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PARP family enzymes: regulation and catalysis of the poly(ADP-ribose) posttranslational modification

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Abstract

Poly(ADP-ribose) is a posttranslational modification and signaling molecule that regulates many aspects of human cell biology, and it is synthesized by enzymes known as poly(ADP-ribose) polymerases, or PARPs. A diverse collection of domain structures dictates the different cellular roles of PARP enzymes and regulates the production of poly(ADP-ribose). Here we primarily review recent structural insights into the regulation and catalysis of two family members: PARP-1 and Tankyrase. PARP-1 has multiple roles in the cellular response to DNA damage and the regulation of gene transcription, and Tankyrase regulates a diverse set of target proteins involved cellular processes such as mitosis, genome integrity, and cell signaling. Both enzymes offer interesting modes of regulating the production and the target site selectivity of the poly(ADP-ribose) modification.

The ADP-ribose modification and the PARP family of enzymes

ADP-ribose is a posttranslational modification and signaling molecule produced by the ADP-ribosyltransferase (ART) activity of PARP enzymes using NAD^+ as a substrate (Fig. 1a). ADP-ribose is primarily attached to proteins, but can also be found on DNA and small chemical groups [1]. The ADP-ribose modification is important for the regulation of multiple cellular processes including DNA repair, transcription, cell fate decisions, and the stress response [2]. The poly(ADP-ribose) polymerase (PARP) family of proteins is responsible for catalyzing the production of the ADP-ribose modification in cells. The PARP family has 17 members that all share a conserved catalytic domain fold that is homologous to the diphteria toxin ART fold [3](Fig. 1a). Hence, the PARP family of proteins are also referred to as ART diphteria toxin-like (ARTD) enzymes [4]. Among the 17 PARP family

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Conflict of interest

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members, PARP-1, PARP-2 and PARP-5a/PARP-5b (the Tankyrases) synthesize polymers of ADP-ribose (Fig. 1b), while other PARPs catalyze a mono ADP-ribose posttranslational modification [5]. In addition to linear chains of ADP-ribose, poly(ADP-ribose) can also include branch points (Fig. 1b) [6,7]. Like other posttranslational modifications, poly(ADP-ribose) can alter the biochemical properties and activities of proteins targeted for modification. Moreover, there are a number of protein modules that recognize and bind to poly(ADP-ribose), and an assortment of enzymes that can trim and/or reverse the complex structure of poly(ADP-ribose) [8,9].

The PARP family members are NAD^+ consumers and have the capacity to dramatically influence cellular levels of NAD^+. PARP enzyme activities are thus tightly regulated to control the activation of cell signaling pathways but also in order to avoid depletion of the cellular energy supplies. This review presents recent structural insights into the regulation and catalysis of PARP enzymes involved in the production of poly(ADP-ribose): PARP-1, PARP-2, and the Tankyrases.

PARP regulation and catalysis in response to DNA damage

Poly(ADP-ribose) catalysis by PARP-1, PARP-2 and PARP-3 is robustly activated through binding to DNA strand breaks. These enzymes are thereby involved in various pathways of the cellular response to DNA damage [10–12]. The catalytic domain of these PARP enzymes includes a regulatory helical domain (HD) that is closely coupled to the ART fold (Fig. 2a). Outside of the catalytic domain, PARP-1, PARP-2 and PARP-3 share a common Trp-Gly-Arg (WGR) domain that interacts with DNA and acts as a key regulator of catalytic activity in response to DNA damage [13]. In addition, PARP-1 bears three zinc finger domains (Zn1, Zn2, Zn3) and a BRCA-C-terminus (BRCT) domain, whereas PARP-2 and PARP-3 have more compact structures with only short N-terminal extensions from the WGR domain. The N-terminal extensions of PARP-2 and PARP-3 contribute to overall DNA binding affinity, but are not strictly required for catalytic activity [14] [15]. Thus, the WGR domain serves as the central regulatory feature of PARP-2 and PARP-3. Notably, the WGR domains of PARP-2 and PARP-3 are sensitive to the nature of the DNA break. For example, both PARP-2 and PARP-3 are preferentially activated by DNA breaks carrying a 5’ phosphate group (5’P) [15–17], and PARP-3 activity is most responsive to 5’ phosphorylated single strand nicks [15]. In contrast, PARP-1 is relatively insensitive to the phosphorylation state of the DNA breaks that it binds and is activated by. A recent crystal structure of the WGR domain of PARP-2 bound to DNA has provided the first insights into the structural basis for selectivity toward 5’ phosphorylated DNA and for the detection of DNA breaks (Fig. 2b) [18]. Interestingly, the WGR domain of PARP-2 spans two DNA ends in the crystal structure (Fig. 2b), leading to the proposal that PARP-2 might serve a role in bridging DNA ends in pathways of DNA repair [18].

PARP-1 detection of DNA strand breaks

PARP-1 binds in a sequence-independent manner to a variety of DNA strand break structures, including nicked and gapped single strand breaks (SSBs), and overhang and blunt ended double strand breaks (DSBs). Zinc fingers Zn1 and Zn2 serve as the primary sensors

of DNA strand breaks. Crystal structures of the individual Zn1 and Zn2 domains bound to blunt-ended, double-strand DNA breaks indicated similar modes of interaction, with the zinc fingers contacting a continuous segment of the DNA backbone (“the backbone grip”) and the nucleobases that were exposed at the DNA end (“the base-stacking loop”) [19](Fig. 2c). A crystal structure containing the domains of PARP-1 that are essential for DNA-dependent catalysis (Zn1, Zn3, WGR and CAT) in complex with a DNA double-strand break indicated how multiple domains of PARP-1 could assemble around the Zn1 domain bound to the end of the DNA [13](Fig. 2d). The essential domains of PARP-1 exhibit mutually compatible DNA binding contacts and collectively provide high affinity interaction with a DNA break. Moreover, the contact points between the domains are critical for poly(ADP-ribose) production in response to DNA double-strand breaks [13,20].

