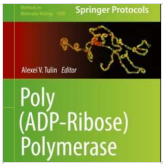

Sep 02, 2021

Identifying and Validating Tankyrase Binders and Substrates: A Candidate Approach

Book Chapter

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ABSTRACT

The poly(ADP-ribose)polymerase (PARP) enzyme tankyrase (TNKS/ARTD5, TNKS2/ARTD6) uses its ankyrin repeat clusters (ARCs) to recognize degenerate peptide motifs in a wide range of proteins, thereby recruiting such proteins and their complexes for scaffolding and/or poly(ADP-ribosyl)ation. Here, we provide guidance for predicting putative tankyrase-binding motifs, based on the previously delineated peptide sequence rules and existing structural information. We present a general method for the expression and purification of tankyrase ARCs from *Escherichia coli* and outline a fluorescence polarization assay to quantitatively assess direct ARC–TBM peptide interactions. We provide a basic protocol for evaluating binding and poly(ADP-ribosyl)ation of full-length candidate interacting proteins by full-length tankyrase in mammalian cells.

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KEYWORDS

Tankyrase, PARP, Poly(ADP-ribosyl)ation, Tankyrase-binding peptide motif, Enzyme–substrate relationships, Protein-protein interactions, Protein expression, Protein purification, Structural biology, Fluorescence polarization (FP)

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GUIDELINES

1. Introduction

Poly(ADP-ribosyl)ation (PARylation) constitutes a striking posttranslational modification (PTM) catalyzed by poly(ADP-ribose)polymerase (PARP) enzymes, which serially transfer ADP-ribose from NAD^+onto substrate proteins [1]. The resulting long, strongly negatively charged PAR chains provide attachment sites for proteins endowed with PAR-binding modules or directly affect substrate function [2]. PAR can thus act as a scaffolding component as well as a regulatory PTM. PARylation is most commonly associated with nuclear events such as gene regulation and DNA damage repair, but PAR is found in both the nucleus and the cytoplasm [1,3]. The PARP tankyrase, of which there are two human paralogs (TNKS/ARTD5, TNKS2/ARTD6, *see* Fig. 1a), contributes to both the nuclear and cytoplasmic pools of PAR [4,5]. The biological roles of the two tankyrases are largely redundant [6], pointing to shared molecular mechanisms. The recruitment of tankyrase to different protein complexes associated with specific cellular processes and situated at different subcellular locations defines its diverse functions. All tankyrase binders characterized to date bear a tankyrase-binding motif (TBM), which in its simplest form consists of six to eight consecutive amino acids [4,7,8]. TBMs are recognized by tankyrase's ankyrin repeat clusters (ARCs) [7,9,10]. Out of the five ARCs, the central one (ARC3) is devoid of a known peptide-binding function while the other four (ARCs 1, 2, 4, and 5) each feature a highly conserved peptide-binding pocket with similar specificities [7,10] (Fig.1a). Given four peptide-binding ARCs, tankyrase recognizes its binders multivalently .

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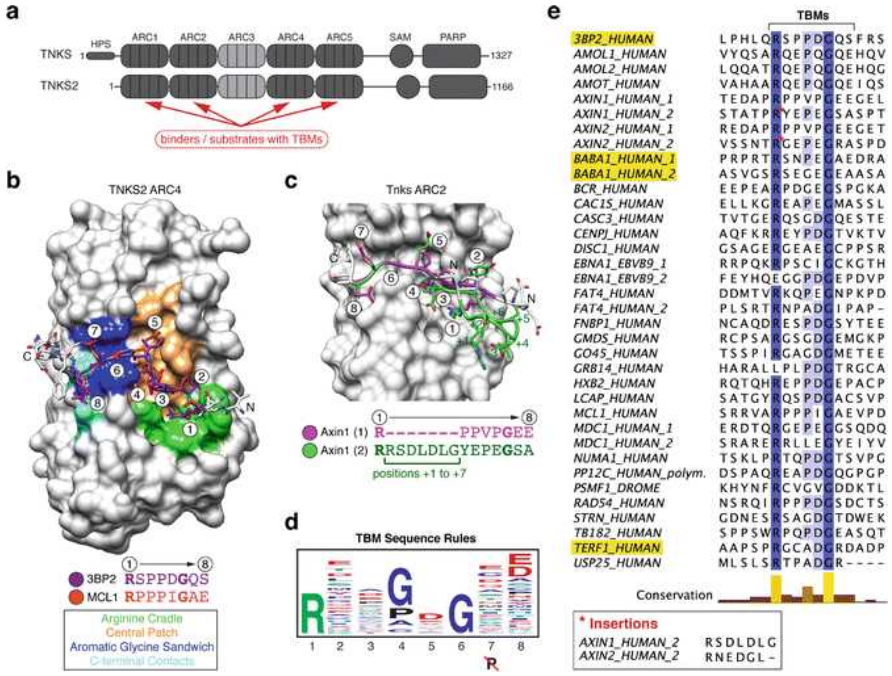

