Crystallization and X-ray diffraction of LGN in complex with the actin-binding protein afadin

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Asymmetric stem-cell divisions are fundamental for morphogenesis and tissue homeostasis. They rely on the coordination between cortical polarity and the orientation of the mitotic spindle, which is orchestrated by microtubule pulling motors recruited at the cortex by NuMA–LGN–Gai complexes. LGN has emerged as a central component of the spindle-orientation pathway that is conserved throughout species. Its domain structure consists of an N-terminal TPR domain associating with NuMA, followed by four GoLoco motifs binding to Gai subunits. The LGN^{TPR} region is also involved in interactions with other membrane-associated proteins ensuring the correct cortical localization of microtubule motors, among which is the junctional protein afadin. To investigate the architecture of LGN^{TPR} in complex with afadin, a chimeric fusion protein with a native linker derived from the region of afadin upstream of the LGN-binding domain was generated. The fusion protein behaves as a globular monomer in solution and readily crystallizes in the presence of sulfate-containing reservoirs. The crystals diffracted to 3.0 Å resolution and belonged to the cubic space group $P2_13$, with unit-cell parameter $a = 170.3$ Å. The structure of the engineered protein revealed that the crystal packing is promoted by the coordination of sulfate ions by residues of the afadin linker region and LGN^{TPR}.

1. Introduction

Asymmetric cell divisions regulate the position and the fate choice of daughter cells, with an impact on numerous phenotypes of multicellular organisms both during development and in adulthood (Knoblich, 2010). Asymmetric cell divisions are attained by the unequal segregation of cell-fate defining components and by differential positioning of siblings within the tissue. In several stem-cell systems, only daughters retaining contact with a specialized microenvironment called the niche will maintain stemness. The asymmetric outcome of a cell division requires tight coordination between cellular polarity and the division plane, and hence the mitotic spindle axis (Morin & Bellaïche, 2011). Spindle coupling to polarity cues involves the recruitment at spatially restricted cortical sites of molecular devices known as force generators, the main task of which is to capture astral microtubules emanating from the spindle poles and to establish pulling forces. One of the main players in the spindle-orientation pathway is the scaffolding protein LGN (where LGN stands for Leu-Gly-Asn repeat-enriched protein), which is found to be conserved from nematodes to mammals. From a topological standpoint, LGN

Table 1

Macromolecule-production information.

Source organism	<i>Homo sapiens</i>
DNA source	Synthetic
Forward primer	GAAATTTCAAGAGAGGTTGGGGATCAGCGAAACGCCTC- CTACCTCAAA
Reverse primer	AAACAAATATTACTAGTGTGCGACTTATCTGCTAGG- CTGCAGCTCCTC
Cloning vector	pGEX-6P1
Expression vector	pGEX-6P1
Expression host	<i>E. coli</i> BL21 Rosetta
Sequence of LGN ^{TPR} - afadin ^{PEPT} †	GPLGS ASCLELALAGERLCKSGDCRAGVSFFEEAAVQVG- TEDLKTLSAIYSQLGNAYFYLDYAKALEYHHDLT- LARTIGDQLGEAKASGNLNTLKVLFNDEAIVCCQ- RHLDISRELNDKVGEARALYNLGNVYHAKGKSFQCP- GPQDVGEFPEEVRDALQAAVDYFEEENLSLVTALGDR- AAQGRAFGNLGNTHYLLGNFRDAVIAHEQRLLIAKE- FGDKAAERRAYSNLGNAYIFLGEFETASEYKKTL- LARQLKDRAVEAQSCYSLGNTYTLLQDYEKADYHL- KHLAIAQELNDRIGEGRACWSLGNAYTALGNHDQAM- HFAEKHLEISREVGDQRNASYLKTVLSPDSLFTAK- FVAYNEEEEEEDCSLAG

† The afadin^{PEPT} sequence is underlined.

has been depicted as a molecular switch (Du & Macara, 2004) that is held inhibited in interphase by intramolecular interactions and opens up in mitosis to bind several interactors including the G α i subunits of heterotrimeric G proteins, the stem cell-specific cortical adaptor Inscuteable (Schaefer *et al.*, 2000; Mapelli & Gonzalez, 2012) and the major regulator of the microtubule motor dynein/dynactin, NuMA (nuclear mitotic apparatus protein; Du *et al.*, 2001). Less characterized ligands of LGN such as the actin-binding protein afadin have recently been identified in *Drosophila* (Wee *et al.*, 2011). In vertebrates, afadin is known as a junctional protein that is localized at the zona adherens and is required for epithelial organization (Ooshio *et al.*, 2007). Whether afadin is the positional cue restricting LGN at the lateral membrane during oriented epithelial divisions is an open issue, the elucidation of which will benefit from structural studies of the LGN–afadin interaction.

