Molecular Glues

Ciaran P. Seath

MacMillan Group Meeting

4/20 2021
Outline

What is a molecular glue?

Why are they important

Background/History

Case study: Hijacking the ubiquitination pathway

Highlights in molecular glue research

Conclusions
What are Molecular Glues?

Molecular glues are small molecules that induce protein-protein interactions.
What are Molecular Glues?

binding is initiated through a small molecule ligand

two proteins without binding affinity
What are Molecular Glues?

a new protein-protein interaction is initiated

binding is initiated through a small molecule ligand
What are Molecular Glues?

BenchMarks

The Rise of Molecular Glues

Stuart L. Schreiber1,2,*
1Department of Chemistry & Chemical Biology, Harvard University, 12 Oxford St, Cambridge, MA 02138, USA
2Chemical Biology & Therapeutics Science Program, Broad Institute, 415 Main St, Cambridge, MA 02142, USA
*Correspondence: stuart_schreiber@harvard.edu

2021 marks the 30th anniversary of the revelation that cyclosporin A and FK506 act in a way previously not seen—as “molecular glues” that induce neo-protein–protein associations. As a torrent of new molecular-glue probes and medicines are fueling interest in this field, I explore the arc of this story.
What are Molecular Glues?

### A. Proximity in nature

i. Cell signaling
- PDGF
  - Phosphate
- Kinase
- Phosphorylation
- Gene expression

ii. Chromatin remodeling
- Methyl
- Methyl-transferase
- Transcriptional silencing

iii. Immune response
- MHC
- Antigenic peptide
- T cell receptor
- T cell

### B. Natural product-based glue

i. With linkers, genetic fusions
- FK1012
- Ronacidin
- Camptothecin

ii. Without linkers, genetic fusions
- Rapamycin
- FKBP12
- FRB/mTOR

iii. Without linkers, genetic fusions
- Chitin remodeling
- EIF

iv. Without linkers, native proteins
- α-tubulin
- β-tubulin
- Taxol
desmodermide
- Microtubule stabilization
- MitoC

### C. Hybrid natural product/synthetic glue

i. With linker, genetic fusion and native protein
- Ubiquitin
- Fusion protein
- Native protein

ii. Without linker, native protein
- Adenosine
- PKG
- Rapamycin

### D. Synthetic glue

i. Without linker, native proteins
- Ubiquitin
- Thalidomide
- Meladomide

ii. With linker, native proteins
- SHP2 and PP2A
- PROMAC

iii. Without linker, one native protein

iv. Synthal
What are Molecular Glues?

Hormones, antigenic peptides and PTMS all induce PPIs in nature
What are Molecular Glues?

Natural products are known to induce protein dimerization and can be exploited through genetic fusions.
What are Molecular Glues?

Synthetic hybrids have been used as tools to control biology.
What are Molecular Glues?

Molecular glues for therapeutic intervention
Why are they Important?

44% of the “druggable” proteome currently have FDA approved compounds.

This accounts for 3% of the total proteome.

Or 0.5% when you include protein-protein interactions.
Why are they Important?

Accessing these PPIs in drug discovery would be extremely powerful.

enabling interactions for gain of function

blocking interactions for loss of function
Why are they Important?

Most glues provide gain-of-function (new interaction)

Molecular Glues (this presentation)

- Small molecules  
- Numerous mechanisms of action  
- Several blockbuster drugs and developing area  
- New research area

thalidomide derivatives  
rapamycin analogs  
SHP099
**Why are they Important?**

Most glues provide gain-of-function (new interaction)

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**Molecular Glues (this presentation)**

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- Numerous mechanisms of action  
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- New research area

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**Degraders of undruggable proteins**

- thalidomide derivatives  
- rapamycin analogs  
- SHP099
A Quick Look at the Ubiquitin Proteosome System

Nerve cell

Muscle cell

Cells have same genome - but vastly different constituents
A Quick Look at the Ubiquitin Proteosome System

different cells have different proteomes

they *make* different proteins and *degrade* different proteins
A Quick Look at the Ubiquitin Proteosome System

Ubiquitination by sequential E1/2/3 ligases leads to altered function or degradation.
A Quick Look at the Ubiquitin Proteosome System

Ubiquination by sequential E1/2/3 ligases leads to altered function or degradation
A Quick Look at the Ubiquitin Proteosome System

Ubiquitination by sequential E1/2/3 ligases leads to altered function or degradation

At least 600 known E3 ligases - combinations allow discrete function

E2 binding site
E3 ligases and molecular glues were first linked in 2007 in the context of plant biology.
A Historical Perspective

