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Pioneering science delivers vital medicines

TARGETED PROTEOLYSIS AND RNA INTERFERENCE – EMERGING MODALITIES IN MEDICINAL CHEMISTRY

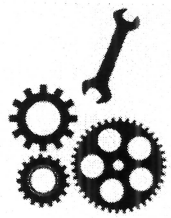
Philip Tagari – VP Research

ISAOC Napoli. September, 2018

THE GOALS OF MEDICINAL CHEMISTRY – MOLECULES THAT:

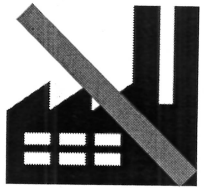
ACTIVATE*

INHIBIT*



DISPOSE OF

PREVENT PRODUCTION



*INTRACELLULAR & EXTRACELLULAR

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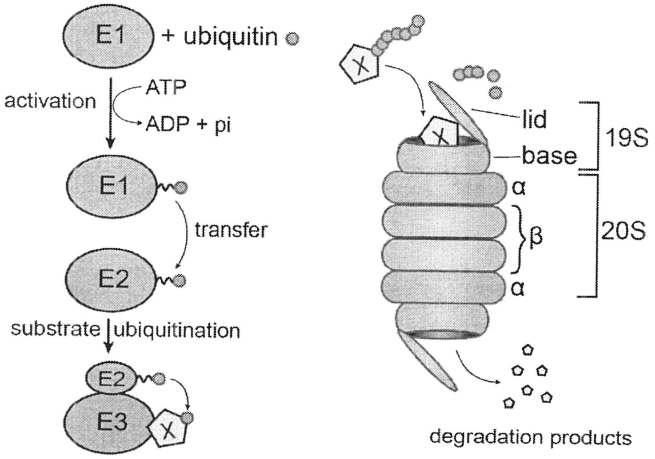
PROTEINS* ARE NATURALLY DISPOSED OF BY UBIQUITINATION



*overproduced, misfolded, mistranscribed, inappropriately modified etc

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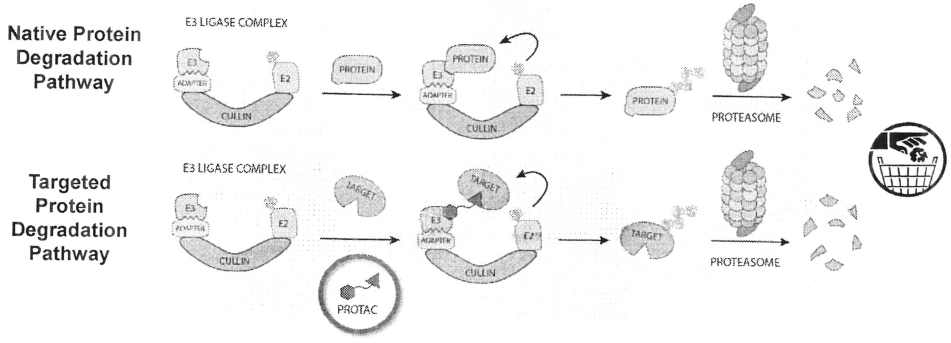
CAN WE HIJACK THE UBIQUITIN LIGASE MACHINERY TO TAG "UNDRUGGABLE" PROTEINS FOR DEGRADATION?



ProTaC: Proteolysis Targeting Chimera

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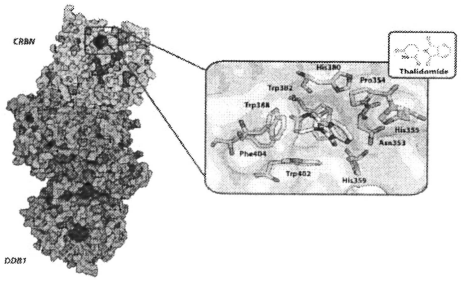
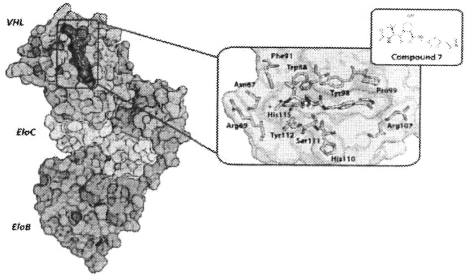
TWO LOW AFFINITY LIGANDS FOR THE E3 LIGASES CRBN AND VHL HAVE BEEN DESCRIBED

