

Antagonistic interactions between odorants alter human odor perception

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- 4 Antagonistic interactions between odorants alter human odor perception.
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23 Abstract

24 The olfactory system detects a vast number of odorants using hundreds of olfactory receptors 25 (ORs), the largest group of the G protein-coupled receptor (GPCR) superfamily. Each OR is 26 activated by specific odorous ligands. Like other GPCRs, activation of ORs may be blocked 27 through antagonism. Recent reports highlight widespread antagonisms in odor mixtures 28 influencing olfactory neuron activities. However, it is unclear if and how these antagonisms 29 influence perception of odor mixtures. Here we show that odorant antagonisms at the receptor 30 level alter odor perception. Using a large-scale heterologous expression, we first identified a set 31 of human ORs that are activated by methanethiol and hydrogen sulfide, two extremely potent 32 volatile sulfur malodors. We then screened odorants that block activation of these ORs and 33 identified a set of antagonists, including β -ionone. Finally, human sensory evaluation revealed 34 that odor intensity and unpleasantness of methanethiol were decreased by β -ionone. Odor 35 intensity of β -ionone itself is not correlated with the degree of suppression of malodor 36 sensation. Suppression was also not observed when methanethiol and β -ionone were 37 simultaneously introduced to different nostrils. Together, our data supports the model that odor 38 sensation is altered through antagonistic interactions at the level of the ORs. 39

39

40

42 Introduction

43	Animals detect and discriminate numerous environmental odorants through combinatorial
44	activation of membrane receptors expressed in olfactory sensory neurons (OSNs). In humans,
45	the predominant receptors are the olfactory receptors (ORs), which are the largest protein-
46	coding gene family of Class A G-protein coupled receptors (GPCRs) with ~400 members ¹⁻⁵ .
47	When an odorous agonist binds to an OR, the OR favors an active conformation that initiates a
48	signal transduction cascade, leading to depolarization of the OSNs. Each OSN expresses only
49	one type of OR, meaning that the activation of an OSN is determined by the activation of the
50	OR ^{6,7} . A single OR can respond to various odorants, and one odorant activates multiple ORs.
51	Thus, the combinatorial pattern of OR activation is foundational for the detection and
52	discrimination of a given odor ⁸⁻¹¹ .

53 In addition to acting as an agonist, comparative to ligands for canonical GPCRs, some 54 odorants can function as antagonists or inverse agonists. By binding to ORs, odorants can 55 inhibit the activation by agonists in a competitive manner or stabilize the receptor in the 56 inactive conformation. Previous in vivo and ex vivo studies as well as in vitro research using 57 heterologously expressed ORs have shown that odorants are often capable of acting as antagonists for ORs ¹²⁻¹⁹. Natural odors are usually mixtures of odorants, so antagonistic 58 59 interactions between odorants at ORs are likely. Mixture suppression, in which odor intensity is 60 less than a linear sum of component odorants, is commonly observed ^{20,21}. Previous studies 61 highlighted the role of central olfactory processing in mixture suppression²²⁻²⁴. Yet peripheral 62 events, such as antagonistic interactions of odorants at the ORs, were also proposed as an 63 underlying mechanism of mixture suppression ^{24,25}. Despite widespread antagonism on OSNs in

64	odor mixtures, there is scare evidence supporting a role of odorant antagonism in odor
65	perception ^{20,26} . This is partly because OR antagonists also act as agonists for other ORs,
66	making it challenging to distinguish the role of antagonism in odor perception from central
67	processing.
68	In this study, we addressed the effects of odorant antagonism in odor perception by
69	focusing on a set of ORs that are activated by extremely potent and unpleasant volatile sulfur
70	compounds (VSCs), including methanethiol (methyl mercaptan, CH ₃ SH). Antagonists, such as
71	β -ionone, blocked the VSC OR response and modified the sulfur odorants odor perception.
72	

73 **Results**

74 Screening human ORs that respond to gaseous sulfur compounds

75 Previously, we developed a method to detect *in vitro* OR responses to odors in the vapor

phase ^{11,27}. Using this technique with modifications (see Methods), we performed an OR

screening assay for two highly volatile sulfur compounds (VSCs), methanethiol and hydrogen

sulfide (Fig. 1A and Extended data Fig. 1).

79 By screening the response of 359 human ORs, representing the vast majority of intact ORs 80 encoded on the human genome, to methanethiol and hydrogen sulfide, we identified several 81 candidate hits that exhibited strong responses (p<0.001, one-way ANOVA followed by Dunnett's 82 test) (Fig. 1B and Supplementary Data). A secondary screening confirmed concentration-83 dependent responses of OR2T11 and OR2T1 to methanethiol and hydrogen sulfide as well as 84 the response of OR2T6 to hydrogen sulfide (Fig. 1C). These three ORs share relatively high 85 amino acid sequence similarities among the OR family members (Identities; 73% (222/305), 86 66% (206/310), and 63% (193/304) in OR2T1/OR2T6, OR2T1/OR2T11, OR2T6/OR2T11, 87 respectively) (Fig. 1D). 88 OR2T11 was previously shown to respond to various thiol molecules in a copper- and silver 89

ion-dependent manner ²⁸⁻³⁰. We examined the metal dependence of the ORs' responses to
methanethiol and hydrogen sulfide. Consistent with the previous reports, the addition of copper

91 in the media dramatically enhanced the response to both VSCs. The addition of silver produced

92 moderate response enhancement, while the addition of zinc showed no effect (Extended data

93 Fig. 2A). The effect of copper was diminished by the addition of the copper chelator

94 tetraethylenepentamine (TEPA) (Extended data Fig. 2B). Our data supports the previously

95 suggested model that metal-sulfur complexes activate sulfur-responsive ORs.

