Crystallographic Insight into Collagen Recognition by Discoidin Domain Receptor 2

Federico Carafoli,1 Dominique Bihan,2 Stavros Statthopoulos,3 Antonios D. Konitsiotis,3 Marc Kvansakul,1,4 Richard W. Farndale,2 Birgit Leitinger,3 and Erhard Hohenester1,*

1Department of Life Sciences, Imperial College London, London SW7 2AZ, UK
2Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, UK
3Division of National Heart and Lung Institute, Imperial College London, London SW7 2AZ, UK
4Present address: Structural Biology Division, The Walter and Eliza Hall Institute, 3050 Parkville, Victoria, Australia
*Correspondence: e.hohenester@imperial.ac.uk
DOI 10.1016/j.str.2009.10.012

SUMMARY

The discoidin domain receptors, DDR1 and DDR2, are widely expressed receptor tyrosine kinases that are activated by triple-helical collagen. They control important aspects of cell behavior and are dysregulated in several human diseases. The major DDR2-binding site in collagens I–III is a GVMGFO motif (O is hydroxyproline) that also binds the matricellular protein SPARC. We have determined the crystal structure of the discoidin domain of human DDR2 bound to a triple-helical collagen peptide. The GVMGFO motifs of two collagen chains are recognized by an amphiphilic pocket delimited by a functionally critical tryptophan residue and a buried salt bridge. Collagen binding results in structural changes of DDR2 surface loops that may be linked to the process of receptor activation. A comparison of the GVMGFO-binding sites of DDR2 and SPARC reveals a striking case of convergent evolution in collagen recognition.

INTRODUCTION

Supramolecular collagen assemblies are crucial for the mechanical stability of animal bodies (Myllyharju and Kivirikko, 2004). The basic collagen structure is a triple helix of three chains containing multiple Gly-X-X' repeats; X and X' are often proline and 4-hydroxyproline (Hyp, O), respectively (Brodsky and Persikov, 2005). Apart from their prominent structural roles, collagens have fundamental functions in cell adhesion and signaling, by serving as ligands for a diverse set of cellular receptors (Heino, 2005). Apart from their prominent structural roles, collagens have fundamental functions in cell adhesion and signaling, by serving as ligands for a diverse set of cellular receptors (Heino, 2005). The discoidin domain receptors, DDR1 and DDR2, are highly expressed receptor tyrosine kinases that are activated by triple-helical collagen. They control important aspects of cell behavior and are dysregulated in several human diseases. The major DDR2-binding site in collagens I–III is a GVMGFO motif (O is hydroxyproline) that also binds the matricellular protein SPARC. We have determined the crystal structure of the discoidin domain of human DDR2 bound to a triple-helical collagen peptide. The GVMGFO motifs of two collagen chains are recognized by an amphiphilic pocket delimited by a functionally critical tryptophan residue and a buried salt bridge. Collagen binding results in structural changes of DDR2 surface loops that may be linked to the process of receptor activation. A comparison of the GVMGFO-binding sites of DDR2 and SPARC reveals a striking case of convergent evolution in collagen recognition.

RESULTS

Crystal Structure of a DDR2 DS Domain-Collagen Peptide Complex

During the course of our previous study (Konitsiotis et al., 2008), we discovered that substitution of methionine in GVMGFO by the isosteric amino acid norleucine (Nle) increases DDR2 binding in a solid-phase assay ~10-fold (Figure 1A). We synthesized a number of short triple-helical peptides for co-crystallization with the DDR2 DS domain. The peptides contained the DDR2-binding sequence, GPRGQGVNleGFO, flanked by 2–3 GPO repeats at either end; the GPRGQ sequence was included because it is required for DDR2 activation in cells (Konitsiotis et al., 2008). Since we obtained crystals with the first peptide tested, Ac-GPOGQGVGPRGQGVNleGFO, we did not perform a systematic analysis of the remaining peptides. We used analytical size exclusion chromatography to demonstrate peptide binding to the DDR2 DS domain in solution and binding to DDR2 receptor tyrosine kinases in cells (Konitsiotis et al., 2008).
A peptide complex was solved by molecular replacement (Figure 2; synchrotron radiation and the structure of the DDR2 DS-collagen Table 1).

