

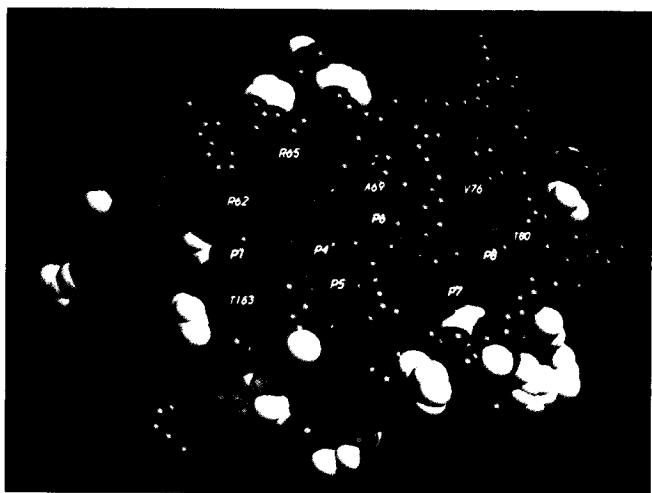


FIG. 4 The 'antigenic' surface composed of Np 91-99 peptide (orange) and MHC atoms (blue, conserved; red, polymorphic; light blue, not conserved or polymorphic). N-terminal of peptide is to the left, α_1 domain α -helix top, α_2 domain α -helix bottom. Figure generated with RASTER3D. (Of the 12 polymorphic residues facing into the binding site, 8 contact the peptide directly (9, 45, 66, 70, 74, 77, 95, 116) and four do not (67, 97, 114, 156), but 11 of the 12 (66 excluded) are nevertheless completely buried by the bound peptide. These polymorphic positions must therefore, as anticipated²³, have their primary effect on T-cell recognition of HLA-Aw68 through the choice of peptides that can bind. Of the six polymorphic residues that face more directly toward solvent (62, 65, 69, 76, 80, 163), four also contact the peptide (62, 69, 80, 163) but all have atoms accessible to direct recognition by the TCR and therefore represent polymorphism recognizable by TCRs in the presence or absence of peptide.)

may not be a major factor in the creation of novel antigenic surfaces recognized by T cells. On the basis of the number of atomic contacts, Np 91-99 appears to be bound to HLA-Aw68 predominantly by two main features of the MHC molecule: (1) conserved MHC residues hydrogen bond to the peptide termini; (2) polymorphic MHC residues bury the two 'anchor' peptide side chains. Although both of these sets of interactions would also provide for the peptide-dependent stabilization of the MHC molecule, only the peptide termini binding sites are conserved in class I histocompatibility antigen sequences. The overall mode of peptide binding observed here seems to be a general mechanism for class I MHC presentation now visualized in three human alleles and one murine allele: HLA-B27³⁻⁵, HLA-Aw68¹⁰, HLA-A2¹² and H-2K^b (refs 13, 24). □

Received 4 August; accepted 2 October 1992.

1. Townsend, A. & Bodmer, H. A. *Rev. Immun.* **7**, 601-624 (1989).
2. Brodsky, F. M. & Guagliardi, L. E. A. *Rev. Immun.* **9**, 707-744 (1991).
3. Madden, D. R., Gorga, J. C., Strominger, J. L. & Wiley, D. C. *Nature* **253**, 321-325 (1991).
4. Jardetzky, T. S., Lane, W. S., Robinson, R. A., Madden, D. R. & Wiley, D. C. *Nature* **253**, 326-329 (1991).
5. Madden, D. R., Gorga, J. C., Strominger, J. L. & Wiley, D. C. *Cell* **70**, 1035-1048 (1992).
6. Turner, M. J. *et al. J. biol. Chem.* **265**, 4512-4519 (1990).
7. Bjorkman, P. J., Strominger, J. L. & Wiley, D. C. *J. molec. Biol.* **186**, 205-210 (1986).
8. Silver, M. L., Parker, K. C. & Wiley, D. C. *Nature* **350**, 619-622 (1991).
9. Cerundolo, V., Tse, A. G. D., Salter, R. D., Parham, P. & Townsend, A. *Proc. R. Soc. Lond. B* **244**, 169-177 (1991).
10. Guo, H.-C., Jardetzky, T. S., Lane, W. S., Strominger, J. L. & Wiley, D. C. *Nature* **360**, 364-366 (1992).
11. Garrett, T. P. J., Saper, M. A., Bjorkman, P. J., Strominger, J. L. & Wiley, D. C. *Nature* **342**, 692-696 (1989).
12. Saper, M. A., Bjorkman, P. J. & Wiley, D. C. *J. molec. Biol.* **219**, 277-319 (1991).
13. Fremont, D. H., Matsumura, M., Stura, E. A., Peterson, P. A. & Wilson, I. A. *Science* **257**, 919-927 (1992).
14. Teng, T.-Y. *J. appl. Crystallogr.* **23**, 387-391 (1990).
15. Durbin, R. M. *et al. Science* **232**, 1127-1132 (1986).
16. Blum, M., Metcalf, P., Harrison, S. C. & Wiley, D. C. *J. appl. Crystallogr.* **20**, 235-242 (1987).
17. Fox, G. C. & Holmes, K. C. *Acta Crystallogr.* **A34**, 517 (1966).
18. Brünger, A. T. XPLOR (version 2.1) Yale University, New Haven, (1990).
19. Silver, M. L. thesis, Harvard University (1992).
20. Jones, T. A. *J. appl. Crystallogr.* **11**, 268-272 (1978).
21. Parham, P. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 4005-4009 (1988).

