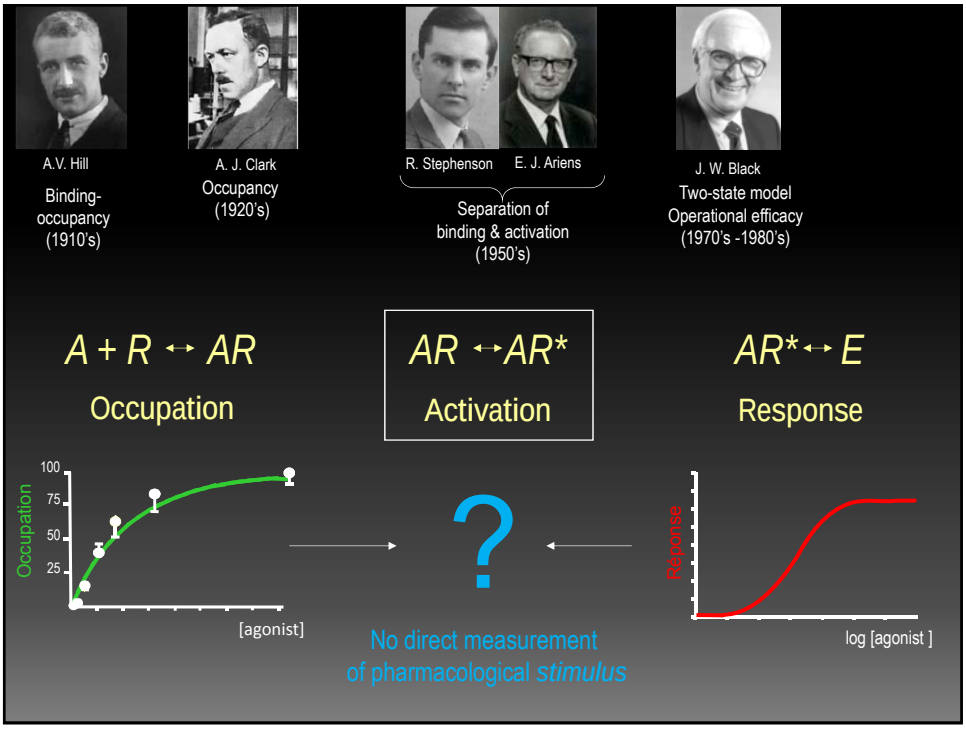
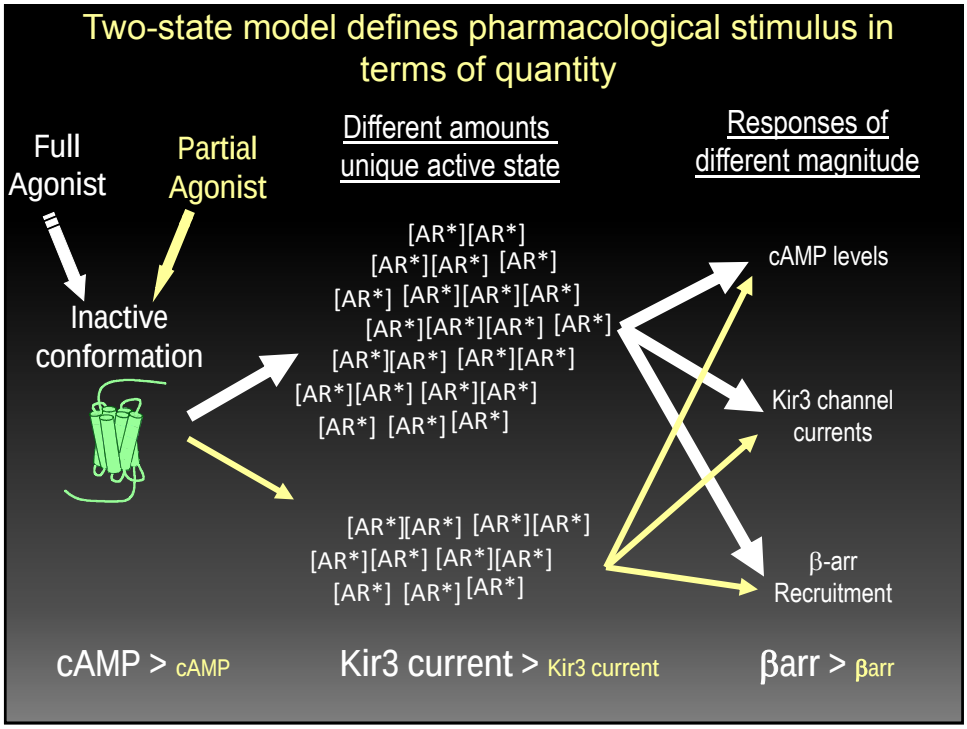
