### Title

RNA-TAG Mediated Protein-RNA Conjugation.




### Journal

ChemBioChem, 24(18)



### Authors

Tota, Ember

Devaraj, Neal



### Publication Date

2023-09-15

### DOI

10.1002/cbic.202300454

Peer reviewed

Published in final edited form as:

*Chembiochem*. 2023 September 15; 24(18): e202300454. doi:10.1002/cbic.202300454.

# RNA-TAG Mediated Protein-RNA Conjugation**

**Ember M. Tota**[a], **Prof. Neal K. Devaraj**[a]

[a]Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, Natural Sciences Building 3328, La Jolla, CA 92093 (USA)

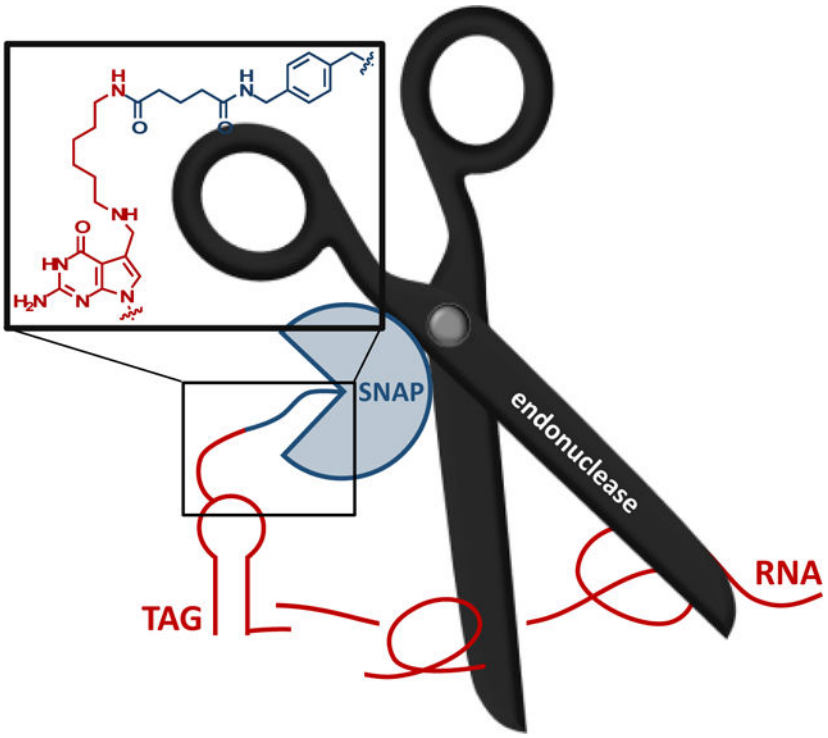
## Abstract

Combinations of biological macromolecules can provide researchers with precise control and unique methods for regulating, studying, and manipulating cellular processes. For instance, combining the unmatched encodability afforded by nucleic acids with the diverse functionality of proteins has transformed our approach to solving several problems in chemical biology. Despite these benefits, there remains a need for new methods to site-specifically generate conjugates between different classes of biomolecules. Here we present a fully enzymatic strategy for combining nucleic acids and proteins using SNAP-tag and RNA-TAG technologies via a bifunctional preQ1-benzylguanine small molecule probe. We demonstrate the robust ability of this technology to assemble site-specific SNAP-tag – RNA conjugates with RNAs of varying length and use our conjugation strategy to recruit an endonuclease to an RNA of interest for targeted degradation. We foresee that combining SNAP-tag and RNA-TAG will facilitate researchers to predictably engineer novel macromolecular complexes.

## Graphical Abstract

[**]TAG = transglycosylation at guanosine

ndevaraj@ucsd.edu .




Here we present an enzymatic, covalent, protein-nucleic acid conjugation method. This method precisely links two classes of biological macromolecules using SNAP-tag and RNA-TAG labeling technologies and a bifunctional small molecule probe. We demonstrate the utility of this method by recruiting an endonuclease to an RNA of interest for targeted degradation.

**Keywords**

conjugation; RNA; RNA degradation; RNA modification; SNAP-tag

## Introduction

Assemblies of the four macromolecular building blocks make up all vital cellular components. As scientists continue to uncover the complex functions of these naturally occurring assemblies, the utility of developing artificial counterparts has become increasingly apparent. Combining the programmability of nucleic acids with the specificity and activity of polypeptides, lipids, and glycans has begun to transform the way we approach biological problem solving and therapeutic intervention.[1–3] For instance, scientists have repurposed CRISPR/Cas systems, native to bacteria and archaea, to develop a set of technologies that employ the encodability of nucleic acids to direct the function of enzymes.[4–8]

Naturally occurring macromolecular assemblies maintain order and structure through highly evolved intermolecular interactions; synthetic assemblies, on the other hand, necessitate programming of the desired order and structure, sometimes using known affinity interactions, but often requiring covalent linkages between different biomolecules. Many

current technologies facilitating the association of DNA and RNA with other biomolecules rely on chemical conjugations or noncovalent interactions, and enzymatic strategies are generally limited to conjugation at the ends of the oligonucleotide of interest. These constraints limit the utility of such techniques, and their availability is often restricted by synthetic challenges and a high price tag.

