SUPPLEMENTARY INFORMATION

High resolution biosensor to test the capping level and integrity of mRNAs

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1. SUPPLEMENTARY METHODS

1.1. Plasmid construction for protein expression and purification

Plasmid pET28a+ (Novagen, UK) was used for protein expression. The synthetic gene encoding B4E was PCR amplified with primers 1 and 2 (Supplementary Table 1), the pET28a+ backbone was amplified with the primers 3 and 4 and both parts assembled via Gibson DNA assembly (40), to generate the plasmid pET28a-B4E-ⅰ. A second version of the expression plasmid, pET28a-B4E-ⅱ, was prepared by deleting some additional residues from N- and C-termini of B4E-ⅰ. Here primers 5 and 6 were used to amplify B4E from the original gene synthesis vector and primers 7 and 8 to amplify the pET28a+ plasmid backbone. Both fragments were joined using Gibson assembly. For the construction of the plasmid pET28a-βLac the ampR gene was amplified with primers 9 and 10 from the pET-15b plasmid and the pET28a+ backbone was amplified with primers 11 and 12. The fragments were joined using Gibson DNA assembly.

1.2. SDS-PAGE analysis of B4E

B4E was expressed and purified as described in the main manuscript. Figure S1 shows, the SDS-PAGE analysis of the various fractions obtained from his-tag purification. Elutions 2, 3 and 4 (lanes 8-10) were combined and buffer exchanged using a centrifugal concentrator with a 30 kDa MWCO (Vivaspin). 200 µL of purified B4E at 663 µg/mL were obtained (as measured by absorbance at 280 nm).
Figure S1. 10 % SDS-polyacrylamide gel showing the different fractions for the purification of B4E protein, PageRuler (Thermo) is used as a molecular weight marker. Lane 1: cell lysate; lane 2: soluble fraction; lane 3: insoluble fraction; lanes 4: flow through (fraction not bound to the resin); lanes 5 and 6: washes; lanes 7 to 11: elutions 1 to 5. The expected size of the B4E protein is 56.2 kDa.

1.2. Purification of *in vitro* transcribed (IVT) RNAs

Figure S2. 1.5 % agarose gel showing the different IVT RNAs used in this study (~600 ng of sample loaded).
Table S1. Yields and purity of the IVT RNAs used in this study.

|         | Concentration (ng/µL) | Yield (µg) | 260/230 | 260/280 |
|---------|------------------------|------------|---------|---------|
| RNA₁    | 857.6                  | 30         | 2.831   | 2.393   |
| cRNA₁   | 820                    | 28.7       | 2.228   | 2.032   |
| RNA₂    | 1047.2                 | 36.22      | 2.012   | 1.838   |
| cRNA₂   | 632.8                  | 22.15      | 2.782   | 2.401   |
| RNA₃    | 550.2                  | 35.22      | 2.454   | 2.209   |
| cRNA₃   | 781.6                  | 25.01      | 2.49    | 2.186   |
| RNAᵥ    | 3100                   | -          | 2.194   | 2.137   |
| cRNAᵥ   | 3100                   | -          | 2.471   | 2.298   |

1.3. Decapping reaction of mRNA from CHO cells

An aliquot of eukaryotic mRNA (~ 800 ng) extracted from 10⁶ CHO cells following the method described in the main text, was digested with 7.5 U of Cap-Clip™ acid phosphatase (CellScript, US) in a 50 µL reaction volume at 37 °C for three hours. Prior to the addition of the enzyme and reaction buffer, the mRNA sample was heated at 60 °C for 10 min and then snap cooled in an ice bath. The decapped sample was purified and concentrated using the RNA Clean & Concentrator kit (Zymo Research).

1.4. Total RNA extraction from CHO cells for biosensor control experiments

The total RNA from CHO cells was extracted from 10⁶ CHO cells collected on day 4 of culture produced as indicated in the materials and methods section. The RNA was purified from 0.5 mL of cell lysate with a RiboPure™ RNA purification kit (Thermo), with the addition of the Phenol:Chloroform:IAA step (omitting the use of zirconia beads) and continuing with the purification as indicated by the manufacturer.

