

## **O6-Methylguanosine 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<sup>6</sup>GUG Val**

A Schematic of initiation complexes containing [<sup>35</sup>S]-fMet, 70S ribosomes, and intact or m<sup>6</sup>G mRNAs. Experimental m<sup>6</sup>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<sup>6</sup>G: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<sup>6</sup>GAA Glu**

A Schematic of initiation complexes containing [<sup>35</sup>S]-fMet, 70S ribosomes, and intact or m<sup>6</sup>G mRNAs. Experimental m<sup>6</sup>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<sup>6</sup>G: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<sup>6</sup>GGC Gly**

- A Schematic of initiation complexes containing [<sup>35</sup>S]-fMet, 70S ribosomes, and intact or m<sup>6</sup>G mRNAs. Experimental m<sup>6</sup>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<sup>6</sup>G: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<sup>6</sup>GC Arg**

- A Schematic of initiation complexes containing [<sup>35</sup>S]-fMet, 70S ribosomes, and intact or m<sup>6</sup>G mRNAs. Experimental m<sup>6</sup>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<sup>6</sup>G: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 Am<sup>6</sup>GC Ser**

- A Schematic of initiation complexes containing [<sup>35</sup>S]-fMet, 70S ribosomes, and intact or m<sup>6</sup>G mRNAs. Experimental m<sup>6</sup>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<sup>6</sup>G: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<sup>6</sup>G Met**

A Schematic of initiation complexes containing [<sup>35</sup>S]-fMet, 70S ribosomes, and intact or m<sup>6</sup>G mRNAs. Experimental m<sup>6</sup>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<sup>6</sup>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<sup>6</sup>G Trp**

A Schematic of initiation complexes containing [<sup>35</sup>S]-fMet, 70S ribosomes, and intact or m<sup>6</sup>G mRNAs. Experimental m<sup>6</sup>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<sup>6</sup>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. m<sup>6</sup>G alters proofreading during tRNA selection**

A, B and C End-point dipeptide formation efficiencies for initiation complexes with m<sup>6</sup>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<sup>6</sup>GA Stop**

A Schematic of initiation complexes containing [<sup>35</sup>S]-fMet, 70S ribosomes, and intact or m<sup>6</sup>G mRNAs. Experimental m<sup>6</sup>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<sup>6</sup>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<sup>6</sup>AC Asp**

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

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

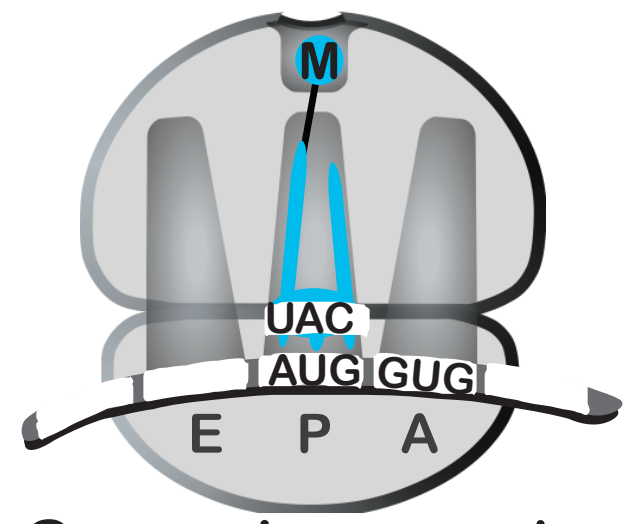
C Phosphorimage of resolved reactants and products. The cognate aa-tRNA reaction is shown in green.

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

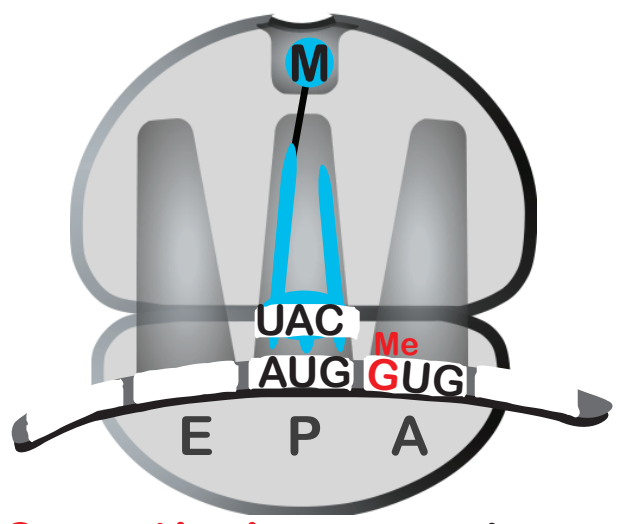


A

Initiation complexes



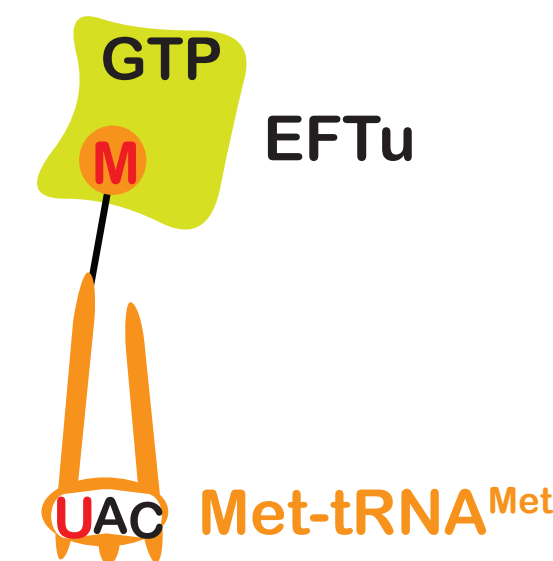
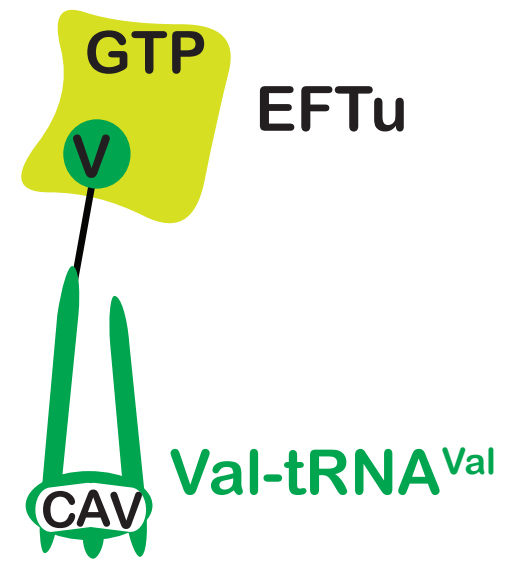
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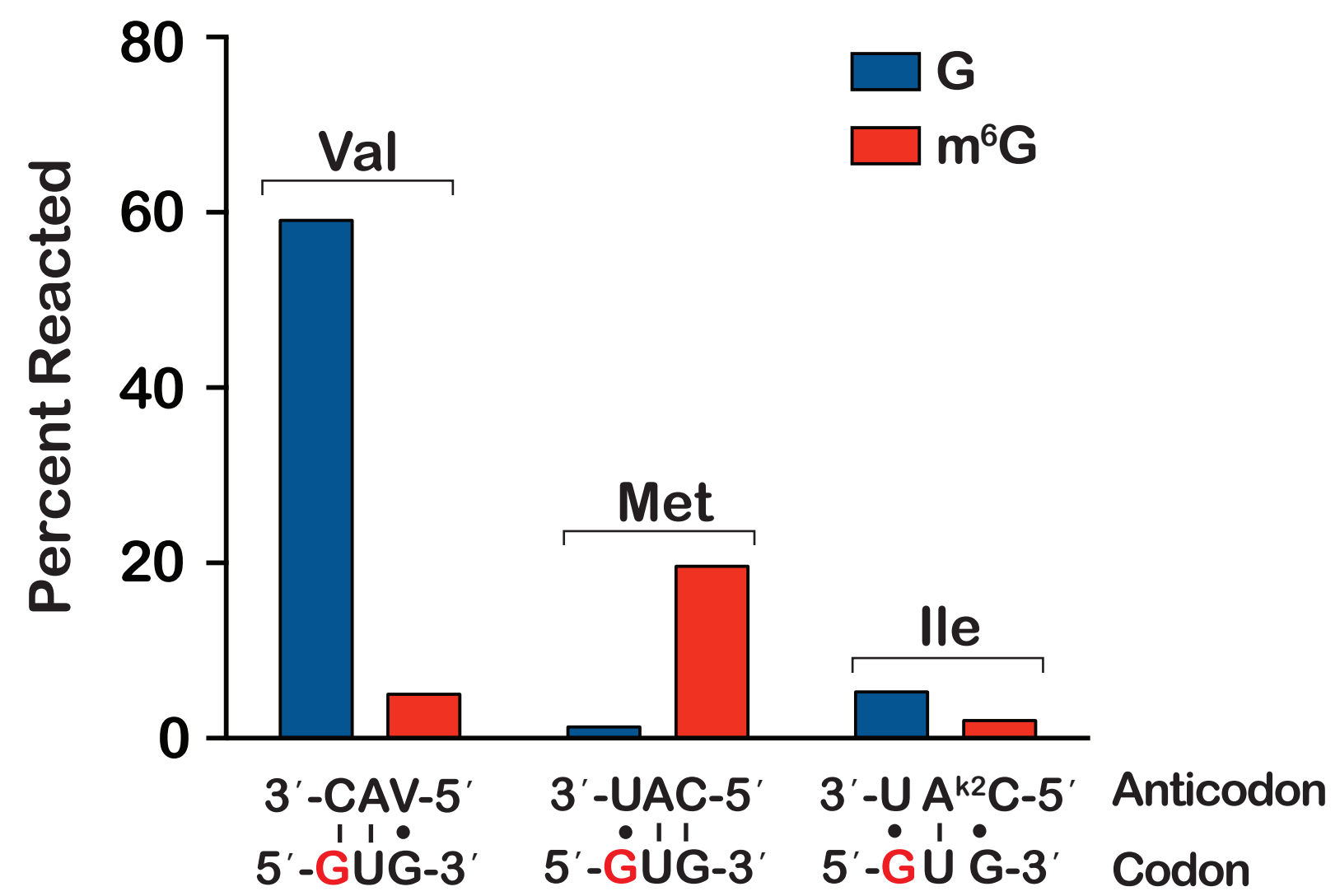
O6-methylguanosine complex

