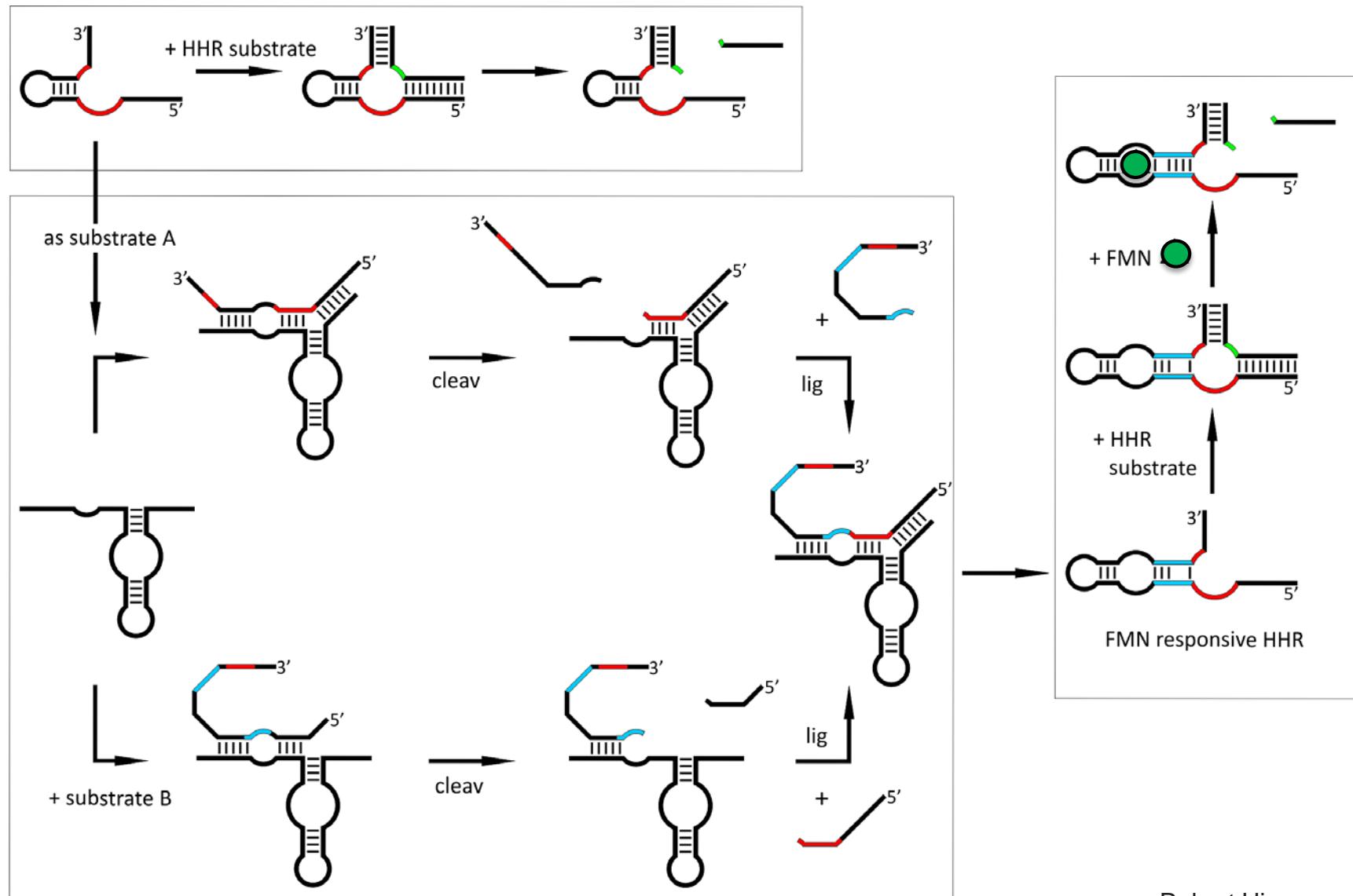
Redox-responsive RNA switch



Strohbach D, Novak N, Müller S., *Angew Chem Int Ed Engl.* **2006**, 45: 2127-9

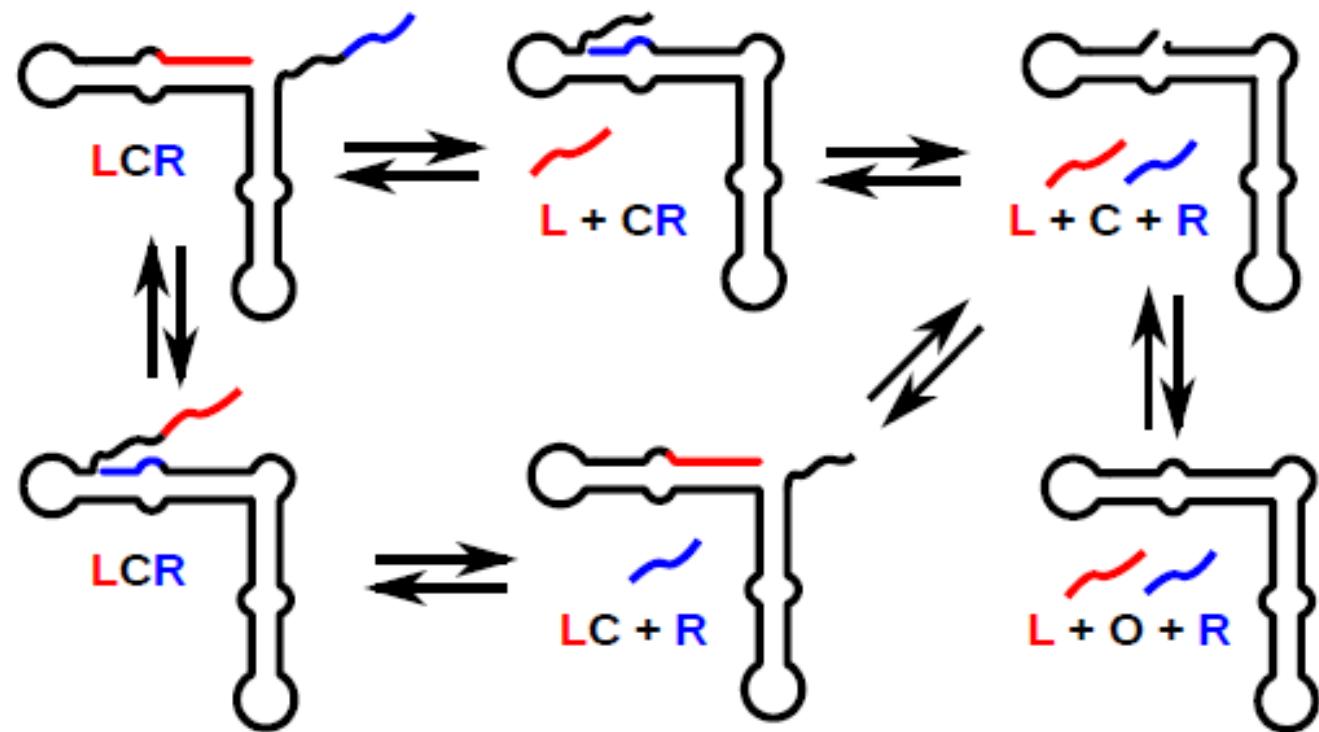
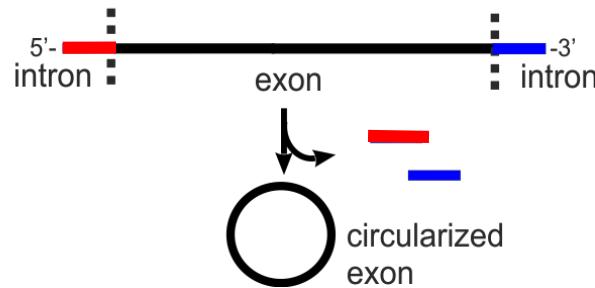
D. Strohbach, F. Turcu, W. Schuhmann, S. Müller., *Electroanalysis* **2008**, 20, 935-40

