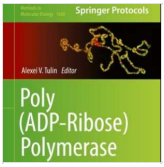

Sep 02, 2021

Predicting Tankyrase Binders

Book Chapter

In 1 collection

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ABSTRACT

This protocol is part of a collection: Identifying and Validating Tankyrase Binders and Substrates: A Candidate Approach

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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COLLECTIONS

Identifying and Validating Tankyrase Binders and Substrates: A Candidate Approach

KEYWORDS

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

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Identifying and Validating Tankyrase Binders and Substrates: A Candidate Approach

GUIDELINES

The details of many standard experimental methods not addressed here can be found in [21].

Notes

1. 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].

2. 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.

3. 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.

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

5. 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.

6. 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.

7. Detergent is used to reduce nonspecific binding and surface tension that may interfere with fluorescence

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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.

8. 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 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].

9. 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.

10. Compared to LB, TB is richer and enables higher cell densities in the log phase of growth. Cooling the cultures before IPTG induction slows down expression, thereby facilitating correct protein folding and increasing protein solubility.

11. 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].

12. For large volumes, it may be easier to lyse cells in two batches. Alternative disruption techniques can be used, such as homogenization by an Avestin EmulsiFlex homogenizer.

13. If available with the FPLC setup, a superloop or, preferably, a sample pump can be used to load the column.

14. Depending on the ARC construct and the final concentration of imidazole in the dialysis buffer, ARCs can bind weakly to the Ni^{2+}column even after tag cleavage . They can be eluted with a further imidazole gradient. While this adds one more step to the purification protocol, it enables even higher purities to be achieved.

15. The Q column step can also be performed before concentration, directly using the flow-through from the second Ni^{2+} affinity column; however, prior concentration saves time in loading the column.

16. Confirm the pH of the peptide stock solution before measuring the concentration. Acidity, for example due to residual trifluoroacetic acid from the peptide synthesis, will strongly affect fluorophore absorption.

17. We recommend leaving wells in the outermost rows and columns empty to reduce the microplate “edge effect,” a discrepancy in readings between the central and peripheral wells [36].

18. It is important to add the different transfection reagents in the specified order to ensure proper calcium phosphate–DNA particle formation.

19. Avoid using micropipette tips to transfer the cell suspension after scraping. The small opening of the tips can cause cells to break due to shearing. You can cut off the tips to avoid this risk.

20. For directly HRP-coupled antibodies, azide as a preservative should be avoided as it inhibits HRP activity .

21. The Western blot protocol can be adapted for film-free chemiluminescence detection or fluorescence-based detection.

22. Attributing a PAR signal to a particular protein by molecular weight may be challenging, in part due to possible PAR-induced mobility shifts in SDS-PAGE. Since the tankyrase substrate candidates are immunoprecipitated from cell lysates, it is possible that the observed PAR signal corresponds to other PARylated proteins in a protein complex. Ultimate confirmation of substrates can be obtained from experiments with purified proteins or PAR site mapping by mass spectrometry, for example.

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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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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]

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).

We recommend using one 5 mL column per 4 L expression culture. If using larger volumes, connect additional

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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 peptide synthesis is available. To save costs, peptides can be used at non-HPLC-purified grade, in which case

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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-conjugated antibody (here 1 mg/mL), rabbit polyclonal anti-PAR (4336-BPC-100, Trevigen, concentration not specified by

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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.

BEFORE STARTING

Experimental approaches such as yeast-two-hybrid analysis [22] or co-immunoprecipitation coupled with tandem mass spectrometry [23] have been used to identify proteins present in complexes with tankyrase. It is desirable to pinpoint those proteins that directly interact with tankyrase. The existence of putative TBMs is the most reliable known indicator for direct interaction. Based on previously identified TBM sequence rules and structural information, we provide a guide to identifying strong TBM candidates for further validation. A previously generated list of ranked octapeptide motifs across the entire human proteome can be used [7]. Here, we recapitulate a simple step-by-step approach for identifying candidate TBMs without the need for specialized bioinformatics beyond the use of established programs and databases.

Predicting Tankyrase Binders

1 Screen your protein of interest for potential TBMs. In an initial step, a search for Arg and Gly spaced 6 amino acids apart (i.e., with four amino acids in between, RxxxxG) will suffice. See step 4 below for atypical TBMs.

2 Using UniProt (http://www.uniprot.org), assess the topology of the protein and whether the candidate motif would be located in the cytoplasm or nucleoplasm, accessible to tankyrase [24]. The relevant information can be found under *Subcellular location > Topology*.

