Identification And Characterization Of Novel Inhibitors Of HIV Tat Protein

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CONFLICTS OF INTEREST

No conflicts of interests.

COMMUNITY SUMMARY

- **Key question(s) being asked:**

The HIV Tat protein is indispensable for HIV replication and pathogenesis but there are no clinically available antiretrovirals that block Tat. Our goal is to discover new HIV Tat inhibitors.

- **Key finding(s) and take-home message:**

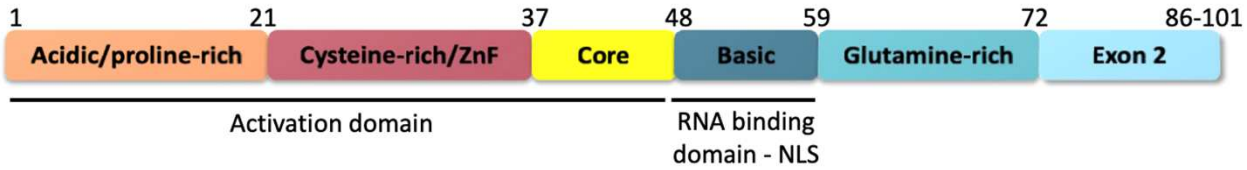
A high throughput screening of ~580K small molecules identified three distinct Tat inhibitors. These inhibitors trigger Tat breakdown to enforce deep-latency/sleep needed in HIV cure efforts.

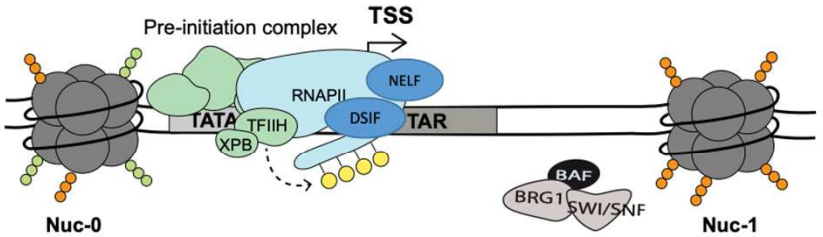
- **What are the next steps?:**

Medicinal chemistry is ongoing to improve the pharmacological properties of these molecules.

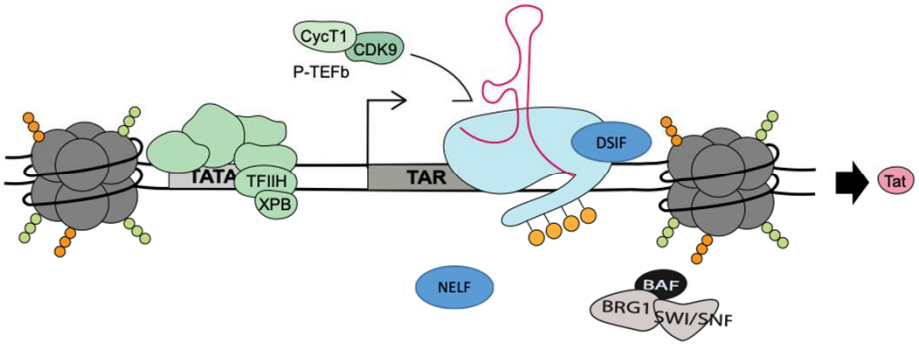
Their potency and efficacy will be tested in preclinical models of HIV infection.

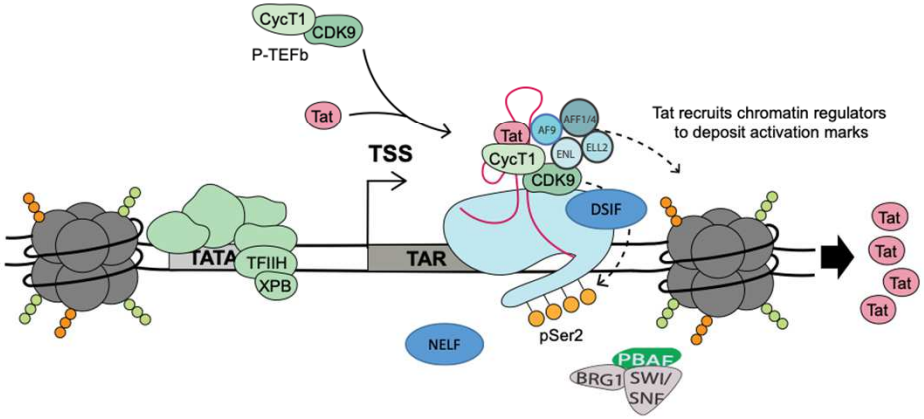
HIV Tat protein is an attractive target for therapeutic intervention





