

June 26 - 30, 2023  
Smolenice Castle, Slovakia

BenBedPhar Training School 2023

NRF2 in noncommunicable diseases:  
From bench to bedside

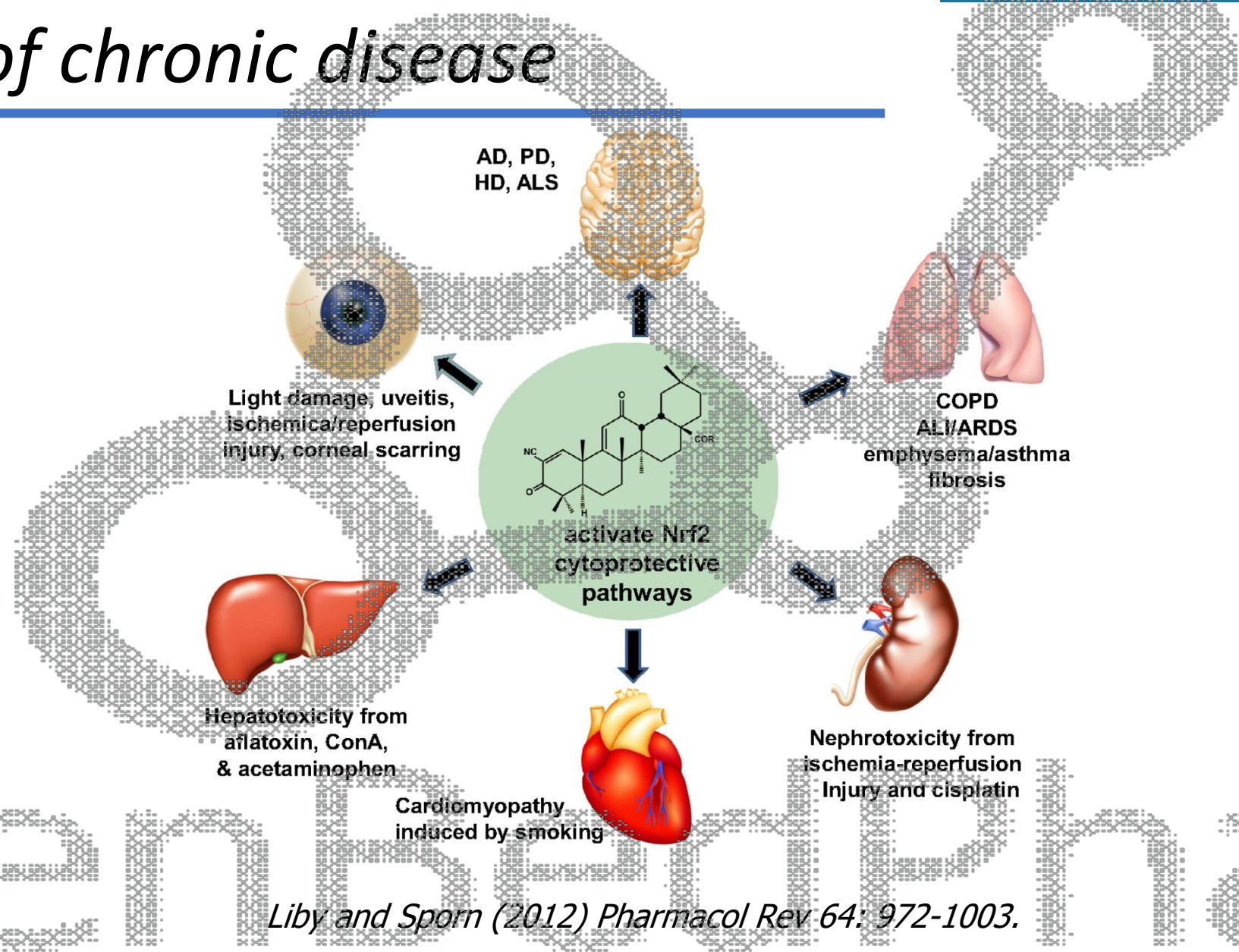


**Nrf2 pharmacology**

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University of Dundee  
United Kingdom

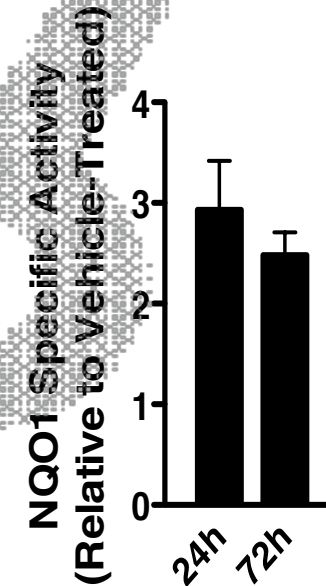
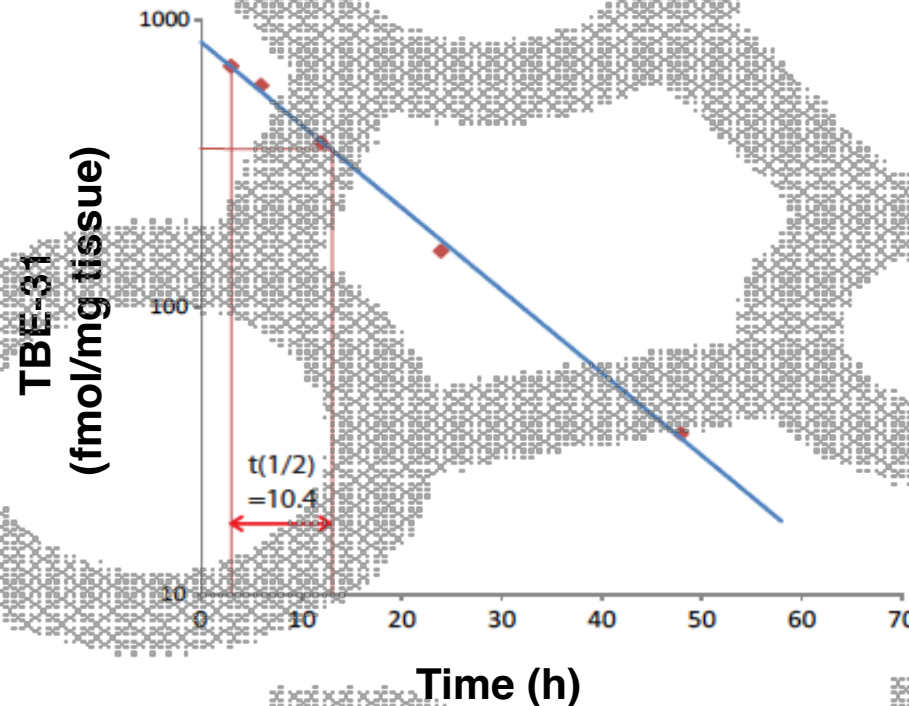
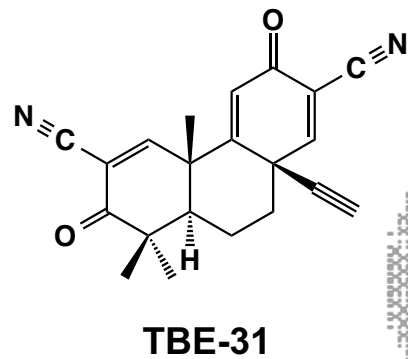
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# Nrf2 activation is protective in many models of chronic disease



*Liby and Sporn (2012) Pharmacol Rev 64: 972-1003.*

# The consequences of pharmacological Nrf2 activation are long-lasting and exceed the half-life of the drug



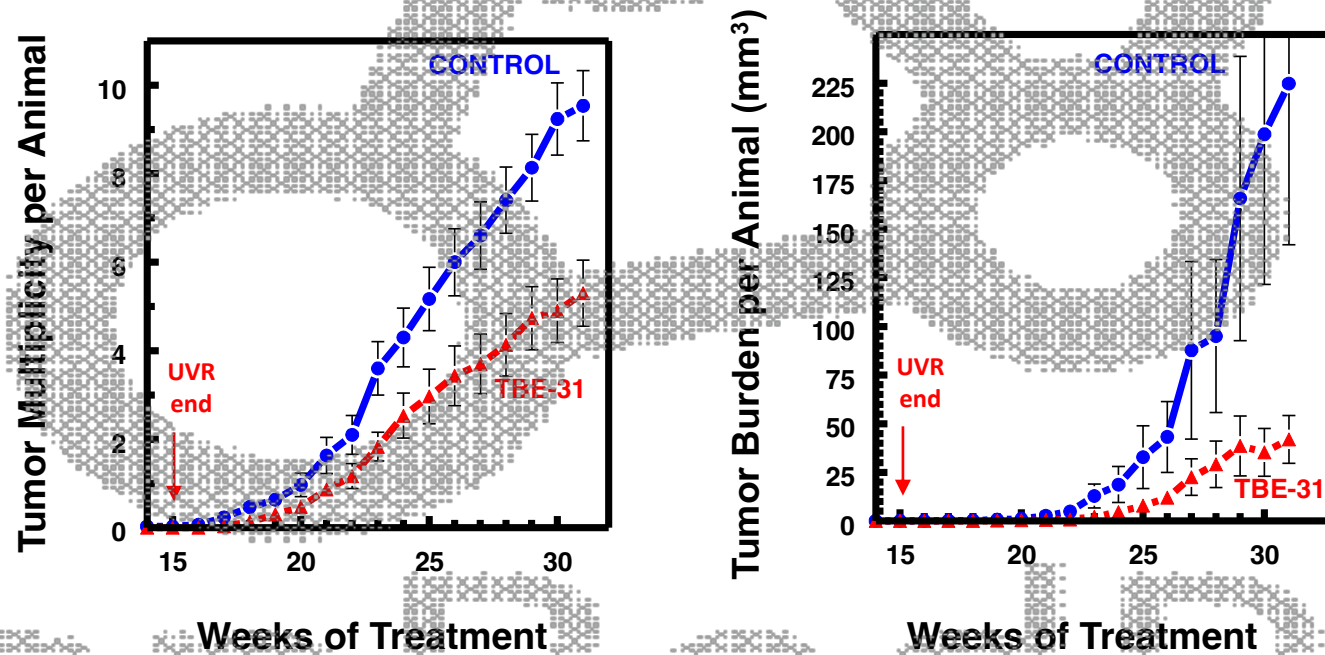
Knatko et al. (2015) *Cancer Prev Res* 8: 475-86.



