Reducing stress in honey bees

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Reduction of stress responses in honey bees by synthetic ligands targeting an allatostatin receptor.

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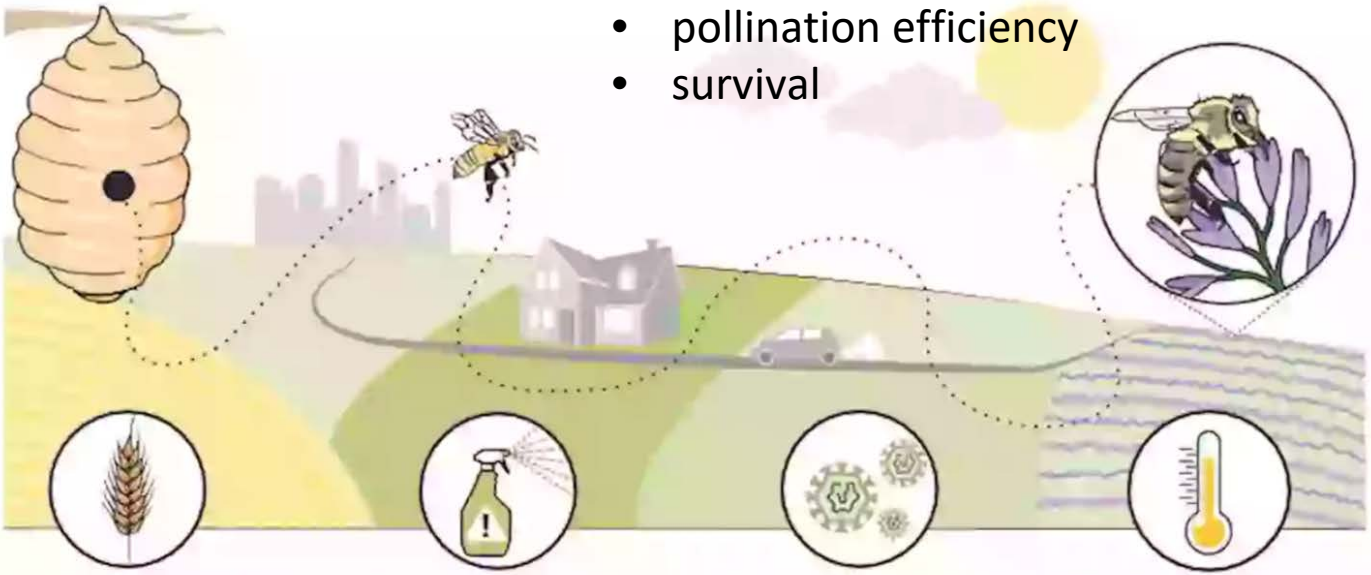
Pollination of up to a third of all the food we eat

Anthropogenic factors that affect bee foraging efficiency

Anthropogenic changes can affect the ability of bees to efficiently forage for food, which can reduce the survival of solitary and social bees.

- pollination efficiency
- survival



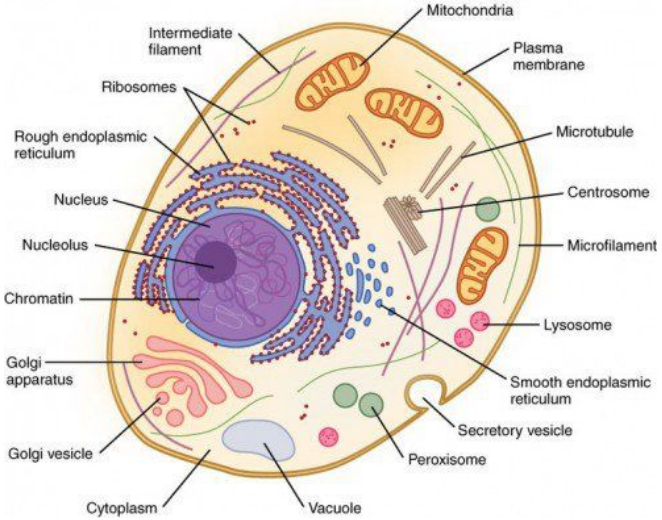
Habitat fragmentation
Agriculture and/or urbanization cause bees to travel further from their nest to find food. This could especially affect smaller species or those exposed to pesticides and/or disease.

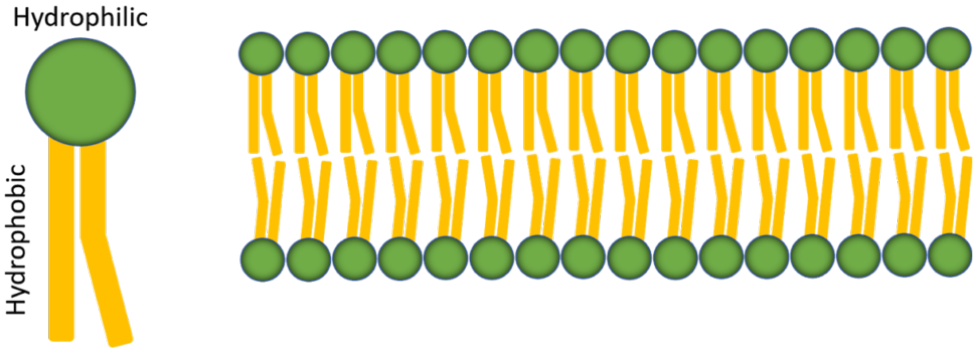
Exposure to pesticides and other chemicals
Pesticides can have sublethal effects on bee behavior that impair navigation, memory, and learning. So-called "inert" ingredients can also affect bee foraging.

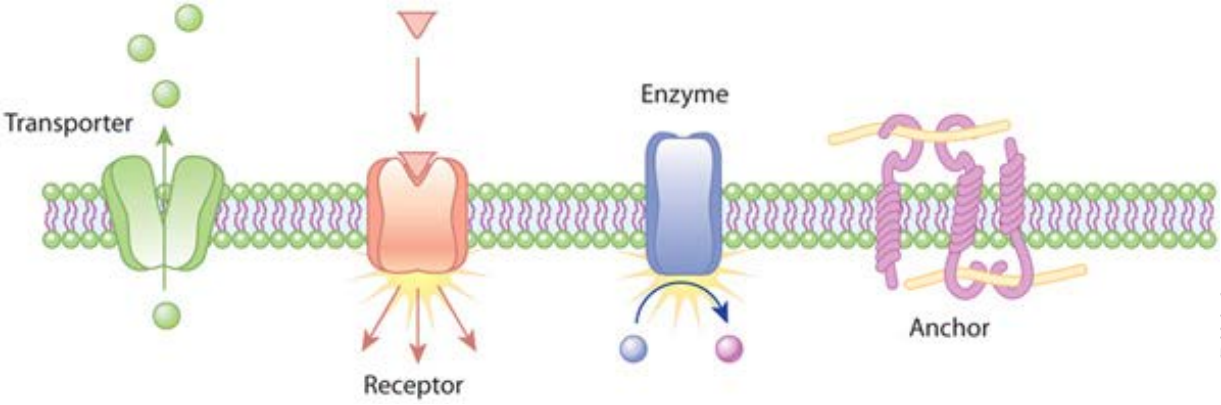
Exposure to pollutants
Airborne pollutants affect the detection of floral odors and learning about floral rewards. They can also impair flight capacity and navigation.

Climate change
Higher temperatures and rising CO_2 affect floral traits, such as flower number, nectar production, and protein content of pollen, which influence bee foraging choices.