The structural data described above for PARP-1 interaction with DNA double-strand breaks established a model for PARP-1 activation, but there were still questions regarding PARP-1 interaction with other types of DNA damage, and whether the same assembly of domains and mode of activation would be utilized. Given PARP-1 involvement in SSB repair pathways [10], the mode of activation by single-strand break DNA damage was of particular interest and importance. Recently, the Neuhaus group determined the NMR structure of the Zn1-Zn2 fragment of PARP-1 detecting a single-strand DNA break [21] (Fig. 2c), indicating how the PARP-1 zinc fingers are utilized to specifically engage this type of DNA damage. Briefly, the NMR study indicated that the two zinc fingers are distributed to separate sides of the DNA break site, with Zn1 engaging the 5′ stem, and Zn2 engaging the 3′ stem (Fig. 2c). Moreover, NMR analysis with the Zn1–Zn2–Zn3 fragment bound to the SSB, and with the WGR domain titrated into the Zn1–Zn2–Zn3/DNA complex, indicated that the same domain interfaces formed in the PARP-1 complex with a DSB are also utilized in the complex with an SSB (e.g. Zn3 contacts with Zn1, and WGR contacts with Zn1 and Zn3). Notably, the specific positioning of the Zn1 domain on the 5′ stem of the SSB allows the other domains of PARP-1 to collectively assemble on a single-strand break in the same manner observed on a double-strand break, thus providing a unified model for PARP-1 detection of DNA damage (Fig. 2e).

The structural data summarized above together with other recent studies involving sedimentation analysis, small-angle x-ray scattering, single molecule tracking, and cell-based functional assays [13,21–25] indicate a monomeric, intramoleculer mechanism for PARP-1 detection of DNA strand breaks and subsequent activation. Collectively, these data argue strongly against studies that had suggested a dimeric interaction of PARP-1 with DNA [26–28]. One feature of the monomeric, intramolecular activation mechanism is that it positions PARP-1 for self-modification (automodification) *in cis*, as recently demonstrated biochemically [21]. The biochemical analysis of DNA strand-break activation of PARP-1 has highlighted the critical importance of the interfaces that form between PARP-1 domains after detecting DNA damage [13,19–21,29]. More recently, cell-based CRISPR-Cas9 screens have identified PARP-1 point mutations that lie at these same domain interfaces and give rise to PARP inhibitor resistance, as well as a patient-derived example of a clinical resistance mutation that targets the WGR interface with Zn1 [30].

Opening the HD for NAD+

The crystal structure of PARP-1 bound to a DNA double-strand break founded an allosteric activation mechanism in which the detection of DNA damage organized PARP-1 domains into a configuration that imposed a de-stabilizing structural transition in the HD domain [13]. However, the structural transition in the HD observed in the crystal structure did not offer an obvious mechanistic explanation for 1000-fold increase in poly(ADP-ribose) production in response to DNA breaks. A critical advance in the model of PARP-1 allosteric activation came from the analysis of PARP-1 dynamics using hydrogen/deuterium exchange coupled to mass spectrometry (HXMS) [31](Fig. 2d). HXMS identified that specific regions of the HD that form helices in the absence of DNA become unfolded when PARP-1 binds to DNA damage. Deletion of the HD led to constitutive PARP-1 activity at a level that matched DNA break-induced activation, thus revealing an inhibitory function for the HD [31]. The HD inhibitory mechanism was proposed either (i) to force bound NAD^+ into a conformation that was incompatible with efficient catalysis, or (ii) to completely block NAD^+ binding to the active site. This next critical element of the allosteric activation mechanism was recently provided from a study using the non-hydrolyzable NAD^+ analog called BAD (benzamide adenine dinucleotide)[32]. This study revealed that the HD fully blocks binding of substrate NAD^+ to the active site of PARP-1, thereby inhibiting poly(ADP-ribose) production by restricting access to substrate (Fig. 3). The HD provides a *selective* steric block of the active site, since smaller compounds such as benzamide and nicotinamide are still able to bind to the catalytic active site in the presence of a folded HD structure. Only larger compounds like NAD^+ that extend toward the HD are prevented from binding (Fig. 3). When PARP-1 binds to DNA damage, the block on substrate NAD^+ is relieved through local unfolding of the HD that is induced through contacts with the regulatory domains bound to DNA. In the absence of DNA, PARP-1 can only bind to NAD^+ when the HD transiently samples the unfolded conformation, thus explaining the very low basal activity of PARP-1.

The same allosteric mechanism of activation exists in PARP-2 and PARP-3, where the HD also serves an inhibitory function that blocks NAD^+ binding [31,32]. The need to tightly control the activity of PARP-1, PARP-2 and PARP-3 is likely to be related to the fact that they are NAD^+ consumers with the capacity to globally effect the cellular energy pool and metabolic state, and with the potential for dramatic cellular consequences [33]. Indeed, it was shown that the PARP-1 HD mutant L713F, with elevated constitutive activity, leads to NAD^+ depletion and apoptosis when expressed in human cells [34]. Importantly, the HD does not influence the NAD^+ binding site conformation, but instead provides a blockage that can be reversibly controlled in order to achieve different levels of activation that allow PARP-1 to perform cellular housekeeping functions as well as acute responses to cellular stress.

Active site communication to the DNA binding domains – allostery in reverse

PARP-1 DNA binding domains communicate the detection of a DNA strand break to the catalytic active site (Fig. 2d). Analysis of PARP-1 binding to the non-hydrolyzable NAD^+

analog BAD indicated that active site occupancy can be communicated back to the DNA binding domains, thereby influencing PARP-1 persistence on DNA [32](Fig. 3). BAD binding to the catalytic domain increased the affinity of PARP-1 for a DNA strand break and the retention of PARP-1 on a DNA probe in a competition experiment. At the structural level, a decrease in hydrogen/deuterium exchange was observed in PARP-1 peptides involved in domain-domain and DNA contacts, indicating that these interactions were strengthened in the presence of BAD [32], This effect was specific to a compound like BAD with a binding mode that extends to the HD and can therefore influence the distribution of HD conformations; the smaller compound benzamide was not able to influence active site communication to the DNA binding domains.