A plant hormone that controlled plant development

Implicated in degradation of transcription factors

A report in 2001 showed that auxin was key for inducing SCF\textsuperscript{TIR1} degradation of AUX/IAA

Still no molecular basis for this - SAR not instructive

A Historical Perspective

Structural biology from the Zheng group revealed the answer

A Historical Perspective

Auxin ligand binds to peptide recognition pocket, promoting a PPi

Structural biology from the Zheng group revealed the answer

A Historical Perspective

Auxin ligand binds to peptide recognition pocket, promoting a PPi

no auxin - no binding - no degradation

Structural biology from the Zheng group revealed the answer

A Historical Perspective

Auxin ligand binds to peptide recognition pocket, promoting a PPi

**Auxin binding - recruits IAA/AUX - degradation**

Structural biology from the Zheng group revealed the answer

This discovery prompted a closer look at this mechanism in humans
Case Study - Thalidomide as a molecular glue
Case Study: The Thalidomide Story - From Villain to Hero

How did thalidomide turn around its image? Once a poster child for bad Pharma, now the genesis of the hottest area in small molecule drug discovery.

15 years of world class structural and molecular biology
Case Study: The Thalidomide Story - From Villain to Hero

How did thalidomide turn around its image? Once a poster child for bad Pharma, now the genesis of the hottest area in small molecule drug discovery.

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Scifinder search “molecular glues”
Case Study: The Thalidomide Story - Highjacking the Ubiquitination pathway

**Thalidomide**

**Glutethimide**

**Phenobarbital**

Developed by Chemie Grunenthal in 1953 as a safer alternative to barbiturates

Marketed heavily the drug was widely prescribed, including to pregnant women
Case Study: The Thalidomide Story - Highjacking the Ubiquitination pathway

Developed by Chemie Grunenthal in 1953 as a safer alternative to barbiturates

This led to the a terrible medical tragedy as 80,000 babies died of teratogenic effects
Case Study: The Thalidomide Story - Highjacking the Ubiquitination pathway

Given a new lease of life by Dr Judah Folkman who found it was an effective treatment for multiple myeloma

at the time multiple myeloma was incurable by conventional chemotherapy

33% of patients responded to the therapy

Case Study: The Thalidomide Story - From Villain to Hero

- Following thalidomide, two more IMiDs were approved for MM and other indications

### Thalidomide

- **Celgene, 2006**
- ENL, MM

### Lenalidomide

- **Celgene 2006**
- MM, amyloidosis, mantle cell lymphoma. In the clinic for many other cancers

### Pomalidomide

- **Celgene, 2013**
- Primary myelofibrosis, MM
**Case Study: The Thalidomide Story - Mechanistic Hypothesis**

Older reviews (into early 10’s) provide numerous differing hypotheses to explain the therapeutic effects of thalidomide (FDA approval 2006).

- **Anti-angiogenesis**: FGF and Shh signaling decreased, modulates expression of growth factors and immune-modulatory proteins. TNF-α inhibition.

- **Tubulin binding**: Thalidomide metabolites bind tubulin altering cell division.

- **ROS**: O₂ leads to abnormal growth. Induces oxidative stress. Upregulation of Bmp and dkk1 both prevented by spin trapping agents.
Case Study: The Thalidomide Story - Mechanistic Hypothesis

Mechanistic target and molecular mechanism were unknown until recently
Case Study: The Thalidomide Story - CRBN

A race to understand Thalidomide

2010: Handa, Science

2014: Ebert, Science and Kaelin, Science

2015: Ebert, Nature

2016: Chamberlain, Nature/Thoma, Nature


Most of the key data gathered at Celgene or Dana-Farber
**Case Study: The Thalidomide Story - CRBN**

2010: Hiroshi Handa and co-workers show CRBN is the primary target of thalidomide

**Hypothesis:** Thalidomide blocks natural degradation pathways

*Science* 2010, 327, 1345–1350
**Case Study: The Thalidomide Story - CRBN**

2014: IMiD binding to CRBN leads to degradation of Ikaros transcription factors

**Hypothesis:** CRBN binders also provide a gain-of-function, a new MOA in cancer DD

**Case Study: The Thalidomide Story - CRBN**

2014: IMiD binding to CRBN leads to degradation of Ikaros transcription factors

Confirmed by WB in MM1S cells and in cancer tissue

**Hypothesis:** CRBN binders also provide a gain-of-function, a new MOA in cancer DD

Case Study: The Thalidomide Story - CRBN

2014: Thalidomide binds the surface of CRBN - stabilizing PPIs

Case Study: The Thalidomide Story - CRBN

2014: Thalidomide binds the surface of CRBN - stabilizing PPIs

IMiDs all bind the same surface of CRBN

Case Study: The Thalidomide Story - CRBN

2014: Thalidomide binds the surface of CRBN - stabilizing PPIs

IMiDs binding surface is conserved across species

2014: Thalidomide binds the surface of CRBN - stabilizing PPIs

Protein microarrays suggest MEIS2 as a native substrate for CRBN (no ligand).

