Kinetic and Dynamic Aspect in Class A GPCR Activation and Implications therein

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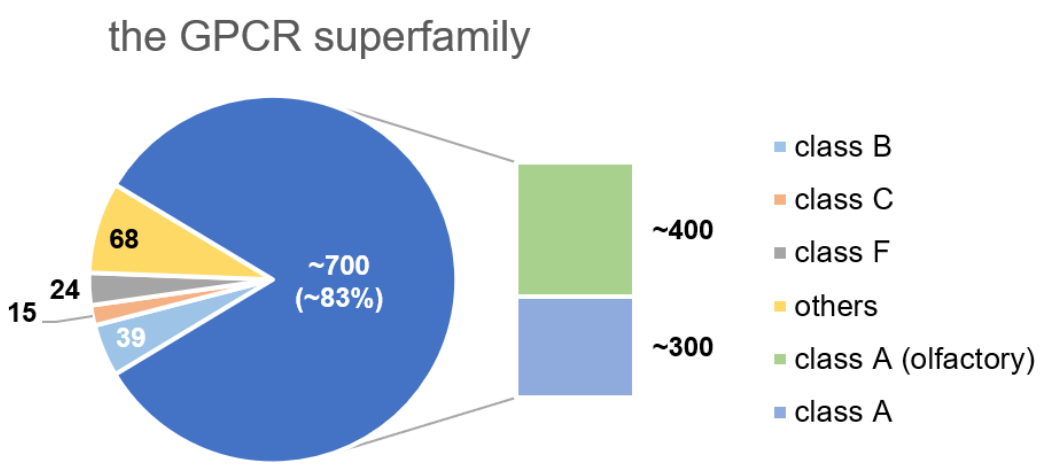
Supervisor: Tuoping Luo
College of Chemistry and Molecular Engineering, PKU
June 22nd 2024

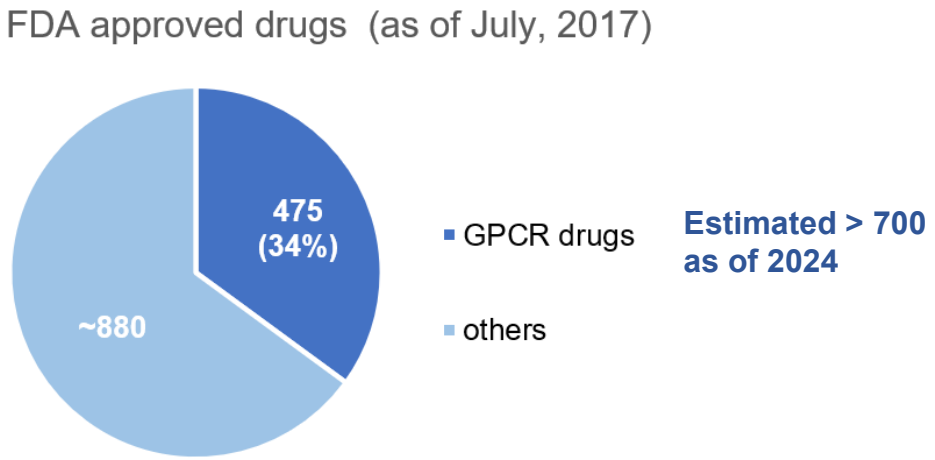
Outline

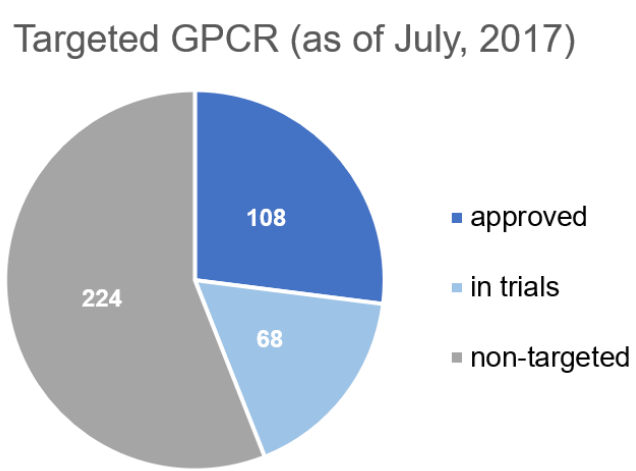
- **Introductions**
  - Class A GPCRs
  - Ligand-target binding kinetics
- **Kinetics and dynamics of GPCR signaling**
  - G-protein pathway
  - β-arrestin pathway
- **Revisiting the ligand-target binding kinetics**
  - Examples
  - Structure-kinetic relationship
- **Summary**

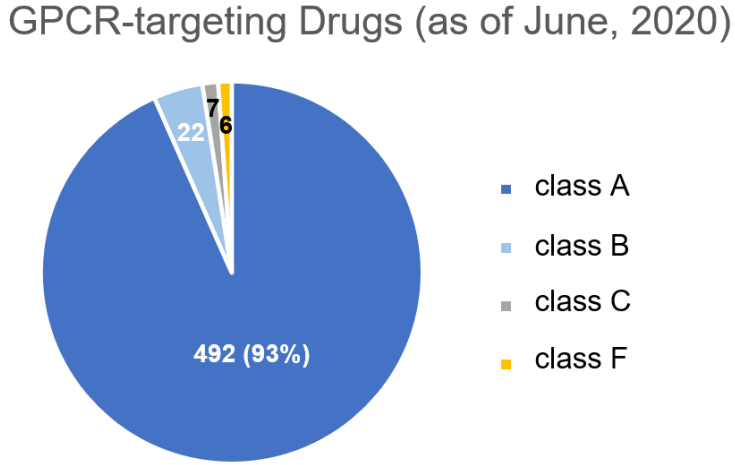
Brief Introduction to Class A GPCRs

- The GPCR superfamily and GPCR drugs





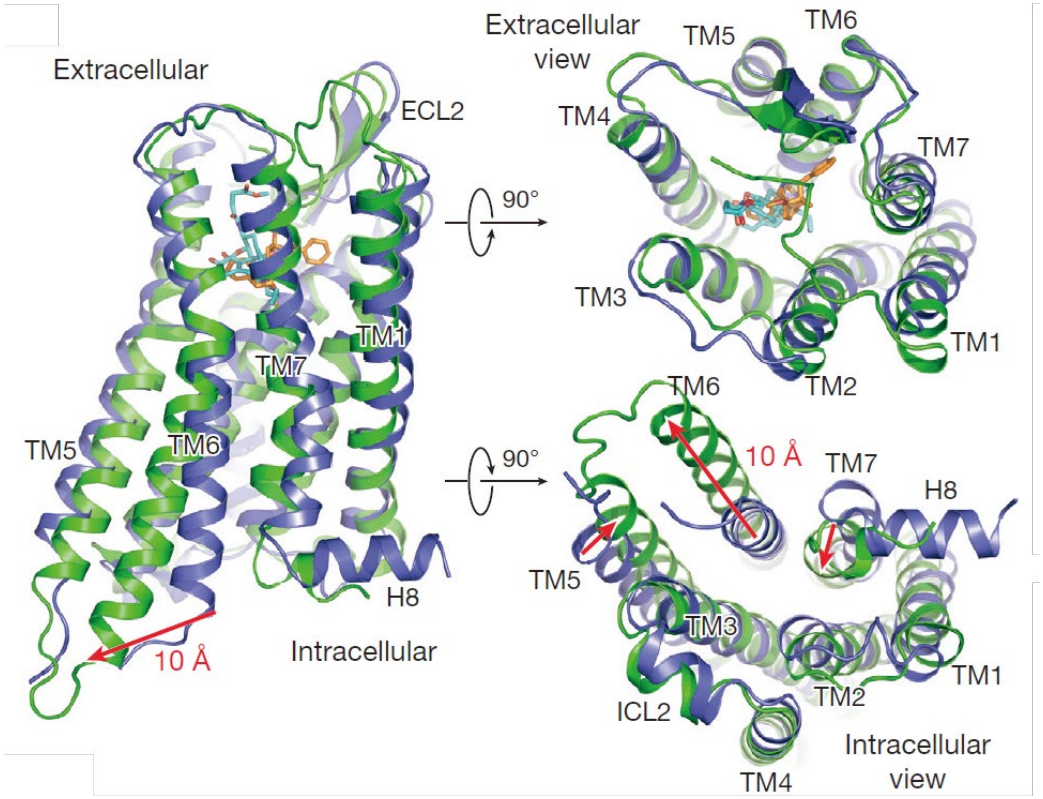




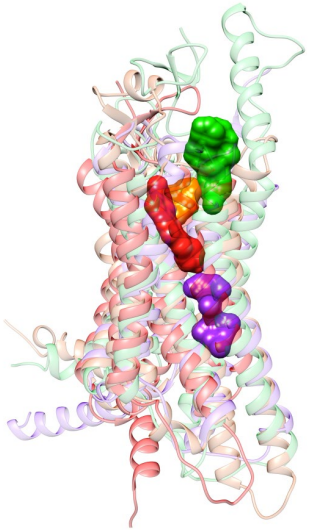
Gloriam, E. T. *et al. Nat. Rev. Drug Discov.* **2017**, *16*, 829.

Brief Introduction to Class A GPCRs

- Structure and activation
  - A 10 Å outward movement of TM6
  - A slight inward movement of TM7



- Differences



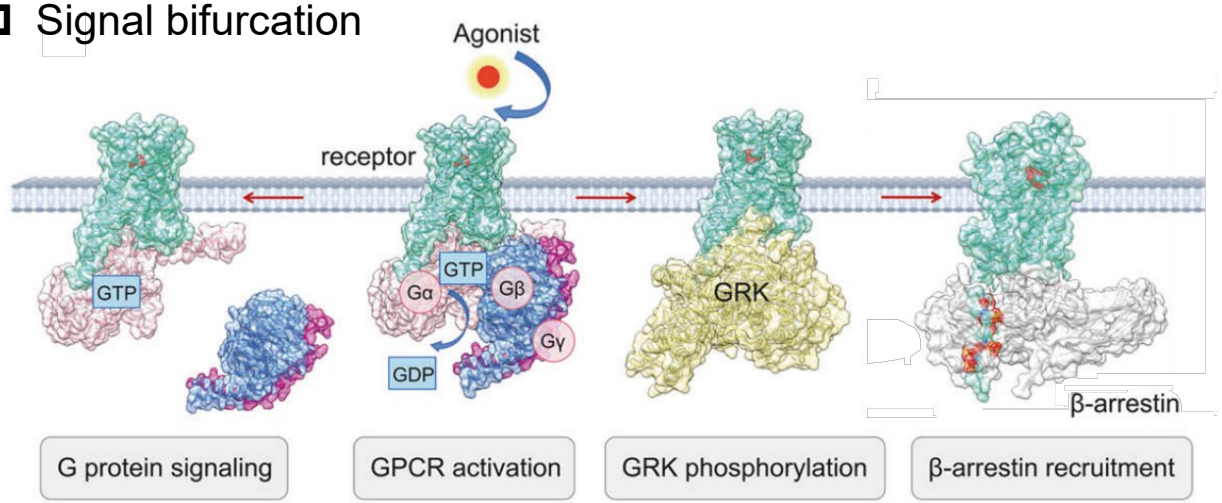
Class A

Class B

Class C

Class F

- Signal bifurcation



Kobilka. B. K. *et al. Nature* **2015**, *524*, 315.

The Ligand Binding Event

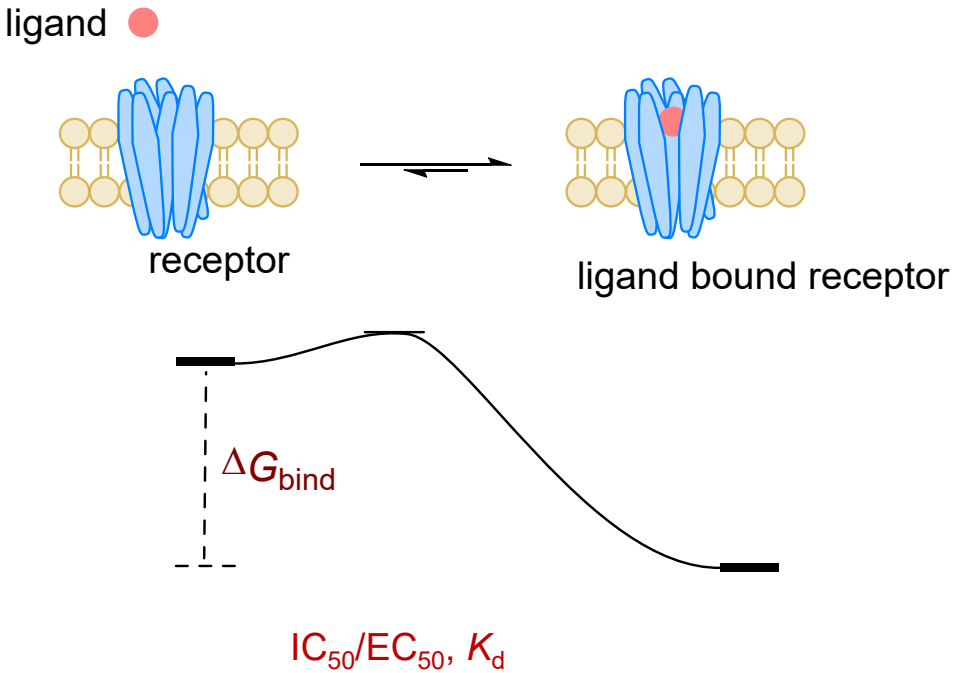
Corpora non agunt nisi fixata

(Agent only works when it is bound)

—— Dr. Paul Ehrlich

Equilibrium vs. Kinetic Parameters

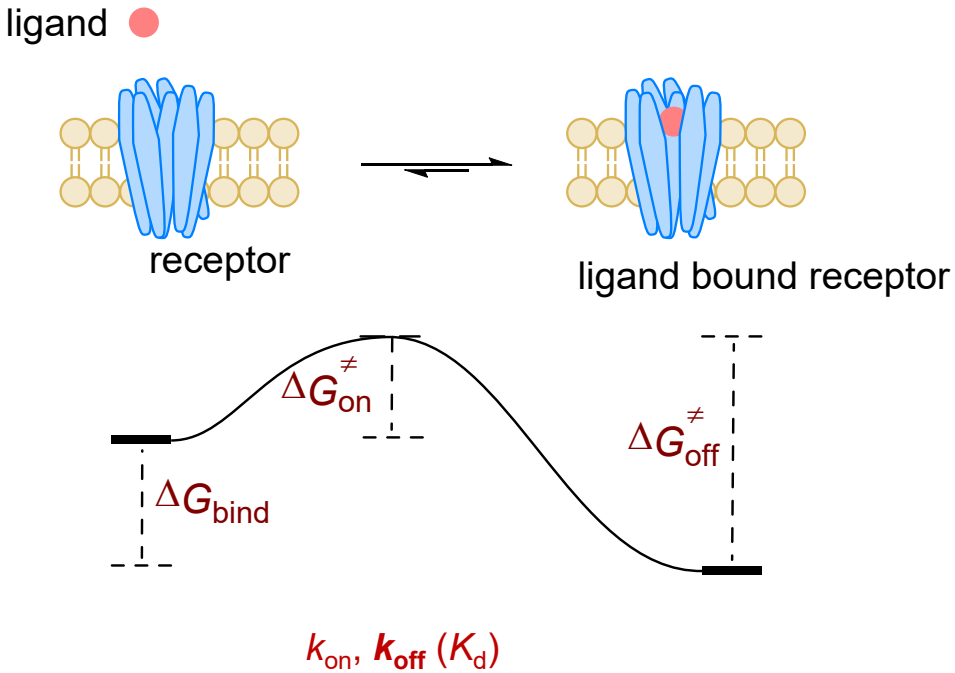
- much of early-phase drug discovery has been focused on the optimization of target **affinity** and **selectivity**.
  - Ligand binding rate was considered diffusion-controlled



IC_{50}/EC_{50}, K_d

- Typical *in vitro* affinity measurements are performed under closed-system conditions, in which the target is exposed to an invariant concentration of the compound.

- Copeland *et al.* suggested that **kinetic parameters**, especially k_{off} being taken into considerations.
  - Binding rates are mostly **not** (99.6%) diffusion-controlled



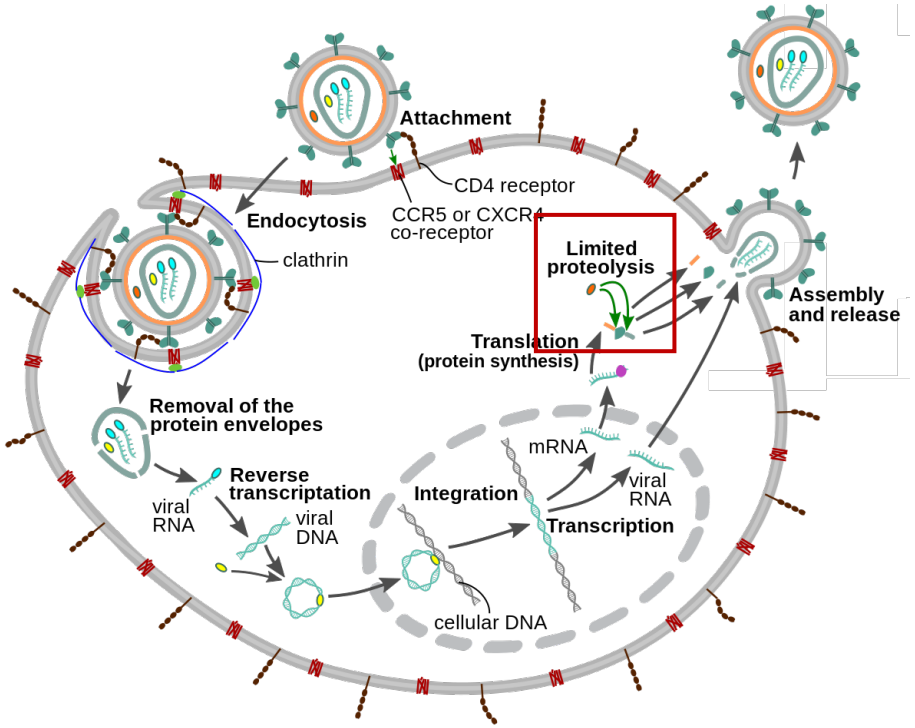
k_{on}, $\boldsymbol{k_{off}}$ (K_d)

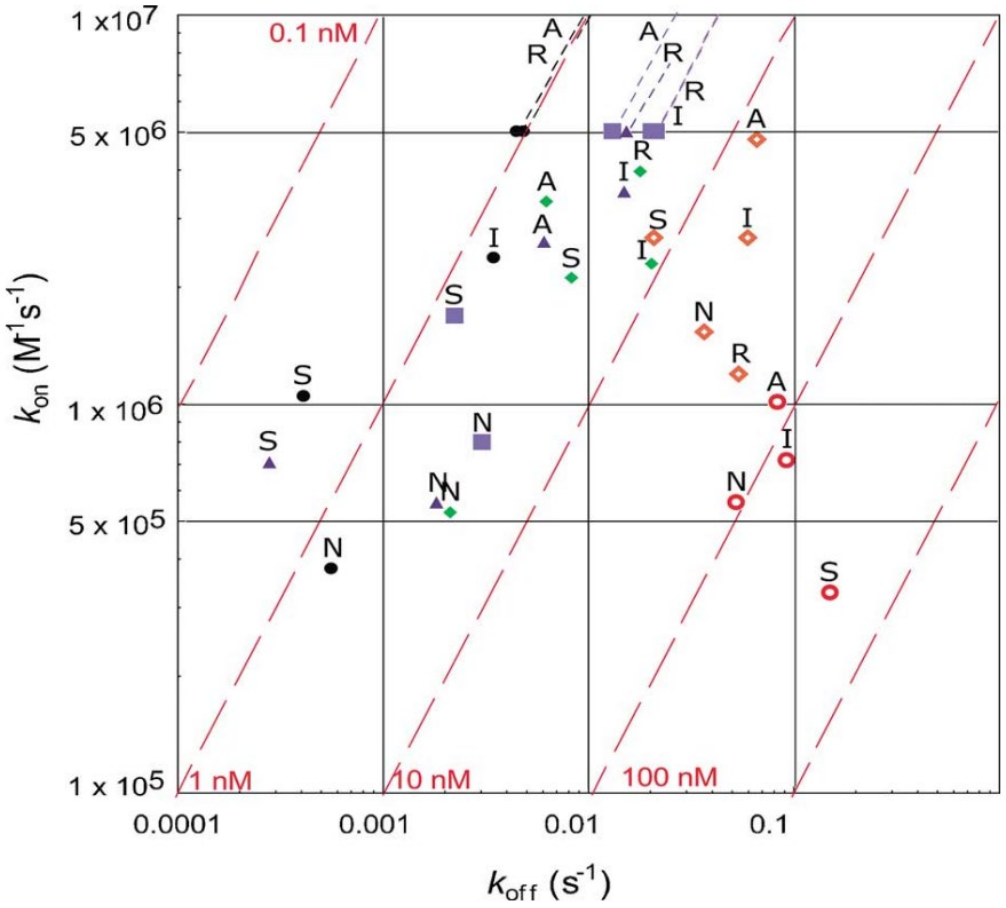
- In the open system of *in vivo* experiments, the concentration of ligand to which a receptor is exposed varies with time.
- **Drug residence time**

Copeland, R. A. *et al. Nat. Rev. Drug Discov.* **2006**, *5*, 730.

Preliminary Discoveries

- The kinetic-activity profile of drugs targeting HIV-1 protease





- Inhibition of HIV-1 protease prevents virus maturation, and mutations at protease confer resistance to drugs.

saquinavir—**S**, nelfinavir—**N**, ritonavir—**R**, indinavir—**I**, amprenavir—**A**

wild-type—●, L90M—■, G48V—◆, V82A—▲, I84V/L90M—◇, G48V/V82A/I84V/L90M—○.

Danielson, U. H. *et al. Antiviral Res.* **2003**, *58*, 235.

