A ribose-functionalized NAD\(^+\) with unexpected high activity and selectivity for protein poly-ADP-ribosylation

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Nicotinamide adenine dinucleotide (NAD\(^+\))-dependent ADP-ribosylation plays important roles in physiology and pathophysiology. It has been challenging to study this key type of enzymatic post-translational modification in particular for protein poly-ADP-ribosylation (PARylation). Here we explore chemical and chemoenzymatic synthesis of NAD\(^+\) analogues with ribose functionalized by terminal alkyne and azido groups. Our results demonstrate that azido substitution at 3\(\text{'}\)-OH of nicotinamide riboside enables enzymatic synthesis of an NAD\(^+\) analogue with high efficiency and yields. Notably, the generated 3\(\text{'}\)-azido NAD\(^+\) exhibits unexpected high activity and specificity for protein PARylation catalyzed by human poly-ADP-ribose polymerase 1 (PARP1) and PARP2. And its derived poly-ADP-ribose polymers show increased resistance to human poly(ADP-ribose) glycohydrolase-mediated degradation. These unique properties lead to enhanced labeling of protein PARylation by 3\(\text{'}\)-azido NAD\(^+\) in the cellular contexts and facilitate direct visualization and labeling of mitochondrial protein PARylation. The 3\(\text{'}\)-azido NAD\(^+\) provides an important tool for studying cellular PARylation.
Numerous biological processes are orchestrated by protein post-translational modifications (PTMs). Among key PTMs is protein ADP-ribosylation catalyzed by a superfamily of enzymes named ADP-ribosyltransferases (ARTs) with nicotinamide adenine dinucleotide (NAD\(^+\)) as a cosubstrate. The human genome is found to encode 20 ART enzymes including intracellular poly-ADP-ribose polymerases (PARPs) and sirtuins (SIRTs) and ecto-ART1-5\(^2\), which possess poly- or mono-ADP-ribosylation activity. Protein ADP-ribosylation plays vital roles in regulating genome stability, protein homeostasis, cell proliferation, differentiation and apoptosis\(^2\)\(^3\)\(^4\). In the human ART superfamily, 17 members (85%) are strongly implicated in various human diseases\(^5\)\(^6\)\(^7\)\(^8\)\(^9\)\(^10\)\(^11\). The activation or enhancement of those ART enzymatic activities positively correlate with pathogenesis and progression of those diseases. Given their importance and emerging roles in a wide range of prevalent human diseases, ART enzymes have attracted considerable interest for early diagnosis and treatment of disease. To study NAD\(^+\) analogues with substituted adenines raise the question of whether native NAD\(^+\) displays excellent substrate activity for protein poly-ADP-ribosylation (PARylation), comparable to that of native NAD\(^+\). Importantly, in comparison to established adenine-modified NAD\(^+\) analogues with strong activities for ADP-ribosylation, the 3'-azido NAD\(^+\) displays unexpected higher activity and selectivity for protein PARylation catalyzed by human PARP1 and PARP2. Moreover, the 3'-azido NAD\(^+\)-based poly-ADP-ribose (PAR) polymers reveal improved resistance to degradation by human poly(ADP-ribose) glycohydrolase (PARG). These unique properties for the 3'-azido NAD\(^+\) collectively result in more significant labeling of protein PARylation in the cell lysates relative to NAD\(^+\) and adenine-substituted NAD\(^+\) analogues and enable direct visualization and labeling of mitochondrial protein PARylation. This ribose-functionalized NAD\(^+\) with marked activity and specificity for protein PARylation not only offers an important tool for studying post-translational ADP-ribosylation but also may pave the ways

![Diagram](image-url)

**Fig. 1** Biosynthesis of NAD\(^+\) and ribose-functionalized NAD\(^+\) analogues. a NRK- and NMNAT-catalyzed formation of NAD\(^+\). b Enzymatic synthesis of NAD\(^+\) analogues with functionalized ribose for protein PARylation. c Chemical structures of NAD\(^+\) analogues 1-6. PPI: pyrophosphate; Nam: nicotinamide; Nu: nucleophile.
toward the creation of bi- and multi-functional NAD$^+$ molecules for investigating PARylation-dependent signaling and processes.

### Results

**Chemical and enzymatic synthesis of NAD$^+$ analogues.** Inspired by NRK- and NMNAT-mediated biosynthesis of NAD$^+$ from NR (Fig. 1a), we envisioned that ribose-functionalized NR may allow facile chemoenzymatic synthesis of NAD$^+$ analogues (Fig. 1b). Human NRK1 and NMNAT1 display adequate catalytic activities for NR + ATP and nicotinamide mononucleotide (NMN) + ATP, respectively, and promiscuously toward other substrate analogues.28-30 X-ray structures of human NRK1 and NMNAT1 support that ribose-functionalized NR and NMN are likely recognized for catalysis.31,32 (Supplementary Fig. 1). Moreover, previous studies showed that leaving group activation is an important catalytic force for reactions catalyzed by multiple N-ribosyltransferases.33-38 Combined with structural analysis of PARP active sites, these results suggest that modifications of NR ribose 2'-OH and 3'-OH with terminal alkyne and azido groups can possibly result in bioorthogonal NAD$^+$ with considerable substrate activities and a facile approach for the production of NAD$^+$-based chemical tools. Importantly, such ribose-functionalized NAD$^+$ may allow incorporation of additional functional groups at distinct positions without significant loss of substrate activity for the development of NAD$^+$ molecules with dual or multiple functions.

To test this notion, propargyl, pentynyl, and azido were chosen for modifications of NR 2'-OH and 3'-OH to generate 1–6 (Fig. 1c and Supplementary Figs. 2–8). The stereochemistries of the generated intermediates O-benzoyl protected NR1-6 were determined as β-isomers on the basis of $^1$H−$^1$H COSY experiments to confirm proton assignments and subsequent NOESY experiments (Supplementary Figs. 9–21). The resulting NR1-6 were then subject to 5'-phosphorylation and pyrophosphate coupling with adenosine monophosphate (AMP) for chemical synthesis of 1–6 (Supplementary Figs. 2–8).

