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Crystal structure of a putative transcriptional regulator SCO0520 from *Streptomyces coelicolor A3(2)* reveals an unusual dimer among TetR family proteins

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Abstract

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A structure of the *apo*-form of the putative transcriptional regulator SCO0520 from *Streptomyces coelicolor A3(2)* was determined at 1.8 Å resolution. SCO0520 belongs to the TetR family of regulators. In the crystal lattice, the asymmetric unit contains two monomers that form an Ω-shaped dimer. The distance between the two DNA-recognition domains is much longer than the corresponding distances in the known structures of other TetR family proteins. In addition, the subunits in the dimer have different conformational states, resulting in different relative positions of the DNA-binding and regulatory domains. Similar conformational modifications are observed in other TetR regulators and result from ligand binding. These studies provide information about the flexibility of SCO0520 molecule and its putative biological function.

Keywords

Helix-turn-helix DNA-binding motif; Structural genomics; TetR transcriptional regulator; X-ray crystal structure

Introduction

The filamentous, soil-inhabiting bacterium *Streptomyces coelicolor* exhibits an unusual and developmentally complex life cycle, adapting to a wide range of environmental conditions. The bacterium is also a source of many important secondary metabolites, such as antibiotics, immunosuppressants, insecticides, and antitumor agents [1–3]. To adapt to disparate environments and produce potentially toxic metabolites, members of genus *Streptomyces* require extensive collections of resistance genes, and the corresponding regulatory genes, to protect themselves from these metabolites as well as those produced by competing species. *sco0520* is thought to act as a regulatory gene, which encodes for the SCO0520 protein, a 194 amino acid putative transcriptional regulator with a molecular weight of 21 kDa. While the exact function of SCO0520 is not yet known, sequence comparisons suggest that the protein is likely to bind DNA and regulate transcription.

The SCO0520 protein is a member of the tetracycline family of regulators (TetRs), all of which contain a helix-turn- helix (HTH) DNA-binding motif [4]. All members of the TetR family of known function act as repressors, and many of them regulate the transcription of genes involved in antibiotic resistance or biosynthesis [4]. Proteins of known structure in the TetR family form all-helical structures with two domains. The smaller, N-terminal DNA-binding domain is highly conserved, while the larger C-terminal domain, which binds small molecule ligands, is less so. Three-dimensional structures of several members of the family have been determined and characterized: TetR from *Escherichia coli* [5–8], QacR from *Staphylococcus aureus* [9–12], CprB from *S. coelicolor A3(2)* [13], EthR from *Mycobacterium tuberculosis* [14–16], YfiR from *Bacillus subtilis* [17], AcrR from *E. coli* [18], ActR from *S. coelicolor A3(2)* [19], TetR from *Thermotoga maritima* [20, 21] and TetR from *S. coelicolor A3(2)* [22]. For TetR and QacR, structures of both DNA-bound and ligand-bound states are known [6–11]. For EthR and ActR, structures of protein complexed with ligands were obtained [14, 16, 17, 19]. Biochemical and crystal structure analyses of TetR family proteins indicate that the functional unit of the proteins is a dimer which adopts an “Ω” shape with approximate dimensions of 60 Å × 50 Å × 25 Å [6, 11, 13, 14, 20]. The

B-DNA motif bound by the two TetR DNA-binding domains comprises two adjacent 6-nucleotide- long major groove regions [4]. Both TetR and QacR bind to DNA in the absence of an inducer molecule, and binding of the inducer to the regulatory domain causes conformational changes in the DNA-binding domain, and results in release of the protein from DNA and subsequent transcription initiation from the cognate promoter.

In this paper, we describe the crystal structure of the *apo*-form of the TetR transcriptional regulator SCO0520 from *S. coelicolor A3(2)*. The details of the dimer architecture, the ligand binding site, and the conformational differences between the subunits of SCO0520 are presented. In addition, the structure is compared to structures of other TetR family members. The analysis indicates that the distances between the DNA-recognition domains vary widely and correlate with the surface area of the dimerization contact for the dimer of TetR proteins. The results presented here suggest that the DNA-binding domains possess conformational flexibility, which is important for the biological function of TetR regulators.

