On the dimerization of CCR5

To the editor:

In the February 2004 issue of *Nature Immunology*, Hernanz-Falcon *et al.* reported that the combination of two point mutations (I52V and V150A) in the first and fourth transmembrane domains of the CCR5 chemokine receptor disrupted its ability to form dimers, as shown by both biochemistry and resonance energy transfer–based approaches[1]. The authors also showed that the formation of oligomers of CCR5 is essential for triggering signaling but is dispensable for its correct expression on the cell surface and its ability to bind chemokines. The properties of this form of CCR5 (CCR5mut) offered unique perspectives regarding the mechanism and function of seven-transmembrane receptor dimerization. They were also of considerable interest for studies of human immunodeficiency virus 1 (HIV-1) cell entry, because CCR5 is the principal CD4-associated HIV-1 coreceptor, and dimerization has been proposed to modulate its activity[2]. The availability of CCR5mut therefore allowed us to address the coreceptor activity of a strictly monomeric form of CCR5.

We have expressed wild-type CCR5 and CCR5mut in $CD4^+$ target cells and found no differences in their ability to mediate HIV-1 entry or fusion with cells expressing HIV-1 envelope proteins, in agreement with their similar cell surface receptor expression (**Supplementary Table 1** online). Notably, incubation with the chemokine MIP-1β promoted efficient endocytosis of CCR5mut, confirming that the mutations did not impair ligand binding (**Supplementary Table 1** online). Based on the findings of Hernanz-Falcon *et al.*, these results would indicate that putative CCR5mut monomers remain functional HIV-1 coreceptors. To confirm this conclusion, we directly addressed the oligomeric status of CCR5mut by coimmunoprecipitation and bioluminescence resonance energy transfer (BRET) experiments, which have been used to demonstrate that dimerization of CCR5 is a constitutive process[3]. The association of epitope-tagged forms of CCR5mut was demonstrated by coimmunoprecipitation of lysates of HEK 293 cells and was comparable to that of wild-type CCR5 (**Supplementary Fig. 1** online). BRET assays of the same cells showed that wild-type CCR5 and CCR5mut formed homo- or heterodimers with a similar efficiency in intact cells but did not associate with an unrelated seven-transmembrane receptor (**Supplementary Fig. 1** online). These experiments indicated that the mutations I52V and V150A do not impair the formation of CCR5 dimers, which is in disagreement with the report of Hernanz-Falcon *et al.*

At present the precise reason for these discrepancies cannot be ascertained, but it could relate to the use of different techniques to detect dimers. The numerous causes for potential misinterpretations have been reviewed[4,5]. The loss of FRET signal, which was considered by Hernanz-Falcon *et al.* as a strong evidence of impaired dimerization, can be found after conformation changes affecting the spatial orientation of donor and acceptor without disturbing dimerization. Thus, it could be envisioned that the mutations I52V and V150A influence the conformation of CCR5 in a relatively subtle way, thereby affecting its coupling to the cell signaling machinery, but do not abrogate its dimerization. Although the issue of possible functional differences between CCR5 (or other seven-transmembrane receptors) monomers or dimers remains important, it may well be elusive, because a growing number of observations[5] indicates that early dimerization of seven-transmembrane receptors is a prerequisite for their proper targeting to the cell surface.

Note: Supplementary information is available on the Nature Immunology website.

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Hernanz-Falcón *et al.* reply:

Resonance energy transfer (RET) methods, including BRET and fluorescence RET (FRET), are now widely used to study protein-protein interactions in living cells[1]. The nature of BRET limits its use to the study of cell populations; it cannot be applied to single-cell analysis. It is thus impossible to determine the cell site at which a signal is generated, and any intracellular associations of the energy transfer partners would be measured as BRET. In contrast, FRET permits visualization of subcellular protein-protein interactions in a single living cell; in this case, background is easily eliminated by the use of an unlabeled cell region as an internal autofluorescence reference.

There is no perfect RET method, and each is accompanied by specific advantages and drawbacks. A common concern when measuring the RET of overexpressed fusion proteins is that large amounts of protein can increase the incidence of random collisions; such nonspecific interactions would be attributed to RET[2]. Furthermore, high protein expression can overwhelm the glycosylation capacity of a cell. It is thus not unusual to find a substantial fraction of BRET-competent G protein–coupled receptors at intracellular membranes[3]. This is especially pertinent in the case of chemokine receptors, as only a small fraction of total receptors reach the extracellular membrane. In cells with receptor overexpression, we