# Pharmacological activation of Nrf2 by TBE-31 (topically, 2 times per week) protects against UV radiation-induced cutaneous squamous cell carcinoma in mice



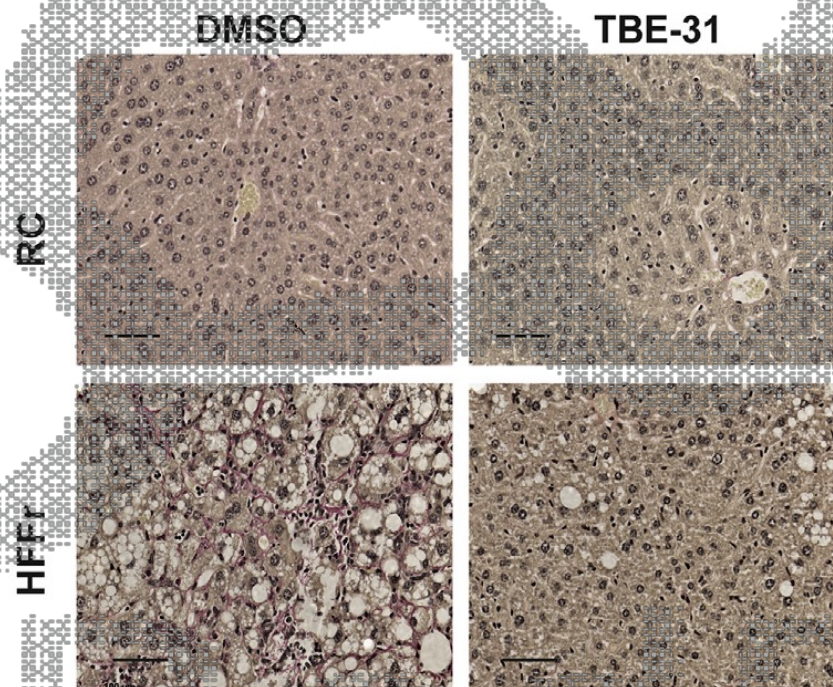
Elena Knatko



*Knatko et al. (2015) Cancer Prev Res 8: 475-86.*



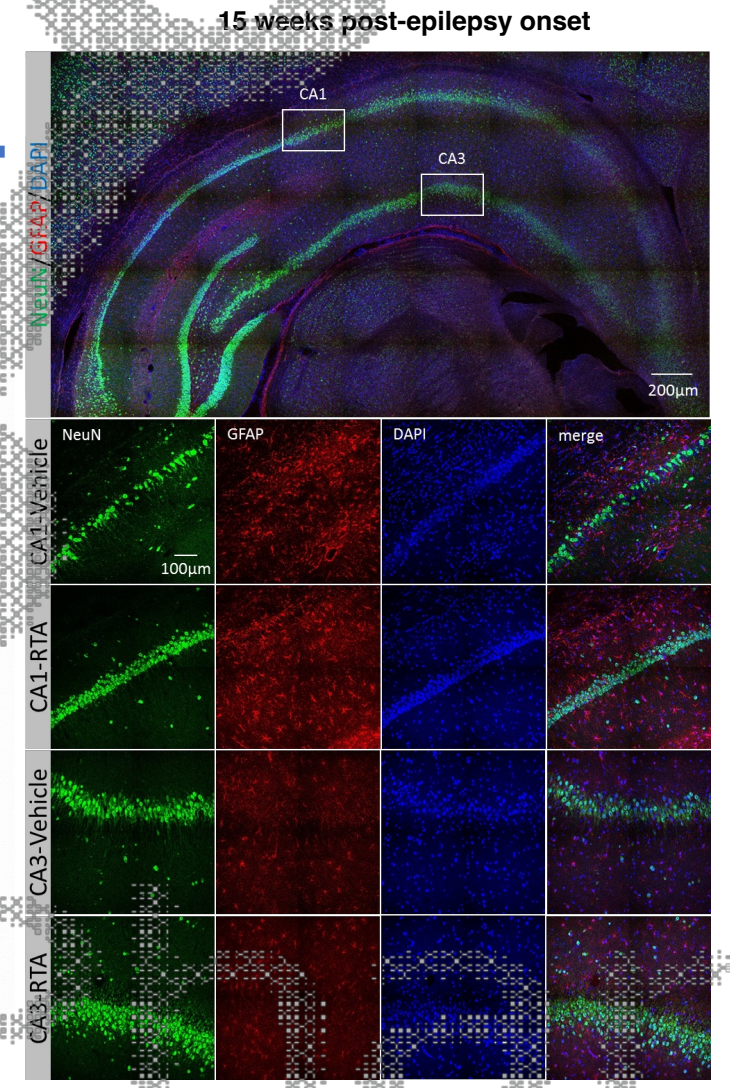
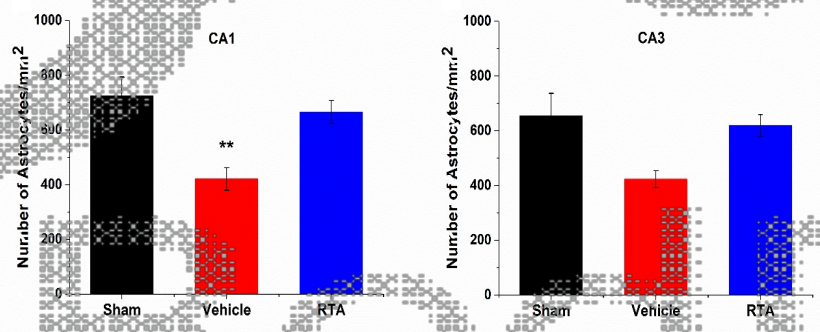
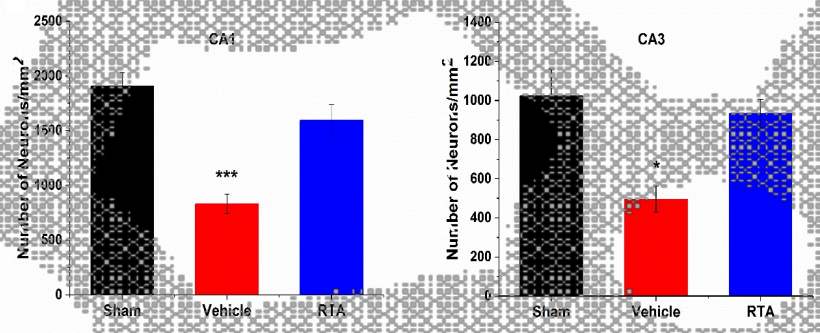
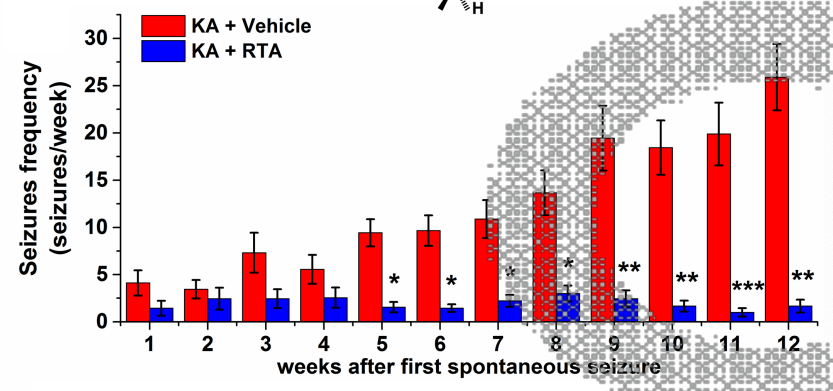
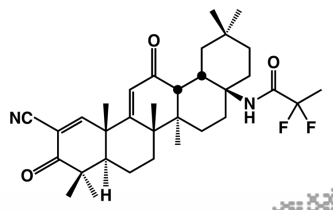
*Pharmacological activation of Nrf2 by TBE-31 (orally, 3 times per week) protects against high fat+high fructose (HFFr) diet-induced liver fibrosis in mice*



*Sharma et al. (2018) Cell Mol Gastroenterol Hepatol 5: 367-98.*



# *Omaveloxolone preserves neurons and astrocytes in the hippocampus and prevents seizure progression in a rat model of epilepsy*



Shekh-Ahmad et al. (2018) Brain 141: 1390-1403.