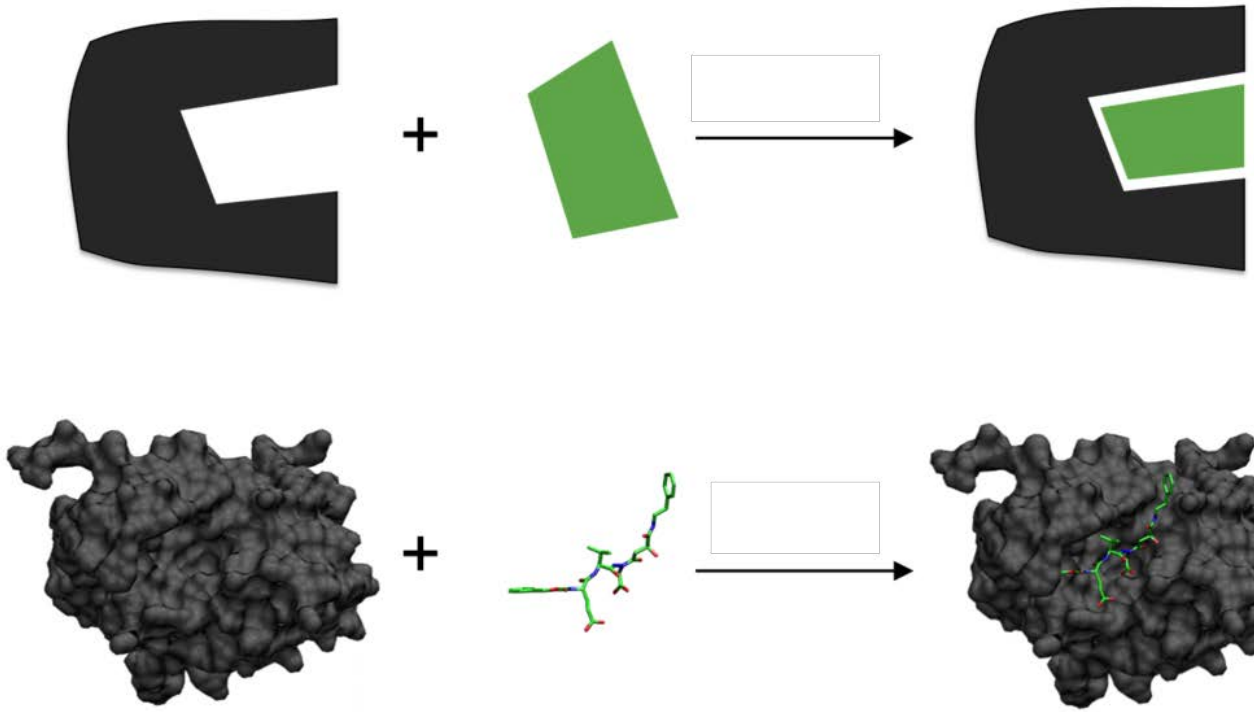
The cell membrane





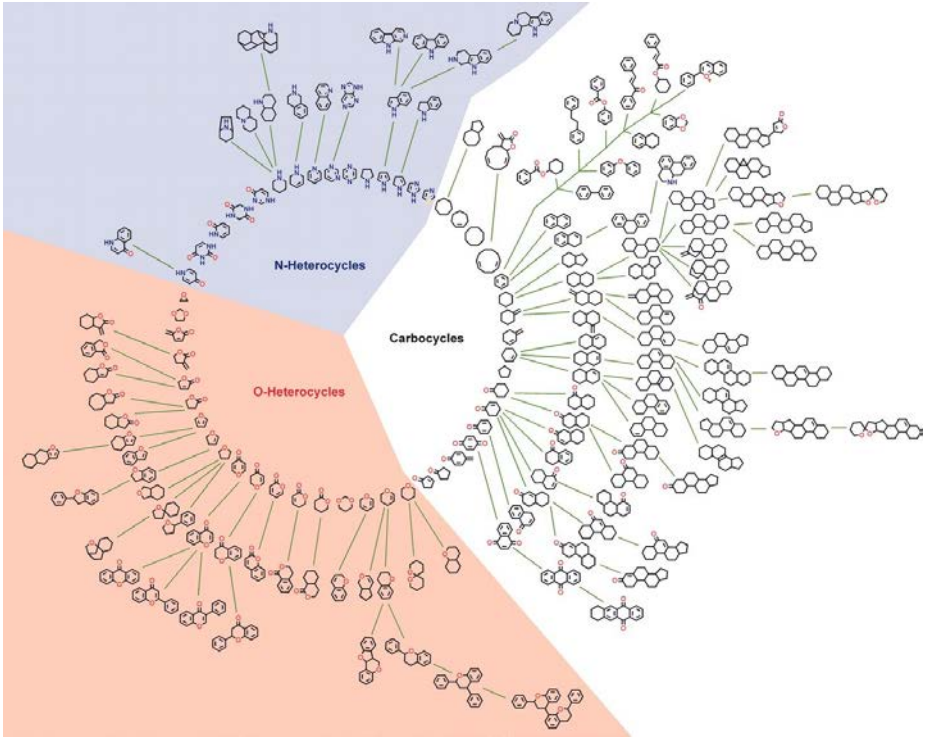


- 25% of proteins in the human genome are membrane proteins
- 50% of drugs target membrane proteins



By Scigenis - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=45515965

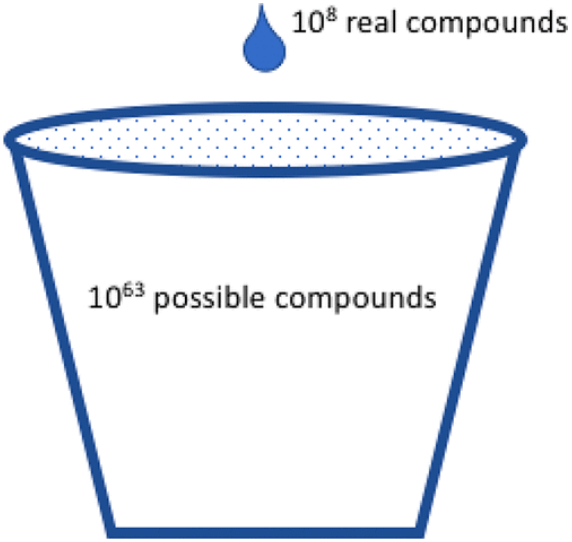
The chemical space



https://www.pnas.org/doi/full/10.1073/pnas.0503647102

C, H, O, N, S, P, (F, Cl, Br, I)

