Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Electronic Supplementary Information

Promoters vs. Telomeres: AP-Endonuclease 1 interactions with abasic sites in G-quadruplex folds depend on topology

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Additional Methods

The plasmids were constructed from the psiCHECK2 plasmid (Promega) that has coding sequences for the Renilla luciferase (Rluc) and firefly luciferase (luc) genes. The luc gene is regulated by the HSV-TK promoter and was not modified allowing expression from this luciferase to be used as the internal standard. The data were collected via a dual-glo luciferase assay (Promega). The Rluc gene was originally regulated by the SV40 early enhancer/promoter, which we modified by removing the TATA box and replacing it with the cruciform-forming sequence. Additionally, the cruciform-forming sequence was flanked by recognition sequences for the Nt.BspQ1 nicking endonuclease. Insertion of the cruciform-forming sequence and nicking endonuclease recognition sequences was achieved using restriction-free cloning. The cloning experiment was conducted in a 25-µL reaction with 1x Phusion reaction buffer (NEB), 200 µM dNTPs, 1 µM each of the forward and reverse primers, 1,000 ng of psiCHECK2 plasmid, 3% DMSO, and 0.5 units of Phusion DNA polymerase. The PCR was initiated with a 98 °C denaturation step for 30 sec followed by 30 cycles of PCR consisting of a 98 °C denaturation step for 20 sec, a 55 °C annealing step for 30 sec, a 72 °C extension step for 5 min, a post step consisting of 98 °C for 8 sec followed by 72 °C for 5 min. After the 30 cycles, a final extension step of 72 °C for 5 min was conducted. Following PCR, the samples were digested with Dpn1 by adding 2.3 µL of Dpn1 reaction buffer and 5 units of Dpn1 to the PCR reaction vessel. The Dpn1 reaction was conducted for 2 hr at 37 °C followed by heat quenching at 80 °C for 20 min.

Following the PCR and Dpn1 reactions, the sample was then transformed into NEB 5-alpha competent *E. coli* cells following the manufacturer's protocol. After transformation, 50-100 µL from the transformation was spread on a pre-warmed agar plate containing ampicillin (100 µg/mL). The plates were incubated overnight at 37 °C. Next, individual colonies were picked and each grown overnight at 37 °C in 5 mL of lysogeny broth media containing ampicillin (100 µg/mL). The plasmid DNA from each grown sample was then purified using a miniprep kit (Qiagen) following the manufacturer's protocols. Last, ~200 ng of plasmid and 1 µM sequencing forward primer in 15 µL of 10 mM Tris (pH 8.0) was submitted for Sanger sequencing at the DNA Sequencing Core facility at the University of Utah.

Insertion of site-specific modifications into the plasmids was achieved following literature protocols.[1,2] Specifically, 5 µg of plasmid with Nt.BspQ1 recognition sequences flanking the site in which the DNA base modification will be inserted were placed in 50 µL of 1x Nt.BspQ1 reaction buffer with 5 U of Nt.BspQ1. The reaction was placed at 50 °C for 60 min followed by heat quenching at 80 °C for 20 min. Next, 1 nmole (~1000x) of 5`-phosphorylated synthetic oligomer with the site-specific modification was added to the quenched reaction. The modified oligomers were synthesized by standard solid-phase synthesis in the DNA/Peptide Core Facility at the University of Utah. The oligos were cleaved, deprotected, and HPLC purified following protocols previously outlined.[3] The plasmid oligomer mixture was thermal cycled by heating at 80 °C for 2 min followed by cooling on ice for 2 min, which was repeated four times. The nicks were then sealed using T4 DNA ligase, by adding 6 µL of 10x ligase buffer and 800 units of ligase. The ligation reaction was left at 20 °C for 4 h. The modified plasmids were purified from the protein and excess insertion oligomer using an Ultra Clean PCR cleanup kit (Mo Bio) following the manufacturer's protocol. Plasmid concentrations were determined by nanodrop UV-vis measurements.

To confirm the DNA modifications were introduced into the plasmid, we applied a protocol established in our laboratory, in which the modification was removed by a DNA glycosylase to yield a ligatable gap for sequencing.[4] By ligating the gap, Sanger sequencing provided a characteristic nucleotide loss at the modification site to confirm the presence of the modification.

