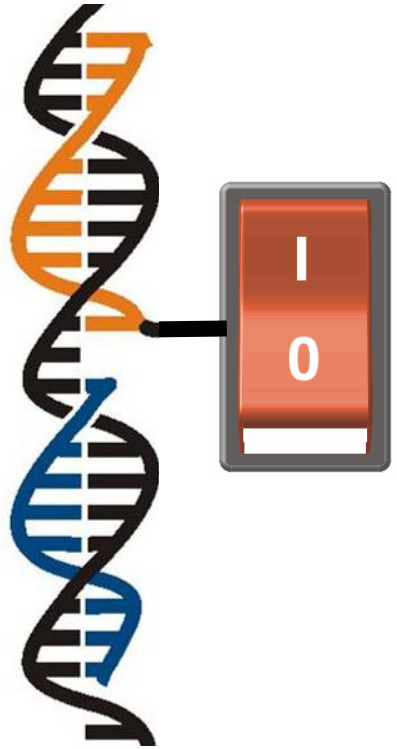
Switching with nucleic acid hybridization



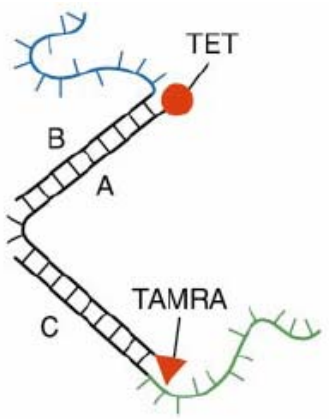
Who pushs the button?

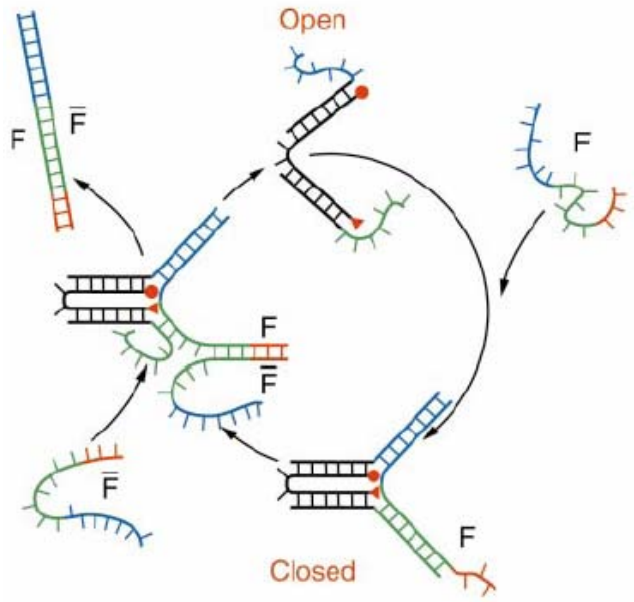
→ **Response**

DNA/RNA	→ Light
Protein	→ Light
DNA/RNA	→ Protein activity

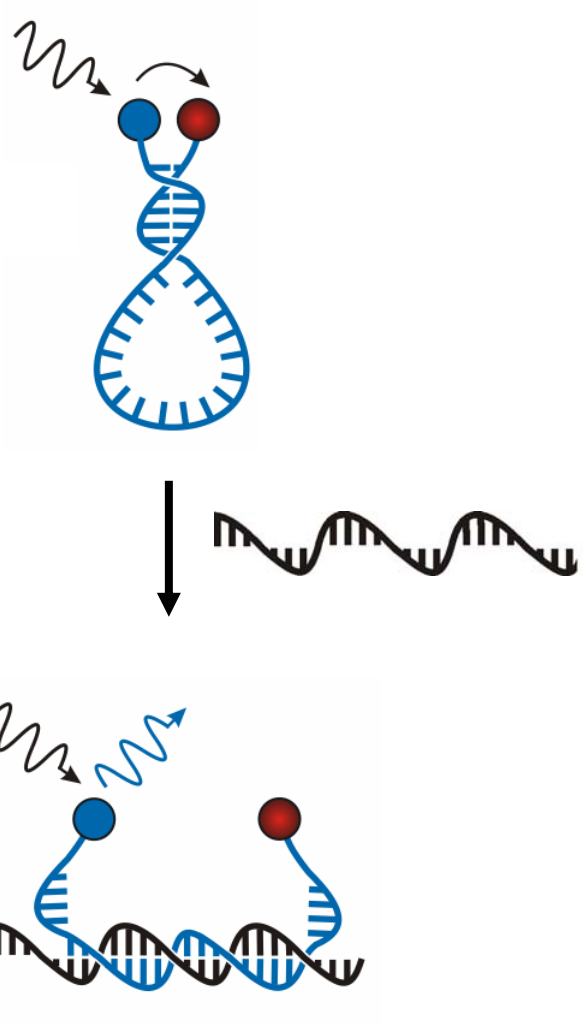
Trigger: Nucleic Acid ; Response: Light

DNA-driven light switch





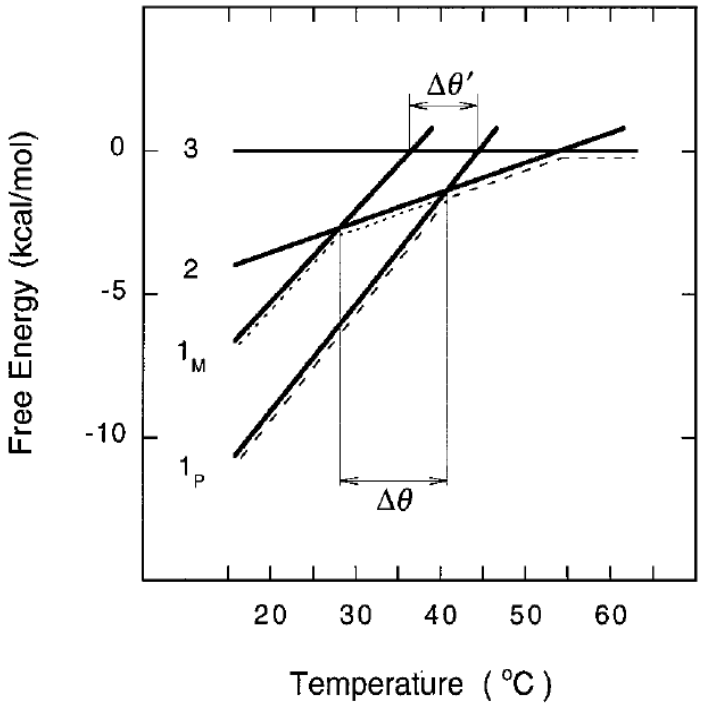
Molecular Beacons



Yurke, *Nature* **2000**

Tyagi, Kramer, *Nat. Biotechnol*. **1996**

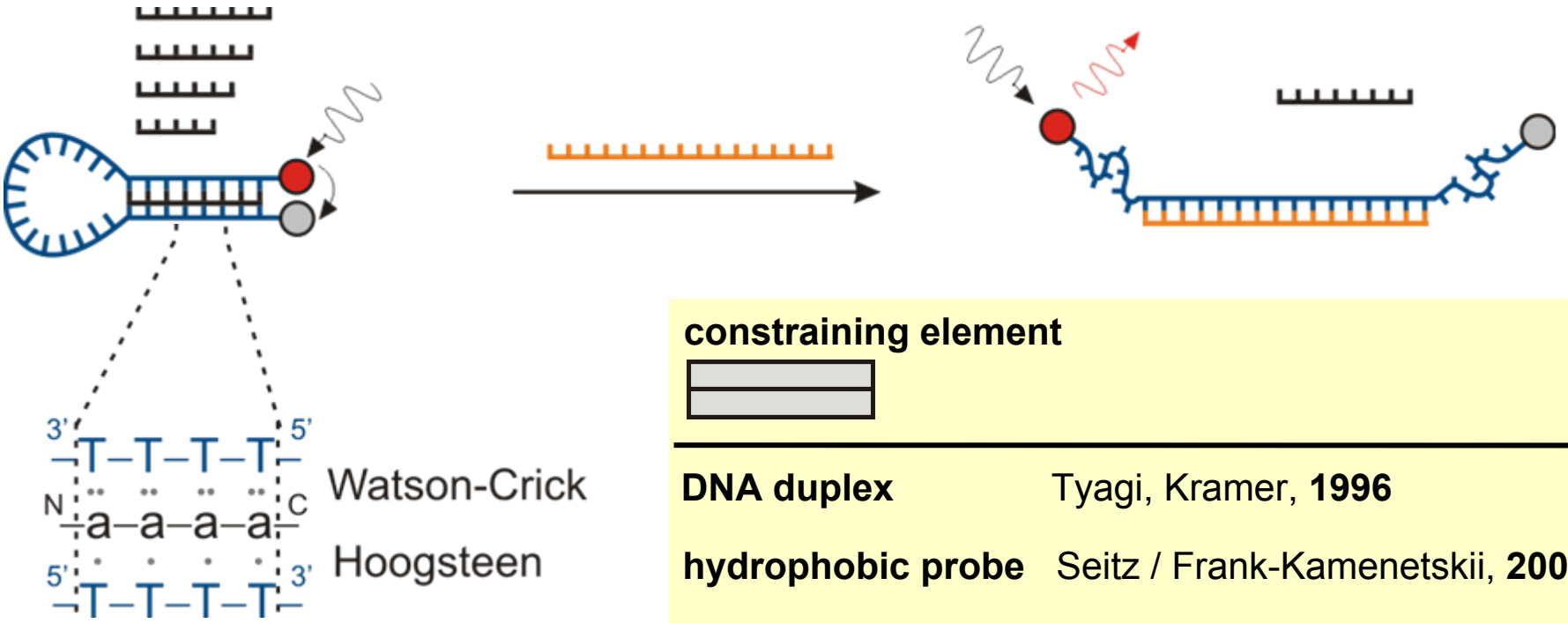
Molecular Beacons: Enhanced specificity through constraint



constraining element

DNA duplex	Tyagi, Kramer, **1996**
hydrophobic probe	Seitz / Frank-Kamenetskii, **2000**
homo-DNA duplex	Leumann, **2005**
LNA	Benner, Tan, **2005**
DNA quadruplex	Jullien, Mergny, **2006**
?	

Molecular Beacons: Enhanced specificity through constraint



Modular system:

one dual labeled oligonucleotide

tuning of restoring force through unlabeled stem forming strand

constraining element

DNA duplex	Tyagi, Kramer, **1996**
hydrophobic probe	Seitz / Frank-Kamenetskii, **2000**
homo-DNA duplex	Leumann, **2005**
LNA	Benner, Tan, **2005**
DNA quadruplex	Jullien, Mergny, **2006**
DNA_2·PNA triplex	Grossmann, Röglin, Seitz, *Angew. Chem. Int. Ed.* **2007**, *46*, 5223