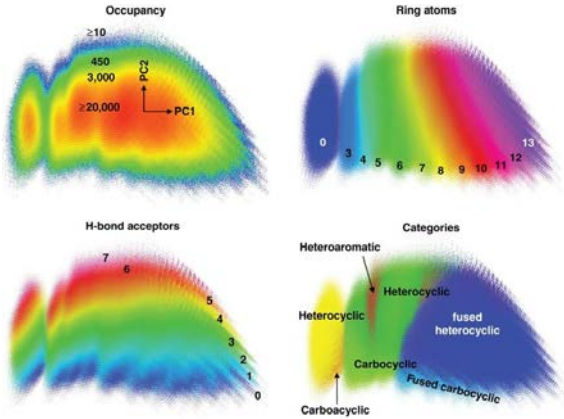
The chemical space



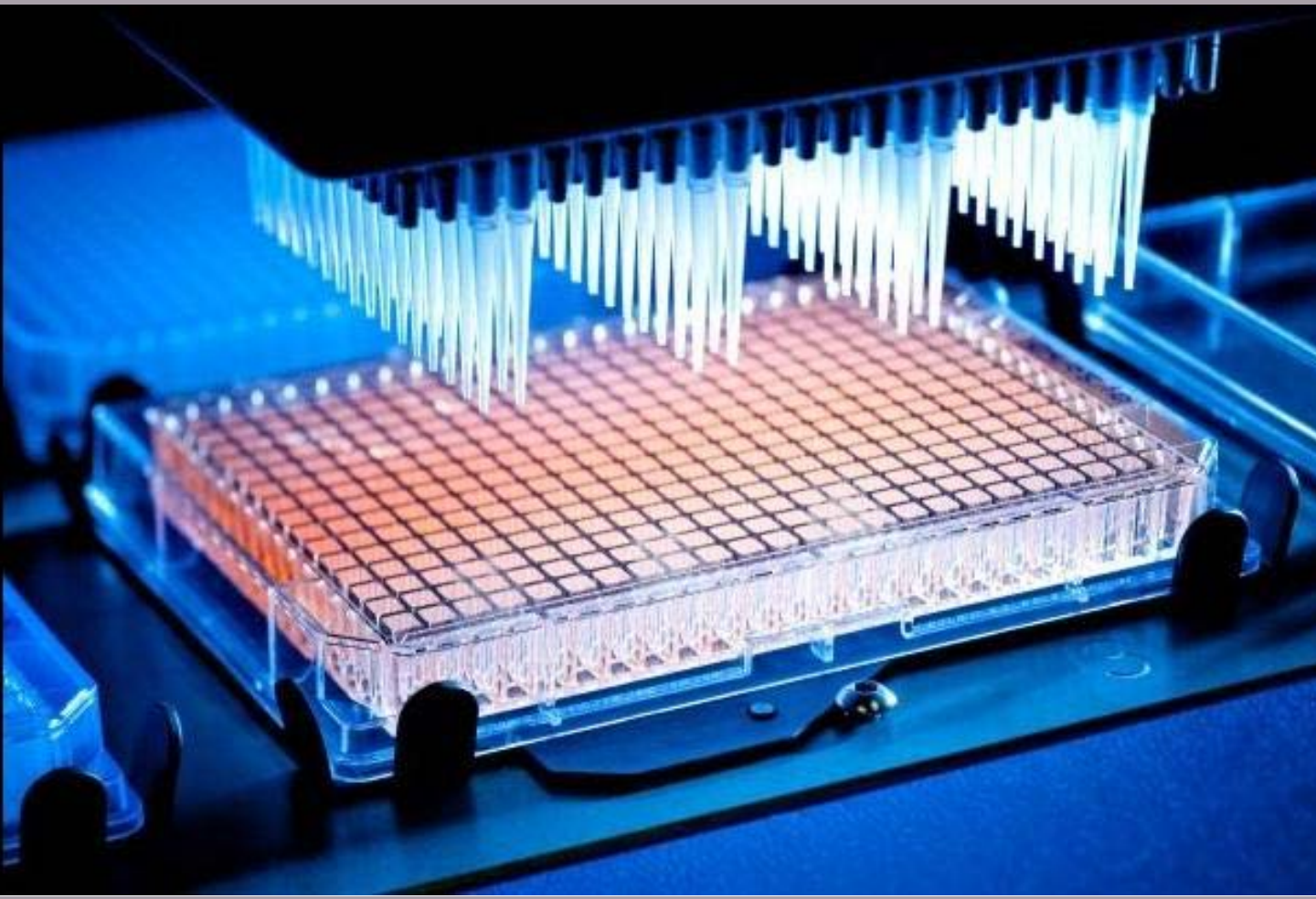
https://pubs.acs.org/doi/10.1021/acs.jmedchem.8b01048

Molecules up to 13 atoms of C, N, O, Cl, S (GDB-13 database)