Recombination towards higher complexity

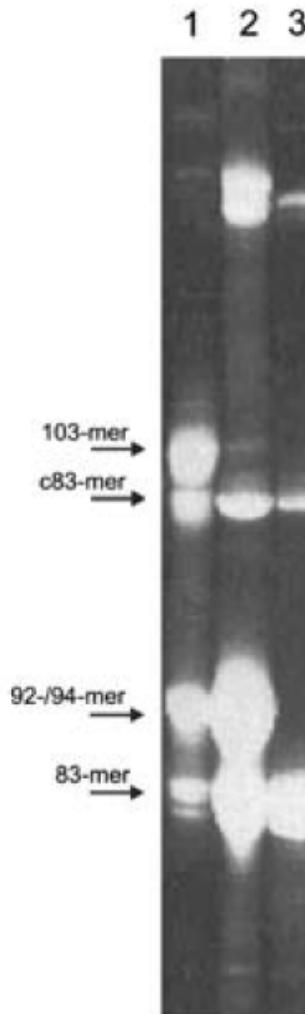
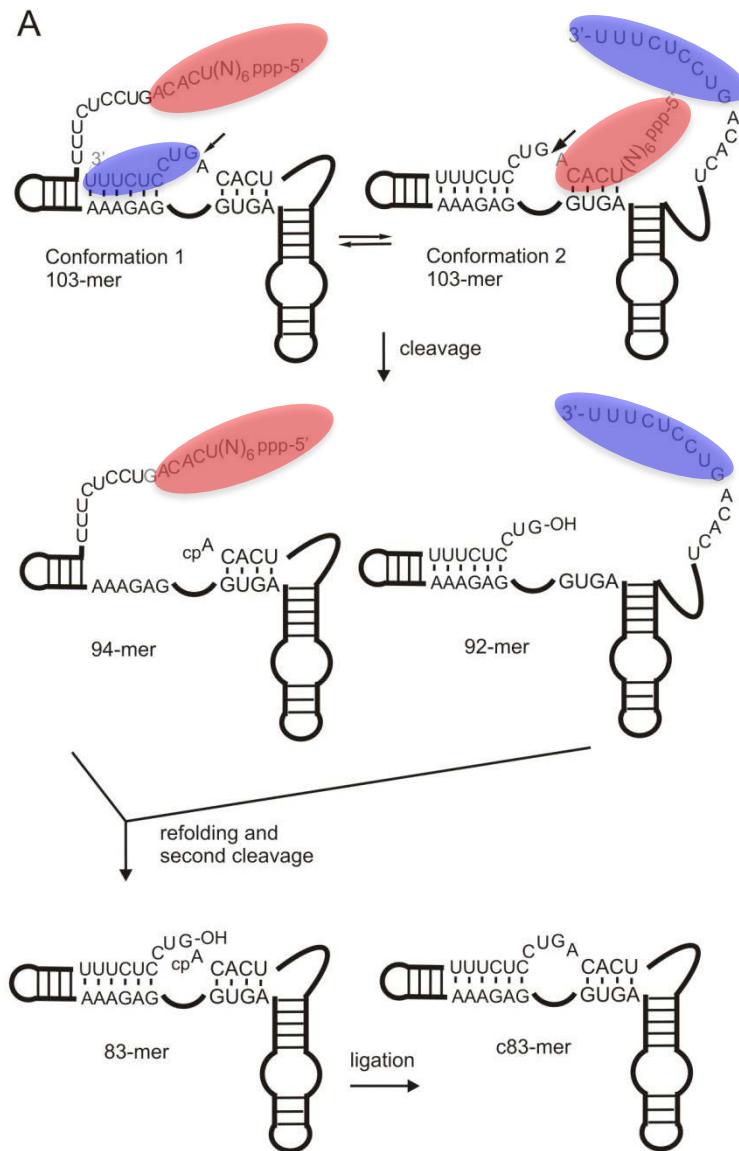


Robert Hieronymus

Hairpin ribozyme mediated back splicing ?



