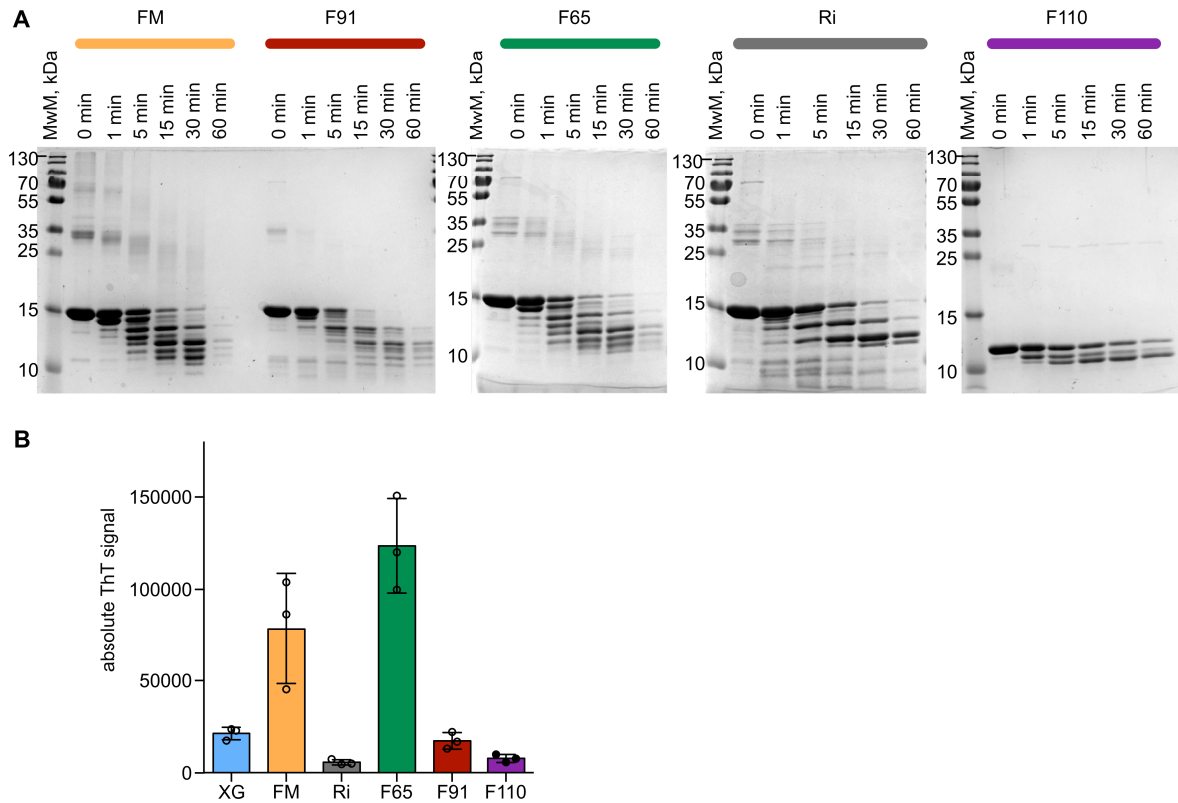


**Appendix for “Structural polymorphism of  $\alpha$ -synuclein fibrils alters pathway of Hsc70 mediated disaggregation”**