Cereblon (CRBN) E3 Ligase



VHL (Von Hippel-Lindau) E3 Ligase



Emil Bulatov, and Alessio Ciulli Biochem. J. 2015;467:365-386

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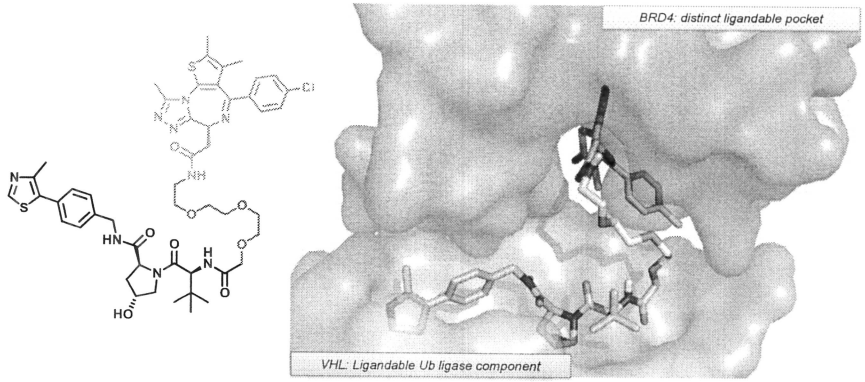
CONVERSION OF A VHL BINDER TO A BRD4 PROTAC



VHL/MZ1/BRD4P
DB ID: 5T35

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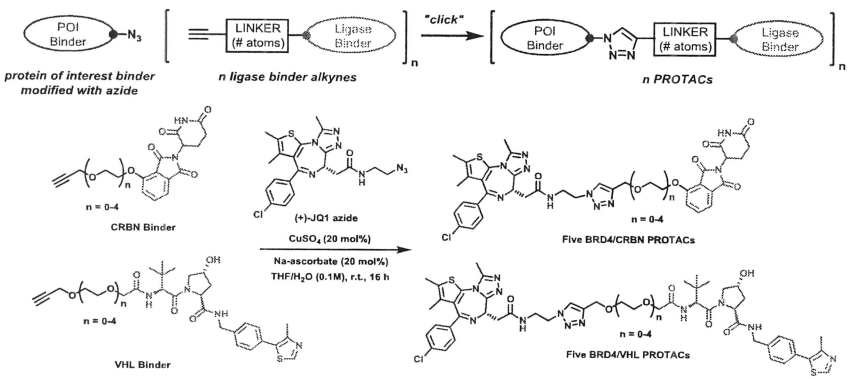
CLICK CHEMISTRY PLATFORM ENABLES RAPID PROTAC SYNTHESIS



Journal of Medicinal Chemistry. 2017, DOI: 10.1021/acs.jmedchem.6b01781

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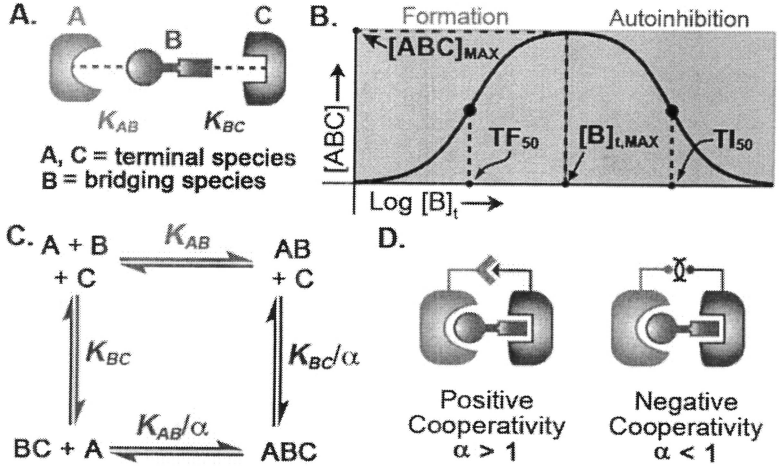
MODEL OF PROTAC COMPLEX FORMATION



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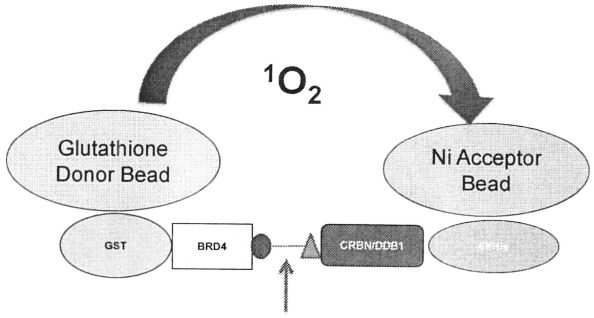
ALPHASCREEN ASSAY FOR PROTAC COMPLEX ASSEMBLY



