# Linear Scorpion Peptides: An unexplored pool for peptide hydrogels

Constantinos Avraamides [1], Spyridoula Diavoli [1], Ariana Robertson [2], Manos Vlasiou [1], Elena Mourelatou [1], Christos Petrou [1], Yiannis Sarigiannis *[1]

1. Department of Life & Health Sciences, University of Nicosia, Nicosia, Cyprus
2. Department of Molecular and Cell Biology, University of California Berkeley, USA

Corresponding author: Dr Yiannis Sarigiannis, email: sarigiannis.i@unic.ac.cy

## Introduction

Non-Disulfide Bridged Peptides (NDBPs), around 40, are a second class of peptides and their study has been of great interest in recent years because these peptides have a variety of biological properties. Most of them consisting of 13-56 amino acid residues have been classified in 5 categories using the criteria of pharmacological activity, peptide length and sequence similarity firstly by Zeng et al [1] and later by Almaaytah and Abalas [2] [UniProtKB/Swiss-Prot]. The majority exhibit a-helix secondary structure, except for the peptides T and K-12. Over the past decade there is a great enthusiasm in the study of peptides not only as therapeutics but as biomaterials [3], especially hydrogels [4]. The economic viability, the variability in chemistry as well as the ease of the synthesis and mainly their properties; most of them are non-toxic, biocompatible, biodegradable, and applicable to localized therapies have increased the research interest for natural and synthetic peptides acting as hydrogelators [5]. The process of hydrogelation, which involves the confinement of water within the highly cross-linked network structures formed from the hierarchical arrangement of molecules, is driven enthalpically by several non-covalent forces like hydrogen bonding, Van der Waals forces, hydrophobic forces, π–π stacking and cation –π interactions [6].

**Aim: The main objective of this study is to investigate the structures of the linear scorpion peptides and their physicochemical properties involved in gel formation. We used the library of the linear scorpion peptides with 13-19 amino acids (Group 4 – Antimicrobial Peptides) from UniProtKB/Swiss-Prot. In addition to the previous calculations, we used as a model the linear scorpion peptide Mucroporin consisting of 17 AA, isolated from the venom of *Lychas Mucronatus*.**

**Table 1. List of Group 4 – Antimicrobial Peptides obtained from UniProtKB/Swiss-Prot**

| Systematic Name | Name | Peptide Properties (Sequence) | Length | Gravy | Hydrophobicity (Kcal*mol-1) | pI | Net Charge |
|---|---|---|---|---|---|---|---|
| NDBP-4.1 | IsCT | ILGKIWEGIKSLF | 13 | 0.77 | 10.23 | 9.74 | 1 |
| NDBP-4.2 | ISCT2 | IFGAIWNGIKSLF | 13 | 1.14 | 4.69 | 9.93 | 1 |
| NDBP-4.3 | BmKb1 | FLFSLIPSAISGLISAFK | 18 | 1.54 | 2.59 | 9.8 | 1 |
| NDBP-4.4 | BmKn2 | FIGAIANLLSKIF | 13 | 1.67 | 4.88 | 9.93 | 1 |
| **NDBP-4.5** | **Mucroporin** | **LFGLIPSLIGGLVSAFK** | **17** | 1.62 | 4.59 | 9.8 | 1 |
| NDBP-4.6 | Meucin-13 | IFGAIAGLLKNIF | 13 | 1.7 | 5.57 | 9.93 | 1 |
| NDBP-4.7 | Imcroporin | FFSLLPSLIGGLVSAIK | 17 | 1.59 | 3.9 | 9.8 | 1 |
| NDBP-4.8 | StCT1 | GFWGSLWEGVKSVV | 14 | 0.51 | 10.18 | 6.81 | 1 |
| NDBP-4.9 | HP1090 | IFKAIWSGIKSLF | 13 | 1.08 | 5.95 | 10.6 | 2 |
| NDBP-4.10 | Ctriporin | FLWGLIPGAISAVTSLIKK | 19 | 1.16 | 6.74 | 10.6 | 2 |
| NDBP-4.11 | AamAP1 | FLFSLIPHAIGGLISAFK | 18 | 1.43 | 5.15 | 9.8 | 1 |
| NDBP-4.12 | AamAP2 | FPFSLIPHAIGGLISAIK | 18 | 1.23 | 7.13 | 9.8 | 1 |
| NDBP-4.13 | VmCT1 | FLGALWNVAKSVF | 13 | 1.21 | 5.23 | 9.93 | 1 |
| NDBP-4.14 | VmCT2 | FLSTLWNAAKSIF | 13 | 0.82 | 4.59 | 9.93 | 1 |
| NDBP-4.15 | StCT2 | GFWGKLWEGVKSAI | 14 | 0.14 | 12.82 | 9.94 | 1 |
| NDBP-4.16 | UyCT1 | GFWGKLWEGVKNAI | 14 | **-0.05** | 13.21 | 9.94 | 1 |
| NDBP-4.17 | UyCT2 | FWGKLWEGVKNAI | 13 | **-0.02** | 12.06 | 9.94 | 1 |
| NDBP-4.18 | UyCT3 | ILSAIWSGIKSLF | 13 | 1.39 | 4.07 | 9.93 | 1 |
| NDBP-4.19 | UyCT5 | IWSAIWSGIKGLL | 13 | 1.14 | 4.38 | 10.1 | 1 |
| NDBP-4.20 | Pantinin-1 | GILGKLWEGFKSIV | 14 | 0.67 | 12.04 | 9.93 | 1 |
| NDBP-4.21 | Pantinin-2 | IFGAIWKGISSLL | 13 | 1.42 | 4.76 | 10.1 | 1 |
| NDBP-4.22 | Pantinin-3 | FLSTIWNGIKSLL | 13 | 0.94 | 4.08 | 10.1 | 1 |
| NDBP-4.23 | TsAP-1 | FLSLIPSLVGGSISAFK | 17 | 1.32 | 5.61 | 9.8 | 1 |
| NDBP-4.24 | TsAP-2 | FLGMIPGLIGGLISAFK | 17 | 1.55 | 5.2 | 9.8 | 1 |