Fig. 1
Substrate binding by tankyrase. (**a**) Domain organization of human tankyrase and tankyrase 2 (modified from [31]). (**b**) and (**c**) Examples for ARC–TBM interactions studied by X-ray crystallography. (**b**) Human TNKS2 ARC4 is shown in surface representation with bound TBM peptides from 3BP2 and MCL1 shown in stick representation with the core TBM octapeptide colored *purple* and *orange*, respectively, and by heteroatom. TBM amino acid positions (1–8) and sequences shown. The figure was generated by superimposing the ARCs of the ARC4-3BP2 and ARC4-MCL1 crystal structures (PDB accession codes 3TWR and 3TWU, respectively) onto each other and showing ARC4 of the former [7]. The *colored* surface areas represent different contact areas, as indicated, that mediate binding of the TBM peptides (Modified from [7] with permission from Elsevier/Cell Press). (**c**) ARC2 (from ARC2–3) of murine Tnks bound by the N-terminus of murine Axin1 (PDB accession code 3UTM), which contains two TBMs [11]. Each TBM binds one copy each of ARC2 in a dimeric ARC2-3 assembly. The figure was generated by superimposing the two ARC2-3 copies onto each other; the surface of ARC2 bound by the first TBM is shown. TBMs are shown and labeled as in (**b**). The first TBM, shown in *magenta*, consists of a continuous stretch of eight amino acids. In the second TBM, shown in green, the Arg at position 1 is followed by a seven-amino-acid insertion (positions +1 to +7), as indicated in the sequences shown. The peptide insertion forms a loop. (**d**) TBM sequence rules represented by a sequence logo. (Reprinted from [7] with permission from Elsevier/Cell Press.) (**e**) Sequence alignment of known example TBMs ([7] and references therein, [11,22,23,37,38,39]), colored by identity with conservation graph, generated with ClustalX and Jalview [40,41]. UniProt IDs are indicated [24]. The *asterisk* indicates insertion sequences in AXIN1 and AXIN2. The TBMs of 3BP2, TRF1 (TERF1_HUMAN), and MERIT40 (BABA1_HUMAN), studied as model TBMs here, are *highlighted*

The TBM has been characterized extensively and a consensus sequence of R-x-x-[small hydrophobic or G]-[D/E]-G-[no P]-[D/E] identified through positional scanning and evaluation of known TBMs [7] (Fig. 1d and e). Paired with structural information from ARC–peptide complexes [7,11,12,13], these “sequence rules” provide a valuable tool for identifying tankyrase binders/substrates. Fig.1b-e gives examples for X-ray crystal structures describing TBM-ARC interactions, summarize the experimentally derived TBM sequence rules, and list known TBMs. Within the motif, the Arg residue at position 1, bound in an extensive “Arg cradle,” is essential, as is Gly at position 6, owing to its unique geometry that is adopted when the residue is sandwiched between two aromatic side chains in the ARC (Fig.1b, d). At positions 2 and 3, where side chains point away from the ARC, a wide range of amino acids is tolerated. At position 4, a small and/or hydrophobic residue binds a tight hydrophobic sub-pocket. Gly, Pro, Ala, and Cys (in this order of preference) have been found suitable by positional scanning [7], and known TBMs mostly feature Pro and Gly, followed by Ala, Val, Leu, and Cys (Fig.1e). At position 5, Asp is clearly preferred, with Glu ranked second, but Val, Gln, Tyr, Ile, and Cys were all allowed in positional scanning experiments (Fig.1b, d) [7]. In line with these observations, Asp and Glu are found at position 5 in the vast majority of known TBMs, followed by Gln, Pro, Ile, and Val (Fig.1e). Positions 7 and 8 tolerate a wide range of amino acids, with the exception of Pro at position 7, where Pro causes an unfavorable distortion of the peptide backbone (Fig.1d). At position 8, Asp and Glu are most favored, owing to an electrostatic contact with the ARC, and can balance out the negative contribution made by suboptimal residues at other positions (Fig.1b, d) [7]. Recent structural and biophysical studies suggest that the spatial organization of the five ARCs and the positioning of multiple TBMs within a subset of tankyrase binders encode another determinant of tankyrase binding [13].

