A Spectroscopic Mechanism for Primary Olfactory Reception

Luca Turin

Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK

Correspondence to be sent to: Luca Turin, Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK

Abstract

A novel theory of primary olfactory reception is described. It proposes that olfactory receptors respond not to the shape of the molecules but to their vibrations. It differs from previous vibrational theories (Dyson, Wright) in providing a detailed and plausible mechanism for biological transduction of molecular vibrations: inelastic electron tunnelling. Elements of the tunnelling spectroscope are identified in putative olfactory receptors and their associated G-protein. Means of calculating electron tunnelling spectra of odorant molecules are described. Several examples are given of correlations between tunnelling spectrum and odour in structurally unrelated molecules. As predicted, molecules of very similar shape but differing in vibrations smell different. The most striking instance is that of pure acetophenone and its fully deuterated analogue acetophenone-d8, which smell different despite being identical in structure. This fact cannot, it seems, be explained by structure-based theories of odour. The evidence presented here suggests instead that olfaction, like colour vision and hearing, is a spectral sense. Chem. Senses 21: 773–791, 1996.

Introduction

Putative olfactory receptors have been identified previously (Buck and Axel, 1991; Buck, 1993; Ngai et al., 1993; Raming et al., 1993). Signal transduction is known to involve a G-protein-coupled adenylate cyclase mechanism (for review see Shepherd, 1994). However, the mechanism by which receptors detect odorants, and thus the molecular basis of odour, remain unclear. As has been repeatedly pointed out, structure–odour relations provide conflicting evidence (Beets, 1971, 1978; Klopping, 1971; Ohloff, 1986; Weyerstahl, 1994). On the one hand, molecules of widely different structures can have similar odours, e.g. the bitter almond character, shared by as many as 75 molecules, including the triatomic molecule HCN. On the other hand, minor changes to the structure of a molecule can alter its smell character completely. For example, isomers such as vanillin (3-methoxy-4-hydroxy-benzaldehyde) and isovanillin (3-hydroxy-4-methoxybenz-aldehyde), and enantiomers such as R- and S-carvone smell very different (Arctander,
Furthermore, olfaction is often 'analytical': the presence in a molecule of certain chemical groups such as aldehydes, carbonyls, nitriles, isothiocyanates, thiols, esters and ethers is frequently correlated with a particular odour. The example best known to non-specialists is thiol (SH), which gives any odorant molecule a unique 'sulphuraceous' (rotten egg, garlic) odour character. Structure–odour theories tend to reflect one or the other line of evidence: 'functional group' theories emphasize the analytical view, but do not provide a mechanism by which individual groups can be detected regardless of molecular shape. Theories based exclusively on shape enjoy some success within specific odorant groups, but do not account for the striking chemical group regularities. Nor do they account for the fact that very different structures give similar odours.

The present work originates from a theory first put forward by Dyson (1938). He suggested that olfactory organs might somehow detect molecular vibrations. If the whole vibrational range to 4000 cm⁻¹ were detected as by a Raman or IR spectroscope, detection of functional groups would be explained, since many have distinctive vibrational signatures, usually above 1000 cm⁻¹. This theory was modified in a series of articles by Wright and co-workers (Wright, 1977 and references therein). They argued that since optical means were out of the question, detection of molecular vibrations must necessarily be mechanical in nature, and therefore only vibrational modes excited at body temperature could be detected. This meant that the range of detectable vibrations must lie below ~2 kT, or 500 cm⁻¹. Correlations between spectrum and odour in that range were reported, but they were achieved by dismissing discrepant peaks as 'anosmic', and adding some at computed 'difference' frequencies. In any event, it was always clear that the region below 500 cm⁻¹ could not determine odour (Klopping 1971): many inorganic molecules with strong, distinctive odours, such as NH₃, H₂S, O₃ and HCN, vibrate only in the range 700–3500 cm⁻¹. Furthermore, the basic idea of vibrational spectroscopy could never account for the different odours of enantiomers. Lastly, no plausible mechanism was ever found by which a biological system could perform vibrational spectroscopy by mechanical means, and the theory eventually fell from favour.

This article will cover the following topics: inelastic electron tunnelling spectroscopy; its implementation in olfactory receptors; evidence from neurophysiology; the calculation of structure–odour relations; the problem of odourless molecules; applications of the theory to a few selected structure–odour problems; and isotope replacement.

A biological 'spectroscope'

Inelastic electron tunnelling spectroscopy (IETS) is a non-optical form of vibrational spectroscopy (Jaklevic and Lambe, 1966; Hansma, 1982; Adkins and Phillips, 1985). It relies on the interaction between electrons tunnelling across a narrow gap between metallic electrodes and a molecule in the gap. When the gap is empty, tunnelling electrons cross the gap at constant energy and the tunnelling current is proportional to the overlap between filled and empty electronic states in the metals. Thus, if the density of filled and empty states is constant, the junction is ohmic. If a molecule is present in the gap, tunnelling electrons will be scattered by the partial charges on the molecule's energy gap.

