- **GPCRs**
- Binding/unbinding protocol
  - Small ligands
  - Extension to peptides
- Activation/ Deactivation protocol
- **Conclusions**

Ligand Binding/Unbinding and Activation/Deactivation Profiles for G-Protein Coupled Receptors

Tim Clark

Jacqueline Calderón, Dr. Passainte Ibrahim — Friedrich-Alexander-Universität Erlangen-Nürnberg

Prof. Francesco L. Gervasio, Dr. Dorothea Gobbo — University of Geneva

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GPCR Versatility

- **GPCRs**
- Binding/unbinding protocol
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  - Extension to peptides
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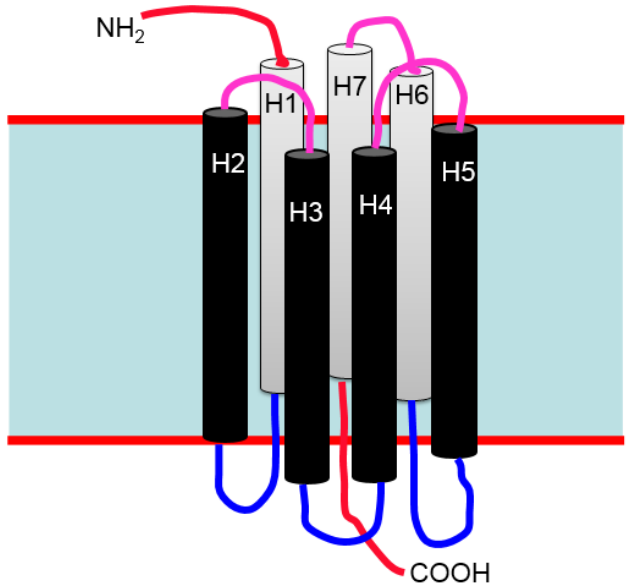
- **Conclusions**

The ligand may be a sensory switch, an agonist, antagonist, inverse or partial agonist, ***and may act differently for different intracellular binding partners (IBPs).***

There is usually either positive or negative feedback (cooperativity) between the ligand and the IBP.



The IBP may be one or more of three different G-Proteins or β-Arrestin

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G-Protein Activation: *Apo*-GPCR

- **GPCRs**
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  - Small ligands
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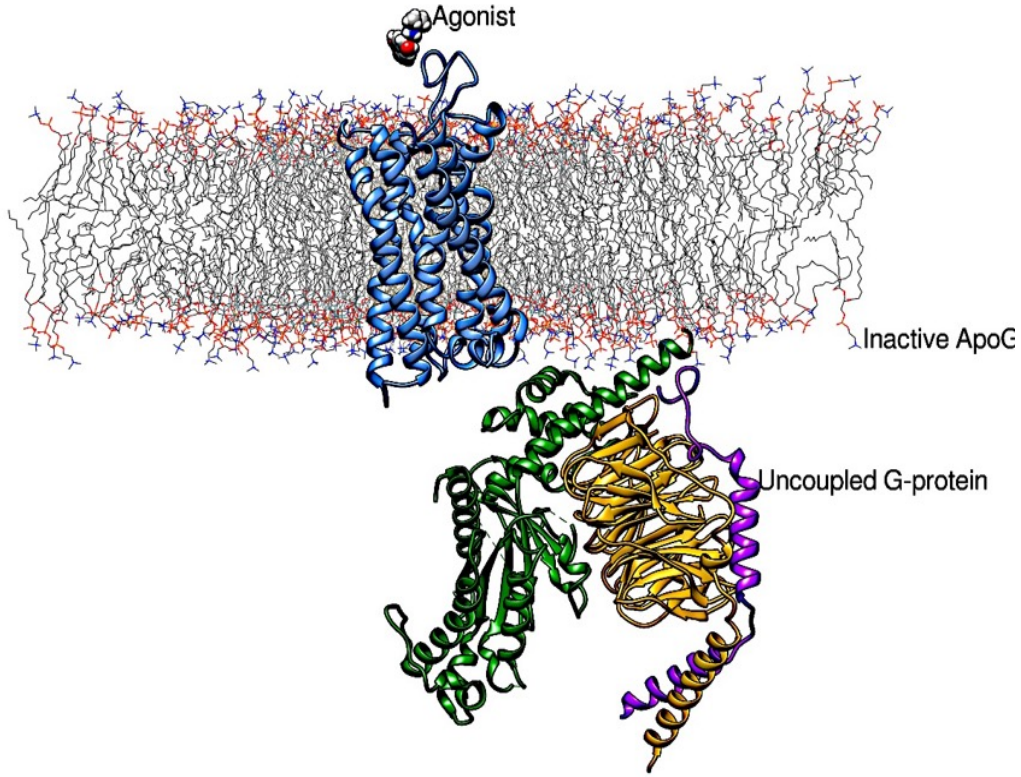
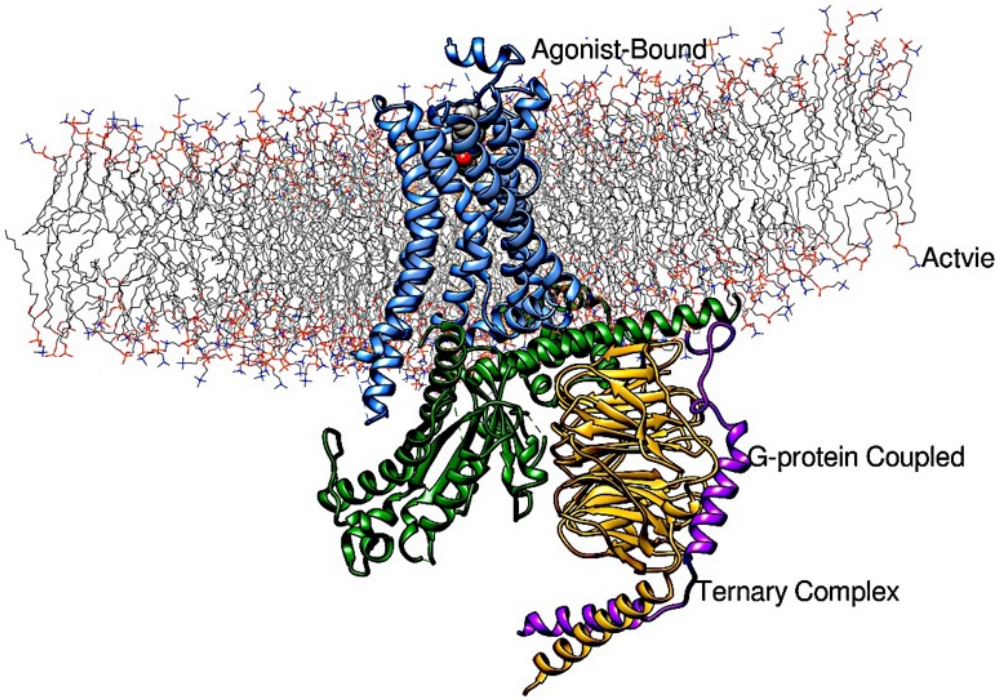
- **Conclusions**

