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Studies of BTB domain polymerisation through integrative structural biology

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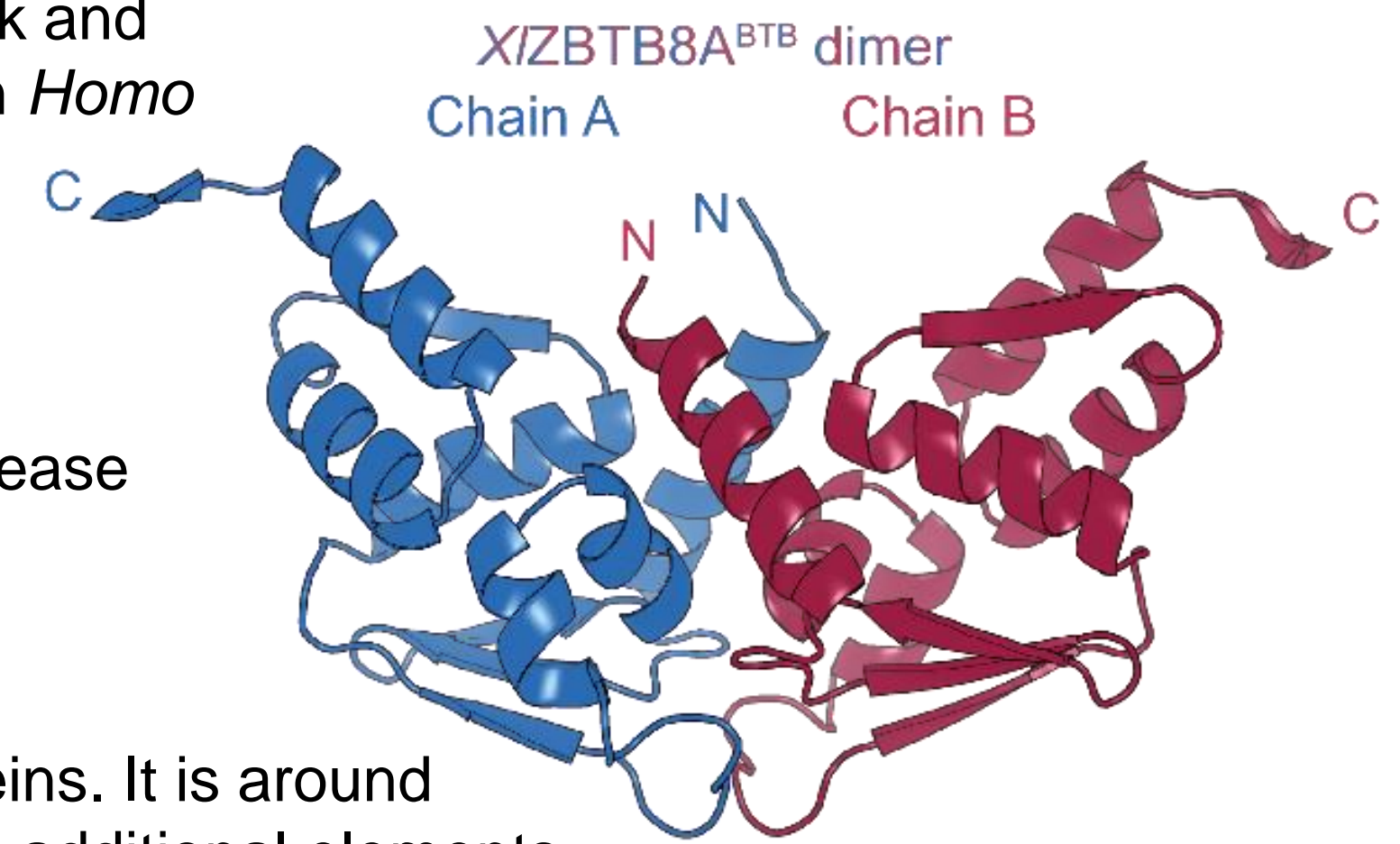
ZBTB family

The ZBTB (ZF and BTB domain-containing) family is a group of proteins defined by the presence of a single N-terminal Broad-complex, Tramtrack and Bric-à-brac (BTB) domain and multiple copies of a zinc finger (ZF) domain at the C-terminus. It is among the largest transcription factor families in *Homo sapiens*, with around 50 canonical members. Besides being involved in the repression of transcription, mainly in the context of differentiation and development, many members of the ZBTB family are implicated in development of cancer and other pathologies. In this study, we focus on two proteins from the ZBTB family - **ZBTB8A** (BTB/POZ and Zinc finger domains Factor on chromosome 1 (BOZF1)) and **ZBTB18** (58 kDa Repressor Protein (RP58) or Zinc Finger-containing protein 238 (ZNF238)). While both ZBTBs function as downregulators of transcription, ZBTB8A is shown to be involved in development of prostate and breast cancer, and ZBTB18 is related to neural development disease intellectual disabilities and cancer.

