Ubx-homeodomain electron density, and polyalanine helices were placed into the Eox-homeodomain density. The MIRAS map was further improved by cycles of solvent flattening with program DM11. Using these and $F_o - F_c$ and $2F_o - F_c$ maps, interspersed with positional and individual $B$-factor refinement using X-PLOR12, the model was rebuilt, side chains were added, and the Eox loops and N-terminal arms were built with the program O (ref. 27). The first four residues of Eox, the residues from $-7$ to 4 of Ubx and the first 6 residues of Ubx were disordered. There was clear density for the YPWM motif; but it was not readily interpretable for residues other than the tryptophan side chain. To obtain a more interpretable map, we refined and improved the MIRAS phases by solvent flattening and extended them to 2.8 Å using the program SHARP15.

This map was greatly improved and showed us how to fit the YPWM motif. The YPWM fit was further verified by an anomalous-difference Fourier map calculated with data measured from selenomethionine (SeMet)-substituted protein; this map showed the positions of the three substituted seleniums, including the one in the YPWM motif. The refinement of the structure was extended to 2.4 Å resolution using the native 1 data, and the structure verified through extensive simulated annealing omit maps. Finally, 110 water molecules were added from the inspection of $F_o - F_c$ maps. The final refined structure has good stereochemistry, with the Ramachandran plot showing 84.5% of the residues in the core allowed regions and no residues in the disallowed or generously allowed regions.

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Structural basis for activation of the titin kinase domain during myofibrillogenesis

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The giant muscle protein titin (connectin) is essential in the temporal and spatial control of the assembly of the highly ordered sarcomeres (contractile units) of striated muscle. Here we present the crystal structure of titin’s only catalytic domain, an autoregulated serine kinase (titin kinase). The structure shows how the active site is inhibited by a tyrosine of the kinase domain. We describe a dual mechanism of activation of titin kinase that consists of phosphorylation of this tyrosine and binding of calcium/calmodulin to the regulatory tail. The serine kinase domain of titin is the first known non-arginine-aspartate kinase to be activated by phosphorylation. The phosphorylated tyrosine is not located in the activation segment, as in other kinases, but in the P + 1 loop, indicating that this tyrosine is a binding partner of the titin kinase substrate. Titin kinase phosphorylates the muscle protein telethonin in early differentiating myocytes, indicating that this kinase may act in myofibrillogenesis.

Protein kinases are important in controlling cell proliferation and differentiation and they require specific mechanisms of regulation and substrate recognition1–3. Tight control of the enzymatic activity of protein kinases is achieved by phosphorylation of specific residues in the activation segment of the catalytic domain, sometimes combined with reversible conformational changes in carboxy-terminal autoregulatory tails, induced by effector molecules such as Ca2+/calmodulin. In all available structures of protein kinases regulated by phosphorylation, one or more phosphorylated residues are always located in the activation segment (Fig. 1). These kinases contain a strictly conserved arginine preceding the catalytic aspartate, and are hence known as RD kinases. The arginine interacts with a phosphorylated residue from the activation segment2. In the non-RD kinases of the myosin-light-chain kinase (MLCK) family, this pattern is not found (Fig. 1), and activation of these kinases by phosphorylation has not been predicted.

The assembly of striated myofibrils in differentiating myocytes involves the controlled integration of hundreds of proteins into the highly ordered macromolecular complex of the sarcomere. The giant protein titin extends over one half of the sarcomeric unit and is crucial in this control4,5. Close to its C terminus, titin contains a MLCK-like kinase domain. Although sequence similarity of the catalytic domain of titin to that of its invertebrate analogue, the serine/threonine kinase domain of twitchin6, has indicated that titin and twitchin may have similar function, the differential localization of the kinase domains7, their distinct C-terminal regulatory tails8,9,10 and the presence of several titin-specific residues in the active site11.