The ability to join nucleic acids with proteins could facilitate the assembly of biomolecular machines. Available strategies require chemical modification of the enzyme, the oligonucleotide, or both, while noncovalent strategies include transient nucleic acid-protein interactions, such as the MS2 coat protein, which recognizes and associates itself with a stem-loop structure from the phage genome.[9–14] Self-labeling proteins (SLPs) such as SNAP-tag, CLIP-tag, and HaloTag have been engineered to selectively recognize, and covalently attach to, their small molecule substrate, making them useful tools for labeling or functionalizing a protein of interest[15–17]. Chemical-enzyme hybrid strategies employing modified oligonucleotides and enzyme linked-SLPs have been employed to recruit an enzyme to a transcript of interest via a covalently bound guide sequence. For instance, a guide functionalized SNAP-ADAR (adenosine deaminase acting on RNA) construct was used to facilitate the conversion of adenosine to inosine, and a sgRNA guided Cas fused PIN (PilT N-terminus) endonuclease was employed to direct RNA degradation.[18,19]

Previous work in our lab has established enzymatic nucleic acid modification strategies, RNA- and DNA-TAG (transglycosyl-ation at (deoxy)guanosine).[20,21] These technologies rely on the tRNA guanine transglycosylase (TGT) enzyme from *Escherichia coli* (*E. coli*), which exchanges a specific guanine for derivatives of the 7-deazaguanine small molecule substrate, prequeuosine1 (preQ1), in nucleic acids containing a cognate hairpin loop. TGTs are tRNA modifying enzymes that are present across all three kingdoms of life, differing slightly in their small molecule and RNA substrate specificities.[22] Bacterial and mammalian TGTs recognize and exchange guanine for the 7-deazaguanine derivatives, preQ1 and queuine, respectively, in their cognate tRNAs (aspartate, histidine, asparagine, and tyrosine). RNA-TAG was developed with the 17-nucleotide anticodon loop of tyrosine tRNA from *E. coli*, ECYA1 (TAG2; GCAGACTGTAAATCTGC), and the extended stem 25-nucleotide minihelix hairpin, ECYMH (TAG3; GGGAGCAGACTGTAAATCTGCTCCC). We have demonstrated the utility of RNA-TAG by attaching these hairpins to longer nucleic acid substrates for light controlled CRISPR editing, light-activated translation, fluorogenic and wash-free mRNA imaging, and proteomics.[23–28] The more recently developed DNA-TAG technology employs the same enzyme to modify a set of DNA hairpin substrates and has been used to quickly and inexpensively generate fluorescence in situ hybridization (FISH) probe sets and a near-infrared (IR) fluorescent Northern blot (irNorthern) probe.[21]

Both RNA-TAG and SNAP-tag technologies rely on enzymes which recognize specific small molecule substrates. We speculated that combining the function of these two enzymatic tools would enable a straightforward, fully enzymatic methodology to site specifically conjugate nucleic acids to proteins. We designed a bifunctional preQ1-benzylguanine (BG) small molecule probe, with either end being a substrate for one of the enzymes (Figure 1). Here, we develop a strategy for the RNA-TAG mediated assembly of the SNAP-tag SLP with RNAs of various sizes (Figure 2). Next, we demonstrate the

applicability of the approach by functionalizing an RNA of interest with preQ1-BG and subsequently recruiting a PIN-SNAP construct to initiate transcript degradation *in vitro* (Figure 3).

## Results and Discussion

### Enzyme-Nucleic Acid Conjugation

The synthesis of preQ1-BG was carried out using NHS coupling chemistry. A commercial BG-NHS ester was reacted with a commercially acquired preQ1-C6-NH2 probe precursor as previously described (Scheme S1).[21] The resulting small molecule was tested for TGT recognition by inserting it into the 17 nucleotide TAG2 RNA-TAG hairpin substrate, yielding the TAG2-BG modified RNA. The modification was detected via urea PAGE gel shift analysis, where an upward shift in the product band due to an increase in molecular weight was clearly visible (Figure 1).[20]

The TAG2-BG hairpin was quickly purified via spin column and incubated with commercially available SNAP-tag protein. The SNAP-RNA conjugate was confirmed via SDS PAGE gel shift, with the modified protein appearing higher on the gel due to an increase in molecular weight (Figure 1). Both the labeling and conjugation steps go to near completion as evidenced by the loss of the starting material in the product lanes of the gels.