Initial purpose: making circular RNA



1: Incubation of 103mer at 50 mM MgCl₂

2: Incubation of 94/92mer at 50 mM MgCl₂

3: Incubation of 83mer at 50 mM MgCl₂

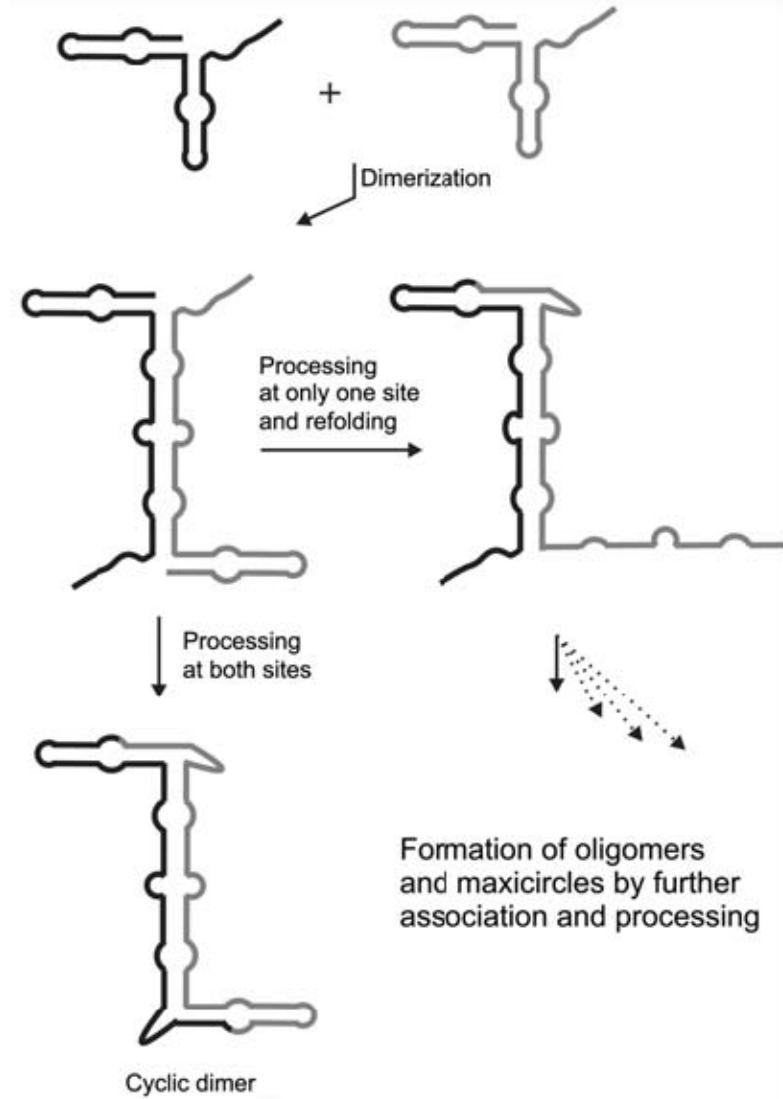
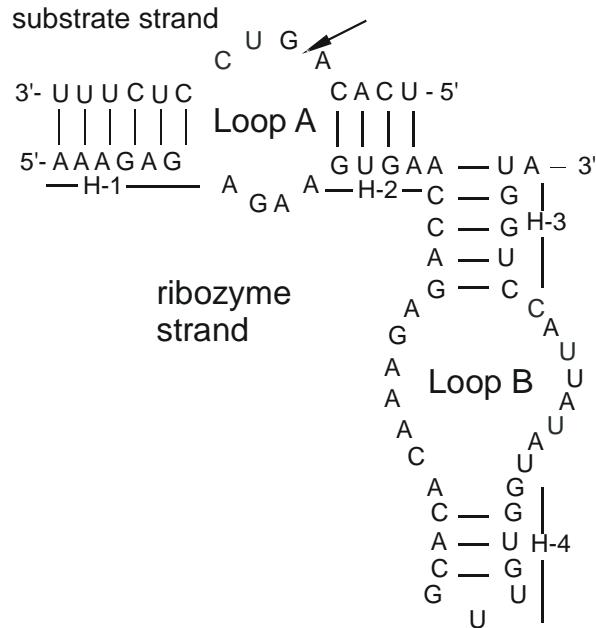
Pieper S, Vauléon S, Müller S., *Biol Chem.* **2007**, 388: 743-6.

Hairpin Ribozyme Dimerization ... and Concatemerization?

ERNST MORITZ ARNDT
UNIVERSITÄT GREIFSWALD



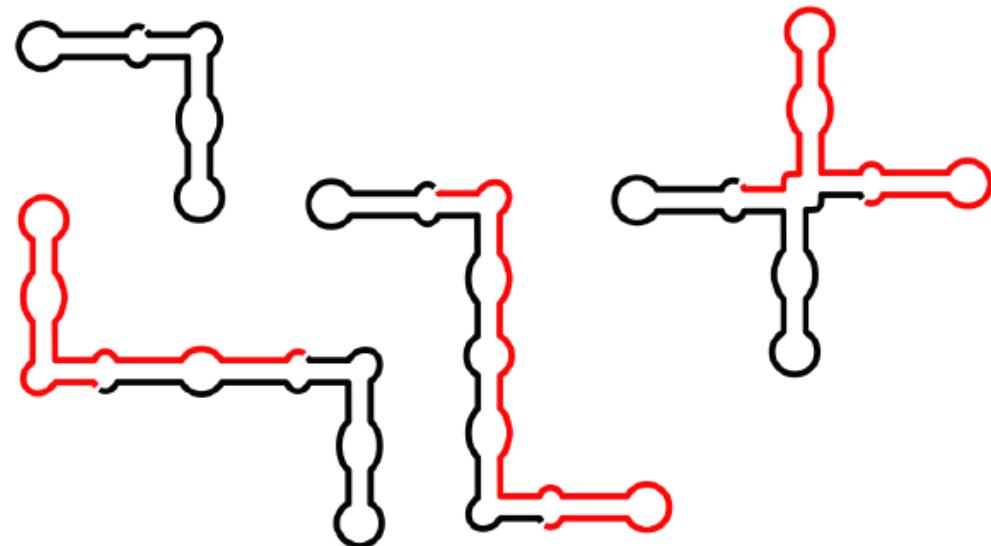
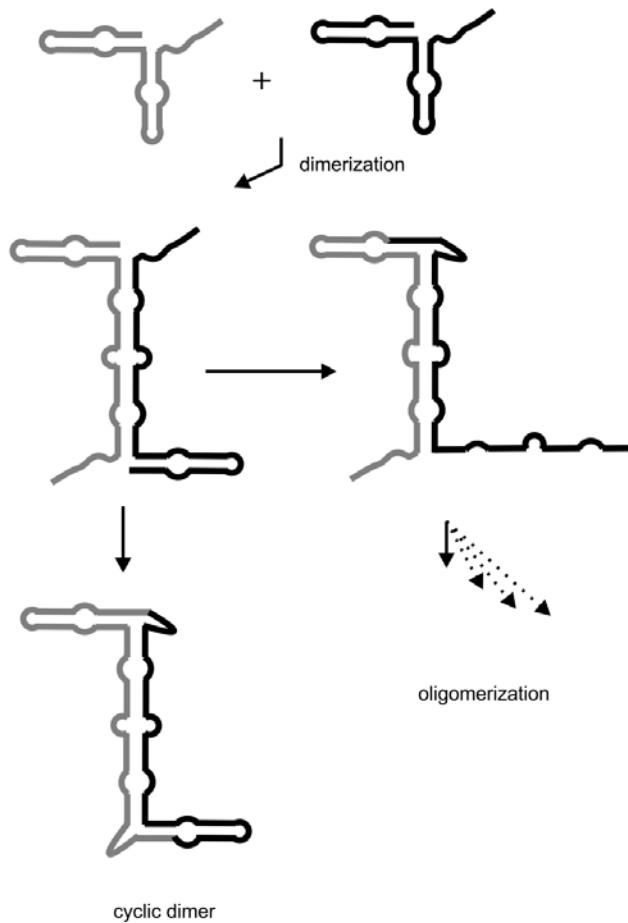
Wissen
leckt.
Seit 1456