- **Expressed early during virus replication**
- **Detected during suppressive ART**
- **No host cellular homologs**
- **Interacts with more than ~ 700 partners to mediate HIV replication and HIV-associated pathologies**





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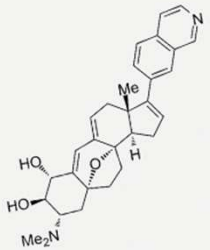
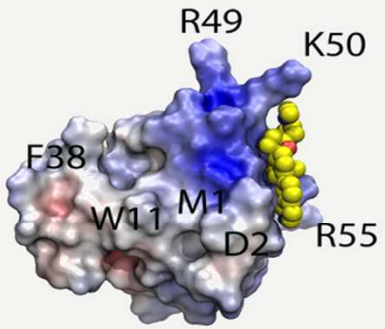
Tat inhibitors will help develop Block-and-Lock approaches

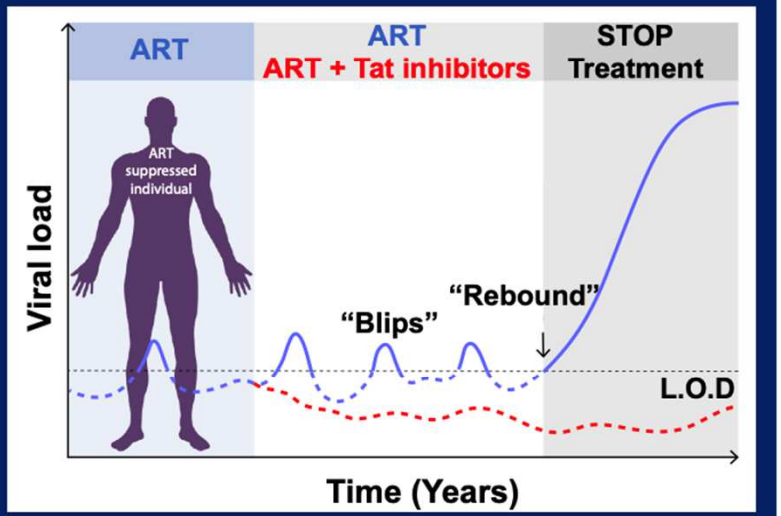
didehydro-Cortistatin A (dCA)

