Abstract. Synucleinopathy, a class of neurodegenerative diseases best known for Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA), is characterized by pathological aggregation of alpha-synuclein (aSyn) in neurons and glial cells. Misfolded aSyn aggregated into different fibril species that have been shown to have distinct spreading patterns, contributing differently to the pathogenesis of the diseases. Structural information of different aSyn fibrillar species has come from electron microscopy (EM), solid-state nuclear magnetic resonance (ssNMR), and crystallography. However, little is known about how aSyn fibril polymorphs differ in atomic structure and seeded spreading behavior in cells and brains. We characterized two fibril preparations of recombinant aSyn (1-140): narrow and broad fibrils. Cryo-EM study of the narrow fibril preparation revealed the near-atomic structures of the two polymorphs (rod and twister) at the resolution of 3.7Å. Both polymorphs consist of two protofilaments sharing the same kernel structure but are constructed around different contact interfaces between the two protofilaments. Negative stain EM images of the broad fibril preparation demonstrated a third polymorph, striated ribbon. The Cryo-EM structures of the rod and twister polymorphs together suggested a hypothetical model of the striated ribbon polymorph, supported by the fiber powder diffraction data. Furthermore, the biological experiments of the narrow and broad fibril preparations showed different seeding efficiencies in cells and distinct spreading patterns in mouse brains. Different packing arrangements among identical protofilament kernels in all three polymorphs may thus underlie the distinct in vitro and in vivo spreading patterns. Drug design efforts targeting aSyn fibril aggregation in synucleinopathy may necessarily involve consideration of pathogenic contributions from all concurrent fibril polymorphs.