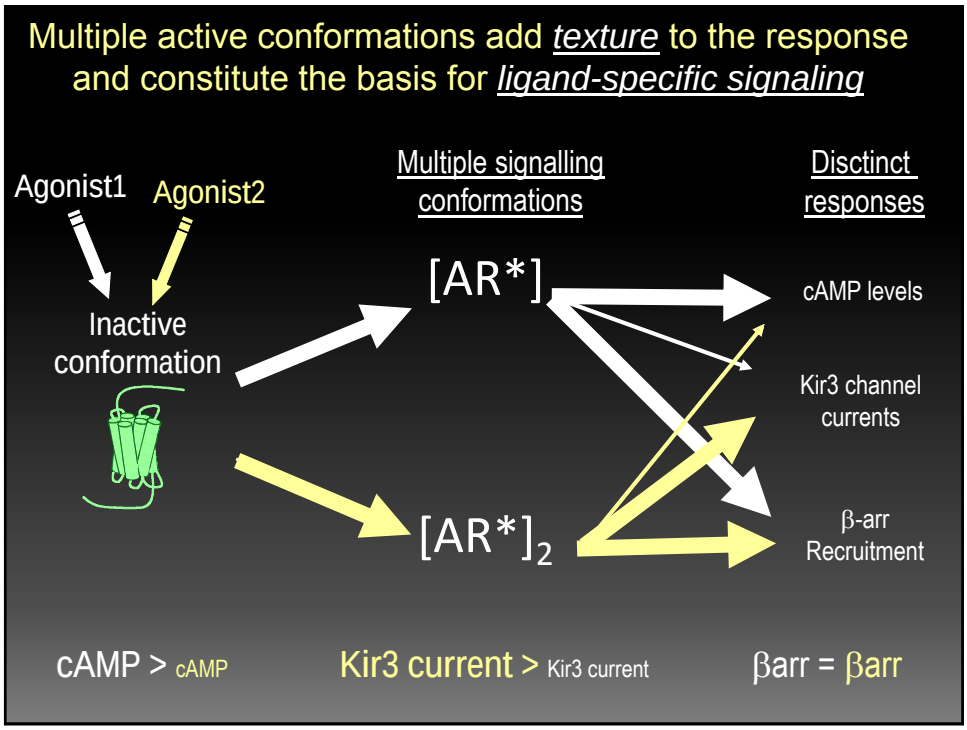
Analysing signalling data to quantify pharmacological bias

