**4.1.3.2 Rvs161 and Rvs167 are recruited as dimers to endocytic sites**

FCCS and genetic studies have both proposed that the Rvs complex is recruited to endocytic sites as heterodimers, although the deletions of one do not exactly match the deletion of the other. Deletion of Rvs161, for example, confers a defect in cell fusion, that is not present in the rvs167deletion. FCCS measurements have indicated that Rvs167 and Rvs161 form stable heterodimers in the cytoplasm, although they appear to be expressed at different concentration. Cytoplasmic concentration of 161 is expressed at 721nM by FCS, while 167 is measured at 354nM, suggesting a nearly two-fold increase in expression of 161. Overexpression of 167 in the duplicated strain, however, does not lead to increased recruitment of that protein to endocytic sites, unless matched by overexpression of Rvs161. In the case of overexpression of BAR, without overexpression of 161, however, there is an increased recruitement of BAR to sites. Cytoplasmic background quantification has shown only a moderate decrease in the expression of BAR, compared to full-length Rvs167. This leads to a conundrum of recruitment: how does more BAR lead to more protein, while more 167 does not?

**4.1.3.3 Contribution of the N-amphiphatic helix and GPA region**

The influence of the N-terminal amphiphatic helix (N-helix) and the unstructured GPA region have not been studied in this work. Preliminary results (not shown) indicate that removal of the N-helix does not prevent recruitment of endocytic sites. Other work has shown that N-helix is not necessary for localization of the protein, except in high salt conditions, and could aid clustering of the protein at endocytic sites in normal growth conditions. The GPA region is thought to function as the linker between the BAR and SH3 regions, and no other specific function is known.