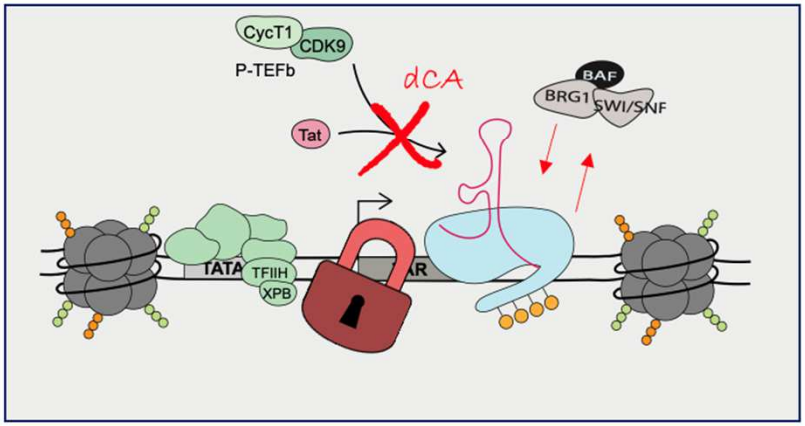


EC_{50} = 0.1-1 nM , CC_{50} > 20 μM

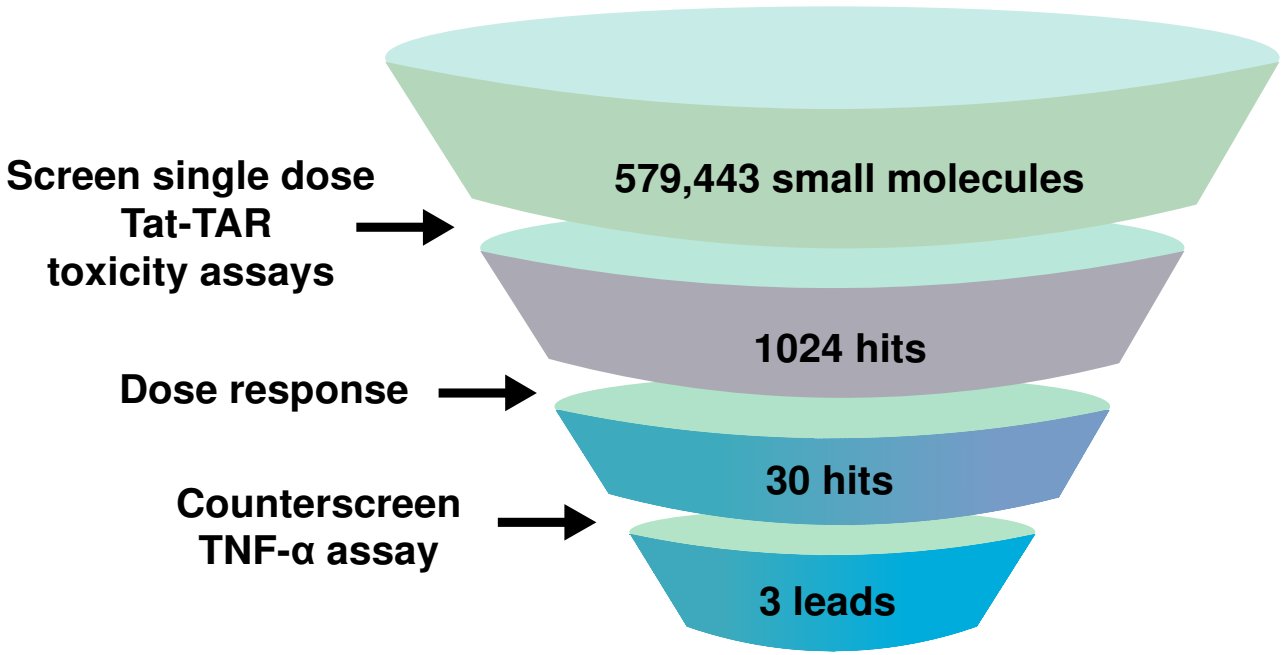


Block-and-Lock approach

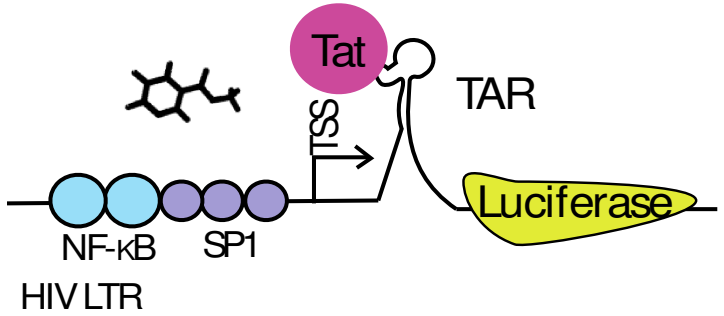




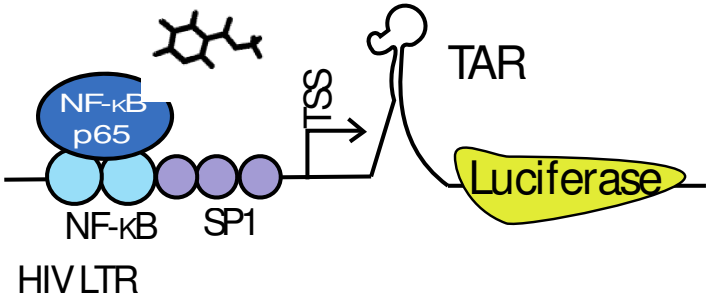
HTS of ~580k small molecules reveal 3 potential Tat inhibitors



Screen: Tat-TAR assay
Tat dependent reactivation



Counterscreen: TNF-α assay
Tat independent reactivation



SR SOUTHERN RESEARCH

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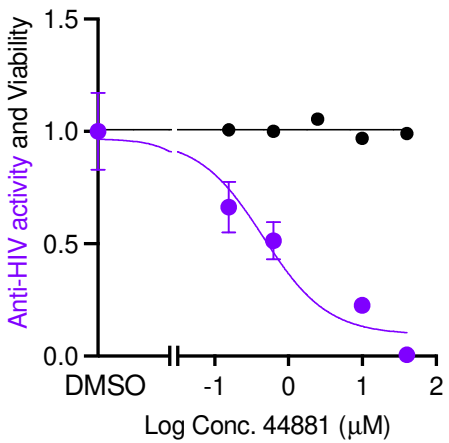
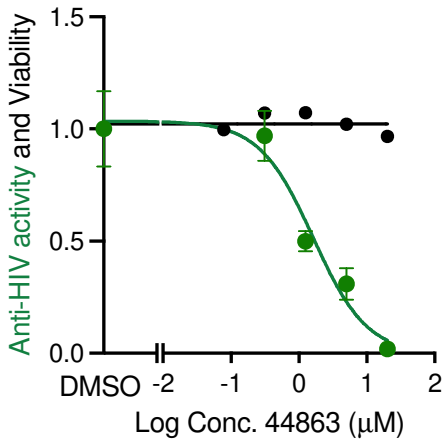
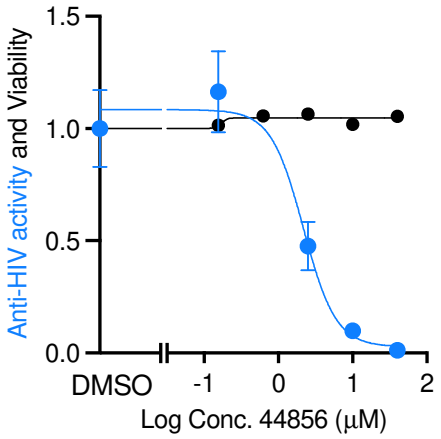
Activity of the 3 selected hits