96

97 Identification of antagonists

98 Antagonists or inverse agonists of ORs that are potently activated by the sulfur odorants 99 may block the perception of their unpleasant odor, potentially leading to the development of 100 novel deodorants. To identify antagonists that block the activity of VSC-responding ORs, we 101 screened the effects of 100 odorants on OR2T11 response to methanethiol and hydrogen 102 sulfide. OR2T11-expressing cells in a buffer containing each compound at a final concentration 103 of 100 µM were stimulated by 7 ppm methanethiol and 41 ppm hydrogen sulfide. A subset of 104 ketones, including specific ionones and damascones, showed inhibitory effects on the OR 105 responses against the tested VSCs (Fig. 2A). β -ionone was the most potent antagonist among 106 the tested odorants (LogIC50 = -4.85) (Fig. 2B and Extended data Fig. 3A) and did not alter the 107 activity of OR2T11 by itself when tested without VSCs (Fig. 2C). In contrast, Iso E super (tetramethyl acetyloctahydronaphthalen) 31,32 , which is one of the most commonly used ketone 108 109 fragrances for deodorant, did not show potent antagonistic effects on OR2T11 (Fig. 2B). 110 Next, we examined the inhibitory effects of the ionone and damascone molecules on the 111 responses of OR2T11, OR2T1, and OR2T6 to the VSCs. We observed that all tested ORs were 112 similarly inhibited by a given antagonist, suggesting a common blocking mechanism. (Fig. 2D 113 and Extended data Fig. 3B). The possibility that the inhibition of the odorants was caused by 114 adverse effects on the assay system, such as cytotoxicity, was excluded because another OR, 115 mouse Or2aj6 (also known as Olfr171 and MOR273-1), was activated by these compounds in 116 the same assay (Extended data Fig. 3C). In the vapor stimulation assays, a mixture of

117 methanethiol and β-ionone also showed a concentration-dependent and molecule-specific 118 inhibitory effect against OR2T11 and OR2T6 (Fig. 2E and Extended data Fig. 4A). Since the 119 concentration of methanethiol did not decrease by mixing with β-ionone in the sampling bag 120 (Extended data Fig. 4B), chemical reactions between methanethiol and β-ionone is unlikely to 121 be a cause of suppression of OR activations.

122 To gain insight into mechanism underlying the antagonisms, we performed docking of the ligands on the structural models of OR2T families based on AlphaFold2³³ (Extended data Fig. 123 124 5). Following previous publications and our data suggesting copper as an essential co-factor to 125 antagonism (Extended data Fig. 2), OR bound to sulfur odorants by coordinating with a copper cation at the level of sulfur residues ^{28-30,34,35}. The docking of copper resulted in a binding close 126 to C^{BW5.43} and M^{BW5.39}. These residues, conserved in a small subset of ORs including OR2T1, 127 128 OR2T6, and OR2T11, pointed towards the odorant binding cavity (Fig. 3F and Extended data 129 Fig. 6A). The bottom of this cavity was defined by the OR toggle switch, the "FYG" motif (BW6.47 to BW6.49), in the transmembrane helix (TM) 6³⁶. Hydrogen sulfide and 130 131 methanethiol were docked on the copper bound OR structures. All poses were located in 132 between the copper and the toggle switch (Fig 3F and Extended data Fig. 6), suggesting a 133 possibility of effective binding for these two molecules in OR2T1, OR2T6, and OR2T11. The 134 β-ionone binding location overlapped with that of the Cu-sulfur odorant in all three structures 135 when it was docked into the cavity of OR2T1, OR2T6, and OR2T11. This model is consistent 136 with the idea that β -ionone antagonizes OR activation by competitively blocking the binding of 137 Cu-sulfur odorant.

139 **Human sensory evaluation test**

140 Thus far, we have identified that OR2T family members robustly respond to the tested 141 VSCs and that certain ketones, including β -ionone, act as antagonists. If antagonists inhibit the 142 response of all receptors that respond to particular VSCs, it is expected that inhibition of 143 olfactory perception will occur. To test whether the antagonistic interactions affect olfactory 144 perception, we designed a set of human sensory evaluation tests aiming to distinguish 145 antagonistic effects and central processing in mixture suppression. We selected β -ionone 146 (floral/violet/woody smell), the most potent antagonist we identified, in parallel with Iso E 147 Super (woody/floral/amber/violet smell), which does not show potent antagonistic effects (Fig. 148 2B). While perceived odor intensities of β -ionone and Iso E Super varied among subjects, we 149 adjusted their concentrations so that the intensities were matched as a group (Fig. 3A and 150 Extended data Fig. 7 and 8). Iso E Super is more pleasant than β -ionone at the adjusted 151 concentrations (***p<0.001, non-parametric Wilcoxon test) (Extended data Fig. 8B). Sensory 152 evaluation tests were conducted by asking subjects to rate total odor intensity, malodor 153 intensity, and pleasantness of methanethiol and its mixtures (methanethiol and β -ionone, 154 methanethiol and Iso E Super). The subjects were separated into two groups with different 155 evaluation orders (Extended data Fig. 8C). In order to clarify the evaluation criteria for the 156 subjects, we presented the odors in a non-random order. We first conducted a non-blind test for 157 methanethiol and asked subjects to score one value for both total odor intensity and malodor 158 intensity. Subsequently, two odor mixtures (methanethiol with either β -ionone and 159 methanethiol with Iso E Super) were presented in a random order to separately evaluate total 160 odor intensity, malodor intensity, and pleasantness in the blind condition (Extended data Fig.