To explore enzymatic synthesis of 1–6 from their corresponding NR and NMN analogues, human NRK1 and NMNAT1 were expressed and purified from *Escherichia coli* (Supplementary Table 1 and Supplementary Fig. 22). In vitro biosynthesis of NAD$^+$ from NR was first carried out using purified NRK1 and NMNAT1. In the presence of NRK1 and NMNAT1 and ATP, a substantial amount of NAD$^+$ was formed from NR after 40 h incubation (Fig. 2a, b). Then, enzymatic syntheses of 1–6 were attempted under the same conditions. It was found that a significant amount of 5 and 6 could be generated by incubating NR5 and NR6 with ATP and NRK1 and NMNAT1 at room temperature for 24 or 40 h (Supplementary Fig. 23 and Fig. 2c, d), while incubation of other NR analogues NR1-4 with ATP and the purified enzymes gave no formation of NAD$^+$ analogues 1–4 (Supplementary Fig. 23). Compared with an 83% isolated yield for biosynthesis of NAD$^+$ from NR (Supplementary Fig. 2a, b), the two-step enzymatic approach gave rise to a 68% isolated yield for the production of 6 starting from NR6 (Supplementary Fig. 25 and Fig. 2c, d). Using this enzymatic method, 12.2 mg of 6 was facilely produced and purified for the later experiments. In contrast, chemical synthesis of 6 from NR6 revealed a combined yield of 32% (Supplementary Fig. 8) and the pyrophosphate coupling step needs four days plus tedious and challenging HPLC purification. These results demonstrate a facile and efficient chemoenzymatic approach for generating 3'-azido NAD$^+$.

Additionally, NR1-6 and NMN1-6 were examined separately with purified NRK1 and NMNAT1 to determine their substrate activities for enzymatic conversions. Compared with NRK1 that could only catalyze conversion of NR5 and NR6, NMNAT1 displayed higher tolerance to these ribosyl modifications and was shown to catalyze formation of 1, 2, 5 and 6 from respective NMN precursors (Supplementary Figs. 26–28). HPLC analysis revealed that NMN6 could be rapidly converted to 6 within 4 h in a 74% yield at the milligram level (Supplementary Fig. 29 and Fig. 2e). These results indicate that the azido substitution at NR 3'-OH position allows efficient enzymatic synthesis of 6, in particular from its NMN analogue precursor.

#### Substrate activities of NAD$^+$ analogues for human PARP1.

To evaluate substrate activities of 1–6 for protein PARylation, full-length human PARP1 was expressed and purified from *E. coli*. Human PARP1 is a well-characterized enzyme for protein PARylation. In addition to catalyzing PARylation on various types of cellular proteins, PARP1 also undergoes robust auto-PARylation in the presence of NAD$^+$ and DNA fragments. The substrate activities of 1–6 were first evaluated by performing PARP1-catalyzed automodification with highly pure 1–6 (Supplementary Fig. 30 and Supplementary Table 2). It was shown that both NAD$^+$ and 6 resulted in strong auto-PARylation of PARP1, which could be potently suppressed by veliparib, an inhibitor of PARP1-PARP4 (Fig. 3a). This also indicates that the
anti-PAR antibody can recognize the 3’-azido NAD$^+$-derived PAR polymers. Since 6 contains an azido group, which is bioorthogonal to biotin-conjugated alkynes through click chemistry, auto-modified PARP1 by NAD$^+$ and 6 were then clicked with biotin and detected by a streptavidin-HRP conjugate. Consistent with the immunoblot results using the anti-PAR antibody, PARP1 modified by 6 revealed strong PARylation signals that were sensitive to veliparib treatment (Fig. 3b). Due to the lack of azido group for NAD$^+$, no PARylation signals were observed for NAD$^+$-modified PARP1 on this biotinylation-based immunoblot. In comparison, 5 with an azido group attached to NR 2'-OH displayed no PARylation signals (Supplementary Figs. 31 and 32). Similarly, 1 and 3 containing 2'-substitutions were shown to have no substrate activities for PARP1-catalyzed PARylation. In contrast, 2 and 4 with terminal alkyne groups modified at NR 3'-OH display strong to moderate activities for PARP1 auto-modification, respectively (Supplementary Figs. 31 and 32).

Next, kinetic parameters of NAD$^+$, 2, and 6 for PARP1-catalyzed auto-modification (PARP activity) and hydrolysis (NADase activity) were determined by HPLC-based activity assays (Table 1). The $k_{cat}$ of 6 for PARP1 auto-PARylation is 4.1 ± 0.6 min$^{-1}$ (mean ± standard deviation of three replicates), only slightly lower than that of NAD$^+$ (4.7 ± 0.4 min$^{-1}$). The $K_m$ (370.5 ± 104.8 µM) of 6 is higher than that (145.4 ± 36 µM) of NAD$^+$. The $k_{cat}$ of 2 is significantly lower than that of NAD$^+$ and 6. Same as NAD$^+$, 2 and 6 could undergo slow hydrolysis catalyzed by PARP1. Similar to their PARP activities, the NADase activity for 6 is comparable to that of NAD$^+$. These results are consistent with immunoblot analyses and support 3’-azido NAD$^+$ as an excellent substrate for PARP1-catalyzed PARylation.

### Biological activities of 2 and 6 in cellular context
To evaluate their biological activities in the cellular context, 2 and 6 were introduced into HeLa cells through transient permeabilization of the cell membrane, which has no significant effect on cell viability$^{27,39,40}$ (Supplementary Fig. 33). Cells without transient permeabilization were used as controls (Supplementary Figs. 34 and 35). Confocal microscopic analysis of cells treated with 6 clearly revealed the extent and location of cellular ADP-ribosylation (Fig. 4a). Hydrogen peroxide (H$_2$O$_2$) can induce oxidative DNA damage in the nucleus, leading to activated PARP1 and PARP2 for DNA repair. Consistent with previous results, treatment of cells with H$_2$O$_2$ resulted in significantly increased ADP-ribosylation in the nucleus, which were suppressed by veliparib. In addition, immunoblot analyses of the lysates of HeLa cells showed marked protein labeling for cells treated with 6 (Fig. 4b and Supplementary Fig. 36). Consistent with the confocal imaging study, treatment with H$_2$O$_2$ caused significantly increased protein labeling, possibly due to the activated PARP1 and PARP2. Additions of veliparib inhibited protein labeling detected in the absence and presence of H$_2$O$_2$. Compared with imaging and labeling signals derived from 2 (Supplementary Fig. 36), the analogue 6 exhibited improved signal-over-background and sensitivity for both imaging and protein labeling studies, consistent with its excellent substrate activity for PARP1 as determined from immunoblot and enzyme kinetic assays. Collectively, these results support 3’-azido NAD$^+$ as a valuable tool for studying cellular PARylation.