Materials and methods

Protein cloning, expression, purification and crystallization

The *sco0520* gene was cloned, expressed and purified using a protocol described previously [23], which was developed at the Midwest Center for Structural Genomics (MCSG). Crystals of selenomethionine-incorporated SCO0520 were grown by hanging-drop vapor-diffusion methods at 293 K. Crystallization drops were composed of a 1:1 mixture of reservoir solution and protein solution. The protein solution contained 20.4 mg/mL of protein, 0.5 M sodium chloride, and 0.5 mM tris (2-carboxyethyl) phosphine (TCEP) in a 0.01 M HEPES buffer (pH 7.5). The reservoir solution contained 0.2 M ammonium acetate, 26% w/v PEG 3350, 4% w/v sucrose, and 2.5 mM tylosin tartrate in a 0.1 M sodium acetate buffer (pH 4.0). Crystals selected for data collection were transferred into paratone-N oil and flash cooled in liquid nitrogen.

Data collection, structure determination and refinement

A low-temperature (100 K) X-ray diffraction data set was obtained from a single crystal using 0.9791 Å radiation at the 19-BM beamline of the Structural Biology Center [24] at Argonne National Laboratory. The diffraction data were processed with the HKL-2000 program suite [25]. X-ray data-collection statistics are summarized in Table 1.

The SCO0520 structure was solved by single-wavelength anomalous dispersion (SAD). Initial phases were obtained with HKL-3000 [26]. HKL-3000 is a software package that interacts with SHELX [27], MLPHARE [28], DM [29, 30], CCP4 [31], SOLVE [32], RESOLVE [33], ARP/wARP [34], O [35] and COOT [36]. During substructure solution, 6 out of the 8 selenium sites predicted by the sequence were identified and their positions, occupancies and displacement factors were refined. Subsequent electron density modification followed by initial model building was done using HKL-3000. The rest of the model was constructed iteratively, by cycles of manual building with COOT and least-squares refinement with REFMAC [37].

The crystal structure contains two protein chains per asymmetric unit. The refinement statistics and characteristics of the model of SCO0520 are given in Table 1. The model of the SCO0520 contains 173 and 175 residues in monomers A and B, respectively. Fifteen N-terminal residues in both monomers and 6 C-terminal residues in monomer A (and 4 in monomer B) are not ordered in the electron density map. One Cl^- ion and one acetate ion, which were components of the crystallization buffer, were found to be ordered in the structure.

The structure was validated with SFCHECK [38], PROCHECK [39], ADIT [40], MOLPROBITY and KING [41]. The atomic coordinates and structure factors for the SCO0520 structure have been deposited in the Protein Data Bank (PDB) [42] with accession code 2Q24. Figures were produced using PyMOL [43] and CCP4MG [44].

Dynamic light scattering

Dynamic light scattering (DLS) measurements were performed using a Zetasizer Nano-S Zen 1600 instrument at 25°C. The protein sample was in a solution containing 0.1 M HEPES (pH 7.5), 0.02 M sodium chloride, and 0.5 mM TCEP. For DLS measurements, the concentration of SCO0520 was 1 mg/mL. Data analyses were performed with the Zetasizer Nano software.

Sequence and structural analysis

Sequenced-based searches for homologs of SCO0520 from *S. coelicolor A3(2)* were performed using PSI-BLAST [45]. Homologous sequences were aligned using CLUSTALW [46] and the resulting alignment was formatted using ESPript [47].

Searches for structural homology of SCO0520 by comparison of three-dimensional protein structures was performed using the DALI [48], VAST [49, 50] and Pro- Func [51] web services.

Results and discussion

Overall structure

Sequence and structural analysis suggested that SCO0520 is a member of the TetR-family of transcriptional regulators and forms a homodimer similar to those of TetR, QacR, EthR, CprB, YfiR and ActR (Figs. 1, 2).

