49-6

Application of Molecular Imprinting for Improving Odorant Detection by Peptide Libraries

Heau-Shan Gao*, Jen-Yu Chen, Yi-Shan Chien, Chuang-Fu Ha
Department of Chemistry, Fu-Jen Catholic University, Taipei County, Taiwan, R.O.C.

Abstract

A novel method for odorants detection is developed by integrating the functional peptides from combinatorial chemistry and molecular imprinting technology. The molecular imprinting technology is applied to enhance the sensitivity and specificity of binding ability of functional peptide with the target odorant. In the study, the hexapeptide library was prepared by using the combinatory chemistry. The peptides were mixed with the target odorant to prepare the molecularly imprinted peptides, then were coated on a piezoelectric quartz crystal. Based on the positional screening strategy, the imprinted peptides with high binding ability of butyric acid odorant was determined then the best amino acid candidates of each position of hexapeptide were identified. The binding affinity of the odorant with the imprinted peptides and non-imprinted peptides were also determined. The result was shown that the imprinted peptides had higher sensitivity than the non-imprinted peptides. The binding ability of imprinted peptide was 1.2~5.5 fold higher than non-imprinted peptide. The sensitivity of binding between the peptides and the odorants was significantly enhanced by the effect of the molecular imprinting. The combination of peptide library and molecular imprinting technology can be used for development of odorant sensing tools.

Keywords: combinatorial chemistry, peptide library, molecular imprinting, odorant

1. Introduction

The mammalian olfactory system can recognize and discriminate large number of different odorant molecules.[1] The odor discrimination occurs during the association of odorous ligands with specific receptors on olfactory sensory neurons. The receptor protein plays an important role in odorant recognition and cell signaling. The literature reports suggest that the extracellular loop and transmembrane domain might be the major part of the odorant-binding domain in olfactory receptors.[2,3] The design of functional polymers that can selectively recognize molecules has become an active area of research in recent years.[4,5]

The recognition and subsequent complementary binding between a substrate and an odorant molecule is the key step in the odor discrimination process. To mimic the olfactory sensing system, a series of synthetic peptides as sensing materials were designed and studied. The peptide library that is composed of thousands or millions of peptide sequences offers a high throughput for drug screening or compound screening.[6,7] However, the peptide library screening approach may have low selectivity and sensitivity to the target compound. Since the conformations of peptides are considered as one of the key factors for absorption effect between peptides and odorant molecules, integrating the molecular imprinting technology shall create the selective recognition sites for the template molecule.[8, 9] These recognition sites have a complementary shape and size with the template compound which can enhance the sensitivity and specificity of binding ability of functional peptide with the target odorant. An imprinted functional peptide can form a specific configuration with a complementary target odorant and increase the binding ability with the target odorant.

The objective of our research is to develop a novel method for odorants detection by integrating the functional peptides from combinatorial chemistry and molecular imprinting technology. The butyric acid is selected as a target odorant to investigate the sensitivity and specificity effect of imprinted functional peptide and non-imprinted peptide on their sensing ability for odorant detection. The imprinted peptides with high binding ability of butyric acid are selected by the positional screening process. The potential amino acid candidates corresponded to six positions of hexapeptide are identified.

2. Method

This study integrates the peptide library and molecular imprinting technologies. The hexapeptide library was synthesized by solid phase peptide synthesis method.[10,11] Based on the positional screening strategy,[12, 13] the 114 hexapeptide combinatory libraries were prepared (Fig. 1).

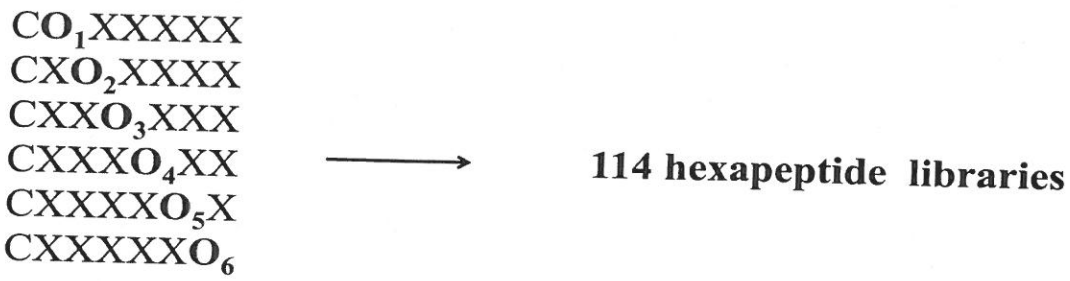


Fig. 1 Positional Scanning of Synthetic Peptide Combinatorial Libraries

O: "Known position" represents a known amino acid which is one of the 19 natural L-amino acids (cysteine was excluded).