### Comparison of 6 and 2 with adenine-modified NAD$^+$ analogues
The substrate activity of 6 and 2 were then compared with previously reported NAD$^+$ analogues with modified adenines (Fig. 5). For this purpose, 6-alkyne-NAD$^+$ (6-a-NAD$^+$) and 2-alkyne-NAD$^+$ (2-a-NAD$^+$) were synthesized according to published methods (Fig. 5b)41,42, which show strong activities for PARP1$^{24,27,41,42}$. Relative to 6, 6-a-NAD$^+$ and 2-a-NAD$^+$ exhibit comparable $K_m$ but significantly lower $k_{cat}$ for PARP1 auto-PARylation (Table 1). The immunoblot analyses of their substrate activities for PARP1-catalyzed auto-PARylation showed consistent results that PARP1 modified by 6 displayed significantly higher PARylation signals (Fig. 5c). In addition to PARP1, these NAD$^+$ analogues were examined for substrate activities for its close relative PARP2 that catalyzes protein PARylation. Similar to PARP1, human PARP2 auto-PARYlated by 6 showed significantly higher signals relative to 6-a-NAD$^+$ and 2-a-NAD$^+$ (Fig. 5d). Furthermore, the substrate activities of 2, 6, 6-a-NAD$^+$, and 2-a-NAD$^+$ for catalytic domains of human PARP5a and PARP10 were compared (Fig. 5e, f), which catalyze protein PARylation and mono-ADP-ribosylation (MARylation), respectively. Unlike 6-a-NAD$^+$ and 2-a-NAD$^+$ that displayed strong auto-modification activities for both enzymes, 2 and 6 revealed little activities.

### Table 1 Kinetic parameters of NAD$^+$ and its analogues for human PARP1

| Substrate | $k_{cat}$ (min$^{-1}$) | $K_m$ (µM) | $k_{cat}/K_m$ (min$^{-1}$ µM$^{-1}$) |
|-----------|----------------|----------|----------------------------------|
| PARP      |                |          |                                  |
| NAD$^+$   | 4.7 ± 0.4      | 145.4 ± 36.0 | 3.2 × 10$^4$                     |
| 2         | 0.06 ± 0.01    | 218.3 ± 81.7 | 2.8 × 10$^2$                     |
| 6         | 4.1 ± 0.6      | 370.5 ± 104.8 | 1.1 × 10$^4$                     |
| 6-a-NAD$^+$ | 1.9 ± 0.5     | 603.1 ± 307.9 | 3.2 × 10$^3$                     |
| 2-a-NAD$^+$ | 1.4 ± 0.4     | 400.8 ± 238.2 | 3.5 × 10$^3$                     |
| NADase    |                |          |                                  |
| NAD$^+$   | 0.39 ± 0.08    | 471.5 ± 187.4 | 8.3 × 10$^2$                     |
| 2         | 0.02 ± 0.01    | 661.1 ± 489.0 | 0.3 × 10$^2$                     |
| 6         | 0.46 ± 0.17    | 326.9 ± 278.8 | 1.4 × 10$^4$                     |
| 6-a-NAD$^+$ | 0.36 ± 0.02   | 370.8 ± 47.5  | 9.7 × 10$^2$                     |
| 2-a-NAD$^+$ | 0.47 ± 0.13   | 545.9 ± 313.5 | 8.6 × 10$^2$                     |

*Data are shown as mean ± standard deviation of three replicates.*
for auto-ADP-ribosylation catalyzed by PARP5a and PARP10 under the same conditions. These results reveal that 6 is a more selective substrate with excellent activity for PARP1 and PARP2.

In addition to their substrate activities, the stability of the PAR polymers formed by these NAD⁺ analogues were compared upon treatment by human PARG, one of the major enzymes responsible for degrading PAR polymers (Fig. 6). In contrast to NAD⁺- and 6-a-NAD⁺-derived PAR polymers that were rapidly removed by PARG, 2-a-NAD⁺- and 6-based PAR polymers show comparable resistance to PARG-mediated degradation.

Next, 6 was applied to label protein PARylation in cell lysates for comparison with those labeled by NAD⁺ and 2-a-NAD⁺, which causes higher level of labelling than 6-a-NAD⁺ (Fig. 7a, b). By using a WWE domain-based reagent 49 that exhibits comparable binding toward PAR polymers formed by NAD⁺, 6, and 2-a-NAD⁺ (Supplementary Fig. 37), immunoblot analyses indicated that 6 results in more significant labelling of protein PARylation in cell lysates relative to NAD⁺ and 2-a-NAD⁺ (Fig. 7c, d).

Analysis of mitochondrial protein PARylation by 6. To further demonstrate its utility, 6 was applied to examine mitochondrial protein PARylation. In addition to the nucleus as the predominant subcellular location for protein PARylation, multiple studies indicated the presence of PARylation in mitochondria 49–54. In vitro biochemical assays also confirmed several mitochondrial proteins as PARP1 substrates 24. Despite emerging but debating roles of PARylation in regulating mitochondrial DNA metabolism, limited approaches are available for studying mitochondrial PARylation and activity-based probes have yet to be developed for analyzing mitochondrial PARylation. Considering its high activity and specificity for protein PARylation, 6 was attempted to label mitochondrial PARylation in cells permeabilized with 0.025% Triton X-100. Confocal microscopy indicated that in addition to the predominant PARylation in nucleus, considerable PARylation signals were colocalized with mito.

Discussion

In summary, a 3’-azido NAD⁺ with unexpected high activity and selectivity for protein PARylation was generated. By exploiting recombinant human NRK1 and NMNAT1, this ribose-functionalized NAD⁺ molecule could be enzymatically synthesized in high efficiency and yields from its NR and NMN analogue precursors, establishing a facile approach for the production of 3’-azido NAD⁺. In contrast to established NAD⁺ analogues, 6 may represent a robust tool with enhanced activity and sensitivity for studying cellular PARylation. The enhanced labelling by 6 likely results from faster kinetics for PARylation and increased resistance to PAR removal by PARG.