Preliminary Discoveries

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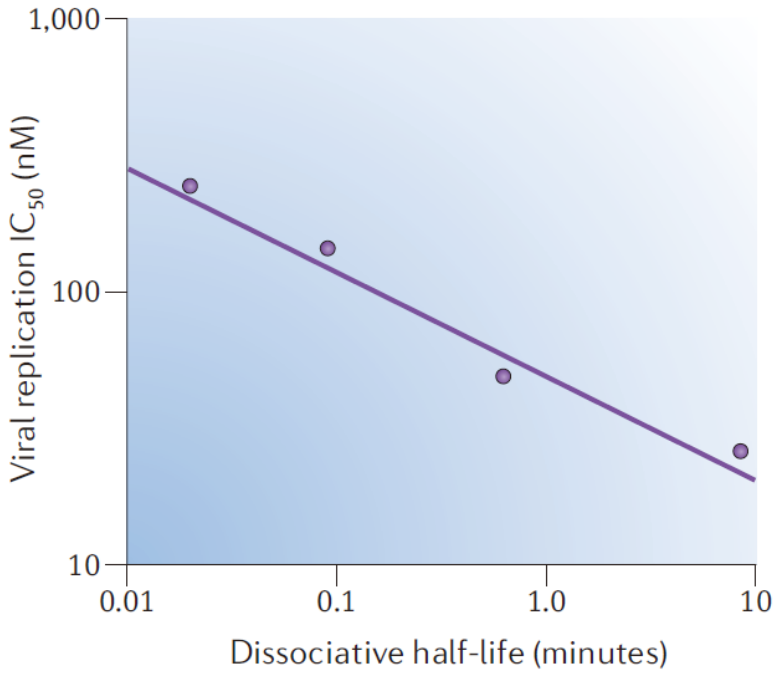
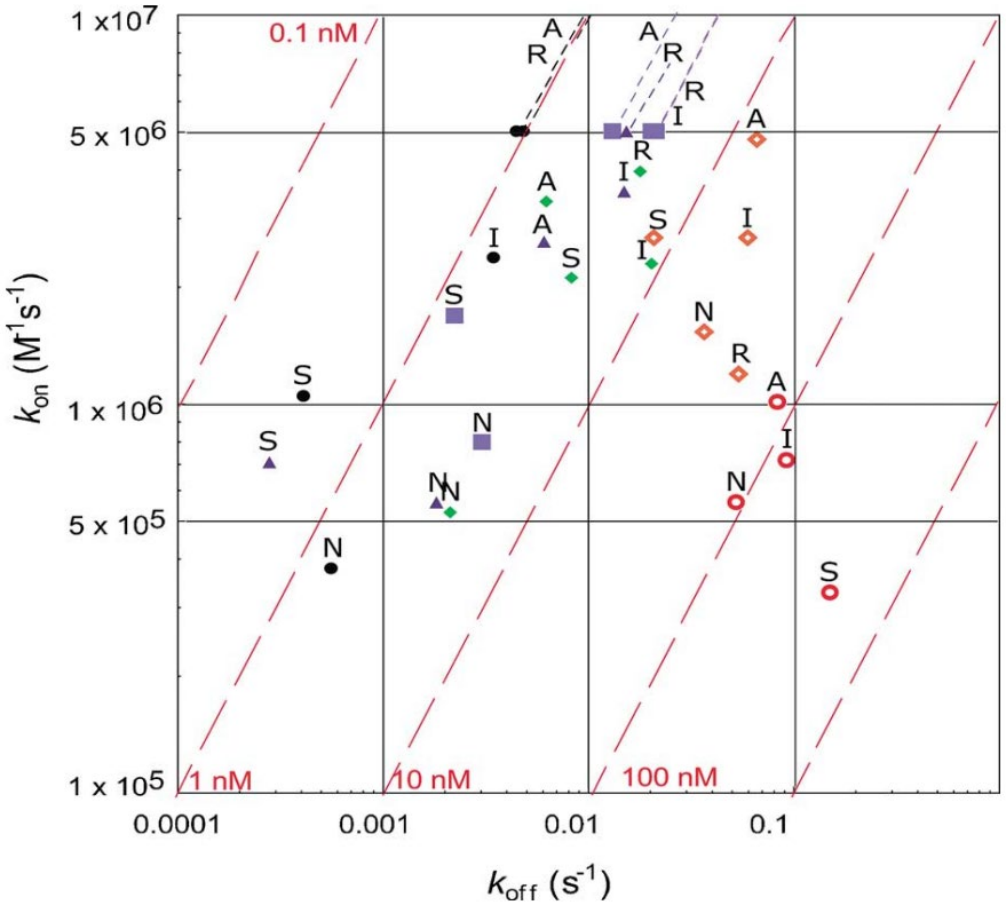


$$[\mathrm{E}] = -\frac{[\mathrm{I}]_{\mathrm{tot}} - [\mathrm{E}]_{\mathrm{tot}} + \frac{k_{\mathrm{off}}}{k_{\mathrm{on}}}}{2} + \sqrt{\left(\frac{[\mathrm{I}]_{\mathrm{tot}} - [\mathrm{E}]_{\mathrm{tot}} + \frac{k_{\mathrm{off}}}{k_{\mathrm{on}}}}{2}\right)^2 + \frac{k_{\mathrm{off}}}{k_{\mathrm{on}}} \times [\mathrm{E}]_{\mathrm{tot}}}$$

Danielson, U. H. *et al. J. Med. Chem.* **2004**, *47*, 5953.



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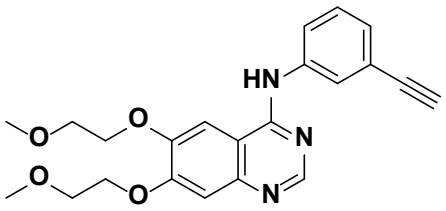
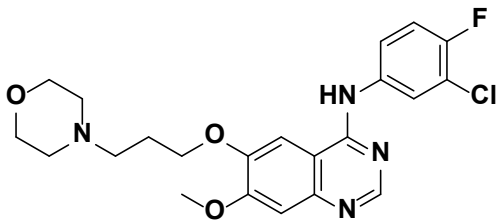
Danielson, U. H. *et al. Antiviral Res.* **2003**, *58*, 235.

Preliminary Discoveries

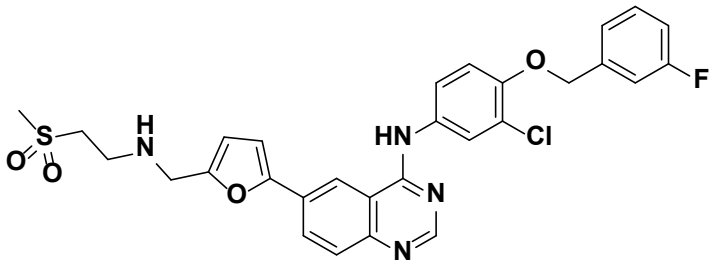
- Pronounced kinetic-activity relationship in an **open-system** assay



OSI-774

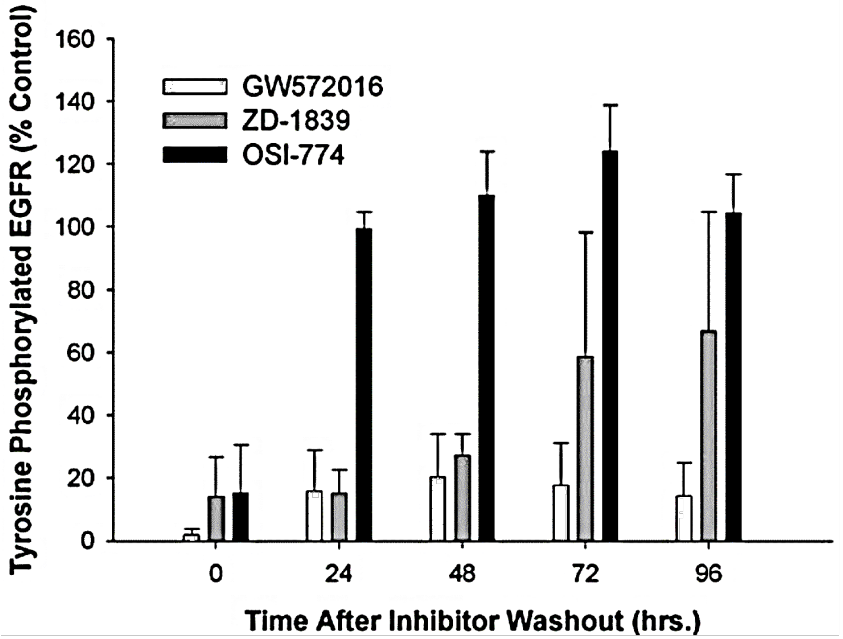


ZD-1839



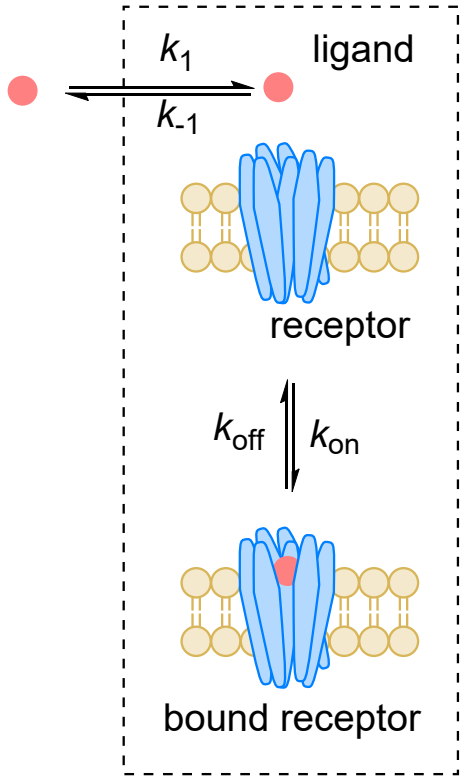
GW572016

	OSI-774	ZD-1839	GW572016
K_i	0.7 nM	0.4 nM	3.0 nM
Dissociation $t_{1/2}$	< 10 min	< 10 min	~ 300 min



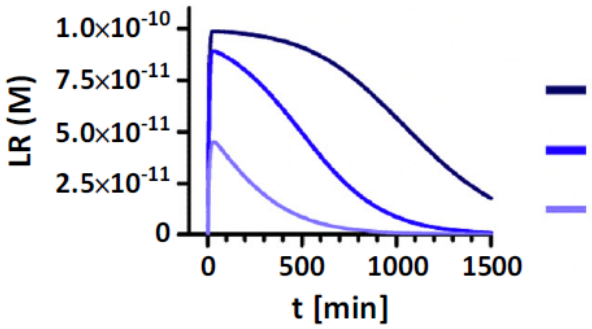
Danielson, U. H. *et al. J. Med. Chem.* **2004**, *47*, 5953.

Hypothetical Models in Two-state Open System

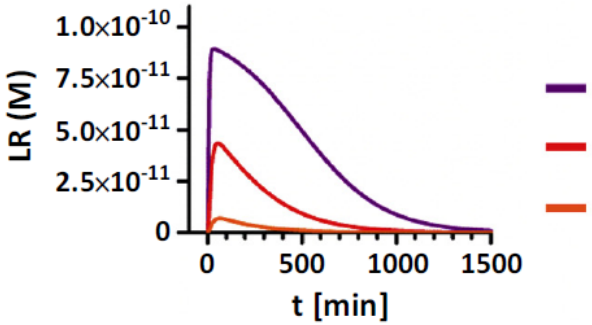


$[R]_0$ = 1 nM, $[L]_0$ = 100 nM, $[L]_{max}$ = 37 nM

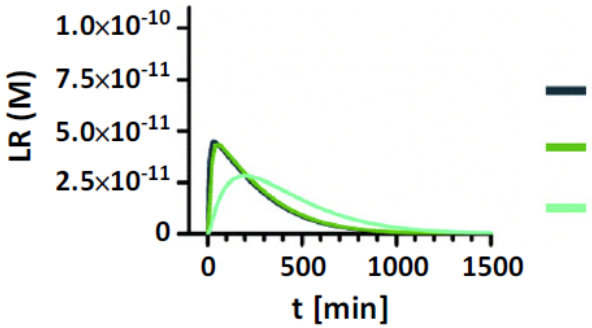
K_d = 1 nM, $k1$ = 0.0167 min^{-1}, k_{-1} = 0.0167 min^{-1}



k_{on} (nM^{-1} min^{-1})	k_{off} (min^{-1})	τ (min)	K_i (nM)	AVG *c* (pM)
0.1	0.005	200	0.05	11
0.1	0.05	20	0.5	5.1
0.1	0.5	2	5	1.4



k_{on} (nM^{-1} min^{-1})	k_{off} (min^{-1})	τ (min)	K_i (nM)	AVG *c* (pM)
0.1	0.05	20	0.5	5.1
0.01	0.05	20	5	1.4
0.001	0.05	20	50	0.19

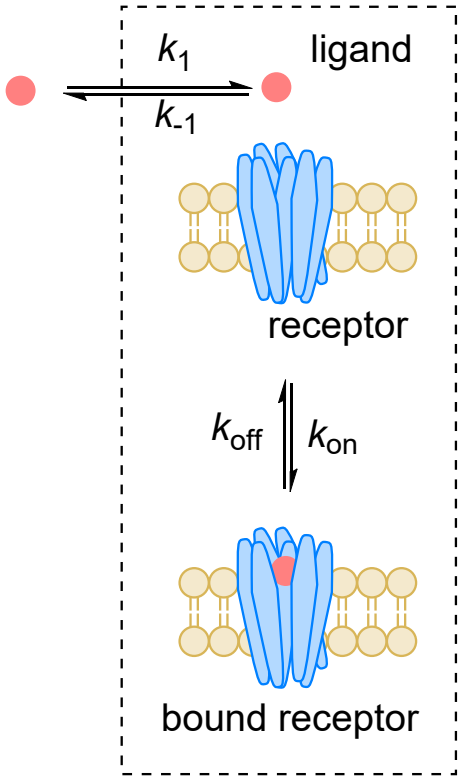


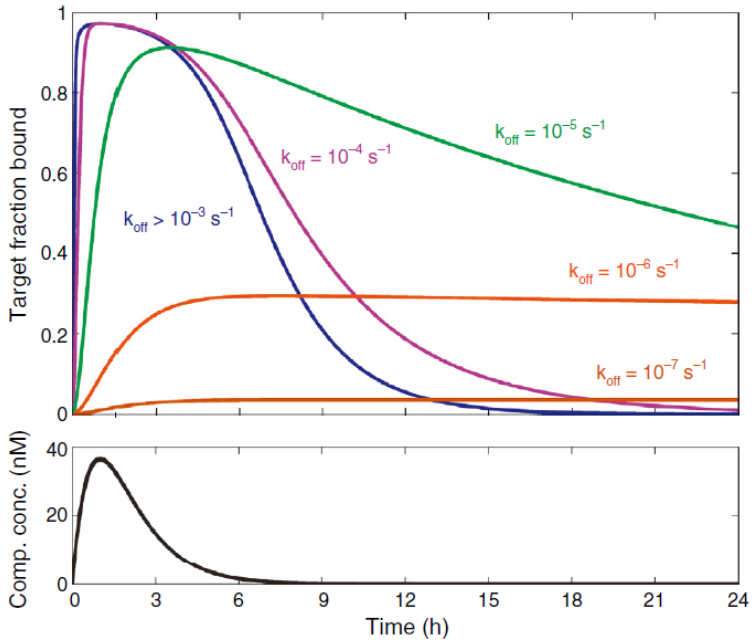
k_{on} (nM^{-1} min^{-1})	k_{off} (min^{-1})	τ (min)	K_i (nM)	AVG *c* (pM)
0.1	0.5	2	5	1.4
0.01	0.05	20	5	1.4
0.001	0.005	200	5	1.6

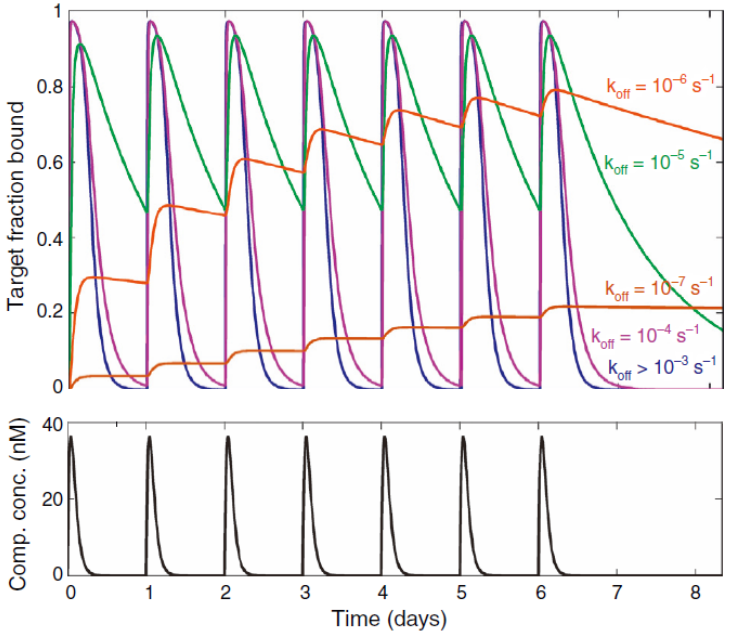
Strasser, A. *et al. Trends Pharmacol. Sci.* **2017**, *38*, 717.

Thomas, A. *et al. Drug Discov. Today* **2013**, *18*, 697.

Hypothetical Models in Two-state Open System







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Strasser, A. *et al. Trends Pharmacol. Sci.* **2017**, *38*, 717.

Thomas, A. *et al. Drug Discov. Today* **2013**, *18*, 697.

Outline

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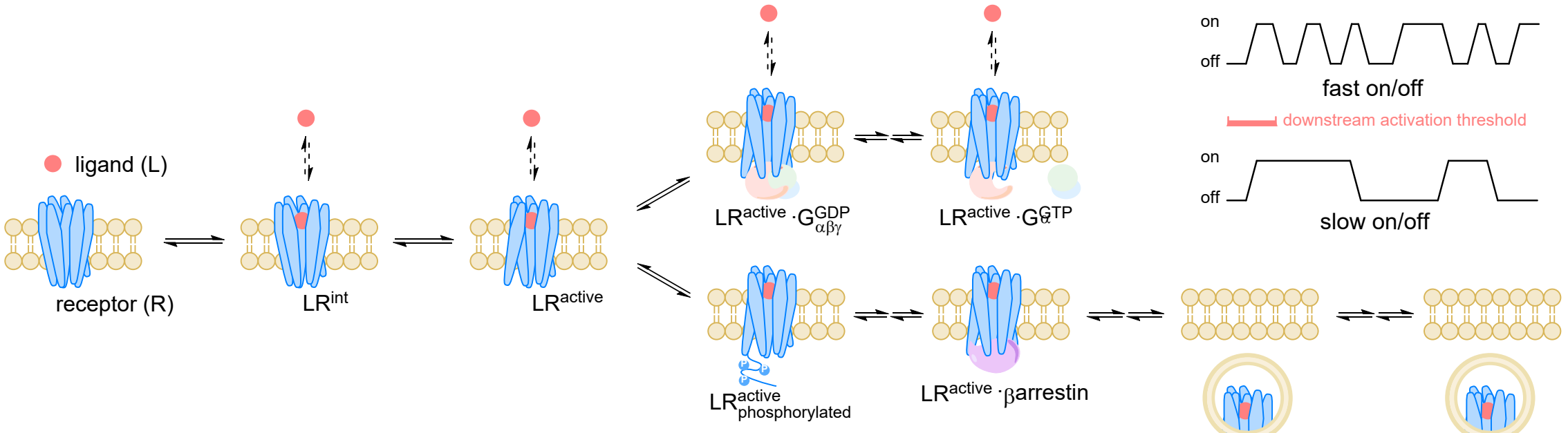
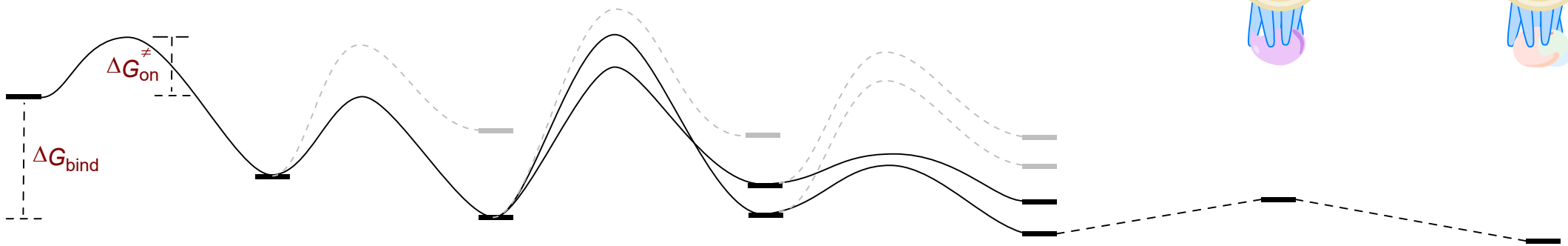
  - Ligand-target binding kinetics
- **Kinetics and dynamics of GPCR signaling**
  - G-protein pathway
  - β-arrestin pathway
- Revisiting the ligand-target binding kinetics
  - Examples
  - Structure-kinetic relationship
- Summary

Spatiotemporal Signaling at GPCRs

- Spatiotemporal signaling complicated kinetic analysis at GPCRs





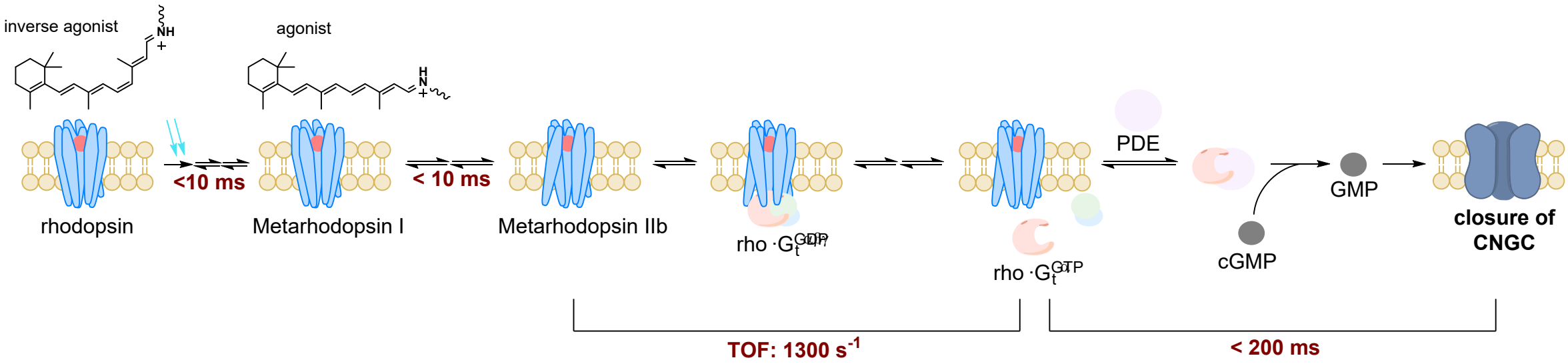
- Spatiotemporal "barcode"

Kostenis, E. *et al. Trends Pharmacol. Sci.* **2017**, *38*, 1110.

