Dimerization properties of a *Xenopus laevis* kinesin-II carboxy-terminal stalk fragment

Valeria De Marco¹, Ario de Marco¹, Kenneth N. Goldie¹, John J. Correia² & Andreas Hoenger^{1*}

¹European Molecular Biology Laboratory, Heidelberg, Germany, and ²Department of Biochemistry, University of Mississippi Medical Center, Jackson, Mississippi, USA

We have analysed the structural and physical properties of the carboxy-terminal stalk region of a kinesin-II, *Xenopus* kinesin-like protein 3A/B (Xklp3A/B), which we showed to be essential for heterodimerization in a previous work (De Marco *et al.*, 2001). We expressed the corresponding A-stalk and B-stalk fragments and investigated their modes of interaction by analytical ultracentrifugation (AUC), circular dichroism spectroscopy, denaturation assays and electron microscopy. Co-expression of the A-stalk and B-stalk produced the properly folded, hetero-dimeric coiled coil at high yields. The dimeric nature of the complex was confirmed by AUC. We also found that the isolated A-stalk fragment forms a stable helix by itself and shows a significant tendency towards homodimer and higher-order complex formation. In the absence of the corresponding A-stalk fragment, the isolated B-stalk fragment remains partially unfolded, which suggests that the A-stalk provides a template structure for the B-stalk in order to recompose the complete heterodimeric coiled coil.

EMBO reports 4, 717–722 (2003)

doi:10.1038/sj.embor.embor884

INTRODUCTION

Kinesins are a large family of molecular motors that mediate microtubule-based motility in cells. They are composed of an ATP- and microtubule-binding motor-head, a stalk region and a cargo-binding domain. Whereas the motor-head domains are well conserved among all kinesin family members, the stalk and cargo-binding regions are highly variable and are individually adapted to their tasks in cells (for a review, see Hirokawa, 1998; Vale, 2003).

kinesin-II family members (for a review, see Marszalek & Goldstein, 2000) are heterodimeric complexes composed of two different heavy chains and a non-motor, kinesin-associated polypeptide (KAP). In mammals, kinesin-II members have a unique modular ability to dimerize with various partners, adapting to specialized

transport tasks in different tissues (Muresan *et al.*, 1998; Yang & Goldstein *et al.*, 1998). Dimerization of the motor subunits is mediated by a heterodimeric coiled-coil interaction of their stalk regions.

In our previous work on kinesin-II (De Marco *et al.*, 2001), we analysed the dimerization properties of a kinesin-II motor unit from *Xenopus laevis*, *Xenopus* kinesin-like protein 3A/B (Xklp3A/B; Tuma *et al.*, 1998). kinesin-II family members have a unique charged region in their stalk, at the carboxy-terminal end of the neck region. This region has opposite charges in the corresponding partners, which suggested an involvement in the process of heterodimerization and partner recognition (Rashid *et al.*, 1995; Chana *et al.*, 2002). However, our results described in De Marco *et al.* (2001) suggest a different mechanism. We showed that the crucial regions for heterodimerization of Xklp3A and Xklp3B are located at the C-terminal end of the stalk. Immunoprecipitation experiments showed that the removal of the neck and the charged region did not affect heterodimerization or induce homodimerization of the two subunits. Unlike conventional kinesins, the regions essential for dimerization extend over two adjacent coiled-coil stretches (De Marco *et al.*, 2001).

This work presents a follow-up investigation of the results published in De Marco *et al.* (2001) and consists of an analysis of the structural and physical properties of the C-terminal stalk regions. We cloned and expressed two C-terminal coil fragments, of 112 and 113 residues, of the Xklp3A and Xklp3B stalks, respectively, which are hereafter referred to as the A-coil and B-coil (Fig. 1A). Using circular dichroism (CD) spectroscopy, we found that the isolated A-coil is able to form a stable helix that seems to function as a template for the B-coil to fold into a heterodimeric complex. The B-coil alone is unstable. Co-expression of the two stalks revealed a properly folded, heterodimeric coiled-coil fragment at high yields, suitable for ultracentrifugation, denaturation experiments, crystallization trials and visualization by electron microscopy (EM).