Primer Sequences

Primers utilized that enabled synthesis of modifications in the coding strand

hTelo-5-1
5`- CAG TTC CGC CCA TTC TCC GCC CCA TGG CTG ACG CTC TTC C TAGGG TTA GGG TTA GGG TTA GGG TT GCT CTT CTG CAG AGG CCG AGG CCG CCT CGG CCT CTG AGC T

hTelo-5-2

5`-AGC TCA GAG GCC GAG GCG GCC TCG GCC TCT GCA GAA GAG CAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TAG GAA GAG CGT CAG CCA TGG GGC GGA GAA TGG GCG GAA CTG

hTelo-4-1
5`- CAG TTC CGC CCA TTC TCC GCC CCA TGG CTG ACG CTC TTC C TAGGG TTA GGG TTA GGG TTA GGG TT GCT CTT CTG CAG AGG CCG AGG CCG CCT CGG CCT CTG AGC T

hTelo-4-2
5`-AGC TCA GAG GCC GAG GCG GCC TCG GCC TCT GCA GAA GAG CAA CCC TAA CCC TAA CCC TAA CCC TAG GAA GAG CGT CAG CCA TGG GGC GGA GAA TGG GCG GAA CTG

Sequencing Primer
5`- TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTC ATG CAT CTC AAT TAG TCA GCA ACC ATA GT

The OG-containing insert sequences studied (**O** = 8-oxo-dG)

hTelo-5OG insert

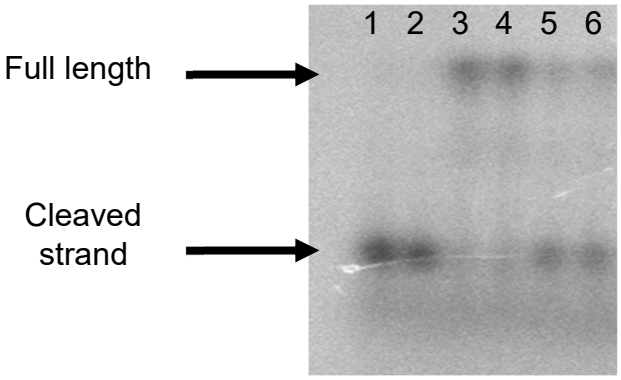
5′-p TAGGG TTA GGG TTA **O**GG TTA GGG TTA GGG TT GCT CTT CT

hTelo-4OG insert

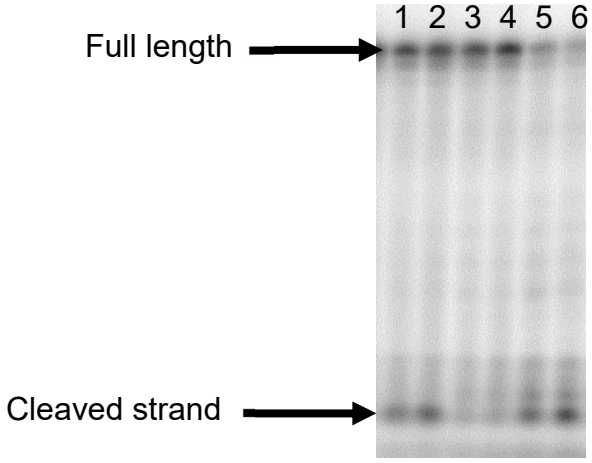
5′-p TAGGG TTA **O**GG TTA GGG TTA GGG TT GCT CTT CT

Figure S1. Analysis of APE1 cleavage of ^{32}P-labeled F-containing DNA by denaturing PAGE (in Li^+ buffer and K^+ buffer)

1 – hTelo F duplex in Li^+ buffer
2 – hTelo F duplex in K^+ buffer
3 – no APE1 in Li^+ buffer
4 – no APE1 in K^+ buffer
5 – hTelo 4F in Li^+ buffer
6 – hTelo 4F in K^+ buffer