BTB domain

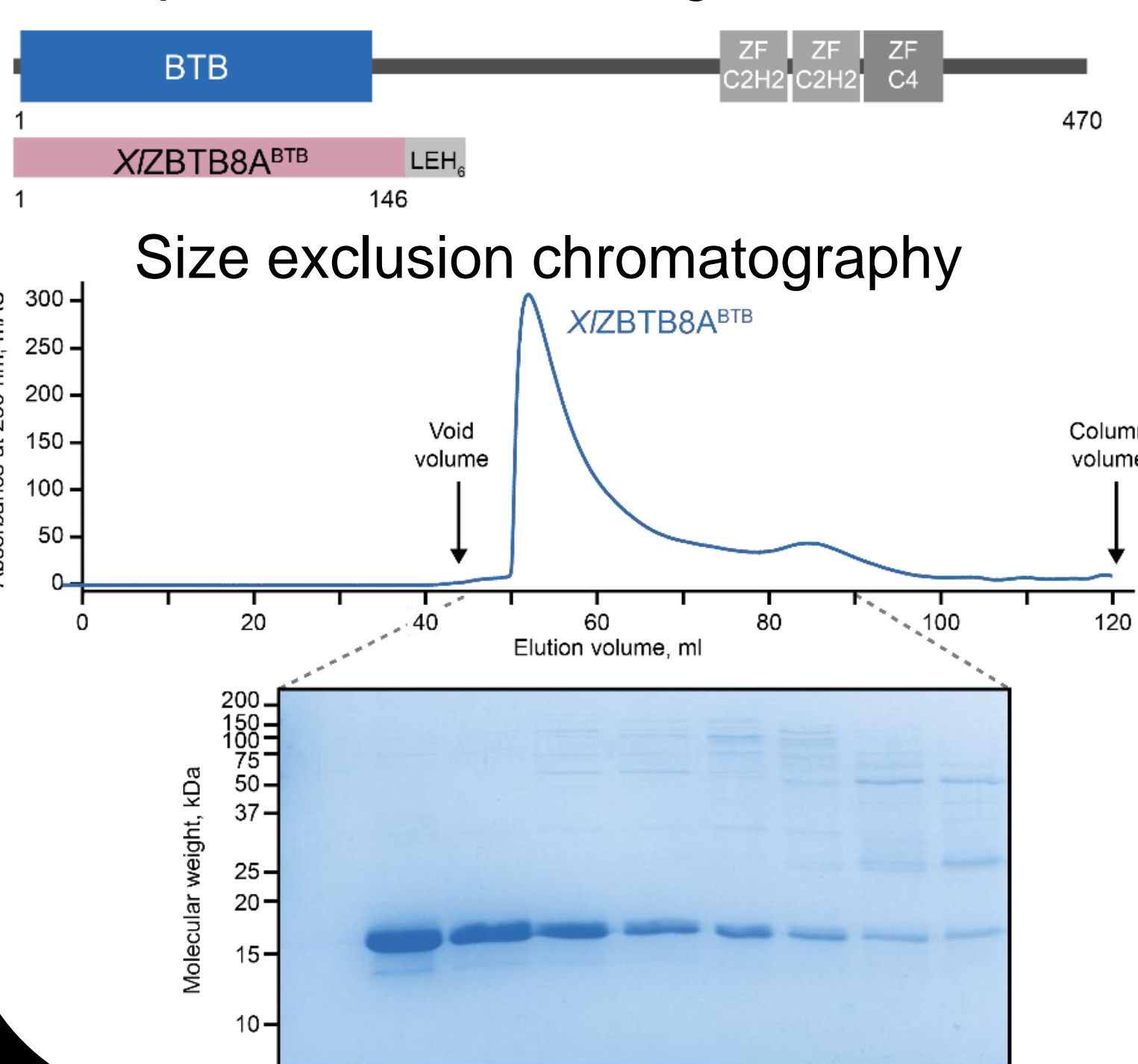
The BTB (or Pox-virus and Zinc-finger (POZ)) is a domain present not only in ZBTB family members, but also in various eukaryotic and viral proteins. It is around 120 amino-acids in length and it comprises a core fold composed of five α -helices and three β -strands, which can be extended on either side with additional elements. BTB domain mediates formation of homo and hetero dimers, as well as protein-protein interactions.