	Assays	µM	44881	44863	44856
HTS DATA	Tat-TAR, Tat 101 a.a	IC_{50}	8.1	3.5	7.1
	Toxicity	CC_{50}	> 50	> 50	> 50
	TNF-α	IC_{50}	> 100	> 100	> 100
CONFIRMATION	Tat-TAR, Tat 86 a.a	IC_{50}	9.6 ± 1.6	4.4 ± 0.7	7.8 ± 1.8
	Toxicity	CC_{50}	597	> 800	> 400
	Therapeutic Index CC_{50}/IC_{50}	**TI**	**62.2**	**> 181.8**	**> 51.3**
NL4.3	Jurkat	**IC_{50}**	**0.45**	**1.06**	**2.39**
		CC_{50}	> 40	> 20	> 40
		TI	> 88.9	> 18.9	> 16.7

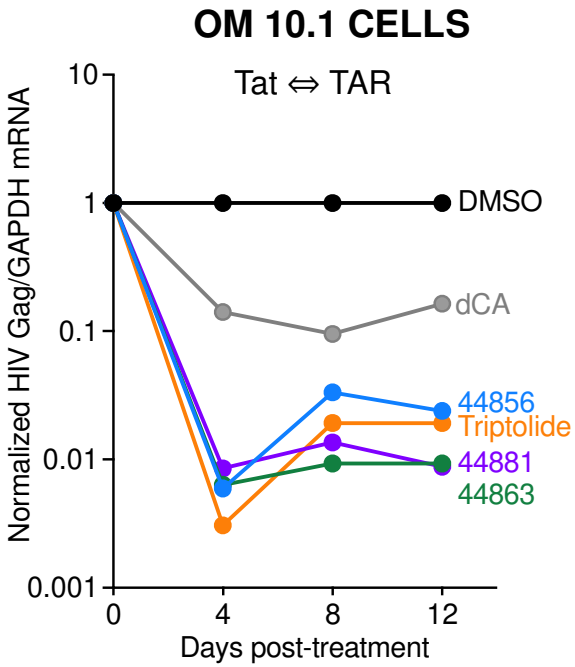