Lessons from delta opioid receptor (DOR) signaling

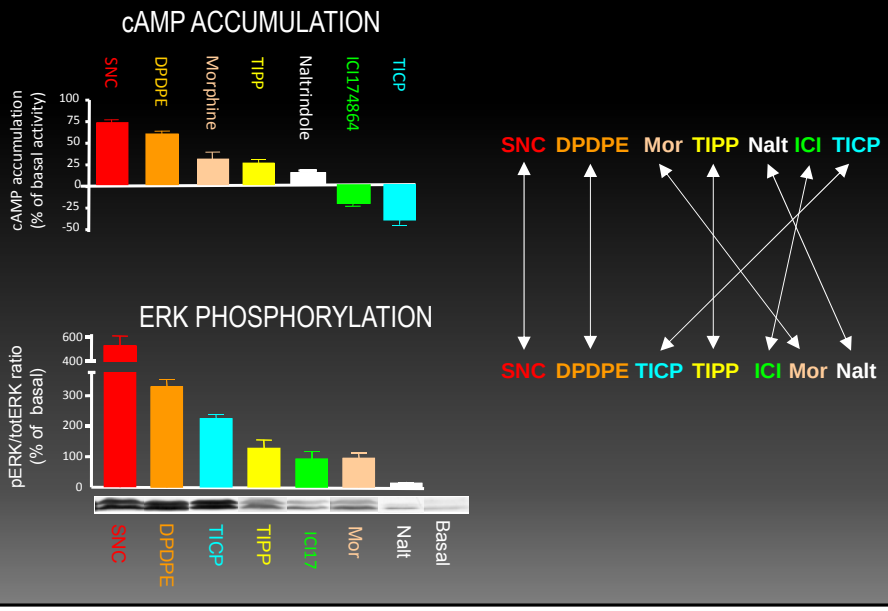
Graciela Piñeyro
Université de Montréal



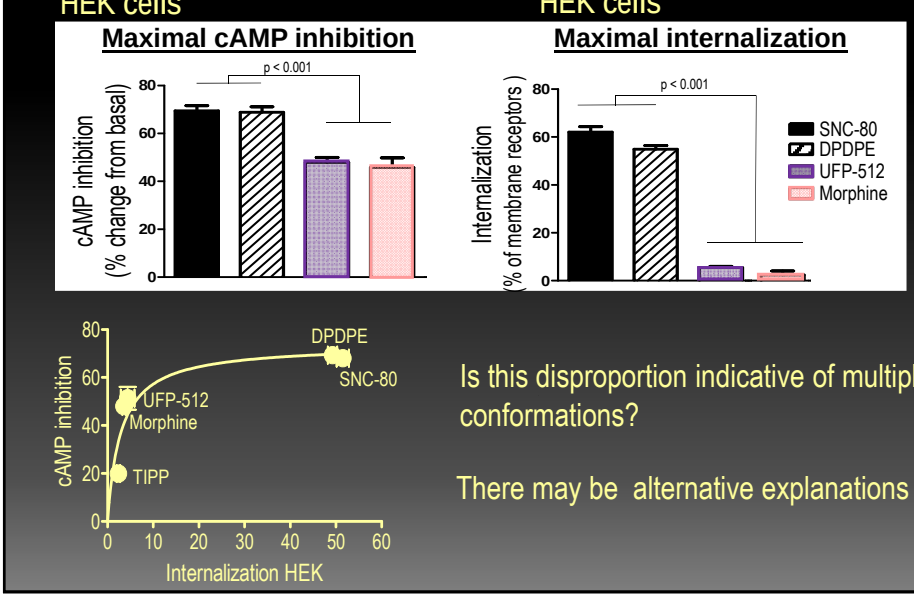

Two-state model defines pharmacological stimulus in terms of quantity
Full Agonist
Partial Agonist
Inactive conformation
Different amounts unique active state
Responses of different magnitude
[AR*][AR*] [AR*][AR*] [AR*] [AR*] [AR*][AR*][AR*] [AR*][AR*][AR*] [AR*] [AR*][AR*] [AR*][AR*] [AR*][AR*] [AR*][AR*] [AR*] [AR*] [AR*]
[AR*][AR*] [AR*][AR*] [AR*][AR*] [AR*][AR*] [AR*] [AR*] [AR*]
cAMP levels
Kir3 channel currents
β-arr Recruitment
cAMP > cAMP
Kir3 current > Kir3 current
βarr > βarr


Multiple active conformations add texture to the response and constitute the basis for ligand-specific signaling
Agonist1
Agonist2
Inactive conformation
Multiple signalling conformations
Disctinct responses
[AR*]
[AR*]2
cAMP levels
Kir3 channel currents
β-arr Recruitment
cAMP > cAMP
Kir3 current > Kir3 current
βarr = βarr

In some cases ligand-specific signaling can be easily identified from signaling responses