Figure 1 Sequence alignment of active-site regions. Serine/threonine kinases activated by calmodulin or by other calcium-binding proteins: human cardiac titin kinase (TK), twitchin (TwK) from Aplysia californica, rat skeletal myosin-light-chain kinase (MLCK), and human calcium/calmodulin-dependent protein kinase-I (CAMK-I); serine/threonine kinases activated by phosphorylation in the activation segment: human cell-division-protein kinase 2 (CDK2), human extracellular-signal-regulated kinase 2 (ERK2), and human cAMP-dependent protein kinase α-catalytic subunit (cAPK); tyrosine kinases: fibroblast growth factor receptor kinase 3 (FGFR3), insulin-receptor inase (IRK3), and proto-oncogene tyrosine kinase protein kinase c-Src (C-SRC). Yellow, identified phosphorylation sites; red, RD motif; cyan, deviations from the RD motif; green, conserved basic residues (K, R) in the +2 or +4 position relative to the RD motif. The secondary-structure elements shown at the top relate to the three-dimensional structure of titin kinase. The limits of the activation segment and the P + 1 loop are defined by the sequence motifs DFG (EFG in titin kinase) to APE2.
hint at a different function for titin. Twitchin is a Ca\textsuperscript{2+}/S100A1-regulated MLCK\textsuperscript{15}. Despite biochemical evidence for Ca\textsuperscript{2+}/calmodulin binding\textsuperscript{7}, however, the activation process and function of titin kinase have remained unknown.

The crystal structure of autoinhibited titin kinase shows how a tyrosine of the P + 1 loop inhibits the active site. The titin kinase structure has provided the basis for determining its dual activation mechanism, which comprises phosphorylation of Y170 and Ca\textsuperscript{2+}/calmodulin binding. Titin kinase is activated in differentiating myocytes, where it phosphorylates the muscle protein telethonin, indicating its probable importance in myofibrillogenesis.

**Unusual autoinhibited conformation**

The complete autoinhibited form of titin kinase, including the catalytic domain and the regulatory tail (kin1; Fig. 2), could be grown in thin-plate crystals <5 μm thick. We used these crystals to determine the atomic structure of titin kinase at 2.0 Å resolution from synchrotron radiation X-ray data. The overall fold shows the catalytic domain and the autoregulatory C-terminal tail, which wraps around the lower lobe and the active site of the catalytic domain (Fig. 3a, b). The amino-terminal helix of this tail (\(\alpha R1\)) is in a similar location to the equivalent helices in twitchin\textsuperscript{10} and calcium/calmodulin-dependent kinase-1 (CaMK-1)\textsuperscript{11}. The C-terminus of this tail superimposes with that of twitchin (Fig. 3c) but not with the tail of CaMK-I. The second helix of the tail, \(\alpha R2\), binds into the ATP-binding site and is followed by a β-sheet (BR1) which forms an antiparallel β-sheet with strands \(\beta C10\) and \(\beta C11\) in the lower lobe of the catalytic domain. Specific interactions to the catalytic domain are mostly restricted to the two termini, \(\alpha R1\) and \(\beta R1\), of the regulatory tail (Fig. 3c). The C-terminal regulatory tail of titin kinase provides the calmodulin-binding site, which mapped to a segment that covers helix \(\alpha R1\) (ref. 9). The surface of this helix exhibits a cluster of basic and hydrophobic residues that is characteristic of Ca\textsuperscript{2+}/calmodulin-binding sequences\textsuperscript{14}. The analogous segment is also involved in Ca\textsuperscript{2+}/calmodulin binding of other MLCK-like kinases\textsuperscript{13,15,16}.

Unlike other kinases, in which the activation segment undergoes a switch from a ‘closed’ to an ‘open’ conformation after serine and/or tyrosine phosphorylation\textsuperscript{5}, the activation segment of titin kinase is in an open conformation in the autoinhibited structure (Fig. 4). In the active site, however, the carboxyterminal group of the invariant catalytic base D127 is involved in a hydrogen-bonding network to R129, Q150 and Y170 (Fig. 4a, d), blocking the active site of access to its protein substrate and thus inhibiting catalysis. In turn, the hydroxyl group of Y170 is embedded in a network of hydrogen bonds to residues D127, R129 and the main-chain carbonyl group of F126. The important residue in this network is R129, a conserved lysine in all other MLCK-like kinases, which acts as a bridge between D127 and Y170. Steric inhibition of the catalytic base by a tyrosine in its vicinity is reminiscent of the inhibition of the inactive forms of insulin-receptor kinase (IRK)\textsuperscript{17} (Fig. 4c) and the MAP kinase ERK2 (ref. 18). These kinases are activated by phosphorylation of at least one tyrosine residue in the activation segment, indicating that tyrosine phosphorylation may be involved in activation of titin kinase. In contrast to ERK2 and IRK, the important inhibitory residue of titin kinase, Y170, is located in the P + 1 loop that connects \(\beta C10\) and \(\alpha C4\) in the lower lobe of the kinase. This region forms the pocket that accommodates the P + 1 position of the substrate in other kinase peptide-substrate structures\textsuperscript{19,20}.