The free DS domain, which is not very soluble, the DS-collagen volume is consistent with a complex of 1:1 stoichiometry. Unlike does not contribute to the absorption at 280 nm). This elution plex was formed that eluted at 12.0 ml (note that the peptide was added in a two-fold molar excess, a protein-peptide com-plex was added in a two-fold molar excess, a protein-peptide com-

Figure 1. Collagen Peptide Binding by the DDR2 DS Domain

(A) Solid-phase binding assay with recombinant DS2-Fc protein (Leitinger, 2003) added to 96-well plates coated with triple-helical collagen peptides at 10 µg/ml: GPC-(GPP)_2-GPRGQGQVXGFO-(GPP)_2-GPC-NH₂, where X is either methionine or norleucine. Shown is a representative of three independent experiments, each performed in duplicate.

(B) Analytical size exclusion chromatograms of the free DDR2 DS domain and its complex with the triple-helical collagen peptide Ac-GPOGPOGPOGPR-

GQGKVNeGFPQGPGOPOG-NH₂. The DS domain and peptide were mixed in the indicated molar ratios. A globular molecular mass standard of 29 kDa, carbonic anhydrase, elutes at 12.3 ml from this column.

The collagen peptide in the DDR2 DS-collagen complex is completely straight (Figure 2B), and the helical parameters of the three collagen chains are therefore essentially identical. The GPO-rich N-terminal region is close to a 7/2 helical symmetry, whereas the GVNeGFO motif approximates a more relaxed 10/3 symmetry (Figure 3). A relaxation of the helical twist in regions lacking imino acids has been observed in several model peptide structures (Kramer et al., 1999; Okuyama et al., 2006). The observed transition in helix parameters is thus likely to be an inherent feature of the collagen peptide itself, rather than a consequence of DDR2 binding.

The details of collagen binding revealed by our structure are in excellent agreement with biochemical results showing that M, F, and O of the GVMGFO motif are critical for DDR2 binding (Konitsiotis et al., 2008). Why the substitution of methionine by norleu-
cine enhances DDR2 binding is not evident from the structure, as the slightly longer methionine side chain is readily modeled into the amphiphilic pocket without steric clashes (not shown). It may be significant, however, that there is a close (3.5 Å) contact between C of Nle21 (leading chain) and Phe23 (middle chain). This contact may be less favorable when the corresponding atom is sulfur, as it is in methionine. In any case, the effect is subtle, and we think that it is highly unlikely that methionine is
recognized in a radically different manner. We will therefore make no distinction between norleucine and methionine in the following discussion.

An arginine four residues upstream of the GVMGFO motif has been shown to contribute to DDR2 binding, and the GPR triplet containing this arginine is strictly required for signaling (Konitsiotis et al., 2008). In our structure, the side chain of Arg15 (trailing chain) points toward an acidic patch on the DS domain formed by Glu66, Glu67, and Asp69, but it is too distant (8 Å) to form any specific interactions (not shown). This long-range electrostatic interaction may explain why Arg15 contributes to DDR2 binding. However, further studies are required to understand the apparently critical role of this arginine in receptor activation (Konitsiotis et al., 2008).

Comparison with the Solution Structure of the Free DDR2 DS Domain

The solution structure of the free DDR2 DS domain has been determined, and the collagen-binding site has been identified by transferred cross-saturation experiments and mutagenesis (Ichikawa et al., 2007). Although there is good general agreement with our DS-collagen complex structure regarding the identity of the major collagen-binding residues (Figures 4A and 4B), the mode of collagen binding was not predicted correctly. The presence of Arg105 and Glu113 in the collagen-binding site of DDR2 led Ichikawa et al. (2007) to predict that complementary charges must exist in collagen. However, the GVMGFO motif is notably apolar, and charge compensation in the DDR2-collagen complex is, in fact, achieved by a buried salt bridge between Arg105 and Glu113 (Figure 2C). A comparison of our complex structure with the NMR ensemble of the free DS domain shows that collagen binding leads to a restructuring of loops L1 (bearing the critical Trp52) and L4 (bearing at its base Arg105 and Glu113). L1 and L4 appear to move in a concerted manner to clamp down on Phe23 of the collagen middle chain (Figure 4C). The movement of L4 is followed by L5 at the side of the DS domain β-barrel (not shown). A caveat of this comparison is that there are very few long range NOEs that determine the conformations of L1 and L4 in the NMR ensemble. Nevertheless, it is likely that collagen binding leads to a freezing of the mobile loops surrounding the collagen-binding trench.

