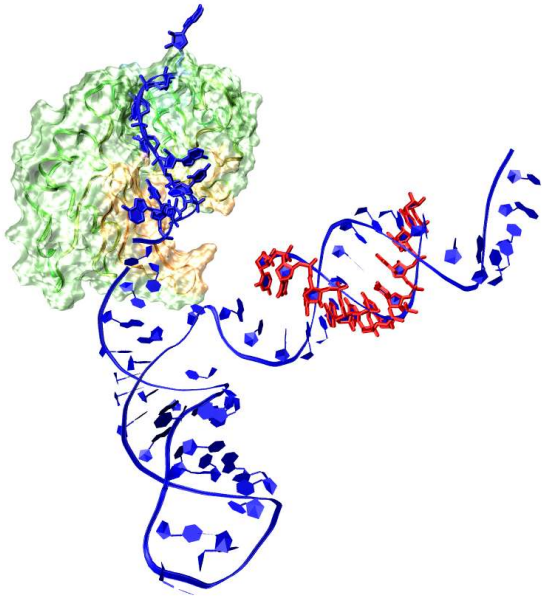


The RNA secondary structure dependence of RNA protein interactions and its implications for the post transcriptional regulation of gene expression

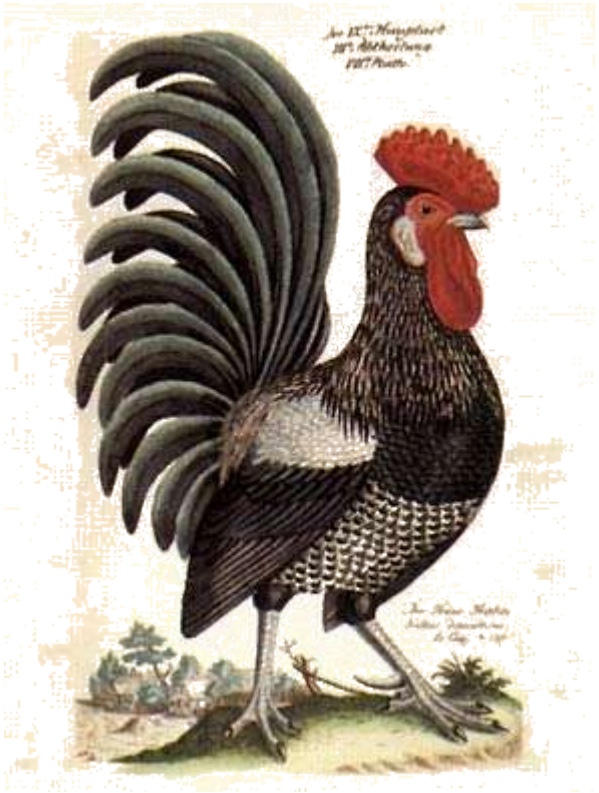
Defensio Dissertationis

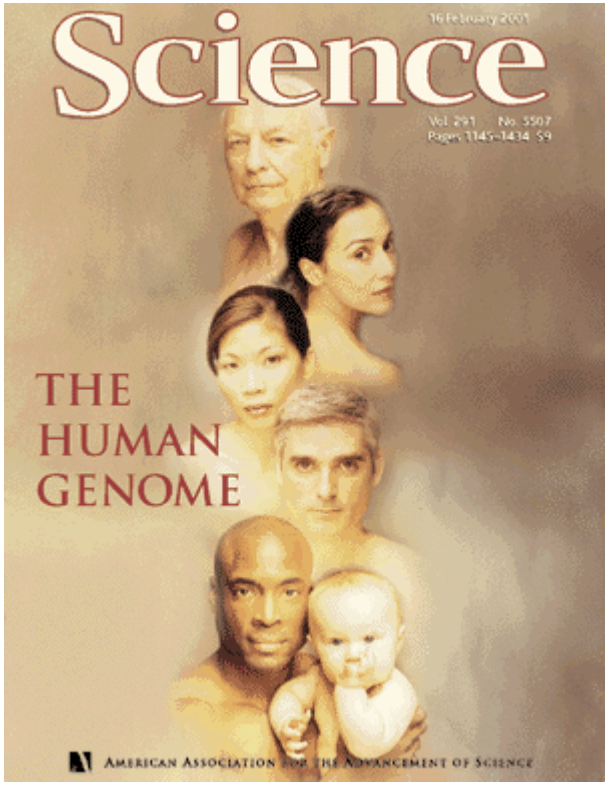
Jörg Hackermüller

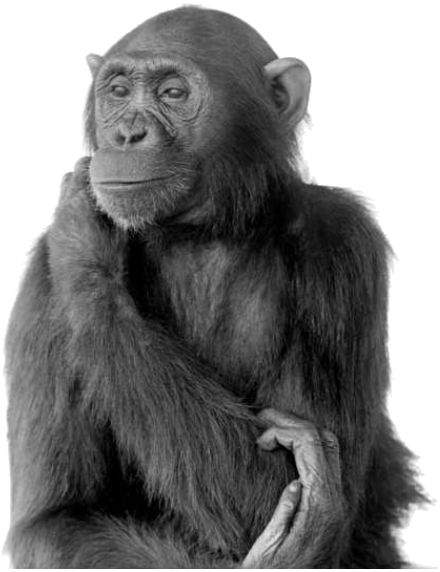
IK@N Informatics and Knowledge Management at Novartis Research