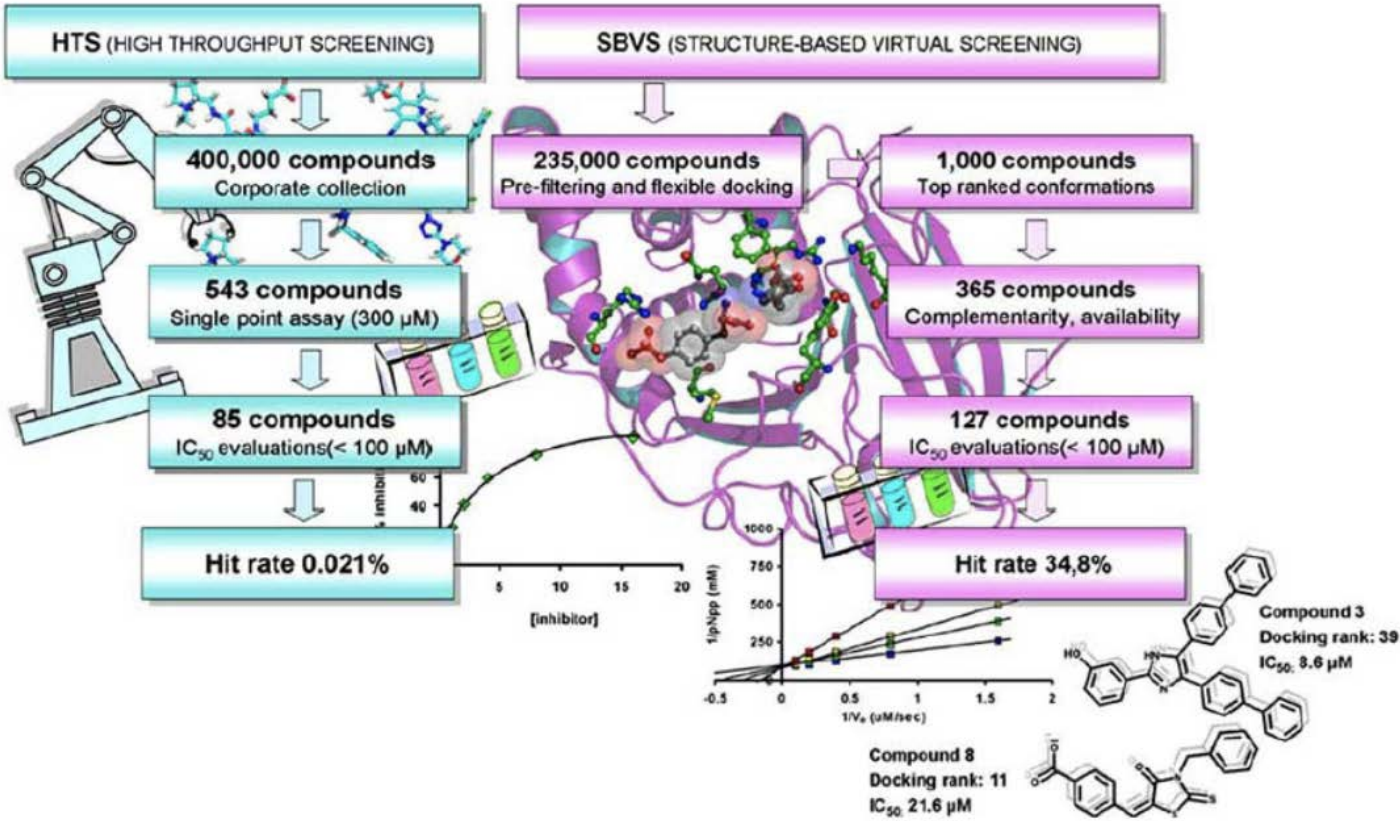
10^9 million compounds



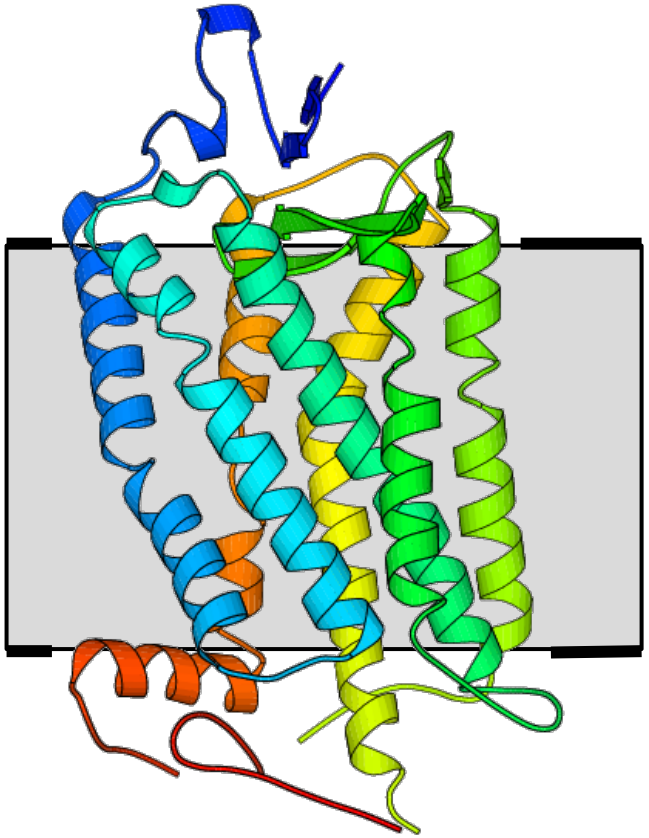
High throughput screening



High throughput screening vs Virtual Screening

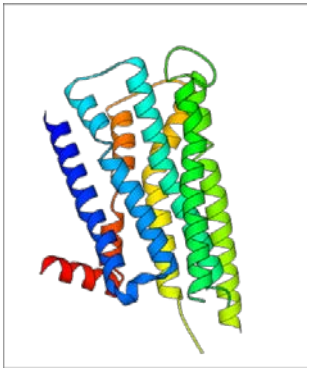


GPCRs

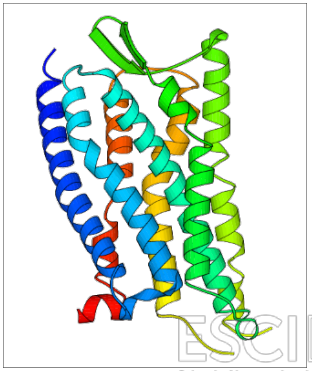


rhodopsin

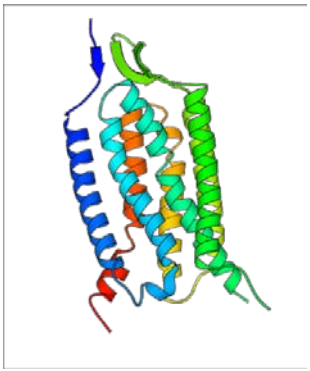
https://zhanggroup.org/GPCR-EXP/



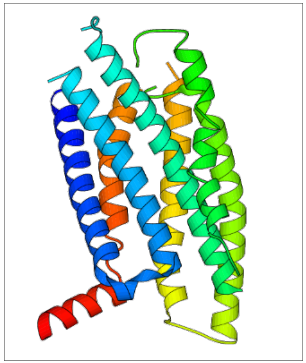
Dopamine R



Opioid R



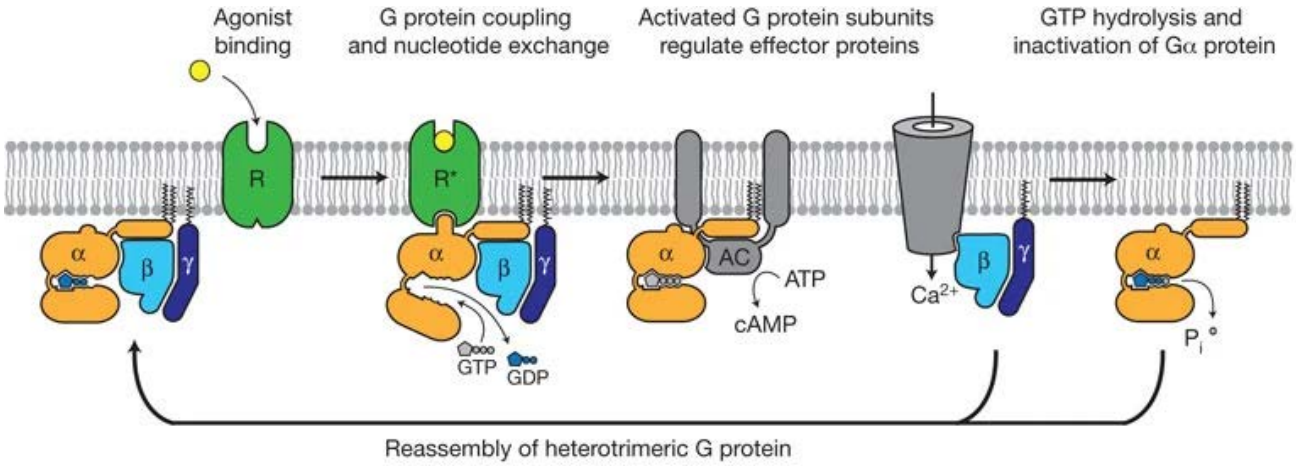
Glutamate R



Glucagon R

Many proteins, same mechanism

800 human GPCRs

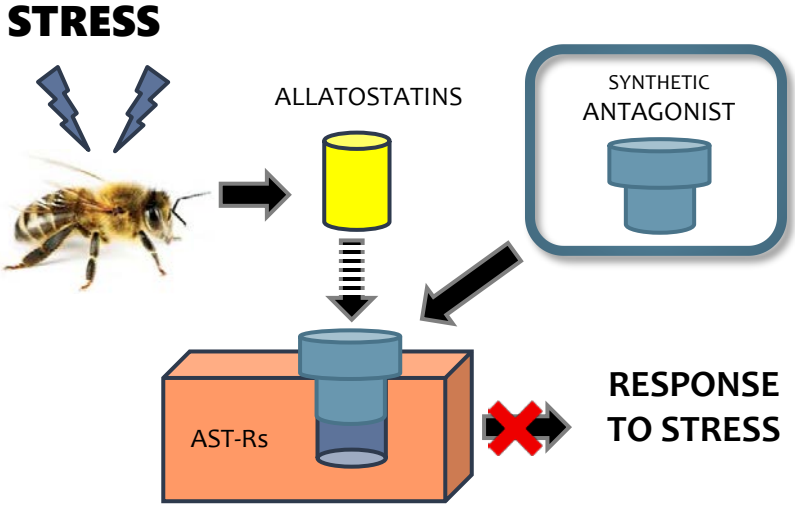


https://www.nature.com/articles/nature10361/figures/1

Allatostatin A an its receptor

Allatostatin A receptor		300-260 amino acids
Allatostatin A	*Neuropeptide*	GRQPYSFGL-amide

