Human Olfactory Receptors: A journey from cell engineering for efficient *in vitro* functional assays to effective antagonists in human sensory assay

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			Cell lines		
Entry	ORs	Literature data	HEK293T	HANA3	ChemCom's proprietary
1	OR56A1	Adipietro et al., 2012			
2	OR10J5	Saito et al., 2009			
3	ORX116	-			
4	ORX069	-			
5	OR10H5	-			
6	OR8B3	Mainland et al., 2013			
7	OR6P1	Mainland et al., 2013			
8	ORX074B	-			
9	ORX126	-			
10	ORX189	-			
11	ORX081	-			
12	ORX213	-			
13	OR1D2	Spehr et al., 2003			
14	OR7C1	Mainland et al., 2013			
15	OR8H1	Kato et al., 2012			
16	ORX083	-			
17	OR7D4	Keller et al., 2007			
18	ORX159	-			
19	ORX200	-			
20	OR10H2	Mainland et al., 2013			
21	ORX299	-			
22	ORX039	-			
23	ORX097	-			
24	ORX350	-			
25	OR10G9	Adipietro et al., 2012			
26	ORX162	-			
27	OR52D1	Sanz et al., 2005			
28	ORX035	-			
29	OR5K1	Kato et al., 2013			
30	ORX405	-			
31	OR56A5	Adipietro et al., 2012			
32	ORX406	-			
33	ORX173	-			
34	OR4Q3	Mainland et al., 2013			
35	OR5A2	Jaeger et al., 2013			
36	OR52A5	Chatelain and Veithen, 2013			
37	ORX372	-			
38	OR5D3P	Veithen et al., 2009			
39	OR2B11	Adipietro et al., 2012			
40	OR2C1	Saito et al., 2009			
41	ORX308	-			
42	ORX376	-			
43	OR2AT4	Busse et al, 2014			
44	ORX351	-			
45	ORX182	-			
46	ORX370	-			
47	OR5P3	Saito et al., 2009			
48	OR8D1	Adipietro et al., 2012			
49	hpfh1OR	-			
50	ORX386	-			
51	OR5AN1	Shirasu et al, 2014			
52	ORX103	-			
53	OR11A1	Adipietro et al., 2012			
54	ORX073	-			
55	ORX282	-			
56	OR1A1	-			
57	OR10A6	Mainland et al., 2013			
58	OR10G7	Adipietro et al., 2012			
59	ORX082	-			
60	ORX017	-			
61	OR52E8	Chatelain and Veithen, 2013			
62	ORX089	-			
63	ORX379	-			
64	OR11H4	Menashe et al., 2007			
65	ORX150	-			
66	ORX259	-			
67	ORL309	-			
68	OR2J2	Saito et al., 2009			
69	OR10G4	Adipietro et al., 2012			
70	OR2A25	Adipietro et al., 2012			
71	ORX388	-			
72	ORX137	-			
73	OR4D1	-			
74	ORX398	-			
75	ORX205	-			
76	ORX261	-			
77	ORL307b	-			
78	OR2J3	McRae et al., 2012			
79	OR2M4	Gonzalez-Kristeller et al., 2015			
80	ORX183	-			
81	ORX317	-			
82	ORX052	-			
83	ORX247	-			
84	ORX349	-			
85	OR5A1	Jaeger et al., 2013			
86	ORL312	-			
87	ORX313	-			
88	ORX080	-			
89	ORX360	-			
90	ORL481	-			
91	ORX106	-			
92	ORX186	-			
93	ORX397	-			
94	ORX076	-			
95	ORX195	-			
96	OR2AG1	Mashukova et al., 2006			
97	ORX197	-			
98	ORX330	-			
99	ORX281	-			
100	ORX095	-			
101	ORL362	-			
102	ORX217	-			
103	ORX322	-			
104	OR4E2	-			
105	ORX320	-			
106	ORX219	-			
107	ORX129	-			
108	ORX348	-			
109	ORX319	-			
110	ORX367	-			
111	ORX218	-			
112	ORX362	-			
113	OR4D1	-			
114	OR51E1	Fujita et al., 2007			
115	OR2W1	Saito et al., 2009			
116	OR51L1	Saito et al., 2009			
117	OR51E2	Saito et al., 2009			
118	ORX366	-			
119	ORX149	-			
120	OR2M2	-			
		TOTAL	18	68	120
		%	15%	57%	100%

Table 1. Comparison of the potency of different cell lines. Green indicates positive dose-response analysis. Red indicates a lack of activation.

Introduction:

Many odorant compounds are perceived as unpleasant. They can be present in different contexts such as body-, home-, factory-, material-, or fabric-emitted odors, so that humans are daily exposed to this olfactory pollution. In addition, odorant compounds can also taint food or beverages. Malodor and off-note counteraction is a daily challenge for many different industries.
The first step of the odor perception corresponds to the interaction of olfactory receptors (ORs) with odorant molecules. Therefore, a selective inhibition of the ORs by weakly odorant or odorless antagonists represents an innovative solution to malodor issues. The identification of such odor blockers requires an efficient technological platform to first fish out the receptors that interact with a malodor of interest and second, to screen libraries of potential antagonists of these ORs.