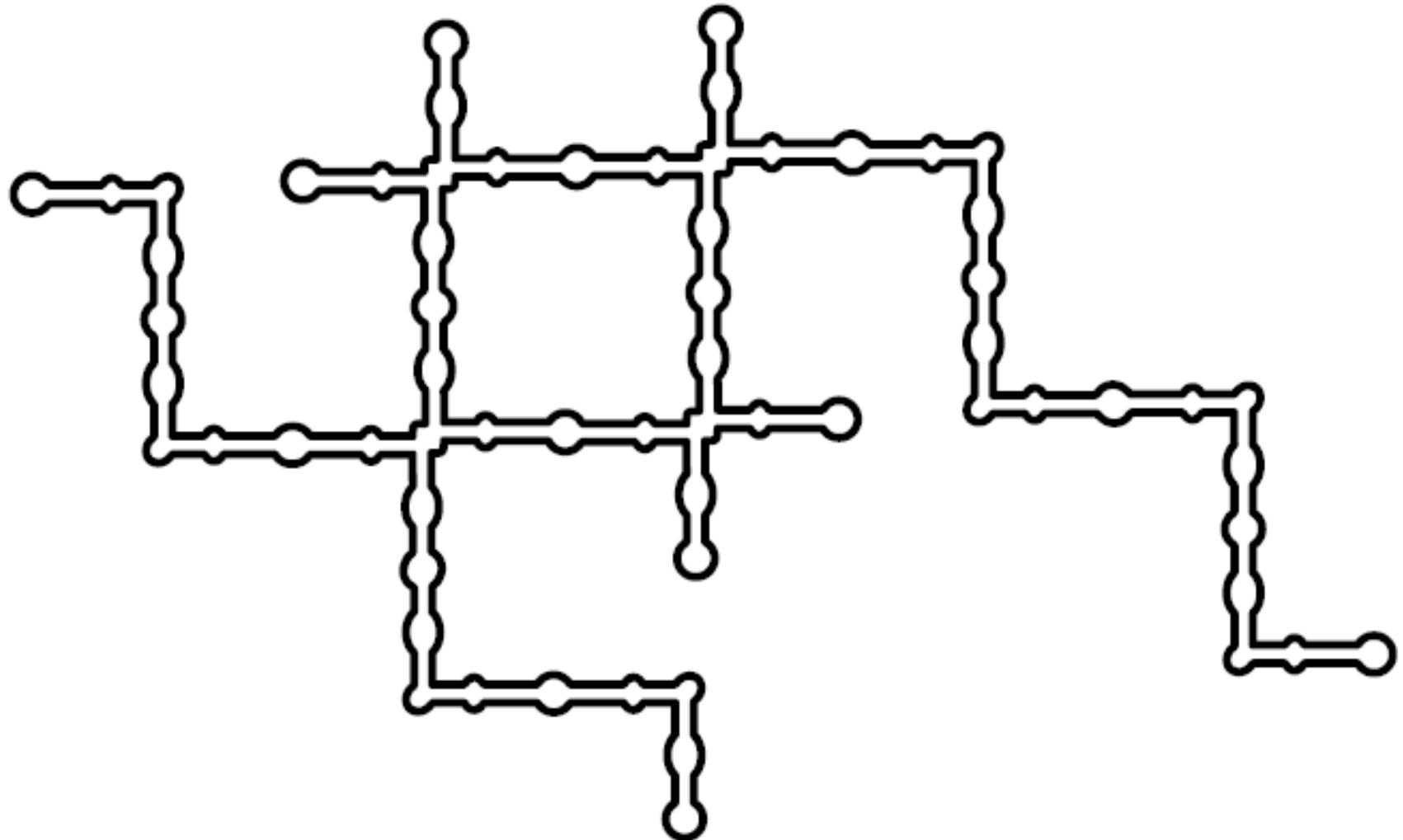
Hairpin ribozyme dimers have been identified previously!
(Burke, 1994)

Pieper S, Vauléon S, Müller S., *Biol Chem.* 2007, 388: 743-6.

Formation of dimers

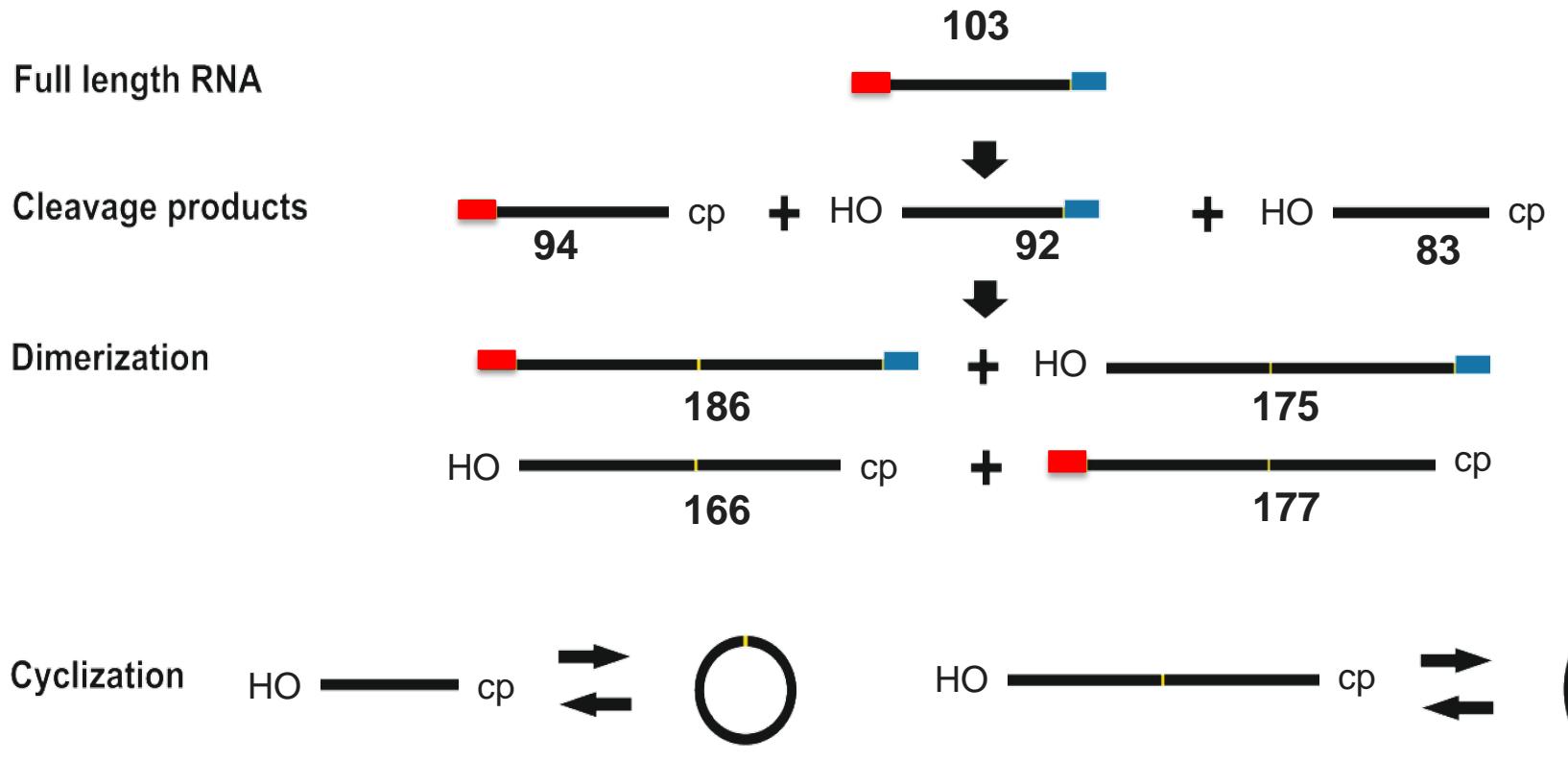


...and oligomers



Complex product spectrum!