The SNAP-tag enzyme was also able to conjugate to longer RNAs. Three TAG3 containing RNA constructs of varying sizes were *in vitro* transcribed (IVT) from a cut plasmid template: a random ~200 nucleotide sequence with the TAG sequence near the center, a ~400 nucleotide 7SK transcript with the TAG sequence near the 5′ end, and a ~1000 nucleotide mCherry transcript with a TAG sequence in the 3′ UTR (Figure 2). Each RNA was efficiently conjugated to the SNAP-tag protein, as evidenced by urea PAGE gel shift analysis. Since the insertion of the preQ1-BG small molecule into the RNA is not large enough to create a gel shift on its own, the constructs were analyzed for labeling after SNAP-tag protein conjugation and the apparent efficiency is a combination of both steps (Figure 2B).

### PIN-SNAP RNA Degradation

Directing the function and specificity of enzymes with the programmability of nucleic acids leads to powerful chemical biology tools. By using macromolecular conjugation strategies, nucleic acid base pairing can provide a template to direct the activity of enzymes, such as nucleases, to a specific location in RNA and DNA. PIN endonuclease domains are present across all kingdoms of life and are involved in non-sense mediated mRNA decay.[29] The PIN domain from *SMG6,* the nonsense mediated mRNA decay factor from *chlorocebus sabaeus*, has been conjugated to RNA binding proteins such as the PUF domain of human pumilio1 and dCas9 to direct its nuclease activity.[19,30] Using RNA-TAG and SNAP-tag, we initiated recruitment of the PIN endonuclease domain to direct RNA transcript degradation.

The PIN endonuclease domain was inserted 5′ to the SNAP-tag protein in a SNAP-tag containing plasmid. Subsequently, the SNAP-tag protein was cloned out for the PIN only construct. Both the PIN and PIN-SNAP constructs were recombinantly expressed and

purified in *E. coli* (Figure S1). IVT mCherry-TAG3 was modified with preQ1-BG (mCherry-BG) and both the unmodified and BG functionalized transcripts were incubated with 0, 0.05, 1, or 5 μg of either the PIN or PIN-SNAP construct for 4 hours at 37°C. As expected, RNA degradation was seen in all reactions, even without recruitment (Figure 3). This suggests that, even under recruitment conditions, the endonuclease activity of unconjugated PIN constructs likely results in degradation before the RNA-enzyme conjugation is complete, limiting the recruitment effect for this particular enzyme. Regardless, no difference in degradation was detected between the PIN and PIN-SNAP nucleases when incubated with the unmodified RNA transcript (Figure 3A). Conversely, targeted degradation is observed with 1 μg (1.3uM) PIN-SNAP, while untargeted degradation with 1 μg (2.5uM) PIN is not as efficient (Figure 3B). In fact, far more units of PIN only vs SNAP-PIN enzyme are needed to more units of PIN only vs SNAP-PIN enzyme are needed to see efficient degradation, supporting that SNAP/BG recruitment to an RNA of interest can direct enzymatic activity. This evident increase in nuclease activity suggests that our recruitment approach could be used for targeted RNA degradation in more complex environments, such as live cells, and extended to direct alternative targeted enzymatic activities to transcripts of interest.

## Conclusion

Combining biological molecules to assemble macromolecular machines is of interest in synthetic and chemical biology. Proteins, lipids, glycans, and nucleic acids all play important and unique roles in biological processes and merging their abilities to generate useful new tools has the potential to impact biomedical applications, fundamental research, engineering, and clinical tools and therapeutics. The nucleic acid-protein conjugation system outlined here is efficient and simple to execute. We successfully and site-selectively modified a variety of RNAs with a benzylguanine handle, and, using a well characterized self-labeling protein system, created RNA-enzyme conjugates. We further demonstrated the utility of this system by designing a functional nuclease construct, that, when recruited to an RNA of interest, improves degradation efficiency.

We envision the continued development of this robust macromolecular assembly technique. For instance, expanding its application scope by conjugating proteins to DNA species using this strategy in tandem with DNA-TAG. Additionally, the selectivity of different SLPs toward their small molecule substrates should allow for their simultaneous use and future work will expand the system by creating bifunctional preQ1 probes compatible with other SLP systems such as CLIP-tag (preQ1-benzylcytosine) and HaloTag (preQ1-chloroalkane) to facilitate multiplexing. Lastly, while this work demonstrates the utility of this assembly technique *in vitro*, the bioorthogonal nature of SLP systems warrants exploration of their use as a recruitment tool for acting on transcripts in live cells.

