Bonvin P, Dunn SM, Rousseau F, Dyer DP, Shaw J, Power CA, Handel TM, Proudfoot AE. (2014). Identification of the pharmacophore of the CC chemokine-binding proteins Evasin-1 and -4 using phage display. *J. Biol. Chem*. Nov 14;289(46):31846-55.

To elucidate the ligand-binding surface of the CC chemokine-binding proteins Evasin-1 and Evasin-4, produced by the tick Rhipicephalus sanguineus, we sought to identify the key determinants responsible for their different chemokine selectivities by expressing Evasin mutants using phage display. We first designed alanine mutants based on the Evasin-1·CCL3 complex structure and an in silico model of Evasin-4 bound to CCL3. The mutants were displayed on M13 phage particles, and binding to chemokine was assessed by ELISA. Selected variants were then produced as purified proteins and characterized by surface plasmon resonance analysis and inhibition of chemotaxis. The method was validated by confirming the importance of Phe-14 and Trp-89 to the inhibitory properties of Evasin-1 and led to the identification of a third crucial residue, Asn-88. Two amino acids, Glu-16 and Tyr-19, were identified as key residues for binding and inhibition of Evasin-4. In a parallel approach, we identified one clone (Y28Q/N60D) that showed a clear reduction in binding to CCL3, CCL5, and CCL8. It therefore appears that Evasin-1 and -4 use different pharmacophores to bind CC chemokines, with the principal binding occurring through the C terminus of Evasin-1, but through the N-terminal region of Evasin-4. However, both proteins appear to target chemokine N termini, presumably because these domains are key to receptor signaling. The results also suggest that phage display may offer a useful approach for rapid investigation of the pharmacophores of small inhibitory binding proteins.