All 2-step synthesis

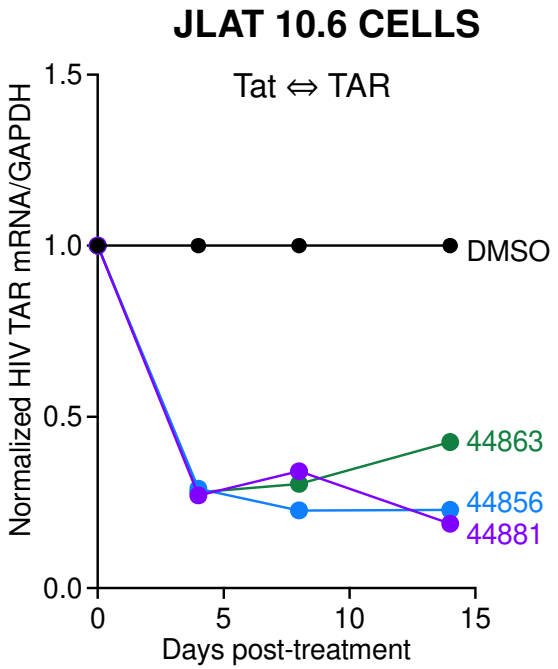


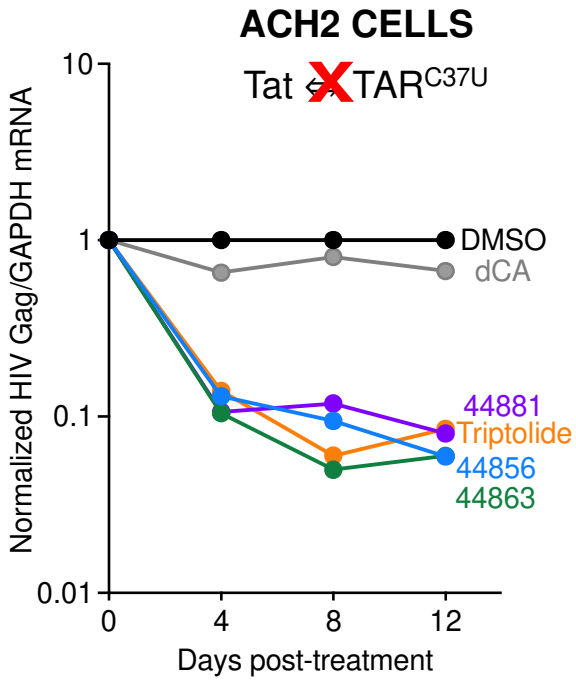


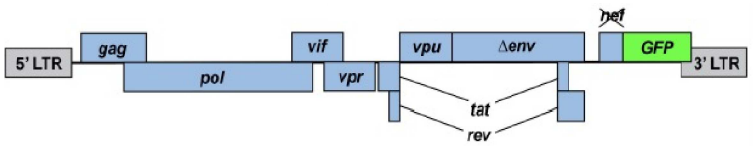


Leads inhibit HIV transcription in models of HIV latency



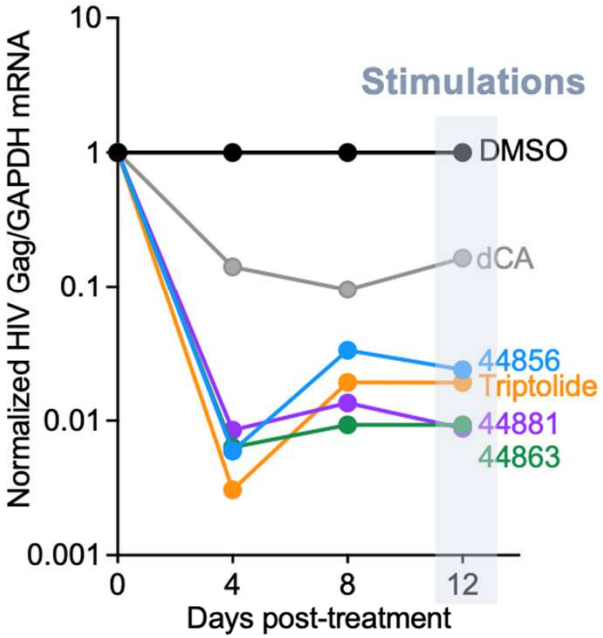


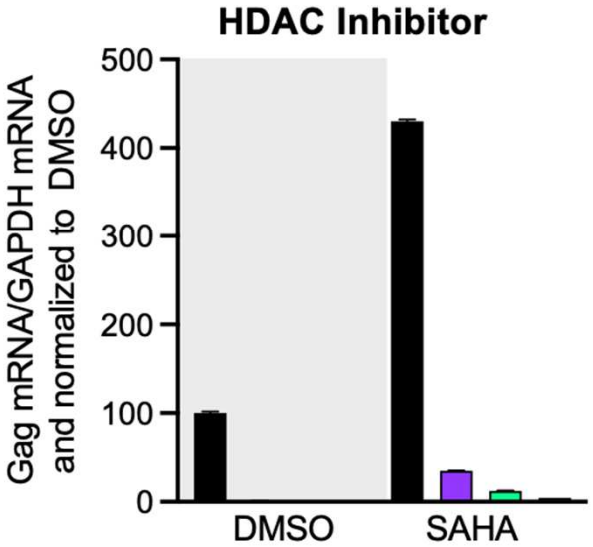


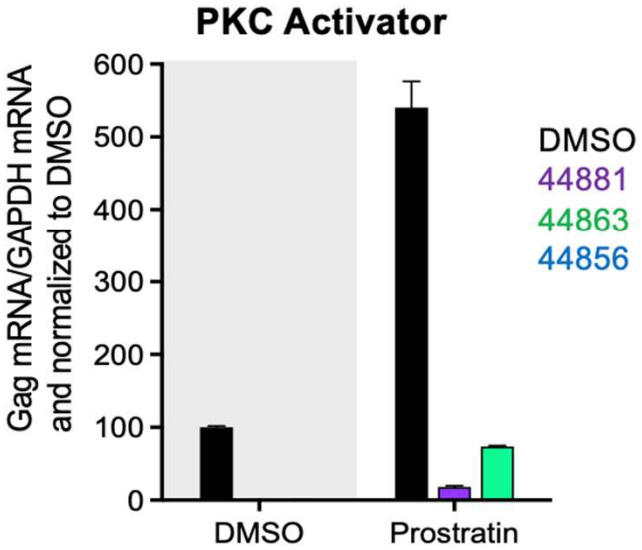


OM 10.1 - ACH2 cells: (15 µM leads, 10 nM dCA, 1 nM Triptolide) + cocktail ARVs. JLAT 10.6 cells: 20 µM leads