22. Johnson, C. K. ORTEP Manual (Oak Ridge National Laboratory, Tennessee, 1965).
23. Bjorkman, P. J. *et al. Nature* **329**, 512-518 (1987).
24. Zhang, W., Young, A. C. M., Imarai, M., Nathenson, S. G. & Sacchettini, J. C. *Proc. natn. Acad. Sci. U.S.A.* **89**, 8403-8407 (1992).

ACKNOWLEDGEMENTS. We acknowledge the contributions made by T. P. J. Garrett, M. A. Saper, and P. J. Bjorkman to the determination of the structure of HLA-Aw68 to 2.6 Å resolution¹¹. We thank J. Gorga for supervising the protein purifications and A. Haykov, K. Svenson and R. Crouse for technical assistance. H.-C.G. is a Cancer Research Institute fellow. J.L.S. and D.C.W. acknowledge support from the NIH. D.C.W. is supported by the Howard Hughes Medical Institute.

The three-dimensional structure of an intact monoclonal antibody for canine lymphoma

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CRYSTAL structures of Fab antibody fragments determined by X-ray diffraction characteristically feature four-domain, β -barrel arrangements¹⁻³. A human antibody Fc fragment has also been found to have four β -barrel domains⁴. The structures of a few intact antibodies have been solved⁵⁻⁸; in two myeloma proteins, the flexible hinge regions that connect the Fc to the Fab segments were deleted^{5,6} so the molecules were non-functional, structurally restrained, T-shaped antibodies; a third antibody, Kol, had no hinge residues missing but the Fc region was sufficiently disordered that it was not possible to relate its disposition accurately with respect to the Fab components^{7,8}. Here we report the structure at 3.5 Å resolution of an IgG2a antitumour monoclonal antibody which contains an intact hinge region and was solved in a triclinic crystal by molecular replacement using known Fc and Fab fragments. The antibody is asymmetric, reflecting its dynamic character. There are two local, apparently independent, dyads in the molecule. One relates the heavy chains in the Fc, the other relates the constant domains of the Fabs. The variable domains are not related by this 2-fold axis because of the different Fab elbow angles of 159° and 143°. The Fc has assumed an asymmetric, oblique orientation with respect to loosely tethered yet almost collinear Fabs. Our study enables the two antigen-binding segments as well as the Fc portion of a functional molecule to be visualized and illustrates the flexibility of these immune response proteins.

The specific murine antibody described here reacts with cells of canine lymphoma⁹, the most common haemopoietic tumour in the dog, which resembles human non-Hodgkin's lymphoma. This antibody can participate in antibody-dependent cellular cytotoxicity as well as complement-dependent cytotoxicity¹⁰ and is used as an anticancer therapeutic¹¹ by veterinarians. The immunoglobulin crystallizes from a low concentration of polyethylene glycol at slightly alkaline pH (ref. 12).

