14-3-3 proteins bind a variety of molecules involved in signal transduction, cell cycle regulation and apoptosis. 14-3-3 binds ligands such as Raf-1 kinase and Bad by recognizing the phosphorylated consensus motif, RSX-pSXP, but must bind unphosphorylated ligands, such as glycoprotein Ib and *Pseudomonas aeruginosa* exoenzyme S, via a different motif. Here we report the crystal structures of the ξ isoform of 14-3-3 in complex with two peptide ligands: a Raf-derived phosphopeptide (pS-Raf-259, LSQRQRSTpSTPNVHM) and an unphosphorylated peptide derived from phage display (R18, PH-CVPRLDSTRPLEANMCLP) that inhibits binding of exoenzyme S and Raf-1. The two peptides bind within a conserved amphipathic groove on the surface of 14-3-3 at overlapping but distinct sites. The phosphoserine of pS-Raf-259 engages a cluster of basic residues (Lys49, Arg56, Arg60, and Arg127), whereas R18 binds via the amphipathic groove, with its two acidic groups coordinating the same basic cluster. 14-3-3 is dimeric, and its two peptide-binding grooves are arranged in an antiparallel fashion, 30 Å apart. The ability of each groove to bind different peptide motifs suggests how 14-3-3 can act in signal transduction by inducing either homodimer or heterodimer formation in its target proteins.

14-3-3 proteins are a family of highly conserved dimeric molecules associated with numerous biological activities in eukaryotes (reviewed in Ref. 1). 14-3-3 binds a variety of regulatory proteins, including receptors, such as insulin-like growth factor I receptor (2); kinases, such as Raf (3–7), Bad (10) and Cdc25 (11). A phosphopeptide comprising residues 251–265 of Raf-1, named pS-Raf-259 (LSQRQRSTpSTPNVHM), binds several isoforms of 14-3-3 (estimated $K_d \approx 120$ nm for 14-3-3ξ, inhibits the association of 14-3-3 with Raf-1 in vitro and, when injected into *Xenopus laevis* oocytes, inhibits Raf-dependent maturation (14).

Phosphoserine recognition cannot account for all 14-3-3-ligand interactions, however. A different recognition motif must exist for unphosphorylated ExoS, as well as for glycoprotein Ib, which when expressed in unphosphorylated form binds 14-3-3 (16). Recently, Wang *et al.* screened random peptide phage display libraries,2 and isolated a series of unphosphorylated peptides containing a consensus sequence, WLDL, which bind 14-3-3 with high affinity. One of these, termed “R18” (PH-CVPRLDSTRPLEANMCLP), binds 14-3-3 with a $K_d \approx 80$ nm, inhibits 14-3-3-Raf-1 complex formation, reduces Raf-1-dependent transcriptional activity in cells, and blocks the activation of ExoS by 14-3-3. Like pS-Raf-259, the R18 peptide binds to different 14-3-3 isoforms with equal affinities.

In 1995, we reported the crystal structure of the ξ-isoform of 14-3-3 (17), and Xiao *et al.* reported the structure of the highly homologous ζ-isoform (18). The molecule consists of a bundle of nine α-helices organized in an antiparallel fashion, with the four N-terminal α-helices participating in dimer formation. The dimeric molecule has a cup-like shape with a conserved inner surface and a variable outer surface. A striking feature of the inner surface is a groove roughly 25 Å long formed by the four parallel helices α3, α5, α7, and α9. This groove is amphipathic, with a cluster of basic and polar residues (from α3 and α5) on one side and a cluster of hydrophobic residues (from α7 and α9) on the other. Based on the crystal structure, we predicted that this amphipathic groove would form the principal binding site for 14-3-3 ligands, with the cluster of basic residues engaging the phosphoserine (17). We have since shown that mutations affecting either the basic or hydrophobic face of the groove do indeed disrupt the ability of 14-3-3 to bind ligands (19, 33).

