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Flavours, fragrances and force fields

BY **MARTIN SLATER** AND **KATRIONA SCOFFIN**

Computational methods that analyse how drugs interact with protein targets are also effective for flavour and fragrance molecules.

Flavours and fragrances traditionally depend on essential oils drawn from naturally occurring products. Camphor oil is obtained by steaming the wood of the camphor tree; saffron is the stigma plucked from the flower of *Crocus sativus*.

Natural products are often subject to short or erratic supply owing to weather and other natural events. Environmental or preservation considerations also come into play. Up to 50 male musk deer are killed to produce 1 kilogram of musk, highly sought after for perfumes. Ambergris, produced in the digestive system of the sperm whale, was formerly used as a fixative to make scents last longer. Its use is now illegal in Australia and the US and the synthetic ambroxide is widely used as a substitute.

Synthetic substitutes that retain the same odour and flavour characteristics as the natural compound have been developed for many flavours and fragrances. Degradation is a particular factor for flavours, and synthetic versions are often chosen because of their longer shelf life. Health considerations drive other synthetic choices; for example, low calorie sweeteners as alternatives to sugar.

Companies are continually looking

for new and improved versions of these substitutes, synthetic or otherwise. In some cases manufacturers are keen to identify alternative natural products that have a similar effect to hard-to-obtain flavours and fragrances. Molecular modelling plays an important role in identifying possible alternatives.

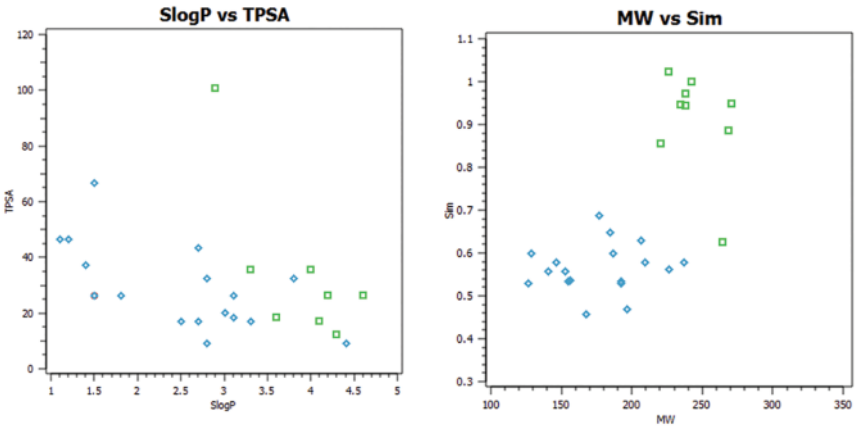
Applying pharmaceutical discovery methods to flavours and fragrances

There are many well-established computational techniques for modelling the interactions between pharmaceuticals and proteins. Since flavour and fragrance molecules interact with specific protein targets to exert their effects, many of these computational techniques are directly transferable from pharmaceuticals to flavours and fragrances.

When looking for new synthetic flavours or fragrances, the starting point could be an existing natural or synthetic compound, or a taste or odour receptor. Perhaps surprisingly, flavours are more diverse and complex in their interactions than pharmaceuticals. Some components of taste perception also include the simultaneous triggering of olfactory receptors, which belong to the wider, therapeutically important, G-protein-coupled receptors (GPCR) family.

Clearly, there are many differences between flavour and fragrance molecules compared with their drug counterparts. The pharmacological effects of pharmaceuticals cover a wide spectrum of therapeutically relevant biological targets and also require physiochemical properties in keeping with their use; for example, oral, central nervous system, inhaled or topical applications.

Fragrance molecules need to be both non-toxic and volatile and as such are restricted by tight chemical and physical property considerations. In addition, the mode of action of fragrance molecules is generally accepted to be through GPCR agonism and therefore the spectrum of size, shape and molecular weight is accordingly further narrowed (see graphs above). It is an incredible observation that the huge spectrum of fragrance perception is dictated by the precise 3D distribution of typically between only 2 and 20 atoms of only five elements – carbon, hydrogen, nitrogen, oxygen and sulfur. Possibly unsurprisingly, this translates into very compact electrostatic, shape and chirality profiles for each of these special molecules.

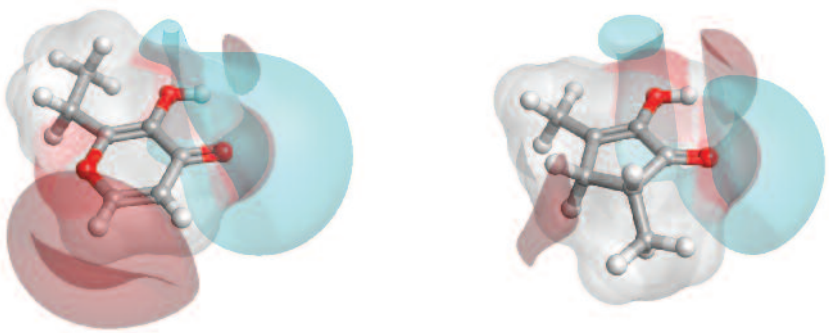


The physiochemical properties of 18 diverse fragrance molecules (blue diamonds) and 9 diverse musks (green squares), showing the narrow spectrum of size, shape and molecular weight that these molecules occupy.