161	8C). Regardless of the order of odor presentation, the subjects' evaluations were similar,
162	excluding the role of odor adaptation in our tests (p >0.05, Mann-Whitney tests) (Extended data
163	Fig. 7B). The total odor intensity of methanethiol and β -ionone mixtures was less than those of
164	the methanethiol only, indicating mixture suppression (p <0.001, Wilcoxon test) (Fig. 3C Left).
165	Additionally, the malodor intensity and unpleasantness of methanethiol was significantly
166	reduced by mixing it with β -ionone (<i>p</i> <0.001, Kruskal-Wallis test) (Fig. 3C Center and Right).
167	To test whether odor intensity of β -ionone alone predicts its suppressive effects on
168	methanethiol, we first focused on the group with low odor intensity against β -ionone (6 out of
169	20 subjects) and found that the suppressing effect was evident ($p=0.03$, nonparametric
170	Wilcoxon multiple comparison test) (Fig. 3D). When we compare the suppressing effects
171	between this group and the group with higher odor intensity against β -ionone (14 out of 20
172	subjects), there was no statistically significant difference in methanethiol suppression ($p=0.98$,
173	Mann-Whitney test). Moreover, both of the odor intensities and the pleasantness of β -ionone
174	showed no significant correlation with the degree of suppressing effects against methanethiol
175	(p=0.58 and 0.84, respectively, Spearman's tests) (Fig. 3E). Together, the data suggests that
176	odor intensity of β -ionone alone is independent from it's odor suppressing effects. In the
177	Relative rank, mixture of β -ionone was significantly higher than one of Iso E Super in the
178	evaluation of malodor intensity ($p=0.02$, nonparametric Friedman's multiple comparison test)
179	(Fig. 3F). Iso E Super also reduced total odor intensity, malodor intensity, yet it was less
180	effective in suppressing malodor intensity than β -ionone (Fig. 3F and Extended data Fig. 8).
181	Similarly, damascones (α -damascone, β -damascone, δ -damascone) and β -damascenone that
182	inhibited OR response to the VSCs were effective in reducing the odor intensity and

183	unpleasantness to methanethiol (Extended data Fig. 9). δ -damascone, which showed a low
184	inhibitory effect in in vitro assay (Fig. 2D), also had the lowest suppressing effect among the
185	tested antagonists in the sensory evaluation test. Together, the data is consistent with our
186	hypothesis that antagonistic effects on ORs change odor perception.
187	
188	Nostril-specific stimulation
189	Finally, to differentiate the impact of peripheral antagonism vs central processing in
190	the malodor suppression, we compared the impact of β -ionone on the perception of
191	methanethiol when β -ionone was inhaled via the same versus a different nostril (Fig. 4A). The
192	subjects were unable to distinguish which nostril the tested odor was presented to (Fig. 4B).
193	This is consistent with previous reports stating that humans cannot identify the directionality of
194	OSNs when OSNs are stimulated and somatosensory nerves in the nasal cavity are not ³⁷ . For
195	β -ionone single stimulation at the adjusted concentrations, subjects tended to evaluate that the
196	malodor intensity and pleasantness were neither strong nor weak, and those who felt a strong
197	odor evaluated the malodor intensity higher. (Fig. 4C). Despite being performed in the blind
198	test, all the subjects gave the same score for total odor intensity and malodor intensity for
199	conditions contained methanethiol (Fig. 4D and Supplementary Data). When premixed gas of
200	methanethiol and β -ionone was inhaled from one nostril, total odor intensity, malodor intensity
201	and unpleasantness was reduced with compared to that of each of the components inhaled
202	individually (* p =0.035, * p =0.035 and p =0.031, respectively, Kruskal-Wallis test) (Fig. 4D and
203	4E). In a striking contrast, when methanethiol and β -ionone were simultaneously inhaled
204	through different nostrils, no significant suppression effect was observed in total odor intensity,

205	malodor intensity and pleasantness ($p=0.27$, $p=0.27$ and $p=0.072$, respectively, Kruskal-Wallis
206	test) (Fig. 4D and 4E). In relative ranking, it is clear that premixed gas showed a significant
207	change in malodor intensity, and that individual stimulation did not (* $p \leq 0.05$, nonparametric
208	Friedman's multiple comparison test) (Fig. 4F). Altogether, these results suggest that
209	antagonistic effects of β -ionone on methanethiol-responsive ORs altered odor perception.
210	