B

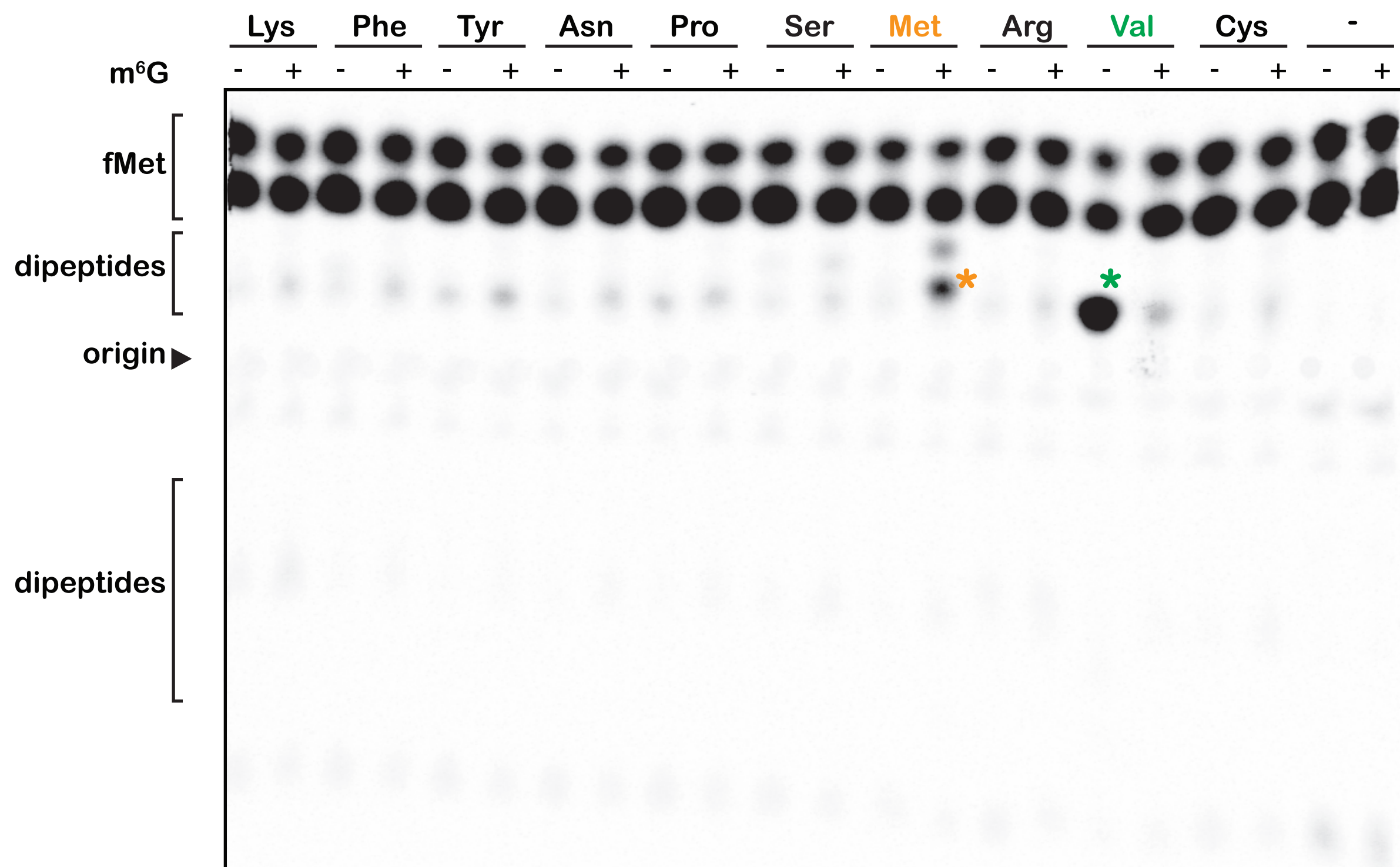
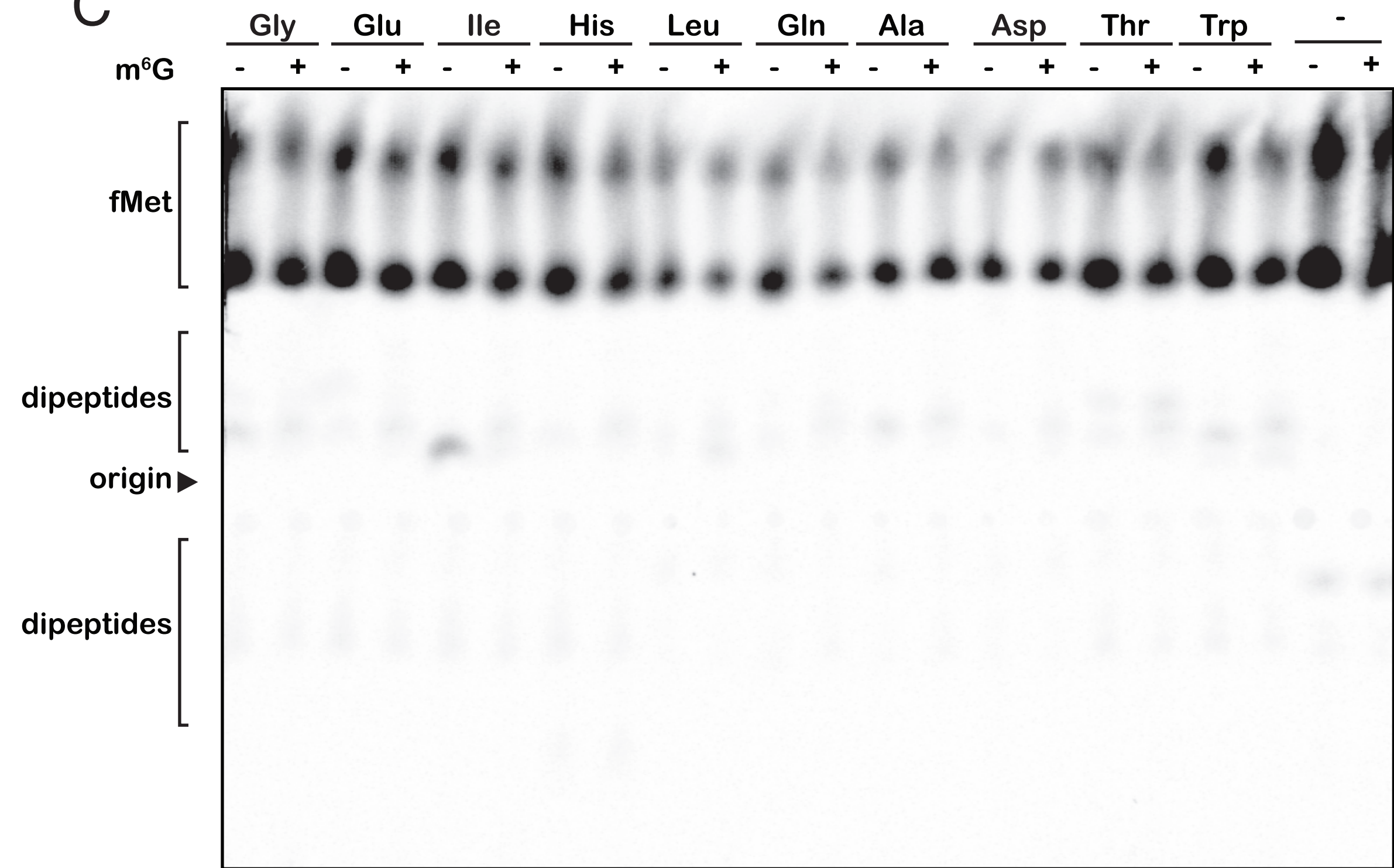
Ternary complexes



D



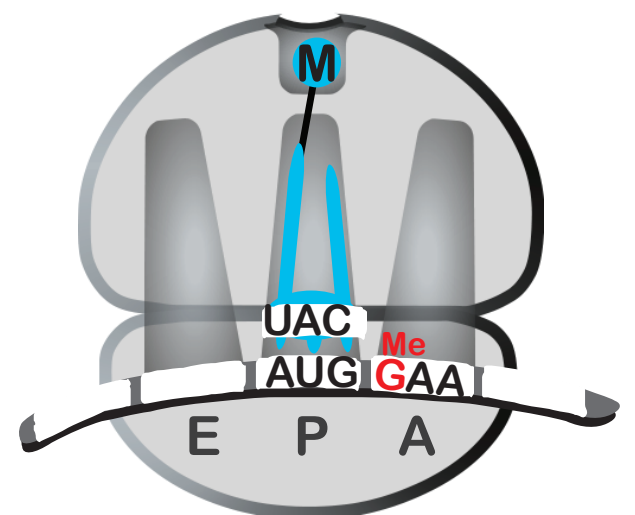
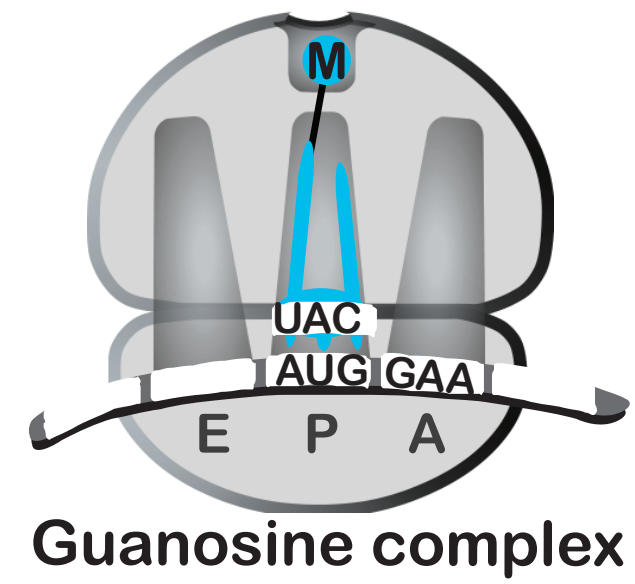
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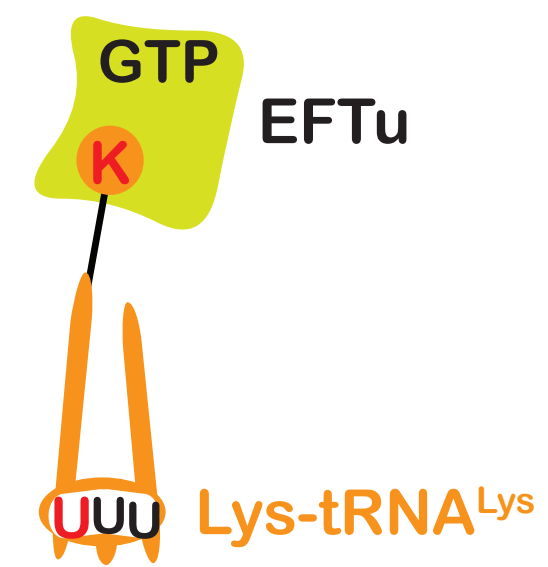
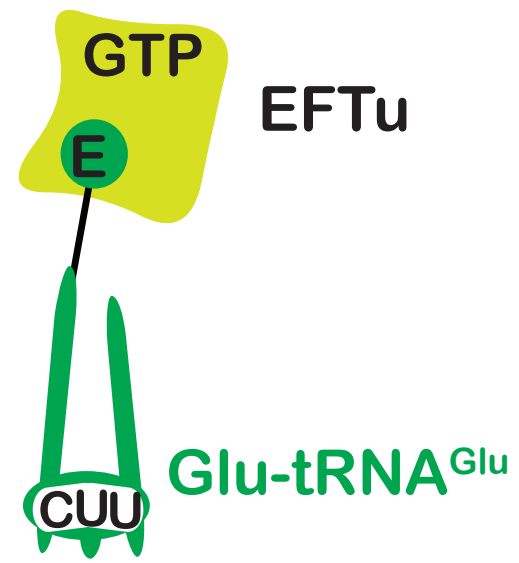
A

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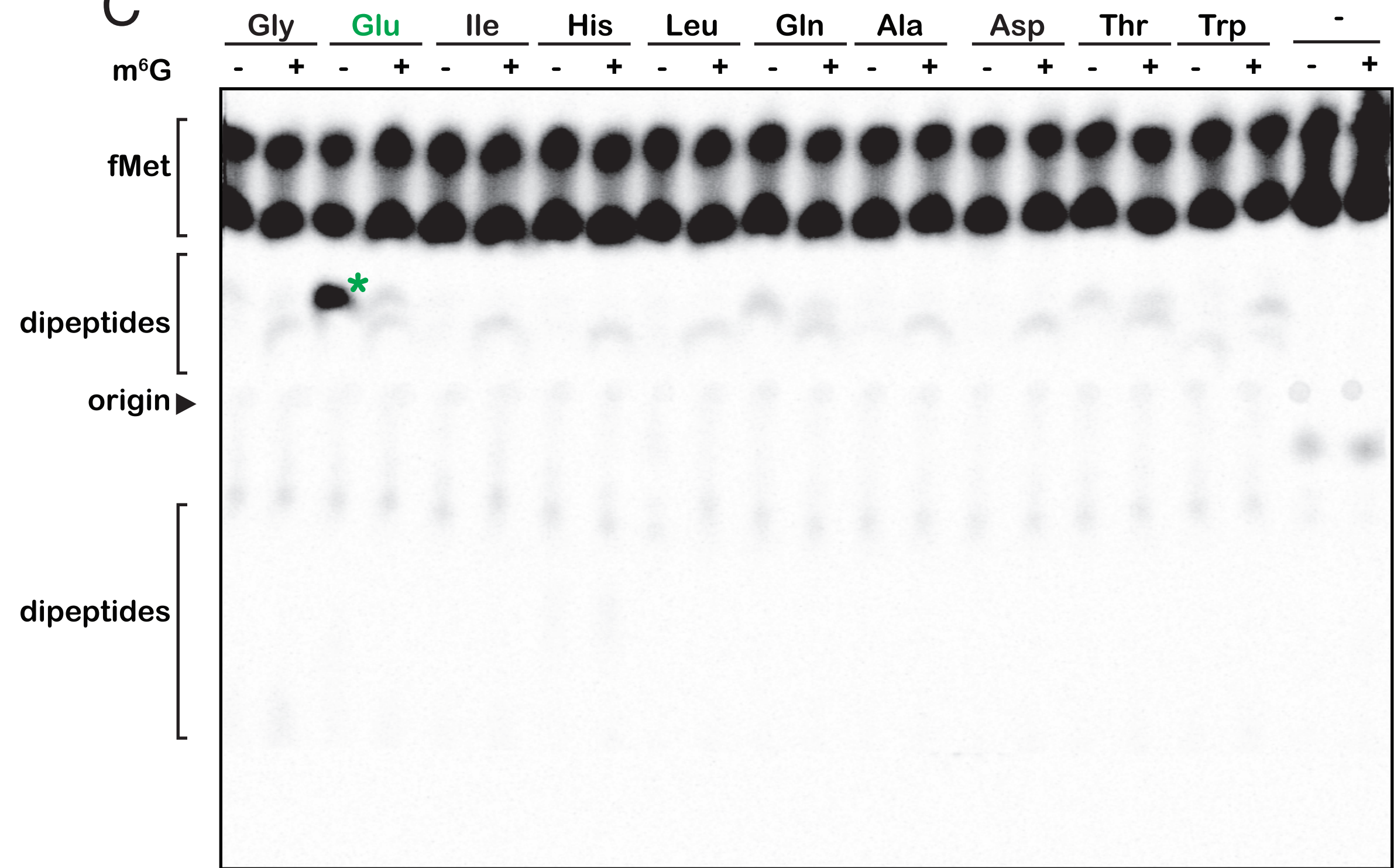