(Basal Activity)



Agonist-Induced Activity



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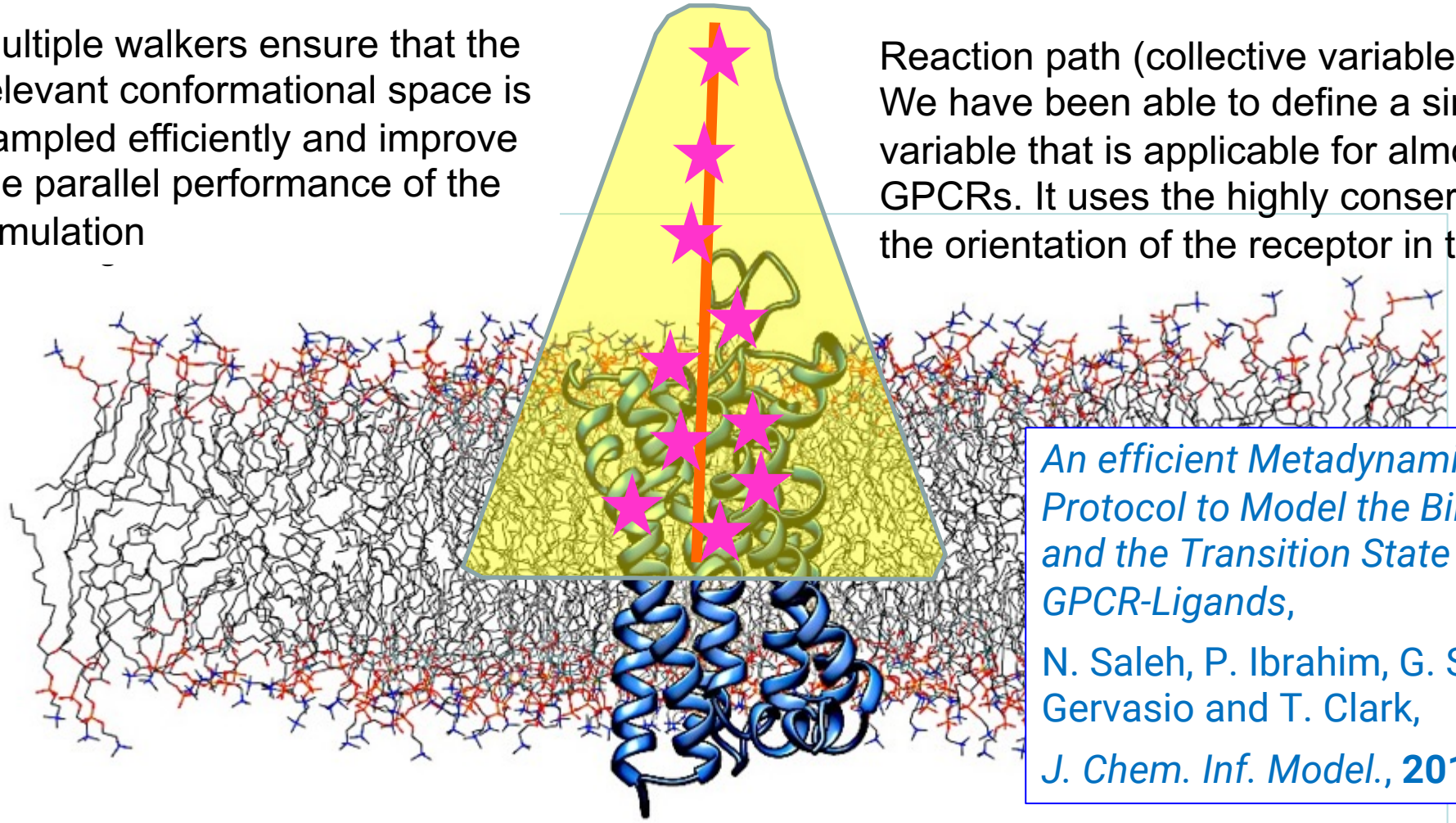
Binding/Unbinding Metadynamics Protocol