Strasser, A. *et al. Trends Pharmacol. Sci.* **2017**, *38*, 717.

Kinetics at the Prototypical “Light Receptor”

- Rhodopsin mediates signal transduction of **high-fidelity**, **high-speed** and **high-amplification ratio**.

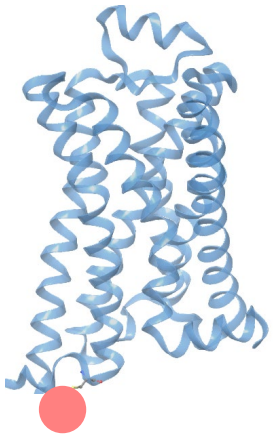


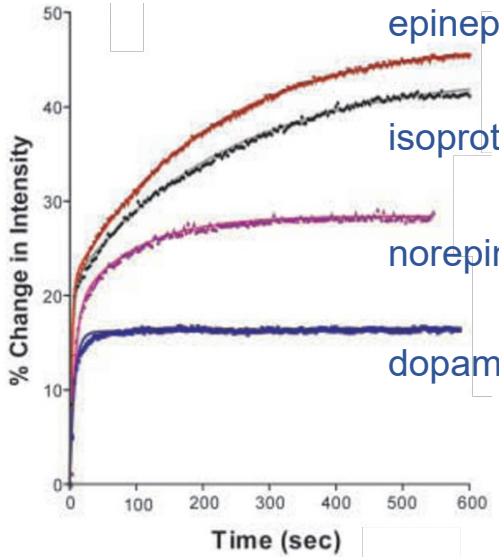
Ernst, O. P. *et al. Proc. Nat. Acad. Sci .* **2007**, *104*, 20290.
Hofmann, K. P. *et al. J. Bio. Chem.* **2001**, *276*, 10000.
Heyn, M. P. *et al. Biochemistry.* **2008**, *47*, 11518.

Non-rhodopsin Receptors

Conformational activation of GPCR

- An environmentally sensitive FL probe was attached to C^{265} at the end of TM6 (β_2AR)

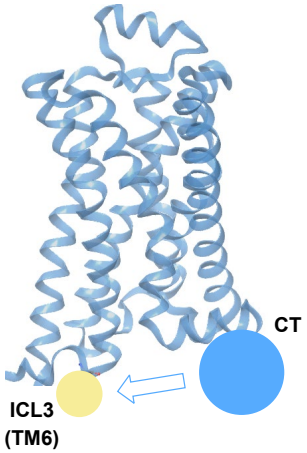


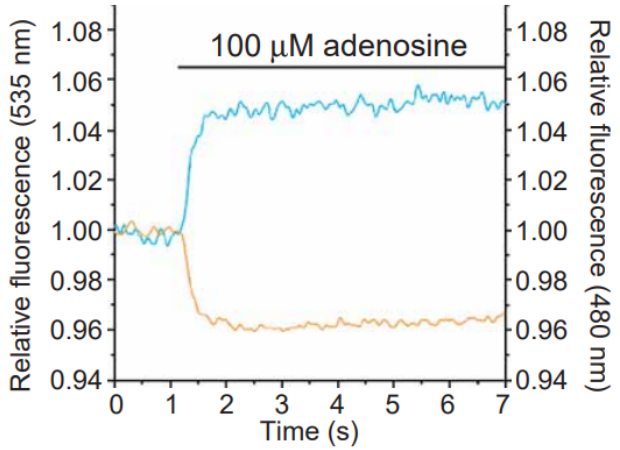


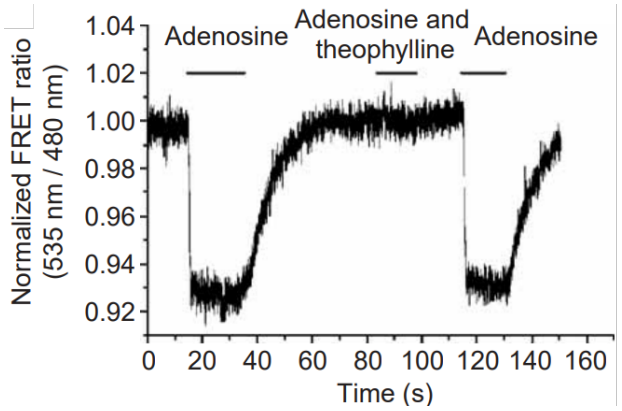
Drugs	Fast component, $t_{1/2}$	Slow component, $t_{1/2}$
	s	*s*
Isoproterenol	2.6 ± 0.2	147 ± 10
Epinephrine	4.5 ± 0.6	164 ± 20
Norepinephrine	2.8 ± 0.4	70.2 ± 6.1
Dopamine	4.2 ± 0.3	

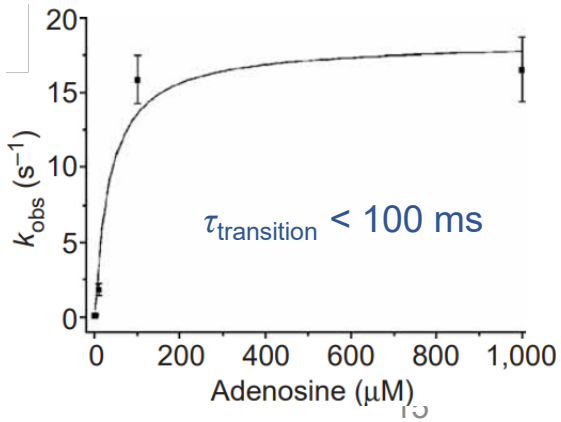
Kobilka, B. K. *et al. J. Bio. Chem.* **2004**, *279*, 686.

- For living cell analysis, FRET/BRET based methods are usually used.





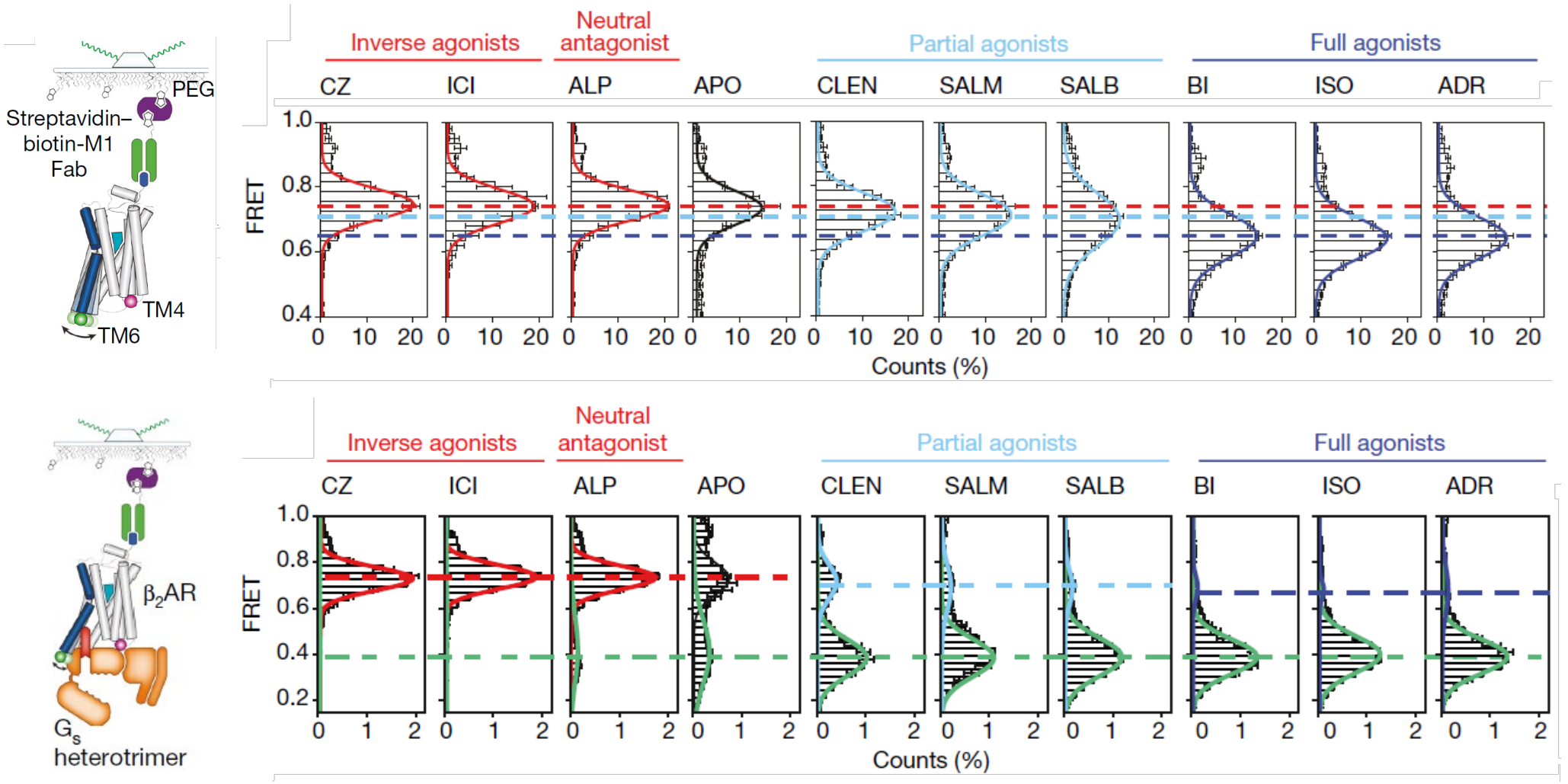




Lohse, M. J. *et al. Nat. Methods* **2005**, *2*, 171.

smFRET Analysis of GPCR Dynamics

- **Conformational activation and G protein binding**



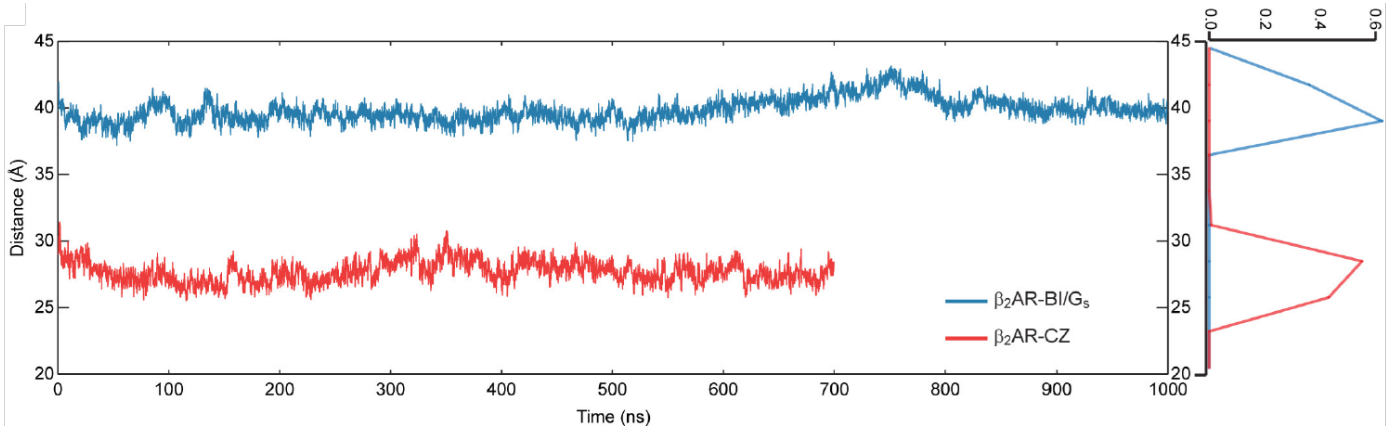
$\tau_{transition}$ ~ 2-10 ms

Kobilka, B. K.; Blanchard, S. C. *et al. Nature* **2017**, *547*, 68.

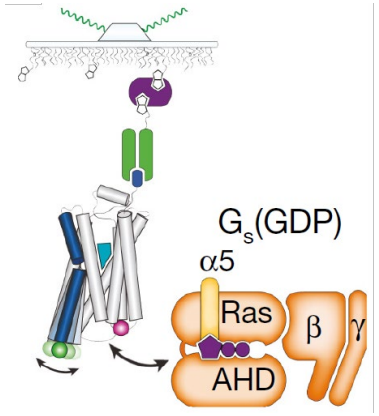
G Protein Association

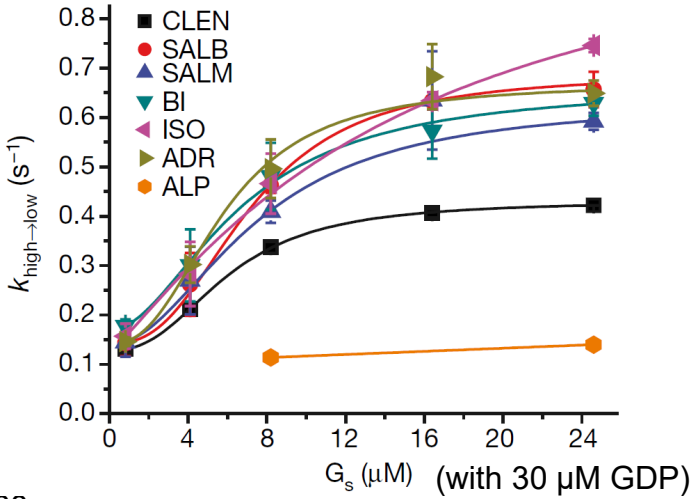
G protein binding

- FRET reflects similar inter-dye distance with MD simulation



- G_s titration





Apparent k_{on} ~ 0.03-0.05 $\mu M^{-1}\ s^{-1}$

[G protein] ~ 0.2-3 μM

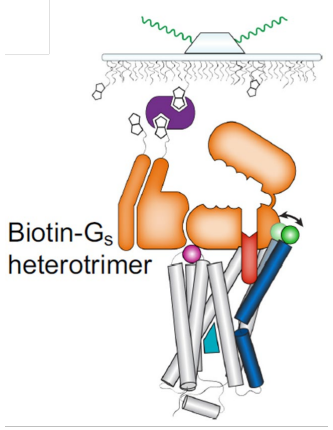
k_{on} ~ 0.05 s^{-1}, $k_{on,\ max}$ ~ 0.4-1.1 s^{-1}

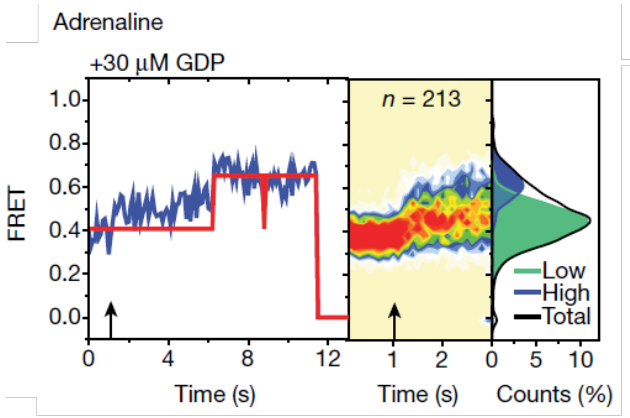
- A rate limiting conformational change was postulated to precede G_s binding

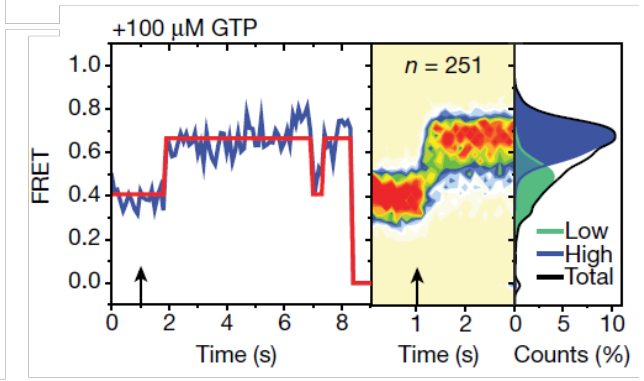
Kobilka, B. K.; Blanchard, S. C. *et al. Nature* **2017**, *547*, 68.

G Protein Dissociation

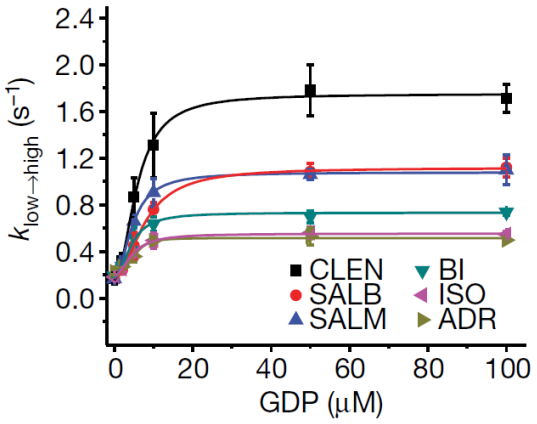
- **GDP/GTP association and G_s(GDP/GTP) dissociation**



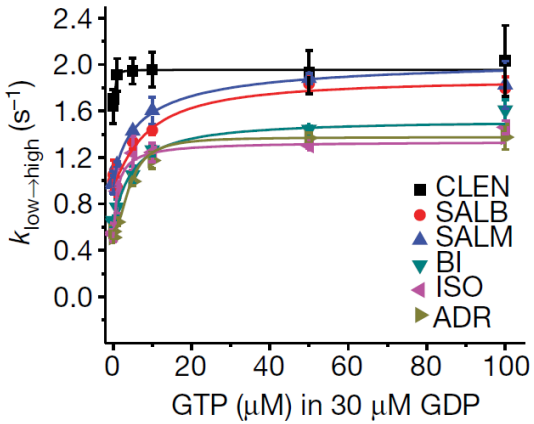




$k_{on(GDP/GTP)}$ ~ diffusion controlled

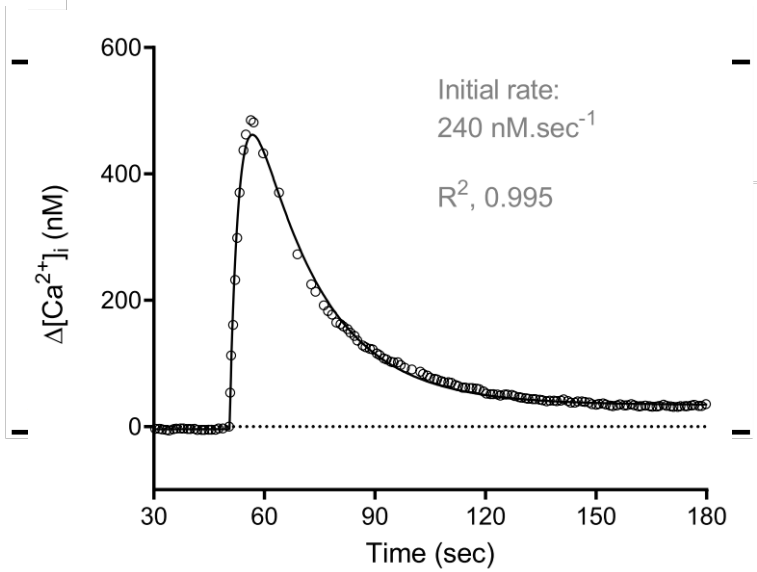


$\tau_{transition}$ ~ 0.6-2 s



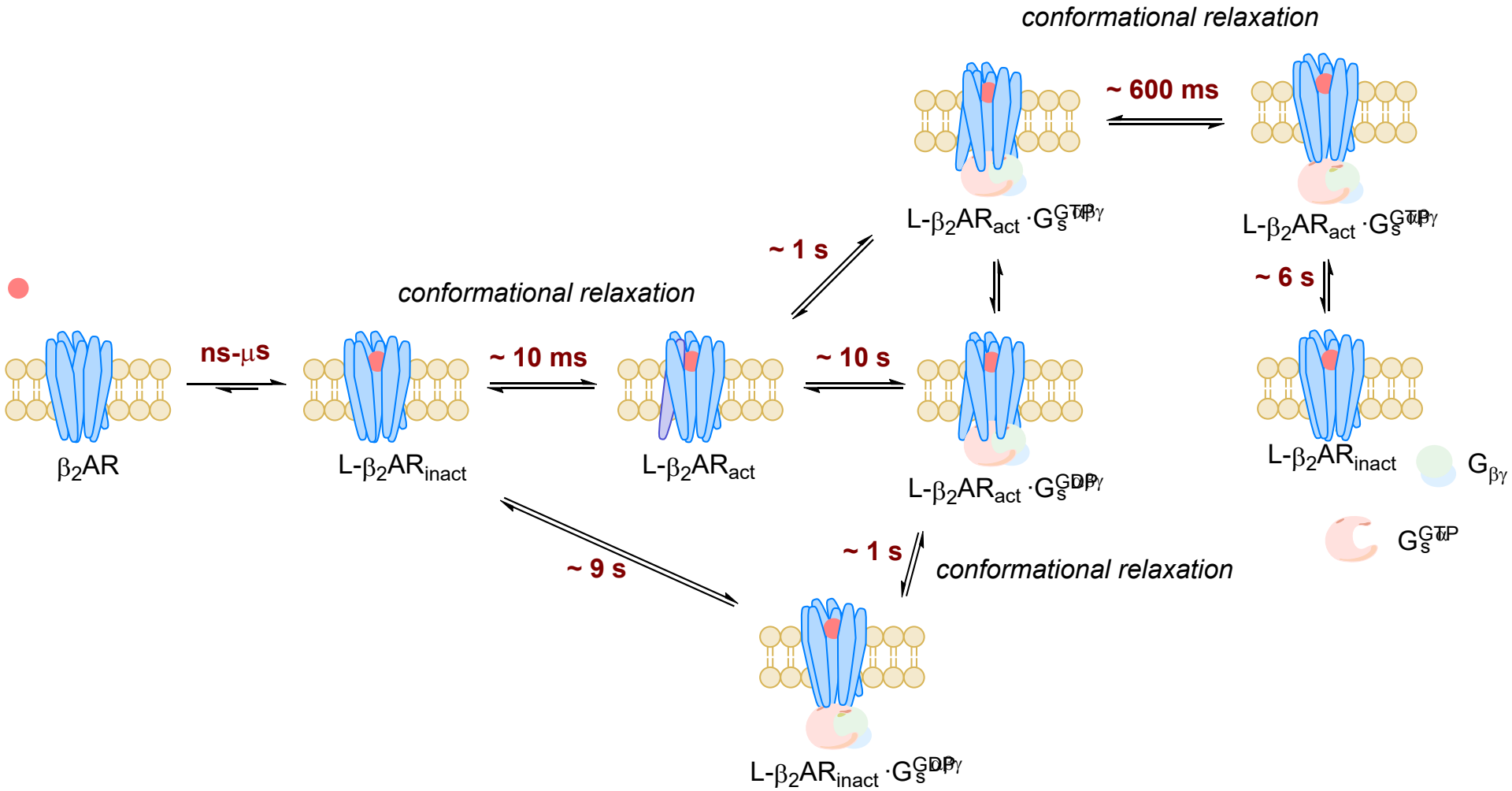
$\tau_{transition}$ ~ 0.5-0.8 s

Ca^{2+} mobilization, $GnRH_1$ receptor, GnRH



Kobilka, B. K.; Blanchard, S. C. *et al. Nature* **2017**, *547*, 68.