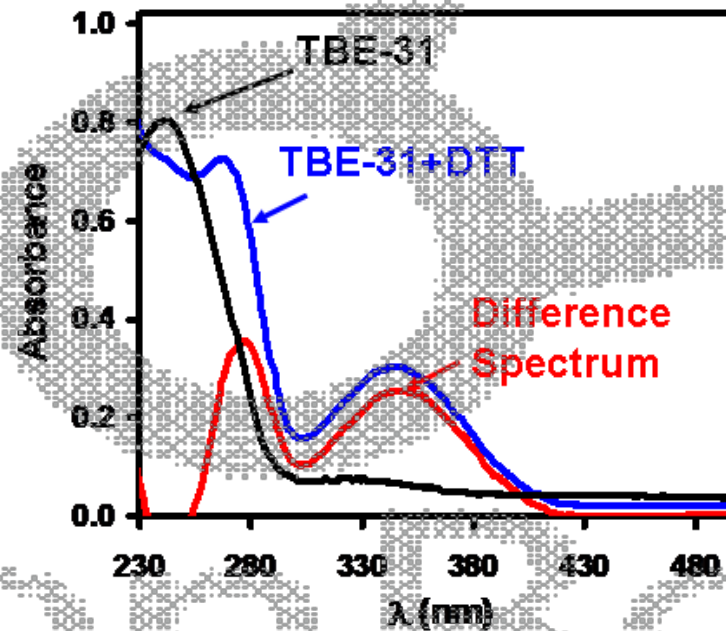
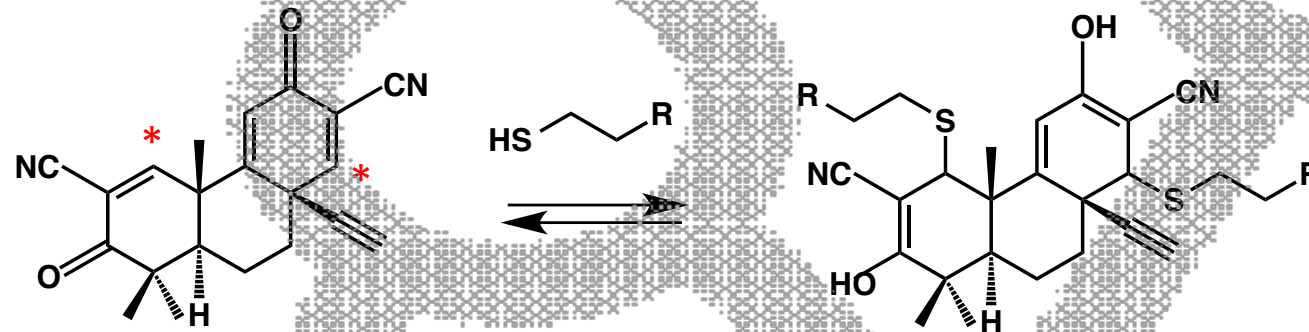
# *How do the cyanoenones activate Nrf2?*

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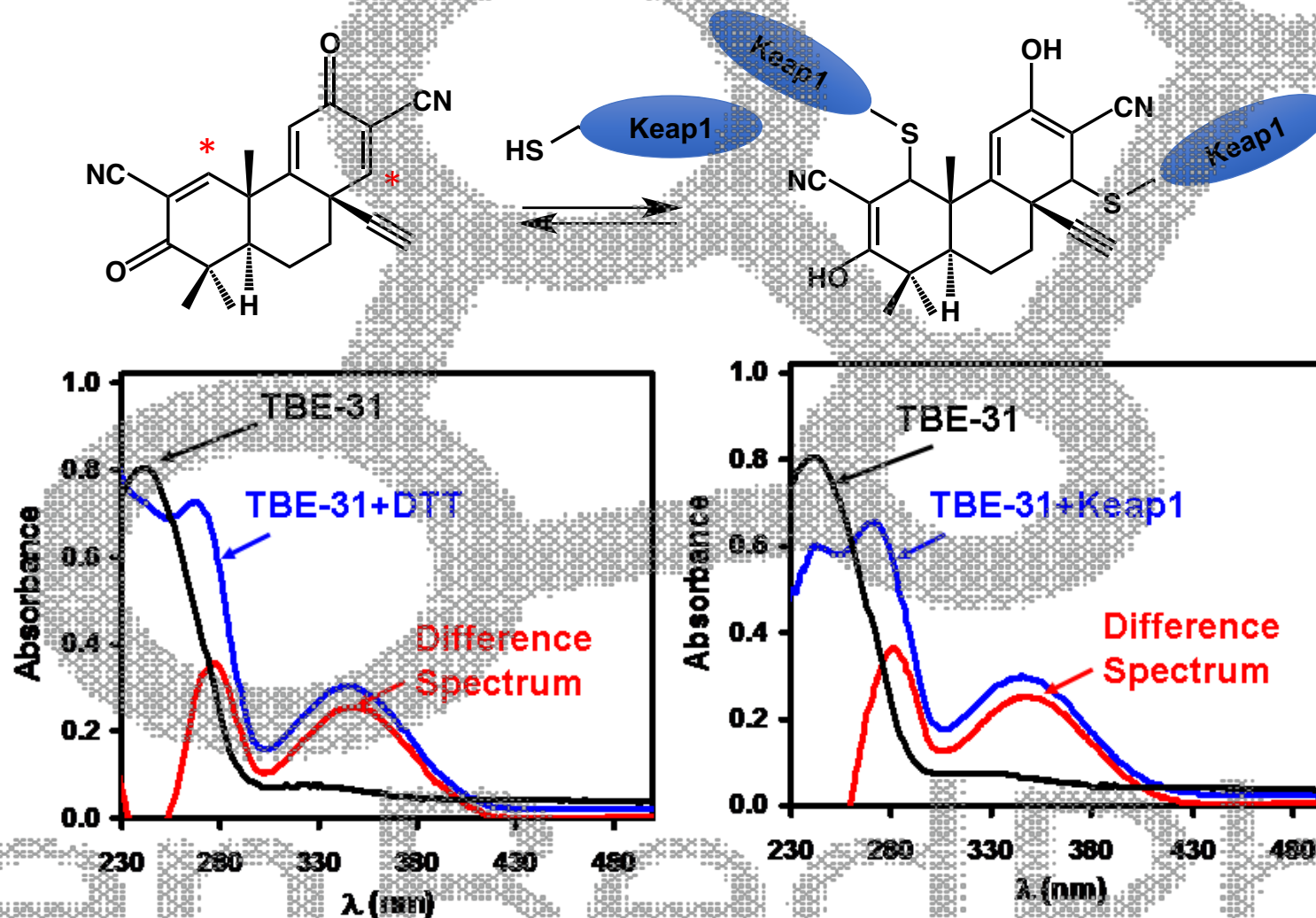


# TBE-31 reacts with sulfhydryl groups



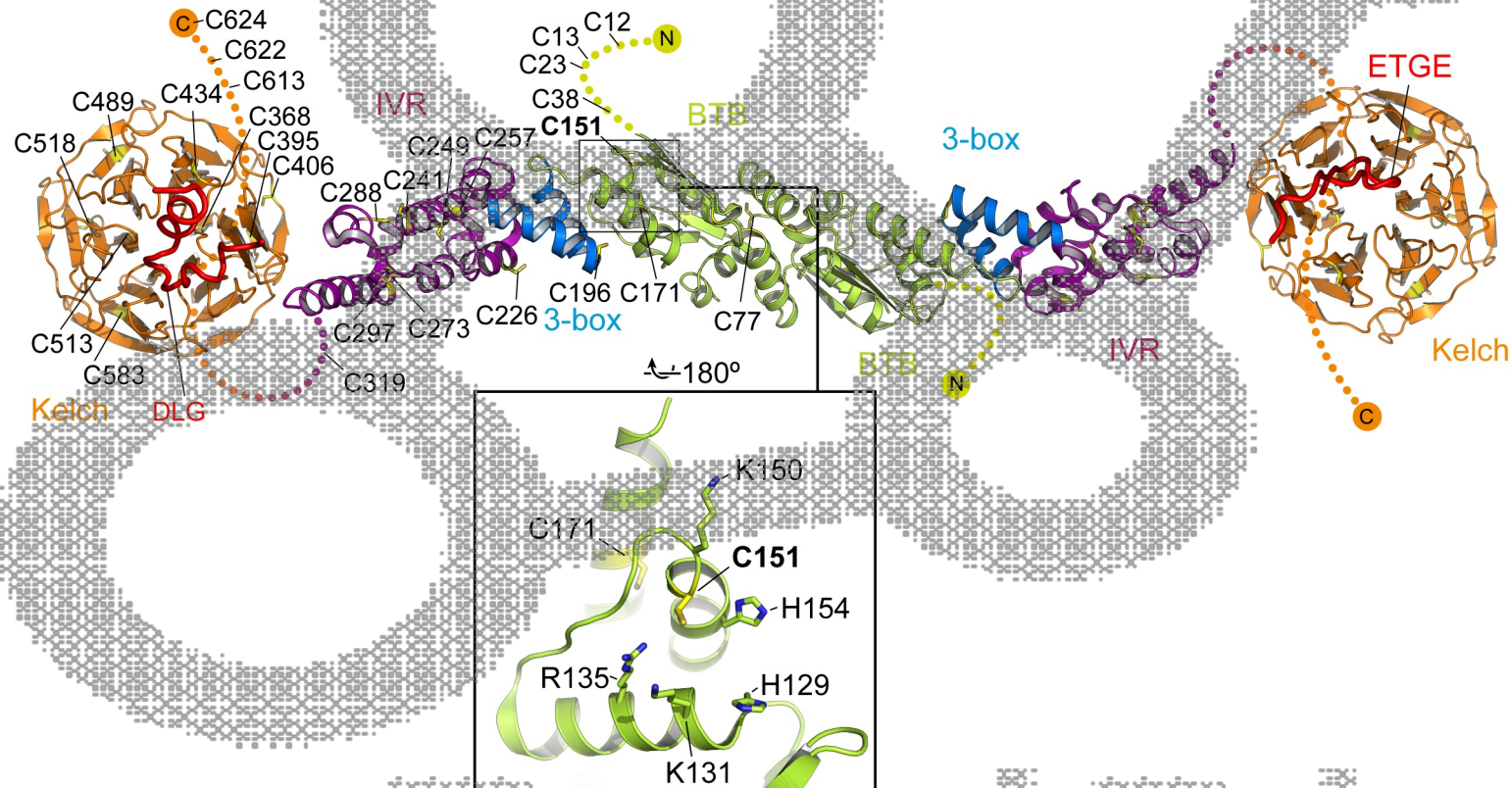
Dinkova-Kostova et al. (2010) *J Biol Chem* 285: 33747-55.