![Figure 1](http://chemse.oxfordjournals.org/)

Figure 1: Schematic of the proposed transduction mechanism: the receptor protein accepts electrons from a soluble electron donor (NADPH). When the receptor binding site is empty (top), electrons are unable to tunnel across the binding site because no empty levels are available at the appropriate energy. The disulphide bridge between the receptor and its associated G-protein remains in the oxidized state. When an odorant (here represented as an elastic dipole) occupies the binding site (bottom), electrons can lose energy during tunnelling by exciting its vibrational mode. This only happens if the energy of the vibrational mode equals the energy gap between the filled and empty levels. Electrons then flow through the protein and reduce the disulphide bridge via a zinc ion, thus releasing the G-protein for further transduction steps.
constituent atoms, and lose energy to the molecule by exciting one of its vibrational modes. When this happens, electrons can follow an indirect path, first exciting the molecular vibration and then tunnelling to the second metal at a lower energy. The new tunnelling path causes an increase in the conductance of the junction. Successive vibrational modes are excited as junction voltage is increased. Differentiating the current–voltage relationship twice yields narrow peaks at energies corresponding to those of the vibrational modes of the molecule in the gap.

Metallic conductors are absent in biology, but electron transfer is ubiquitous. Doing IETS with proteins (Figure 1) would involve addition and removal of electrons at well-defined energy levels on either side of an odorant-sized (<400 daltons) binding site which serves as the tunnelling gap. On one side of the gap a donor site with occupied donor levels is present, while an acceptor site with empty acceptor levels is on the other side. If there is nothing between the electron source (donor) and sink (acceptor), then for direct tunnelling to occur there must be an (occupied) energy level in the source which matches the energy of an (empty) state in the sink.

If there is a molecule between the electron source and electron sink, and if that molecule vibrates, then (taking the energy of the vibrational quantum as \( E \)) indirect tunnelling can occur by an additional channel if there is an energy level in the source with energy \( E \) above that in the sink. After tunnelling, the molecule will have a vibrational energy higher by \( E \). In other words, tunnelling occurs only when a molecular vibrational energy \( E \) matches the energy difference between the energy level of the donor and the energy level of the acceptor. The receptor then operates as a spectrometer which allows it to detect a single well-defined energy, \( E \). If the change in energy between donor and acceptor levels is sufficiently large, tunnelling current flows across the device only when a molecule with the appropriate vibrational energy is present in the gap. If there are several vibrational modes, which one(s) will get excited will depend on the relative strengths of the coupling, and that may be expected to depend, among other things, on the partial charges on the atoms and the relative orientation of the charge movements with respect to the electron tunnelling path.

Unlike conventional IETS, ‘biological IETS’ does not involve scanning of the energy range, which would probably be unfeasible in a biological system. Instead, the range of vibrational energies is covered piecewise by a series of receptors tuned to different energies. The energy range is limited only by the e.m.f. (reducing power) of the electron source. An estimate of biological reducing power is 500 mV (1 eV = 8086 cm\(^{-1}\)) (Frauesto da Silva and Williams, 1993), which means that the entire vibrational range to 4000 cm\(^{-1}\) could be sampled. To cover the vibrational spectrum, several receptor classes would be required, each tuned to a different segment of the vibrational spectrum. A small number might be sufficient, much as three pigments with broad, partially overlapping absorption spectra suffice for colour vision. One essential feature of the biological spectrometer is its relatively poor resolution. Vibronic coupling, which results in a broadening of acceptor and donor levels across the tunnelling gap, is present in all tunnelling systems, be they metallic, semiconducting or chemical. When IETS is performed between metal electrodes, the tunnelling junction is cooled to cryogenic temperatures to increase resolution. A biological system must work at ambient or body temperature, i.e. at \( \approx 300^\circ\text{K} \). Donor and acceptor levels across the tunnelling gap will therefore have a minimum width of 2 kT \( \approx 400 \text{ cm}^{-1} \). The range 0–4000 cm\(^{-1}\) could therefore be covered by 10 or so receptor types. A similar arrangement exists in the other spectral senses, vision and hearing, in which broadly tuned receptor classes cover segments of the complete spectrum.

Evidence for electron tunnelling in putative olfactory receptors

If olfactory receptor proteins function as a tunnelling spectroscopy, their primary amino acid sequences may contain some telltale features, provided these are not too widely scattered and brought together only by folding. Firstly, since most odorants are redox-inactive, the receptor protein must obtain electrons from either a soluble electron carrier or possibly a reductase. If no intermediate enzyme is involved, one would expect to find a motif designed for binding to soluble electron donors. Secondly, the presence of a metal-binding site would not be unexpected, since many electron-transfer enzymes require metal cofactors. Thirdly, since the electron current must be turned into a chemical reaction at some point to connect it to the conventional signal transduction pathway, one would expect an electrochemical (e.g. a reduction) step to be the final stage in the transduction.

The sequences of 25 putative olfactory and eight
gustatory receptors have been published. They have strong homologies with G-protein-coupled receptors, are specific to olfactory neuroepithelia and contain a variable hydrophobic region which may be involved in odorant binding. The first step was to test for the presence of an NAD(P)H binding site motif in these sequences. The searches were performed using the Blitz server at Heidelberg. NAD(P)H binding motifs are highly conserved (Lawton et al., 1990; Scrutton et al., 1990). Remarkably, an exact match to an NAD(P)H binding site sequence (GLGLLA) highly conserved in dehydrogenases and flavine monoxygenases is found in one of the olfactory receptors, olf8 mouse, and related sequences are found in all the others. Since according to this theory the receptor functions as an 'NADPH diaphorase', it may be significant that high levels of diaphorase activity have been detected in olfactory receptor neurons (Zhao et al., 1994).