Jean-Marc Devaud, Paul Sabatier
University - Toulouse III



A pharmacological approach

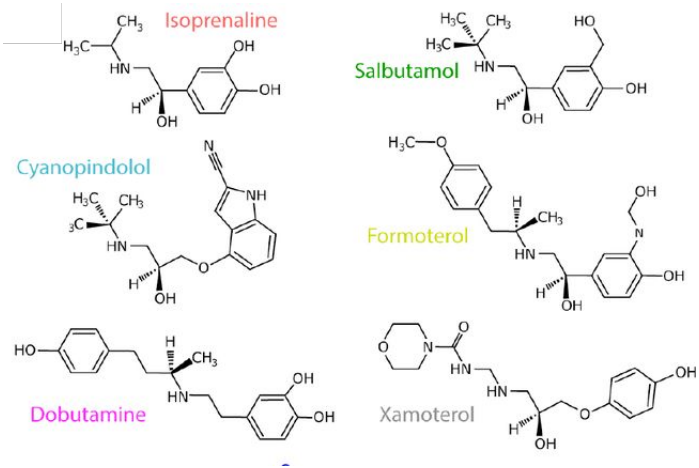
~~Drugs~~

~~Pesticides~~

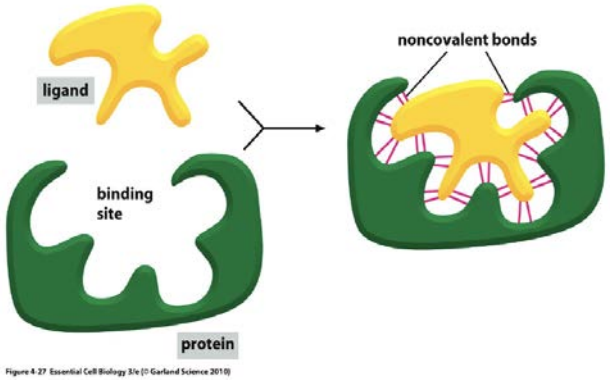
Make bees more active (foraging)

Computer-aided drug design

Ligand based



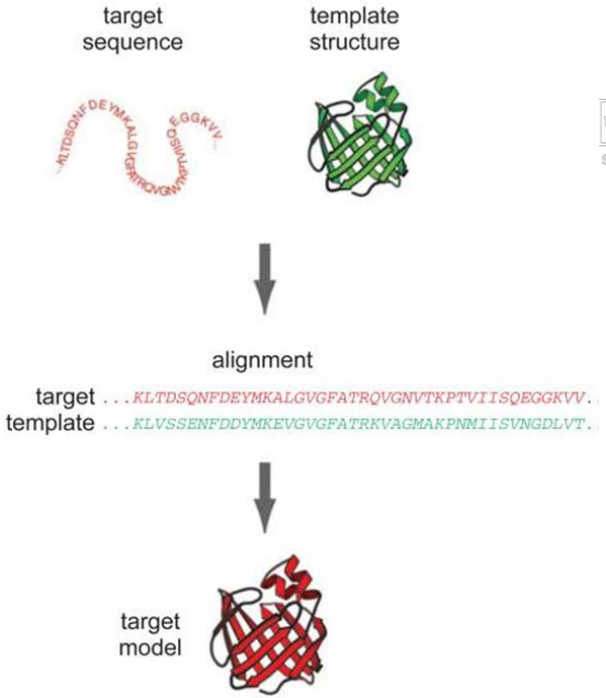
Structure based



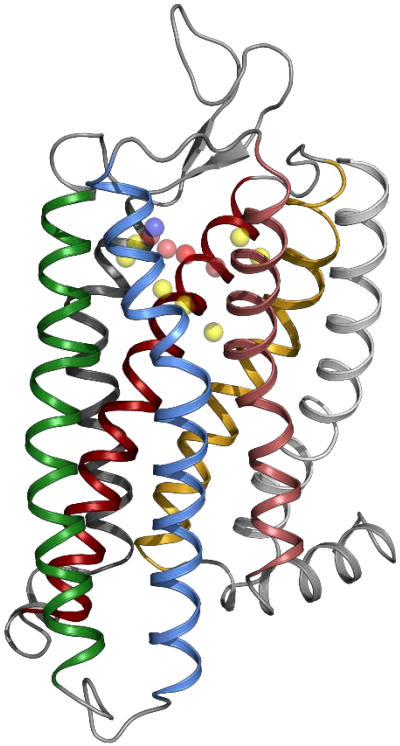
Homology modeling

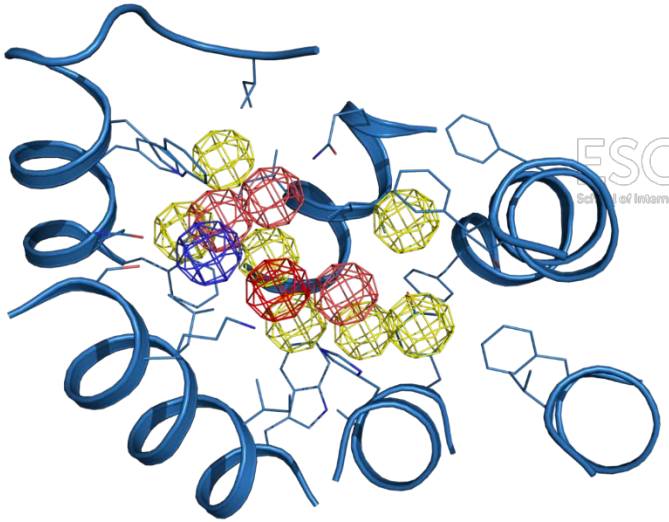
- Known natural ligand (peptide) / No known small-molecule ligand
- Not known how the peptide binds the receptor

- No receptor structure
  - 36% identity with the human galanin receptor (no structure)
  - 28% identity with the human delta-opioid receptor (**solved structure**)

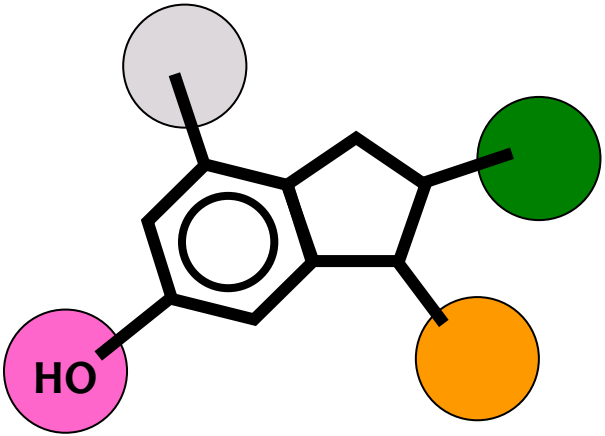


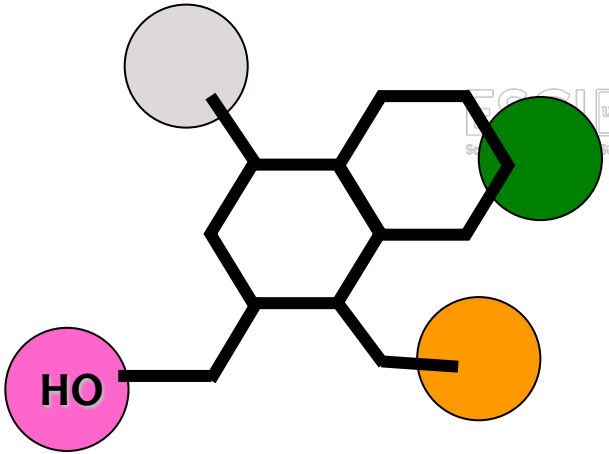
Homology model + Probes + Pharmacophore model