The polypeptide chain of the monomer of SCO0520 forms an all-helical, two-domain molecule (Fig. 2a). The smaller, N-terminal domain likely to be responsible for DNA binding comprises residues 1–56. The larger, C-terminal domain, which in homologs determines regulatory properties of the protein and binds small molecule ligands, comprises residues 57–189. The putative DNA-binding domain comprises three α-helices: α1 (16–32), α2 (36–44), and α3 (47–55). The regulatory domain comprises eight α-helices: α4 (57–75), α5 (77–84), α6 (85–106), α7 (108–117), α8 (124–143), α9 (151–167), and α10 (169–186).

Within the TetR family, the DNA-binding domain is highly conserved (Fig. 1). Helices α2 and α3 of the DNA-binding domain of SCO0520 form the typical HTH motif. It was

demonstrated that target DNA sequences are recognized by the HTH motifs of the structures of TetR and QacR [6, 10]. Calculated electrostatic potentials for the solvent accessible surfaces of SCO0520 show a concentration of positively charged residues in the area of helices α2 and α3 (Fig. 2b). We hypothesize that SCO0520 binds DNA in a manner similar to that of TetR and QacR. Furthermore, comparison of the structures of the complexes of TetR and QacR with their corresponding operators suggests that residues His36, Leu37, Glu38, Gly47, Ser48, Gly49, Thr50, Tyr52, Arg53, Asn54 and Arg58 in SCO0520 may also be involved in interactions with DNA (Fig. 1). The structure of the DNA-binding domain in the SCO0520 molecule is stabilized by hydrophobic contacts between residues Ile20, Leu21, Ala23, Ala24, Val27, Ala35, Ile40, Ala41, Ala44 and Leu51, which form the core of the domain and are largely conserved within the TetR family (Fig. 1). All helices of the regulatory domain of the SCO0520 structure, with the exception of α7, form an antiparallel bundle of six helices. In contrast, the regulatory domain is significantly less conserved than the DNA-binding domain with greater variation among specific TetR regulators (Fig. 1). The DNA-binding and regulatory domains of the SCO0520 molecule are connected by helix α4 (Fig. 2a). The following residues: 17–32 (α1), 36–44 (α2), 51–57 (α3), 57–76 (α4), 103–107 (α6), 109–113 (α7), 123–127 (α8) are involved in the interactions between the two domains.

The C-terminal domain contains a large cavity that is the predicted ligand binding site; it is bordered by helices α4, α6, α7, α8, α9 and has a volume of 780 $Å^3$ and average depth of 8 Å (Fig. 3a).

Comparison of the cavity to the known binding sites in TetR member structures shows that this site is very similar to the cavity seen in the complex of QacR containing ethidium [9]. The residues that comprise the inner surface of the possible ligand binding pocket in SCO0520 monomer are presented in Fig. 3b. Almost all hydrophobic residues are located on one side of the cavity. The inner surface of the pocket has a positive charged region that is created by the completely buried residues Lys104 and Arg126. This implies that SCO0520 may bind neutral and negatively charged ligands more favorably. None of residues composing the cavity are conserved among related regulators, suggesting that the ligand-binding pocket for each regulator is designed to recognize different specific ligands.

Dimer architecture

The asymmetric unit of the SCO0520 structure contains an Ω-shaped dimer (dimer I) that is similar to those observed for other TetR family members (Fig. 2). The interface between the subunits forming dimer I has a mostly hydrophobic character, and dimerization occurs at the surface formed by helices α9, α10 and the interconnecting loop (Fig. 2a). The area of the solvent-accessible surface of a monomer buried upon dimerization is about 880 $Å^2$.