MEIS2 involved in transcriptional regulation – important for human development

**Case Study: The Thalidomide Story - CRBN**

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**Case Study: The Thalidomide Story - CRBN**

2014: Thalidomide binds the surface of CRBN - stabilizing PPIs

MEIS2 levels are stabilized by Len exposure

MEIS2 involved in transcriptional regulation – important for human development

Hypothesis: IMiDs act as both agonists and antagonists

**Case Study: The Thalidomide Story - CRBN**

**2015: Lenalidomide provides GoF over other IMiDs, and cancer specific degradation**

Lenalidomide also effective treatment for MDS with chromosome 5q deletion

*Small changes in IMiD structure can have dramatic effects*

Ebert: *Nature* 2015, 523, 183–188
2015: Lenalidomide provides GoF over other IMiDs, and cancer specific degradation

*Case Study: The Thalidomide Story - CRBN*

Ebert: *Nature* 2015, 523, 183–188
**Case Study: The Thalidomide Story - CRBN**

2015: Lenalidomide provides GoF over other IMiDs, and cancer specific degradation

<table>
<thead>
<tr>
<th>Drug (μM)</th>
<th>Lenalidomide</th>
<th>CC-122</th>
<th>Pomalidomide</th>
<th>Thalidomide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0  1  10</td>
<td>0  1  10</td>
<td>0  1  10</td>
<td>0  10  50</td>
</tr>
</tbody>
</table>

*IB: CK1α, IKZF1, GAPDH*

*Lenalidomide and not pomoloidomide or thalidomide degrades CK1α*

Ebert: *Nature* 2015, 523, 183–188
When designing mouse models the authors observed some interesting differences.
Case Study: The Thalidomide Story - CRBN

2015: Lenalidomide provides GoF over other IMiDs, and cancer specific degradation

Mouse CRBN does not respond to Lenalidomide

Valine to isoleucine change shown to be responsible

Ebert: Nature 2015, 523, 183–188
Case Study: The Thalidomide Story - CRBN

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Case Study: The Thalidomide Story - CRBN

CC-885 shown to have clinical potential in phenotypic screens

Potent anti-cancer effect vs Acute Myeloid Leukemia

GSPT1 shown to be degraded by CC-885

Case Study: The Thalidomide Story - CRBN

4 Proteins now identified as Neo-substrates – no identifiable sequence homology

What is going on?

Case Study: The Thalidomide Story - CRBN

Structural biology reveals the loop required for degradation

Xray of CRBN/DDB1 in complex with CC-885 and GSPT1

Case Study: The Thalidomide Story - CRBN

Structural biology reveals the loop required for degradation

Interactions with W400, H357, N351 are key

Case Study: The Thalidomide Story - CRBN

Structural biology reveals the loop required for degradation

Overlay with Ikaros shows same loop. Only homologous AA G141.

Case Study: The Thalidomide Story - CRBN

Structural biology reveals the loop required for degradation

This degron hypothesis was further strengthened by the structure of CK1α with CRBN and lenalidomide

Case Study: The Thalidomide Story - CRBN

Structural biology reveals the loop required for degradation

Case Study: The Thalidomide Story - CRBN

Back to the beginning - how does Thalidomide cause teratogenic birth defects?

Back to the beginning - how does Thalidomide cause teratogenic birth defects?

Mus musculus

Mice are resistant to thalidomide teratogenic effects

Case Study: The Thalidomide Story - CRBN

Back to the beginning - how does Thalidomide cause teratogenic birth defects?

Mice are resistant to thalidomide teratogenic effects

Mouse CRBN does not induce degradation or Ikaros etc with IMiDs

Case Study: The Thalidomide Story - CRBN

Back to the beginning - how does Thalidomide cause teratogenic birth defects?

Mice are resistant to thalidomide teratogenic effects

Mouse CRBN does not induce degradation or Ikaros etc with IMiDs

Maybe Ikaros degradation is responsible?