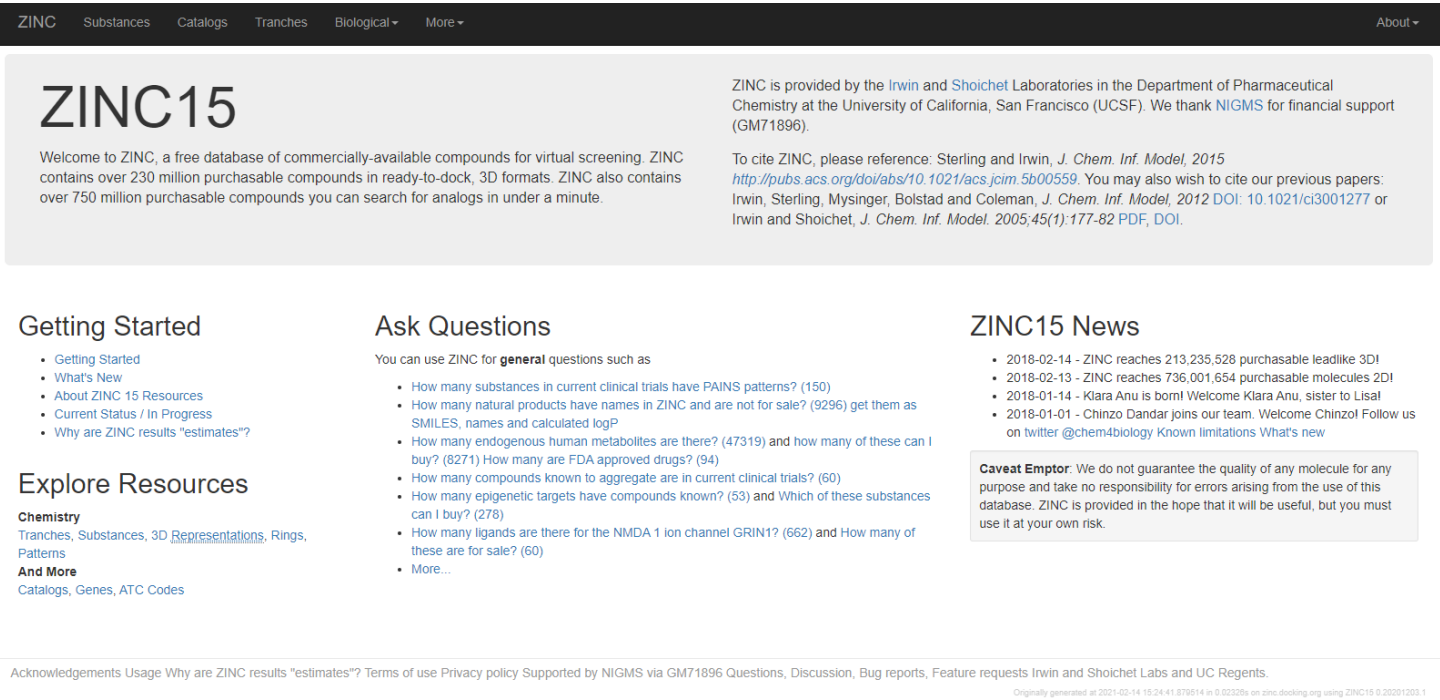


Similarity at recognition (mould)





ZINC (all purchasable compounds)



https://zinc.docking.org/

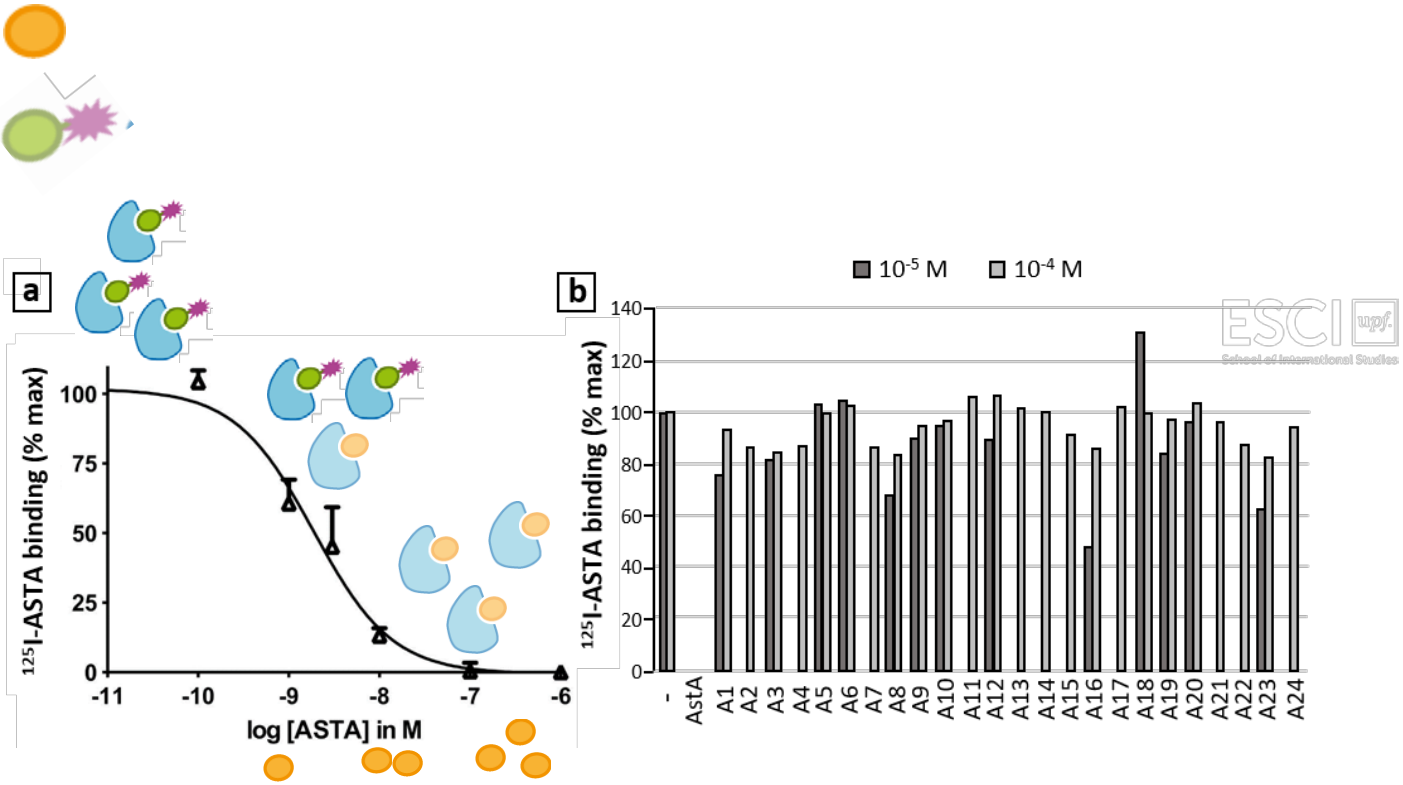
750 million purchasable compounds

Virtual screening

- Subset of ZINC ~5.5 million compounds
- Defined 13 features
- Hits: molecules with combinations of 5-7 features
- Rank best 1000 compounds
- Ordered 24 compounds for testing (~1000€)