Conservation of Collagen-Binding Residues in DDR1 and DDR2

The central collagen-binding residues of DDR2 delineated by our structure (Trp52, Thr56, Asp69, Arg105, Glu113, and
GVMGFO motif is also the major binding site for DDR1 (authors’ unpublished data), we find it difficult to believe that the corresponding tryptophan in DDR1, Trp53, is not required for receptor activation.

**Convergent Evolution of GVMGFO-Binding Sites**

The GVMGFO motif is a recently defined hotspot in collagens I–III that binds not only DDR2, but also von Willebrand factor (vWF) (Lisman et al., 2006) and the matricellular protein SPARC (Giudici et al., 2008). A comparison of the collagen complexes of DDR2 and SPARC (Hohenester et al., 2008) (a complex of vWF does not exist) reveals a remarkable case of convergent evolution. The GVMGFO-binding site of SPARC is created by a long α helix and an adjacent helical hairpin, in sharp contrast to the irregular loops that make up the binding site of DDR2 (Figure 7). Despite their different structures, however, both proteins feature similar amphiphilic specificity pockets, sandwiching the critical Phe23 side chain (middle chain in DDR2, trailing chain in SPARC) between a tryptophan and a salt bridge between arginine and glutamic acid, with the latter also mediating recognition of Hyp24. Another interesting parallel is that the Phe23 phenyl ring does not form any stacking interactions in either structure but is bound in a manner favoring the formation of C–H···π hydrogen bonds (Hohenester et al., 2008).

**DISCUSSION**

Cell-collagen interactions are critical for tissue stability and function, but structural studies are difficult because of the large size and structural complexity of collagens. Comprehensive sets of synthetic triple-helical peptides (“Collagen Toolkits”) have been invaluable in defining specific receptor-binding sites in collagens (Farndale et al., 2008) and have made possible crystallographic studies of receptor-collagen complexes. However, to date, α2 integrin has been the only collagen receptor for which the mode of collagen binding was understood in atomic detail (Emsley et al., 2000).

We have determined a high-resolution crystal structure of the DDR2 DS domain in complex with a 28-residue collagen peptide, revealing how DDR2 recognizes a conserved GVMGFO motif present in the fibrillar collagens I–III (note that in our peptide methionine is replaced by norleucine; see above). The two large apolar residues of this motif, M and F, are inserted into a
specificity pocket at the top of the DDR2 DS domain. This pocket is surprisingly polar on one side, allowing multiple hydrogen-bonding interactions with the O of the GVMGFO motif. An important feature of the DDR2-collagen interaction, correctly predicted from modeling (Konitsiotis et al., 2008), is that the key collagen residues are not provided by the same chain, explaining why a triple-helical conformation is required for binding (Leitinger, 2003; Vogel et al., 1997).

Most remarkably, an essentially identical collagen-binding mode to DDR2 is employed by SPARC, an x-helical matricellular protein unrelated to DDR2 that also recognizes the GVMGFO motif in collagen (Giudici et al., 2008; Hohenester et al., 2008). The convergence of binding mechanisms suggests that the GVMGFO motif may have been selected as a binding site because of its unique properties: the presence of two large apolar residues separated by a glycine is rare in collagens and results in pronounced hydrophobic knobs on the triple helix surface.

Apart from the GVMGFO motif, which is present in Collagen II Toolkit peptides 22 and 23, additional DDR2-binding sites have been observed (but not yet characterized) in peptides 13 and 44 (Konitsiotis et al., 2008). A GIVGLO motif in peptide 44 may bind DDR2 in a similar way as the GVMGFO motif, but there are no analogous candidate motifs in peptide 13. Thus, alternative modes of collagen recognition by DDR2 may exist.

The major binding site in collagens I–III for x1|1 and x2|1 integrins is a GFOGER motif (Knight et al., 2000; Xu et al., 2000). In contrast to the situation with DDR2 and SPARC, all three phenylalanine side chains of the triple-helical GFOGER peptide remain substantially solvent-accessible in the complex with the integrin x2 I domain (Emsley et al., 2000), consistent with the finding that the requirement for phenylalanine is not strict (Kim et al., 2005; Raynal et al., 2006). The invariant residue of all integrin-binding sites in collagen is a glutamic acid, which coordinates the magnesium ion bound to the integrin I domain (Emsley et al., 2000). Thus, the two major classes of collagen receptors in animals, integrins and DDRs, have evolved to bind collagen by very different mechanisms despite their shared affinity for GFO triplets.