The structure of the triclinic crystals, having one entire antibody as the asymmetric unit, was solved using the method of molecular replacement as implemented in the programs MERLOT¹³ for rotation functions and XPLOR^{14,15} for translation functions. By virtue of the triclinic cell there were no symmetry constraints on the molecule, immediately suggesting that the antibody has an asymmetric conformation. Molecular probe coordinates for Fab fragments and the Fc fragment were obtained from the Brookhaven Data Bank¹⁶. Sometimes cross-rotation¹⁷ searches were done with probes representing one third of the asymmetric unit, and in other cases the search probes comprised only one sixth of the asymmetric unit. Convincing



FIG. 1 Ribbon representation of the structure of the murine antibody against canine lymphoma determined by X-ray analysis of the triclinic crystals. The heavy chains are shown in yellow and blue, the light chains in red. The Fc stem of the molecule projects towards the viewer and assumes an asymmetric, oblique orientation with respect to the Fabs. This orientation illustrates the large difference in hinge angles of about 65° and 115° . The local dyad relating the heavy chains of the Fc is that dyad indicated by the primary solution of the self-rotation function. Fab2 is viewed along the axis through the switch peptides. Fab2 has an elbow angle of 143° , in contrast to Fab1, which has an elbow of 159° . Twenty-three residues in each heavy chain, comprising the hinge regions seen here, were built into the model with idealized geometry using both FRODO²⁹ and XPLOR^{14,15}. These residues were missing from the fragment models taken from the Brookhaven Data Bank.