Materials and Methods:

In vitro functional assay
Dilution-response analysis were performed in HEK293T, HANA3 and HEK293T-hRTP1S/hRTP2 cells using the CRE-luciferase reporter assay system. Briefly, each cell plated one day before was transfected with deorphanized ORs (identified at Chemcom S.A.) or empty vector plasmids using TransIT®-LT1 (Mirus) according to the manufacturer's protocol. Twenty hours after transfection and four hours after incubation with tested compounds, cells were lysed and processed for luminescence measurement using a Spectra Max M5 reader (Molecular Devices). To find antagonist(s), cells expressing the OR of interest and the reporter construction are exposed to the reference agonist in the presence or absence of the candidate modulator. The receptor activity is revealed using the CRE-luciferase reporter assay. Results of agonist concentration-response analyses are expressed as the percentage of the response induced by 10 µM of Forskolin (FSK) and were fitted to the Hill's equation. For antagonist concentration-response, a presentation as percentage of the maximal response has been preferred to allow an easier comparison of the different conditions.
Sensory assessment:
Tedlar bags (previously conditioned 24 hours in a ventilated oven at 90°C to eliminate intrinsic plastic smell) were first loaded with a known volume of pure air (from oil free compressor with additional charcoal filters). Then the compound of interest (EtOH solution) were injected with a high accuracy micro-syringe through a septum placed on the tap-valve. In case of deposit, visible haloes could be observed on the bag walls. Gentle heating was then applied until no more haloes were visible. We assumed a complete vaporisation of the solute. The so prepared tedlar bags are presented to expert panel, trained to ISO5496 and the "**field of odor**"® but also used to ISO12219-7. ®

A proprietary cells line, expressing hRTP1A1/hRTP2, is the best model to identify OR agonists.

ChemCom has developed a unique and proprietary cell line (worldwide exclusive license from Duke University technology) highly efficient for the expression of ORs as well as high-throughput screening systems (HTS). The assays are based on rapid measurements of second messenger production that allows the qualitative and quantitative characterization of the interaction of olfactory receptors with their ligands. This proprietary cells line coupled with our HTS capabilities facilitates massive deorphanisation campaigns with large chemical libraries available at ChemCom. Today, ChemCom has deorphanized more than 120 hORs.
To demonstrate the efficiency of our proprietary cell line for human ORs functional characterization, the responses of 120 deorphanized hORs were compared in either ChemCom's proprietary cell line, HANA3 or HEK293T cells, using the best agonist identified at ChemCom (Table 1). It appeared that the activity of only 57% of the hORs expressed in HANA3 cells could be detected. This value dropped down to 15 % in HEK293T. The obtained responses can be clustered in three different scenarios as exemplified on Fig. 1:

A: All tested cell lines were responsive to the agonist. However, responses observed in HEK293T cells were always lower than those measured in HANA3 cells and ChemCom's proprietary cells line. This situation is observed for 15% of the tested receptors.

B: Receptor activation is detected in HANA3 cells and ChemCom cell line but not in HEK293T cells. Responses elicited in HANA3 cells were always lower than in ChemCom's proprietary cells line. This scenario prevails for 42 % of the receptors.

C: Only ChemCom's cell line was efficient for the functional expression of the receptor, as it is the case for 43% of the tested ORs.

