

Novel Insulin Sensitizers

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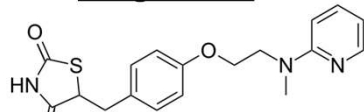
Presentation

- PPAR γ Modulators devoid of classical Agonism
 - Insulin sensitizers and nuclear receptors
 - PTM control of NR Signaling and SR1664
 - Structural aspects of SR1664 Action
 - Potential to develop novel non-agonists PPAR γ modulators for use in the clinic?

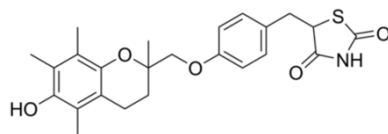
Discovery of Insulin Sensitizing TZDs

Thiazolidinediones (TZDs)

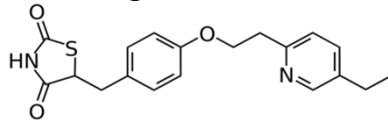
Rosiglitazone



Troglitazone



Pioglitazone

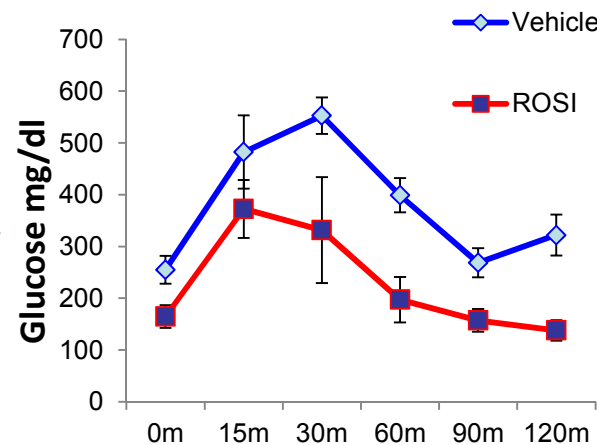


Wistar Fatty Rats



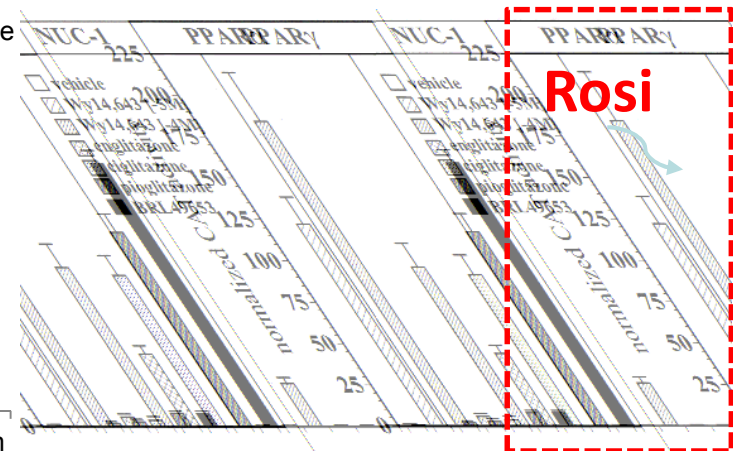
Ikeda et al. Diabetes 1981

Glucose Tolerance Test



Rosi: Clark et al. JMC 1991

PPARG Transactivation Assay



Lehmann J M et al. JBC 1995

Classical Model of NR Signaling

Corepressors

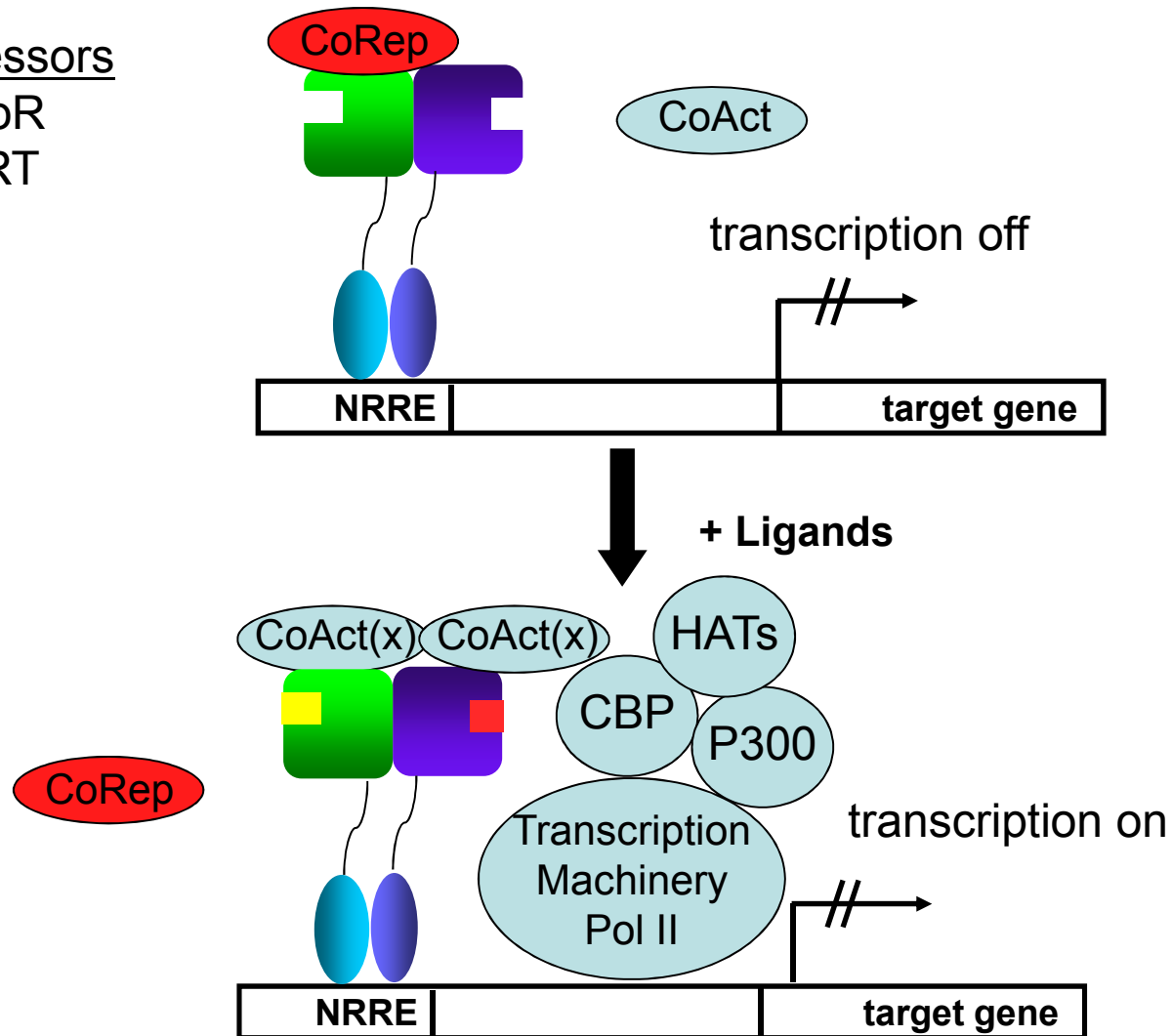
N-CoR
SMRT

Coactivators

p160 family
SRC1,2,3
TRAP220
RIP140
PGC1 α

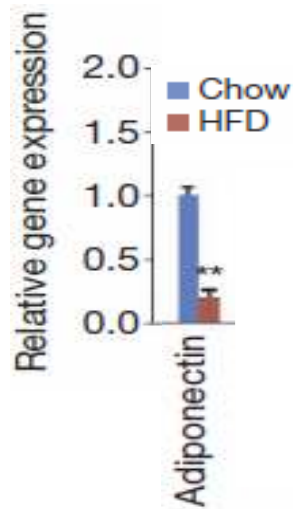
Cointegrators

p300
CBP
pCAF



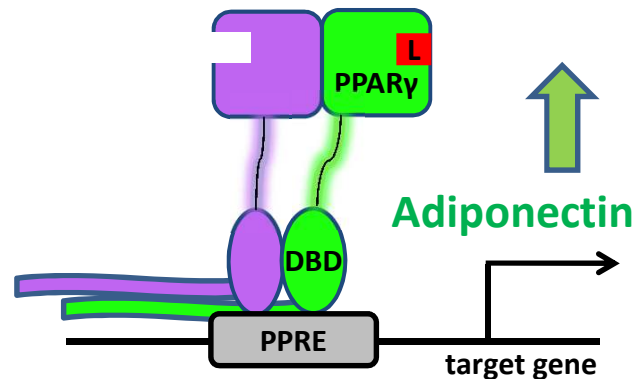
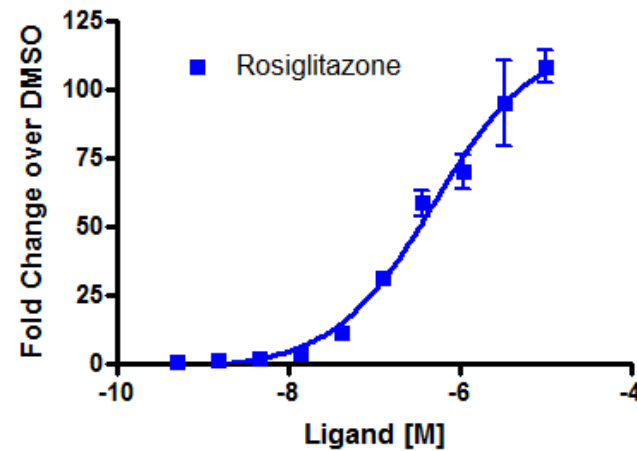
Proposed Mechanism of Action of TZDs

Ob/Ob AdipoQ

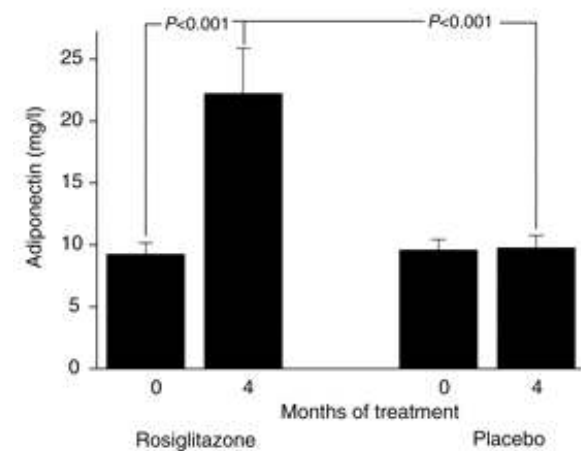


Choi et al. Nature 2010

PPARG Transactivation Assay



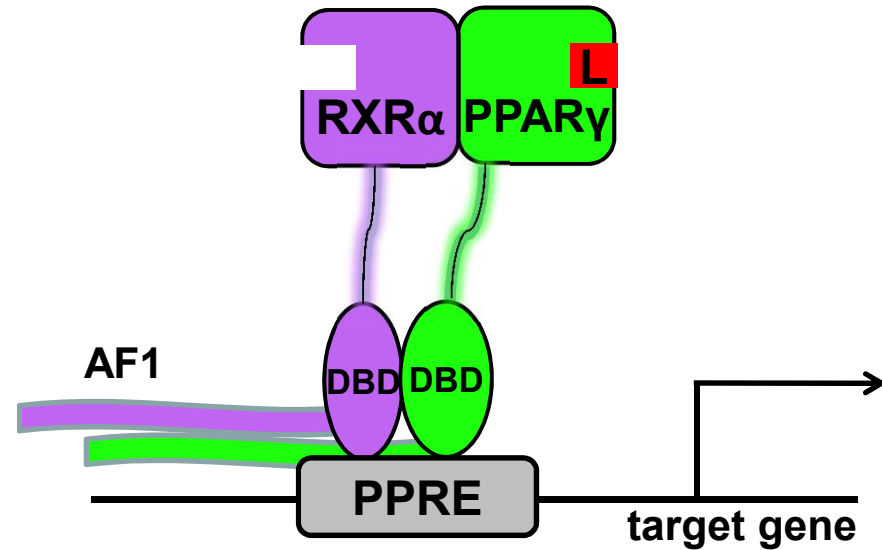
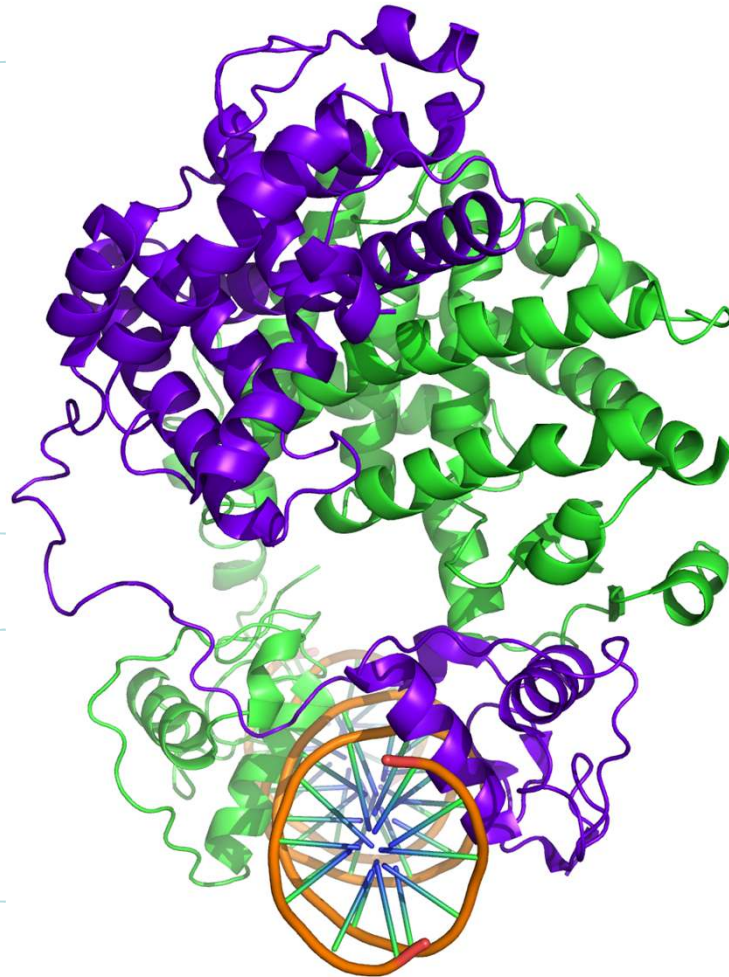
Adiponectin Expression



Majuri et al. Eur J Endocrinol 2007

PPARG:RXR Crystal Structure

DNA Binding Domain Ligand Binding Domain

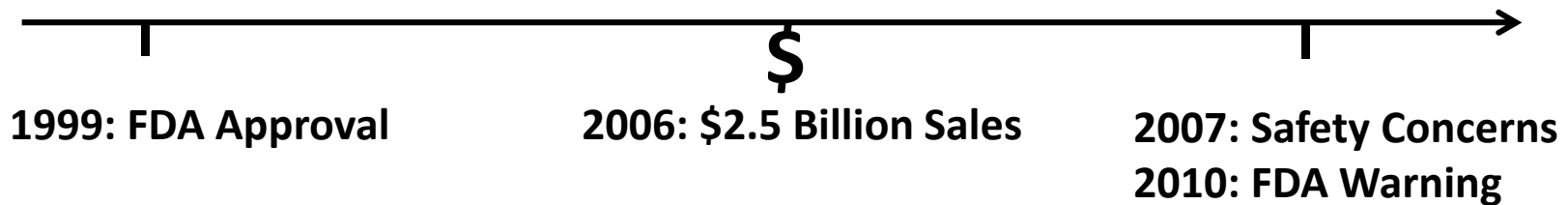


PPARG: ~10,000 PubMed Hits

Chandra et al. Nature 2008

TZDs Run into Problems

- Reported *in vivo* efficacy for Alzheimer's Disease, Cancer, Atherosclerosis, Browning of fat, Inflammation, etc.



Heart Failure, Weight Gain, Bone Density

PPARG Ligands and Insulin Resistance

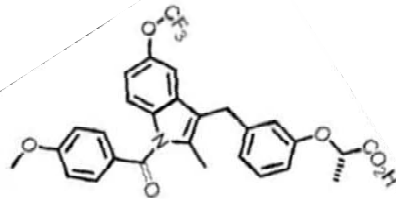
Partial loss of function mutations in PPAR γ in humans unambiguously cause severe insulin resistance. But....

PPAR γ agonists improve insulin-resistance and diabetes, but most PPAR γ target genes are already fully “on” in obesity – and typically there is no defect in receptor.

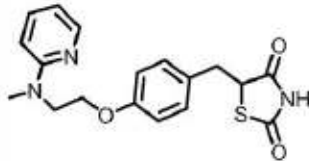
Some PPAR γ ligands with poor agonist activity still have *marked* anti-diabetic actions (MRL24, Mbx-102, INT131).