One of the most interesting features of the SCO0520 dimer I is the large center-to-center distance (63 Å) between the HTH motifs, as measured from the Cα atom of the most highly conserved residue Tyr52 from helix α3 of one monomer to the corresponding atom in the other subunit (Fig. 2a). The HTH distances in the DNA-bound forms of QacR and TetR are 37 and 35 Å, respectively, and those of the ligand-bound dimers of QacR, TetR, YfiR and ActR are 45, 38, 54 and 43 Å, respectively. Comparative structural analyses of more than 80

dimers of TetR family members reported in the PDB were performed. In this set, the HTH distances vary widely, between 31 and 73 Å, and among the deposited structures of TetR regulators, five structures of proteins similar to SCO0520 (TetR regulator from *Pseudomonas aeruginosa* (PDBID 2OER), TetR regulator from *Mycobacterium vanbaalenii* (PDBID 2QWT), TetR/AcrR regulator from *Novosphingobium aromaticivorans* (PDBID 2RAS), TetR regulator from *Rhodospirillum rubrum* (PDBID 3CWR), and TetR regulator from *Cytophaga hutchinsonii* (PDBID 3F0C)) also have a large HTH distances similarly to that of SCO0520 (Fig. 4).

In general, most TetR dimers have similar distances to QacR and TetR regulators. We also observed a negative correlation between the HTH distances and the surface areas of the dimerization contact for the dimers of TetR regulators. The area of contact surfaces for SCO0520 and for transcriptional regulators with large HTH distances are smaller in comparison to other TetR dimers (Fig. 4).

In another view of the SCO0520 crystal packing (Fig. 5), an alternative dimer arrangement (dimer II) can be identified.

The surface area buried by the contact of the monomers of the dimer II is substantially larger than any other contact surface areas with symmetrically equivalent monomers. Dimer II is formed by applying the symmetry operation X, Y, Z and translation along *c* axis to an SCO0520 monomer. The solvent-accessible surface area buried upon formation of dimer II is 950 $Å^2$, which is 19% of the total surface area of the dimer. This value correlates well with the mean distribution of dimerization surface areas of homodimeric structures in the PDB as calculated by Wang et al. [52].

Without experimental confirmation, it is not possible to determine which dimerization arrangement is physiologically relevant (or if neither or both are). Both types of dimers have contact areas greater that the 856 $Å^2$ threshold proposed by Postingl et al. [53, 54], who used that threshold to discriminate between monomeric and homodimeric proteins. Dynamic light scattering data indicate that monodisperse solutions of SCO0520 have a species with an exclusion radius of 9.8 nm. If the protein polymerizes in a globular arrangement, this indicates that the protein may form a multimer as large as a hexamer in solution (Supplementary material Fig. S1). However, there is no clear hexameric arrangement seen in the crystal lattice.

Conformational changes of monomers

The Ω-shaped dimer of SCO0520 comprises two monomers that adopt different conformations (Fig. 6). The root mean square deviation (RMSD) between the coordinates of all Cα atoms of the two monomers in the SCO0520 structure equal 1.2 Å. The largest conformational changes are observed in the fragments formed by residues 25–49 (α1-α3), which are part of the putative DNA-binding domain, and in the regions of the regulatory domain formed by residues 105–119 (located on α6 and α7) and 163–171 (located on α9 and α10; Fig. 6). Similar conformational flexibility is observed in the structures of other TetR regulators, and it seems that ligand binding limits this flexibility by locking the structure into a more rigid state. It was proposed based on analysis of structures [5] that

ligand binding to TetR and QacR causes the shift of helix α6 (analogous to α7 in SCO0520), which in turn induces the relocation of helix α4 (analogous to α4 in SCO0520) and the DNA-binding domain.

A simulation of the domain motion needed to change the conformation in one monomer of SCO0520 to the conformation of the other was calculated by the DynDom server (http://sys.uea.ac.uk/dyndom). The shifts of helices α6, α7, α9 and the putative DNA-binding domain seem to occur concomitantly. In the simulation, the DNA-binding domain rotates about 12º around a hinge axis comprising residues 55–56 (mechanical hinges), which is involved in interdomain bending.