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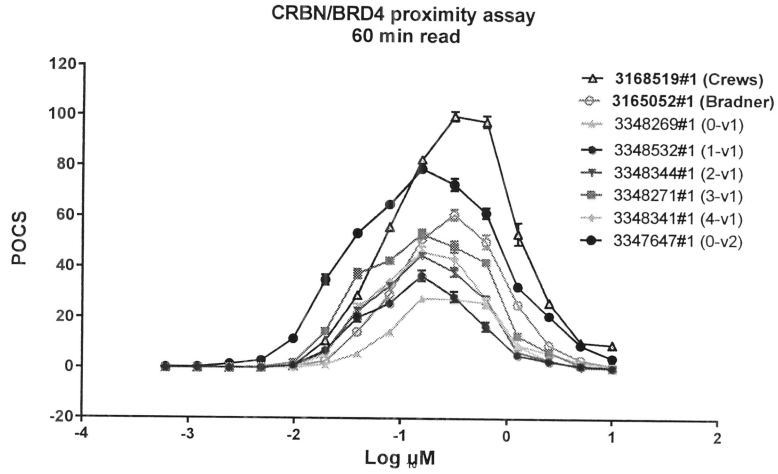
MAGNITUDE AND POTENCY OF COMPLEX FORMATION *IN VITRO*



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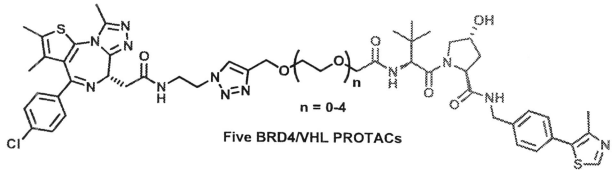
CLICK CHEMISTRY PROOF OF CONCEPT - VHL



BRD4/VHL Click PROTACs					
n	0	1	2	3	4
4 h H661 Cell BRD4 Degradation IC50 (uM)	0.059	0.083	0.24	0.46	0.63

Journal of Medicinal Chemistry. 2017, DOI: 10.1021/acs.jmedchem.6b01781

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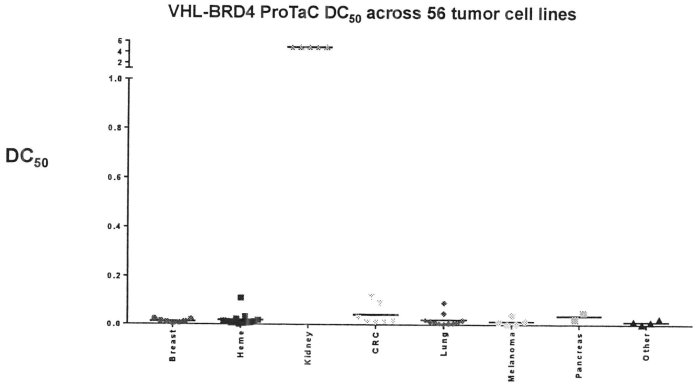
VHL- BROADLY ACTIVE AS A PROTAC-LIGASE ACROSS TUMOR TYPES (except RCC)



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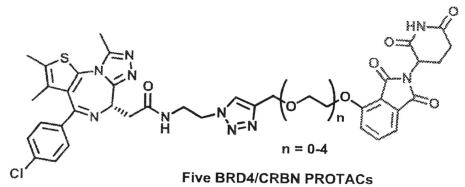
CLICK CHEMISTRY PROOF OF CONCEPT - CRBN



Five BRD4/CRBN PROTACs

BRD4/CRBN Click PROTACs					
n	0	1	2	3	4
4 h H661 Cell BRD4 Degradation IC50 (uM)	0.49	>5	>5	0.45	0.2

Journal of Medicinal Chemistry. 2017, DOI: 10.1021/acs.jmedchem.6b01781

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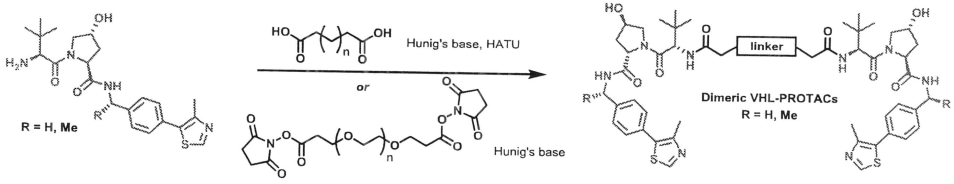
PROTEIN SELF-DESTRUCTION: DIMERIC PROTACS