Leads block reactivation from latency in OM 10.1 cells







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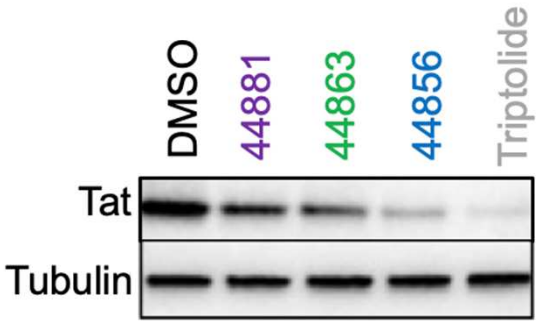
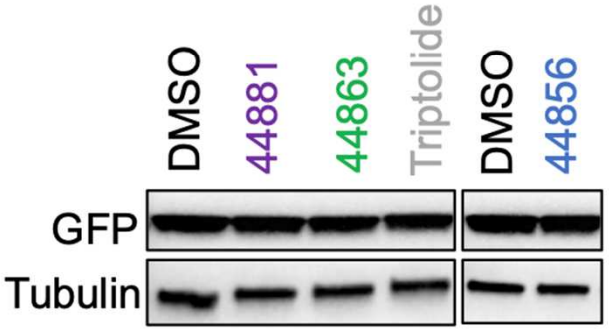
OM 10.1 cells: 15 μM leads + cocktail ARVs

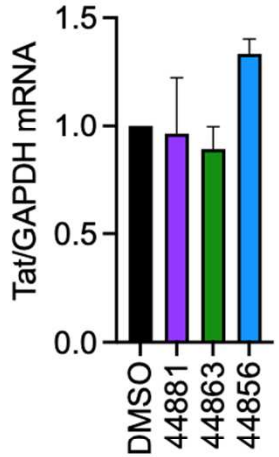
Leads mediate Tat degradation without affecting Tat mRNA

Tat overexpression in HEK293T cells

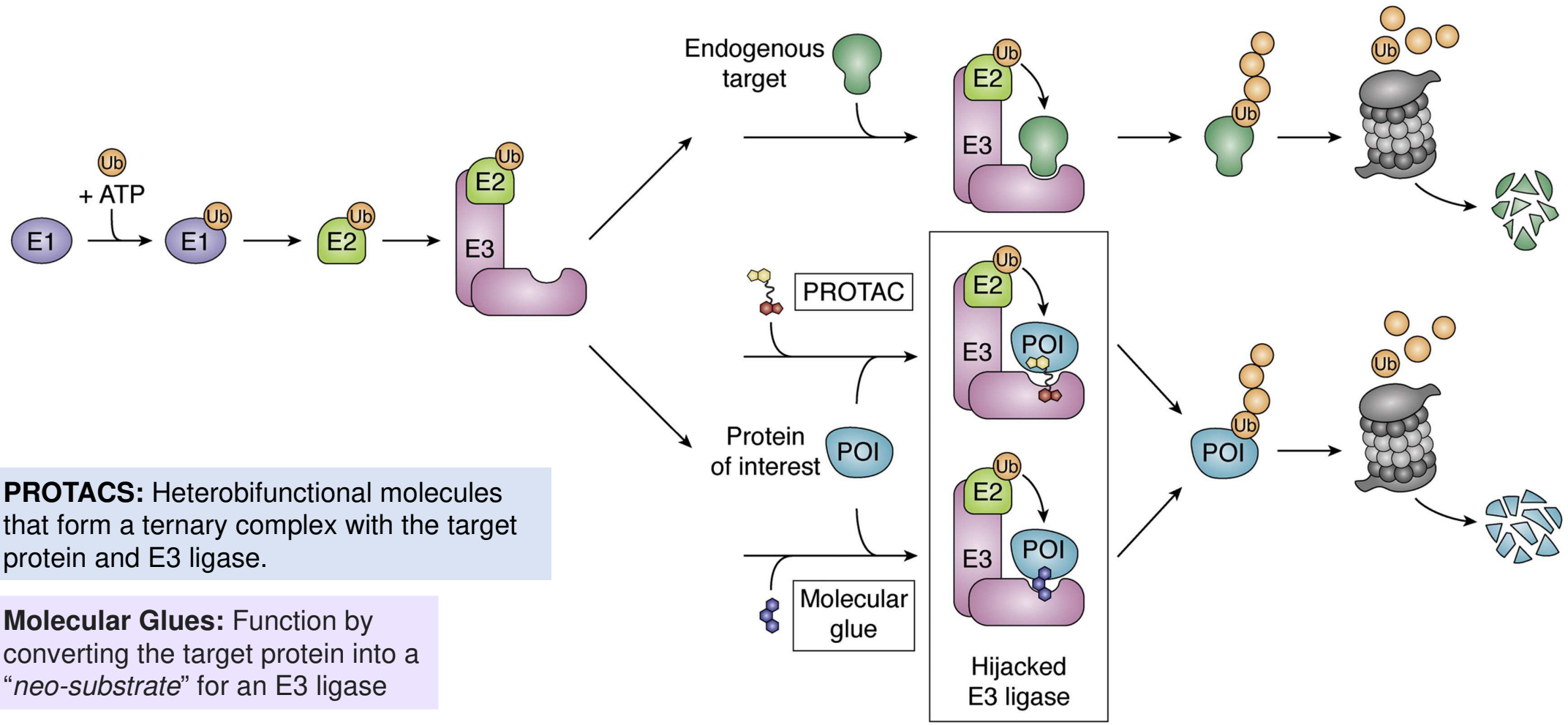
"Degradation assay"