1.5. Capping efficiency of IVT mRNAs

To estimate the percentage of capped RNAs incorporated either co-transcriptionally for cRNA₁, cRNA₂ and cRNA₃ or post-transcriptionally for mRNAᵥ, 5 µg of RNA was first digested with an RNA 5’ polyphosphatase (Lucigen, UK), leaving 5’ monophosphorylated chains on the uncapped molecules of the RNA sample. Reactions were performed in a 20 µL final volume, at 37°C for 45 min. The digested samples were purified with the RNA Clean & Concentrator kit (Zymo Research). Subsequently, 500 ng of the purified RNA was digested with Terminator™ 5’ to 3’ ribonuclease (Lucigen), to degrade the 5’ monophosphorylated RNAs. Reaction were performed in a 20 µL final volume at 30°C for 30 min for cRNA₁ and for 15 min for the other RNAs. Reaction were stopped by mixing with an equal volume of 2x RNA loading dye (NEB) in an ice bath. Samples were run on a 1.5 % agarose gel in TBE buffer at 110V (Figure S3). The percentage of capped RNA was calculated by densitometry by comparison with a control reaction lacking the ribonuclease using the image analysis software Fiji 2.
2. **SUPPLEMENTARY RESULTS**

2.1. Biosensor optimization

Several alternative assay conditions were studied to decrease non-specific binding between B4E and RNA. The pH was increased in order to decrease positive charges on the surface of the protein. However, increasing pH was not effective, since even though the non-specific binding was decreased, the B4E binding to capped RNA was also drastically reduced (Figure S8). The optimal pH for the interaction between eIF4e and m\(^{7}\)GTP is approximately 7.25, and is influenced by zwitterionic nature of the m\(^{7}\)GTP group, which binds in its cationic form to eIF4e (1).

Alternatively, altering the reducing conditions in the buffer was a more effective way to reduce non-specific binding. Figure S9 shows the response of the biosensor with and without addition of DTT to buffer B. Adding DTT considerably reduces non-specific binding, likely by preventing intermolecular B4E oligomerisation via disulfide bond formation (2) and electrostatic interaction between the positively charged region of the oxidised cysteines of the B4E and the negatively charged mRNA, pdT\(_{25}\) oligo and beads. This observation is also in agreement with the fact that eIF4E is naturally present in the cell nucleus and cytoplasm, which are reducing environments. Finally, further reduction of non-specific binding to an almost complete reduction of the background signal was achieved by the removal of non-functional amino acids on the C and N termini of the B4E (Figure S10).

2.2. Biosensor m\(^{7}\)GTP inhibition analysis

A biosensor assay using a buffer with a low level of stringency to permit non-specific binding further confirmed the specificity of the interaction between B4E and the mRNA cap during the actual biosensor assay. The presence of the m\(^{7}\)GTP is expected only to affect the B4E-cap 0 interaction, since some of the eIF4e peptides will bind the methylated nucleotide instead of capped RNA, while the controls should remain unaffected. This was confirmed experimentally, as shown in Figure S11. The presence of m\(^{7}\)GTP leads to a decrease in the signal of the biosensor when cRNA\(_{1}\) is used, while the signal due to non-specific binding observed for RNA\(_{1}\) or the pdT\(_{25}\) oligo-functionalised beads was unchanged. This further confirms the existence of a specific interaction between the cap binding region of B4E and the cap 0 of mRNA while immobilised on the beads used for the biosensor assay. In addition, this experiment suggests that the background signal originates from non-specific interactions with other regions of the protein.

3. **OLIGOS AND SEQUENCES**

Table S2. Oligos used for PCR amplification and latter Gibson assembly.

