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The Structure of a C_H2-Deleted Humanized Antibody. Steven B. Larson, John S. Day, Alexander McPherson, Univ. of California, Irvine, CA 92697, Scott Glaser and Gary Braslawsky, Biogen Idec Corp, San Diego, CA.

C_H2 domain-deleted CC49 (ddCC49), a recombinant humanized antibody that recognizes the tumor associated TAG72 antigen expressed on a variety of human carcinomas, is secreted from cultured cells as a mixture of two homodimeric isoforms. One isoform, referred to as Form A, contains a covalent interchain disulfide bond at heavy chain positions 239 and 242. Form B fails to develop an interchain disulfide bond. The A and B isoforms can be separated by hydrophobic interaction chromatography. Form A is currently in preclinical development as a therapeutic for treating colorectal carcinoma.

ddCC49 Form B has been crystallized in the presence of various detergents. ALS Synchrotron data were collected on a single cryo-cooled crystal grown with triton X100 detergent. The crystal belonged to the space group P2₁2₁2 with cell dimensions of a=82, b=224, and c=167 Å. The structure was solved by MR and the model has refined to R=0.260 [R_{free}=0.328] for 2.8 Å data.

The antibodies pack in the crystal around crystallographic 2-fold axes as tetramers with approximate 222 symmetry. AFM studies suggest that this tetrameric structure is the crystal building block. The tetramer is composed of two rings back-to-back with a thickness of ~82 Å. Each ring is composed of two antibodies with the CDR regions of the two Fabs of one antibody interacting with the CDR regions of the second antibody in a head-to-head fashion. These rings are approximately 167 Å long and 112 Å wide. The C_H3 domain is inverted with respect to the Fabs when compared to the usual orientation found in conventional antibodies. The lack of disulfide bridges in the hinge region endows the C_H3 domains with considerable mobility.