- **GPCRs**
- Binding/unbinding protocol
  - Small ligands
  - Extension to peptides
- Activation/ Deactivation protocol
- **Conclusions**

Multiple walkers ensure that the relevant conformational space is sampled efficiently and improve the parallel performance of the simulation



Reaction path (collective variable)
We have been able to define a single collective variable that is applicable for almost all class A GPCRs. It uses the highly conserved $Trp^{6.48}$ and the orientation of the receptor in the membrane.

An efficient Metadynamics-based Protocol to Model the Binding Affinity and the Transition State Ensemble of GPCR-Ligands,

N. Saleh, P. Ibrahim, G. Saladino, F. L. Gervasio and T. Clark,

J. Chem. Inf. Model., **2017**, *57*, 1210

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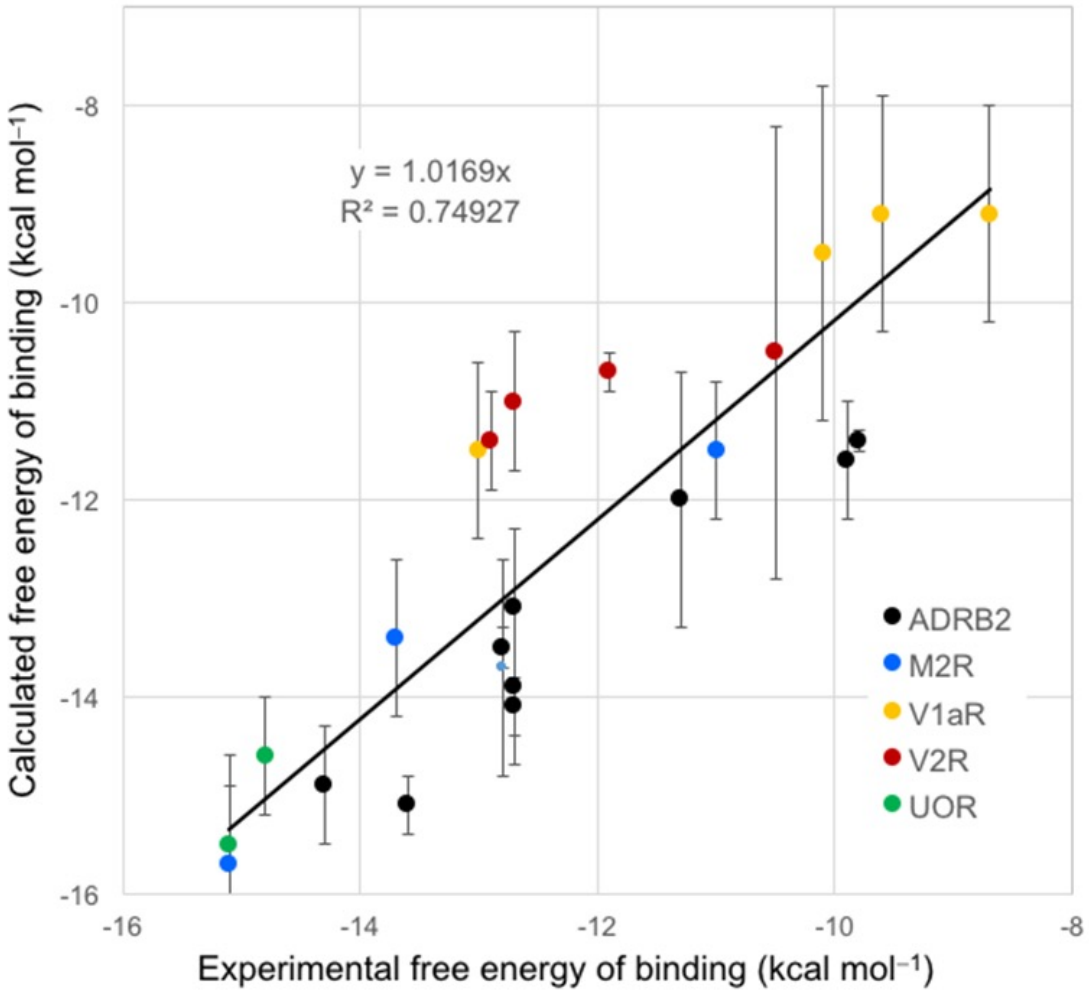
Predicting binding free energies

- **GPCRs**
- Binding/unbinding protocol
  - Small ligands
  - Extension to peptides
- Activation/ Deactivation protocol
- **Conclusions**