RESULTS AND DISCUSSION

The motor unit of Xklp3A/B forms a heterodimeric complex that is mediated by a coiled-coil interaction in the stalk domains. The ATP-binding Xklp3A and Xklp3B motor domains are followed by a short neck, a stretch of charged residues and an α -helical coiled coil, which can be divided into three discrete subdomains

¹European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany

²Department of Biochemistry, University of Mississippi Medical Center, 2500 North State Street, Jackson, Mississippi 39216, USA

*Corresponding author. Tel: +49 6221 387453; Fax: +49 6221 387519; E-mail: hoenger@embl-heidelberg.de

Received 1 April 2003; revised 8 May 2003; accepted 20 May 2003

Fig. 1 | Domain organization of Xklp3A/B. **(A)** Xklp3A/B is a heterodimeric kinesin-II motor protein composed of an A-chain and a B-chain. The carboxy-terminal region shown between the red lines is essential for heterodimerization (De Marco *et al.*, 2001). Here, we constructed two peptides that included the C-terminal ends of the stalk domains, as outlined in **(A)** and shown in detail in **(B)**. **(B)** The polypeptide sequence of the A-coil and B-coil analysed in this study. Red lines indicate potential attractive interactions between the two coils. Blue bars mark the 'a' and 'd' positions of the coiled-coil core, which form strong hydrophobic interactions. A FIP motif on both strands marks the transition into the tail domain. Grey bars indicate core 'a' and 'd' positions; the green bar indicates the stutter ('h').

separated by stutters (Fig. 1A). The elements responsible for heterodimer formation are two coiled-coil segments, which are located at the C-terminal end of the stalk (shown in red in Fig. 1A; De Marco *et al.*, 2001). The arrangement of heptad repeats in this segment is shown in Fig. 1B. Red lines indicate the extensive network of interhelical salt bridges that extend along coiled-coil region II, which promote heterospecific pairing and prevent homodimerization. Here, we expressed two constructs, composed of amino acids 485–597 for the A-fragment and 479–592 for the B-fragment (Fig. 1A). Positions 597 in the A-coil and 592 in the B-coil are both prolines (Fig. 1B) and mark the termination of the coiled-coil interaction and the transition into the tail domains.

The A-coil and B-coil form a stable dimer

The A-coil and B-coil fragments were both cloned in a pET-derived vector that was designed specifically for the expression of small protein domains. The polypeptide chains were expressed as fusions with N-utilization substance A (NusA) in *Escherichia coli* strains that co-express chaperones (Tomoyasu *et al.*, 2001). To facilitate the study of the heterodimeric coiled coil, the A-coil and B-coil fragments were cloned in a polycistronic vector (Tan, 2001) and co-expressed with chaperones to promote dimer formation and improve heterodimer stability (see the Methods section). The homogeneity of the recombinant proteins after the purification procedure was assessed by tricine-SDS-polyacrylamide gel electrophoresis (PAGE; Fig. 2A), which gave single bands for the A-coil and B-coil that were consistent in

size with their predicted molecular masses (A-coil, 13.9 kDa; B-coil, 14.3 kDa; Fig. 2A, lanes 1 and 2). Similarly, analysis of the co-expressed recombinant complex by tricine-SDS-PAGE indicated the presence of two components of the predicted molecular masses and in the expected 1:1 ratio. For a preliminary evaluation of the composition and quaternary structure of the recombinant proteins, samples were analysed by native PAGE on a gradient gel. In the moderate salt concentration used for our analysis, the individually expressed A-coil runs as a smeared band under native conditions (Fig. 2B, lane 1), indicating the co-existence of different oligomerization states. The B-coil, however, runs as a sharp band (Fig. 2B, lane 2), indicating a single association state. The co-expressed complex runs as a single band under non-denaturing conditions (Fig. 2B, lane 3), which confirms the association of the two subunits in a stable complex.

