sonication and cleared by centrifugation for 6 h at 40,000*g*. The SH2 protein was contained in the supernatant and was purified by affinity chromatography on a phosphotyrosine–Sepharose column prepared by reacting two bed volumes of 20 mM *o*-phospho-L-tyrosine with CNBr-activated Sepharose beads in $NaHCO_3$ buffer at pH 8.3. Bound SH2 domain was eluted with the addition of 20 mM free phosphotyrosine to the wash buffer (200 mM NaCl, 2 mM DTT, 50 mM Tris, pH 7.4); gel filtration on a Superose-12 FPLC column in storage buffer (200 mM NaCl, 2 mM DTT, 20 mM BES, pH 7.0) removed phosphotyrosine and a high-molecular-weight contaminant from the affinity eluent. Crystallization and data collection: For crystallization, SH2 protein in storage buffer was concentrated to 50 mg ml^{-1} in a Centricon-10 concentration device (Amicon) and combined with an equimolar amount of the phosphotyrosyl peptide EPQpYEEIPIYL (10 mg ml^{-1} in 40 mM BES, pH 7.0), which was synthesized and purified as described[9]. Crystals were grown using the hanging drop/vapour diffusion method by combining 2 μl protein/peptide solution with 2 μl of a well solution containing 25–35% PEG 2,000, 4,000 or 8,000, 50 mM $MgCl_2$ and 100 mM sodium acetate, pH 4.6. Clusters of long, thin rod-shaped crystals of orthorhombic space group $P2_12_12$ (a = 61.31, b = 57.31, c = 31.18) grew overnight at 22 °C to maximum dimensions of 0.1 × 0.03 × 1.0 mm. Essentially complete data were collected from two crystals to 2.2 and 1.8 Å resolution using a MAR Research image plate scanner mounted on an Elliot GX-13 rotating anode generator equipped with double focusing mirror optics. Images were processed with the image plate version of the MOSFLM package[32]. 47,097 fully recorded observations of 9,956 unique reflections were scaled and merged with CCP4 programs[33], yielding a merging R-factor of 8.4% on intensities for all data to 1.8 Å. Merged data were 99% complete to 2.2 Å and 85% complete between 2.2 and 1.8 Å. The mean $I/\sigma(I)$ was greater than 2 in the highest resolution shell. Structure determination: Structure factor phases were determined by molecular replacement[34] using the Src SH2/peptide A structure of Waskman *et al.* as a search model[18]. Solvent molecules and the phosphopeptide were omitted from the model. Refined atomic temperature factors were retained. Rotation and translation functions and Patterson-correlation (PC) refinement[35] were carried out using X-plor[36]. A cross-rotation function using 8–20 Å vectors and data to 3.5 Å resolution yielded a 9σ peak. The PC-refined rotation solution was used in a translation search, which yielded an unambiguous 12σ peak. Inspection of a packing model and of an electron density map, calculated after rigid body refinement, revealed density for the phosphopeptide that had not been included in the model. The model was rebuilt to reflect the amino-acid sequence of the Lck SH2 domain and refined by repeated cycles of manual rebuilding using the program O (ref. 37) and simulated- annealing and positional refinement using X-plor[36]. The entire phosphopeptide was excluded from the model until after the first cycle of simulated annealing and positional refinement; at this stage the 11-residue peptide was readily built into a 2.2 Å electron density map. Simulated-annealing omit maps[36] were used to rebuild several segments of the polypeptide chain, including the N terminus, and the CD and DE turns, where the Lck backbone diverges from Src. The model contains 104 residues (123–226 of intact Lck), the 11 residues of the phosphopeptide and 65 water molecules. The first five residues of the SH2 construct are not visible in the electron density, and E123, the first residue for which main-chain density is visible, is modelled as an alanine. After restrained individual temperature factor refinement, R_{cryst} = 22% for 2σ data to 1.8 Å resolution. The corresponding 'free' R value[38], calculated using 10% of the diffraction data (selected randomly from eight resolution bins) that had been excluded from all previous refinement calculation, was 31%. The model has reasonable stereochemistry (r.m.s. bonds 0.021 Å, angle 3.7°). It superimposes on the Src SH2 structure[18] with an r.m.s. deviation for Cα positions of 1.12 Å.

sequences containing pYMXM or pYVXM (refs 3–7). That is, it appears to have strong specificity for pY+3 and additional selectivity for pY+1. Residue βD5 may be particularly central for peptide recognition (Fig. 1*a*). The tyrosine in Lck contacts the side chains of pY+1 and pY+3; it constrains the BG loop conformation by accepting a hydrogen bond from the main-chain amide of residue BG5; and it is a ligand of the water that bridges to carbonyl pY+2. A tyrosine is present at βD5 in all members of the Src family, as well as in Abl, Arg, Csk, ZAP70 (C-terminal) and Syk[1,25–28]. All these proteins have similar sequences in their BG loops (a characteristic Asp-Gly-Leu at BG 2–4) and Ile or Val at βE4 (Fig. 2). We therefore expect that they will have similar pY+3 pockets and a strong preference for hydrophobic side chains of about the same size. Where alignments are clear for other families with more divergent sequences (Fig. 2), the characteristics of the pocket also appear to favour hydrophobic residues at pY+3.

It is clear that a more complete understanding of comparative specificities will require additional structures. An NMR study of the unliganded Lck SH2 domain is in progress (J. Lee, personal communication), and a structure for the Src SH2 domain in complex with the same phosphopeptide used here has been determined at lower resolution[29]. □

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ERRATUM

The earliest Acheulean from Konso-Gardula

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THE cat specimen featured on the cover of the issue of 24/31 December 1992, originally referred to *Homotherium* as detailed in the cover caption, was changed to *Dinofelis* sp. aff. *piveteaui* while the paper was in press. The cover shows the cranium listed as *Dinofelis* in Table 2 of this letter.