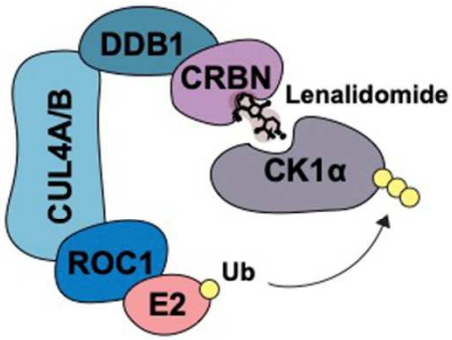
Ubiquitin protein system hijacked by PROTACS and Molecular Glues

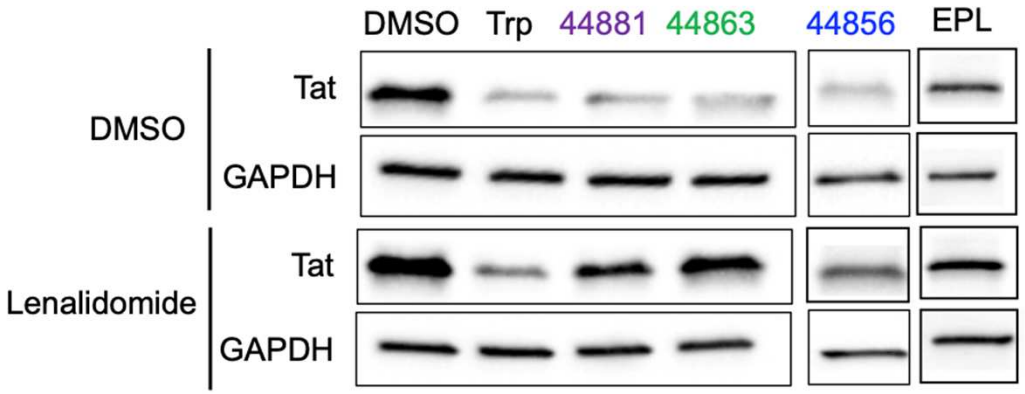


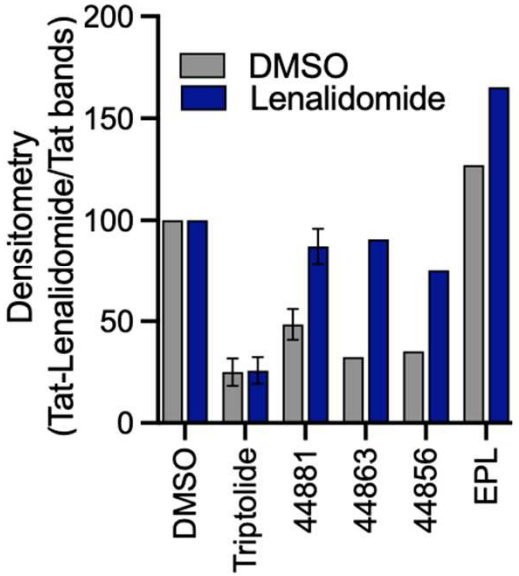
PROTACS: Heterobifunctional molecules that form a ternary complex with the target protein and E3 ligase.