X: "Mix position" represents an equimolar mixture of the 19 natural L-amino acids (cysteine was excluded).

C: Additional cysteine residue provides a thio (-SH) group to form a chemical adsorption between the peptide and the gold surface of the piezoelectric crystal.[14]

These hexapeptide libraries were mixed with the target odorant to prepare the molecularly imprinted peptides then coated on a piezoelectric (PZ) quartz crystal.[15,16]

Imprinted functional peptide preparation:

(a) The hexapeptide was dissolved in the butyric acid solution to form the covalently interaction between peptide and the template butyric acid.

(b) The hexapeptide - butyric acid complex was coated on the surface of piezoelectric quartz crystal.

(c) The template butyric acid was removed from surface of piezoelectric quartz crystal.

Non-imprinted peptide preparation:

(a) The hexapeptide was dissolved in the 75% ethanol solution.

(b) The hexapeptide solution was coated on the surface of piezoelectric quartz crystal.

The PZ crystal was served as a signal transducer to determine the binding affinity of synthetic peptides for odorants (Fig. 2).

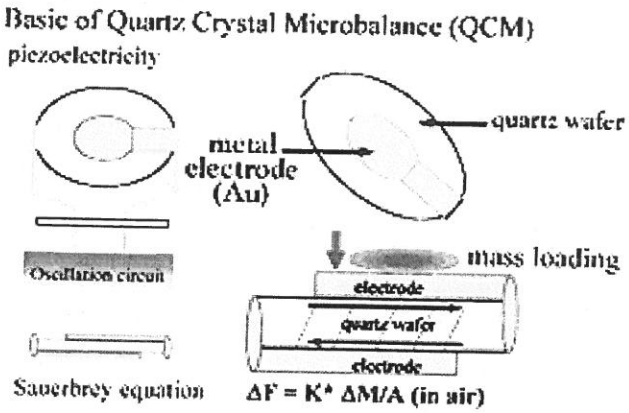


Fig. 2 The Piezoelectric Crystal Served As a Signal Transducer

The sensitivity between peptide and butyric acid on a piezoelectric quartz crystal was determined.

$$\textbf{Sensitivity} = \frac{\textbf{The change of frequency } \Delta F \textbf{ [Hz]}}{\textbf{Coating amount of peptide } \Delta M \textbf{ [}\mu g\textbf{]}}$$

3. Results and Discussion

The conformations of peptides are considered as one of the key factors for absorption effect of peptides on odorant molecules. The molecular imprinting process is applied to enhance the binding ability of peptide with the target odorant (Fig. 3). An imprinted functional peptide can form a specific configuration with a complementary target odorant and enhance the binding ability with the target odorant.