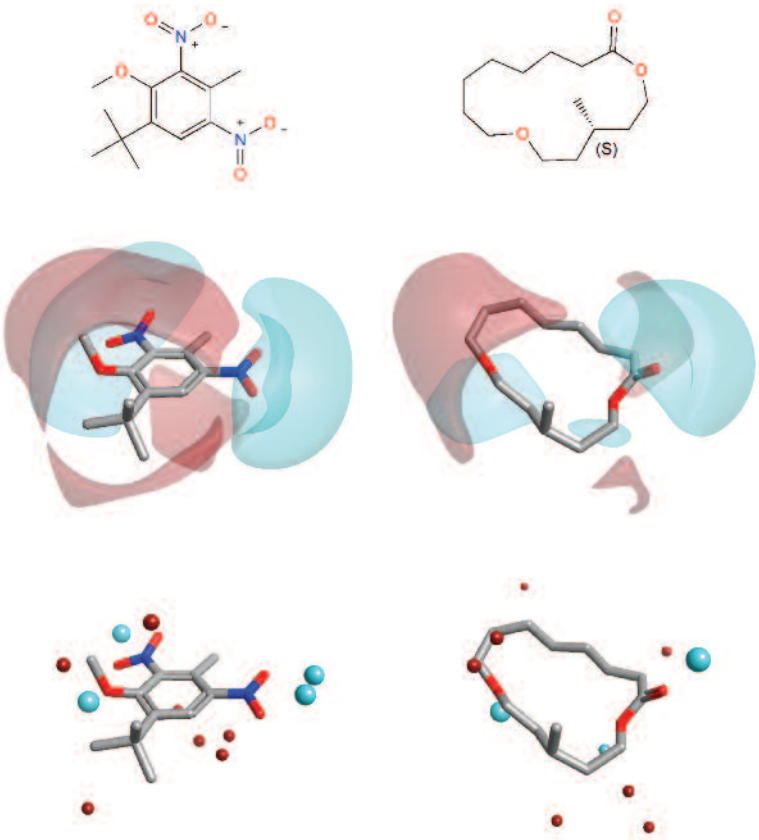
Molecular fields and virtual screening

Small molecules are recognised by and bind to proteins on the basis of their 3D electrostatic, shape and hydrophobic properties, otherwise known as their molecular fields. It is possible to calculate detailed computational models of these fields that provide a highly accurate description of the interactions between a small molecule and its protein binding site. In this way, the active profile of a compound can be calculated, visualised and analysed, giving a 'protein's eye view' of the compound (see below). These calculations are complex and long if carried out by using quantum mechanics methods, but a comparable level of quality can be obtained in a fraction of the time using a force field approach. The molecular fields shown here were calculated using the Cresset XED force field, which combines electrostatic calculations in combination with Van der Waals forces.

However, molecular field calculations are computationally



The molecular fields around two caramels, ethyl maltol and furaneol. The surfaces show the shape (grey) and the positive (red) and negative (blue) electrostatic isopotentials, giving a 'protein's eye view' of the compound.



Two structurally diverse musks, nitro musk (left) and ambrette (right), with their electrostatic isopotential surfaces positive (red) and negative (blue). The molecular fields (centre) reveal similar bioactivity that is not evident from their 2D or 3D structures. Field points (bottom) make it possible to compare thousands of structures and fragments on the basis of biological similarity.

intensive. It is unfeasible to use an entire molecular field profile to search through large collections of molecules. Such a search would take more computing power and time than any project would have available. Instead, methods have been developed to condense molecular fields down to 'field points' that encode the fields accurately enough to make biologically useful comparisons between the profiles of different compounds (see above). Field points are an elegant and computationally viable way of expressing the molecular field patterns of molecules and they unlock tremendous computational potential.

These field point profiles can be used as molecular fingerprints to search databases of active molecules to return compounds that are likely to have similar activity. Field points make it possible to search a database of about 10 million compounds in 48 hours on a 30 CPU cluster. This method of virtual screening has proved highly successful in pharmaceutical research in identifying compounds with similar activity profiles, despite being from different chemical classes and having markedly different 2D structures.

The same method has been successfully applied to fragrance and flavour molecules in order to compare their active profile. Field-based models of active flavour and fragrance ingredients can be developed in the same way and used to search for synthetic alternatives for natural products. Once a range of alternative compounds has been identified, the individual alternatives can be modelled and compared in greater detail by using a more accurate calculation of the molecular fields.

Utility of molecular fields for analysing flavour and fragrance bioactivity

To illustrate the usefulness of electrostatic descriptions for fragrance molecule space, a small set of 27 diverse fragrance molecules were built and analysed (Kraft P., Bajgrowicz J.A., Denis C., Frater G., *Angew. Chem.* 2000, vol. 112, p. 3106; *Angew. Chem., Int. Ed.* 2000, vol. 39, p. 2980; www.leffingwell.com/chirality/chirality.htm). The set included an extended group of musks.