3 Assess the occurrence of disordered stretches in your protein of interest. TBMs must be present in unstructured regions of the protein to be accessible to tankyrase and must not be part of a stable α-helix or β-strand. Secondary structure information, obtained from the Protein Data Bank [25], is available in UniProt (http://www.uniprot.org) under *Structure > Secondary structure* [24]. It remains possible that TBMs are present within loops of structured protein domains. If no structural information is available, a disorder predictor such as IUPred (http://iupred.enzim.hu) may be used to obtain an indication of protein disorder [26].

4 Assess the short-listed sequences for their suitability as TBMs. REAGDGEE has been found to be the optimal 8-amino-acid TBM by positional scanning [7], but many TBMs only span six amino acids (positions 1–6). While the sequence rules from positional scanning prove valuable in predicting TBMs, it should be noted that some of their aspects may be specific to the context of the 3BP2 TBM peptide , which positional scanning was performed on [7]. Whereas Arg and Gly at positions 1 and 6, respectively, are essential, other residues are important as well, with the exception of positions 2 and 3, which can likely be ignored since they do not contribute to binding and mainly fulfil spacing roles (Fig.1b, d). Please refer to the motif description under Subheading 1 (Introduction) for the assessment. Of note, a large or strongly charged amino acid at position 4 very likely disqualifies the TBM candidate as a genuine tankyrase binder. Furthermore, Asp and Glu at position 8 can compensate for unfavorable amino acids at other positions [7], as seen in one of the TBMs of AXIN1/2, where a suboptimal Val at position 4 appears to be counterbalanced by a Glu at position 8 (Fig.1e) [7, 11]. Interestingly, a second TBM in AXIN1/2 shows that the Arg, normally at position 1, can be placed further N-

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terminal, resulting in looping-out of the “inserted” residues (Fig.1c, e). Likewise, the E3 ubiquitin ligase RNF146 has been proposed to contain numerous atypical TBMs with a nonconventional positioning of the Arg [27], and it is possible that a distal Arg is required for weak binding of the TBM of GRB14 to tankyrase (Fig.1e) [7, 8, 28]. Thus, the possibility of a distal Arg residue should be taken into consideration. It is likely that such extended TBMs display a weaker affinity for tankyrase. Numerous tankyrase binders contain multiple TBMs, such as AXIN1/2 [11] and MERIT40 [7] (*see* below). Since avidity effects likely contribute to binding of these proteins to tankyrase, the affinity of some of these individual TBMs for the ARC may be lower, and this may be reflected in deviations from the “optimal” sequence.

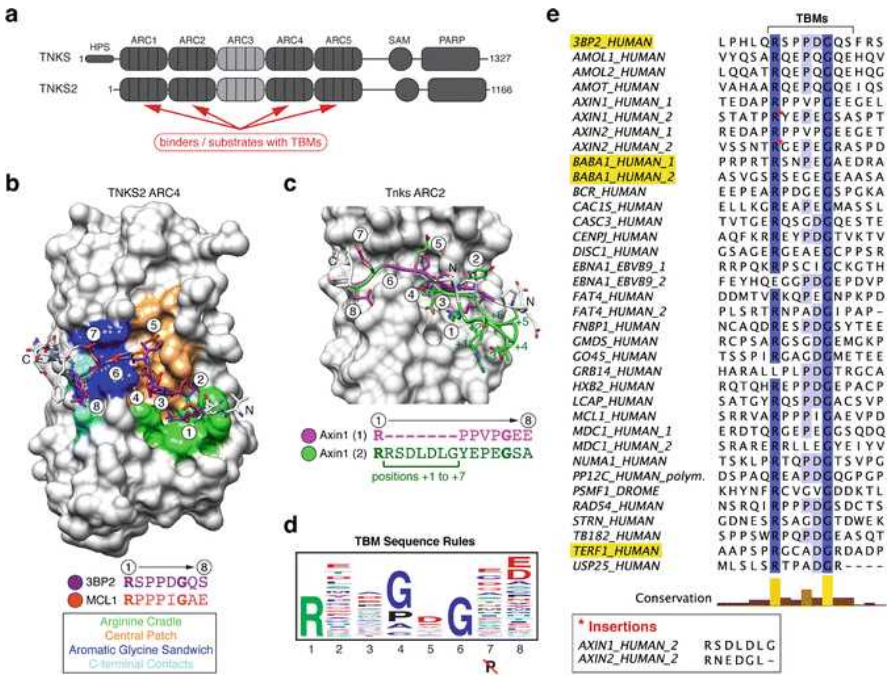

Fig. 1
Substrate binding by tankyrase. (**a**) Domain organization of human tankyrase and tankyrase 2 (modified from [31]). (**b**) and (**c**) Examples for ARC–TBM interactions studied by X-ray crystallography. (**b**) Human TNKS2 ARC4 is shown in surface representation with bound TBM peptides from 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*

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