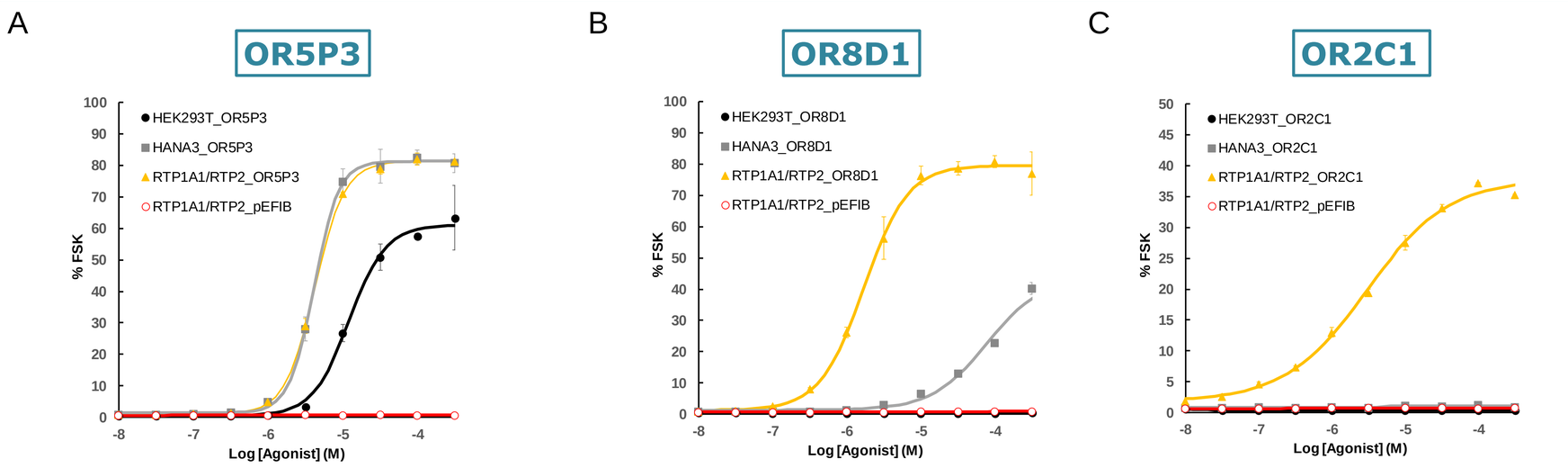

Figure 1. Examples of differential activation profiles in the 3 different cell lines. Responses are monitorated using the luciferase-based gene reporter assay

This proprietary cell line not only permits massive deorphanization campaigns with large chemical libraries available at ChemCom, but also allows modulators screening (i.e. odorless or odorant antagonists and positive allosteric modulators). One example is given here with a potent antagonist of OR7D4, **CC-049-893,** which completely suppresses OR7D4 activation induced by its classical agonists (i.e. Androstenone and Androstadienone, see Fig. 2 and 3). When assessed by human panelists, **CC-049-893** nicely suppresses the OR7D4-mediated sweat/urineous malodor of Androstenone and Androstadienone (Fig. 4).
With its proprietary cells line, ChemCom is therefore able to identify, for more than 120 hORs, *in vitro* modulators (antagonists and enhancers) that will efficiently reduce or stimulate the perception of odorants *in vivo*.

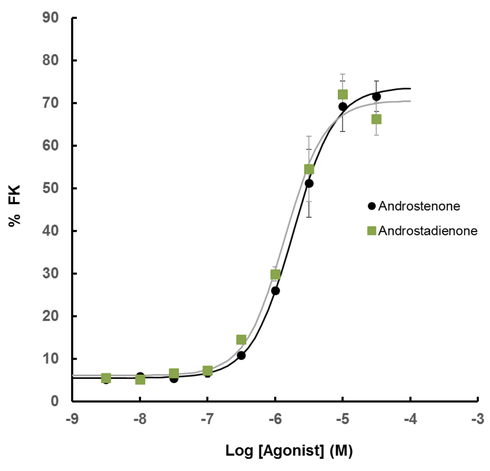

Figure 2. Dose-response activation of OR7D4 by different agonists

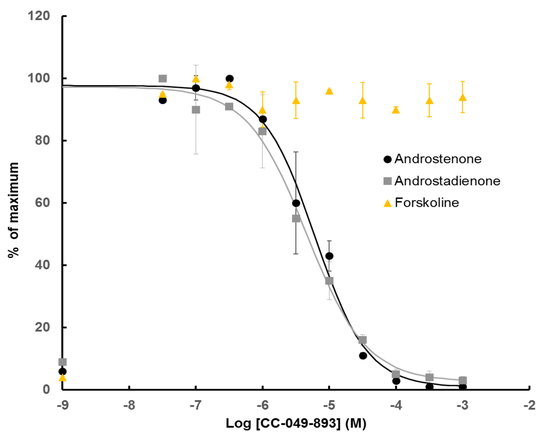

Figure 3. Dose-response inhibition of OR7D4 activated by different agonists

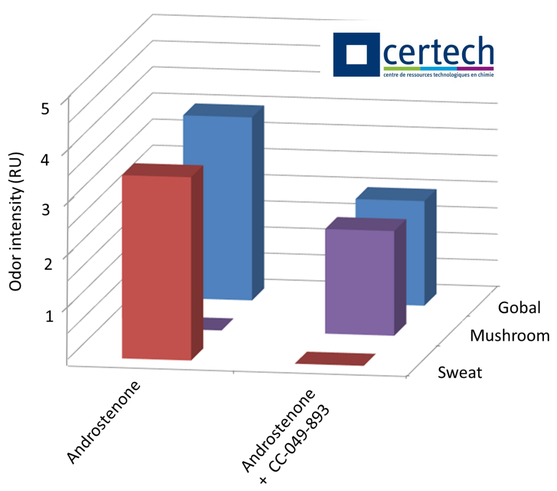

Figure 4. Suppression of urineous/sweat odor perception evoked by OR7D4 agonists in human sensory assessement using CC-049-893

Conclusion.

ChemCom is in a privileged position whereby it can offer unique combinations of knowledge, unique tools and expertise focused on the needs of its business partners. This allows us to work together to co-create innovative solutions to delight consumers around the world. Through an in-depth understanding of how the human sense of smell works, we are able to determine the olfactory receptors that are triggered by particularly offensive malodors e.g. sweat, body odor, smoke, urine and several others as well as by off-flavors that taint food and beverages. Working with our business partners, we aim to reduce not only the perceived intensity of these malodors or off-notes, but also to enhance the perception of preferred fragrances or aroma molecules – thus we offer the capability to explore and develop routes to optimize perfume and flavour performances.

Acknowledgements.

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