| Nº-Orientation | Target | Sequence |
|---------------|--------|----------|
| 1-Fwd         | pRSET-B4E.  Amplification of B4E gene. | CTTAAGAAGGAGATATCCATCGGGGTTCATCATCA |
| 2-Rev | | TTGTTAGCAGCCGGATCTCACTACTCGAGAACAACAAAACG |
| 3-Fwd | pET28a+ backbone amplification | GTTTTGTGTGTTCTGAGTAGTGAGATCCCGGCTGCTAACAA |
| 4-Rev | | TGATGATGAGAACCACCACACACACCACACACATGACATCCGGAACACTGTTT |
| 5-Fwd | pRSET-B4E. Amplification of B4E gene. | GGAGATATACACATGCCACCACACCACACACCACATGACATCCGGAACACTGTTT |
| 6-Rev | | TTGTTAGCAGCCGGATCTCATAACAAAAACGATTATTTGGTGG |
| 7-Fwd | pET28a+ backbone amplification | CCAAAATCCGTTTTGTTGTTTATCAGATCCCGGCTGCTAACAA |
| 8-Rev | | CGGATGCATGTGGTGGTGGTGGTGCATTGGTATATCTCCTTCTTAAAGTTAAAC |
| 9-Fwd | pET15b. Amplification of AmpR gene | CAGTGGTGTTGTTGTTGTTGTTGTTGTTGTTGTTGCTTACATCAGTGG |
| 10-Rev | | AAGAAGGAGATATACCAATGCACCCAGAAAGCCTGGTG |
| 11-Fwd | pET28a+ backbone amplification | CCTCACTGATTAGCATTGGCCACCAACCACACCACCACCTG |
| 12-Rev | | ACCACCGTTTCGGTGACATTGTTATATCTCCTTCTTAAAGTTAAAAAA ATTATTT |

- DNA sequence of BAE-v.i. Red font indicates the nucleotides removed to generate B4E-v.ii:

  ATGGCTAGCATGACTGGATCCATGCATCCGGGAAACACTGGTTAAGTTAAGATGCCGAAGATCA
  GCTGAGGTGACATGTTGTTATATGGAATCCTGGATCTGAAATCCTGGGAGAGCTTGGTTTGGTGCT
  GTCCCCGAAGAAGCCTTTCCGATGATGACCCACCTTTAAAGCTTCTGTCTGTGATGTCAGTCTTCGAG
  CCGTGGATGATGCCAGGGCGGCCGACTGGGCGGTGTTTACATCTGGCTCCAAATGCATGACCAGATCTGTT
  GAATATAGTCGCGTACCCGGAAAACACATCTGGATGACCGGCGTCTGGATGTTGAGTCGGGGAAGGTGTTG
The following are the sequences of the mRNAs transcribed in vitro and tested with the biosensor.

- **mRNA$_1$**

```
GGGGAAUUGUGAGCGGAUAACAAUUCCCCUCUAGAAAUAAUUUUGUUUAACUUUAAGAAGGA
GAUAUACCAUGCCAGCGCAAGCCAUCAUCAUCAUCAUCACAGCAGCGGCCUGGUGCCGCGCGGC
AGCCAUAUGGCUAGCAUGACUGGUGGACAGCAAAUGGGUCGCGGAUCCGAAUUCGAGCUCCG
UCGACAAGCUUGCGGCCGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

- **mRNA$_2$**

```
GGGGAAUUGUGAGCGGAUAACAAUUCCCCUCUAGAAAUAAUUUUGUUUAACUUUAAGAAGGA
GAUAUACCAUGCCAGCGCAAGCCAUCAUCAUCAUCAUCACAGCAGCGGCCUGGUGCCGCGCGGC
AGCCAUAUGGCUAGCAUGACUGGUGGACAGCAAGCGGGAUAUAACAUGAGCUGUCUUCGGUA
UCGACAAGCUUGCGGCCGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

The following are the sequences of the mRNAs transcribed in vitro and tested with the biosensor.

- **mRNA$_1$**

```
GGGGAAUUGUGAGCGGAUAACAAUUCCCCUCUAGAAAUAAUUUUGUUUAACUUUAAGAAGGA
GAUAUACCAUGCCAGCGCAAGCCAUCAUCAUCAUCAUCACAGCAGCGGCCUGGUGCCGCGCGGC
AGCCAUAUGGCUAGCAUGACUGGUGGACAGCAAAUGGGUCGCGGAUCCGAAUUCGAGCUCCG
UCGACAAGCUUGCGGCCGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

- **mRNA$_2$**

```
GGGGAAUUGUGAGCGGAUAACAAUUCCCCUCUAGAAAUAAUUUUGUUUAACUUUAAGAAGGA
GAUAUACCAUGCCAGCGCAAGCCAUCAUCAUCAUCAUCACAGCAGCGGCCUGGUGCCGCGCGGC
AGCCAUAUGGCUAGCAUGACUGGUGGACAGCAAGCGGGAUAUAACAUGAGCUGUCUUCGGUA
UCGACAAGCUUGCGGCCGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

The following are the sequences of the mRNAs transcribed in vitro and tested with the biosensor.