Molecule	ZINC Nbr.	MolPort Ref. Nbr.	Provider – Catalog Nbr.
A1	08961583	003-113-947	Life Chemicals Inc. - F2277-0085
A2	00343628	002-799-596	Specs - AC-907/34130057
A3	06938450	004-093-769	ENAMINE Ltd. - Z45611413
A4	60362419	010-428-507	ENAMINE Ltd. - Z1020952700
A5	04957299	002-136-046	ChemBridge Corporation - 5211388
A6	12787609	005-668-019	ENAMINE Ltd. - Z30141626
A7	65194116	010-912-878	ChemDiv, Inc. - M952-1127
A8	22617467	006-045-762	ENAMINE Ltd. - Z24861695
A9	00246283	000-162-802	ChemBridge Corporation - 5316696
A10	13894175	002-943-804	Vitas-M Laboratory, Ltd. - STK087888
A11	13183644	005-454-560	ENAMINE Ltd. - Z85907627
A12	04276367	002-247-882	Life Chemicals Inc. - F1407-0215
A13	35397617	010-737-204	ChemDiv, Inc. - F293-0690
A14	69622155	020-019-052	ENAMINE Ltd. - Z1138601758
A15	67641273	019-798-932	ChemBridge Corporation - 10257373
A16	02236226	000-830-195	InterBioScreen Ltd. - STOCK3S-72356
A17	13186976	005-458-297	ENAMINE Ltd. - Z85969560
A18	63211889	002-010-141	ENAMINE Ltd. - Z56971771
A19	08754917	003-097-856	ENAMINE Ltd. - Z237523122
A20	04868082	002-101-186	ChemBridge Corporation - 9007792
A21	15774319	007-850-500	ChemDiv, Inc. - BB01-3683
A22	00870369	001-908-122	Vitas-M Laboratory, Ltd. - STK077856
A23	12997949	009-624-345	ENAMINE Ltd. - Z238539738
A24	01282655	000-696-223	Vitas-M Laboratory, Ltd. - STK541026

In vitro assay



***In vitro* competitive binding assays for the A-series molecules. (a)** Competition curve for the native ligand ASTA on HEK cells. **(b)** Results (% max) obtained with the 24 tested A molecules (positive control: ASTA 10^{-6}M). Only A8, A16 and A23 showed dose-dependent binding.

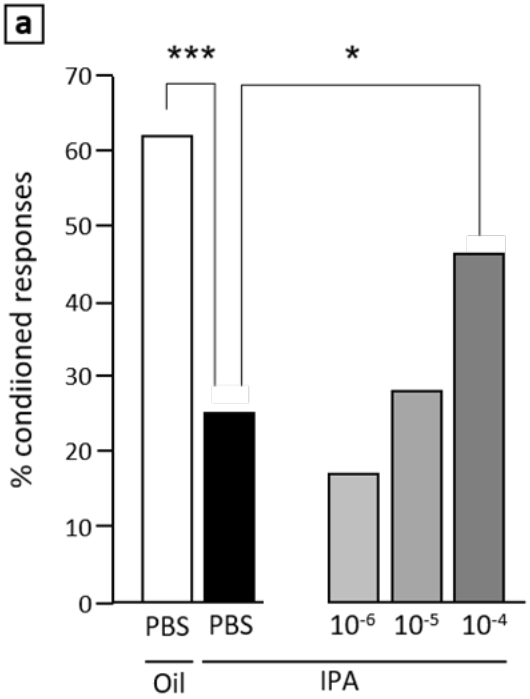
Sting alarm pheromone

- Sting alarm pheromone (SAP)
  - main component isopentylacetate (IPA)

- inducing the recruitment of nestmates to defend the colony
- increasing aggressiveness

- increased respiratory rate
- reduced sensitivity to noxious stimuli (‘analgesia’)
- decreased motivation for food
- learning impairment …

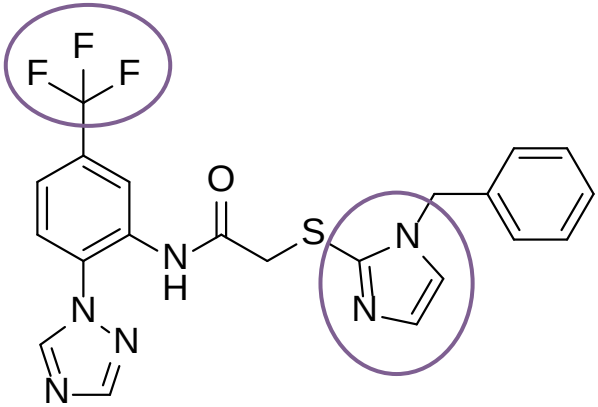
In vivo experiments (learning)



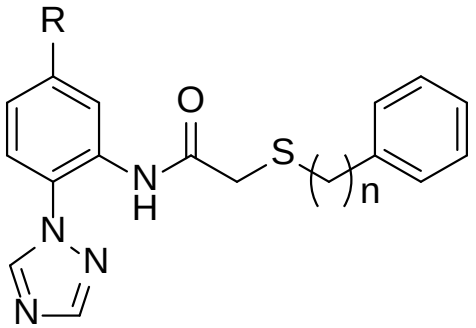
Effects of A8 on stressed bees. Learning: proportions of individuals showing a conditioned response to the odorant paired with sucrose, at the end of the conditioning session (third trial). Bees were injected either with PBS or A8, then exposed to IPA or paraffin oil only (negative controls). While exposure to IPA significantly reduced learning performance in PBS-injected bees, this effect was restored by an injection of 10^{-4} M A8**.** *: $p<0.025$; ***$p<0.0005$ (PBS: n=100; A8: n=47-99/group).

Synthesis

Adrià Sánchez, Félix Busqué, Ramon Alibés
Departament de Química, UAB



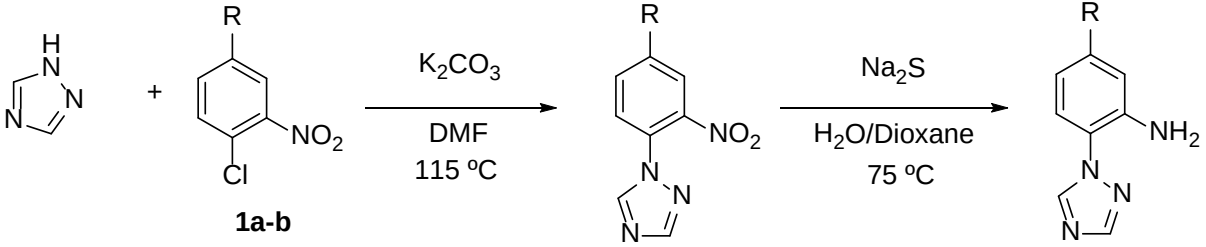
A8



B1: R = $-CF_3$, n = 1; **B4**: R_1 = $-CH_3$, n = 1
B2: R = $-CF_3$, n = 2; **B5**: R_1 = $-CH_3$, n = 2
B3: R = $-CF_3$, n = 3; **B6**: R_1 = $-CH_3$, n = 3

Structures of A8 and the related synthesized compounds B1-6.