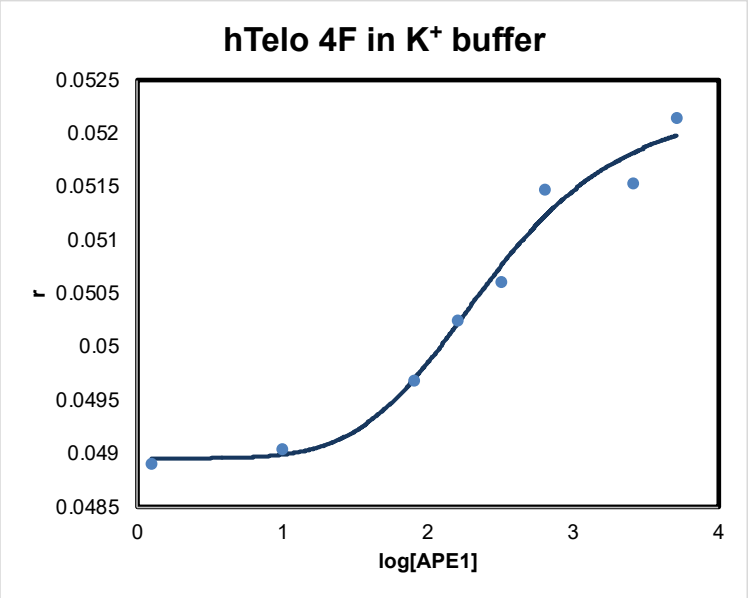


1 – hTelo-5F 3`-ext in Li^+ buffer
2 – hTelo-5F 3`-ext in K^+ buffer
3 – no APE1 in Li^+ buffer
4 – no APE1 in K^+ buffer
5 – hTelo-5F 5`-ext in Li^+ buffer
6 – hTelo-5F 5`-ext in K^+ buffer

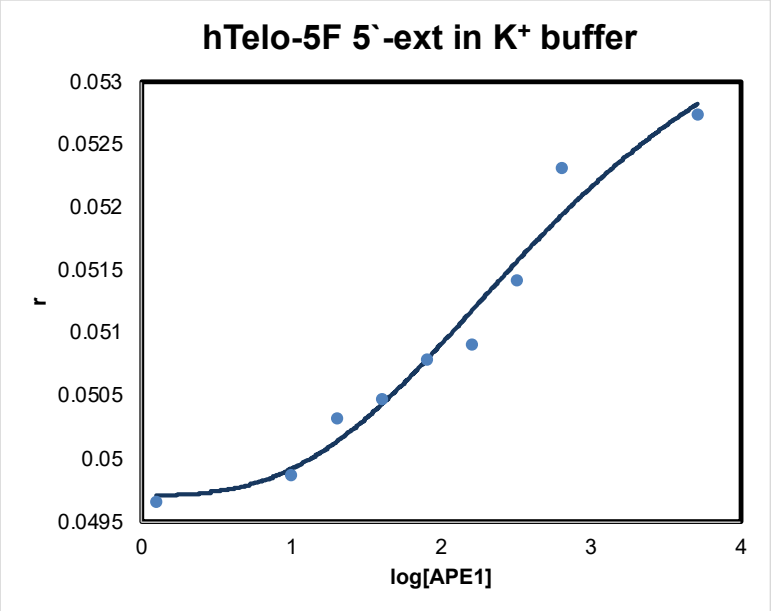


Conditions: 50 mM potassium or lithium acetate, 20 mM Tris-acetate (pH 7.4 at 37 °C), 10 mM magnesium acetate, 1 mM DTT, 37 °C, 1 h, 3 nM APE1, and 10 nM DNA.

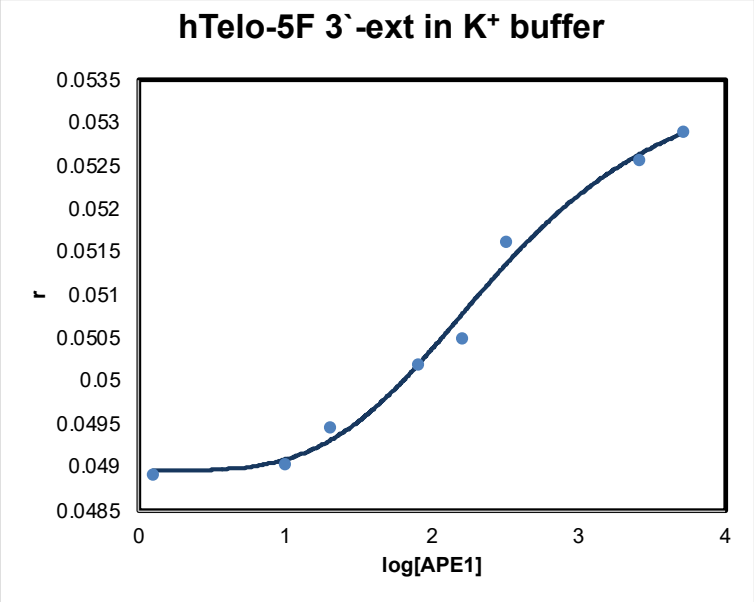
Figure S2. Example analysis of APE1-G4 dissociation constants measured by fluorescence anisotropy.