Is the GVMGFO motif also the major DDR2-binding site in collagen fibrils? In this regard, it is worth noting that DDR2 binding to fibrillar collagen has yet to be demonstrated by direct observation. However, fibrillar and nonfibrillar collagen have been shown to act differently on cells in a DDR2-dependent manner (Wall et al., 2005). A low-resolution structure of the collagen I microfibril has been reported recently (Orgel et al., 2006). Two alternative models of a collagen fibril have been generated from this structure (Herr and Farndale, 2009; Perumal et al., 2008), with the GVMGFO motif being surface-exposed only in the model of Herr and Farndale (2009). However, the binding mode observed in our DDR2 DS-collagen peptide structure is not compatible with the crystalline structure of Orgel et al. (2006). It is possible that DDR2 binds to the more disordered, fluid-like regions that are known to exist in collagen fibrils (Huines, 1995).

How does collagen binding to the DDR2 DS domain lead to receptor activation? Many RTKs are believed to be dimerized by their ligands, which brings the cytosolic kinase domains into close proximity and facilitates the autophosphorylation reaction that is the first step in RTK signaling (Schlessinger, 2000). Certain RTKs, such as the epidermal growth factor (EGF) receptor, appear to become activated by structural rearrangements within a preformed dimer (Jura et al., 2009). The DDRs are constitutive dimers at the cell surface (Abdulhussein et al., 2008; Mihal et al., 2009; Noordeen et al., 2006). Furthermore, collagen peptides containing the GVMGFO motif activate DDR2 with the same slow kinetics as native collagen, suggesting that receptor clustering is unlikely to be the main mechanism of DDR activation (Konitsiotis et al., 2008). We envisage an activation mechanism that involves collagen-induced changes within a DDR dimer. It should be noted that our discussion only refers to the first step of transmembrane signaling, not the slow process by which full DDR phosphorylation eventually is achieved (which minimally also involves Src kinase) (Ikeda et al., 2002; Yang et al., 2005).

We can think of two ways in which collagen binding could activate DDR (Figure 8). A single collagen triple helix could interact...
with both DS domains in the DDR dimer (“composite binding site”) and thereby activate the receptor, similar to the situation exemplified by the growth hormone-growth hormone receptor complex (de Vos et al., 1992). The key collagen residues in our crystal structure are Nle21 and Hyp24 of the leading chain and Phe23 of the middle chain. Because of the helical symmetry of the homotrimeric collagen peptide, an equivalent constellation of residues occurs again on the middle and trailing chains (Figure 1B). However, it is impossible to replicate the interactions of the first DS domain at this second, vacant, site without causing major steric clashes between the two DS domains (data not shown). Thus, the complex would have to be asymmetric with two distinct receptor-ligand interfaces. The high-affinity DS-collagen interface would correspond to the interaction seen in our crystal structure, whereas the second interface may be weaker and only form when the two DS domains are joined in a stable DDR dimer.

In the alternative scenario, collagen binding to two independent sites would trigger the transition from the inactive to the active DDR dimer, conceivably by amplifying the small changes in the collagen-binding loops of the DS domain (Figure 3). This situation would be more akin to the EGF-EGF receptor complex, in which two EGF molecules bind to equivalent sites on the outside of an active receptor dimer (Burgess et al., 2003). It should be noted that there is no formal requirement for both DS domains to be occupied by ligand in the active DDR complex: binding of collagen to one DDR DS domain may be sufficient to “unlock” the inactive dimer. Structures of the full-length receptor will now be required to gain further insight into the mechanism of DDR activation. Our structure of a DDR2 DS-collagen complex provides the foundation for such future studies.