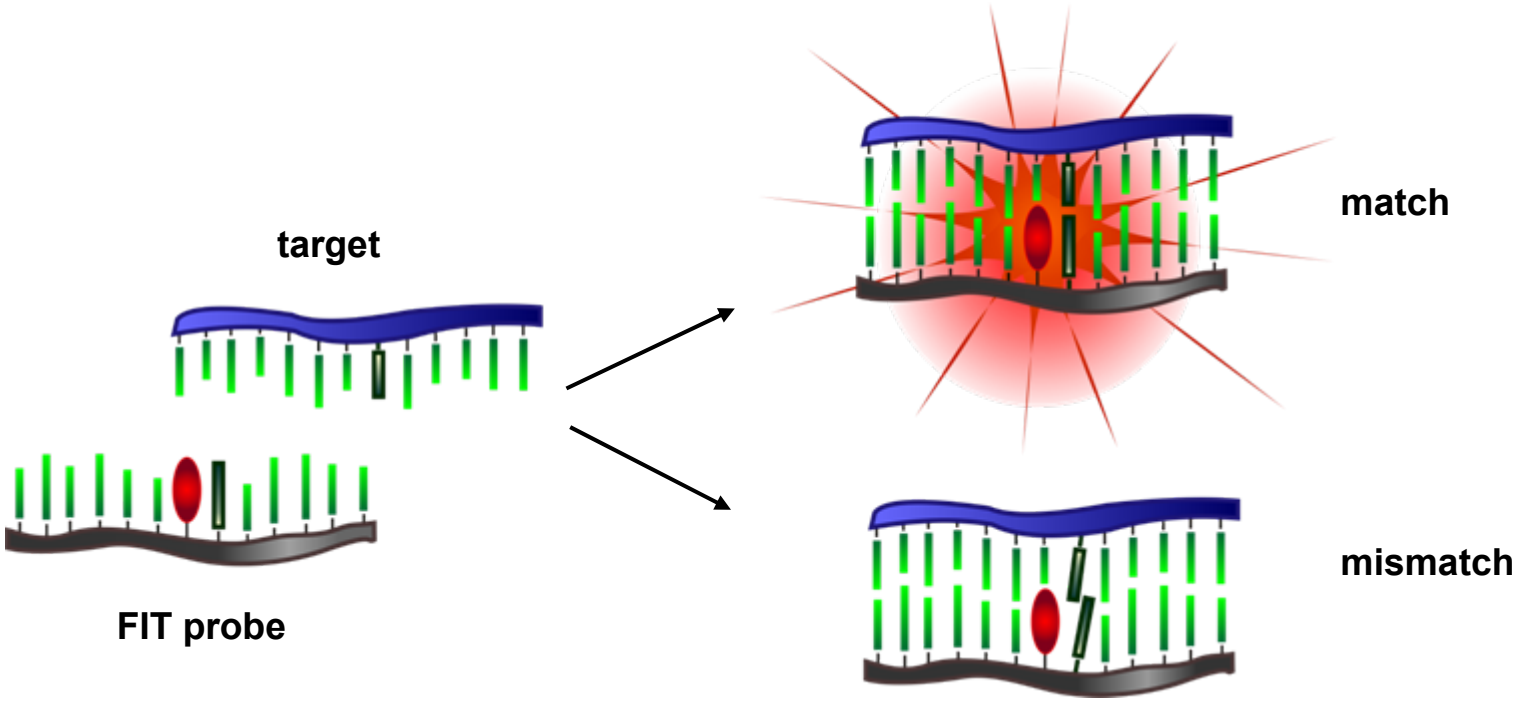
High fidelity probes

<u>Aim</u>: **Improve target specificity**

1) improve hybridization selectivity of probes

2) add another sequence discriminating event

FIT probes



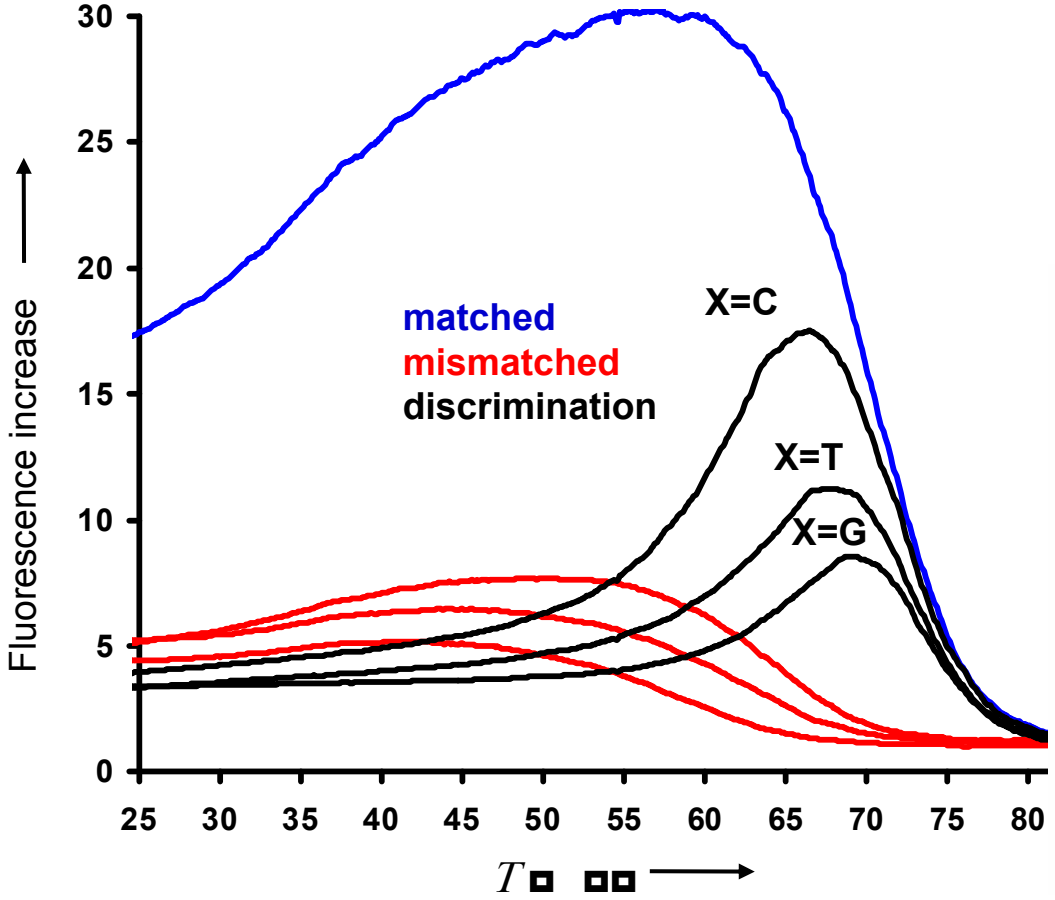
Forced intercalation (FIT): **fluorescent dye serves as base surrogate**

responds to perturbations

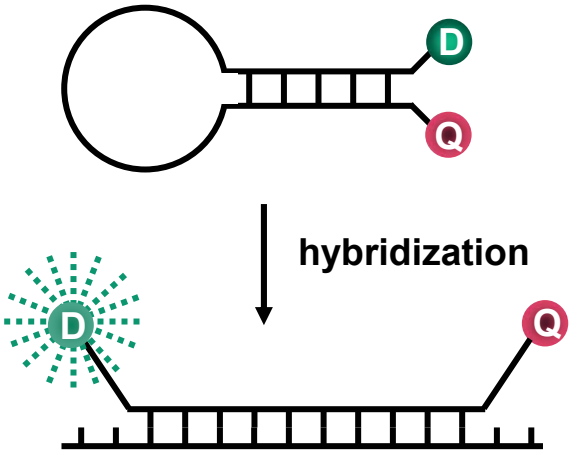
Temperature dependence of fluorescence increase

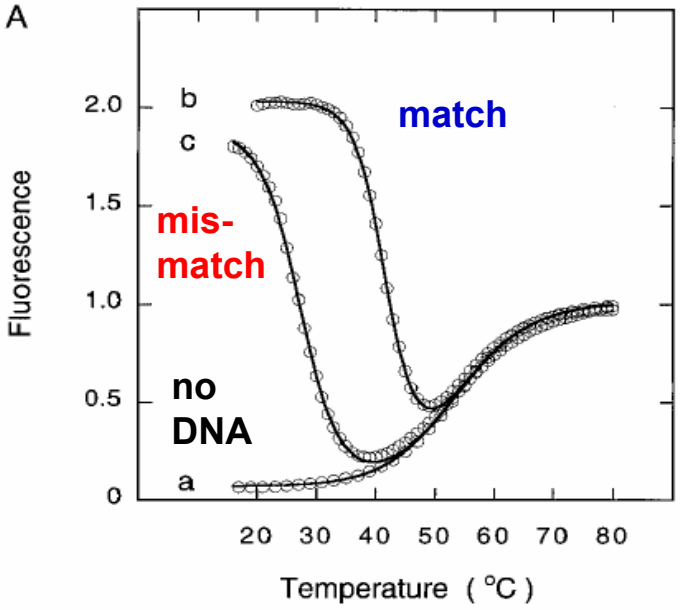
FIT PNA

5´-CGGCT XT TACGGC-3´
C-Gly-gccga tO atgccg-*N*



Molecular Beacon



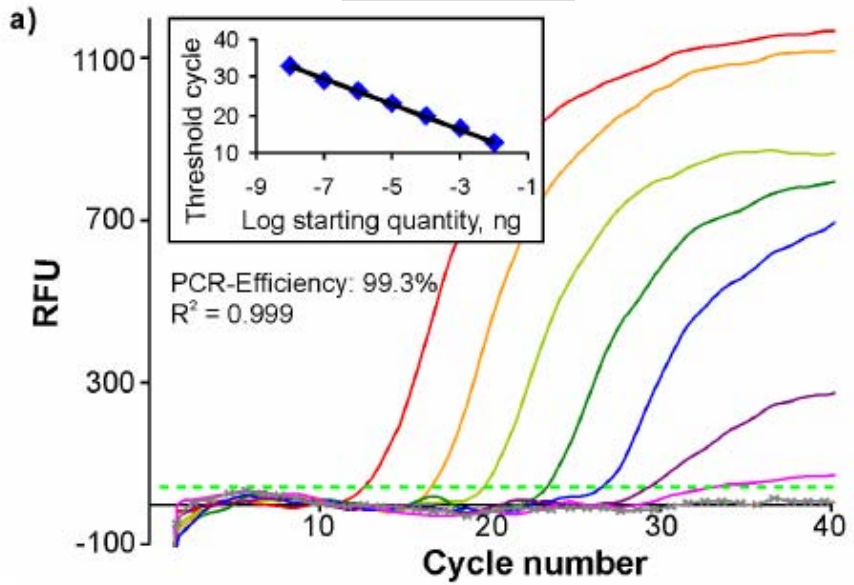


Tyagi, Kramer, *Nat. Biotechnol.* **1996**, *14*, 303

Detection of Her2-neu

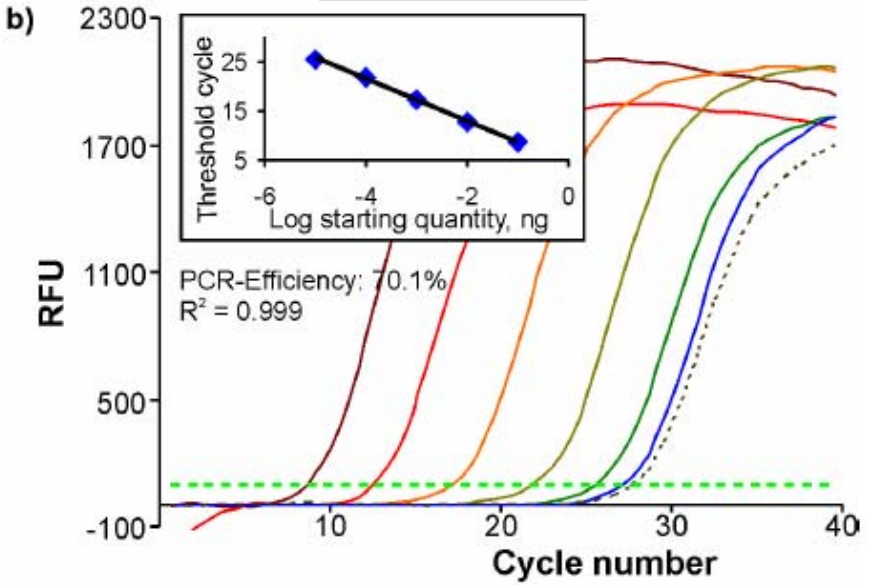
FIT probe: ^{AcNH}gtactg Orn(**T0**) aagcct-Gly^{CONH2}
target DNA : 3′-CATGAC T TTCGGA- 5′

FIT probe



Linear range: 7 orders of magnitude
Sensitivity: 10^2 copies

SYBR-Gold



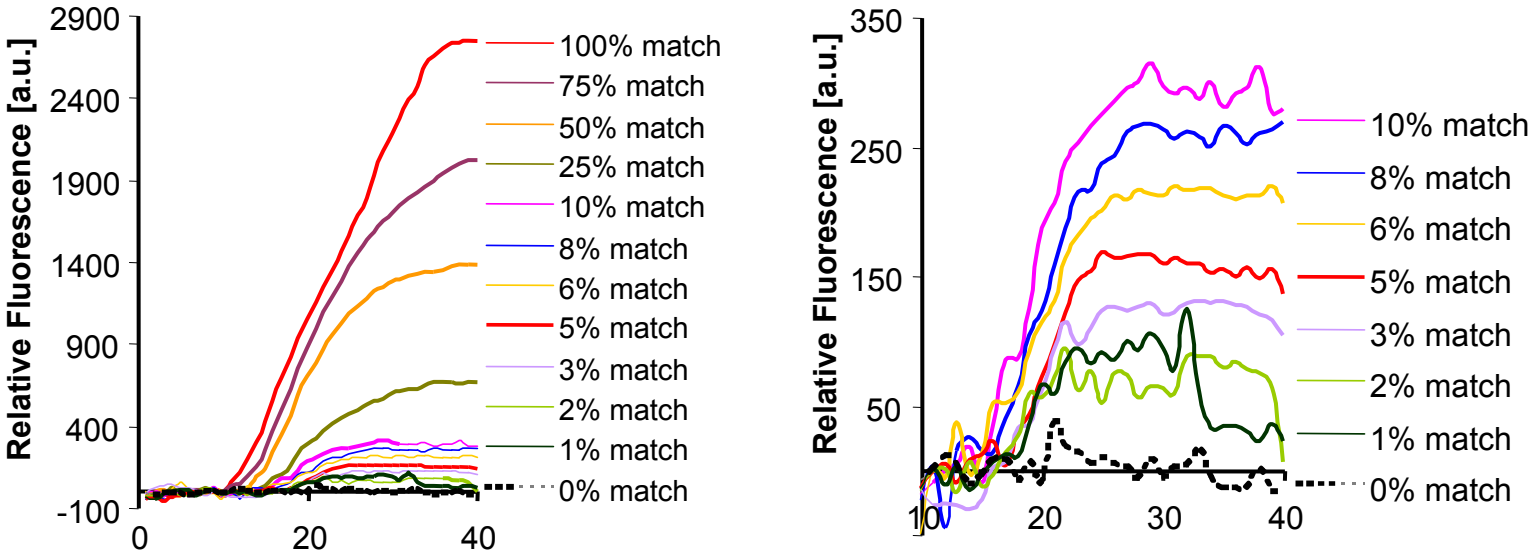
Linear range: 5 orders of magnitude
Sensitivity: 10^5 copies

Improved sensitivity compared to DNA-stain

Socher, Jarikote, Knoll, Röglin, Burmeister, Seitz, *Anal. Biochem.* **2008**, *375*, *2*, 318-330

Single base mutation analysis with wildtype background

Probe: ^{Ac}gccgta**TO**atagccg; **target:** 3‘---CGGCATT**T/A**ATCGGC---5‘



FIT Probes: **High sequence specifity**

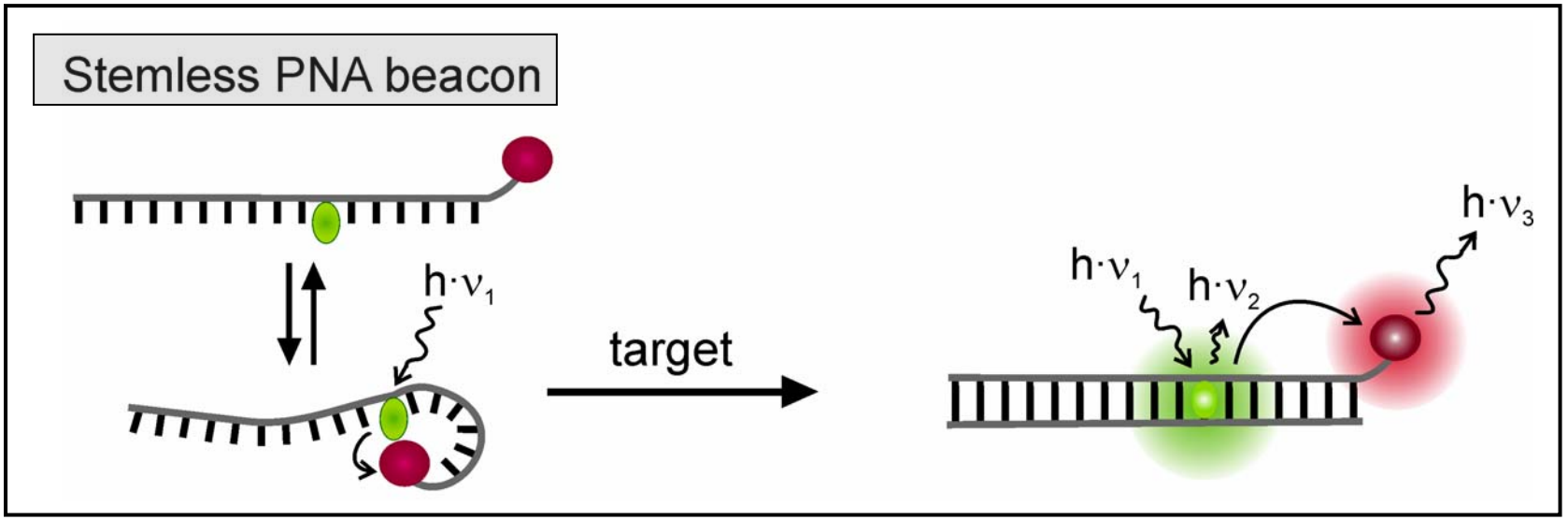
$(F_{ma}-F_0)/(F_{mi}-F_0) \leq 10$ at non-stringent conditions