Analytical ultracentrifugation results

To establish precisely the native state of the A-coil, the B-coil and the heterodimeric complex, we performed sedimentation velocity runs in an analytical ultracentrifuge. Under the conditions of our analysis (similar to the ones used for native PAGE), the A-coil showed a non-homogeneous composition. The fitting of the runs suggested the presence of a homodimeric species ($S_{20,w} \sim 2.94$; molecular weight $\sim 28,600$ Da) that co-exists with a tetrameric complex ($S_{20,w} \sim 4.60$; molecular weight $\sim 54,100$ Da). At the concentrations used in our study, the homodimer is the predominant form and the dimer-tetramer reaction seems to be reversible, although the kinetics may be slow (data not shown). Due to the low absorbance at 280 nm, the B-coil

Fig. 2 | Gel electrophoresis and analytical ultracentrifugation analysis of the heterodimeric A/B complex. **(A)** 10% Tris-tricine gel showing the isolated A-coil (lane 1), B-coil (lane 2) and the A/B complex (lane 3). **(B)** Native polyacrylamide gel electrophoresis using an 8–25% gradient gel on which the isolated A-coil (lane 1), B-coil (lane 2) and the heterodimeric A/B complex (lane 3) were run. **(C)** Sedimentation velocity runs of three independently purified A/B complex samples at different concentrations at 24.7 °C. The green curve shows a Cys-light mutant, which was created to demonstrate that dimer formation is not caused by cysteine bridges. Data were analysed as described in the Methods section. This confirmed that the complex behaves as a tight dimer composed of A-coils and B-coils in a 1:1 ratio, with an average $S_{20,w}$ of 2.286 ± 0.061 and a molecular weight of $29,160 \pm 596$ Da. The sedimentation coefficient ($C(S)$) distribution was determined by a method in Sedfit, as described in the Methods section.

was analysed as a fusion with NusA. Analysis of the NusA–B-coil data showed a weakly concentration-dependent zone with a slight tendency (dissociation constant (K_d) $\sim 100 \mu M$) to self-associate in a dimeric form. Extrapolation of the peak position suggests a monomeric $S_{20,w}$ value of 3.822. Sedimentation runs of the co-expressed A/B-complex confirmed the presence of a unique species of the predicted size for a heterodimer ($S_{20,w}$ 2.286 ± 0.061 ; molecular weight $29,160 \pm 596$ Da) composed of the A-coil and B-coil in a 1:1 ratio (Fig. 2C).

A-coils and B-coils show different folding properties

Far-ultraviolet CD spectroscopy analysis was used to investigate the folding patterns of the isolated A-coils and B-coils as well as that of the heterodimeric AB-coil (Fig. 3A–C; Table 1). The CD spectrum for the A-coil alone shows a profile typical of predominantly α -helical folding, with double minima at 208 nm and 222 nm (Fig. 3A–C). The helical content of the A-coil fragment in benign buffer is 75% (Fig. 3A) and can be increased to a completely α -helical peptide by adding 50% trifluoroethanol (TFE; Sönnichsen *et al.*, 1992). By contrast, the isolated B-coil fragment shows a significantly lower α -helical content (40%; Fig. 3B), which can be increased only to 67% in the presence of 50% TFE. As expected, the heterodimeric A/B coiled coil has an even higher α -helical content than the A-coil (Fig. 3C). Addition of TFE destroys the coiled-coil interaction, as indicated by the reversed ratio of $[\Theta]_{208}/[\Theta]_{222}$ (where $[\Theta]$ is the mean residue ellipticity at a given wavelength; Fig. 3C); the increased overall ellipticity of the sample reflects the increase in helical content of two single subunits.