# TBE-31 reacts with cysteines in Keap1



Dinkova-Kostova et al. (2010) *J Biol Chem* 285: 33747-55.

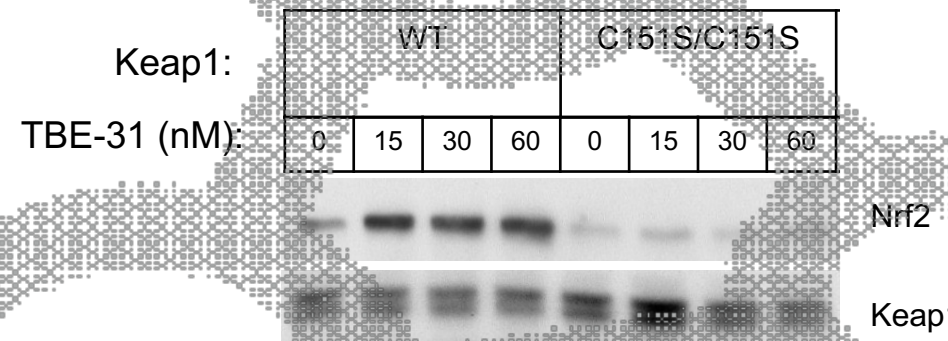
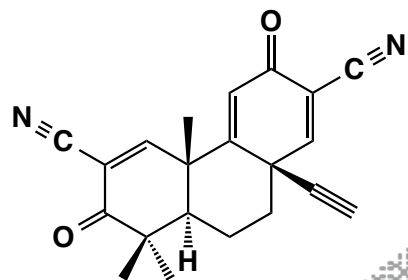
# Keap1 is a cysteine-based sensor



*Dinkova-Kostova et al. (2017) Arch Biochem Biophys 617: 84-93.*



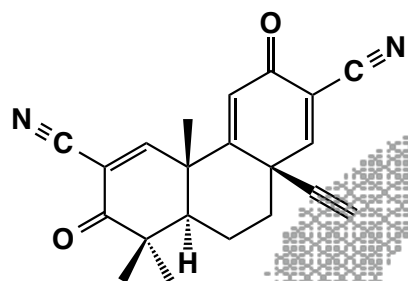
# C151 in Keap1 is the sensor for TBE-31



Sharadha Dayalan Naidu

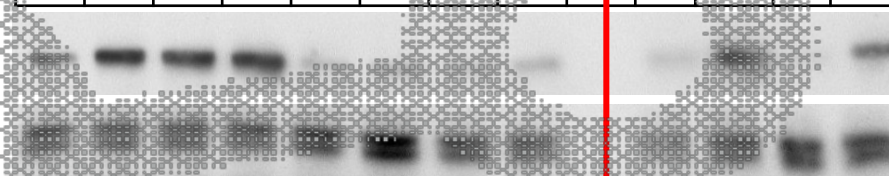
Dayalan Naidu et al. (2018) *Sci Rep* 23: 8037.

*C151 in Keap1 is the sensor for TBE-31.  
However, at higher inducer concentration,  
other cysteines in Keap1 can be modified*



Keap1:  
TBE-31 (nM):

WT				C151S/C151S				WT		C151S/ C151S	
0	15	30	60	0	15	30	60	0	120	0	120

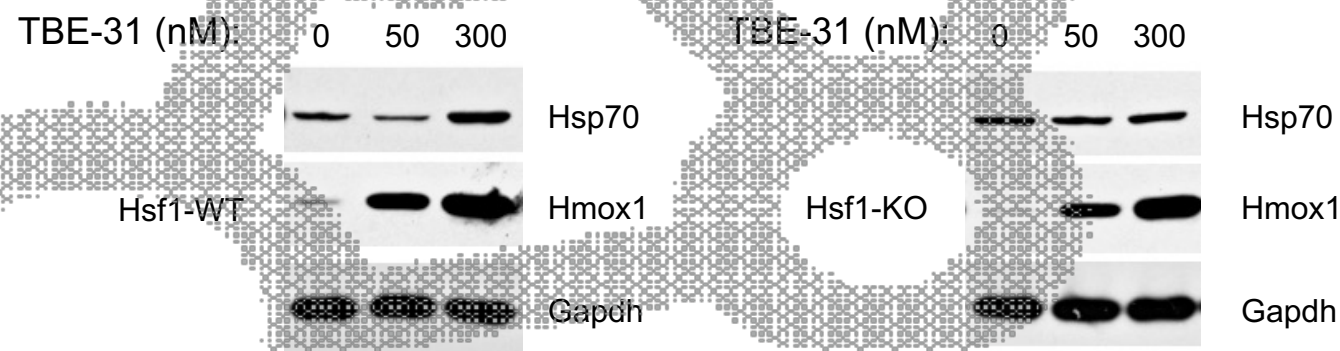
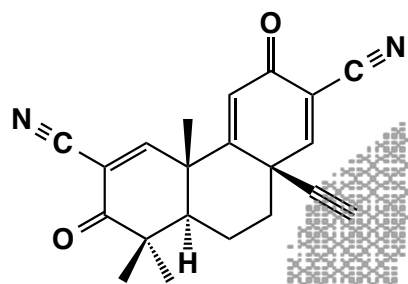


Nrf2

Keap1

*Dayalan Naidu et al. (2018) Sci Rep 23: 8037.*

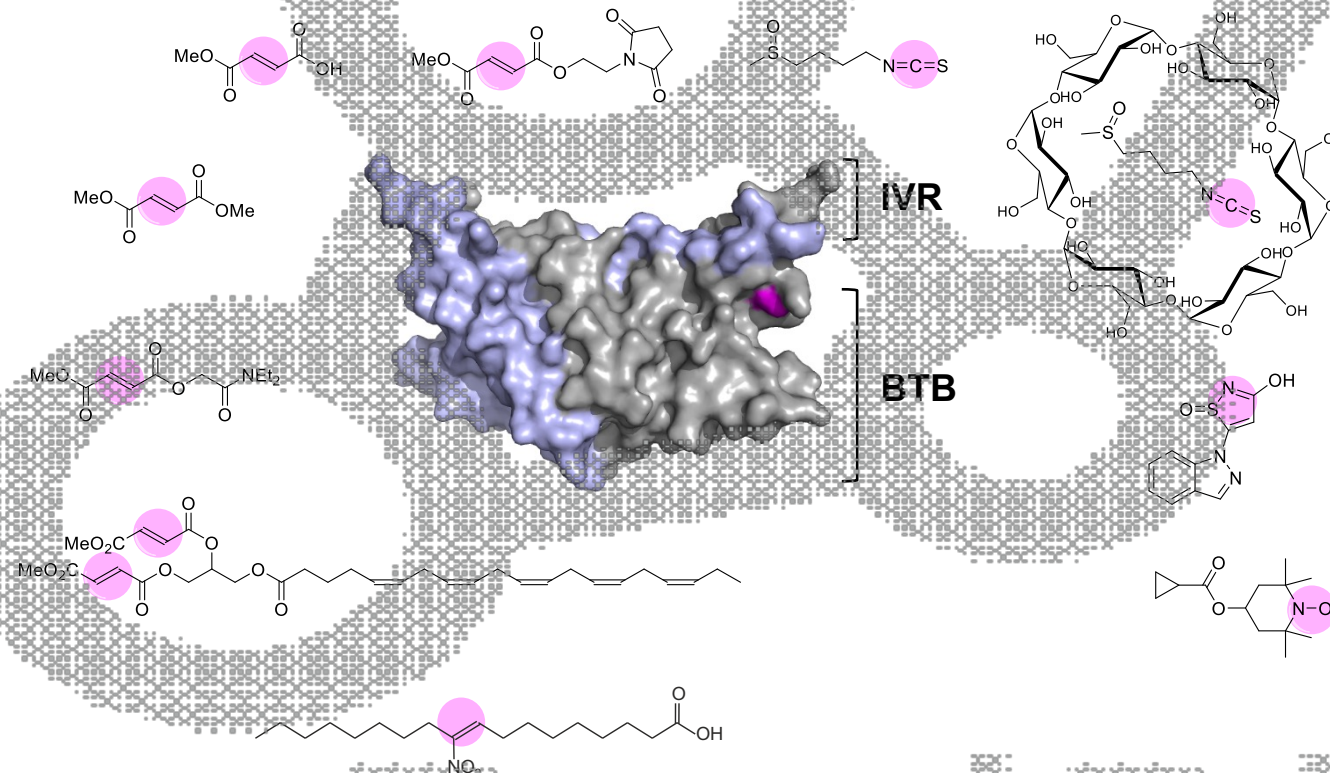
*C151 in Keap1 is the sensor for TBE-31.  
However, at higher inducer concentration,  
cysteines in other proteins can be modified*



