"First Principle" Concept in Designing Small Molecules for Targeting RNA Expansion Repeats

Alexei Pushechnikov, Volodymyr Kysil, Roman Timakhov

E-mail: apushechnikov@chemdiv.com Company Web Site: www.chemdiv.com

The massive of the genome sequencing data has established a clear connection between expansions of short nucleotide repeats and several neurological and neuromuscular disorders[1]. To name few, these maladies include Myotonic Muscular Dystrophy, Machado–Joseph disease, Huntington disease, Lou Gehrig's disease. In numerous instances it was demonstrated that the small organic molecules therapeutics – a well-proven tool of modern medicine – also can be utilized for treatment these, otherwise non-curable, disorders by targeting specific RNA sequences of the overexpressed expansions repeats[2,3].

The ligand-target mechanism when the RNA expansion repeats are targeted with small molecules has been shown working in multiple research studies including animal models. However, one of the limiting obstacles for finding new high-quality lead molecules is that most of the commercial high throughput screening (HTS) libraries are biased towards the protein affinity chemical space. The unique distinction of the RNA affinity chemical space requires different weight factors that contribute into physicochemical interactions of a ligand-target ensemble in the RNA chemical space vs in the protein one.

One of the most advantageous approaches is to target RNA's with designer macrocyclic compounds[4]. The use of macrocycles for therapeutic RNA targeting is especially alluring as selective G-Quadruplex RNA (as well as DNA) ligands[5]. As a back-end selectivity reinforcement approach, we propose the 3D shape and electrostatic field similarity virtual screening method[6] using the complimentary trinucleotide sequences as reference training sets.

Disease Type	Gene	RNA Repeat	Normal	Pathogenic
DRPLA (Dentatorubropallidoluysian atrophy)	ATN1/DRPLA	CAG	6 - 35	49 - 88
HD (Huntington's disease)	HTT	CAG	6 - 35	36 - 250
SBMA (Spinal and bulbar muscular atrophy)	AR	CAG	9 - 36	38 - 62
SCA1 (Spinocerebellar ataxia Type 1)	ATXN1	CAG	6 - 35	49 - 88
SCA2 (Spinocerebellar ataxia Type 2)	ATXN2	CAG	14 - 32	33 - 77
SCA3 (Machado-Joseph disease)	ATXN3	CAG	12 - 40	55 - 86
FRAXA (Fragile X syndrome)	FMR1	CGG	6 - 53	> 230
FRAXE (Fragile XE mental retardation)	AFF2/FMR2	CCG	6 - 35	> 200
FRDA (Friedreich's ataxia)	FXN or X25	GAA	7 - 34	> 100
DM1 (Myotonic dystrophy Type 1)	DMPK	CUG	5 - 34	> 50
ALS (Amyotrophic lateral sclerosis) and FTLD (Frontotemporal lobar degeneration)	C9orf72	GGGGCC	2 - 30	> 250

Search in PDB for 3D structures

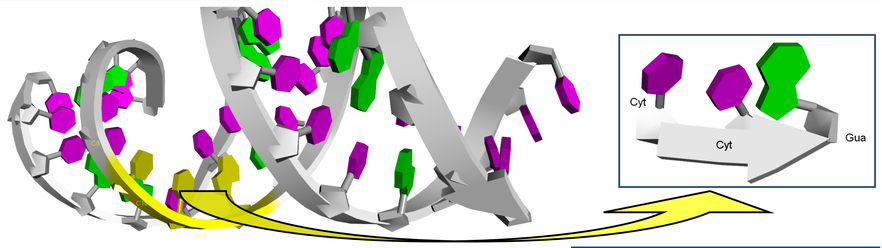
RNA Repeats	Complementary Repeats
CAG	GUC
CGG	GCC
CCG	GGC
GAA	CUU
CUG	GAC
GGGGCC	CCC, CCG, CGG

