hydration pattern around the ice-binding side chains (Fig. 2*d*) shows no specific pattern but instead forms an elongated surface around the polar groups that is limited only by accessibility. This is consistent with the variation from ideal geometry observed for the hydrogen-bonding of water to the side chains of other proteins[13]. It is conceivable that less than perfect hydrogen-bonding geometry is employed in the AFP's selection of a target site on ice.

Current ice-binding models[14,15] position binding groups to extend from the AFP's structure to occupy ice-lattice sites. In support of this, molecular modelling has identified a spatial match between the binding groups of the AFP and water molecules on the ice planes to which the AFP binds[14,15]. This spacial match is also consistent with the AFP's $\langle 01\bar{1}2 \rangle$ axial alignment on the binding planes[1], although several features of our crystal structure appear to contradict this view. First, a sufficiently close spatial match between the AFP and ice is not apparent: whereas the average separation between equivalent ice-binding groups in adjacent IBMs closely matches the 16.7 Å $\langle 01\bar{1}2 \rangle$ axial repeat distance of water molecules on the binding plane, the closest match on ice found to fit the spacing of binding groups within an IBM is 4.5 Å. Second, the ice-binding groups do not protrude sufficiently from the AFP's ice-binding surface to clear sterically hindering groups; the Cγ atoms of the threonine residues and the Cβ atoms of alanines 9, 20 and 31 would prevent binding groups from entering into ice even if a congruent lattice match were available. Third, the trigonal planer (SP2) coordination of asparagine and aspartate hydrogen-bonding groups differ from the tetrahedral (SP3) coordination of water molecules in ice.

In contrast, we envisage that the binding of AFP to ice is defined by a less stringent hydrogen-bonding criterion. This forms the basis of an ice-binding model (Fig. 3). A characteristic feature of the $\{20\bar{2}1\}$ binding plane is a ridge-and-valley topology[15–17] which subtends the $\langle 01\bar{1}2 \rangle$ binding axis by 66°. This topology complements the AFP structure when the AFP is aligned along the binding axis. Considering that the hydrogen-bonding groups extend minimally from the AFP's flat ice-binding surface, water molecules on the ridges of the $\{20\bar{2}1\}$ ice plane are the most probable binding sites. The 4.5 Å spacing of water molecules along the ridges provides accessible hydrogen-bonding targets for both ice-binding groups within an IBM.

In conclusion, we believe that the relative flatness of the AFP's ice-binding surface and the rigidity of the side chains are critical to the AFP's ice-binding mechanism. The latter maintains the AFP in its ice-binding conformation while the former maximizes the accessibility of binding groups to an ice surface. Together these characteristics allow for the concerted formation of many hydrogen bonds between the AFP and ice, giving permanence to binding. Whereas the spatial arrangement of ice-binding residues contributes to the binding ability and specificity of the AFP, the underlying hydrogen-bonding interactions are likely to be more liberally defined than previously proposed[14,15]. The finding that all of the AFP's structure appears to be dedicated to ice binding and helix stabilization strongly suggests that ice binding is the only essential characteristic required for the AFP to inhibit ice crystal growth. Our ice-binding model is amenable to testing and we are currently designing HPLC6 mutants for this purpose. A flat and rigid ice-binding surface, albeit with different spacial arrangements of ice-binding groups, may be a general feature of all AFPs. □

Received 16 February; accepted 6 April 1995.