The lack of substrate activity for 5 could be possibly caused by the blocked formation of branched PAR, which requires access to free 2'-OH of NR for covalently attaching ADP-ribose units, and/or the significantly decreased cleavage rate of the N'-glycosidic bond resulting from adjacent electron-withdrawing 2’-azido group 55. Substitutions of NR 3’-OH for terminal alkyne and azido groups result in NAD⁺ analogues recognized by PARP1 for PARylation. The extended loop (GL76-1895) at PARP1 active site may accommodate 3'-OH modifications (Fig. 5a) and the mitochondrion inner membrane protein (Fig. 8a), suggesting the presence of PARylation in mitochondria. Consistent with confocal imaging results, immunoblot analysis of the isolated mitochondria fractions clearly revealed significant protein labeling in the presence of 6. And treatment of cells with H₂O₂ resulted in increased protein labeling by 6 in the mitochondria fractions, which were suppressed by veliparib inhibitor (Fig. 8b). Moreover, a considerable amount of PARP1 was detected in mitochondrial fractions (Fig. 8b), which is consistent with previous studies 52–54 and suggests its important role in catalyzing protein PARylation in mitochondria. Taken together, these results provide direct evidence for mitochondrial PARylation through an activity-based probe, demonstrating 6 as a valuable tool for studying cellular PARylation.

![Fig. 4 Visualization and labeling of cellular protein PARylation by 6.](image-url)
increased size and/or flexibility of the functional groups could cause decreased substrate activities for PARP1.

The high specificity and activity of 6 for PARP1 and PARP2 could be attributed to the extended loop (G876-I895) and (G442-I461) at PARP1 and PARP2 active sites, respectively, which possibly involve in the interactions with the NR 3’-OH modification and are nearly identical for PARP1 and PARP2, but show large variations in length and sequence among PARP1/ PARP2, PARP5a, and PARP10 (Fig. 5a, g). It should be noted that in addition to this extended loop, more subtle differences at the catalytic sites of these PARP enzymes could result in different activities with 6. In addition, further work is required to characterize the PAR polymers formed by 6, including the individual steps of initiation, elongation, and branching for PARP1-catalyzed PARylation with 6 as the substrate.

Additionally, the substrate activity of 6 for recombinant human sirtuin 2 (SIRT2), an NAD+-dependent protein deacetylase, was examined by using a trypsin-coupled fluorescence-based activity assay. It was found that in overnight reactions 6 displays no substrate activity for human SIRT2, suggesting that 6 is not a substrate of SIRT2 and azido substitution at 3’-OH of NR moiety is not tolerated by SIRT2 (Supplementary Fig. 38).
This work provides a valuable tool for studying NAD\(^+\)-dependent PARylation. Notably, the success in generation of this ribose-functionalized NAD\(^+\) with robust biological activities potentially enabled further development of bi- and multi-functional NAD\(^+\) molecules as innovative research tools for investigating cellular signaling and processes mediated by ADP-ribosylation.

**Methods**

**General materials and methods.** \(^1\)H NMR spectra were recorded on an Oxford AM-400 spectrometer for solution in CDCl\(_3\), CD\(_3\)OD or D\(_2\)O. Coupling constants \(J\) are shown in Hz. \(^1\)C NMR spectra were recorded on an Oxford AM-400 spectrophotometer (100 MHz) with complete proton decoupling spectrophotometer (CDCl\(_3\); 77.0 ppm). Flash column chromatography was performed using 230–400 mesh silica gel (Sigma–Aldrich, St. Louis, MO). For thin-layer chromatography (TLC), silica gel plates (Sigma–Aldrich GF254) were used. HPLC was performed on a Waters 2487 series with a C18 Inertex column (5 \(\mu\)m, 100 A, 150 x 10.0 mm, from Phenomenex Inc, Torrance, CA). cDNA of human NRK1 (accession number: BC036804) and human NMNAT1 (accession number: BC014943) were purchased from GE Dharmacon (Lafayette, CO). TAMRA-alkyne, azide-fluor 545 (N–Fluor 545), DAPI, β-NAD\(^+\), azide-PEG3-biotin and alkyne-PEG4-biotin, and Calf thymus DNA (activated) were purchased from Sigma–Aldrich. HeLa and NRK cells were obtained from the ATCC (Manassas, VA) and tested negative for mycoplasma. All other reagents were purchased from readily available commercial sources and used without further purification.

**Synthesis and characterization of NAD\(^+\) analogues.** The experimental details and results for synthesis of the NAD\(^+\) analogues 1–6, 2-a-NAD\(^+\), and 6-a-NAD\(^+\) are provided in the Supplementary Information.

**Cloning and generation of NRK1 and NMNAT1.** Protein sequence of human NRK1 (528 bp) and NMNAT1 (840 bp) with C-terminal His\(_6\)-tags were amplified through polymerase chain reaction (PCR) using primers of NRK1-Fw/Rv and NMNAT1-Fw/Rv, respectively (Supplementary Table 1). NcoI and XhoI restriction enzyme sites were placed at 5' and 3'-end of the primers, respectively. After digestion with NcoI and XhoI restriction enzymes, the amplified DNA fragments and backbone of pET-28a (+) vector were purified using gel DNA recovery kits (Zymo Research, CA), followed by ligation using the T4 DNA ligase. All constructed bacterial expression vectors were verified by DNA sequencing (Genewiz LLC, NJ). To express human NRK1 and NMNAT1, BL21 (DE3) cells were electroporated with the expression constructs and grown in LB Broth containing 50 μg mL\(^{-1}\) of kanamycin. After overnight growth, 5 mL of culture was transferred into 1 liter of LB Broth containing 50 μg mL\(^{-1}\) of kanamycin and grown in an incubator shaker (250 rpm; Series 25, New Brunswick Scientific, NJ) at 37 °C until the OD\(_{600}\) nm reached 0.6–0.8. After addition of 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) to induce protein expression, the bacterial culture was grown in an incubator shaker (250 rpm) overnight at 18 °C, followed by centrifugation at 4500 x g (Beckman J6B Centrifuge, J6-4.2 rotor) and resuspension in lysis buffer (20 mM Tris–HCl, pH 8.0, 200 mM NaCl, 20 mM imidazole). Cells were then lysed by passing three times through French Press (Glen Mills, NJ) at 25,000 psi and centrifuged at 4 °C for 1 h at 14,000 x g (Beckman Coulter centrifuge, JA-17 rotor) to remove debris. The supernatants were filtered using 0.45 μm membranes and loaded on gravity flow columns with 1 mL of Ni-NTA agarose resin (Thermo Fisher Scientific, Waltham, MA). After washing the columns with 15 column volumes of elution buffer (20 mM Tris–HCl, pH 8.0, 200 mM NaCl, 20 mM imidazole), the NRK1 and NMNAT1 were eluted using 15 column volumes of buffer (20 mM Tris–HCl, pH 8.0, 200 mM NaCl, 400 mM imidazole). Fractions containing of eluted proteins were combined and dialyzed in the storage buffer (20 mM Tris–HCl, pH 8.0, 300 mM NaCl, 1 mM DTT, 10% glycerol) overnight at 4 °C. After another 6-h dialysis in fresh storage buffer, the purified proteins were concentrated by Amicon centrifugal concentrators (10 kDa cutoff; EMD Millipore, Temecula, CA) and analyzed by SDS-PAGE. Protein concentrations were determined by NanoDrop 2000C spectrophotometer (Thermo Scientific).
Fisher Scientific, Waltham, MA) using calculated molecular extinction coefficient (1.537 for NRK1-His6 and 1.592 for NMNAT1-His6). Aliquots of proteins were flash-frozen in liquid nitrogen and stored at −80 °C.