To demonstrate directly the phosphoserine recognition site on 14-3-3 and to determine the binding site for the unphosphorylated R18 peptide, we have solved crystal structures of 14-3-ζ in complex with the pS-Raf-259 and R18 peptides at a resolution of $\sim 3.5$ Å. At this relatively low resolution, the general course of the peptide backbone within the amphipathic groove is clear, as is the location of the phosphoserine phos-
phate group. However, the side chain density is ambiguous for many peptide side chains, such that we cannot clearly distinguish between the two possible directions of the peptide chain through the electron density. Our ability to describe detailed protein-peptide interactions is consequently limited. Nevertheless, we show unambiguously that both peptides bind in the amphipathic groove at overlapping but distinct sites, with significant implications for the mechanism of signal transduction by the 14-3-3 family.

EXPERIMENTAL PROCEDURES

Protein Purification and Peptide Synthesis—Recombinant 14-3-3ζ protein was expressed in Escherichia coli strain BL21 (DE3) as a hexahistidine-tagged product and isolated on a Ni²⁺-charged imidodic acid-Sepharose column (Hi-Trap Chelating; Amersham Pharmacia Biotech) as described (11, 19). After removing the tag, 14-3-3ζ was further purified on a Mono-Q column (Amersham Pharmacia Biotech) using a gradient of NaCl. The final product was estimated to be ≥99% pure by SDS-polyacrylamide gel electrophoresis and silver staining.

The pS-Raf-259 and R18 peptides were assembled by solid phase peptide synthesis as described (20). The cleaved, deprotected peptides were purified by reverse-phase HPLC on a Zorbax-SBC18 silica column and desorbed using a gradient of acetonitrile in trifluoroacetic acid (0.1%). The purity of the peptides (≥98%) was confirmed by analytical reverse-phase HPLC, and the masses of the peptides were confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The pS-Raf-259 peptide is identical to the peptide used by Muslin et al. (14) except that it contains an additional Val at the C terminus.

Crystallization and Diffraction Data Collection—Initial attempts to crystallize the 14-3-3ζ-pS-Raf-259 and 14-3-3ζ-R18 complexes were made by incubating 14-3-3ζ with the relevant peptide overnight before setting up crystallization trials. Crystals of both complexes were readily grown under conditions similar to the native protein (17). Unfortunately, these crystals did not diffract beyond 4 Å spacing, and the resolution limit could not be improved by varying the crystallization conditions or the protein/peptide ratio nor by using synchrotron radiation. The co-crystals were isomorphous with the native crystals, indicating that the peptides were compatible with the native crystal lattice. Native 14-3-3ζ crystals were therefore soaked in solutions containing 1.5 mM pS-Raf-259 or R18 (a concentration ~10000 times the estimated K_d) for 9 or 13 days. Soaking with pS-Raf-259 induced a 6% increase in the c axis (a = b = 94.7 Å, c = 250.9 Å), whereas soaking with R18 (a = b = 95.5 Å, c = 235.4 Å) had little effect on cell dimensions. Diffraction data were collected from pS-Raf-259-soaked crystals using synchrotronradiation at Daresbury beam line 7-2 (λ = 1.488 Å) with a MarResearch image plate. Diffraction was somewhat anisotropic, varying between 3.3 and 3.6 Å resolution. The R18 data set was collected on an Raxis IV image plate system mounted on a Rigaku RU200HB rotating anode generator with a copper anode. These crystals diffracted to 2.3 Å resolution.

The data were processed using DENOVO and SCALEPACK (21). The data statistics are summarized in Table I.