The second step was to look for a metal-binding site. A clue as to which metal may be involved is provided by the evidence of a strong link between zinc and olfaction. Zinc deficiency, whether dietary (Alpers, 1994) or caused by treatment with histidine (Henkin et al., 1975), thiocarbamides (Erikssen et al., 1975) or captopril (Zumkley et al., 1985), is unique in causing a complete and rapidly reversible anosmia. A search for zinc-binding sites found in enzymes using Prosite gave no good matches. A search for homologies was then performed using small segments of the primary sequence close to the flavin-binding site, the rationale being that the electron circuit is likely to be compact in size to avoid long-range transfer, and that this may be reflected in the primary structure. This search turned up a motif C[GATV]SH[L], present in all the receptors, most commonly as CGSHL (Cys-Gly-Ser-His-Leu) seven amino acids away from the 'flavin-binding' region. This motif lies near the cytoplasmic end of the (predicted) sixth transmembrane domain and is absent from other G-protein-coupled receptors (Figure 2). CGSHL is the zinc-binding motif of insulins (Hodgkin, 1974). In insulin, several molecules coordinate around a single zinc ion via the histidine in the motif to form oligomers during storage in the pancreas. Perhaps surprisingly, this five-residue sequence is rare. In a search in the complete Swissprot database, 64 proteins gave an exact match to CGSHL. Ten of these were olfactory or gustatory receptors and 51 were insulins or proinsulins. Only three were neither olfactory receptors nor insulins: bombyxin B-8 precursor, alpha methyl dopa hypersensitive protein and chitinase. These three extraneous proteins are unrelated to one another. Searches with the alternative sequences found in the olfactory receptors CASHL, CVSHL CTSHEL and CSSHEL give figures for unrelated/total of 5/11, 0/2, 0/6 and 8/10 respectively. With these few exceptions, therefore, the motif appears to be diagnostic of olfactory receptors and the zinc-binding site of insulins.

Zinc's involvement in olfaction, its ability to form bridges between proteins, its presence in electron transfer enzymes such as alcohol dehydrogenase (Frausto da Silva and Williams, 1993) and the presence of the redox-active amino acid cysteine in the receptor's zinc-binding motif all point to a possible link between electron flow and G-protein transduction. In the closely related adrenergic receptors, a role for cyclic reduction and oxidation of disulphide bridges has been suggested (Kuhl, 1985). It involves cross-linking of the G-protein to the receptor by an S-S bridge which is then reduced upon binding of the (redox-active) catecholamine to the receptor, thereby releasing the G-protein. I propose that a similar mechanism may be at work in olfaction (Figure 1). Suppose that the zinc-binding motif on the olfactory receptor is involved in docking to the olfactory G-protein g(olf), and that the docking involves formation of a disulphide bridge between the receptor and the g-protein. One would expect to find on g(olf) the other half of a zinc coordination site, e.g. two histidines in close proximity and a cysteine nearby. A search in the primary sequence of g(olf) (Jones and Reed, 1989) found the motif HYCYPH (His-Tyr-Cys-Tyr-Pro-His) at positions 343–351. This motif has the requisite properties for docking (Figure 3A): it is exposed on the surface of the g-protein (Coleman et al., 1994; Lambright et al., 1996) and is known to interact with g-protein-coupled receptors (Rasenick et al., 1994).

The docking scheme was tested by molecular modelling of the two sequences. Figure 3 shows that when the three histidines coordinate a zinc ion, the minimum-energy configuration incorporating a disulphide bridge puts the two sulphurs very close to the zinc with no intervening group. The proline bend in the g(olf) motif helps bring the two histidines close together without strain. Reduction of the disulphide bridge followed by energy minimization causes little change in the structure, indicating that the redox reaction could take place reversibly at near-constant configuration energy. I have modelled every one of the zinc-binding motif variants in the proposed zinc binding configuration and found that the different side chains makes little difference to zinc binding and overall geometry. This
Figure 2  Sequence homologies between insulin (top line) and olfactory and gustatory receptors. The motif indicated in red (box) is the zinc-binding motif of insulin, which is also present in all the olfactory and gustatory receptors with small variations. The blue motif of olf8_mouse is a highly conserved NADPH binding sequence.

锌—硫醇位点可能作为电子出口，是嗅觉感受器的正极连接器。

气味-结合位点可能位于黄素和锌结合位点之间。注意其胞内位置并不影响气味的接近性：它们是小的疏水性分子，其分配系数通常在10^3数量级。这些发现表明未来尝试表达或重建受体应包括锌和胞浆中的电子供体在胞浆相位之前进行测试。目前还不清楚其他受体的相应序列是否能结合电子受体。可能需要一系列能够以不同的氧化还原电位结合供体的接受位点来探测不同段的光谱，而没有一个序列能适合所有。