**HPLC of enzymatic conversion of NR and NMN analogues.** NRK1 and NMNAT1 were used to catalyze the conversion processes from NR analogues to NMN analogues and from NMN analogues to NAD⁺ analogues, respectively. Both enzymes were used in the conversion from NR analogues to NAD⁺ analogues. All the conversion reactions were performed at room temperature in 100 µL assay solutions containing 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 20 mM MgCl₂, 1 mM DTT, 5 mM ATP, 1 mM NR/NMN analogues and 5 μM purified enzymes. After the indicated incubation times, the reactions were stopped by adding 50% TCA to a final concentration of 10%. After centrifugation at 12,000 × g for 5 min, the supernatants of reaction mixtures were analyzed by reverse-phase HPLC using a semipreparative C18 Kinetex column (5 µm, 100 Å, 150 × 10.0 mm, from Phenomenex Inc, Torrance, CA) with a gradient of methanol (0–40% in 20 min) in water containing 0.1% formic acid.

**Purity analysis of NAD⁺ analogues 1–6.** Each NAD⁺ analogue (500 µM) was analyzed separately by reverse-phase HPLC using a semipreparative C18 Kinetex column (5 µm, 100 Å, 150 × 10.0 mm, from Phenomenex Inc, Torrance, CA) (mobile phase A: 0.1% formic acid (aq); mobile phase B: 0.1% formic acid in methanol; flow rate = 2.0 ml min⁻¹; 0–2 min: 0–4% B, 2–4 min: 4–10% B, 4–6 min: 10–20% B, 6–12 min: 20–50% B, 12–14 min: 50–0% B) with detection of UV absorbance at 260 nm. The purity of 1–6 was calculated based on the proportion of their corresponding integrated peak areas in the total integrated peak areas.

**Cloning and generation of human PARP1.** cDNA of human PARP1 (accession number: BC037545) was purchased from GE Dharmacon (Lafayette, CO). Full-length human PARP1 with a C-terminal His₆-tag was amplified through PCR using primers PX (5′-TGGTGCTCGAGCCACAGGGAGGTCTTAAAATTGAATTTCA GT-3′) and PY (5′-CCCTCTAGAATAATTTGTTTAACTTTAAGGAGATATACCATGGCGGAGTCTTCGGATAAGC-3′) purchased from Integrated DNA Technologies (IDT) (Coralville, IA). The amplified full-length PARP1 and pET-28a (+) vector were digested by XhoI and XbaI restriction enzymes, purified using gel DNA recovery kits (Zymo Research, CA), and then ligated using the T4 DNA ligase. The resulting expression construct for full-length human PARP1 was verified by DNA sequencing (Genewiz LLC, NJ).

The expression and purification of full-length human PARP1 were performed on the basis of a previous report with minor modifications.²⁶ Briefly, BL21 (DE3) cells were electroporated with the constructed pET-28a vector encoding full-length PARP1. cDNA of human PARP1 (accession number: BC037545) was purchased from GE Dharmacon (Lafayette, CO). Full-length human PARP1 with a C-terminal His₆-tag was amplified through PCR using primers PX (5′-TGGTGCTCGAGCCACAGGGAGGTCTTAAAATTGAATTTCA GT-3′) and PY (5′-CCCTCTAGAATAATTTGTTTAACTTTAAGGAGATATACCATGGCGGAGTCTTCGGATAAGC-3′) purchased from Integrated DNA Technologies (IDT) (Coralville, IA). The amplified full-length PARP1 and pET-28a (+) vector were digested by XhoI and XbaI restriction enzymes, purified using gel DNA recovery kits (Zymo Research, CA), and then ligated using the T4 DNA ligase. The resulting expression construct for full-length human PARP1 was verified by DNA sequencing (Genewiz LLC, NJ).

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human PARP1 and grown in LB Broth media containing 50 µg mL⁻¹ of kanamycin for overnight. Each liter of LB Broth with 50 µg mL⁻¹ of kanamycin was then inoculated with 10 mL of overnight bacterial culture and grown at 37 °C in an incubator shaker (250 rpm; Series 25, New Brunswick Scientific, NJ) until the OD₆₀₀ nm reached 0.6–0.8, followed by addition of ZnSO₄ (final concentration: 0.1 mM). At an OD₆₀₀ nm of 0.8–1.0, the flasks were removed and incubated on ice for 1 h, followed by induction of protein expression with 0.5 mM IPTG for overnight at 16 °C, centrifugation at 4550 × g at 4 °C, and resuspension in lysis buffer (25 mM HEPES pH 8.0, 500 mM NaCl, 1 mM PMSF). Cells were lysed by passing a French Press (Glen Mills, NJ) for three times at 25,000 psi and then centrifuged at 27,000 × g for 100 min (Beckman Coulter centrifuge, JA-17 rotor) at 4 °C. The collected supernatants were then filtered by passing through 0.45 µm membranes, followed by loading on a gravity flow column with 5 mL of Ni-NTA agarose resins (Thermo Fisher Scientific, Waltham, MA), washing with 50 mL of low-salt wash buffer (25 mM HEPES pH 8.0, 500 mM NaCl, 20 mM imidazole), 50 mL of high-salt wash buffer (25 mM HEPES pH 8.0, 1 M NaCl, 20 mM imidazole) and 50 mL of low-salt wash buffer. Proteins were then eluted with 25 mL elution buffer (25 mM HEPES pH 8.0, 1 M NaCl, 20 mM imidazole) and 50 mL of low-salt wash buffer. The concentrated proteins were injected on to a size-exclusion chromatography column Superdex 200 Increase 10/300 GL (GE Healthcare, Princeton, NJ), and eluted using gel filtration buffer (25 mM HEPES, pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.1 mM DTT). Purified PARP1 was examined by SDS-PAGE and a NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA), then aliquoted and flash-frozen in liquid nitrogen for storage at −80 °C. Calculated molecular extinction coefficient value for human PARP1 with a C-terminal His₆-tag is 1.052.