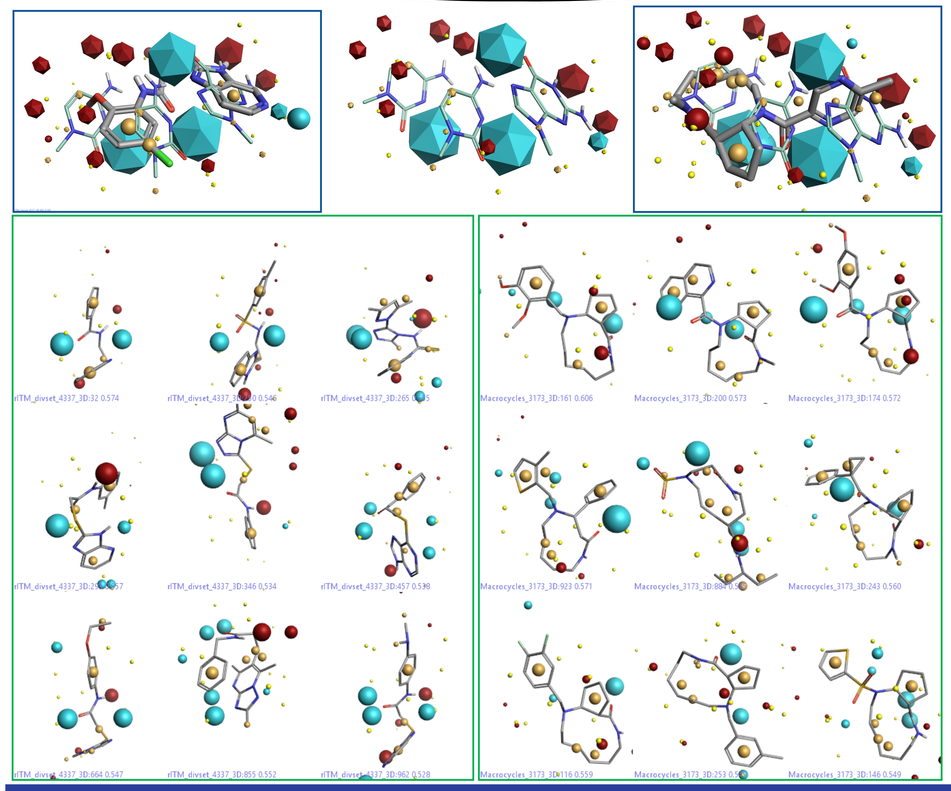
Templates for Virtual Screening of Small Molecules

- Pool of Fragments
- HTS Libraries
- Macrocycles Libraries (Static and Evolutionary)

Integral Multiparameter Scoring Function of Alignments:

- 3D Shape Molecular Surfaces
- 3D Electrostatic Potential Surfaces
- 3D Pharmacophores





Design and Refinement of RNA-Targeted Macrocyclic Libraries

- Decorating macrocycles with prioritized fragments
- Morphing synthetically compatible high-scored macrocycles with selected small molecules
- Incorporating high-scored small molecules structural motifs into new designer macrocyclic libraries

Macrocycle 1	Small molecule 1	Morphing poses	Morphed structure 1
343.5	291.4	**MW**	386.5
33.5	56.1	**TPSA**	67.2
4.3	2.5	**cLogP**	2.8
296.1	261.2	**Area, Å^2**	329.7
321.0	250.9	**Volume, Å^3**	342.8
0.571	0.547	**Score**	0.571

Macrocycle 2	Small molecule 2	Morphing poses	Morphed structure 2
329.5	327.4	**MW**	435.5
24.8	73.9	**TPSA**	91
4.0	2.8	**cLogP**	3.2
301.1	286.5	**Area, Å^2**	342.1
330.7	279.4	**Volume, Å^3**	373.1
0.549	0.528	**Score**	0.537

	Example 1	Example 2	Example 3
MW	452.6	406.5	480.6
TPSA	98.7	92.0	95.1
cLogP	2.3	1.7	2.5
Area, Å^2	369.1	338.8	372.9
Volume, Å^3	415.9	357.3	418.3
Score	0.600	0.576	0.561