Among the tankyrase–substrate relationships most extensively studied to date are those with AXIN (axis inhibition protein, AXIN1 , AXIN2), scaffolding proteins involved in Wnt signaling [11, 14], the telomeric protein TRF1 (telomeric repeat-binding factor 1, official gene name TERF1) [12, 15], and the signaling adaptor protein

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3BP2 (SH3 domain-binding protein 2, official gene name SH3BP2) [7, 16]. Many more tankyrase binders/substrates have been identified, and the list of known interactors keeps expanding [5]. An *in silico* prediction of tankyrase binders gives reason to anticipate a broad involvement of tankyrase in a wide range of biological functions [7]. To understand the complex biological roles of tankyrase, also in light of the considerable interest in tankyrase as a potential therapeutic target [5, 17], we require insights into the complement of tankyrase-binding proteins in the proteome.

Here, we outline a hierarchical three-step candidate approach for identifying tankyrase binders and substrates, providing further experimental detail on the method reported previously [7]. **Step 1** constitutes TBM prediction, **step 2** the evaluation of TBMs as direct ARC binders by fluorescence polarization (FP), and **step 3** the validation of tankyrase binding and tankyrase-dependent PARylation in the full-length protein context. We chose two model proteins: the first identified tankyrase binder, TRF1 [15], and a novel tankyrase binder, MERIT40 (Mediator of RAP80 interactions and targeting subunit of 40 kDa, official gene name BABAM1), which was identified by the approach presented here [7]. The TBM from 3BP2 serves as an additional example in the FP assay [7]. As part of **step 2**, we present a general method for the expression and purification of TNKS and TNKS2 ARCs from *Escherichia coli* (Table 1). ARCs 1, 4, and 5 can be produced as individual domains . ARCs 2 and 3 are insoluble when produced independently; however, they can be produced as a double ARC2-3 construct. Moreover, the entire tankyrase N-termini with all five ARCs can be generated [7, 13]. Proteins are expressed with a cleavable N-terminal His_6-GST tag , which enables simple affinity purification, minimally followed by size exclusion chromatography upon tag removal. The subsequent FP assay uses a candidate TBM peptide, synthesized with a fluorescent label such as fluorescein, to directly measure the binding affinity to a tankyrase ARC or a set of ARCs. In this assay, the fluorescent peptide probe is excited by polarized light. The light emitted by an unbound probe loses most of its polarization due to its rapid motion in solution. When bound to an ARC, movement of the peptide is slowed down and a high degree of polarization retained in the emitted light. Titration of tankyrase ARCs at a constant probe concentration allows the dissociation constant (K_d) to be determined [7, 18, 19, 20]. Upon confirmation of the isolated TBM, further validation of the candidate tankyrase binder requires an assessment of tankyrase binding and substrate PARylation using full-length proteins (**step 3**). We present details for a straightforward assay based on co-expression in HEK293T cells , co-immunoprecipitation and the detection of PAR in the immunoprecipitates, using PAR-binding antibodies [7].