INTRODUCTION



Polymerisation of BTB domain from ZBTB8A

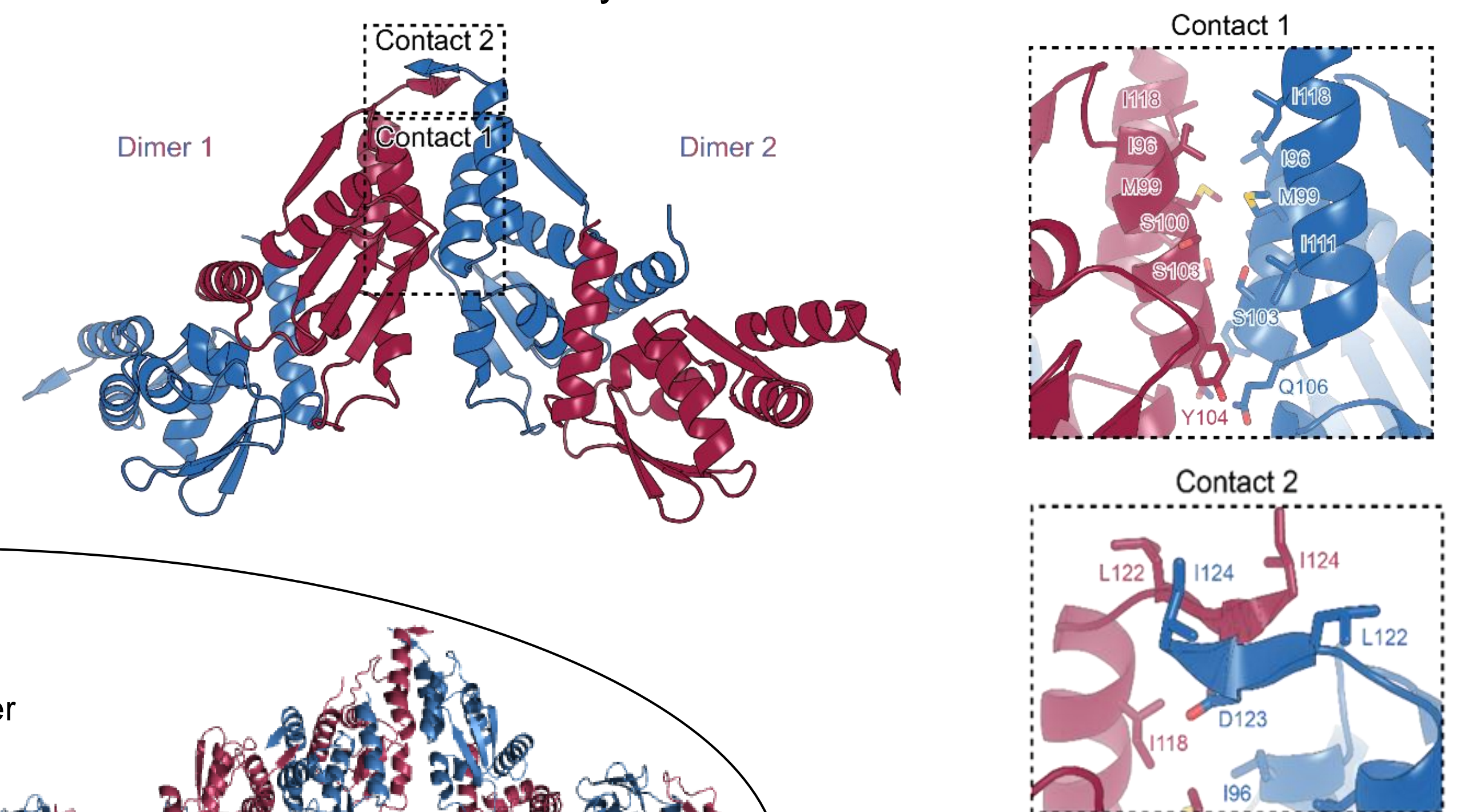
Xenopus laevis ZBTB8A gene scheme



Crystals of **BTB domain of ZBTB8A** from *Xenopus laevis* were obtained at a resolution of 3.10 Å. In the structure, BTB dimers from neighbouring asymmetric units are connected through a noncovalent interface, leading to the formation of linear filaments. The observed interdimer interface has an area of around 630 Å².