The PPAR γ Paradox

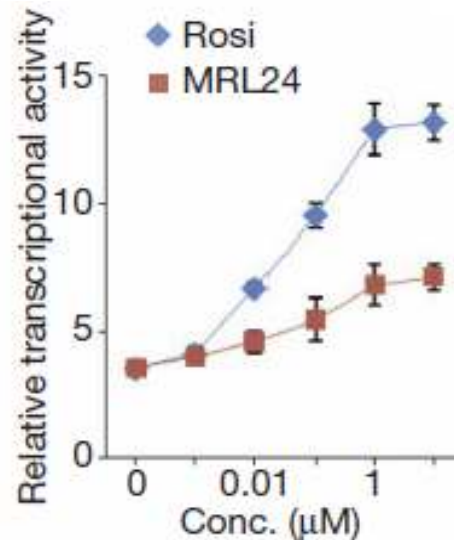
MRL24



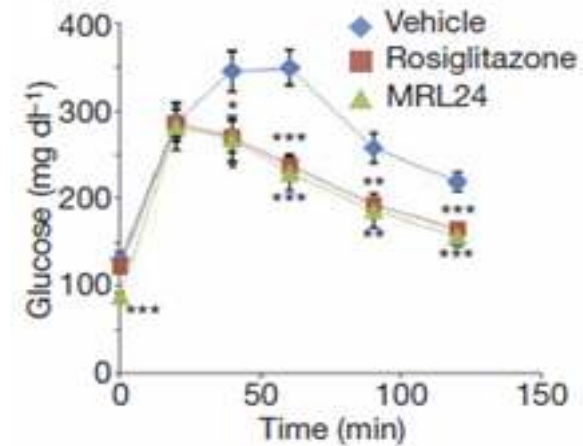
Rosiglitazone



PPAR γ Transactivation Assay



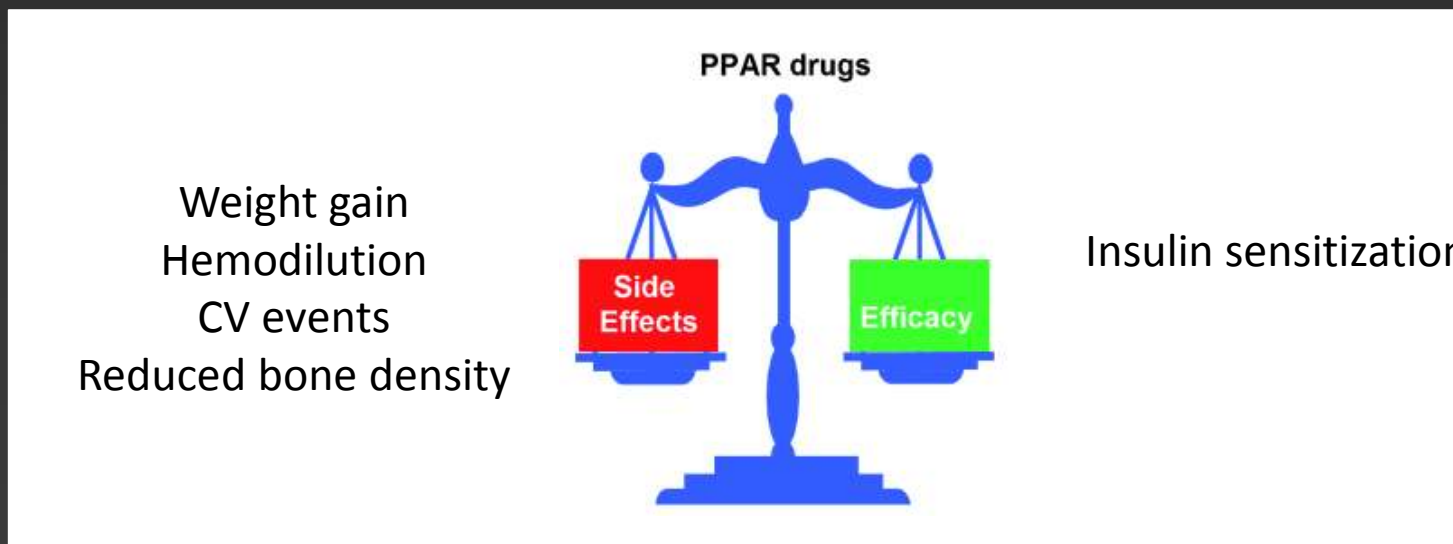
Glucose Tolerance Test



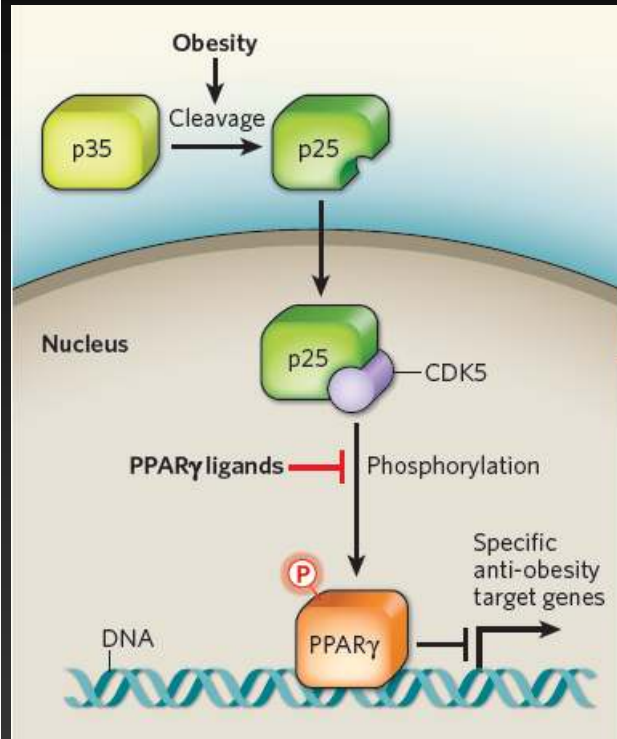
Paradox: If agonism of PPAR γ drives adipokine expression then why are partial agonists equally efficacious as full agonists?

Questions about Partial Agonists

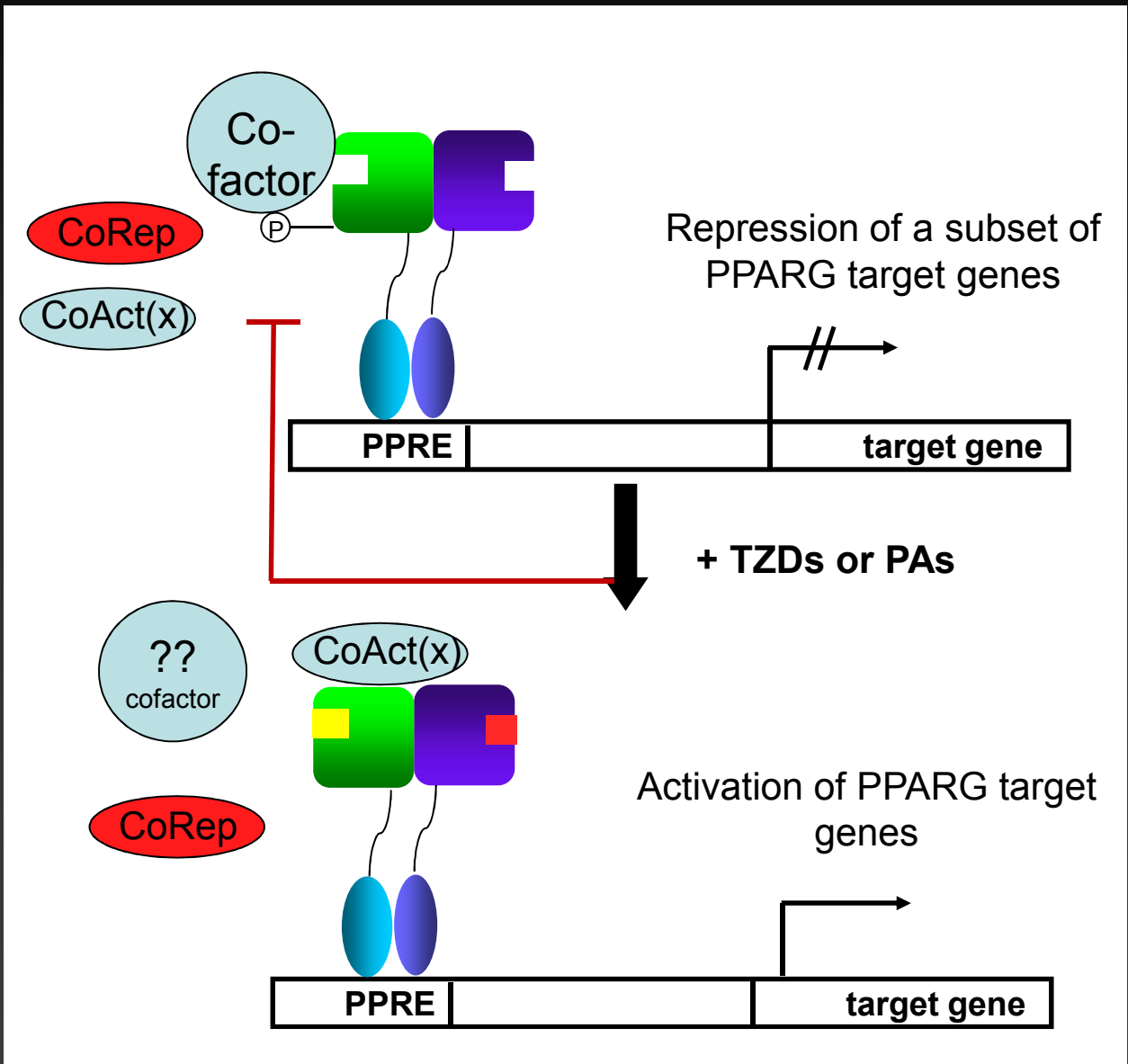
- Do PAs regulate expression of adipokines via a different mechanism than TZDs?
- Do PAs afford separation of insulin sensitization pathways from pro-adipogenic, fluid expansion/retention, cardiohypertrophy?



PTM status impacts PPARG function

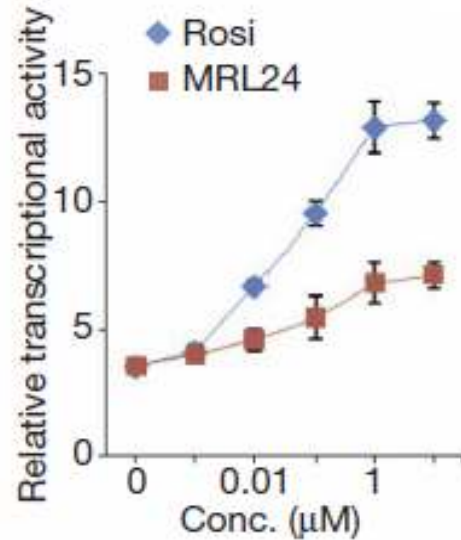


Ligands bind to PPARG and interfere with the ability of kinase(s) to PO3 the receptor

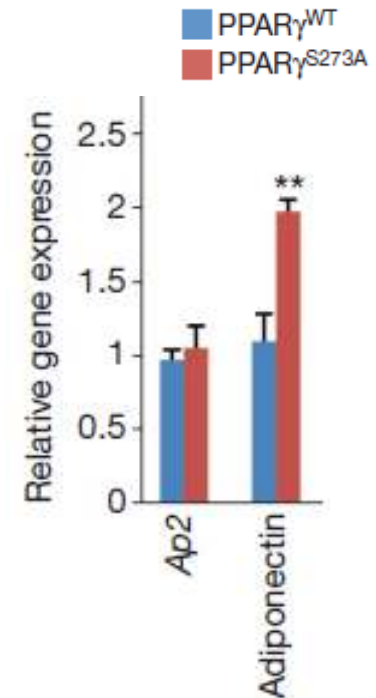
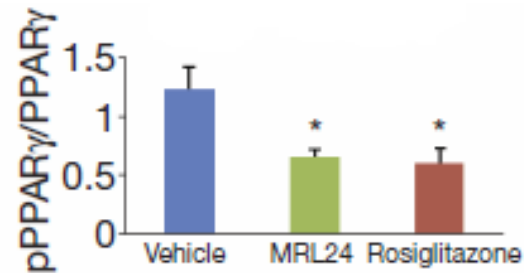
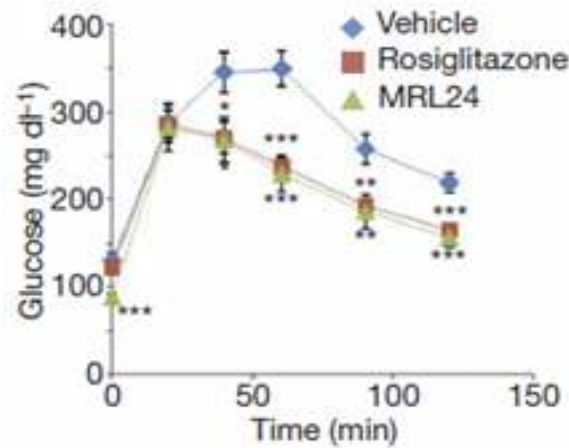


PTM status impacts PPAR γ function

PPAR γ Transactivation Assay

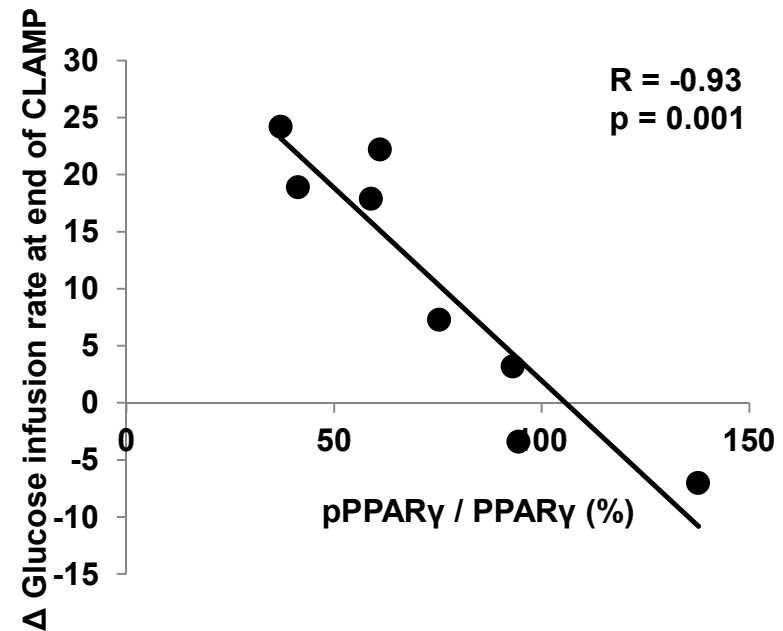
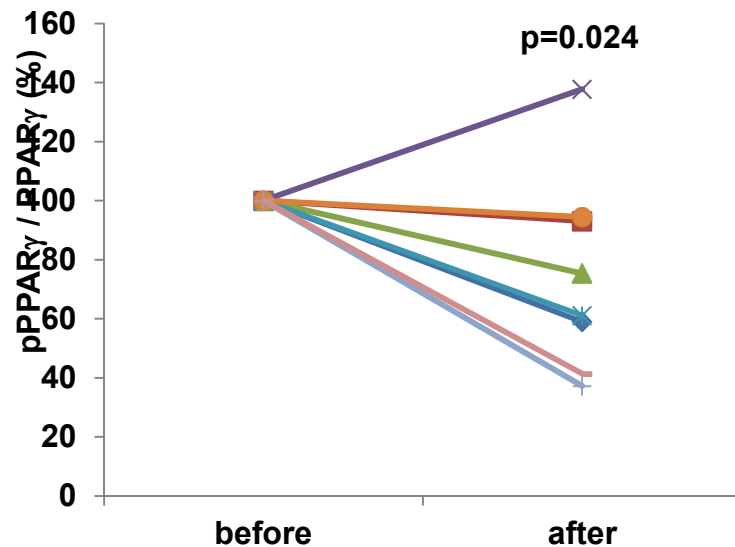


Glucose Tolerance Test

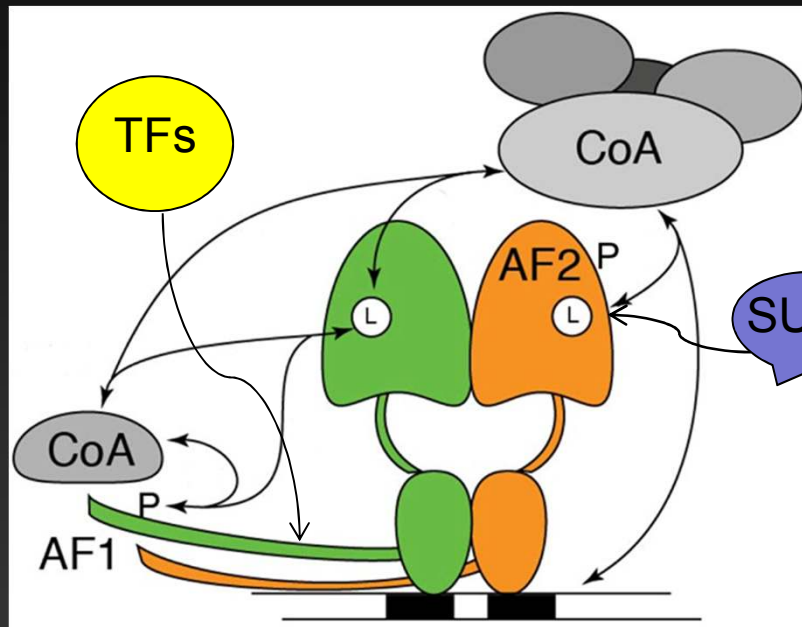


Modulation of PPAR γ phosphorylation by rosiglitazone during therapy of human type 2 diabetics

Newly diagnosed T2D, male, 6 months treatment, 4mg/day



PTM Control of NR Signaling



Combinatorial Control of NR Function

Functional Interactions

Ligand – AF2

Ligand – dimer partner

AF1- AF2

Ligand – AF1

Ligand – PTM

PTM – co-regulator

DNA – co-regulator

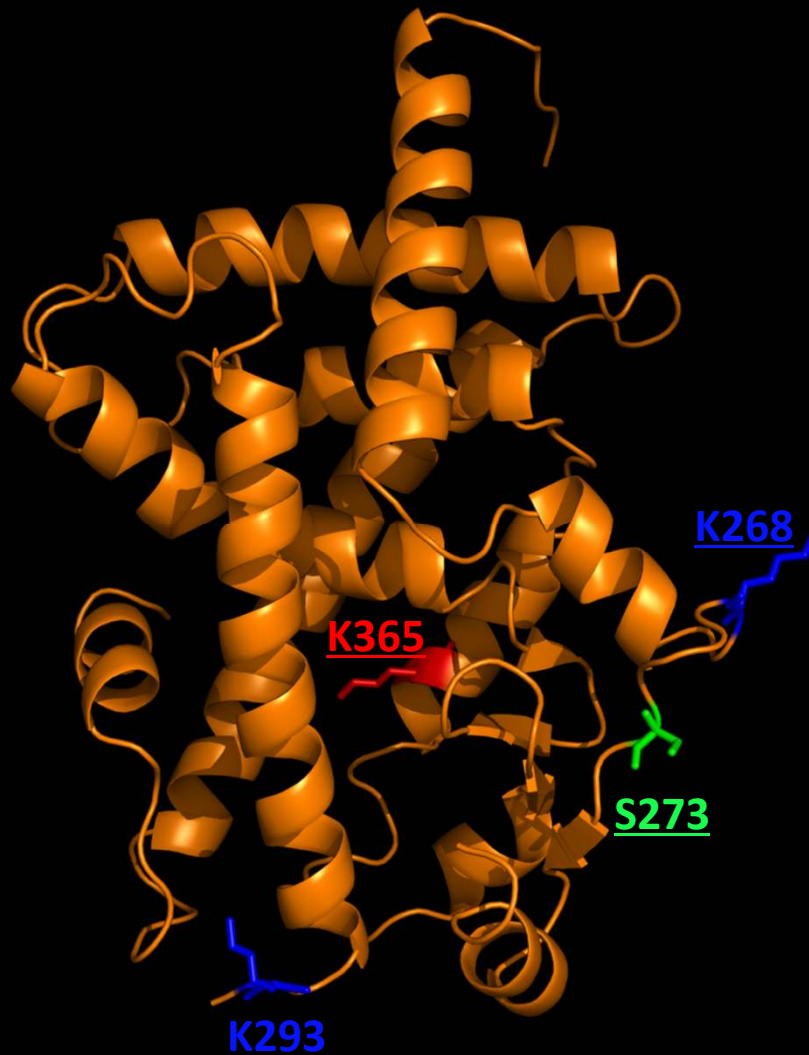
PTM – DNA

Ligand – DNA

Ligand-mediated translocation

Goal of our lab – to develop functionally selective modulators of nuclear receptors