The differences between the monomers in the SCO0520 structure may be explained both by their different packing environments in the crystal and the differences in the mobility of individual structural elements (i.e., differences in B factors). In fact, the DNA-binding domain of chain B of the Ω-shaped dimer forms more contacts with symmetrically equivalent molecules than with chain A. Conversely, the regions formed by residues 147–166 and 174–187 (which are part of the regulatory domain) in chain A form more contacts with symmetrically equivalent molecules than do the corresponding regions in chain B. In the regions of SCO0520 where the conformations of the two monomers differ the most, the temperature factors have larger values (Fig. 2c). Conformational flexibility of these helices is a dynamic feature of TetR regulators and important for their biological function [55].

Conclusion and discussion of the biological role

Structural and bioinformatics analysis show that SCO0520 from *S. coelicolor A3(2)* is a putative transcriptional regulator that belongs to the TetR regulator family. Chromosomal mapping indicates that the *sco0520* gene is located near two neighboring genes *sco0521* and *sco0522*, which encode for a putative transcriptional regulator (SCO0521) and a putative oxidoreductase (SCO0522), respectively. Sequence analysis shows that the oxidoreductase SCO0522 belongs to the short-chain dehydrogenase/reductase (SDRs) family [56] and shares similarity with the short-chain alcohol dehydrogenase. SDRs are one-domain NAD(P)(H)-dependent enzymes of (typically) 250 amino acid residues. SDRs display a wide substrate spectrum, ranging from steroids, alcohols, sugars, and aromatic compounds to xenobiotics. The close distance between genes *sco0520* and *sco0521* suggests the hypothesis that the gene cluster *sco0520*, *sco0521* and *sco0522* may comprise a functional operon. Therefore, SCO0520, perhaps together with SCO0521, may regulate the *sco0522* gene which encodes for the putative oxidoreductase. A similar type of gene organization and regulation, by the transcriptional regulator SCO0332 from *S. coelicolor A3(2)*, is described by Okada et al. [22].

The observed sequence similarity within HTH DNA-binding motifs, structure identity and conformational modifications strongly suggest that SCO0520 is a transcriptional repressor analogous to other TetR repressors. Binding of an inducer molecule to the regulatory domain of TetR proteins causes conformational changes in the DNA-binding region, which result in the release of the repressor from the target DNA. The functional unit of TetR regulators is the homodimer. Two conserved tyrosine residues from each monomer

recognize thymine bases in the major groove of DNA [6, 10]. Thus, the operator sequence is restricted to the pattern 5′-A-X_n-T-3′, where *n* 7 in TetR and *n* 8 in QacR. Most of the structures of TetR regulators deposited in the PDB have an HTH distance ranging from 36 to 45 Å range (Fig. 4). However, structure analysis of the *apo*-form of SCO0520 reveals that the distance between the two recognition helices is significantly larger (63 Å) than the corresponding distances in most other known TetR regulators. Moreover, several structures of TetR regulators deposited in PDB with similar distances could be identified (Fig. 4). In fact we note that the structure of another transcriptional regulator of the TetR/ AcrR family from *Novosphingobium aromaticivorans DSM* (PDB accession code 2RAS) has even larger distance between HTH motifs at 73 Å. We assume that the large separation between the HTH motifs in TetR regulators can be explained by the flexibility of the DNA-binding domains and ligand-binding helices in the TetR structures, which allows for a large conformational change between the ligand-bound and the DNA-bound states. Further analyses of the ligand and DNA-bond forms of SCO0520 will facilitate the comprehensive functional characterization of TetR family regulators.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

DLS	Dynamic light scattering
HTH	Helix-turn-helix
MCSG	Midwest Center for Structural Genomics
PDB	Protein Data Bank
RMSD	Root mean square deviation
SDR	Short-chain dehydrogenase/reductase
TCEP	Tris (2-carboxyethyl) phosphine
TetRs	Tetracycline family of regulators

