# New and Notable

## Lateral Exchange Smooths the Way for Vimentin Filaments

Laurent Kreplak[1,*] and Andrew D. Rutenberg[1]
[1]Department of Physics and Atmospheric Science, Dalhousie University, Halifax, Nova Scotia, Canada

Intermediate filaments (IFs), such as vimentin, do not assemble with end-directed addition of monomeric or dimeric subunits such as seen with filamentous actin or microtubules, respectively. Instead, IF assembly has been shown to involve short multisubunit unit-length filaments (ULFs) that assemble end-to-end (1). This first assembly step is well established in vitro for several proteins of the IF family (1). It is followed by a more elusive annealing process thought to involve tetramer exchange (2). The in vitro results of Nöding et al. (3) on vimentin IF confirm and extend earlier in vivo studies that indicated tetramer exchange occurs within cells. Using an elegant microfluidic approach and fluorescently labeled filaments assembled in different ways (see Fig. 1 *A*). Nöding et al. have shown that tetramer exchange occurs both between and along filaments, and that it is fastest when the number of tetramers per filament cross-section varies significantly along the filaments' length. Because initial lateral heterogeneity of IF cross-sections occurs during rapid filament assembly, we now have a picture of vimentin filaments in dynamic exchange with a soluble pool of tetramers, driving toward a network with uniform radius filaments.

The microfluidic platform described by Nöding et al. (3) (Fig. 1 *A*) could be extended to investigate the role of shear flow in modulating tetramer exchange; this would have direct implication for IF network remodeling in epithelial and endothelial cells exposed to shear stresses (4). With the existing platform, it will also be worthwhile to directly compare the dynamics of various types of IF proteins. Keratins are especially promising for in vitro studies of this type, because their networks are continually turned over in epithelial cells (in contrast to relatively stable vimentin networks in mesenchymal cells (4)). Microfluidic platforms also let you test many conditions at once while using very small amounts of material. For IFs, this approach is ideal to systematically quantify the effect of phosphate solutions (5) and of individual protein kinases (6) on tetramer exchange. These quantitative experiments will be essential to confirm that the intrinsic tetramer exchange process observed by Nöding et al. (3) coexists with phosphorylation-mediated filament disassembly and turnover observed in vivo.

The results of Nöding et al. strongly imply the existence of an equilibrium state for vimentin filaments, with a well-defined radius, which can be reached within several hours even without cellular helpers. What determines the equilibrium IF radius? If we view filaments as simple laterally packed uniform rods, then ever-larger radii will both satisfy local binding and minimize surface energy costs. However, vimentin dimers are long double-stranded $\alpha$-helical coiled-coils (7) that are qualitatively similar to the tropocollagen molecules inside a collagen fibril. For such packed helical molecules we have recently shown how equilibrium radius control could be achieved through the competition of lateral twisting and bending of molecules into a double-twist architecture (Fig. 1 *B*) (8). Our computational approach and an earlier analytical study of chiral packing by Grason and Bruisma (9) both rely on coarse-grained or continuum descriptions of elastic energies. Quantitative corrections will occur when the filament radius is comparable to the radius of its chiral building block, as it is for vimentin IFs (9). Nevertheless, we expect that the equilibrium picture of chiral filament radius control will remain qualitatively intact.

Equilibrium radius control implies that filaments of radii different (either larger or smaller) than the minimal free-energy radius will more rapidly turn over tetramers, which agrees with the observations of Nöding et al. (3). For radii close to the equilibrium, turnover will also be associated with net growth or shrinkage toward the equilibrium radius. Conversely, equilibrium radii filaments should have the lowest turnover rates. Qualitative descriptions of IF insolubility, and the relatively slow turnover seen in the cellular context (2), therefore suggests close-to-equilibrium radii in those conditions. Interestingly, the equilibrium vimentin cross-section appears to be eight tetramers (10) whereas initial in vitro filament assembly proceeds with ULF intermediates that have a range of tetramers per cross-section between 5 and 15, but with an average above 8 (1). This implies that tetramers released from thicker sections of filaments may also extend existing filaments, and not just be exchanged with other preexisting sections.

Microfluidic approaches like the one developed by Nöding et al. (3) will significantly further our understanding of IF assembly and dynamics in vitro, and in the cellular context as well. We now know that tetramer exchange is an intrinsic property of vimentin filaments and does not require cellular helpers. Due to their propensity for end-to-end fusion, IFs are efficient at forming continuous networks (2). This network-forming ability is essential for IFs to function as regulators of intracellular mechanics (11), but requires some mechanism(s) to ensure network integrity over longer timescales of several days or more. Intrinsic

*Submitted September 19, 2014, and accepted for publication October 27, 2014.*

*Correspondence: kreplak@dal.ca

Editor: Nathan Baker.