PPARG LBD PTMs



S273: phosphorylation correlates with obese gene expression.
Choi et al. Nature 2010

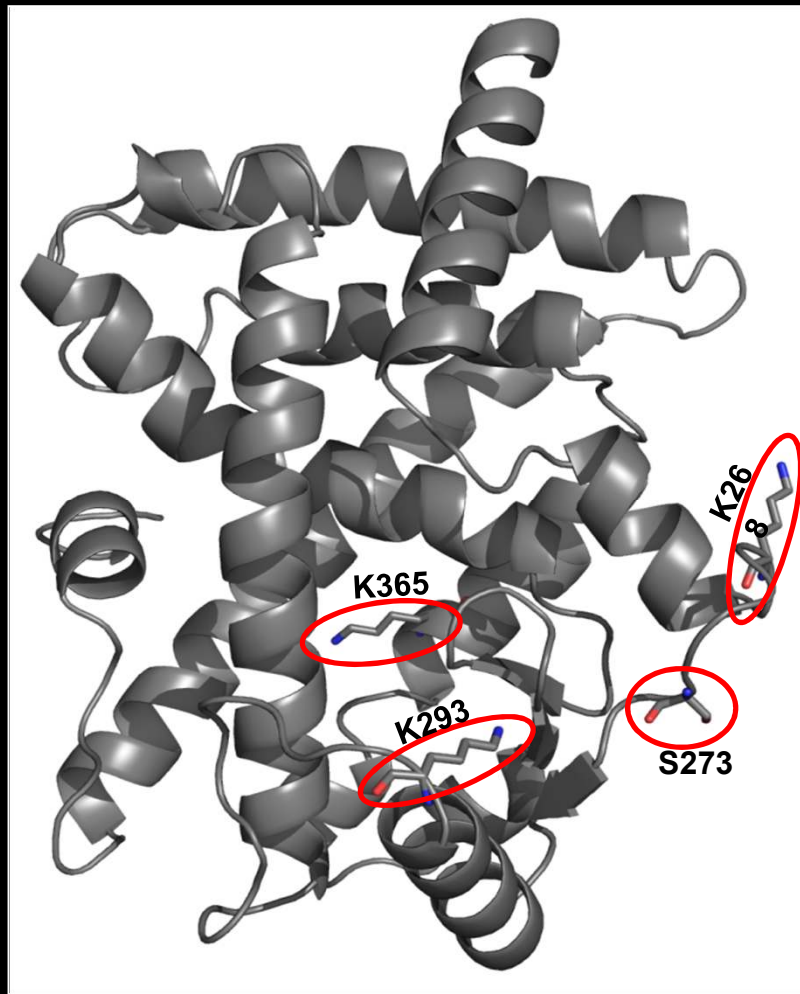
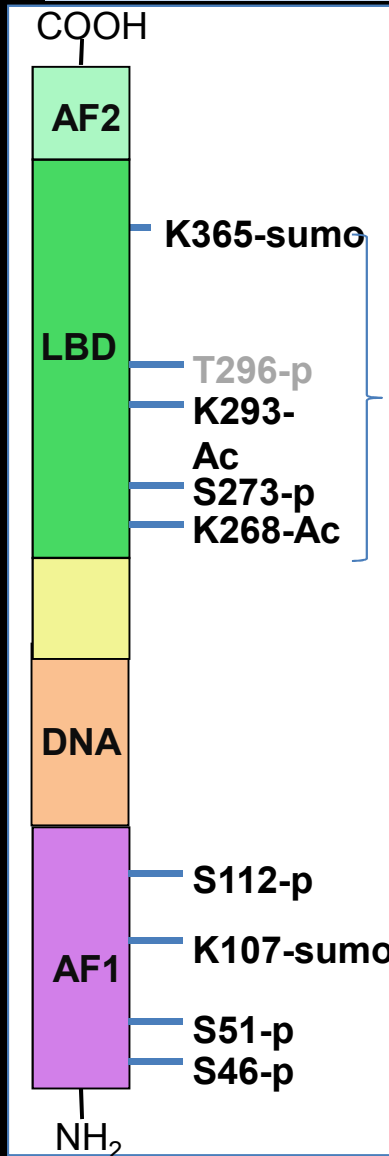
K365: SUMOylation leads to repression of NFkB target genes.
Pascual et al. Nature 2005

K293: 'Ser273 phosphorylation correlates with Lys293 acetylation'
Qiang et al. Cell 2012

K268 & K293: 'Deacetylation of PPPARG on Lys293 is required to recruit coactivator Prdm16, while deacetylation on Lys268 and Lys293 is required to clear corepressor NCoR.'
Qiang et al. Cell 2012

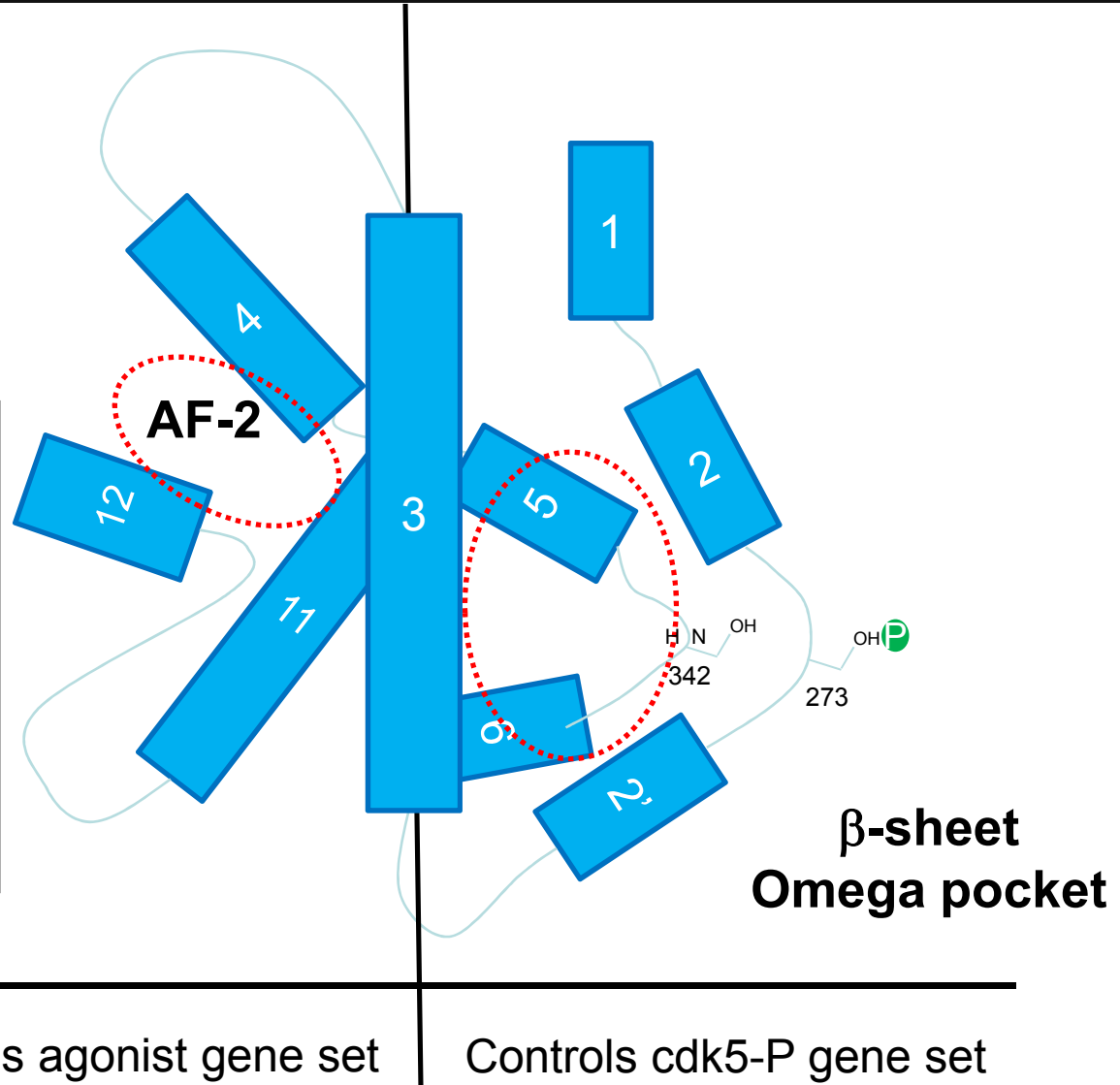
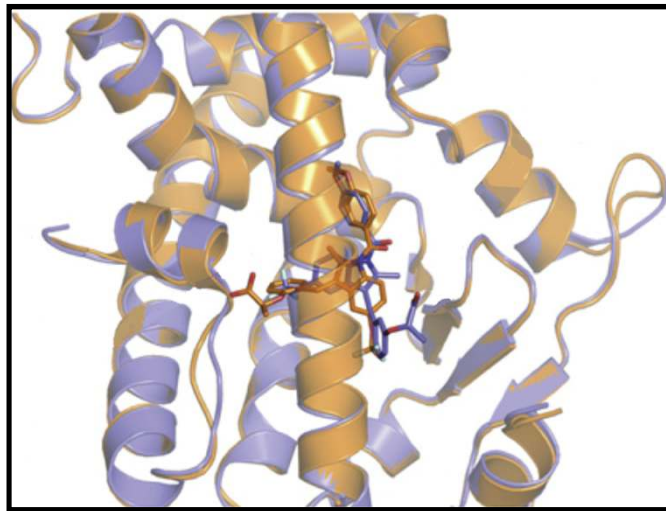
Paradigm Shift?

Modulate PTMs and not Receptor Activation

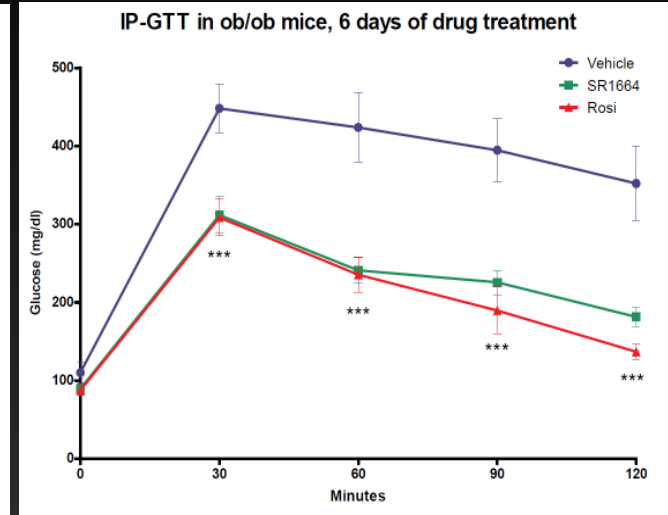
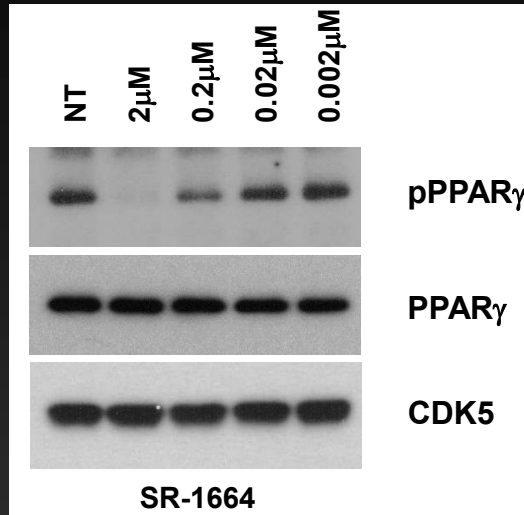
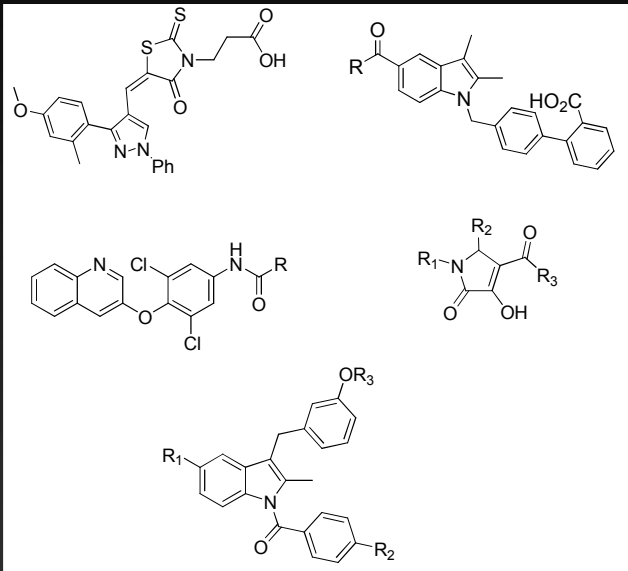


PTM	Rosi	1664
K268	✓	?
S273	✓	✓
K293	✓	?
K365	✓	?
Agonism	✓	✗

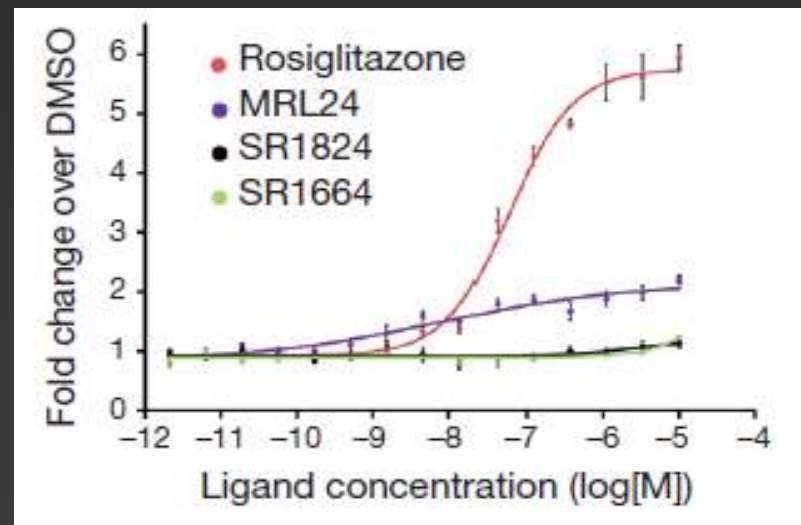
Can Agonism and Blocking S273-P be separated?



Ligand Discovery



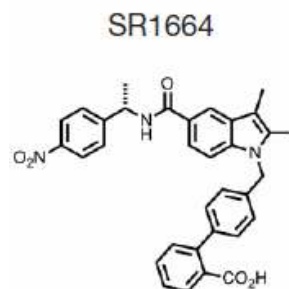
Compound	IC ₅₀ (competition assay)
rosiglitazone	60nM
SR1664	80nM
MRL24	20nM
SR1824	28nM
SR2227	10nM
SR2278	10nM
SR1707	>10 μ M
SR1708	10nM
SR1713	>10 μ M
SR1714	17nM



Choi et al Nature 2011

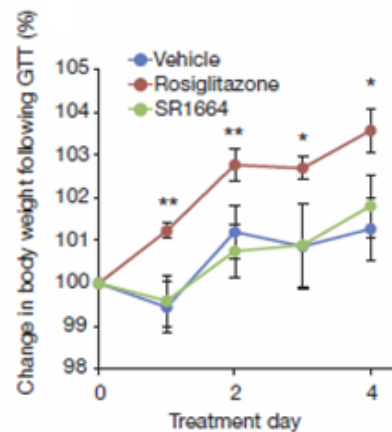
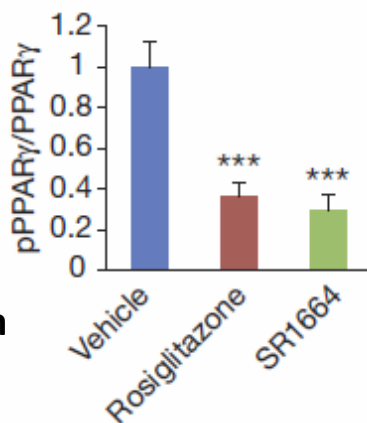
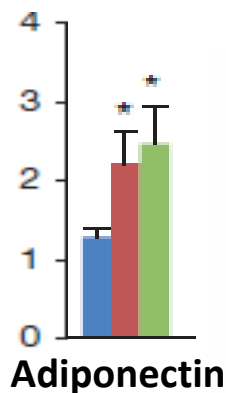
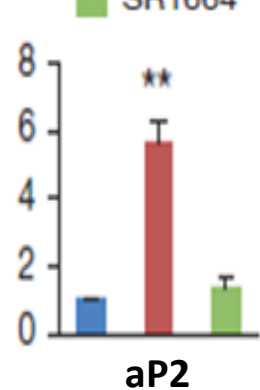
Antidiabetic actions of a non-agonist PPAR γ ligand blocking Cdk5-mediated phosphorylation

Jang Hyun Choi^{1*}, Alexander S. Banks^{1*}, Theodore M. Kamenecka^{2,4*}, Scott A. Busby^{3*}, Michael J. Chalmers³, Naresh Kumar³, Dana S. Kuruvilla³, Youseung Shin², Yuanjun He², John B. Bruning⁵, David P. Marciano³, Michael D. Cameron^{2,3,4}, Dina Laznik¹, Michael J. Jurczak⁶, Stephan C. Schürer⁷, Dušica Vidović⁷, Gerald I. Shulman⁶, Bruce M. Spiegelman¹ & Patrick R. Griffin^{2,3,4}