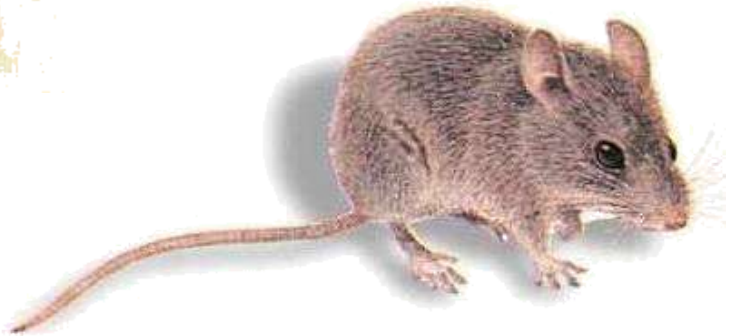
In Silico Sciences

NOVARTIS



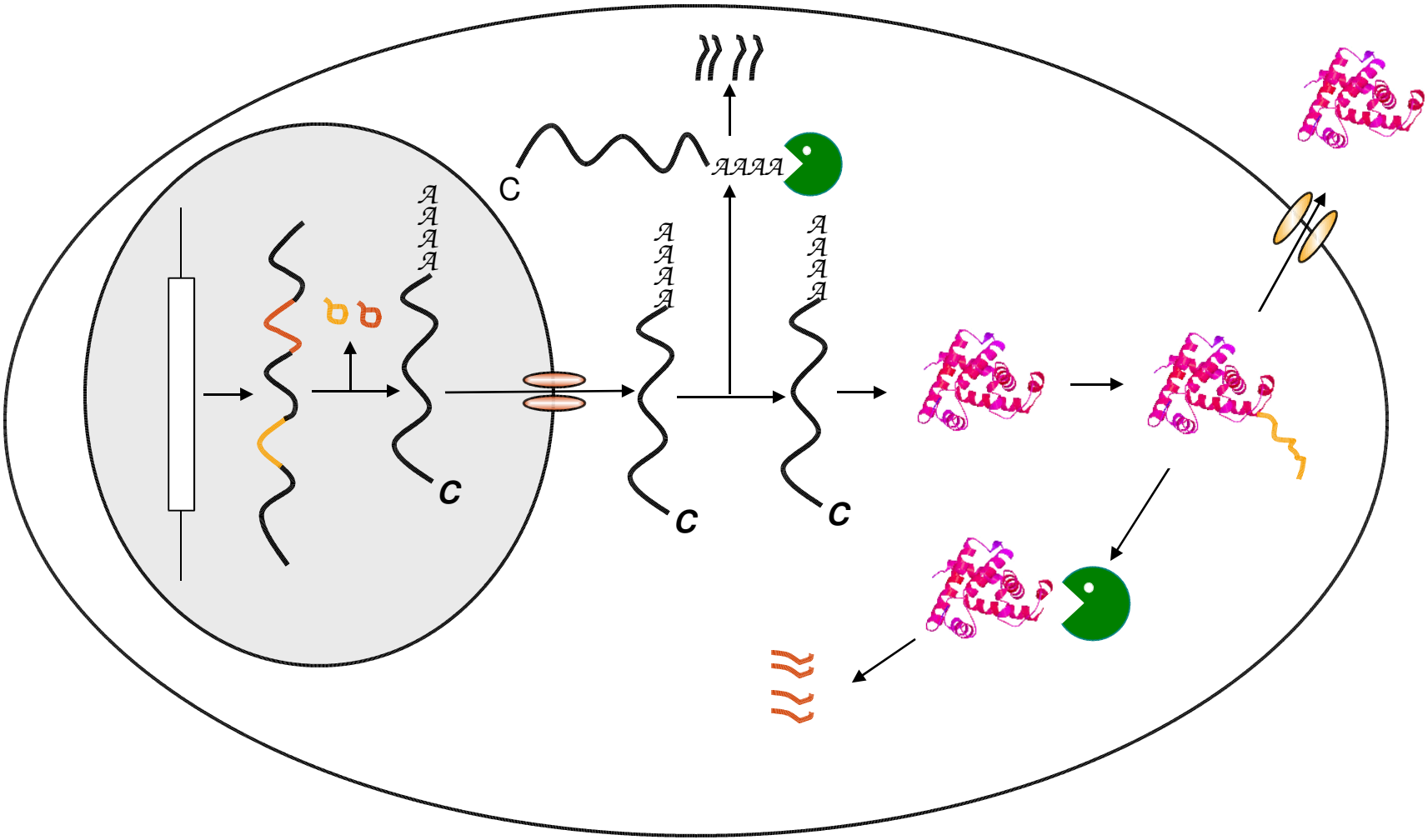

Science
Vol. 291 No. 5507
Pages 1145–1434 S9
THE HUMAN GENOME
American Association for the Advancement of Science





NOVARTIS

Regulation of Eukaryotic Gene Expression

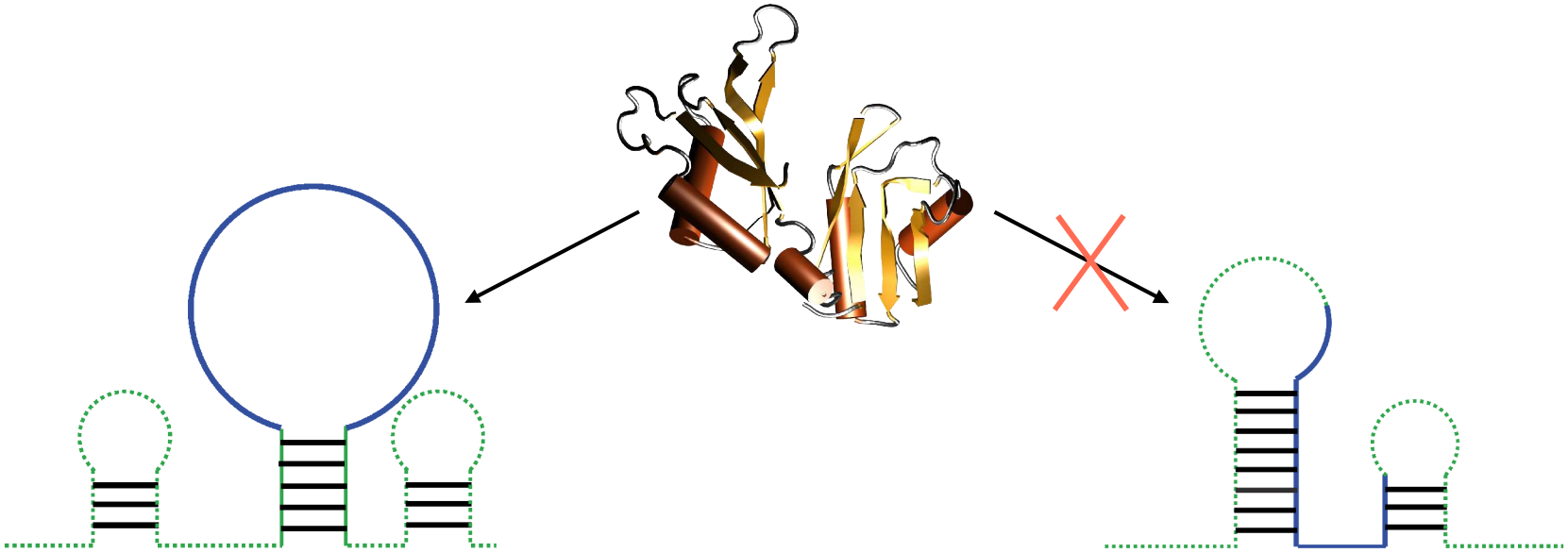


degradation

NOVARTIS

RNA-protein interactions

- highly relevant (involved in many biological pathways – particularly in post transcriptional regulation of gene expression)
- often dependent on recognition of RNA (secondary) structure features



- despite the importance no systematic investigation of RNA secondary structure dependence

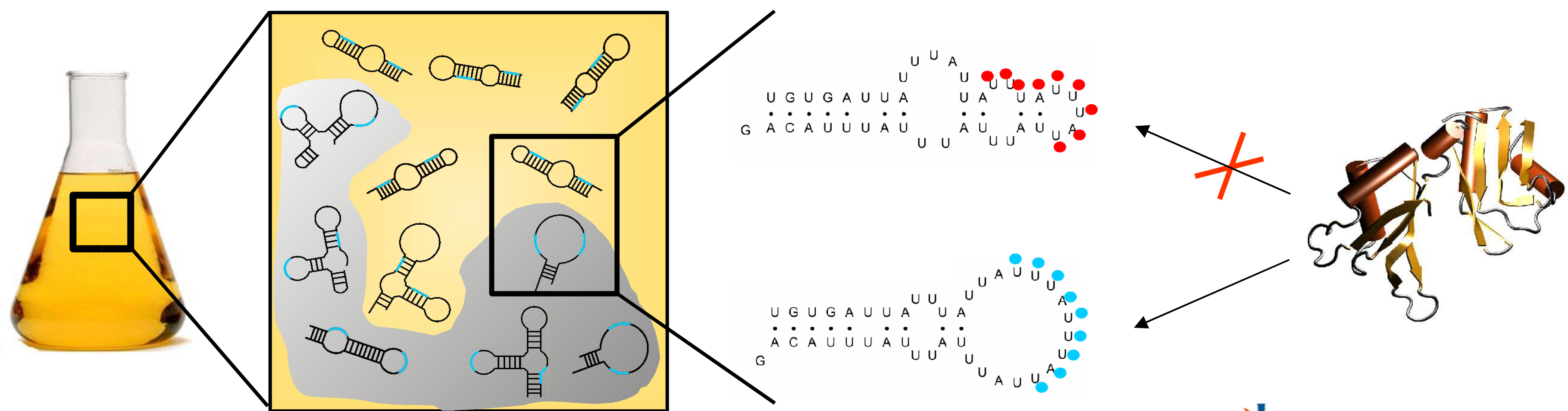
NOVARTIS

Quantitative model of RPIA

Model:

- proteins binds only RNAs that form secondary structure feature
- 1:1 binding (can be extended at cost of more complex expressions)
- non pseudo-knotted RNA secondary structures (can be extended at cost of higher computational effort)