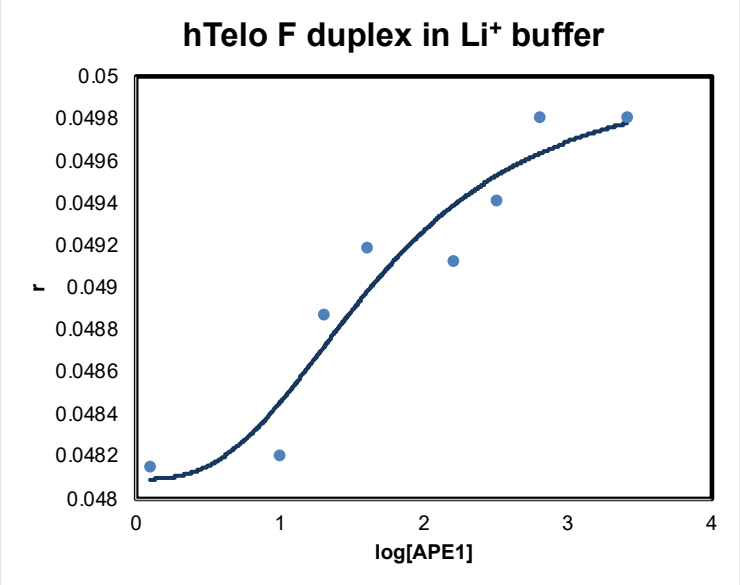
START	0.04895 ± 1.67043E-4
END	0.05233 ± 5.20061E-4
k	2.566 ± 1.4990809
n	1.512679
Reduced Chi-Sqr	4.92E-08
R-Square (COD)	0.98052
Adj. R-Square	0.96592



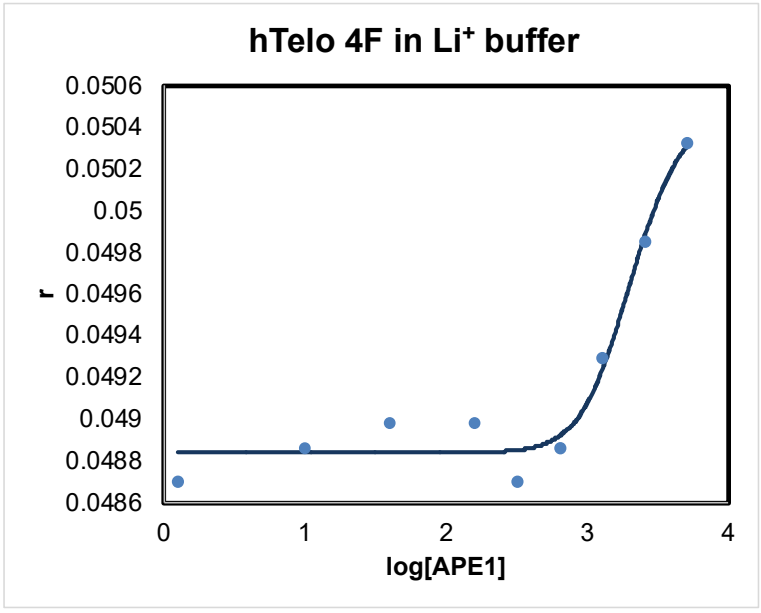
START	0.04971 ± 2.19677E-4
END	0.05427 ± 0.0019
k	2.8139 ± 1.4325
n	1.290587
Reduced Chi-Sqr	5.70E-08
R-Square (COD)	0.96754
Adj. R-Square	0.94806