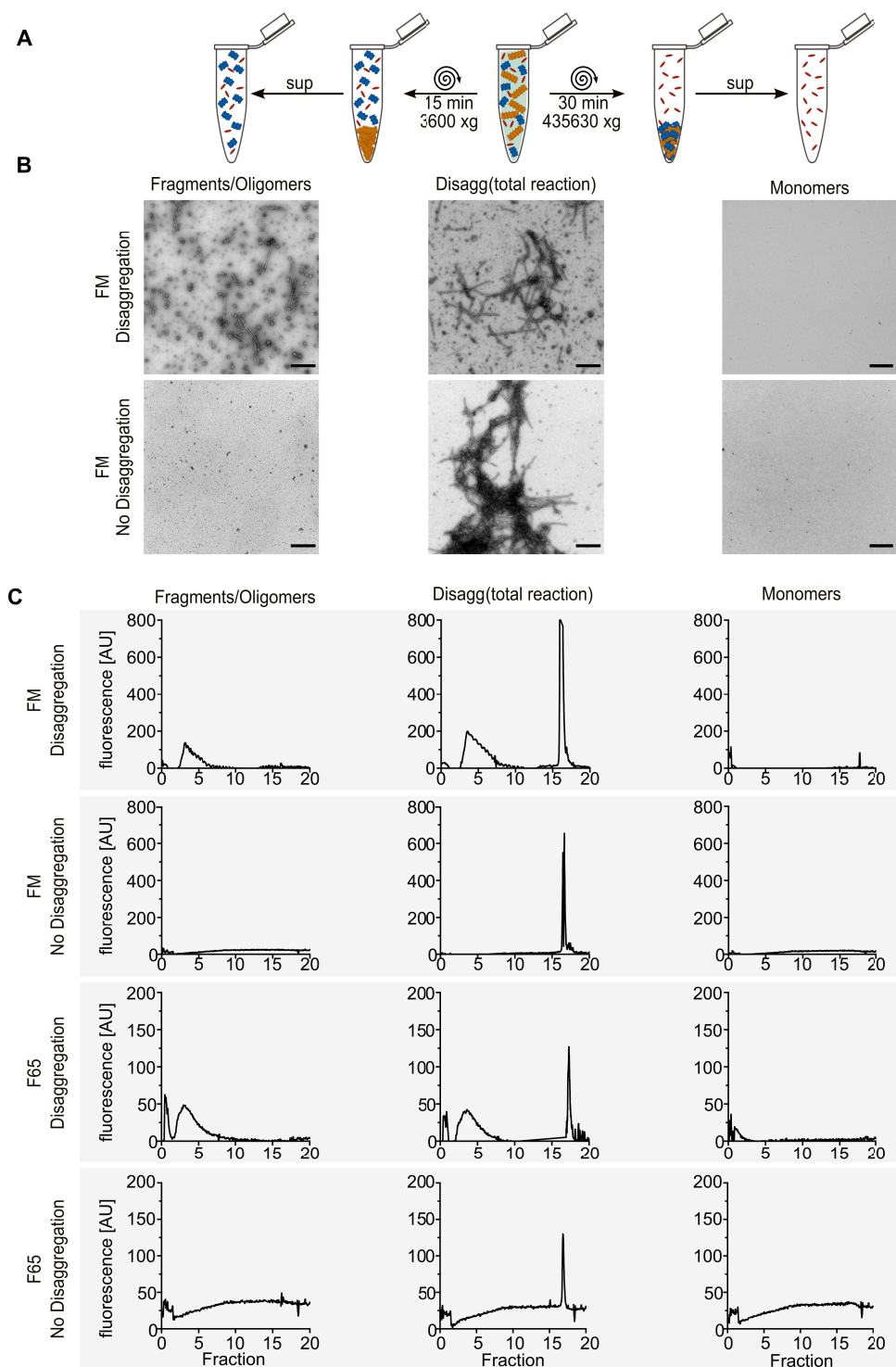
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**Appendix Figure S1 |  $\alpha$ -syn polymorphs differ in sensitivity to proteolysis and reactivity to Thioflavin T.**

**A** SDS-PAGE gels of limited proteolysis of FM (yellow), F91 (red), F65 (green), Ri (grey), and the C-terminally truncated F110 (purple) by proteinase K at timepoint 0, and after 1, 5, 15, 30 and 60 min. **B** Absolute ThT signal of  $\alpha$ -syn fibrillar polymorphs (XG (blue), FM (yellow), Ri (grey), F65 (green), F91 (red), and the C-terminally truncated F110 (purple)) at the same concentration (2  $\mu$ M protein – 30  $\mu$ M ThT). Data are mean  $\pm$  s.e.m. of three biological replicates.



### Appendix Figure S2 | Disaggregation reaction products can be fractioned based on differential centrifugation.

**A** Centrifugation procedure as shown in Fig. 4C to separate different disaggregation reaction products. A total disaggregation reaction is centrifuged at 3,600g for 15 min, fibrils (orange) are separated in the pellet and small fragments/oligomers remain in the supernatant (left). By centrifugation of the total reaction at a higher speed (435,630g) for 30 min, small fragments/oligomers (blue) and fibrils are pelleted and only monomers (red) remain in the supernatant (right). **B** Representative electron micrographs of fibrils incubated with the chaperone machinery in the presence (Disaggregation) and absence (No Disaggregation) of ATP for the polymorph FM. Scale bar 500 nm. **C** Sucrose density gradient (10-85%) profile of AF555-labelled fibrils incubated with the active (Disaggregation) and inactive (No Disaggregation) chaperone machinery and similar prepared as in panel A.