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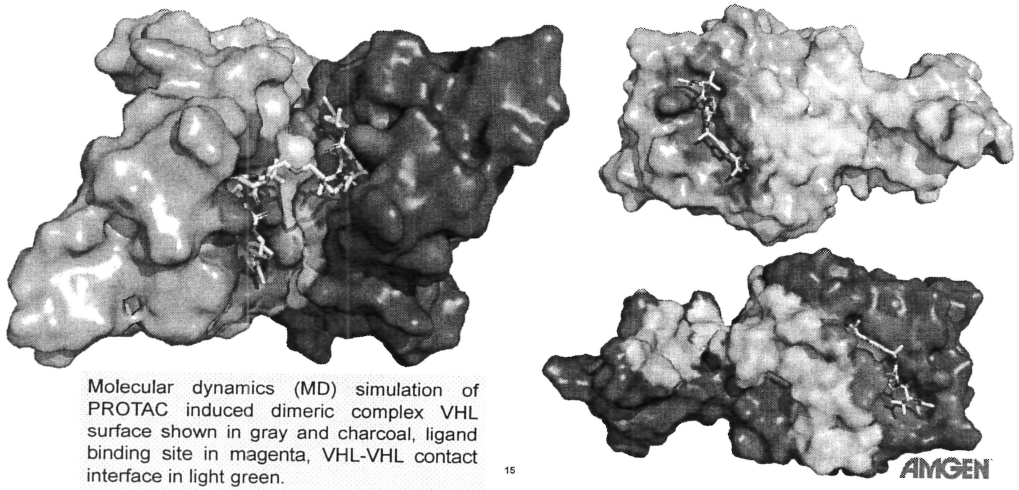
DIMERIC PROTAC INDUCES VHL SELF-ASSOCIATION



Molecular dynamics (MD) simulation of PROTAC induced dimeric complex VHL surface shown in gray and charcoal, ligand binding site in magenta, VHL-VHL contact interface in light green.

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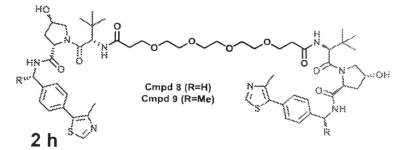
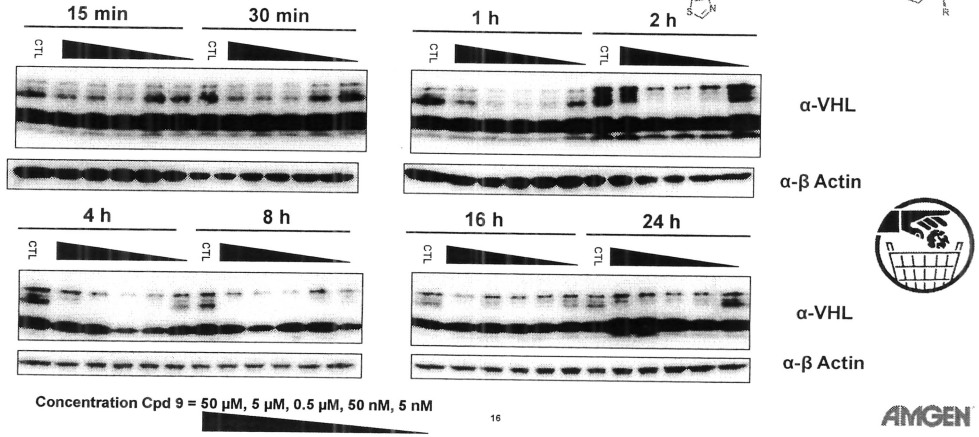
DIMERIC PROTAC INDUCES VHL SELF-DESTRUCTION





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SUMMARY - 1

- Ubiquitination is nature's way of removing surplus or misfolded proteins.
- Ubiquitination is a result of formation of a complex containing the target and E1, E2 and E3 ubiquitinylation proteins.
- Hetero - bivalent molecules that bind a target protein and an E3 ligase can induce ubiquitinylation and destruction of the target (essentially inhibiting its activity).
- Homo – bivalent E3 binders can catalytically induce destruction of the E3 ligases themselves.