Inhibition of PARP enzymes has emerged as a strategy for targeting the inherent genomic instabilities of certain cancer cells [35,36]. A reverse allostery mechanism has been proposed to explain why some PARP-1 inhibitors are more efficient at trapping PARP-1 on DNA and exhibit correspondingly greater cytotoxicity, despite having similar inhibitory potential [37,38]. However, some studies have suggested that reverse allostery does not play a significant role for current clinical inhibitors in PARP-1 trapping on DNA and that the observed differences in trapping abilities might solely be explained by differences in inhibitory potential and inhibitor dissociation rates [3 9–41]. Moving forward, a deeper understanding of inhibitor influence on PARP-1 allostery is necessary and could provide important insights into the continued development and clinical use of PARP inhibitors.

In addition to a better understanding of the intramolecular activation mechanism of PARP-1, it will be important to understand how other cellular factors influence the PARP-1 catalytic output. An interesting recent example is histone PARylation factor 1 (HPF1), a binding partner of PARP-1 (and PARP-2) that modulates the output of PARP-1 catalytic activity [42], HPF1 interacts with the catalytic domain of PARP-1 and shifts the distribution of the poly(ADP-ribose) modification toward heteromodification of histones (rather than just PARP-1 automodification). Moreover, HPF1 steers PARP-1 to create serine-linked ADP-ribose modifications over other residues such as glutamic acid [43]. Structural studies will be necessary to determine how exactly HPF1 affects PARP-1/PARP-2 conformation and/or their interaction with histones to operate this switch in target residues. Notably, recent studies have highlighted an underappreciated prevalence of serine-linked ADP-ribosylation in cells [44,45]. These studies underscore the need to better understand the specificity underlying the modification of serine residues, and to understand the differences in biological outcomes associated with serine-linked ADP-ribosylation *versus* other types of modification, such as glutamate-linked ADP-ribosylation.

Tankyrase enzymes

The PARP enzymes known as Tankyrases (also referred to as PARP5a/PARP5a and PARP5b/PARP6) participate in diverse cellular functions, for example telomere maintenance, mitosis, and β-catenin/Wnt signaling [46–49]. There are two human Tankyrase enzymes, Tankyrase-1 and Tankyrase-2 (here we will use the term Tankyrase to refer to both enzymes). Tankyrase modification of target proteins with poly(ADP-ribose) frequently marks these proteins for processing by the ubiquitin ligase/proteosomal degradation system

[50,51], thus allowing Tankyrase to regulate cellular protein levels and thereby influence biological pathways. In a notable example, Tankyrase catalytic activity regulates the canonical Wnt signaling pathway by binding and modifying Axin with poly(ADP-ribose) [49,52–54]. Axin is the limiting factor of a multicomponent assembly of proteins referred to as the “destruction complex,” [55,56], which degrades β-catenin in the cytoplasm to prevent its movement to the nucleus where it activates gene transcription [56]. Tankyrase modification of Axin with poly(ADP-ribose) lowers Axin levels, protects β-catenin from destruction, and thereby increases β-catenin dependent transcription [49].

Tankyrase-1 and Tankyrase-2 have the same overall domain organization composed of an ankyrin repeat region, a sterile alpha motif (SAM) fold, and a catalytic domain with the ART fold (Fig. 4a). The N-terminus of Tankyrase-1 additionally bears a region of low complexity sequence with runs of histidine, proline, and serines (HPS). In contrast to the autoinhibited catalytic domain of PARP-1, Tankyrase catalytic domains do not contain an HD and are largely accessible to bind NAD^+ substrate. The principal Tankyrase regulatory mechanisms appear to be ankyrin repeat selection of binding partners, and SAM domain modulation of Tankyrase polymer formation. Consistent with the diverse cellular function of Tankyrase, the ankyrin repeats interact with a variety of partners proteins: e.g. telomere repeat factor 1 (TRF1), nuclear mitotic apparatus protein (NuMA), the β-catenin/Wnt signaling protein Axin, and DNA repair factor MERIT40.

Tankyrase ankyrin repeats – a flexible, multivalent binding platform

The ankyrin repeat region mediates interaction with Tankyrase binding partners. It consists of 25 copies of the ankyrin motif, a ~33 amino acid structure composed of a helix-loop-helix that can form extended solenoid-like configurations. The N-terminal repeats of Tankyrase are segmented into 5 ankyrin repeat clusters (ARCs) owing to the regularly-spaced positioning of variant ankyrin repeats that form N-terminus-capping and C-terminus-capping structures, and thereby disrupt the otherwise continuous extension of the repeats [57,58](Fig. 4a, b). The ARCs form the basic unit for recognizing peptide motifs in Tankyrase binding partners. ARCI, ARC2, ARC4, and ARC5 are capable of interacting with Tankyrase peptide motifs, whereas ARC3 lacks key peptide-interacting residues and therefore does not bind to Tankyrase peptide motifs [58–60]. Tankyrase-interacting peptide motifs generally span a segment of 8-amino acids and exhibit degenerate sequence requirements in most positions, with the exception of a strict requirement for an arginine at position 1 and a glycine at position 6 [58](Fig. 4b).

X-ray crystallography has provided a firm structural basis for ARC interaction with peptides derived from binding partners [58–63]. In contrast, much less is understood about Tankyrase interaction with these peptide motifs in the context of the complete tertiary/quaternary structures of diverse binding partners. Most, if not all, Tankyrase binding partners exists as multimers (e.g. dimeric TRF1, polymeric Axin), and thus present multiple peptide motifs with different spatial positioning. Moreover, some binding partners present consecutive peptide motifs, such as the two motifs identified in Axin [60], which provide the potential for multivalent binding. Likewise, the relative positioning of the five ARCs will also play a role in the mode of Tankyrase interaction with binding partners. Together, these structural

determinants are likely to play a major role in the overall affinity and stability of Tankyrase interaction with binding partners.