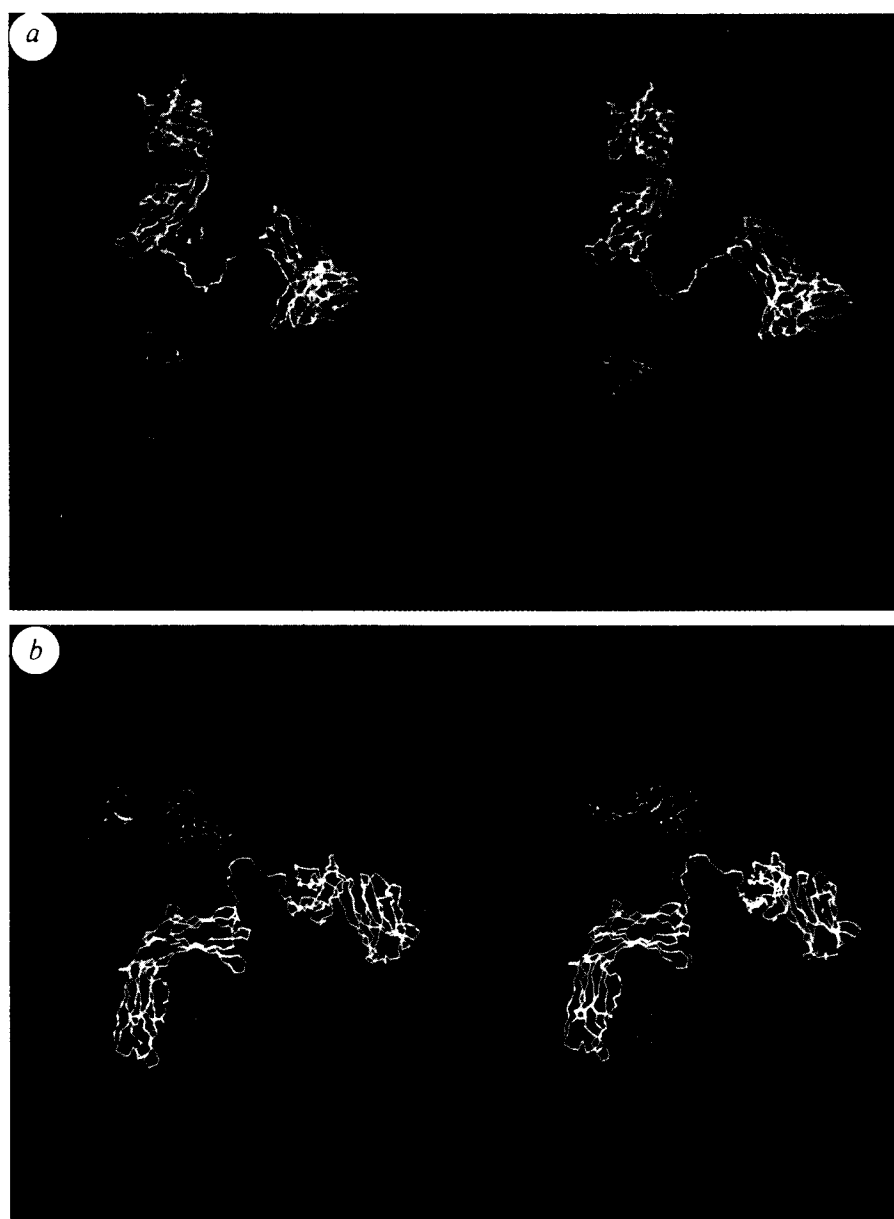


FIG. 2 *a*, stereo diagram of the monoclonal antibody viewed perpendicular to the approximate 2-fold axis relating the constant domains of the Fabs. This dyad was that indicated by the secondary solution of the self-rotation function. Apparent here is the difference in the two elbow angles and the consequent failure of the variable domains to maintain this relationship. Also apparent in this view is the failure of the Fc dyad to intersect the 2-fold axis relating the constant domains of the Fabs. Both symmetry axes are apparently independent local dyads. *b*, Stereo diagram of the IgG2a antibody showing the region between the CH2 domains. In the human IgG1 Fc fragment⁴, carbohydrate was located in this area between the two CH2 domains and is probably in a similar location in this antibody. No attempt has yet been made to include the carbohydrate component in the model. It can also be seen here that the dyad axis of the Fc does not intersect the approximate long axis of the Fabs. Colour coding is the same as in Fig. 1.

TABLE 1 Crystallographic data, structure solution and refinement

Crystal data: Space group $P1$; $a=66.39$ Å, $b=77.34$ Å, $c=101.42$ Å, $\alpha=87.6^\circ$, $\beta=92.6^\circ$, $\gamma=97.5^\circ$, $Z=1$; resolution ~ 3.0 Å. Data collection: SDMS (Xuong-Hamlin) detectors, Rigaku Ru-200 source, frame size 0.12° , counting time 60–120 s. Total observations, 114,867; at 3.5 Å unique reflections, 24,808; 98.7% complete; $R_{\text{sym}}=0.098$. After $F/\sigma=4.0$ cutoff, unique reflections at 3.5 Å = 20,964

Operation	Result	
Self-rotation function	Two consistent dyad solutions in several resolution ranges	
Fc rotation function	Two peaks related by pseudodyad; r.m.s.* = 6.03 and 5.54	
(1) Search probe entire Fc	same solutions as (1); r.m.s. = 5.66 and 5.44	
(2) CH3 domains	same solutions as (1); r.m.s. = 3.41 and 3.14	
(3) CH2 domains		
Fab1 rotation function	Exceptional peak with constant domains of HYHEL-5; r.m.s. = 6.42	
(1) Search probes were constant domains of 7 different Fabs	Solution optimal for variable domains of McPC603; r.m.s. = 4.35	
(2) Probes were variable domains of 7 different Fabs	Outstanding solution consistent with (1) and (2); r.m.s. = 7.98	
(3) An intact Fab1 constructed according to above results		
Fab2 rotation function	Unambiguous solution for probe with elbow angle 140° ; r.m.s. = 6.27	
Range of Fab models with elbows of 120° – 180° constructed using XPLOR by altering Fab1 elbow every 5°		
Translation function	A self-consistent set of solutions	
(1) Fc fixed, Fab1 moving	from all searches (1)–(4);	
(2) Fc fixed, Fab2 moving	$cc^\dagger=0.157$ – 0.249 for 4–8 Å	
(3) Fab1 fixed, Fab2 moving	resolution	
(4) Fab1 and Fab2 fixed, Fc moving		
Refinement		
(1) Rigid body with twelve β -barrel domains; 3.5–12 Å	$R=0.386$	$cc^\dagger=0.529$
(2) Powell minimization and simulated annealing after insertion of correct amino-acid sequence (occupancy of 46 hinge residues set to zero); 3.5–8 Å	$R=0.188$	$cc^\dagger=0.876$
	r.m.s. deviations	
	Bonds	0.017 Å
	Angles	4.038°
	Dihedrals	28.597°
	Impropers	0.686°

* All r.m.s. values are stated for 4–8 Å resolution searches. † cc, Correlation coefficient.