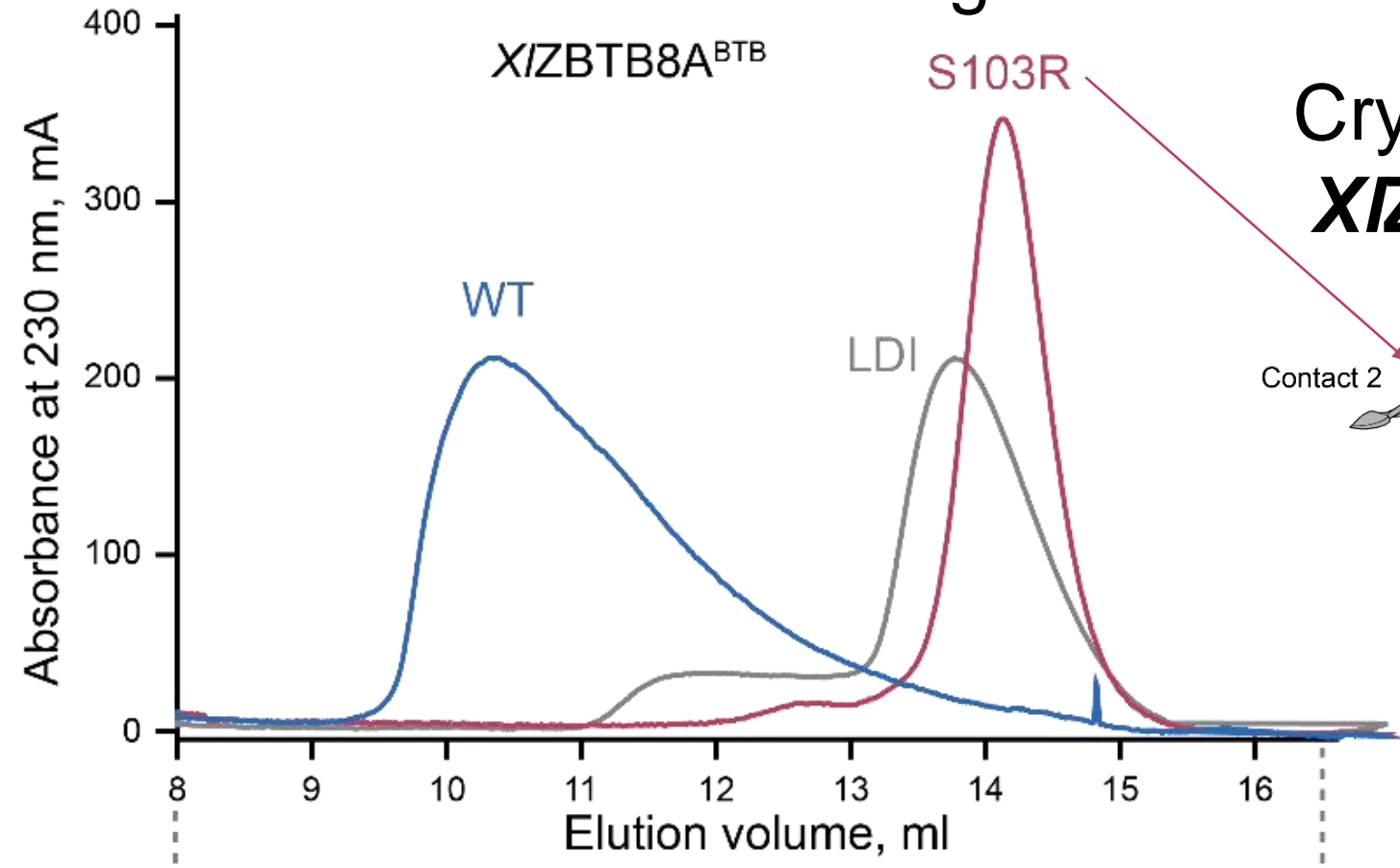
Structural insights into BTB interdimer interface

The BTB interdimer interface is symmetrical and it consists of two contacts.