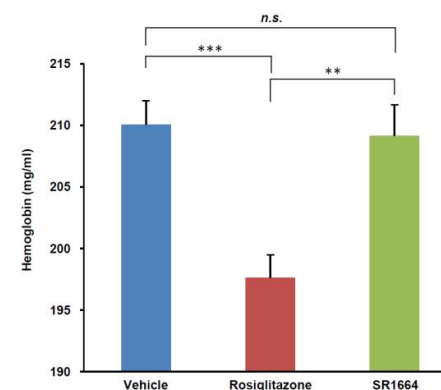


Relative gene expression

■ NT
■ Rosiglitazone
■ SR1664

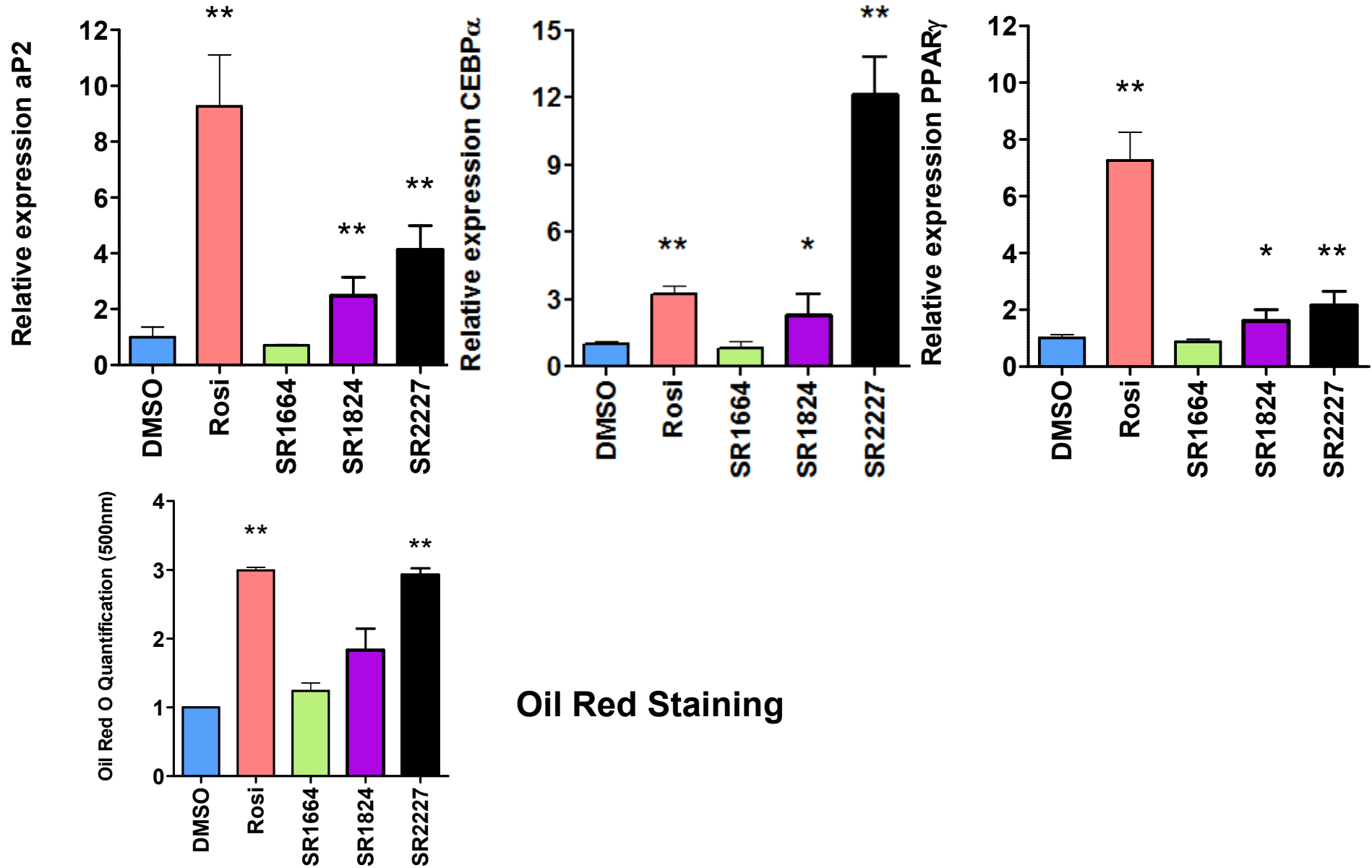


Body Weight

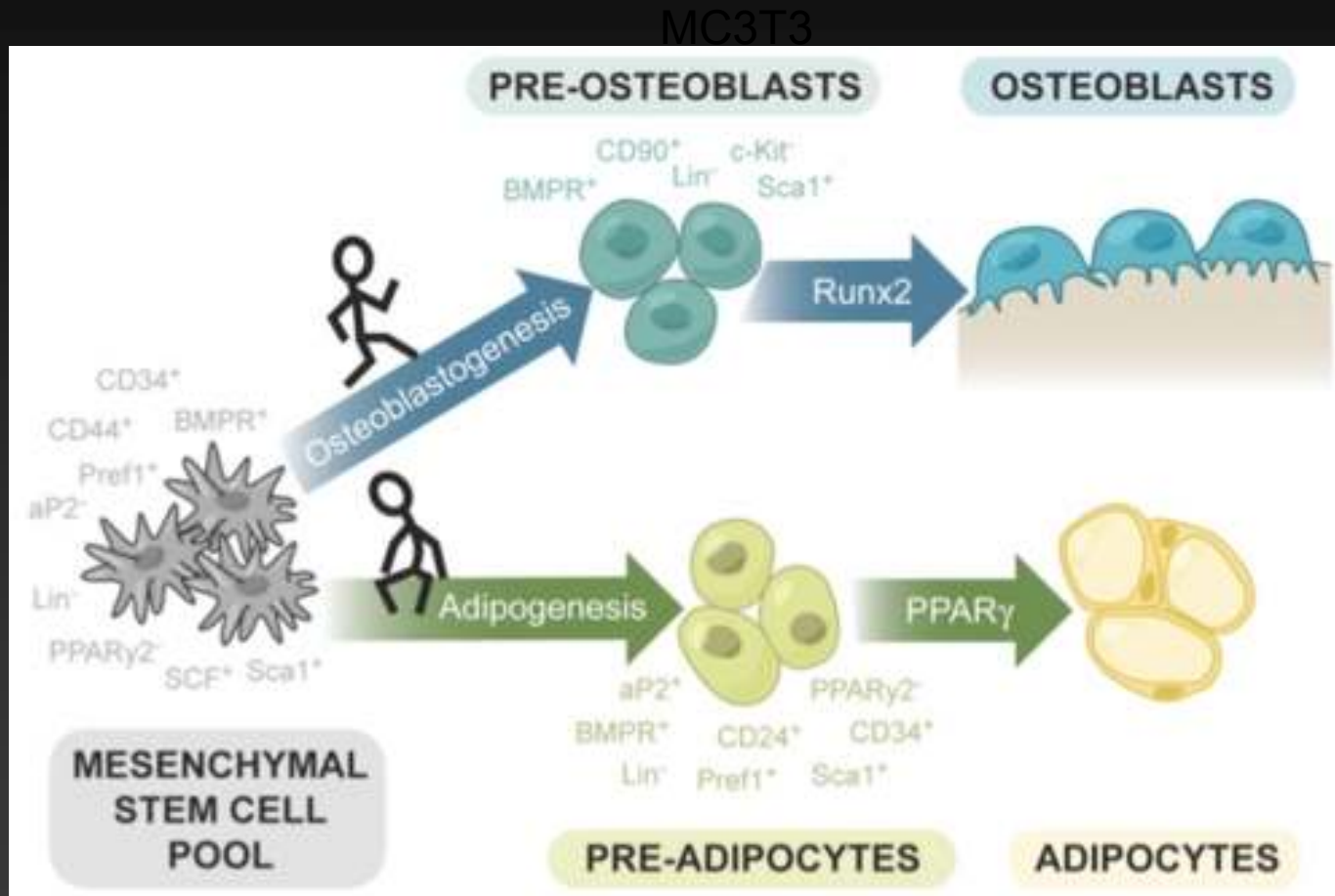


PVE

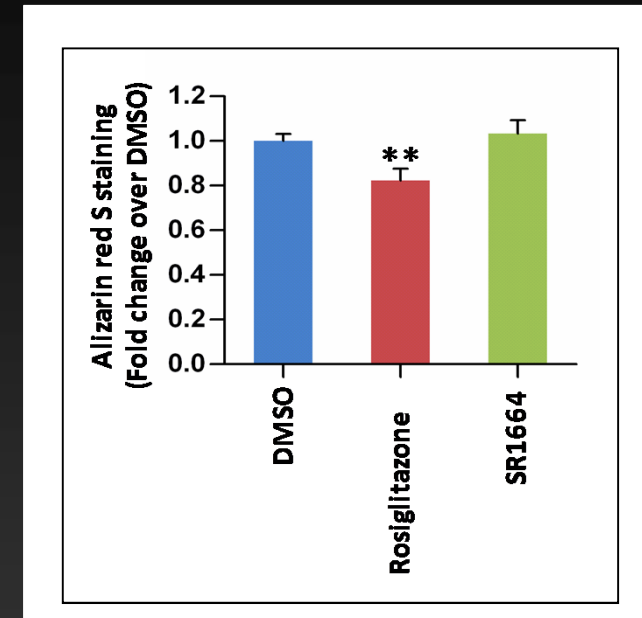
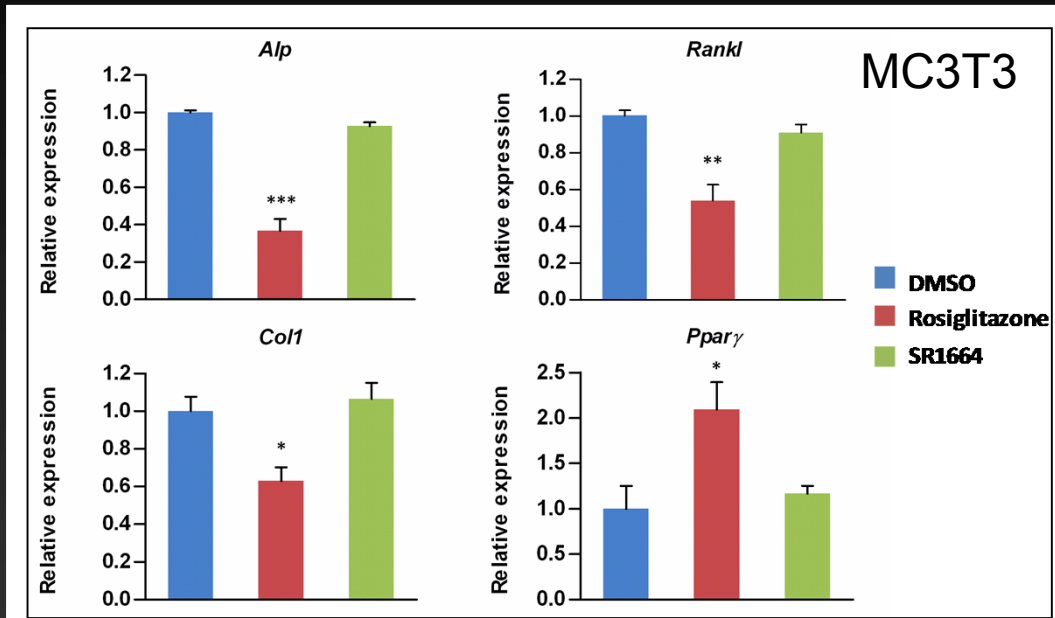
SR1664 effect on Adipocytes



SR1664 and Analogs on Bone



SR1664 and Analogs on Bone



		ALP activity	p	Fold change
		uU/min		
	DMSO	3.80		
Full Agonist	RSG/10uM	0.54	1.0E-07	0.141
	RSG/1uM	0.69	2.4E-07	0.182
Partial Agonist	1824/10uM	5.08	0.01	1.337
	1824/1uM	4.78	0.02	1.258
Full Agonist	2227/10uM	1.16	0.00	0.305
	2227/1uM	2.25	0.05	0.593
Partial Agonist	MRL24/10uM	2.19	0.08	0.576
	MRL24/1uM	2.02	0.01	0.533
Non-Agonist	1664/10uM	4.66	0.56	1.226
	1664/1uM	4.54	0.32	1.194
Non-Agonist	2539/10uM	4.14	0.71	1.089
	2539/1uM	3.54	0.78	0.933

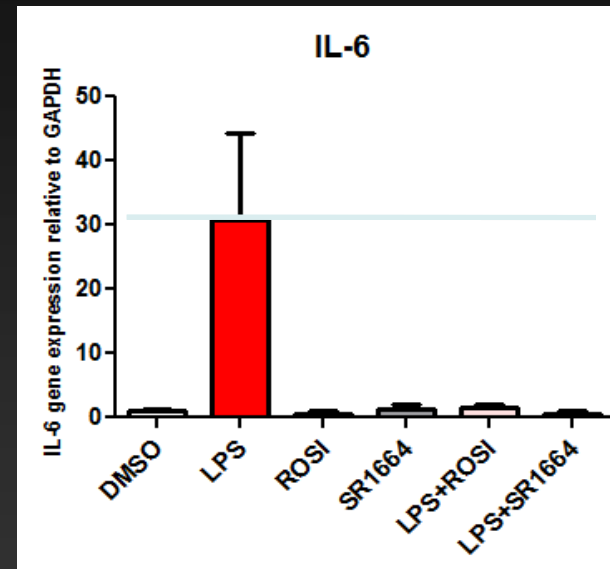
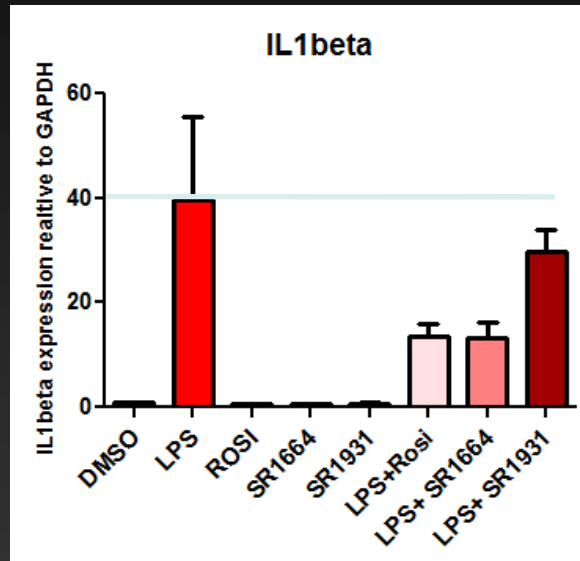
Beata Lecka-Czernik in U-33/PPARg2 cells

Adipogenesis only seen with rosi

activity of pro-osteoblastic signaling – repression%

Rosi	50%
SR1824	30%
SR2227	30%
MRL24	20%
SR1664	~2%
SR2539	0%

Inhibition of inflammatory cytokines

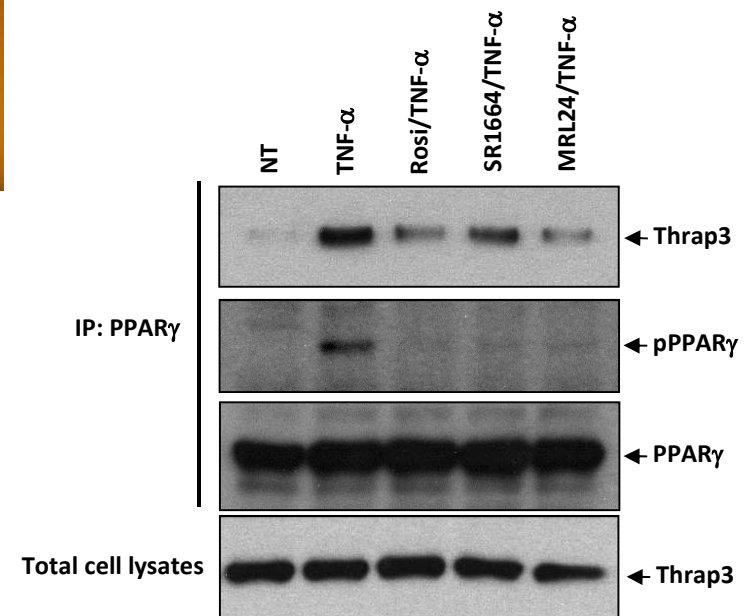
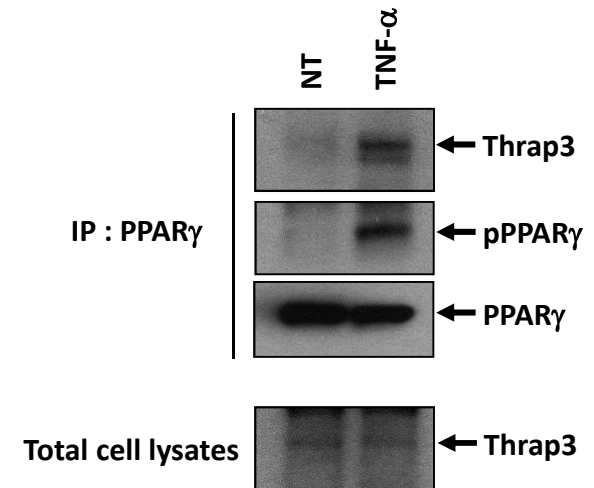
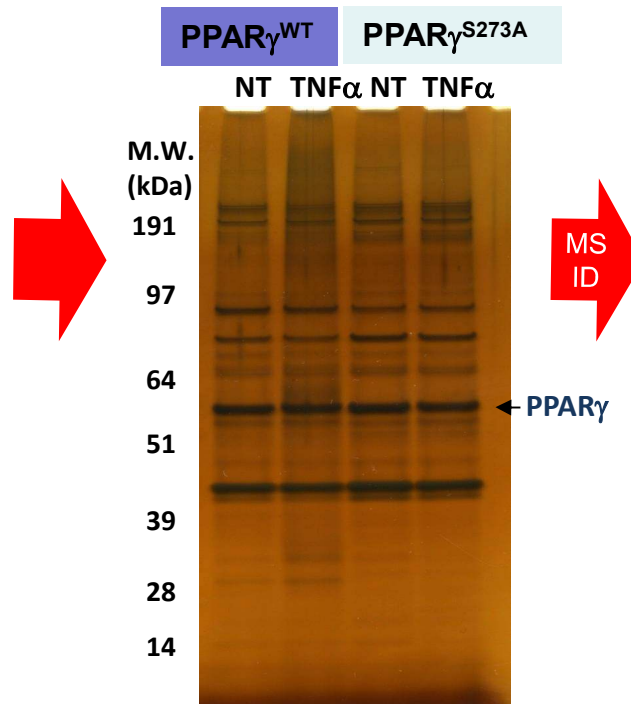
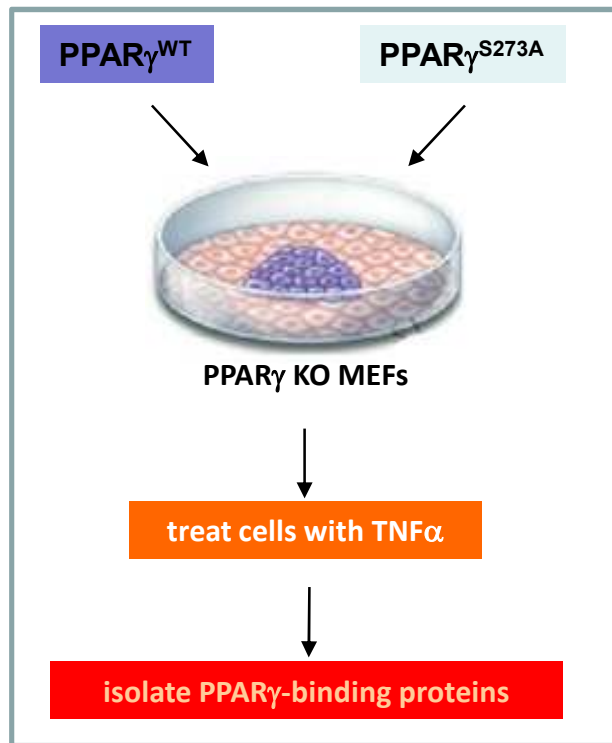