**Tyrosine phosphorylation of titin kinase**

To determine whether Y170 is a site of phosphorylation in titin kinase, we made a truncated titin kinase construct (kin4) lacking the regulatory C-terminal tail. Kin4 is predicted to be constitutively active, like C-terminal-truncated twitchin\textsuperscript{15}. In a yeast two-hybrid analysis, kin4 interacts specifically with overlapping peptides from the regulatory region of titin kinase (Fig. 2b), thus showing the structural integrity of this construct. We introduced a mutation of Y170 (Y170E) into kin4 to abolish the inhibitory tyrosine and, at the same time, to mimic the phosphorylated state\textsuperscript{18}. The K36A mutation is predicted to disrupt catalytic activity, by analogy to K72A in cAMP-dependent protein kinase\textsuperscript{21}. When expressed in C2C12 myocytes, phosphoryrosine was detected in the wild-type kin4 and in the K36A mutant. No phosphotyrosine signal could be detected for the Y170E mutant (Fig. 5a). Similarly, the full-length kinase (kin1) was tyrosine-phosphorylated by extracts from differentiating myocytes, but not by extracts from adult muscle. Again, tyrosine phosphorylation in the mutant kin1(Y170E) was markedly reduced (Fig. 5b). These results indicate that titin kinase is transphosphorylated by kinase activities in differentiating muscle at an unusual site in the P + 1 loop, where Y170 is the major phosphorylation site.

Y170 is buried in the autoinhibited, unphosphorylated structure of titin kinase (Fig. 4) like in ERK2 (ref. 18) in which the residues phosphorylated upon activation are equally inaccessible. To make the peptide-binding site accessible, major structural rearrangements are predicted to take place during, or following, tyrosine phosphorylation in both ERK2 (ref. 18) and titin kinase. We immunoprecipitated the native, *in vitro* phosphorylated titin kinase with anti-phosphotyrosine antibodies (Fig. 5c). This indicates that the P + 1 loop may undergo major structural rearrangements involving the exposure to solvent of phosphorylated Y170.

**Activated titin kinase phosphorylates telethonin**

To study kinase activity of titin kinase, we expressed the truncated titin kinase construct, kin4, in C2C12 myocytes. When we used kin4 in phosphorylation assays with different substrates, we found no myosin light-chain-kinase activity. We detected specifically increased phosphorylation of a protein of relative molecular mass 22,500 (M, 22K) in day 2 myocyte extracts in the presence of wild-type kin4, but not in the presence of the catalytically inactive kin4 mutant K36A (Fig. 6a). We used mass-spectrometrical microsequencing to determine that this band was telethonin, a protein of cardiac and skeletal muscle\textsuperscript{22}. By using phosphorylation assays with recombinant subfragments of telethonin and subsequent sequencing by tandem mass spectrometry, we identified a single phosphorylated serine in the recognition sequence\textsuperscript{23} RRSLS (phospho) RSMSSQAERG, close to the C terminus of telethonin. The identification of telethonin as a sarcomeric Z-disk protein\textsuperscript{24} indicates that its phosphorylation may be involved in the control of myofibrillogenesis. This is supported by the observation that C2C12 myocytes exposed to solvent of phosphorylated Y170.
cells expressing the constitutively active kin4 show a breakdown of normal cytoskeletal architecture (Fig. 6b). In the differentiated myofibril, titin kinase is located ~1 μm from the Z-disk, at the edge of the M-band. However, both the titin C terminus and telethonin can be detected by immunofluorescence in dot-like aggregates on stress-fibre-like structures in day 2 myocytes (Fig. 6c). These data indicate that the activation of titin kinase in differentiating myocytes and the resulting phosphorylation of telethonin are involved in the reorganization of the cytoskeleton during myofibrillogenesis. During these events, the titin C terminus is transiently in close proximity to the titin kinase substrate telethonin.