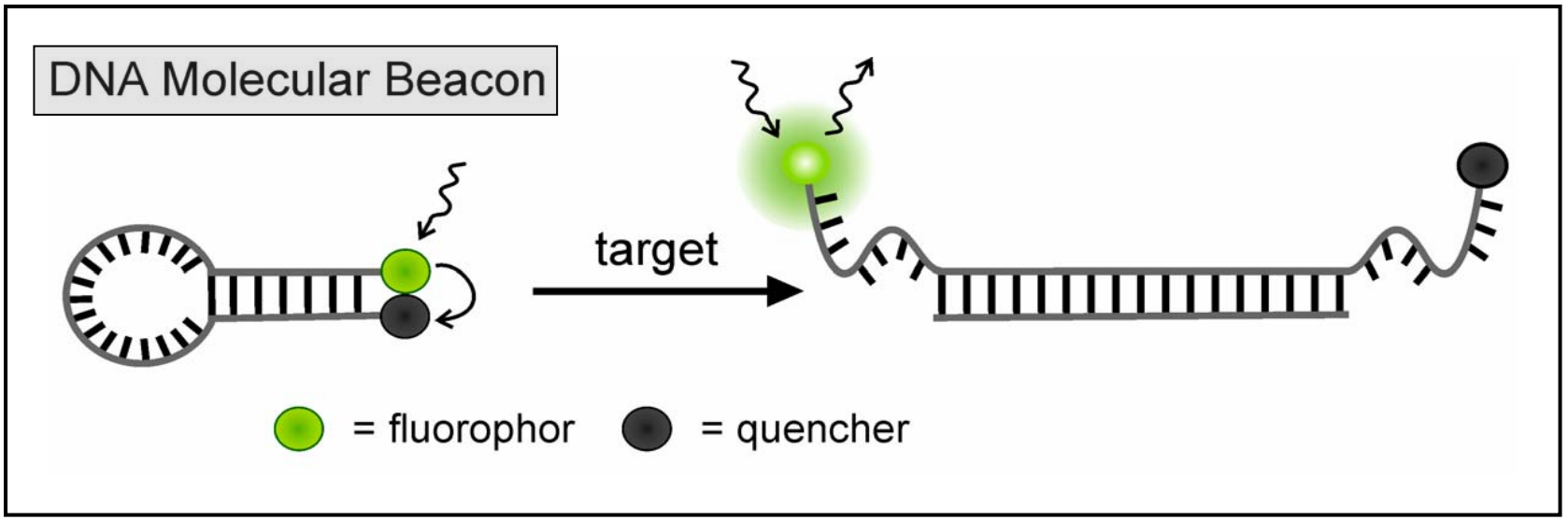
$(F_{ma}-F_0)/(F_{mi}-F_0) \leq 200$ at stringent conditions

High dynamic range of fluorescence signaling

$F_{ma}/F_0 \leq 30$

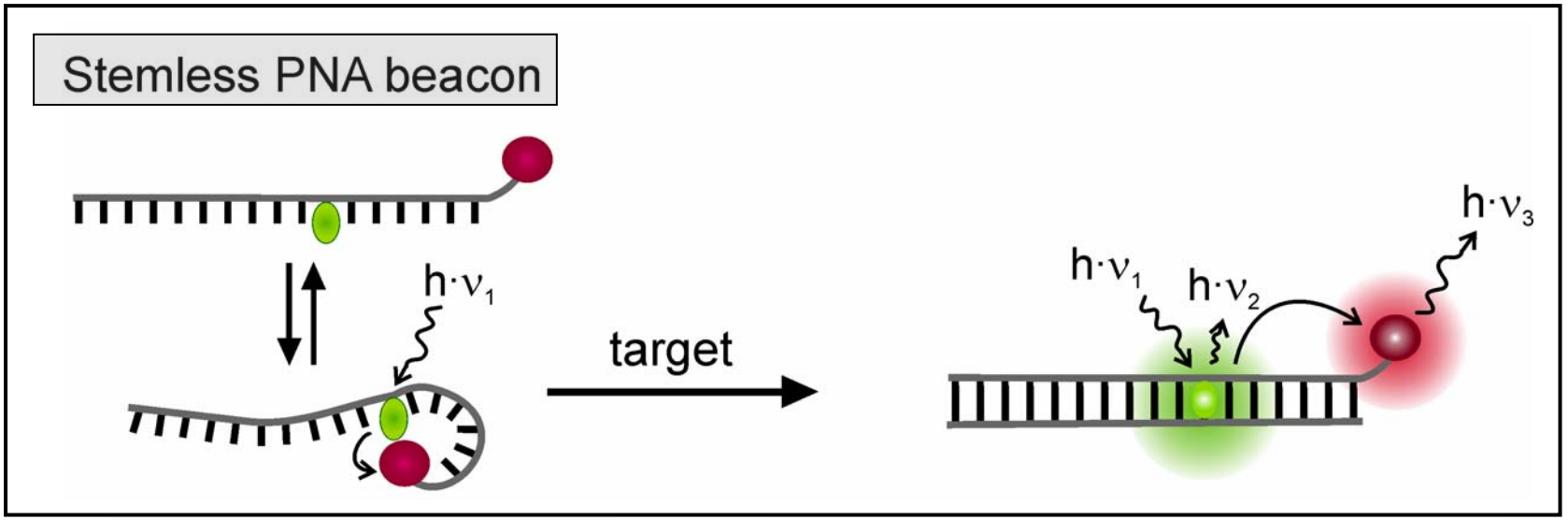
Next generation: Low noise stem-less PNA beacons

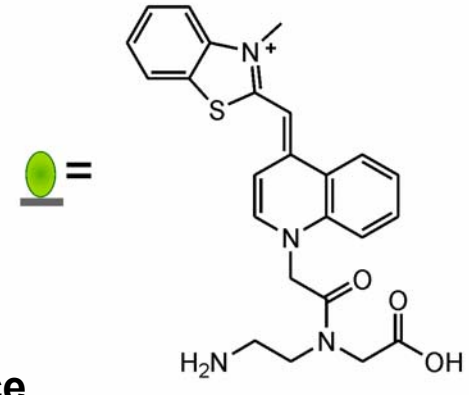




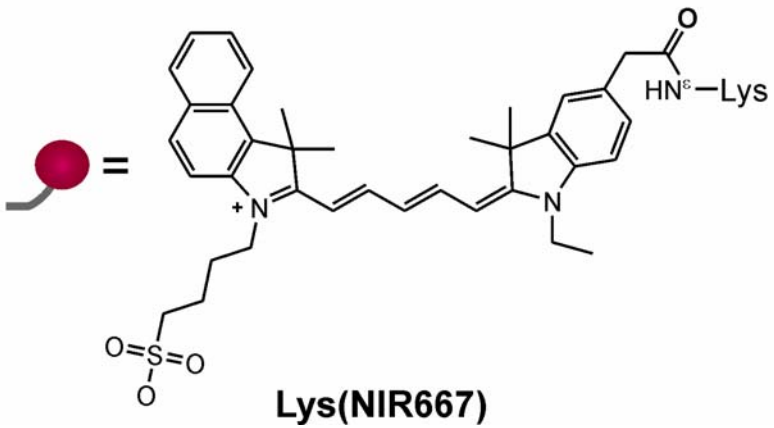
Socher, Bethge, Knoll, Jungnick, Herrmann, Seitz, *Angew. Chem. Int. Ed.* **2008**, *47*, 9555-9559

Next generation: Low noise stem-less PNA beacons





Aeg(TO)



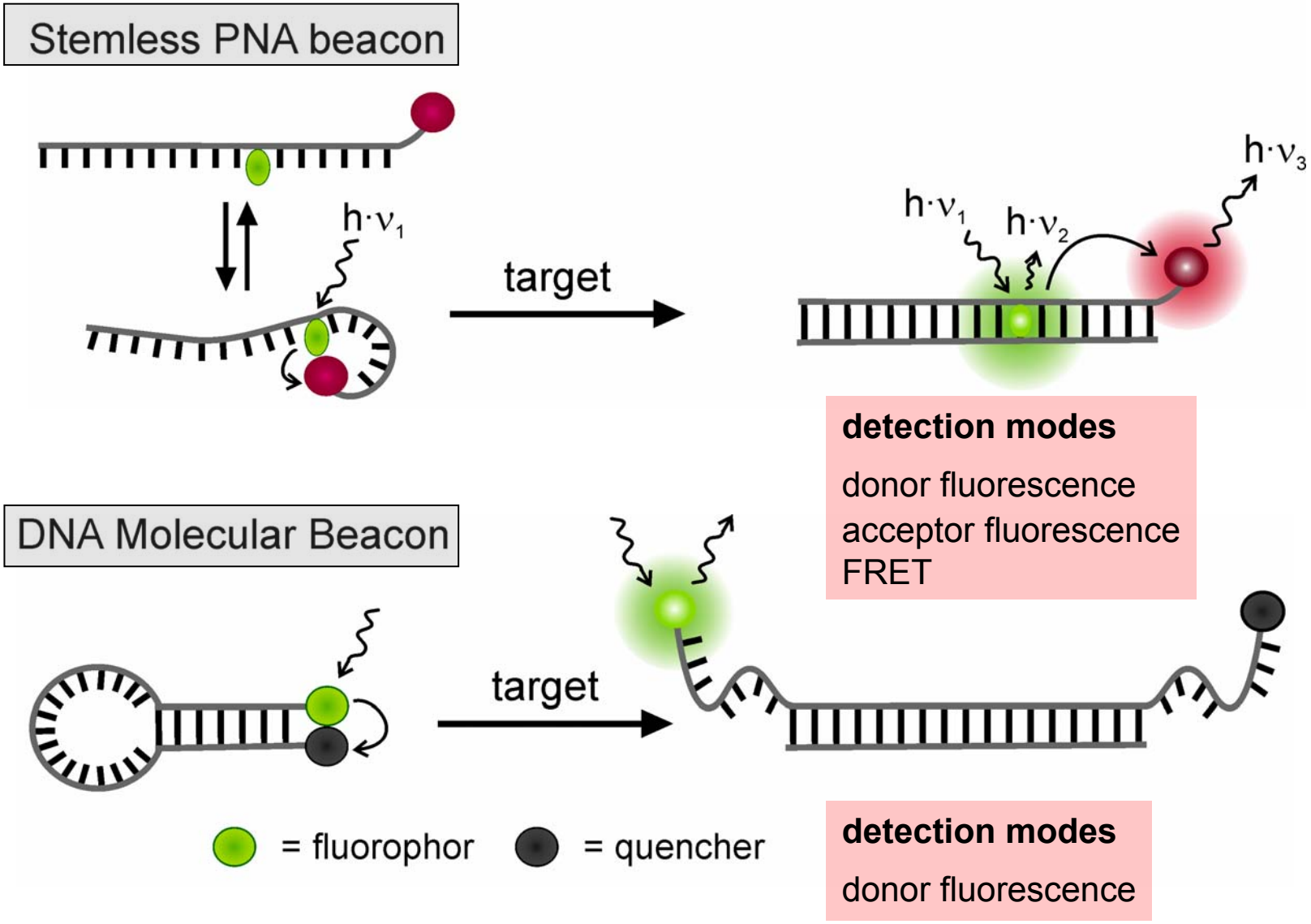
Lys(NIR667)

Fluorescence

Single strand:	**low** → twisting, FRET, contact	**low** → contact
Double strand:	**high**	**high** → FRET

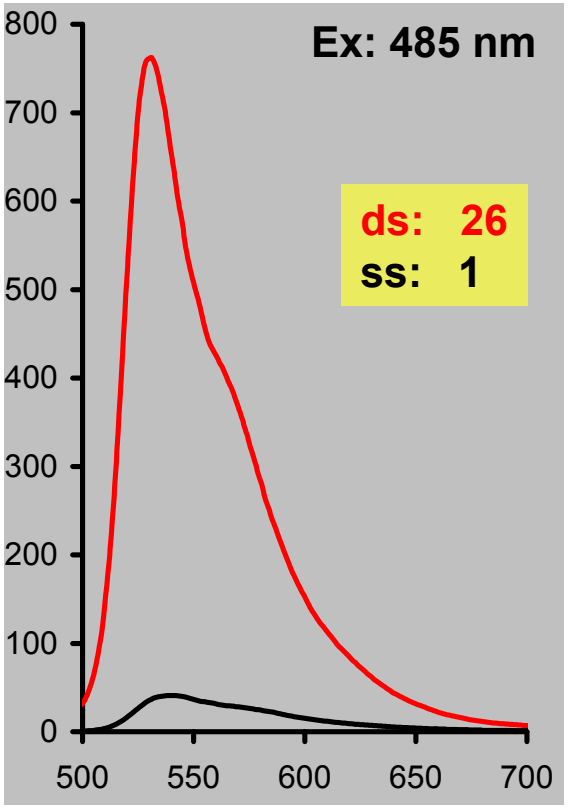
Socher, Bethge, Knoll, Jungnick, Herrmann, Seitz, *Angew. Chem. Int. Ed.* **2008**, *47*, 9555-9559