- PPAR γ non agonist (SR1664) inhibited inflammatory cytokines as well as TZD
- Different scaffold SR1931 did not - compound is a weak binding non-agonist

LPS- RAW264.7 cells

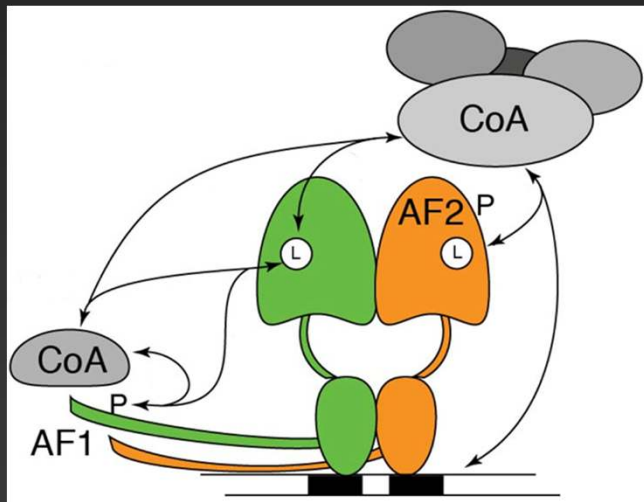
- preincubation with compounds for 18 hrs
- then LPS stimulation for 6 hrs

PTM status impacts PPAR γ function: How?

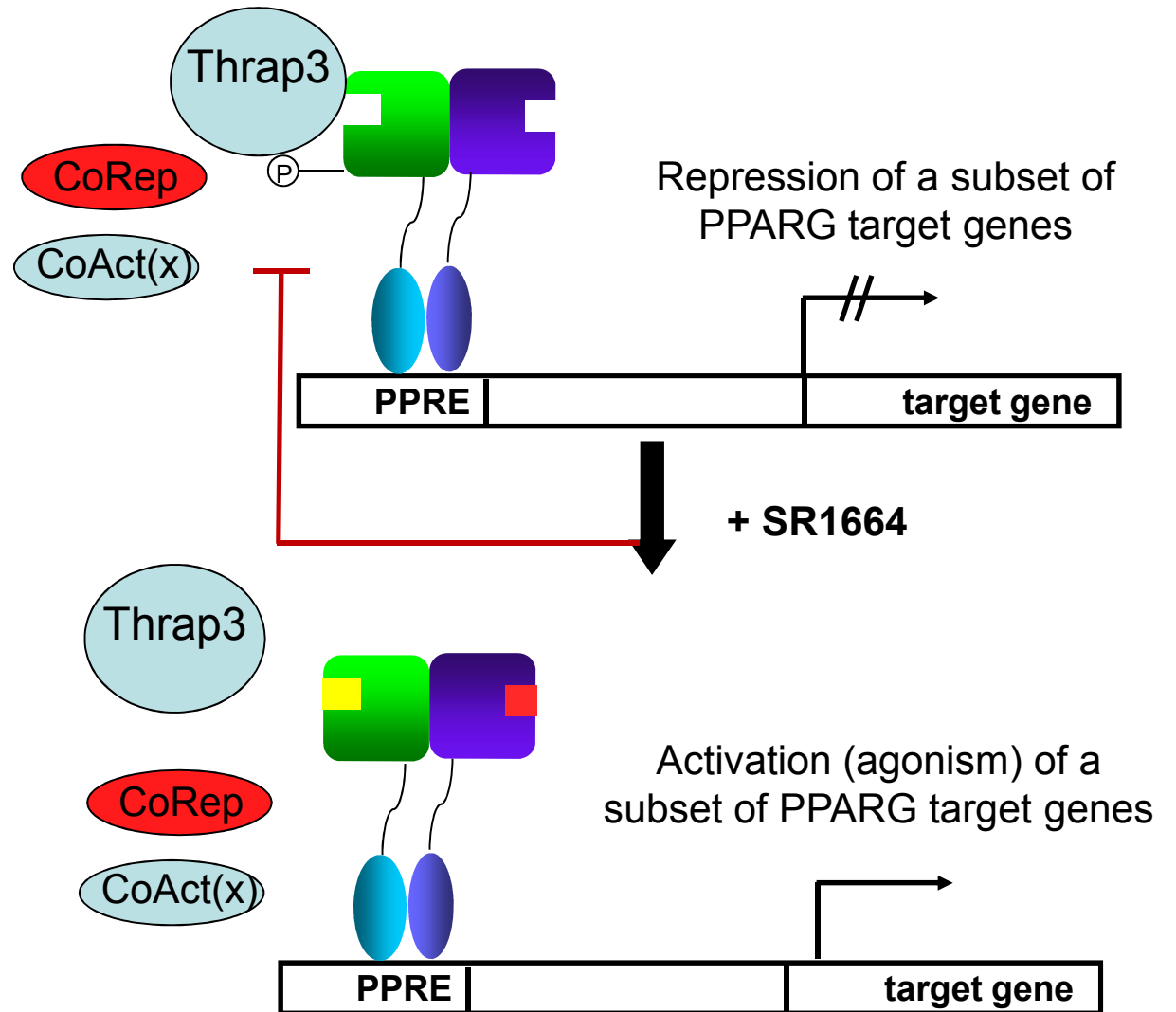


Thrap3 is a factor that binds to PPAR γ when S273 is phosphorylated.
Functional studies ongoing.

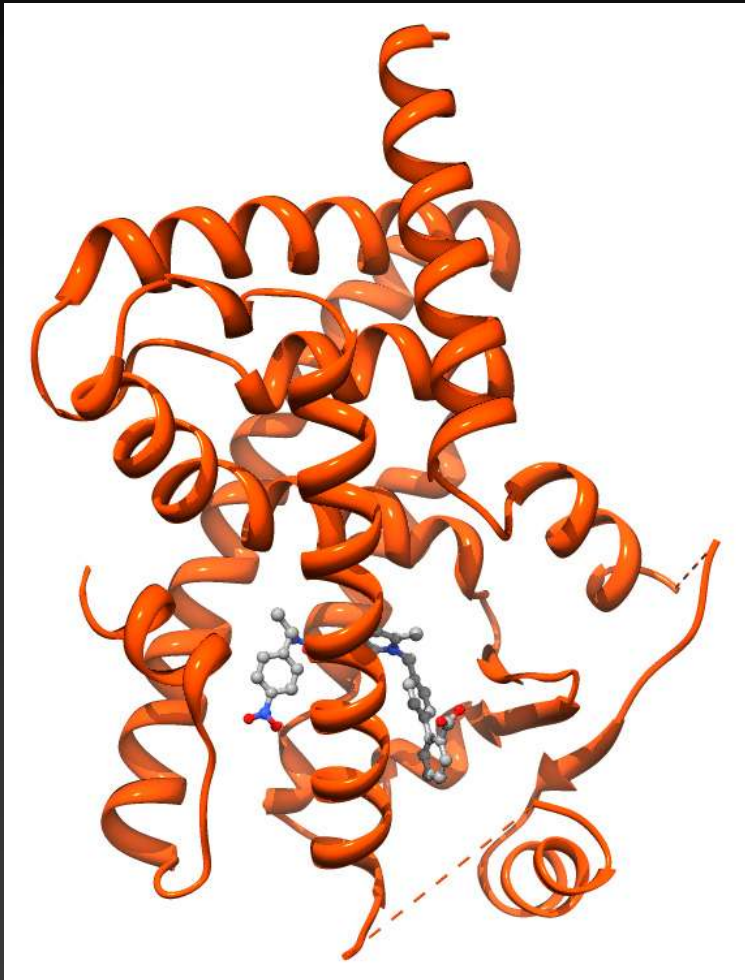
SR1664 POC



Ligand – PTM
PTM – co-regulator



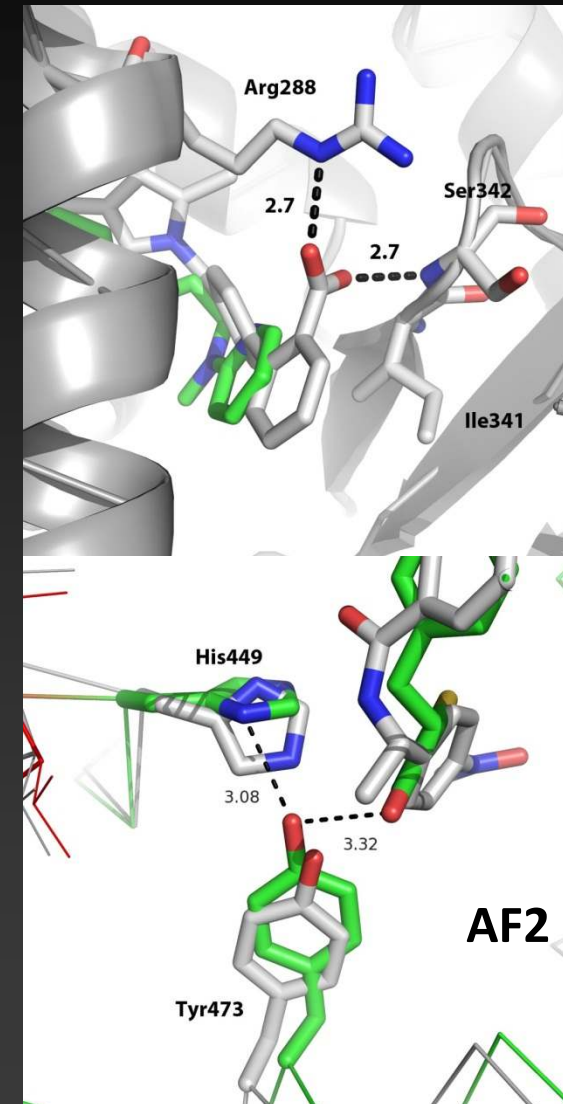
Crystal Structure of PPAR γ :SR1664



SR1664 stabilizes the β -sheet region

SR1664 Abrogates full agonist H-Bonds

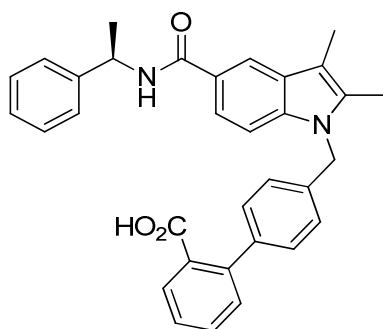
- **No Large Changes in Global Fold**
- **So what is SR1664 doing to the receptor?**



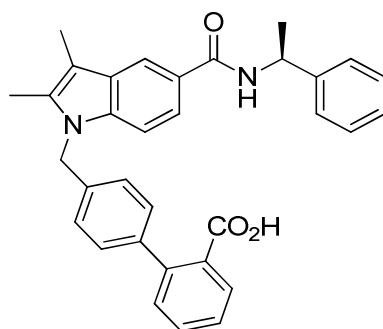
Rosiglitazone structure* = green
SR1664 structure = grey *PDB:2PRG

Structure of Enantiomers SR1663 & SR1664

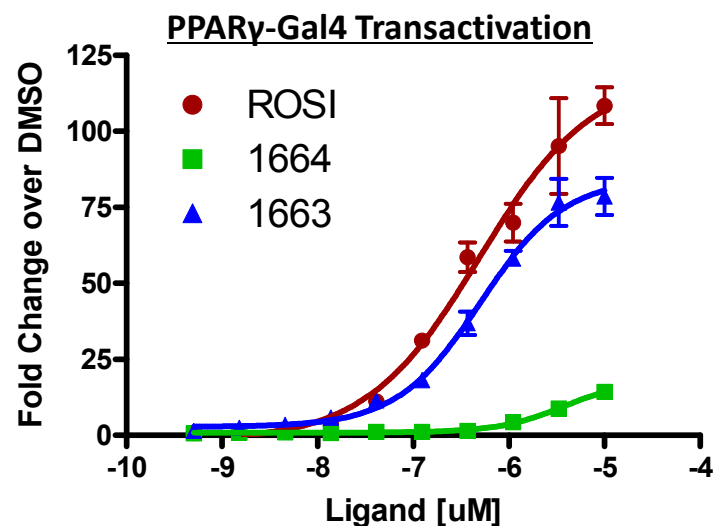
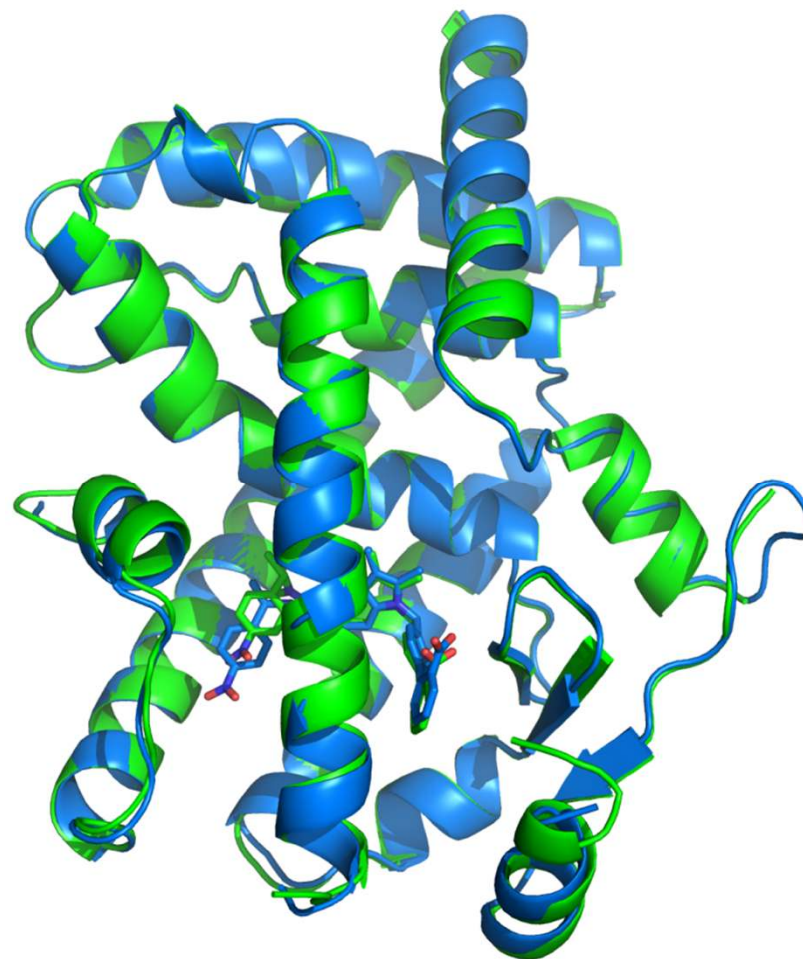
SR1663:
Partial Agonist