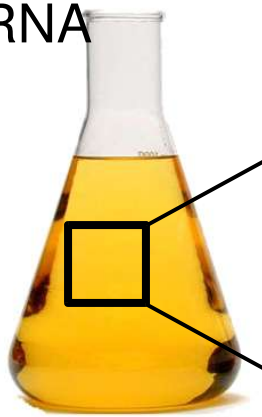
$$\frac{[\mathrm{RNA}]\ [\mathrm{Ligand}]}{[\mathrm{Ligand}\cdot\mathrm{RNA}]} = \frac{K_d}{p_*} =: K_d^{\mathrm{app}}$$

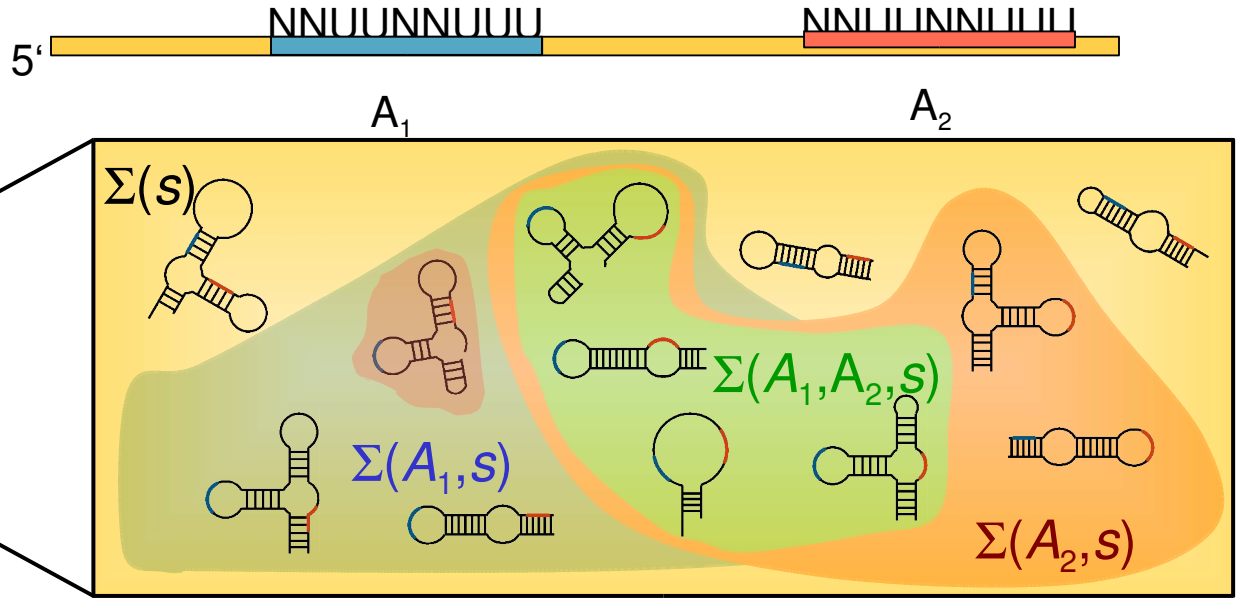


NOVARTIS

Ways to calculate p

HuR target mRNA





$$p_* = \frac{1}{Z}\left(\right.$$

$$p_* = \frac{1}{Z}\left(Z_{*A_1} + Z_{*A_2} - Z_{*A_1,A_2}\right)$$

$$1 - p_* = \sum_{\ell=0}^{M} (-1)^\ell \sum_{\substack{\mathcal{A} \\ |\mathcal{A}|=\ell}} p(\mathcal{A})$$

NOVARTIS

A statistical test for the influence of secondary structure

$$K_d^{\mathrm{app}} = \frac{K_d}{p_*[\Xi]}$$

(i) calculate $p_*[\Xi]$ for any test conformation Ξ for all sequences in the set

(ii) calculate correlation coefficient between K_d^{app} and $p_*[\Xi]$

(iii) test whether this correlation is significant:

$$\sqrt{\frac{(k-2)r^2}{1-r^2}} \geq t_{(k-2)}(1-\frac{\alpha}{2})$$

k number of sequences in set

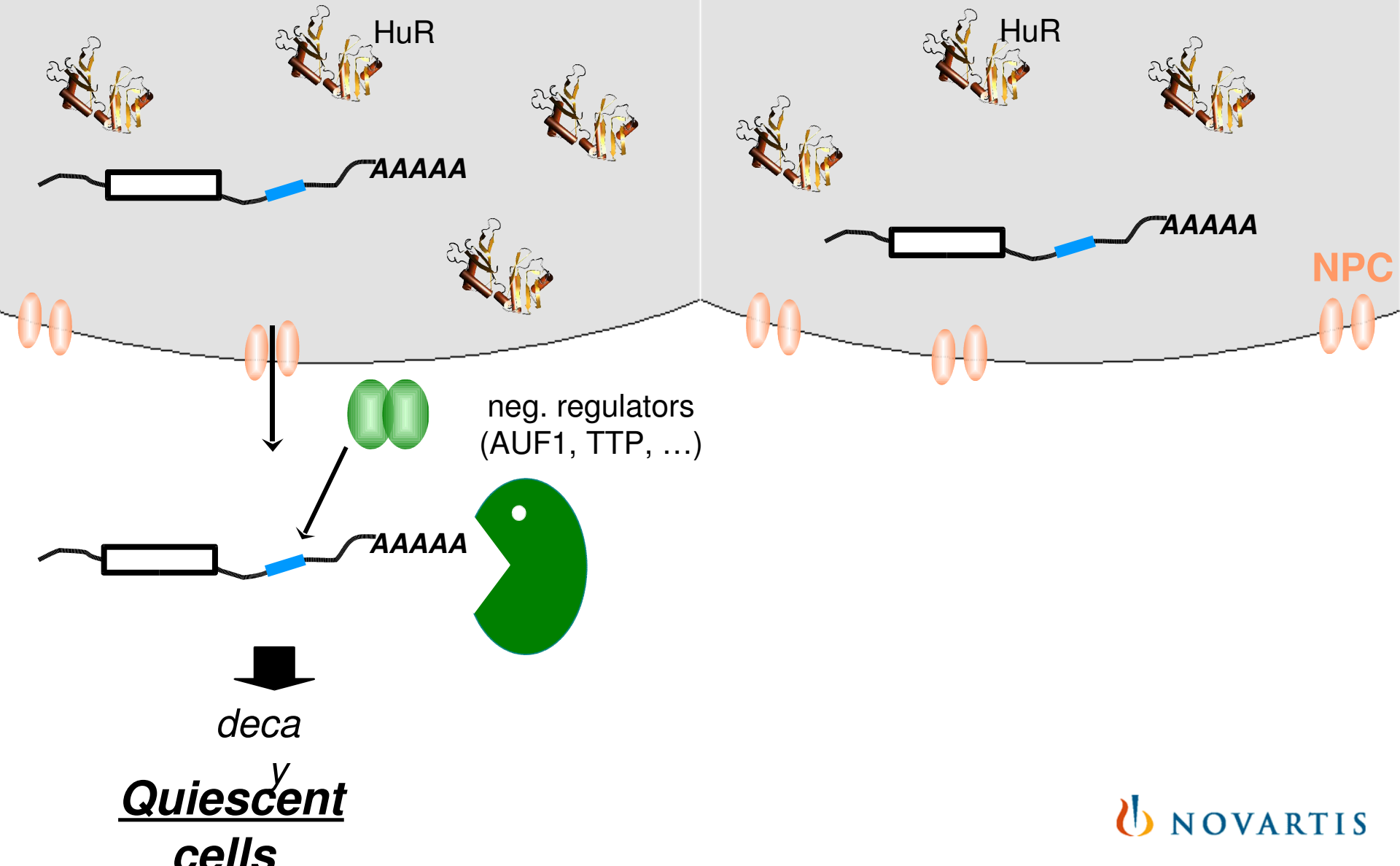
r empirical correlation coefficient

t(.) Student's t-distribution for (k-2) degs. of freedom

α significance level

NOVARTIS

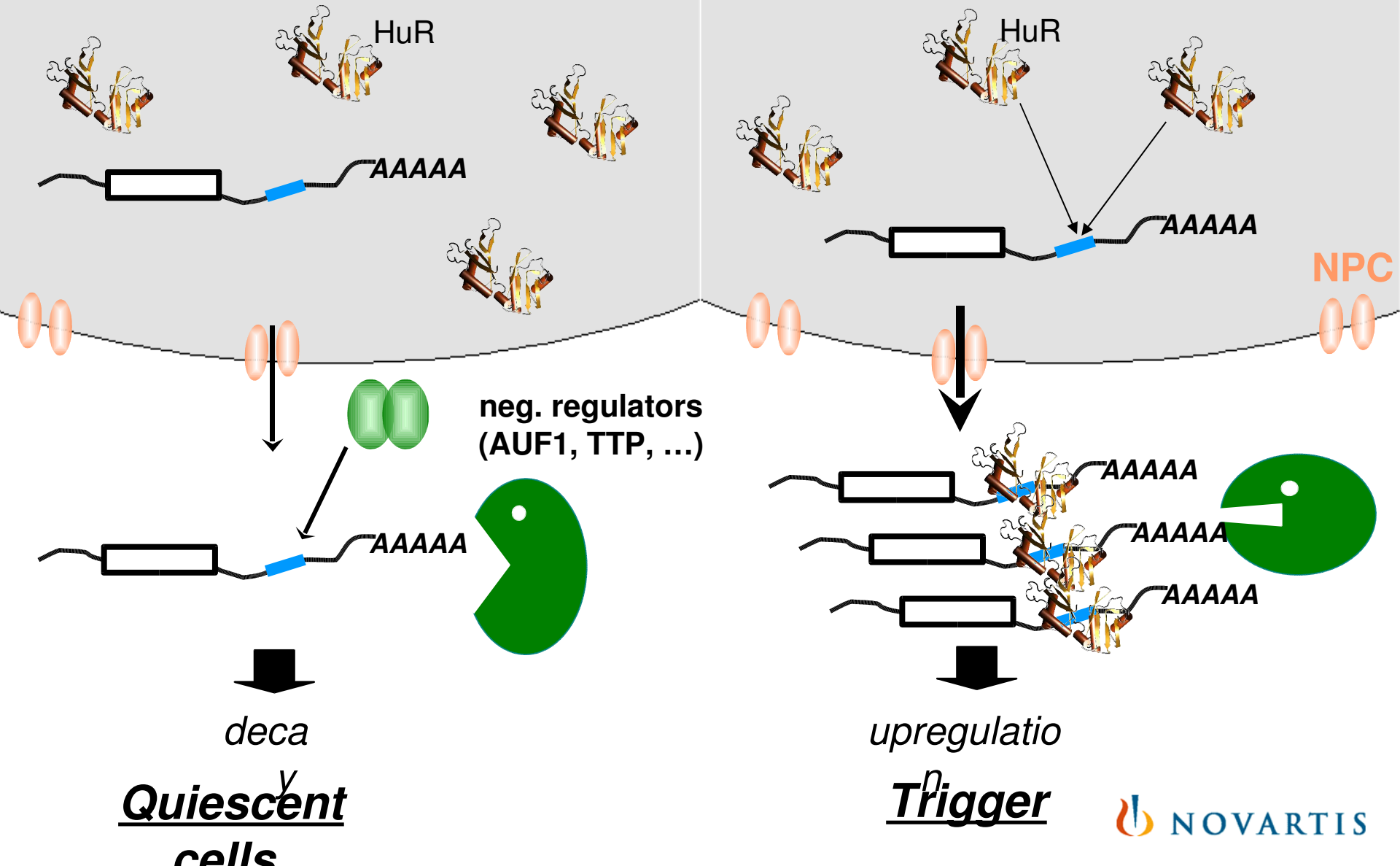
mRNA stability regulation by HuR



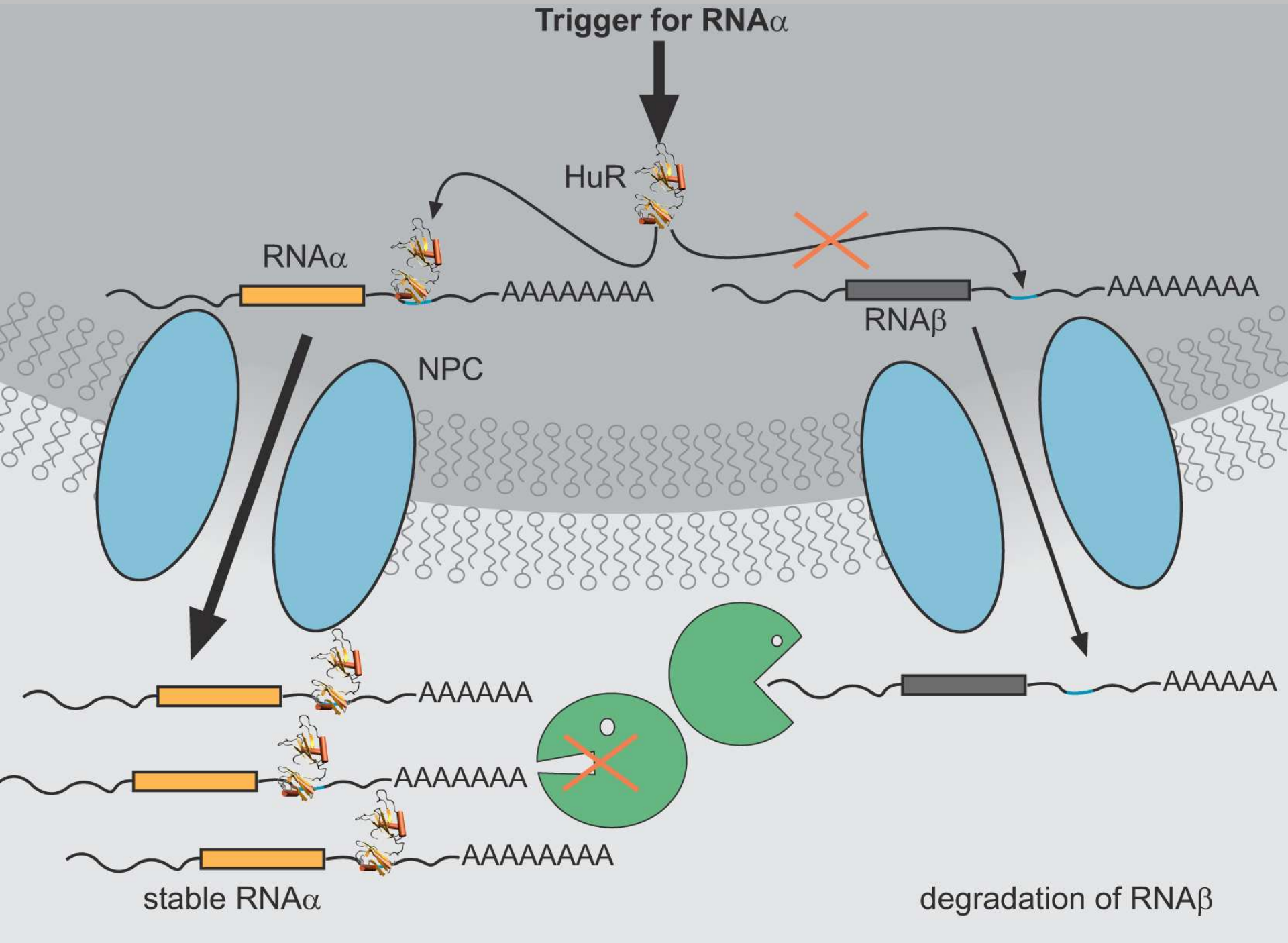
Quiescent cells

NOVARTIS

mRNA stability regulation by HuR



NOVARTIS


Trigger for RNAα
HuR
RNAα
AAAAAAAA
RNAβ
AAAAAAAA
NPC
AAAAAA
AAAAAAA
AAAAAAAA
AAAAAA
stable RNAα
degradation of RNAβ