Structure Solution and Refinement of the 14-3-3ζ-Peptide Complexes—Because the unit cell dimensions differed significantly from those of native crystals, the 14-3-3ζ-pS-Raf-259 structure was solved by molecular replacement. Using the two dimers in the asymmetric unit of the native 14-3-3ζ crystal as a search model, a cross-rotation function was calculated with 10–3.6 Å data using AMoRE (22) from the CCP4 program suite (23). This gave an unambiguous solution at 10.9 Å above the mean with the highest noise peak at 3.1 σ. A translation search gave a clear solution at 9.2 Å with the highest noise peak at 5.7 σ. Rigid body refinement of the search model as a single rigid body caused a drop in the crystallographic R-factor from 0.48 to 0.44 and an increase in correlation coefficient from 0.44 to 0.53 (10–3.6 Å), thereby confirming the solution to be correct. The model was refined in XPLOR (24) using all reflections from 20 to 3.6 Å with no sigma cut-off. Progress in refinement was monitored by following the free R-factor, calculated on eight thin resolution shells comprising ~1450 reflections (10% of unique data) omitted from all steps of refinement. Because of the limited resolution of the data, a conservative strategy was maintained throughout the course of refinement. The four molecules in the asymmetric unit were first refined as rigid bodies to better define the non-crystallographic symmetry operators. All subsequent refinement steps were carried out using strict noncrystallographic symmetry constraints. Bulk solvent correction, torsional angle dynamics, and grouped B factor refinement caused the R_factor to drop from 0.51 to 0.40, yielding a model

| Resolution shell | R_ref | Unique | Completeness | Redundancy | I/σ | Refinement statistics |
|------------------|-------|--------|--------------|------------|-----|----------------------|
| 20.0–3.6 Å       | 25.8  | 2108   | 99.3         | 3.7        | 7.1 | rms bond lengths, 0.02 Å |
|                  | 99.8  |        |              |            |     | rms angles, 2.9 σ     |

### Table I

**Summary of crystallographic statistics**

| Resolution shell | R_ref | Unique | Completeness | Redundancy | I/σ | Refinement statistics |
|------------------|-------|--------|--------------|------------|-----|----------------------|
| 20.0–3.6 Å       | 25.8  | 2108   | 99.3         | 3.7        | 7.1 | rms bond lengths, 0.02 Å |
|                  | 99.8  |        |              |            |     | rms angles, 2.9 σ     |

### Table II

**Residues invariant across 84 sequences and 30 species**

| Residues exposed in the amphipathic groove are underlined. | α2 | Met22 | Met26 | Leu36 | Leu90 | Ala188 | Phe196 |
|-----------------------------------------------------------|----|-------|-------|-------|-------|--------|-------|
| Residues exposed in the amphipathic groove are underlined. | α3 | Gly28 | Val116 | Gly123 | Gly128 | Val116 | Gly123 |

| Residues exposed in the amphipathic groove are underlined. | α4 | Tyr82 | Tyr82 | Tyr149 | Tyr149 | Tyr82 | Tyr82 |
|-----------------------------------------------------------|----|-------|-------|--------|--------|-------|-------|
| Residues exposed in the amphipathic groove are underlined. | α6 | Arg127 | Arg127 | Arg127 | Arg127 | Arg127 | Arg127 |

| Residues exposed in the amphipathic groove are underlined. | α8 | Ala188 | Ala188 | Ala188 | Ala188 | Ala188 | Ala188 |
|-----------------------------------------------------------|----|--------|--------|--------|--------|--------|--------|
| Residues exposed in the amphipathic groove are underlined. | Leu36 | Leu36 | Leu36 | Leu36 | Leu36 | Leu36 | Leu36 |

| Residues exposed in the amphipathic groove are underlined. | Leu36 | Gly35 | Gly35 | Gly35 | Gly35 | Gly35 | Gly35 |
|-----------------------------------------------------------|-------|-------|-------|-------|-------|-------|-------|
| Residues exposed in the amphipathic groove are underlined. | Tyr211 | Tyr211 | Tyr211 | Tyr211 | Tyr211 | Tyr211 | Tyr211 |

| Residues exposed in the amphipathic groove are underlined. | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 |
|-----------------------------------------------------------|-------|-------|-------|-------|-------|-------|-------|
| Residues exposed in the amphipathic groove are underlined. | Tyr211 | Tyr211 | Tyr211 | Tyr211 | Tyr211 | Tyr211 | Tyr211 |

| Residues exposed in the amphipathic groove are underlined. | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 |
|-----------------------------------------------------------|-------|-------|-------|-------|-------|-------|-------|
| Residues exposed in the amphipathic groove are underlined. | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 |