- **mRNA$_1$**

```
GGGGAAUUGUGAGCGGAUAACAAUUCCCCUCUAGAAAUAAUUUUGUUUAACUUUAAGAAGGA
GAUAUACCAUGCCAGCGCAAGCCAUCAUCAUCAUCAUCACAGCAGCGGCCUGGUGCCGCGCGGC
AGCCAUAUGGCUAGCAUGACUGGUGGACAGCAAAUGGGUCGCGGAUCCGAAUUCGAGCUCCG
UCGACAAGCUUGCGGCCGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

- **mRNA$_2$**

```
GGGGAAUUGUGAGCGGAUAACAAUUCCCCUCUAGAAAUAAUUUUGUUUAACUUUAAGAAGGA
GAUAUACCAUGCCAGCGCAAGCCAUCAUCAUCAUCAUCACAGCAGCGGCCUGGUGCCGCGCGGC
AGCCAUAUGGCUAGCAUGACUGGUGGACAGCAAGCGGGAUAUAACAUGAGCUGUCUUCGGUA
UCGACAAGCUUGCGGCCGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```
CUGCAUGCUUUUAUAUGCUUGGUCGGGUGAACCUUGGAGUUGAGUUAAACGA
CAAUGGCAACCUUGGAGCAGCGAAAGCUAACUGGGAUGACUGGUUGCCUGAAG
GAAAACCAUCGUGGUCAGUUCAUUCGGGCGGAAGUGUGGCUAGCUUAACUUUGGUGA
AACAUAGGUAUGGGAUGUUCGAUUCAGUACCUGUAACUGGUACUGGACUUUGAAGAUAUUGCAC
GUGCAGUUUGGGCAUUGUUUGGCAAGAGUGGAAGAAUGGCGCAACAGUGGAGCCUUAAAGGCACU
GUAGUGUUCGGCUAUCAGACUGCAGCGCCGAGGUUUCUAGUCGGCAGCCAGACUCUGCGACG
AACUGGGAUGAAGAUAACCGAUAUCGGUUCUGGUCUAAUGUAAUUGUGAGCUCAAGAAG
AUUGAAGUGCGAUGUUCGAUCGGCGCGCACAAACAAAAAAGGGGGGGAAUUGUGAGCGGAUAACAA
UUCCCCUCUAGAGCGCCGAACUGGGCCUCGGAAGGAAGGGCCCAUAUGGCUAGCAUGACUGGUGGACAGCAAAUGGGUCGCGGAUCCGAAUUCGAGCUCCGU
CUGACAAGCUUGCAGCGCCGCAAQGGGGAUGGAGAAACUUGGACAUGGAGCGGCAUAACAUUCCCUAAGAAUAAUUGUUGUUAACUUUAAGAAGGA
GAUUAACCGAAGGCGACAGCAUCUAUAUCUAACACAGCGCCGCGCGCCGCGCCGCG
AGCCAUAAUGGCUAGCAUGACUGGUGGACAGCAGAAAUGGUGUCGGCAGAUCGGGAUUCUGACUCUG
UCGACAAGCUUGCAGCGCCGAC

• 5'-UTR of mRNA1B:

TTAAAACAGCTGTGGGTTGTCACCCACCCACAGGGTGCCACTGCGGCTACTCTCTGATCTC
GGTAACCTTTGTGCGCCTTTTATACCCCCCCCCCAGTTTTGAACCTTGAAAGCAGCGCAAAACAG
ATCAATAGCAGGCATACGCTCCAGTTATGCTTGTGATCAAGCAGACTTTCTGTCTTCCGGACTGAG
ATCAAT

• mRNA1B

GGGGAAUUGUGAGCGGAUAACAAUUCUCUCUCUCAGACGCGGGCCGCAGAAUGGACAGGCG
CCCAUAUGGCUAGCAUGGUGGACAGCAGAAAUGGUGUCGGCAGAUCGGGAUUCUGACUCGUG
CGACAAGCUUGCAGCGCCGCAAQGGGGAUGGAGAAACUUGGACAUGGAGCGGCAUAACAUUCCCUAAGAAUAAUUGUUGUUAACUUUAAGAAGGA
GAUUAACCGAAGGCGACAGCAUCUAUAUCUAACACAGCGCCGCGCGCCGCGCCGCG
AGCCAUAAUGGCUAGCAUGACUGGUGGACAGCAGAAAUGGUGUCGGCAGAUCGGGAUUCUGACUCUG
UCGACAAGCUUGCAGCGCCGAC