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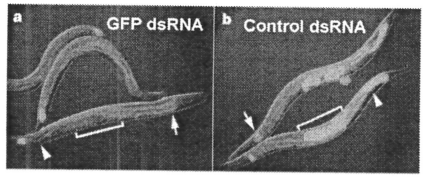
DISCOVERY OF RNA INTERFERENCE (RNAi)

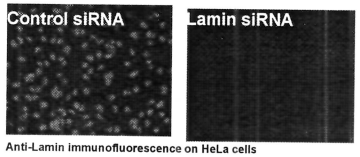
Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas†, Samuel E. Driver‡ & Craig C. Mello‡

* Carnegie Institution of Washington, Department of Embryology, 115 West University Parkway, Baltimore, Maryland 21210, USA
† Biology Graduate Program, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, USA
‡ Program in Molecular Medicine, Department of Cell Biology, University of Massachusetts Cancer Center, Two Biotech Suite 213, 373 Plantation Street, Worcester, Massachusetts 01605, USA

Nature, 1998





Anti-Lamin immunofluorescence on HeLa cells

Nature, 2001

Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells

Sayda M. Elbashir*, Jens Harborth*, Winfried Lendeckel*, Abdullah Yalcin*, Klaus Weber† & Thomas Tuschl*

* Department of Cellular Biochemistry and † Department of Biochemistry and Cell Biology, Max-Planck-Institute for Biophysical Chemistry, Am Fassberg 11, D-37077 Göttingen, Germany

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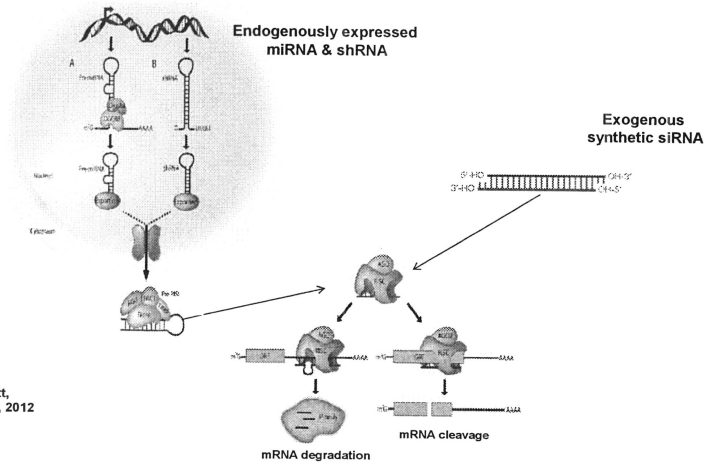
GENE REGULATION BY siRNA, shRNA, OR miRNA



Adapted from Burnett, Chemistry & Biology, 2012

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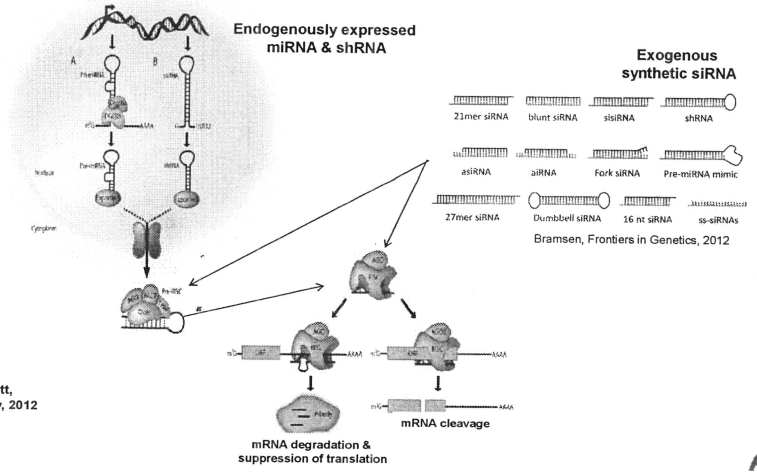
ALTERNATE FORMS OF SYNTHETIC siRNA



Adapted from Burnett, Chemistry & Biology, 2012

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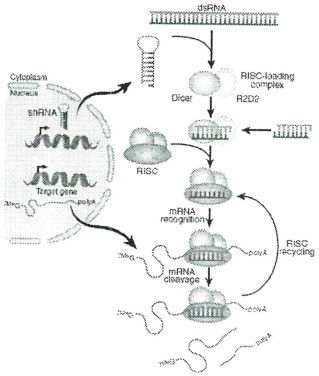
WHAT IS RISC?