| Residues exposed in the amphipathic groove are underlined. | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 |
|-----------------------------------------------------------|-------|-------|-------|-------|-------|-------|-------|
| Residues exposed in the amphipathic groove are underlined. | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 | Thr226 |
with an $R_{\text{cryst}}$ of 0.35. Phases calculated from the model were improved by noncrystallographic symmetry averaging, solvent flattening, and histogram matching using the CCP4 program DM (25). Inspection of $2F_o - F_c$ and $F_o - F_c$ maps calculated with the improved phases revealed an extra turn at the N terminus of helix $\alpha 9$ and showed that rebuilding was required in the $\alpha 6-\alpha 7$ and $\alpha 7-\alpha 8$ loops. These modifications were made, and further cycles of XPLOR refinement, DM phase improvement, and manual model rebuilding were carried out. In the last cycles the density in the groove was modeled as an (Ala)$_2$-phosphoSer-(Ala)$_3$ peptide, allowing for a more accurate molecular mask for density modification techniques. The final model has $R_{\text{cryst}}$ and $R_{\text{free}}$ of 0.31 and 0.36, respectively (20–3.6 Å), reasonable B factors for all residues, and no residues in disallowed regions of the Ramachandran plot. Similar density is observed in maps calculated from two other crystals soaked in peptide (for either 12 h or 3 days) and for a third crystal obtained by cocrystallizing 14-3-3 with pS-Raf-259 peptide (data not shown).

A similar strategy was employed for the 14-3-3-R18 complex. Following molecular replacement and rigid body fitting, XPLOR refinement was carried out using data from 20–3.35 Å resolution with no sigma cut-off. In the final cycles, the density in the groove was modeled as a WLDLE peptide, with the N-terminal Trp at the bottom of the groove (see Fig. 2B). There is sufficient side chain density to account for the bulky Trp side chain, and its position is stereochemically reasonable. We cannot completely rule out the tracing DLEAN (with the N-terminal Asp at top) on the basis of stereochemistry, but the small Ala side chain is less consistent with the electron density than the corresponding Leu. The final model has $R_{\text{cryst}}$ and $R_{\text{free}}$ of 0.30 and 0.35, respectively (20–3.35 Å), reasonable B factors for all residues, and no residues in disallowed regions of the Ramachandran plot.

**Sequence Homologs**—The sequence data bank maintained at the National Center for Biotechnology Information (Swissprot + PIR + GenBank$^\text{TM}$ data bases) at the United States National Library of Medicine was searched for homologs of 14-3-3$z$. This yielded a set of 98
sequences, including 11 redundant and 3 partial sequences. The 84 sequences that were both unique and complete belonged to 30 different species, including 6 vertebrates (human, mouse, rat, sheep, bovine, and X. laevis), 3 invertebrates (Drosophila melanogaster, Caenorhabditis elegans, and Schistosoma mansoni), 14 plants (barley, fava bean, maize, pea, potato, rice, soybean, tomato, pumpkin, Arabidopsis thaliana, Oenothera hookeri, Mesembryanthemum crystallinum, and Chlamydomonas reinhardtii), 2 yeast (Saccharomyces cerevisiae and Schizosaccharomyces pombe), and 5 others (Trichoderma harzianum, Entamoeba histolytica, Dictyostelium discoideum, Fucus vesiculosus, and Neospora caninum). Sequences were aligned using ClustalW (26).

We found that 46 residues are strictly invariant. 35 of these are clustered in the four helices ($\alpha_3$, $\alpha_5$, $\alpha_7$, and $\alpha_9$) that define the amphipathic groove (Table II), with 21 of these being solvent-accessible and thus available in principle to bind ligand (Table II and Fig. 3B). Three of the four basic residues next to the pS-Raf-259 phosphate (Lys49, Arg60, and Arg127) are invariant, and the fourth (Arg56) is an Arg in 83 of the 84 sequences. By contrast, of the 21 residues in the dimer interface, only Tyr82 is invariant.