Synthetic routs



1a-b

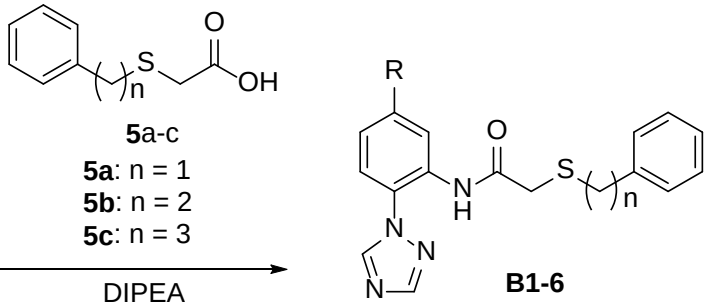
1a: R = -CF_3
1b: R = -CH_3

2a-b

2a: R = -CF_3, 94%
2b: R = -CH_3, 82%

3a-b

3a: R = -CF_3, 51%
3b: R = -CH_3, 68%

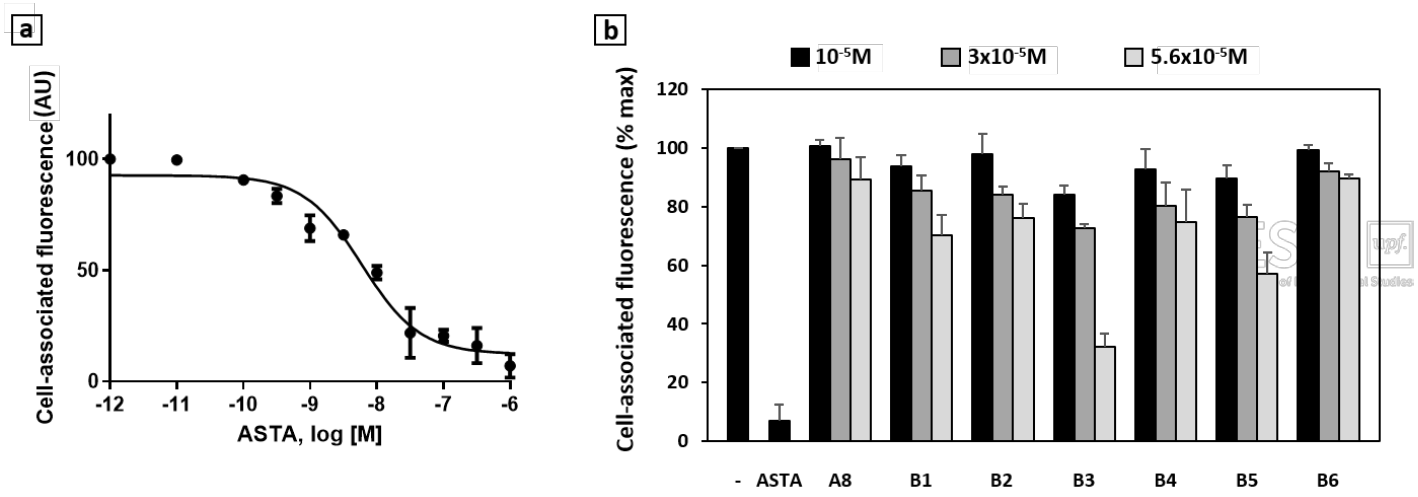


5a-c
5a: n = 1
5b: n = 2
5c: n = 3

B1-6

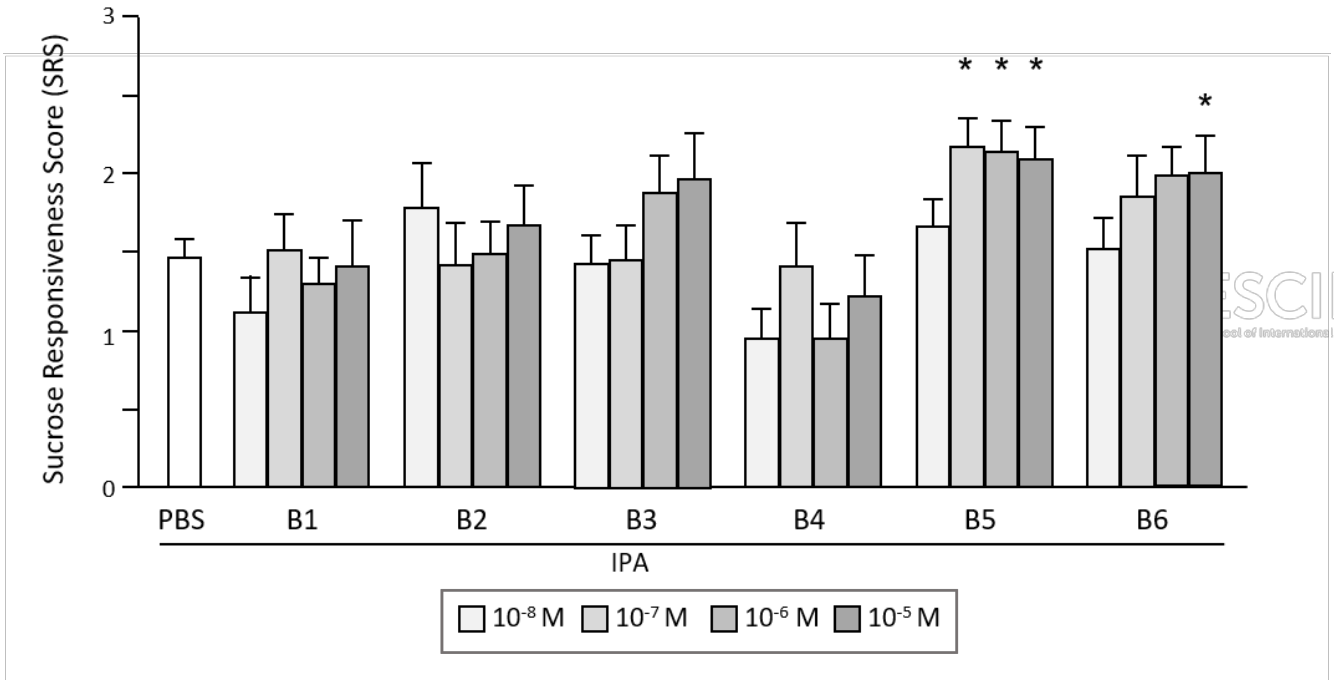
B1:R = -CF_3, n = 1, 83%; **B4**: R = -CH_3, n = 1, 60%
B2:R = -CF_3, n = 2, 60%; **B5**: R = -CH_3, n = 2, 83%
B3:R = -CF_3, n = 3, 56%; **B6**: R = -CH_3, n = 3, 50%

In vitro assay



***In vitro* competitive binding assays for the B-series molecules. (a)** Competition curve for the native ligand ASTA on HEK cells. **(b)** Results (% max) for the 6 tested B molecules, as compared with those of A8 and ASTA (10^{-6} M) (5 replicates).

In vivo experiments



Compared effects of B-series molecules on sucrose responsiveness. Mean sucrose responsiveness score values in bees injected with PBS or either molecule of the B series (between 10^{-8} and 10^{-4} M), then exposed to IPA. Treatment with B5 or B6 significantly improved sucrose responsiveness, with a greater efficiency for B5. *: $p<0.0125$ (PBS: n=114; B compounds: 28-41/group).

Conclusions

- Synthetic compounds targeting specific bee receptors
- First step alleviating stress in honey bees