A	B	C
ARC construct	**Construct boundaries**	**Molecular weight (kDa)**
TNKS ARC1–5	178-958	85.0
TNKS ARC1	178-336	17.2
TNKS ARC2-3	331-645	34.7
TNKS ARC4	646-807	18.0
TNKS ARC5	799-958	17.5
TNKS2 ARC1-5	20-800	85.3
TNKS2 ARC1	20-178	17.7
TNKS2 ARC2-3	173-487	35.3
TNKS2 ARC4	488-649	17.9
TNKS2 ARC5	641-800	17.3

Table 1
Human tankyrase (TNKS/TNKS2) ARC constructs for biophysical assays. The proteins include a non-native, vector-derived GAMGS sequence at the N-terminus that is retained upon cleavage of the affinity tag [7]

Acknowledgements

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(WT102360/Z/13/Z).

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MATERIALS TEXT

Unless the supplier is explicitly mentioned, chemicals are typically obtained from Sigma-Aldrich.

2.1 Protein Expression

- Inducible bacterial expression constructs for affinity-tagged tankyrase ARCs (TNKS: NM_003747.2; TNKS2: NM_025235.2); see Table 1 for construct details:

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TNKS2 ARC2–3	173–487	35.3
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Table 1
Human tankyrase (TNKS/TNKS2) ARC constructs for biophysical assays. The proteins include a non-native, vector-derived GAMGS sequence at the N-terminus that is retained upon cleavage of the affinity tag [7]

We recommend using vectors with a kanamycin selection marker. Ampicillin hydrolysis by secreted β-lactamase and under low pH increases the proportion of cells lacking the plasmid, which decreases protein yield [33].

- BL21-CodonPlus (DE3)-RIL *E. coli* chemically competent cells (Agilent Technologies)

RIL cells contain additional tRNAs for codons of Arg, Ile, and Leu that are otherwise rare in *E. coli*. The plasmid bearing these genes contains a chloramphenicol selection marker.

- “Lysogeny Broth” (LB) agar plates , supplemented with kanamycin (50 µg/mL) and chloramphenicol (34 µg/mL).
- LB medium (100 mL for overnight starter culture).
- “Terrific Broth” (TB) medium (4–8 L for large-scale expression).
- 1000× stock solutions of antibiotics: 50 mg/mL kanamycin (in H_2O) and 34 mg/mL chloramphenicol (in isopropanol).
- Shaking incubators capable of a temperature range of at least 18–37 °C.
- Erlenmeyer flasks (250 mL) for pre-cultures, baffled Erlenmeyer flasks (2 L) for large-scale expression.
- Isopropyl β-d-1-thiogalactopyranoside (IPTG , 1 M stock solution)
- Refrigerated centrifuge for harvesting large volumes of bacterial cultures (4000 ×*g*, e.g., Beckman Coulter Avanti J-26XP with JLA 8.1000 rotor).
- Liquid nitrogen bath.
- 50 mL Falcon tubes or plastic film with thermal sealer for storage of bacterial pellets.

2.2 Protein Purification

- Protease inhibitors , such as Pierce protease inhibitor tablets, EDTA-free (Thermo Fisher Scientific).
- Lysozyme, 40 mg/mL stock.
- Sonicator fitted with a large probe or homogenizer capable of breaking bacterial cells .
- Ultra-filtered H_2O.
- Cell lysis buffer: 50 mM Tris–HCl pH 7.5, 500 mM NaCl, 5 mM β-mercaptoethanol . Add protease inhibitor tablets and lysozyme (100 µg/mL final concentration) immediately before use.
- Refrigerated centrifuge for removing insoluble lysate fraction (30,000 × *g*, e.g., Beckman Coulter Allegra 64R with F0650 rotor).
- 5.0 µm syringe filter units.
- 5 mL HisTrap HP Ni^{2+}affinity column (GE Healthcare).

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We recommend using one 5 mL column per 4 L expression culture. If using larger volumes, connect additional column(s) in series. Ni^{2+}affinity columns can easily be reconstituted by stripping from and re-charging with Ni^{2+}as per the manufacturer's instructions.