We further tested the unfolding properties of the A-coil, B-coil and the heterodimeric complex by monitoring the change in mean residue ellipticity at 222 nm with increasing amounts of urea (0–5 M; Fig. 3D). These comparisons show that the isolated B-coil is unstable and unfolds quickly. The isolated A-coil is more stable and shows an unfolding transition typical of a monomeric event, with a $[\text{urea}]_{1/2}$ (the concentration of urea that produces 50% unfolding) of 1.75 M and a calculated free energy of unfolding of 9.1 kcal mol^{−1} (Table 1). These values indicate that the homo-oligomerization forces are relatively weak. The urea denaturation curve for the heterodimeric A/B-complex was initially fitted assuming a two-state process, in which only the folded dimer or two unfolded monomers exist. The analysis indicated a $[\text{urea}]_{1/2}$ of 1.55 M. This value is lower than that calculated for the A-coil, suggesting that a transition intermediate might exist, which could be an A-coil in a fully or partially folded state. This is confirmed by the overlapping profiles of denaturation of the A-coil and the heterodimeric complex at a urea concentration higher than 1.5 M (Fig. 3D), due to the dominating effect of A-coil denaturation on the entire process.

Co-expression of the A-coil and B-coil showed stable heterodimeric complexes at high yields suitable for crystallization trials, which have so far yielded preliminary crystals (Fig. 4B) that diffract to only 0.7 nm. Nevertheless, the potential to form crystals indicates the presence of a uniform species. The length and shape of the heterodimeric complex can be estimated from EM images of low-angle rotary-shadowed preparations. According to such estimations, the length of the complex is in the order of 16 nm, consistent with the calculated length of a coiled-coil stretch composed of 16 heptad repeats (Fig. 4A).

Fig. 3 | Circular dichroism spectroscopy and urea denaturation profiles of the A-coil, B-coil and the heterodimeric A/B-complex. **(A)** Circular dichroism (CD) spectroscopy shows the differences in helical propensities of the isolated constructs. The A-coil has a helical content of 75% (solid line), which increases to 90% after adding 50% trifluoroethanol (TFE; dotted line). **(B)** By contrast, the B-coil has only 40% helical content, which increases to 70% in the presence of TFE. **(C)** As expected, the heterodimeric complex (blue) showed the highest helical content (80%). Adding 50% TFE disrupted the coiled-coil interaction. **(D)** Denaturation profiles of the A-coil, B-coil and the heterodimeric A/B-complex, measured by monitoring the change of mean residue ellipticity at 222 nm ($[\Theta]_{222}$) by adding urea. As expected, the B-coil (green) showed a rapid transition to a completely unfolded form, whereas the A-coil (red) unfolds as expected for a monomeric event. The curve of the heterodimeric complex (blue) shows a slight shoulder at the beginning (arrow), but from there it seems to unfold similarly to a monomeric event. See Table 1 for more details.

CONCLUSIONS

Here, we demonstrate that a C-terminal Xklp3A/B stalk fragment composed of coiled-coil segments II and III (according to De Marco *et al.*, 2001) forms a stable, heterodimeric coiled coil (Fig. 4A). On the basis of the different stabilities and folding properties of the isolated A-coil and B-coil, we suggest the following mechanism: the A-coil folds into a stable α -helical coil by itself, which is then used by the B-coil as a template to complete the heterodimeric complex, overriding the slight tendency of the A-coil to form a homodimer.

Our findings confirm that the charged regions (Rashid *et al.*, 1995; Chana *et al.*, 2002) are not necessary for inducing specific hetero pairing. At present, the three-dimensional structures of the charged regions within the entire molecule are unknown and the opposite charge distribution that is observed in their primary sequences might not be relevant for heterodimer recognition in the folded molecule. In any case, we have shown here that the C-terminal regions of the A-stalk and B-stalk have the potential to fold into a stable, heterodimeric coiled coil (Figs 1B,2). This C-terminal region of kinesin-II family members is highly

Table 1 | Summary of circular dichroism analysis

Polypeptide	[Θ] ₂₂₂ (deg × cm ² × dmol ⁻¹)		Helical content* (%)	No. res.	[Urea] _{1/2} (M)	ΔG ^{H₂O} (kcal mol ⁻¹)
	Benign	50% TFE				
A-coil	-28,750	-35,160	75	112	1.75	9.1
B-coil	-16,500	-25,590	42	113	–	–
AB-coil	-31,450	-38,870	82	112 + 113	1.55	n.d.