## Experimental Section

### General Information.

All reagents used for buffers and coupling reactions were purchased from Sigma-Aldrich. Benzylguanine-NHS was purchased from New England Biolabs. Urea PAGE gels were prepared using the 19:1 sequagel system from National Diagnostics, separated using

the Mini-Protean system from Biorad, and stained with GelRed from Biotium. All polyacrylamide gels were imaged on a Bio-Rad ChemiDoc-MP gel imager. Gels images were analyzed using imageJ software from the NIH. His purification was conducted using the His Pur resin from Thermo Fisher and the His-Spin buffers from Zymo. Protein gels were run using the Mini-Protean gel system and precast gels from Bio-Rad and stained with instant blue Coomassie stain from Abcam. All restriction enzymes, bio-reagents, and competent bacterial strains were purchased from New England Biolabs, Promega, or Life Technologies. Absorbance measurements were obtained with a Thermo Scientific Nano-Drop 2000c UV-Vis spectrophotometer. High resolution mass spectrometry (HRMS) of the small molecule probe was collected by the UCSD Department of Chemistry and Biochemistry Molecular Mass Spectroscopy Facility on an Agilent 6230 time-of-flight mass spectrometer (TOFMS) with JetStream electrospray ionization source (ESI).

### PIN plasmids.

A plasmid containing the PIN domain was ordered from (Addgene 104184). Another lab member gifted me a bacterial expression plasmid containing the SNAP-tag sequence. The PIN domain was cloned into the SNAP-tag plasmid for the PIN-SNAP construct and the SNAP-tag was subsequently cloned out for the PIN only construct. The resulting plasmids were verified via Sanger sequencing by Eton biosciences (Table S1).

### In vitro transcription (IVT) of RNAs.

The mCherry and 7SK templates (Table S2) used for IVT were cloned into the pcDNA3.1-empty-TAG3 plasmid (Addgene #138209) containing the 189 nt random sequence. The mCherry-TAG3 and empty-TAG3 templates were prepared by linearization using XbaI, purified by phenol chloroform extraction to ensure removal of RNAses, and ethanol precipitated. PCR amplification was used to attach a T7 promoter and prepare the 7SK template with the following primers: forward: AAGCTGTAATACGACTCACTATA GGGGATCCCCGGGAGCAGAC reverse: AAAAGAAAGGCAGA CTGCCACATG. Transcription reactions were set up with rNTPs (5 mM each ATP, CTP, UTP, 9 mM GTP) from NEB, 0.004 U/μL Thermostable Inorganic Pyrophosphatase from NEB 0.15 μg/μL, T7-RNAP made in house, and 0.05% Triton X-100 from Sigma Aldrich in 1X T7 Reaction Buffer. Approximately 4 μg of cut plasmid or 1 μg of purified PCR product was used for a 100 μL transcription reaction. The reactions were run at 37°C for 4 hours. The transcripts were purified by addition of LiCl to a final concentration of 2.5 M and precipitated overnight at −20°C. The LiCl solution was removed and the precipitated RNA was resuspended in water. The size was verified via 4% urea PAGE and the transcripts were stored at −20 °C.

### PreQ1-benzylguanine synthesis.

PreQ1 benzylguanine was synthesized as described previously by removing a tert-butyloxycarbonyl (boc) protecting group from a preQ1 derivative and coupling it to commercially available NHS functionalized benzylguanine (Scheme S1).[20, 31] Briefly, the boc protected starting material was deprotected in 10% TFA/DCM for one hour. The TFA was neutralized 3 times with 10% TEA in DCM, rotoevaporating the solution to near dryness between each wash. The crude oil product was taken to the next step where it was dissolved in dry DMF followed by the dropwise addition of TEA. Benzylguanine-NHS

probe was dissolved in dry DMF and added to the reaction dropwise with stirring. The reaction was allowed to proceed for 2 hours at room temperature, the DMF evaporated, and the product purified by HPLC. The final product was confirmed by LMCS.

### Expression of enzymes.

*E. coli* TGT was expressed as previously described.[20] The PIN and PIN-SNAP enzymes were also expressed using the same protocol. Briefly, the plasmids were transformed into BL21(DE3) competent *E. coli* cells (NEB C2527) and a starter culture was grown overnight. Approximately 5 mL of starter culture was transferred into 200 mL of kanamycin or carbenicillin containing LB and allowed to grow to an optical density of ~0.6. At this point, expression was induced with by adding IPTG to a final concentration of 1 mM and shaken at 37°C for 4 hours. The bacteria were pelleted by centrifugation (6,000xg for 20 minutes at 4°C). Cell pellets were either resuspended and purified immediately or stored at −20°C if desired.