211 **Discussion**

212 OR antagonisms are hypothesized to play an essential role in odor perception, based on 213 previous reports showing widespread antagonistic interactions of odor mixtures at the level of OSNs and ORs in rodents ^{15,16,18,38,39} in conjunction with widespread mixture suppression of 214 215 odors in humans^{24,40}. This study shows that OR2T1 and OR2T11 are activated by methanethiol 216 and antagonized by β-ionone *in vitro*. This corroborates our psychophysics studies showing that 217 β-ionone reduces the intensity and unpleasantness of methanethiol in mixtures. Our results 218 imply that activation of specific OR2T members by certain VSCs induces a characteristic foul 219 odor sensation. Blocking OR2T activation using specific ketone antagonists, such as β -ionone, 220 results in lower odor intensity and unpleasantness of the VSCs, which suggests that OR 221 antagonism plays a prominent role in odor perception 222 Previous studies show that genetic variations of human ORs can cause changes in OR 223 function when tested in vitro 5,41-43. Loss-of-function variants of ORs are often associated with 224 reduced odor sensation to their ligands, suggesting direct connections between OR activation 225 and odor perception⁴⁴. Consistent with the role of OR2T family members in sulfur odor 226 perception, genetic variation of OR2T6 is associated with liking onions, whose key aroma

227	components are sulfur-containing volatiles ⁴⁵ . Additionally, mouse homologs of OR2T family
228	members are among the most significantly activated ORs by sulfur-containing odorants in vivo
229	⁴⁶ , supporting the notion that OR2T members are among the most potent ORs against the VSCs.
230	The binding of sulfur odorants with copper has been studied in silico using homology
231	models, which suggested that residues interact with the odorants ^{28-30,34,35} (Extended data Fig.
232	6). Here, using AlphaFold structural models, we showed that specific residues within the
233	transmembrane domain 5 ($C^{BW5.43}$ and $M^{BW5.39}$) are critical for OR2T1, OR2T6, and OR2T11
234	binding to hydrogen sulfide and methanethiol complexed with copper. Our models are
235	consistent with those of Haag et al., ³⁵ and Vihani et al., ⁴⁶ which noted the potential importance
236	of TM5 in copper-OR complex, notably by a ^{5.39} MYxCC ^{5.43} motif that is more prevalent in
237	sulfur-activated ORs ⁴⁶ . This motif is composed of sulfur-containing amino acids that can
238	coordinate copper and may be how many sulfur-specific ORs bind to sulfur odorants. Our
239	docking simulations also suggest that the β -ionone complex with these ORs occur at this same
240	location, preventing the effective binding of the sulfur agonists.
241	The use of OR antagonists in evaluating activation/inactivation of specific ORs in odor
242	perception has several advantages over genetic studies. Firstly, a given antagonist can block the
243	activation of multiple related ORs that may have redundant functions. In our study, β -ionone
244	antagonizes OR2T1 and OR2T11 that are activated by methanethiol. If these ORs have similar
245	roles in malodor sensation, it would be difficult to apply genetic methods due to the limited
246	effect size of each genetic variant without assessing a very large number of subjects, as done
247	with the UK biobank study ⁴⁷ . Secondly, we can test the same subject to evaluate the effects of

an antagonist (and a control) in odor perception, mitigating non-specific effects caused by

249 individuals' genetic backgrounds and other non-genetic variations. However, a given 250 antagonist for ORs may also act as an agonist for other ORs, creating a potential disadvantage 251 in distinguishing the effects of OR antagonisms from central processing in odor perception. We 252 addressed this challenge by selecting β -ionone, which shows wide variations in perceived odor 253 intensities among subjects 44,48,49 . We demonstrated that intensity or pleasantness of β -ionone 254 has no correlation with odor suppressive effects against methanethiol. Crucially, the malodor 255 blocking effect was more effective when odorants were presented as a mixture in one nostril 256 than when they were presented separately in each nostril. This strongly supports our hypothesis 257 that β -ionone reduces methanethiol odor intensity and unpleasantness by antagonizing OR2T 258 family members activity.

259 Our study does not exclude the role of central processing in mixture suppression. In the case 260 of odor masking between 1-propanol and n-amyl, the masking effects were similar when two 261 substances were mixed or presented separately to the two nostrils simultaneously, indicating that the central processing affects odor masking 23 . In the present study, Iso E Super, which does not 262 263 potently block OR2T activities, also reduces malodor intensity albeit at lower efficacy, 264 suggesting a role of central processing in masking sulfur odors. Determining the relative roles of 265 antagonistic interactions between odorants and central processing in odor masking in different 266 odor mixtures is a critical step for the future.

Finally, our study highlights a potentially efficient strategy to identify novel deodorants via *in vitro* screening of ORs. This strategy avoids biases, the limited throughput associated with odor adaptation, and the fatigue of human sensory subjects during the initial screening phase. Future studies should determine if this method is fruitful in discovering deodorants for various

- environmental malodors.
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- 276

277 Author Contribution

- 278 Y.F. conceived and designed the project. Y.F., M.A. and H.S. performed the ligand assay. R.E.
- and T.T. conducted sensory evaluation test. C.D.M. performed ligand docking and binding
- analysis, Y.F., H.M. and M.Y. carried out the analysis and wrote the paper with inputs from all

authors. Y.F., H.M. and Y.M. supervised the project.

282

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289 CADM.