*Calculated from the ratio of the observed [Θ]₂₂₂ divided by the predicted molar ellipticity × 100. The predicted molar ellipticity was calculated from [Θ]₂₂₂ = 40 × 10³ × (1 – 4.6/*n*), where *n* is the number of residues in the peptide. [Urea]_{1/2} is the transition midpoint. ΔG^{H₂O} is the free energy of unfolding in the absence of denaturant (according to Tripet & Hodges, 2002). Deg, degrees; no. res., number of residues; TFE, trifluoroethanol.

Fig. 4 | Imaging of A/B heterodimeric carboxy-terminal coiled-coil fragments. (A) Low-angle rotary shadowing of A/B heterodimeric complexes reveals their elongated shape, with an estimated length of ~16 nm. Red arrows indicate selected, well-stained examples. (B) Crystallization trials produced crystals, which diffract to 0.7 nm. The potential to form ordered crystals indicates the presence of a uniformly shaped A/B heterodimeric complex. Scale bar, 50 nm.

conserved (>95% sequence identity) among the corresponding partners (see figure 2 in De Marco *et al.*, 2001), indicating that our findings represent a general property of kinesin-II family members rather than an Xklp3A/B-specific phenomenon.

SPECULATION

It is not yet clear why heterodimeric motors evolved and what the mechanistic advantages for transmitting force and movement could be. Unlike homodimeric coiled coils, heterodimeric coiled coils require a complicated pattern of positively and negatively charged partners on both sides, which is repulsive for homodimer formation and attractive for heterodimer formation. However, as a side-effect, this enables these complexes to change partners. This seems to be an economic approach of the cell to creating two different heterodimeric motors with only three chains, allowing for a quick adaptation to changes in the cellular environment (Muresan *et al.*, 1998; Yang & Goldstein, 1998). These complexes can be disrupted rapidly by taking advantage of the lower stability of one of the two components. The unfolding of the A/B complex seems to be induced by the rapid unfolding of the B-chain, whereas the A-coil maintains its structured conformation, functioning as a template for either of the partners.

METHODS

Cloning, expression and purification of the A-coil, B-coil and A/B-coil. Full-length complementary DNA clones of *X. laevis* Xklp3A (de Marco *et al.*, 2001) and Xklp3B (Le Bot *et al.*, 1998) were used as templates for the PCR amplification of DNA fragments that encode residues 485–597 (A-coil) and 479–592 (B-coil), respectively. For expression of the single subunits, oligonucleotides were designed to introduce *Nco*I and *Xho*I sites at the 5' and 3' ends, respectively. The amplified products were ligated into a pET-derived vector downstream of the *NusA* gene (Davis *et al.*, 1999) and sequenced.

For co-expression, oligonucleotides were designed to contain *Nco*I and *Bam*HI sites at the 5' and 3' ends of the A-coil construct and *Nde*I and *Bam*HI sites at the 5' and 3' ends, respectively, of the B-coil construct. The B-coil amplified product was ligated into a pET3aTr vector (Tan, 2001), excised by digestion with *Eco*RI and *Hind*III and recloned in the second cassette of the polycistronic vector pST39, which was modified to contain the His₆ tag, the tobacco etch virus (TEV) cleavage site and the multiple cloning site of the pETM60 vector in the first cassette. The A-coil amplified fragment was subcloned directly into pST39 by using the *Nco*I and *Bam*HI sites of the introduced pETM60 multiple cloning site. All constructs were sequenced. Plasmids were used to transform *E. coli* strains that co-expressed chaperones (Tomoyasu *et al.*, 2001). Crude extracts were poured onto HiTrap Chelating HP (Amersham Bioscience) charged with Co²⁺. The NusA A-coil and B-coil and the histidine-tagged A/B-coil affinity eluates were incubated with TEV protease (0.01 mg per mg of target protein) for 2 h at 30 °C, diluted three times in water and loaded onto a HiTrap Q HP (Amersham Bioscience) and eluted with a linear gradient of 0–500 mM NaCl, supplemented with 10mM sodium phosphate, pH 7. A-coils and B-coils were typically eluted at 250 mM salt, and the complex at ~300 mM salt. The A/B-complex was also loaded onto a Superose-12 (Amersham Bioscience) run with 10 mM sodium phosphate, 300 mM NaCl and 1 mM DTT. Purity and homogeneity were assessed by tricine–SDS–PAGE. Protein concentration was calculated by measuring the absorbance at 280 nm.