- Peristaltic pump.
- Vacuum pump and bottle filters (0.22 µm) for filtering and degassing buffers.
- Buffer A for Ni^{2+}affinity column: 50 mM Tris–HCl pH 7.5, 500 mM NaCl, 5 mM β-mercaptoethanol , 10 mM imidazole pH 7.5—filtered and degassed.
- Buffer B for Ni^{2+}affinity column: 50 mM Tris pH 7.5, 500 mM NaCl, 5 mM β-mercaptoethanol, 250 mM imidazole pH 7.5—filtered and degassed.
- FPLC system with buffer gradient capabilities , UV absorbance detector and fraction collector (e.g., ÄKTA Purifier, GE Healthcare).
- 5 mL HiTrap Q HP column (GE Healthcare).
- Buffer A for Q column: 50 mM Tris–HCl pH 7.5, 100 mM NaCl, 5 mM β-mercaptoethanol—filtered and degassed.
- Buffer B for Q column: 50 mM Tris–HCl pH 7.5, 1.5 M NaCl, 5 mM β-mercaptoethanol—filtered and degassed.
- Dialysis buffer: 50 mM Tris–HCl pH 7.5, 100 mM NaCl, 5 mM β-mercaptoethanol.

For ARC2-3 and ARC1-5 constructs described here, a minimal NaCl concentration of 300 mM needs to be maintained, compared to 100 mM for all single-ARC constructs, and glycerol may further help stabilize the protein [7].

- Dialysis tubing, 3500 Da molecular weight cutoff (MWCO).
- Dialysis tubing clips.
- 2 L beaker, magnetic stirrer plate, stirrer bar.
- TEV protease , 5 mg/mL stock.
- 15 mL spin protein concentrator, 3000 Da MWCO for single-ARC constructs, 10,000 Da MWCO for double-ARC constructs, 30,000 Da MWCO for ARC1-5 constructs .
- Refrigerated centrifuge for concentrating protein (3200 × *g*, e.g., Beckmann Coulter Allegra X12-R centrifuge with SX4750 swinging bucket rotor).
- Refrigerated centrifuge for removing precipitate prior to size exclusion chromatography (18,000 × *g*, e.g., Eppendorf 5417R with F45-30-11 rotor).
- HiLoad 16/600 Superdex 75 or 200 pg size exclusion column (GE Healthcare).

Use a Superdex 200 column for ARC1-5 constructs. All other ARC constructs are sufficiently small for the Superdex 75 column.

- Tris(2-carboxyethyl)phosphine (TCEP), 0.5 M stock.

TCEP is more stable than DTT and not volatile, unlike β-mercaptoethanol or DTT. However, TCEP is acidic and will affect the final pH of the buffer unless the 0.5 mM stock solution is pH-adjusted with NaOH. To keep costs down, TCEP is only used in the final purification step.

- Size exclusion buffer: 25 mM HEPES-NaOH pH 7.5, 100 mM NaCl, 2 mM TCEP—filtered and degassed.

HEPES is preferred for the final protein and in experiments due to its lower temperature dependency compared with Tris [34]. Tris was chosen for the affinity purification step due to its weak interaction with Ni^{2+}, which would help decrease background (contaminant) binding [35]. If HEPES buffer is used in the affinity purification step, the imidazole concentration may need to be increased to achieve comparably low background binding.

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For ARC2-3 and ARC1-5 constructs described here, a minimal NaCl concentration of 300 mM needs to be maintained, compared to 100 mM for all single-ARC constructs, and glycerol may further help stabilize the protein [7].

- 96-deep-well blocks for fraction collection, or fraction collector tubes (depending on the format of the fraction collector).
- UV spectrophotometer.
- 4× SDS sample buffer.
- 15% polyacrylamide gels for SDS-PAGE.
- Protein standard for SDS-PAGE.
- Coomassie stain for SDS-PAGE gels.
- 15 mL and 50 mL Falcon tubes .
- Thin-walled individual 0.2 mL PCR tubes for flash-freezing protein aliquots.
- Liquid nitrogen bath.