• cRNAΔpA

GGGGAAUUGUGAGCGGAUAACAAUUCUCUCUCAGACGCGGGCCGCAGAAUGGACAGGCG
CCCAUAUGGCUAGCAUGGUGGACAGCAGAAAUGGUGUCGGCAGAUCGGGAUUCUGACUCGUG
CGACAAGCUUGCAGCGCCGCAAQGGGGAUGGAGAAACUUGGACAUGGAGCGGCAUAACAUUCCCUAAGAAUAAUUGUUGUUAACUUUAAGAAGGA
GAUUAACCGAAGGCGACAGCAUCUAUAUCUAACACAGCGCCGCGCGCCGCGCCGCG
AGCCAUAAUGGCUAGCAUGACUGGUGGACAGCAGAAAUGGUGUCGGCAGAUCGGGAUUCUGACUCUG
UCGACAAGCUUGCAGCGCCGAC
4. SUPPLEMENTARY TABLES AND FIGURES

Table S3. Parameters of the lines of best fit for the transfer functions displayed in Figure 4.

|          | Intercept | Slope (x10³) | R²     |
|----------|-----------|--------------|--------|
| cRNA1    | 0.253     | 3.78         | 0.994  |
| cRNA2    | 0.273     | 3.73         | 0.989  |
| cRNA3    | 0.246     | 3.42         | 0.984  |

Table S4. Amounts of RNAs bound to the beads during the first incubation step of the biosensor assay. RNA concentrations of the samples before and after binding to the beads were measured on a BioDrop DUO+ microspectrophotometer. The percentage of the bound mRNA that is then bound to an E4B molecule was estimated using a calibration curve of the activity of the free protein. The calibration curve is shown in Figure S4.

|          | Initial concentration (µM) | Mass bound (ng) | pmol bound | % bound  | % coverage with E4B |
|----------|---------------------------|-----------------|------------|----------|---------------------|
| cRNA₁    | 0.6                       | 346.67 ± 51.43  | 4.69 ± 0.69| 68.42 ± 10.15 | 20.65              |
| cRNA₂    | 0.6                       | 853.3 ± 68.97   | 2.60 ± 0.21| 20.70 ± 1.67 | 37.25              |
| cRNA₃    | 0.3                       | 960 ± 42.23     | 1.10 ± 0.04| 16.50 ± 0.72 | 57.74              |
| cRNA₅    | 0.3                       | 1254.67 ± 108.24| 0.60 ± 0.05| 11.11 ± 0.96 | 75.85              |

Table S5. Amounts of mRNA (ng/µL) extracted from 10⁶ CHO-S cells, for different days of cell culture.

|          | Culture 1 | Culture 2 |
|----------|-----------|-----------|
| Day 2    | 41.6      | 76.8      |
| Day 4    | 40.0      | 42.4      |
| Day 4 (extraction 2) | 53.2 | 41.7      |
| Day 8    | 3.2       | 3.4       |
Figure S3. Estimation of the capped fractions of the mRNAs used in this study. Products of the 5' to 3' ribonuclease digestion of co-transcriptionally capped RNAs (RNA₁, RNA₂ and RNA₃) and post-transcriptionally capped RNAᵥ. The fractions of capped mRNA estimated by densitometry analyses comparing the digested samples with the reference ones are: cRNA₁ 93.3%, cRNA₂ 85.9%, cRNA₃ 82.5 % and cRNAᵥ 82.6%

Figure S4. Calibration curve for free B4E. Spectroscopic measurement of nitrocefin hydrolysis at different concentrations of free B4E. The calibration curve (insert) is built by plotting the response at 1200 s versus the amount of free protein in the reaction.
Figure S5. Biosensor assay with m^7GTP-functionalised resin loaded with B4E (a) and β-lactamase (c) and the elution fractions after addition of m^7GTP (b and d, respectively). Protein was bound to the resin and washed four times with buffer B (solid lines on “a” and “c”, labelled as “Before elutions”). Subsequently, three elutions were performed using m^7GTP as the elution agent. Dashed lines in “a” and “c” correspond to assays performed with the resin post-elution. Figures “b” and “d” correspond to assays performed on the elution fraction. For the colorimetric assay, 5 µL of each elution fraction or 5 µL of resin resuspended in 100 µL of buffer B were used. The presence of protein (either bound to the resin or free in the elution fractions) can be detected by the hydrolysis of nitrocefin and the consequent increase in absorbance at 492 nm.