神经生理学证据

有证据表明单个嗅觉受体神经元（ORNs）能对多种气味作出反应（Sicard and Holley, 1984）。这与它们对多个分子的共同特征作出反应的想法是一致的。这种特征是结构的还是振动的？如果是后者，不同气味对ORNs的反应应与结构有关，还是与振动有关？如果后者，我们预期ORNs对不同气味的响应是否可能？
vibrational spectra. Furthermore, ORNs are known to express only one or a small number of receptor genes (Ressler et al., 1994). Their response patterns should therefore be consistent with each ORN responding to one, or possibly a few, vibrational bands. It has recently become possible to record from isolated olfactory receptor neurons using patch-clamp methods. The most direct evidence (Firestein et al., 1993) for comes from a study of isolated salamander olfactory neurons exposed to three odorants: cineole, isoamyl acetate and acetophenone. Cells were found that responded in all the possible patterns to one odorant, two odorants or all three. The response pattern of three cells, taken from figure 1 of Firestein et al.'s paper, is as follows: one cell responded to all three odorants, one cell to acetophenone and isoamyl acetate but not to cineole, and one to cineole alone. When the spectra of the three odorants are calculated using an algorithm which simulates tunnelling spectra (see below), the results of Firestein et al. are consistent with their sensing three different vibrational bands (Figure 4). The cell responding only to cineole could have been tuned to the band at \( \sim 1200 \text{ cm}^{-1} \) which is largely absent in the other two odorants. The cell responding to all three may have been sensitive to the band at \( \sim 1000 \text{ cm}^{-1} \), and the cell responding only to acetophenone and isoamyl acetate may have been responding to the C=O stretch at \( \sim 1800 \). By contrast, it is far from clear which structural features would account for this pattern. The vibrational theory could readily be tested by further experiments: the cells responding to the C=O stretch should respond to a nitrile, while the cell responding only to acetophenone should also respond to benzene and possibly to an alkene.

Calculating odours: a first approximation

The proper test of a structure-odour theory is odour prediction. If electron tunnelling is the means by which molecules are recognized, then their tunnelling spectra as
measured by the biological detector should correlate with their odour. Spectral comparison requires three independent pieces of information: the frequency of a given vibrational mode, its intensity as perceived by the sensor and the resolution of the detector system. The first is reasonably easy to obtain: semi-empirical quantum chemistry programs calculate most vibrational mode frequencies to an accuracy of the order of 50 cm\(^{-1}\), i.e. much greater than the assumed sensor resolution. As was discussed before, a resolution of the order of \(~400\) cm\(^{-1}\) is the best that can be expected. Line intensities are more of a problem: if, for example, Raman spectra of dyes with absorption lines in the visible were compared with perceived colour, no correlation would be found. This is because the pigments in the photoreceptors operate by a mechanism involving absorption, not Raman scattering. Furthermore, prediction of the perceived colour of a dye from its absorption spectrum requires knowledge of the number of pigments present in the retina and their spectral resolution.

IETS spectra of different compounds in metal–insulator–metal junctions have been measured, and a theory for calculating their line intensities has been developed. Unfortunately odorants are not well-suited to conventional IETS measurements, because they tend to evaporate during the vacuum deposition steps used in the manufacture of the tunnelling junctions. From a theoretical standpoint, another problem exists. Line intensities in conventional IETS are determined in part by the image charges caused by the close proximity of metal surfaces with their effectively infinite dielectric constant. In a biological sensor, dielectric constants will vary over a much smaller range, bounded by 3–5 for protein and 80 for water. Therefore, even if measured IETS spectra of odorants were available, they would not necessarily correlate well with those detected by a biological sensor.

I have developed an algorithm for the calculation of 'biological' IETS spectra called CHYPRE (for CHaracter PREdiction). Three steps are involved: the first is calculation of the \(6N - 6\) (where \(N\) is the number of atoms) vibrational mode frequencies and \(N(6N - 6)\) atom displacements, using MOPAC with PM3 parameters. This will be most reliable when calculating molecules which do not contain nitrogen, which fortunately is the case for most odorants. The second step involves calculation of partial charges. This is important because electron scattering is proportional not to overall dipole moments but to the square of the partial charge. Partial charges are not direct observables, and therefore the choice of algorithm and parameters for their calculation is to some extent arbitrary. In all the calculations shown below Zindo with INDO/1 parameters was used because it gives the best correlation between the computed spectrum and smell (see below). Both MOPAC and Zindo form part of the CaCHE Worksystem (Oxford Molecular) which was used in all the calculations.

The core of the CHYPRE algorithm calculates a 'biological IETS' spectrum according to the theory developed by Sleigh et al. (1986), modified to take into account the fact that rotation of the molecule is likely to occur because of the weakness of the forces (hydrogen bonds and dispersion forces) binding it to the receptor, and neglecting image charges. Details of the algorithm will be published at a later date. For the purpose of the present article, it should be emphasized that all molecules were calculated using identical parameters and that, unless otherwise indicated, no scaling of the spectra was involved. To mimic the thermal broadening of lines in a biological system, spectra were convolved with a Gaussian blurring function with a standard deviation of \(200\) cm\(^{-1}\). The most obvious difference between computed IR and CHYPRE spectra is that the fingerprint region below \(1500\) cm\(^{-1}\), used by spectroscopists to determine molecular structure, is emphasized more by CHYPRE than by IR. This fits empirical odour data, because most of the differences between odorants with similar elemental composition and different structures will be concentrated in this region.

**Two examples of vibrational structure-odour relations**

A good test of the CHYPRE algorithm would be comparison of the spectra of two or more molecules with very similar odours and very different structures. Such an occurrence is rare, but enquiries among perfumers elicited one example, that of the chemically unrelated ambergris odorants cedramber, karanal, Jeger's ketal and timberol (Figure 5). The ambergris note of the four is very similar, karanal being slightly greener than Jeger's ketal, with cedramber and timberol somewhat woodier. The upper graph in Figure 5 illustrates the raw (unconvolved) data from CHYPRE calculations. Each point on the graph represents a vibrational mode, with wave number along the abscissa and intensity along the ordinate. A considerable amount of scatter is present, showing that these three
Figure 5 Structures (top), raw vibrational spectra from CHYPRE calculations (middle) and convolved spectra (bottom) for four ambergris odorants with closely related odours and very different structures. Each point in the raw spectra represents the energy and amplitude of a single vibrational mode. A considerable degree of scatter is present, reflecting the structural diversity of the molecules. By contrast, the convolved CHYPRE spectra, like the odours, are remarkably similar.

