

Design of oligonucleotides as RNA-based drugs prerequisites and opportunities for *in silico* methods within the framework of fragment-based approaches

Fabrice Leclerc, July 1st 2020

Mini-symposium: RNA structure, design and interactions with proteins



Introduction

- RNA-based drugs
- Fragment-based approaches
- Proof of concept for RNA molecules: pros & cons
- Preliminary results and lessons learned
 - 3 examples of RNA-binding proteins (RBPs)
- Benchmarking for single nucleotide fragments
- 120 nucleotide-protein complexes
- Conclusions

Outline

RNA or nucleotide based drugs **Modes of action**

• hybridisation-dependent (RNA target)

- siRNA (small interfering RNA)
- miR (microRNA)
- ASO (Antisense Oligonucleotides)
- hybridisation-independent (targets)
 - aptamers (protein targets, ...)
 - immunostimulatory (CpG) oligos
 - mRNA

•••

...

P. Andersson, OTS seminar (2020)





Aptamers in the rapeutics **RNA drugs and clinical trials**

Name	Form	Target	Condition
Pegaptanib sodium (Macugen)	27-nt RNA	VEGF (Vascular Endothelial Growth Factor)	Age-related macular degeneration
E10030	29-nt DNA	PDGF (Platelet-Derived Growth Factor)	Age-related macular degeneration
REG1 (RB006 and RB007)	37-nt RNA	Coagulation factor IXa	Coronary artery disease
ARC1905	38-nt RNA	C5 (Complement component 5)	Age-related macular degeneration
AS1411	26-nt DNA	Nucleolin	Acute myeloid leukemia
ARC1779	39-nt DNA	A1 domain of von Willebrand factor	Von Willebrand disease/thrombotic thrombocytopenic/purpura
NOX-E36	40-nt RNA	CCL2 (Chemokine C-C motif Ligand 2)	Chronic inflammatory diseases/type 2 diabetes mellitus/systemic lupus erythematous
NOX-A12	45-nt RNA	CXCL12 (Chemokine C-X-C motif Ligand 12)	Multiple myeloma and non-Hodgkin lymphoma/autologous or hematopoietic stem cell transplantation
NU172	26-nt DNA	Thrombin	Heart disease
NOX-H94	44-nt RNA	Hepcidin peptide hormone	Anemia/end-stage renal disease/inflammation
ARC19499	32-nt RNA	TFPI (Tissue Factor Pathway Inhibitor)	Hemophilia

Zhuo *et al.*, 2017





Fragment-based design/discovery (FBD) approaches

High-throughput screening (a)

Diverse screening library





Biochemical assay

Hit identification



Schmidt & Rademann, 2009

- **Pros & Cons**
- (b) Fragment-based drug discovery





FBD applied to RNA molecules **Proof of concept**



discrete representation of 3-mers (fragment=trinucleotide)

Chauvot de Beauchene *et al.*, 2016a - 2016b



MCSS-based Modeling of RNA Ligands

single-nucleotide fragment & forcefield based strategy

MCSS (CHARMM)

Multiple Copy Simultaneous Search





Leclerc & Karplus, 1999

Simoes & Leclerc, Molpy package





MCSS-based workflow for FBD of oligos Reproduce oligonucleotides binding on RBPs



connectivity



constraint satisfaction



building



extension of dinucleotides

optimisation ranking





n-mer oligonucleotides



Preliminary analysis and MCSS results on 3 RBPs

optimal/sub-optimal binding in RRM, KH and CCCH families





KH (5wwx) 5'-AGA-3'

A1

691

100

90

80

70

60

50

40

30

20

10

0

A1

contacts

5

ρ

z











Zn-CCCH (5elh) 5'-UUA-3'





Preliminary MCSS+Molpy results on 3 RBPs 2-mers/3-mers oligonucleotides

200U & 200C 2XNR



200 initial poses



RMSD = 1,3 Å

~5000 dincleotides

Top 1 2-mers



Preliminary MCSS+Molpy results on 3 RBPs **3-mers oligonucleotides & nucleotide-type screening**

PDB selected poses		sequence	generated	native chains (≤2 Å)				
ID	Α	С	G	U	constraint	chains	rank	RMSD (Å)
2XNR	/	155	/	155	UCU	13 782	1	1,48
5ELH	490	/	/	490	UUA	1 584 619	ND	ND
5WWX	691	/	691	/	AGA	2 433 270	ND	ND









Preliminary MCSS+Molpy results on 3 RBPs sequence-free predictions



2XNR

score-based nucleotide preferences

5ELH

5WWX





Nucleotide-Protein Complexes



high resolution, non-redundant, etc

120 benchmark

5' patches **R010 R110 R210 R**310 **R410** R=A,C,G,U **17Å**³

Minimized protein without ligand

native pose: RMSD_(mcss/exp) ≤ 2.0Å

Chevrollier & Leclerc, (bioRXiv preprint), 2019







Chevrollier & Leclerc, (bioRXiv preprint), 2019



MCSS Charges & Solvent Models: Implicit/Explicit **Hard/Soft Predictions**







Implicit/Explicit Models & Hard/Soft Predictions **Optimal (hard) / Good (soft) / Poor / No prediction Top 10 Top 100** 100 Good Poor Optimal Good Optimal 80 60 -40 soft 20 hard 0 explicit implicit implicit licit

Predicted Proteins (%)





No (soft, poor) Predictions: Structural Features A qualitative analysis 20



Soft Predictions: Structural Features A qualitative analysis

Illustration of soft (poor) predictions Why soft (poor, no)?

Illustration of soft (poor) predictions **Binding modes**

implicit

PDB ID: 3EWY (U)

explicit

Illustration of soft (poor) predictions Loss of native contacts

Illustration of soft (poor) predictions **Non-native contacts**

– Arg451P

implicit

Conclusions strengths & weaknesses

- **fragment-based** approach applied to single nucleotide binding • explicit solvent improve predictions of nucleotide binding
- - improved docking power (native poses)
 - improved screening power ("true" binders)
- pitfalls:
 - protein flexibility in the binding site
 - binding thermodynamics
 - binding kinetics

Perspectives and opportunities in silico approach to fragment-based design

IN SILICO

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