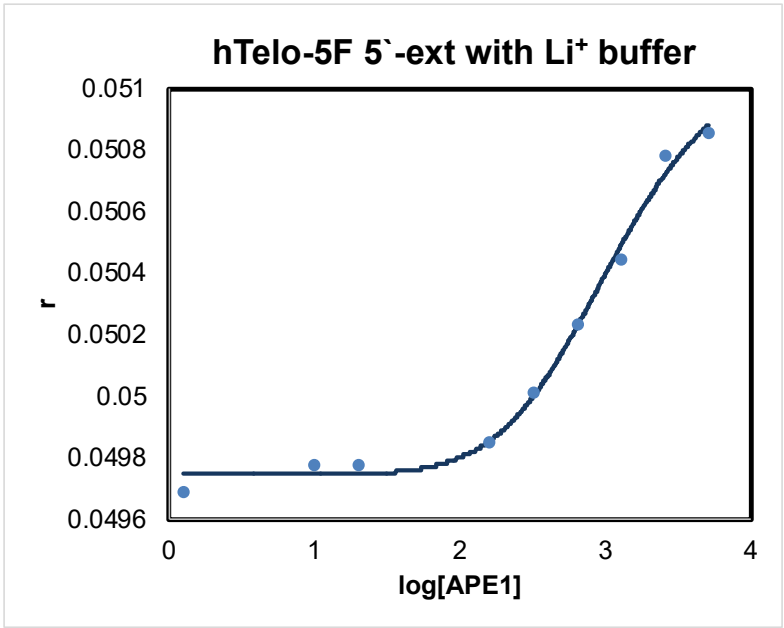
START	0.04897 ± 1.80077E-4
END	0.05371 ± 8.89439E-4
k	2.475173 ± 1.499
n	1.391146
Reduced Chi-Sqr	4.53E-08
R-Square (COD)	0.98926
Adj. R-Square	0.98121



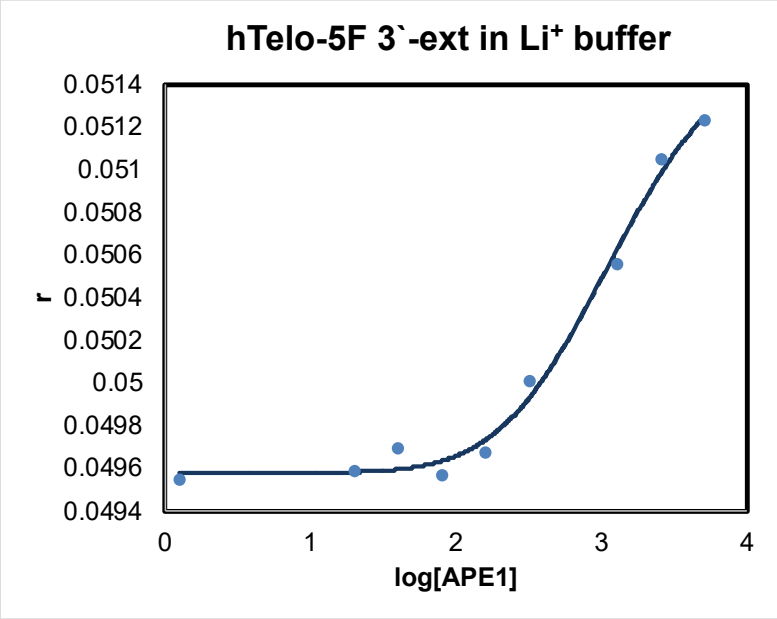
START	0.04809 ± 2.46562E-4
END	0.05003 ± 7.34202E-4
k	1.7459 ± 0.50561
n	1.27468
Reduced Chi-Sqr	6.13E-08
R-Square (COD)	0.91394
Adj. R-Square	0.84939



START	0.04884 ± 6.10165E-5
END	0.05051 ± 3.69133E-4
k	3.36348 ± 2.107
n	1.79046
Reduced Chi-Sqr	1.84E-08
R-Square (COD)	0.96331
Adj. R-Square	0.94129