### Purification of enzymes.

The *E. coli* TGT and PIN enzymes were purified via C-terminal His tags and 10μL aliquots were taken at each step for analysis via 4–20% SDS PAGE (Figure S1). His purification was carried out using HisPurTM Ni-NTA Resin spin columns (Thermofisher 88224) and His purification buffers (Zymo P2003–4; P2003–5). The cell pellet was resuspended in lysis buffer and sonicated on ice using the following settings: 4 cycles (30 sec on / 2 min off); output control = 4; duty cycle% = 30–40. The lysate was centrifuged at 10,000xg for 30 min at 4°C to remove cellular debris. In tandem, the resin from the spin column was equilibrated 2 times with 400 μL of lysis buffer. The supernatant of the centrifuged cell lysate was transferred to the capped spin column and incubated for at least one hour at 4°C with end over end mixing. The resin was then washed and the protein eluted according to the HisPur manual. The lysis and wash buffer are the same as it was noted that a higher imidazole concentration during binding reduced nonspecific carry over into the final elutions. After elution from the His column, the enzyme preps were dialyzed against storage buffer to remove imidazole and concentrated using a 10 kDa (Millipore UFC5010; PIN) or 30 kDa (UFC5030; PIN-SNAP and TGT) cut off spin filter. Enzyme concentration was determined using a BCA assay kit (Thermo Fisher 23227). The final proteins were aliquoted in 20 μL fractions and stored at −80°C.

### TGT labeling reaction.

TGT modification of RNA substrates was carried out at 37°C for 4 hours in a thermalcycler with a heated lid[20, 31] using the following reaction conditions: 10 μM enzyme, 5 μM nucleic acid substrate, and 50 μM of preQ1-BG (benzylguanine), 1X TGT reaction buffer, and 1-unit RNAsin from Promega. Components were mixed together, gently vortexed, and briefly spun down.

### Labeled oligo purification.

TAG2-BG was purified using the oligo clean and concentrator kit from Zymo (D4060) and the product eluted in pure water.

### SNAP – RNA-BG (benzylguanine) conjugation.

Benzylguanine modified RNA substrates were incubated with commercial SNAP-tag enzyme (NEB P9312S): 2 μM RNA was incubated with 18 μM SNAP-tag in ultra-pure water at 37°C for 4 hours. Reactions were analyzed via 4% urea PAGE without purification.

### Urea PAGE analysis.

Urea PAGE was used to assess TGT modification of TAG2 and SNAP-tag conjugated RNA-BG constructs. For all gels, we used the 19:1 Acrylamide:Bisacrylamide SequaGel® UreaGel System (National Diagnostics EC-833) and the Mini-PROTEAN Tetra Vertical Electrophoresis Cell from Bio- Rad. Modification of the 17 nucleotide TAG2 transcript was analyzed using 20% urea PAGE and longer transcripts were analyzed using 4% or 5% urea PAGE. Briefly, 1.5 mm plates were assembled on the Bio-Rad gel casting stand, 10 mL of the desired percentage gel was freshly prepared in a 15 mL centrifuge tube followed by the addition of 14 μL of TEMED and 80 μL of 10% ammonium per sulfate. After gentle, brief mixing, the solution is poured between the plates, a comb inserted, and allowed to solidify (~20–30 min). RNA samples were prepared for gel electrophoresis using 2X RNA loading dye (NEB B0363S). The solutions were denatured at 98°C for 5 minutes on a thermocycler and 150–200 ng of the construct was loaded onto the gel for visualization. The electrophoresis cassette was assembled, and the chamber filled with 1X TBE. After thorough washing of the wells to remove leached urea, the samples were loaded into the wells and the gel was run for 10 min at 100V to allow the samples to enter then 90 min at 200V. Afterward, the gel was stained with gel red in TBE (1:1000 dilution; Biotium 41011) for ~5 min and imaged on a Bio-Rad ChemiDoc gel imager system.

### PIN degradation.

Unlabeled or benzyl guanine modified in vitro transcribed mCherry-TAG3 were incubated with varying amounts of recombinantly expressed PIN or PIN-SNAP enzymes under the following conditions: 0, 0.05, 1, or 5 μg PIN construct; 650 μM RNA; 1X PIN reaction buffer; 4 hours 37°C. Reactions were run on 4% urea PAGE gel for 25 minutes at 100V followed by 75 minutes at 200V. Afterward, the gel was stained with gel red in TBE (1:1000 dilution; Biotium 41011) for ~5 min and imaged on a Bio-Rad ChemiDoc gel imager system. Remaining RNA was quantified via densitometry using imageJ.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

The authors acknowledge the UCSD Molecular Mass Spec- trometry Facility for small molecule high resolution mass spectrometry support. This work was supported by the National Institutes of Health [R35 GM141939]. The content of the information does not necessarily reflect the position or the policy of the government, and no official endorsement should be inferred.

