## Probing Intermediate Filament Structure and Assembly with Small-angle X-ray Scattering

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Intermediate filaments (IFs) together with microtubules and actin filaments, form the cytoskeleton of most living cells. IFs are long macromolecular aggregates, with about 10 nm cross-section. While crystallographic data on the dimer representing the elementary IF 'building block' have recently become available, little structural detail is known on both the mature IF architecture and their assembly pathway.

We have applied small angle X-ray scattering (SAXS) to investigate the *in vitro* assembly of human IF protein vimentin in varying pH and ionic strength conditions. SAXS is a method allowing one to analyze protein structure in solutions at different external conditions and also to quantitatively characterize of mixtures of different oligomeric states. We demonstrate that formation of tetramers, octamers and IFs represent the principal steps along the vimentin assembly pathway. By combining the SAXS data with the atomic structures and additional structural restraints, threedimensional models of these assembly intermediates are constructed and refined. These results are further confirmed by electron microscopy observations.

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