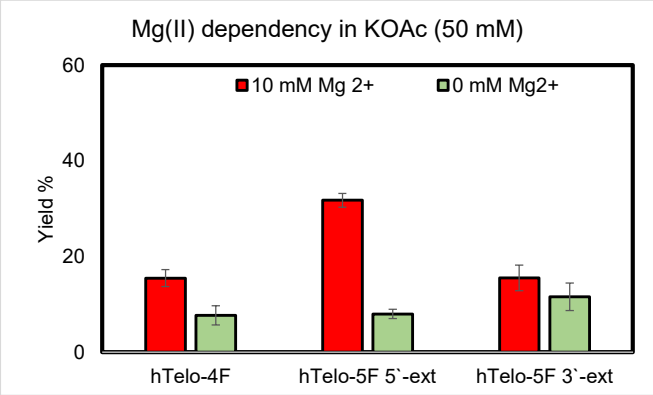


START	0.04975 ± 2.8222E-5
END	0.05114 ± 1.93814E-4
k	3.05172 ± 2.10755
n	1.762753
Reduced Chi-Sqr	2.41E-09
R-Square (COD)	0.99244
Adj. R-Square	0.98791



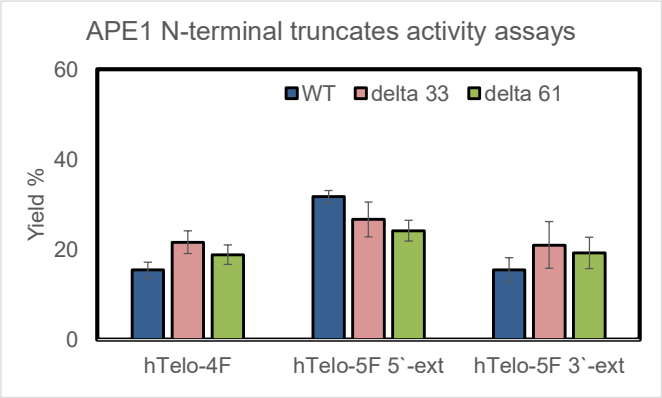
START	0.04958 ± 4.89361E-5
END	0.05174 ± 4.25931E-4
k	3.062 ± 2.105943
n	1.732135
Reduced Chi-Sqr	7.01E-09
R-Square (COD)	0.99025
Adj. R-Square	0.9844

Figure S3. The magnesium dependency for APE1 to cleave an F in the hTelo G4 folds.



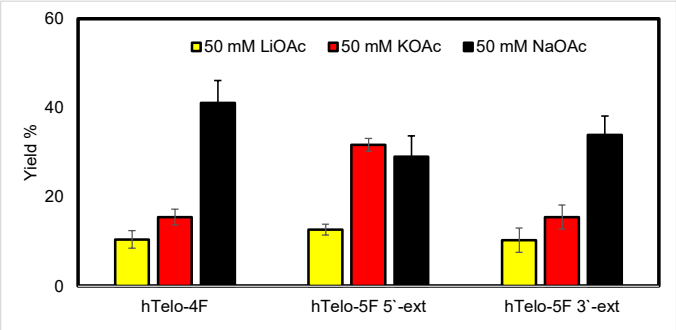
Reaction conditions: 50 mM potassium acetate, 20 mM Tris-acetate (pH 7.4 at 37 °C), 0 or 10 mM magnesium acetate, 1 mM DTT, 37 °C, 1 h, 3 nM APE1, and 10 nM DNA.

Figure S4. The N-terminal domain dependency for APE1 cleavage an F site in the hTelo G4 folds.



Reaction conditions: 50 mM potassium acetate, 20 mM Tris-acetate (pH 7.4 at 37 °C), 10 mM magnesium acetate, 1 mM DTT, 37 °C, 1 h, 3 nM WT-APE1, delta 33-APE1, or delta 61-APE1, and 10 nM DNA.

Figure S5. The APE1 cleavage yields of an F site in the hTelo G4s in Li^+-containing buffers.



Reaction conditions: 50 mM acetate salt with lithium, sodium or potassium, 20 mM Tris-acetate (pH 7.4 at 37 °C), 10 mM magnesium acetate, 1 mM DTT, 37 °C, 1 h, 3 nM APE1, and 10 nM DNA.

References

1. J. Allgayer, N. Kitsera, S. Bartelt, B. Epe, et al., *Nucleic Acids Res.*, 2016, **44**, 7267.
2. C. You, X. Dai, B. Yuan, J. Wang, et al., *Nat. Chem. Biol.*, 2012, **8**, 817.
3. A. M. Fleming, J. Zhou, S. S. Wallace and C. J. Burrows, *ACS Cent. Sci.*, 2015, **1**, 226.
4. J. Riedl, A. M. Fleming and C. J. Burrows, *J. Am. Chem. Soc.*, 2015, **138**, 491.