**EXPERIMENTAL PROCEDURES**

**Peptide Synthesis**

Peptides were synthesized as C-terminal amides by the solid-phase method on a 9050 Plus PepSynthesizer (Perseptive Biosystems). The peptides were prepared on a 0.1 mmol scale, using Fmoc (9-fluorenylmethoxycarbonyl) chemistry and TentaGel R RAM (Rapp Polymere) resin (0.18 mmol/g). Fmoc deprotection was performed using 2% (v/v) piperidine and 2% (v/v)
1,8-diazabicyclo[5.4.0]undec-7-ene in dimethylformamide (DMF). Coupling of Fmoc-amino acids (0.4 mmol) was performed in DMF using HCTU (2-(6-Chloro-1-H-benzotriazole-1-yl)-1,3,3-tetramethyluronium hexafluorophosphate) (0.4 mmol) with N,N-diisopropylethylamine (0.8 mmol). Cleavage of the peptides from the resin and simultaneous side-chain deprotection was done by treatment of the peptide-resin with a trifluoroacetic acid (TFA), water, and trisopropylsilane mixture (95:2.5:2.5 v/v, 10 ml) containing Dl-dithiothreitol (0.25 g), for 3 hr. The resin was filtered, and the filtrate was concentrated under reduced pressure to ~ 1 ml volume, after which the crude peptides were precipitated with ice-cold ether. The filtered crude peptides were ether-washed (twice), dissolved in 5% acetonitrile in water containing 0.1% TFA, and then lyophilized. Crude peptides were purified by reverse-phase high-performance liquid chromatography (PerkinElmer Life Sciences LC200) using ACE diphenyl columns (Hichrom Ltd) and a linear gradient of 5–45% acetonitrile in water containing 0.1% TFA. The pure peptides were characterized by matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometry and then lyophilized.

Acetylation of the peptide GPGOPGPOGPRQGQNleGPGOPGOGNH2 (Nie, norleucine) was performed manually. Following solid-phase synthesis and removal of the final Nα-Fmoc protecting group, the prewashed resin was treated for 45 min with acetic anhydride (10 ml) and N,N-diisopropylethylamine (3 ml) in dichloromethane (10 ml). The filtered peptide resin was then washed with ether (four times). Cleavage from the resin, simultaneous side-chain deprotection, isolation, and purification were performed as for the other peptides.

**Protein Expression Vectors**

DNA coding for the DS domain of human DDR2 was obtained by PCR using the His-DDR2 vector (Leitinger, 2003) as a template. The DS domain boundaries used were NPAICR...CVWLGD, corresponding to residues 26–190 of Swiss-Prot entry Q18832. The PCR product was cloned into a modified pCPE-Pu vector (Kohfeldt et al., 1997), which adds a His-tag (APLVHHTHHHALA) at the N terminus. The expression vector for the Fc-tagged DS domain (DS2-Fc) has been described elsewhere (Leitinger, 2003). The W52A mutation in full-length DDR2 was introduced by strand overlap extension PCR as described elsewhere (Leitinger, 2003); the PCR product containing the mutation was inserted into pcDDR2 using EcoRI and NarI. To create wild-type and W52A ectodomain constructs tagged with human Fc, cDNAs encoding the respective DDR2 ectodomains (Lys22-Thr398) were obtained by PCR amplification and cloned into a modified pCPE-Pu vector containing a human Fc sequence (Hussain et al., 2006). All expression vectors were verified by DNA sequencing.

**Protein Expression and Purification**

All soluble DDR2 constructs were purified from the conditioned serum-free medium of episomally transfection 293-EBNA cells. Cells were cultured in Dulbecco’s modified Eagle medium containing 10% fetal calf serum, (Invitrogen), transfected using Fugene reagent (Roche Applied Science), and selected with 1 μg/ml of puromycin (Sigma). Proteins were purified by a combination of affinity and size exclusion chromatography on an Äkta platform (GE Healthcare). The Fc-tagged proteins were purified using 1 ml rProtein A FF HiTrap columns according to the manufacturer’s instructions (GE Healthcare) and were dialyzed into phosphate-buffered saline (PBS) buffer (140 mM NaCl, 10 mM Na2PO4, and 3 mM KCl [pH 7.45]). The conditioned medium containing the His-tagged DS domain was loaded onto a 5 ml HiTrap column (GE Healthcare) was equilibrated in PBS, and the DS domain was eluted with 500 mM imidazole in PBS. The eluate was concentrated using a Vivaspin centrifugal device (Sartorius AG), and the DS domain was further purified by size exclusion chromatography on a 24 ml Superdex 75 size-exclusion chromatography column (GE Healthcare) with 20 mM MES and 100 mM NaCl (pH 6.5) as the running buffer. The final yield from one liter of cell culture medium was 5 mg of DDR2 DS domain. The protein was only moderately soluble in PBS and a number of other buffers tested (≤ 1 mg/ml).

**Collagen Binding and Activation Assays**

The solid-phase assay with immobilized collagen (peptides) and the DDR2 activation assay were performed as described elsewhere (Konitsiotis et al., 2008; Leitinger, 2003). For analytical size exclusion chromatography (column and running buffer as above), 16 nmol of purified DDR2 DS domain in PBS was mixed with varying amounts of collagen peptide, diluted from a concentrated stock solution, and incubated for 30 min in a total volume of 0.5 ml.