## Results & Discussion

The library of the linear scorpion peptides with 13-19 amino acids from UniProtKB/Swiss-Prot (Group 4 – Antimicrobial Peptides) is presented in Table 1. Systematic name NDBP – x.y where x and y stand for the subfamily and peptide number within the subfamily, which should be assigned chronologically. Several interesting findings, common sequences of three to fourteen amino acids, are assigned with different colors. Interestingly, the peptide **Pantinin-1 (NDBP-4.20)** has a common hexapeptide, **GKLWEG**, with the peptides **StCT1 (NDBP-4.8), StCT2 (NDBP-4.15), UyCT1 (NDBP-4.16) and UyCT2 (NDBP-4.17).** The hexapeptide **GIKSLF** is common in the C-terminal site of **four peptides**. The tetrapeptide **SAFK** and **IGGL** are present in **five peptides** while the tripeptide **LIP** is occurred in **seven peptides.**

## Results & Discussion

We used the web application **PepDraw** to calculate the net charge of the peptide, the pI point, the hydrophobicity. The pI is an important property of a peptide because at this point the peptide is almost insoluble. At this point the probability of a peptide to crystalize is increased dramatically. Hydrophobicity is the free energy associated with transitioning a peptide from an aqueous environment to a hydrophobic environment like octanol.

The scale used is the Wimley-White scale [7], an experimentally determined scale, where the hydrophobicity of the peptide is the sum of Wimley-White hydrophobicities. The units of measure is in Kcal per mol while the pH is assumed neutral. Moreover, we calculated the Grand Average of Hydropathy Value for protein sequences (GRAVY) by using another web application. The GRAVY value is defined by the sum of hydropathy values of all amino acids divided by the protein length. Positive GRAVY values indicate hydrophobic; negative values mean hydrophilic. **Table 1** summarizes the calculated physicochemical properties of the peptides. Two peptides exhibit net charge +2 while the rest display +1. None of them display negative net charge. Except **StCT1** which displays a pI around 7 (pI = 6.81) the rest of the peptides exhibit pI in the range of 9.74 to 10.59. That means that in neutral pH (~7.4) the peptides are *positively charged* developing electrostatic interactions with the negatively charged phospholipid heads of the lipid membranes of the target cells. Most of the peptides present positive GRAVY values (0.51 – 1.67) indicating the *hydrophobic peptide backbones* while the peptides *StCT2, UyCT1 and UyCT2* exhibit values close to 0. The *most hydrophobic peptide* is BmKn2. In addition, we used the web application Net Wheels, the software Avogadro and Samson software platform as well as PEP-FOLD 3.5 [8] to predict and visualize 3D helical structures of the peptides.

