

## DESIGNER MOLECULES FOR NON-LETHAL PEST MANAGEMENT

Richard W. Watkins and Nick R. Price from the UK's Defra / Central Science Laboratory (CSL) at Sand Hutton, York, UK, discuss the use of molecular modelling in the exploitation of natural products for the management of problem wildlife

The pest control industry will have to find ever more sophisticated solutions to conflicts between wildlife and ourselves, if it is going to meet the needs of the agricultural community for safe and efficient food production and demands from the wider public for improved animal welfare. Traditional pest control methods, especially those involving lethal agents, are becoming increasingly unacceptable to a public concerned about humaneness and risks to non-target wildlife. Alternatives have to be found, and to meet these demands the Central Science Laboratory (CSL) of the UK Department for Environment, Food and Rural Affairs (Defra) is developing a range of non-lethal control techniques to resolve wildlife conflicts (Gurney *et al.*, 1996; Gill *et al.*, 2000; Grey *et al.*, 1997). Wherever possible these techniques have been derived from natural systems in the belief that these will be effective, pest-specific and have a minimal impact on the environment (Watkins *et al.*, 1996).

Pheromones play a significant role in controlling both the behaviour and physiology of mammals (Gosling, 1982). Rodents, being largely nocturnal and often moving through dense cover, rely heavily on these species-specific chemical signals to attract mates, warn rivals, aid navigation and control the sexual development of other members of the colony (Hurst, 1987). The reliance placed upon these pheromones by rodents means that through careful manipulation we may be able to develop powerful and species-specific techniques for managing problem populations.



Figure 1 | The house mouse, *Mus domesticus*, and one of its urine 'posts' in breeze block.

Both mice and rats secrete pheromones within their urine and deposit these signals as scent marks throughout their home ranges (Collins *et al.*, 2001). Mice often deposit urine repeatedly at the same location, producing, overtime, 'posts' of partially dried urine that may often be seen along any projecting surfaces inside buildings occupied by these animals (Figure 1) (Robertson *et al.*, 1993). Rats and mice excrete a considerable amount of protein in their urine (Finlayson *et al.*, 1965) with males excreting far more than females. In mice these proteins are known as Major Urinary Proteins (MUPs) and in rats as Alpha<sub>2u</sub>-globulins (Bocskei *et al.*, 1992). The pheromones are held tightly within the core of these sponge-like proteins: for example, the urinary proteins of male mice contain 2-*sec*-butyl-4,5-dihydrothiazole (Figure 2) and 3,4-dehydro-*exo*-brevicomin (Novotny *et al.*, 1990). These species-specific compounds are repellent to male mice and are attractive to females controlling their sexual development. The proteins play a crucial role in protecting these pheromones from being rapidly lost by evaporation or degradation. They thereby extend the 'shelf-life' of the scent mark, releasing the pheromones slowly as the proteins dehydrate or degrade (Robertson *et al.*, 1993).

As part of an investigation into the use of mammalian chemical signals as a novel pest management technique, CSL is examining the relationship between the protein carrier (MUP) and its pheromone cargo. This project seeks to 'explain' the binding interactions between the pheromones and their MUP carriers and to use this information to help us identify new 'chemical stimuli' with which to control mouse behaviour. These agents could be used to disrupt MUP-pheromone communication systems or to produce a means to deliver super-normal attractive or repellent stimuli to problem animals.

In the past, screening for new bioactive molecules involved a series of expensive and laborious laboratory studies but we are now using new computer-aided molecular design (CAMD) technology to assist in this discovery process. Here computer-generated models of both proteins and pheromones are used to study the interaction between the pheromone molecule and the barrel-like cavity in which it is bound. This approach is used by the pharmaceutical and food industries for product identification, and has already been successfully used by CSL to identify and predict the activity of bird repellents (Watkins *et al.*, 1999).

Initially we used CAMD to identify which of the MUP amino acid residues present in the protein formed the binding pocket and which residues were within contact distance of the pheromones. It was important to identify