**Complex Formation and Crystallization**

The DDR2 DS-collagen complex for crystallization was formed by dissolving the lyophilized peptide Ac-GPGOPGPOGPRQGQNleGPGOPGOG-NH2 in 6 ml of a diluted protein solution (0.5 mg/ml protein; molar peptide:protein ratio ~1.5:1). After incubation for 30 min, the solution was concentrated to a volume of 0.5 ml and subjected to size exclusion chromatography (column and running buffer as above). The DDR2 DS-collagen complex eluted as a single peak and was concentrated to 10 mg/ml. Crystals were obtained by hanging drop vapor diffusion at room temperature using 0.1 M PCIB (pH 7.0) and 25% PEG1500 as precipitant. The PCIB buffer system was produced by mixing sodium propionate (40 mM), sodium cacodylate (20 mM), and bis-Tris propane (40 mM) in a molar ratio of 2:1:2. Crystals grew as clusters that could be dissected into single crystals.

**Data Collection and Structure Determination**

Crystals were flash-frozen in liquid nitrogen after a brief soak in mother liquor supplemented with 25% glycerol. Diffraction data were collected at 100 K on station i-02 at the Diamond Light Source (Oxfordshire, UK) at a wavelength of 0.980 Å, and processed with MOSFLM (www.mrc-lmb.cam.ac.uk/harry/mosflm) and programs of the CCP4 suite (CCP4, 1994). The DDR2

---

**Figure 7. Comparison of Collagen Recognition by DDR2 and SPARC**

DDR2 (A) (this work) and SPARC (B) (Hohenester et al., 2008) are in cyan and shown as cartoons with semitransparent surfaces. The leading, middle, and trailing chains of the collagen peptides are in yellow, orange, and red, respectively. Selected residues are shown as sticks. X denotes norleucine. Dashed lines indicate hydrogen bonds.

---

**Figure 8. Possible mechanisms of DDR activation**

DDR1 and DDR2 are dimeric in the absence of collagen (Noordeen et al., 2006). The mechanism of autoinhibition in the inactive dimer is unknown, but is likely to involve the second domain of the ectodomain and/or the large cytosolic juxtamembrane domain. DDR activation may result from the simultaneous binding of both DS domains in the dimer to a single collagen triple helix (“composite binding site”), or the DS domains may bind collagen independently (“independent binding sites”). In any case, collagen binding is proposed to release the autoinhibition, resulting in activation of the cytoplasmic tyrosine kinase domains.
DS-collagen structure was solved by molecular replacement with PHASER (McCoy et al., 2005; Storoni et al., 2004) using the first DS domain (b1 domain) of neuropilin-2 (pdb 2qqj) (Appleton et al., 2007) and a truncated collagen peptide from the SPARC-collagen complex (pdb 2v3s) (Hohenester et al., 2008) as search models. Completion of the model was aided by the solution structure of the free DDR2 DS domain (pdb 2zf4) (Ichikawa et al., 2007). Multiple rounds of rebuilding with O (Jones et al., 1991) and refinement with CNS (Brunger et al., 1998) resulted in a R-factor of 0.204 (Rfree 0.230). The final model comprises DDR2 residues 27 to 187, all collagen residues except for Gly31 of the trailing chain, and 166 water molecules. Analysis with MOLPRO-BITY (Davis et al., 2004) shows that 98% of residues are in favored regions of the Ramachandran plot and that there are no outliers. Crystallographic statistics are summarized in Table 1. The figures were made with PyMOL (www.pymol.org).

**ACCESSION NUMBERS**

Atomic coordinates and structure factors have been deposited in the Protein Data Bank under the ID code 2wuh.

**ACKNOWLEDGMENTS**

We thank Michael Mrosek (Diamond Light Source) for help with data collection, Jordi Bella (University of Manchester) for a CNS script to analyze the collagen helix parameters, Steve Matthews (Imperial College London) for help with the comparison of the crystal and NMR structures, and Irina Zalivina (Imperial College London) for technical assistance. This study was supported by grants from the Wellcome Trust (E.H.) and UK Medical Research Council (B.L. and R.W.F.). E.H. is a Wellcome Trust Senior Research Fellow.

Received: August 24, 2009
Accepted: October 9, 2009
Published: December 8, 2009

**REFERENCES**


Structure
Structure of a DDR2-collagen complex


