Abstract

Selective Inhibition of Erk-2 Signaling by Engineered Monobody

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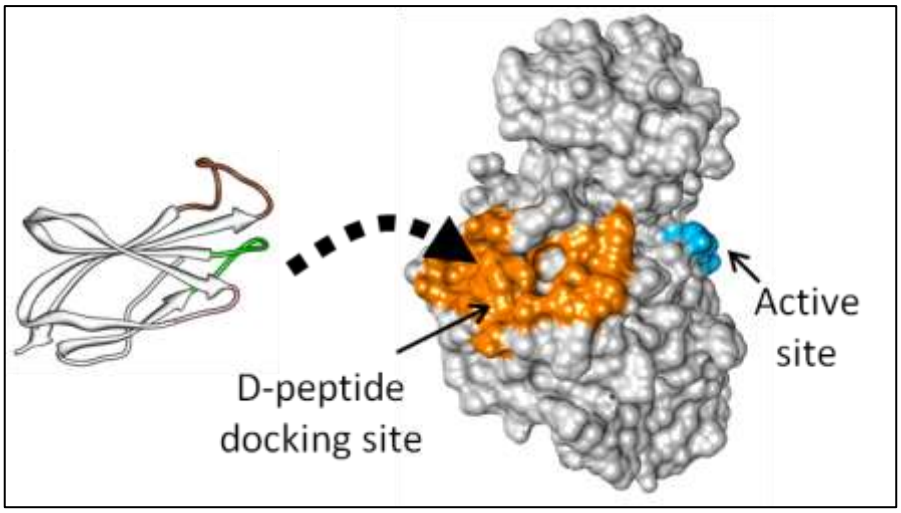
The phosphorylation networks are central to intracellular signaling and orchestrate a broad range of cellular responses to environmental cues. The mitogen activated protein kinases (MAPK) form evolutionarily conserved pathways that are activated by stimuli at the cell surface and induce changes in gene expression. In human, there are at least four MAPK pathways, including the Erk1/2, p38, JNK, and Erk5 pathways that mediate cellular responses to growth factors, inflammatory cytokines, and stress signals, and regulate embryogenesis, and cell fate determination. Dysregulation of MAPK pathways is associated with various diseases, including cancer, diabetes, neurological and inflammatory diseases. Therefore, engineered MAPK inhibitors would be useful tools for studying the molecular basis of signaling in model organisms and for developing therapeutics against overactive signaling networks. In the current study, we describe the engineering of monobody inhibitors that selectively block the interaction between Erk-2 and its *in vivo* substrates, and activators. MAPKs use the docking of a D-motif peptide found on their interactors and conserved "CD" docking domain to confer specificity of interaction and increase the efficiency of signaling. Since the docking interaction is essential for the function of Erk-2, the designed monobody should reduce the efficiency of Erk-2 activation and its catalytic activity. We screened a combinatorial fibronectin type III (FN3) monobody library expressed on the yeast surface using magnetic and fluorescence activated cell sorting. To isolate the monobodies that specifically target the CD domain, affinity maturation was alternated with a negative sort using mutant Erk-2 containing mutations in the docking site. The clones identified from the sort bind Erk-2 at the docking site with low nM affinity and inhibit its phosphorylation by MEK2 as well as phosphorylation of the transcription factor Elk1. Some of the clones exhibit high target specificity and can discriminate against p38 and JNK with > 100 fold selectivity. The inhibitors strongly inhibit the Erk-2 pathway in mammalian cells and can modulate the physiological processes in yeast and worms by inhibiting the activity of Erk-2 orthologs in each organism, demonstrating that the engineered inhibitors are useful in a variety of cellular context. Since the design strategy used in our study is general, a similar approach should be useful to engineer inhibitors against other MAPKs and intracellular enzymes in the future.



Engineering monobodies to target the docking domain of Erk-2