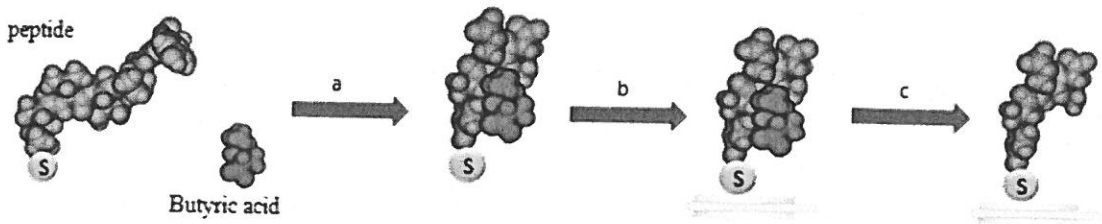
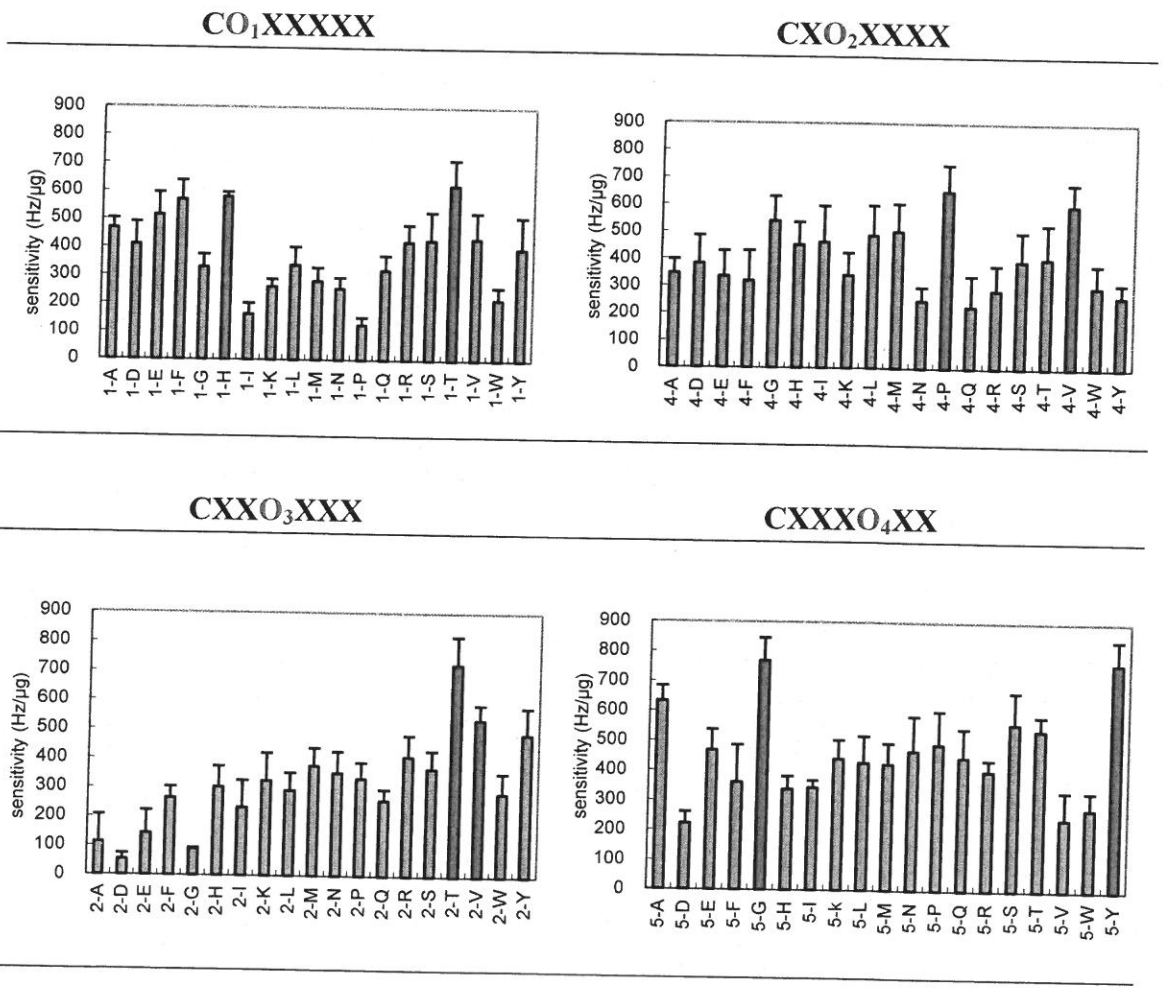


Fig. 3 Schematic Representation of Peptide Molecular Imprinting Process

The binding affinity of the odorant for the imprinted peptides were studied. The imprinted peptides with high binding ability to butyric acid odorant were selected by the positional screening process. The best amino acid candidates corresponded to six positions of hexapeptide were identified.

On the basis of the positional screening approach of the 114 hexapeptide libraries, the result was shown that His and Thr have higher sensitivity to odorant butyric acid in the first position of hexapeptide library (CO_1XXXXX). Therefore, the best amino acid candidate in the first position of hexapeptide library is His or Thr. In the second position of hexapeptide library (CXO_2XXXX), the best amino acid candidate is The or Val. In the third position of hexapeptide library ($CXXO_3XXX$), the best amino acid candidate is Leu or Trp. In the fourth position of hexapeptide library ($CXXXO_4XX$), the best amino acid candidate is Pro or Val. In the fifth position of hexapeptide library ($CXXXXO_5X$), the best amino acid candidate is Gly or Tyr. In the sixth position of hexapeptide library ($CXXXXXO_6$), the best amino acid candidate is Tyr (Fig. 4). The top two amino acid candidates at six positions for hexapeptide are shown in Table 1.