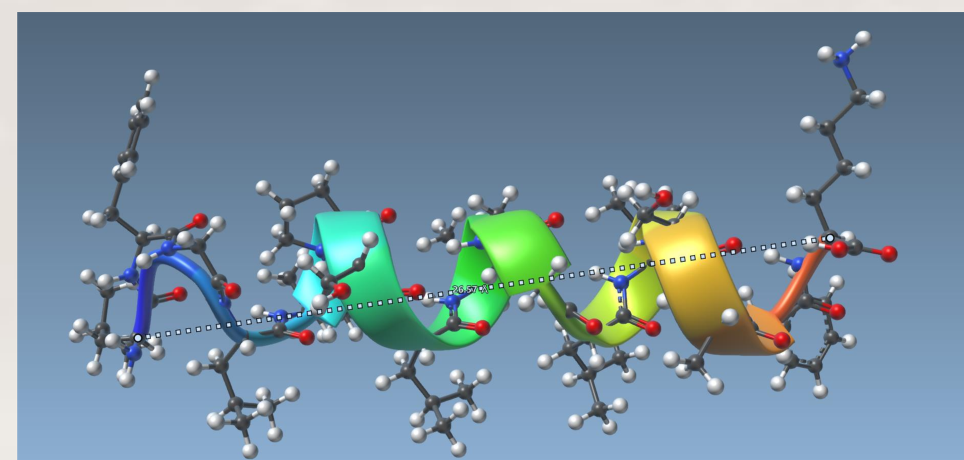

**Figure 1**: 3D visualization of the Mucroporin by using SAMSON software

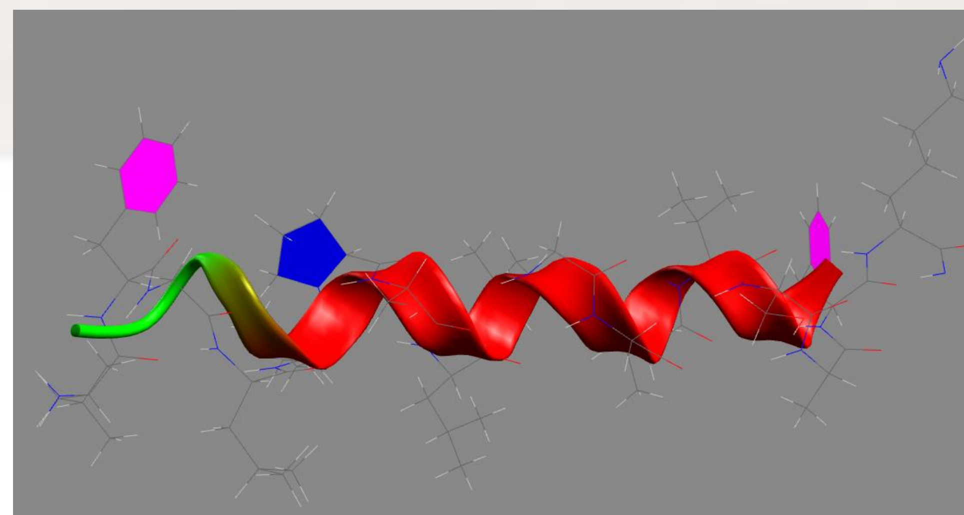

**Figure 2:** 3D visualization of the Mucroporin by using AVOGADRO

**Figure 1 and 2** displays the helices of our peptide model mucroporin as they were predicted by the AVOGADRO and the SAMSON software. All the software predicted the same 3D visualization. The distance between the N and the C terminal site was calculated on **26.57 Å**

### *Synthesis of Mucroporin and its analogs*

We synthesized the peptide and its synthetic analogs by Solid Phase Peptide Synthesis techniques and Fmoc/tBu methodology; we replaced the $Lys^{17}$ with Asp at the C-terminal site of the peptide altering the net charge of the peptide from +1 to -1 and we also deleted an aliphatic amino acid Ile at position 5 (next to Pro). The replacement of Lys with Asp alters the net charge of the peptide from +1 to -1. As a result, the pI of the new peptide drops to 3.2. The changes in the hydrophobicity or GRAVY are rather not significant. After the synthesis and the purification of the peptides $^{1}H$ NMR spectrums were recorded on a Bruker Avance 300 spectrometer operated at 300 MHz, with a 30° pulse with and a 0.2 s relaxation delay