Auto-PARylation of PARP1 with 1–6. Auto-PARylation of purified PARP1 was performed at room temperature in 100 µL assay solutions containing 100 mM Tris-HCl, pH 8.0, 50 mM NaCl, 20 mM MgCl₂, 1 mM DTT, 100 ng mL⁻¹ activated DNA, 0.4 mM NAD⁺ or NAD⁺ analogues 1–6, and 1 µM purified PARP1 enzyme. PARP1 autoamidation controls were performed with 100 µM veliparib inhibitor. After indicated incubation time, the reactions were stopped by adding 100 µM veliparib.

The reaction mixtures were further labeled with alizarin-biotin (for 1–4) or alizarin-biotin (for 5 and 6) through copper(I)-catalyzed azide alkyne cycloaddition (CuAAC). Click reactions were performed for 2 h at room temperature in 45 µL volume, which contain 30 µL PARP1 autoamidation mixtures, 2 mM THPTA, 1 mM CuSO₄, 100 µM alizarin-alkyne-biotin, and 10 mM sodium ascorbate in PBS.

The levels of auto-PARylation were evaluated by immunoblot using an anti-PAR monoclonal antibody (clone: 10H, from Santa Cruz Biotechnology sc-56198; 1:300 dilution) for detection of the formation of ADP-ribose polymer, a streptavidin-HRP conjugate (R&D Systems: DY998; 1:200 dilution) for detection of the biotinylated PARP1 via click chemistry, and an anti-His₆ antibody (clone: H16E8, from Thermo Fisher Scientific MA1-21315; 1:2000 dilution) for detection of PARP1 as loading control. The goat anti-mouse IgG antibody-HRP conjugate (Thermo Fisher Scientific: G-21040; 1:1000 dilution) was used as the secondary antibody for the anti-PAR antibody and anti-His₆ antibody. Uncropped and unprocessed scans of the blots are provided as a Source Data file.

**HPLC-based kinetic assays.** Auto-PARylation of PARP1 was carried out in 80 µL assay solutions (30 mM HEPES, pH 8.0, 5 mM MgCl₂, 5 mM CaCl₂, 1 mM DTT, 100 ng mL⁻¹ activated DNA, 100 µg mL⁻¹ BSA) containing varied concentrations of NAD⁺ (30, 100, 250, 450, 600 and 750 µM) or NAD⁺ analogues (2, 6, 6-a-NAD⁺, and 2-a-NAD⁺) at 30 °C with purified PARP1 enzymes. The reactions were quenched at different time points (NAD⁺: 0, 2.5, 5, 10, 15, and 20 min; 2, 0, 60, 120, 180, 240 and 300 min; 6: 0, 5, 10, 15, 20 and 30 min; 6-a-NAD⁺ and 2-a-NAD⁺: 0, 10, 20, 30, and 40 min) using 20% ice-cold TCA. After centrifugation, the reaction mixtures were analyzed by reverse-phase HPLC using a semi-preparative C18 Inosil column (5 µm, 150 x 10.0 mm, from Phenomenex Inc, Torrance, CA) (mobile phase A: 0.1% formic acid (aq)); mobile phase B: 0.1% formic acid in acetonitrile; flow rate: 2.0 mL min⁻¹; 0–8 min: 0% B; 8–12 min: 0%–50% B; 12–13 min: 50–2.5% B, 13–18 min: 2.5–40% B, 18–20 min: 40–0% B) with detection of UV absorbance at 260 nm. The retention times for NAD⁺, 2, 6, 6-a-NAD⁺ and 2-a-NAD⁺ were 13.0, 13.6, 13.6, 14.0 and 13.8 min, while for ADPγS, ADPγE, ADPβS, ADPβS-a-NAD⁺, and ADPβS-a-NAD⁺, the retention times were 6.3, 12.9, 9.0, 13.0, and 14.2 min. Standard curves for NAD⁺ and NAD⁺ analogues together with ADPβS and ADPβS analogues were constructed by linear
correlations of concentrations and corresponding integrated peak areas. NADase action rates were determined based on the increase in peak areas of the assigned peaks of ADP and ADP analogues, while reaction rates for PARP with 13 were measured based on the decrease in peak areas of the assigned peaks of NAD+ and NAD− analogues excluding NADase activity. Kinetic parameters were determined by fitting data to the Michaelis-Menten model implemented in GraphPad Prism (La Jolla, CA).

**Cellular PARylation assays.** Detection of PARylation activities in oxidatively stressed cells was performed by following a previously established assay with minor modifications. HeLa cells were first incubated with 500 μM H2O2 in PBS for 20 min. As negative controls, cells were pre-incubated for 2 h with 1 μM veliparib prior to the additions of H2O2, followed by washing once with PBS. For control experiments, veliparib was added in all experimental steps.

For confocal imaging analysis, cells were fixed with 4% paraformaldehyde (PFA) for 20 min at room temperature, followed by washing twice with 3% BSA/PBS. CuAAC reactions were performed with 10 μM CuSO4, 5 μM alkyne-TAMRA or azide-Fluor 545, 50 mM sodium ascorbate in PBS at room temperature for one hour, followed by washing three times with PBS. Cell nuclei were stained with DAPI (1 μg/mL −1 in PBS) for 20 min at room temperature, followed by washing three times with PBS and confocal microscopic analysis using a Leica SP8 confocal laser scanning microscope (Leica Microsystems Inc., Buffalo Grove, IL).