290

291 **Declaration of interests**

292 Y.F., M.A., R.E. and T.T. filed patent applications relevant to this work. R.E. and T.T. are full-

293	time employee of S.T. Corporation. H.M. has received royalties from ChemCom, research
294	grants from Givaudan, and consultant fees from Kao Corporation. The remaining authors
295	declare no competing interests.
296	
297	Correspondence
298	Correspondence should be addressed to Y.F., H.M. and M.Y
299	
300	Methods
301	DNA and vector preparation.
302	Open reading frames of human OR genes were subcloned into pCI (Promega, WI, USA)
303	with a Rho-tag (the sequence encoding the first 20 amino acids of rhodopsin) at the N terminal.
304	To generate mutants of ORs, DNA fragments of OR genes were amplified by PrimeStar MAX
305	polymerase (Takara bio, Shiga, Japan). The fragments were mixed and amplified by PCR reaction
306	to obtain full sequences. The plasmid for the expression of human ORs, RTP1S ^{50,51} , and
307	pGlosensor F-22 (Promega) were amplified and purified by Nucleospin plasmid TF grade
308	(Takara bio, Shiga, Japan). All plasmid sequences were verified using Sanger sequencing (3100
309	Genetic Analyzer, Applied Biosystems).
310	
311	Cell culture.
312	HEK293T and Hana 3A cells ⁵² were grown in Minimal Essential Medium (MEM)
313	containing 10% FBS (vol/vol) with penicillin-streptomycin and amphotericin B. Hana 3A cells

314 were authenticated using polymorphic short tandem repeat (STR) at the Duke DNA Analysis

Facility using GenePrint 10 (Promega) and shown to share profiles with the reference (ATCC).
All cell lines were incubated at 37°C, saturating humidity, and 5% CO2. No mycoplasma
infection was detected in all cell cultures.

318

319 Vapor Glosensor assay

320 In the volatile sulfur detection test, Vapor Glosensor cAMP Assay (Promega) was used to measure the changes in cAMP levels caused by receptor activation upon ligand binding¹¹. 321 322 Hana3A cells were plated on poly-D-Lysine coated 96-well plates. 18-24 hours after plating, cells 323 were transfected with 80 ng/well of plasmids encoding ORs, 5 ng/well of RTP1S ⁵², and 10 324 ng/well of Glosensor plasmid (Promega). 18-24 hours later, the medium was replaced with 25 325 µL of HBSS (Gibco) containing 10 mM HEPES and 1 mM Glucose, followed by 25 µL of the 326 HBSS containing GloSensor cAMP Reagent (Promega). Plates were kept in a dark place at room 327 temperature for two hours to equilibrate cells with the reagent. The test plate was inserted into 328 the plate reader. The luminescence derived from basal activity in each ORs was measured. Before 329 odor stimulation of the cells expressing individual ORs on testing 96 well plate by odorants, a 96 330 well plate put into the 5L PET film sampling bag (Flek-Sampler, Omi odor air service Co., Shiga, 331 Japan) with a small fan. After 5L of pure air gas was inserted into the bag containing the assay 332 plate, H₂S or CH₃SH gas was added into same bag to make the desired gas phase concentration. 333 Based on a previous report that an odor response of the heterologous cells expressing ORs is 334 more than 1000 times weaker than that of olfactory sensory neuron cells¹², the stimulation 335 concentration for 1st screening (7 ppm for CH₃SH and 41 ppm for H₂S) was set to 10,000 times 336 the human olfactory threshold concentration (0.00007 ppm for CH₃SH and 0.00041 ppm for

337	H_2S) ⁵³ . Concentration of VSCs in sampling bag was measured with the Gas detector tube system
338	and detector tubes (No.70L for CH ₃ SH and No.4LL for H ₂ S) (GASTEC Corporation, Kanagawa,
339	Japan). Then the assay plate was incubated in the bag for 10 minutes. Immediately, the test plate
340	was inserted in the plate reader GloMAX discover (Promega) to measure the luminescence by
341	VSC stimulation. The luminescence in each well was measured. Response was evaluated by the
342	fold change in luminescence value before and after odor stimulation.

When the screening and evaluating antagonist was conducted, before putting the assay plate into the sampling bag, tested fragrance solutions were put into each well on the assay plate. When evaluating the response to each fragrance against ORs, all luminescence values were divided by the value obtained from the cells transfected with the empty vector at the same cycle. Multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by Dunnett's test with the target group set to control condition.

349

350 Docking

351 Structures were downloaded from the Alfaphold 2 database (https://alphafold.ebi.ac.uk/, 352 downloaded 02/17/2022) ⁵⁴. The most variable parts of the receptor were mainly located at the 353 Nter (Fig S12). OR2T1 possesses an amino acid abnormally longer Nter loop than mammals OR 354 (Fig S12 and S13) that is modelled with low confidence by Alphafold. In consequence, we 355 decided to truncate the Nter parts of the models as indicated in Fig S13. The structures of OR2T1, 356 OR2T6, and OR2T11 are very close as the Root Mean Square Deviation (RMSD) between the 357 structures are very low (Fig S12). Odorant (hydrogen sulfide, methanethiol and β -ionone) and copper SDF files were downloaded from Pubchem⁵⁵ and converted in pdb with Open Babel 2.3.2 358

on PyRx 0.8 ⁵⁶ and in pdbqt with Autodock Tools 1.5.7 ⁵⁷. The truncated Alfaphold models were
converted in pdbqt files and the grid for docking ligands and copper was generated with Autodock
Tools (Fig S14). Docking was realized with Vina 1.2.0 ^{58,59}. On a rigid receptor, 20 poses were
generated with an energy range of 15 kcal/mol and an exhaustiveness of 10. Results were
analyzed on Autodock Tools, and visualized on VMD 1.9.3⁶⁰ and UCSF Chimera 1.15⁶¹.

