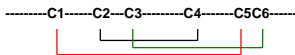


## What are Beta Defensins?

- A family of cationic, cysteine rich antimicrobial peptides that are constituents of the innate immune response to microbial infection.
- Members of the family have low sequence homology but characteristic disulfide bridge connectivity<sup>2</sup>.
- Expressed systemically in vertebrates, insects and plants with activity against gram negative and gram-positive bacteria as well as some enveloped viruses<sup>1</sup>.
- Mechanism of action is not well understood. Current thinking suggests electrostatic binding events of defensins with key residues at the bacterial cell surface direct cataclysmic membrane destabilisation, ionic imbalance and subsequent bacterial cell death<sup>3</sup>.

### Figure 1. Typical Beta Defensin connectivity

The backbone is shown linearly with cysteine alpha carbon atoms. Solid lines represent disulfide bridge connections.



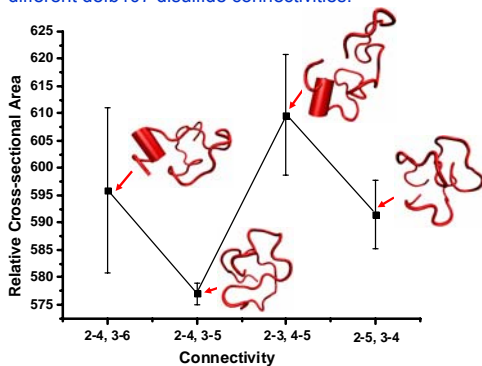
### Figure 2. A typical beta Defensin structure



1. Guo L, Lim KB, Poduje CM, et al. Lipid A acylation and bacteria resistance against vertebrate antimicrobial peptides. *Cell* 1998; 95:189-98
2. Guo L, Lim KB, Gunn JS, et al. Regulation of Lipid A of lipid A modification by *Salmonella* Typhimurium virulence genes *phoP-phoQ*. *Science* 1997; 276:250-253.
3. Paschel A, and Collins, L.V. (2001) Staphylococcal resistance to antimicrobial peptides of mammalian and bacterial origin. *Peptides* 22, 1651-165.

## Results for defb107

Figure 4. Predicted Cross-sectional areas for different defb107 disulfide connectivities.



### 2. Experimental Cross-Sectional Area

• Results pending: Experimental cross-sectional areas that map to calculated cross-sectional values will be assumed to share the same connectivity.

## Acknowledgements

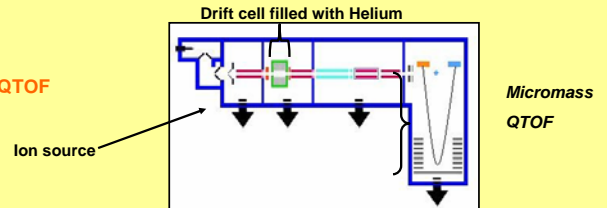
This work would not have been possible without help from the following people and institutions. Thank you very much:

1. Waters MS technologies: Bob Bateman, Kevin Giles, Steve Pringle and Jason Wildgoose
2. UCSB: Paul Kemper, Thomas Whyttenbach and Mike Bowers
2. EPSRC, Edinburgh University, British Mass Spectrometry Society (BMSS)

## What is Ion mobility and what does it tell us?

- Ion Mobility spectrometry measures the ability of molecules to travel through a helium filled drift cell<sup>4</sup>. The velocity of each molecule within the cell depends largely on its shape and therefore information about conformation can be established.
- The Mobility Quadrupole time of flight (MoQTOF) spectrometer performs both ion mobility and mass analysis of samples.

Figure 3. A schematic representation of the MoQTOF A modified QTOF



• Here we propose that ion mobility combined with molecular modelling, could be used to assist determination of disulfide bridge connectivity in synthetic defensins.

<sup>4</sup> Thomas Wyttenbach and Michael T. Bowers *Top. Curr. Chem.* 2003, 225, 207-232

## But why is it necessary to know the disulfide bridge connectivity?

- The canonical disulfide bridge connectivity is based on solved structures for 6 defensins. But does this connectivity apply to all defensins?
- Which disulfide bridge topology presents higher antimicrobial activity?
- Which enhances the cell signalling properties of defensins?
- How does disulphide bridge location effect the conformation of defensins, and does this affect their aggregation propensity?

## So how does molecular modelling come in to it?

The general strategy for determining structures is as follows:

### 1. Generate candidate theoretical structures with different connectivities

- AMBER is a software package that models molecular properties<sup>5</sup>.
- The energy of the system within AMBER is calculated by considering the sum of contributions from electrostatic, Van der Waals, bond angle and dihedral angle energy terms.
- The package allows a set of structures to be computed for every conceivable disulfide connectivity.

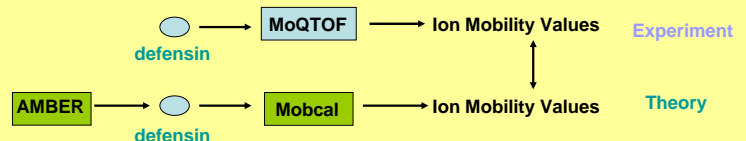
### 2. Calculate the Ion mobility of the theoretical structures

The program sigma calculates the mobility of ions using a projection approximation approach as follows<sup>6</sup>:

- The calculated 3-Dimensional structure of the ion is projected into a randomly chosen 2-Dimensional plane.
- The projected image is then replaced by a circle of equivalent area
- A square in area equivalent to the radius of gyration is then superimposed on top of this circle.
- A series of random points are then chosen from the square, and if these points correspond to a region within the circle, then a 'hit' is registered.
- The number of hits are proportional to the mobility and thus the approximation provides a basis for relating disulfide connectivity to structure.

### 3. Compare experimental mobilities with calculated mobilities

- The mobility values for the calculated structures are compared to the experimentally determined ones. Similarity in mobility is presumed to map to similarity in structure (and therefore to connectivity).



5. Pearlman, D. A., Case, D. A., Caldwell, J. W., Cheatham, T. E., Wang, J., Ross, W. S., Simmerling, C. L., Darden, T. A., Merz, K. M., Stanton, R. V., Cheng, A. L., Vincent, J. J., Crowley, M., Tsui, V., Gohlke, H., Radmer, R. J., Duan, Y., Pitera, J., Massova, I., Seibel, G. L., Singh, U. C., Weiner, P. K., Kollman, P. A. (2002) AMBER 8, University of California, San Francisco.
6. Thomas Wyttenbach, Gert von Helden, Joseph J. Batka, Jr., Douglas Carlat, and Michael T. Bowers *J. Am. Soc. Mass Spectrom.* 1997, 8, 275-282