0006-3495/14/12/2747/2 $2.00

http://dx.doi.org/10.1016/j.bpj.2014.10.050

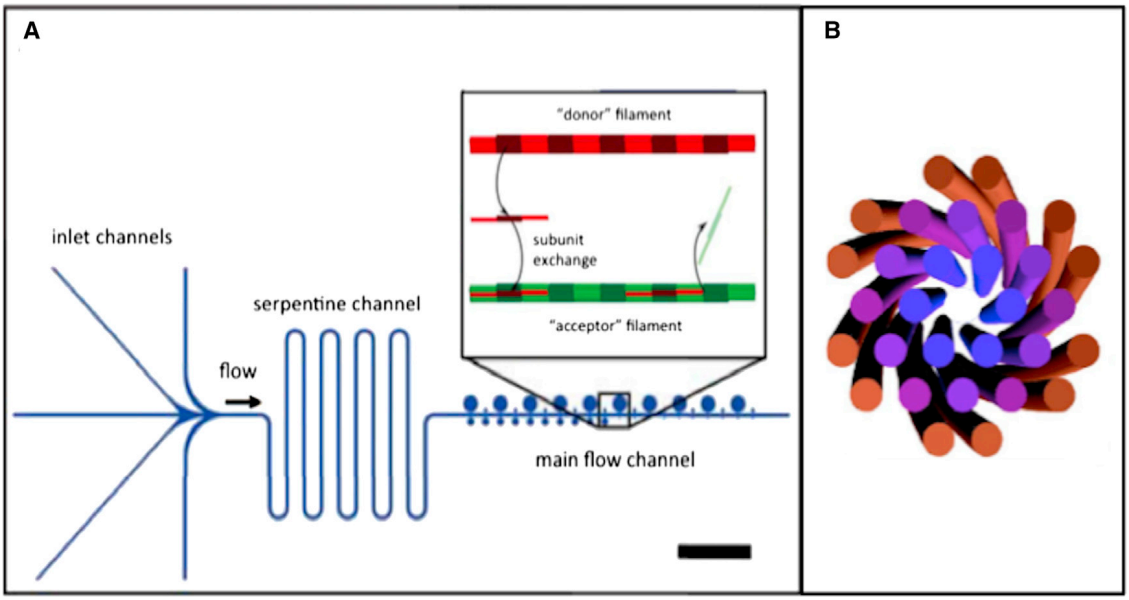


FIGURE 1 (*A*) Geometry of the microfluidic device used by Nöding et al. (3), adapted from their Figs. 1 and 2 with scale bar of 1 mm. (*Left*) Inlet channels are used to combine components at defined mixing ratios; the downstream serpentine channel mixes these components before they enter the main channel (*right*). Round diffusion chambers, used for imaging the dynamics of fluorescently labeled vimentin over the course of several hours, are coupled to the main channel without significant flow. (*Inset*) Two vimentin filaments inside a diffusion chamber. By mixing red- and green-labeled filaments, Nöding et al. (3) can visualize exchange as well as discern that the exchange is of a few tetramers rather than entire unit-length filaments (ULF, spanning the filament cross-section). (*B*) Double-twist model of vimentin IF structure, in a cross-sectional end-view, adapted from Grason and Bruinsma (9). The twisted cylinders represent the local orientation of vimentin tetramers, where the twist angle increases away from the core of the IF (*blue*) toward the filament surface (*brown*). To see this figure in color, go online.

tetramer exchange of IF could be important in this respect, with phosphorylation-driven disassembly (6) used to drive network remodeling. With the results and techniques of Nöding et al., we anticipate that further research on IF dynamics will be smooth.

## REFERENCES

1. Herrmann, H., M. Häner, …, U. Aebi. 1999. Characterization of distinct early assembly units of different intermediate filament proteins. *J. Mol. Biol.* 286:1403–1420.

2. Colakoğlu, G., and A. Brown. 2009. Intermediate filaments exchange subunits along their length and elongate by end-to-end annealing. *J. Cell Biol.* 185:769–777.

3. Nöding, B., H. Herrmann, and S. Koester. 2014. Direct observation of subunit exchange along mature vimentin intermediate filaments. *Biophys. J.* 107:2923–2931.

4. Windoffer, R., M. Beil, …, R. E. Leube. 2011. Cytoskeleton in motion: the dynamics of keratin intermediate filaments in epithelia. *J. Cell Biol.* 194:669–678.

5. Aebi, U., W. E. Fowler, …, T. T. Sun. 1983. The fibrillar substructure of keratin filaments unraveled. *J. Cell Biol.* 97:1131–1143.

6. Sihag, R. K., M. Inagaki, …, H. C. Pant. 2007. Role of phosphorylation on the structural dynamics and function of types III and IV intermediate filaments. *Exp. Cell Res.* 313:2098–2109.

7. Chernyatina, A. A., S. Nicolet, …, S. V. Strelkov. 2012. Atomic structure of the vimentin central α-helical domain and its implications for intermediate filament assembly. *Proc. Natl. Acad. Sci. USA.* 109:13620–13625.

8. Brown, A. I., L. Kreplak, and A. D. Rutenberg. 2014. An equilibrium double-twist model for the radial structure of collagen fibrils. *Soft Matter.* 10:8500–8511.

9. Grason, G. M., and R. F. Bruinsma. 2007. Chirality and equilibrium biopolymer bundles. *Phys. Rev. Lett.* 99:098101.

10. Strelkov, S. V., H. Herrmann, and U. Aebi. 2003. Molecular architecture of intermediate filaments. *BioEssays.* 25:243–251.

11. Guo, M., A. J. Ehrlicher, …, D. A. Weitz. 2013. The role of vimentin intermediate filaments in cortical and cytoplasmic mechanics. *Biophys. J.* 105:1562–1568.