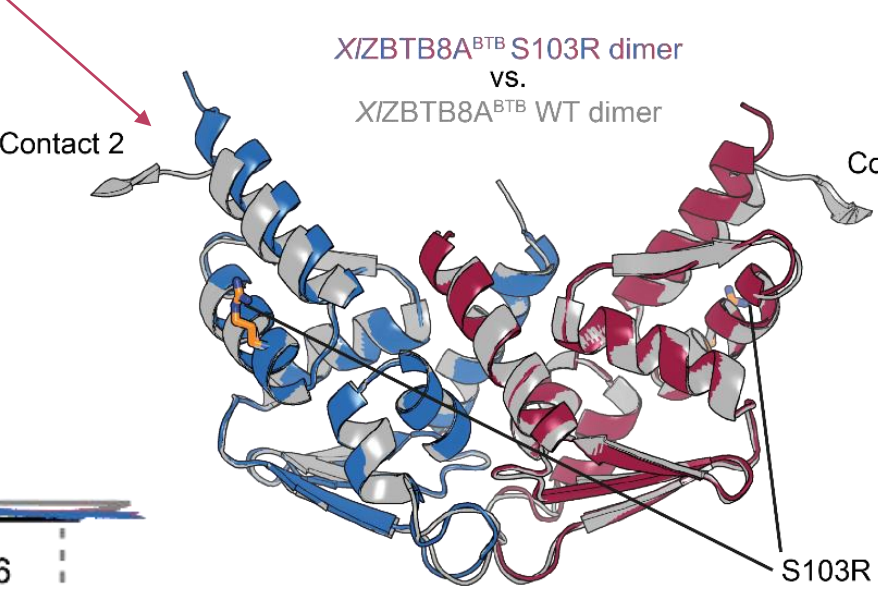


BTB-mediated polymerisation confirmed in solution

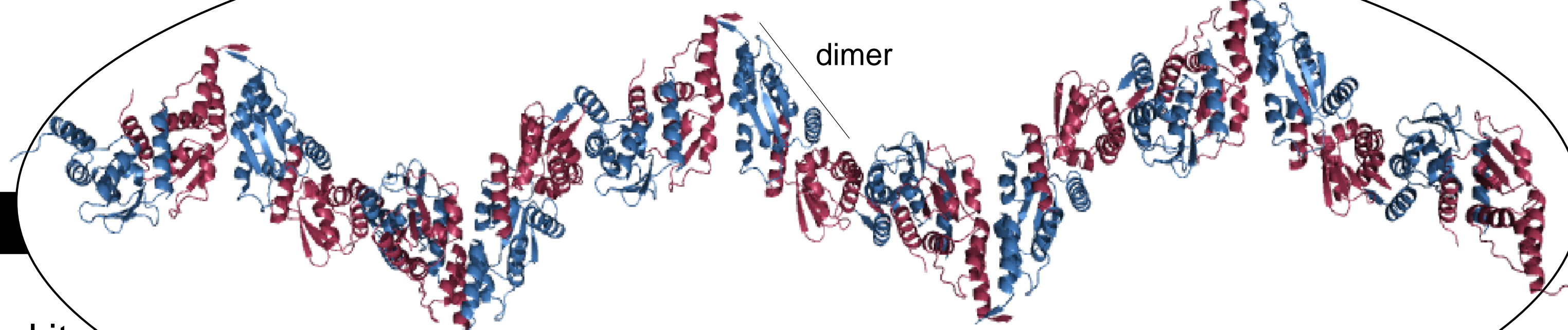
Analytical size exclusion of XIZBTB8A wt and its two structure-guided mutants