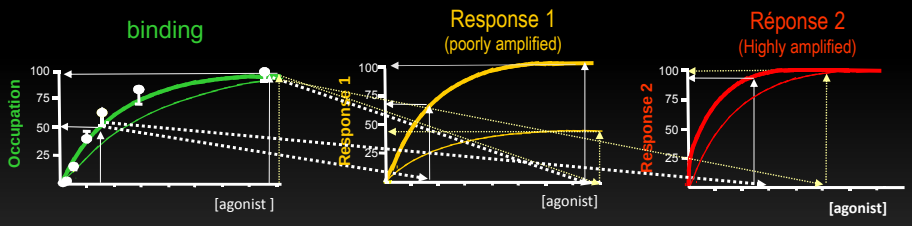
Certain DOR ligands display disproportionately low internalization as compared to cyclase responses

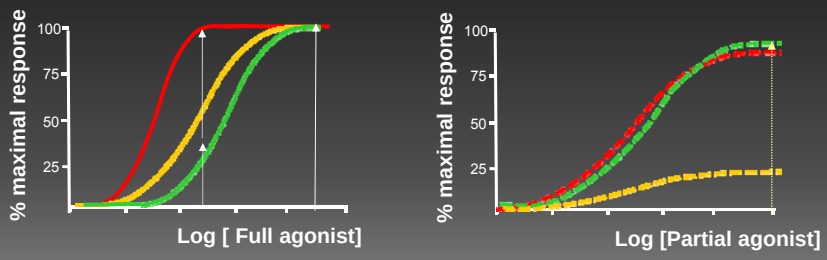


Is this disproportion indicative of multiple conformations?

There may be alternative explanations

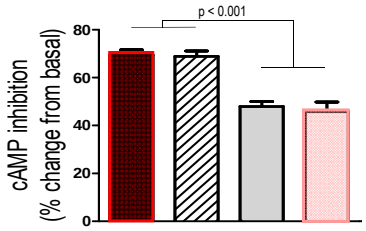
Differential amplification and 'ceiling effect' may contribute to disproportion in maximal responses