Next generation: Low noise stem-less PNA beacons

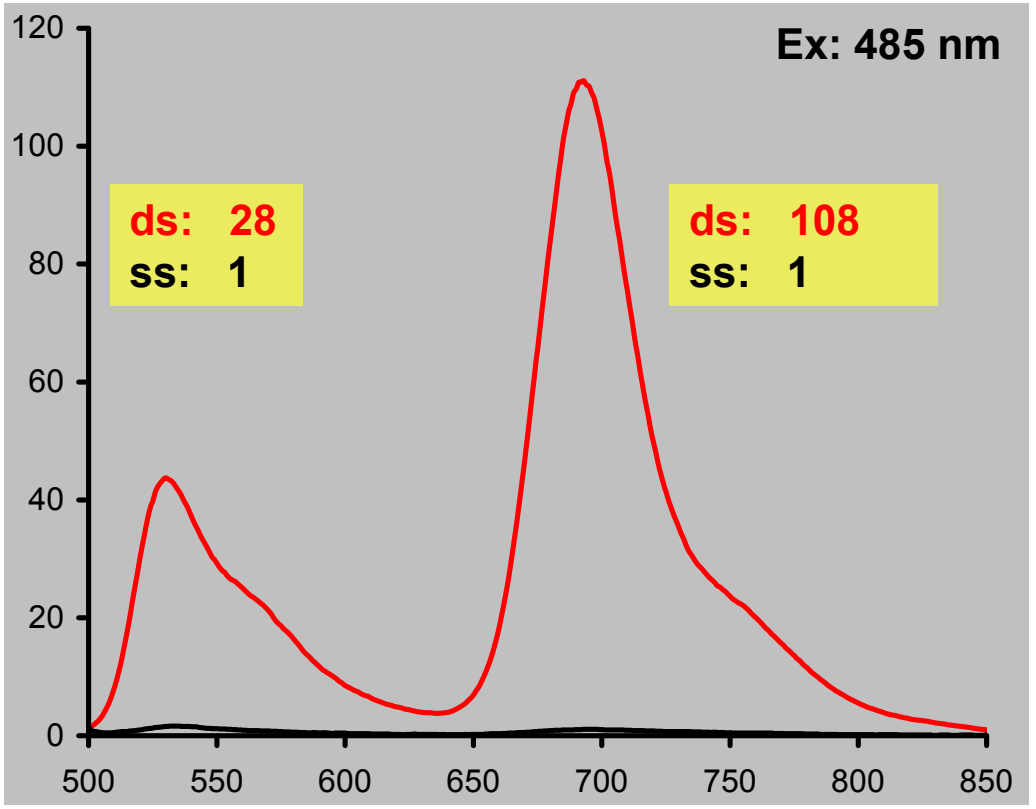


Stem-less PNA beacons provide for high sensitivity

5´-CGGCTATTTAGGC-3´
C-gccgata**O**atgccg-*C*



5´-CGGCTATTTAGGC-3´
C-gccgata**O**atgccg-**NIR**

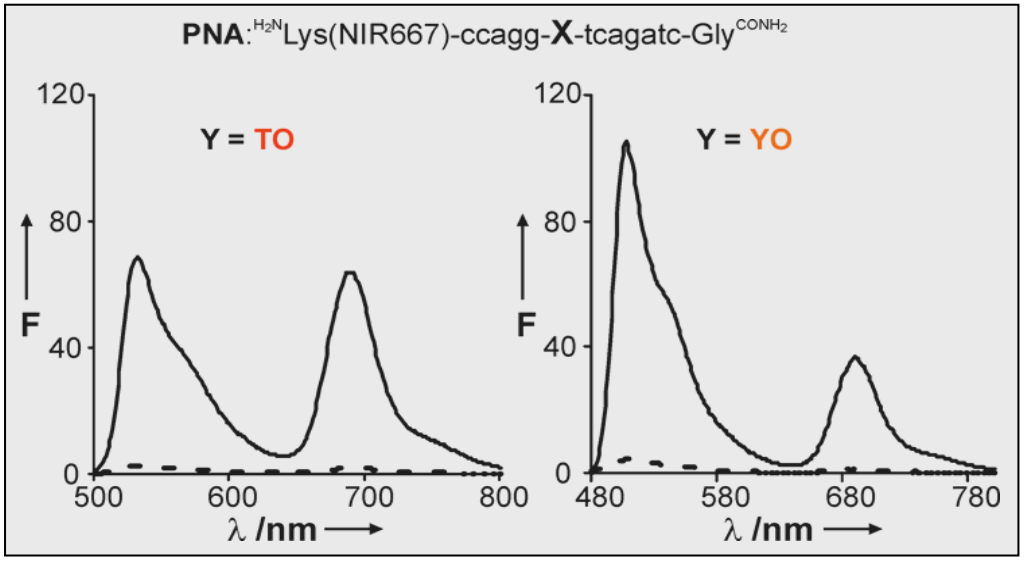


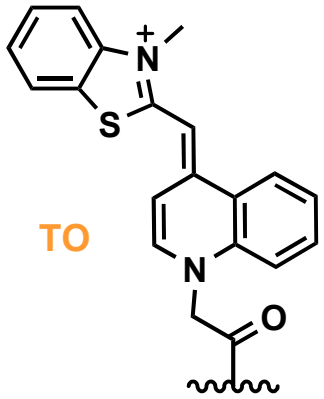
Socher, Bethge, Knoll, Jungnick, Herrmann, Seitz, *Angew. Chem. Int. Ed.* **2008**, *47*, 9555-9559

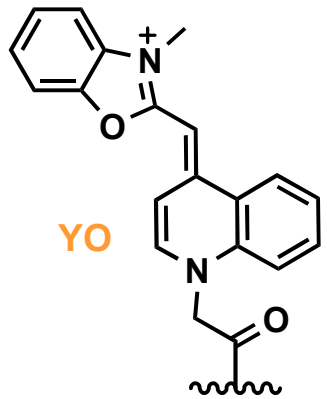
RNA Detection

Short synthetic RNA

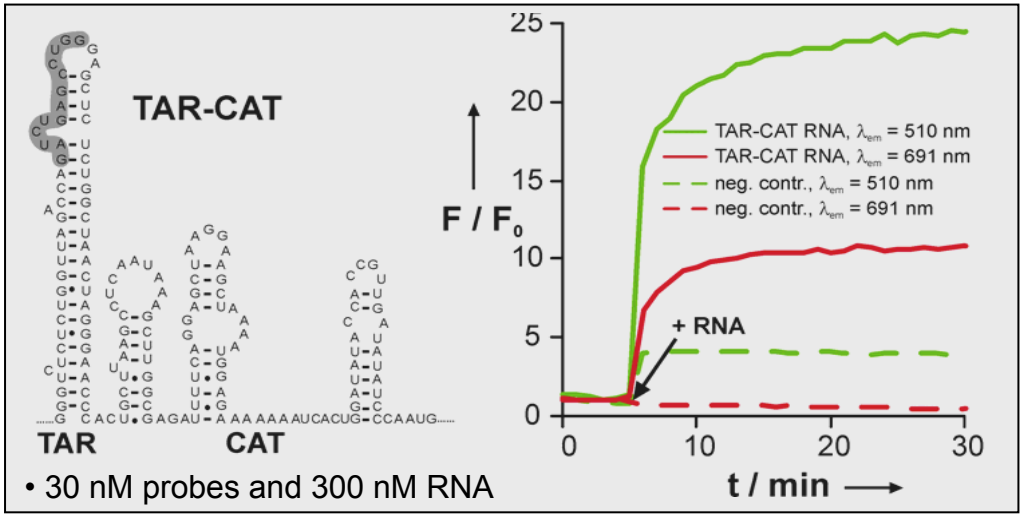
• 1µM probes and 2µM RNA





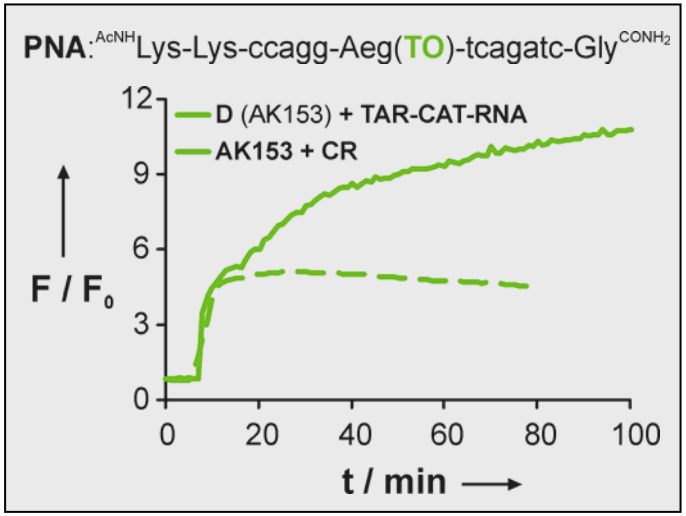


In vitro transcribed 650 nt RNA

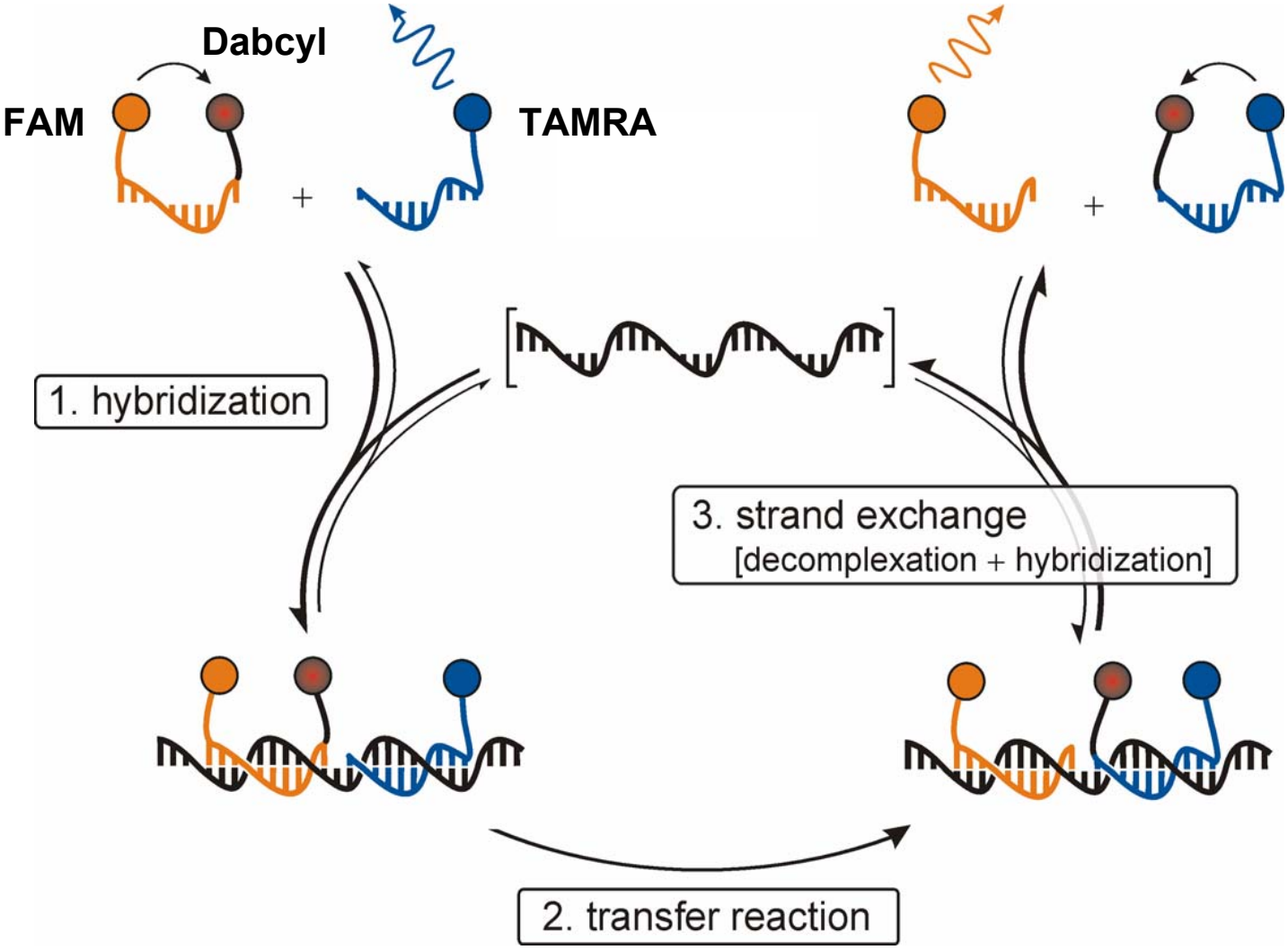


• 30 nM probes and 300 nM RNA

Compared to FIT-probe



DNA-catalyzed transfer

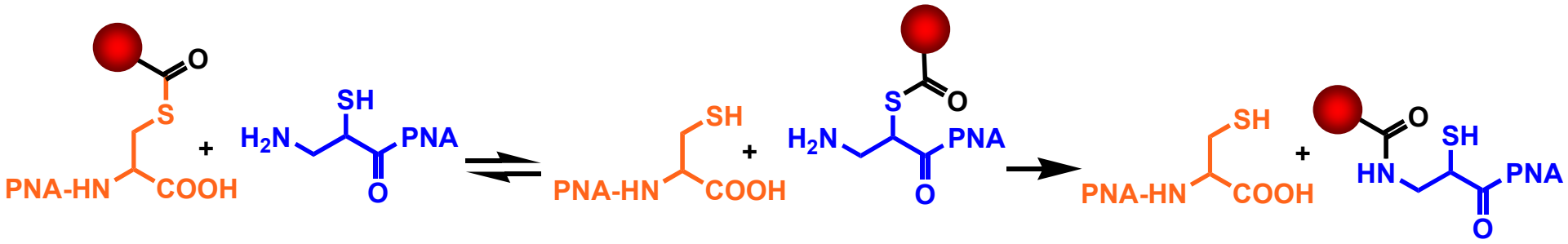


Großmann, Seitz, *J. Am. Chem. Soc.* **2006**, *128*, 15596-15597

The chemistry

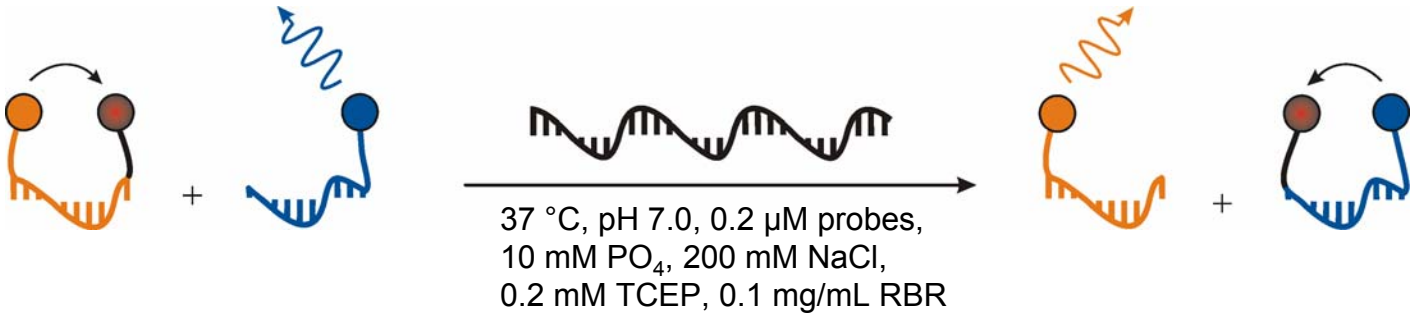
Transfer: **Entropy** **3** ⟶ **3**

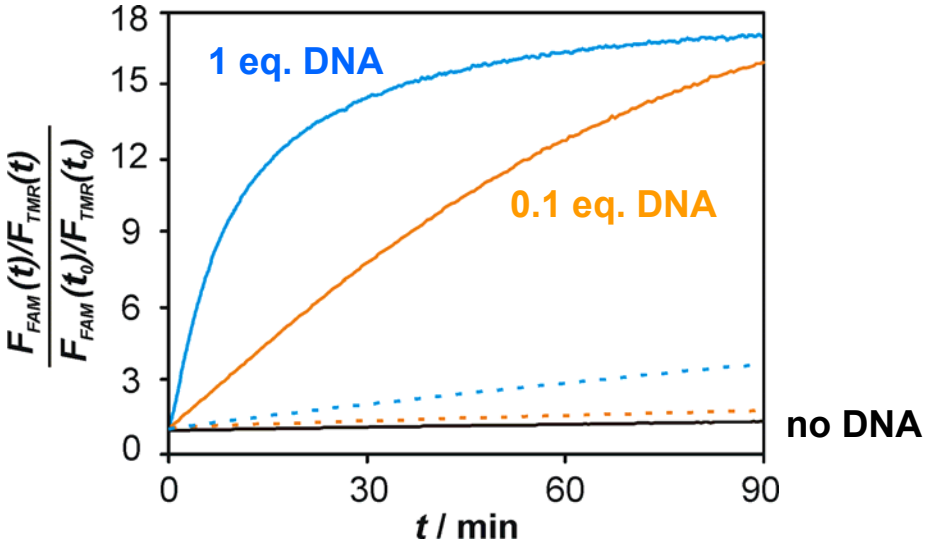
Affinity T_M = 38°C T_M = 38°C ?