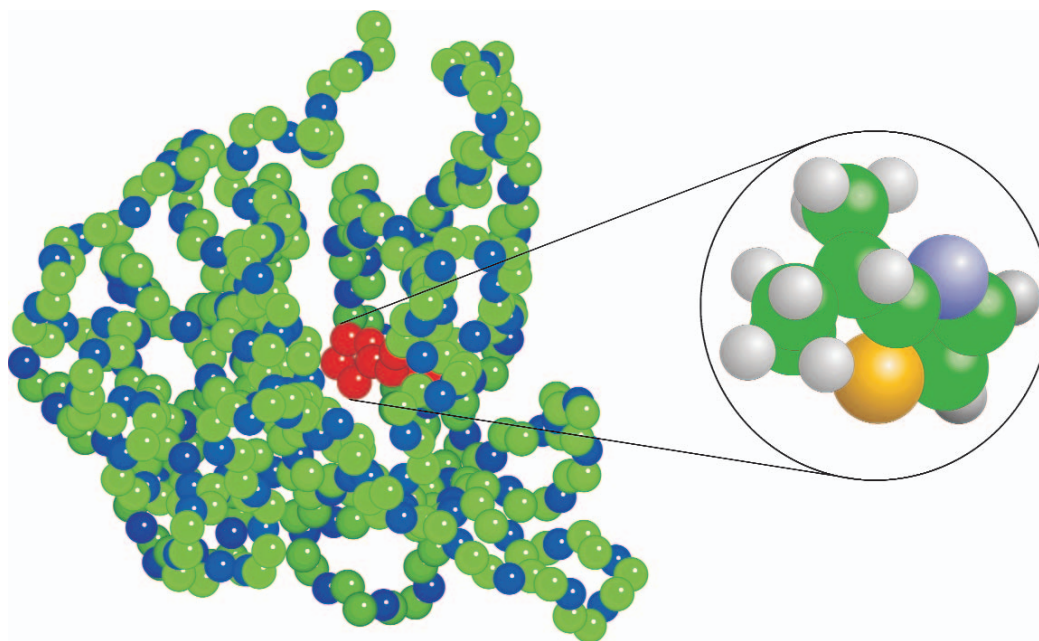


Figure 2. Computer-generated model of MUP showing the pheromone 2-sec-butyl-4,5-dihydrothiazole (red) at its core site, together with an enlargement of the pheromone itself.

these residues as these amino acid contacts are vital in determining the trajectory of any molecule entering or leaving the binding pocket.

A series of molecular dynamics simulations were run to examine the movements of atoms and molecules at real-life temperatures and thereby determine the 'comfort factor' of a range of pheromones and other molecules in the binding site of MUP. These simulations revealed the route by which the pheromones 'escape' from the binding pocket. The results of these studies were consistent with the hypothesis that pheromones move out of the protein as the urine matrix dries and that, with the loss of both pheromone and internal water molecules, the binding site collapses. The results of these simulations are in agreement with the work of other authors on the homologous Retinol-Binding Protein (Sandblom *et al.*, 1986; Aqvist *et al.*, 1986) and are consistent with the role of the MUP as a 'delayed' release mechanism for pheromones that can extend the effective 'life' of the scent mark (Robertson *et al.*, 1993).

The conclusions of these simulations were that molecules sit 'comfortably' within the binding site of the MUP with the following criteria:

- a volume of about 190 cubic Angstroms
- an inflexible structure
- a hydrophobic surface area
- at least one hydrogen bonding atom
- the hydrogen bonding atom should be perpendicular to the bulk of the molecule

Allied to this approach we have developed a conventional quantitative structure activity relationship (QSAR) from published data on a series of ligands with known affinities to MUP. The model (which accounts for over 80% of the variation in the data) provides a quantitative prediction of a

molecule's affinity for the protein core. Using both molecular dynamics simulations and our QSAR model we were able to search chemical databases (some containing over 40,000 organic molecules) for compounds, 'super-ligands', that have a higher affinity for the MUP than its 'natural' cargo of pheromones. The products of this search are six 'super-ligands' with the potential to oust the pheromones from the protein core and thereby disrupt this mouse-specific communication system.

Studies to quantify the impact of the 'super-ligands' on the MUP pheromonal communication system are currently underway. Preliminary research, using high-resolution gas chromatography-mass spectrometry to study the rate of release of pheromones from artificial scent marks, has shown that the marks still contain measurable quantities of pheromone when 24 hours old. Application of the super-ligands results in the displacement of the entire pheromone cargo of the scent mark within 6 hours of treatment, with 90% of the content being lost within the first 30 minutes. Further studies to quantify the power of these materials in modifying mouse behaviour and sexual maturation are now in progress.

CAMD and QSAR techniques are now routinely used in the discovery of new compounds with desired properties, where the molecules may be pharmaceuticals, proteins, polymers, catalysts, pesticides and so on. CAMD and QSAR have been credited in the design of the drug norfloxacin, the herbicides metamitron and bromobutide and the fungicide myclobutinail, which are marketed around the world (Boyd, 1990).

The study reported here used CAMD to examine the structure-function relationship of MUPs to (a) gain an insight into the mechanisms involved in pheromone release and (b) by investigating the principal amino acid residues

involved in molecular recognition within the core binding site, identify new ligands with high affinity for MUPs that can be used as new tools in the management of problem wildlife.

