Structural analysis and protein-protein interactions of the multidomain PARP14 enzyme

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PARP14 is one of the 17 members belonging to the poly-ADP-ribose polymerase (PARP) family. The family has a homologous ADP-ribose transferase (ART) domain and catalyses the conversion of β-NAD+ to ADP-ribosyl groups on molecular targets. PARP14 is an 1801 amino acid multidomain protein harbouring two opposing catalytic activities. In addition to the ART domain, macrodomain-1, a glycohydrolase, removes the same modification. PARP14 also contains two further macrodomains that recognize and bind mono-ADP-ribosylation, as well as several RNA and protein interacting domains, namely RNA recognition motifs (RRM) and K-homology domains. PARP14 is associated with the anti-viral response and is overexpressed in a variety of cancers and inflammatory diseases. As such, PARP14 is a pharmacological target, with several ART domain inhibitors currently in clinical trials. Available structures of single domain PARP14 constructs enable the design of potent inhibitors. However, they fail to demonstrate how the protein's dual catalytic activities and its multidomain context can be reconciled. We designed and expressed in *E.coli*, an artificial protein construct containing both catalytic activities that has improved solubility, purity and yield over the wild-type PARP14. The aim was to study the PARP14 multidomain arrangement in association to the two catalytic activities and upon interaction with protein binding partners. Following initial SAXS studies with synchrotron and in-house X-ray sources, we showed that our PARP14 mutant is a flexible-multidomain protein according to Kratky analysis, has a preference for compact conformations as shown with maximum parsimony ensemble selection method, and is possibly monomeric as revealed by concentration series showing similar *Rg* and molecular weight. In addition, the ensemble selection method indicates that with increasing concentration, there is minor compaction (possibly crowding effect), but flexibility remains the same, indicating absence of strong interactions between PARP14 molecules. We expect that SAXS analysis of different states of ADP-ribosylation and protein-protein interaction will yield important functional and structural information as well as guide us toward conditions that are amenable to single particle cryo-EM analysis.