Chemically unrelated molecules have very different underlying vibrational modes and partial charges. After convolution, however (lower graph), the spectra become very similar, consistent with their similar smells. The relationship between structure and odour is thus subject to two encoding steps: from structure to vibrational modes, and from vibrational modes to perceived convolved spectrum.
The converse experiment should also be true, namely that molecules with very similar structures and different smells should have different spectra. Figure 6 illustrates the CHYPRE spectra of the well-known example (Ohloff, 1986) of three undecanones differing in the position of the carbonyl group. 2-Undecanone smells of ruewort, 6-undecanone smells fruity and 4-undecanone is reportedly intermediate in odour between the two. Despite the close structural similarity, the spectra of 2- and 6-undecanone are clearly different. Peak 1 differs in amplitude and is shifted by 100 cm\(^{-1}\), and peaks 2 and 3 differ in amplitude. This suggests that the ability of the olfactory system to discriminate closely related molecules could be achieved by a vibrational transduction mechanism.

Six ways of being odourless
A peculiar feature of a vibrational theory is that since all molecules vibrate, they should all have an odour (provided of course they are volatile) unless some other factors peculiar to the function of a biological spectroscope prevent electron tunnelling from taking place.

No partial charges
A typical example of this are the homonuclear diatomic gases such as H\(_2\), N\(_2\) and O\(_2\). Molecules made up of two identical atoms can have no partial charges on their atoms for reasons of symmetry and would be predicted to be odourless, which they are. The apparent exception of...
halogen gases, e.g. Cl₂, cannot be settled at present, because they cause an intense trigeminal sensation of pungency and immediately react with biological molecules to form chloramines, which themselves have an intense ‘chlorine’ smell.

Too big
Odorants are all <400 daltons or so in molecular weight. Larger molecules, while they may develop appreciable partial pressures, are odourless. Near the maximum size, many specific anosmias are known to exist. For example, most subjects are anosmic to one or another of the large musks such as pentadecanolide or Tonalide. According to this theory, if a small change to a large molecule renders it completely odourless, as opposed to less odorant or nondescript in character, it is likely to be because it no longer fits any receptor. Note that a maximum size follows naturally from a tunnelling theory. All other things being equal, tunnelling probabilities across a gap of length $d$ are proportional to $\exp(-t^2/2)$, so that the size requirement may reflect the inability of a tunnelling sensor to give appreciable currents over distances $>1-2$ nm.

Blind spots
The tunnelling gap must contain something, either water molecules or more probably side chains of hydrophobic amino acids. If the latter is the case, then receptors tuned to the broad C–H stretch frequency would be fully switched on all the time, which would probably cause rapid habituation. A more likely possibility, since C–H stretch will be too common in any event to distinguish molecules from one another, is that such a band is simply not represented in the spectrum, and that one or more receptor bands are missing. This would account, for example, for the fact that CH₄ is odourless, though weak scattering (see below) may also contribute. A second type of blind spot might arise if a band is never represented in odorants, thus making the receptor class redundant. One example might be the 2600–2800 band between SH stretch and the lowest aldehyde CH stretches.

Frequencies too low
Vibrational modes below kT will not be detected by a biological spectroscope for two reasons: firstly, they will already be thermally excited, and electrons will be as likely to gain energy from them as to excite them to a higher vibrational level. This will reduce the tunnelling current.

Secondly, if the difference in energy between donor and acceptor levels is of the order of kT, thermal broadening of the levels will mean that electrons will flow whether or not an odorant is present, and the problem reduces to that of the blind spot discussed above. The tetrahalomethanes CCl₄, CBr₄ and Cl₄ provide a test of this idea. The first two have (different) distinctive haloalkane odours, but Cl₄ is odourless and merely smells weakly of its decomposition product, iodine. CCl₄ (459 and 790 cm⁻¹) and CBr₄ (267 and 671 cm⁻¹) have modes above kT, but all the modes of Cl₄ (90, 123 and 178 cm⁻¹) except one lie below kT (Dollish et al., 1974). This is consistent with the lower limit of frequencies being between kT and 2kT.

Scattering too weak
Taking the simplest case of a diatomic molecule as an example, scattering will be proportional to the square of atom displacements. Stiff bonds carrying small partial charges will have low line intensities. For example, CHYPRE calculates very low line intensities for the C=C stretch (see the discussion of bitter almonds below) and a low intensity for the stretch band in carbon monoxide, which may explain why it is odourless despite being a heteronuclear diatomic and a potential odorant.
Wrong relative group orientation

An unusual feature of a protein-based spectrocope is that it exhibits polarization effects. When scattered, electrons change direction as well as momentum. For example, they will be scattered sideways if the scattering vibration takes place at right angles to the tunnelling path (Figure 7). In conventional IETS, this lengthens their path across the insulating gap and reduces their tunnelling probability. In a biological sensor, where donor and acceptor sites on either side of the tunnelling gap are likely to have a small cross-section, electrons scattered in the wrong direction may fail to reach the acceptor site altogether, and the sensor will not respond. The effect will be clearest when the vibration involves only two atoms, with a well-defined direction of charge movement. This will ensure that electrons are scattered in a preferred direction. The scattering group must be fixed with respect to the rest of the molecule, which must in turn assume a preferred orientation with respect to the sensor. Flat rings bearing intense scatterers as substituents are ideal in this respect. What will happen in this case is that only part of the molecule will be odorant, and that the smell character will resemble that of the molecule with the 'hidden' (transverse scattering) group removed. Examples of this effect are discussed in the next section.