- RISC = RNA-induced silencing complex, mediates post-transcriptional gene silencing
  - Dicer – RNase III family member
  - TRBP (HIV transactivating response RNA-binding protein) – orientates siRNA loading
  - Ago2 – endonuclease, catalytic core of RISC
- Phase I: Programming – siRNA loading into Ago2
- Phase II: Execution – cleavage of mRNA target complimentary to antisense (guide) strand of siRNA

Journal of Cell Science 123:1183-1189 (2010)



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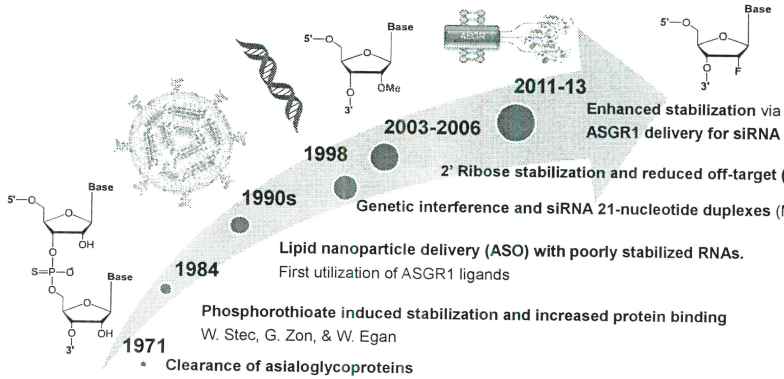
BACKBONE PHOSPHOROTHIOATES, HEAVY RIBOSE MODIFICATION, & ASGR1 HAVE DRIVEN siRNA EVOLUTION



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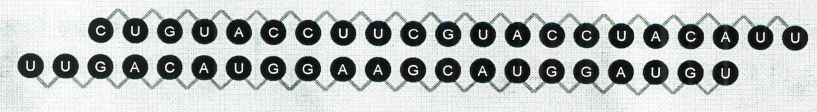
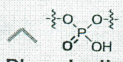
BUILDING THERAPEUTIC siRNAS

Unmodified siRNA

14 kDa RNA duplex
21 base pairs
2-nucleotide overhangs





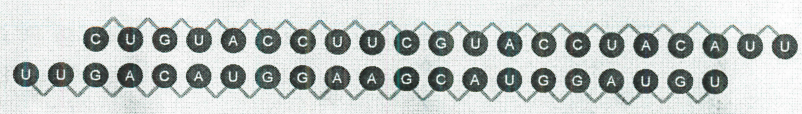
Phosphodiester

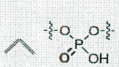
AMGEN

BUILDING THERAPEUTIC siRNAS

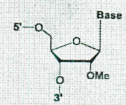
2'-Ribose (OMe, F) Modified siRNA

14 kDa RNA duplex
21 base pairs
2-nucleotide overhangs
No natural ribose nucleotides

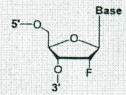




Phosphodiester



2'-Methoxy



2'-Fluoro

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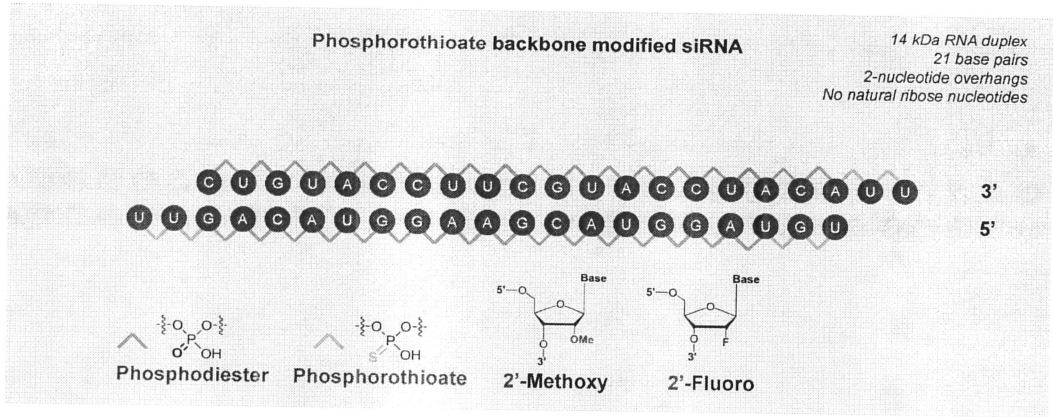
BUILDING THERAPEUTIC siRNAS



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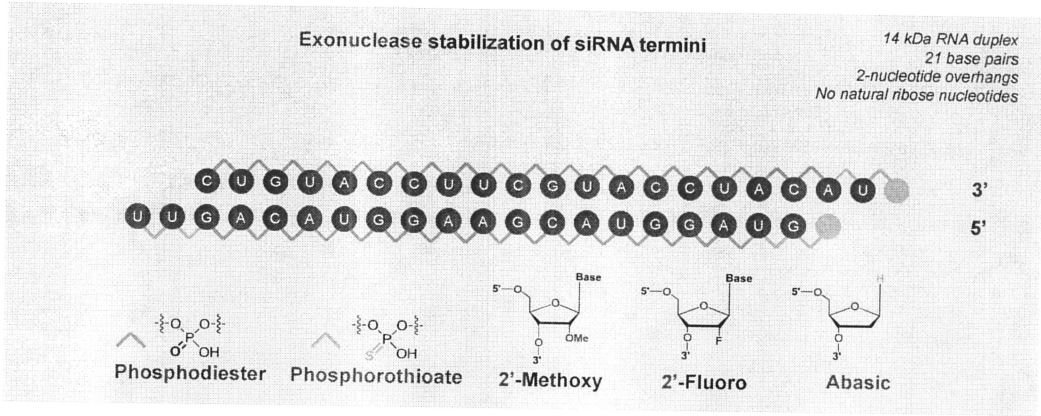
BUILDING THERAPEUTIC siRNAS