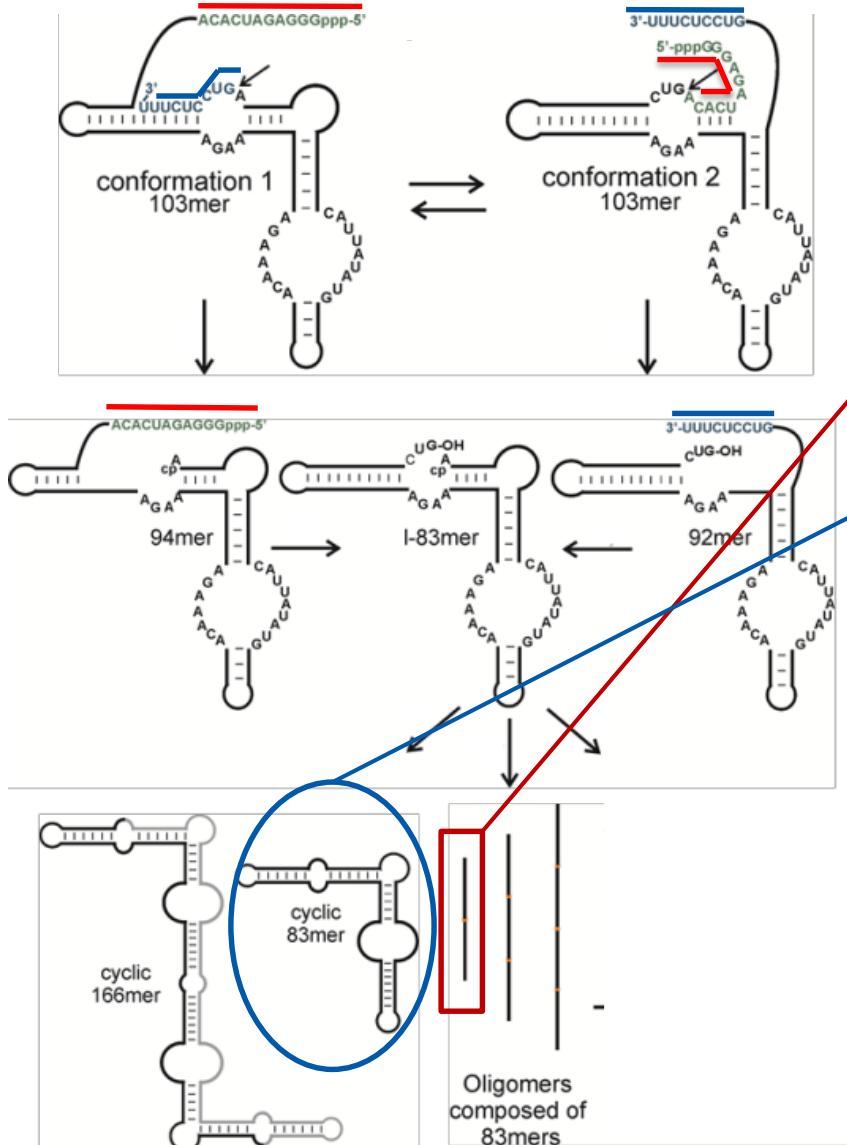
For dimers:



A complex mixture of oligomers

Dimers:	Trimers:	Tetramers:	Maxi circles:
166 cp	249 cp	332 cp	166
175	258	341	249
177cp	260 cp	343 cp	332
186	269	352	.
			.
			.

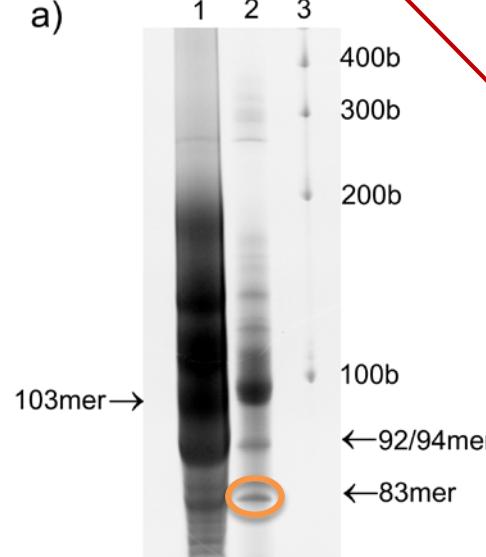
Linear and circular products



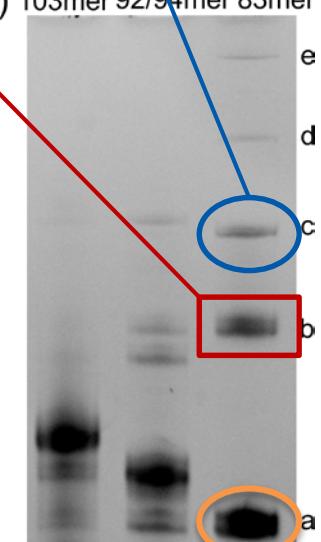
Linear dimer

Circular 83mer

a)

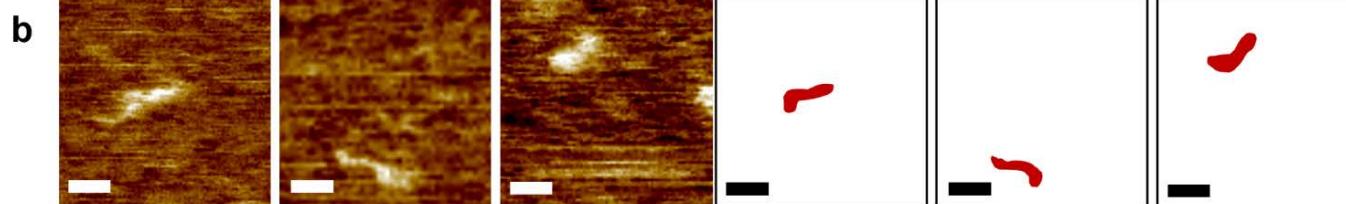
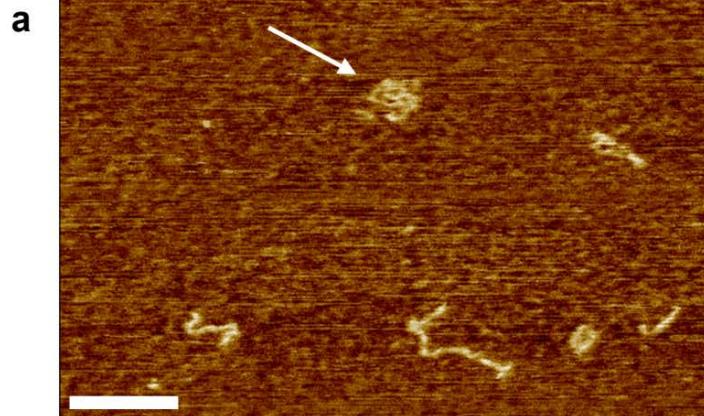