A crystal structure of the ARC1-ARC2-ARC3 (ARC1–3) segment of Tankyrase-1 represents the largest portion of the ankyrin repeats analyzed to date and provides new insights into the overall organization of the ankyrin repeat region [59] (Fig. 4b). The X-ray structure and solution structure from small-angle X-ray scattering (SAXS) indicated a rigid conformation for the ARC1–3 section of the ankyrin repeat region, with defined transitions from ARC1 to ARC2 (“broken helix”) and ARC2 to ARC3 (“continuous helix”) giving rise to a distinctive C-shape (Fig. 4b). The positioning of the peptide binding pockets on ARC1 and ARC2 are thus held in a fixed relative conformation. A SAXS-derived model for the full ankyrin repeat region, ARC1–5, indicated that ARC4 and ARC5 are flexibly positioned relative to the more rigid ARC1–3 segment (Fig. 4c). Binding analysis with a divalent peptide derived from Axin was used to test the importance of the relative positioning of the two Axin motifs, and the ability of ARCs to simultaneously engage the two motifs. Consistent with the fixed relative spacing of ARC1 and ARC2 observed in the crystal structure, the binding analysis indicated that the affinity of the Tankyrase-1/Axin interaction is sensitive to the spacing between the two peptide motifs in Axin1, in that a shortened linker between the motifs lowered the affinity of interaction [59]. Tankyrase-1 mutagenesis indicated that specific pairs of ARCs can bind simultaneously to the two motifs in Axin: ARC1/ARC2, ARC4/ARC5, and ARC1/ARC5, but not ARC2/ARC4 (Fig. 4d). ARC3 likely serves as a fixed structural element that prevents ARC2 and ARC4 from simultaneously engaging Axin1. The pairing of ARC1 and ARC5 is quite interesting since it suggests a high level of overall flexibility, since ARC1 and ARC5 are located at opposite ends of the ankyrin repeat region (Fig. 4d). Unlike the pairing of ARC1/ARC2 and ARC4/5, the pairing of ARC1/ARC5 was not sensitive to the shortened linker between the two peptide motifs in Axin. The SAXS analysis also indicated that the conformation of the ankyrin repeats is altered upon binding Axin, suggesting that Tankyrase is an adaptable binding platform that responds to the nature of the interacting partner.

Structural analysis of Tankyrase bound to complete binding partners will ultimately be required to answer questions regarding the specificity of ankyrin repeat interactions with proteins of varied structures, and the potential for the binding interaction to be coupled to delivery to the catalytic domain for ADP-ribose modification. It is noteworthy that not all Tankyrase binding partners are modified with ADP-ribose [64,65], potentially indicating that the mode of interaction could influence catalysis, for example by imposing an inactive Tankyrase conformation.

SAM domain regulation of Tankyrase catalysis and polymer formation

The SAM domain is a ~70 amino acid helical protein module that is commonly involved in homo- and hetero-dimerization/oligomerization, but is also used to mediate other functions such as RNA binding [66]. Tankyrase-1 and Tankyrase-2 polymerize through their SAM domains, and SAM domain polymerization is required for full catalytic activity [67]. Recent X-ray crystallographic studies and structural modeling have provided detailed insights into the interfaces that form in the isolated SAM domain polymer [68–70](Fig. 3e), allowing structure-based mutagenesis to illustrate the essential role of Tankyrase polymerization in

promoting β-catenin/Wnt signaling [68,69]. Polymerization-deficient mutants were not able to stimulate Wnt signaling and exhibited greatly reduced catalytic activity. In contrast to the disruption of SAM domain polymerization, neither Tankyrase catalytic inhibitor treatment nor catalytic active site mutations were able to completely abolish Tankyrase stimulation of Wnt signaling, at least under Tankyrase overexpression conditions [68]. These results suggest that Tankyrase plays a protein scaffolding role that supports Wnt signaling, in addition to the role that catalytic activity plays in regulating Wnt signaling. The components of the Wnt signaling destruction complex reversibly and dynamically associate into cytoplasmic signaling complexes [71]. Axin self-polymerizes through a Dishevelled and Axin (DIX) domain (Fig. 4f) and interacts with destruction complex components, thereby playing a pivotal role in regulating the assembly and stability of the destruction complex [72]. The polymeric structure of Tankyrase could play a similar role in organizing cytoplasmic signaling complexes through its proposed scaffolding function (Fig. 4f)[68].

A complete understanding of Tankyrase regulation and catalysis will clearly require further structural insights into its multidomain architecture. There are several key questions to be addressed. For example, what is the mechanism by which Tankyrase oligomerization contributes to catalytic activity? The linker connecting the SAM domain to the CAT domain is fairly short, perhaps allowing an interaction between these domains that could influence catalytic output, and possibly be reversed through SAM polymerization. Zhang and colleagues have recently suggested that dimerization of the Tankyrase catalytic domain regulates catalytic output [73]. In this model, catalytic domain dimerization depends on SAM-mediated polymerization, thus providing a potential basis for SAM polymerization to regulate poly(ADP-ribose) production. Another plausible mechanism is that the oligomeric form of Tankyrase serves to optimally position the CAT relative to a target protein(s) bound to the ankyrin repeats. Indeed, the N- and C-termini of the SAM domain both face the outside surface of the polymeric structure, thus supporting this type of communication between the ARCs and the CAT (Fig. 4e, f).

Conclusion

The PARP family has a variety of regulatory domains and structural features that matches the diverse aspects of cellular biology that PARP enzymes regulate. As highlighted here, PARP-1 and Tankyrase offer a glimpse of the interesting mechanisms that regulate PARP enzymes. PARP inhibitors have gained considerable interest for targeting cancers with genomic instabilities that depend on PARP-1 function and are sensitive to PARP-1 being trapped on DNA [74,75], and also for treating cancers with abnormal Wnt signaling, where Tankyrase can be targeted for its substantial regulatory role in this pathway [49–51]. Thus, the structural analysis of PARP-1 and Tankyrase will not only yield interesting insights into unique modes of enzyme regulation and catalysis, but could also contribute to the development of inhibitors that specifically target these unique regulatory mechanisms.