Case Study: The Thalidomide Story - CRBN

Do humanized mice respond to thalidomide?

Mouse expressing hCRBN

hCRBN mouse displays degradation of Ikaros/Aiolos but remains resistant to teratogenicity

Ikaros is not the neosubstrate that leads to teratogenic effects!

Case Study: The Thalidomide Story - CRBN

SALL4 was considered as a possible target

ORIGINAL ARTICLE
Mutations at the SALL4 locus on chromosome 20 result in a range of clinically overlapping phenotypes, including Okihiro syndrome, Holt-Oram syndrome, acro-renal-ocular syndrome, and patients previously reported to represent thalidomide embryopathy

J Kohlhase, L Schubert, M Liebers, A Rauch, K Becker, S N Mohammed, R Newbury-Ecob, W Reardon

TF that displays correct degron motif

**Case Study: The Thalidomide Story - CRBN**

Thalidomide degrades SALL4 in a CRBN dependent manner.

<table>
<thead>
<tr>
<th>CRBN WT</th>
<th>CRBN --/--</th>
</tr>
</thead>
<tbody>
<tr>
<td>- 0.02 0.2 2 20</td>
<td>- 0.02 0.2 2 20</td>
</tr>
<tr>
<td>Thal (μM)</td>
<td></td>
</tr>
<tr>
<td>Sall4</td>
<td></td>
</tr>
<tr>
<td>Cereblon</td>
<td></td>
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<tr>
<td>Actin</td>
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</table>

SALL4 localizes to limb buds decreases with thalidomide treatment.

Case Study: The Thalidomide Story - CRBN

Mouse immunity can be explained by SALL4 sequence homology models

4 AA changes in ZF2

Case Study: The Thalidomide Story - CRBN

X-ray consistent with all of the work performed so-far

Case Study: The Thalidomide Story - CRBN

What’s coming next in the Thalidomide story?
Case Study: The Thalidomide Story - CRBN

Native ligands for CRBN?

- Uridine
- Arginine: $R = \text{Me}_n$
- Lysine: $R = \text{Ac}, \text{Me}_n$

Other proteins containing degron

Methods for discovery of new glues

*Science* 2018, 362, eaat0572
Case Study: The Thalidomide Story - CRBN

Thalidomide has changed the industry
Case Study: The Thalidomide Story - CRBN

Clinical regulation changed overnight

Thalidomide has changed the industry
Case Study: The Thalidomide Story - CRBN

Clinical regulation changed overnight
Highlights in Molecular Glue Research

The Bradner lab describe the quintessential PROTAC

Highlights in Molecular Glue Research

Bradner and Fischer lab report the first BRD4 selective degrader

Indisulam recruits RBM39 to the DCAF15 E3 ligase complex

(In clinic vs AML and MDS)
**Highlights in Molecular Glue Research**

**New E3 Ligases**

Indisulam recruits RBM39 to the DCAF15 E3 ligase complex

*(In clinic vs AML and MDS)*

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CR8 recruits CDK12/Cyclin K complex to CRL4 E3 ligase (without CRBN)
Highlights in Molecular Glue Research

Protein-RNA Molecular Glues: A treatment for Spinal Muscular Atrophy

SMA affects 1 in 10,000

Treatments are broadly supportive

Protein-RNA Molecular Glues: A treatment for Spinal Muscular Atrophy

Highlights in Molecular Glue Research

NVS-SM2

Highlights in Molecular Glue Research

Intramolecular Glues: SHP2

Auto-inhibited conformation

Open/active conformation

Phosphatases “undruggable targets” - anionic molecules make poor medicines

Nature 2016, 535, 148–152
Highlights in Molecular Glue Research

Intramolecular Glues: SHP2

SHP099 locks SHP2 in closed conformation

First clinical phosphatase inhibitor

6 ongoing clinical trials for advanced solid tumors

SHP2 IC₅₀: 0.071 μM

**Nature** 2016, 535, 148–152
Conclusions

- This fast moving topic isn’t finished, the best is yet to come

- Proteomics has been at the forefront of this research, new innovations will accelerate discovery
  - Industry driven fundamental research

- Has the potential to unlock 1000’s of “undruggable” targets
Resources

Schreiber Cell 2021

Dana-Farber Targeted Degradation Webinar Series: Bradner, Chamberlain, Koduri and others

novartis.com

Evolution of Cereblon-Mediated Protein Degradation as a Therapeutic Modality

ACS. Med. Chem. Lett. 2019, 10, 1592
Thanks for listening! Questions?