O6-Methyguanosine leads to position-dependent effects on ribosome speed and fidelity

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Supplemental Figure Legends

Figure S1. aa-tRNA reactivity profile for GUG and m^6GUG Val

A Schematic of initiation complexes containing [^{35}S]-fMet, 70S ribosomes, and intact or m^6G mRNAs. Experimental m^6G residue is highlighted in red.

B Schematic of cognate and predicted near-cognate aa-tRNAs with respective anticodon stem loops.

C Phosphorimage of resolved reactants and products. The cognate aa-tRNA reaction is shown in green, while the predicted m^6G:U near-cognate is orange.

D Quantification of the end-point dipeptide production for the most abundant cognate and near-cognate reactions.

Figure S2. aa-tRNA reactivity profile for GAA and m^6GAA Glu

A Schematic of initiation complexes containing [^{35}S]-fMet, 70S ribosomes, and intact or m^6G mRNAs. Experimental m^6G residue is highlighted in red.

B Schematic of cognate and predicted near-cognate aa-tRNAs with respective anticodon stem loops.

C Phosphorimage of resolved reactants and products. The cognate aa-tRNA reaction is shown in green, while the predicted m^6G:U near-cognate is orange.

D Quantification of the end-point dipeptide production for the most abundant cognate and near-cognate reactions.
Figure S3. aa-tRNA reactivity profile for GGC and m^6GGC Gly

A Schematic of initiation complexes containing [^{35}S]-fMet, 70S ribosomes, and intact or m^6G mRNAs. Experimental m^6G residue is highlighted in red.

B Schematic of cognate and predicted near-cognate aa-tRNAs with respective anticodon stem loops.

C Phosphorimage of resolved reactants and products. The cognate aa-tRNA reaction is shown in green, while the predicted m^6G:U near-cognate is orange.

D Quantification of the end-point dipeptide production for the most abundant cognate and near-cognate reactions.

Figure S4. aa-tRNA reactivity profile for CGC and Cm^6GC Arg

A Schematic of initiation complexes containing [^{35}S]-fMet, 70S ribosomes, and intact or m^6G mRNAs. Experimental m^6G residue is highlighted in red.

B Schematic of cognate and predicted near-cognate aa-tRNAs with respective anticodon stem loops.

C Phosphorimage of resolved reactants and products. The cognate aa-tRNA reaction is shown in green, while the predicted m^6G:U near-cognate is orange.

D Quantification of the end-point dipeptide production for the most abundant cognate and near-cognate reactions.

Figure S5. aa-tRNA reactivity profile for AGC and A m^6GC Ser

A Schematic of initiation complexes containing [^{35}S]-fMet, 70S ribosomes, and intact or m^6G mRNAs. Experimental m^6G residue is highlighted in red.

B Schematic of cognate and predicted near-cognate aa-tRNAs with respective anticodon stem loops.

C Phosphorimage of resolved reactants and products. The cognate aa-tRNA reaction is shown in green, while the predicted m^6G:U near-cognate is orange.
D Quantification of the end-point dipeptide production for the most abundant cognate and near-cognate reactions.

**Figure S6. aa-tRNA reactivity profile for AUG and AUm^6^G Met**

A Schematic of initiation complexes containing [^35^S]-fMet, 70S ribosomes, and intact or m^6^G mRNAs. Experimental m^6^G residue is highlighted in red.

B Schematic of cognate and predicted near-cognate aa-tRNAs with respective anticodon stem loops.

C Phosphorimage of resolved reactants and products. The cognate aa-tRNA reaction is shown in green, while the predicted m^6^G:U near-cognate is orange.

D Quantification of the end-point dipeptide production for the most abundant cognate and near-cognate reactions.

**Figure S7. aa-tRNA and release factor reactivity profile for UGG and UGm^6^G Trp**

A Schematic of initiation complexes containing [^35^S]-fMet, 70S ribosomes, and intact or m^6^G mRNAs. Experimental m^6^G residue is highlighted in red.

B Schematic of cognate and predicted near-cognate aa-tRNAs with respective anticodon stem loops.

C Phosphorimage of resolved reactants and products. The cognate aa-tRNA reaction is shown in green, while the predicted m^6^G:U near-cognate is orange.

D Quantification of the end-point dipeptide production for the most abundant cognate and near-cognate reactions.

**Figure S8. m6G alters proofreading during tRNA selection**

A, B and C End-point dipeptide formation efficiencies for initiation complexes with m^6^G at the first, second, or third codon position, respectively. Codons and predicted anticodons are shown
below the graph with the experimental residue highlighted in red. Error bars represent the standard error of curve fitting from a single representative time course. 23 of the 28 time courses were performed in at least duplicates with less than 10% variability between samples.

**Figure S9.** Formaldehyde agarose electrophoresis of the different mRNA reporters used to follow the construction of the reporters and the addition of the polyA.

**Figure S10.** aa-tRNA and release factor reactivity profile for UGA and Um\(^6\)GA Stop

A Schematic of initiation complexes containing \[^{35}\text{S}]\text{-fMet}, 70S ribosomes, and intact or m\(^6\)G mRNAs. Experimental m\(^6\)G residue is highlighted in red.