1. Knight, C. A., Cheng, C. C. & DeVries, A. L. *Biophys. J.* **59,** 409–418 (1991).
2. Chakrabartty, A. & Hew, C. L. *Eur. J. Biochem.* **202,** 1057–1063 (1991).
3. Wen, D. & Laursen, R. A. *J. biol. Chem.* **267,** 14102–14108 (1992).
4. Jorgensen, H. *et al. Protein Engng* **6,** 19–27 (1993).
5. Ananthanarayanan, V. A. & Hew, C. L. *Biochem. biophys. Res. Commun.* **74,** 685–689 (1977).
6. Chakrabartty, A., Ananthanarayanan, V. A. & Hew, C. L. *J. biol. Chem.* **264,** 11307–11312 (1989).
7. Richardson, J. S. & Richardson, D. C. *Science* **240,** 1648–1652 (1988).
8. Presta, L. D. & Rose, G. D. *Science* **240,** 1632–1641 (1988).
9. Baker, E. N. & Hubbard, R. E. *Progr. Biophys. molec. Biol.* **44,** 97–179 (1984).
10. Hew, C. L. *et al. Eur. J. Biochem.* **160,** 267–272 (1986).
11. Piela, L., Nemethy, G. & Scheraga, H. A. *Biopolymers* **26,** 1273–1286 (1987).
12. McGregor, M. J., Islam, S. A. & Sternberg, M. J. E. *J. molec. Biol.* **198,** 295–310 (1987).
13. Thanki, N., Thornton, J. M. & Goodfellow, J. M. *J. molec. Biol.* **202,** 637–657 (1988).
14. Knight, C. A., Driggers, E. & DeVries, A. L. *Biophys. J.* **64,** 252–259 (1993).
15. Wen, D. & Laursen, R. A. *Biophys. J.* **63,** 1659–1662 (1992).
16. Lal, M., Clark, A. H., Lips, A., Ruddock, J. N. & White, D. N. J. *Faraday Discuss.* **95,** 299–306 (1993).
17. Madura, J. D. *et al. J. Am. chem. Soc.* **116,** 417–418 (1994).
18. Yang, D. S. C., Chung, Y. J., Chen, P., Rose, J. P. & Hew, C. L. *J. molec. Biol.* **189,** 725 (1986).
19. Brunger, T. A., Kuriyan, J. & Karplus, M. *Science* **235,** 458–460 (1987).
20. Fitzgerald, P. M. D. *J. appl. Crystallogr.* **21,** 273–278 (1988).
21. Yang, D. S. C., Sax, M., Chakrabartty, A. & Hew, C. L. *Nature* **333,** 232–237 (1988).
22. Hew, C. L., Chakrabartty, A. & Yang, D. S. C. *Integration and Control of Metabolic Processes: Pure and Applied Aspects* (eds Kon, O. L. *et al.*) 299–309 (ISCU, Cambridge, 1987).
23. Jones, T. A., Zou, J. Y., Cowan, S. W. & Kjeldgaard, M. *Acta crystallogr.* A**47,** 110–119 (1991).

ACKNOWLEDGEMENTS. We acknowledge the Canadian Space Agency for funding and the National Cancer Institute for allocation of computing time and staff support at the Biomedical Supercomputing Center of the Frederick Cancer Research and Development Center. We thank C. Hew and G. Fletcher for partially purified protein, and K. Miyata and S. Hemming for critically reviewing the manuscript.

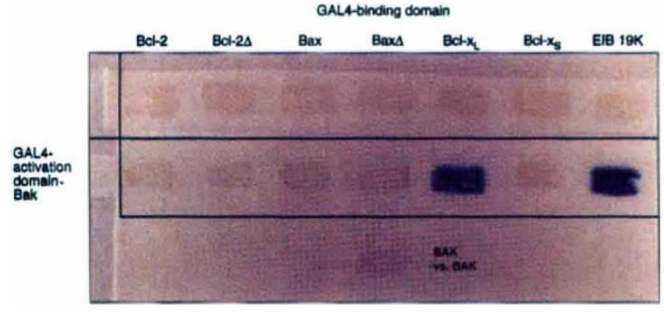
ERRATA

Cloning of a *bcl-2* homologue by interaction with adenovirus E1B 19K

Stuart N. Farrow, Julia H. M. White, Isabelle Martinou, Thomas Raven, Kwok-Tao Pun, Christine J. Grinham, Jean-Claude Martinou & Robin Brown

Nature **374,** 731–733 (1995)

FIGURE 4 of this Letter should have been reproduced in colour, as shown here. The last sentence of the caption is accordingly amended to read "A blue colour indicates interaction". □



Silica aerogel films prepared at ambient pressure by using surface derivatization to induce reversible drying shrinkage

Sai S. Prakash, C. Jeffrey Brinker, Alan J. Hurd & Sudeep M. Rao

Nature **374,** 439–443 (1995)

PARTS *c* and *d* of Fig. 4*A* of this Letter were incorrectly labelled: part *c* should have been labelled as *d* and part *d* as *c*. □