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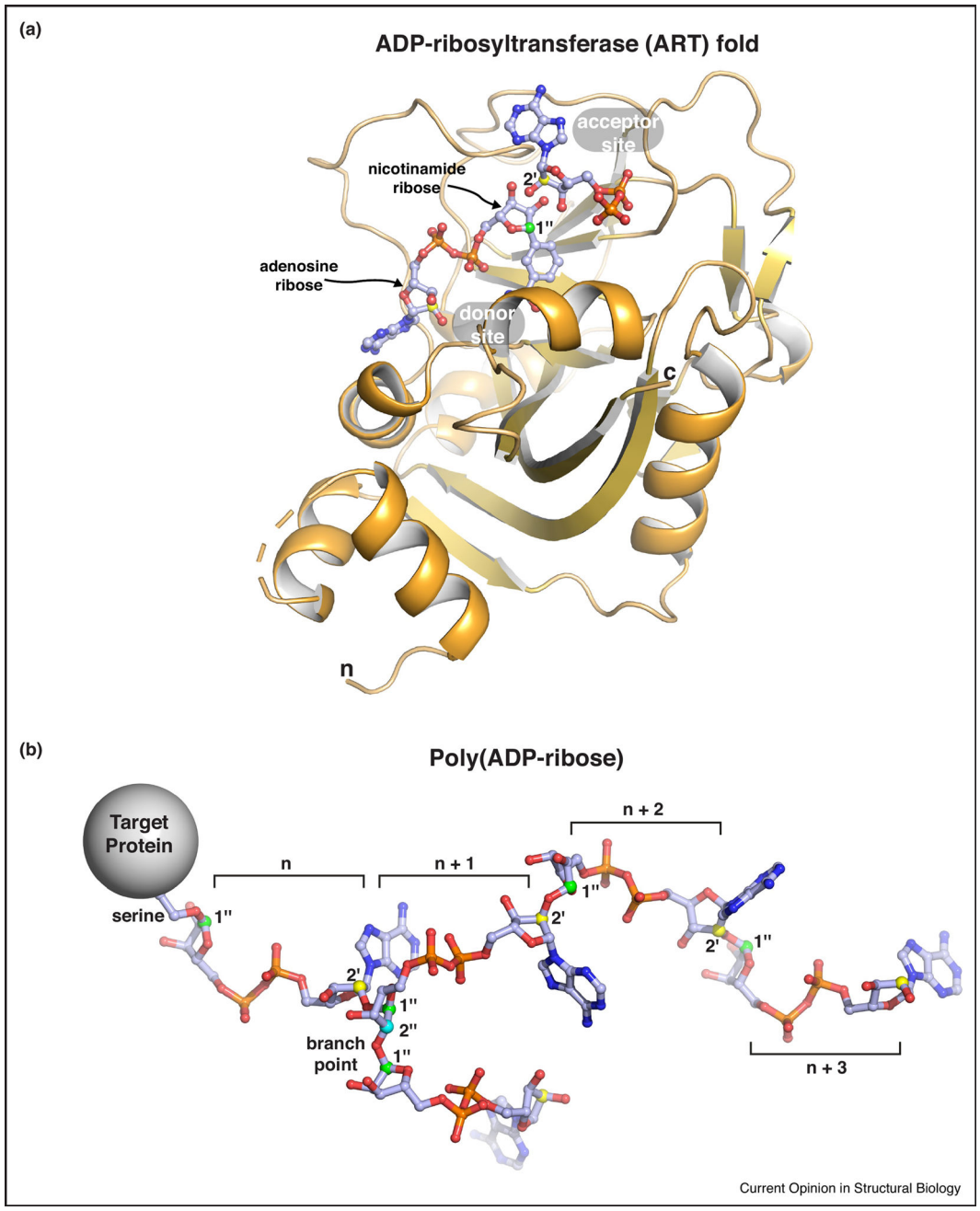

Figure 1. Poly(ADP-ribose) and the ADP-ribosyltransferase (ART) fold.

(a) The ADP-ribosyltransferase (ART) fold of PARP family enzymes. The minimal catalytic region of human PARP-1 is shown bound to a non-hydrolyzable NAD^+ analog (PDB code 6bhv; [32]). Substrate NAD^+ binds to the "donor site." The adenosine ribose and the nicotinamide ribose are indicated. Adenosime ribose atoms are labeled as single prime (') and nicotinamde ribose atoms are labeled as double prime ("). Protein side chains to be modified with ADP-ribose attack the 1 " carbon of nicotinamide ribose (labeled and colored green), thus releasing nicotinamide. The current model for the extension of the primary ADP-ribose group is based on an "acceptor site" identified in the crystal structure of chicken

PARP-1 (PDB code la26; [76]). The ADP group from the chicken PARP-1 structure was overlayed on the structure of human PARP-1. Extension of the ADP-ribose chain is proposed to arise from the acceptor site adenosine ribose 2' hydroxyl (2' carbon is labeled and colored yellow) attacking the donor site NAD^+ on the nicotinamide ribose 1" carbon (creating a 1" to 2' linkage; see panel b). Branches are proposed to arise from an ADP-ribose that binds in a reversed orientation in the "acceptor site," thus instead placing the nicotinamide ribose 2" hydroxyl in position to attack the donor site NAD^+ on the nicotinamide ribose 1" carbon (creating a 1" to 2" linkage; see panel b). Structures that capture reaction intermediates are required to fully understand the catalysis of poly(ADP-ribose). The termini of the ART fold are labeled (n and c).

(b) The poly(ADP-ribose) posttranslational modification. Four linear units of ADP-ribose (denoted n, n+1, …) are shown attached to a serine side chain on a target protein. A single branch point is shown extending from the n + 1 ADP-ribose of the linear chain. The serine sidechain oxygen is attached to the 1" carbon of the nicotinamide ribose (colored green as above). Linear chains of ADP-ribose units are formed through a ribose-ribose linkage: nicotinamide ribose 1" carbon linked to the adenosine ribose 2' carbon (colored yellow as above). A branch in the poly(ADP-ribose) chain forms a different ribose-ribose linkage: nicotinamide ribose 1" carbon linked to a nicotinamide ribose 2" carbon (colored cyan on the n + 1 ADP-ribose unit).