**Activation of titin kinase**

To further characterize the mechanism of activation of titin kinase, we used purified, autoregulated kin1 (Fig. 1). Kin1 showed only weak basal kinase activity towards the substrate telethonin (residues 104–167) (Fig. 7a). Addition of the muscle calcium-binding proteins calmodulin, S100A12, CACY or CAPL did not increase kinase activity significantly (Fig. 7a). In contrast, the basal activity...
of the phosphate-mimickry mutant kin1(Y170E) towards telethonin was more than tenfold higher than that of wild-type and was stimulated by up to 100-fold by Ca\(^{2+}\)/calmodulin (Fig. 7b). None of the S100 proteins (S100A1, CACY or CAPL) activated the mutant titin kinase (Fig. 7b). These results show that two different autoinhibitory mechanism must be overcome to fully activate titin kinase: the P\(^{1}\) loop must be released from the substrate-binding site by tyrosine phosphorylation, mimicked in kin1(Y170E), and the conformation of the C-terminal tail must change as a result of binding of Ca\(^{2+}\)/calmodulin. This novel dual autoregulatory mechanism is likely to provide tight control for titin kinase activity during muscle differentiation.

**Substrate recognition by titin kinase**

In contrast to the RD kinases\(^2\), the active site of titin kinase does not contain a pocket composed of basic residues that could accommodate a phosphorylated tyrosine. Replacement of the phosphorylated tyrosine of the kinase with substrate, which occurs in IRK\(^{25}\), is unlikely because of the catalytic specificity of titin kinase for serine. None of the other MLCK-like kinases contains a tyrosine in the P\(^{1}\) loop (Fig. 1), excluding the possibility of a related activation mechanism. The most significant difference in the phosphorylation site on telethonin, as compared with the sites of phosphorylation of substrates for MLCK and twitchin\(^{26}\) is the presence of an arginine in the P\(^{1}\) position. A potential function of the phosphorylated Y170 could, therefore, be to directly bind this arginine, explaining the unusual presence of arginine in the P\(^{1}\) position of the titin kinase substrate and the location of the titin kinase phosphorylation site, Y170, in the P\(^{1}\) loop. Furthermore, Q150 in the activation segment of titin kinase is replaced by a hydrophobic residue in other MLCK-like kinases (Fig. 1). This glutamine is located in the pocket that accommodates the P\(^{1}\) position of the substrate in known kinase peptide-substrate structures\(^27\). Therefore, Q150 might be another ligand for the P\(^{1}\) arginine of telethonin. The only other known protein kinase that shows the same pattern of a preferred arginine in the substrate P\(^{1}\) position and a tyrosine in the kinase P\(^{1}\) loop is NIMA\(^{19}\). The significance of this observation is unknown.

![Figure 4](image_url)  
Figure 4. Active-site conformation of the autoinhibited forms of titin kinase, twitchin and IRK. a, The active site of titin kinase. The guanidinium group of R129 forms short hydrogen bonds with the side chains of D127 and Y170 from the P\(^{1}\) loop. There is a weak direct hydrogen bond between D127 and Y170 (3.1 Å in length). D127 forms further hydrogen bonds with Q150. b, Twitchin active site\(^10\). The catalytic aspartate, D174, forms hydrogen bonds with K176, Q200 and R355 from the regulatory tail. At the position of Y170 in titin kinase, there is an alanine in twitchin. In the autoinhibited twitchin structure\(^10\), the catalytic aspartate is blocked by a salt bridge with an arginine (R355 in twitchin) of the regulatory tail, suggesting a different activation mechanism than for titin kinase. In titin kinase, the equivalent arginine, R306, does not interact with the catalytic aspartate. c, Active site of the autoinhibited form of IRK\(^{17}\). The catalytic aspartate, D1132, is bound to Y1162. This bond is disrupted after phosphorylation of Y1162, accompanied by phosphorylation of two other tyrosines and induces a conformational change of the activation segment from a closed to an open conformation\(^25\). The colour codes of the tubes are as in Fig. 3a. d, Stereo view of a 2\(\Delta F_0 - \Delta F_1\) electron-density map, using phases of the final model, contoured at 1.3\(\sigma\). The electron density shown covers several active-site residues and solvent molecules. Some titin-kinase residues are labelled. a–c were prepared with GRASP\(^6\) and d with program O (ref. 46).
Figure 5 Titin kinase is tyrosine-phosphorylated on Y170 in differentiating C2C12 cells. a. Transfected in4 (lane 1), kin4(K36A) (lane 2) and kin4(Y170E) (lane 3) were immunoprecipitated with a rabbit antibody against titin kinase (α-TK-ra; ref. 9) and phosphorytosine was detected on blots of the immunoprecipitates with the monoclonal antibody 4G10 (anti-PY). The immunoprecipitated kinase was detected with the anti-T7 tag antibody (anti-T7). The absence of a phosphotyrosine signal in kin4(Y170E) indicates that Y170 is the major tyrosine phosphorylation site. The phosphorylation of the catalytically inactive kin4(K36A) indicates that titin kinase is transphosphorylated by upstream kinase activities rather than intramolecularly autophosphorylated. b. Similarly, the recombinant full-length kinase kin1 (WT) is markedly tyrosine-phosphorylated in vitro by cytosolic extracts from differentiating day 2 C2C12 cells (lane 1) but only weakly from adult psoas muscle extracts (lane 3) as visualized in blots with 4G10. The phosphorytosine signal of the kin1(Y170E) mutant is markedly reduced (lane 1, bottom), indicating that Y170 is the major phosphorylation site in the autoinhibited form of titin kinase also. Lanes 2 and 3, bottom: control without cell extracts; 1 μg of enzyme was loaded per lane. c, the phosphorylated tyrosine in titin kinase is accessible to anti-phosphotyrosine antibodies in the native state of the enzyme, indicating that it is exposed to solvent and that major structural rearrangements of the P + 1 loop occur in the activated kinase. Kin1 was phosphorylated as in a in the presence of [γ-32P]ATP (lane 1) and immunoprecipitated with the 4G10 antibody. The autoradiograph shows the presence of phosphorylated titin kinase in the immunoprecipitated fraction (lane 2) and its near absence in the unbound supernatant (lane 3).