For immuno blot analysis, cells were harvested and lysed on ice for 15 min using M-PER Mammalian Protein Extraction Reagent (Thermo Fisher Scientific, Waltham, MA) with protease inhibitors. After centrifugation at 12,000 × g for 10 min at 4 °C, cell supernatants were loaded onto a gel for subsequent separation. The electrophoretic separation was followed by transferring the gel onto a nitrocellulose membrane. The membrane was blocked with 5% milk in TBS containing 0.1% Tween-20 (TTBS) and incubated with primary antibodies for overnight. The membrane was subsequently incubated in secondary antibodies conjugated with horseradish peroxidase (HRP). After washing with TTBS, a chemiluminescence substrate was added, and the reaction was monitored using a ChemiDoc XRS+ system (Bio-Rad Laboratories, Hercules, CA). Bands were quantified using ImageJ software (LSI Microscopy Systems Inc., Buffalo Grove, IL).

**Generation of human PARP5a and PARP10 catalytic domain.** cDNAs of human PARP10 (accession number: BC144235) and PARP5a (GE Dharmacon: MH56279-208068376) were used as templates for polymerase chain reaction to amplify the catalytic domains of PARP10 and PARP5a, respectively, and a His6-tag at the N-terminus of C-terminus. Primers #1 (5′-GCCGGATCTCAGGTTGTGGTGGTGTTGTTGTCGTTAGGATTATACCCAGAGGGTCGTCGGG-3′) and #2 (5′-AAGGAGATAGTACATACGCACATCATATCATTGAGGTTGGCGGCGGCGACGGCTGAAGG-3′) were used to amplify PARP10, and primers #3 (5′-GTTGGCTCCTGAGTTATTACCCAGAGGGTCGTCGGG-3′) and #4 (5′-TTAACCTTAAAAAGAAGGATATACCTGACCACTACCATG-3′) were used to amplify PARP5a. PCR products were verified by agarose gel electrophoresis and an additional round of PCR was performed. The PCR products were cloned into pET-28a (+) plasmids were digested overnight using XbaI and Xhol at 37 °C and cut vector was purified from agarose gel electrophoresis. Digested PCR fragments and vector were ligated using T4 DNA Ligase (New England Biolabs) for 1 h at room temperature prior to transformation into DH10B electrocompetent cells.

**Expression and purification of PARP5a and PARP10 catalytic domains.** Cells were grown in 2 L LB (DE3) cells for bacterial expression and purification. 5 mL of LB broth with 50 μg/ml −1 kanamycin were inoculated with transformed BL21 (DE3) cells for overnight culture and grown to OD600 nm = 0.8 at 37 °C with 250 rpm. Protein expression was induced using a final concentration of 500 μM IPTG and grown overnight at 22 °C in 250 rpm. Cells were harvested by centrifuging for 30 min at 2,700 × g and discarding the supernatant. Cell pellets were resuspended in 30 mL equilibrium buffer (20 mM Tris pH 7.5, 500 mM NaCl, 20 mM imidazole) with 10% glycerol and 10% DTT. Cell lysates were sonicated for 30 s on ice and centrifuged at 100,000 × g for 1 h. Pellets were removed by centrifugation and the supernatant was concentrated by the centrifugal filter unit. Protein concentrations were determined by SDS-PAGE and spectrophotometry, analyzing the wavelength at 280 nm and 260 nm, respectively.

For immunoblot analysis, cells were harvested, and mitochondria fractions were isolated using OptiPrep Mitochondria Isolation Kit (from QIAGEN, Cat No. 77632) following the manufacturer's protocol. Mitochondria were lysed on ice for 15 min using lysis buffer (25 mM Tris-HCl pH 7.5, 50 mM NaCl, 10% glycerol, 1% Nonidet P-40) with protease inhibitors. After centrifugation at 12,000 × g for 10 min at 4 °C, proteins in supernatants were loaded with an equal amount of protein for 2 h at 4 °C before loading on the gel. Proteins were separated by SDS-PAGE using a Mini-Protean III Cellars gel (Bio-Rad Laboratories, Hercules, CA) and transferred to Immobilon-P membranes (Millipore, Billerica, MA). Membranes were blocked with 5% milk in TBST and probed with overnight at 4 °C with primary antibodies against PARP10 (clone: GA1R, from Thermo Fisher Scientific MA-51738; 1:3000 dilution) for COX IV as mitochondrial protein loading control. PARP10 was detected using a polyclonal anti-Histone 2A.Z antibody (from Cell Signaling Technology product #2718S; 1:500 dilution) for Histone 2A.Z as a nuclear protein marker, and a monoclonal anti-PARP1 antibody (clone: 46D11, from Cell Signaling Technology #2123; 1:2000 dilution) for PARP1. The goat anti-mouse IgG-HRP conjugate (Thermo Fisher Scientific: G-21040; 1:3000 dilution) was used as the secondary antibody for the anti-PARP1 antibody and the goat anti-rabbit IgG-HRP conjugate (Thermo Fisher Scientific: G-21234; 1:3000 dilution) was used as the secondary antibody for the anti-COX IV antibody, anti-Histone 2A.Z antibody and anti-PARP1 antibody. Uncropped and unprocessed scans of the blots are provided as a Source Data file.

**Expression and purification of PARP5a catalytic domain.** cDNAs of human PARP5a (GE Dharmacon: MH56279-208068376) were used as templates for polymerase chain reaction to amplify the catalytic domain of PARP5a and PARP10, respectively, and a His6-tag at the N-terminus and a G-21040-c antibody and anti-PARP1 antibody. Uncropped and unprocessed scans of the blots are provided as a Source Data file.
using extinction coefficients of 0.762 and 0.753 for PARP10 and PARP5a, respectively.

Substrate activities of NAD$^+$ analogues for PARPs. Auto-PARylation of purified PARP1 was performed at room temperature in 100 µL of assay solution containing 100 mM Tris-HCl pH 8.0, 50 mM NaCl, 20 mM MgCl2, 0.2 mM DTT, 100 ng µL$^{-1}$ activated DNA, 0.4 mM NAD$^+$ or NAD$^+$ analogues 2, 6, 6-a-NAD$^+$, 2-a-NAD$,^+$ and 1 µM purified PARP1 enzyme. PARP1 autомodification controls were performed with 100 µM olarip ab inhib. After indicated incubation time, the reactions were stopped by adding 100 µM oarip ab. Auto-PARylation of recombinant human PARP2 was performed at room temperature in 10 µL of assay solutions containing 100 mM Tris-HCl pH 8.0, 50 mM NaCl, 20 mM MgCl2, 0.2 mM DTT, 100 ng µL$^{-1}$ activated DNA, 0.1 mM NAD$^+$ or NAD$^+$ analogues 6, 6-a-NAD$^+$, 2-a-NAD$^+$, and 200 ng purified PARP2 enzyme. PARP2 autомodification controls were performed with 100 µM olarip ab inhibitor. After indicated incubation time, the reactions were stopped by adding 100 µM oarip ab.