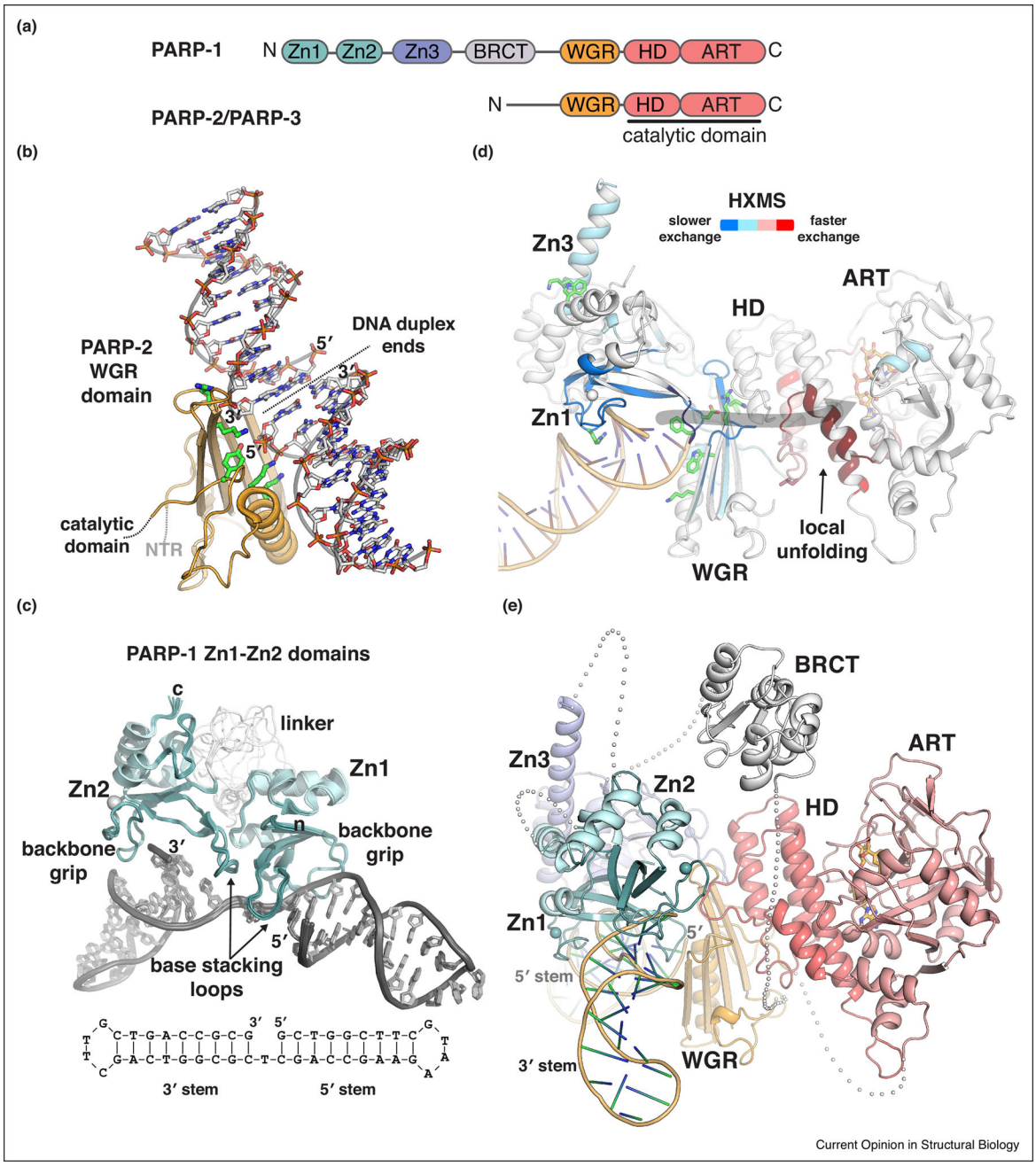

Figure 2. Structural insights into PARP-1 and PARP-2 DNA damage detection.

(a) Schematic representation of human PARP-1, PARP-2, and PARP-3 domain organization. Zinc fingers: Zn1, Zn2, and Zn3; BRCT – BRCA C-terminus; WGR: Trp-Gly-Arg domain; HD –helical domain; ART – ADP-ribosyltransferase.

(b) Structure of the human PARP-2 WGR domain bound to 5’ phosphorylated DNA (PDB code 6f5b; [18]). The crystal asymmetric unit contains two DNA duplexes. The WGR binds where the two DNA ends meet, which mimics a DNA strand break. Thus, the WGR spans the DNA break, engaging the 5’ phosphorylated end of one DNA duplex (labeled 5’), and the 3′OH terminated end of the second duplex (labeled 3’). Some of the key esidues involved

in binding the 5’ phosphate, or the second duplex, are shown as sticks and colored green. A second WGR domain is present in the crystal asymmetric unit, but has not been illustrated for clarity. The second WGR domain is bound to the opposite face of the DNA in the same manner as shown.