2.3 Fluorescence Polarization (FP) Assay

- Plate reader capable of taking FP measurements (e.g., BMG Labtech POLARstar Omega).
- Appropriate wavelength filters for chosen fluorophore, one corresponding to excitation wavelength, and two (ideally a matched pair with identical optical properties), corresponding to the emission wavelength. Here, we use a 485 nm excitation filter and two matched 520 nm emission filters for fluorescein.
- Opaque, black, 384-well, non-binding, flat-bottom plates, either in standard format (e.g., 781,900, Greiner Bio-One) or in small-volume format (e.g., 784,900, Greiner Bio-One). The latter are particularly useful if limited protein is available.
- Microplate centrifuge (1000 × *g*, e.g., Beckman Coulter Allegra X-12R with SX4750 swinging bucket rotor, fitted with microplate inserts)
- FP assay buffer: 25 mM HEPES–NaOH pH 7.5, 100 mM NaCl, 1 mM TCEP , 0.05% w/v 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate (CHAPS).

Detergent is used to reduce nonspecific binding and surface tension that may interfere with fluorescence intensity readings in the plate format. The choice of detergent and its concentration is empirical. We have also had good experience with using 100 µg/mL bovine serum albumin (BSA), but use CHAPS here since it is more effective at reducing nonspecific binding.

For ARC2-3 and ARC1-5 constructs described here, a minimal NaCl concentration of 300 mM needs to be maintained, compared to 100 mM for all single-ARC constructs, and glycerol may further help stabilize the protein [7].

- Fluorescently tagged peptide corresponding to TBM of potential tankyrase binder/substrate, 2× stock (50 nM for a final assay concentration of 25 nM) in FP assay buffer. In the present examples (3BP2, MERIT40, TRF1), fluorescein and 5(6)-carboxyfluorescein are used as fluorophores. The peptides have been described previously [7]. We recommend peptides of minimally the TBM octapeptide with one flanking amino acid on either side. The peptides used here are longer (*see* Fig. 3b for peptide sequences). The fluorescein fluorophore is linked via β-Ala, which also provides an additional linker to minimize potential steric interference of the fluorophore with the peptide-ARC interaction.

Keeping the fluorescently labeled peptides as short as possible increases the ΔFP signal window between the bound and unbound states. Ideally, peptides should be HPLC-purified; however, this is not always realistically achievable, especially if large numbers of peptides are to be compared and no access to in-house solid-state

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peptide synthesis is available. To save costs, peptides can be used at non-HPLC-purified grade, in which case a capping step is strongly recommended after each amino acid coupling reaction to prevent peptide synthesis intermediates from being linked to the fluorophore in the final coupling step and affecting the assay [7].

- Tankyrase ARC protein: 2× stock of twofold dilution series, 0–400 µM, (final assay concentrations of 0–200 µM protein) in FP assay buffer .
- Software for curve fitting and analysis (e.g., GraphPad Prism 6).

2.4 Binding and PARylation of Full-Length Candidate Proteins by Tankyrase in Cells

- Mammalian expression constructs for epitope-tagged (e.g., MYC_2) tankyrases (TNKS: NM_003747.2; TNKS2: NM_025235.2) and epitope-tagged (e.g., FLAG) tankyrase-binding protein candidates (here for TRF1 /TERF1: NM_017489.2 and MERIT40/BABAM1: NM_001033549.2), corresponding empty vectors as controls .
- QuikChange Lightning mutagenesis kit (Agilent Technologies) or individual components from other sources for site-directed mutagenesis.
- Mutagenesis primers to mutate putative TBM (recommended mutation: G6R).
- PCR thermal cycler, standard setup and reagents for recombinant DNA techniques .
- Human Embryonic Kidney (HEK) 293T cells (ATCC).
- 10 cm and 15 cm cell culture dishes.
- Hemocytometer or automated cell counter.
- Dulbecco's Modified Eagle's Medium (DMEM).
- Fetal bovine serum (FBS).
- Humidified cell culture incubators at 37 °C, 5% CO_2.
- 0.2% Versene in PBS (137 mM NaCl, 2.7 mM KCl , 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 537 µM EDTA, 80 µM phenol red, final pH adjusted to 7.2, sterilization-autoclaved; all reagents cell-culture grade).
- 0.05% trypsin in Versene (137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 5.5 mM D-glucose , 1.5 mM KH_2PO_4, 25 mM Tris, 1% phenol red, 0.5 g trypsin (1:250) per 1 L, 137 µM streptomycin sulfate, 168 µM benzyl penicillin, final pH adjusted to 7.5, filter-sterilized using a 0.22 µm filter).
- Ultra-filtered sterile H_2O .
- Calcium phosphate transfection reagents (all cell culture grade):