Structure–function analysis conducted in chicken neuro-epithelial cells (Peyre *et al.*, 2011), skin progenitors (Williams *et al.*, 2011) and monolayered epithelia (Zheng *et al.*, 2010) revealed that the function of LGN as a hub conveying mitotic signals that instruct spindle orientation is intimately connected to its domain structure. The C-terminal half of the molecule harbours four GoLoco motifs responsible for the recruitment of LGN at the membrane by direct binding to G α i subunits anchored to the lipid bilayer *via* myristoyl groups. The N-terminal domain of LGN consists of a contiguous array of eight tetratricopeptide repeats (TPRs) stacking in a super-helical arrangement. TPR motifs are degenerate 34-amino-acid units characterized by two antiparallel α -helices, generally referred to as A and B, which form the inner and outer surface of the superhelix, respectively (Das *et al.*, 1998). Intriguingly, most of the structurally characterized LGN^{TPR} interacting partners recognize the inner surface of the TPR scaffold *via* an intrinsically unstructured stretch that aligns perpendicularly with all eight A helices (Culurgioni *et al.*, 2011; Zhu *et al.*, 2011; Takayanagi *et al.*, 2015; Yuzawa *et al.*,

2011). Spiralling of the TPR α -solenoid around the elongated peptides results in a reduction of the intrinsic conformational flexibility of the helical array, which has proven to be a prerequisite for crystallizability of the domain. An analogous conformational stabilization of the TPRs has been observed in the complex of LGN^{TPR} with the last two LGN–GoLoco motifs, which recapitulates the architecture of the inhibited closed form of the molecule (Pan *et al.*, 2013).

Similar to other ligands such as Inscuteable and NuMA, the minimal LGN^{TPR}-binding region of afadin consists of a 37-residue peptide, which we will refer to as afadin^{PEPT}. Initial attempts to crystallize a complex reconstituted from purified LGN^{TPR} and a synthetic version of afadin^{PEPT} were not successful. Therefore, to gain insights into the architecture of the LGN^{TPR}–afadin^{PEPT} interaction, we engineered a chimeric protein carrying the LGN-binding region of afadin fused C-terminally to the LGN^{TPR} domain. We show that the afadin peptide of this chimeric protein is recognized intramolecularly by the TPR domain and that it stabilizes a compact conformation of the LGN^{TPR} superhelix required for the growth of diffraction-quality crystals. We also show that the amino-acid composition of the linker between the minimal interacting fragments of the two proteins is crucial in promoting crystal packing in sulfate-containing crystallization conditions.

2. Materials and methods

2.1. Generation of the chimeric fusion protein and macromolecule production

To reconstitute the interaction between human LGN (UniProt code B2RAL8) and afadin (UniProt code P55196, isoform 4), we used an LGN^{TPR} construct encompassing residues 15–350 and a fragment of afadin spanning residues 1709–1746 (afadin^{PEPT}). To stabilize the LGN^{TPR}–afadin^{PEPT} interaction, the sequence of afadin^{PEPT} was covalently linked to the C-terminus of LGN^{TPR} by means of a native polypeptide sequence derived from the ten residues of afadin upstream of afadin^{PEPT}. The entire sequence of the fusion protein is shown in Table 1, with afadin^{PEPT} underlined. To generate the chimeric construct, a two-step restriction-free cloning approach was employed (van den Ent & Löwe, 2006; Supplementary Fig. S1). Firstly, the coding sequence of the linker-afadin^{PEPT} cDNA was amplified using 50-base primers designed for the insertion of the PCR product into a previously generated pGEX-6P1-LGN^{TPR} vector. The forward primer carried a 24-base overlap with the vector, which was complementary to the 3'-end of the LGN^{TPR} construct, followed by 25 bases of linker-afadin^{PEPT}. The reverse primer annealed on the pGEX-6P1 vector with 24 bases complementary to the 3'-end of the point of insertion. The amplified PCR fragment was cleaned using a PCR purification kit (Qiagen) and diluted to 100 ng μ l⁻¹. A second amplification was conducted using 2–4 μ l of the PCR fragment previously produced to prime the PCR reaction of *PfuTurbo* polymerase (Agilent Technologies) on 50 ng pGEX-6P1-LGN^{TPR} vector in 50 μ l reaction mixture. The amplification

protocol consisted of 35 cycles, with an elongation step of 15 min. Once completed, 9 μ l of the PCR reaction was treated