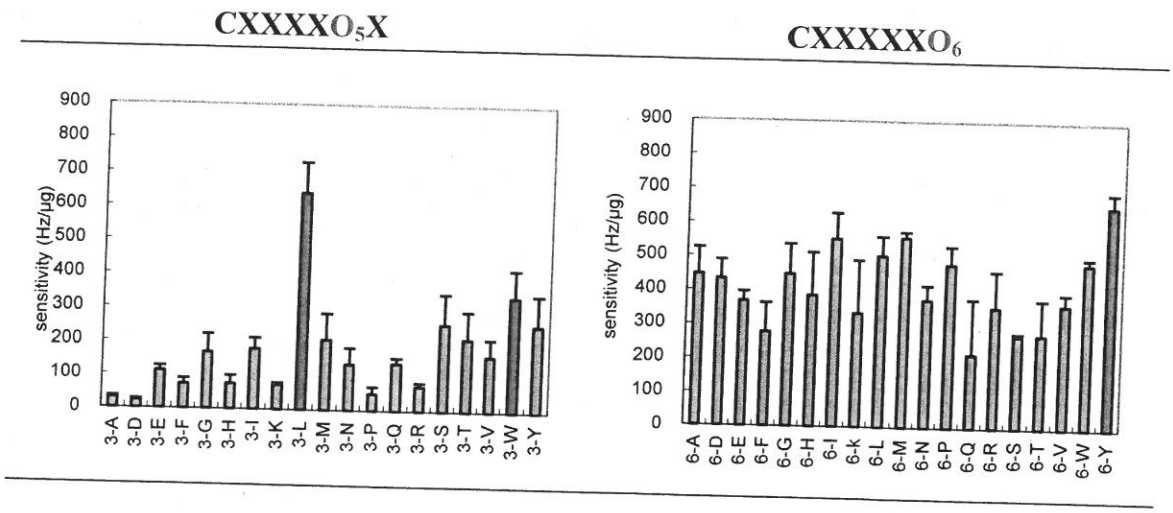


Fig. 4 Binding Ability of Imprinted Hexapeptide Library with Butyric Acid

	X_1	X_2	X_3	X_4	X_5	X_6
1st amino acid candidate	H	T	L	P	G	Y
2nd amino acid candidate	T	V	W	V	Y	

Table 1 The best amino acid candidate in hexapeptide

Based on the data of the amino acid candidates in Table 1, it was shown that the butyric acid molecule was interacted with peptide through the hydrogen bonding between the hydroxyl group of Thr/Tyr and butyric acid.

Assessment of the selectivity and specificity effect of imprinting functional peptides on the odorant, the binding affinity of butyric acid for the imprinted and non-imprinted peptides were studied (Fig. 4). The less sensitivity peptides (1-P, 2-G, 3-D, 4-H, 5-K, and 6-F) at the sixth position of the amino acid candidates were served as a negative control. The result demonstrated that the imprinting functional peptides (red bar) have higher affinity for odorant butyric acid than the non-imprinted peptides (blue bar) at each position of the best amino acid candidate. The sensitivity of imprinted peptide is 1.2~5.5 fold higher than that of non-imprinted peptide in those position of the best amino acid candidate (Table 2). These data indicate that the imprinted peptide have selective recognition.