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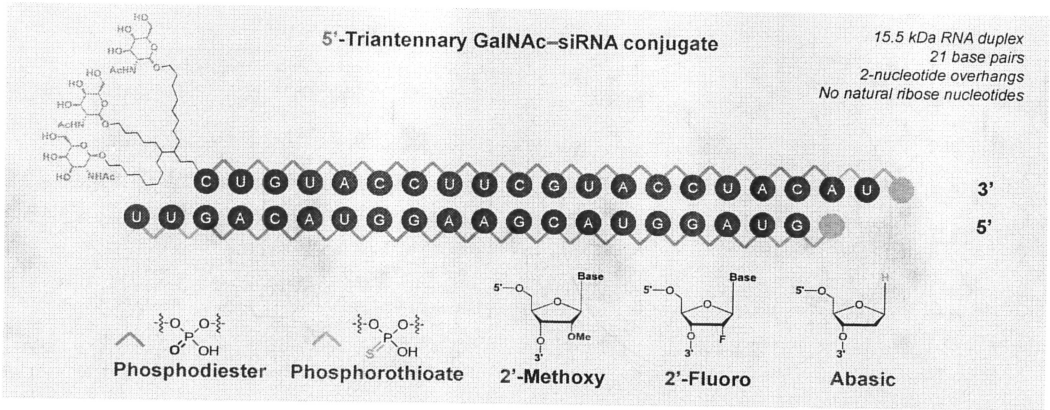
BUILDING THERAPEUTIC siRNAS



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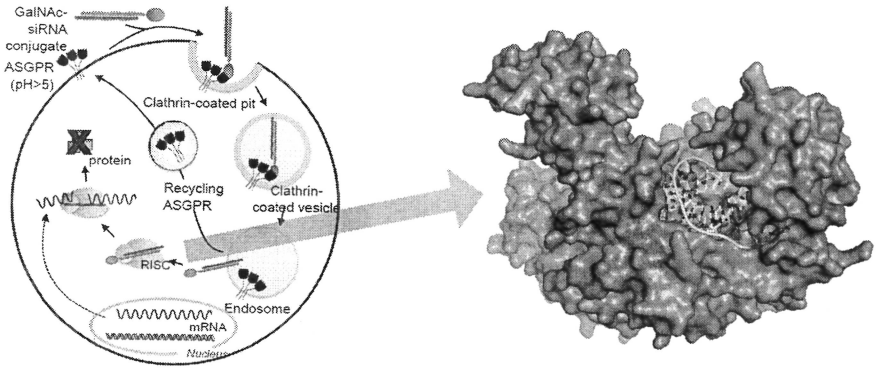
siRNA DELIVERED TO AGO IN LIVER CELLS BY ASGR1



PDB: 4W5O; hAgo2 with siRNA fragment bound

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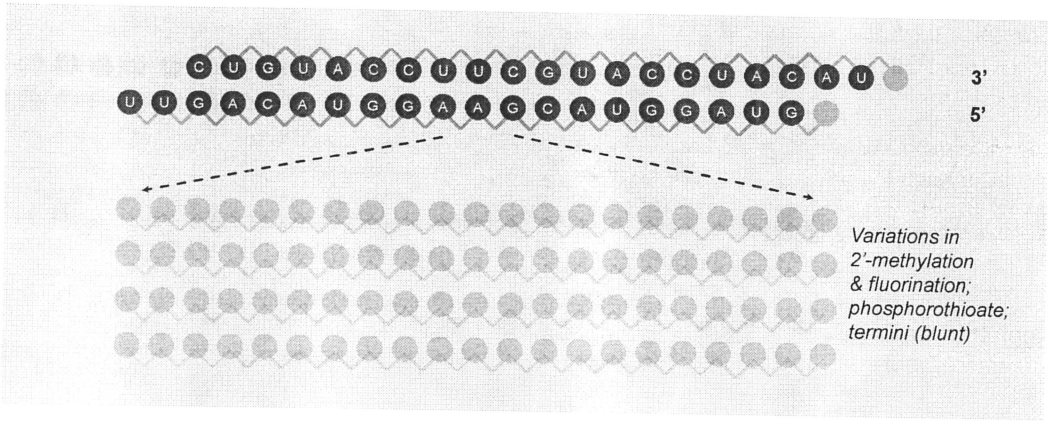
SUBTLE CHANGES IN siRNA DESIGN CAN HAVE SIGNIFICANT IMPACT ON FUNCTIONAL AND PHYSIOCHEMICAL PROPERTIES