364

365 Sensory Evaluation test

366 All procedures involving human subjects were approved by the ethics committee of ST 367 Corporation. All subjects gave informed consent to participate. A filter paper placed in a beaker 368 was impregnated with 1g of undiluted solution of fragrances. The beaker was placed in a 10L 369 sampling bag filled with non-odor pure air gas filtered through silica and activated charcoal. 370 The scented air was adjusted by allowing the bag to stand overnight at room temperature. A 371 new 3L sampling bag was willed with pure air gas. Then, 200ml of the scented air and 0.4 mL 372 of 2% CH₃SH gas (final concentration of 0.3 ppm) were injected into the bag. In the same 373 process each fragrance and CH₃SH gas alone were adjusted. Each odor bag was prepared for 374 the whole nose test and single-nostril test (Extended data Fig. 10). Subjects evaluated the total 375 intensity, malodor intensity and pleasantness against each condition. The example of answer sheet shown in Extended data Fig. 10 was used for the evaluation. In order to clarify the 376 377 evaluation criteria, a non-blind test was conducted for Methanthiol alone and the other test in 378 the blind condition. In the test for β -ionone and Iso E Super, panelists (n=20) were divided into 379 two, and the order of the scents was changed for each. No grouping is performed for sensory 380 evaluation using other fragrances. The panelists were men and women in their 20s and 30s.

381	Detailed data of each panelist was also shown in Supplementary Data. The panelists evaluated		
382	the degree of pleasant/unpleasant, the intensity of malodor, and the intensity of total odor.		
383	Single nostril evaluation tests were performed in non-blind condition against the control bag		
384	containing only pure air and the other test in the blind condition. Panelists were instructed to		
385	sniff their hands and arms and reset their noses between samples. Nonparametric multiple		
386	comparisons were performed using one-way analysis of variance (ANOVA) following Dunn's		
387	or Friedman's multiple comparisons test using the GraphPad Prism. Correlation values were		
388	also calculated by nonparametric Spearman correction test.		
389			
390	Statistical analysis.		
391	Multiple comparisons were performed using one-way analysis of variance (ANOVA)		
392	using the GraphPad Prism. Analysis methods for each data were also described in each methods		
393	and figure legends.		
394			
395	Data availability		
396	All relevant data are available within the manuscript and its supplementary information or		
397	from the authors upon reasonable request.		
398			
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58 Figure 1 | Screening of human ORs responding volatile sulfur compounds.

559 A) Schematic representation of the vapor stimulation assay with the OR signal transduction pathway. 560 AC; adenvlyl cyclase, ATP; adenosine triphosphate, cAMP; cyclic adenosine monophosphate, 561 RTP1S; receptor transporting protein 1 short. B) Response of 359 unique human ORs against 7 ppm 562 CH₃SH and 41 ppm H₂S in vapor phase. Multiple comparisons were performed using one-way 563 analysis of variance (ANOVA) followed by Dunnett's Test. C) Vapor dose response analysis of 564 identified human ORs. The normalized luminescence value indicates the S/N ratio before and after 565 stimulation of CH₃SH or H₂S. Multiple comparisons were performed using one-way analysis of 566 variance (ANOVA) followed by Dunnett's Test (*p<0.05. **p<0.01, ***p<0.001). D) Similarity of OR2T1, OR2T6 and OR2T11 in phylogenic tree of human ORs. 567



570 571

Figure 2 | Antagonists of VSC responding human ORs

572 A) Screening for 100 odorants that inhibit the VSC response of OR2T11. B) Dose response inhibition 573 of β -ionone (red) and Iso E Super (blue) against OR2T11 with the vapor concentration of CH₃SH 574 held constant at 7ppm. IC50 value was calculated using Graph pad Prism software. C) Dose-response 575 curves of OR2T11 against β -ionone. D) Antagonistic effect of damascone & ionone analogs. 7ppm 576 CH₃SH stimulation of human OR2T1 and OR2T11 masked by 100µM odorants. Multiple 577 comparisons were performed using one-way analysis of variance (ANOVA) followed by Dunnett's 578 multiple comparison test (**p < 0.01, ***p < 0.001). E) Inhibitory effect in vapor phase. Soon after 579 adding 25 μ L of β -ionone (red) solution between the wells of the 96-well plate. OR2T11 expressing 580 cells were quickly stimulated by CH₃SH gas (blue). The normalized luminescence value indicates the S/N ratio before and after stimulation of CH₃SH. Multiple comparisons were performed using one-581 582 way analysis of variance (ANOVA) followed by Dunnett's Test (p<0.05. p<0.01) F). Left. View of the OR2T1, 2T6 and 2T11 Alphafold models. Residues M^{BW5.39} and C^{BW5.43} are represented for the 583 584 three ORs while the rest of the structure is only shown for OR2T1 for clarity. The docked copper is 585 shown in orange Van der Waals volume. The toggle switch of mammals ORs, the FYG motif at positions F^{BW6.47} to G^{BW6.49}, is also developed and serves as reference for the bottom of the odorant 586 binding site. Right. Top. All the docking position of H₂S and methanethiol in the binding cavity of 587 588 the three OR2T are represented in transparent volume. Bottom. The docking position of β -ionone in 589 the three OR2T binding cavity are represented in blue licorice.