Crystal structure of dimeric XIZBTB8A S103R mutant

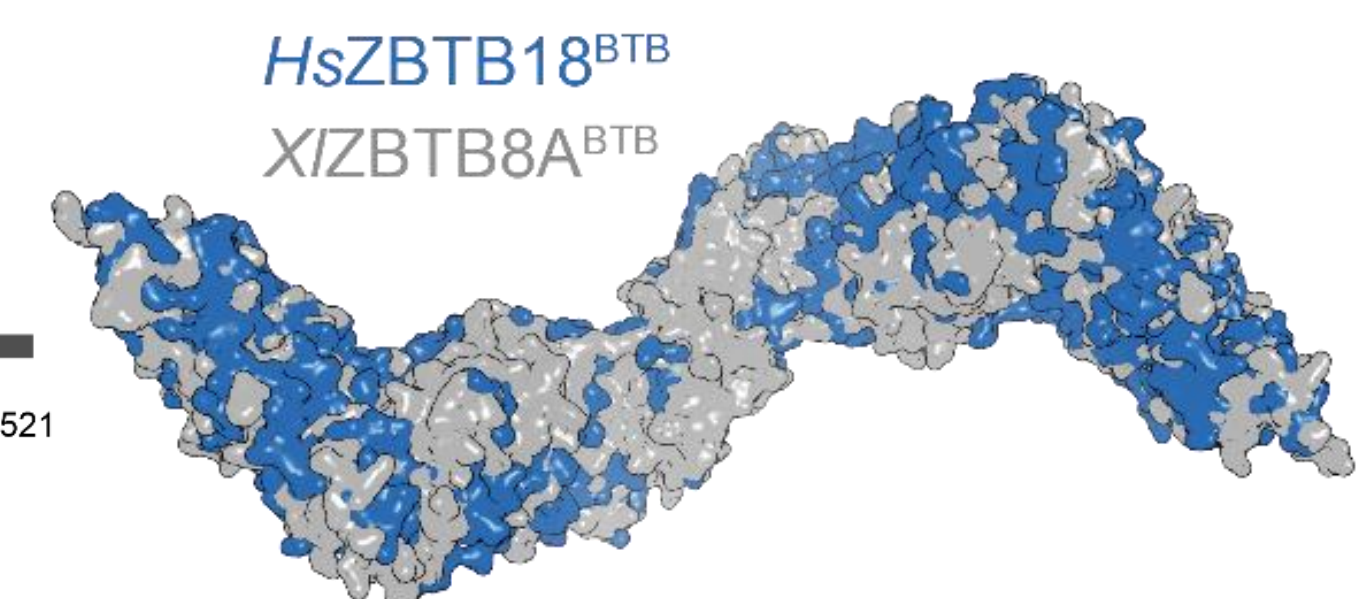


XIZBTB8A BTB domain filament

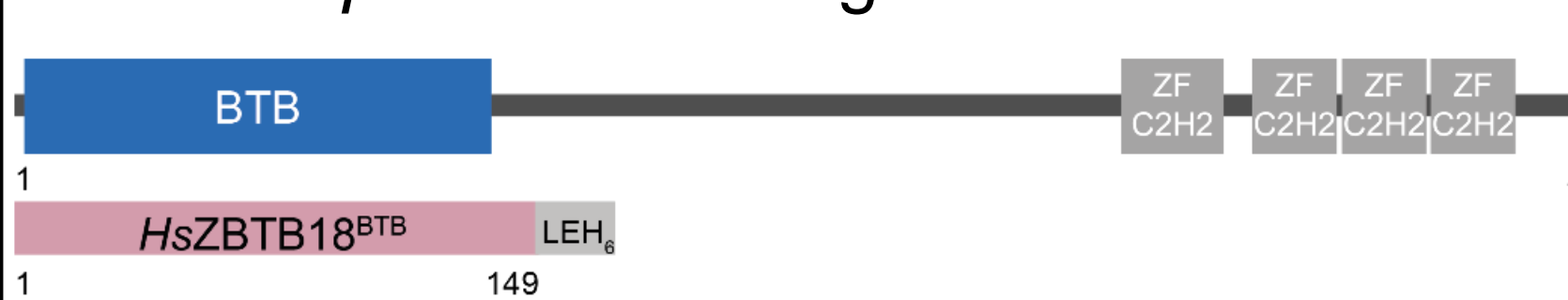


BTB polymerisation is a property of other ZBTBs

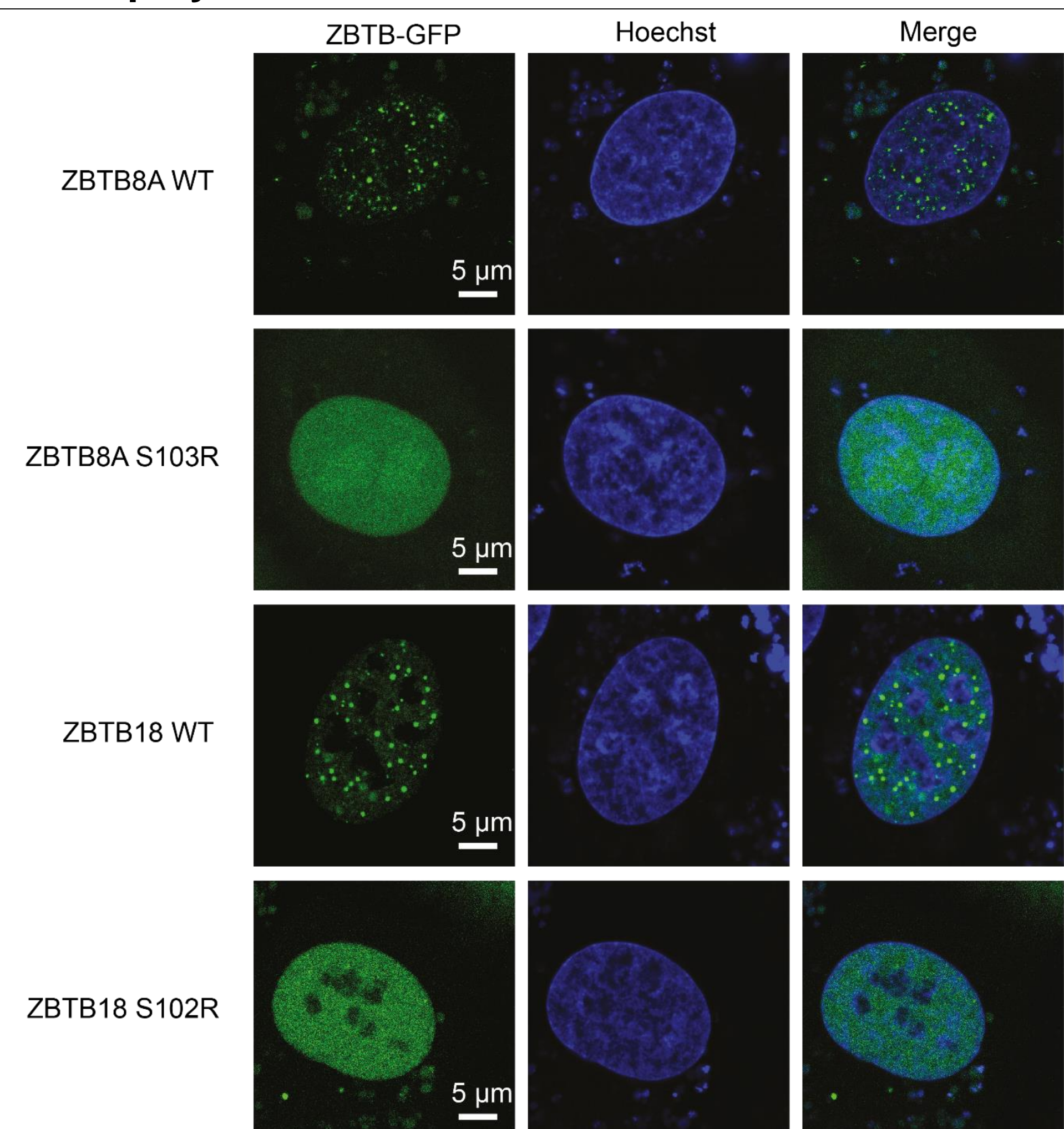
HsZBTB18 BTB domain forms similar filaments as observed for XIZBTB8A