Auto-PARylation of the purified PARP5a catalytic domain was performed at room temperature in 100 µL of assay solutions containing 100 mM Tris-HCl pH 8.0, 200 mM NaCl, 20 mM MgCl2, 0.2 mM DTT, 100 ng µL$^{-1}$ NAD$^+$ or NAD$^+$ analogues 2, 6, 6-a-NAD$^+$, 2-a-NAD$,^+$, and 20 µM purified PARP10 catalytic domain. PARP5a autомodification controls were performed with 100 µM olarip ab inhibitor. After indicated incubation time, the reactions were stopped by adding 100 µM oarip ab.

Auto-PARylation of the purified PARP10 catalytic domain was performed at room temperature in 100 µL of assay solutions containing 100 mM Tris-HCl pH 8.0, 200 mM NaCl, 20 mM MgCl2, 0.2 mM DTT, 100 ng µL$^{-1}$ NAD$^+$ or NAD$^+$ analogues 2, 6, 6-a-NAD$^+$, 2-a-NAD$,^+$, and 10 µM purified PARP10 catalytic domain. PARP5a autомodification controls were performed with 100 µM olarip ab inhibitor. After indicated incubation time, the reactions were stopped by adding 100 µM oarip ab.

The levels of automodification were evaluated by immunoblotting using a streptavidin-HRP conjugate (R&D Systems, MN) and 45.4 nM recombinant human SIRT2 (R&D Systems, MN) in buffer containing 25 mM Tris pH 8.0, 150 mM NaCl, 1 mM DTT for overnight incubation at room temperature. Reactions without SIRT2 were included as controls. Following the overnight reactions, 50 nM bovine trypsin (Sigma-Aldrich, MO) was added to reaction mixtures for cleaving the deacetylated peptide substrate and releasing NAD$^+$ cations: the chemistry of proteome diversification. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Statistical analysis. One-tailed unpaired t tests were performed for comparison between two groups. A $P < 0.05$ was considered statistically significant. Significance of one test was determined as: ns = not significant, $P < 0.05$; *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$. Data are shown as mean ± SD. All statistical analyses were calculated using GraphPad Prism (GraphPad Software, CA).

Binding of WVE domain to auto-modified PARP1. Auto-PARylation of PARP1 was performed at 30 °C overnight using 3 µM purified PARP1 and 400 nM NAD$^+$ or NAD$^+$ analogues in a reaction buffer containing 100 mM Tris-HCl pH 8.0, 10 mM MgCl2, 50 mM NaCl, 1 mM DTT, and 100 ng µL$^{-1}$ activated DNA. 10 ng µL$^{-1}$ of Biotinylated PARP1/2/5a/10 via click chemistry, an anti-His6 antibody (clone: HIS.H8, from Thermo Fisher Scientific MA: 211315: 1:2000 dilution) for detection of the anti-His6 antibody and anti-PARP2 antibody. Uncropped and unprocessed scans of the blots are provided as a Source Data file.

Preparation of HeLa cell lysates. HeLa cells were grown in T25 flasks to 90% confluence and then collected using trypsin to detach the cells from the flasks before spinning down and washing the cell pellet with PBS. Two hundred microliter of lysis buffer (25 mM Tris-HCl pH 7.5, 30 mM NaCl, 10% glycerol, 1% Nonidet P-40 (VWR: 97064-734), and Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific: 78430)) was used to resuspend the cell pellets. Cells were shaken for 10 min at room temperature before spinning down cellular debris at 14,000 × g at 4 °C for 15 min. Protein concentrations of the cell lysates were measured using Bradford reagent (Thermo Fisher Scientific: 23236).

Labeling of protein PARylation in cell lysates. Two hundred micromolar of NAD$^+$ or NAD$^+$ analogue was added to 10 µg of lysate diluted in PBS to a final volume of 10 µL. Reactions were incubated at 30 °C for 1 h. Five microgram of lysate was quenched by the addition of LDS loading dye and analyzed on PAGE gels; the remaining 5 µg was subjected to CuAAC before quenching with LDS dye and running on PAGE gels. Proteins were transferred onto PVDF membranes and blocked with 5% nonfat milk or 3% BSA. Protein PARylation was detected using the streptavidin-HRP conjugate for 2-a-NAD$^+$ and 6-a-NAD$^+$ or FC-WVE antibody for 6 and 2-a-NAD$. The anti-rabbit antibody-HRP conjugate was used as the secondary antibody for the FC-WVE antibody. The anti-GAPDH antibody was used for a loading control at 1:3000 in PBS, and the anti-mouse antibody-HRP conjugate was used at 1:3000 as the secondary antibody. Detection was done as described above. Images were analyzed by ImageJ.

Substrate activities of NAD$^+$ and 6 for human SIRT2. 0.5 mM NAD$^+$ or 6 was incubated with 0.25 mM acetylated peptide substrate Ac-Ary-Gly-Lys(Ac)-AMC (R&D System, MN) and 45.4 mM recombinant human SIRT2 (R&D System, MN) in buffer containing 25 mM Tris pH 8.0, 150 mM NaCl, 1 mM DTT for overnight incubation at room temperature. Reactions without SIRT2 were included as controls. Following the overnight reactions, 50 mM bovine trypsin (Sigma-Aldrich, MO) was added to reaction mixtures for cleaving the deacetylated peptide substrate and releasing fluorescent 7-amino-4-methylcoumarin (AMC) group (excitation 380 nm; emission 460 nm). The reactions were monitored by using Synergy H1 microplate reader (Biotek, VT) on the basis of fluorescence intensity.

Data availability

The authors confirm that the data supporting the findings of this study are available from the corresponding author upon request. The source data underlying Figs. 3, 4b, 5c–f, 6, 7 and 8b and Supplementary Figs. 22, 31–33, 34b–36b, 37, and 38 are provided as a Source Data file.

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**Author contributions**

X.N.Z., Q.C. and Y.Z. designed research; X.N.Z., Q.C., J.C., A.T.L., Y.L., Z.D. and N.M.E. performed research; H.P. and S.G.L. provided resources and critical insights; X.N.Z., Q.C., J. C., A.T.L., Z.D. and Y.Z. analyzed data; and X.N.Z., Q.C. and Y.Z. wrote the manuscript.

**Additional information**

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