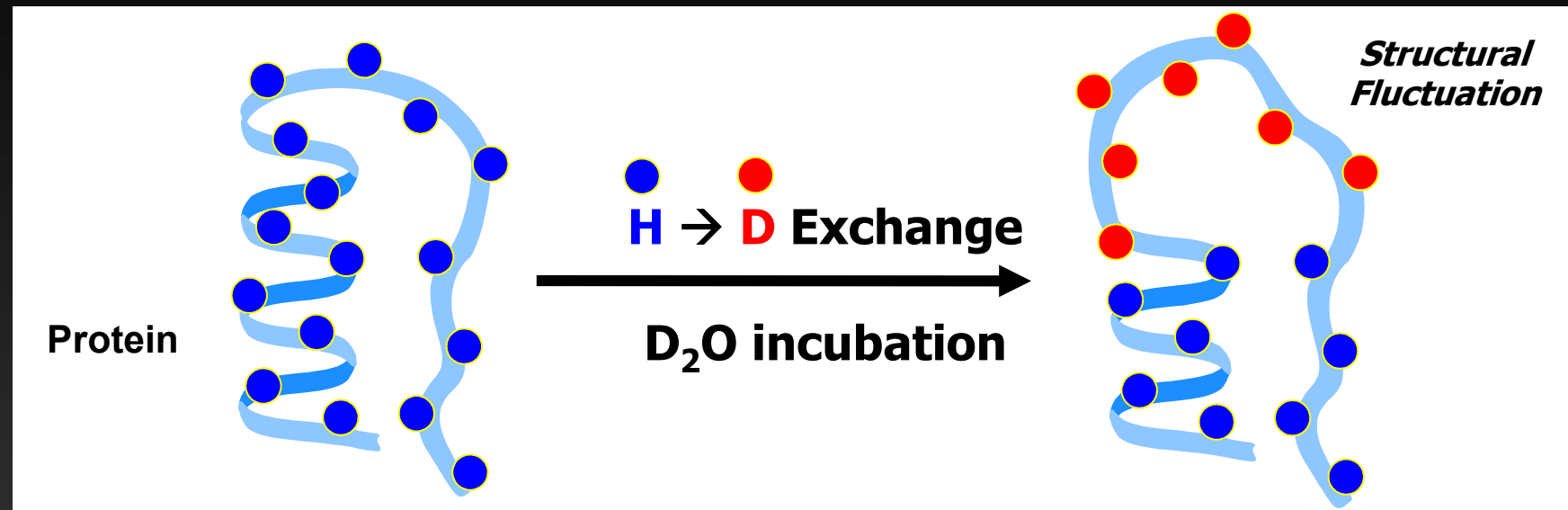
SR1664:
Non Agonist



SR1663 vs. SR1664: RMSD = 0.41



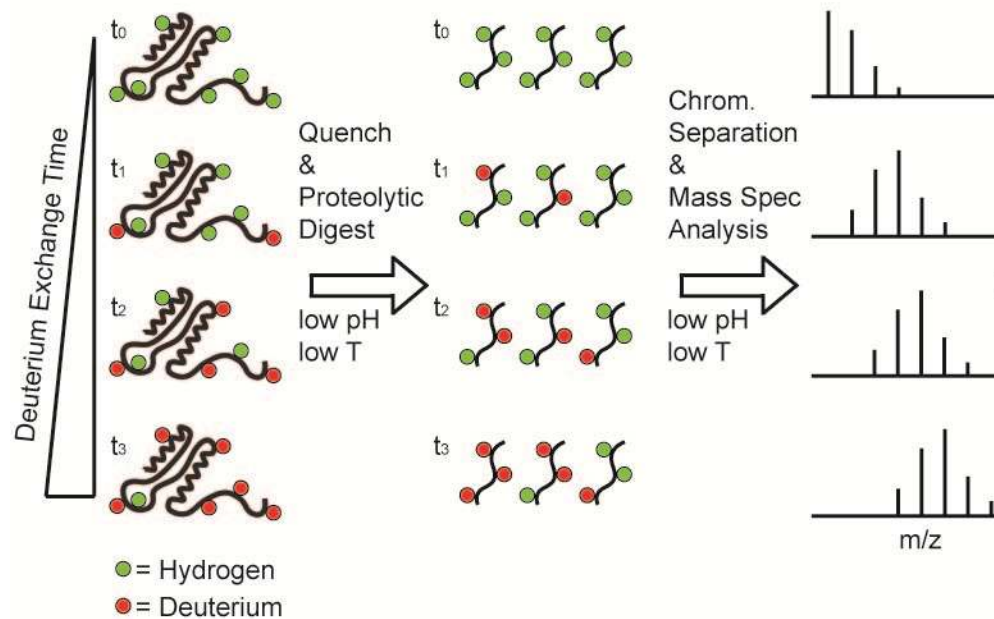
HDX Analysis of Proteins



- Protein conformational mobility influences rate of amide **H** atoms to exchange with solvent **D** atoms.
- Solution based fully automated system; LC-MS LTQ-Orbitrap with ETD to combine bottom-up HDX with ETD sub-localization.

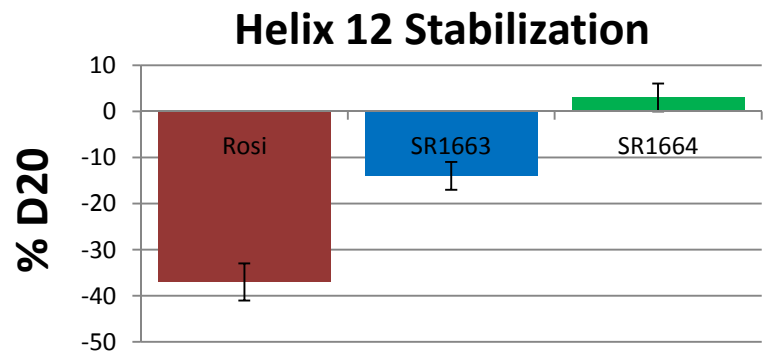
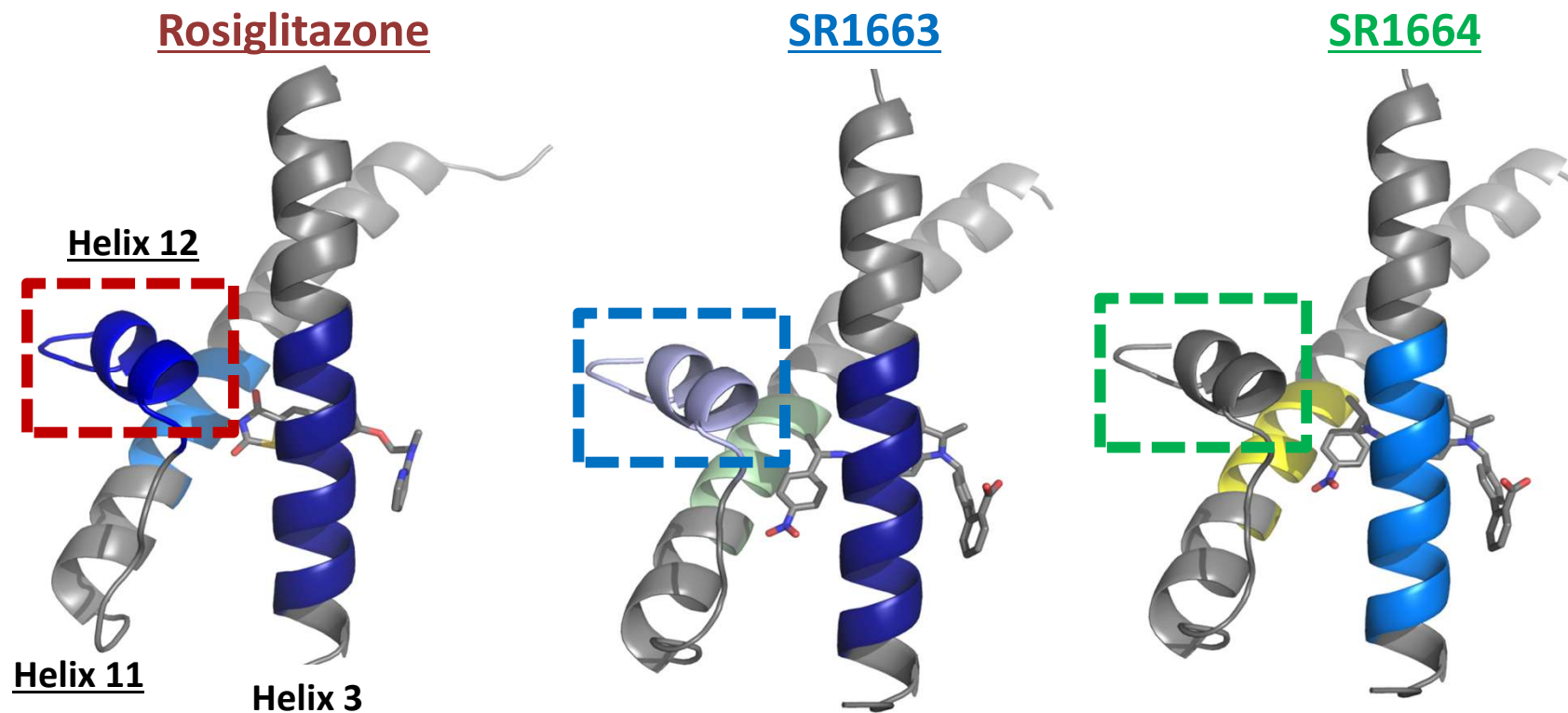
Differential HDX Work Flow

Incubation with D₂O buffer



Chalmers et al JBT 2007
Chalmers et al Exp Rev Prot 2011

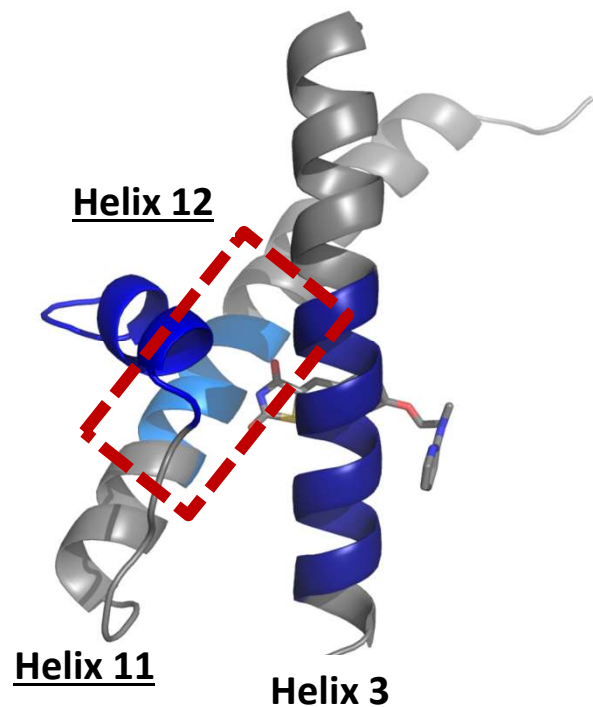
HDX Differentiates Functionally Distinct Enantiomers SR1663 & SR1664



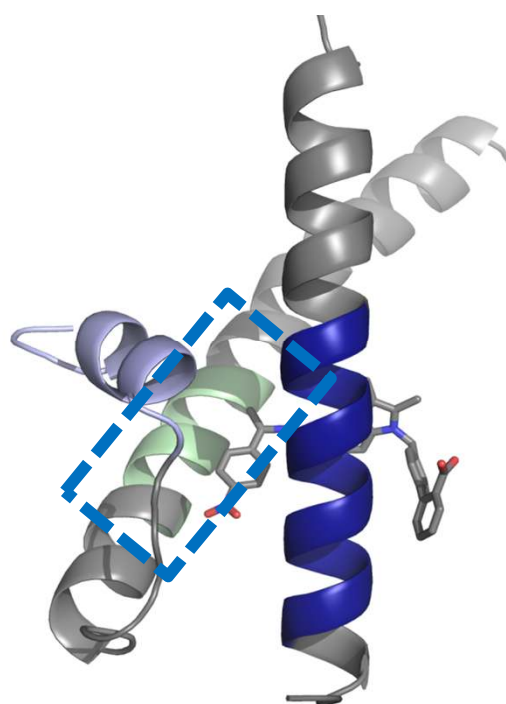
Compound	IC ₅₀ (binding affinity)	EC ₅₀ (PPRE) (% relative to rosiglitazone)
rosiglitazone	18nM	7.4nM (100%)
SR1663	2nM	20nM (23%)
SR1664	80nM	Not active (0%)

HDX Differentiates Functionally Distinct Enantiomers SR1663 & SR1664

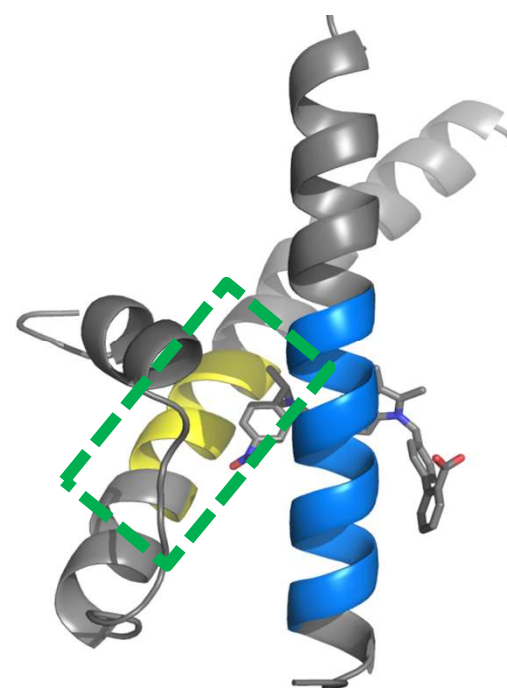
Rosiglitazone



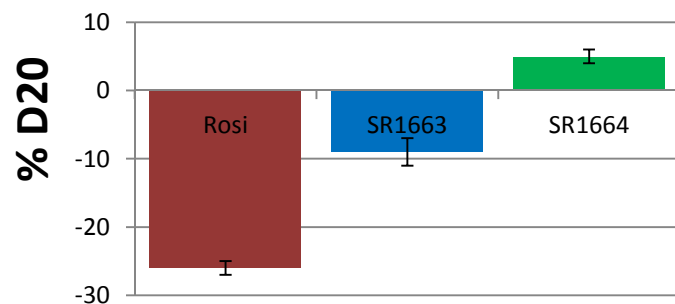
SR1663



SR1664

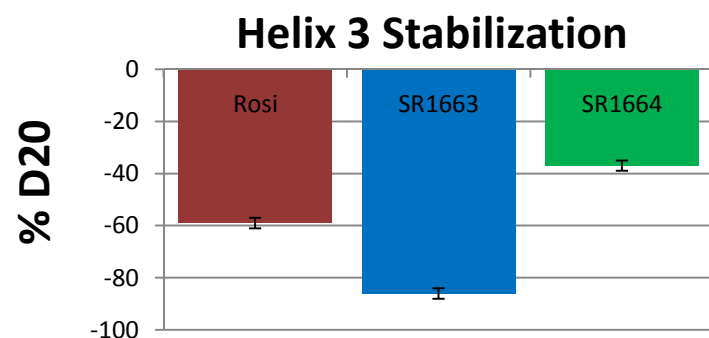
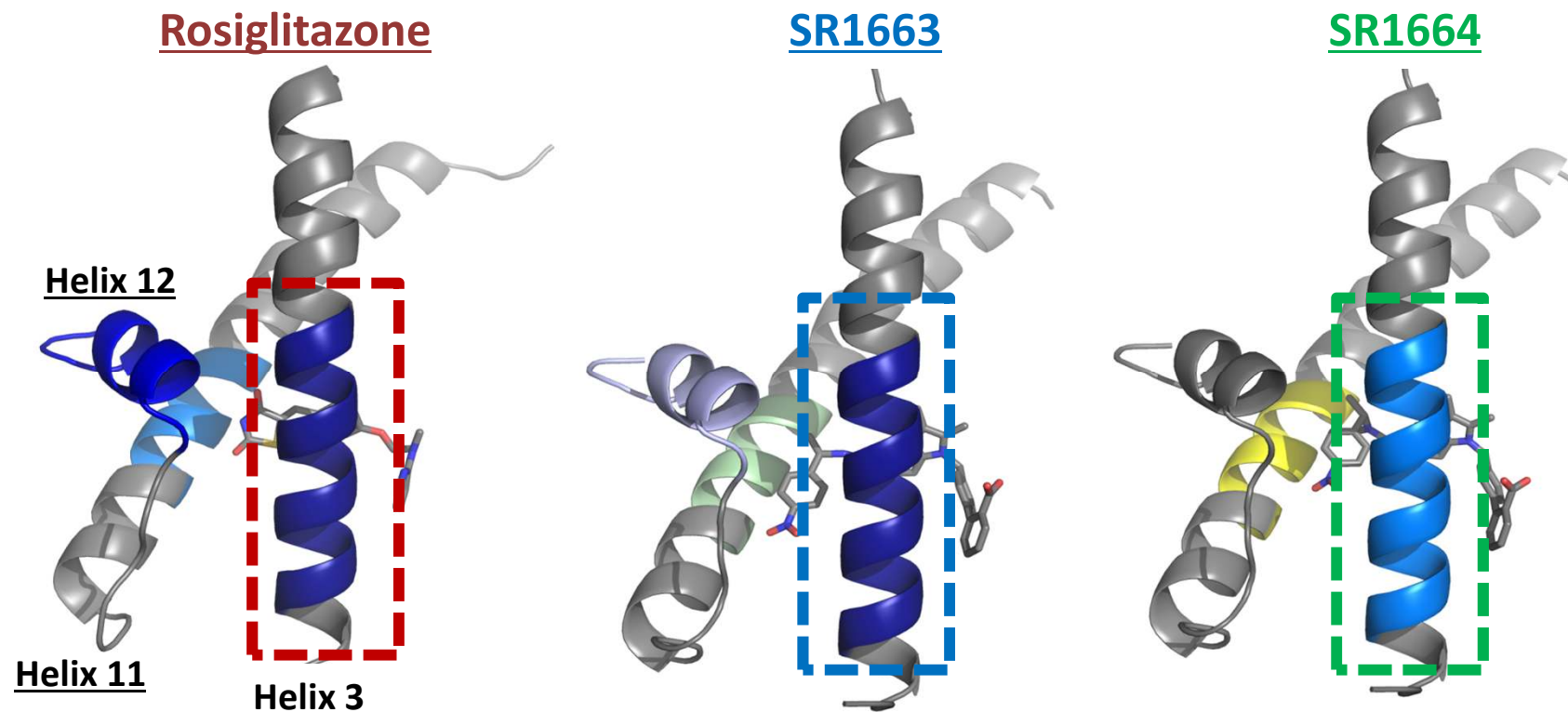


Helix 11 Stabilization



Compound	IC ₅₀ (binding affinity)	EC ₅₀ (PPRE) (% relative to rosiglitazone)
rosiglitazone	18nM	7.4nM (100%)
SR1663	2nM	20nM (23%)
SR1664	80nM	Not active (0%)