*Zhang et al. (2011) Chem Biol 18: 1355-61.*

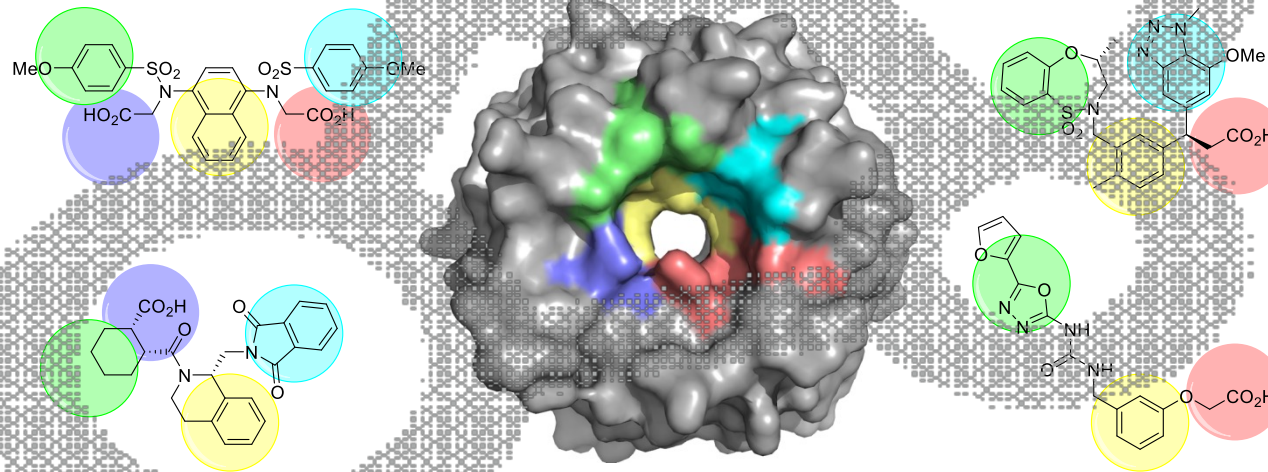


# Targeting Keap1 with electrophiles



*Cuadrado et al. (2019) Nat Rev Drug Discov. 18: 295-317.*

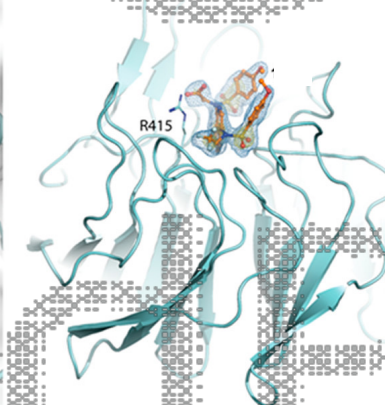
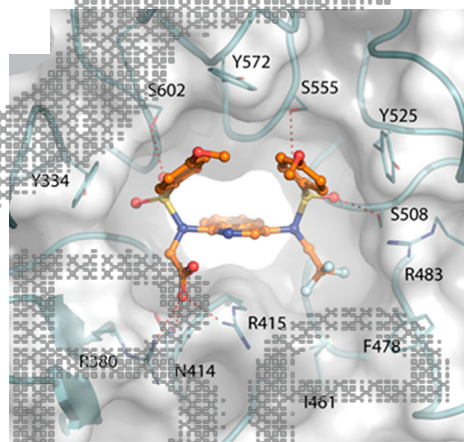
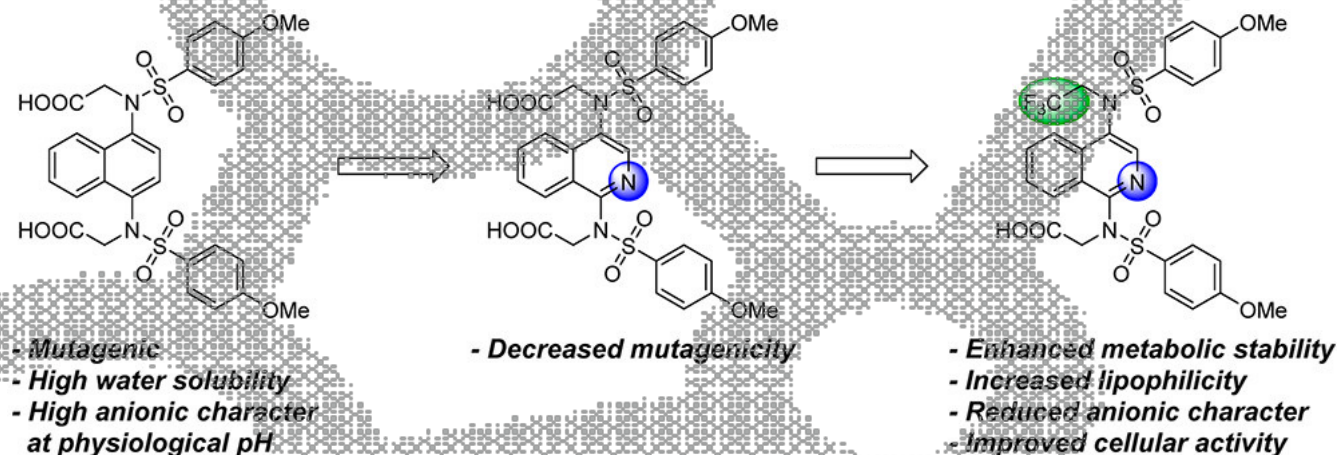
# Targeting Keap1-Nrf2 with non-electrophilic protein-protein interaction inhibitors



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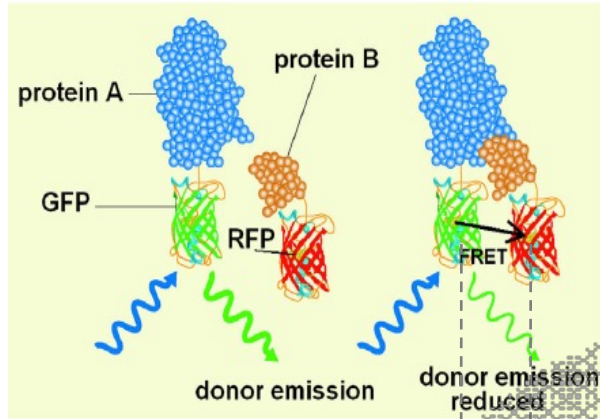
*Cuadrado et al. (2019) Nat Rev Drug Discov. 18: 295-317.*

# Targeting Keap1-Nrf2 with non-electrophilic isoquinoline protein-protein interaction inhibitors





# Using FRET/FLIM to detect the interactions between Nrf2 and Keap1 in single live cells

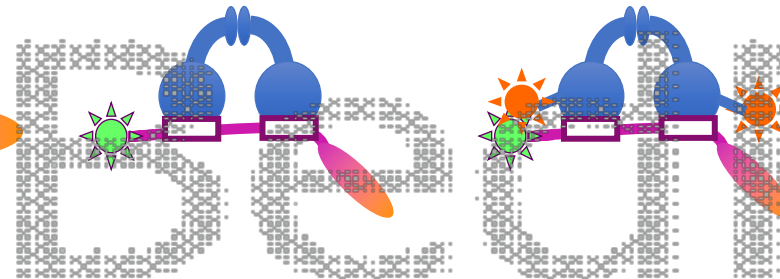
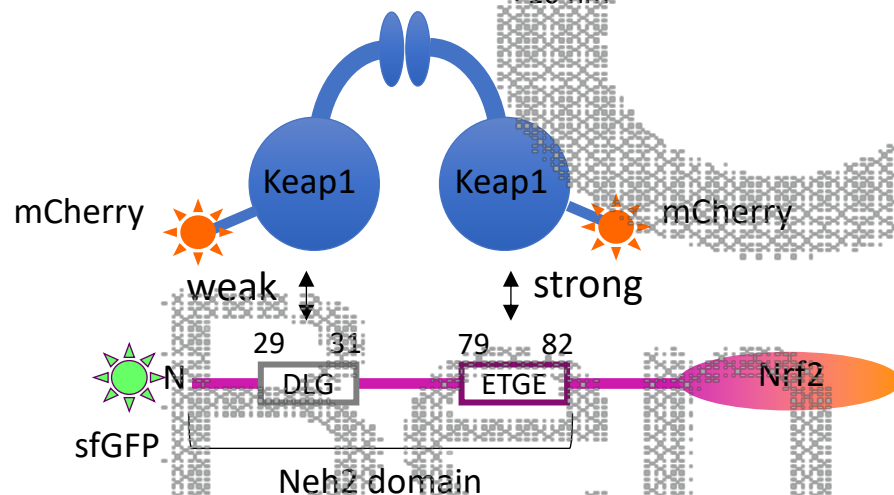
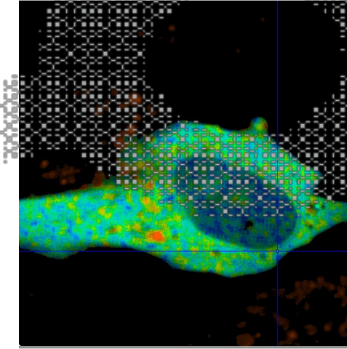
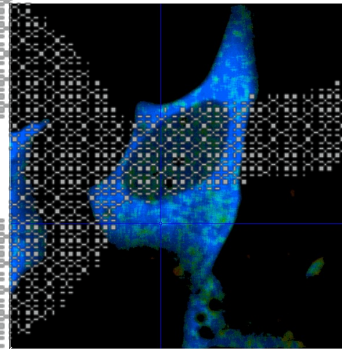