592 Figure 3 | β-ionone changes odor perception against VSC

593 Human Sensory evaluation test against CH₃SH gas containing β -ionone, an effective antagonist. A) 594 Odor intensity value of β -ionone evaluated by 20 subjects. B) Pleasantness value of β -ionone. Color 595 matched the odor intensity score of each subjects representing in Fig 3A. Comparison test was 596 performed using the non-parametric Wilcoxon test Test (***p < 0.001). C) Results of evaluation test 597 Total odor intensity Malodor intensity and Pleasantness. Line thickness: The number of subjects who 598 made the same evaluation transition in mixture gas. Median showed the white circle in each condition. 599 D) Results of evaluation test limited to subjects who is difficult to feel β -ionone. Blue :Weak, 600 purple:Extremely weak in Fig. 3A. Each line indicates one subject. Comparison test was performed 601 using the non-parametric Wilcoxon test Test (*p < 0.05). E) Correlation analysis between relative rank 602 change in malodor intensity by mixing with β -ionone (X-axis) and total odor intensity (Upper) or 603 pleasantness (Lower) score of the β -ionone (Y-axis). Plot size: number. Correlation values were 604 calculated by nonparametric Spearman correction test. F) Relative rank in Total intensity, malodor 605 intensity and pleasantness. Median showed the color arrows in each condition. Both are shown when 606 the 10th and 11th are different. Multiple comparison test was conducted using nonparametric 607 Friedman's test (*p < 0.05)

608



609 610

611 Figure 4 | Single nostril sensory evaluation

612 A) Schematic image of single nostril stimulation in human sensory evaluation test and test odor 613 combinations. B) Test score of whether the malodor comes from the left or right direction in nostril 614 stimulation. Subjects presented the direction they felt strongly. C) Results of evaluation test malodor 615 intensity and total intensity for β -ionone single condition. Comparison test was performed using the 616 non-parametric Wilcoxon test. D) Results of evaluation test for Odor intensity, Malodor intensity and 617 Pleasantness, Center panel: CH₃SH only, Left panel: mixture gas of CH₃SH and β -ionone, Right 618 panel: single nostril stimulation by CH₃SH and β -ionone separately. Each color indicated the score of 619 one subject. Median showed the white circle in each condition. Nonparametric multiple comparisons 620 against mean values were performed using one-way analysis of variance (ANOVA) followed by 621 Dunn's multiple comparisons test (*p < 0.05). E) Comparison of which of the stimulus conditions gave 622 the better score. Odor intensity (Left), Malodor intensity (middle) and Pleasantness (Right). F) 623 Relative rank in odor intensity (Upper), malodor intensity (middle) and pleasantness (Lower). Median

- 624 showed the color arrows in each condition. Both are shown when the 7th and 8th are different.
- 625 Multiple comparison test was conducted using nonparametric Friedman's test (*p<0.05)

Supplementary Figures

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Extended data Figure 1 | Photo image of the vapor stimulation assay with the cell culturing 96 well assay plate and the small fan in the sampling bag



Extended data Figure 2 | VSC binding on odorant receptors via copper ions. A metal ion is essential for CH₃SH/H₂S responding ORs. A) Dose-response curves of 3 ORs against increasing concentrations of metals with the concentration of vapor H₂S constant at 20 ppm. B) Cu²⁺ Cheater TEPA diminished the response of ORs

against both of H₂S and CH₃SH. The y-axis indicates normalized response± s.e.m (n=3). Multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test (*p<0.05. **p<0.01, ***p<0.001).



Extended data Figure 3 | Dose response analysis of candidate antagonists. A) Dose-response curves of OR2T11 against increasing concentrations of ionones and damascones, with the vapor concentration of CH₃SH held constant at 7 ppm. IC50 value was calculated using Graph pad Prism software. B) Antagonistic effect of damascone & ionone analogs. 41 ppm H₂S stimulation on human OR2T1, OR2T6 and OR2T11 were masked by 100 μ M fragrance compounds. Multiple comparisons were performed using one-way

analysis of variance (ANOVA) followed by Dunnett's multiple comparison test (***p<0.001). C) Inhibitor odorants did not cause adverse effects on the assay system. OR2T11 and mouse Or2aj6 (Olfr171) (damascones responding receptor) expressing cells were stimulated by 100µM odorants and Glosensor buffer without any odorant as a negative control. Error bars indicate s.e.m (n=3) Multiple comparisons were performed using one-way ANOVA followed by Dunnett's test (***p<0.001).

A



В

Concentration of methanethiol in the mixed gas



Extended data Figure 4 | Inhibitory effects of β ionone in the vapor phase A) Inhibitory effect on vapor phase mixing β -ionone or Iso E super. Soon after adding 25 µL of β -ionone or Iso E Super solution between the wells of the 96-well plate, OR expressing cells was quickly stimulated by 7ppm CH₃SH gas for 10 min. The normalized luminescence value indicates the S/N ratio before and after stimulation of CH₃SH. Multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by Dunnett's Test (*p<0.05. **p<0.01)

B) Concentration of methanethiol in the sampling bag containing β -ionone or Iso E Super. methanethiol gas was injected into the saturated gas phase of β -ionone or Iso E Super in the sampling bag. Concentration of methanthiol in sampling bag was measured with the Gas detector tube system and detector tube No.70L for CH₃SH detection.





