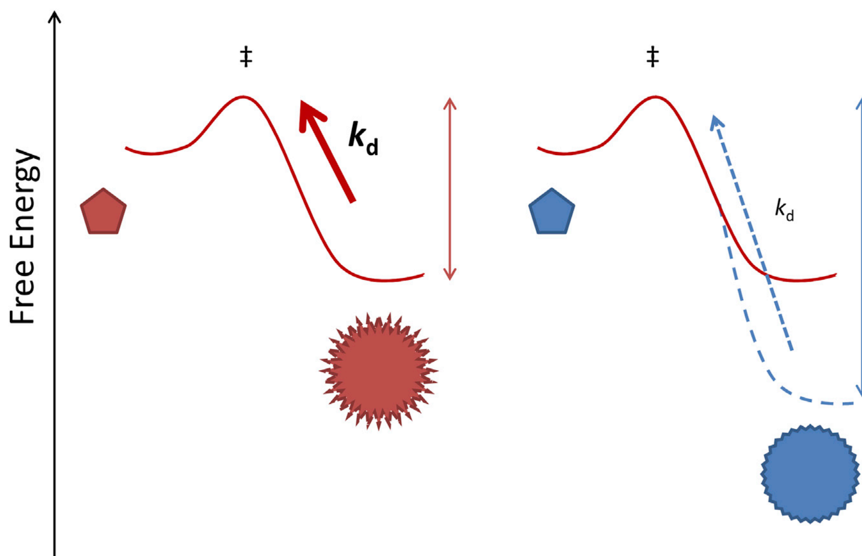

Figure 1. Schematic Showing Kinetic Stabilization of FMDV by Mutation
The parent virus is in red and the virus with acidic-to-neutral mutation is in blue. The associated state, but not the transition state (‡) for irreversible dissociation into pentamers, is stabilized by the mutations, resulting in increased kinetic stability of the capsid.

molecules. More generally, the results provide interesting new insights into how nature has designed giant macromolecular assemblies of incredibly beautiful symmetries that are poised at just the right amount of (in)stability for optimal function. And, for those of us attempting to build biomaterials from self-assembling proteins de novo, it is a humbling view of the design processes involved.

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Chemokine-Receptor Interactions: Solving the Puzzle, Piece by Piece

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In an important addition to the chemokine field, Millard and colleagues, in this issue of *Structure*, report the first structure of a CC chemokine in complex with a sulfated peptide derived from its receptor.

Due to its significant impact on health and disease, the chemokine system has been a target of interest for both academic research and pharmaceutical applications for years. The chemokine system, encompassing about 50 chemokine proteins that selectively bind to one or several cognate chemokine receptors, forms a sophisticated network that is critical in the mammalian immune system, mediating activation and chemotaxis of leukocytes and playing a role in both homing and inflammation. Dysfunction in the chemokine system has been implicated in health issues

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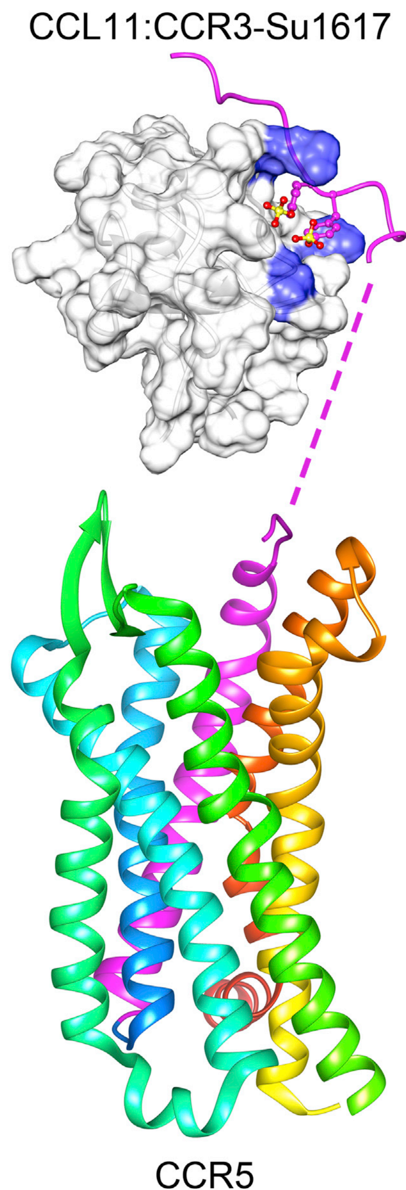

Figure 1. Relative Orientation of a CC Chemokine with Its Receptor, as Suggested by the Structure of Millard et al. (2014)
CCL11 is shown as a space filling model with critical basic residues Arg 16, Arg 22, and Lys 47 highlighted in blue. The receptor peptide from CCR3 (Su1617) is shown in magenta, and its two sulfated tyrosine residues are shown as balls and sticks, clearly interacting with CCL11. The structure of the CCR5 receptor (Protein Data Bank [PDB] ID 4MBS, the only known high resolution CC chemokine receptor structure yet reported) is shown below CCL11 to indicate the orientation of the chemokine relative to its receptor as implied by the structure reported by Millard et al. (2014) (PDB ID 2MPM). The dotted magenta line indicates the possible trajectory of the N terminus of the receptor as it binds a chemokine ligand.

ranging from heart disease to traumatic brain injury to asthma (Charo and Ransohoff, 2006).