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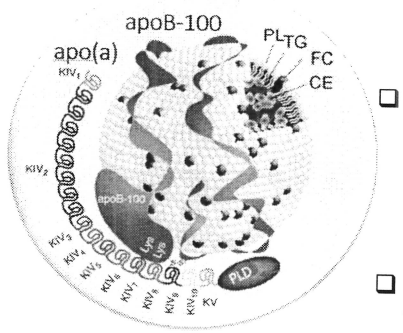
TARGETING Lp(a)* WITH siRNA



- Lp(a) is comprised of apo(a) covalently bound to apoB-100 and bound to a lipid core which includes LDL and oxidized phospholipids
- Lp(a) is an independent risk factor for CVD, however, the precise MOA is unknown
  - Increased Lp(a) levels are genetically determined and are associated with increased risk of CAD, MI, stroke, PAD and aortic stenosis[1-3]
- Pharmacologic reduction of plasma Lp(a) has therapeutic promise for reducing CV events

*Conventionally undruggable

[1]Nordestgaard BG *et al.* (2010) Eur. Heart J. 31:2844-2853
[2]Clarke R *et al.* (2009) N. Engl. J. Med. 361:2518-2528
[3]Kamstrup PR *et al.* (2009) JAMA 310:2331-2339

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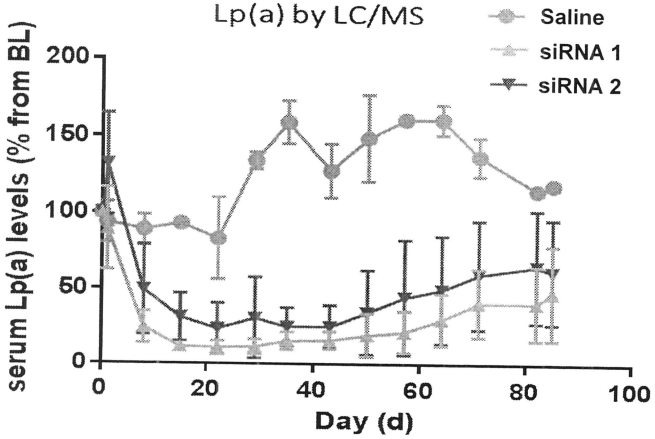
A SINGLE DOSE OF siRNA ACHIEVED >85% KNOCKDOWN OF SERUM Lp(a) IN NONHUMAN PRIMATES FOR >1 MONTH



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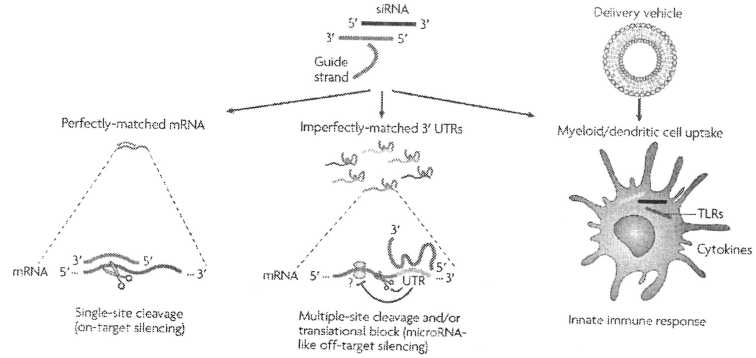
OFF-TARGET EFFECTS OF siRNA



Jackson & Linsley, Nat. Rev. Drug Disc., 2010

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SUMMARY - 2

- Gene expression is natively regulated by siRNA, shRNA, or miRNA.
- There are numerous alternate forms of synthetic (unmodified) siRNA which can also regulate gene expression, but all have poor pharmaceutical properties and minimal biodistribution.
- The last 35 years have seen numerous improvements based on chemical modifications and tissue targeting, allowing novel siRNAs to specifically reduce gene expression.
- ONPATTRO™ was recently the first siRNA approved by the FDA, signaling a new era of nucleotide therapeutics.