RMSD (Å)	OR2T1	OR2T6	OR2T11	OR1A1
OR2T1	-	0.76	0.83	6.46
OR2T6	0.76	-	0.83	6.42
OR2T11	0.83	0.83	-	6.41
OR1A1	6.46	6.42	6.41	-

В



Extended data Figure 5 | 3D model of volatile sulfur responding ORs. A) Superimposition of the Alphafold models of OR2T1, OR2T6 and OR2T11. RMSD

between the backbone of the structures is shown in a table. B) Alphafold confidence score projected on the Alphafold models of OR2T1, OR2T6 and OR2T11.



(Cu) methanethiol -0.9 -1.4 b ionone -0.7 -0.4 -5.6 center-x 7.421 3.2 14.729 -3.701 13.392 -3.759 center-y center-z -5.42 -3.366 0.459

7.045 2.897 11.221 center-x center-v -6.741 9.751 -2.084 -3.07 -1.321 center-z 2.997

Extended data Figure 6 | Simulation analysis of inhibitory effects by β -ionone. A) Alignment of the sulfur responding OR studied in the literature. X.50 positions, TM domains as well as amino acids studied here and previously identified are highlighted. B-D) Docking results for H₂S (B), methanethiol (C) and β ionone (D) for OR2T1 (purple), OR2T6 (yellow) and OR2T11 (pink). Copper is represented in Van der Waals volume colored by OR while the ligand is represented in licorice with sulfur (H₂S and methanethiol) or carbon (β -ionone) atom colored by OR. E) Docking results by affinity represented by the Vina score. F) Box dimension and placement for copper docking. G) Box dimension and placement for ligand docking.



Extended data Figure 7 | Sensory evaluation test A) Score for β -ionone or Iso E Super. Red: Group A (n=10), Blue: Group B (n=10), Dot line: Median value. B) Score for methanethiol only, methanethiol and Iso E Super, and methyl methanethiol and β -ionone. Red: Group A (n=10), Blue: Group B (n=10), Dot line: Median value. The significance analysis between Group A and B was performed using Mann-Whitney test. Nonparametric multiple comparisons among mixed gas were performed using one-way analysis of variance (ANOVA) followed by Dunn's multiple comparisons test (*p<0.05. **p<0.01, ***p<0.001).



Extended data Figure 8 | Human Sensory evaluation test against CH₃SH gas containing β -ionone (an effective antagonist) or Iso E Super. A) Odor intensity value of β -ionone and Iso E Super evaluated by 20 subjects. B) Pleasantness value of β -ionone and Iso E Super. Color matched the odor intensity score of each subject representing in Extended Fig. 8A. Comparison test was performed using the non-parametric Wilcoxon test Test (***p<0.001). C) Test odor combination and order in sensory evaluation test. D) Results of evaluation test Total odor intensity Malodor intensity and Pleasantness. Line thickness: The number of subjects who made the same evaluation transition in mixture gas. Median showed the white circle in each condition. E and F) Results of evaluation test Total odor intensity Malodor intensity and Pleasantness when classified into Odor intensity scores of β -ionone or Iso E super. Color matched the odor intensity score of each subject representing in Extended Fig 8A (E: Extremely weak and Weak, F: Neither weak nor strong and Strong). Line thickness: The number of subjects who made the same evaluation transition in mixture gas. Comparison test was performed using the non-parametric Wilcoxon test Test.



Extended data Figure 9 | Damascones changes odor perception against VSC A and D) Sensory evaluation score for methanethiol only and mixture gas with damascones. A: Odor intensity, D: Pleasantness, Gray dot line: Mean value. Nonparametric multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by Dunn's multiple comparisons test (*p<0.05). B and E) Relative rank in malodor intensity and pleasantness, C and F) Relative rank change in malodor intensity (C) and pleasantness (F) Multiple comparison test was conducted using nonparametric Friedman's test (*p<0.05, **p<0.01).

А

For whole nose stimulation



В

Evaluation sheet in Japanese

				快・	不	夹度					悪	[臭0	り強	度			全	:体0	り強	度	
	極端に不快	非常に不快	不快	やや不快	快でも不快でもない	やや快	快	非常に快	極端に快	無臭	やっと感知できる	弱い	楽に感知できる	強い	強烈	無臭	やっと感知できる	認い	楽に感知できる	強い	強烈
悪臭																					
А																					
В																					
С																					
D																					

Translation

			Р	leas	sant	nes	s			N	lalo	dor	inte	nsi	ty	Odor intensity						
	Extremely Unpleasant	Very Unpleasant	Unpleasant	Slightly unpleasant	Neither Unpleasant Nor Pleasant	Slightly Pleasant	Pleasant	Very Pleasant	Extremely Pleasant	Odorless	Extremely Weak	Weak	Neither Weak Nor Strong	Strong	Extremely Strong	Odorless	Extremely Weak	Weak	Neither Weak Nor Strong	Strong	Extremely Strong	
Malodor																						
А																						
В																						
С																						
D																						

Extended data Figure 10 | Equipment for sensory evaluation test Left) Photographic image of the sampling bags used in sensory evaluation test. Upper) for whole nose stimulation (Results were shown in Figure 3), Lower) for single nostril stimulation test (Results were shown in Figure 4). Each has a suction

port of a suitable size. Right) Score sheet used in sensory evaluation tests. Upper: original score sheet written in Japanese. Lower: English translation version of original.

For single nostril stimulation