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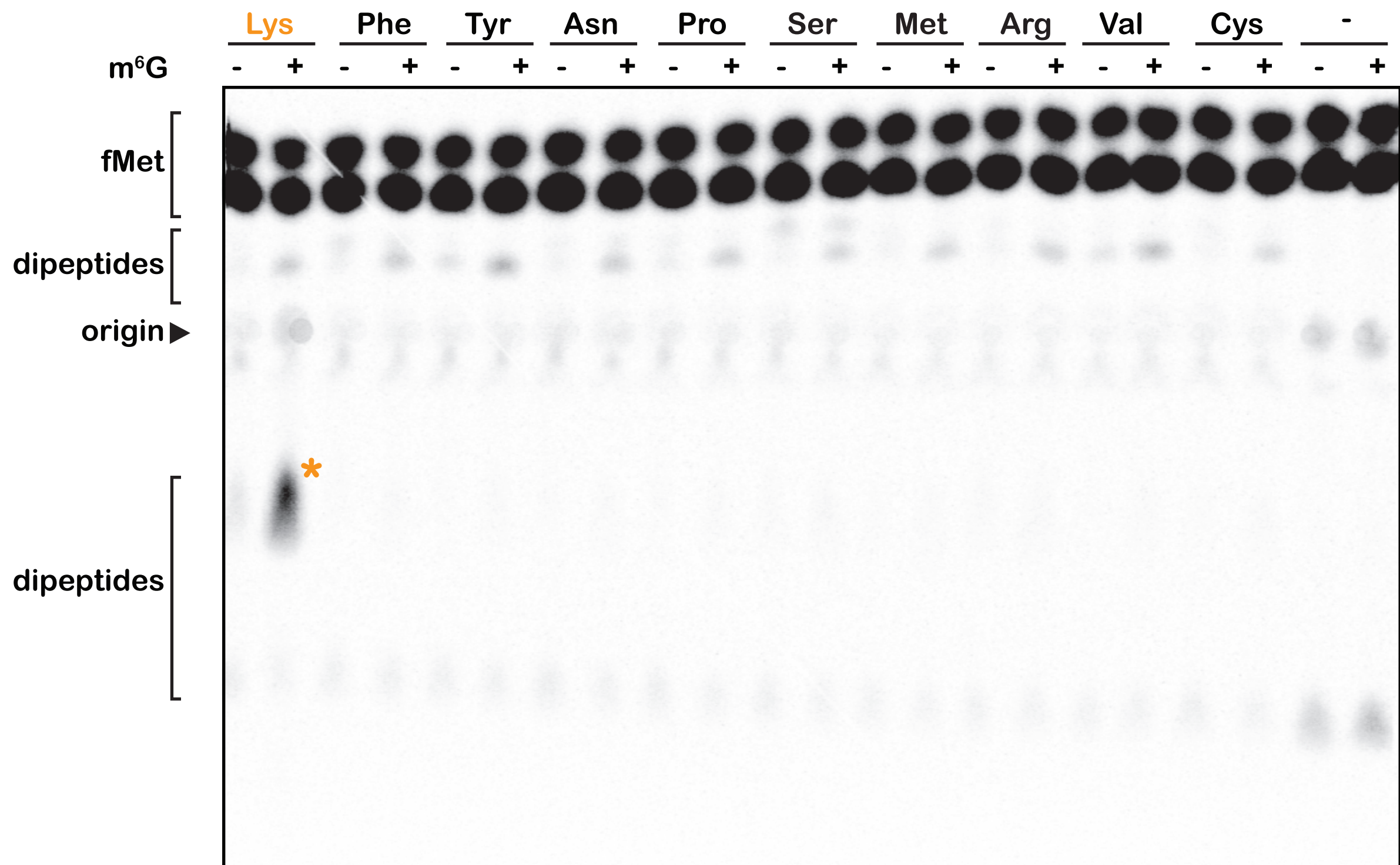
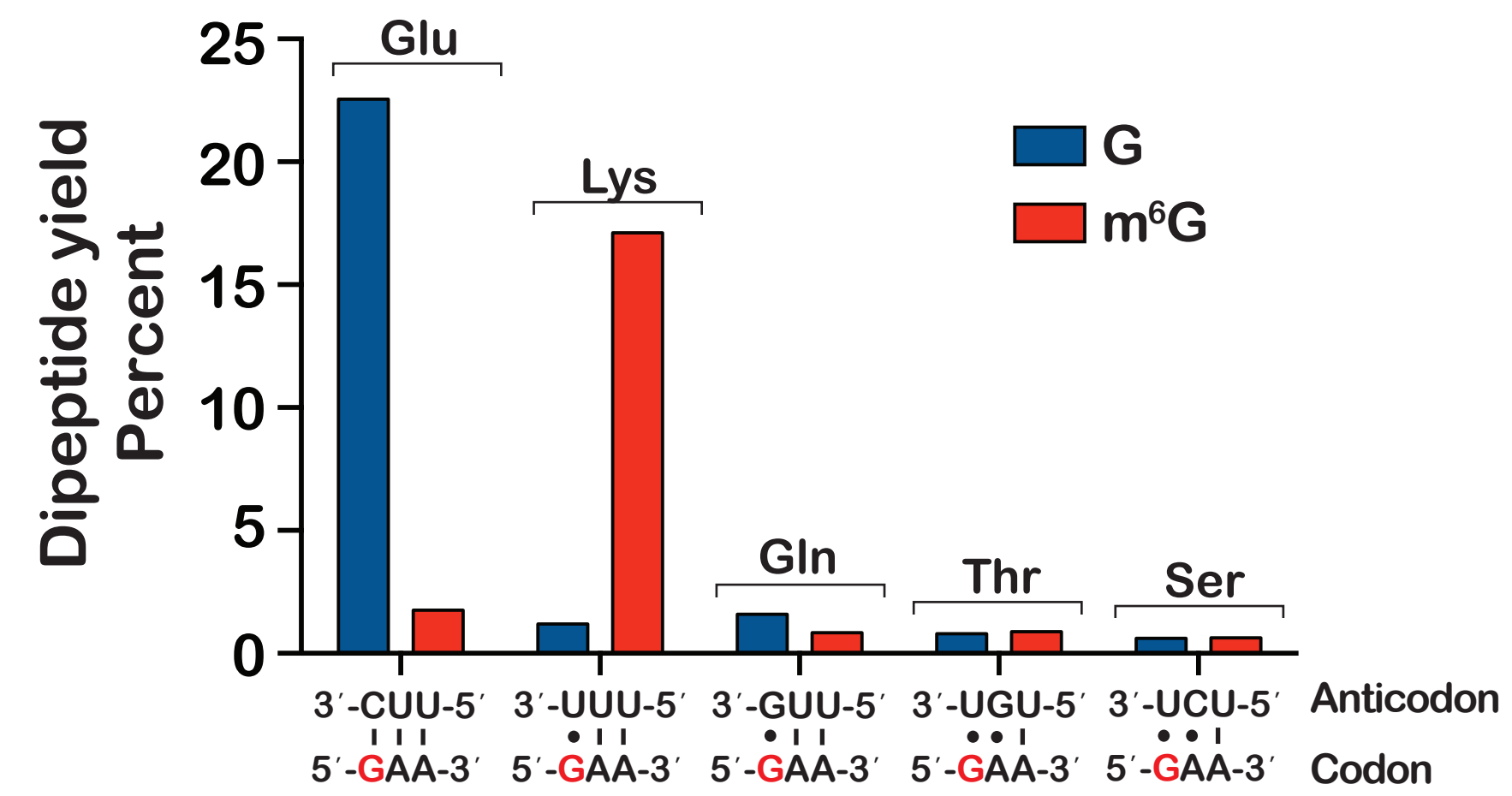
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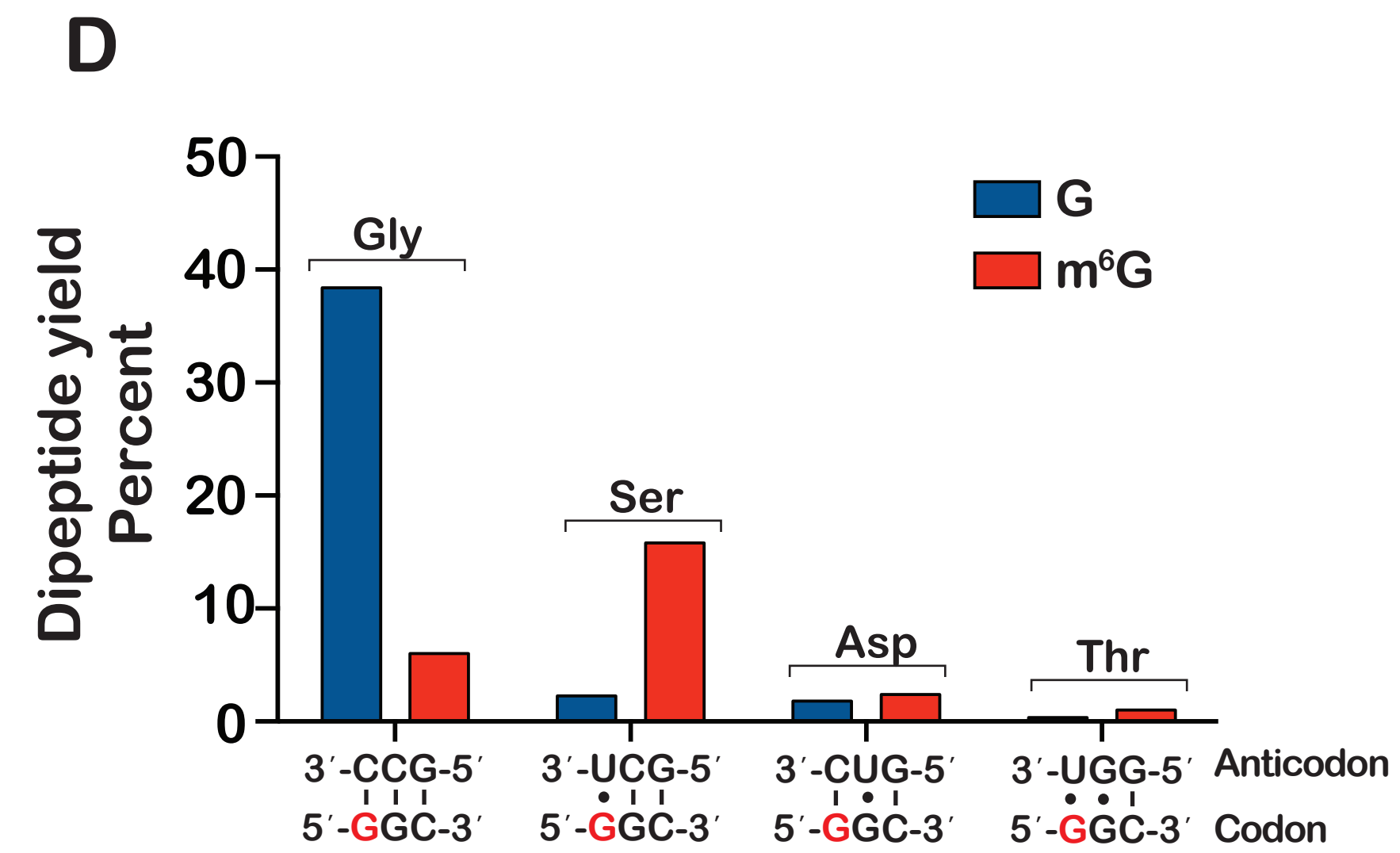
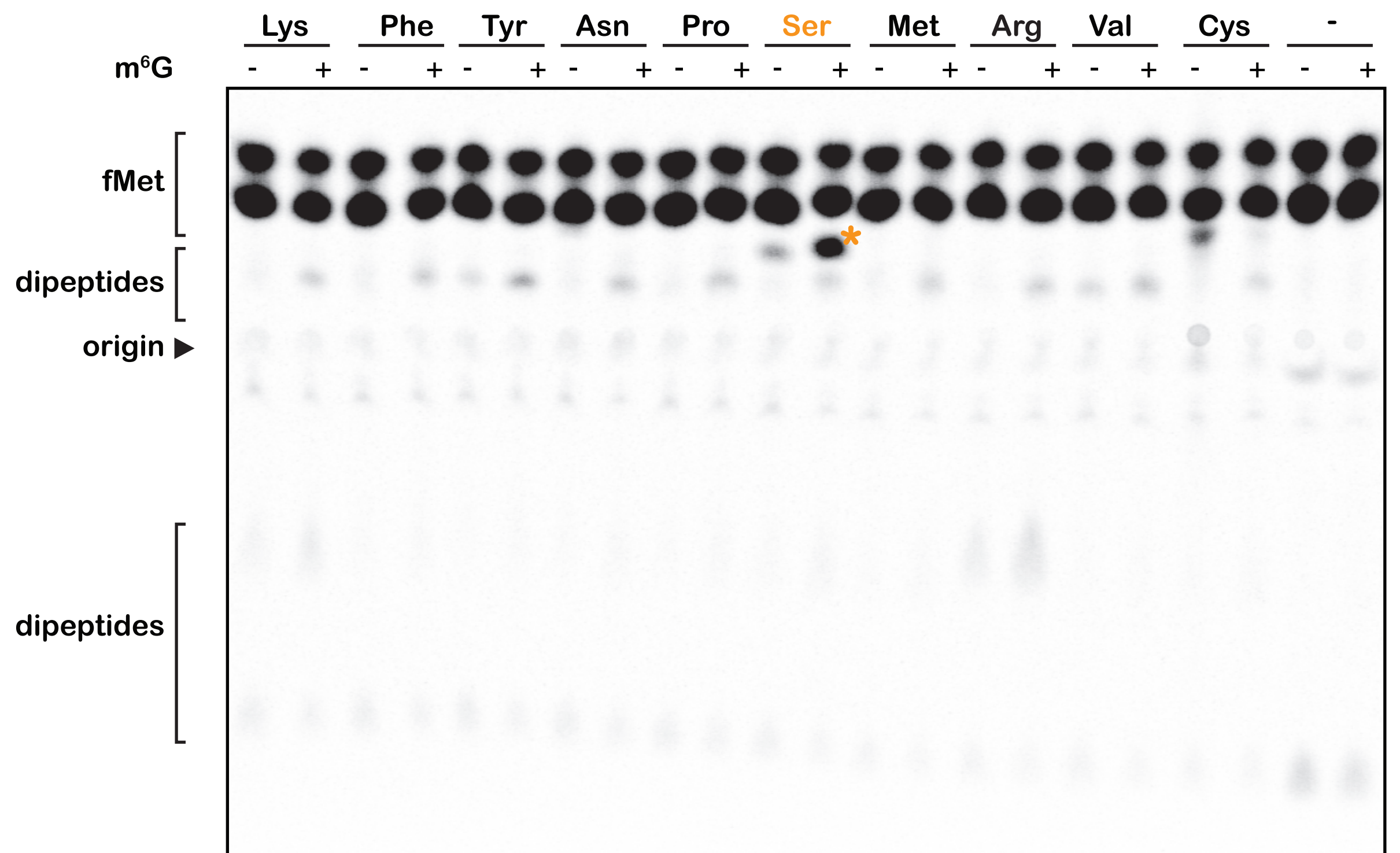
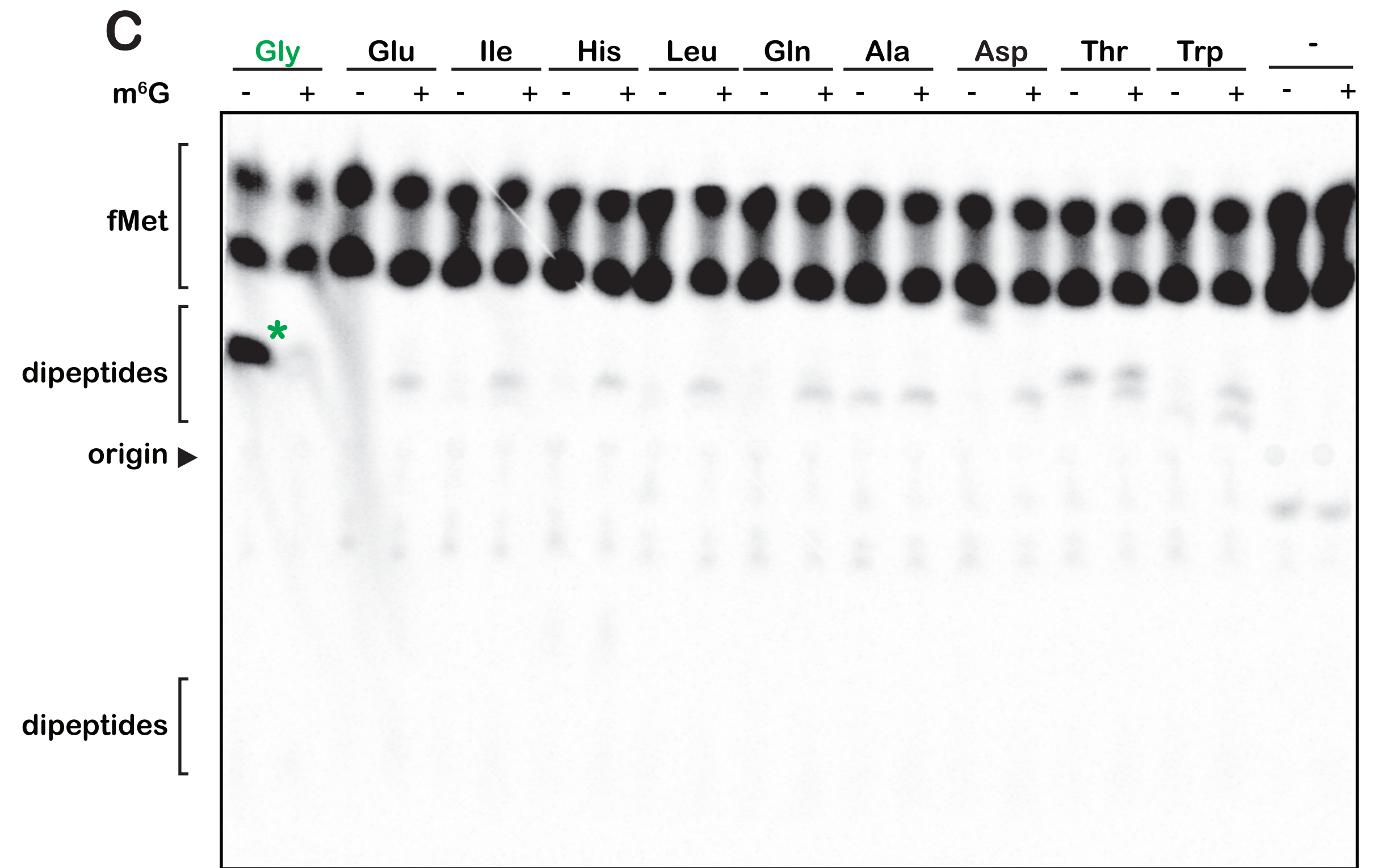
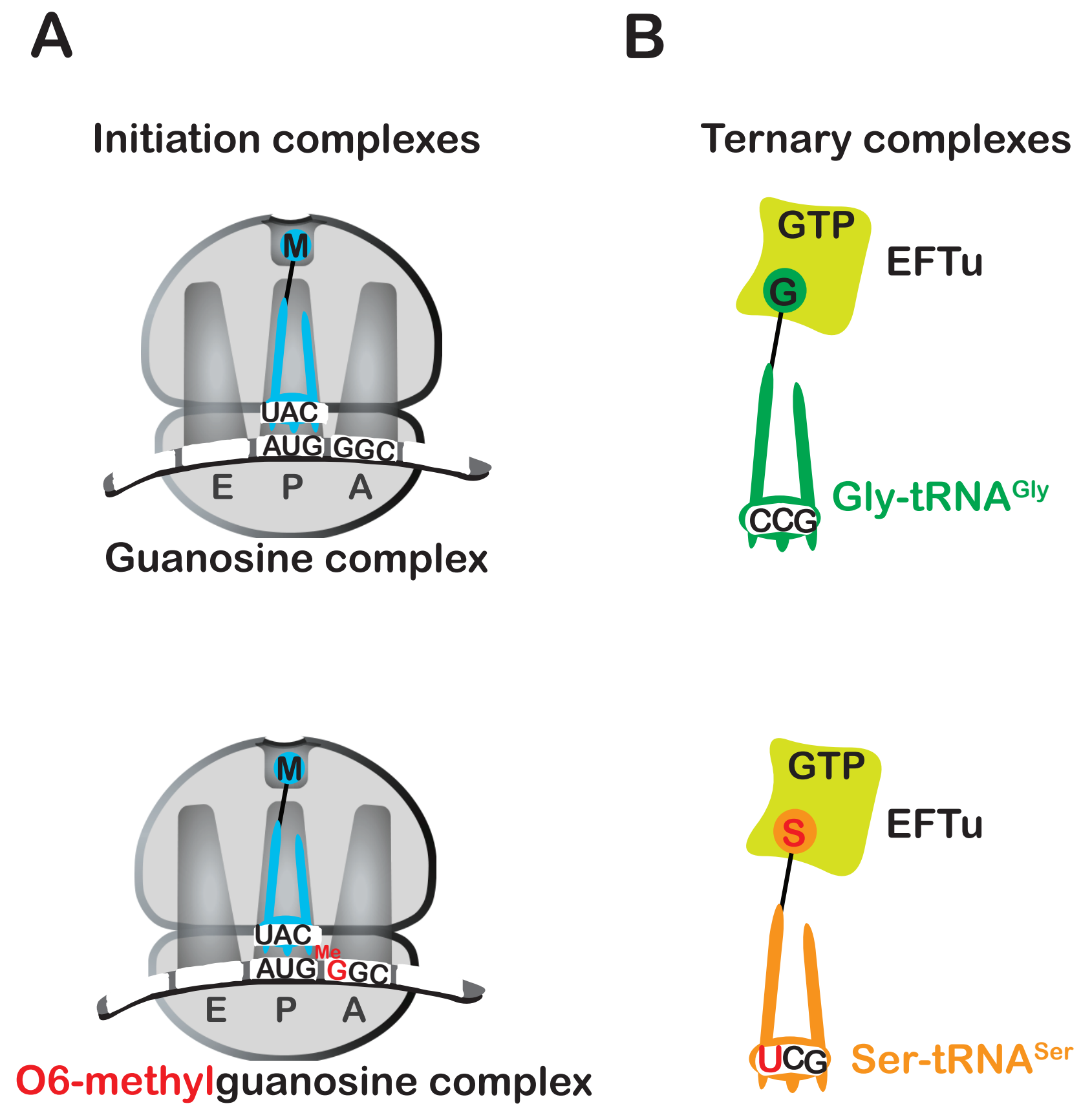
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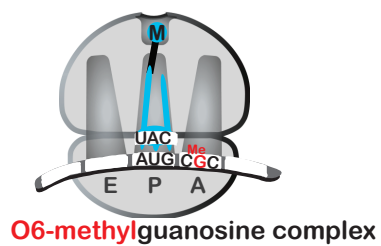
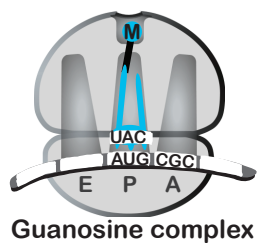