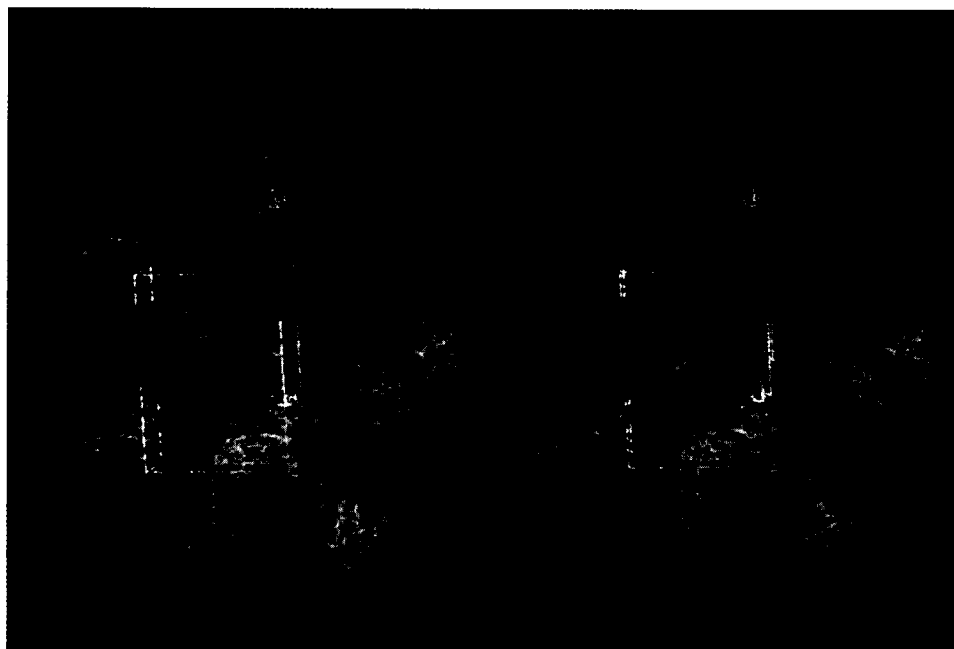
solutions were frequently found even with the probes representing one sixth of the antibody. Rotation function solutions were based on the human Fc fragment⁴, the constant domains of Fab HYHEL-5 (ref. 18), and the variable domains of Fab McPC603 (ref. 19), including the hypervariable regions. Translation searches were performed to determine the relative distances between the three portions of the molecule, the two Fabs and the Fc (Table 1).

The antibody structure was assembled according to essentially independent, but internally consistent, molecular replacement results that ultimately yielded a model fully consistent with the

stereochemistry of an intact antibody. The packing revealed good complementarity of surfaces without interpenetration. Lattice contacts immobilize all segments of the molecule to permit visualization of both Fabs as well as the Fc.

The structure of the antibody is shown in Figs 1 and 2. Its most prominent features are: (1) There is an approximate 2-fold axis relating the heavy chains of the Fc portion of the molecule. The dyad deviates particularly for the CH2 domains; (2) the disposition of the Fc with respect to the Fab portions is quite oblique; (3) the hinge angle between the Fc and Fab1 is approximately 65° and for Fab2 about 115° ; (4) the long axes of the

FIG. 3 Stereo diagram of the packing of four antibodies in the triclinic cell, each of a different colour, showing the intricate network of intermolecular contacts that stabilizes the conformation of the molecule. The Fc segments, which lie more or less along the longest body diagonal, are immobilized by multiple Fab contacts, suggesting why the Fc in these crystals is ordered. The constant domains of an Fab2 of one molecule insert in the elbow region of Fab1 of a different antibody molecule to fix the dispositions of the Fabs. Notable initial exceptions to the otherwise acceptable packing were three hypervariable loops protruding from the variable domains of Fab McPC603. When the correct sequence for the canine lymphoma antibody was examined, it was apparent that the offending residues corresponded to deletions in the latter molecule. Thus, when the correct amino-acid sequence was substituted, virtually all of the packing exceptions were eliminated, as shown in this view.



two Fabs are almost collinear; thus, there is an approximate long axis running through the entire Fab assembly. The angle between the Fabs is $170 \pm 2^\circ$, and the Fab axes are offset by 9° ; (5) Fab1 has an elbow angle of 159° , and Fab2 has an elbow of 143° . These elbow angles are near the middle of the range of values observed for other Fabs¹; (6) the constant domains of Fab1 and Fab2 are related by a near exact dyad axis of symmetry. The variable domains are not so related because of the difference in elbow angles of the Fabs; (7) the dyad of the Fc is at an angle of about 120° with that dyad relating constant Fab domains; (8) the Fc 2-fold axis does not intersect the dyad relating the constant domains of the Fabs, nor does it intersect the approximate long axis of the Fabs; (9) in the crystal, all segments share extensive interfaces which severely restrict the dispositions of neighbours. The contacts, illustrated in Fig. 3, presumably stabilize this particular conformation.

The asymmetric conformation, observed in these crystals of the antitumour antibody, should probably not be considered as a static structure which is maintained in solution. The structure probably represents only one of many possible transient conformations. The unique structure is a product of the intrinsic flexibility of the antibody and the lattice interactions that stabilize this particular distribution of domains. Indeed, electron microscopy²⁰⁻²³, fluorescence polarization^{24,25} and previous X-ray crystallographic studies^{5,26,27} have provided extensive evidence for a wide range of conformations based on segmental flexibility.