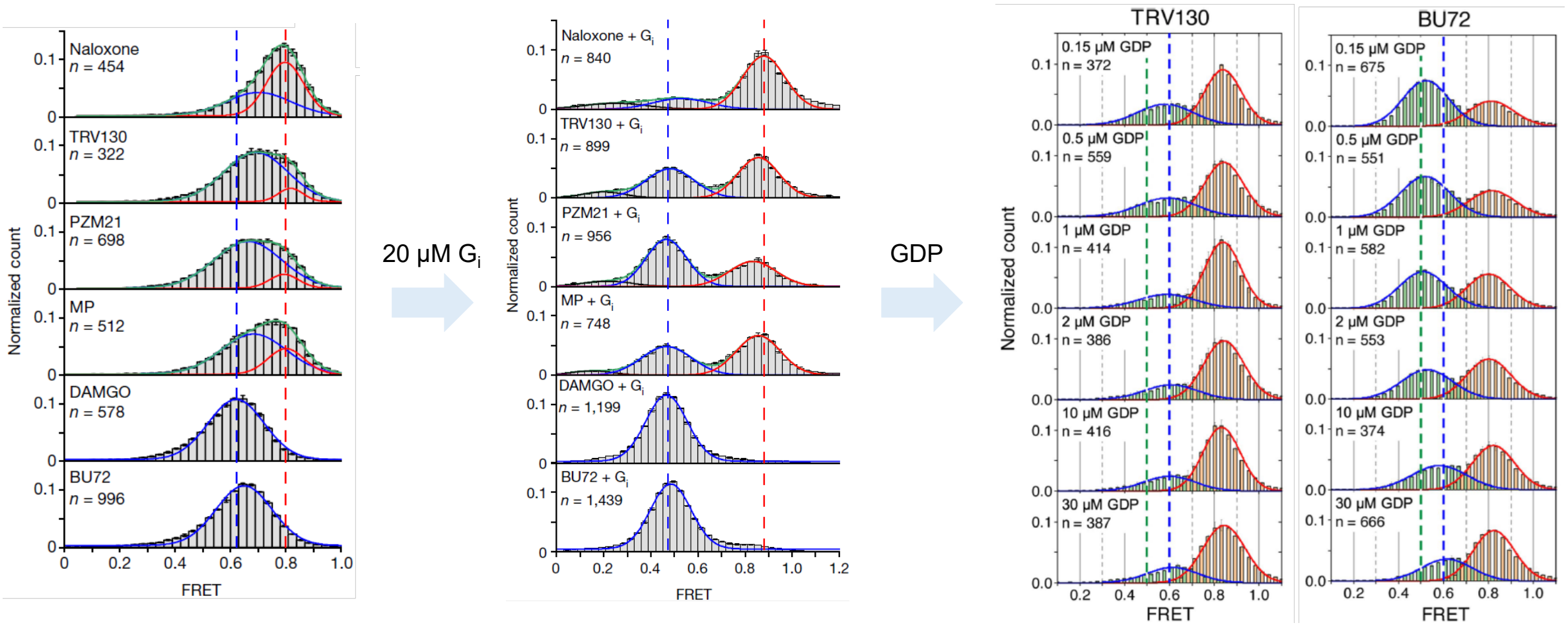
Overall Kinetic Model at β_2AR



Kobilka, B. K.; Blanchard, S. C. *et al. Nature* **2017**, *547*, 68.

On μ-Opioid Receptor

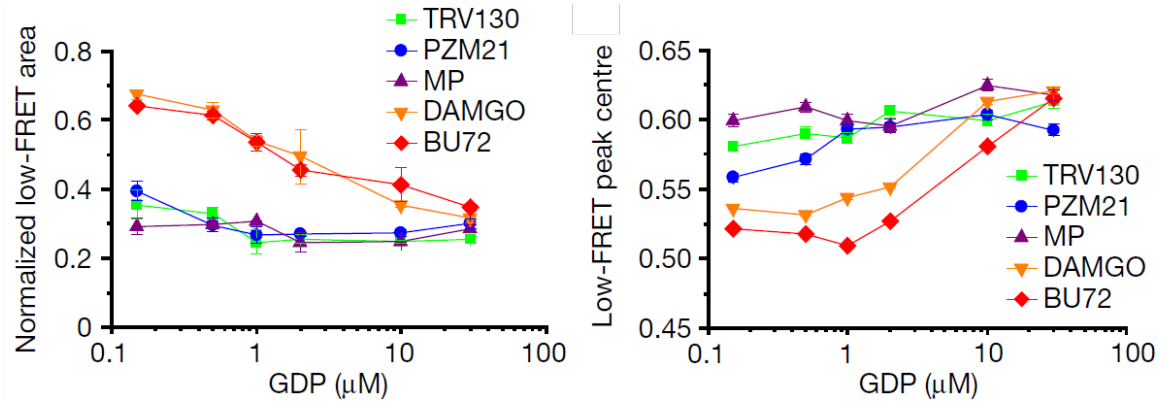
- Conformation dynamics of μOR was studied by DEER and smFRET

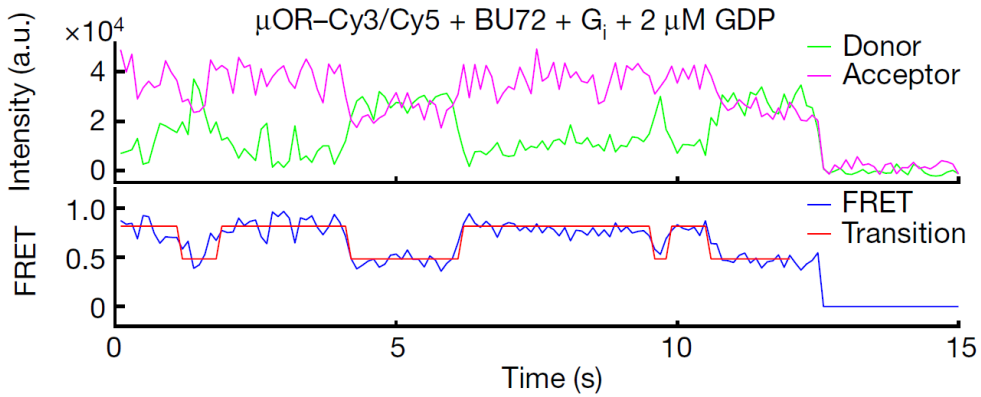


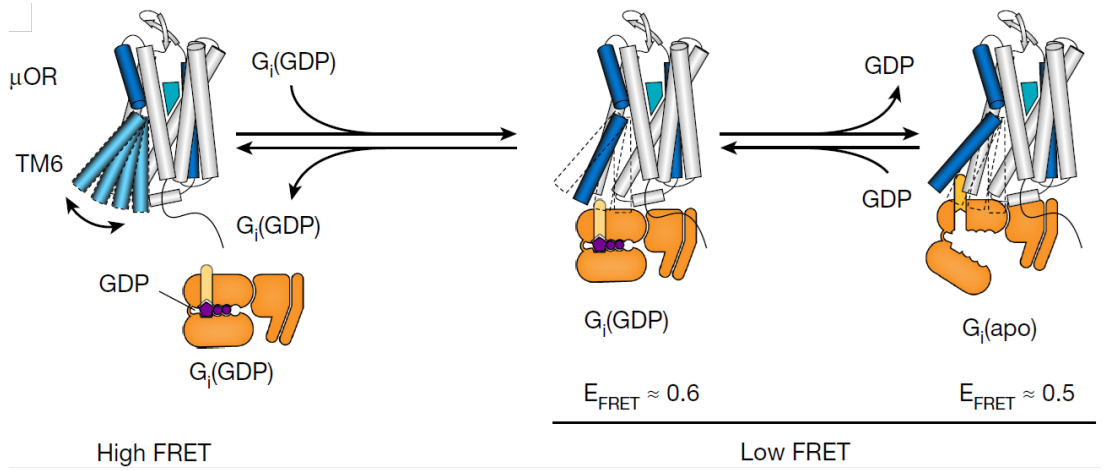
Kobilka, B. K.; Chen, C. *et al. Nature* **2024**, *629*, 474.

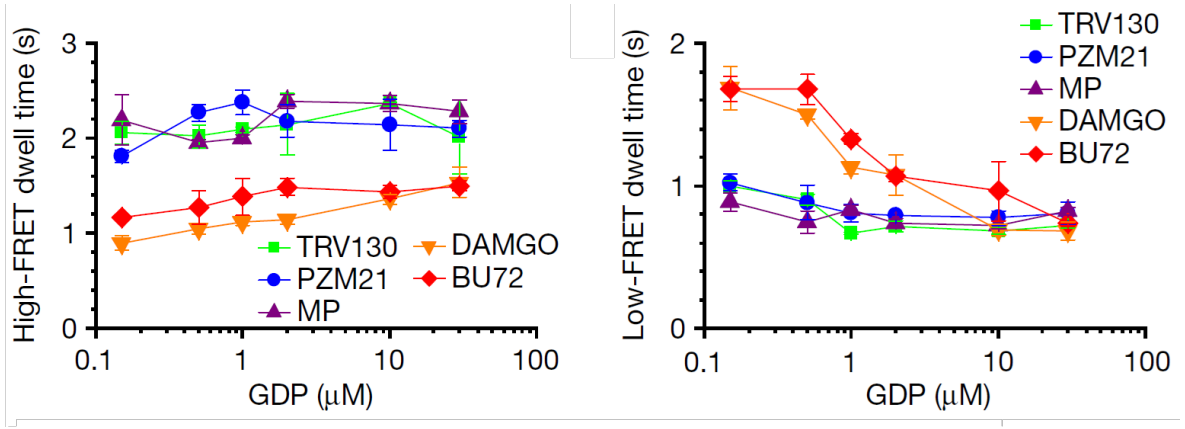
On μ-Opioid Receptor

- Conformation dynamics of μOR was studied by DEER and smFRET









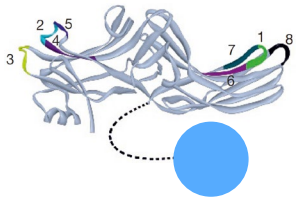
k_{on} ~ 0.5-1 s^{-1}

$\tau_{\mu OR\text{-}Gi(GDP)}$ ~ 1 s ?

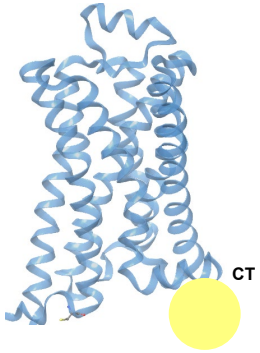
Kobilka, B. K.; Chen, C. *et al. Nature* **2024**, *629*, 474.

Probing the Binding Kinetics of β-arrestin

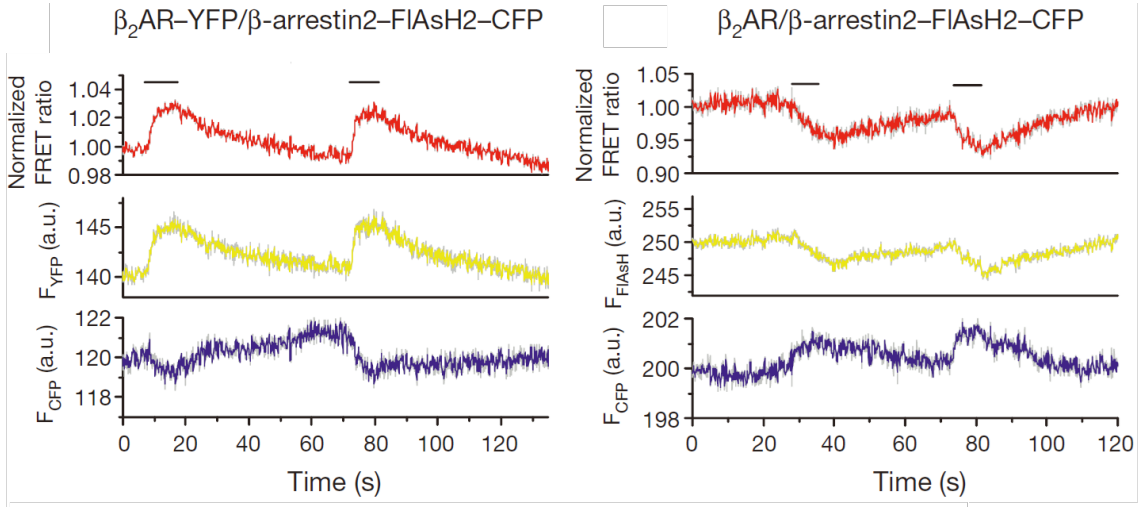
- Binding kinetics of β-arrestin at β_2AR

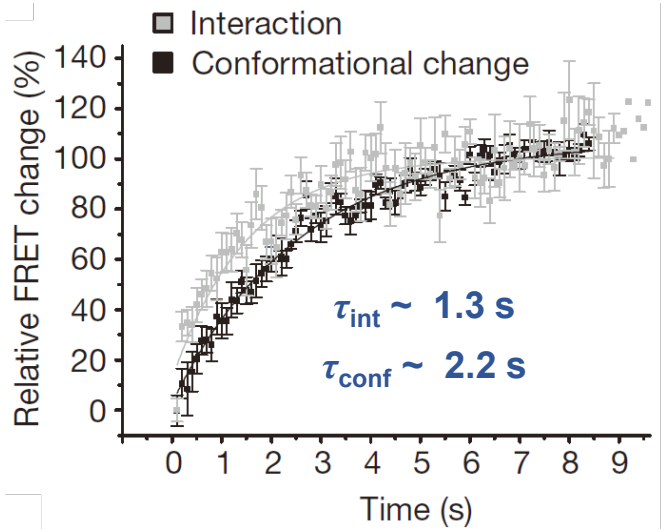


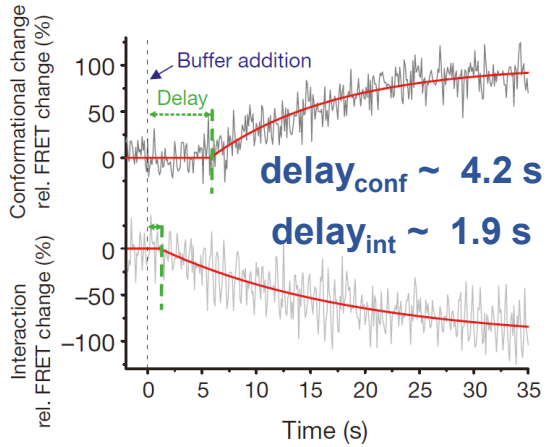
β-arrestin2-FlAsH2-CFP

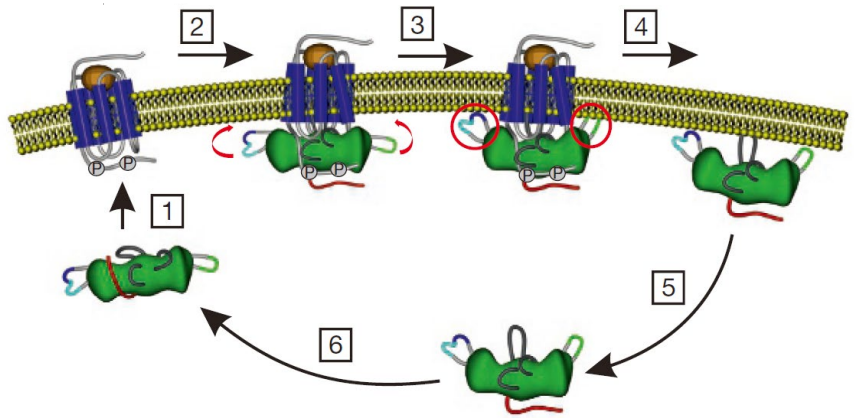


β_2AR-YFP







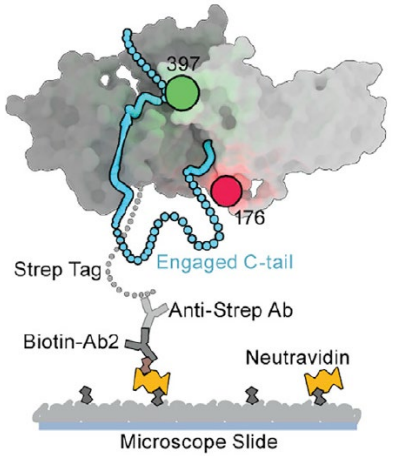


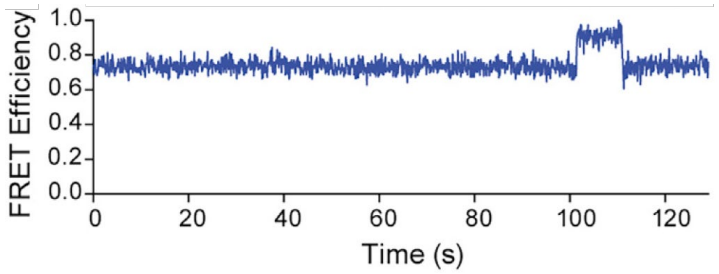
Lohse, M. J.; Hoffmann, C. *et al. Nature* **2016**, *531*, 661.

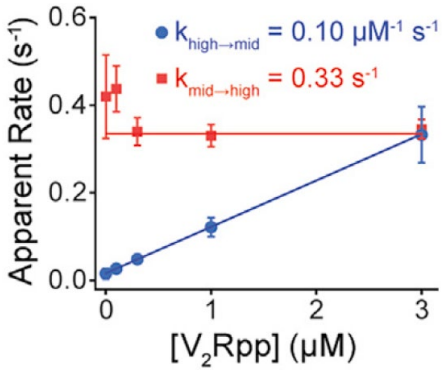
Hinkle, P. M. *et al. Biochem. J.* **2010**, *428*, 235.

Probing the Binding Kinetics of β-arrestin

- Autoinhibition of β-arrestin 1 and β_2AR by C-terminal tail





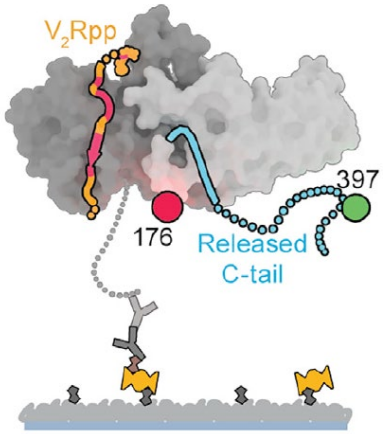


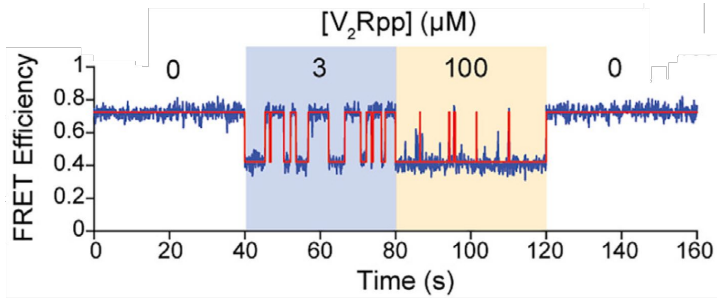
[βarr] ~ 80 nM

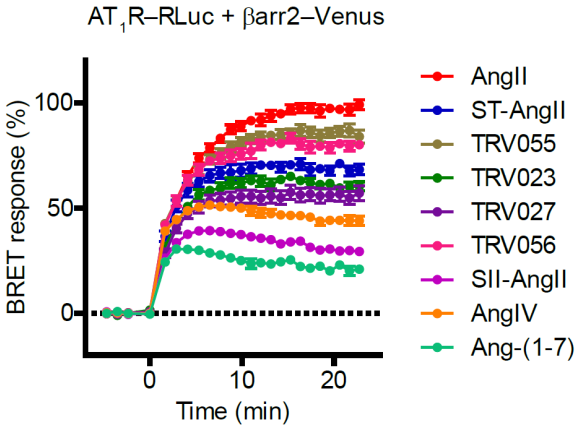
τ_{on} ~ **100 s**

τ_{off} ~ **2 s**

V_2Rpp



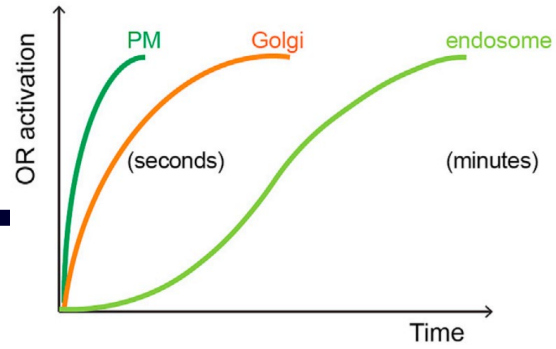




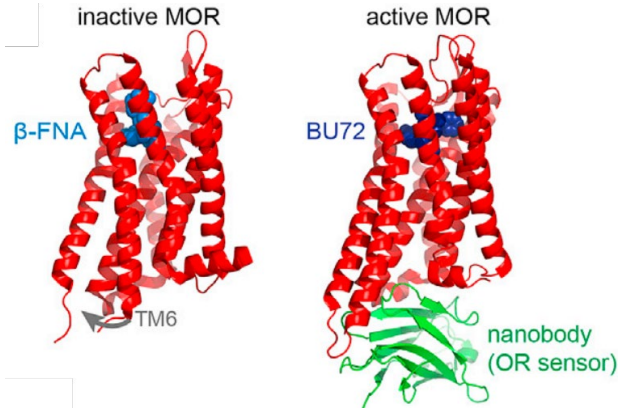
Correlates with the response time scale in most assays

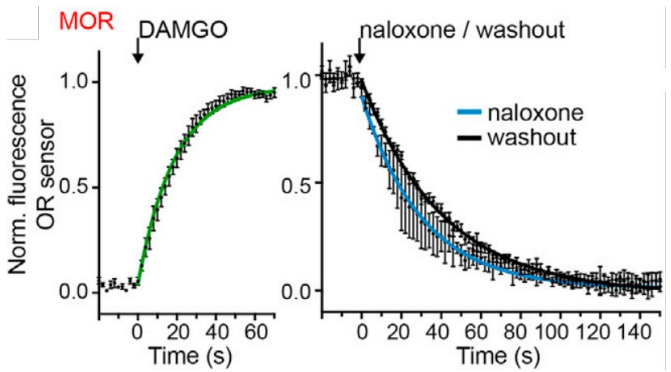
Blanchard, S. C.; Javitch, J. A. *et al. Cell* **2022**, *185*, 1661.