Fluorescence Lifetime



sfGFP-Nrf2  
Keap1

sfGFP-Nrf2  
Keap1-12fl-mCherry



Liam Baird



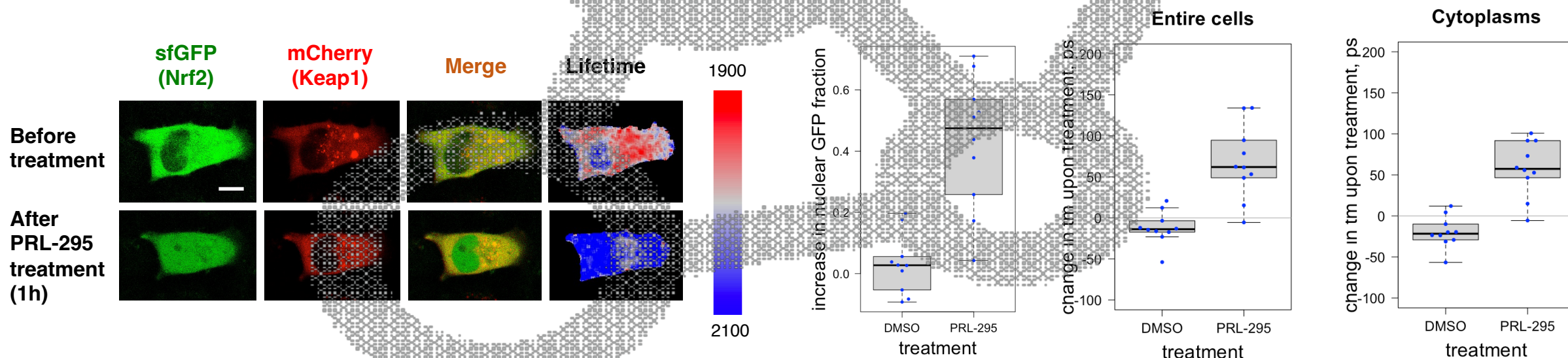
Dina Dikovskaya

Baird et al. (2013) *Proc Natl Acad Sci USA* 110: 15259-64.

Dikovskaya et al. (2019) *Chem Res Toxicol* 32: 500-512.

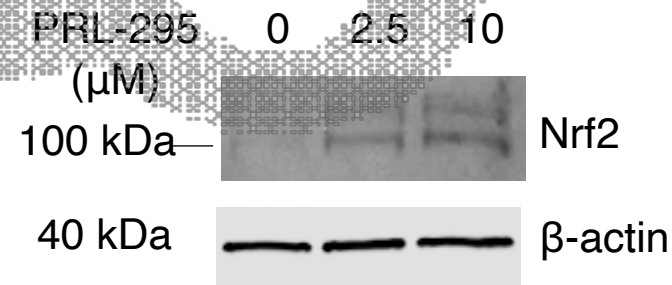
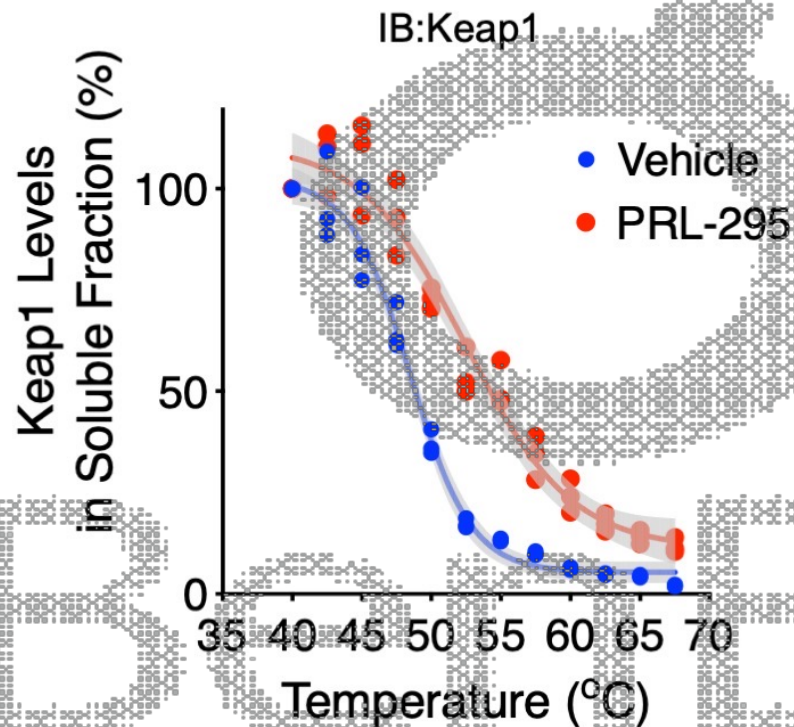
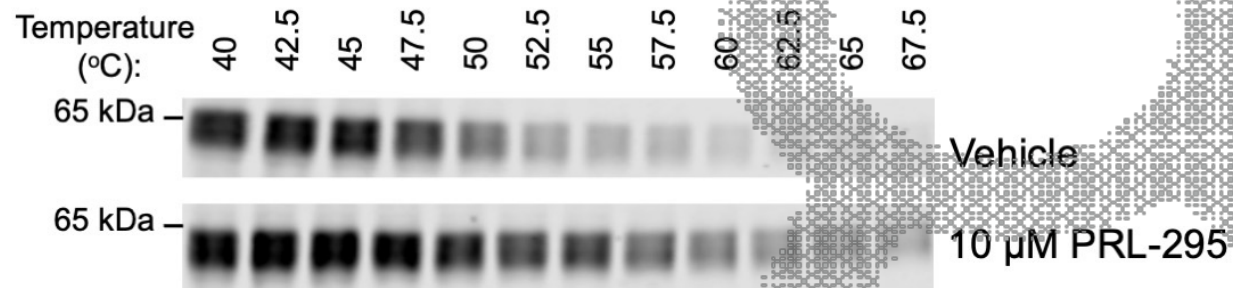
Dikovskaya and Dinkova-Kostova (2020) *Curr Protoc Toxicol* 85: e96.

# *PRL-295 increases the sfGFP-Nrf2 fluorescence lifetime, indicating disruption of its binding to Keap1-mCherry*



*Dina Dikovskaya and Takafumi Suzuki*

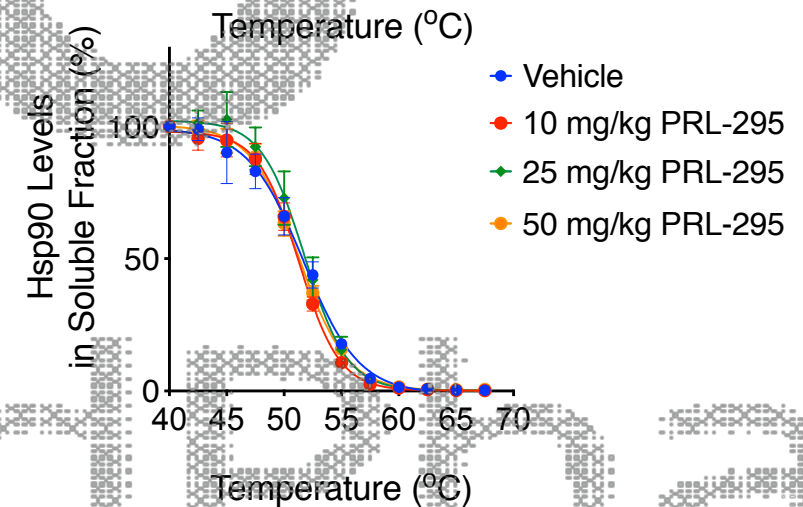
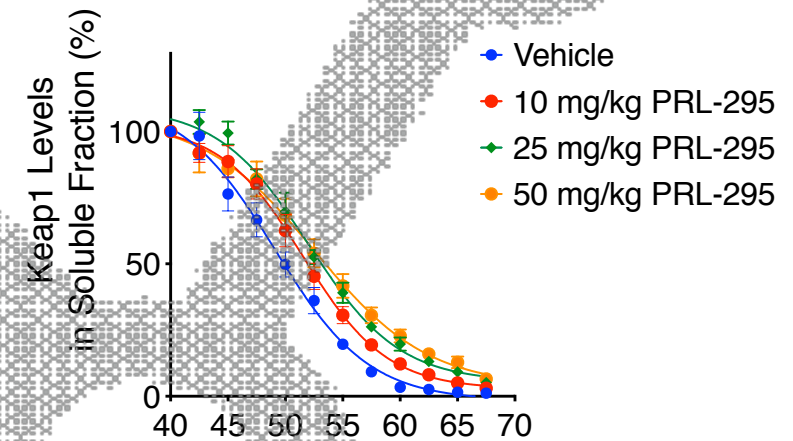
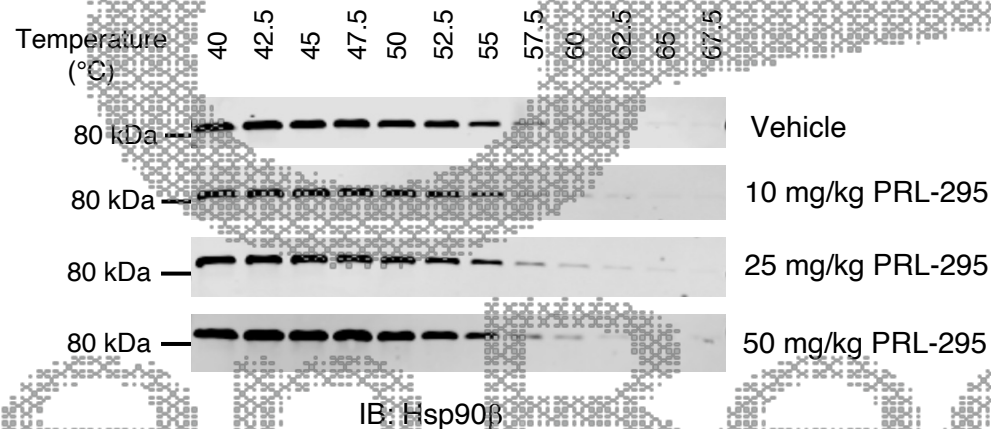
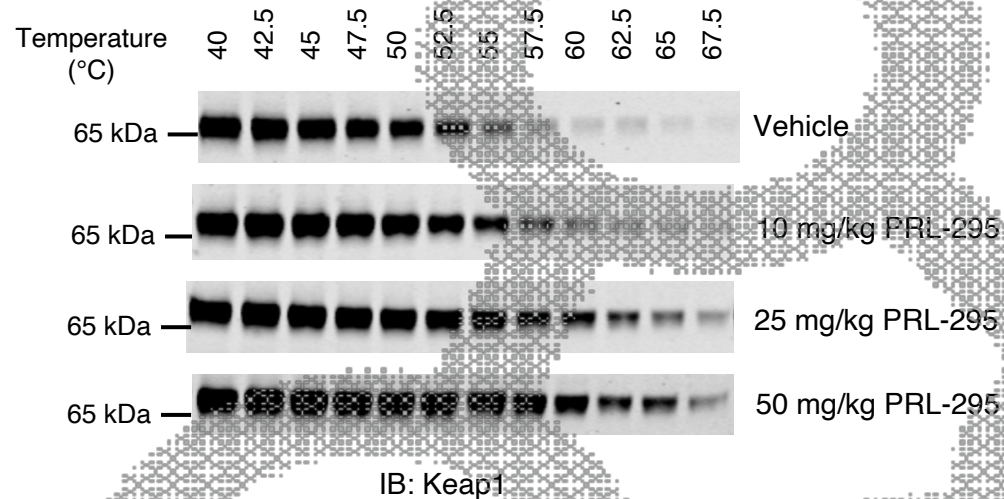
# PRL-295 increases the thermostability of Keap1 in cells