The structure we present is instructive in that it illustrates the nature and extent of this structural variability, or dynamic range, which is inherent in the antibody. The Fabs are loosely tethered to a mobile Fc. Each Fab can assume its own elbow angle as its environment or function requires. Somewhat unexpected is the fact that, were the elbow angles the same, the Fabs would be related by an almost exact 2-fold axis that is quite independent of the Fc. It is the disposition of the Fc that disrupts the overall symmetry of the molecule. This is in keeping with the disorder, or multiple orientations of the Fc, observed in the Kol antibody structure^{7,8}.

The hinge polypeptides are not really hinges, but rather they are tethers that allow the Fab components to drift from the Fc to bind antigen or potentially allow the Fc to move in such a way to trigger effector functions, such as the activation of complement^{25,28}. The connecting polypeptides give the Fabs the freedom to move and twist so as to align hypervariable regions with antigenic sites on large, immobile carriers, in this case tumour cells. The crystal structure visually demonstrates that the antibody is an assembly of units possessing a high degree of flexibility, a molecule suited to the task of scavenging foreign objects or activating a cell lysis system. □

Received 3 August; accepted 9 October 1992.

- Wilson, I. A., Rini, J. M., Fremont, D. H., Fieser, G. G. & Stura, E. A. *Meth. Enzym.* **203**, 153-176 (1991).
- Alzari, P. M., Lascombe, M.-B. & Poljak, R. J. *A. Rev. Immun.* **6**, 555-580 (1988).
- Davies, D. R., Padlan, E. A. & Sheriff, S. A. *Rev. Biochem.* **59**, 439-473 (1990).
- Deisenhofer, J. *Biochemistry* **20**, 2361-2370 (1981).
- Silverton, E. W., Navia, M. A. & Davies, D. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5140-5144 (1977).
- Rajan, S. S. *et al. Mol. Immun.* **20**, 797-799 (1983).
- Colman, P. M., Deisenhofer, J., Huber, R. & Palm, W. *J. molec. Biol.* **100**, 257-282 (1976).
- Marquart, M., Deisenhofer, J., Huber, R. & Palm, W. *J. molec. Biol.* **141**, 369-391 (1980).
- Steplewski, Z., Jeglum, K. A., Rosales, C. & Weintraub, N. *Cancer Immun. Immunother.* **24**, 197-201 (1987).
- Rosales, C., Jeglum, K. A., Obrocka, M. & Steplewski, Z. *Cell. Immun.* **115**, 420-428 (1988).
- Jeglum, K. A. *Proc. Seventh ACVIM Forum, San Diego* (922-925) (Lippincott, Hagerstown, Maryland, 1989).
- Larson, S., Day, J., Greenwood, A., Skaltsky, E. & McPherson, A. *J. molec. Biol.* **222**, 17-19 (1991).
- Fitzgerald, P. M. D. *J. appl. Crystallogr.* **21**, 273-276 (1988).
- Brunker, A. T., Kuriyan, J. & Karplus, M. *Science* **235**, 458-460 (1987).
- Brunker, A. T. *A. Rev. phys. Chem.* **42**, 197-223 (1991).
- Bernstein, F. C. *et al. J. molec. Biol.* **112**, 535-542 (1977).
- Crowther, R. A. in *The Molecular Replacement Method* (ed. Rossman, M. G.) 173-178 (Gordon and Breach, New York, 1972).
- Sheriff, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **84**, 8075-8079 (1987).
- Satow, Y., Cohen, G. H., Padlan, E. A. & Davies, D. R. *J. molec. Biol.* **190**, 593-604 (1986).
- Wrigley, N. G., Brown, E. B. & Skehel, J. J. *J. molec. Biol.* **169**, 771-774 (1983).
- Roux, K. H. *Eur. J. Immun.* **14**, 459-464 (1984).
- Lamy, J. *et al. Biochemistry* **24**, 5532-5542 (1985).

- Wade, R. H., Taveau, J. C. & Lamy, J. N. *J. molec. Biol.* **206**, 349-356 (1989).
- Schneider, W. P., Wensel, T. G., Stryer, L. & Oi, V. T. *Proc. natn. Acad. Sci. U.S.A.* **85**, 2509-2513 (1988).
- Dangl, J. L. *et al. EMBO J.* **7**, 1989-1994 (1988).
- Huber, R., Deisenhofer, J., Colman, P. M. & Matsushima, M. *Nature* **264**, 415-420 (1976).
- Amzel, L. M. & Poljak, R. J. *A. Rev. Biochem.* **48**, 961-997 (1979).
- Oi, V. T. *et al. Nature* **307**, 136-140 (1984).
- Jones, T. A. in *Computational Crystallography* (ed. Sayre, D.) 307-317 (Oxford University Press, New York, 1982).