HuR RNA recognition

HuR to RNA binding:

- diverse affinities to target RNA (sub) sequences (Kd 120 pM to 13 nM)

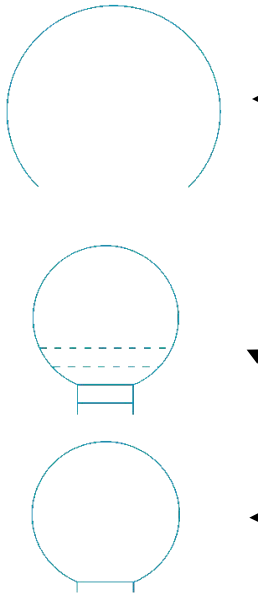
Sequence properties:

- HuR's degenerate binding motif: N-N-U-U-N-N-U-U-U
  - binds in "all or nothing" manner (U_9 1nM, U_8 "nothing"[1])

[1]< 0.01% of RNA bound

NOVARTIS

HuR binds NNUUNNUUU in single stranded conformation



Ξ (NNUUNNUUU)	r	$\sqrt{(k-2)r^2/(1-r^2)}$	p-value
xxxxxxxxx	0.953	9.957	1.65e-06
..xxxxx..	0.366	1.245	2.42e-01
.xxxxxxx.	0.617	2.480	3.25e-02
||.....||	NA	NA	NA
|||||||||	NA	NA	NA
((.....))	NA	NA	NA
(xxxxxxx)	NA	NA	NA

x unpaired

| paired

. No constraint

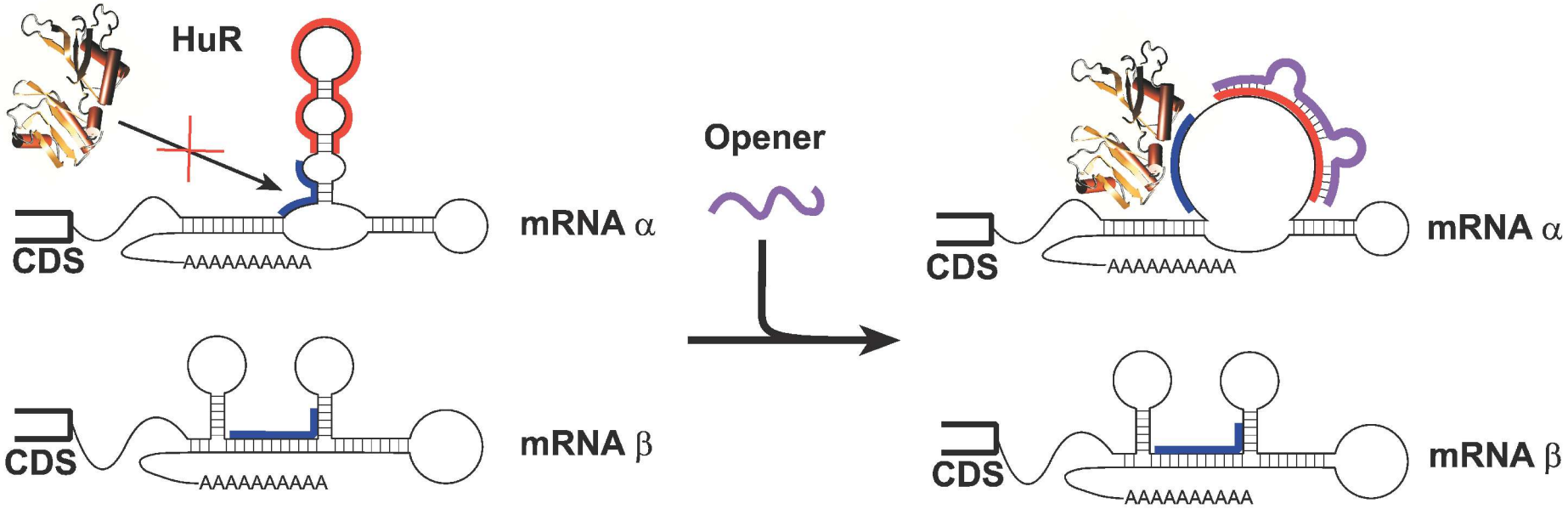
(opening base pair

) closing basepair

NOVARTIS

modRNA mechanism

- manipulate RNA-protein interactions through manipulation of RNA secondary structure
- RNA secondary structure can change significantly upon hybridization of a second RNA (heteroduplex formation)



NOVARTIS

modRNA mechnism II

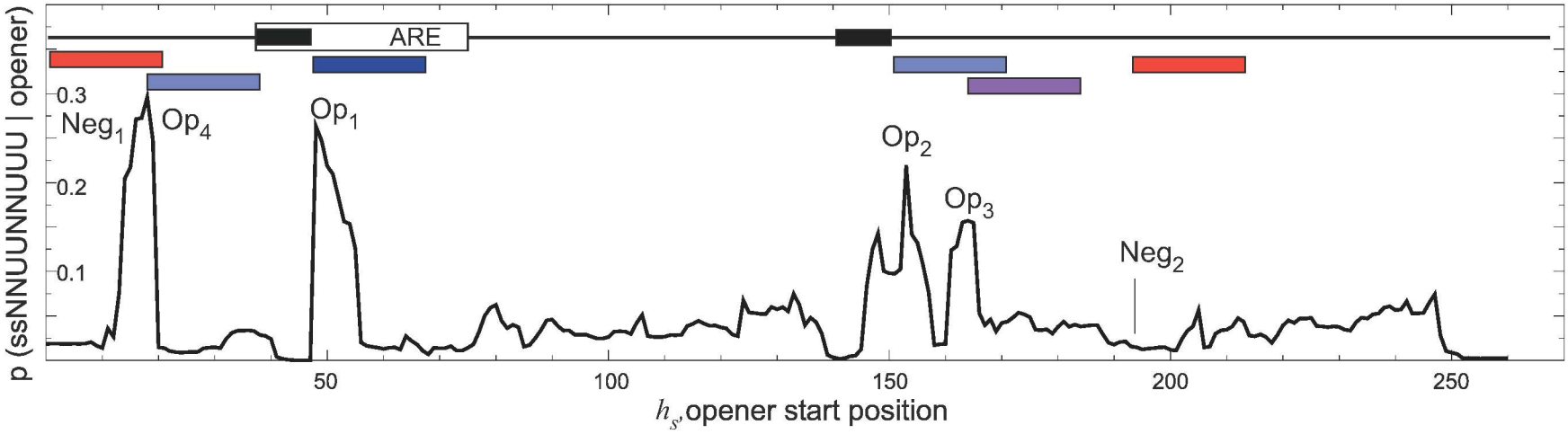
- RNA-RNA hybridization thermodynamically well understood
- no model currently available for influence on RNA-protein interactions
- RNA-RNA hybridization biologically very relevant for RNA-ligand interactions (non-protein coding RNAs)
- approximate model of ternary equilibrium RNA – protein – short RNA