5 Receptors
13 Ligands
23 Data points
Binary and ternary complexes
Up to 3 binding sites per receptor
Ligands act as:

- 10 agonists
- 11 antagonists
- 2 partial agonists

8 receptor structures based on equilibrated homology models

MSE = -0.13 kcal mol^{-1}
MUE = 0.66 kcal mol^{-1}
RMSE = 0.82 kcal mol^{-1}

An efficient Metadynamics-based Protocol to Model the Binding Affinity and the Transition State Ensemble of GPCR-Ligands,

N. Saleh, P. Ibrahim, G. Saladino, F. L. Gervasio and T. Clark,

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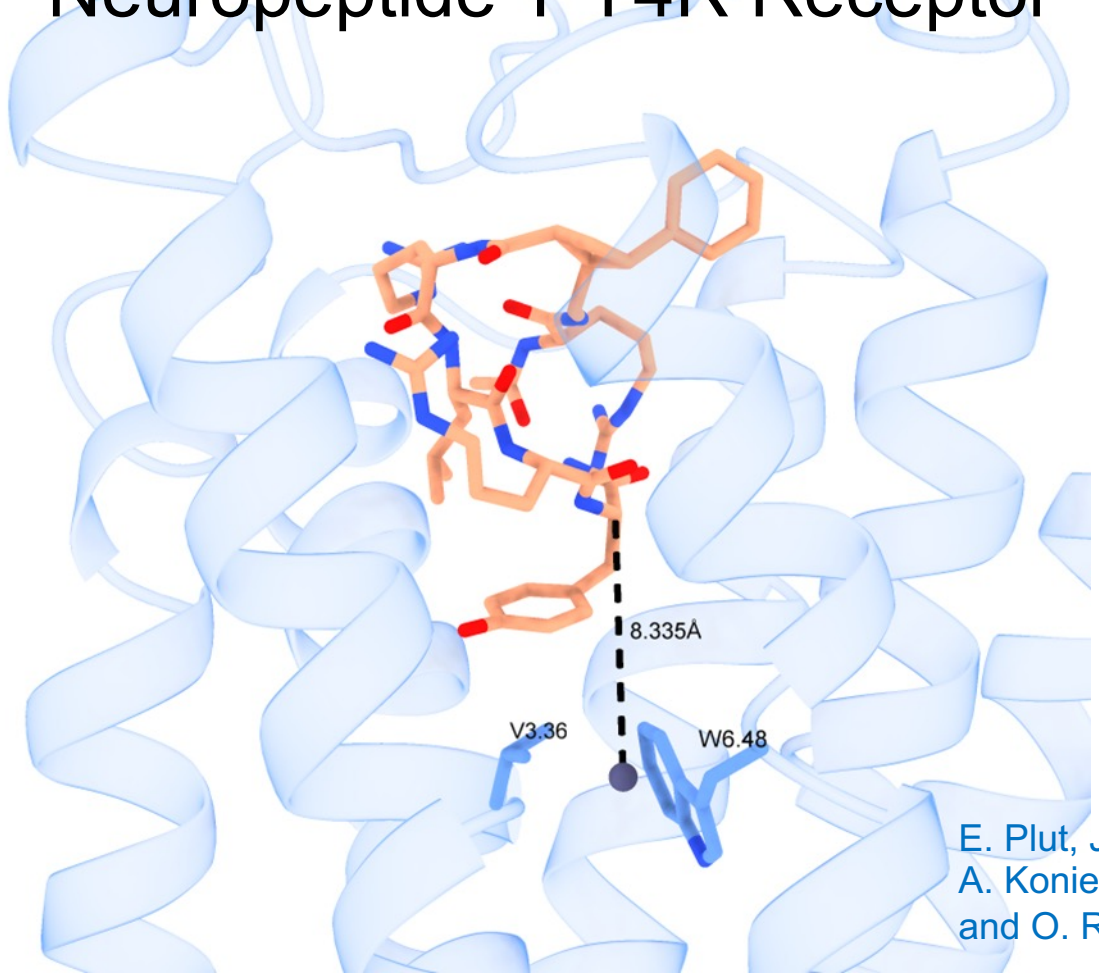
Extension to binding/unbinding of peptides

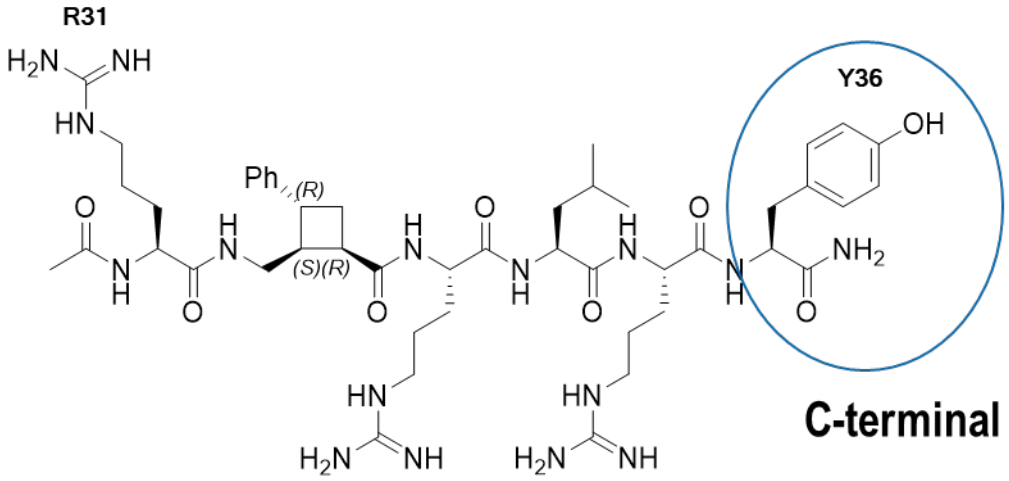
- **GPCRs**
- Binding/unbinding protocol
  - Small ligands
  - Extension to peptides
- Activation/ Deactivation protocol
- **Conclusions**