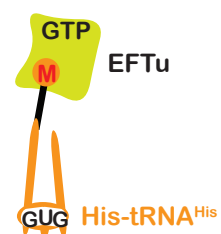
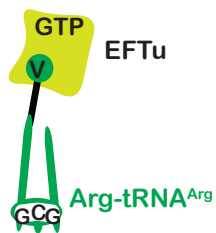
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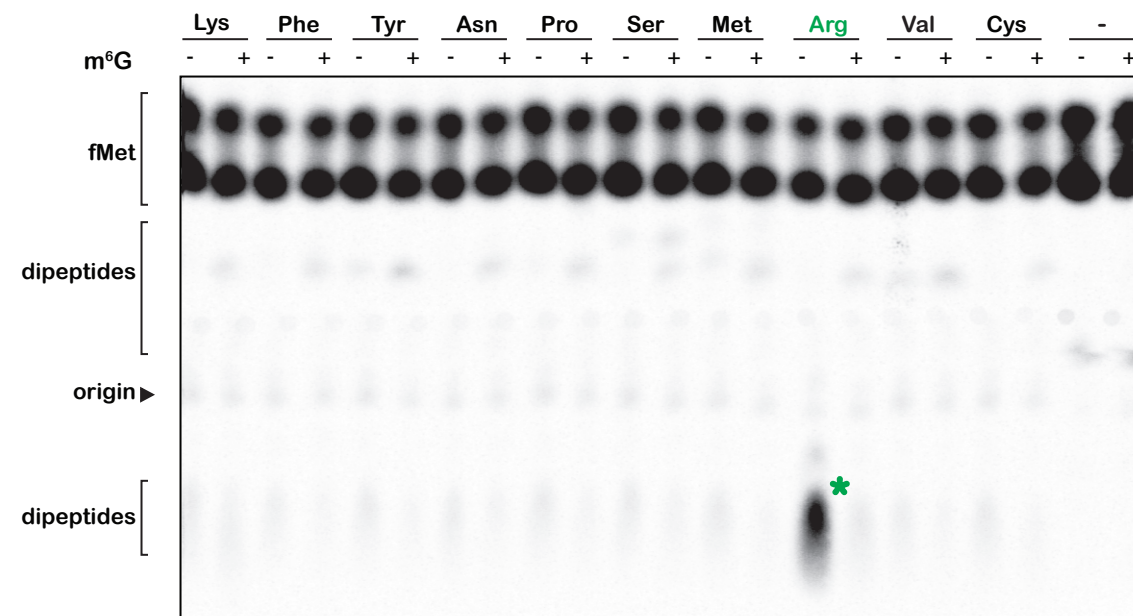
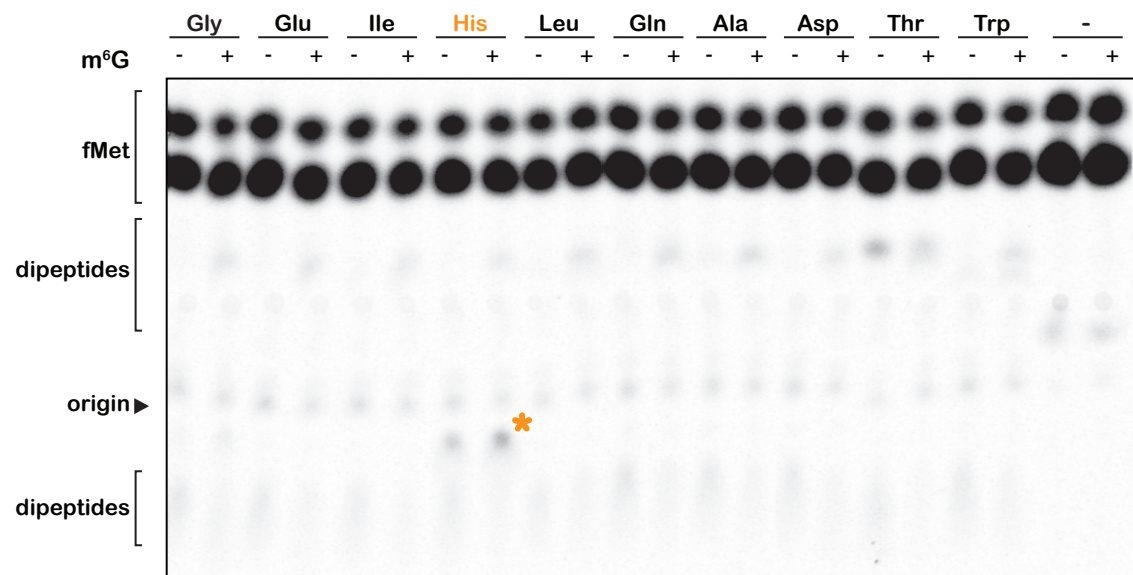


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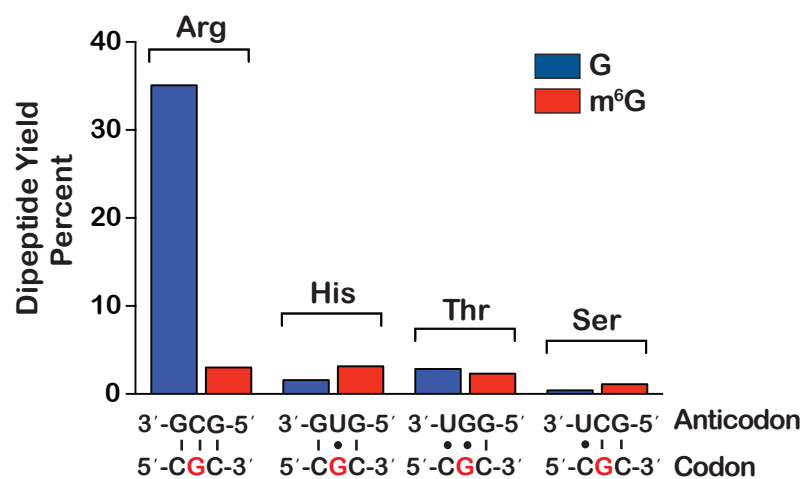
Ternary complexes