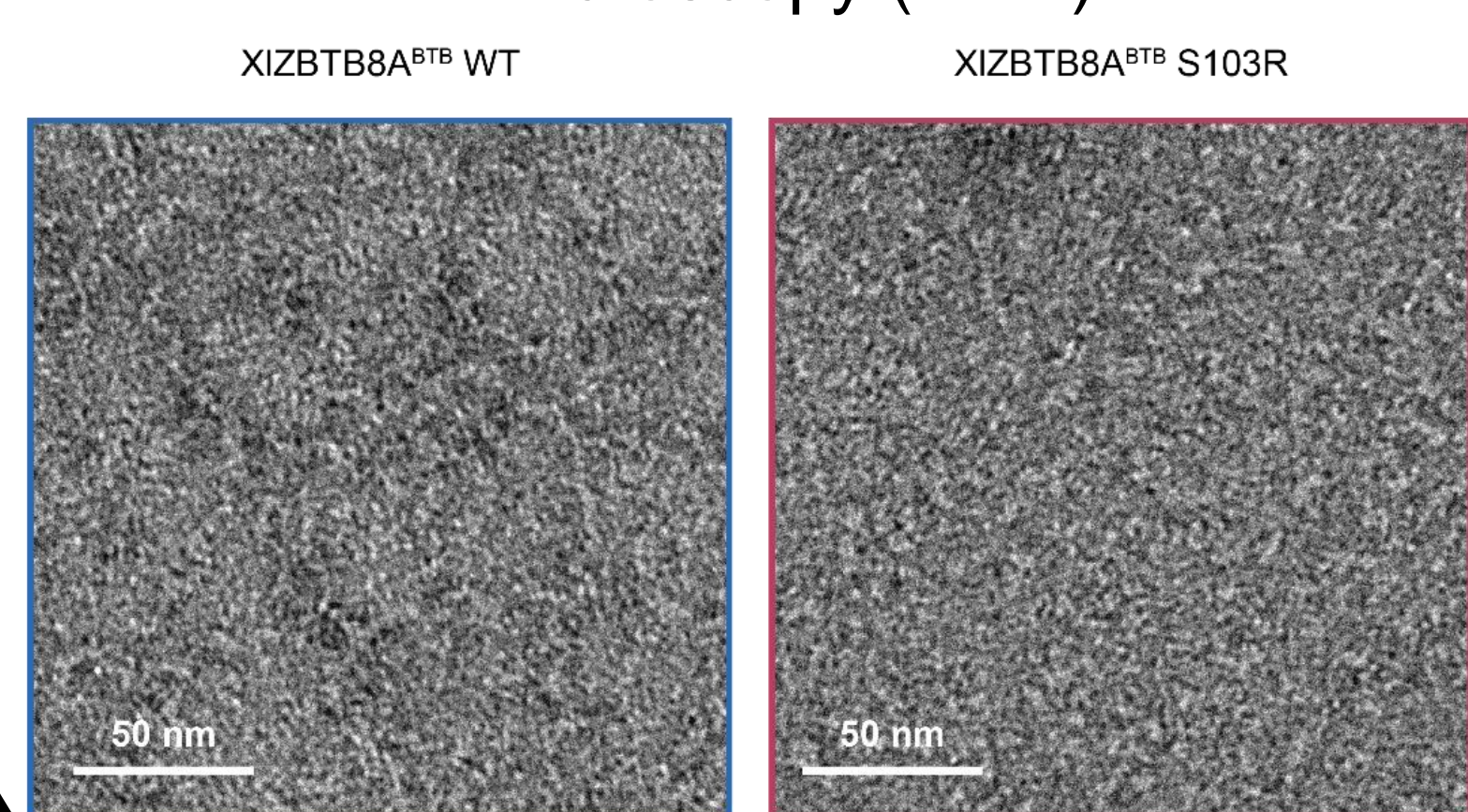
Homo sapiens ZBTB18 gene scheme



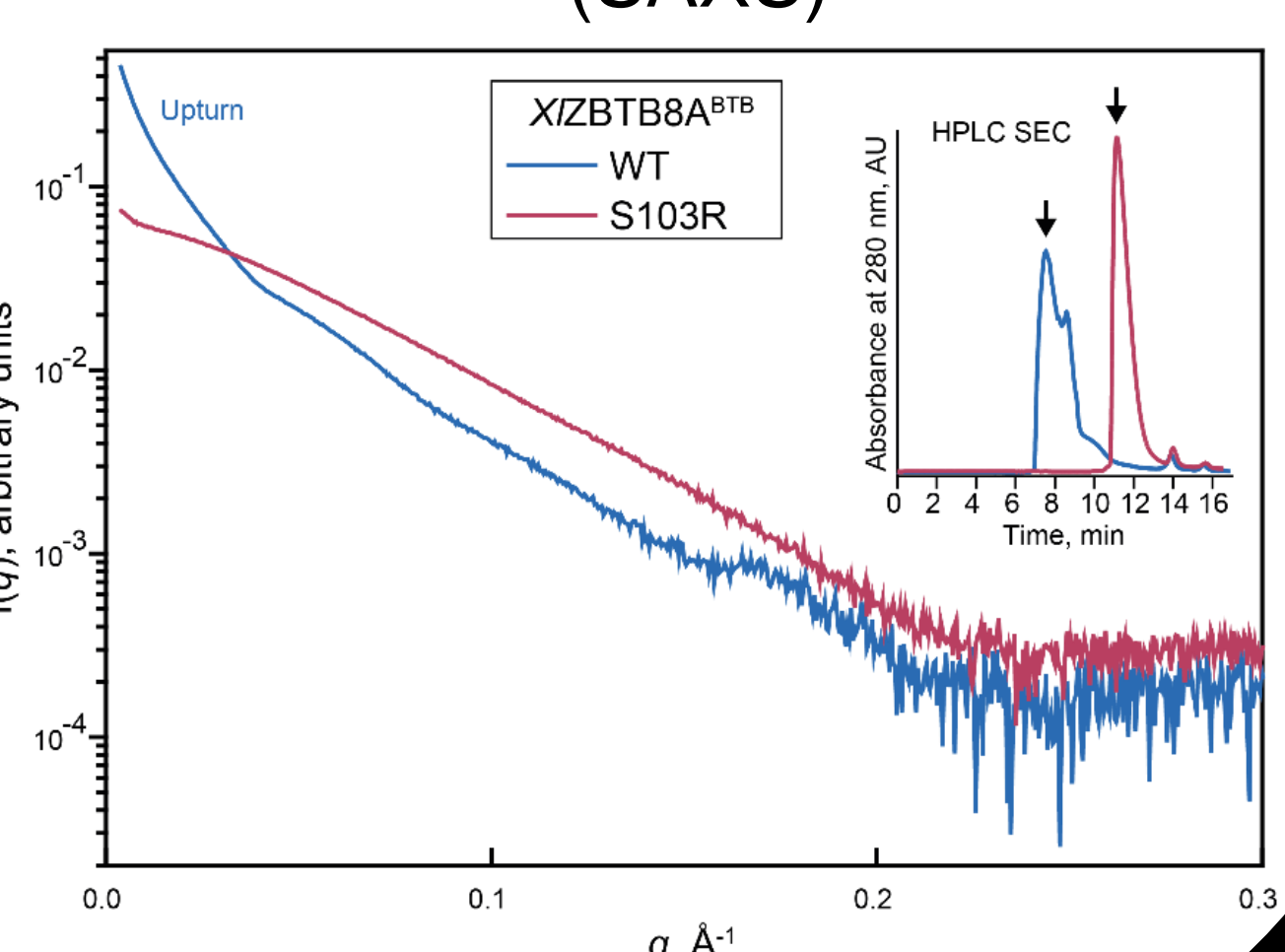
BTB-mediated polymerisation dictates ZBTB subnuclear localisation *in cellulo*



Negative staining Transmission Electron Microscopy (TEM)



Small Angle X-ray light Scattering (SAXS)



CONCLUSIONS

- Our study reveals a previously unreported propensity of BTB domains of **ZBTB8A** and **ZBTB18** to form **noncovalent polymers**
- The propensity for polymerisation among certain human ZBTB transcription factors is required for the localisation of these proteins to nuclear foci *in cellulo*
- AlphaFold2 structural models of ZBTB proteins indicate that the polymerisation property might be extended up to approximately 40% of canonical members of the ZBTB family
- BTB domain-mediated polymerisation might potentially have functional significance in processes such as recognition of specific DNA motifs and transcription repression.