Neuropeptide Y Y4R Receptor



Collective Variable for binding/unbinding peptides

- The z component of the distance between the geometric center of Trp6.48 and V3.36 (Cα) and the peptide C-terminal (Cα)



E. Plut, J. Calderón, V. Stanojlović, A. O. Gattor, C. Höring, L. Humphrys, A. Konieczny, S. Kerres, M. Schubert, M. Keller, C. Cabrele, Timothy Clark, and O. Reiser, submitted to Chem. Sci.

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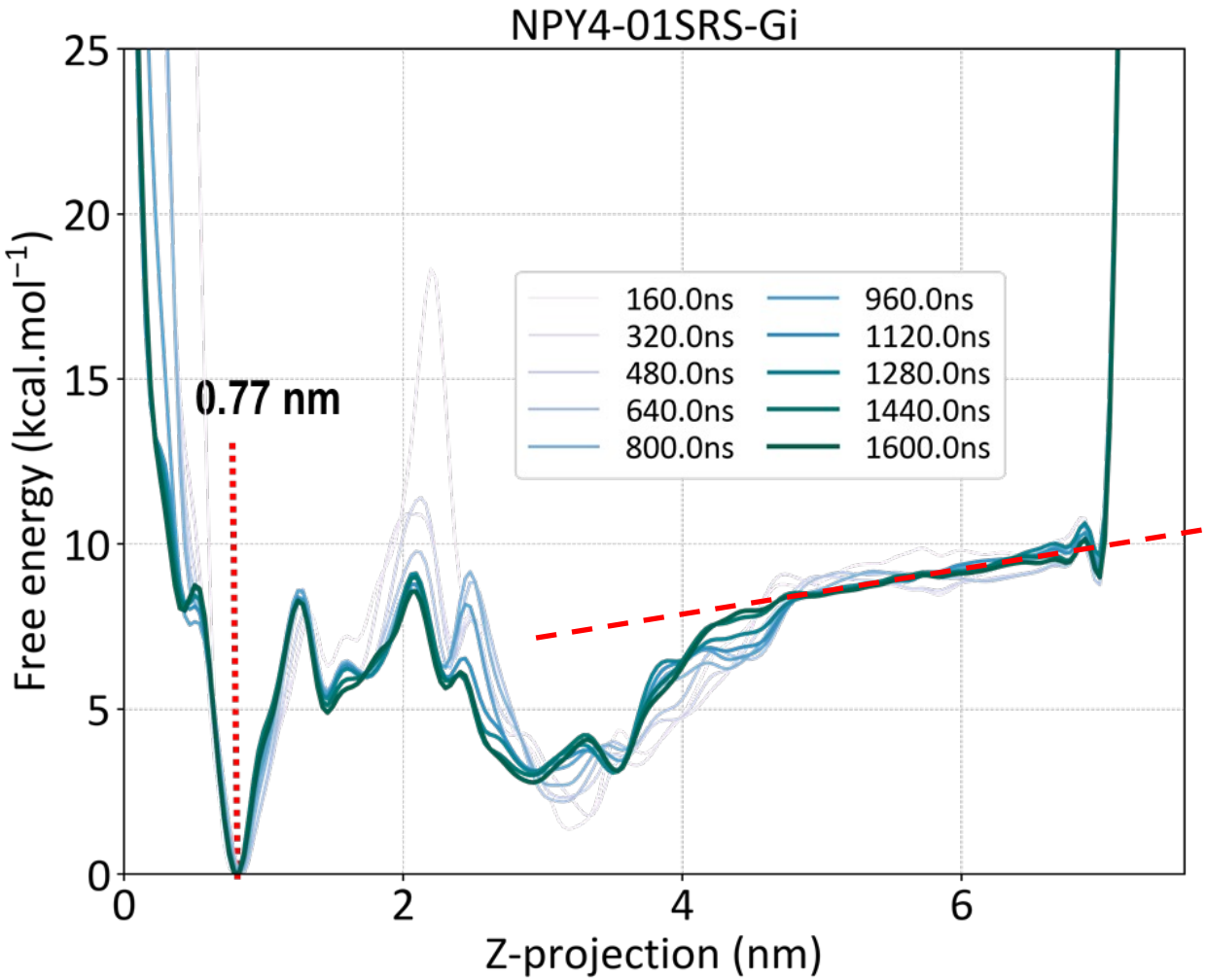
Free-Energy Profile using the Standard Protocol

- **GPCRs**
- Binding/unbinding protocol
  - Small ligands
  - Extension to peptides
- Activation/ Deactivation protocol
- **Conclusions**