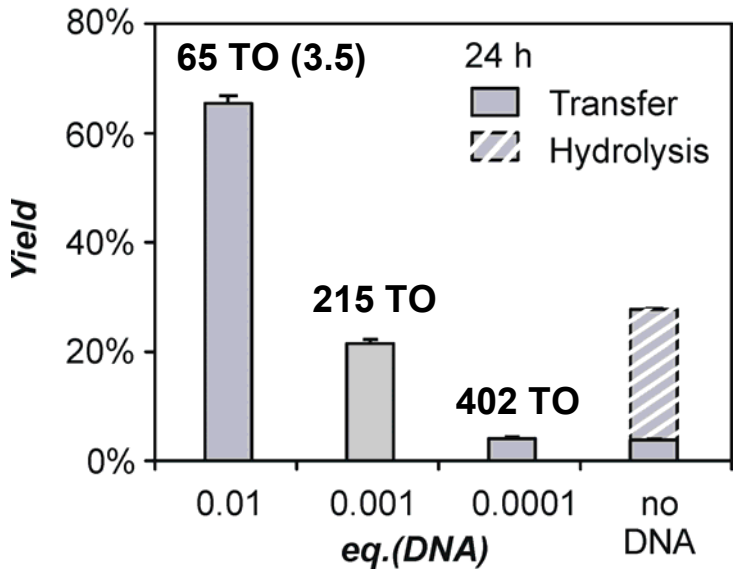


Grossmann, Seitz, *J. Am. Chem. Soc.* **2006**, *128*, 15596-15597

DNA-catalyzed transfer

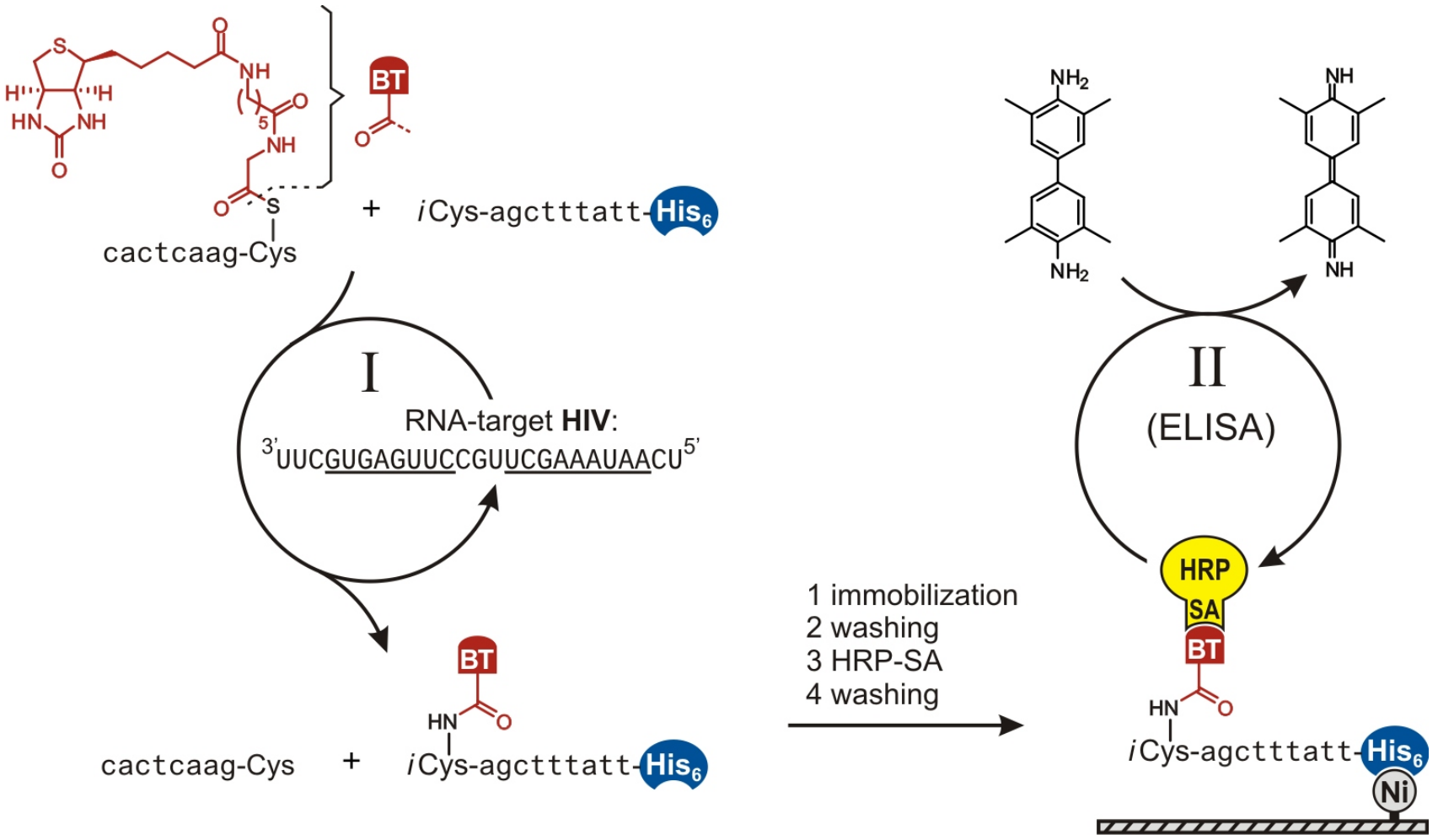






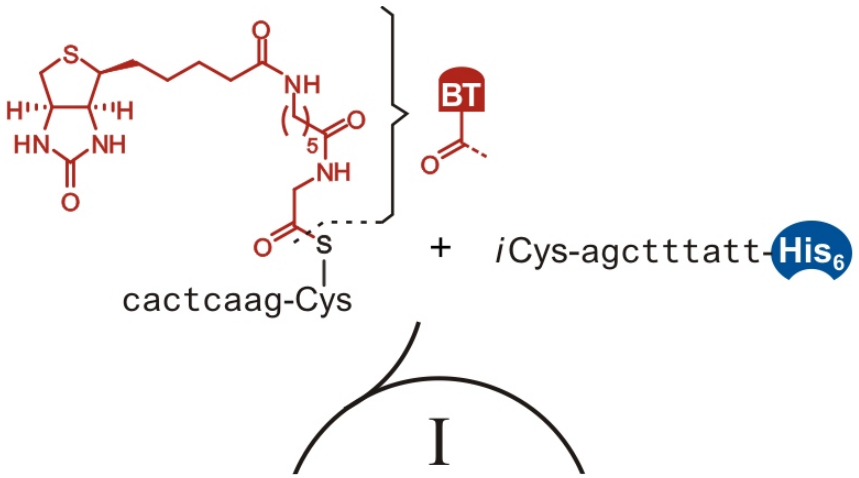
Grossmann, Seitz, *J. Am. Chem. Soc.* **2006**, *128*, 15596-15597

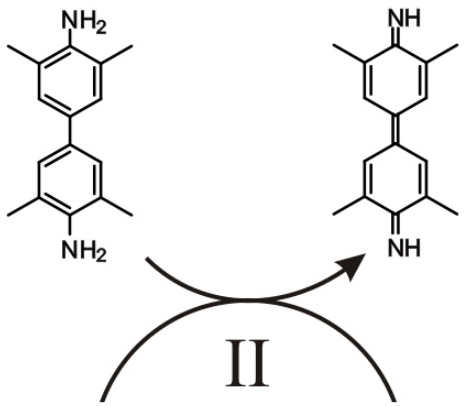
Amplified detection of DNA and RNA



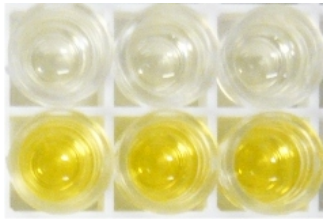
Grossmann, Röglin, Seitz, *Angew.Chem. Int. Ed.* **2008**, *47*, 7119

Amplified detection of DNA and RNA



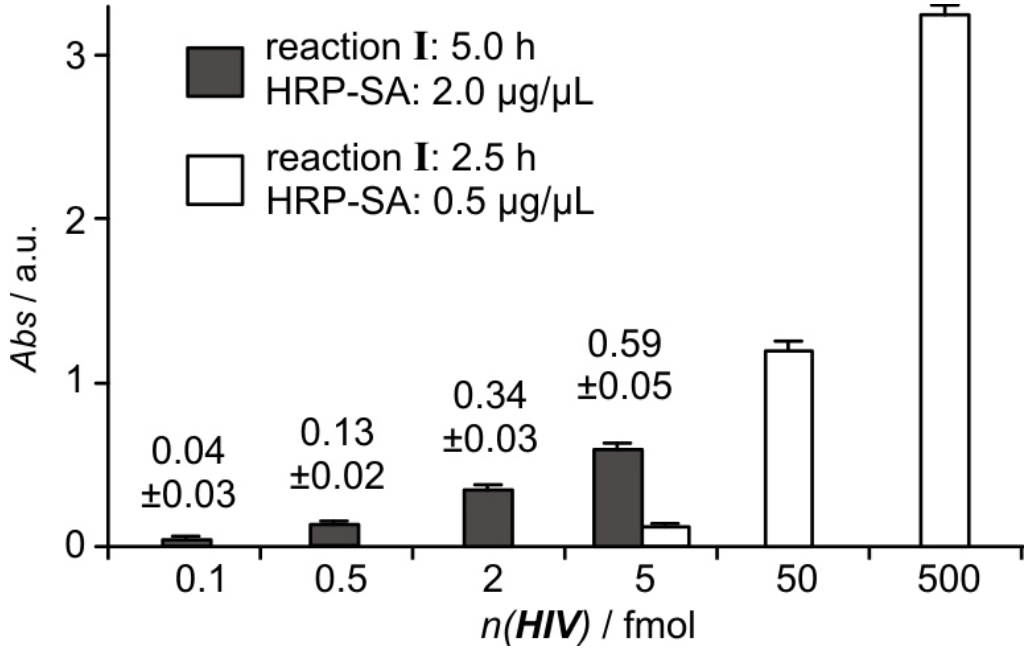


after addition of 1M H_2SO_4:

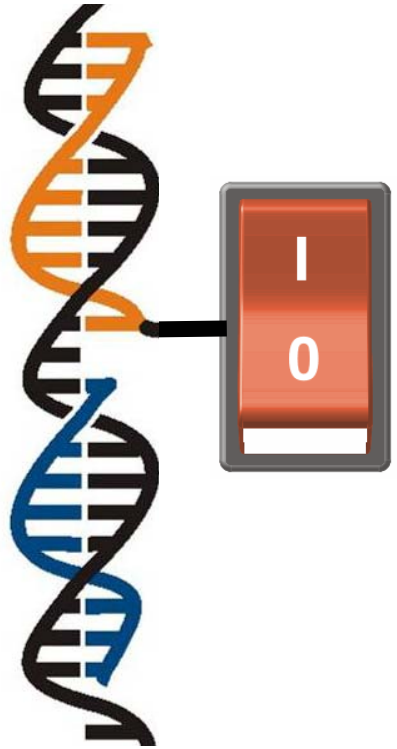


no RNA

50 fmol RNA



Switching with nucleic acid hybridization

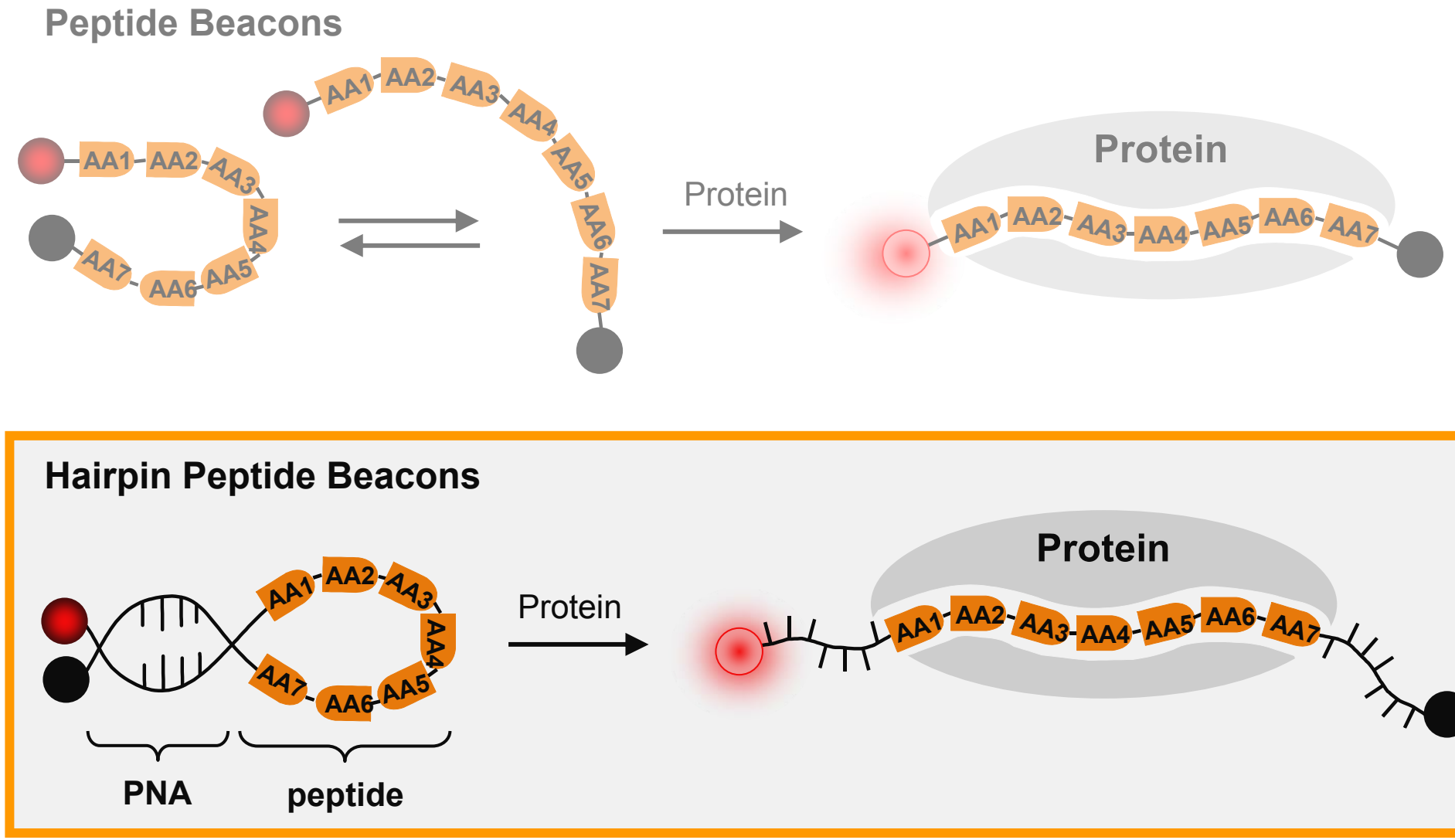


Who pushs the button?

→ Response

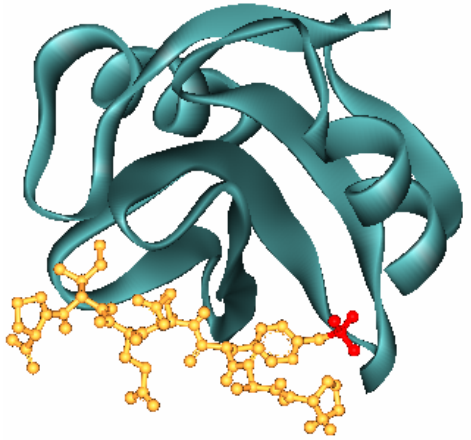
DNA/RNA	→ Light
Protein	→ Light
DNA/RNA	→ Protein activity

Hairpin Peptide Beacons



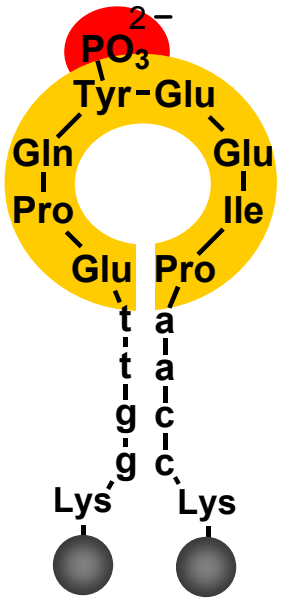
Thurley, Röglin, Seitz, J. Am. Chem. Soc. ***2007****, 129, 12693-12695*

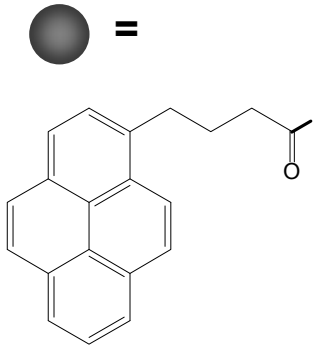
Target: SH2 domain of Src kinase

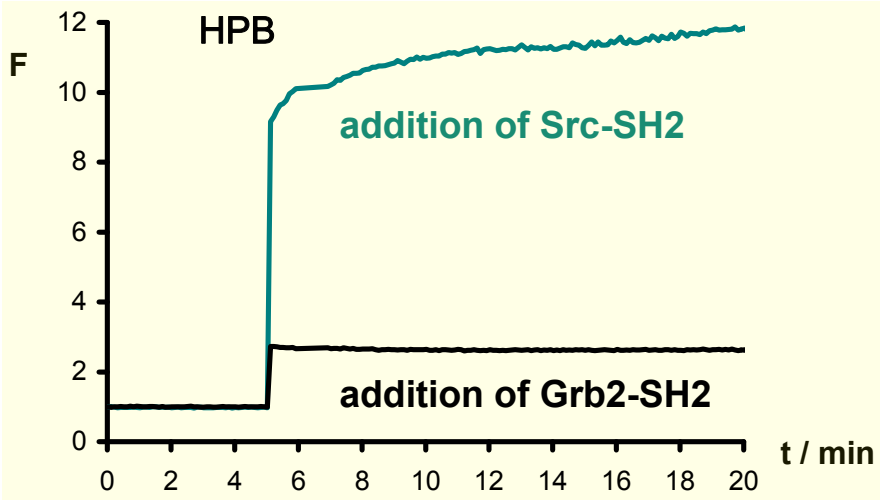


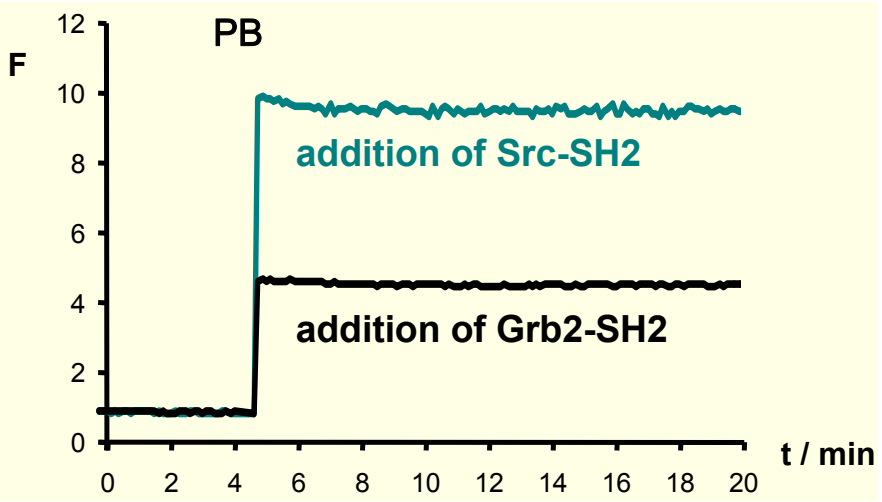
Src-SH2 binds peptide in extended conformation

design, synthesis



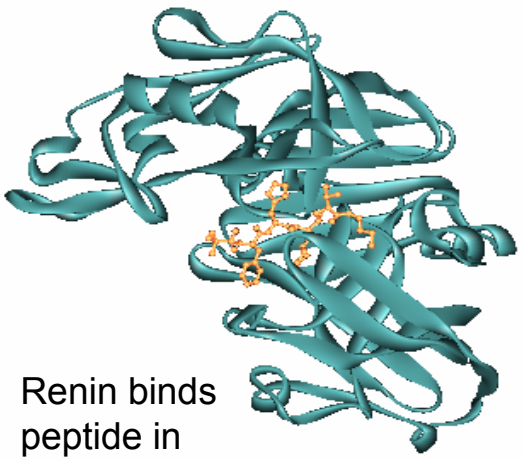




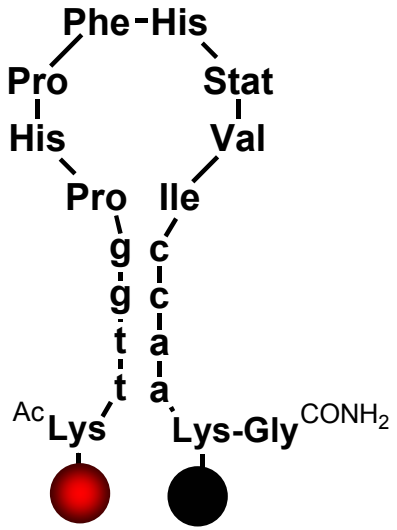


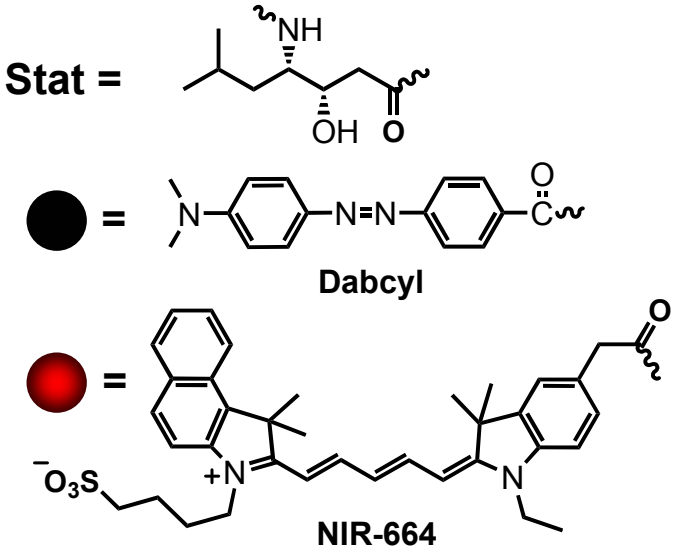
Thurley, Röglin, Seitz, *J. Am. Chem. Soc.* ***2007***, *129, 12693-12695*

Target: active site of protease renin

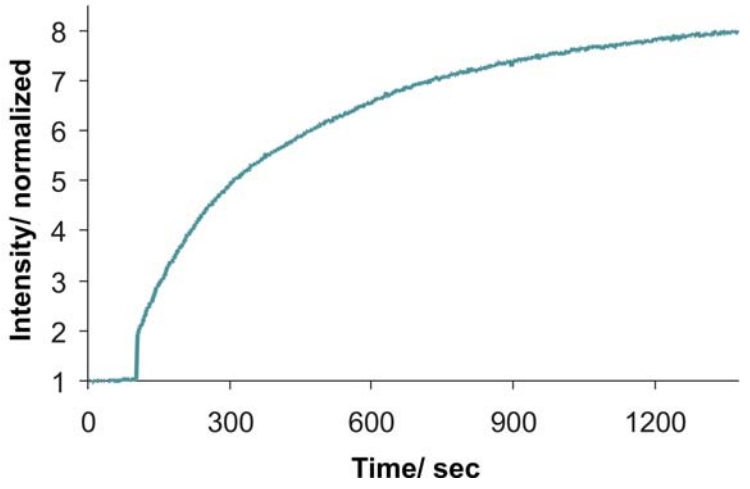


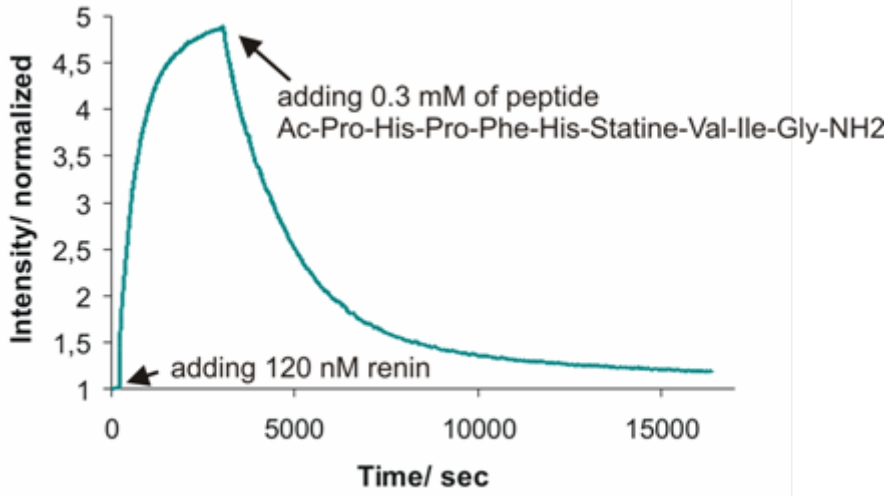
Renin binds peptide in extended conformation





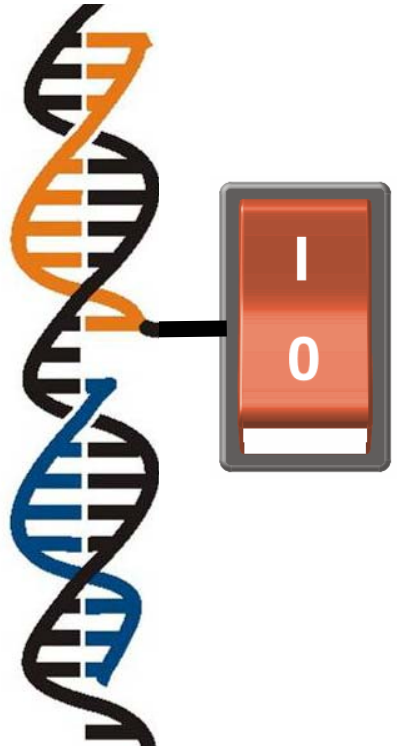
Reversible binding, not cleaved ⇒ increase and decrease of protein activity can be measured