Dayalan Naidu et al. (2021) *iScience* 25: 103703.  
Dayalan Naidu et al. (2022) *STAR Protoc* 3: 101265.



# Oral administration of PRL-295 increases the thermostability of Keap1 in mouse liver

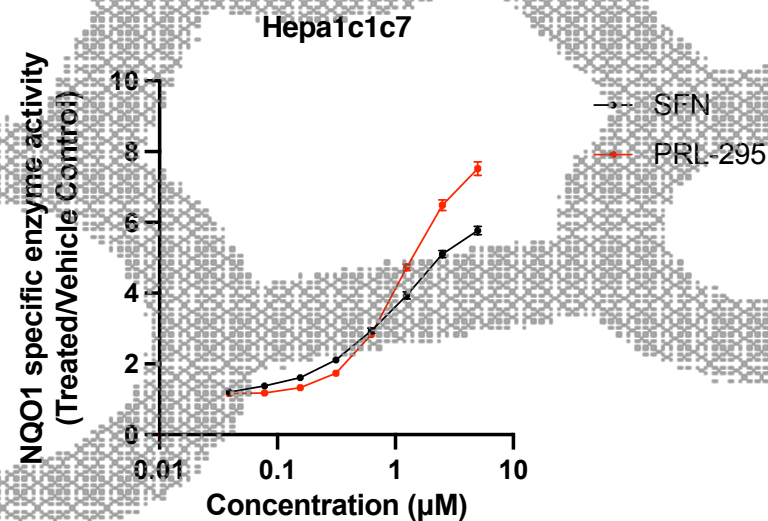




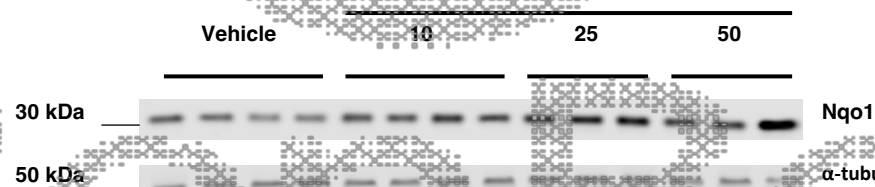
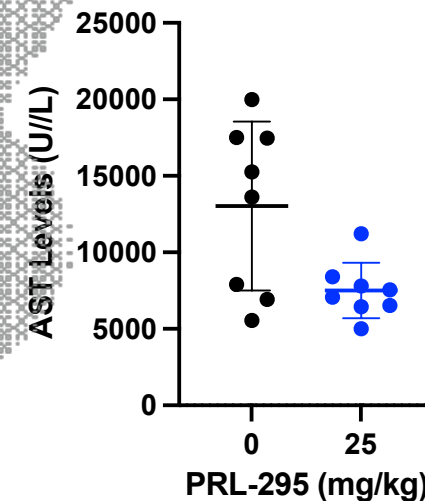
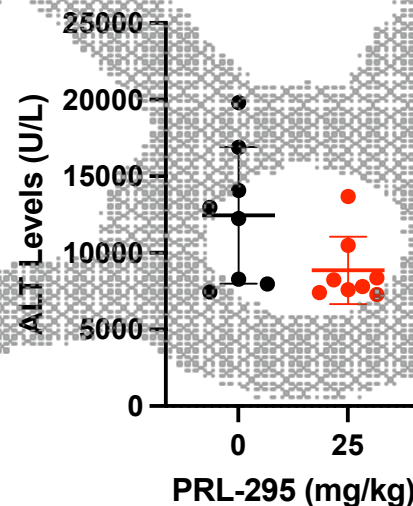
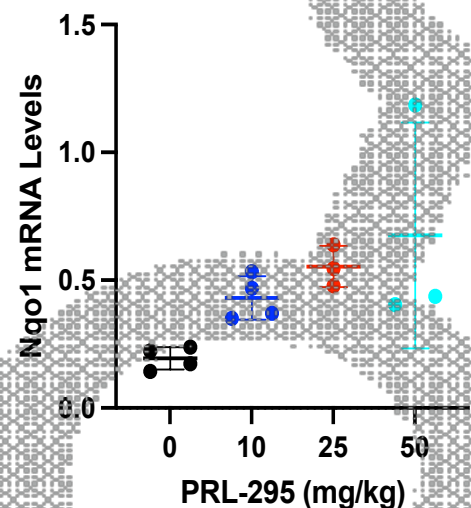
# *PRL-295 activates Nrf2 in cells with potency similar to sulforaphane (SFN)*



