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The role of the C-terminal chemistry in the membrane disrupting activity of the antimicrobial peptide aurein 1.2

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C-terminal amidation is a common feature of wild type membrane disrupting antimicrobial peptides (AMPs). Empirical evidence suggests that this modification increases antimicrobial efficacy. However, the actual role of C-terminal amidation in the mechanism of action of AMPs is not fully understood. Amidation alters two key properties simultaneously: the net charge and helicity of the peptide, both of which are implicated in the mechanism of action. Here the membrane disrupting activity of two aurein 1.2 mutants is compared: one with a free C terminus and one in which a secondary amide was formed with a terminal methyl group, instead of the primary amide as in the wild type peptide. Results of quartz crystal microbalance, dye leakage and circular dichroism experiments show that the activity of each mutant is substantially reduced compared to the wild type peptide; in particular, the modified peptides exhibited a much reduced ability to bind to the membrane. Thus, the primary amide at the C-terminus is required to bind to the membrane, and a secondary amide cannot serve the same purpose. Therefore our results suggests that the role of the C-terminal amidation is not to increase helicity or net charge of the peptide, rather it is a feature required for initial membrane binding; we hypothesize that this function is exerted by controlling the hydration state of the terminus.

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Super-resolution imaging of DNA at low damage levels

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Estimates to the amount of spontaneous DNA damage sustained in mammalian cells are as high as 105 lesions per replicating cell per day [1]. Many of these lesions are relatively easily repaired and generally well tolerated by the cell. The most toxic lesion and the greatest threat to genomic integrity is the DNA double stand break (DSB) [2]. Although researched widely, studies have focussed on long term damage induction and responses, usually at high damage levels.

We have developed single molecule localisation microscopy (SMLM) assays to investigate the immediate cellular responses following low level DSB induction by camptothecin (CPT) treatment. Replication forks and nascent DNA were pulse labelled using the DNA base analogue 5-ethynyl-2'-deoxyuridine (EdU) enabling direct visualisation of DNA. DNA damage response proteins were costained alongside pulsed DNA to visualise sites of DNA damage (Fig. 1). The developed assay was then used to quantify overall replication levels at low CPT concentrations revealing complex cellular responses to damage approaching endogenous levels.

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