RESULTS AND DISCUSSION

pS-Raf-259 Peptide Binds to the Amphipathic Groove of 14-3-3—Electron density maps calculated from crystals of 14-3-3 soaked in the pS-Raf-259 peptide reveal a tube of strong density roughly 20 Å long (Table I and Figs. 1A and 2A). The electron density is consistent with 6 amino acid residues in an extended conformation running along the amphipathic groove. The strongest feature of the difference maps is a large protrusion from the middle of the peptide density (Figs. 1A and 2A). The center of this feature is within 5 Å of the cluster of basic residues, Lys$^{49}$, Arg$^{56}$, Arg$^{60}$, and Arg$^{127}$, two of which (Lys$^{49}$ and Arg$^{56}$) have been implicated in binding Raf-1 kinase by mutagenesis (19). We therefore interpret this density as the phosphoserine phosphate group. Although the course of the pS-Raf-259 peptide backbone is clear, we were unable to choose between two possible peptide orientations, because of the pseudosymmetry of the bound sequence: RSTpSTPN. Nevertheless, the Thr side chains on either side of the phosphoserine clearly point toward the hydrophobic side of the groove, with one next to Leu$^{220}$ and the other next to Asn$^{224}$ of helix $\alpha_9$, whereas the peptide residue 2 positions above the phosphoserine is next to Trp$^{228}$, Val$^{176}$, and Glu$^{180}$.

Following submission of this work, the crystal structure of 14-3-3 in complex with a phosphopeptide derived from polyoma middle T antigen was reported at 2.6 Å resolution (27). The sequence of the middle T peptide, RSHpSYPA, is similar to that of pS-Raf-259 (RSTpSTPN). A comparison of results indicates that the phosphoserine phosphate groups of the middle T and pS-Raf-259 peptides are identically located and that the two peptide backbones follow essentially the same course. Assuming the same orientation as that of the middle T peptide, the pS-Raf-259 peptide would have its N terminus at the top of Figs. 2 and 3, with Ser$^-2$ next to Trp$^{228}$, Thr$^{-1}$ next to Asn$^{224}$, and Thr$^1$ next to Leu$^{220}$ (peptide residues are numbered relative to the phosphoserine).

Decrease in Raf Binding Activity of Asp or Glu Substitution Mutants Varies with Distance to the Peptide Phosphate
Arg60 is further from the phosphate group (binding site). Sequence conservation of the peptide-in the groove are labeled except Gly53 and amphipathic groove. All residues exposed 14-3-3 groove. Ala59 corresponds to residue Ser57 in 14-3-3 chain contacts with the pS-Raf-259 peptide at the top of the pS-Raf-259 peptide backbone with its phosphoserine side chain is shown in yellow. Asp or Glu substitutions leading to reduced Raf binding6 (19) are marked with * (strong effect) or with ± (weak effect). B, the concave inner surface of 14-3-3 with residues invariant across 30 eukaryotic species in red (see also Table II). The R18 peptide is shown in green. None of the residues solvent-exposed on the rear, convex surface are invariant (not shown). C, close-up view of residues from helices a3, a5, a7, and a9 forming the amphipathic groove. All residues exposed in the groove are labeled except Gly55 and Gly69. The viewing orientation and coloring scheme are as in A. Residues boxed in solid or dashed lines correspond to those marked in A by * or ±, respectively. D, schematic of a 14-3-3 dimer with helices as cylinders showing bound Raf peptides with their phosphoserine side chains. The two peptides are oriented in an antiparallel fashion. The view is rotated by 90° around a horizontal axis compared with A–C, so that the dyad axis lies vertically in the plane of the page. The figure was produced with Molscript (32) and Raster3D (31).

**Group**—Substitution of residues in the groove by Asp or Glu yields 14-3-3z mutants whose ability to bind Raf-1 kinase is compromised to varying degrees (19). The loss in Raf binding activity follows the order: V176D > K49E > R56E > L172D > L220D > R60E > L216D > L227D. Inspection of our crystal structure shows that the reduction in Raf binding correlates well with the distance of the residues from the peptide phosphate group (Fig. 3, A and C). Val176 is closest to the phosphate, whereas Lys49 and Arg56 are only ~5 Å away. Slightly further still are Leu172 and Leu220, but they lie next to the phosphoserine a carbon, and steric effects may also play a role here. Arg60 is further from the phosphate group (~8 Å) and would be shielded by Arg56, explaining why R60E behaves more like wild type. The small change in activity of L216D and L227D is consistent with an Asp at these positions being too far (~12 Å) from the phosphate to exert any significant repulsion.