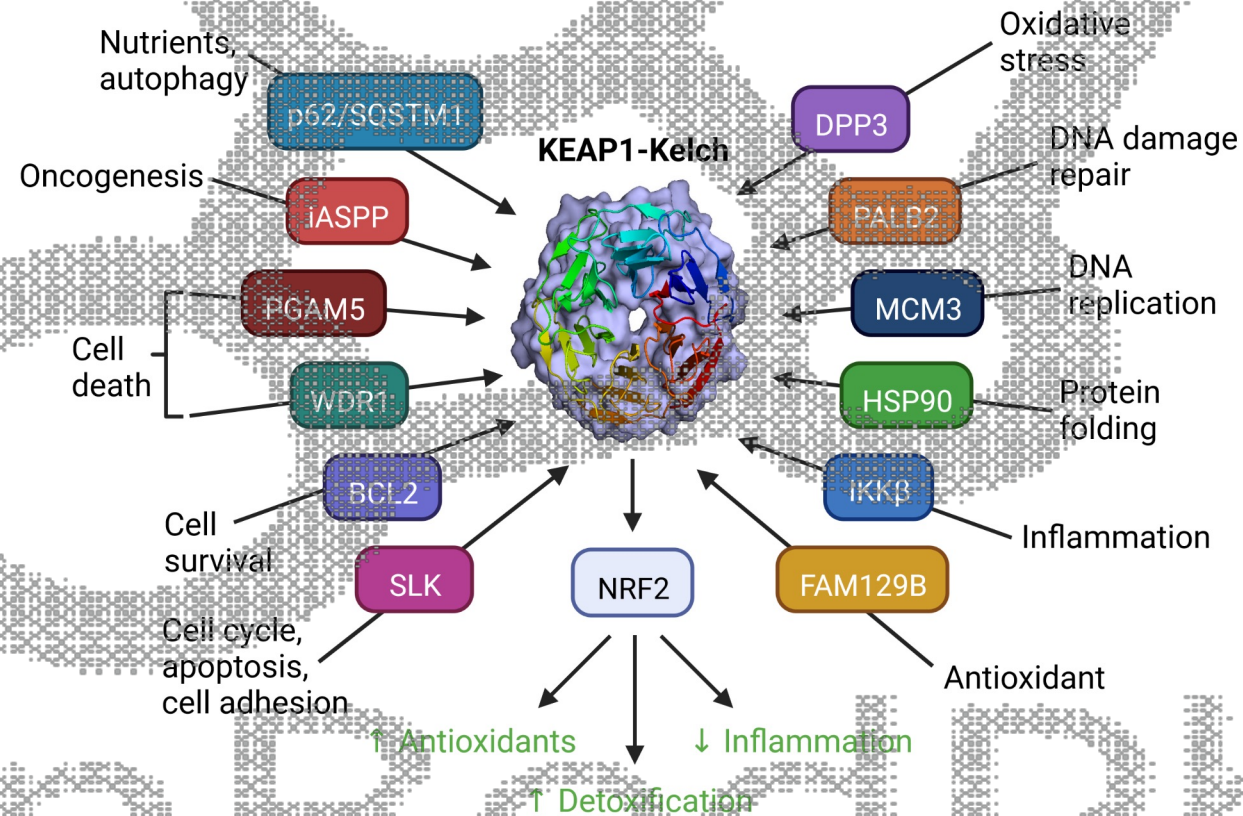
Maureen Higgins



# Oral administration of PRL-295 induces hepatic NQO1 and protects against acetaminophen hepatotoxicity in mice

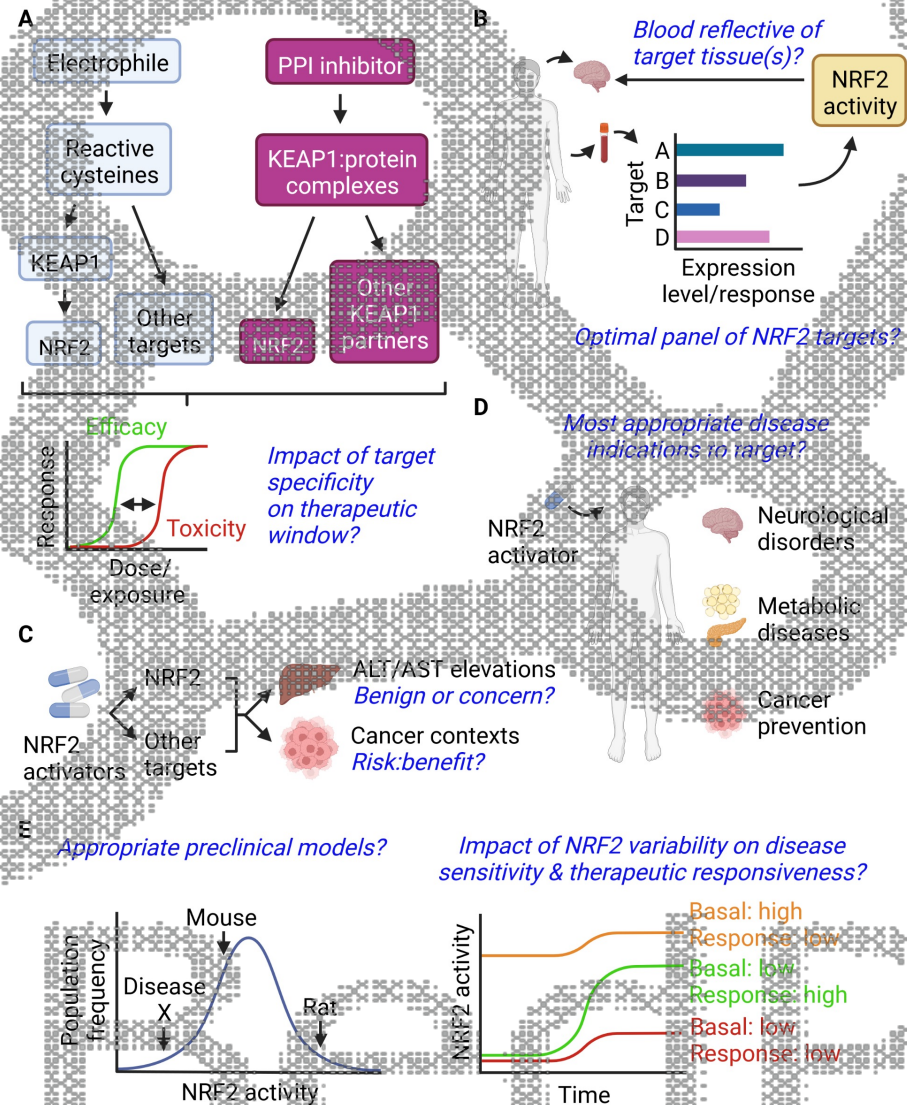


# Does targeting Keap1 with non-electrophilic protein-protein interaction inhibitors affect its interactions with other binding partners?



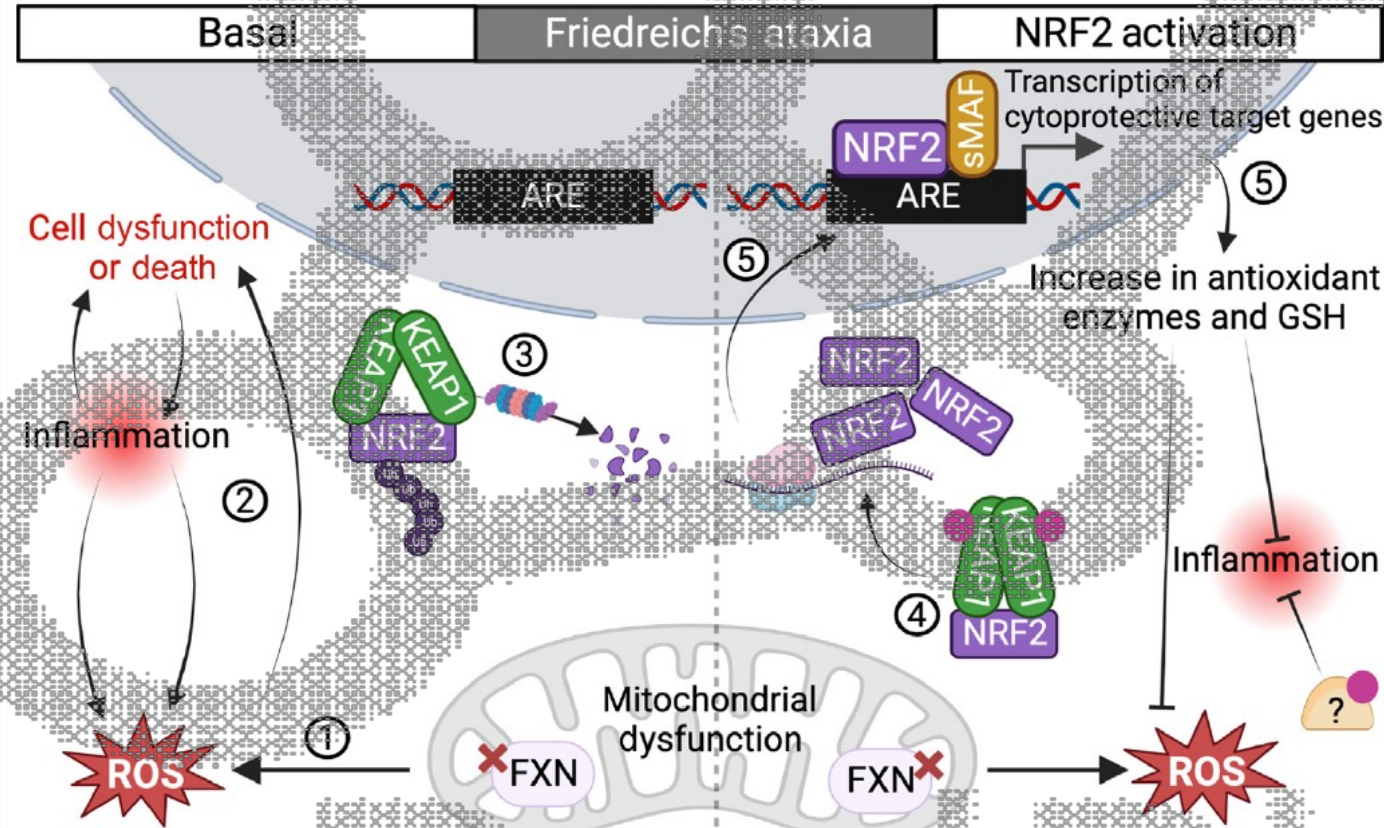
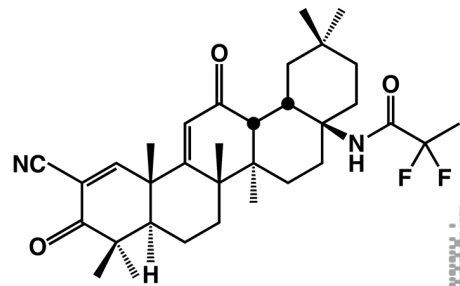
Hayes and Dinkova-Kostova (2017) *Cancer Cell* 32: 539-41.  
Dinkova-Kostova and Copple (2023) *Trends Pharmacol Sci.* 44: 137-149.

# Challenges in therapeutic targeting of Keap1-Nrf2





# Omaveloxolone (Skyclarys™) for patients with Friedreich's ataxia



Trends in Pharmacological Sciences

Dayalan Naidu and Dinkova-Kostova (2023) Trends Pharmacol Sci. 44: 394-395.

# Summary

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- Pharmacological Nrf2 activation is beneficial in numerous animal models of human disease
- The consequences of pharmacological Nrf2 activation are long-lasting and exceed the half-life of the drug
- C151 in Keap1 is the primary sensor for many electrophilic Nrf2 activators
- Non-electrophilic inhibitors of Keap1-Nrf2 protein-protein interactions with comparable potencies to the electrophilic activators are emerging, but their effects on other Keap1 interactors are unclear
- Omaveloxolone (Skyclarys™) is the first and only FDA-approved drug for patients with Friedreich's ataxia

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# Acknowledgments



## Colleagues

Sharadha Dayalan Naidu

Oliver Read

Miroslav Novak

Jialin Feng

Louisa Watt



Medical  
Research  
Council



MEDICAL  
RESEARCH  
SCOTLAND



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Andrey Abramov, Mathew Walker (UCL)

Masayuki Yamamoto, Takafumi Suzuki (Tohoku University)

Tadashi Honda (Stonybrook University)

Terry Moore (University of Illinois at Chicago)



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