B Schematic of cognate and predicted near-cognate release factor and aa-tRNA with respective anticodon stem loops.

C Phosphorimage of resolved reactants and products. The cognate aa-tRNA reaction is shown in green, while the predicted m\(^6\)G:U near-cognate is orange.

D Quantification of the end-point dipeptide production for the most abundant cognate and near-cognate reactions.

**Figure S11.** aa-tRNA reactivity profile for GAC and Gm\(^6\)AC Asp

A Schematic of initiation complexes containing \[^{35}\text{S}]\text{-fMet}, 70S ribosomes, and intact or m\(^6\)G mRNAs. Experimental m\(^6\)G residue is highlighted in red.

B Schematic of cognate aa-tRNA with respective anticodon stem loop.

C Phosphorimage of resolved reactants and products. The cognate aa-tRNA reaction is shown in green.

D Quantification of the end-point dipeptide production for the most abundant cognate and near-cognate reactions.
Hudson and Zaher, Figure S1

A
Initiation complexes

Guanosine complex

O6-methylguanosine complex

B
Ternary complexes

Val-tRNA^{Val}

GTP

EFTu

Met-tRNA^{Met}

C

Percent Reacted

Val

Met

Ile

Gly  Glu  Ile  His  Leu  Gln  Ala  Asp  Thr  Trp

D

Percent Reacted

Val

Met

Ile

3'-CAV-5'  3'-UAC-5'  3'-UA^hC-5'  5'-GUG-3'  5'-GUG-3'  5'-GU G-3'  Codon

Anticodon
A

Initiation complexes

Guanosine complex

O6-methylguanosine complex

B

Ternary complexes

GTP

EFTu

Gly-tRNA\textsuperscript{Gly}

GTP

CU G

Ser-tRNA\textsuperscript{Ser}

C

\begin{tabular}{cccccccccccccccc}
  Gly & Glu & Ile & His & Leu & Gln & Ala & Asp & Thr & Trp & \text{---} \\
  - & + & + & - & + & + & - & + & + & + & + \\
\end{tabular}

Dipeptide yield

Percent

Gly & Ser & Asp & Thr & Lys & Phe & Tyr & Asn & Pro & Ser & Met & Arg & Val & Cys & \text{---} \\
0 & 10 & 20 & 30 & 40 & 50 & + & + & + & + & + & + & + & + & + \\

D

\begin{tabular}{|c|c|c|c|c|}
  \hline
  Anticodon & Codon & G & m\textsuperscript{6}G \\
  \hline
  3'-GGC-5' & 5'-GGC-3' & \text{---} & + \\
  3'-UGG-5' & 5'-GGC-3' & + & \text{---} \\
  3'-UGC-5' & 5'-GGC-3' & + & \text{---} \\
  \hline
\end{tabular}

Hudson and Zaher, Figure S3
A. Initiation complexes

B. Ternary complexes

C. Ternary complex

D. Dipeptide Yield Percent

Hudson and Zaher, Figure S4
A  
Initiation complexes

- MeUAC
- AUG
- AGC
- E
- P
- A

Guanosine complex

- O6-methylguanosine complex

B  
Ternary complexes

- GTP
- EFTu
- Ser-tRNA^Ser

- GTP
- EFTu
- Asn-tRNA^Asn

C  
Hudson and Zaher, Figure S5

D  
Hudson and Zaher, Figure S5

- Percent Reacted

- Ser

- Asn

- 3'-UCG-5'
- 5'-AGC-3'

- 3'-UUG-5'
- 5'-AGC-3'

- Codon

- Anticodon

- fMet

- dipeptides

- origin

- fMet

- dipeptides

- origin
Initiation complexes

Guanosine complex

O6-methylguanosine complex

Ternary complexes

Met-tRNA\textsubscript{Met}

GTP

EFTu

M
tRNA

Ile-tRNA\textsubscript{Ile}

GTP

fMet
dipeptides

origin

Dipeptide yield Percent

Met

Ile

Leu

Ser

Val

3'-UAC-5

3'-UAG-5

3'-UGA-5

3'-CGA-5

3'-GAC-5

Anticodon

Codon

Hudson and Zaher, Figure S6
| Cap, polyA ligated | Stop | HA-Flag | A253G | HA-Flag +1 | HA-Flag +2 | A253m6G | A253m6G +1 | A253m6G +2 |
|-------------------|------|---------|-------|-----------|-----------|---------|------------|------------|
|                   | -    | +       | -     | -         | -         | -       | +          | +          |
|                   | -    | -       | -     | -         | -         | -       | -          | -          |
|                   | -    | -       | -     | -         | -         | +       | -          | +          |
|                   | -    | -       | -     | -         | +         | +       | +          | +          |

Hudson and Zaher, Figure S9
A
Initiation complexes

B
Release Factors and ternary complexes

C

D

Percent Reacted

RF2 RF1

0 20 40 60 80 100

RF2 RF1

G m6G

RF2 RF1

GTP

C Trp-tRNATrp

EFTu A C

Hudson and Zaher, Figure S10