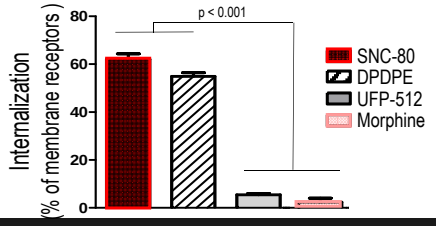
HEK cells

Maximal cAMP inhibition

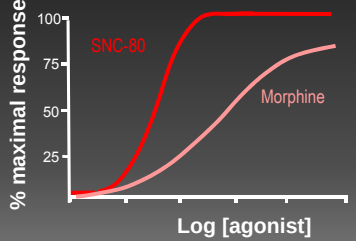


HEK cells

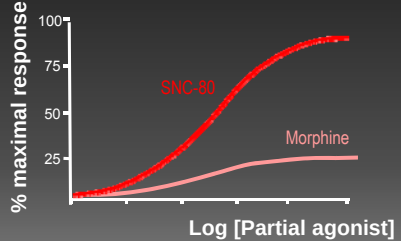
Maximal internalization



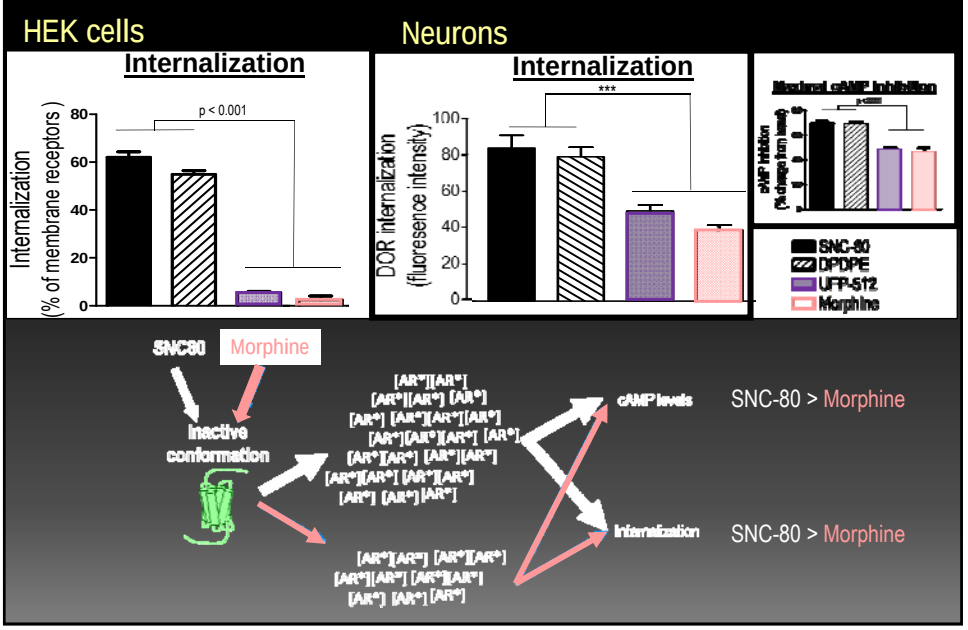
cAMP inhibition
(highly amplified)



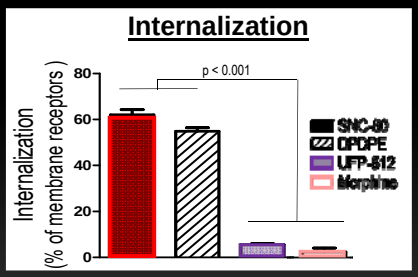
Internalization
(poorly amplified)

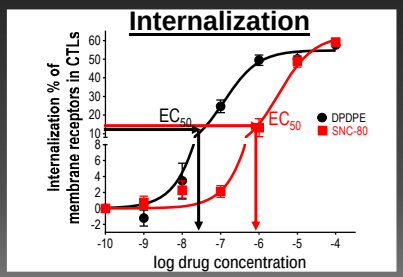


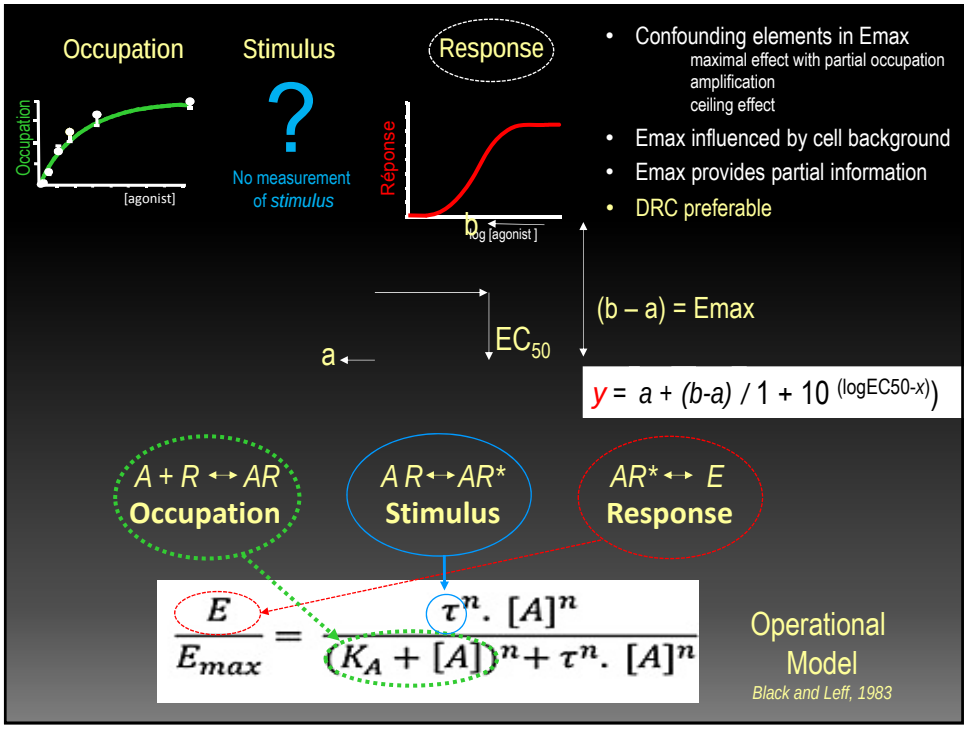
The magnitude of response is influenced by cellular background