Figure 1
Biochemical characterization of LGN^{TPR}-afadin^{PEPT}. (a) Comparative SEC analysis of LGN^{TPR}-afadin^{PEPT}, LGN^{TPR} and the LGN^{TPR}:afadin^{PEPT} complex performed on a Superdex 200 5/150 column (GE Healthcare) at 100 μ M concentration. For this analysis, the LGN^{TPR} complex was assembled by mixing synthetic afadin^{PEPT} with LGN^{TPR} in a 1:1.3 molar ratio. The protein elution profiles are overlaid with the trace of globular molecular-weight markers (grey dashed line). For all samples, the peak fractions were separated by SDS-PAGE and visualized by Coomassie staining. At the top left corner a schematic representation of the domain structure of the chimeric protein is shown, with LGN^{TPR} coloured green and afadin^{PEPT} purple. (b) The conformational heterogeneity of the LGN^{TPR}-afadin^{PEPT} fusion, the LGN^{TPR}:afadin^{PEPT} complex and LGN^{TPR} was assessed by native PAGE followed by Coomassie staining. Native molecular-weight markers (NativeMark, Life Technologies) are displayed in the first lane (labelled in kDa).

with DpnI (New England Biolabs) for 2 h at 310 K to digest the methylated parental plasmid and subsequently transformed into *Escherichia coli* DH10 competent cells (Top10, Invitrogen). Positive clones containing the GST-LGN^{TPR}-afadin^{PEPT} fusion gene were confirmed by DNA sequencing.

The chimeric construct GST-LGN^{TPR}-afadin^{PEPT} was expressed in *E. coli* BL21 Rosetta cells grown to an OD of 0.6 by induction with 0.5 mM IPTG overnight at 291 K (Table 1). Harvested cells were resuspended in 0.1 M Tris-HCl pH 8, 0.3 M NaCl, 10% glycerol, 0.5 mM EDTA, 1 mM DTT and were lysed with a French press. Lysates were cleared for 1 h at 100 000g before incubation with Glutathione Sepharose 4 Fast Flow beads (GE Healthcare) for 2 h at 277 K. After washes in lysis buffer supplemented with 0.7 M NaCl, the GST-fusion protein retained on the beads was incubated with PreScission protease (GE Healthcare) in a 1:50 weight ratio overnight at 277 K to remove the GST tag. The cleaved material, which retained the residues GPLGS preceding the LGN sequence (shown in bold in Table 1), was eluted from the beads in a desalting buffer consisting of 20 mM Tris-HCl pH 8, 40 mM NaCl, 5% glycerol, 1 mM DTT and loaded onto a Resource Q ion-exchange column (GE Healthcare). The protein was eluted with a 40–350 mM NaCl gradient over 20 column volumes. Peak fractions were analyzed by SDS-PAGE and pooled for a subsequent purification step on a preparative Superdex 200 size-exclusion chromatography (SEC) column equilibrated in 10 mM HEPES pH 7.5, 0.15 M NaCl, 5% glycerol. Peak fractions eluted from the SEC column were pooled and concentrated to about 12 mg ml⁻¹ using Vivaspin concentrators (Sartorius). The sample was stored at 193 K prior to use. Typically, about 7 mg pure protein was obtained starting from 1 l culture. The LGN^{TPR} used for SEC and native gel analyses was purified similarly, with a yield of about 3 mg pure protein from 1 l culture.