Figure 6 Titin kinase phosphorylates the muscle protein telethonin. a. Phosphorylation of muscle proteins by constitutively active kin4 was done using tyrosine-phosphorylated enzyme from transfected C2C12 cells. To suppress background calcium-activated kinases in the myocyte extracts, EGTA was used. In extracts of day 2 myocytes, increased phosphorylation of a protein of Mr ~ 22K occurs in the presence of kin4 (lane 1). In the presence of kin4(K36A) (lane 2) or in blank assays (lane 3), phosphorylation of this band is not increased. Kin4 shows no discernible activity towards proteins in adult psoas cytosol (lane 4) or myofibrils (lane 6) compared with controls (lanes 5 and 7), indicating that the activity of titin kinase is directed towards proteins in differentiating myocytes. The constitutively active enzyme has no myosin-light-chain kinase activity (lane 6).

b. The presence of constitutively active kin4 in myogenic cells disrupts myobriillogenesis. Non-transfected cells at day 2 show the typical alignment of titin (top panel, visualized by anti-Z1Z2-ra staining30) along stress-fibre-like structures. In the transfected cells (arrowhead in all three panels), the alignment of titin along the actin cytoskeleton is disrupted and titin (green) and kin4 (red, visualized with the anti-T7 tag antibody in the bottom panel) are randomly distributed. c. The C terminus of titin loops back onto the stress-fibre-associated N-terminal portion, and localizes with telethonin. In day 2 myocytes, C-terminal epitopes of titin (visualized with the anti-titin antibody T30 (ref. 47) localize with telethonin on stress-fibre-like structures in dot-like aggregates. T, Titin; Tth, telethonin; TK, transfected titin kinase. Scale bars: 10 μm.

Figure 7 Full activation of titin kinase requires both tyrosine phosphorylation and Ca2+/calmodulin. Wild-type (WT) kin1 shows very low basal activity towards telethonin[104–167] (lane 1); this activity is not stimulated by Ca2+/calmodulin (CaM), Ca2+/S100A1 (S100), Ca2+/CACY (CACY), or Ca2+/CAPL (CAPL). In these assays, we used 0.1 μg purified enzyme. Autoradiographs were exposed for 12 h. The phosphate-mimicry mutant kin1(Y170E) shows elevated basal activity (lane 1) which is stimulated markedly by Ca2+/calmodulin. The addition of Ca2+/S100A1, Ca2+/CACY or Ca2+/CAPL has no activating effect; the presence of Ca2+/CACY suppresses basal titin-kinase activity by about tenfold, indicating that S100 proteins indeed modulate the activity of titin kinase. The addition of 10 μM Zn2+ in the assays using S100 proteins did not increase kinase stimulation but inhibited kin1(Y170E) as it inhibited C. elegans twitchin."
Discussion
The titin kinase structure exhibits dual inhibition of the active site: the catalytic aspartate is blocked by Y170 from the P + 1 loop, and the ATP-binding site is blocked by helix αR2 from the regulatory tail. Inhibition is removed by a new, dual-activation process, involving phosphorylation of Y170 by an unknown kinase and potential co-factors and the binding of Ca"/calmodulin. Titin kinase is, to our knowledge, the only kinase known to be activated by phosphorylation of a tyrosine from the P + 1 loop and to lack the RD motif. We propose that, on activation of titin kinase, the central part of the regulatory tail is expelled from the active site whereas the flanking interactions of αR1 and βR1 remain in contact with the catalytic domain. It remains to be determined whether activation by phosphorylation in the P + 1 loop will effect substrate specificity, as a general mechanism for kinase regulation, as in protein kinase C.6,24
Although all members of the MLCK family bind Ca"/calmodulin, other calcium-binding proteins are involved in activation; for example, twitchin is activated by the dimeric S100A1 protein.2,16 It will be important to determine the specificity and complementarity of different calcium-binding proteins in the activation of these protein kinases, which appears to be a multistep process in most cases.