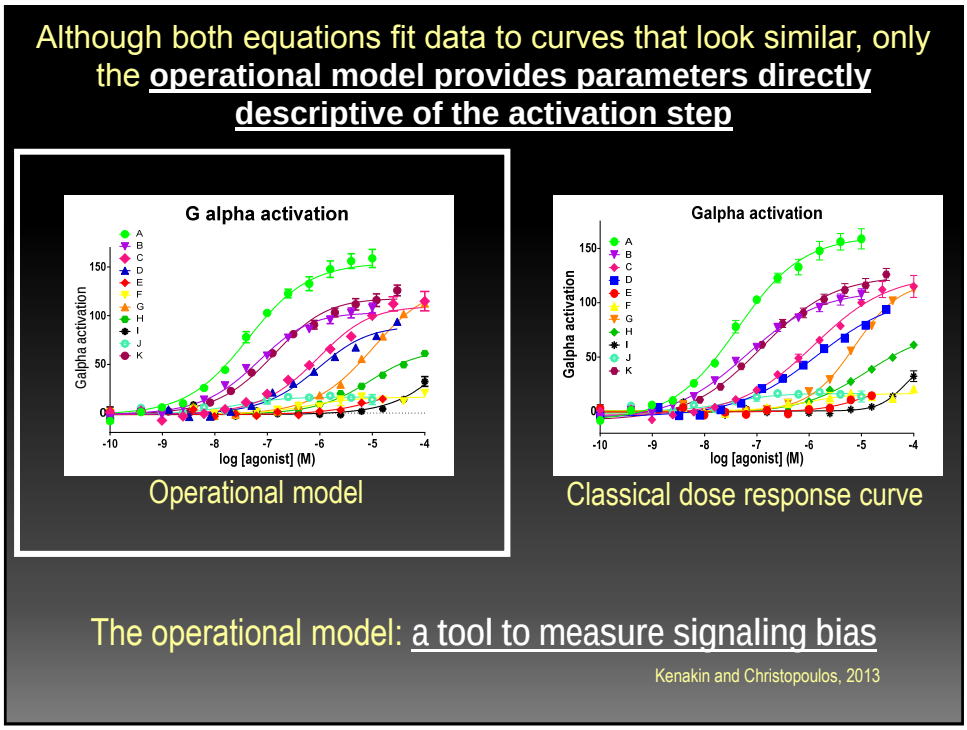


Maximal responses (Emax) provide partial information about a drug's profile






Occupation
Stimulus
Response
Occupation
[agonist]
?
No measurement of stimulus
Réponse
log [agonist]
• Confounding elements in Emax
maximal effect with partial occupation
amplification
ceiling effect
• Emax influenced by cell background
• Emax provides partial information
• DRC preferable
(b – a) = Emax
a
EC50
y = a + (b-a) / 1 + 10 (logEC50-x))
A + R ↔ AR
Occupation
A R↔AR*
Stimulus
AR*↔ E
Response
E/Emax = τ^n . [A]^n / (K_A + [A])^n + τ^n . [A]^n
Operational Model
Black and Leff, 1983


Although both equations fit data to curves that look similar, only the operational model provides parameters directly descriptive of the activation step
G alpha activation
Galpha activation
log [agonist] (M)
Operational model
Galpha activation
Galpha activation
log [agonist] (M)
Classical dose response curve
The operational model: a tool to measure signaling bias
Kenakin and Christopoulos, 2013

Consolidated estimation of *efficiency* to drive response

Tau/KA

Tau (operational efficacy)
Ability of the drug to elicit a response in this particular system