The LGN^{TPR}-afadin^{PEPT} construct has a molecular mass of about 41 kDa. Analytical SEC analysis revealed that the LGN^{TPR}-afadin^{PEPT} fusion elutes at the same volume as the LGN^{TPR}:afadin^{PEPT} complex assembled with a synthetic afadin peptide, but is slightly delayed compared with LGN^{TPR} in isolation (Fig. 1a). This result suggested that the LGN^{TPR}-afadin^{PEPT} chimera runs as a monodisperse monomer in solution, with an overall shape and hydrodynamical properties similar to those of the LGN^{TPR}:afadin^{PEPT} complex. To further corroborate this hypothesis, we compared the behaviour of the three samples on 12% Tris-glycine native PAGE (37:1 acrylamide:bisacrylamide). While LGN^{TPR} in isolation migrates on a native gel as a smear of multiple bands, the LGN^{TPR}-afadin^{PEPT} fusion runs with higher mobility as a single population (Fig. 1b), indicating that the fused afadin^{PEPT} stabilizes a compact conformation of the LGN^{TPR} superhelix. Consistently, the LGN^{TPR}:afadin^{PEPT} complex is less retarded than LGN^{TPR} but is more heterogeneous than the chimeric fusion construct. Collectively, these results provide good evidence that the fused peptide of afadin binds the TPR scaffold intramolecularly, inducing a homogeneous conformational rearrangement, rather than binding to a different molecule and causing heterogeneous aggregation.

Table 2
Crystallization.

Method	Sitting drop	Sitting drop	Hanging drop	Hanging drop, seeding
Plate type	96 × 3 well	96 × 3 well	24 well	24 well
Temperature (K)	293	293	288	277
Protein concentration (mg ml ⁻¹)	10	10	8	5
Buffer composition of protein solution	10 mM Na HEPES pH 7.5, 0.15 M NaCl, 5% glycerol, 4.5 mM TCEP	10 mM Na HEPES pH 7.5, 0.15 M NaCl, 5% glycerol, 4.5 mM TCEP	10 mM Na HEPES pH 7.5, 0.15 M NaCl, 5% glycerol, 4.5 mM TCEP	10 mM Na HEPES pH 7.5, 0.15 M NaCl, 5% glycerol, 4.5 mM TCEP
Composition of reservoir solution	0.1 M Na HEPES pH 7, 1.6 M ammonium sulfate	0.1 M bis-tris propane pH 7, 3.5 M sodium formate	0.1 M Tris-HCl pH 8, 1.4 M ammonium sulfate	0.1 M bis-tris propane pH 7, 1.0 M DL-malic acid
Initial hit	AmSO4 E10	SaltRX C7	AmSO4 E10	SaltRX C9
Volume and ratio of drop	100 nl (1:1 ratio)	100 nl (1:1 ratio)	1 µl (1:1 ratio)	1 µl (1:1 ratio)
Volume of reservoir (µl)	100	100	300	300
Screened using X-rays	No	No	Yes	Yes

These findings are consistent with the micromolar affinity that afadin^{PEPT} displays for LGN^{TPR} (with a dissociation constant K_d of 5.6 µM; Carminati *et al.*, 2016).

2.2. Crystallization

Initial crystallization trials of the LGN^{TPR}-afadin^{PEPT} sample utilized the commercial sparse-matrix screens SaltRX (Hampton Research), AmSO4 (Qiagen), JCSG-plus and Structure Screen 1 + 2 (Molecular Dimensions) and were conducted in sitting-drop vapour-diffusion format using a Cartesian Honeybee nanodispenser (Genomic Solutions) in three-square-well CrystalQuick Greiner plates. At the two concentrations tested (10 and 5 mg ml⁻¹), 100 nl protein solution was mixed with an equal volume of reservoir solution at 293 K. Crystals appeared at 10 mg ml⁻¹ after 1 d in about 20% of the salting-out conditions containing sulfate, phosphate, formate or malic acid ions at pH values between 7.0 and 8.5. The same crystallization trials conducted on a sample of LGN^{TPR} in complex with synthetic afadin^{PEPT} were unsuccessful. Since four of the linker residues present in the chimera have charged side chains, we reasoned that these linker residues could be crucial in promoting nucleation in the presence of high concentrations of multivalent ions. We selected conditions E10 from the AmSO4 kit (0.1 M Na HEPES pH 7,