Genetic analysis in S. cerevisiae identified three mutants (G55D, A59T, and L232S) in the 14-3-3 protein BMH1 that are defective in Ras-mitogen-activated protein kinase signaling during pseudohyphal development (28). These mutations all lie within, or near to, the amphipathic groove, suggesting that they exert their effect by disrupting ligand binding. Gly55 is analogous to Gly53 in 14-3-3z, and the Asp mutant would have its carboxylate group ~6 Å from the peptide phosphate group. Leu232 corresponds to Leu227 in 14-3-3z, which makes side chain contacts with the pS-Raf-259 peptide at the top of the groove. Ala59 corresponds to residue Ser57 in 14-3-3z, it lies at one edge of the groove, although it does not contact any peptide residues in our crystal structure.

The R18-binding Site Overlaps with that of pS-Raf-259 in the Amphipathic Groove—A tube of continuous electron density is readily apparent in a difference Fourier map calculated for 14-3-3z crystals soaked in a solution of the R18 peptide (Fig. 1B). This density, which we ascribe to the R18 peptide, is located in the amphipathic groove and accounts for 5 residues of peptide in an extended conformation. The data for the R18 complex are of higher resolution than for the Raf peptide complex, and we have tentatively traced the density as the pentapeptide WLDLE, which corresponds to the consensus motif found by phage display (Fig. 2B). This tracing places the peptide Asp next to Lys49 and the peptide Glu next to the Arg cluster (Arg56, Arg60, and Arg127), whereas the two Leu residues interact with the hydrophobic side of the groove (Ile166, Gly69, Leu172, Ile217, and Leu220). Thus, R18 appears to bind in an amphipathic conformation to the amphipathic groove. This mode of binding is distinct from that of the Raf peptide, which lacks hydrophobic residues in the sequence flanking the phosphoserine.

**pS-Raf-259 and R18 Peptides Contact Different Sets of Side Chains in the Groove**—The pS-Raf-259 and R18 peptides have unrelated sequences and yet bind 14-3-3 with similar affinities. Each contacts ~20 residues (of a possible 38) in the groove, with the majority of these common to both. The significant overlap between the peptide-binding sites explains why R18
blocks the interaction of 14-3-3 with Raf-1 kinase. The ability of R18 to inhibit the binding of ExoS suggests that ExoS also interacts with residues in the groove. The Raf peptide extends higher in the groove, making unique contacts to Val176 and Glu180 from helix α7, and Leu227 and Trp228 from α9, whereas R18 extends further down the groove, contacting residues from α7 (Pro165 and Ile166) (Figs. 1 and 3, A and B). Below the phosphate-binding site, the Raf peptide lies nearer the hydrophilic face, whereas R18 lies nearer the hydrophobic face.

Thus, although residues in the middle of the groove interact with both peptides, several are uniquely selected by each peptide. The ability to interact via different sets of residues in the groove suggests that 14-3-3 can bind proteins bearing very different sequences. 14-3-3 is dimeric, and the two ligand-binding grooves are located within the same concave surface, about 30 Å apart (Fig. 3D). Our results suggest that a ligand bearing a phosphoserine motif could bind in one groove, with another (perhaps unphosphorylated) ligand bound to the second groove. In this way, 14-3-3 could promote the interaction between proteins that by themselves associate only weakly, as has been found in the case of Raf and Ber (29).

Acknowledgments—We thank Remy Loris for critical reading of the manuscript and the Daresbury Synchrotron Radiation Source for use of its facilities.