	Example 4	Example 5	Example 6
MW	559.7	499.6	458.6
TPSA	104.1	108.0	105.1
cLogP	3.6	2.3	3.1
Area, Å^2	456.3	381.6	386.7
Volume, Å^3	480.2	418.2	421.6
Score	0.559	0.551	0.534

Main Strategies for Synthesis of Macrocycles:

Macrocyclizations

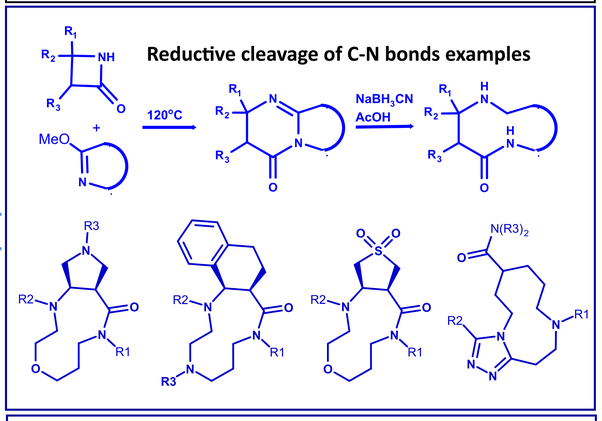
- Ring closure metathesis
- Mitsunobu reaction
- Huisgen 1,3-dipolar cycloaddition
- Lactam and Lactone ring closure

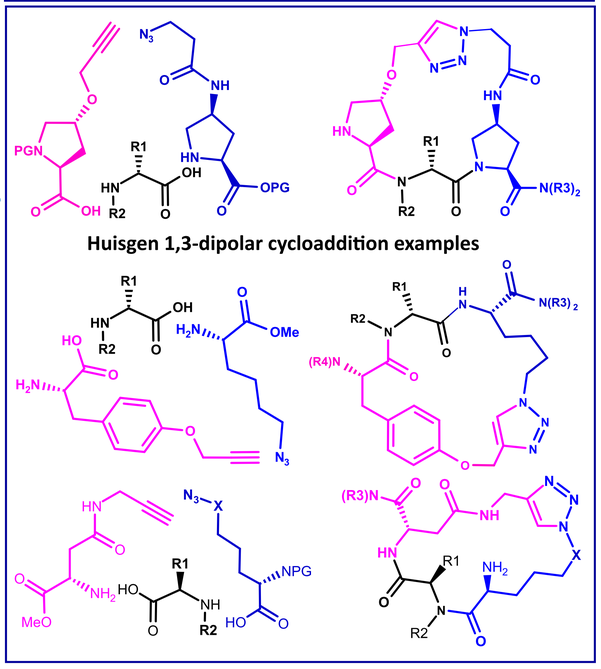
Ring expansions

- Schmidt, Beckmann, and related rearrangements
- Insertion of diazo compounds
- Insertion of activated alkynes

Cleavage of bridged bonds in "hidden macrocycles"

- Oxidative cleavage of double bonds
- Reductive cleavage of C-N bonds





References

1. Angelbello, A.J.; Disney, M.D. et al. *Chem. Rev.* **2018**, *118*, 1599-1663.
2. Deigan Warner, K.; Weeks, K.M. et al. *Nat. Rev. Drug Discov.* **2018**, *17*, 547-558.
3. González, À.L.; Artero, R. et al. PLoS ONE **2017**, *12*(6): e0178931.
4. Huang, Y.; Suga, H. et al. *Chem. Rev.*, ASAP DOI: 10.1021/acs.chemrev.8b00430.
5. Rzuczek, S.G.; Rice, J.E. et al. *J. Med. Chem.* **2010**, *53*, 3632-3644.
6. Forge, 10.6.0 Revision 35953, Cresset®, Litlington, Cambridgeshire, UK; Cheeseright, T.; Mackey, M.; Rose, S.; Vinter, A. *J. Chem. Inf. Model.* **2006**, *46*, 665-676.

ChemDiv, Inc., 12760 High Bluff Dr. Suite 370, San Diego, CA 92130, USA