Native polyacrylamide gel electrophoresis. Samples in benign buffer were loaded onto a PhastGel gradient 8–25 and run in PhastGel Native Buffer Strips (Amersham Bioscience) in accordance with the manufacturer's instructions.

Analytical ultracentrifugation. Sedimentation velocity runs were performed in an XLA analytical ultracentrifuge at 40,000–45,000 r.p.m. at 24.7 °C. Temperature was calibrated by the method of Liu & Stafford (1995). Data were first analysed by the C(S) method using Sedfit (Schuck *et al.*, 2002). When appropriate, data sets were also analysed as one or two non-interacting species to extract best estimates for S_{20,w} and molecular weight. Data from the A-coil

monomer was also analysed with DCDT+ (Philo, 2000) to generate g(s) curves. Buffer densities were measured at the appropriate temperature in an Anton Paar DMA 5000, viscosity and v_{bar} (partial specific volume) were estimated using Sednterp (Laue *et al.*, 1992), and all data were corrected to $S_{20,w}$.

Circular dichroism spectroscopy. Far-ultraviolet CD spectra were recorded using a Jasco-710 spectropolarimeter calibrated with (1S)-(+)-10-camphorsulphonic acid. Protein samples were analysed at a concentration of 10 μM in 10 mM sodium phosphate, pH 7.0, at 5 °C. Data were collected from 190–250 nm at 0.1-nm intervals recorded at 50 nm min⁻¹; measurements were averaged for 20 scans.

The mean residue molar ellipticity $[\theta]$ (deg $\times$ cm² $\times$ dmol⁻¹) was calculated according to the formula:

$$[\theta] = \theta / (N_A \times d \times C_M \times 10)$$

where θ is the measured ellipticity in degrees, N_A is the number of amino acids per protein, d is the path length in centimetres and C_M is the molar protein concentration. The factor 10 originates from the conversion of the molar concentration to the dmol cm⁻³ concentration unit.

For urea denaturation studies, the proteins, at concentrations of 2.5–3.0 μM were dissolved in 10 mM sodium phosphate, pH 7.0. The concentration of urea was increased stepwise from 0 to 5 M. Initial and final denaturant concentrations were determined by refractometry. Denaturation mid-points, slopes and free energy of unfolding were determined according to Tripet & Hodges (2002).

Glycerol-spraying rotary-shadowing electron microscopy. Samples for EM were prepared in 50% glycerol, 0.1 M NH₄Ac, at a concentration of 0.7 mM. This solution was sprayed onto mica using a homemade nebulizer. The mica preparations were air-dried and then rotary shadowed at an elevation angle of 5° in a Balzers BAF-300 using platinum–carbon. Micrographs were taken in a CM120-Biotwin on a GATAN-694 CCD camera.

ACKNOWLEDGEMENTS

We thank P. Burkhard and K. Scheffzek for their help with crystallization trials.

REFERENCES

Chana, M., Tripet, B.P., Mant, C.T. & Hodges, R.S. (2002) The role of unstructured highly charged regions on the stability and specificity of dimerization of two-stranded α -helical coiled-coils: analysis of the neck-hinge region of the kinesin-like motor protein Kif3A. *J. Struct. Biol.*, **137**, 206–219.