(a) 2× HEPES-buffered saline (HBS): 50 mM HEPES , 10 mM KCl, 280 mM NaCl, 1.5 mM Na_2HPO_4, 12 mM glucose, final pH adjusted to 7.05 with KOH , filter-sterilized and stored at 4 °C.
(b) 25 mM chloroquine diphosphate, filter-sterilized and stored at −20 °C (1000× stock).
(c) 2 M $CaCl_2$, filter-sterilized and stored at 4 °C.

- Phosphate-buffered saline (PBS).
- Cell scraper.
- Refrigerated centrifuge for collecting mammalian cells (300 × *g*, e.g., Beckmann Coulter Allegra X-12R with SX4750 swinging bucket rotor).
- Radioimmunoprecipitation assay (RIPA) buffer: 50 mM HEPES–NaOH pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS , 1 mM DTT, 2 µM ADP-HPD PARG inhibitor and protease inhibitors (e.g., Pierce protease inhibitor tablets, EDTA-free, Thermo Fisher Scientific). Add DTT, ADP-HPD, and protease inhibitors immediately before use.
- Sonicator fitted with small probe.
- Refrigerated centrifuge for clearing lysates and settling affinity resin (800–18,000 × *g*, e.g., Eppendorf 5417R with F45-30-11 rotor).
- Anti-FLAG M2 Agarose resin.
- Vacuum pump with inlet trap and collection flask for aspirating buffer.
- SDS-PAGE gels (e.g., 4–15% Tris–glycine polyacrylamide gradient gels for excellent resolution).
- Electrophoresis apparatus for the above and appropriate power supply.
- 4× SDS sample buffer.
- Protein standard for SDS-PAGE .
- Nitrocellulose transfer membrane.
- Wet transfer Western blotting apparatus and appropriate power supply.
- Ponceau S solution.
- Nonfat dry milk powder.
- Mouse monoclonal anti-FLAG M2 HRP-conjugated antibody (here 1 mg/mL), mouse monoclonal anti-MYC (9E10) HRP-

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conjugated antibody (here 1 mg/mL), rabbit polyclonal anti-PAR (4336-BPC-100, Trevigen, concentration not specified by supplier), goat anti-rabbit IgG (H+L) secondary antibody, HRP-conjugate (here 0.8 mg/mL).

Alternatively, fluorescently labeled antibodies can be used for detection with appropriate fluorescence imaging systems. Instead of the anti-PAR antibody, an anti-pan-ADP-ribose or anti-poly(ADP-ribose) binding reagents (MABE1016 and MABE1032, respectively, Millipore) may be explored.

- ECL Western blotting substrate .
- X-ray film for Western blot detection or alternative ECL detection setup.

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

ABSTRACT

The poly(ADP-ribose)polymerase (PARP) enzyme tankyrase (TNKS/ARTD5, TNKS2/ARTD6) uses its ankyrin repeat clusters (ARCs) to recognize degenerate peptide motifs in a wide range of proteins, thereby recruiting such proteins and their complexes for scaffolding and/or poly(ADP-ribosyl)ation. Here, we provide guidance for predicting putative tankyrase-binding motifs, based on the previously delineated peptide sequence rules and existing structural information. We present a general method for the expression and purification of tankyrase ARCs from *Escherichia coli* and outline a fluorescence polarization assay to quantitatively assess direct ARC–TBM peptide interactions. We provide a basic protocol for evaluating binding and poly(ADP-ribosyl)ation of full-length candidate interacting proteins by full-length tankyrase in mammalian cells.

FILES

Predicting Tankyrase Binders
Version 1
by satyavati Kharde

Validating TBMs by Fluorescence Polarization (FP)
Version 1
by satyavati Kharde

Validation of Tankyrase Binding and PARylation in Full-Length Protein Context
Version 1
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