Methods
Plasmids and two-hybrid screens. The titin kinase fragments kin1 (EMBL accession number X90568; amino acids (aa) 24,731–25,054) and kin4 (aa 24,731–25,005) and their mutants were cloned into a modified pCMV5 vector27 with an N-terminal T7-tag (Invitrogen) sequence (MTGGGQMGK). The N-terminal phasing was based on MLCK-like kinases without extra N-terminal domains, for reasons of expression stability. For two-hybrid screens, we inserted kin4 and its mutants into a modified pLexA plasmid.27 Two-hybrid screens and analysis were done as described.27 All cloning and mutagenesis steps followed standard procedures.

Cell culture. We cultured C2C12 cells in DMEM; 20% fetal calf serum and 4.5% glucose at 37°C. Differentiation was induced by moving cells to low-serum medium (DMEM, 4% horse serum). Transfection with lipofectamine was improved by macroseeding and the use of oil layers over the reservoir as described.25 Crystals that grew as thin plates with dimensions of 6 × 10 μm × 300 μm. The thickness of these crystals was slightly improved by macroseeding and the use of oil layers over the reservoir as described.26 Crystals were shock-frozen at 100 K using 12% glycerol as cryoprotectant. A native X-ray data set was collected up to 2.0 Å resolution on the wiggle beamline BW7B (EMBL Hamburg Outstation) using a wavelength of 0.837 Å. The data were recorded in a high-resolution sweep (132 frames, frame width 0.8 degrees, crystal-to-detector distance 180 mm) and a low-resolution sweep (83 frames, frame width 1.2 degrees, detector distance 340 mm) on a 345-mm Mar imaging plate scanner. We used the DENOZ and SCALEPACK software27 for processing and reduction of data, which consisted of 53,151 unique reflections with a redundancy of 3.7, a completeness of 96% and an Rmerge(1) of 7.7% overall (21,052 unique reflections in the highest resolution shell, 2.39–2.0 Å, with a multiplicity of 2.6, a completeness of 94.5% and an Rmerge(1) of 23.9%).

The crystals belong to the P2₁2₁2₁ space group with cell dimensions of a = 78.6 Å, b = 89.9 Å, c = 113.3 Å and two molecules per asymmetric unit. The non-crystallographic two-fold axis is parallel to the crystallographic b axis at x = 0.5 and z = 0.09. Initial phases were obtained from a trimmed model of the catalytic domain of twitchin27 using the molecular-replacement software package AMoRe. A Sigma-A² weighted, 2Fo – Fc electron-density map calculated with the initial molecular-replacement phases was used for NCS averaging using the SOLVER software, with mask creation and manipulation done in MAMA.24 We used the molecular dynamics slow-cooling protocol of X-PLOR for structure refinement. We applied bulk solvent correction, overall anisotropic B-factor scaling, restrained NCS and restrained individual B-factor refinement. The final model includes 5,206 protein non-hydrogen atoms and 514 solvent atoms for the two copies in the asymmetric unit. The overall R-factor is 20.7% and Rfree is 24.8% for all observed data between 40 and 2.0 Å resolution (in the outer-resolution shell, 2.28–2.0 Å, these values are 22.4% and 27.4%, respectively). The test data set corresponds to 5% of the total data and was generated with FREERFLAG.29

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Correspondence and requests for materials should be addressed to M.W. (e-mail: Wilmanns@embl-hamburg.de) or M.G. (e-mail: Gautel@embl-heidelberg.de). Coordinates and structure factors have been deposited at the Protein Data Base (accession number 1TKI).