C



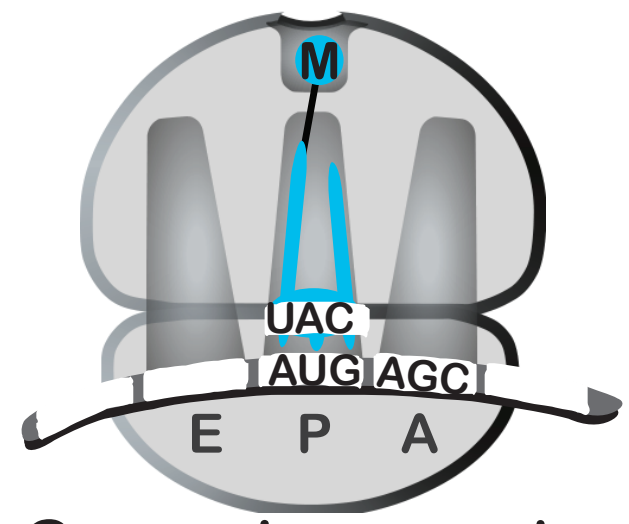
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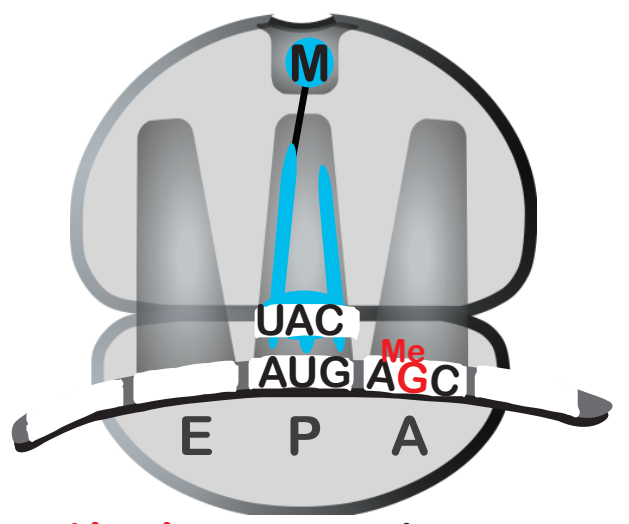


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Initiation complexes



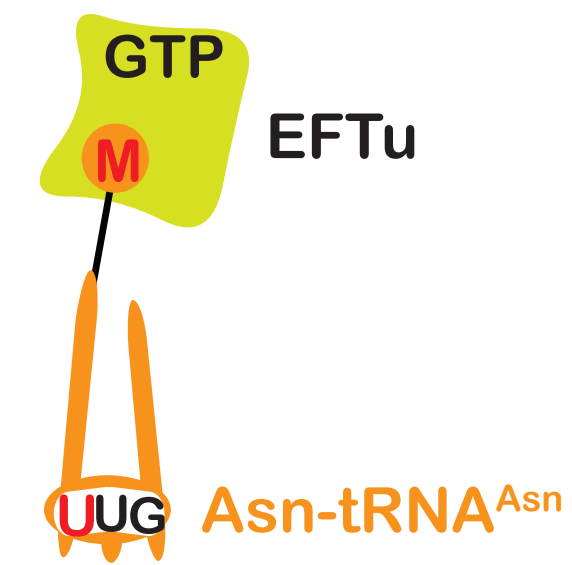
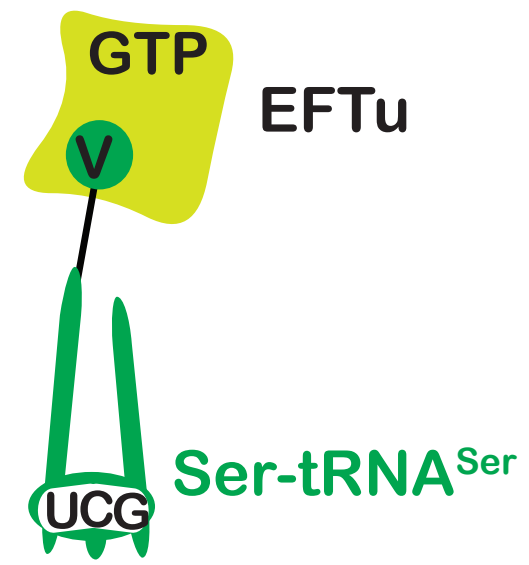
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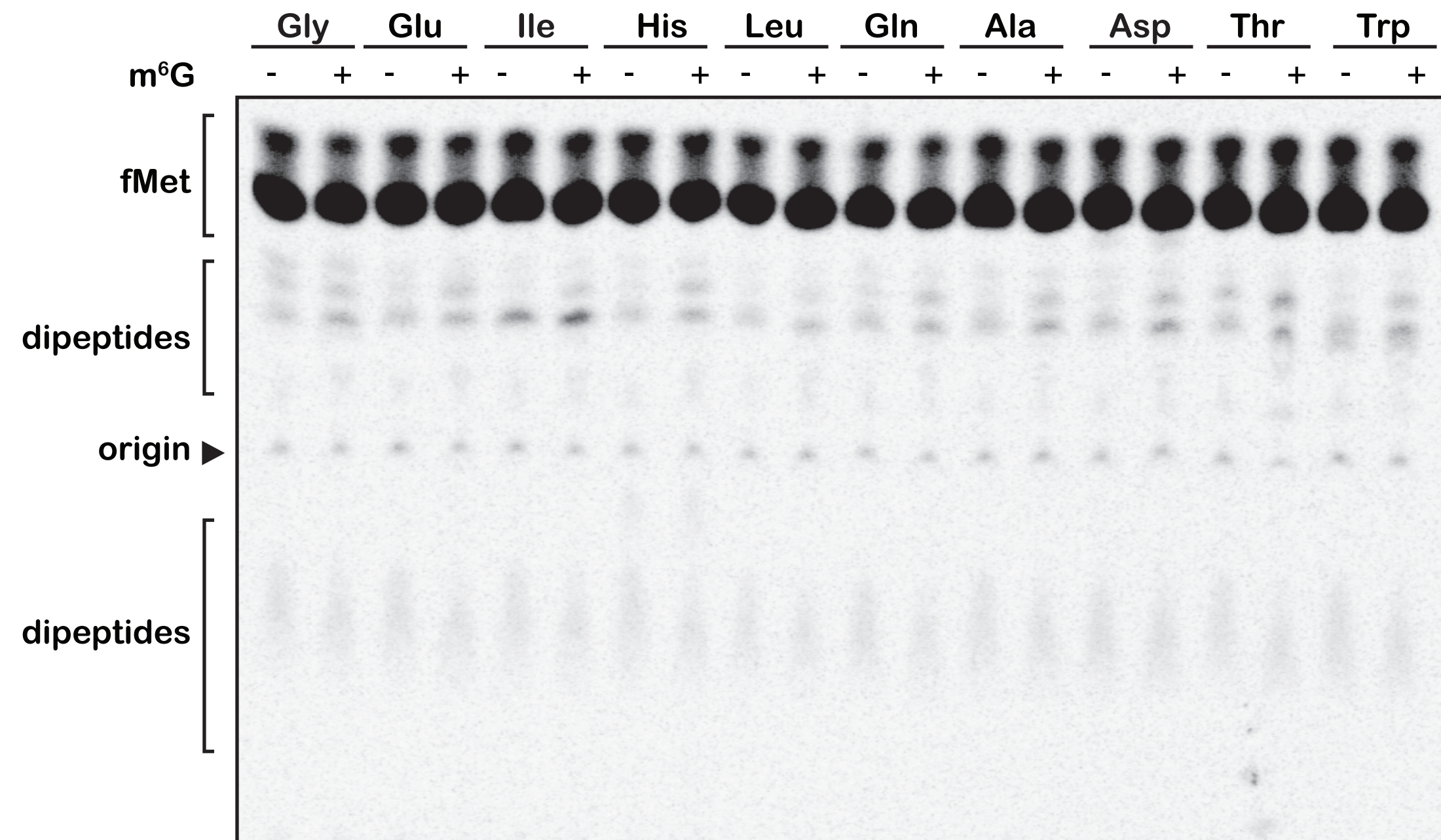
O6-methylguanosine complex

B

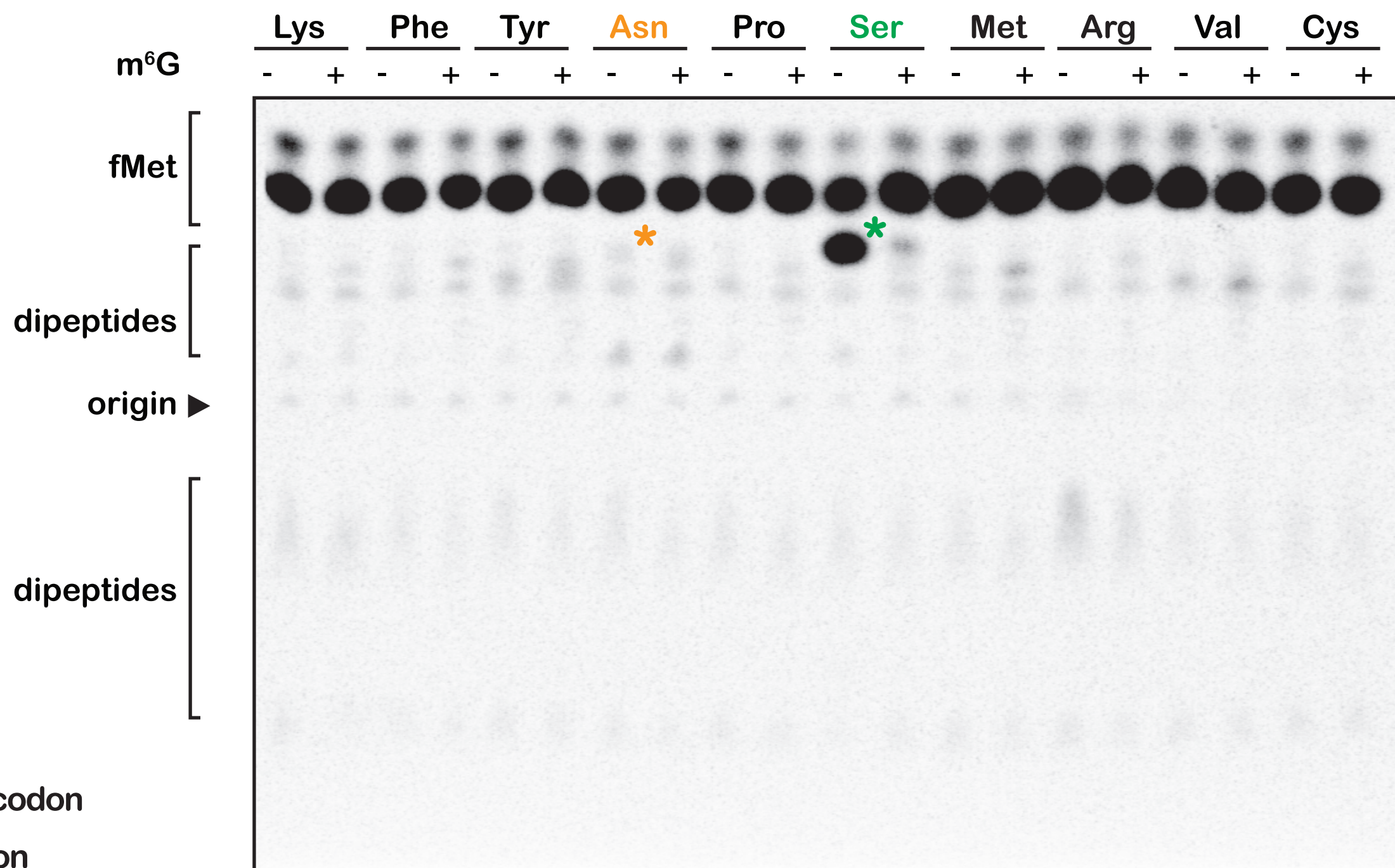
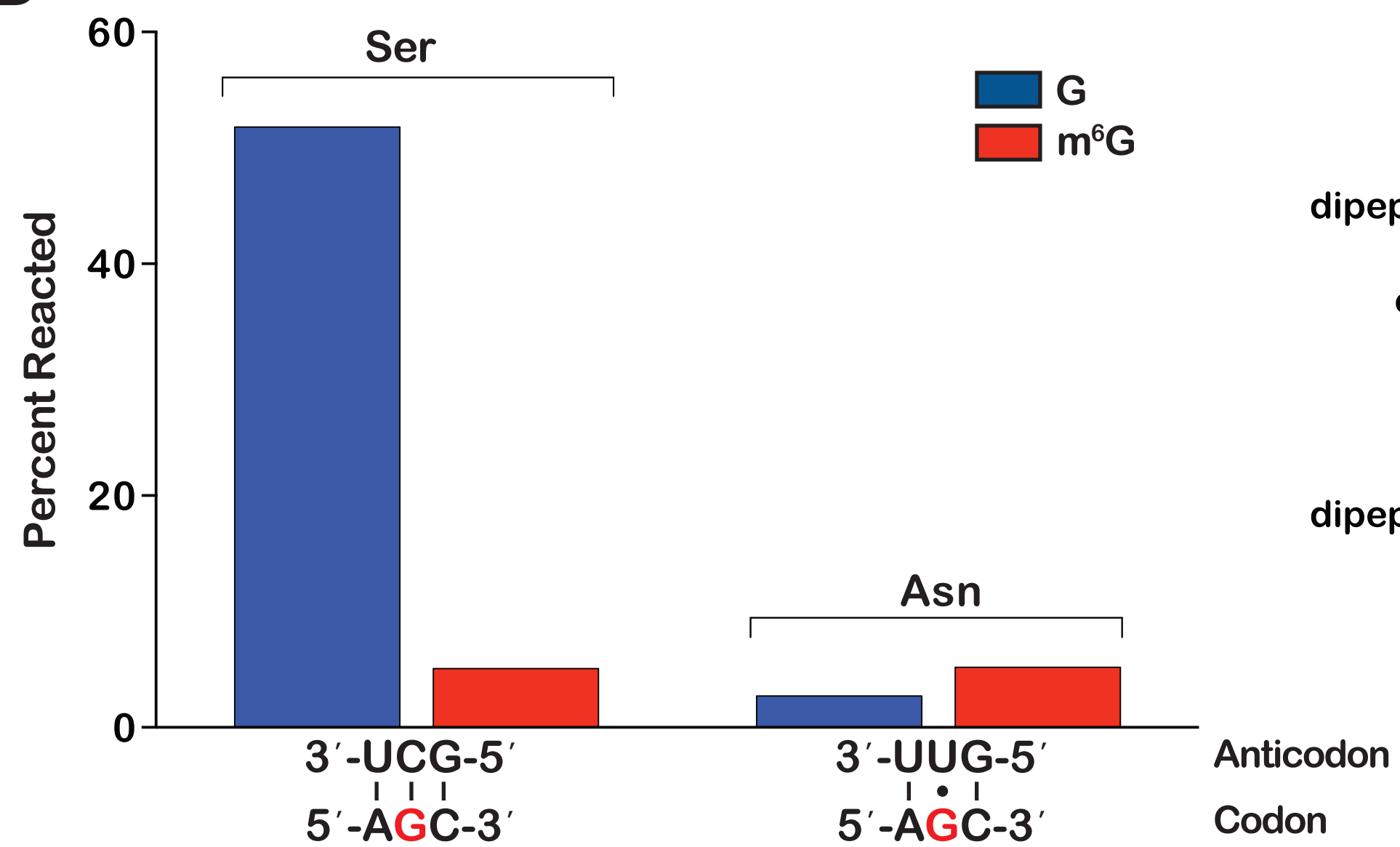
Ternary complexes