Receptor Endocytosis

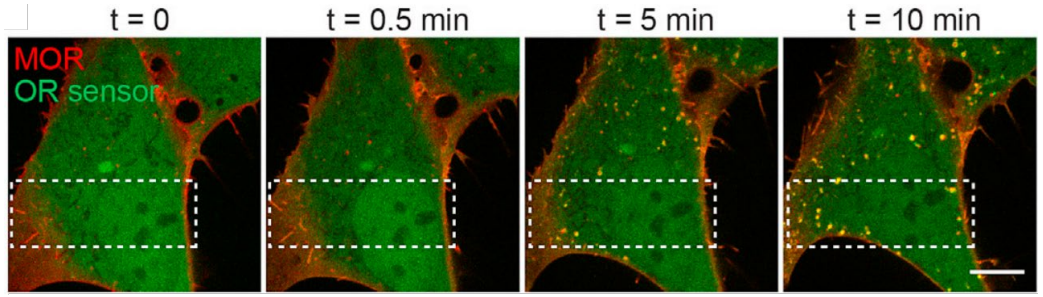


- Spatiotemporally resolved OR activation by conformation-sensitive luminescent nanobody

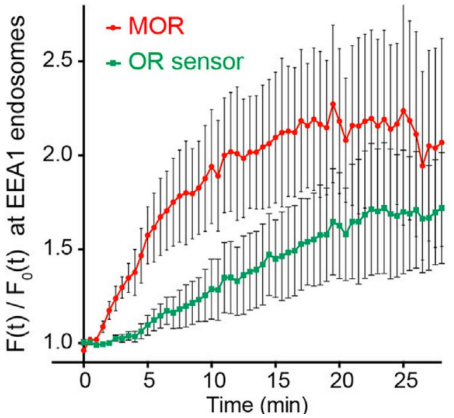


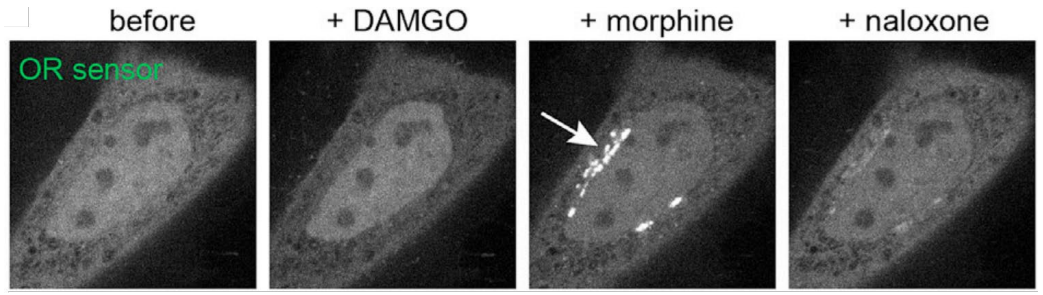


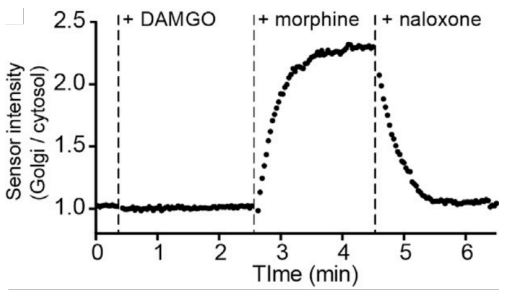
Labeling μOR at PM, then + DAMGO



$t_{1/2}$ ~ 10 min

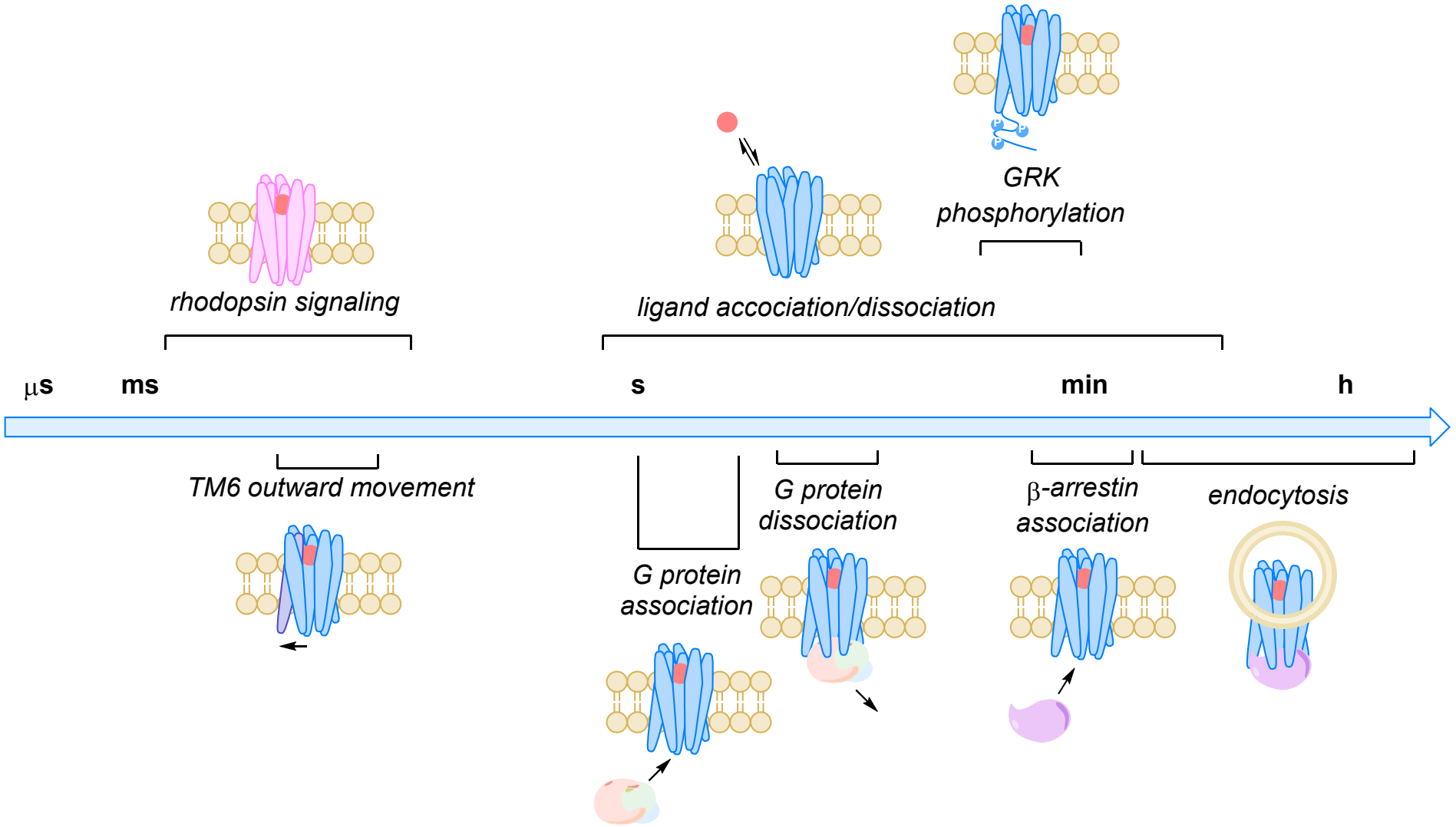




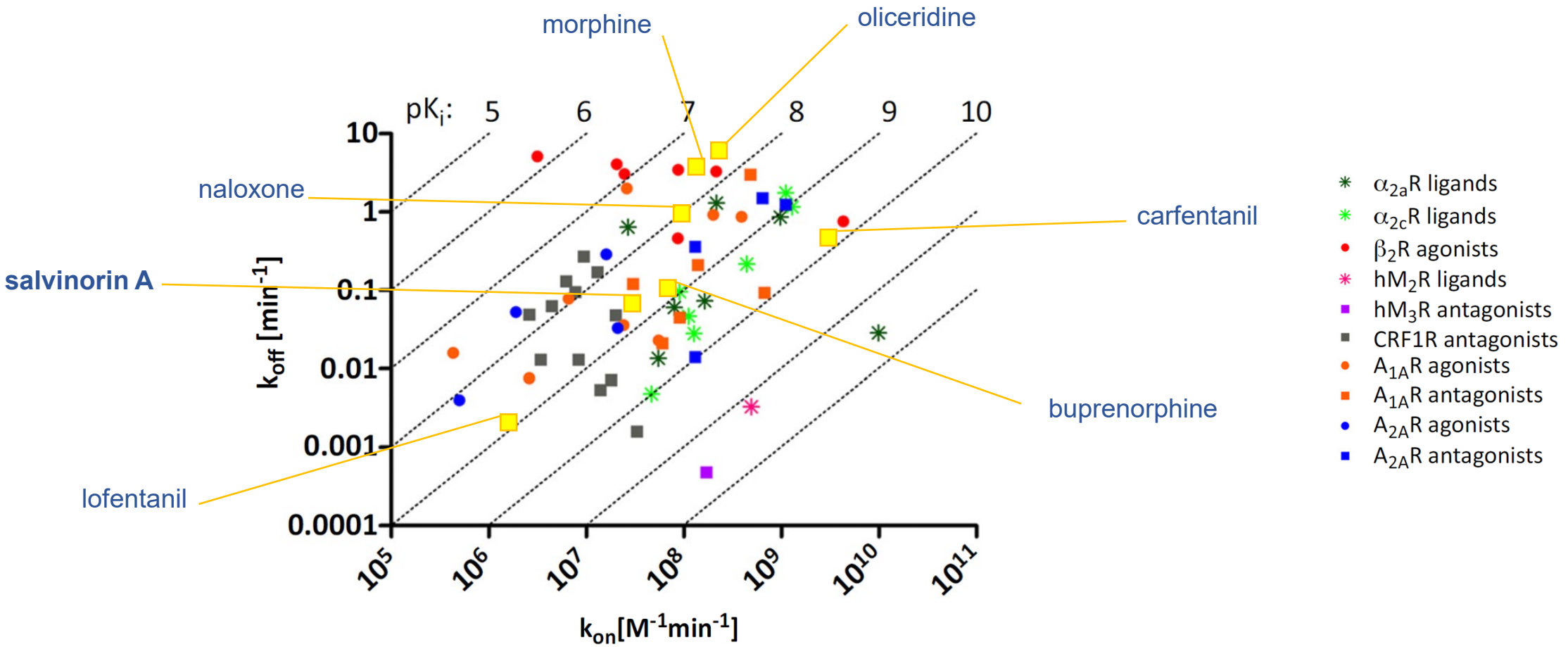


von Zastrow, M. *et al. Neuron* **2018**, *98*, 963.

Time Scale Overview



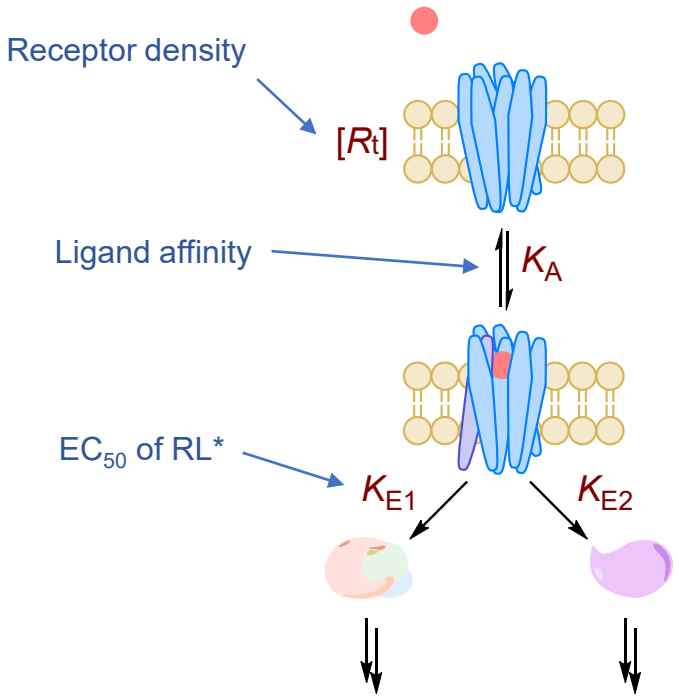
Kinetic Parameters for GPCR Drugs



Hudlicky, T. *et al. Acc. Chem. Res.* **2015**, *48*, 674. Roth, B. L. *et al. Nat. Commun.* **2020**, *11*, 1145. Bouvier, M. *et al.* Neuropharmacology **2020**, *166*, 107718.

Interplay of Ligand Binding and GPCR Signaling Kinetics

- The role of kinetic context in **apparent biased agonism** at GPCRs
- The Black−Leff operational model in quantifying bias



Define: $\tau = [R_t]/K_E$

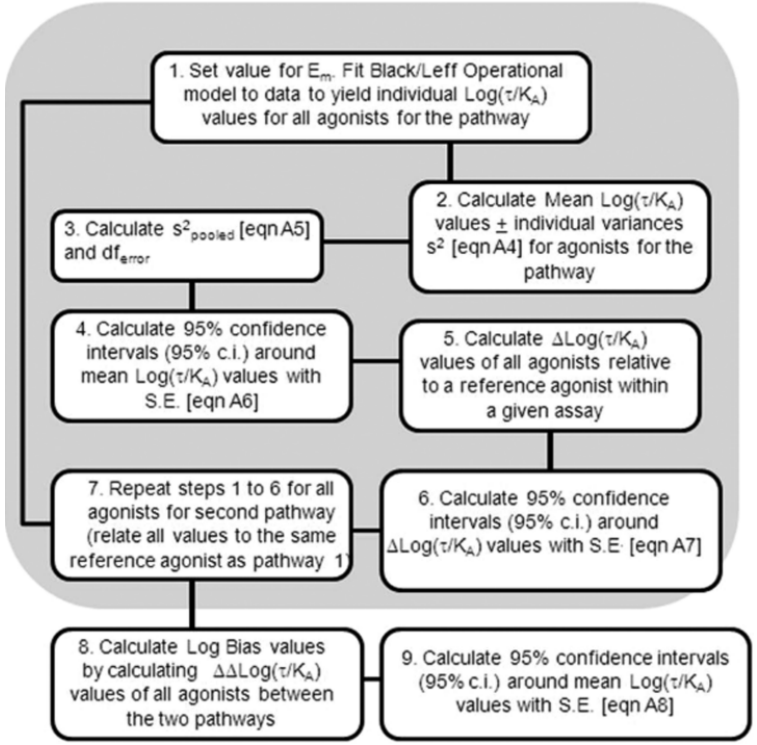
Define: Transduction coefficient = $\log[\tau/K_A]$

Intra-pathway: $\Delta\log[\tau/K_A] = \log[K_{E1}\, K_{A1}/K_{E,\,std}\, K_{A,\,std}]$

Inter-pathway: $\Delta\Delta\log[\tau/K_A]$

bias: 10^($\Delta\Delta\log[\tau/K_A]$)

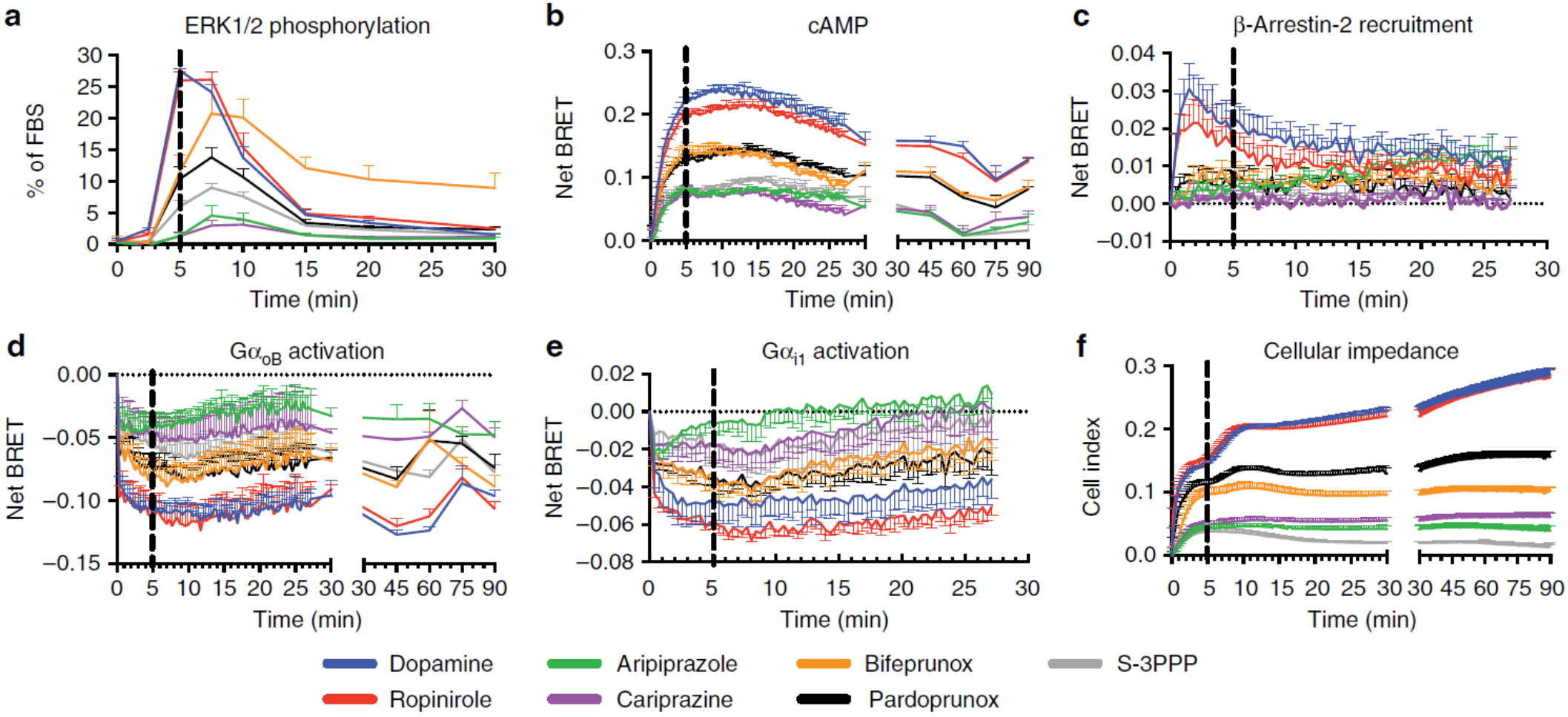
$$\text{response} = \frac{E_m[A]^n\tau^n}{[A]^n\tau^n + ([A] + K_A)^n}$$



Kenakin, T. *et al. ACS Chem. Neurosci.* **2012**, *3*, 193.

Time as an Additional Dimension

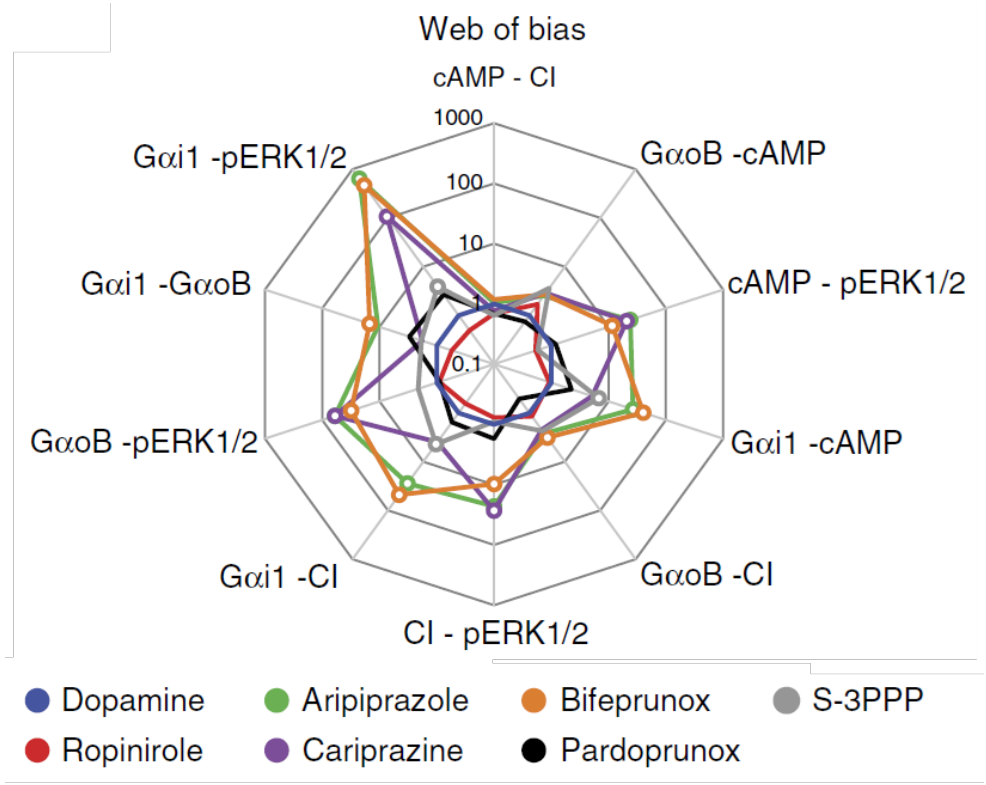
- Time-dependent measurement of response over **7 agonists** and **6 assays** at D_2R

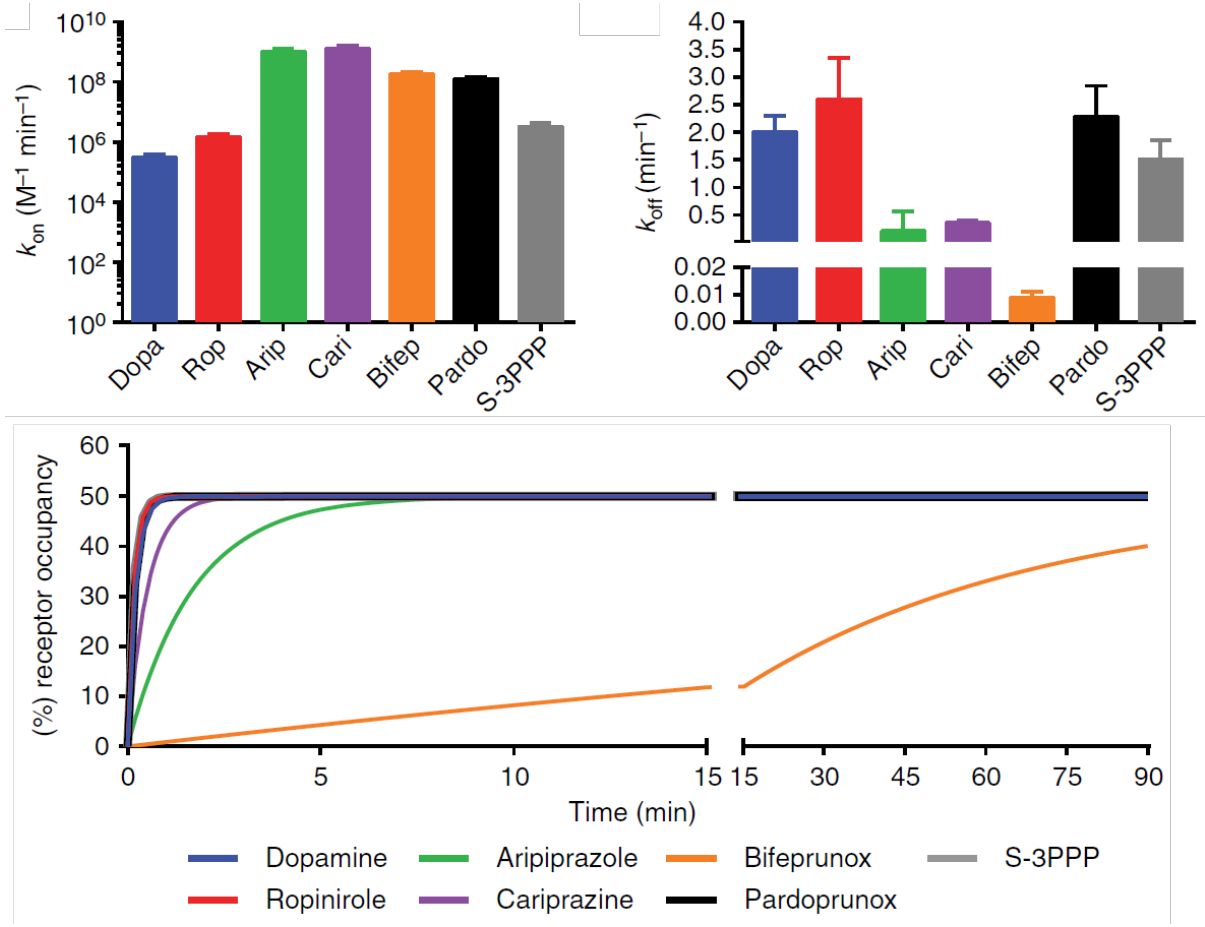


Christopoulos, A.; Lane, J. M. *et al. Nat. Commun.* **2016**, *7*, 10842.