Dependent variable:
Response
… as a fraction of maximum allowed by the system

Functional affinity

Independent variable
Drug concentration

$$\frac{E}{E_{max}} = \frac{\tau^n . [A]^n}{(K_A + [A])^n + \tau^n . [A]^n}$$

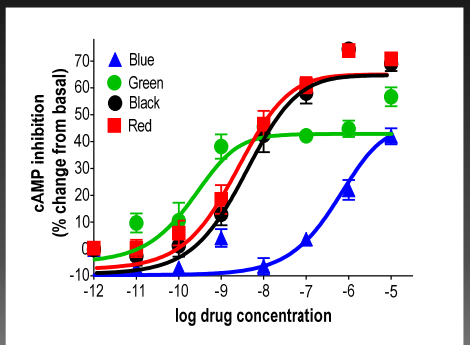
System parameter: shared by all ligands
Maximal response allowed by the system

System parameter: shared by all ligands
Coupling efficiency with which activated receptor elicits response

Operational parameters describing cAMP inhibition

Log (Tau/KA) = LogTau – Log KA

	log KA	SEM	log Tau	SEM	*log(Tau/KA)*	*SEM*
Blue (n=9)	-5,19	0,29	0,34	0,25	5,53	0,38
green n=7)	-8,93	0,18	0,20	0,06	9,13	0,19
Black (n=8)	-6,81	0,13	1,12	0,11	7,93	0,17
Red (n=9)	-7,23	0,18	1,06	0,13	8,29	0,22

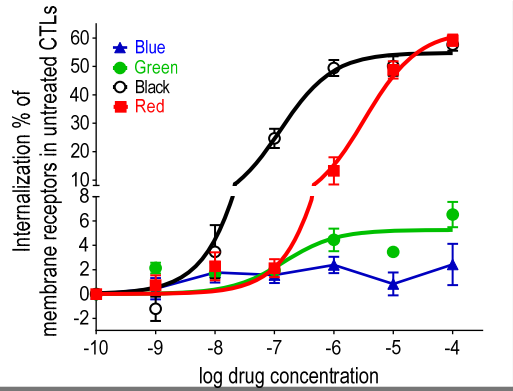


Rank order of efficacy (Tau)
Black = Red > Blue = Green

Rank order of transduction coefficients
log (Tau/KA) or efficiency
green ≥ red = black > blue

Operational parameters describing DOR internalization

	log KA	SEM	log Tau	SEM	log(Tau/KA)	SEM
Blue (n=11)	N/A		N/A		N/A	
Green (n=10)	-6,78	0,24	-1,12	0,05	5,66	0,25
Black (n=11)	-6,02	0,29	0,76	0,20	6,78	0,35
Red (n=12)	-4,15	0,39	1,31	0,35	5,46	0,53



Rank order of efficacy (Tau)
Red ≥ Black > Green

Rank order of transduction coefficients log (Tau/KA) or efficency
Black > Green = Red

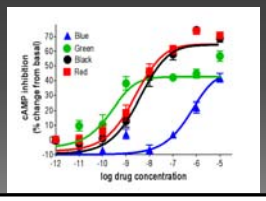
Bias is established by comparing log(Tau/KA) transduction coefficients in different signaling pathways: Need for a standard

	cAMP inhibition log(Tau/KA)	SEM	Internalization log(Tau/KA)	SEM	Normalized cAMP inhibition Δ log(Tau/KA)	SEM	Normalized Internalization Δ log(Tau/KA)	SEM
Blue	5,53	0,38	N/A		-2,40	0,42	N/A	
Green	9,13	0,19	5,66	0,25	1,20	0,25	-1,13	0,43
Black	7,93	0,17	6,78	0,35	0,00	0,24	0,00	0,50
Red	8,29	0,22	5,46	0,53	0,36	0,28	-1,33	0,63

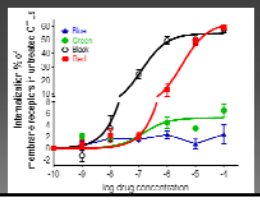
$$\frac{E}{E_{max}} = \frac{\tau^n . [A]^n}{(K_A + [A])^n + \tau^n . [A]^n}$$