Free-energy not constant in the extracellular medium

- Need to extend the CV further
- Requires changes to the funnel constraint

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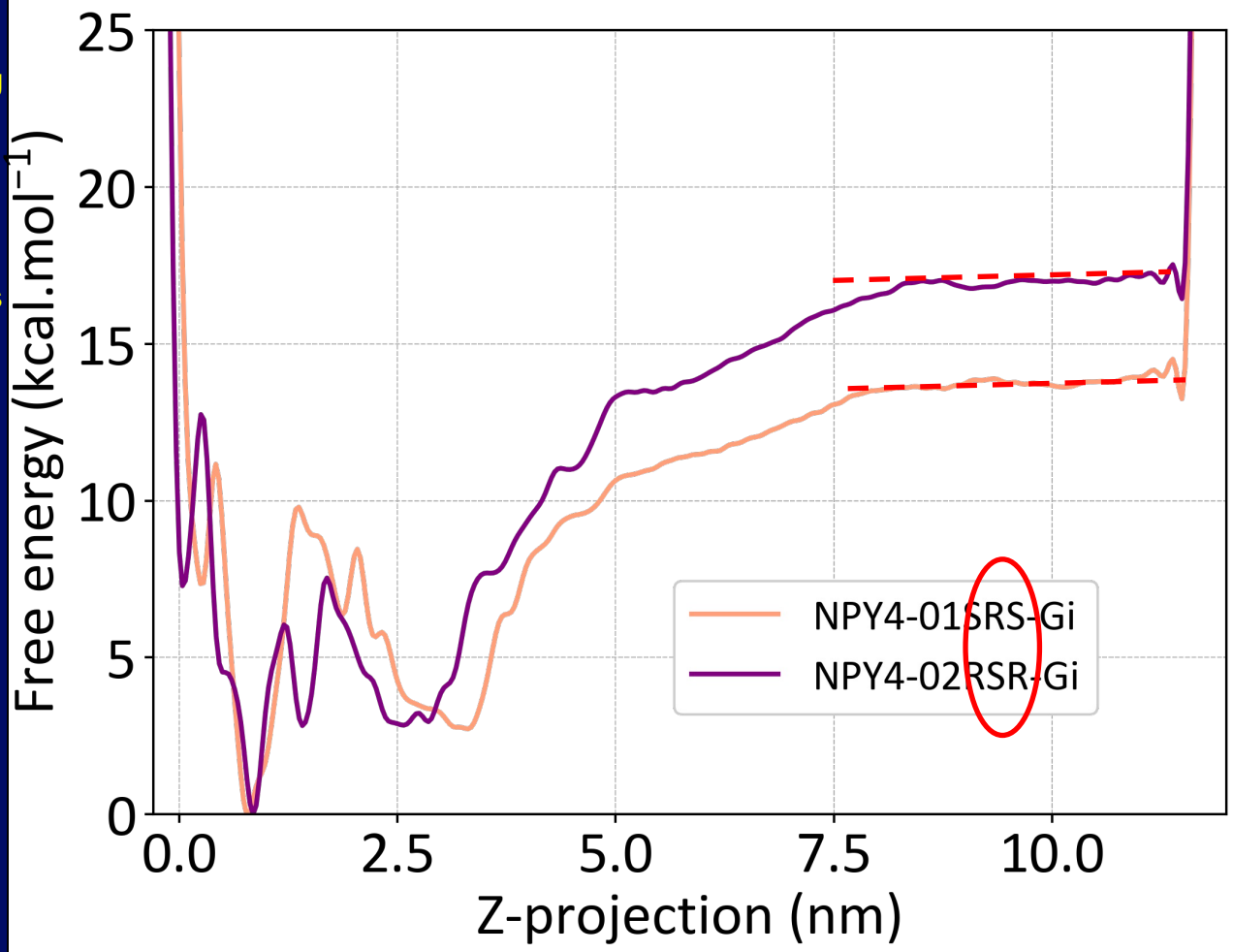
Results: Enantiomeric Y-Peptide analogs

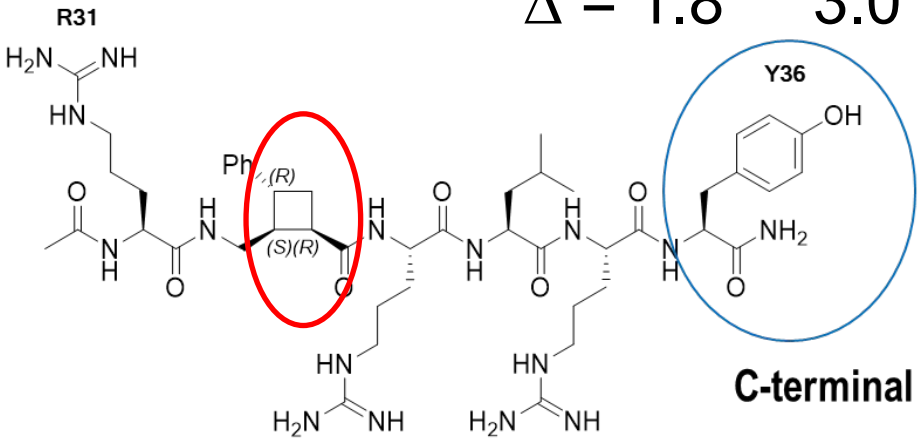
- **GPCRs**
- Binding/unbinding protocol
  - Small ligands
  - Extension to peptides
- Activation/ Deactivation protocol
- **Conclusions**