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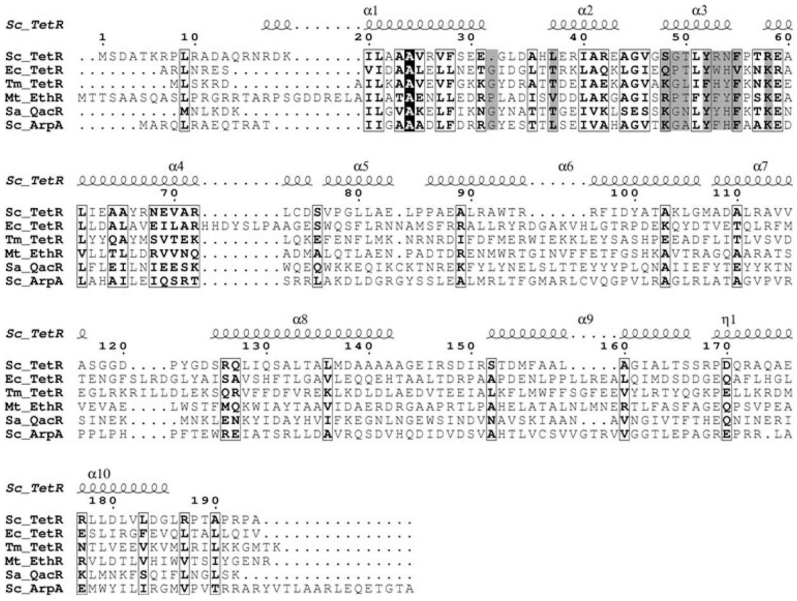

Fig. 1.

Sequence alignment of transcriptional regulator SCO0520 from *Streptomyces coelicolor A3(2)* (Sc_TetR) and other TetR-family members that exemplify the TetR family profile [4]. Abbreviations are as follows: TetR repressor from *Escherichia coli.* (Ec_TetR), TetR repressor from *Thermotoga maritima* (Tm_TetR), EthR repressor from *Mycobacterium tuberculosis* (Mt_EthR), QacR repressor from *Straphylococcus aureus* (Sa_QacR), autoregulator receptor CprB from *Streptomyces coelicolor A3(2)* (Sc_ArpA). Secondary structure elements of SCO0520 are indicated above the sequence. Sequence homologies are highlighted by *black background* (identities) and *boxes* (similarity). The *grey column* indicates residues involved in DNA contacts in the crystal structure of TetR and QacR

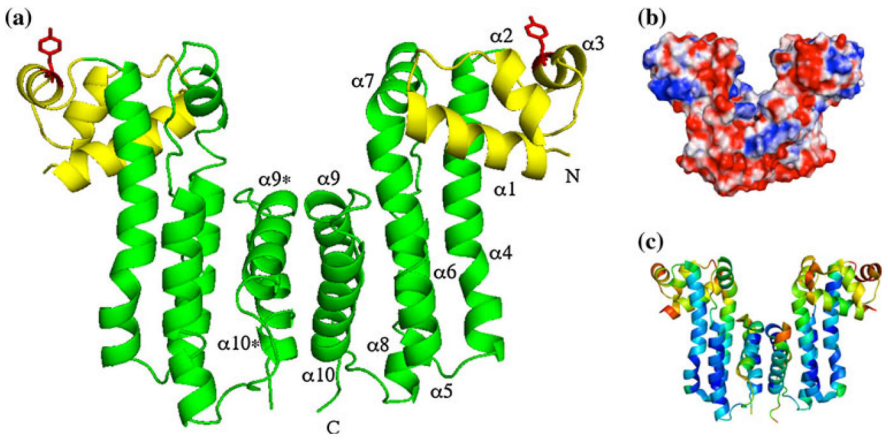

Fig. 2.

a Ribbon diagram of the SCO0520 dimer I. The DNA-binding domains are shown in yellow, regulatory domains in green. Secondary structure elements are labeled in one monomer. Helices involved into the monomers contact are labeled with the star. The conserved tyrosines (*red stick model*) from which HTH distance has being measured. **b** Electrostatic potential mapped on the surface of SCO0520 dimer. *Blue* shows positive potential, *red* negative, and *white* neutral. **c** Ribbon diagram of the SCO0520 dimer, colored by value of temperature factors from *blue* (small values) to *orange* (large values)

