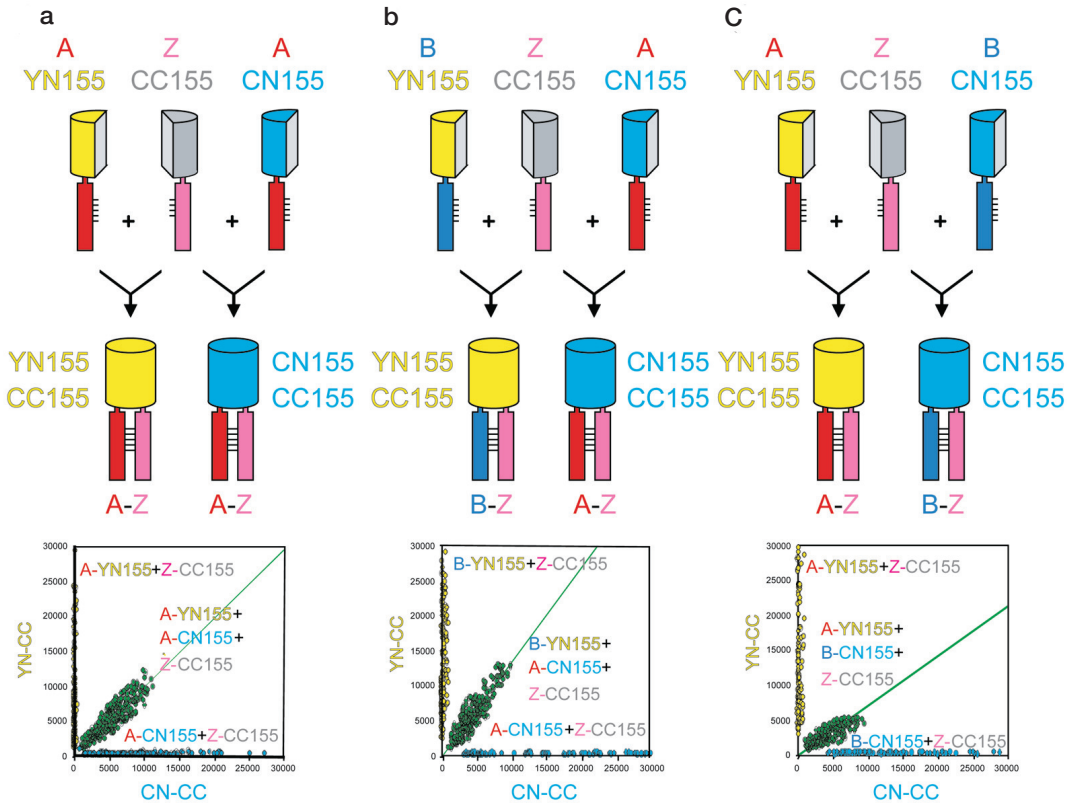

FIGURE 5. Multicolor fluorescence complementation analysis of the competition for dimerization between alternative interaction partners. The relative efficiencies of complex formation between protein Z and two alternative interaction partners, A and B, can be quantified by fusing them to fragments of different fluorescent proteins and expressing them in different combinations. (*a*) BiFC analysis of A-YN155 + Z-CC155, A-CN155 + Z-CC155, and A-YN155 + A-CN155 + Z-CC155. (*b*) BiFC analysis of B-YN155 + Z-CC155, A-CN155 + Z-CC155, and B-YN155 + A-CN155 + Z-CC155. (*c*) BiFC analysis of A-YN155 + Z-CC155, B-CN155 + Z-CC155, and A-YN155 + B-CN155 + Z-CC155. The proteins indicated above each graph were expressed both pair-wise, as shown in the graphs below the diagrams (data shown in *yellow* and *cyan*), as well as in three-way competition (data shown in *green*). Equal concentrations of the plasmids encoding proteins fused to YN155 and CN155 were used in combination with a limiting concentration of the plasmid expressing the protein fused to CC155 (for definitions of fusion proteins, see Table 1). The cells were imaged using filters described in the materials section that distinguish the fluorescence emissions of YN-CC and CN-CC complexes. The fluorescence intensities of YN-CC complexes were plotted as a function of the fluorescence intensities of CN-CC complexes in individual cells. The fluorescence intensities are shown in yellow and cyan for cells that express the interaction partners pair-wise, and in green for cells that express two alternative interaction partners in direct competition. The best fit of a linear function to the data from cells coexpressing three proteins is shown. The slope of this function reflects the relative efficiencies of complex formation between the alternative interaction partners. When B-YN155 and A-CN155 were coexpressed with Z-CC155, the fluorescence intensities produced by B-YN155–Z-CC155 and A-CN155–Z-CC155 exhibited a linear relationship with a slope of 1.3 and a 95% confidence interval of 0.08 (*b*). In comparison, when A-YN155 and A-CN155 were coexpressed with Z-CC155, the fluorescence intensities produced by A-YN155–Z-CC155 and A-CN155–Z-CC155 exhibited a linear relationship with a slope of 0.98 and a 95% confidence interval of 0.05 (*a*). To confirm that the fluorescent protein fragments fused to the alternative interaction partners did not influence the relative efficiencies of complex formation, we exchanged the fragments between the interaction partners (i.e., A-YN155, B-CN155, and Z-CC155) and compared the fluorescence intensities of bimolecular fluorescent complexes formed by these proteins (*c*). The fluorescence intensities of these complexes exhibited a linear relationship with slope of 0.7 and a 95% confidence interval of 0.05. These results are consistent with the interpretation that Z favors complex formation with B over complex formation with A. Data adapted from Grinberg et al. (2004).

Protein–Protein Interactions: A Molecular Cloning *Manual*, 2nd Ed., © 2005 by Cold Spring Harbor Laboratory Press, Chapter 34, Figure 5.