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A functional analysis of PCNA-binding peptides derived from protein sequence, screening and rational design

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The use of small peptide aptamers to competitively inhibit protein function and interaction is becoming increasingly recognised as an exceptionally powerful technique. These peptides may be expressed either as unconstrained fusions or constrained within a scaffold protein. Amongst the advantages of aptamers are their ability to target specific protein-protein interactions and their ready optimisation using genetic techniques. The use of controls with inactivating point mutations provides an excellent control of specificity. RNAi and antisense techniques, while also amenable to genetic manipulation, will lower global protein levels and so non-specifically affect all functions of the target protein.

Here we describe the use of the dual models of *S. pombe* and human cells to develop PCNA-binding peptides. We have compared peptides derived from protein sequence, from two-hybrid screening of random libraries and from rational design. These peptides could inhibit PCNA function by competitive binding in both human and *S. pombe* cells as EGFP fusion proteins and all showed antiproliferative activity.

The peptide derived from rational design (con1) was stable, highly active in inhibiting PCNA function in both *S. pombe* and human cells and showed a high affinity for PCNA both *in vitro* and *in vivo*. These results validate the use of functional screening in yeast to identify peptide aptamers that are functional in mammalian cells. We are currently using the information derived from the con1-PCNA crystal structure to develop small molecule interaction mimics.