C



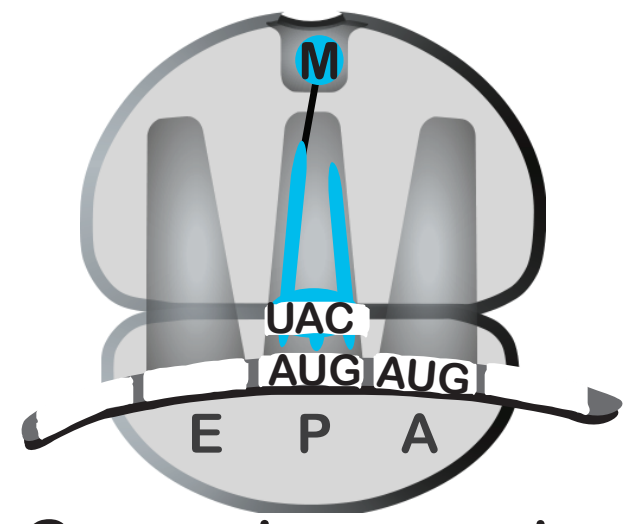
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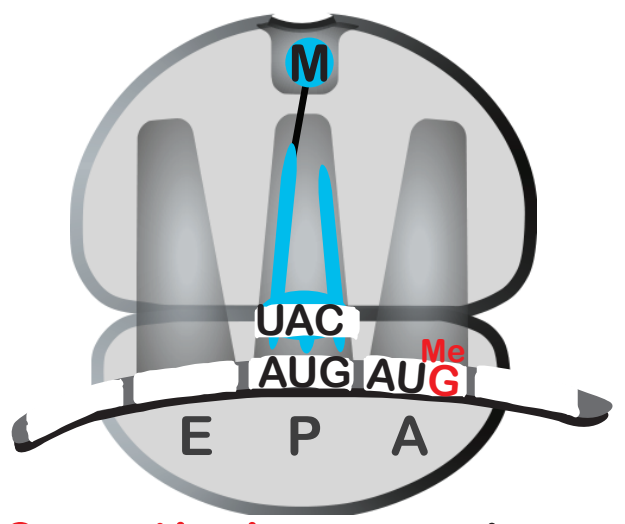


A

Initiation complexes



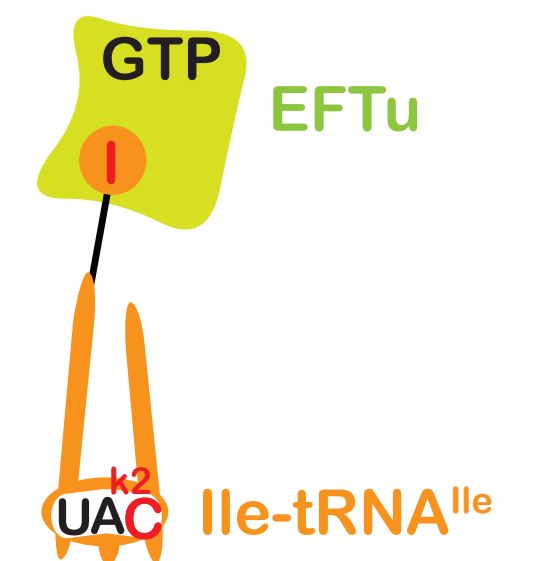
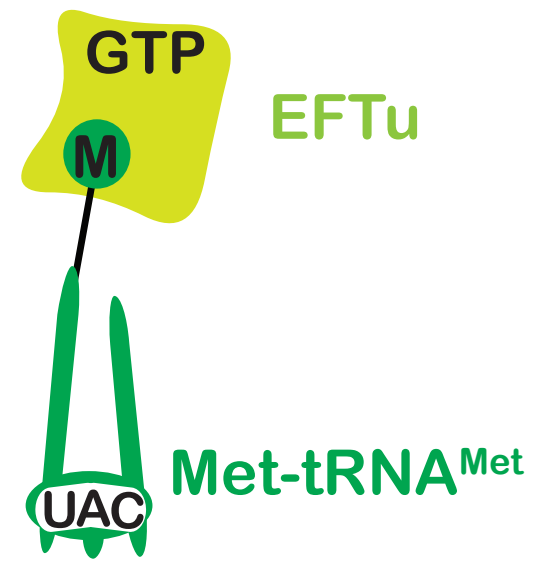
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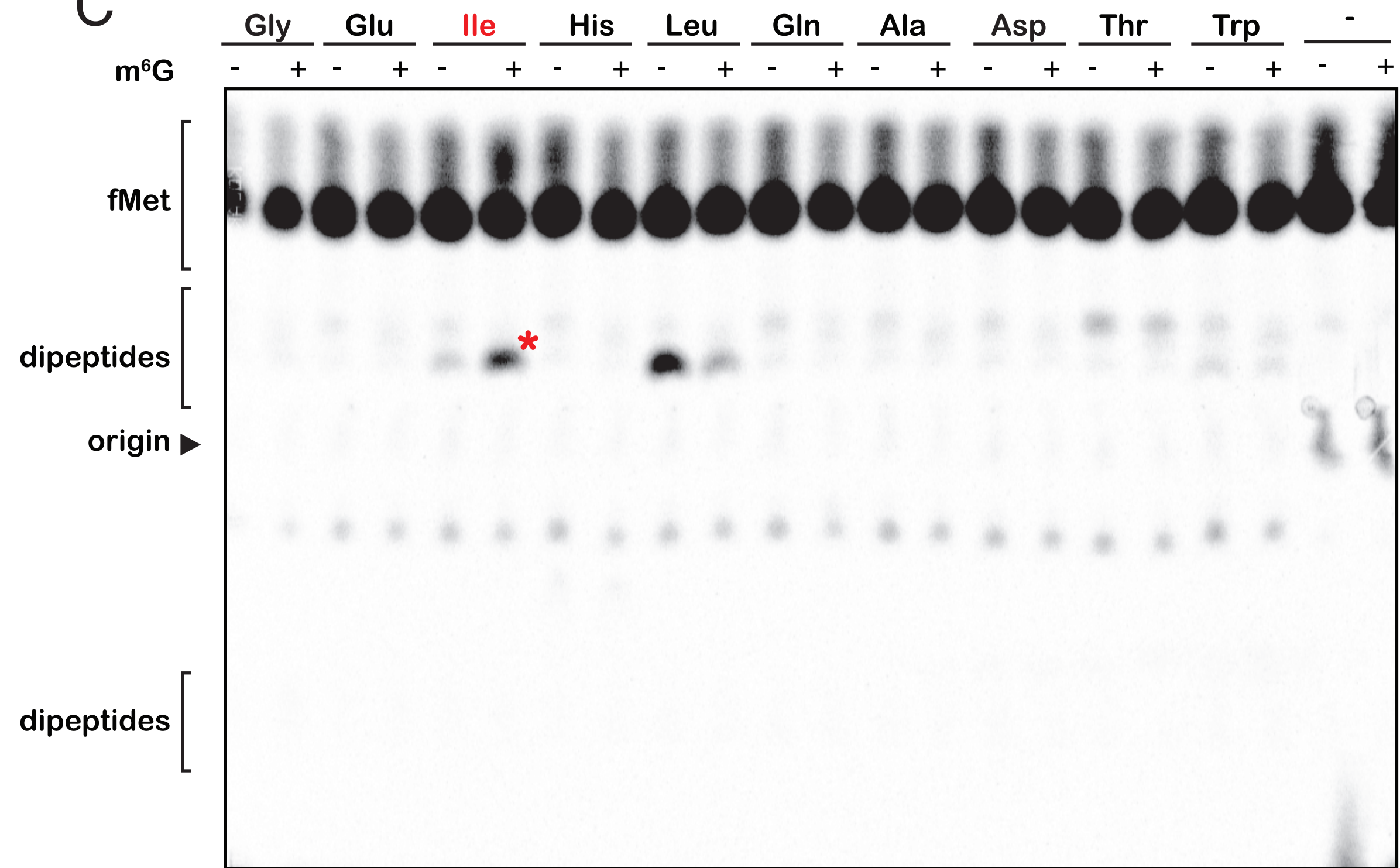
O6-methylguanosine complex