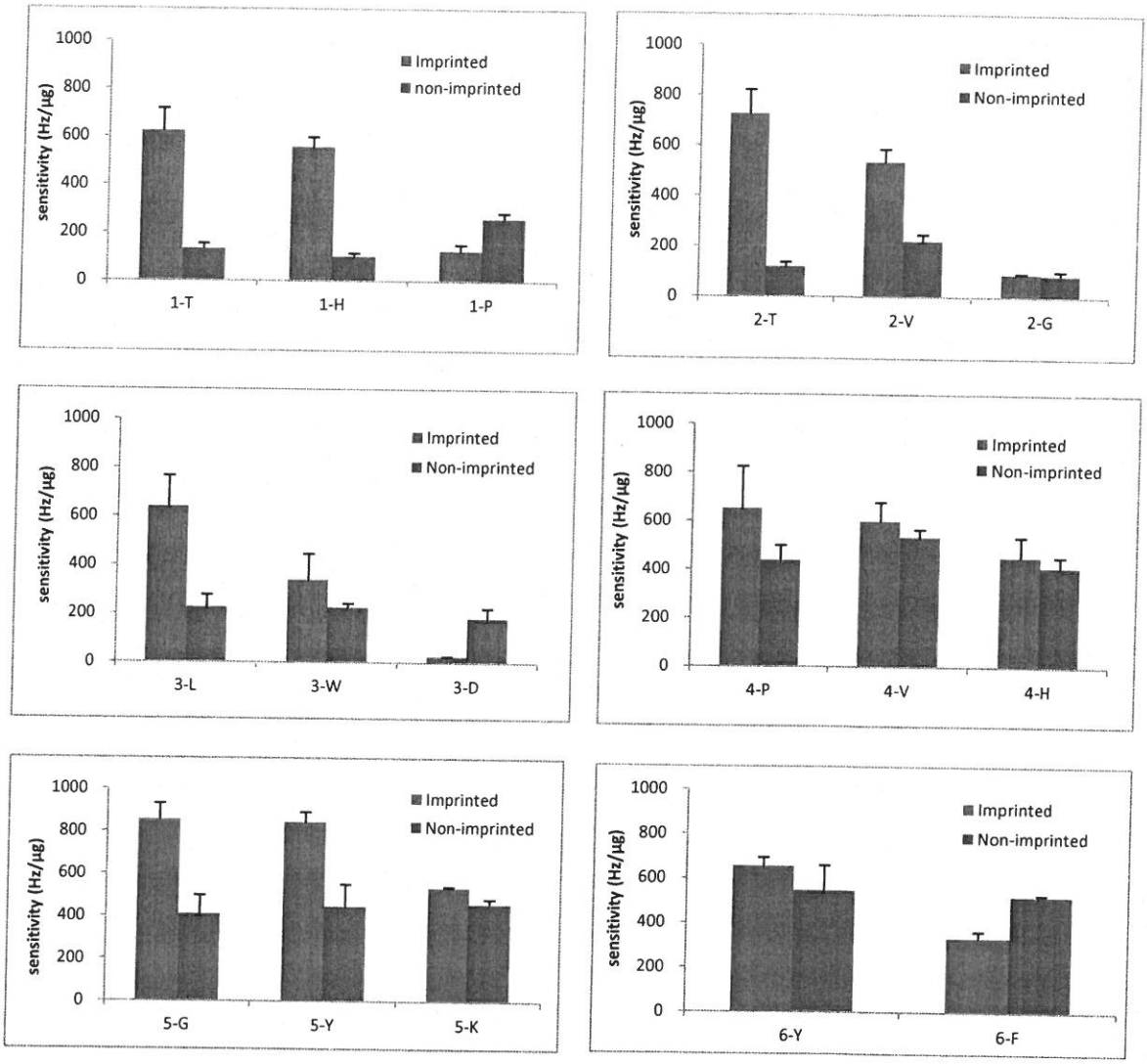


Fig. 5 Binding Affinity of Butyric Acid for the Imprinted and Non-imprinted Peptides

	Sensitivity of Imprinted Peptide (Hz/ug)	Sensitivity of Non-imprinted Peptide (Hz/ug)	Sensitivity Comparison of Imprinted and Non-imprinted Peptide
1-T	624	134	4.7
1-H	557	102	5.5
2-T	725	119	6.1
2-V	536	222	2.4
3-L	640	226	2.8
3-W	338	227	1.5
4-P	652	438	1.5

4-V	660	536	1.2
5-G	855	409	2.1
5-Y	843	447	1.9
6-Y	655	547	1.2

Table 2 Comparison of The Sensitivity of Imprinted and Non-imprinted Peptides

Conclusion

The result showed that the molecular imprinting process can enhance the binding affinity of peptide for the odorant and improve the selectivity and specificity of peptides. The combination of peptide library and molecular imprinting technology can be used for development of odorant sensing tools in environmental, food, and medical sample analysis.