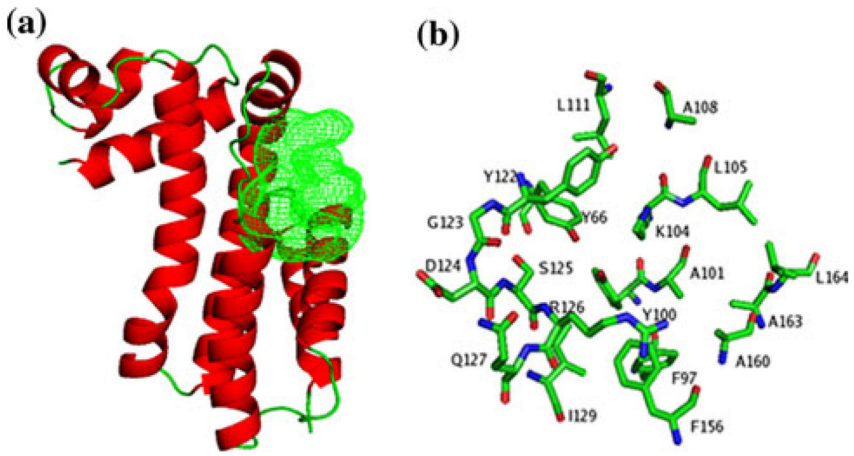

Fig. 3.

a, **b** Surface representation and amino acid composition of the proposed ligand-binding pocket in the SCO0520 structure. In the figure **b** oxygen atoms are shown in *red*, nitrogen atoms in *blue*, and carbon atoms in *green*

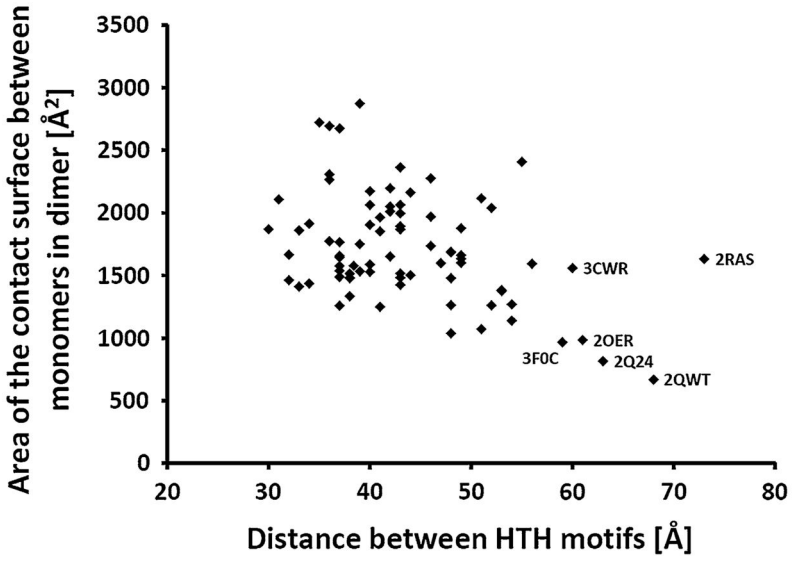

Fig. 4.

Area of the contact surface between monomers in the putative dimer form as a function of the distance between HTH motifs in the TetR regulators deposited to the PDB as of January 2010