B

Ternary complexes

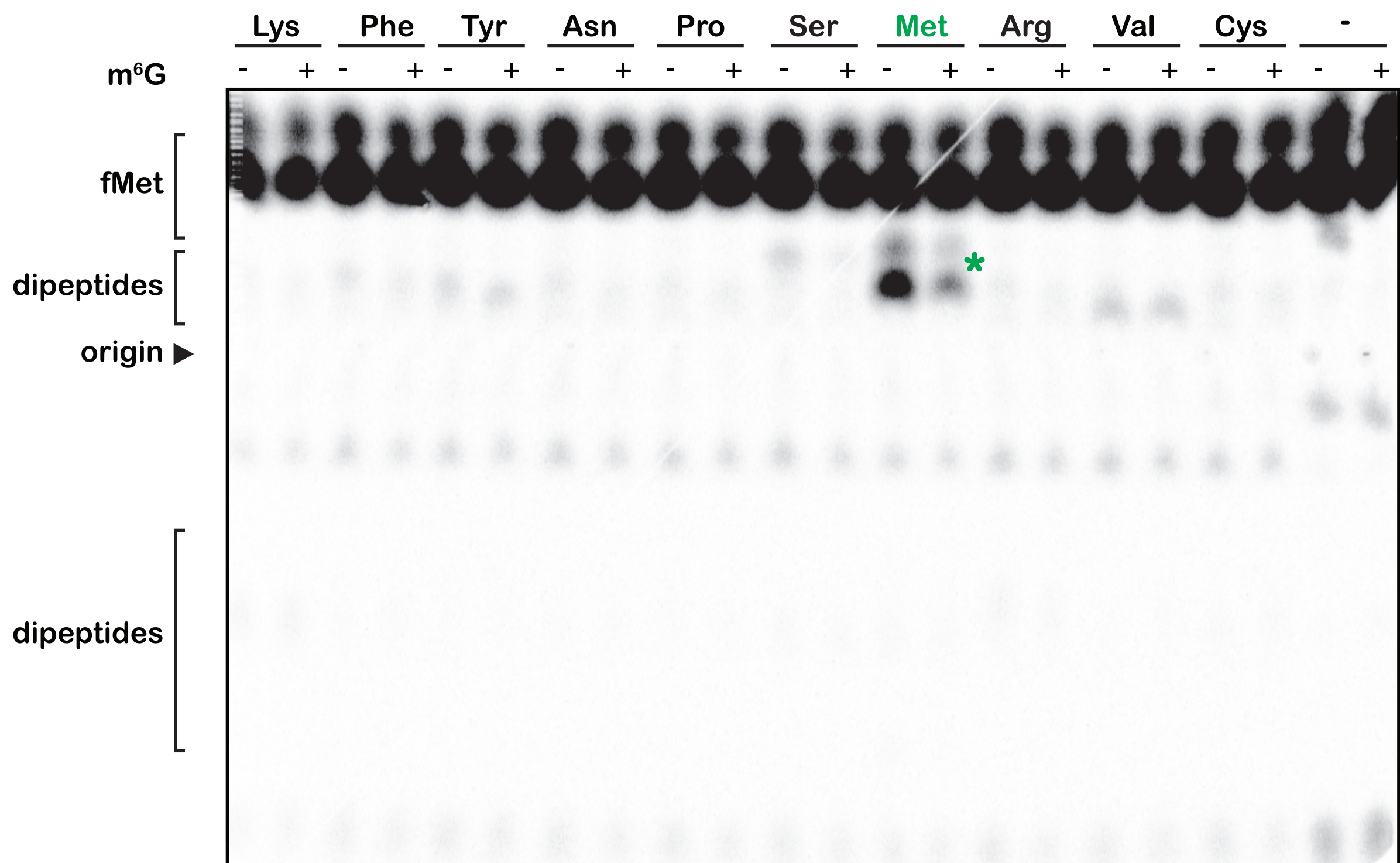
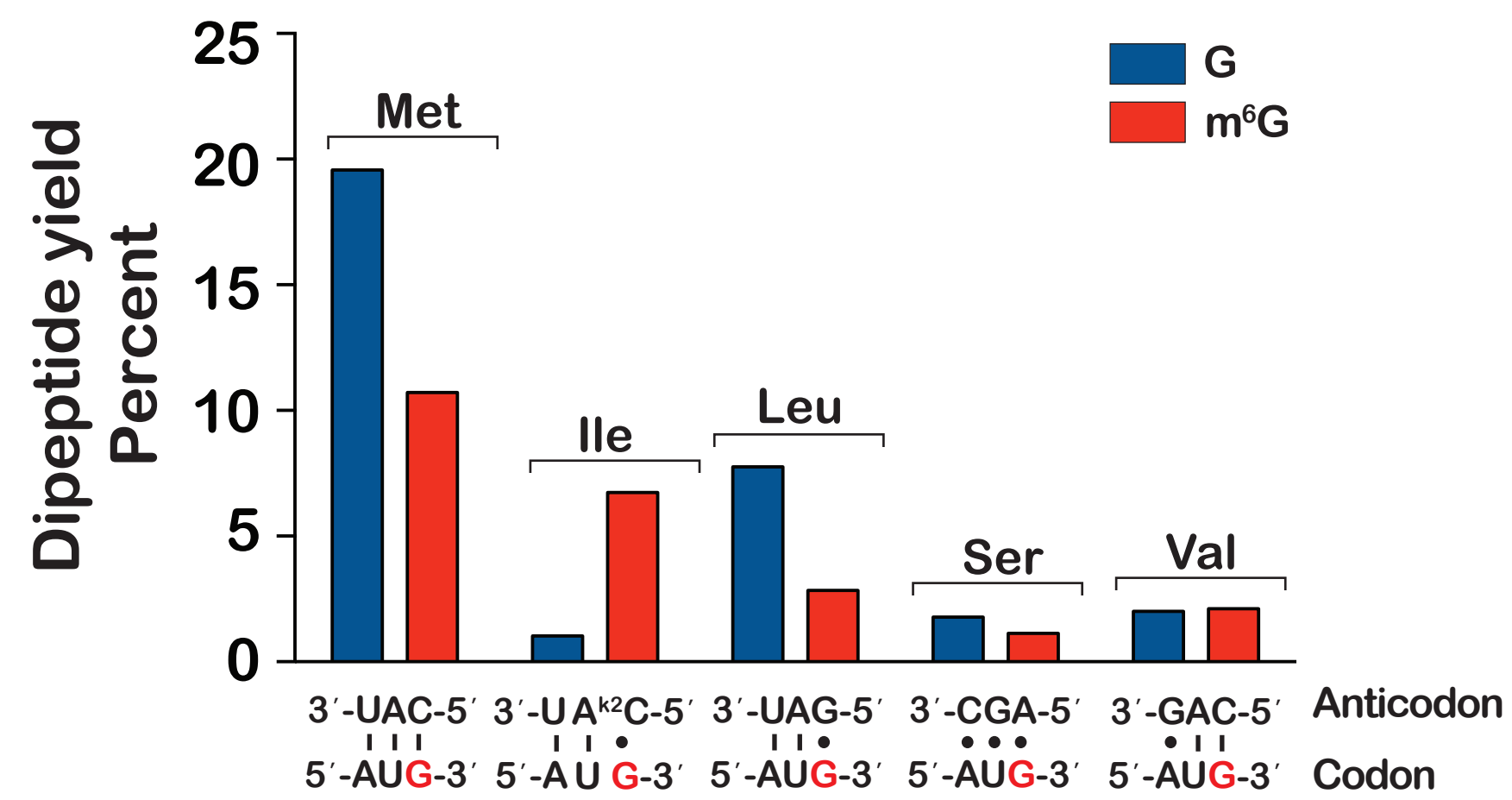


C



dipeptides

D

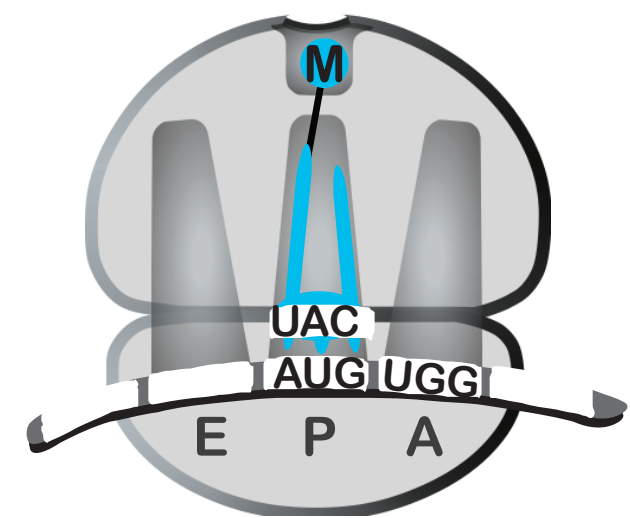


dipeptides

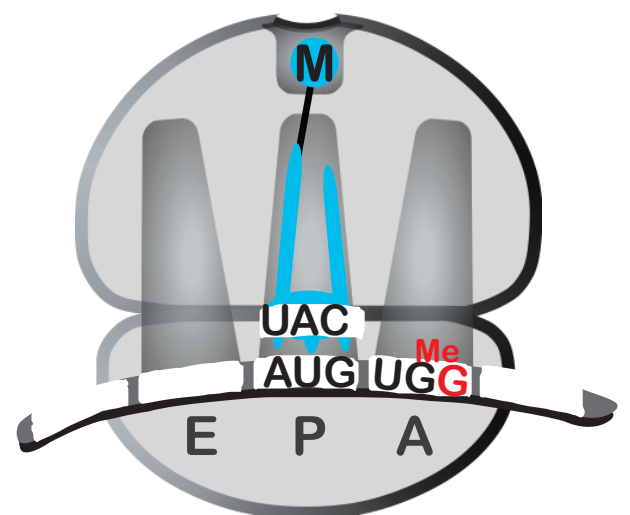


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Initiation complexes



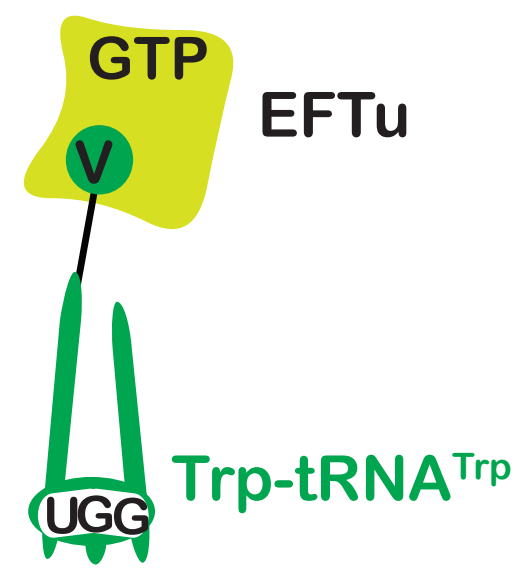
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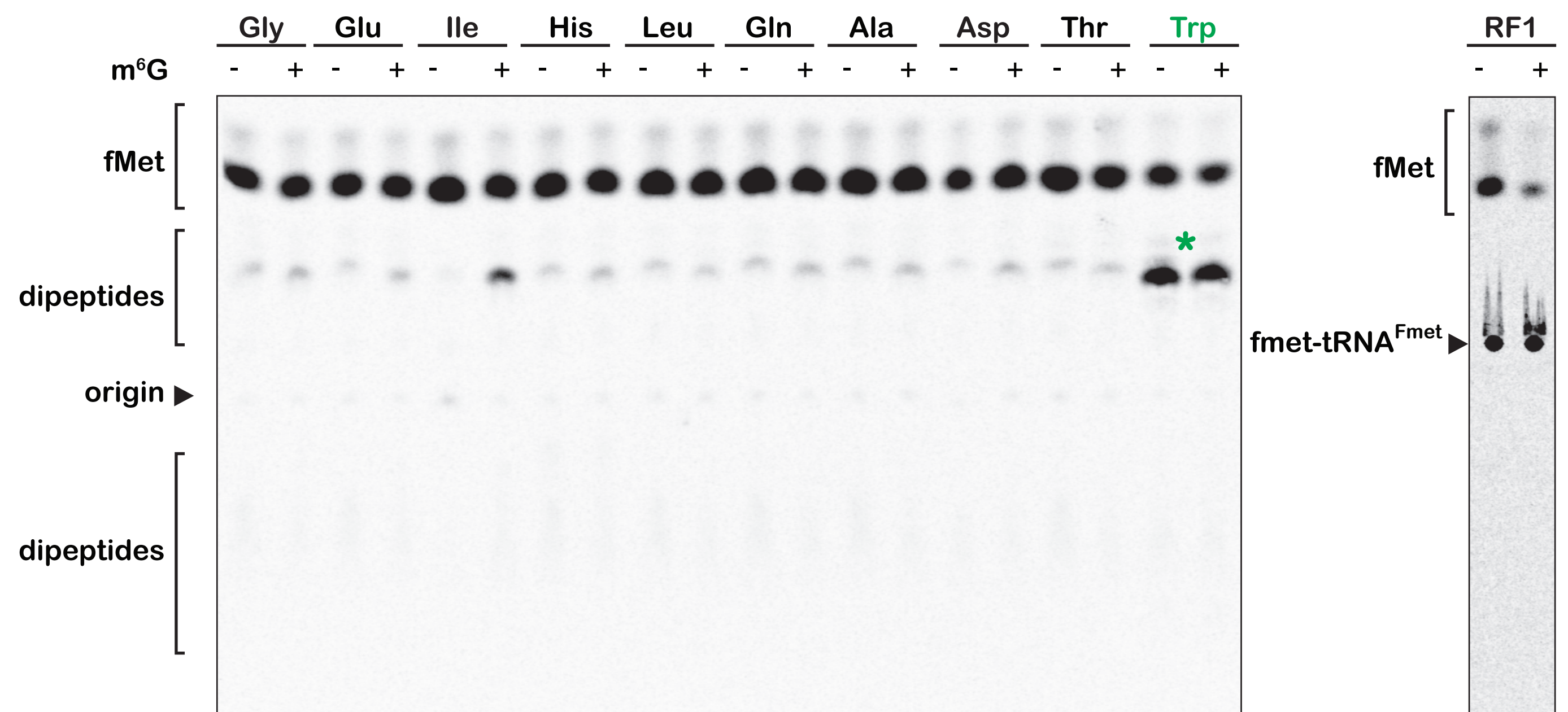
O6-methylguanosine complex