ΔG (kcal.mol^{-1})

System	1st min	Exp	Calc
NPY4-01SRS-Gi	0.77 nm	-11.2	-10.6
NPY4-02RSR-Gi	0.84 nm	-13.0	-13.6

Δ = 1.8 3.0



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A^{100} Activation Index : Protocol

- **GPCRs**
- Binding/unbinding protocol
  - Small ligands
  - Extension to peptides
- Activation/ Deactivation protocol
- **Conclusions**

1. Partially structure-based alignment; helices only without loops/termini. Python script available as SI or interactive A^{100} web site
   - https://www.chemistry.nat.fau.eu/ccc/a100
2. Extract five inter-helix C_α-C_α distances
3. Apply linear combination formula (H4R residues are indicated):

$$A^{100} = -14.43 \times r(V^{1.53}-L^{7.55}) - 7.62 \times r(D^{2.50}-T^{3.37}) + 9.11 \times r(N^{3.42}-I^{4.42}) - 6.32 \times r(W^{5.66}-A^{6.34}) - 5.22 \times r(L^{6.58}-Y^{7.35}) + 278.88$$

- *A Universal Activation Index for Class A GPCRs*, P. Ibrahim, D. Wifling and T. Clark. *J. Chem. Inf. Model.* **2019**, *59*, 3938-3945.

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X-ray Structures: Confusion Matrix

- **GPCRs**
- Binding/unbinding protocol
  - Small ligands
  - Extension to peptides
- Activation/ Deactivation protocol
- **Conclusions**

***n* = 268**		**assigned experimentally**		
Three-State model:		active	intermediate	inactive
Borders		>55	0-55	<0
Predicted	active	42	3	0
	intermediate	24	26	19
	inactive	1	3	150
		62.7%	81.3%	88.8%

Two-State model:		active	inactive
Borders		>25	<25
Predicted	active	63	2
	inactive	4	167
		94.0%	98.8%

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A^{100} as Activation CV

- **GPCRs**
- Binding/unbinding protocol
  - Small ligands
  - Extension to peptides
- Activation/ Deactivation protocol
- **Conclusions**

- A^{100} is a linear combination of five Cα-Cα distances. It can therefore be used as a collective variable for metadynamics simulations of GPCR activation with PLUMED.
- A^{100}, like RMSDs, is not unique to a single structure. It is therefore necessary to use additional sampling techniques (in our case, multiple walkers) and select the walkers for multiple-walker metadynamics very carefully.
- The standard simulation protocol involves:
  - Preliminary single-walker metadynamics simulation to select the walkers for the production simulation
  - Multiple-walker well tempered metadynamics between approximately $-100 < A^{100} > 100$

ADRB2

- **GPCRs**
- Binding/unbinding protocol
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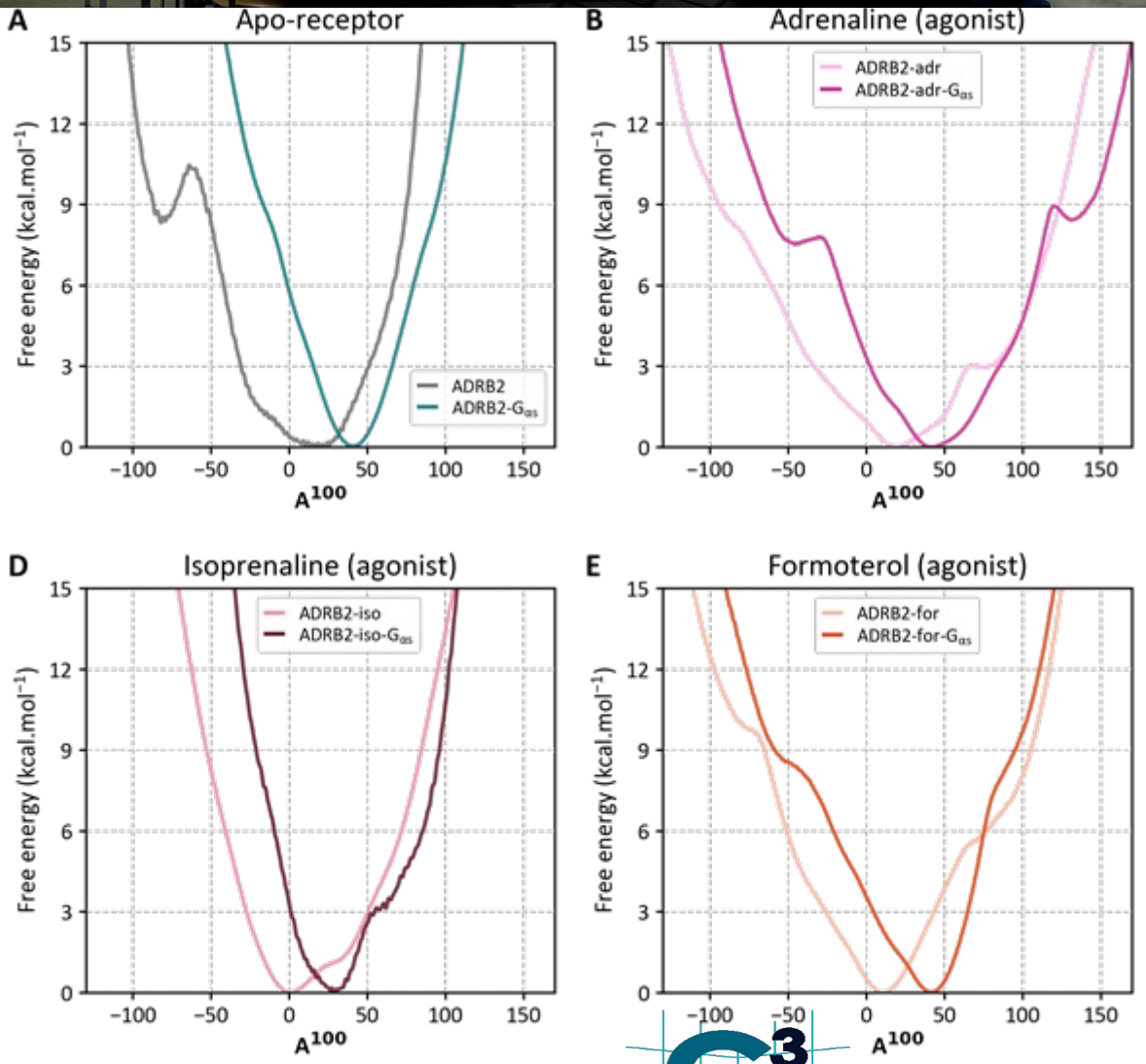
- Activation/ Deactivation protocol
- **Conclusions**