## References


[1]. Esposito CL, Catuogno S, Condorelli G, Ungaro P, de Franciscis V, Genes 2018, 9, 529. [PubMed: 30384431]

[2]. Li X, Feng K, Li L, Yang L, Pan X, Yazd HS, Cui C, Li J, Moroz L, Sun Y, Wang B, Li X, Huang T, Tan W, Natl. Sci. Rev 2020, 7, 1933–1953. [PubMed: 34691533]

[3]. Tesz GJ, Aouadi M, Prot M, Nicoloro SM, Boutet E, Amano SU, Goller A, Wang M, Guo CA, Salomon WE, Virbasius JV, Baum RA, O'Connor MJ, Soto E, Ostroff GR, Czech MP, Biochem. J 2011, 436, 351–362. [PubMed: 21418037]

[4]. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E, Science 2012, 337, 816–821. [PubMed: 22745249]

[5]. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F, Science 2013, 339, 819–823. [PubMed: 23287718]

[6]. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR, Nature 2016, 533, 420. [PubMed: 27096365]

[7]. Gasiunas G, Barrangou R, Horvath P, Siksnys V, Proc. Natl. Acad. Sci 2012, 109, E2579–E2586. [PubMed: 22949671]

[8]. Xu Y, Li Z, Comput. Struct. Biotechnol. J 2020, 18, 2401. [PubMed: 33005303]

[9]. Bertrand E, Chartrand P, Schaefer M, Shenoy SM, Singer RH, Long RM, Mol. Cell 1998, 2, 437–445. [PubMed: 9809065]

[10]. Trads JB, Tørring T, Gothelf KV, Acc. Chem. Res 2017, 50, 1367–1374. [PubMed: 28485577]

[11]. Zhang X, Xu Z, Moumin DS, Ciulla DA, Owen TS, Mancusi RA, Giner J-L, Wang C, Callahan BP, Bioconjugate Chem. 2019, 30, 2799–2804.

[12]. Synakewicz M, Bauer D, Rief M, Itzhaki LS, Sci. Rep 2019, 9, 13820 [PubMed: 31554828]

[13]. Zhao D, Kong Y, Zhao S, Xing H, Top. Curr. Chem 2020, 378.

[14]. Reinking HK, Stingele J, STAR Protoc. 2021, 2.

[15]. Juillerat A, Gronemeyer T, Keppler A, Gendreizig S, Pick H, Vogel H, Johnsson K, Chem. Biol 2003, 10, 53–60. [PubMed: 12573698]

[16]. Los GV, Encell LP, McDougall MG, Hartzell DD, Karassina N, Zimprich C, Wood MG, Learish R, Friedman Ohana R, Urh M, Simpson D, Mendez J, Zimmerman K, Otto P, Vidugiris G, Zhu J, Darzins A, Klaubert DH, Bulleit RF, Wood KV, ACS Chem. Biol 2008, 3, 373–382. [PubMed: 18533659]

[17]. Gautier A, Juillerat A, Heinis C, Corrêa IR, Kindermann M, Beaufils F, Johnsson K, Chem. Biol 2008, 15, 128–136. [PubMed: 18291317]

[18]. Hanswillemenke A, Kuzdere T, Vogel P, Jékely G, Stafforst T, J. Am. Chem. Soc 2015, 137, 15875–15881. [PubMed: 26594902]

[19]. Batra R, Nelles DA, Pirie E, Blue SM, Marina RJ, Wang H, Chaim IA, Thomas JD, Zhang N, Nguyen V, Aigner S, Markmiller S, Xia G, Corbett KD, Swanson MS, Yeo GW, Cell 2017, 170, 899. [PubMed: 28803727]

[20]. Alexander SC, Busby KN, Cole CM, Zhou CY, Devaraj NK, J. Am. Chem. Soc 2015, 137, 12756–12759. [PubMed: 26393285]

[21]. Tota EM, Devaraj NK, J. Am. Chem. Soc 2023, 145, 8099–8106. [PubMed: 36988146]

[22]. Stengl B, Reuter K, Klebe G, ChemBioChem 2005, 6, 1926–1939. [PubMed: 16206323]

[23]. Zhang D, Liu L, Jin S, Tota E, Li Z, Piao X, Zhang X, Fu X-D, Devaraj NK, J. Am. Chem. Soc 2022, 144, 4487–4495. [PubMed: 35257575]

[24]. Zhou CY, Alexander SC, Devaraj NK, Chem. Sci 2017, 8, 7169–7173. [PubMed: 29081948]

[25]. Zhang D, Zhou CY, Busby KN, Alexander SC, Devaraj NK, Angew. Chem., Int. Ed 2018, 57, 2822–2826.

[26]. Zhang D, Jin S, Piao X, Devaraj NK, ACS Chem. Biol 2020, 15, 1773–1779. [PubMed: 32484653]

[27]. Busby KN, Fulzele A, Zhang D, Bennett EJ, Devaraj NK, ACS Chem. Biol 2020, 15, 2247–2258. [PubMed: 32706237]

[28]. Ehret F, Zhou CY, Alexander SC, Zhang D, Devaraj NK, Mol. Pharmaceutics 2018, 15, 737–742.