1.6 M ammonium sulfate) and C7 and C9 from the SaltRX kit (0.1 M bis-tris propane pH 7, 3.5 M sodium formate and 0.1 M bis-tris propane pH 7, 1.2 M DL-malic acid pH 7.0, respectively) for manual optimization. All three conditions could be reproduced in hanging drops in 24-well VDX plates (Hampton Research) by mixing 1 µl protein solution with 1 µl reservoir solution. Under these conditions, crystallogenesis was very rapid at 293 K, yielding showers of small crystals in about 2 h or multiple crystals in the case of malic acid (Figs. 2*a* and 2*b*). To slow the crystal growth, we decided to perform grid screens around 0.1 M Na HEPES pH 7, 1.6 M ammonium sulfate at 288 K. The concentration of ammonium sulfate was varied from 1.35 to 1.6 M in 50 mM increments at pH values ranging from 7.5 to 8.25. Using this approach, single cubic crystals of around 0.5 mm in size (Fig. 2*c*) grew overnight. Further attempts to slow crystal nucleation were conducted at 277 K, seeding drops set up with a protein concentration of 5 mg ml⁻¹ and a reservoir solution consisting of 0.1 M Tris-HCl pH 8.0, 1.4 M ammonium sulfate after 3 h of equilibration (Table 2). Analogous optimizations were conducted around 0.1 M bis-tris propane pH 8.0, 3.5 M sodium formate and around 0.1 M bis-tris propane pH 7.0, 1.2 M malic acid. Single large crystals suitable for diffraction experiments were obtained at 288 K with 1.3 M ammonium sulfate or 1.0 M DL-malic acid or by seeding the same conditions at 277 K. To improve the crystal

Figure 2
(*a, b*) Initial crystals of LGN^{TPR}-afadin^{PEPT} grown manually by hanging-drop vapour diffusion in ammonium sulfate or malic acid. (*c*) After optimization at 288 K or seeding at 277 K, single cubic crystals of about 0.5 mm in size appear after 1 d of crystallization.

packing an additional additive screen was performed starting from these latter conditions, which unfortunately did not change either the crystal morphology or diffraction properties. As the crystals were fragile, they were cryoprotected by stepwise addition of ethylene glycol directly to the crystallization drops to a final concentration of 20% for 10–30 min prior to flash-cooling in liquid nitrogen.

2.3. Data collection and processing

X-ray diffraction data for LGN^{TPR}-afadin^{PEPT} crystals were collected on beamline X06DA at the Swiss Light Source (SLS), Villigen, Switzerland and on beamline ID23-2 at the European Synchrotron Radiation Facility, Grenoble, France. Data were processed using *XDS* (Kabsch, 2010) implemented in *xia2* (Winter *et al.*, 2013). The crystals diffracted to a reso-

lution of about 3.0 Å (Fig. 3*a*) and belonged to the cubic space group *P2*₁3. The unit-cell parameters are consistent with two copies of the fusion protein per asymmetric unit, with a Matthews coefficient of 4.8 Å³ Da⁻¹ and a solvent content of 75%. The high solvent content partly explains the modest resolution of the data despite the relatively large dimensions of the crystals, as often observed by Kantardjieff & Rupp (2003) and Weichenberger & Rupp (2014). Based on the Patterson function, no translational pseudosymmetry exists between the two molecules in the asymmetric unit. The structure of LGN^{TPR}-afadin^{PEPT} was determined by molecular replacement with *Phaser* (McCoy *et al.*, 2007) using the coordinates of mouse LGN^{TPR} solved previously in complex with NuMA (PDB entry 3ro2; Zhu *et al.*, 2011) as a search model. The afadin peptide was built manually by iterative cycles in *Coot* (Emsley *et al.*, 2010) and refinement in *PHENIX* (Adams

Figure 3

Diffraction and packing of the LGN^{TPR}-afadin^{PEPT} crystals. (a) 3.0 Å resolution X-ray diffraction pattern of LGN^{TPR}-afadin^{PEPT} obtained using a synchrotron-radiation source on beamline X06DA at the Swiss Light Source. (b) LGN^{TPR}-afadin^{PEPT} model and composite OMIT electron-density map displayed around the afadin^{PEPT} region contoured at the 1.0σ level. (c) Crystal lattice of LGN^{TPR}-afadin^{PEPT} with the two molecules of the asymmetric unit shown as gold and green cartoons and the sulfate ions shown as red spheres. For all molecules, the native linker of afadin^{PEPT} is displayed in purple. (d) Enlarged view of the crystal contacts contributed to by the side chains of Arg86^{LGN} and Arg120^{LGN} and Gln1718^{afadin} of the two LGN^{TPR}-afadin^{PEPT} fusion proteins present in the asymmetric unit.

