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Using synthetic affinity binders (sABs) as biological tools to study the chromosome passenger complex proteins

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Lack of adequate structural information on Chromosome Passenger Complex (CPC) proteins is mainly attributed to their inherent disordered structures. Thus, there is a significant drive to dissect the structural information that will provide valuable insights into the biological function of these proteins. To cater to the growing demands of tools as probes to delineate the structure and function of the CPC proteins, we generated highly specific, synthetic affinity binders for the different domains of these proteins by phage display using a synthetic Fab library. The selected sABs were very specific for Survivin's N-terminal Baculovirus Inhibitor of Apoptosis-repeat (BIR) domain for the N, C-terminal domain of Borealin as well as for the N-terminal domain of INCENP. High specificity of sABs was evident from ELISA-assays, Dot-blots and Western-blots. Immunofluorescence studies show typical intracellular localization of the sABs bound to the mitotic spindle, the nucleus and the midbody in dividing cells. Interestingly, sABs against the C- and N-terminal domains of Borealin reveal unique cellular patterns not reported to date. These sABs are also validated as customized primary reagents for Western-Blot. The generated high quality antibodies will be used as crystallization chaperones for structure determination of these candidates that are recalcitrant to crystallization by conventional approaches.

Biography

Marcin Lukasz Ura has completed his PhD from Braunschweig University of Technology and he is currently a Postdoctoral Scholar in Tony Kossiakoff Laboratory at the Department of Biochemistry and Molecular Biology of the University of Chicago.

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