[29]. Glavan F, Behm-Ansmant I, Izaurralde E, Conti E, EMBO J. 2006, 25, 5117–5125. [PubMed: 17053788]

[30]. Choudhury R, Tsai YS, Dominguez D, Wang Y, Wang Z, Nat. Commun 2012, 3, 1147. [PubMed: 23093184]

[31]. Busby KN, Devaraj NK, Methods Enzymol. 2020, 641, 373–399. [PubMed: 32713531]

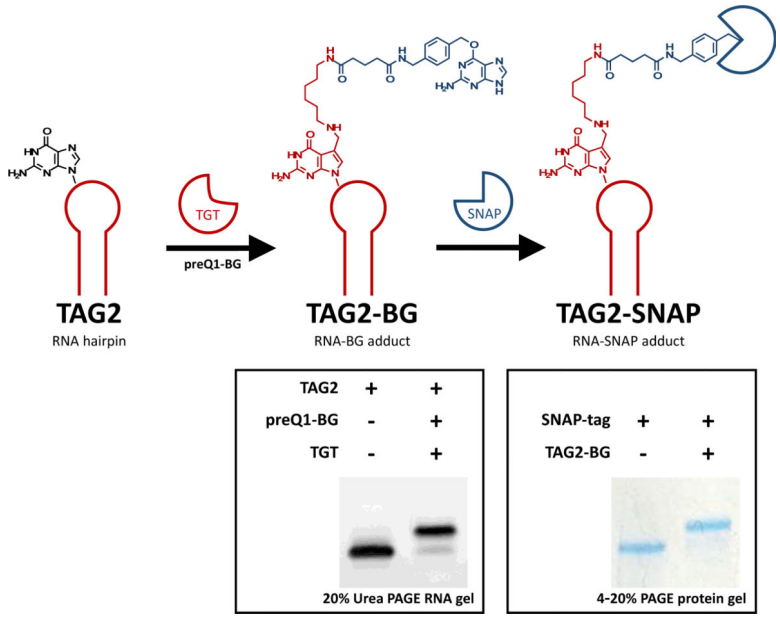



**Figure 1.**

TAG2-BG labelling and conjugation to SNAP-tag protein. TGT efficiently inserts the preQ1-BG substrate into RNA as is evidenced by the upward shift on urea PAGE (left). The BG labelled RNA substrate is treated with commercially available SNAP-tag, forming the macromolecular conjugate in the SDS PAGE gel on the right. Labeling and conjugation reactions are carried out at 37°C for 4 hours.