Time as an Additional Dimension

- Distinct kinetic profiles of agonists

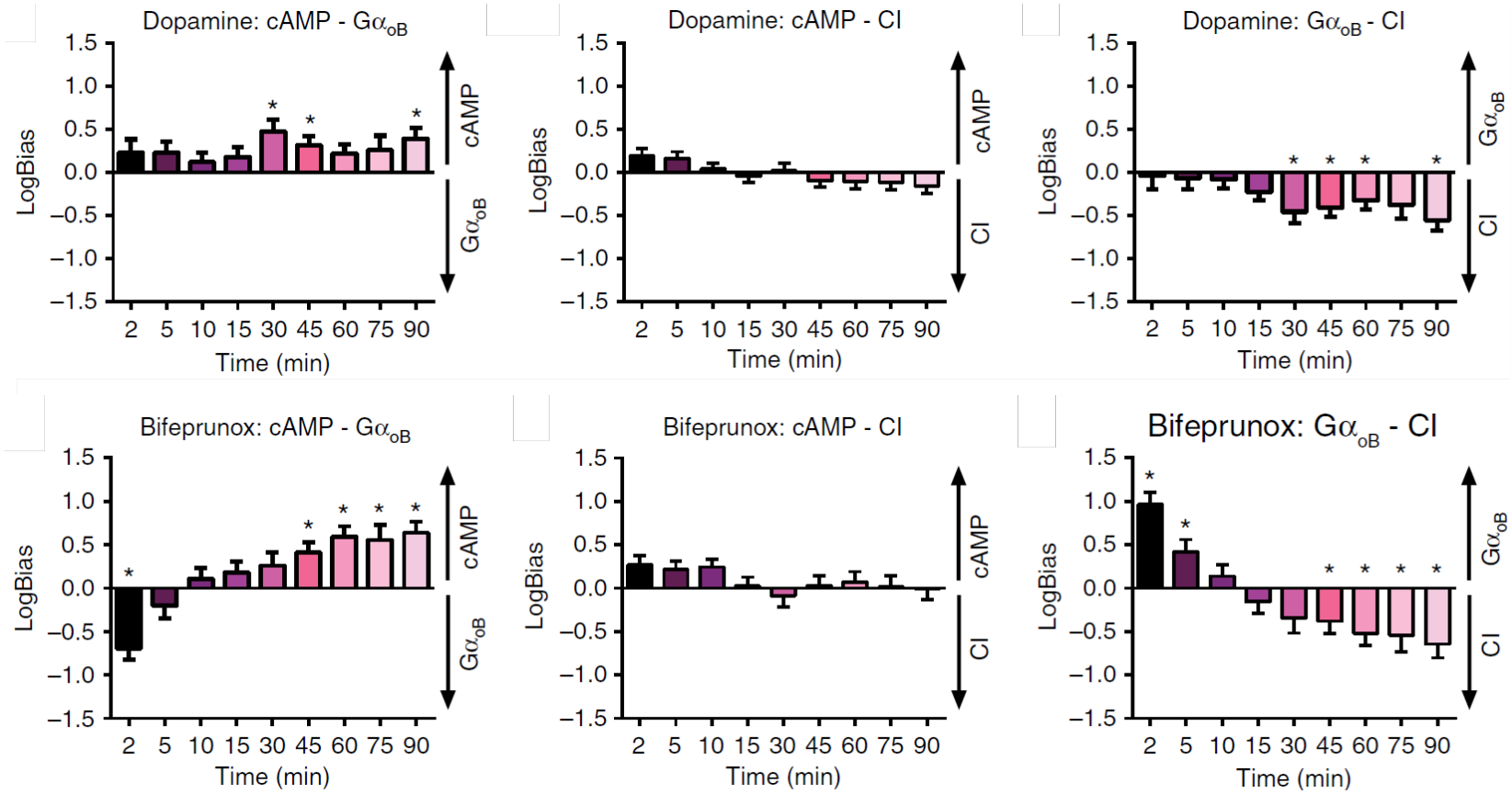




Christopoulos, A.; Lane, J. M. *et al. Nat. Commun.* **2016**, *7*, 10842.

Time as an Additional Dimension for Apparent Bias

- Bias evolves with time for slow-dissociating agonists

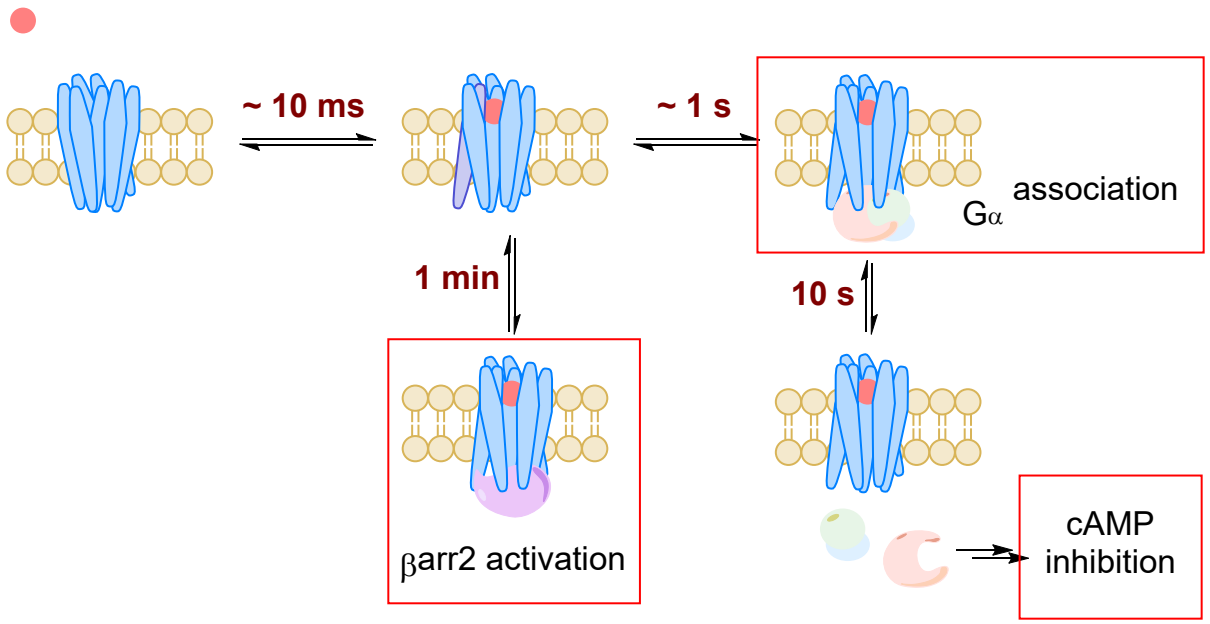


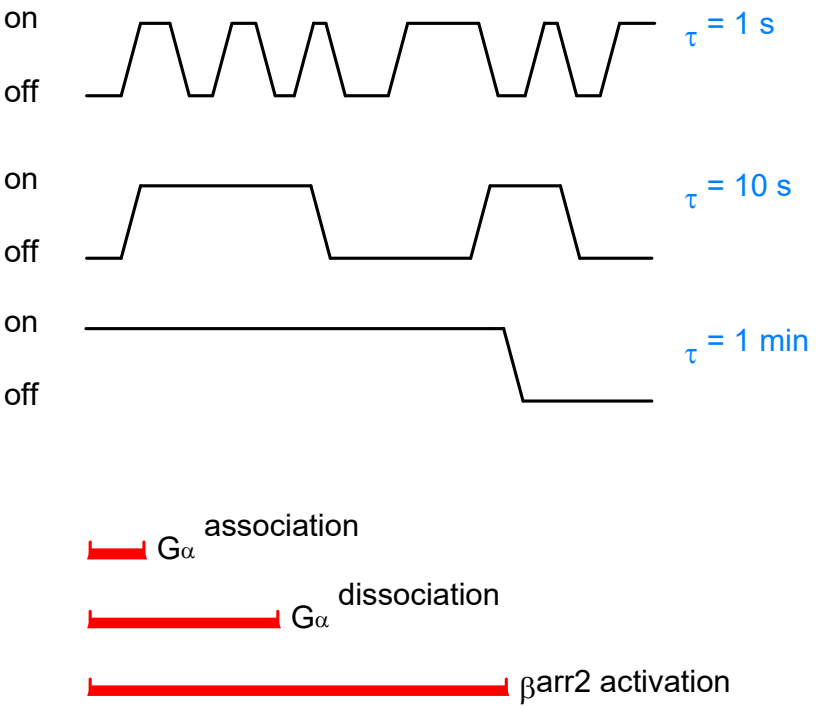
- Is kinetics a “confounding factor” or a important component in bias signaling?

Christopoulos, A.; Lane, J. M. *et al. Nat. Commun.* **2016**, *7*, 10842.

Binding Kinetics and the Intrinsic Bias

- Proofreading by temporal waveform

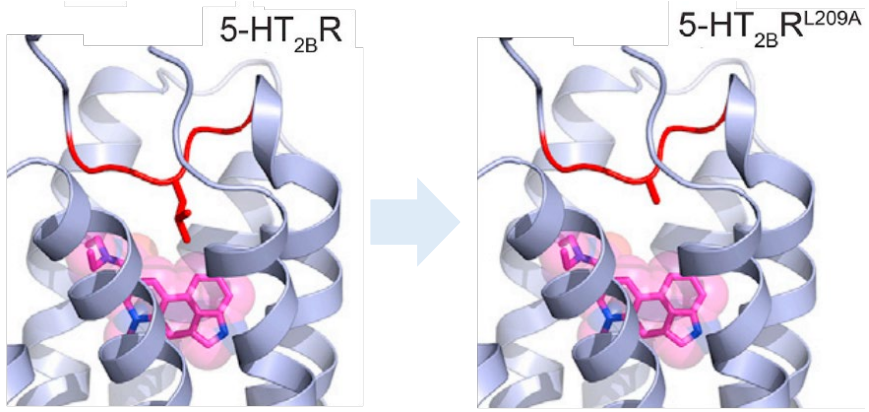




Kiyatkin, A. *et al. Cell* **2016**, *164*, 1172.

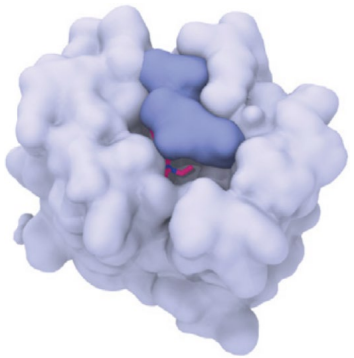
Binding Kinetics and the Intrinsic Bias

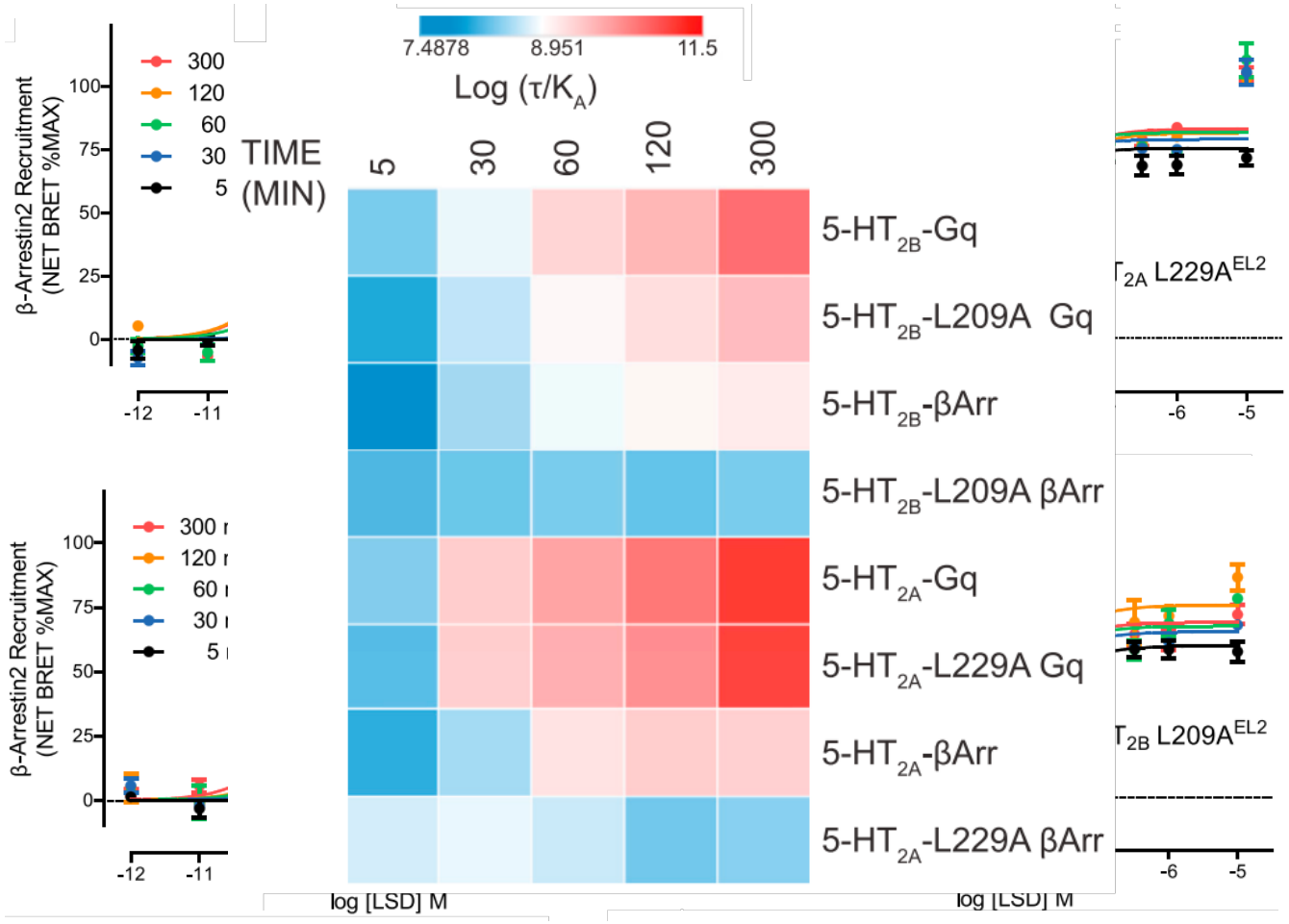
- Slow-off kinetics of LSD at 5-$HT_{2A/B}$R endows β-arrestin bias



$t_{1/2}$ = 221 min (5-HT_{2A}R)
$t_{1/2}$ = 46 min (5-HT_{2B}R)

$t_{1/2}$ = 50 min (5-HT_{2A}R)
$t_{1/2}$ = 4 min (5-HT_{2B}R)





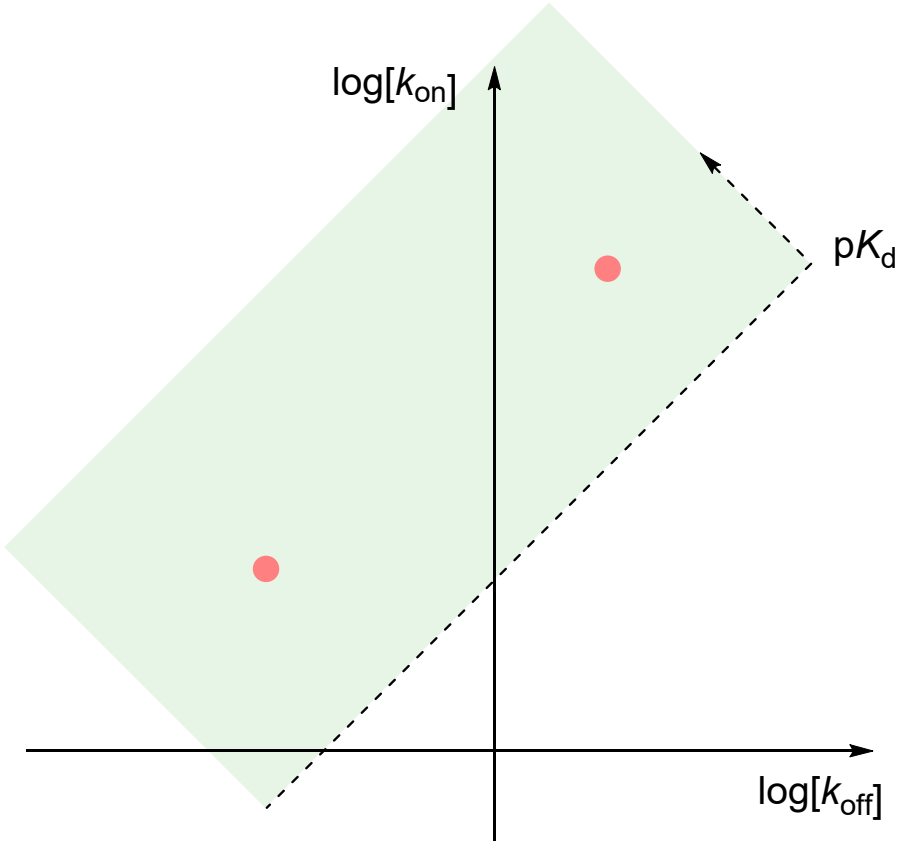
Roth, B. L. *et al. Cell* **2017**, *168*, 377.

Outline

- **Introductions**
  - Class A GPCRs
  - Ligand-target binding kinetics
- **Kinetics and dynamics of GPCR signaling**
  - G-protein pathway
  - β-arrestin pathway
- **Revisiting the ligand-target binding kinetics**
  - Examples
  - Structure-kinetic relationship
- **Summary**

Revisiting the Drug-target Interaction Kinetics

- Which one should dominate?
- Fast-off / slow-off / fast-on / slow-on



Advantages of Fast-off Kinetics

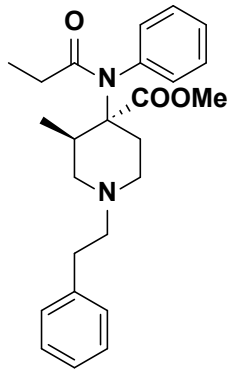
- **Fast-off** / slow-off / fast-on / slow-on
- **Reduce on-target toxicity**

Cmpd[a]	Affinity (K_i, nM)	Dissociation half-life
Haloperidol	1.8	72.4 sec
Nemonapride	0.025 (rat)	355 min (rat)
Spiperone	0.1 (rat)	200 min (rat)
Sertindole	1.2 (rat)	47 min (rat)
Chlorpromazine	1.3 (rat)	35 min (rat)
Raclopride	1.6 (rat)	28 min (rat)
Olanzapine	6.4 (rat)	18 min (rat)
Clozapine	137	14.5 sec
Quetiapine	67.6 (rat)	23 sec (rat)
JNJ-37822681	158	6.5 sec

- D_2R antagonist, antipsychotics
- Risk of extrapyramidal symptoms (EPS) is reduced (the receptors can still sense dopamine level burst)
- **Surmountable antagonism**

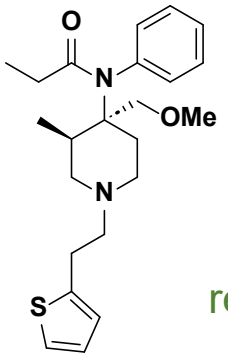
Seeman, P. *et al., N-S. Arch. Pharmacol.* **2012**, *385*, 337.
Seeman, P. *et al., J. Psychiatry. Neurosci.* **2000**, *25*, 161.