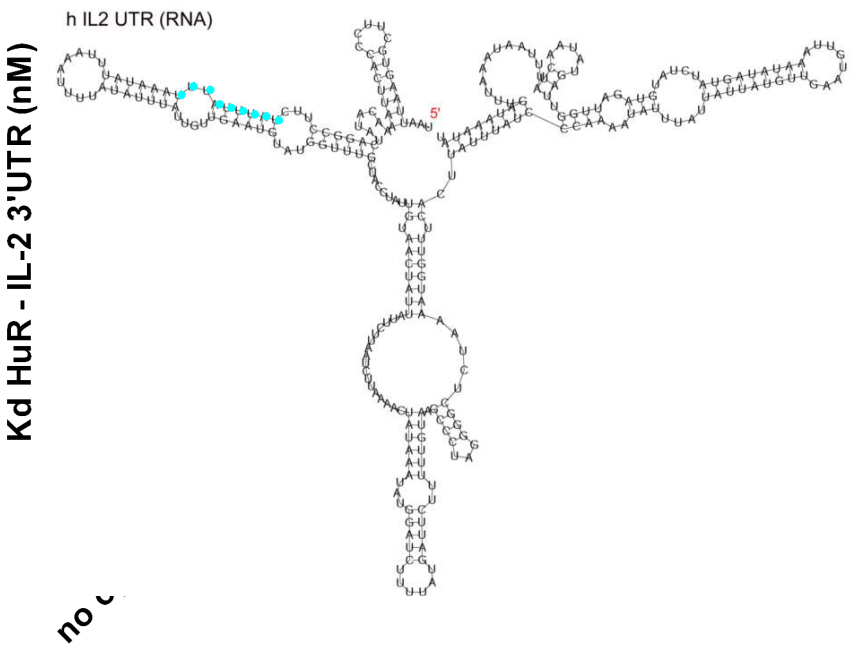
$$K_d^{\text{app}} = K_d \frac{1 + K_{MO}[O]}{p_*^M + p_*^{MO} K_{MO}[O]}$$

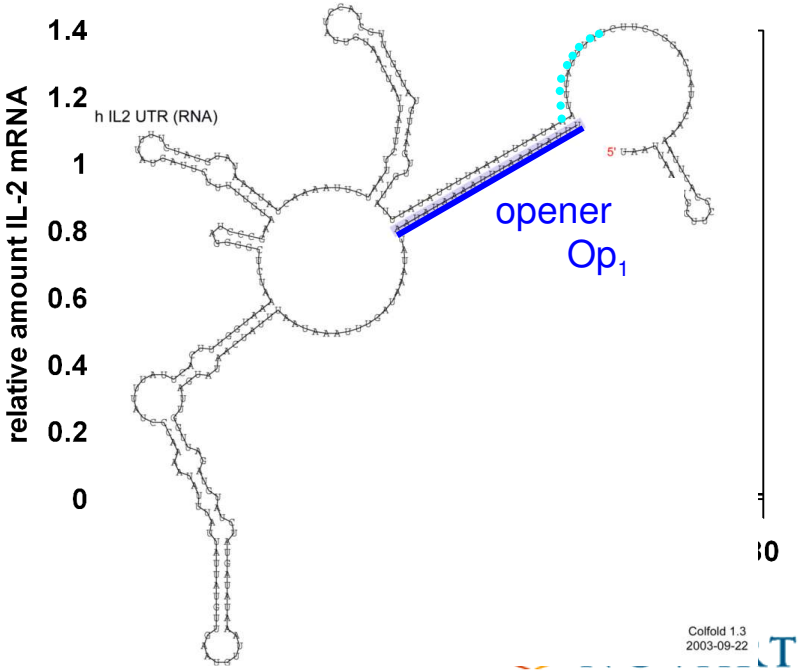
- modRNA selected by calculating p_*^{MO} for any possible exactly reverse complementary modRNA of a given length (20nt)