Thurley, Röglin, Seitz, *J. Am. Chem. Soc.* ***2007***, *129, 12693-12695*

Switching with nucleic acid hybridization

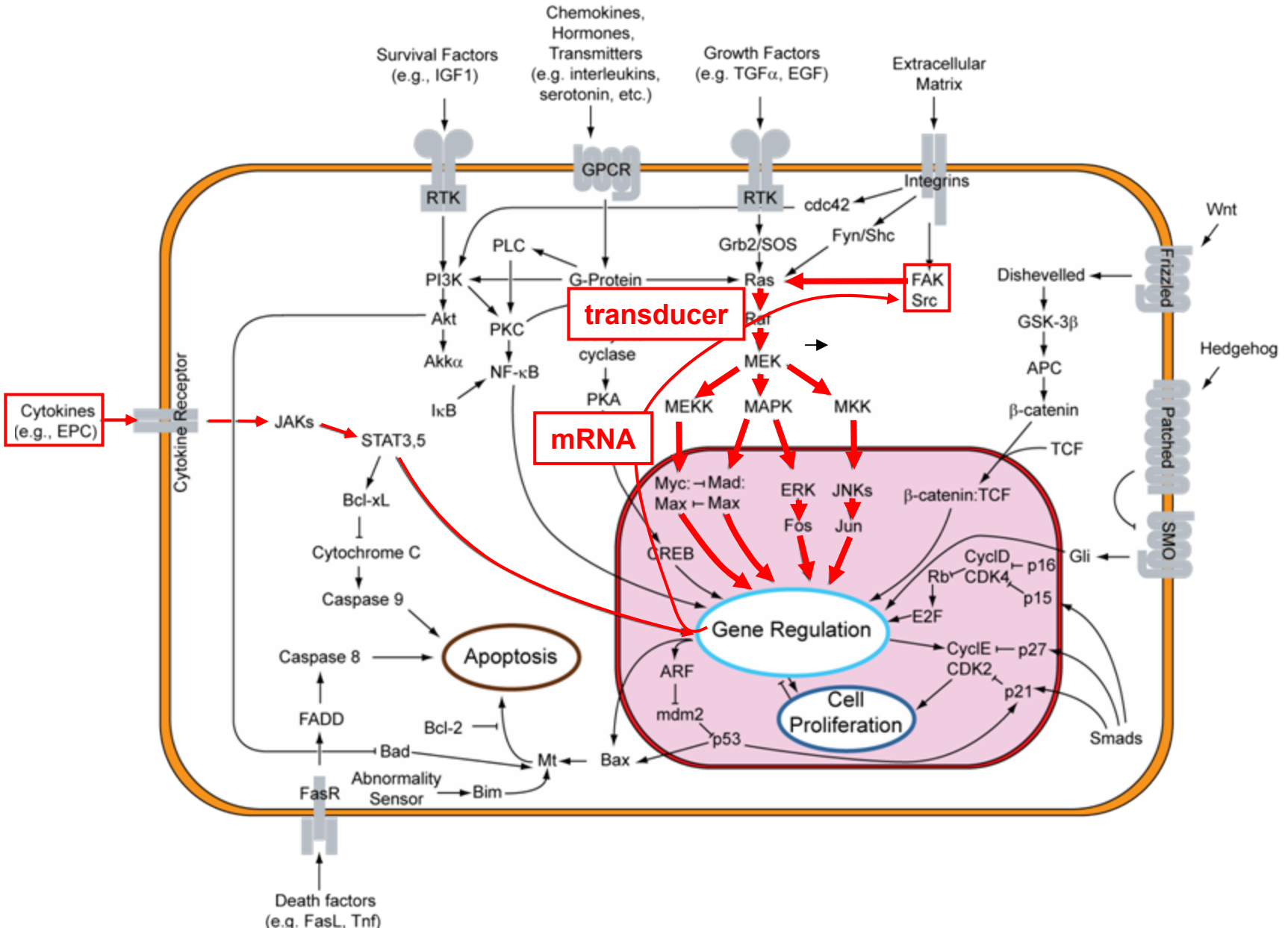


Who pushs the button?

→ Response

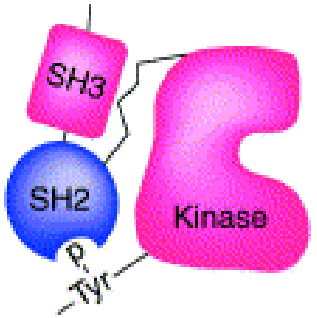
DNA/RNA	→ Light
Protein	→ Light
DNA/RNA	→ Protein activity

Crosslinking signal transduction pathways

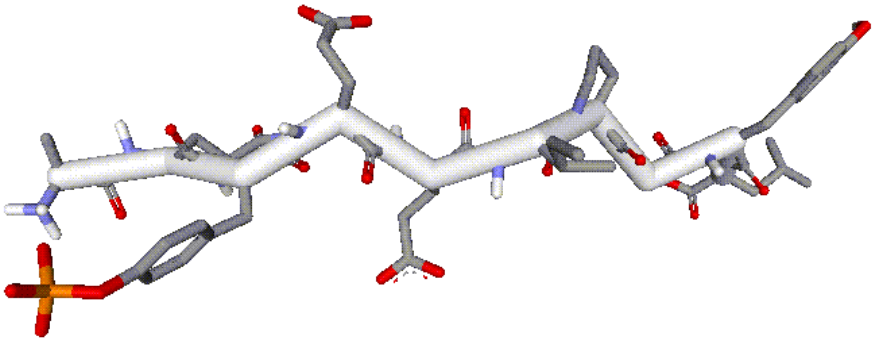


Bioactive peptide conformation of SH2 ligands

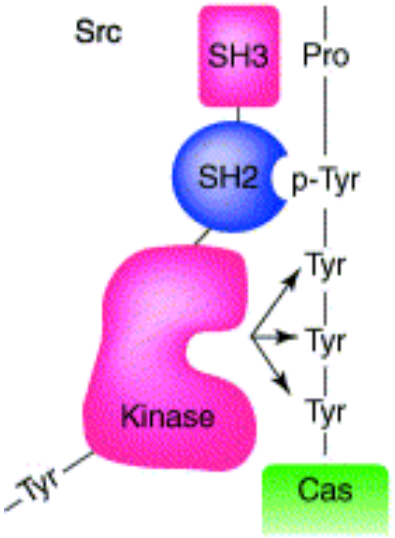
Src SH2 binds extended conformation



inactive



Ala-Gln-pTyr-Glu-Glu-Ile-Pro-Gly-Tyr-Leu

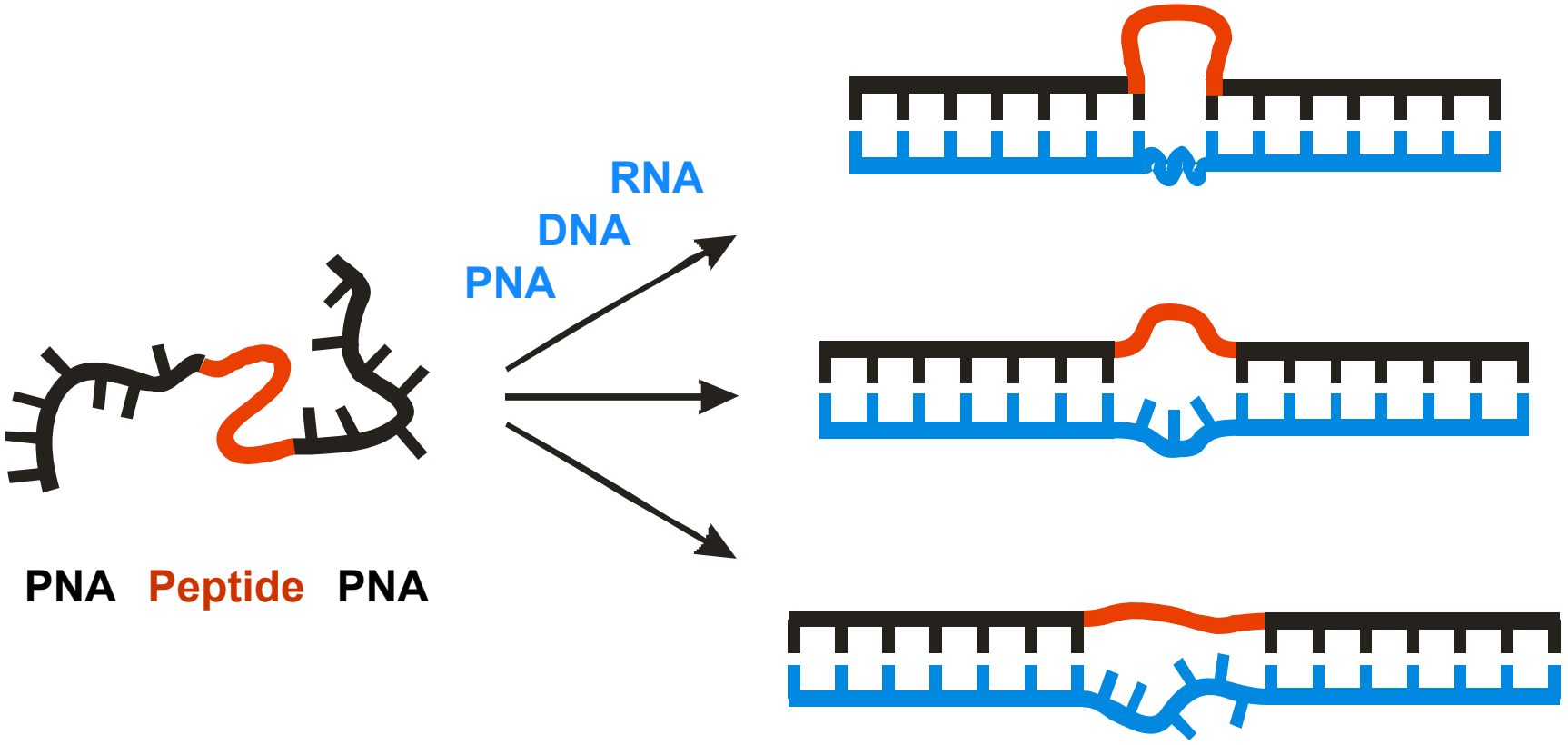


active

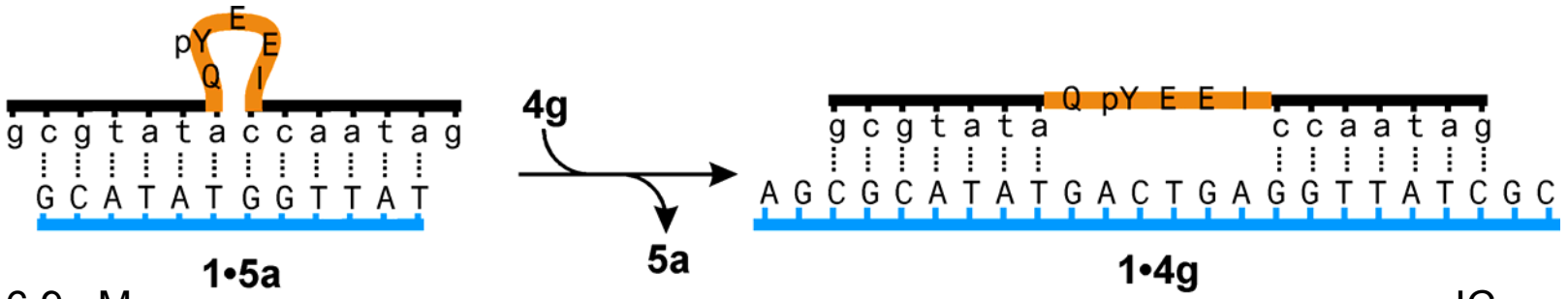
Wakesman et al, *Cell* **1993**, *72*, 779