REFERENCES

1. Aitken, A. (1996) Trends Cell Biol. 6, 341–347
2. Craparo, A., Freund, R., and Gustafson, T. A. (1997) J. Biol. Chem. 272, 11663–11669
3. Fantl, W. J., Muslin, A. J., Kikuchi, A., Martin, J. A., MacNicol, A. M., Gross, E. W., and Williams, L. T. (1994) Nature 371, 612–614
4. Freed, E., Symons, M., MacDonald, S. G., McCormick, F., and Ruggieri, R. (1994) Science 265, 533–535
5. Fu, H., Xia, K., Pallas, D. C., Cui, C., Conroy, K., Narsimhan, R. P., Mamon, H., Collier, R. J., and Roberts, T. M. (1994) Science 266, 126–129
6. Irie, K., Gotoh, Y., Yashar, B. M., Errede, B., Nishida, E., and Matsumoto, K. (1994) Science 265, 1716–1718
7. Reuther, G. W., Fu, H., Cripe, L. D., Collier, R. J., and Pendergast, A. M. (1994) Science 266, 129–133
8. Bonnefoy-Berard, N., Liu, Y.-C., von Vollenbrand, M., Sung, A., Elly, C., Mustelin, T., Yoshida, H., Ishizaka, K., and Altman, A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10342–10346
9. Conklin, D. S., Galaktionov, K., and Beach, D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7892–7896
10. Zha, J., Harada, H., Yang, E., Jockel, J., and Korsmeyer, S. J. (1996) Cell 87, 619–628
11. Fu, H., Coburn, J., and Collier, R. J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2320–2324
12. Michael, N. R., Fabian, J. R., Mathes, K. D., and Morrison, D. K. (1995) Mol. Cell. Biol. 15, 3390–3397
13. Furukawa, Y., Ikuta, N., Omata, S., Yamauchi, T., Isobe, T., and Ichimura, T. (1993) Biochem. Biophys. Res. Commun. 194, 144–149
14. Muslin, A. J., Tanner, J. W., Allen, P. M., and Shaw, A. S. (1996) Cell 84, 889–897
15. Peng, C. Y., Graves, P. R., Thoma, R. S., Wu, Z., Shaw, A. S., and Piwnica-Worms, H. (1997) Science 277, 1501–1505
16. Du, X., Fox, J. E., and Pei, S. (1996) J. Biol. Chem. 271, 7362–7367
17. Liu, D., Bienkowska, J., Petosa, C., Collier, R. J., Fu, H., and Liddington, R. (1995) Nature 376, 191–194
18. Xiao, B., Smerdon, S. J., Jones, D. H., Dodson, G. G., Stone, Y., Aitken, A., and Gamblin, S. J. (1995) Nature 376, 188–191
19. Zhang, L., Wang, H., Liu, D., Liddington, R., and Fu, H. (1997) J. Biol. Chem. 272, 13717–13724
20. Pereira, H. A., Erdem, I., Pohl, J., and Spitznagel, J. K. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 4733–4737
21. Otwinowski, Z. (1993) in Data Collection and Processing (Sawyer, L., Isaacs, N., and Bailey, S., eds) pp. 56–62, Science and Engineering Research Council Daresbury Laboratory, Warrington, UK
22. Navaza, J. (1994) Acta Crystallogr. Sec. A 50, 157–163
23. Collaborative Computational Project No. 4 (1994) Acta Cryst. Sect. D 50, 760–763
24. Brunger, A. T. (1992) X-PLOR Manual, version 3.1, Yale University, New Haven, CT
25. Cowtan, K. (1994) Joint CCP 4 and ESF-EACBM Newsletter on Protein Crystallography 31, 34–38
26. Higgins, D. G., Thompson, J. D., and Gibson, T. J. (1996) Methods Enzymol. 266, 383–402
27. Yaffe, M., Rittinger, K., Valinia, S., Caron, P. R., Aitken, A., Leffers, H., Gamblin, S., Smerdon, S., and Cantley, L. (1997) Cell 91, 961–971
28. Roberts, R. L., Moach, H. U., and Fink, G. R. (1997) Cell 89, 1055–1065
29. Braselmann, S., and McCormick, F. (1995) EMBO J. 14, 4839–4848
30. Esnouf, R. M. (1997) J. Mol. Graphics 15, 133–138
31. Merrit, E. A., and Murphy, M. E. P. (1994) Acta Crystallogr. Sect. D 50, 869–873
32. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
33. Wang, H., Zhang, L., Liddington, R., and Fu, H. (1998) J. Biol. Chem. 273, 16297–16304