References

1. Buck, L., Axel, R. **1991**. A Novel Multigene Family May Encode Odorant Receptors: A Molecular Basis for Odor Recognition. *Cell.*, 65, 175–87.
2. Pimple, Y. and Lancet, D. **1999**. The variable and conserved interfaces of modeled olfactory receptor proteins. *Protein Science.*, 8: 969-977.
3. Freitag, J., Ludwig, G., Andreini, I., Rossler, P. and Breer, H. **1998**. Olfactory receptor in aquatic and terrestrial vertebrates. *J. Comp. Physiol. A.*, 183, 635-650.
4. Sellergren, B. **2001**. Techniques and Instrumentation in Analytical Chemistry: Molecularly Imprinted polymers. *Elsevier Amsterdam*, 23:126-138.
5. Dı´az-Garcı´a, M. E. and Badı´a Laı´no, R. 2005. Molecular Imprinting in Sol-Gel Materials: Recent Developments and Applications. *Microchim. Acta,* 149, 19–36
6. Houghten, R. A., Pinilla, C., Blondelle, S. E., Appel, J. R., Dooley, C.T., Cuervo, J. H., **1991**. Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery. *Nature.*, 354(7), 84-6.
7. Batra, D. and Shea, K. J. **2003**. Combinatorial methods in molecular imprinting. *Curr. Opin. Chem. Biol.*, 7, 434-442.
8. Hart, B. R., Shea, K. J., **2001**. Synthetic Peptide Receptors: Molecularly Imprinted Polymers for the Recognition of Peptides Using Peptide-Metal

Interactions. *J. Am. Chem. Soc.*, 123(9), 2072-3.

9. Haupt, K., Mosbach, K., **2000**. Molecularly Imprinted Polymers and Their Use in Biomimetic Sensors. *Chem. Rev.*, 100(7), 2495-504.
10. Merrifield, R. B., **1963**. Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. *J. Am. Chem. Soc.*, 85(14), 2149-54.
11. Merrifield, R. B., **1986**. Solid phase synthesis. *Science*.,232,341
12. Pinilla, C., Appel, J. R., Blanc, P. and Houghten, R. A. **1992**. Rapid identification of high affinity peptide ligands using positional scanning synthetic peptide combinatorial libraries. *BioTechniques*, 13:6, 901- 905.
13. Dooley, C. T. and Houghten, R. A. **1993**. The use of positional scanning synthetic peptide combinatorial libraries for the rapid determination of opioid receptor ligands. *Life Science,* 52, 1509-1517.
14. Zhou, X. C., Zhong, L., Li, S. F. Y., Ng, S. C. and Chan, H. S. O. **1997**. Organic vapor sensors based on quartz crystal microbalance coated with self-assembled monolayers. *Sensors and Actuators B.*, 42, 59-65.
15. Skladal, P., **2003**. Piezoelectric Quartz Crystal Sensors Applied for Bioanalytical Assays and Characterization of Affinity Interactions. *J. Braz. Chem. Soc.*, 14(4), 491-502
16. O'Sullivan, C. K. and Guilbault, G. G. **1999**. Commercial quartz crystal microbalances – theory and applications. *Biosens. Bioelec.*, 14, 663 - 670.

應用合成胜肽和分子印模對氣味篩選之研究

高華生*，陳貞妤，簡蕙珊，胡創富

輔仁大學 化學系

摘要

本研究應用組合化學的概念及分子印模 (molecular imprinting) 的策略，設計及製備一系列具有氣體辨識能力的胜肽。本研究以化學混合法製備含有六個胺基酸長度的組合胜肽庫 (combinatorial peptide library)。以胜肽庫為模板分子與氣味分子混合，將模板胜肽聯結於壓電晶體訊號轉換元件製成生物晶片，保留被氣味分子印模的特異構形。進而探討胜肽和目標氣味分子間經印模處理後的吸附效能，會否因胜肽與目標氣味分子形成特殊活性構形，能有效提高生物晶片對氣味分子偵測的靈敏性，增強其辨別度。本研究結果顯示經印模處理後胜肽和目標氣味分子丁酸間偵測的靈敏性高過未經印模處理胜肽約 1.2~5.5 倍。顯示分子印模效應能增強胜肽偵測目標氣味分子的靈敏性。本研究的結果可應用於氣味分子檢測工具的開發。

關鍵字: 組合化學、胜肽庫、分子印模、氣味分子