(c) Structure of the Zn1-Zn2 fragment of human PARP-1 detecting a DNA single-strand break (PDB code 2n8a; [21]). A schematic of the DNA is drawn below the complex structure, illustrating the single strand break with a one nucleotide gap. The two sides of the DNA break are denoted 3’ stem and 5’ stem, based on the nature of the DNA terminus. Zn1 engages nucleotide bases on the 5’ stem, and Zn2 engages nucleotide bases on the 3’ stem. The N-terminus of Zn1 is labeled (n), and the C-terminus of Zn2 is labeled (c). The extended linker region connecting Zn1 to Zn2 adopts multiple conformations, and is likely to allow the two zinc fingers to engage a variety of damaged DNA structures in which the relative positioning of the ends could vary [21]. **(c)** Changes in PARP-1 dynamics upon detecting DNA damage were revealed by HXMS [31]. PARP-1 peptides experiencing slower amide hydrogen exchange in the presence of a DNA strand break are indicative of newly formed protein-DNA contacts and/or domain-domain contacts, and the peptides exhibiting slower exchange (colored blue) are consistent with contacts observed in the PARP-1 complex with a DNA double-strand break (shown in cartoon with domains labeled) [13]. Remarkably, several PARP-1 peptides grouped in the HD experienced much faster amide hydrogen exchange in the presence of DNA (colored red). The rate of exchange was much faster than possible for amide hydrogens involved in forming a helix, thus these helical regions of the HD were proposed to unfold in the presence of DNA, or to rapidly sample the unfolded state [31]. Several of the key PARP-1 residues essential for DNA-dependent poly(ADP-ribose) catalysis [13,19,20,29] are drawn as sticks, and they highlight the allosteric network that connects PARP-1 DNA damage detection to the catalytic active site (indicated by grey arrow).

(d) A composite model for full-length PARP-1 detecting a single-strand DNA break. The crystal structure of Zn1-Zn3-WGR-CAT was aligned to the NMR structure of the Zn1-Zn2 fragment bound to a single-strand break [13,21]. The BRCT domain was manually positioned between the C-terminus of the Zn3 domain and the N-terminus of the WGR domain. The linker residues connecting PARP-1 domains are shown as grey spheres, with each sphere representing an amino acid residue. The DNA break site is noted by the labeling of the 5’ terminus adjacent to the WGR domain.

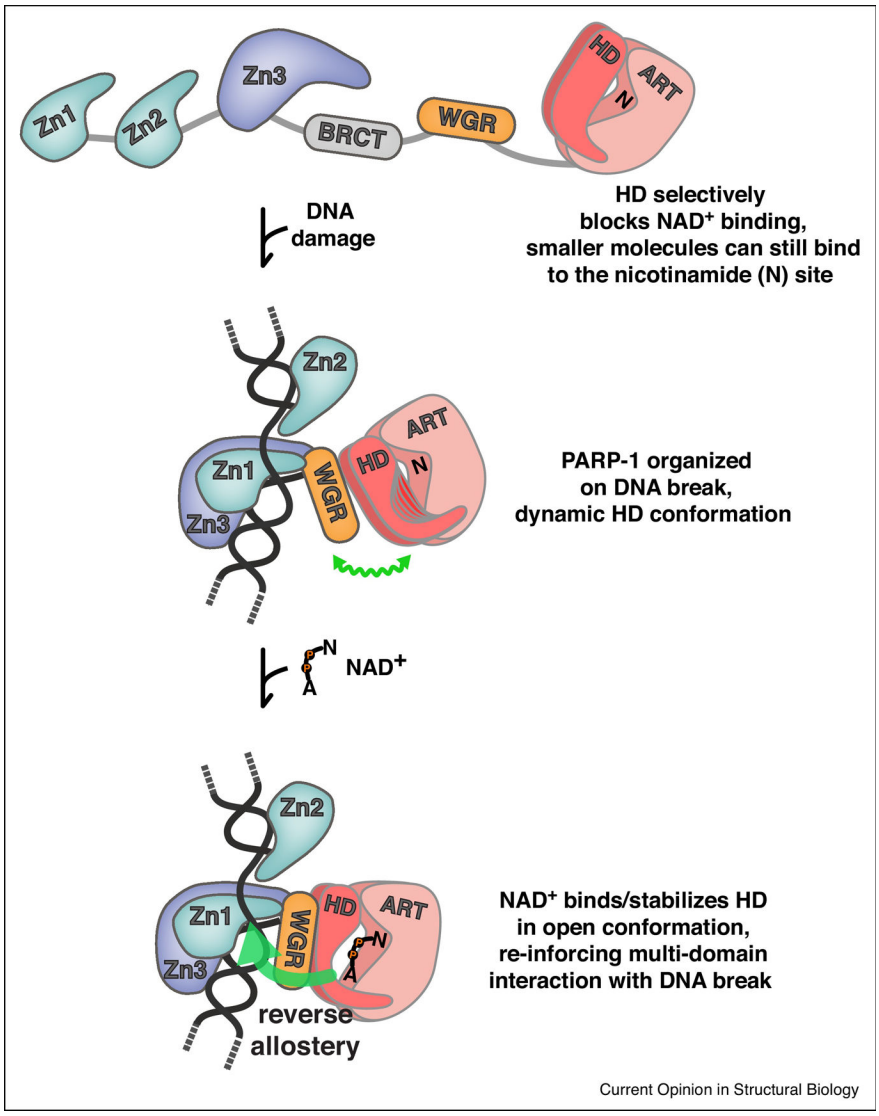

Figure 3. Allosteric regulation of PARP-1.

Regulation of NAD^+ access and reverse allostery. The HD regulates PARP-1 catalytic activity through a substrate-blocking mechanism [32]. In the absence of DNA, the domains of PARP-1 exist in an open configuration. In this state, small molecules can bind to the nicotinamide site (N) of the ART; however, NAD^+ binding is completely blocked. Upon detecting DNA damage, PARP-1 domains are organized around the DNA break, and an allosteric network of contacts destabilizes the HD, leading to a dynamic HD structure, illustrated as multiple potential HD conformations, and accented by a wavy green line that indicates a flexible conformation. NAD^+ is now able to access the catalytic active site, engaging the nicotinamide site (N) and the adenosine site (A) that was previously blocked by the HD. NAD^+ binding pushes the distribution of HD conformations toward the unfolded state, thus promoting the PARP-1 assembly of domains on DNA. Thus, NAD^+ binding can influence PARP-1 interaction with DNA through a reverse allostery mechanism from catalytic active site to DNA binding domain (large green arrow).