b) 103mer 92/94mer 83mer

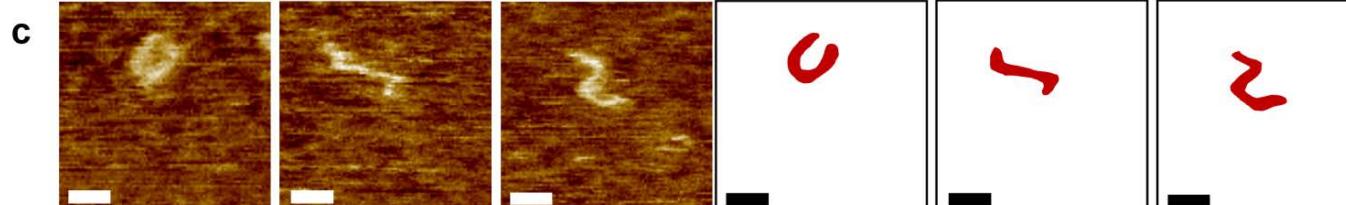


S. Petkovic, S. Müller. FEBS Lett. 2013, 587, 2435-40.

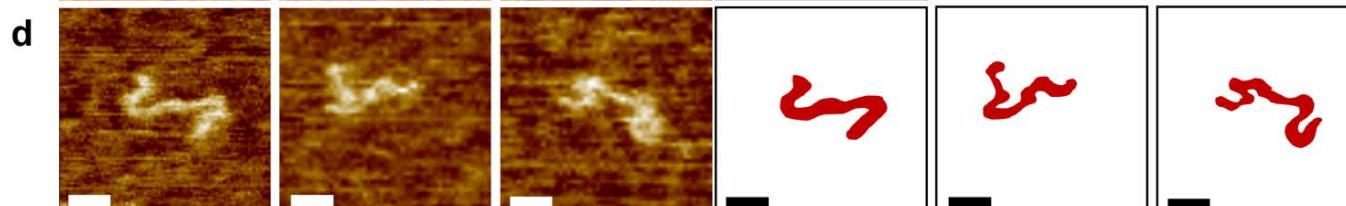
AFM analysis of the re-incubated 83mer



$$L_c = 13 \text{ nm} \pm 1 \text{ nm} \rightarrow 83\text{mer}$$



$$L_c = 24.5 \text{ nm} \pm 1 \text{ nm} \rightarrow \text{dimer}$$

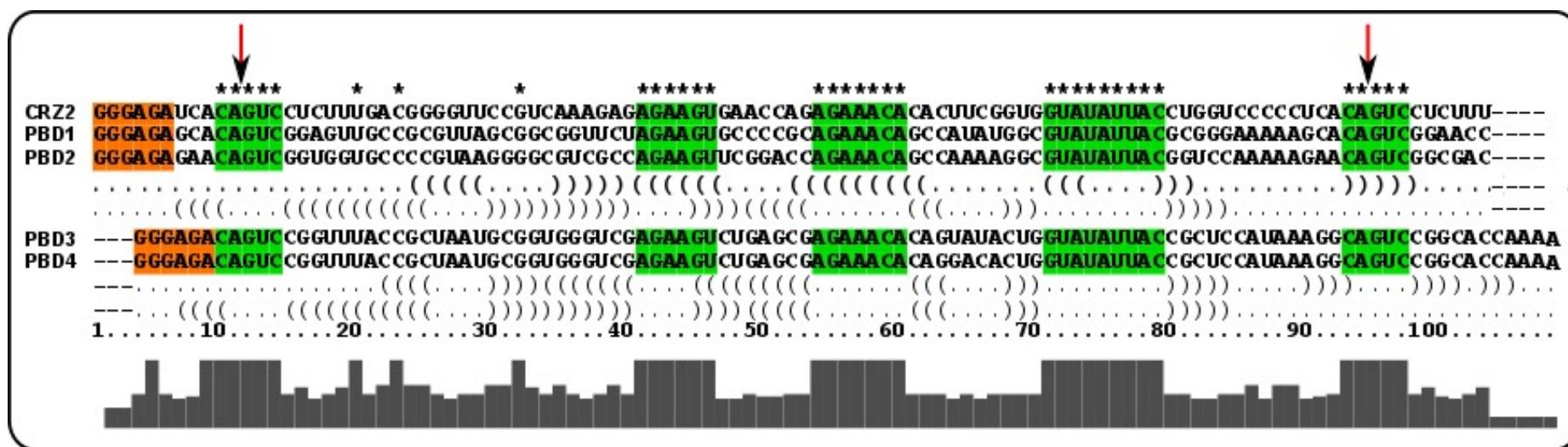


$$L_c = 35 \text{ nm} \pm 2 \text{ nm} \rightarrow \text{trimer}$$

Scale bars: 50 nm (a), 10 nm (b-d)