ACKNOWLEDGEMENTS. This research was supported by grants from the NIH, from the NSF and from NASA, as well as by the Immunopharmaceuticals Company of San Diego. We thank the San Diego Supercomputer Facility for time on the Cray Y-MP.

RETRACTION

Identification by anti-idiotypic antibodies of an intracellular membrane protein that recognizes a mammalian endoplasmic reticulum retention signal

D. Vaux, J. Tooze & S. Fuller

Nature **345**, 495-502 (1990)

OUR further characterization of the M_r 72,000 (72K) protein has shown that the data in Fig. 2 of our paper are erroneous and not repeatable. We retract the statement that the 72K protein is an integral membrane protein. The present evidence is consistent with this protein being associated with the intermediate compartment. We also withdraw our speculation concerning its function. □

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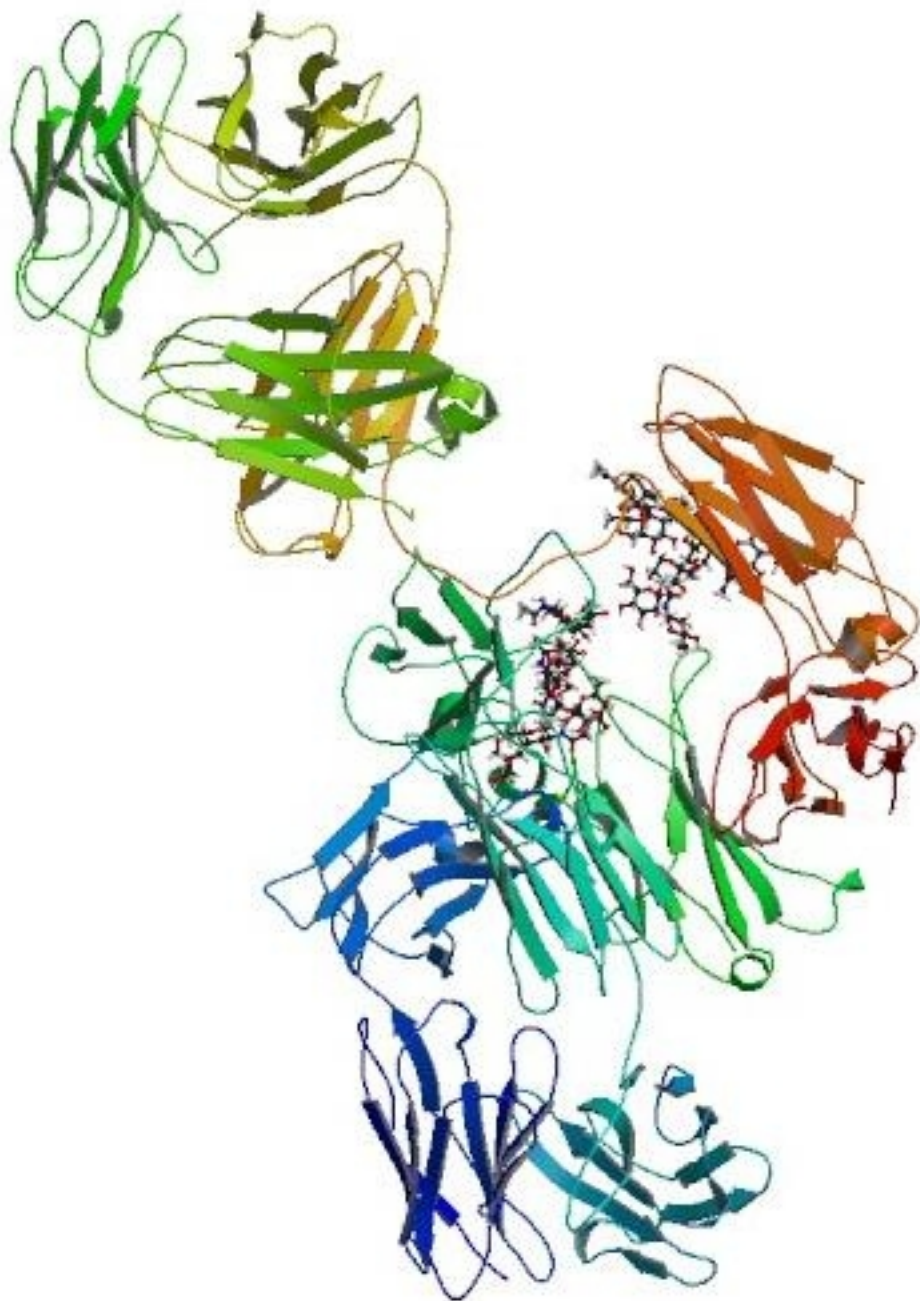
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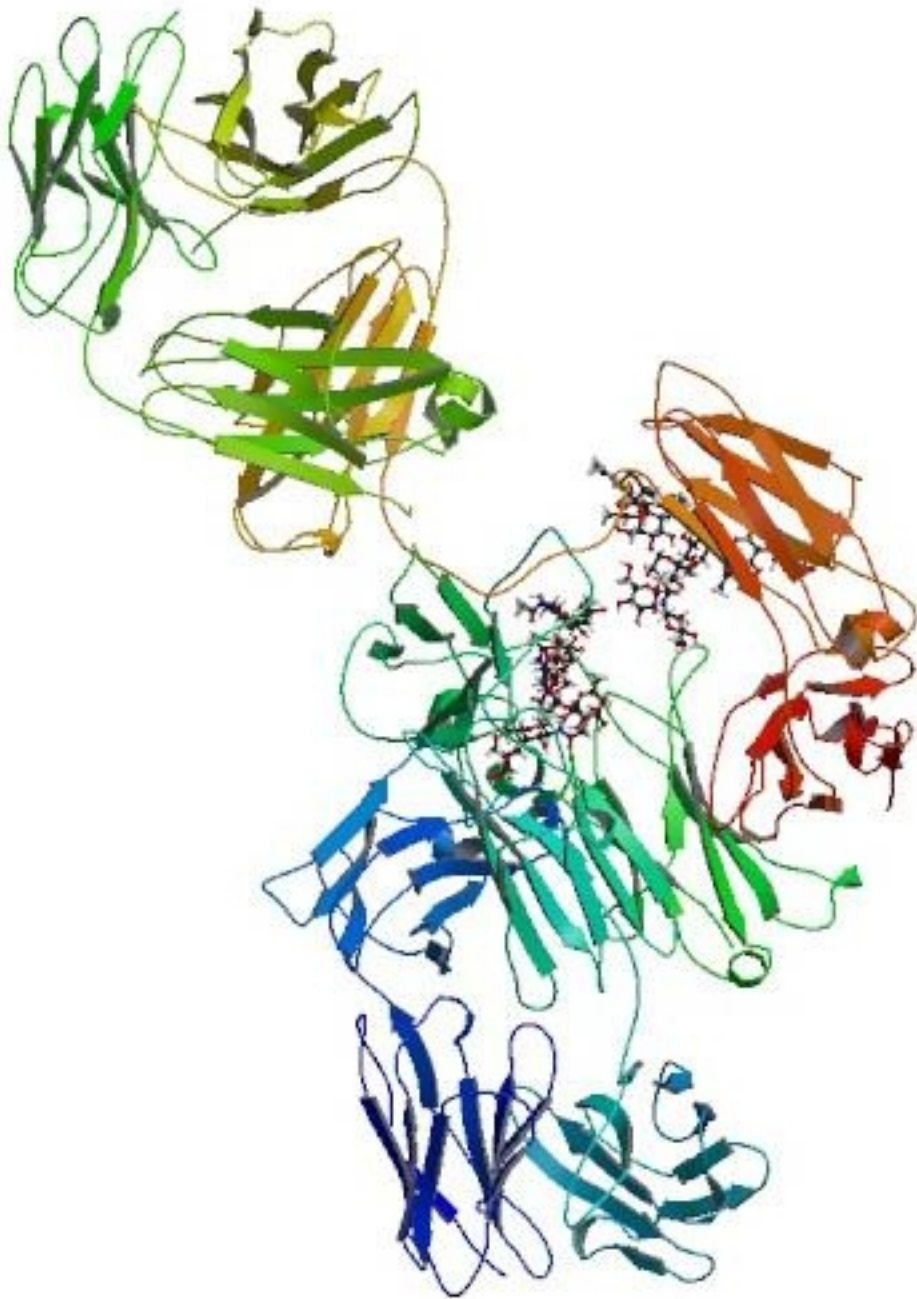
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Biological Assembly 1 of 1IGT - STRUCTURE OF IMMUNOGLOBULIN



Asymmetric Unit of 1IGT - STRUCTURE OF IMMUNOGLOBULIN