- Davis, G.D., Elisei, C., Newham, D.M. & Harrison, R.G. (1999) New fusion protein systems designed to give soluble expression in *Escherichia coli*. *Biotechnol. Bioeng.*, **65**, 382–388.
- De Marco, V., Burkhard, P., Le Bot, N., Vernos, I. & Hoenger, A. (2001) Analysis of heterodimer formation by Xklp3A/B, a newly cloned kinesin-II from *Xenopus laevis*. *EMBO J.*, **20**, 3370–3379.
- Hirokawa, N. (1998) Kinesin and dynein superfamily proteins and the mechanism of organelle transport. *Science*, **279**, 519–526.
- Laue, T.M., Shah, B.D., Ridgeway, T.M. & Pelletier, S.L. (1992) in *Analytical Ultracentrifugation in Biochemistry and Polymer Sciences* (eds Harding, S.E., Rowe, A.J. & Horton, J.C.), 90–125. Royal Society of Chemistry, Cambridge, UK.
- Le Bot, N., Antony, C., White, J., Karsenti, E. & Vernos, I. (1998) Role of Xklp3, a subunit of the *Xenopus* kinesin II heterotrimeric complex, in membrane transport between the endoplasmic reticulum and the Golgi apparatus. *J. Cell Biol.*, **143**, 1559–1573.
- Liu, S. & Stafford, W.F. (1995) An optical thermometer for direct measurement of cell temperature in the Beckman instruments XL-A analytical ultracentrifuge. *Anal. Biochem.*, **224**, 199–202.
- Marszalek, J.R. & Goldstein, L.S. (2000) Understanding the functions of kinesin-II. *Biochim. Biophys. Acta*, **1496**, 142–150.
- Muresan, V., Abramson, T., Lyass, A., Winter, D., Porro, E., Hong, F., Chamberlin, N.L. & Schnapp, B.J. (1998) KIF3C and KIF3A form a novel neuronal heteromeric kinesin that associates with membrane vesicles. *Mol. Biol. Cell*, **9**, 637–652.
- Philo, J.S. (2000) A method for directly fitting the time derivative of sedimentation velocity data and an alternative algorithm for calculating sedimentation coefficient distribution functions. *Anal. Biochem.*, **179**, 151–163.
- Rashid, D.J., Wedaman, K.P. & Scholey, J.M. (1995) Heterodimerization of the two motor subunits of the heterotrimeric kinesin, KRP85/95. *J. Mol. Biol.*, **252**, 157–162.
- Schuck, P., Perugini, M.A., Gonzales, N.R., Howlett, G.J. & Schubert, D. (2002) Size-distribution analysis of proteins by analytical ultracentrifugation: strategies and application to model systems. *Biophys. J.*, **82**, 1096–1111.
- Sönnichsen, F.D., Van Eyk, J.E., Hodges, R.S. & Sykes, B.D. (1992) Effect of trifluoroethanol on protein secondary structure: an NMR and CD study using a synthetic actin peptide. *Biochemistry*, **31**, 8790–8798.
- Tan, S. (2001) A modular polycistronic expression system for overexpressing protein complexes in *Escherichia coli*. *Protein Expr. Purif.*, **21**, 224–234.
- Tomoyasu, T., Mogk, A., Langen, H., Goloubinoff, P. & Bukau, B. (2001) Genetic dissection of the roles of chaperones and proteases in protein folding and degradation in the *Escherichia coli* cytosol. *Mol. Microbiol.*, **40**, 397–413.
- Tripet, B. & Hodges, R.S. (2002) Helix capping interactions stabilize the N-terminus of the kinesin neck coiled-coil. *J. Struct. Biol.*, **137**, 220–235.
- Tuma, M.C., Zill, A., Le Bot, N., Vernos, I. & Gelfand, V. (1998) Heterotrimeric kinesin II is the microtubule motor protein responsible for pigment dispersion in *Xenopus* melanophores. *J. Cell Biol.*, **143**, 1547–1558.
- Vale, R.D. (2003) The molecular motor toolbox for intracellular transport. *Cell*, **112**, 467–480.
- Yang, Z. & Goldstein, L.S. (1998) Characterization of the KIF3C neural kinesin-like motor from mouse. *Mol. Biol. Cell*, **9**, 249–261.