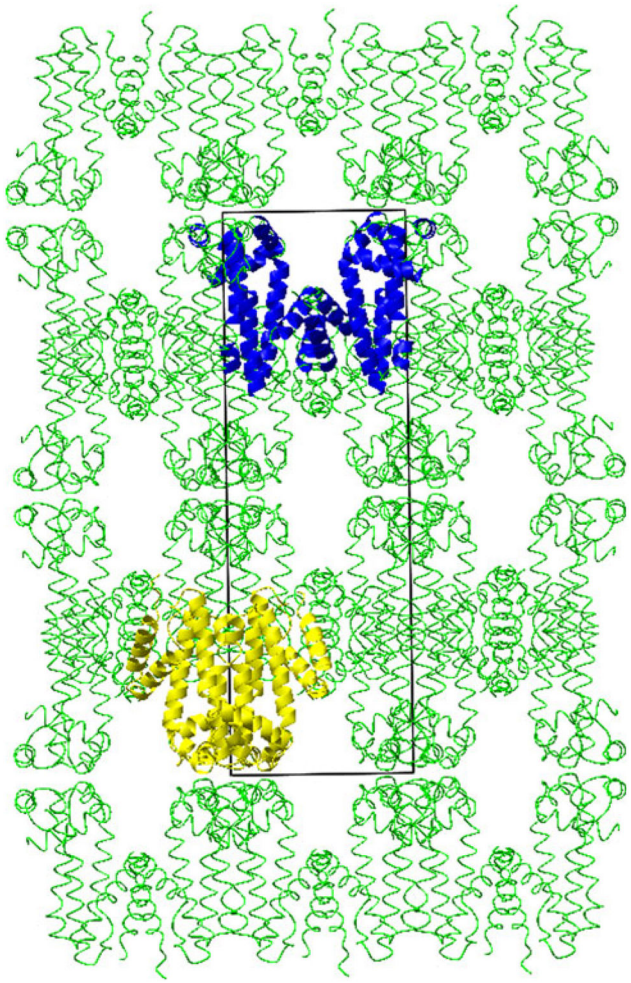

Fig. 5.

Crystal packing of SCO0520 dimers in the unit cell. The dimer I is presented in *blue* and dimer II in *yellow*

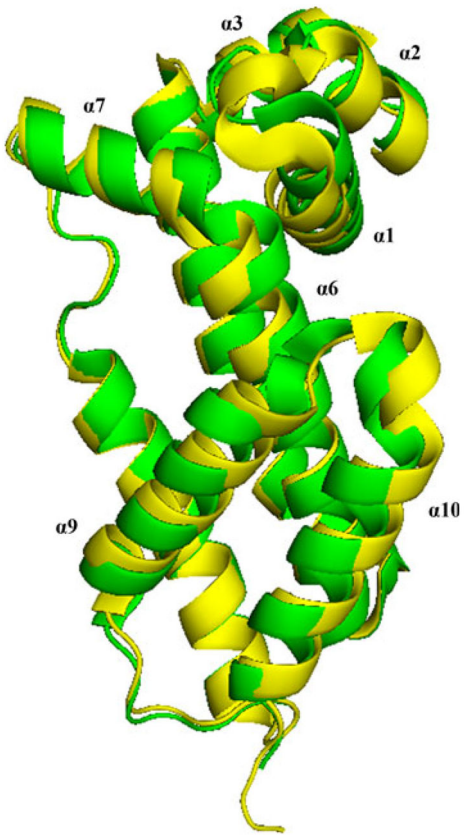

Fig. 6.
Comparison of chains A (*yellow*) and B (*green*) in the asymmetric unit of the SCO0520 structure

Table 1

Crystallographic parameters, data collection and refinement statistics

	SCO0520
Data collection	
Resolution (Å)	50.0–1.8 (1.82–1.80)
Space group	$P2_12_12$
Unit cell parameters:	
a (Å)	52.3
b (Å)	146.2
c (Å)	47.3
Completeness (%)	99.3 (95.4)
Observed/unique reflections	188987/34267
I/σ(I)	33.7 (3.2)
R_{merge} (%)	7.6 (41.6)
Wilson B-factor (Å^2)	26.6
Refinement	
R (%)/R_{free} (%)	18.9/22.3
R.m.s. deviations from idealized geometry:	
Bond lengths (Å)	0.020
Bond angles (◦)	1.6
Mean B value (Å^2)	30.3
Total number of non-hydrogen atoms:	
Protein atoms	2928
Acetate ions	1
Chloride ions	1
Water molecules	272
Ramachandran analysis*	
Favored (%)/n	96.45/296
Allowed (%)/n	3.55/11

Data for the highest resolution shell are given in parentheses

*Pro and Gly residues were excluded from calculation. Analysis was done using SFCHECK and PROCHECK