HDX Differentiates Functionally Distinct Enantiomers SR1663 & SR1664

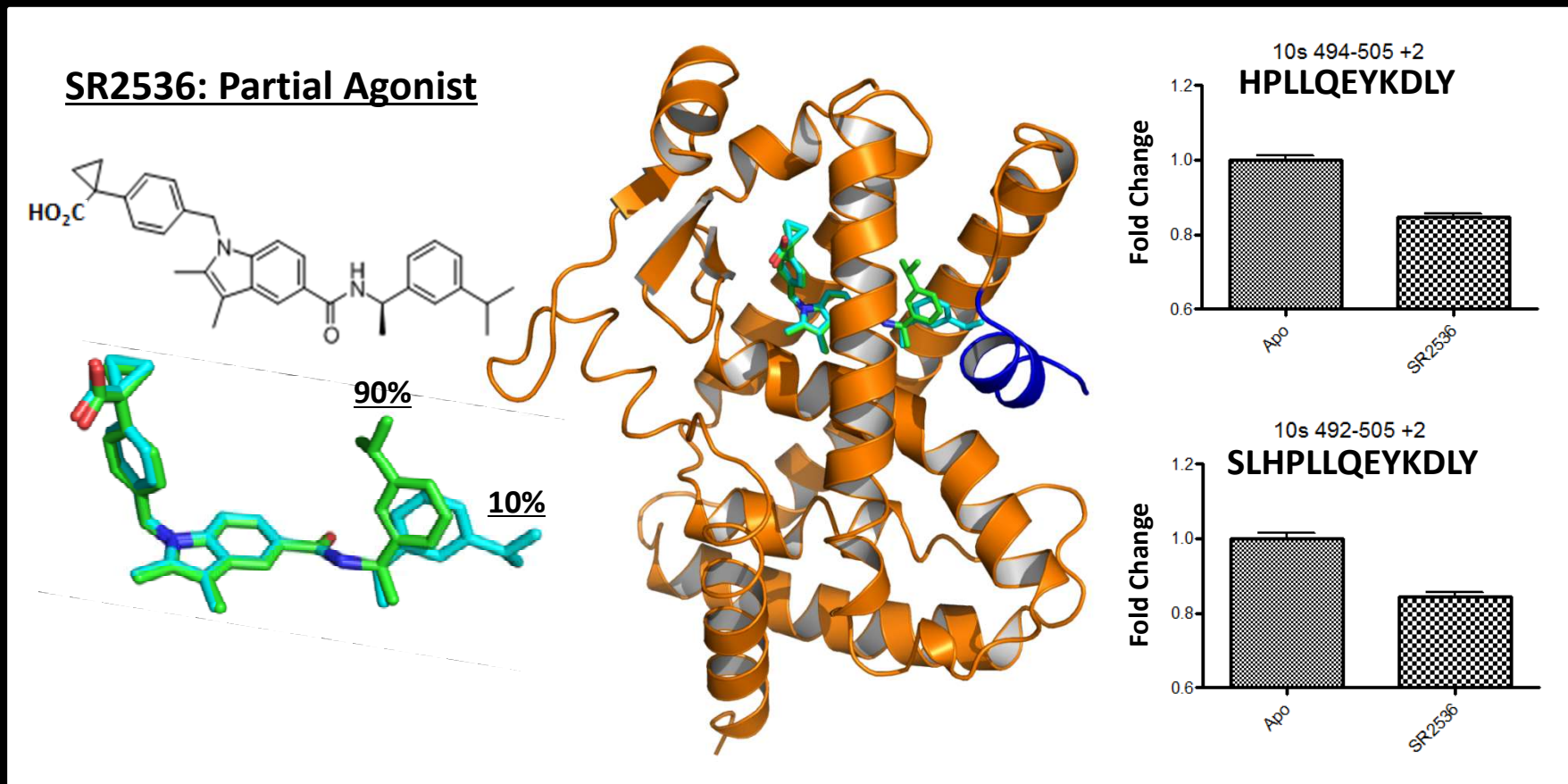


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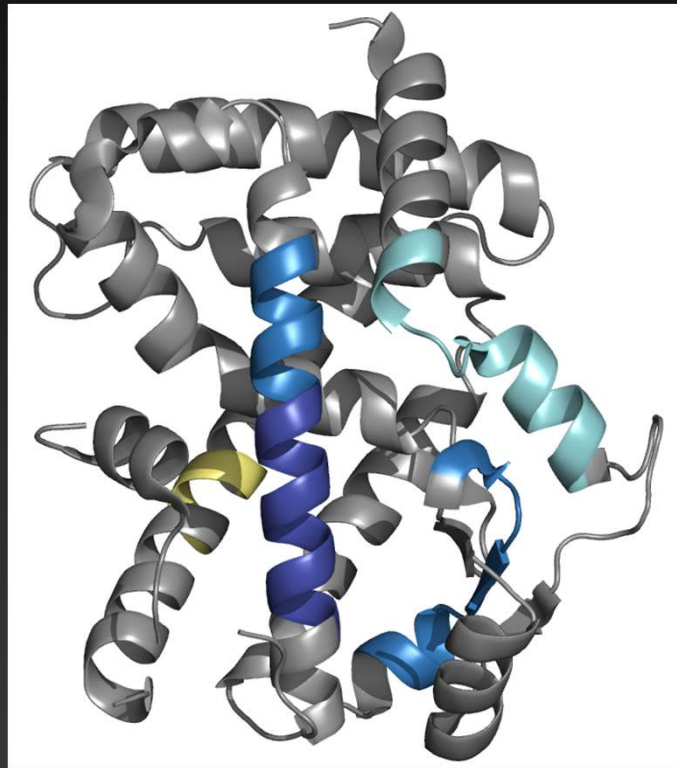
Color scale for %D: +50 (red) to -50 (blue)

Ligand Size and Contacts are Critical

SR2536 Reveals Cause of H12 Stabilization – ‘Flipping’



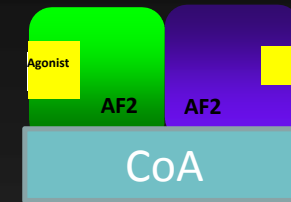
SR1664 Mechanism of Action



Dimer interface H-bonds



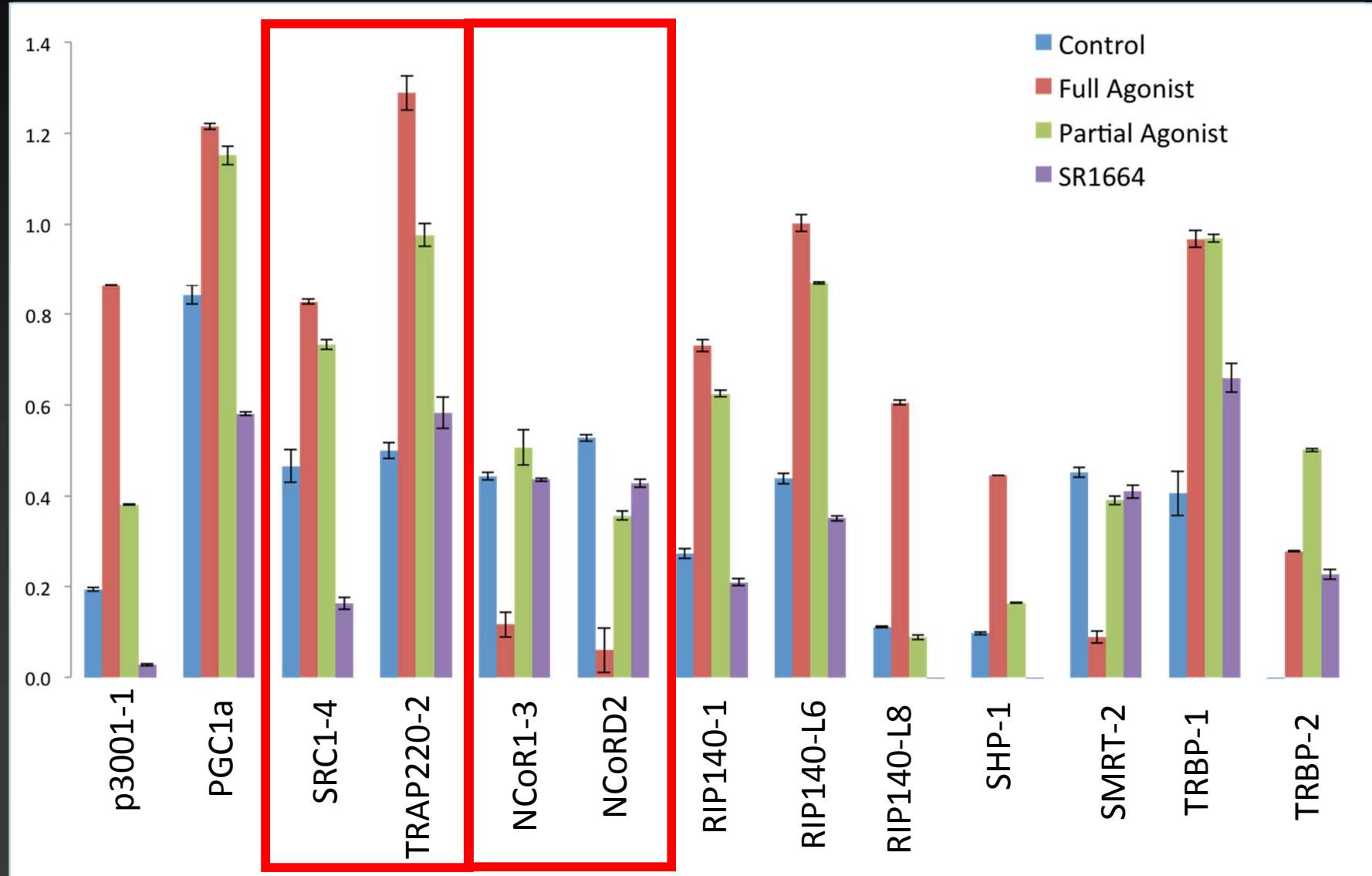
1664 distorts LBD interface



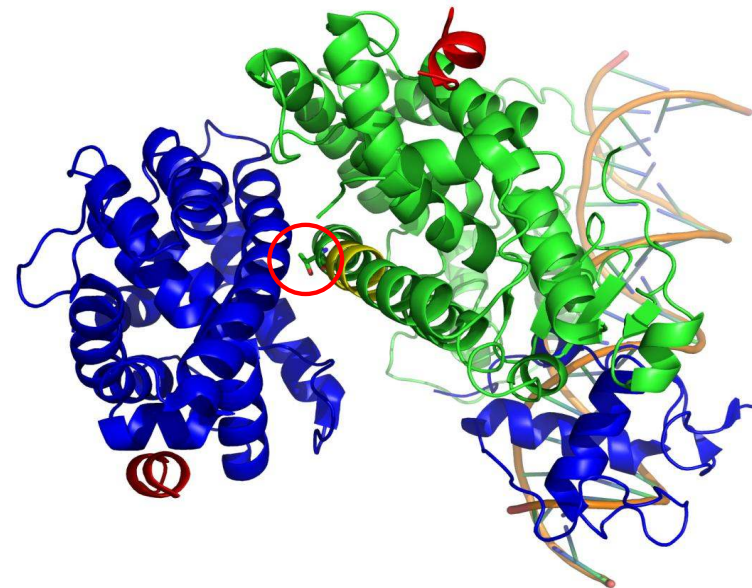
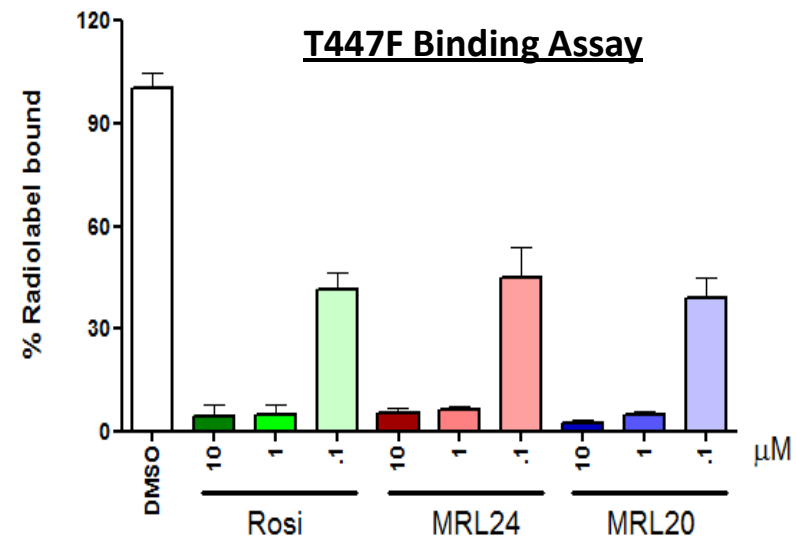
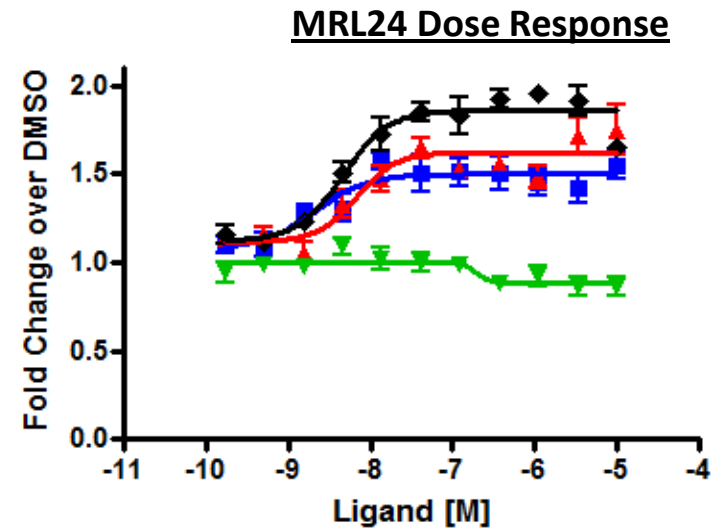
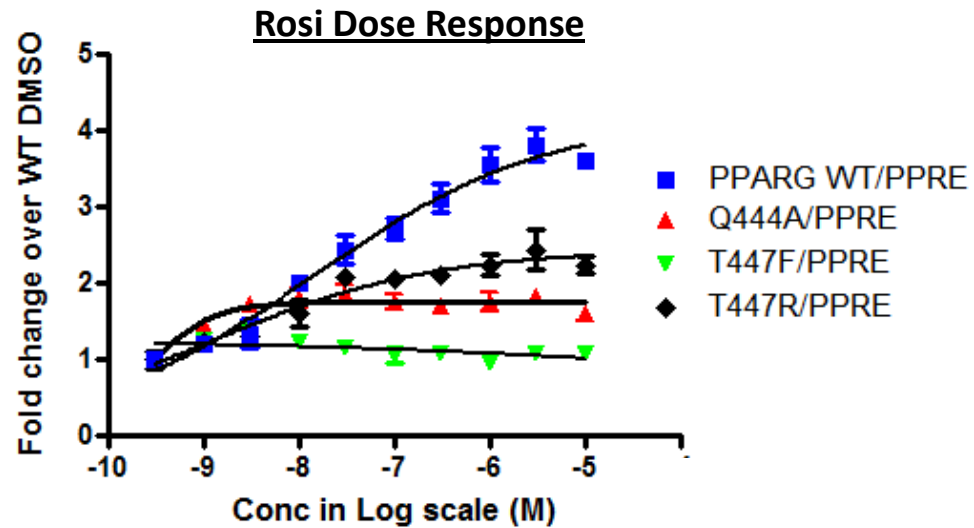
+ SR1664



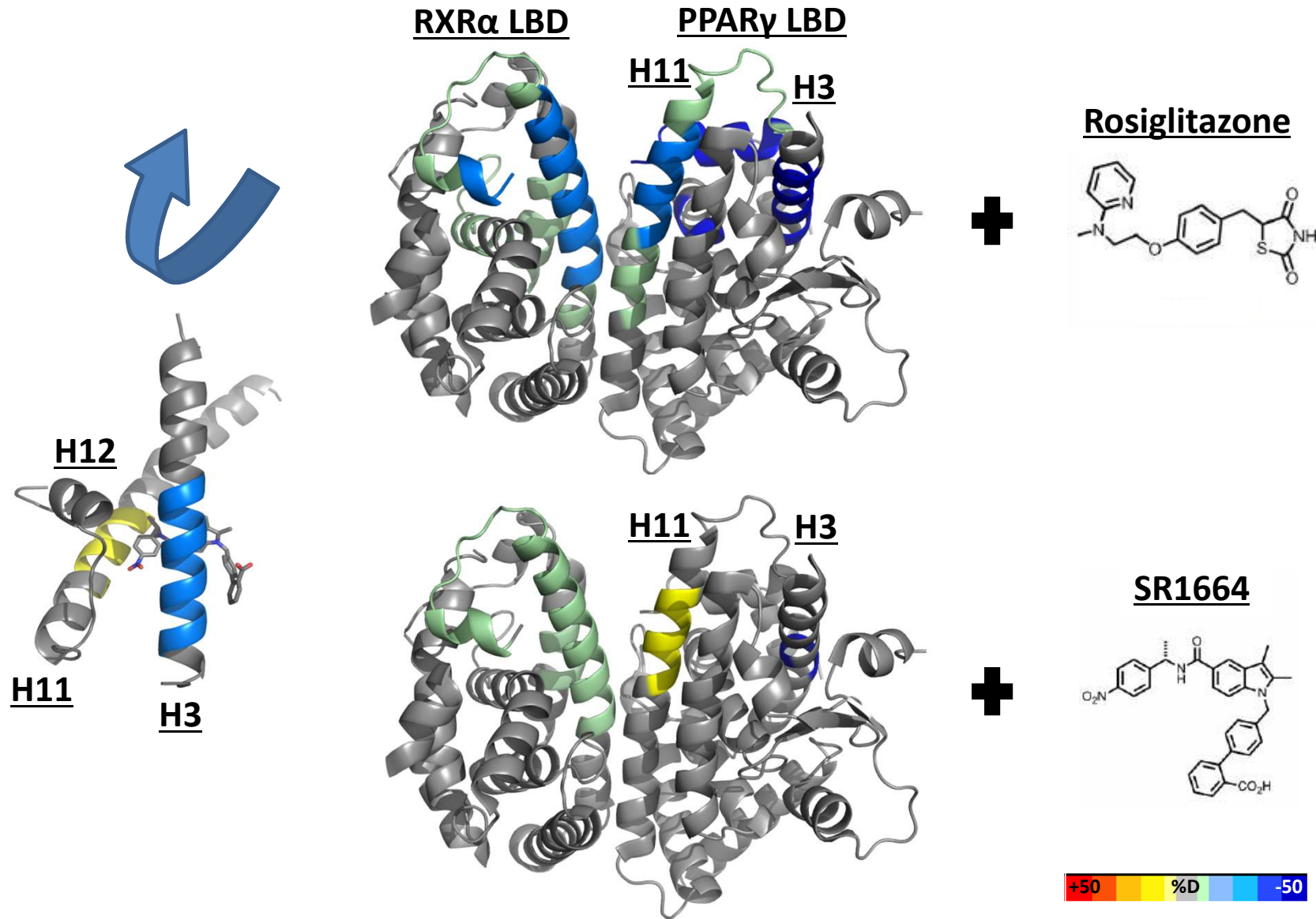
PPARG Co-Regulator Binding



SR1664 Mechanism of Action



Differing Effects of PPAR γ Ligands on RXR α



Summary of Agonism SAR

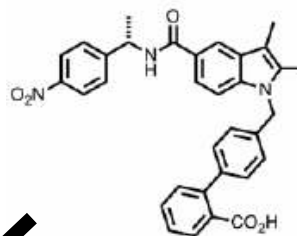
- PPAR γ Paradigm has changed to development of 'Non-agonist' ligands that modulate PTMs.
- HDX allows us to differentiate SR1663 & SR1664 which look identical in static crystal structure but are functionally diverse.
- Degree of H12 stabilization measured by HDX correlates with activity, H3 stabilization correlates with affinity.
- Rosi & SR1664 have different effects on RXR α dynamics which may indicate altered heterodimer affinity/interaction.