One of the compounds, coumarin, was chosen as a starting point for the analysis. The centre of gravity and the overall dipole were fixed relative to this starting point. Some detailed modelling work was carried out on the compounds in the set in order to determine the likely 3D alignments.

Similarity scores were calculated for all of the compounds and each molecule was scored relative to each other to generate an all-by-all matrix

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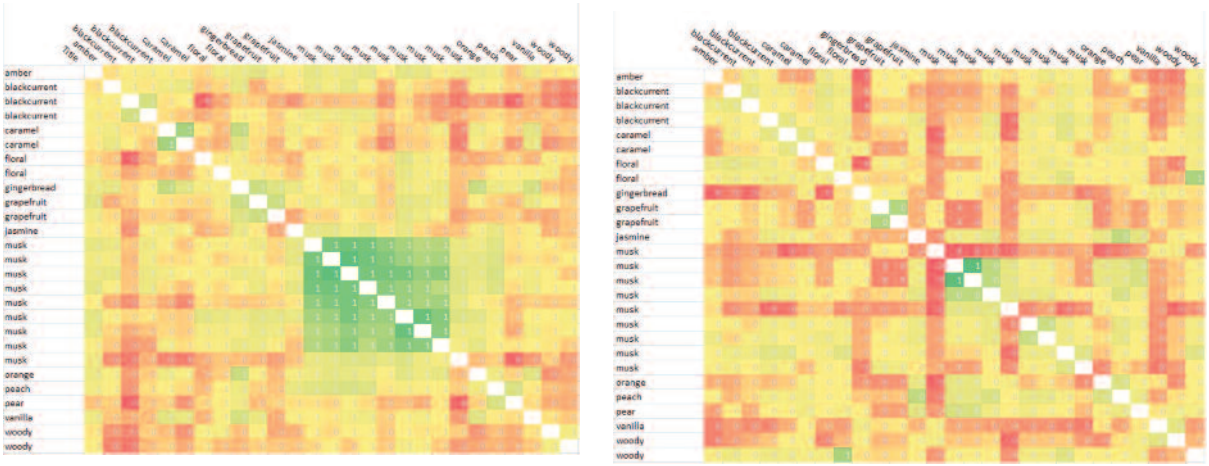


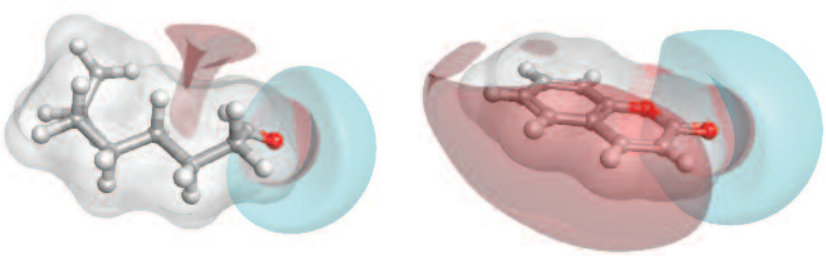
(Left): All-by-all field similarity matrix of 18 diverse fragrance molecules and nine diverse musks (green – yellow – red signifying decreasing similarity), showing significant clustering around fragrance groups.

(Right): All-by-all ECFP4 2D similarity matrix of 18 diverse fragrance molecules and nine diverse musks, showing the low levels of similarity calculated using a purely structure-driven analysis.

(see figure top left). This simple exercise was limited only by the low numbers involved in the analysis, yet still provides a very intuitive picture. From the matrix, brighter green corresponds to higher similarity, and red to a low similarity.

There is a significant cluster around the musks, but also other hot spots of high similarity. For the sake of comparison, a purely chemical structure driven analysis was also carried out using EFCP4 2D similarity. The results are shown in the matrix on the right and they reveal a stark contrast to the force field version, showing mostly low similarity. The relationship of the musk structure activity relationships (SAR) in this example is likely to defeat most 2D similarity methods; therefore, the structurally diverse musk clusters disappear and the caramels blend into the noise.

A hierarchical clustering was also carried out, which provided a different overview of the fragrance set. Interestingly, gingerbread, caramel and orange fragrance compounds, which many would say are highly complementary flavours, showed a high similarity, which suggests that their corresponding targets may be highly similar to each other. The 3D aligned structures of gingerbread and orange with their molecular fields are shown below.



Gingerbread and orange; coumarin (left) and laural aldehyde (right) and positive (red) and negative (blue) electrostatic isopotential surfaces.

Pharma potential

The world of flavours and fragrances holds a wealth of subtle chemistry and huge potential for benefiting from the transfer of computational methods from pharmaceutical discovery.

The analysis discussed here demonstrates the power of a field-based approach for usefully analysing the bioactivity of flavour and fragrance molecules. The fields approach gets to the essence of the unique and precise way that flavours and fragrances molecules interact with their protein targets, independent of their chemical architecture.

Field-based models of active flavour and fragrance ingredients are already being used in industry to provide novel chemotypes, echoing the success of this technology in providing new hits and leads for the pharmaceutical industry.

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