Table 3

Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	X06DA, SLS
Wavelength (Å)	1.008
Temperature (K)	100
Detector	PILATUS 2M
Crystal-to-detector distance (mm)	305
Rotation range per image (°)	0.3
Total rotation range (°)	60.3
Exposure time per image (s)	5
Space group	$P2_13$
a, b, c (Å)	170.3
α, β, γ (°)	90
Mosaicity (°)	0.2
Resolution range (Å)	69.5–3.00 (3.07–3.00)
Total No. of reflections	223474
No. of unique reflections	33516
Completeness (%)	99.9 (100.0)
Multiplicity	6.7 (6.4)
$\langle I/\sigma(I) \rangle$	18.6 (2.5)
R_{meas}	0.109 (1.022)
Overall B factor from Wilson plot (Å ²)	75.6

et al., 2010). The current model was refined at 3.0 Å resolution to an R_{free} of 26.7% and an R_{work} of 23.3%, with good electron density for the afadin^{PEPT} residues (Fig. 3*b*). Data-collection statistics are given in Table 3. The sulfate ions, which were contained in the reservoir, are coordinated by Arg86^{LGN}, Arg120^{LGN} and by Gln1718^{afadin} of afadin^{PEPT} belonging to a symmetry-related molecule, thus promoting crystal packing (Figs. 3*c* and 3*d*). Importantly, direct contacts between the very C-terminus of afadin^{PEPT} of one of the molecules in the asymmetric unit (namely residues Cys-Ser-Leu-Ala) and the TPR repeats of the second molecule in the same asymmetric unit also contribute to the crystal packing. However, crystals of LGN^{TPR} in complex with synthetic afadin^{PEPT} grown by seeding with nuclei of the LGN^{TPR}-afadin^{PEPT} fusion protein, which retain the afadin^{PEPT} contacts but lack those of the linker region, were very tiny (0.03–0.05 µm in size) and diffracted only to about 30 Å resolution. Thus, we conclude that the presence of the afadin linker is the molecular explanation for the good diffraction quality of the crystals of the LGN^{TPR}-afadin^{PEPT} chimera.

3. Results and discussion

In this study, we report the generation and crystallization of a fusion protein between the LGN^{TPR} domain and its binding peptide from afadin as an instrumental step in obtaining well diffracting crystals of the two interacting proteins. Chimeric fusions between low-affinity interactors are known to promote the formation of stoichiometric complexes for structural studies (Pellegrini *et al.*, 2002). Although natively occurring interdomain linkers consist of residues as diverse as Pro, Arg, Phe, Thr, Glu and Gln (George & Heringa, 2002; Kobe *et al.*, 2015), historically for crystallization purposes the linker region between the two binding domains has been chosen as the shortest flexible stretch of glycines and serines that allows unrestrained recognition of the two partners (Reddy Chichili *et al.*, 2013). For our structural investigations, we decided to

covalently join LGN^{TPR} to the LGN-binding domain of afadin using the sequence of afadin upstream of the minimal LGN-binding peptide. When mixed with multivalent anion-containing reservoirs, the resulting chimeric fusion readily yielded cubic crystals. The structure of the fusion construct revealed that crystal packing is contributed to by positively charged residues of LGN^{TPR} and a Gln side chain of the afadin linker, which coordinate sulfate ions. This evidence explains the improved crystallizability of the chimera compared with LGN^{TPR} in complex with synthetic afadin^{PEPT}, which yielded only poorly diffracting crystals upon seeding with nuclei of the chimeric fusion construct. Thus, we can conclude that in this case the use of a native linker sequence coupled the advantage of forming stable complexes between LGN and afadin with the beneficial effect of having chemically active side chains of the linker promoting crystal packing.

Interestingly, the topology of LGN^{TPR}-afadin^{PEPT} is reminiscent of the recognition mode of other helical repeat-containing proteins with their cognate ligands, such as E-cadherin in complex with β -catenin (Huber & Weis, 2001) and karyopherin α bound to NLS peptides (Conti *et al.*, 1998). We predict that the engineering method presented in this study will be particularly successful in investigating the organizational principles of similar α -solenoids in complex with their binding partners.