Conventional strategies for the discovery and screening of novel wildlife management materials, although highly effective, are costly, technically exacting, and test large numbers of animals to ensure statistically robust results. The CAMD approach adopted by CSL and other research organisations promises to increase the speed and efficiency of the discovery process, drastically reducing costs as well as the number of animals required for these studies.

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### References

- Aqvist, J.; Sandblom, P.; Jones, T. A.; Newcomer, M. E.; van Gunsteren, W. F.; Tapia, O. (1986) Molecular-dynamics simulations of the holo and apo forms of retinol binding protein – structural and dynamic changes induced by retinol removal. *Journal of Molecular Biology*, **192**, 593–604.
- Bocskei, Z.; Groom, C. R.; Flower, D. R.; Wright, C. E.; Phillips, S. E. V.; Cavaggioni, A.; Findlay, J. B. C.; North, A. C. T. (1992) Pheromone binding to two rodent urinary proteins revealed by X-ray crystallography. *Nature*, **360**, 186–188.
- Boyd, D. B. (1990) Successes of computer-assisted molecular design. pp 355–371. In: *Reviews in computational chemistry*, K. B. Lipkowitz and D. B. Boyd (eds). VCH Publishers Inc., New York.
- Collins, S. A.; Gosling, L. M.; Watkins, R. W.; Cowan, D. P. (2001) Artificially increasing scent mark rate increases urogenital gland size in mice *Mus musculus*. *Physiology & Behaviour*, **74**, 517–522.
- Finlayson, J. S.; Asofosky, R.; Potter, M.; Runner, C. C. (1965) Major urinary protein complex of normal mice. *Science*, **149**, 981–982.
- Gill, E. L.; Whiterow, A.; Cowan, D. P. (2000) A comparative assessment of potential conditioned taste aversion agents for vertebrate management. *Applied Animal Behaviour Science*, **67**, 229–240.
- Gosling, L. M. (1982) A reassessment of the function of scent marking territories. *Zeitschrift fur Tierpsychologie*, **60**, 89–118.
- Grey, C. B.; Cowan, D. P.; Langton, S. D.; Watkins, R. W. (1997) Systemic application of L-phenylalanine increases plant resistance to vertebrate herbivory. *Journal of Chemical Ecology*, **23**, 1463–1470.
- Gurney, J. E.; Watkins, R. W.; Gill, E. L.; Cowan, D. P. (1996) Non-lethal mouse repellents: evaluation of cinnamamide as a repellent against commensal and field rodents. *Applied Animal Behaviour Science*, **49**, 353–363.
- Hurst, J. L. (1987) The function of urine marking in a free-living population of house mice, *Mus musculus* Ruddy. *Animal Behaviour*, **35**, 1433–1442.
- Novotny, M.; Harvey, S.; Jemiolo, B. (1990) Chemistry of male dominance in the house mouse, *Mus domesticus*. *Experientia*, **46**, 109–113.
- Robertson, D. H. L.; Beynon, R. J.; Evershed, R. P. (1993) Extraction, characterization, and binding analysis of two pheromonally active ligands associated with major urinary protein of house mouse (*Mus musculus*). *Journal of Chemical Ecology*, **19**, 1405–1416.
- Sandblom, P.; Aqvist, J.; Jones, T. A.; Newcomer, M. E.; van Gunsteren, W. F.; Tapia, O. (1986) Structural changes in retinol-binding protein induced by retinol removal – a molecular-dynamics study. *Biochemical and Biophysical Research Communications*, **13**, 564–570.
- Watkins, R. W.; Gill, E. L.; Cowan, D. P. (1996) Plant secondary chemicals as non-lethal vertebrate repellents. *Proceedings of the Vertebrate Pest Conference*. R. M. Timm and A. C. Crabb (eds), pp. 186–192. University of California, Davis, USA.
- Watkins, R. W.; Lumely, J. A.; Gill, E.; Bishop, J.; Langton, S. D.; MacNicol, A.; Price, N. R.; Drew, M. G. B. (1999) Quantitative structure-activity relationships (QSAR) of cinnamic acid bird repellents. *Journal of Chemical Ecology*, **25**, 2825–2845.

Richard Watkins and Nick Price, at the York-based Central Science Laboratory (CSL) of the UK's Department for Environment, Food and Rural Affairs (Defra), are developing novel approaches to manage a range of pest species. As part of this research they are using computational chemistry techniques such as QSAR and protein-ligand docking to study the interaction between pesticides, target organisms and the environment.

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