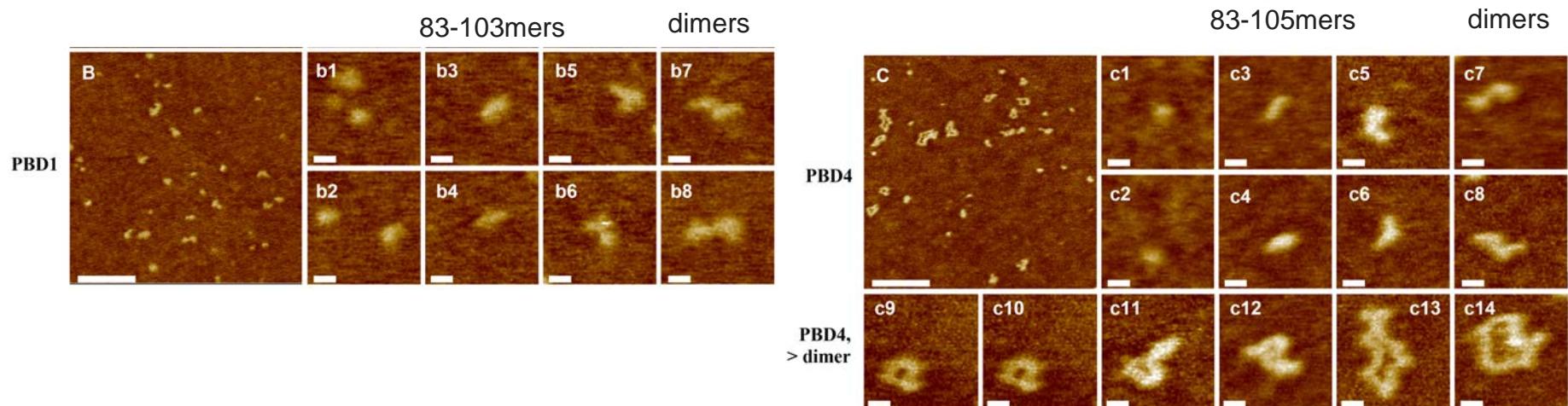
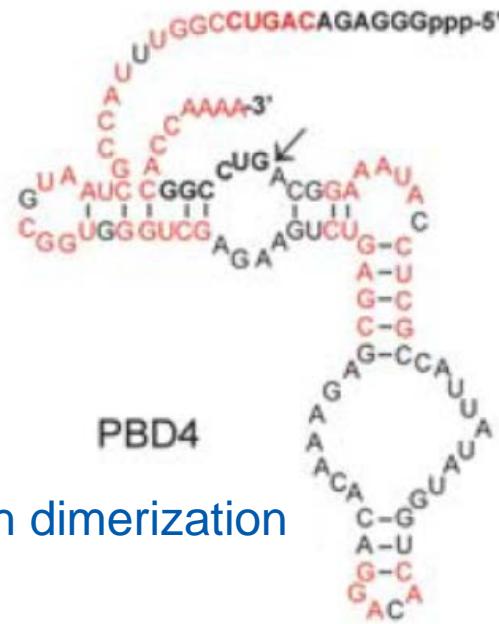
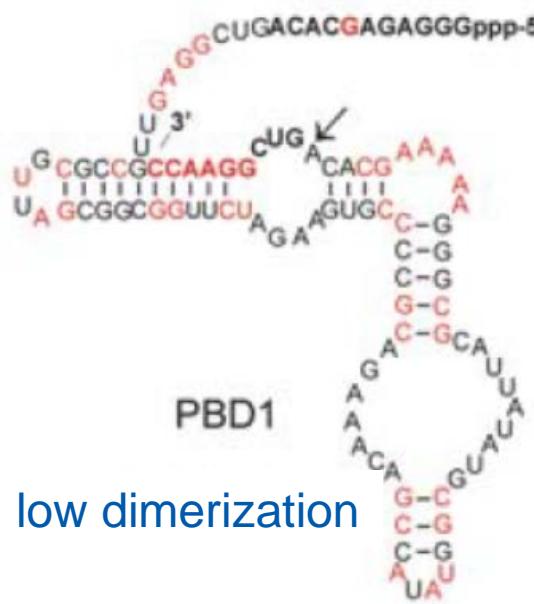
Petkovic S, Badelt S, Block S, Flamm C, Delcea M, Hofacker I, Müller S., *RNA*. 2015, 21: 1249-60.

Computer-aided design of variants



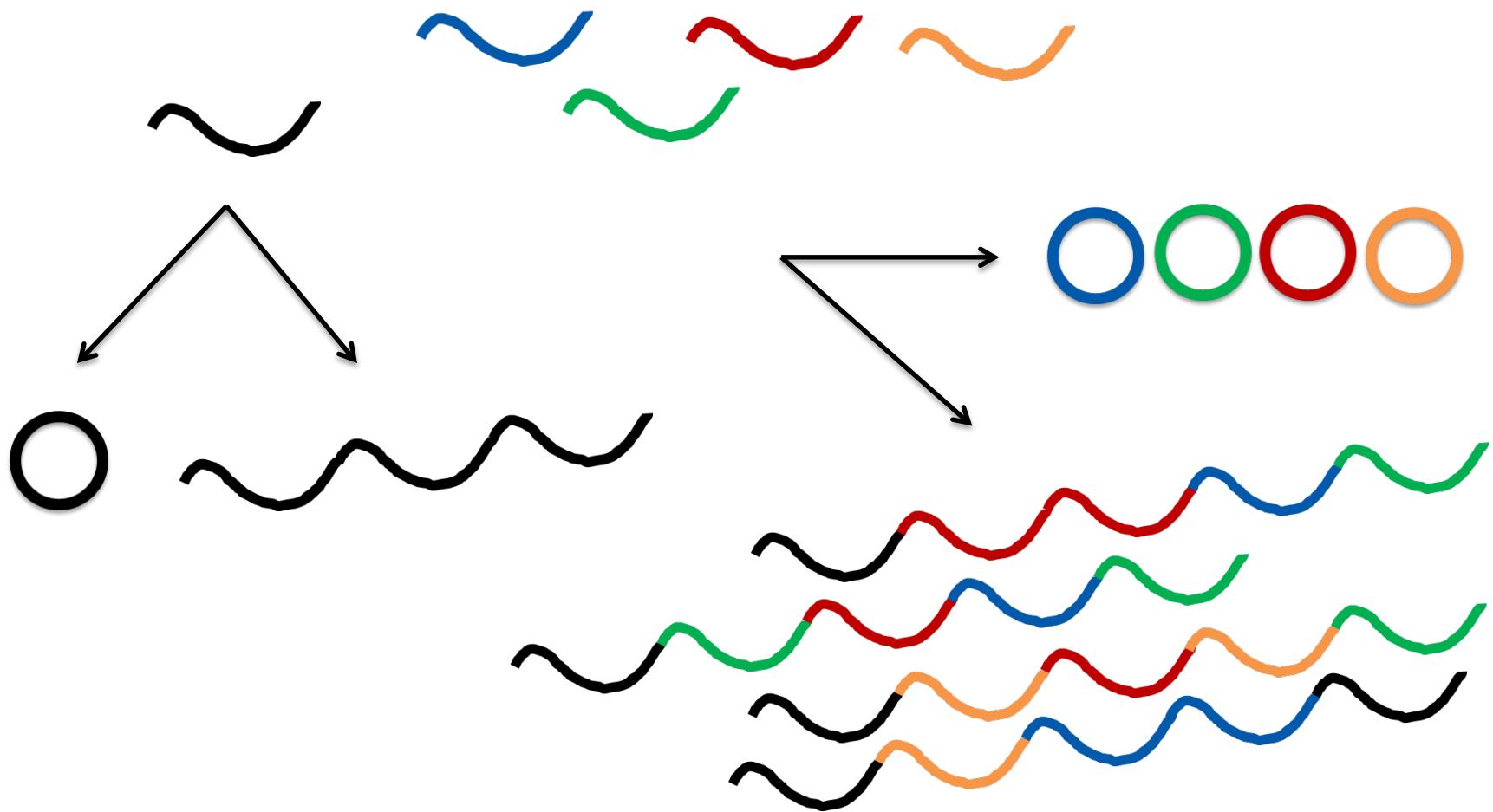
Petkovic S, Badelt S, Block S, Flamm C, Delcea M, Hofacker I, Müller S., *RNA*. 2015, 21: 1249-60.