- **Enable fast reversal of overdose**



Lofentanil

τ_{off} ~ 260 min

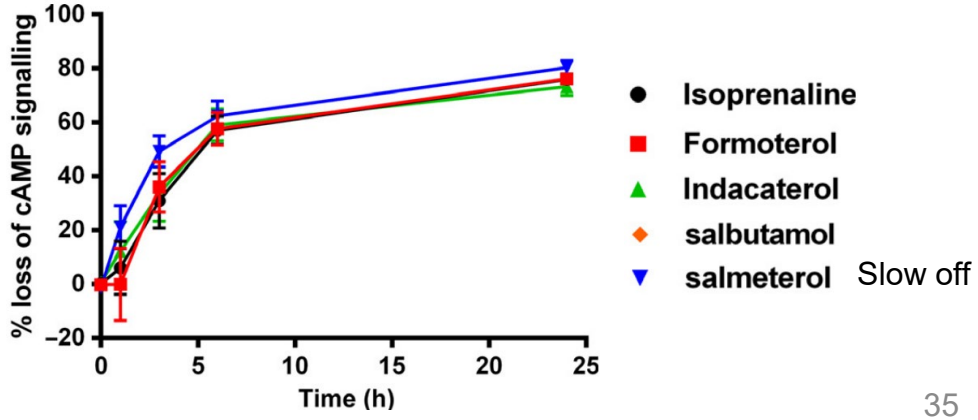


Sufentanil

τ_{off} ~ 16 s

recommended

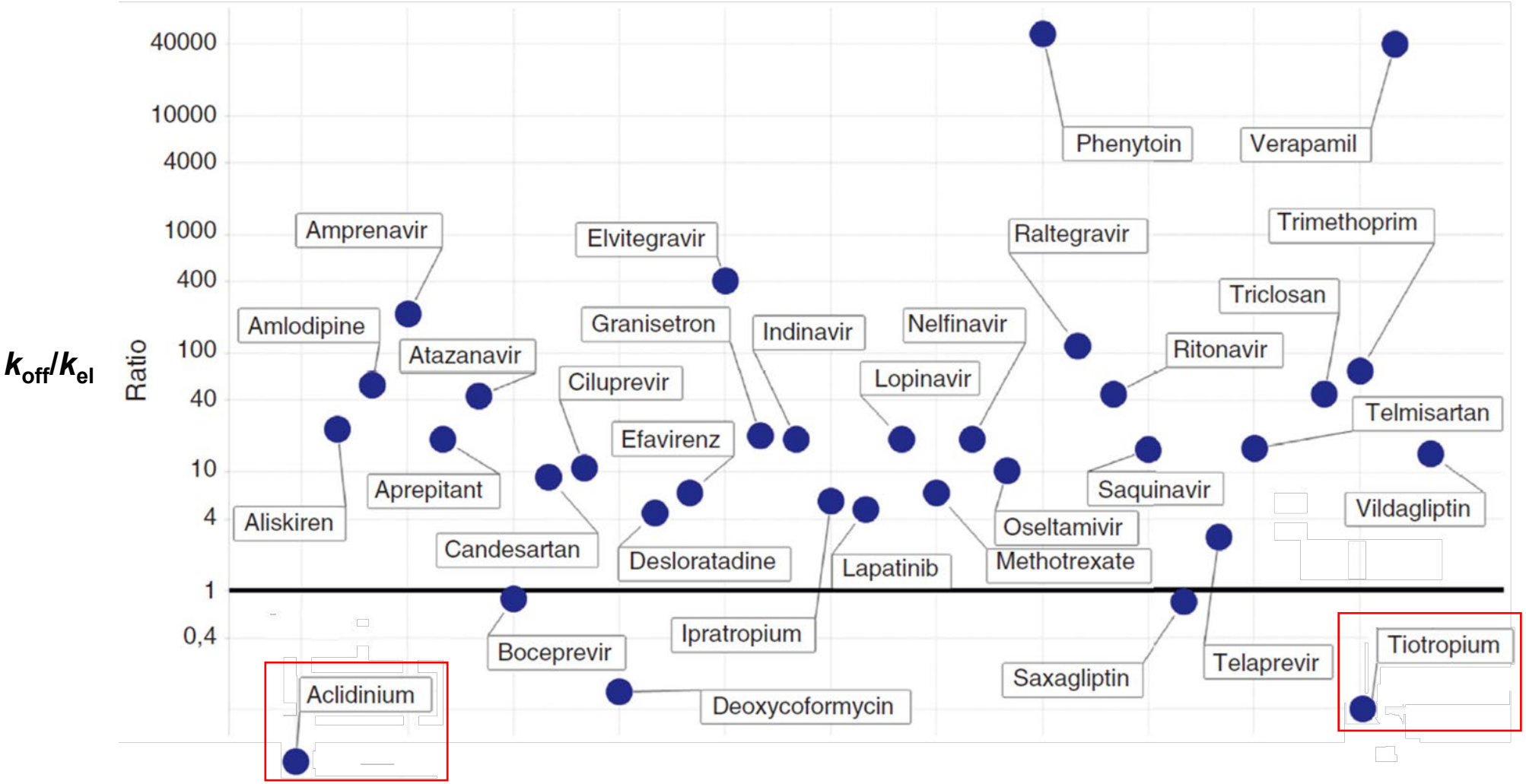
- **Reduce receptor desensitization?**
- In most research, reduced desensitization/internalization seems to correlate with prolonged incubation time.



Carlton, S. J. *et al., Pharma. Res. Per.* **2015**, *3, e000101.*

Advantages of Slow-off Kinetics

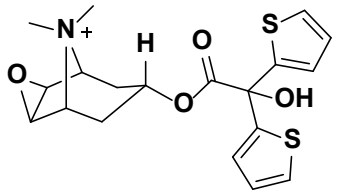
- Fast-off / **slow-off** / fast-on / slow-on



Thomas, A. *et al. Drug Discov. Today* **2013**, *18*, 697. (corrected)

Advantages of Slow-off Kinetics

- Fast-off / **slow-off** / fast-on / slow-on
- **Kinetic selectivity**



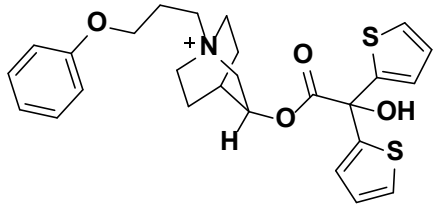
tiotropium

	K_D (nM)	$t_{½}$ (h)
M_1	0.041	14.6
M_2	0.021	3.6
M_3	0.014	34.7

- Long-lasting M_3R antagonist, anticholinergic bronchodilator for COPD.
- Low risk of side effects related to M_2R activation.

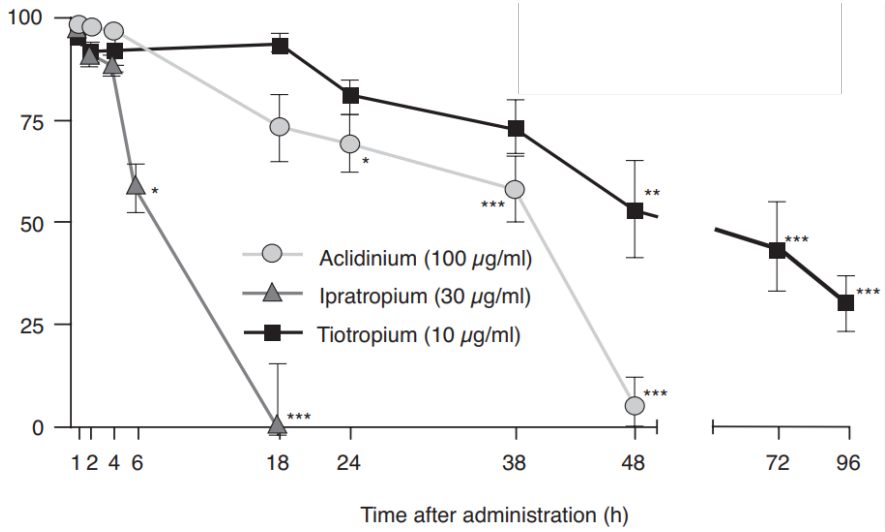
Cerasoli, F. *et al., Expert Opin. Drug Metab. Toxicol.* **2009**, *5*, 417.

- **Elongate duration of action**



aclidinium

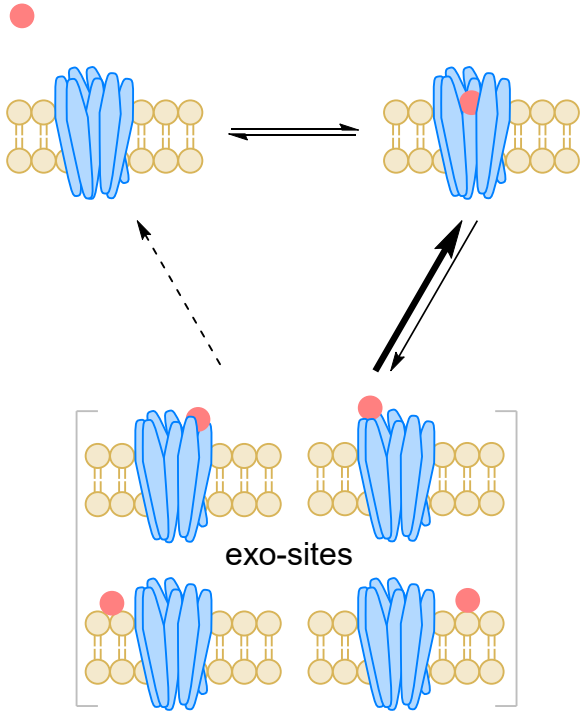
- Designed for **shorter** plasma half-life. ($t_{1/2}$ ~ 3 min!)
- Lower risk of **off-target side effects** while maintaining long half-life.



Hamishi, R. *et al., J. Med. Chem.* **2009**, *52*, 5076.

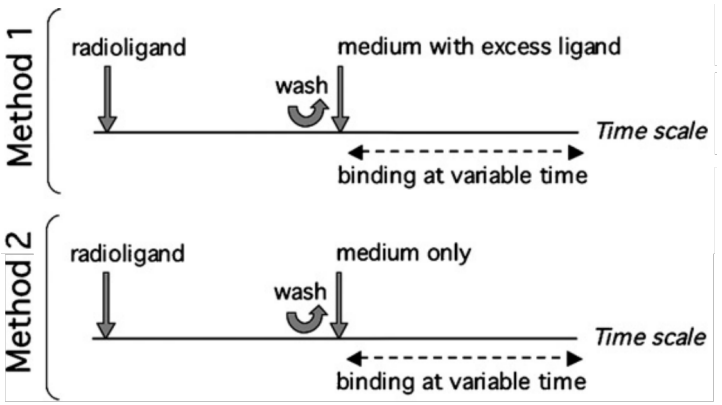
Advantages of Fast-on Kinetics

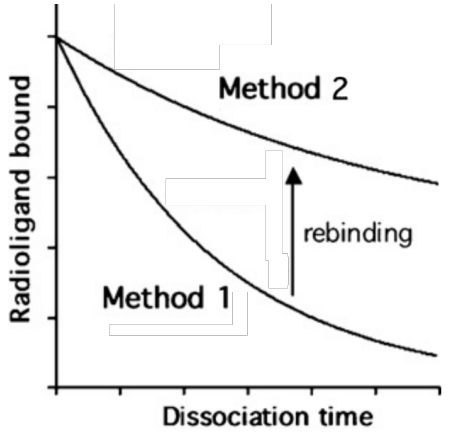
- Fast-off / slow-off / **fast-on** / slow-on
- **Fast onset**
- **Elongate duration of action!**

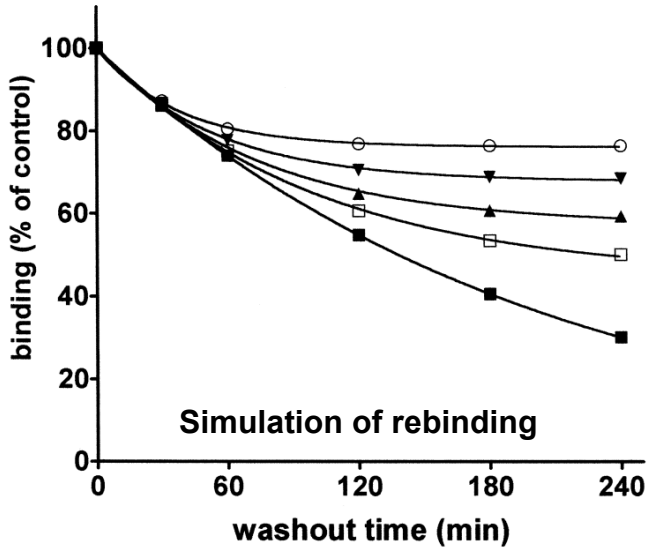


- Ligand rebinding mechanism prolongs drug residence time, especially for **fast-on** drugs.

Seeman, P. *et al., J. Psychiatry. Neurosci.* **2000**, *25*, 161.







accumulation-rebinding ($c_{local} = 8c_{overall}$) ~ Experimental
accumulation-rebinding ($c_{local} = 4c_{overall}$)
accumulation-rebinding ($c_{local} = 2c_{overall}$)
with rebinding

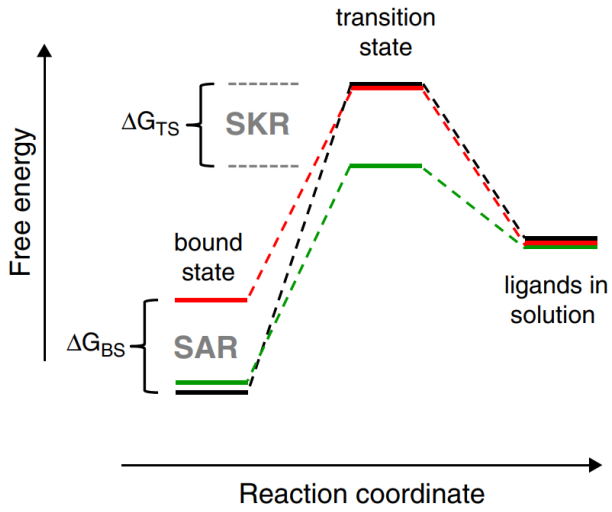
no rebinding

(Candesartan @ angiotensin II AT_1 receptor, CHO cell)

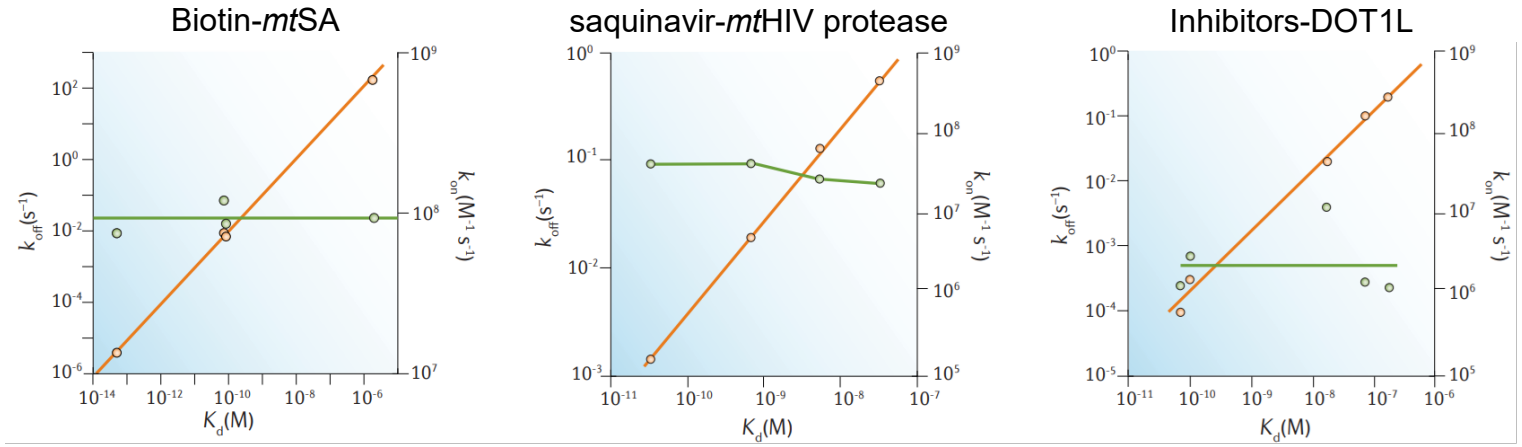
Vauquelin, G. *et al., Neurochem. Int.* **2007**, *51*, 254.
Vauquelin, G. *et al., Eur. J. Pharmacol.* ***1999***, *367*, 413.

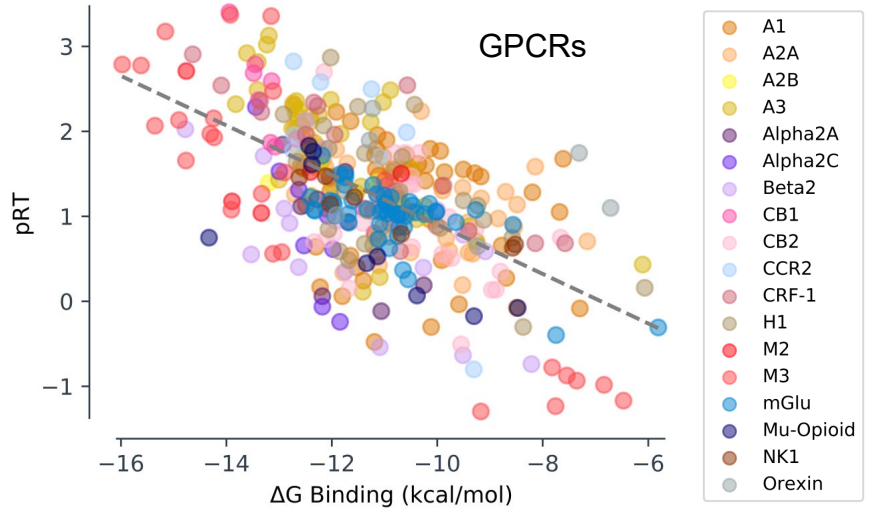
Structure-Kinetic Relationship

- Factors governing binding kinetics

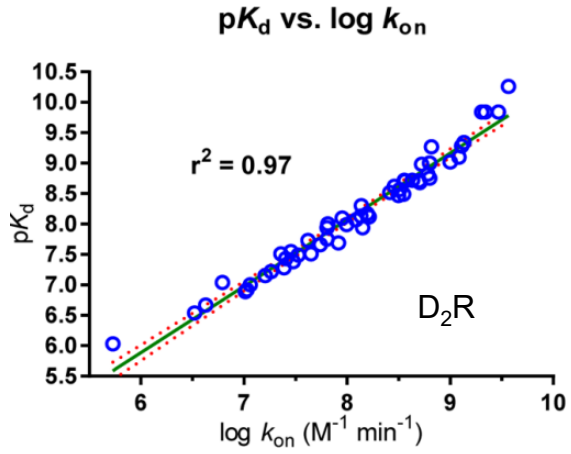


- k_{off} seems to correlate better with K_d





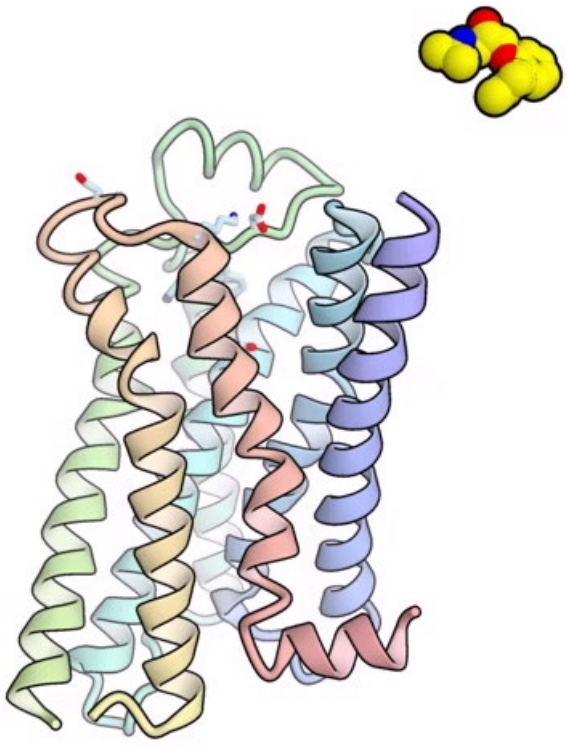
- with exceptions

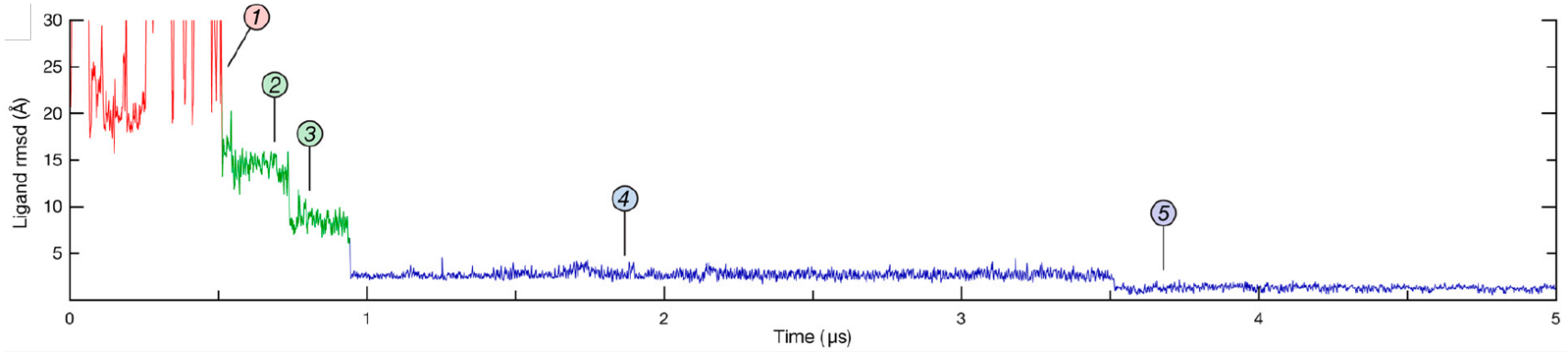


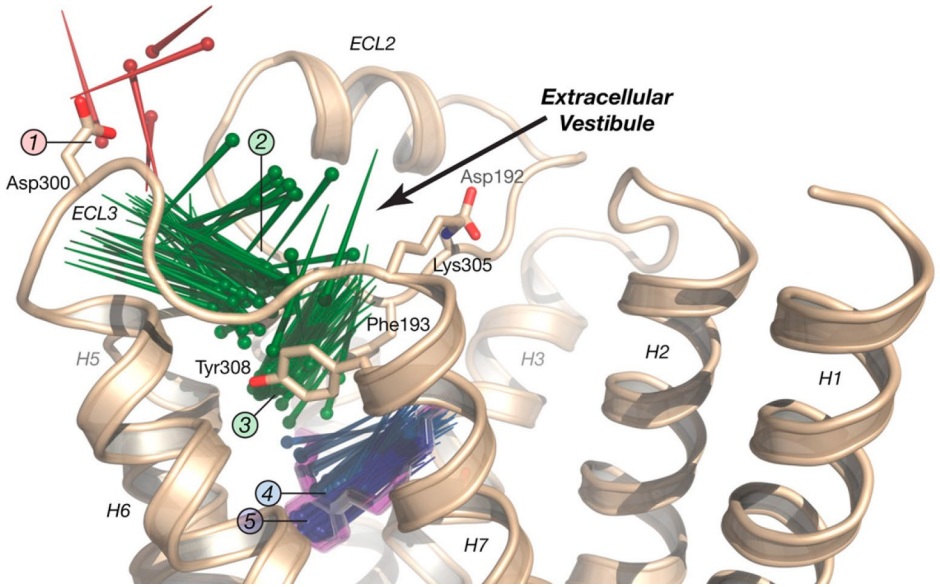
Copeland, R. A. *et al. Nat. Rev. Drug Discov.* **2016**, *15*, 87.
Mistry, S. N. *J. et. al., Med. Chem.* **2019**, *62*, 9488.
Potterton, A. G. *PhD. Thesis, Computational methods that predict residence times of GPCR ligands,* **2020**.