‘Hidden’ dipolar groups

The puzzle of carvone enantiomers

A persistent objection to the infrared-spectrum theory has been that, unlike our nose, it could not resolve enantiomers. While it is true that solution spectra of enantiomers are identical, the objection does not apply to a protein-based sensor. One reason, common to all receptors, is that odorant-binding sites in the sensors are always chiral to some extent, and their relative affinities for enantiomers therefore differ. Interestingly, discrimination between carvone enantiomers in a neurophysiological preparation has been shown to fall with rising temperature (Hanada et al., 1994), consistent with the idea that weak (e.g. Van der Waals) forces hold the odorant in a particular orientation in the receptor.

Another reason, discussed above, is that part of the
The different odours of vanillin and isovanillin are a classic olfactory conundrum. The two molecules differ only in the position of their substituents on the benzene group. Vanillin has a rich vanilla odour whereas isovanillin has a weaker, very different, somewhat phenolic odour. As may be expected from their close similarity of structure, the vibrational spectra of vanillin and isovanillin are very similar. Molecular dynamics simulations of the two molecules, however, reveal an essential difference (Figure 9). In isovanillin the aldehyde group is constrained to lie in the plane of the benzene ring. In vanillin, by contrast, rotation about the bond joining the aldehyde to the benzene ring is easier, and the aldehyde can adopt a range of configurations more nearly orthogonal to the ring. I suggest that it is this rotation that makes detection of the carbonyl possible in vanillin. In isovanillin the carbonyl group is transversely oriented and therefore undetectable. One would therefore expect isovanillin to smell like its aldehyde-less congener guaiacol, i.e. phenolic–medicinal. This is the case: perceptually, isovanillin, though not entirely devoid of vanilla character (Arctander, 1994), possibly reflecting infrequent aldehyde rotations, is closer to guaiacol. As in the case of carvones, an 'additive synthesis' of the vanillin smell is possible by mixing guaiacol (phenolic) and ethylbenzaldehyde (bitter almonds). In the proximity of the 1:1 proportion, a vanillin-like note appears. The illusion is not perfect, but it is striking because the vanilla character is absent from the components. The CHYPRE spectra of benzaldehyde and guaiacol are shown in Figure 10, together with their sum (without scaling) and the spectrum of vanillin. The sum spectrum gives a reasonably good fit to the vanillin spectrum, consistent with the smell character of the mixture.
A Spectroscopic Mechanism for Primary Olfactory Reception

Monochromatic odours: the 2500 and 2300 cm⁻¹ bands

A simple test of a vibrational theory of olfaction would be a 'monochromatic' stimulus, i.e. a stimulus consisting of one frequency only. The region below 1500 cm⁻¹ is unsuited to this approach, because organic molecules have most of their modes in this range and no obvious correlations between the mere presence of a vibration and an odour character exist. By contrast, the region between 1500 and 4000 cm⁻¹ contains the stretch frequencies of various specific groups, and there appears to be a good correlation between some vibrational frequencies and odour character.

The most striking example is the 2500 cm⁻¹ band. Mercaptans, selenomercaptans and telluromercaptans (R-SH, SeH and TeH) have a unique odour character, found in no other organic molecule. Their stretch vibration frequency (~2550 cm⁻¹) is equally unique, and does not overlap with any other common group frequency (Socrates, 1994). Remarkably, terminal boron–hydrogen (BH) group stretch frequencies at ~2600 are the only ones known to overlap S, Se and Te–H stretches (Nakamoto, 1986). Even more remarkably, the stable and relatively non-volatile borane B₁₀H₁₄ (decaborane; Sigma) is to my knowledge the only non-sulphur containing molecule that smells like a mercaptan. Other boranes reportedly smell similar. The next nearest (<2400 cm⁻¹) are the P–H and As–H stretches of phosphine, arsine and their derivatives (Nakamoto, 1986). They reportedly smell of garlic and rotten cabbage (Klopping, 1971)—two sources of complex mercaptans—suggesting that their vibrational frequencies are close enough to excite a putative 2500 cm⁻¹ receptor band. The existence of a congenital specific anosmia to mercaptans

Figure 10. 'Additive' synthesis of the odour of vanillin from vibrational components. The structures of vanillin, benzaldehyde and guaiacol are shown at the top, and the unscaled CHYPRE spectra for the three molecules at bottom. The sum of the spectra of guaiacol (phenolic, medicinal odour) and benzaldehyde (bitter almonds) gives a reasonable approximation to the spectrum of vanillin. A mixture of guaiacol and benzaldehyde is therefore predicted to have a vanilla odour character, which it does.
(Amoore, 1971) is also consistent with a vibrational mechanism. If shape determines odour, it is difficult to understand how a mutation would wipe out a large number of receptors while only affecting a single odour class. By contrast, the absence of a receptor responsible for a unique frequency band will abolish detection, much as the absence of long-wavelength cones gives rise to protanopia. Other congenital anosmias, such as that to amines (Amoore, 1971) (~3300 cm⁻¹), may have a similar origin, and it will be interesting to re-examine them with molecules designed to probe different vibrational bands.