Systems	A^{100} MD	A^{100} MetaD
ADRB2	18.4	20.2
ADRB2-G_{as}	40.4	41.3
ADRB2-adrenaline	-5.2	20.4
ADRB2-adrenaline-G_{as}	34.6	42.2
ADRB2-BI167107	-30.6	-42.3
ADRB2-BI167107-G_{as}	36.2	30.6
ADRB2-isoprenaline	4.2	0.8
ADRB2-isoprenaline-G_{as}	54.5	29.0
ADRB2-formoterol	11.3	10.0
ADRB2-formoterol-G_{as}	43.1	41.6



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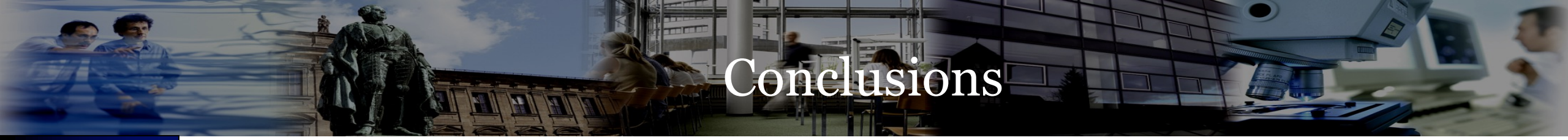

Conclusions

- **GPCRs**
- Binding/unbinding protocol
  - Small ligands
  - Extension to peptides
- Activation/ Deactivation protocol
- **Conclusions**

- Simulations provide comparably accurate and reliable binding free energies for GPCRs as experiment.
- Multiple binding sites are the rule
- The “small molecule” protocol can be extended to peptide ligands
- Activation can be characterized using a simple linear model that relies on five C_α-C_α distances.
- Activation free-energy profiles consistent with experiment
- Partial agonists act as full agonists in ternary complexes but as partial antagonists in binary ligand-receptor complexes

Acknowledgments

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- **Conclusions**

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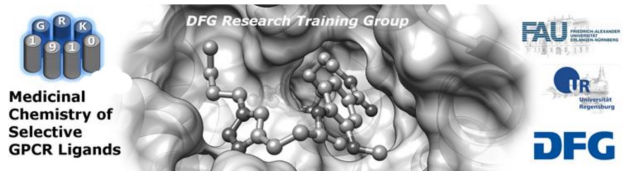
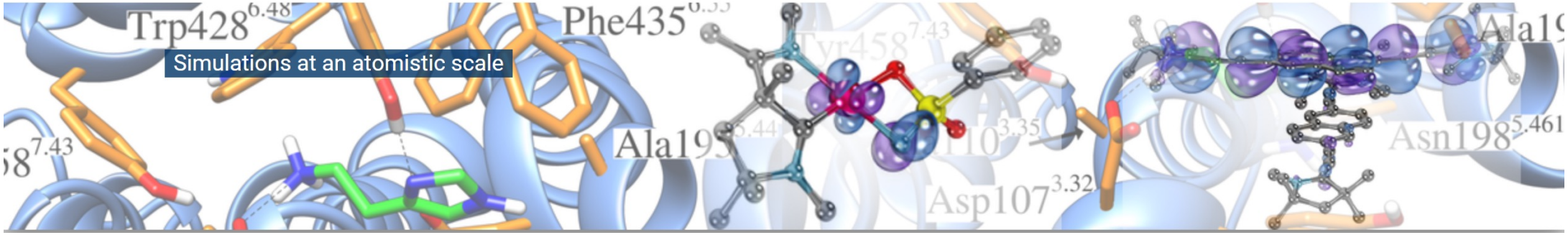
- Prof. Francesco Gervasio, Dr. Dorothea Gobbo, University of Geneva
- Prof. Oliver Reiser, PD Dr. Max Keller, University of Regensburg



FAU Atomic Structure Simulation Lab



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