NOVARTIS

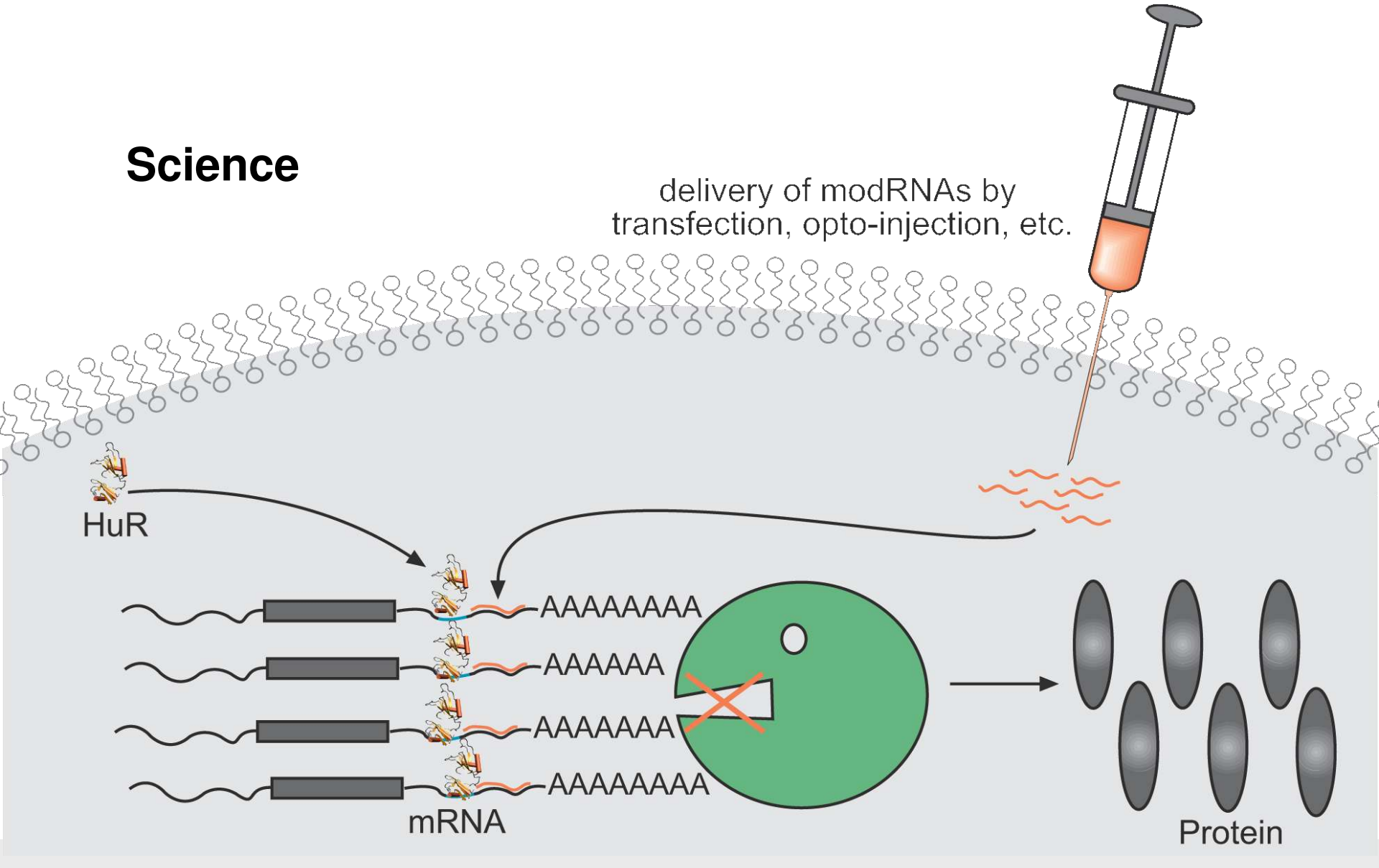
Openers specifically stabilize mRNA



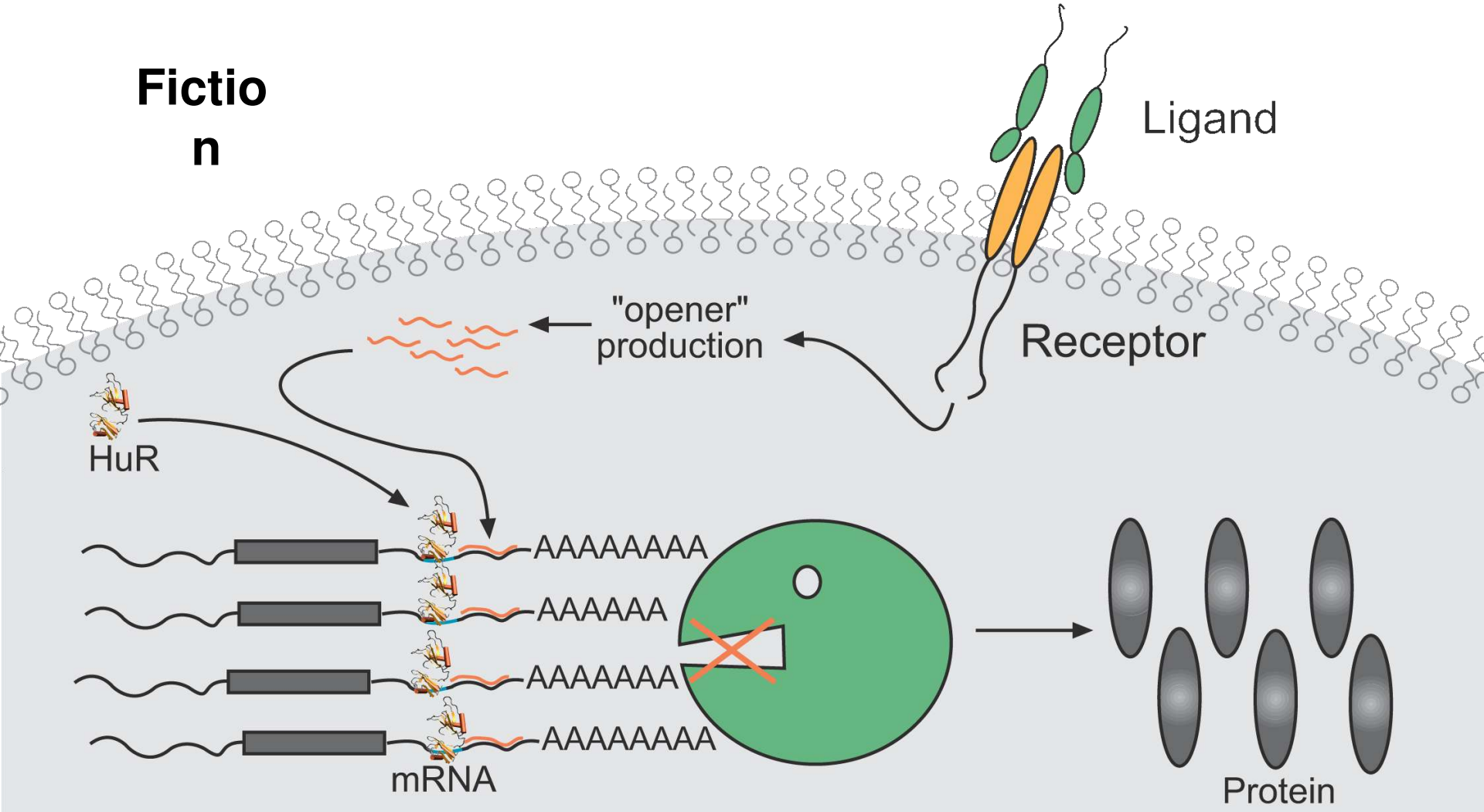




Specificity puzzle and modRNAs



Specificity puzzle and modRNAs



Outlook

- endogenous modRNAs?
  - potential origin
  - bioinformatic strategies to find ‘em
- modRNAs as tools in biology
  - may serve as complementary technique for RNAi
  - issue of delivery
- modRNAs in drug discovery
  - modRNAs as pharmaceutic agents
  - modRNAs as tools in pharma research

NOVARTIS

Scientists involved

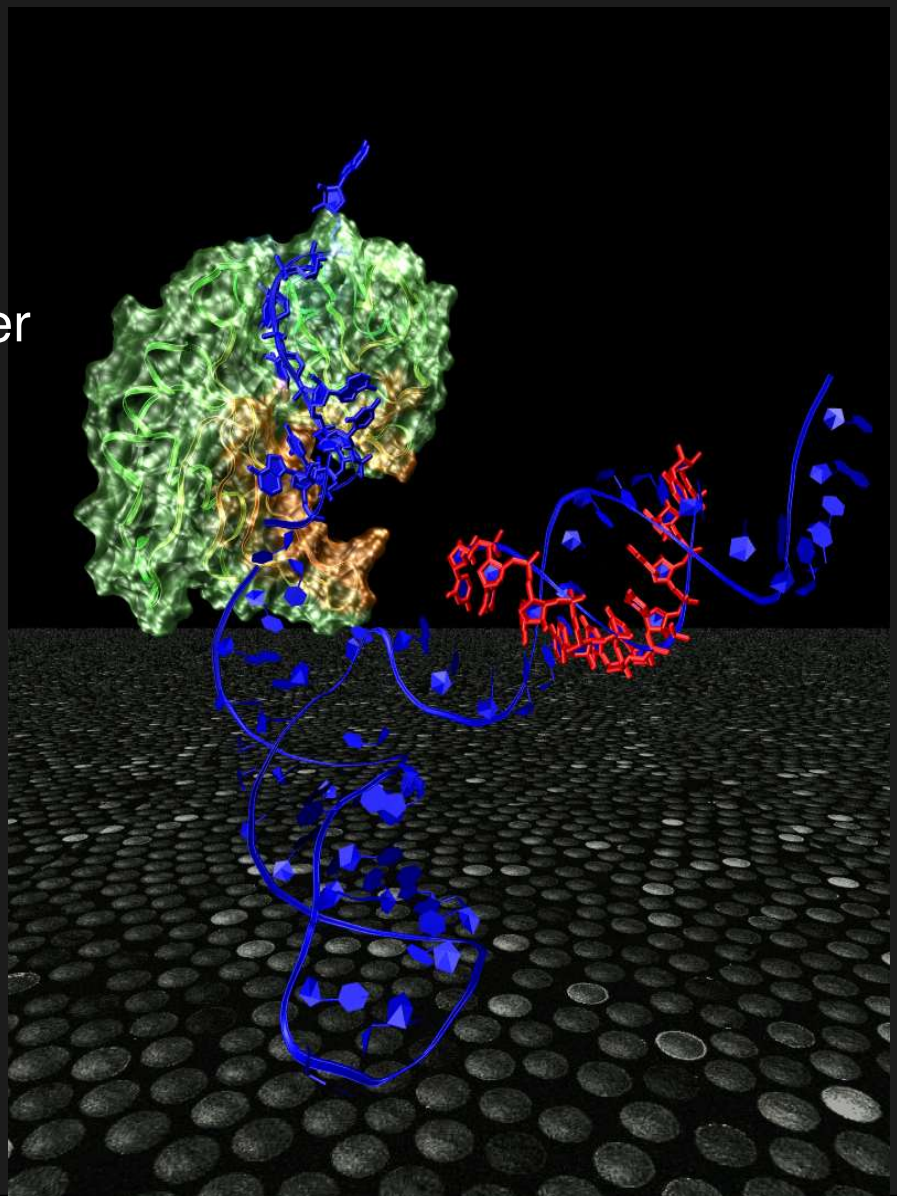
NIBR DT - IST
Volker Uhl
Jan-Marcus Seifert
Martin Hintersteiner