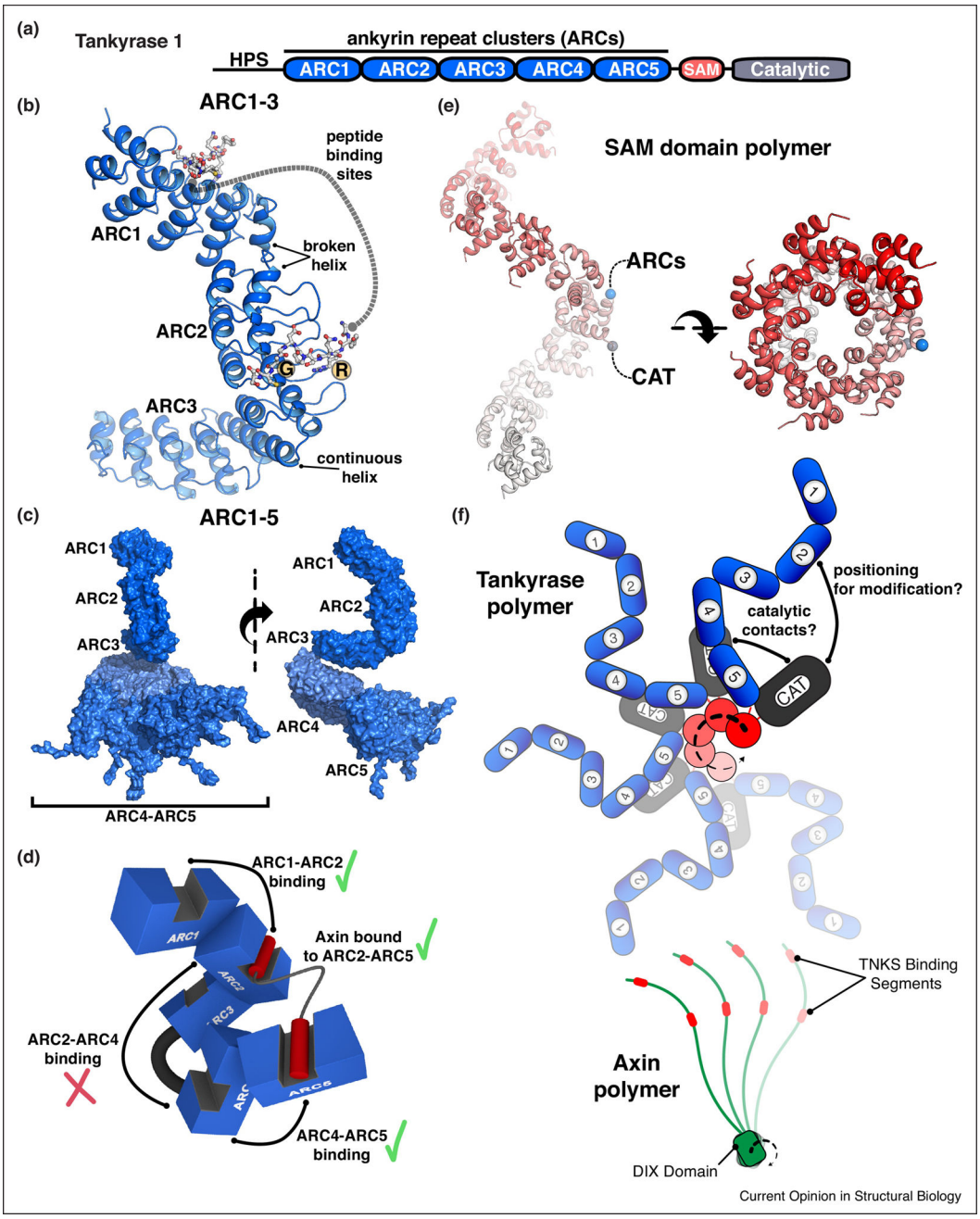

Figure 4. Structural biology of Tankyrase regulation.

(a) Schematic representation of human Tankyrase-1 domain architecture. ARC – ankyrin repeat cluster (ARC); SAM – sterile alpha motif; HPS – histidine/proline/serine-rich region of unknown function. Human Tankyrase-2 has the same domain architecture, but lacks the HPS region.

(b) Crystal structure of the ARC1–ARC2–ARC3 fragment of human Tankyrase-1 bound to a peptide derived from IRAP (insulin regulated aminopeptidase) [59]. Peptide bound to ARC1 and ARC2 are drawn as sticks. The peptide bound to ARC2 indicates the essential contact points – an Arg (R) at position 1 and a Gly (G) at position 6. ARC3 lacks the amino acids

necessary for peptide binding. The structure indicated two types of transitions between consecutive ARCs: a “broken helix” connecting ARC1 to ARC2, and a “continuous helix” connecting ARC2 to ARC3. The relative positioning of the two peptide binding sites is fixed. The ARC1–ARC2–ARC3 conformation was confirmed by SAXS analysis [59].

(c) SAXS analysis of the entire ankyrin repeat region, ARC1–5. In contrast to the rigid conformation of ARC1–3, ARC4 and ARC5 are more flexibly connected. An ensemble of structures was used to model the SAXS data [59], and a selection of ARC4-ARC5 conformations are shown.

(d) Axin interaction with ARC1–5. A cartoon representation of ARC1–5 based on SAXS-based modeling in panel (c). Axin contains two peptide regions that bind to Tankyrase [60] (shown here as red cylinders). Combinatorial mutagenesis of ARC peptide binding sites and Axin binding affinity analysis indicated that specific ARC pairs can function together to bind Axin: ARC1:ARC2, ARC4:ARC5, ARC2:ARC5 (shown as green check mark). Non-binding ARC3 separates ARC2 and ARC4 and prevents them from simultaneously engaging Axin (red check mark.

(e) Tankyrase SAM domain polymer. The crystal structure of the wild-type human Tankyrase-1 SAM domain is shown from two views related by a 90° rotation (PDB code 5kni [69]; see also [68]). For one SAM domain in the polymer, the N-terminus (blue sphere) and C-terminus (grey sphere) are indicated to highlight that the ARCs and the catalytic domain (CAT) will extend from the outside surface of the SAM polymer.

(f) A model for the polymeric from of Tankyrase based on current structural information. A juxtaposed cartoon model of the Axin polymer [72] illustrates the potential for avidity-dependent interaction of these proteins [51,68].