Figure S6. Biosensor response for capped mRNA (cRNA) in the presence some of the components of the T7 RNA polymerase reaction. cRNA_1 at 0.6 µM was mixed with 1 µg/µL linearized DNA template and 1.5 µM of each NTP to simulate a reaction before purification and then mixed with the beads to perform the biosensor assay.
Figure S7. Biosensor assays analysing the effect of the presence of a boxB RNA hairpin in the 5' region of cRNA1 (cRNA1B) and the absence of polyA tail on the mRNA (cRNA1ΔpA). Responses are compared to that obtained for standard cRNA1. The RNA hairpin does not show a detrimental effect on the signal of the biosensor, while the absence of the polyA tail abolishes the signal, as expected from its inability to bind to the oligo dT-functionalised beads.

Figure S8. Biosensor assay for RNA1 analysing the effect of the pH. The response of the sensor using buffer B at pH 7.4 (solid line) and pH 8 (dashed line). The background signal was almost eliminated at pH 8; However, the increase in pH also heavily reduces the specific interaction between B4E and cRNA1.
Figure S9. Biosensor assay analysing the effect of the addition of the reducing agent DTT to buffer B. DTT decreases the non-specific binding between B4E and uncapped RNA and beads. The signal for capped RNA was also reduced, likely due to screening out the non-specific interaction in this case as well.

Figure S10. Biosensor assays comparing versions I and II of the protein (solid and dashed lines, respectively, see supplementary tables for information about the sequence). Standard conditions (those in Figure 3) were used. Both versions of the protein showed a similar affinity towards capped mRNA, while the non-specific binding was significantly reduced for version II.
Figure S11. Biosensor assay for RNA\textsubscript{1} using buffer B' (HEPES 33 mM, KCl 66 mM, 0.1 % BSA, 0.1 % Tween20, pH 7.4). Buffer B' does not include DTT, therefore the non-specific binding between B4E and RNA and beads is higher. Higher non-specific binding allows the observation of the effect of the addition of 10 µM m\textsuperscript{7}GTP to the buffer (dashed line) compared to Buffer B' lacking m\textsuperscript{7}GTP (solid line). Free m\textsuperscript{7}GTP only reduces the signal for the capped RNA (cRNA\textsubscript{1}), highlighting the non-specific nature of the interaction between B4E and uncapped RNAs and beads in buffers lacking DTT.

Figure S12. Biosensor assays of different RNAs produced in vivo. mRNA extracted from 10\textsuperscript{6} CHO cells collected at the day 4 of culture treated with the decapping enzyme Cap-Clip acid phosphatase (blue dashed line) and a mock reaction without the addition of the enzyme (blue straight line). The biosensor was also tested with 50 ng/µL samples of tRNA (grey) total RNA extracted from CHO cells under the same conditions (black). Averages and standard deviations of three experimental replicates are shown.
Figure S13. Visual inspection of the biosensor assay for cRNA (Figure 3A) after 1.5 hours of reaction. Increasing percentage of capped mRNA from left to right. In each column three experimental replicates of the colorimetric assay are shown.

5. SUPPLEMENTARY REFERENCES

1. Niedzwiecka, A., Marcotrigiano, J., Stepinski, J., Jankowska-Anyszka, M., Wyslouch-Cieszynska, A., Dadlez, M., Gingras, A. C., Mak, P., Darzynkiewicz, E., Sonenberg, N., et al. (2002) Biophysical studies of eIF4E cap-binding protein: Recognition of mRNA 5’ cap structure and synthetic fragments of eIF4G and 4E-BP1 proteins. J. Mol. Biol., 319, 615–635.

2. Alcock, L. J., Perkins, M. V. and Chalker, J. M. (2018) Chemical methods for mapping cysteine oxidation. Chem. Soc. Rev., 47, 231–268.