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References

- Adams, P. D. *et al.* (2010). *Acta Cryst.* **D66**, 213–221.
- Carminati, M., Gallini, S., Pirovano, L., Alfieri, A., Bisi, S. & Mapelli, M. (2016). *Nature Struct. Mol. Biol.*, doi:10.1038/nsmb.3152.
- Conti, E., Uy, M., Leighton, L., Blobel, G. & Kuriyan, J. (1998). *Cell*, **94**, 193–204.
- Culurgioni, S., Alfieri, A., Pendolino, V., Laddomada, F. & Mapelli, M. (2011). *Proc. Natl Acad. Sci. USA*, **108**, 20998–21003.
- Das, A. K., Cohen, P. W. & Barford, D. (1998). *EMBO J.* **17**, 1192–1199.
- Du, Q. & Macara, I. G. (2004). *Cell*, **119**, 503–516.
- Du, Q., Stukenberg, P. T. & Macara, I. G. (2001). *Nature Cell Biol.* **3**, 1069–1075.
- Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. (2010). *Acta Cryst.* **D66**, 486–501.
- Ent, F. van den & Löwe, J. (2006). *J. Biochem. Biophys. Methods*, **67**, 67–74.
- George, R. A. & Heringa, J. (2002). *Protein Eng.* **15**, 871–879.
- Huber, A. H. & Weis, W. I. (2001). *Cell*, **105**, 391–402.
- Kabsch, W. (2010). *Acta Cryst.* **D66**, 133–144.
- Kantardjieff, K. A. & Rupp, B. (2003). *Protein Sci.* **12**, 1865–1871.
- Knoblich, J. A. (2010). *Nature Rev. Mol. Cell Biol.* **11**, 849–860.
- Kobe, B., Ve, T. & Williams, S. J. (2015). *Acta Cryst.* **F71**, 861–869.
- Mapelli, M. & Gonzalez, C. (2012). *Open Biol.* **2**, 120102.

- McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
- Morin, X. & Bellaïche, Y. (2011). *Dev. Cell*, **21**, 102–119.
- Ooshio, T., Fujita, N., Yamada, A., Sato, T., Kitagawa, Y., Okamoto, R., Nakata, S., Miki, A., Irie, K. & Takai, Y. (2007). *J. Cell Sci.* **120**, 2352–2365.
- Pan, Z., Zhu, J., Shang, Y., Wei, Z., Jia, M., Xia, C., Wen, W., Wang, W. & Zhang, M. (2013). *Structure*, **21**, 1007–1017.
- Pellegrini, L., Yu, D. S., Lo, T., Anand, S., Lee, M., Blundell, T. L. & Venkitaraman, A. R. (2002). *Nature (London)*, **420**, 287–293.
- Peyre, E., Jaouen, F., Saadaoui, M., Haren, L., Merdes, A., Durbec, P. & Morin, X. (2011). *J. Cell Biol.* **193**, 141–154.
- Reddy Chichili, V. P., Kumar, V. & Sivaraman, J. (2013). *Protein Sci.* **22**, 153–167.
- Schaefer, M., Shevchenko, A., Shevchenko, A. & Knoblich, J. A. (2000). *Curr. Biol.* **10**, 353–362.
- Takayanagi, H., Yuzawa, S. & Sumimoto, H. (2015). *Acta Cryst.* **F71**, 175–183.
- Wee, B., Johnston, C. A., Prehoda, K. E. & Doe, C. Q. (2011). *J. Cell Biol.* **195**, 369–376.
- Weichenberger, C. X. & Rupp, B. (2014). *Acta Cryst.* **D70**, 1579–1588.
- Williams, S. E., Beronja, S., Pasolli, H. A. & Fuchs, E. (2011). *Nature (London)*, **470**, 353–358.
- Winter, G., Lobley, C. M. C. & Prince, S. M. (2013). *Acta Cryst.* **D69**, 1260–1273.
- Yuzawa, S., Kamakura, S., Iwakiri, Y., Hayase, J. & Sumimoto, H. (2011). *Proc. Natl Acad. Sci. USA*, **108**, 19210–19215.
- Zheng, Z., Zhu, H., Wan, Q., Liu, J., Xiao, Z., Siderovski, D. P. & Du, Q. (2010). *J. Cell Biol.* **189**, 275–288.
- Zhu, J., Wen, W., Zheng, Z., Shang, Y., Wei, Z., Xiao, Z., Pan, Z., Du, Q., Wang, W. & Zhang, M. (2011). *Mol. Cell*, **43**, 418–431.