Molecular Glues: Function by converting the target protein into a "*neo-substrate*" for an E3 ligase

Tat degradation by leads is blocked by the Cullin4A/B-Cereblon E3 Ubiquitin Ligase Complex inhibitor Lenalidomide







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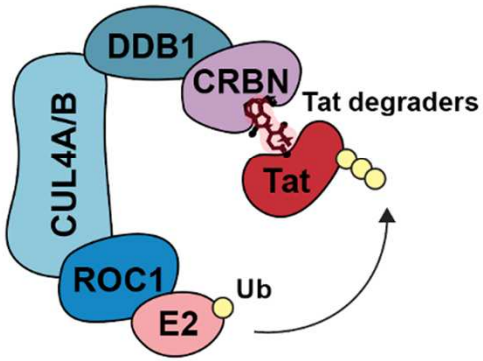
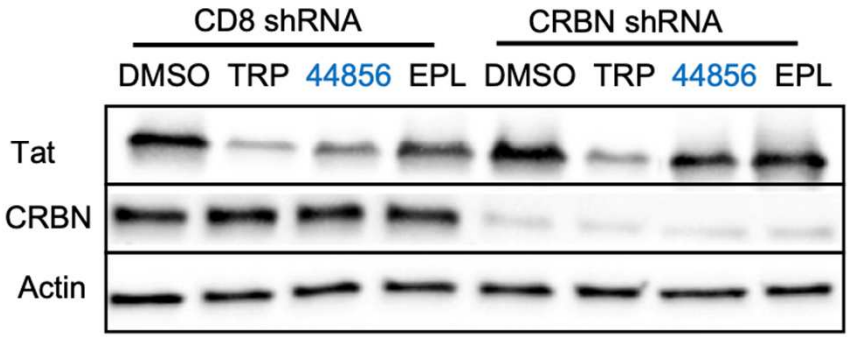
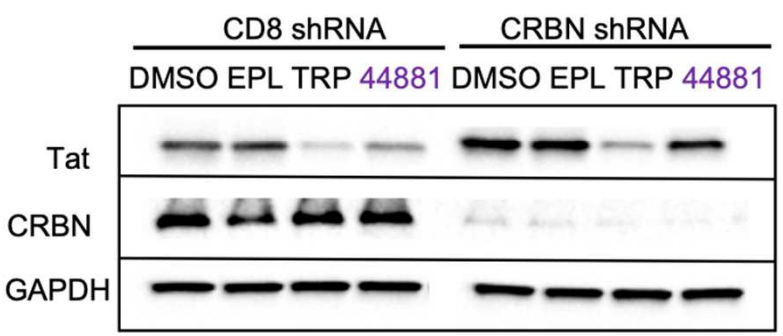
44881: 60 μM, 44863: 40 μM, 44856: 40 μM , Triptolide: 1 nM, EPL: 10 μM, Lenalidomide (Len.): 40 μM

shRNA Knockdown reveals Cereblon E3 Ubiquitin Ligase is needed to engage Tat degradation







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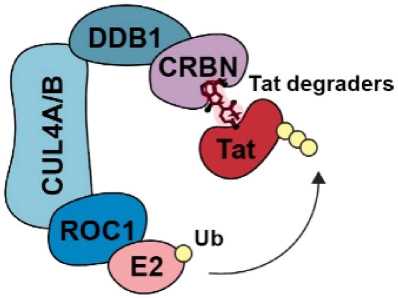
44881: 60 μM, 44863: 40 μM, 44856: 40 μM , Triptolide: 1 nM, EPL: 10 μM

Summary

- The screen of ~580K small molecules identified 3 leads
- Preliminary results suggest all 3 compounds promote block-and-lock
- They act as “molecular glues” to promote Tat recruitment to the Cullin4A/B-Cereblon E3 Ubiquitin Ligase Complex and degradation *via* the 26S proteasomal degradation pathway



BENEFITS:

- Tat degradation limits all Tat pleiotropic activity
- Long efficacy time: function restoration requires protein resynthesis
- One molecular glue degrades proteins sequentially, sub-affinity equilibrium of protein knockdown
- The resistance is less likely given ability to make transient interactions to induce functional knockdown

DRAWBACKS:

- Molecular glues are more difficult to design, although rational design strategies are emerging
- Genomic differences in the ubiquitin-proteasome system may affect activity

THANK YOU !

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Valente Lab
Joe Jablonski
Fermin Ruybal
Shuang Lyu, PhD
Pamela Espinoza-Gonzales
Ana Leda, PhD
Cas Robin Leonard Kranenburg
Luisa Mori
Chuan Li, PhD
Ronald Bronson
Ryan Milione
Andrew McAuley

Chemistry
Thomas Bannister Lab

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Ravi Natarajan

HOPE HIV Obstruction by Programmed Epigenetics

SR SOUTHERN RESEARCH

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Funding for this project:

NIH National Institute of Allergy and Infectious Diseases

UM1 AI164559
R21 AI158296
R41 AI170323
NIAID scholarship award

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