A total of four subfamilies of chemokines have been identified, with the two major ones being the CC and CXC families. Chemokines from different subfamilies bind to a distinct set of receptors to function. Extensive effort has been geared toward understanding the chemokine:receptor interaction. To date, many chemokine structures from all four subfamilies have been reported, because these ligands are generally small proteins that are amenable to structural study. In contrast, the chemokine receptors are seven-transmembrane proteins that have posed major challenges. Recently, the field has been greatly advanced by reports on the high resolution structures of several chemokine receptors bound with a small-molecule antagonist or a peptide inhibitor, including CXCR4 (Wu et al., 2010) and CCR5 (Tan et al., 2013). Still, a critical gap remains in understanding how the receptors interact with their cognate chemokine ligands.

While efforts to obtain a co-structure of a chemokine in complex with its cognate receptor are undoubtedly underway, several groups have adopted a divide-and-conquer strategy, in which chemokines interacting with peptides corresponding to the N-terminal portions of their corresponding receptors were studied. More recent work also recognizes the importance of sulfated tyrosines on the receptors (Choe and Farzan, 2009). Nuclear magnetic resonance (NMR) has been a particularly useful technique in this endeavor, providing information about likely binding sites on the chemokines for the receptor peptides (Duma et al., 2007; Schnur et al., 2013) as well as specific inter-protein contacts, allowing previous NMR structure determinations for CXC subfamily chemokines CXCL8 (interleukin 8 [IL-8]) and CXCL12 (SDF-1) with a peptide from their respective receptors (Skelton et al., 1999, Veldkamp et al., 2008).

In this issue of *Structure*, Millard et al. (2014) report the first structure of a CC chemokine in complex with a receptor peptide, namely the chemokine CCL11 (eotaxin-1) with a sulfated peptide (Su1617) derived from the N terminus of the receptor CCR3. The researchers have obtained 55 intermolecular contacts (as well as numerous intramolecular contacts), leading to a complex structure with a clearly delineated binding surface on the chemokine for its receptor, composed of the N-loop and β2-β3 region from CCL11 (Figure 1). The Su1617-bound CCL11 remained similar to its free form, an observation also noted by previous reports of other chemokine:peptide complexes. Notably, the sidechains of sulfated tyrosine residues 16 and 17 on the receptor peptide are positioned to interact with regions on the chemokine that include important basic, hydrophobic, and aromatic residues that can form salt bridge, hydrophobic, and cation-π interactions, respectively. Further mutagenesis studies by the authors confirmed the importance of the basic residues on CCL11.

This newly described structure extends our understanding of chemokine:receptor interactions, providing an atomic resolution picture as well as validating the importance of tyrosine sulfation of chemokine receptors. It also supports the prevailing "two-site model" of receptor binding, in which the N terminus of the receptor is bound by the chemokine N-loop and β2-β3 regions first, likely followed by the N terminus of the chemokine ligand interacting with the extracellular loops and/or transmembrane segments of the receptor for activation and intracellular signaling.

The authors also gathered recent reports from several groups to summarize and compare chemokine:receptor interactions as they are currently understood. Similarities have been found among these structures, including sulfated tyrosine residues from the receptor peptide interacting with similar binding surfaces on the chemokines. More interestingly, it was noted that there appears to be a clear difference in the orientation of the chemokine relative to its receptor among these structures.

While the present results and contextual comparisons are of great value, some questions remain. For example, probably one of the most important chemokine residues known for receptor affinity for many CC chemokines is the residue immediately following the conserved CC motif, frequently being an aromatic residue (Phe11 in CCL11). In the current structure, the Phe11 residue does not appear to make direct contact with the receptor peptide. It is also noted that chemokines bind to intact receptors

much more tightly than the peptides derived from receptor N terminus. These observations led the authors to suggest a possible role for this residue in "site 2" receptor interactions (as opposed to the "site 1" interactions illustrated by the present structure). Potentially more significant is the apparent variation in the chemokine orientation to the receptor of the three known structures (the current CCL11:CCR3 peptide, CXCL8:CXCR1 peptide, and CXCL12:CXCR4 peptide). One may wonder whether the chemokines would be positioned differently on the full length receptor than with a sulfated peptide. If the orientation difference observed in these studies reflect the actual positioning of these molecules, this suggests that there may be distinct binding patterns by individual chemokine:receptor pairs, which in turn may provide an explanation for the variety of signaling/activation outcomes.

To fully address these questions, structures of chemokines in complex with their full length receptors are needed. As the structure determination of chemokine receptors in complex with their chemokine ligands becomes more likely, this goal may be met in the near future.

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