There may another band of interest just below the SH stretch band, at ~2300 cm⁻¹. Alkyl isothiocyanates (R−N=C=S, stretch frequency ~2200) have a unique mustard odour character, and so does the chemically unrelated carbon suboxide O=C=C=O (C=O stretch frequency ~2200). Another instance of a straightforward correlation between spectrum and smell is the aldehyde−nitrile replacement rule. It is well known to fragrance chemists that the nitrile group can frequently replace an aldehyde with only a minor change of odour character, 'duller' and somewhat 'oily' (Bedoukian, 1986). For example, benzaldehyde and benzonitrile have similar odours, as do agrunitril and citronellal, cinnamaldehyde and cinnamalva. This is consistent with vibrational detection, since the intense nitrile vibration at 2100 could replace the carbonyl band at ~1800 (Socrates, 1994). A receptor band 2kT wide centred at ~1900 cm⁻¹ would cover both easily.

A 'bichromatic' odour: bitter almonds

The bitter almond character is shared by a large number of quite different small molecules with related but not identical odours. A recent study of quantitative structure−activity relationships of 40 bitter almond odorants concludes that the essential features are: small size, one or more C=C bonds and a 'hydrogen-bonding acceptor' group, namely aldehyde, nitrile or (with a more marked change in smell character) nitro (Zakarya et al., 1993). Remarkably, the bitter almonds character is also shared by HCN, which, being a triatomic molecule, has only three vibrational modes, at 712 (bending mode), 2094 (CN stretch) and 3213 cm⁻¹ (C−H stretch), and, of course, no double bonds. Can this be made sense of vibrationally?

The CN stretch of HCN, as we have seen earlier, is a good replacement for aldehydes and nitro groups. The requirement for a double bond is best illustrated by comparing the

CHYPRE spectra of hexanal and trans-2-hexenal. Hexanal has a powerful, fatty green odour, whereas trans-2-hexenal (to most subjects) has a definite bitter almonds note in addition to a green character. The spectra differ chiefly in the amplitude of the two peaks at 1100 and 1400 cm⁻¹. One possibility is that the first harmonic of the v2 fundamental (2ν2 = 1412 cm⁻¹) (Choi and Barker, 1932), which gives an intense IR line, excites the 1400 band. This somewhat ad hoc idea cannot be excluded, but seems hard to reconcile with the fact that CHYPRE calculates rather low intensities for the 1500−1600 cm⁻¹ C=C band (stiff bond, small partial charges). Another possibility is that the bending mode of HCN excites a receptor band lying between 700 and 1000 cm⁻¹. This would be consistent with the fact that the CHYPRE spectrum of cyclohexenal, which does not smell of bitter almonds (and therefore constitutes another exception to the 'structure−odour' rule), has no such peak. It also fits in with the spectra of other odorants with typical bitter almond odours, such as benzonitrile and benzaldehyde, all of which have intense bands at ~1000 cm⁻¹.

A counterintuitive structure−odour relationship: the aliphatic aldehydes

One of the most perplexing structure−odour relationships is that of aliphatic aldehydes. It is well known to perfumers that aldehydes between C-8 and C-12 fall into two groups according to the number of carbon atoms. Though each aldehyde smells different, even numbered ones tend to have fruity, orange-like odours, while odd numbered ones have a more floral (also described as waxy) character (Arctander, 1994). No such pattern is discernible in the corresponding alkanes or alkanols, suggesting that the aldehyde group is responsible. Remarkably, MM2 molecular dynamics reveals a systematic difference in rotational mobility of the aldehyde group around the C−C bond joining it to the rest of the molecule in the two groups (Figure 11a). In heptanal, nonanal and undecanal, the aldehyde rotates only by ~90° on either side of its lowest energy configuration. By contrast, in octanal, decanal and dodecanal it is free to rotate by a full 360°.

This is unlikely to be due to an artefact of MM2 molecular dynamics, because Mopac calculations show that the aldehydes fall into two distinct even and odd groups even when their vibrational properties are calculated from quantum rather than classical methods. Figure 11b shows
Figure 11  Rotational mobility of the aldehyde group in straight-chained aliphatic aldehydes. The histograms depict the distribution dihedral angle between the aldehyde group and the nearest CH2 during a simulation run. Optimal structures and partial charges were calculated using Mopac with AM1 partial charges, and the molecular dynamics were run using MM2 dynamics. Temperature, 300 K; equilibration time, 1 ps; dynamics run, 4 ps; measurement every 20 fs. In the even-numbered aldehydes (left half) the aldehyde is more free to rotate and reaches portions of the phase space (grey histogram bars) which are not accessible to odd-numbered aldehydes (right half). This effect is not likely to be an artefact of the MM2 molecular dynamics. The modes which contribute to aldehyde rotation are low-energy twisting modes involving the whole molecules. The lower graph illustrates the calculated Mopac dipole strength for these modes. Even and odd aldehydes fall into two distinct groups.
Anomalously strong odorants: zinc binding