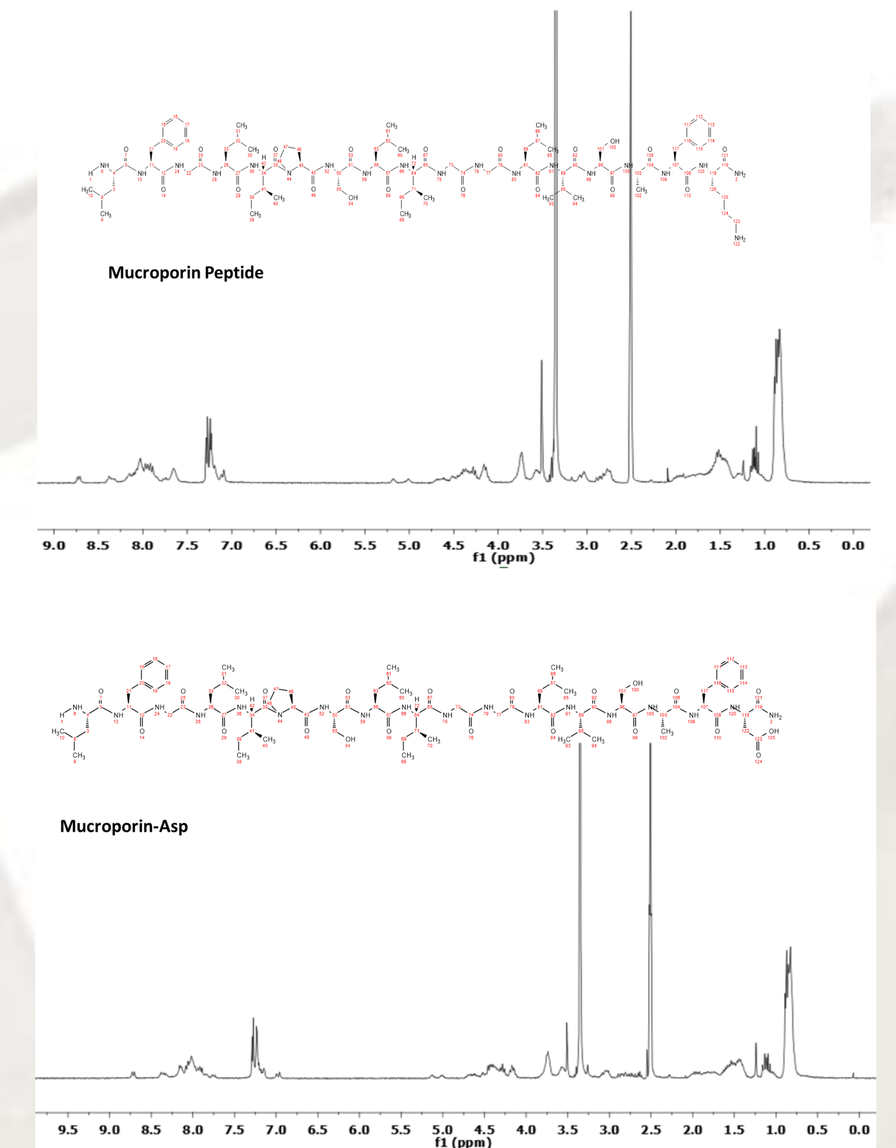


**Figure 3.** $^{1}H$ NMR of the synthesized peptides

**Peptide hydrogels were prepared** by weighing out the appropriate peptide mass (10mM) in 1.5 mL-vials. After the requisite volume of phosphate-buffered saline (0.1M PBS, pH 7.4) was added, the peptide mixture was shaken and sonicated for 10 min. The hydrogels are allowed to anneal for 24 h before they were used for further testing. **Gelation of the final formulations** was assessed by using the test tube inverting method. Briefly, 0,2 ml of the solution prepared were placed in an Eppendorf tube. After annealing, the tube was inverted. *Gelation was determined when no flow was observed over 30s after inversion*.

**Figure 4**. Peptide Hydrogels of Mucroporin (right) and Mucroporin-D (left) formed in 0.1M PBS (pH = 7.4)

## Conclusions

The data from the web applications for the 3D structures of the linear scorpion peptides as well as the predicted toxicity levels in combination with the preliminary experimental results indicate that this type of peptides could be a useful pool of novel peptide hydrogels.

**Conflicts of Interest:** The authors declare no conflict of interest

**References**

[1] X. C. Zeng et al *IUBMB Life*, **2005**, vol. 57, no. 1, pp. 13–21.
[2] A. Almaaytah and Q. Albalas, *Peptides*, **2014**, vol. 51, pp. 35–45.
[3] I. W. Hamley, "*Chem. Rev.*, **2017**, vol. 117, no. 24, pp. 14015–14041.
[4] A. Dasgupta et al *RSC Adv.*, **2013**, vol. 3, no. 24, pp. 9117–9149.
[5] J. Chen and X. Zou, *Bioact. Mater.*, **2019**, vol. 4, no. January, pp. 120–131.
[6] D. Iglesias and S. Marchesan, **2017**, Elsevier Inc.
[7] S. H. White and W. C. Wimley, *Biochim. Biophys. Acta - Rev. Biomembr.*, **1998**, vol. 1376, no. 3, pp. 339–352.
[8] A. Lamiable, P. Thévenet, J. Rey, M. Vavrusa, P. Derreumaux, and P. Tufféry, *Nucleic Acids Res.*, **2016**, vol. 44, no. W1, pp. W449–W454.