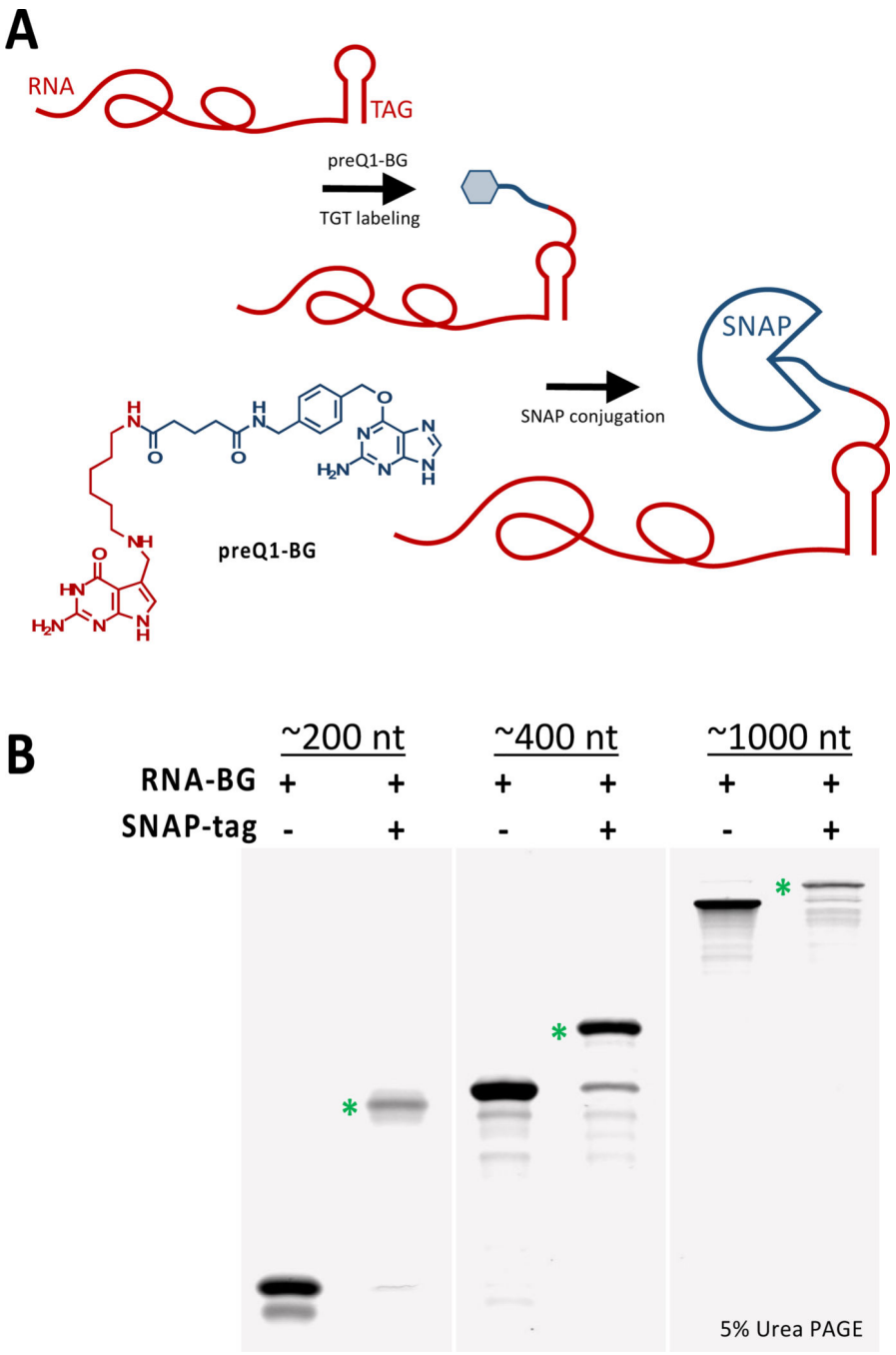



**Figure 2.**

Conjugation of long RNAs with SNAP-tag. (A) Cartoon schematic of RNA-TAG3 modification and conjugation. (B) Three TAG3 containing constructs of varying length are labelled with preQ1-benzylguanine and treated with SNAP-tag. Over two steps, all conjugations go to near completion as evidenced by the upward gel shift of the products (green asterisks). Labeling and conjugation reactions are carried out at 37°C for 4 hours.

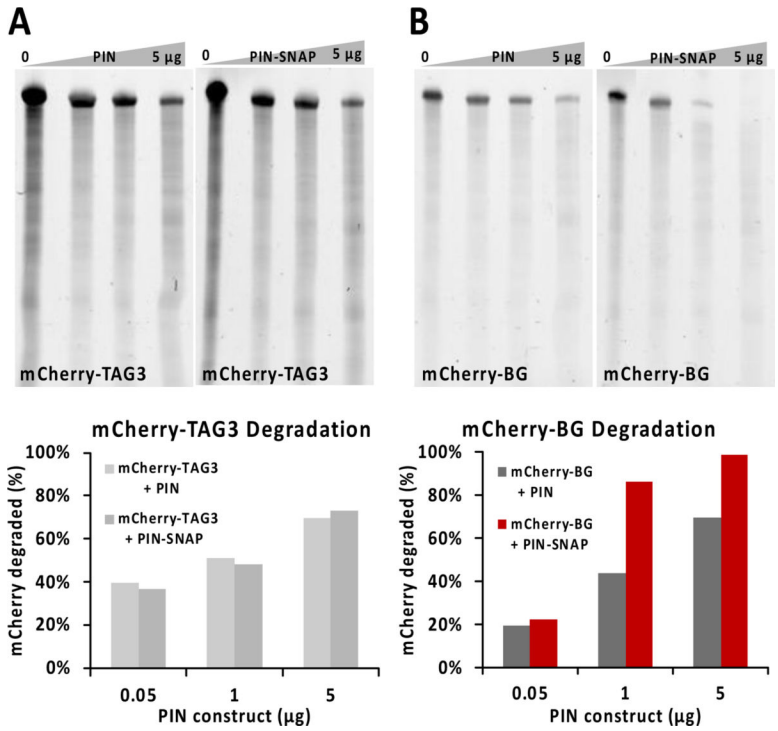



**Figure 3.**

Urea PAGE gel analysis (top) and quantification (bottom) of PIN and PIN-SNAP degradation of mCherry-TAG3 and BG modified mCherry-TAG3 (mCherry-BG) RNA *in vitro*. The reactions were carried out with the indicated amount of enzyme and 0.65 µM RNA. The PIN endonuclease is active in both the PIN only and PIN-SNAP constructs, but degradation is highly improved with recruitment of the endonuclease to the transcript. (A) Degradation of mCherry-TAG3 after treatment with PIN or PIN-SNAP enzymes. (B) Untargeted and targeted degradation of mCherry-BG, respectively, upon SNAP recruitment of the PIN-SNAP endonuclease to the transcript. Labeling and degradation reactions are carried out at 37°C for 4 hours.