Pawson et al, *TICB* **2001**, *11*, 504

Intermolecular hybridization

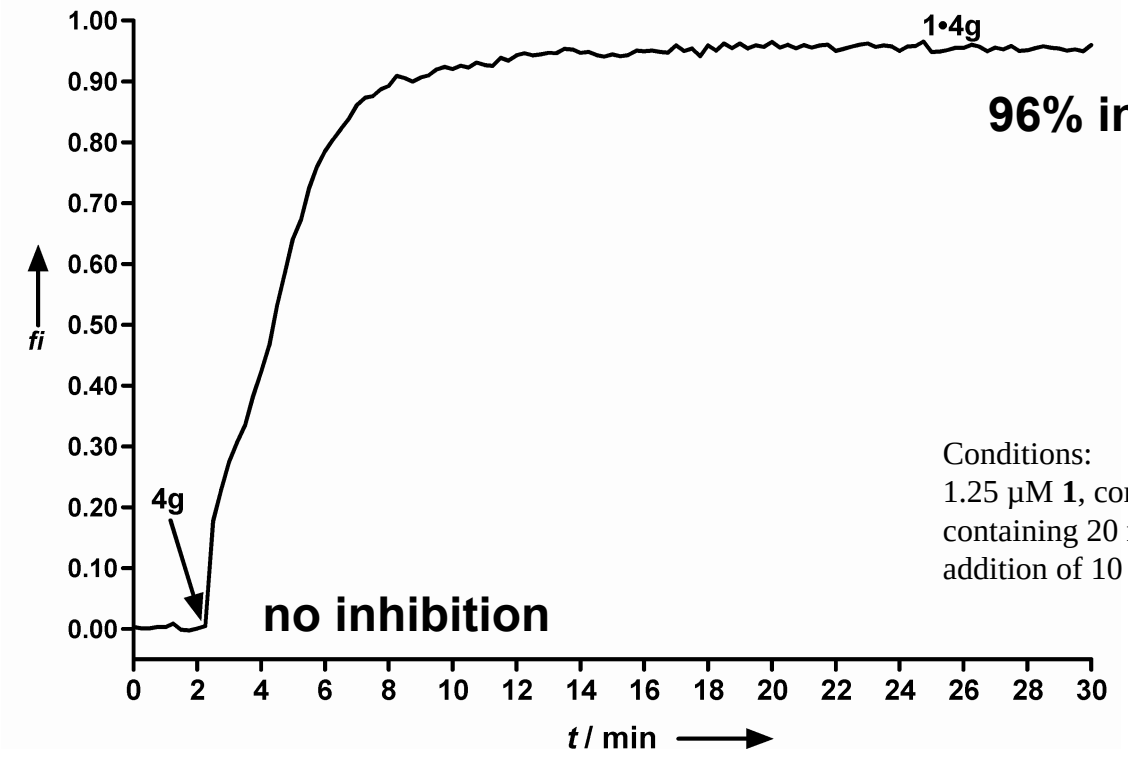


Hybridization triggers inhibition



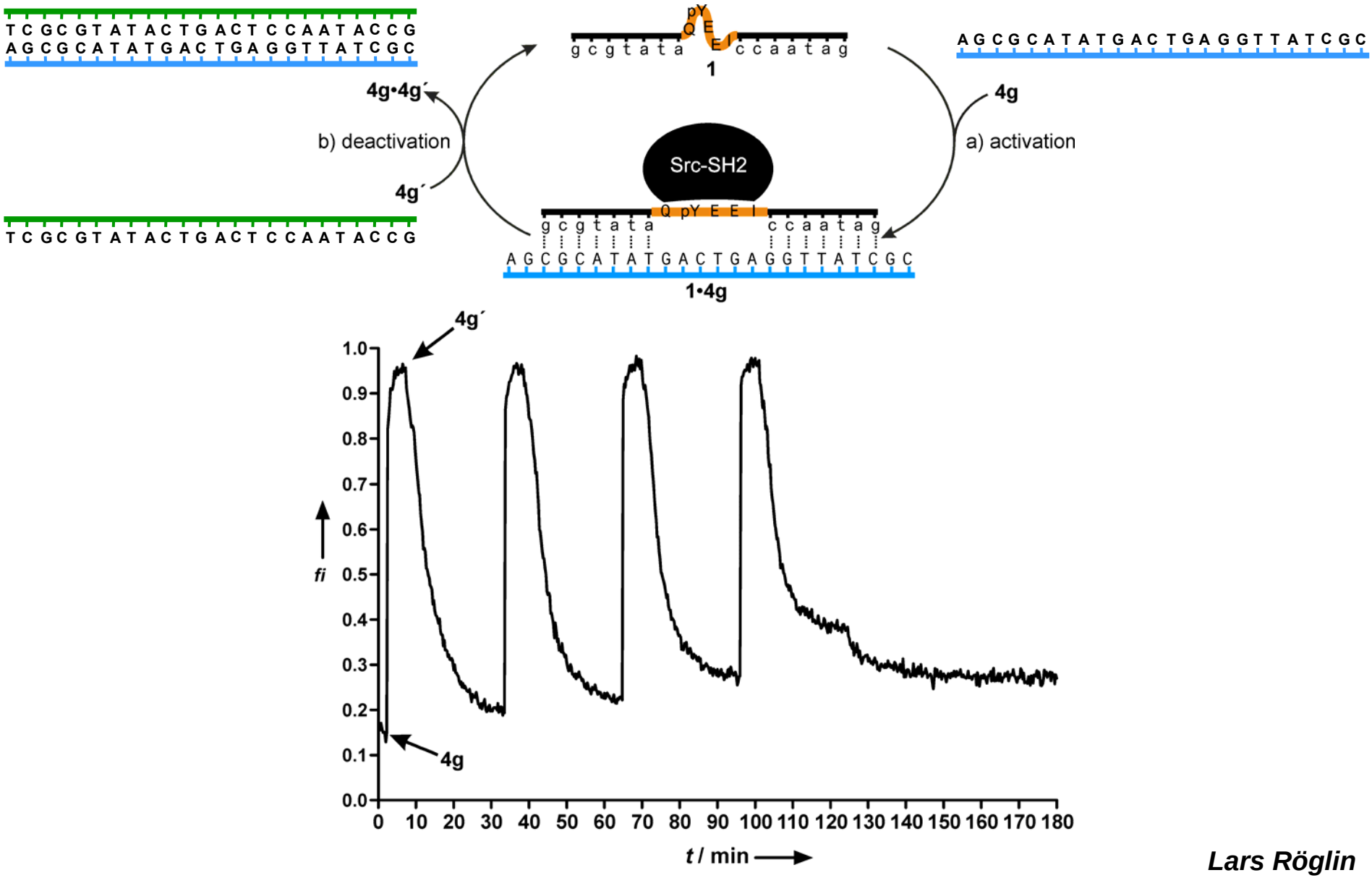
IC_{50} = 6.9 µM

IC_{50} = 0.5 µM



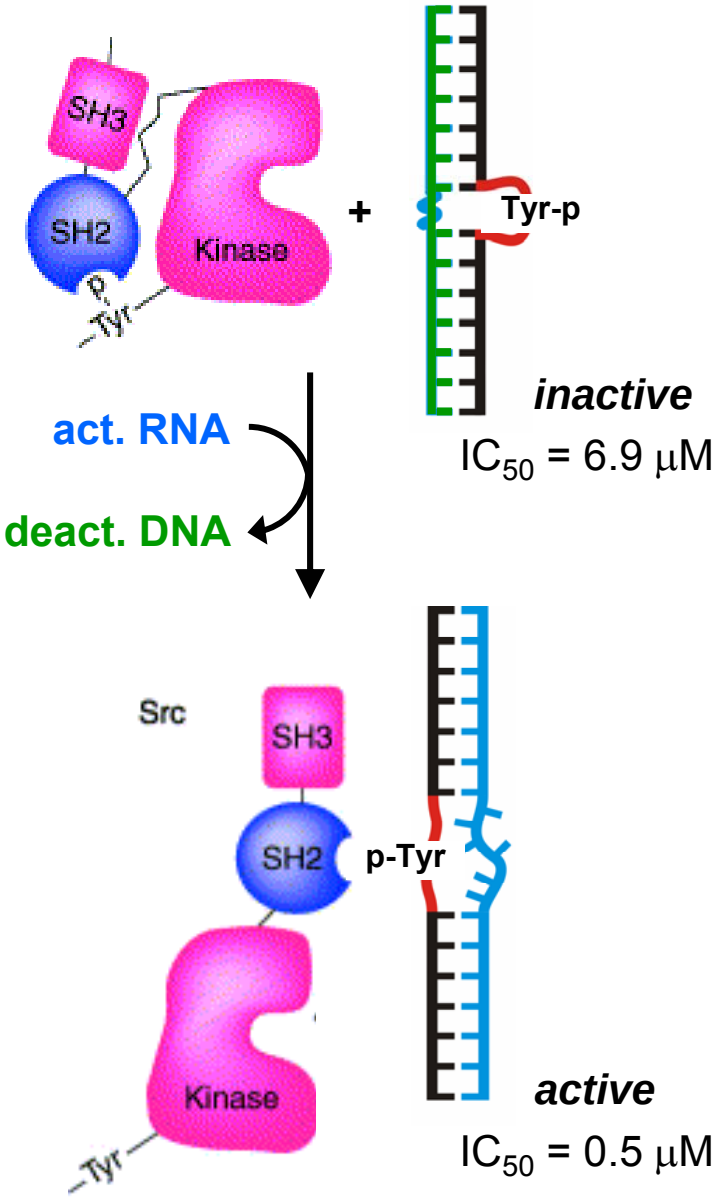
Conditions:
1.25 µM **1**, complexed with 2 µM **5a** in buffer containing 20 nM **6** and 700 nM GST-Src-SH2, addition of 10 µM **4g** after 2.25 minutes.

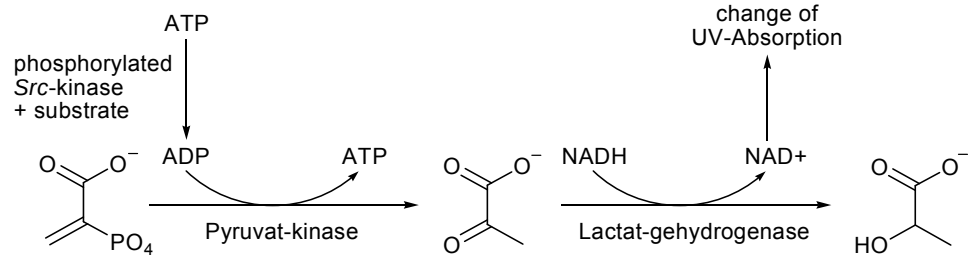
Repeated switching

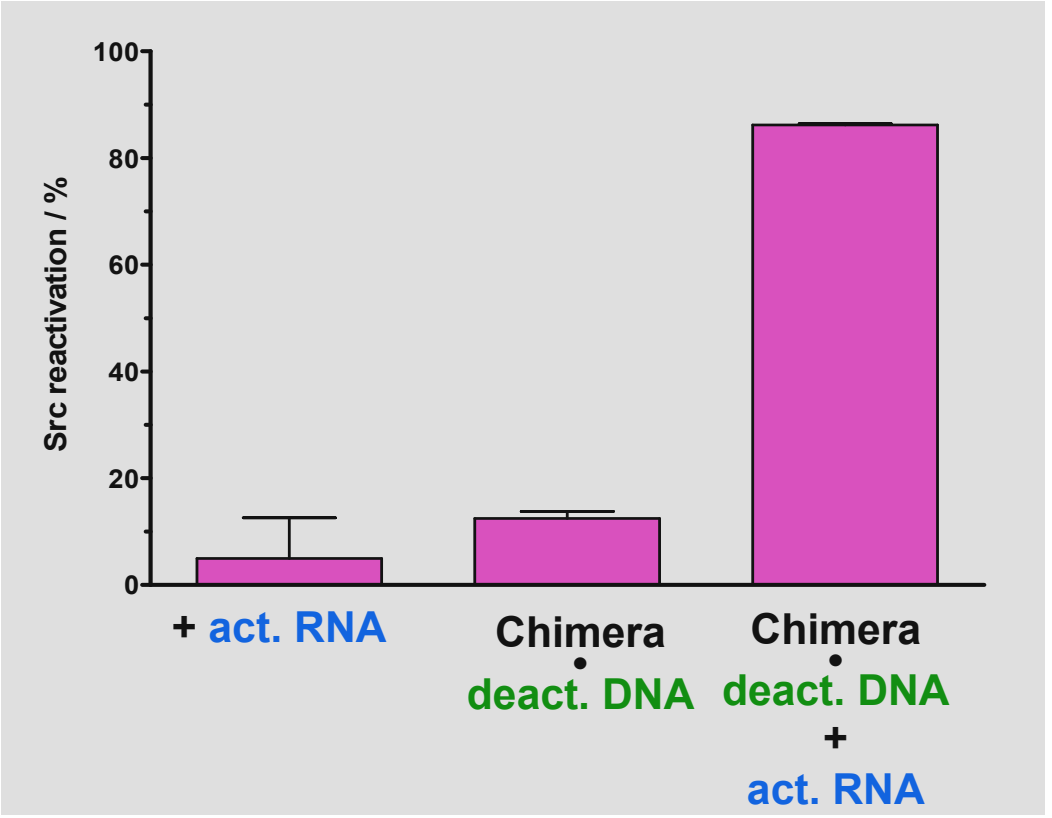


Lars Röglin

RNA-induced activation of Src-kinase



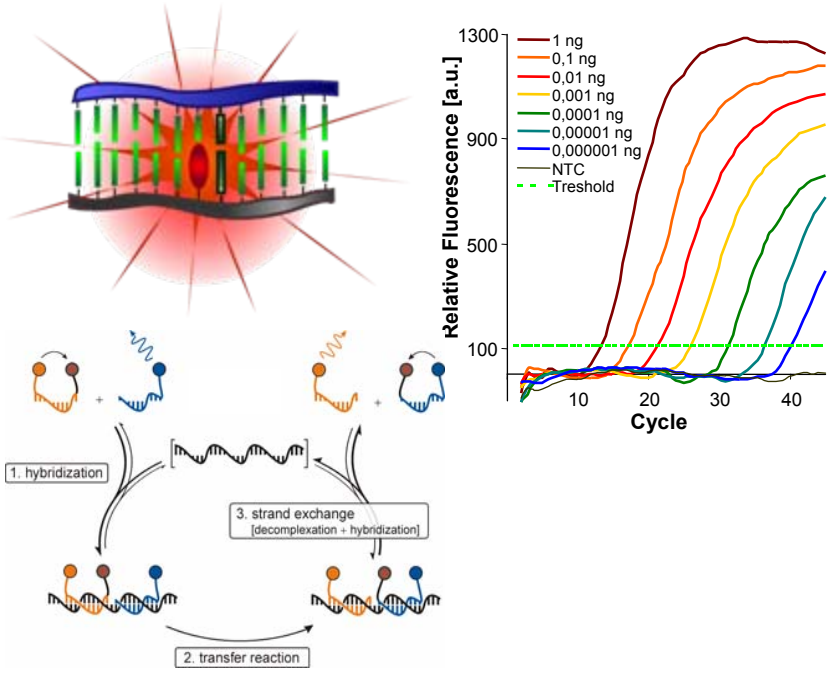




Röglin, Altenbrunn, Seitz, *ChemBioChem* **2009**, *10*, 758

Overview

Nucleic acid detection



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Grossmann et al. *Angew. Chem.* **2007**, *119*, 5315,
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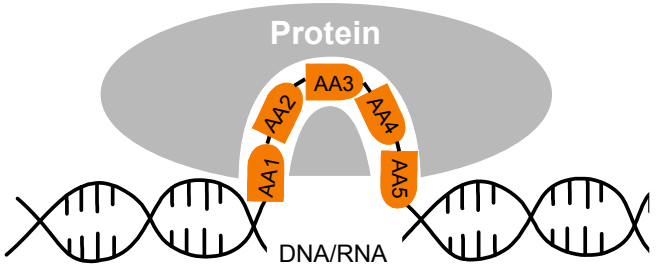
Grossmann et al. *J. Am. Chem. Soc.* **2006**, *128*, 15596.

Protein-protein/DNA interactions



Beuck et al. *Angew. Chem.* **2003**, *115*, 4088,
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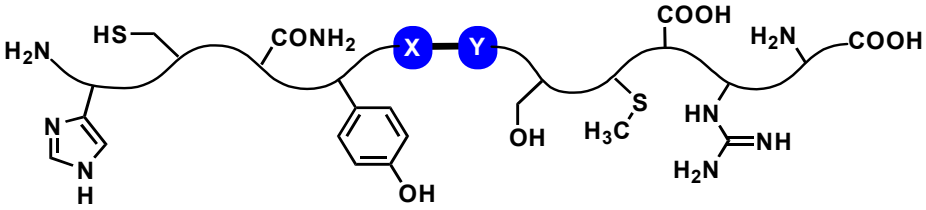
Protein/nucleotide chemistry



Bergmann et *al.Angew. Chem.* **1999**, *111*,
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Haase et al. *Angew. Chem.* **2008**, *120*, 1575,
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Hainke et al. *J. Org. Chem.* **2007**, *72*, 8811

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Christian Stutz
Stefanie Thurley
Tanja Westphalen

Dr. Tom Grossmann
Dr. Lars Röglin

The Collaborators:

Amadian (MPI Dortmund)
Weinhold (RWTH Aachen)
Ernsting (HU Berlin**)**
Röder (HU Berlin)
Herrmann (HU Berlin)