Δ(log Tau/KA) = $\log(Tau/KA)_{drug} - \log(Tau/KA)_{Std}$

*E*max = 75 and *n* = 0.72



Emax = 64 and n = 1.1



By comparing normalized Δlog(Tau/KA) coefficients in different pathways we obtain a bias factor **ΔΔlog(Tau/KA)**

Δ(log Tau/KA)

	Normalized cAMP inhibition		Normalized Internalization		cAMP vs internalization	
	Δ log(Tau/KA)	SEM	Δ log(Tau/KA)	SEM	Bias factor	p value
Blue	-2,40	0,42	N/A		N/A	
green	1,20	0,25	-1,13	0,43	2,33	0,0027
Std Black	0,00	0,24	0,00	0,50		
Red	0,36	0,28	-1,33	0,63	1,69	0,041

ΔΔ(log Tau/KA) = $\log(Tau/KA)_{cAMP} - \log(Tau/KA)_{Int}$

Red is *49 fold* more efficient in eliciting *cAMP inhibition* than *internalization*

Green is *213 fold* more efficient in eliciting *cAMP inhibition* than *internalization*

as compared to Black

Observed bias depends on the choice of standard

BLACK STANDARD

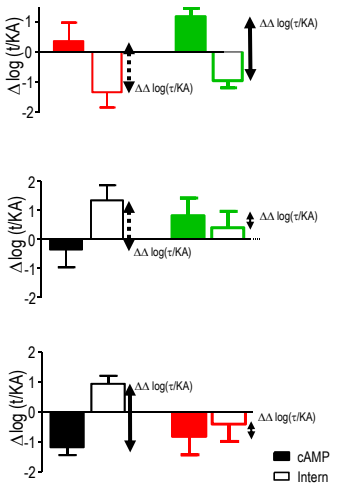
	Bias factor		
	$\Delta\Delta\log(\tau/KA)$	$10^{\Delta\Delta\log(\tau/KA)}$	p value
SNC-80	1.7	50	0.04
DPDPE			
UFP-512	2.33	213	<0.0001

RED STANDARD

	Bias factor		
	$\Delta\Delta\log(\tau/KA)$	$10^{\Delta\Delta\log(\tau/KA)}$	p value
SNC-80			
DPDPE	-1.7	50	0.04
UFP-512	0.42	2.63	0.63

GREEN STANDARD

	Bias factor		
	$\Delta\Delta\log(\tau/KA)$	$10^{\Delta\Delta\log(\tau/KA)}$	p value
SNC-80	-0.42	2.63	0.63
DPDPE	-2.33	213	<0.0001
UFP-512			



Considerations for choosing the standard: Plenary discussion ?

Different methods that use the operational model to quantify bias make different assumptions about KA

$$\frac{E}{E_{max}} = \frac{\tau^n . [A]^n}{(K_A + [A])^n + \tau^n . [A]^n}$$

Model calculates "functional affinity values" from response data

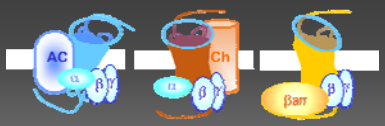
Bias is estimated from Efficacy (τ) and functional affinity (KA)

Kenakin and Christopoulos

Model uses binding affinities obtained in similar conditions as signaling assays and introduces them into the equation

Bias is solely estimated from efficacy (τ)

Rajagopal and Lefkowitz



Conceptually, the calculated KA (or "functional affinity") represents the affinity of the drug for the receptor conformation mediating the response of interest….

Estimation of bias: Summary

- Using Emax concentrations to identify biased responses is not recommended, unless a clear reversal in ligand rank orders is observed
- Bias can be actually measured by applying the operational model to calculate:

  operational efficacies (Tau)
  functional affinities (KA)

  } Consolidated transduction coeffiient: log(Tau/KA)

- Comparisons of log(Tau/KA) ratios for different pathways must always be done with respect to a standard to avoid system confounders
- The selection of the standard will determine the type of bias observed

Thanks!

????

Poster # 8 by Karim Nagi

Remember:

These are models which represent our understanding of drug action…
Reality might be something else!!! Let's keep an open mind…