If an electron tunnelling mechanism is responsible for odorant transduction, one might expect there to be some relationship between atomic partial charges and the strength of an odorant. Empirically, this is the case: 'osmophoric' groups such as ketones, nitro groups, aldehydes, nitriles and ether links are all polar groups. This, however, cannot be the sole reason for differences in odorant strength. For example, vanillin is one of the strongest odorants known, whereas the closely related heliotropin is much weaker despite similar partial charges. In discussions with Dr Charles Sell (Quest International) and Prof. William Motherwell (University College London), it was pointed out to me that the involvement of zinc in or near the active site of the olfactory receptor might account for the anomalous strengths of certain classes of odorants (see Figure 12). Thiols, nitriles and isonitriles, some of which are among the strongest odorants known, coordinate to zinc readily. Indoles bind to zinc and are very strong odorants, as are oxathianes, diketones such as diacetyl, pyrazine esters, and furanones such as emoxyfurone (3-hydroxy-4-methyl-1-5-ethyl-2,5-dihydrofuran-2-one). Binding of these molecules to the zinc ion at or near the electron tunnelling site will increase their effective concentration at the receptor and, all other things being equal, allow their detection at lower partial pressures. Conversely, if zinc binding is a general property of strong odorants, one might expect that it could be used to scavenge strong odorants from ambient air. Indeed, a solution of the zinc salt of ricinoleic acid (Grillocin) has long been used as a very effective deodorizer, and this is likely to be its mode of action. Aside from providing indirect evidence for the involvement of a metal ion in the transduction process, metal binding could be useful in fragrance design if it were possible to build into the molecule to increase odorant potency.

Molecules with near-identical shapes and different odours

Strong evidence for a tunnelling mechanism comes from the ability of electrons to tunnel through a molecule and be
Isotope replacement

The strongest test of a vibrational theory is the comparison of odours of molecules differing only in their vibrational frequencies. This can in principle be done by isotope replacement without altering molecular conformations, because 'chemical' effects of isotope replacement are small. The effects of isotope replacement on vibrational frequencies are complex and depend on the effective masses in motion in a given vibrational mode. The largest effects occur where the relative change in mass of the atom is large, and the motion involves a few atoms. For example, replacement of hydrogen (mass 1.007) by deuterium (mass 2.014) reduces CH stretch frequency from ~3000 to ~2200 cm⁻¹. Similarly, deuteration of the SH group reduces stretch frequency from 2550 to ~1800 cm⁻¹. If the vibrational theory is correct, a deuterated thiol should not have a sulphurous odour. However, the SH–SD replacement is unlikely to give definite answers, because the thiol group is weakly acidic and D–H exchange will occur very rapidly when the odorant dissolves in physiological solution. The same applies to other molecules with rapidly exchangeable protons. For example, the report by Hara (1977) that fish can distinguish between glycine and deuterated glycine is hard to interpret, because glycine contains only exchangeable protons.

Many fully deuterated molecules are commercially scattered by atoms in its interior. Complexes of cyclopentadienyl ions with iron (ferrocene) and nickel (nickelocene) are stable and very similar in size and shape, the metal atoms almost completely encased by the cyclopentadienyl rings (Figure 13). The IR spectra of ferrocene and nickelocene are virtually indistinguishable, save for the position of the intense low-frequency internal metal-ring (M–R) vibrations (Nakamoto, 1986). In ferrocene, the M–R stretch vibration is a broad peak around 478 cm⁻¹, in the aromatic C–Cl stretch region. In nickelocene, the M–R stretch lies at 355, i.e. closer to the predicted lower limit of detectability. Nickelocene has a typical cycloalkene odour, while ferrocene has a camphoraceous odour reminiscent of chloroalkanes. This correlation seems hard to explain other than by tunnelling.

Isotope replacement
available, but few are distinctive, powerful odorants. An attempt with the weak and somewhat nondescript odorant hexane revealed a small difference between its odor and that of the fully deuterated molecule. However, differences in odor between batches of nondeuterated hexane were larger still, suggesting that impurities contributed to the odor character.

A search for a stronger odorant turned up acetophenone, which has a distinctive hawthorn and orange flower smell and is available as a high-purity deuterated compound. Calculation of CHYPRE spectra of acetophenone and acetophenone-d₈ (Figure 14) suggested that odor differences should be perceptible: the peak corresponding to the CH wag modes at ~1400 cm⁻¹ is absent in acetophenone-d₈ and the CH stretches are shifted into the region (~2200 cm⁻¹), which would normally indicate the presence of a nitrile (CN) group. The odors of acetophenone and acetophenone-d₈ were compared undiluted, to make sure that the weakly acidic methyl protons would not exchange with solvent hydrogen. Though both odorants have broadly similar odor profiles, the difference between them is striking: acetophenone-d₈ is fruitier and has less toluene-like character than acetophenone, and also has a much stronger bitter almonds character. The latter is particularly interesting because the spectrum of acetophenone-d₈ is closely related to that of benzonitrile, a strong bitter almond odorant.

In order to test for the possibility that impurities might be responsible for the odor difference, 0.2 µl samples of neat acetophenone and acetophenone-d₈ (Aldrich) were run through a gas chromatograph and smelled as they emerged from the GC capillary column (I am grateful to Ken Palmer at Quest international for help with the GC smelling). The deuterated acetophenone sample was found to contain only one impurity below the p.p.m. level, while the acetophenone had three p.p.m. impurities. The acetophenone-d₈ had the same odor as the bulk sample and was clearly different in odor from the acetophenone peak. In other words, two molecules with identical shapes and different vibrational spectra smell different.

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