Designed variants with distinct dimerization potential

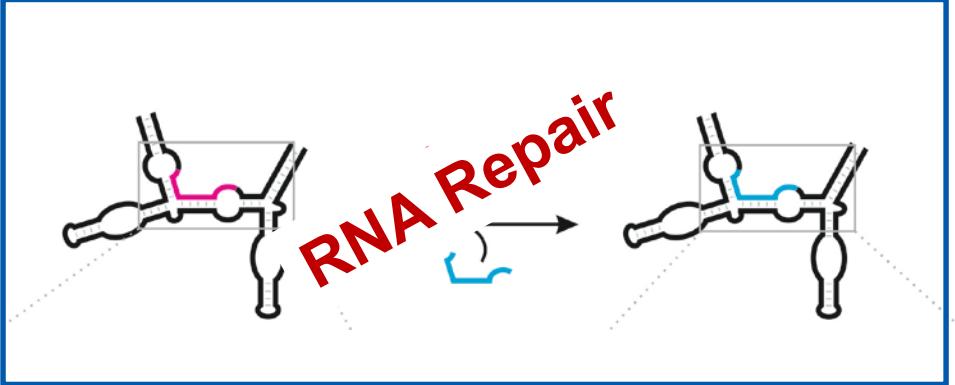


Petkovic S, Badelt S, Block S, Flamm C, Delcea M, Hofacker I, Müller S., *RNA*. 2015, 21: 1249-60.

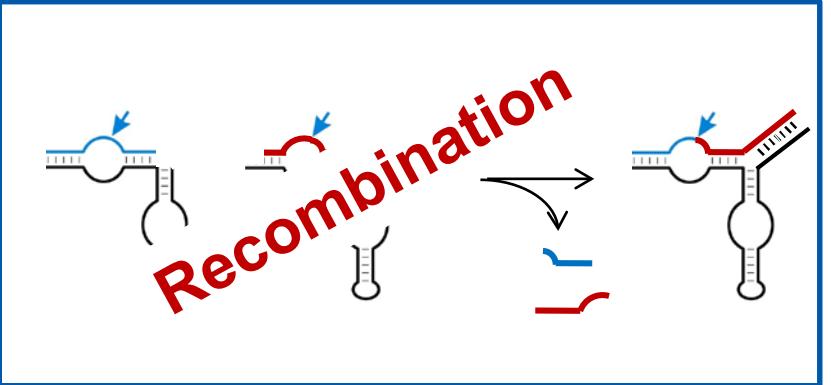
Increase of RNA size and sequence diversity



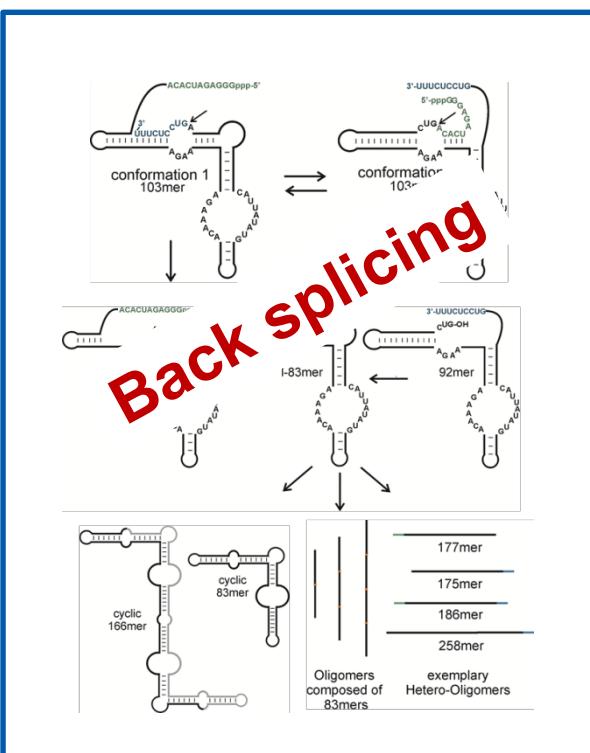
Conclusion: functional diversity of a small RNA



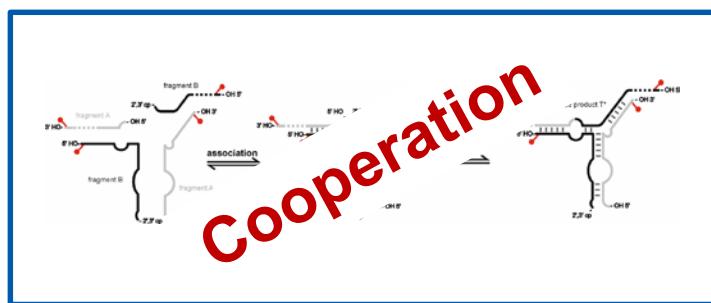
RNA Repair



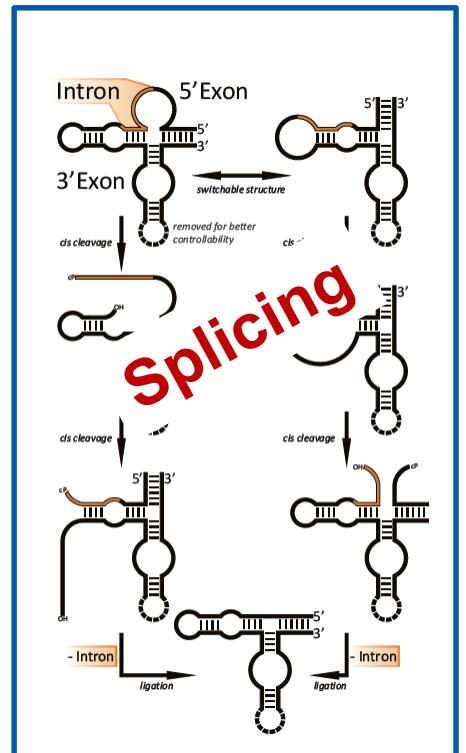
Recombination



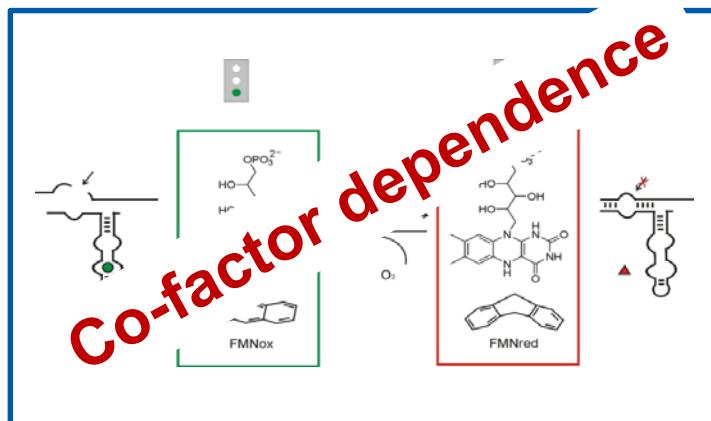
Back splicing



Cooperation



Splicing



Co-factor dependence



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