Summary of SR1664

- .. is a potent binder to PPAR γ – K_d similar to rosiglitazone.
- .. lacks classical AF2 driven agonism – completely inactive in PPRE:Luc assays and no alteration of agonist genes *in vivo*.
- .. blocks S273-P in cells and *in vivo* and is anti-diabetic with improved AE profile versus TZDs.
- .. is an antagonist of natural ligand but agonist of S273-P repressed gene set. The compound disrupts receptor and co-receptor (RXR) conformational dynamics interfering with binding of CoA or release of CoR.
- .. has poor PK and solubility. Some formulations of the compound are toxic.
- We have SAR on partial agonist to non-agonists with over 20 unique non-agonist compounds to date. We have some insight into the molecular mechanism.
- Questions – will non-agonists have similar anti-inflammatory properties as TZDs *in vivo*? TZDs brown fat, and partial and non-agonists do not – will this limit their efficacy?

Lead Optimization

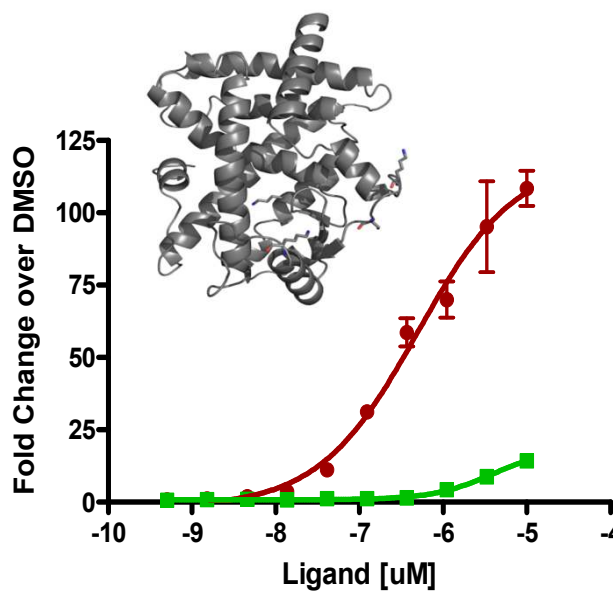
SR1664



Optimization

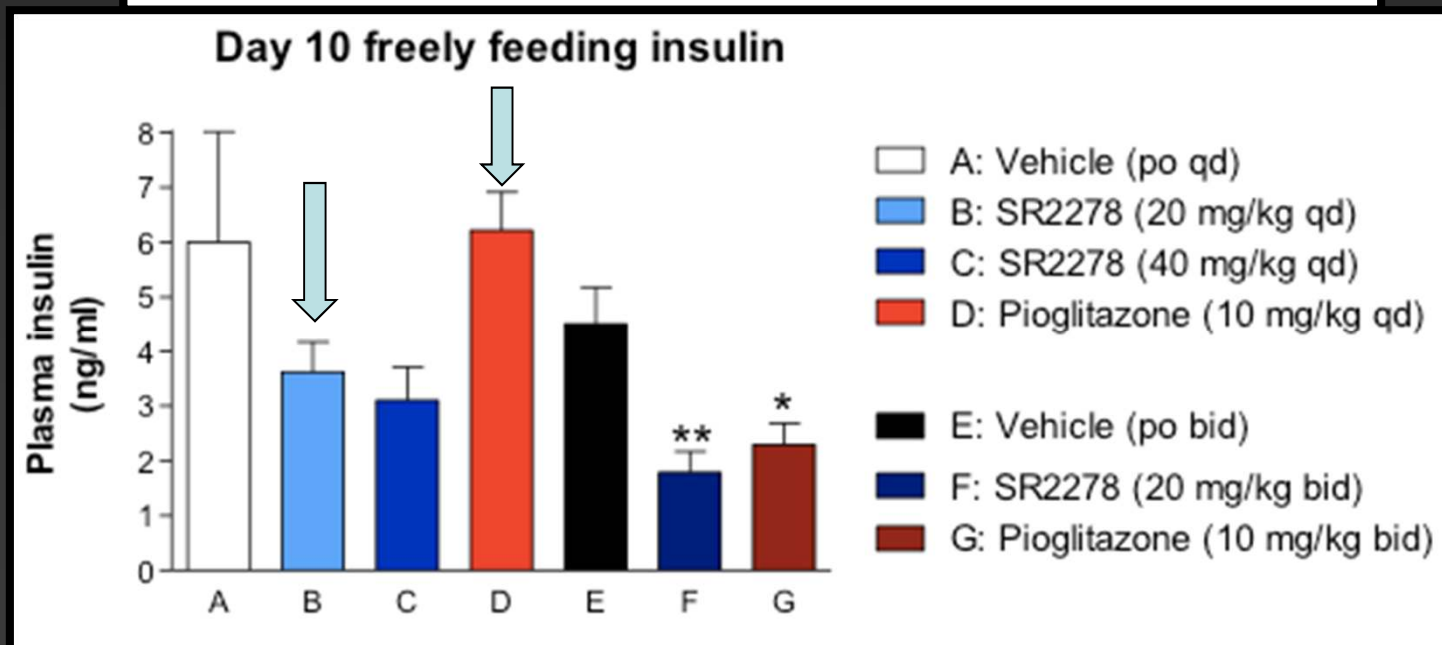
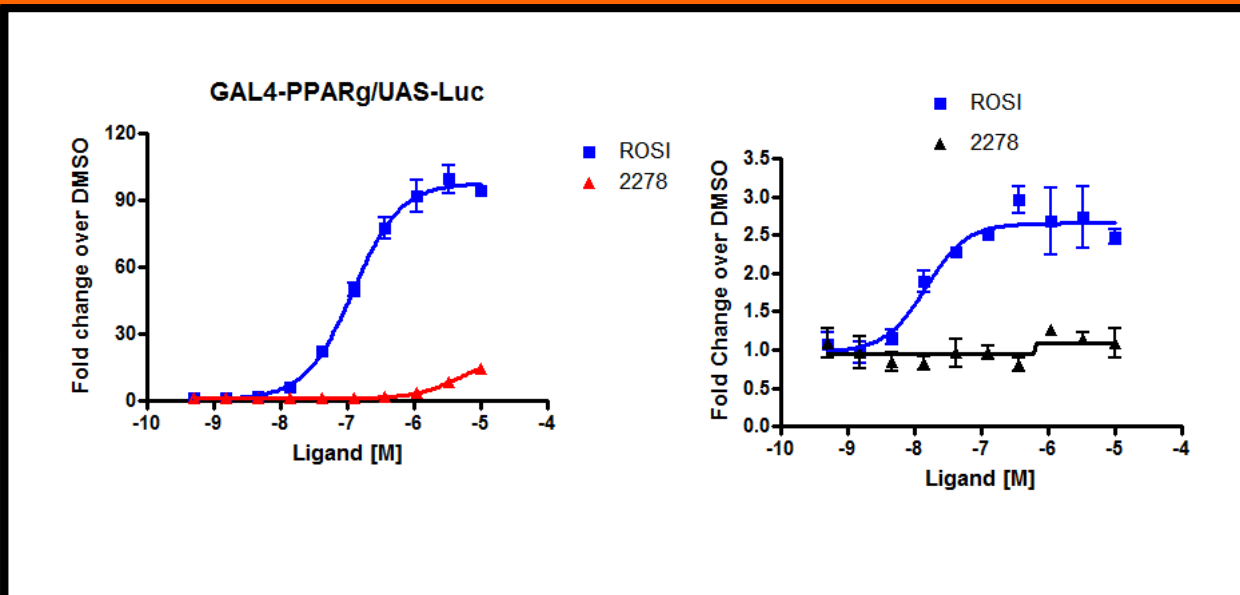
SAR

MOA



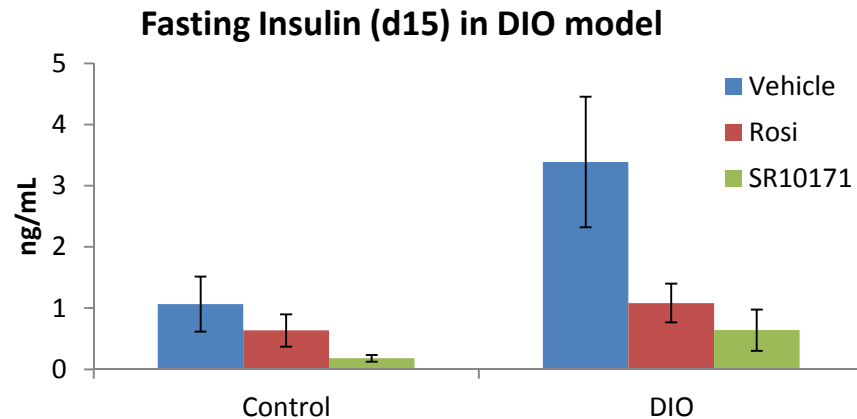
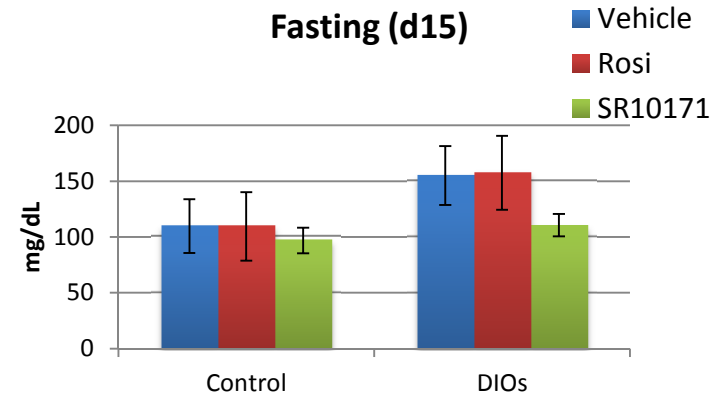
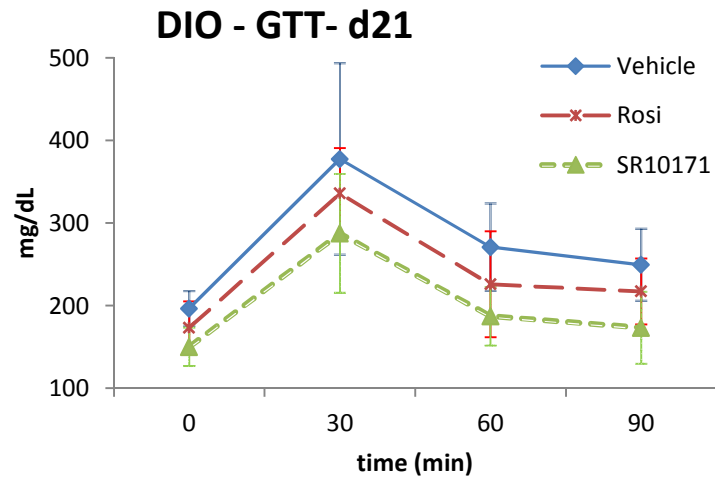
	PTM	Rosi	1664
K268	✓	?	?
S273	✓	✓	✓
K293	✓	?	?
K365	✓	?	?
Agonism	✓		✗

Lead Optimization

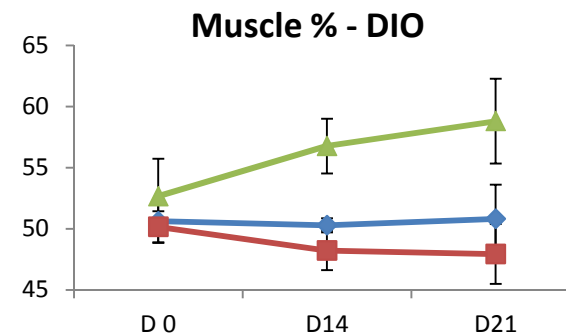
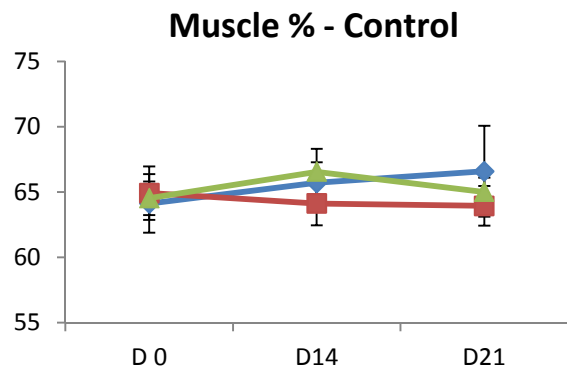
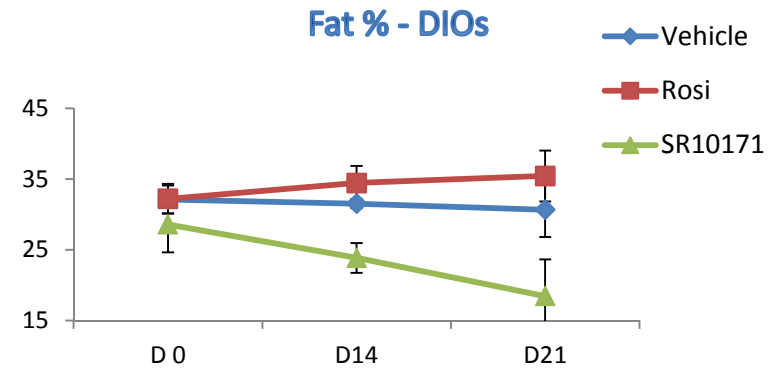
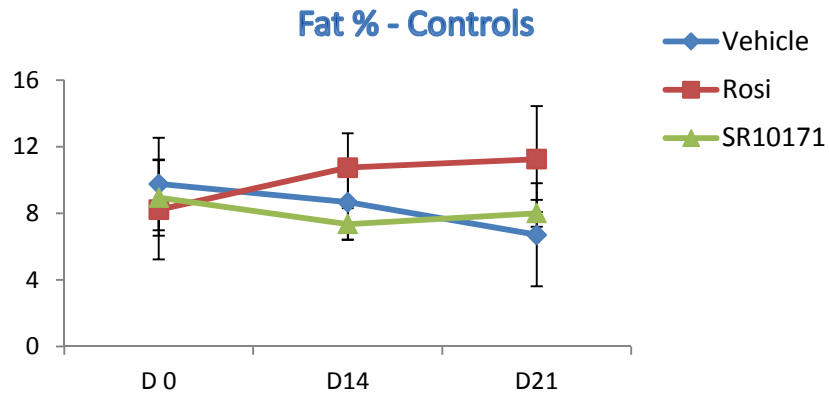


Lead Optimization

Rosiglitazone and SR10171 (20mg/kg P.O.) in DIO model



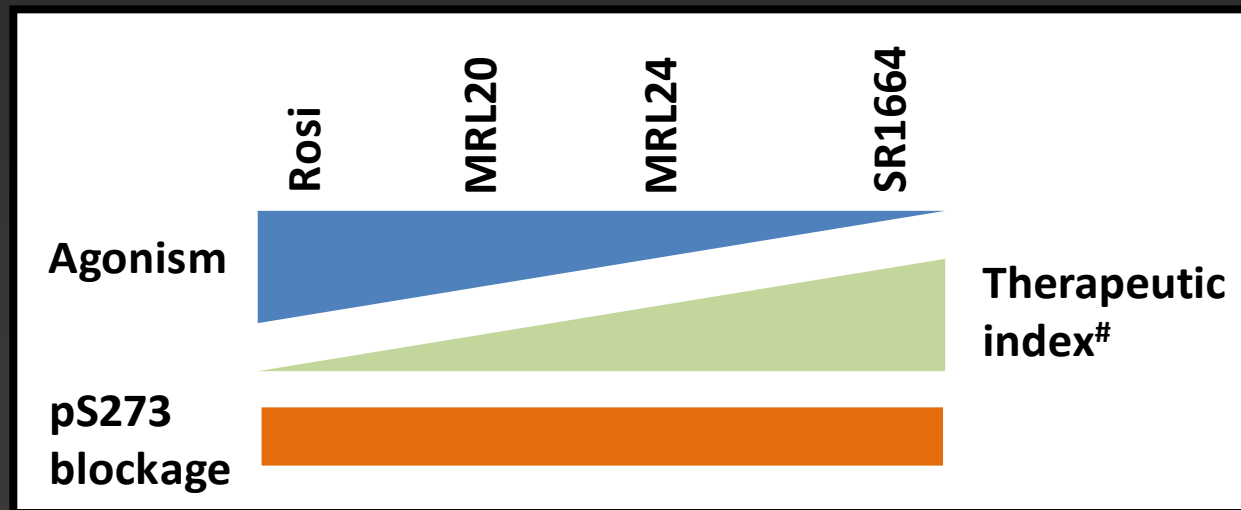
Lead Optimization



BODY WEIGHT PERCENTAGE DATA:
MiniSpec NMR analyzer

Summary

PPARG – Demonstrated that PO3 of PPARG at S273 controls a subset of target genes that are dysregulated in obesity (*Nature* 2010). This led to the discovery of novel modulators that bind to PPARG without inducing AF2-dependent agonism (passive antagonism). These compounds block S273-P and they are efficacious in diabetic mice (*Nature* 2011). But will this be enough for robust efficacy, and can this scaffold be optimized for appropriate pharmaceutical properties?





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<http://griffin.florida.scripps.edu>

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