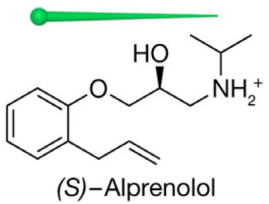
Structure-Kinetic Relationship

- The pathway of ligand binding to β_2AR by MD simulation







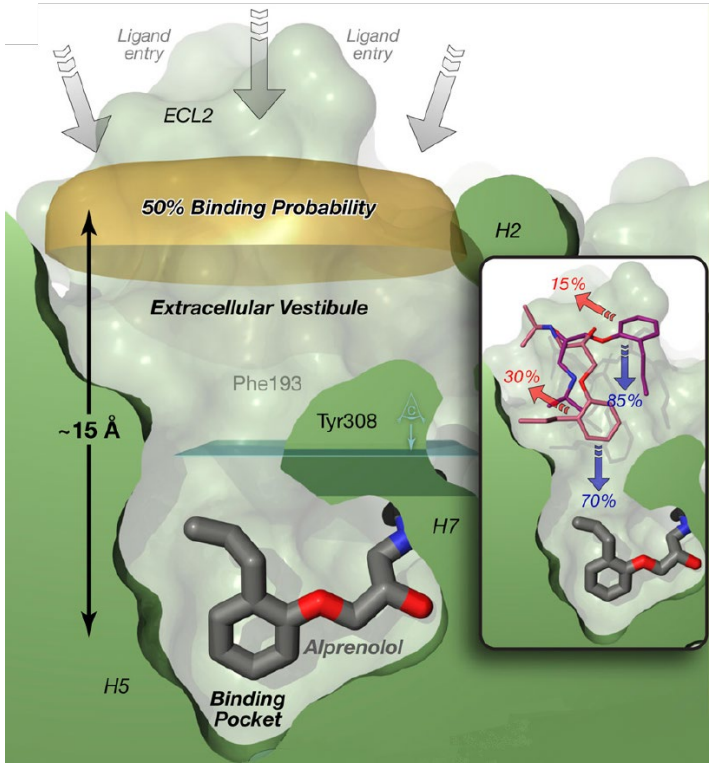


(S)−Alprenolol

Shaw, D. E. *et al. Proc. Nat. Acad. Sci.* **2011**, *108*, 13118.

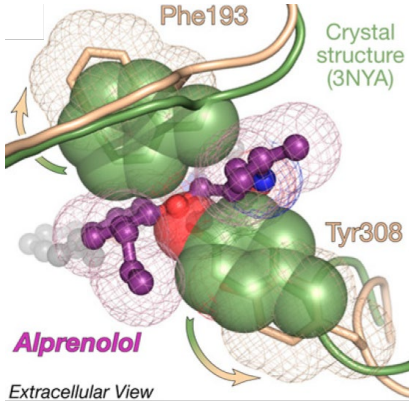
A Two-step Binding of Ligands at β_2AR

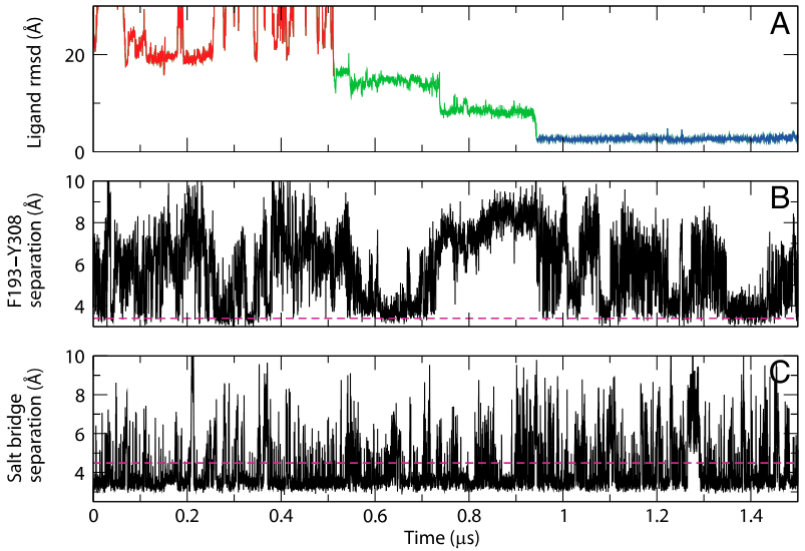
- The 1st barrier: dehydration
  - ~~Conformational change~~, ~~electrostatic interaction~~



  - 63% ligand dehydration
  - +500 Å^2 of hydrophobic surface area

- The 2nd barrier: dehydration ?

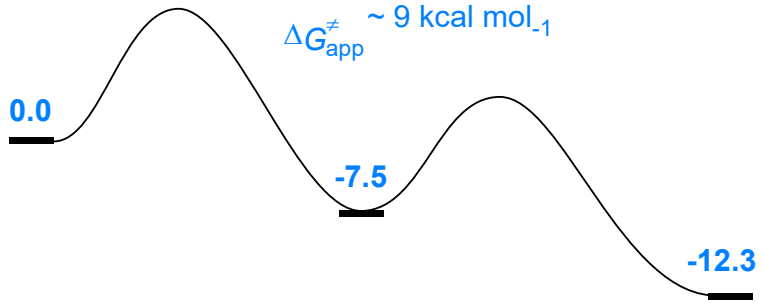




  - Most water molecule evacuates upon binding.

Dihydro Alprenolol-β_1AR dissociation:
ΔH° = −3.5 kcal mol^{-1}, $-T\Delta S^\circ$ = −8.8 kcal mol^{-1}

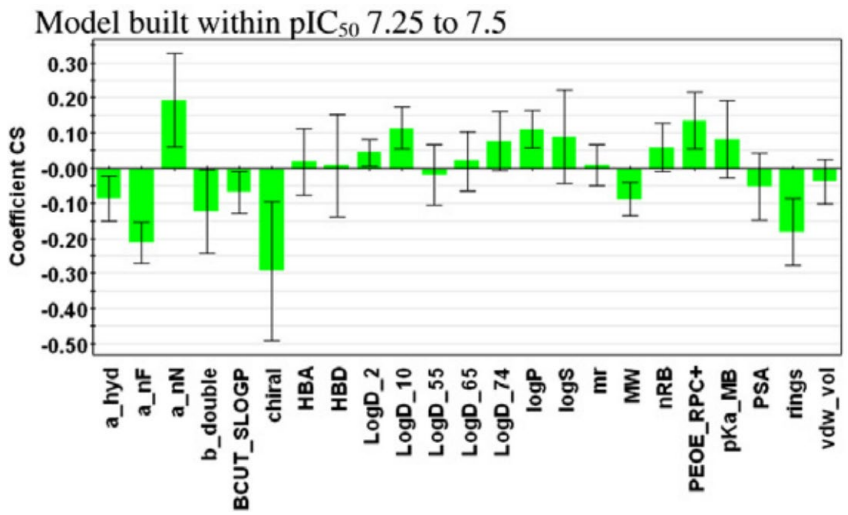
- Overall

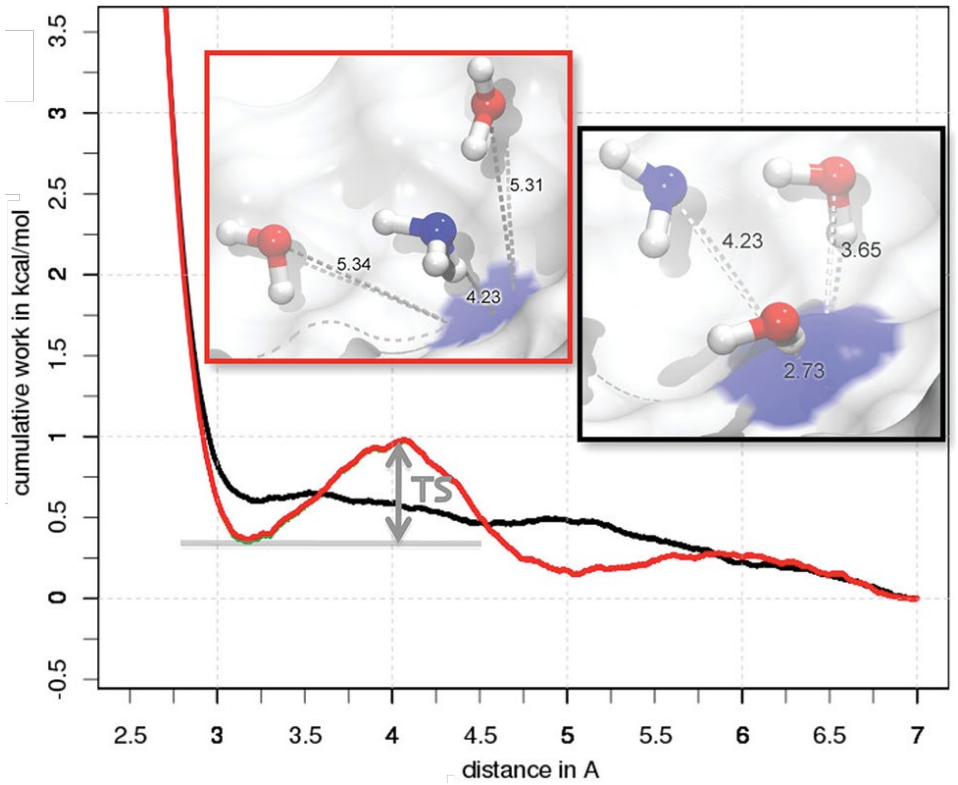


Shaw, D. E. *et al. Proc. Nat. Acad. Sci.* **2011**, *108*, 13118.

Structure-Kinetic Relationship

- Lipophilicity, MW., charge, rigidity
  - Library of D_2R antagonists
- Solvent-inaccessible hydrogen bonds
  - "almost buried polar atoms" (ABPAs)





Langlois, X. *et. al., Bioorg. Med. Chem.* **2011**, *19*, 2231.

Barril, X. *et. al., J. Am. Chem. Soc.* **2011**, *133*, 18903.

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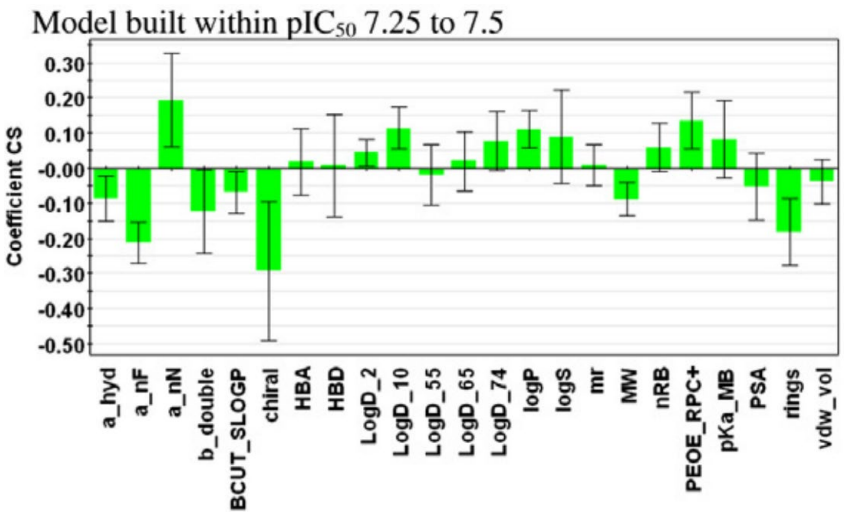
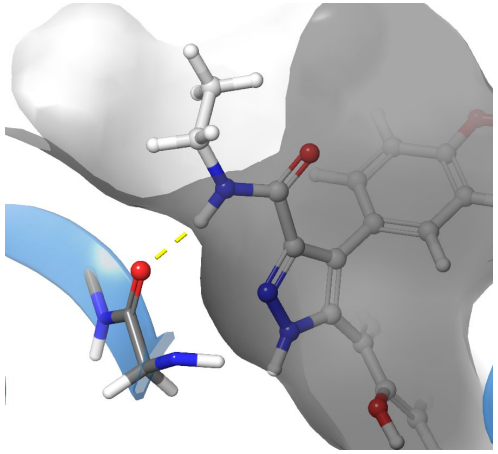
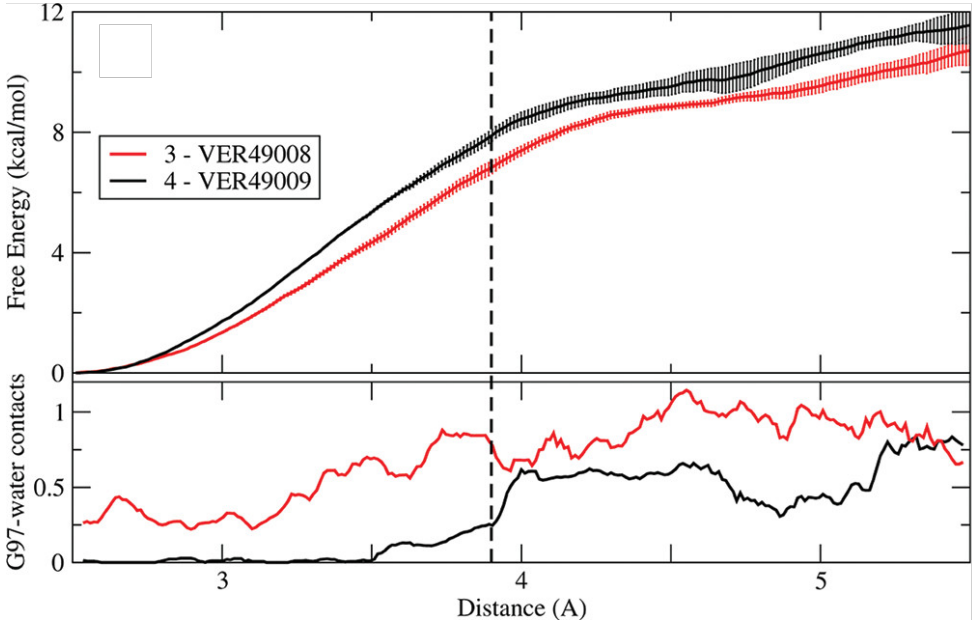




Hsp90-VER9008/9009



~ 0.3 kcal mol^{-1} decrease in ΔG
~ 0.3 kcal mol^{-1} increase in $\Delta G^{\neq}$
1.3/0.7 in case of w *or* w/o

Langlois, X. *et. al., Bioorg. Med. Chem.* **2011**, *19*, 2231.

Barril, X. *et. al., J. Am. Chem. Soc.* **2011**, *133*, 18903.

Structure-Kinetic Relationship

- Receptor-wise

Mechanisms that modulate the life-time of the drug–target complex

Target	Mechanism	Compound
S. aureus FabI	Ordering of the substrate binding loop (SBL).	Alkyl diphenyl ether **PT119**, t_R 12.5 h, 20 °C [15].
M. tuberculosis FabI	Steric clash in the TS between SBL helix-6 and 7 [16].	Triazole diphenyl ether **PT504**, t_R 10 h, 25 °C [17].
Thermolysin	Interaction with Asn112 prevents conformational change required for ligand release [18].	Phosphonopeptide **18**, t_R 168 days.
Purine nucleoside phosphorylase	Gating mechanism involving rotation of Val260 [19].	**DADMe–immucillin-H**, t_R 12 min 37 °C [20•].
Hsp90	Entropically driven binding affects on and off rates [21].	Phenyl-triazole-benzamide **20** t_R 51 min.
IDH2/R140Q	Loop motion associated with an allosteric binding site [22].	**AGI-6780**, t_R 120 min.
DOT1L	Occupancy of binding pocket adjacent to SAM binding site [23].	**EPZ004777**, t_R 55 min, 25 °C.
Soluble epoxide hydrolase	Interactions with Tyr153 and Met189 control TS stability. [24].	**TPPU** t_R 11 min.
Heat shock protein 90	Ligand desolvation: polar substituents decrease k_{on} [25].	Indazole **3c**, t_R 57 min, 25 °C.
CDK2/9	Type I. Conformational change in DFG loop.	**Roniciclib**, t_R 400 min [26].
CDK8/CycC	Type II. H-bonds with hinge and hydrophobic contacts with front pocket.	Pyrazole **2**, t_R 32 h [27].
p38aMAP kinase	Type 1.5. Disruption of the R-spine.	Dibenzosuberone **6g**, t_R 32 h [28].
RIP1 kinase	Type II/III. Increase in cLogP reduced k_{off}.	Benzoxazepine **22**, t_R 5 h [29].
Btk	Reversible covalent. Steric hindrance of α-proton abstraction [30••].	Pyrazolopyrimidine **9**, t_R 167 h.
Btk	Irreversible. Reduced electrophilicity increases selectivity.	**Acalabrutinib** [31].
CCR5	Allosteric ligand with alternative receptor conformation.	**873140**, $t_R >$ 136 h, RT [32].
Muscarinic M3 receptor	Coulomb repulsion between ligand and Lys523 [33•].	**Tiotropium**, t_R 39 h.
β_2 adrenergic receptor	Ligand desolvation [34].	**Alprenolol**, t_R 4 min, 37 °C.
Adenosine A_{2A} receptor	ETH triad forms a lid preventing ligand dissociation [35].	**ZM241358**, t_R 84 min.

- Computation-aided

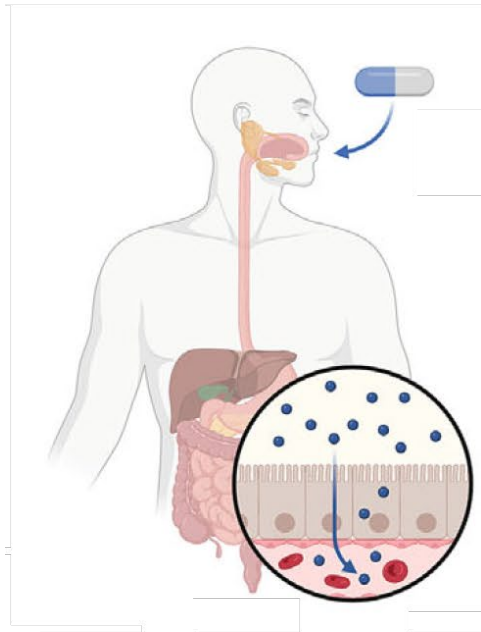
Langlois, X. *et. al., Curr. Opin. Chem. Biol.* **2018**, *44*, 101.

Outline

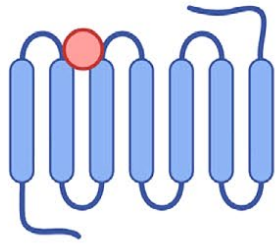
- **Introductions**
  - Class A GPCRs
  - Ligand-target binding kinetics
- **Kinetics and dynamics of GPCR signaling**
  - G-protein pathway
  - β-arrestin pathway
- **Revisiting the ligand-target binding kinetics**
  - Examples
  - Structure-kinetic relationship
- **Summary**

Summary

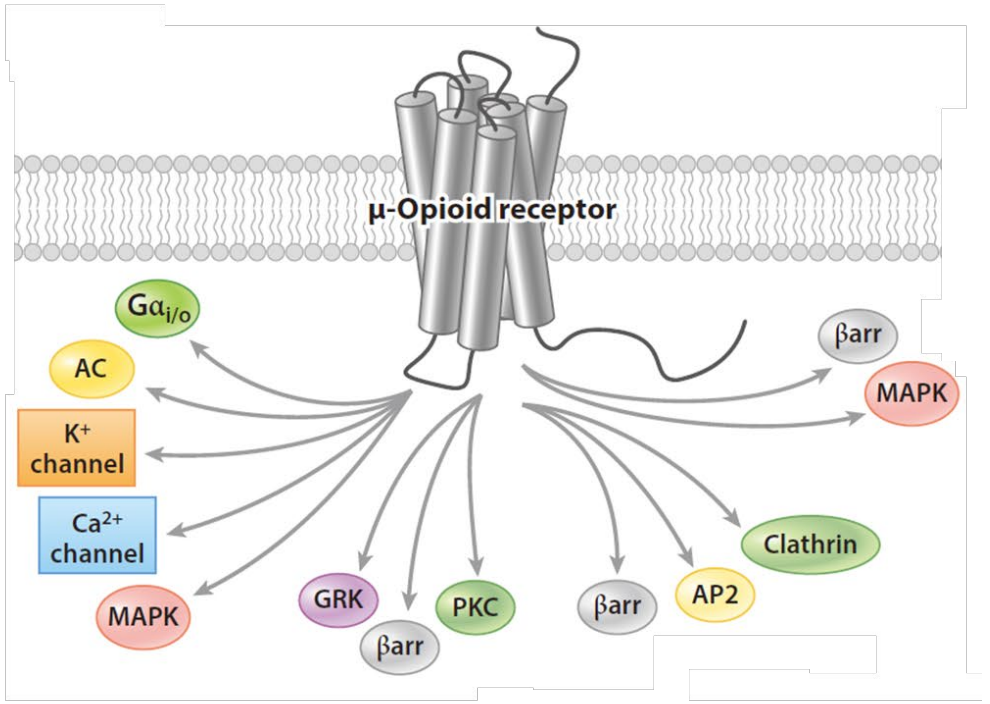
- On the macro-and micro level



Pharmacokinetics



On-target toxicity
Drug resistance
Selectivity
Duration of action
Onset rate
……



Signaling-kinetics

Spatiotemporally biased signaling

- A reproducible approach for **bias** determination is needed
- QSKR