Nicole-Claudia Meisner

Manfred Auer

Univ. Salzburg
Fatima Ferreira

OeAW
DOC 11/2000



NIBR IK@N-ISS
Torsten Schindler
Siegfried Höfinger
Markus Jaritz

Jörg Hackermüller

Andras Aszodi

Univ. Leipzig
Peter F. Stadler

Univ. Vienna
Christoph Flamm
Ivo Hofacker
Kurt Gruenberger

Backup slides

Backup slides

NOVARTIS

A quantitative model for RNA - ligand binding

Ligand binds only to RNA which has a particular *accessible* conformation

$$\text{Ligand} + \text{RNA}_* \rightleftharpoons \text{Ligand} \cdot \text{RNA}$$

$$K_d = \frac{[\text{RNA}_*]\ [\text{Ligand}]}{[\text{Ligand} \cdot \text{RNA}]}$$

$$[\text{RNA}_*] = p_* \, [\text{RNA}]$$

Experiments can not distinguish between accessible and non-accessible

$$\frac{[\text{RNA}]\ [\text{Ligand}]}{[\text{Ligand} \cdot \text{RNA}]} = \frac{K_d}{p_*} =: K_d^{\text{app}}$$

Probability of accessible structures p_* expressed by partition functions:

$$p_* = \sum_{\Psi \in A(s)} p(\Psi) = \frac{1}{Z} \sum_{\Psi \in A(s)} \exp\left(-\frac{F(\Psi)}{RT}\right)$$

$$p(\Psi) = \frac{1}{Z} \exp\left(-\frac{F(\Psi)}{RT}\right)$$

NOVARTIS

modRNA model

- neglect multiple binding of oligo O to mRNA M

$$p_* = p_*(M)\frac{[M]}{[M]_t} + p_*(MM)\frac{[MM]}{[M]_t} + p_*(MO)\frac{[MO]}{[M]_t}$$

- O is exactly reverse complementary to M
- no significant self complementarity in O and M
- excess of O

$$p_* \approx p_*(MO)\frac{[MO]}{[M]_t} \approx p_*(MO)$$

Computation by constrained partition functions:

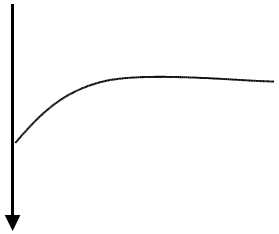
$$p_*^{MO}(\mathcal{A}) = Z(\mathcal{A} \cup \mathcal{T})/Z(\mathcal{T})$$

NOVARTIS

modRNA model II

Effect of modRNAs on affinity

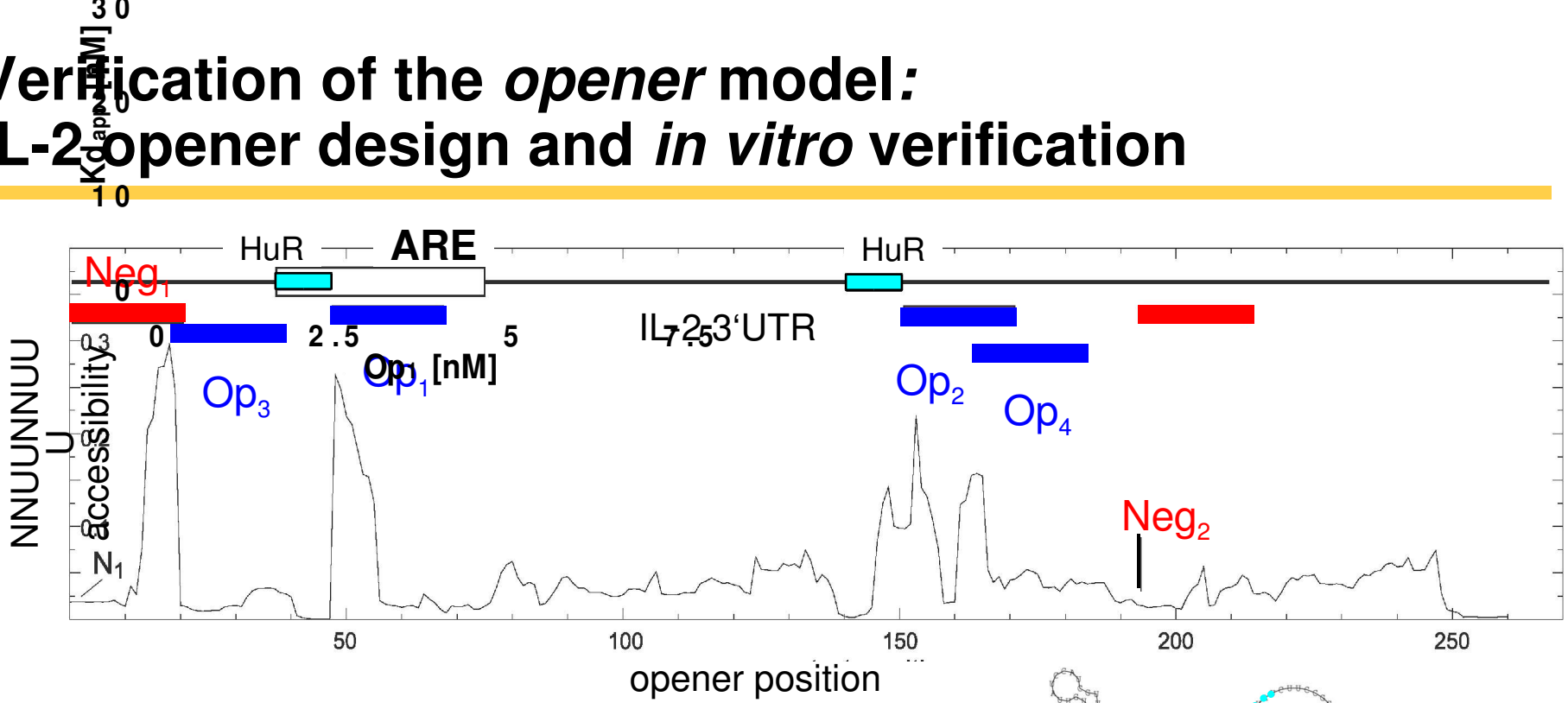
$$K_d^{\text{app}} := \frac{[\text{RNA}]\ [\text{Ligand}]}{[\text{RNA} \cdot \text{Ligand}]} = \frac{[M]\,[\text{Ligand}] + [MO]\,[\text{Ligand}]}{[M \cdot \text{Ligand}] + [MO \cdot \text{Ligand}]}$$



$$\frac{K_d}{p_*} =: K_d^{\text{app}}$$

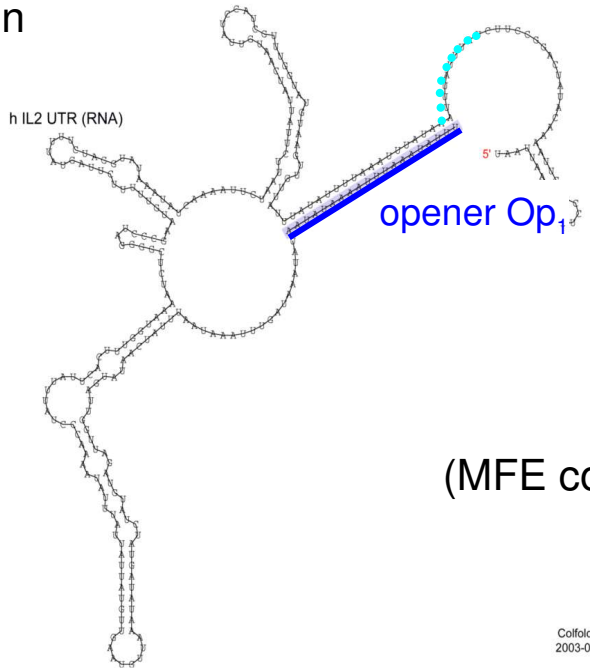
$$K_d^{\text{app}} = K_d \frac{1 + K_{MO}[O]}{p_*^M + p_*^{MO} K_{MO}[O]}$$

Verification of the *opener* model: IL-2 opener design and *in vitro* verification



IL-2 3'UTR

+ Neg_1,Neg_2



(MFE conformation)

Openers increase HuR RNA complexation *in vitro*