B

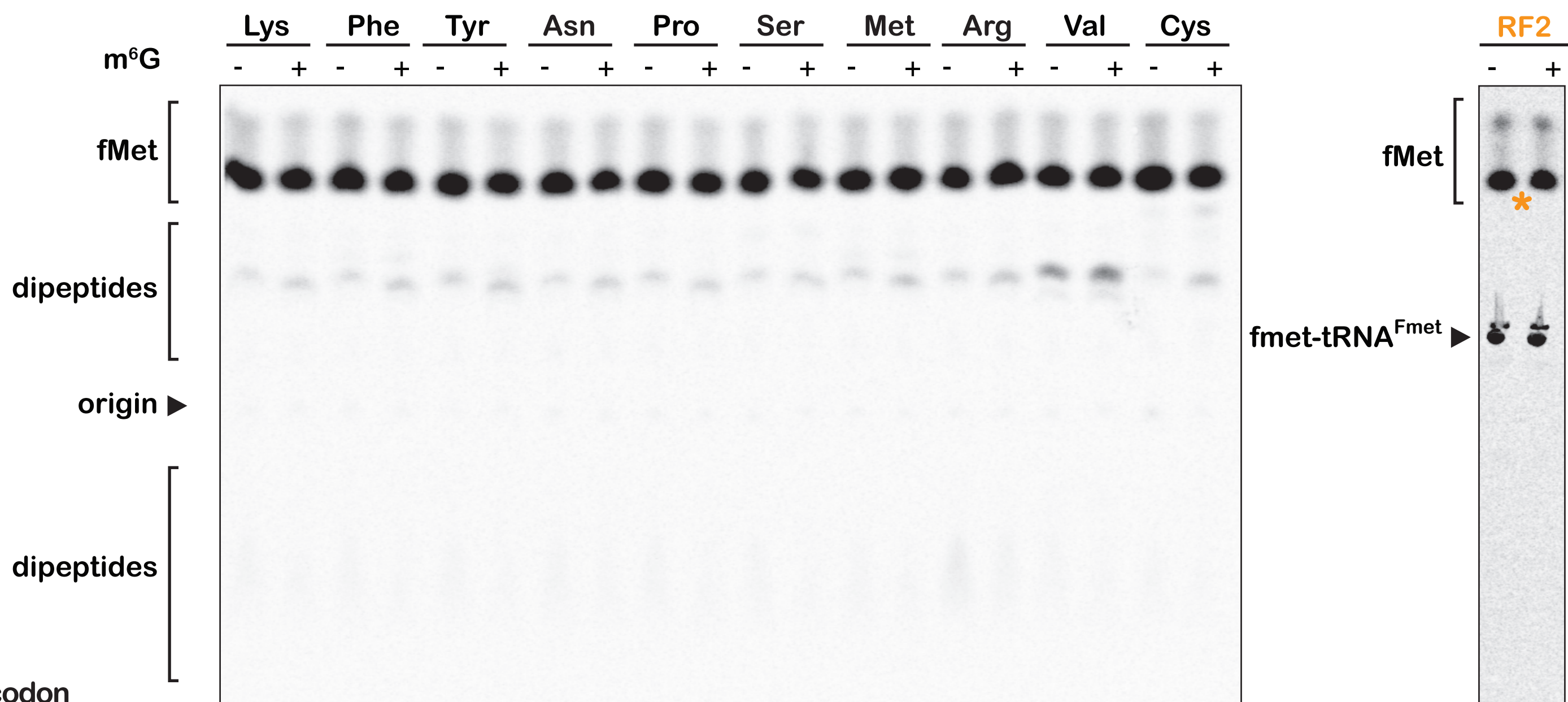
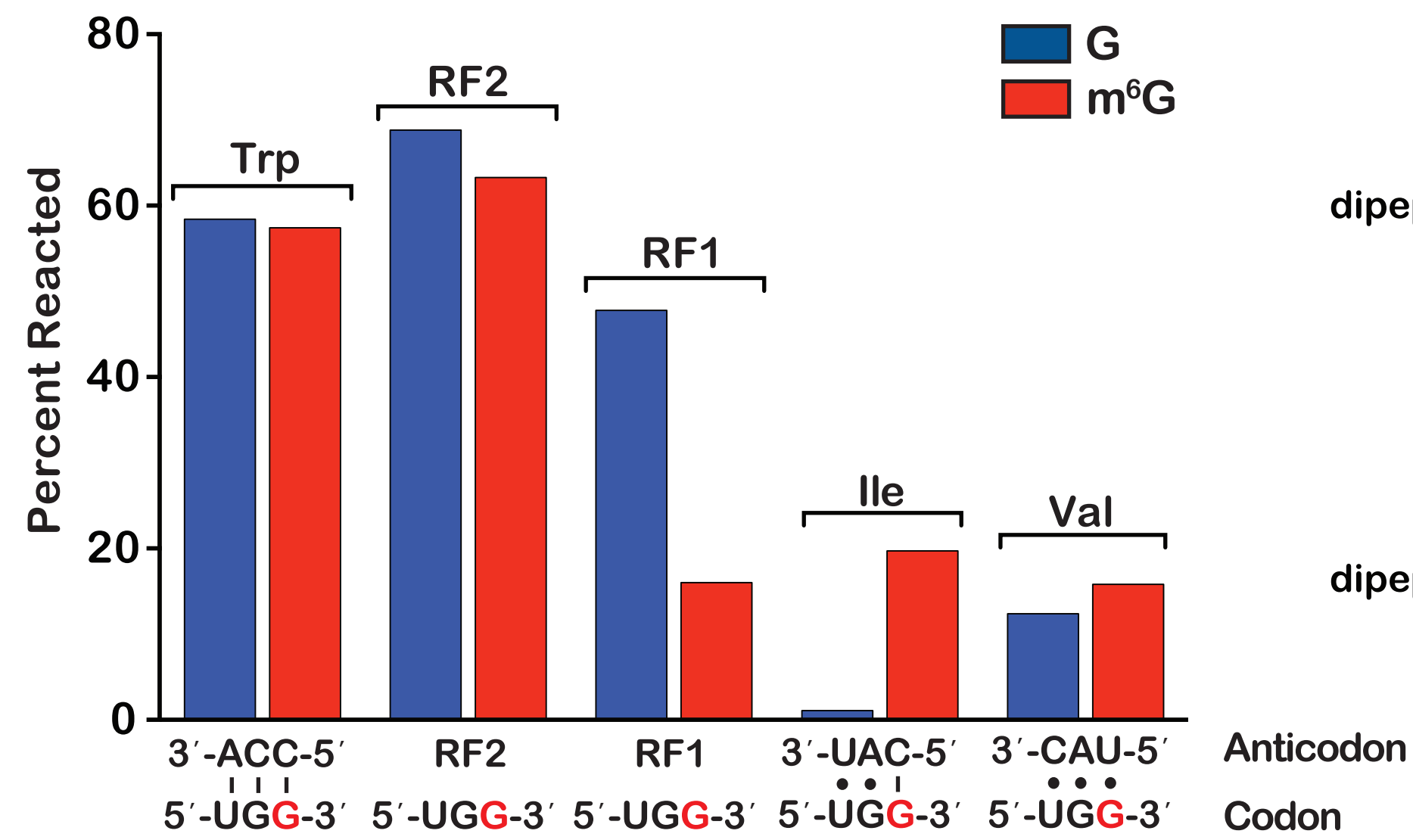
Ternary complexes and release factors



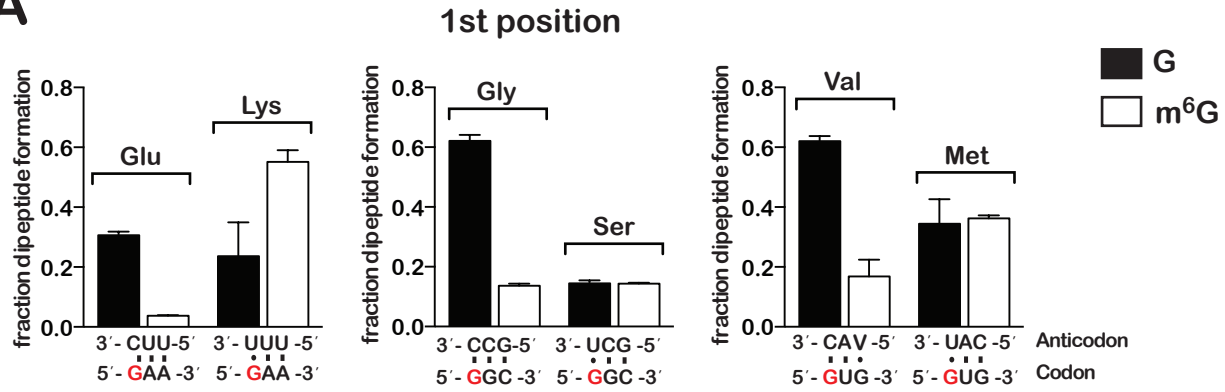
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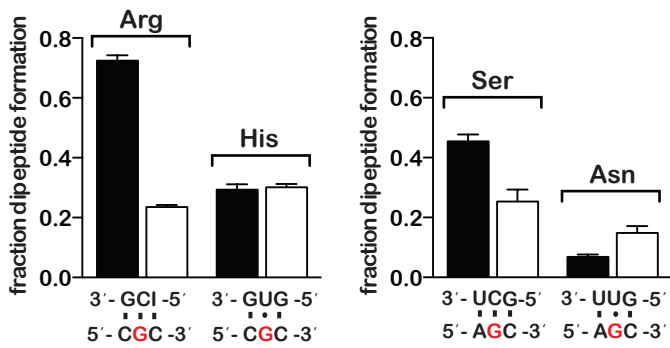


**A**



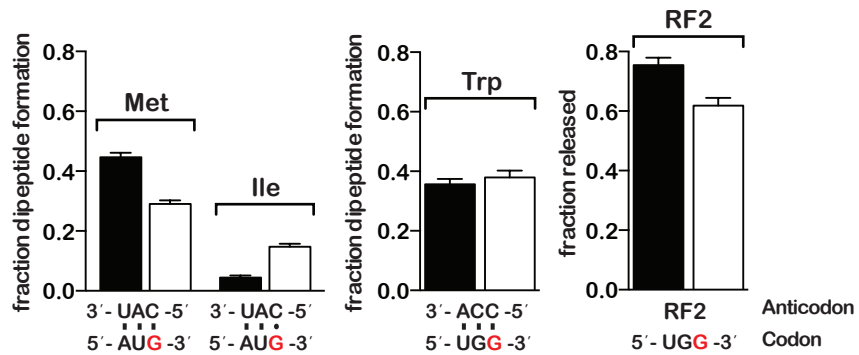
**B**

**2nd position**

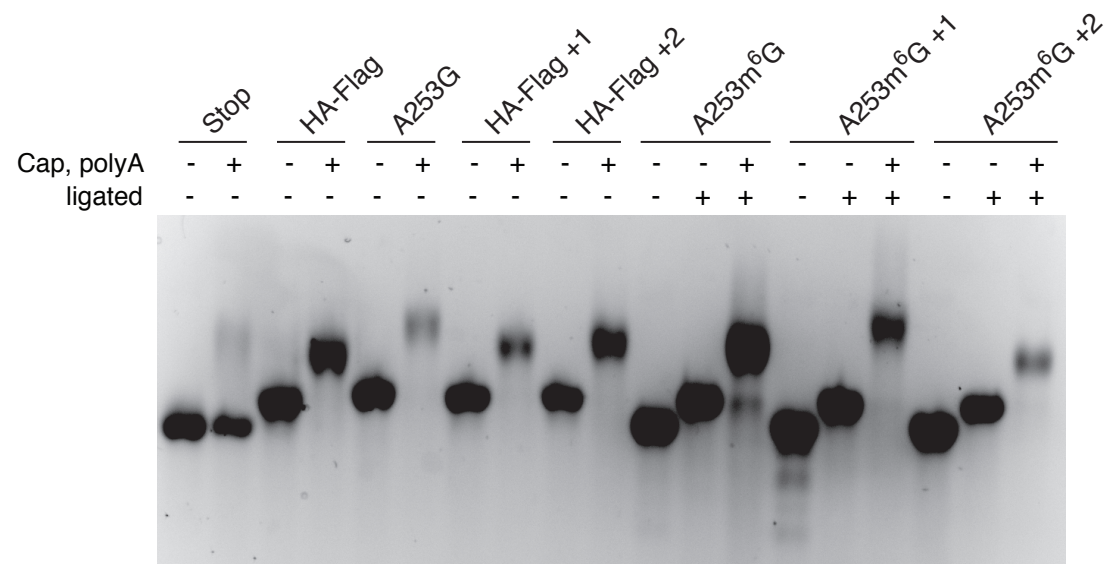


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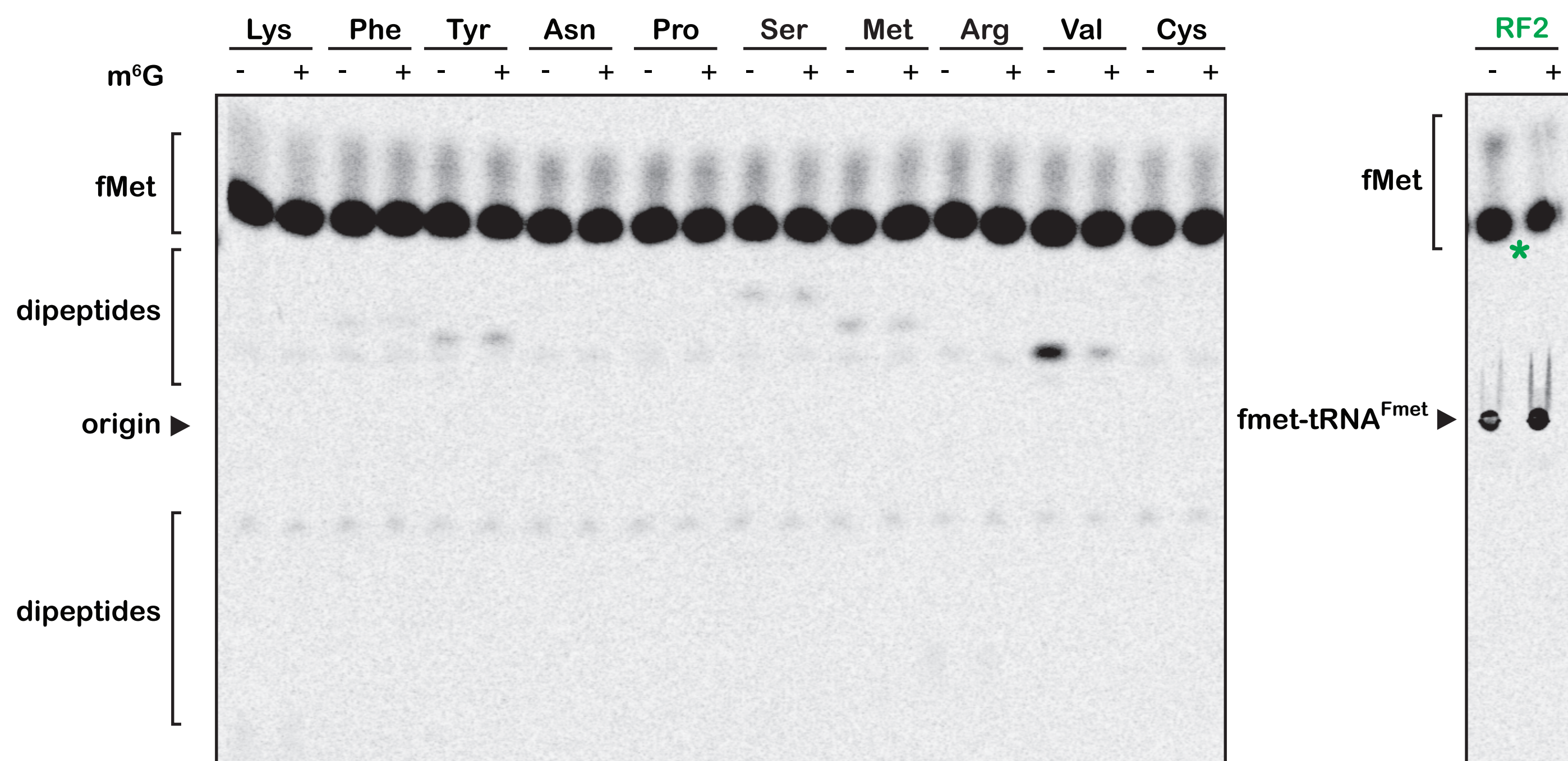
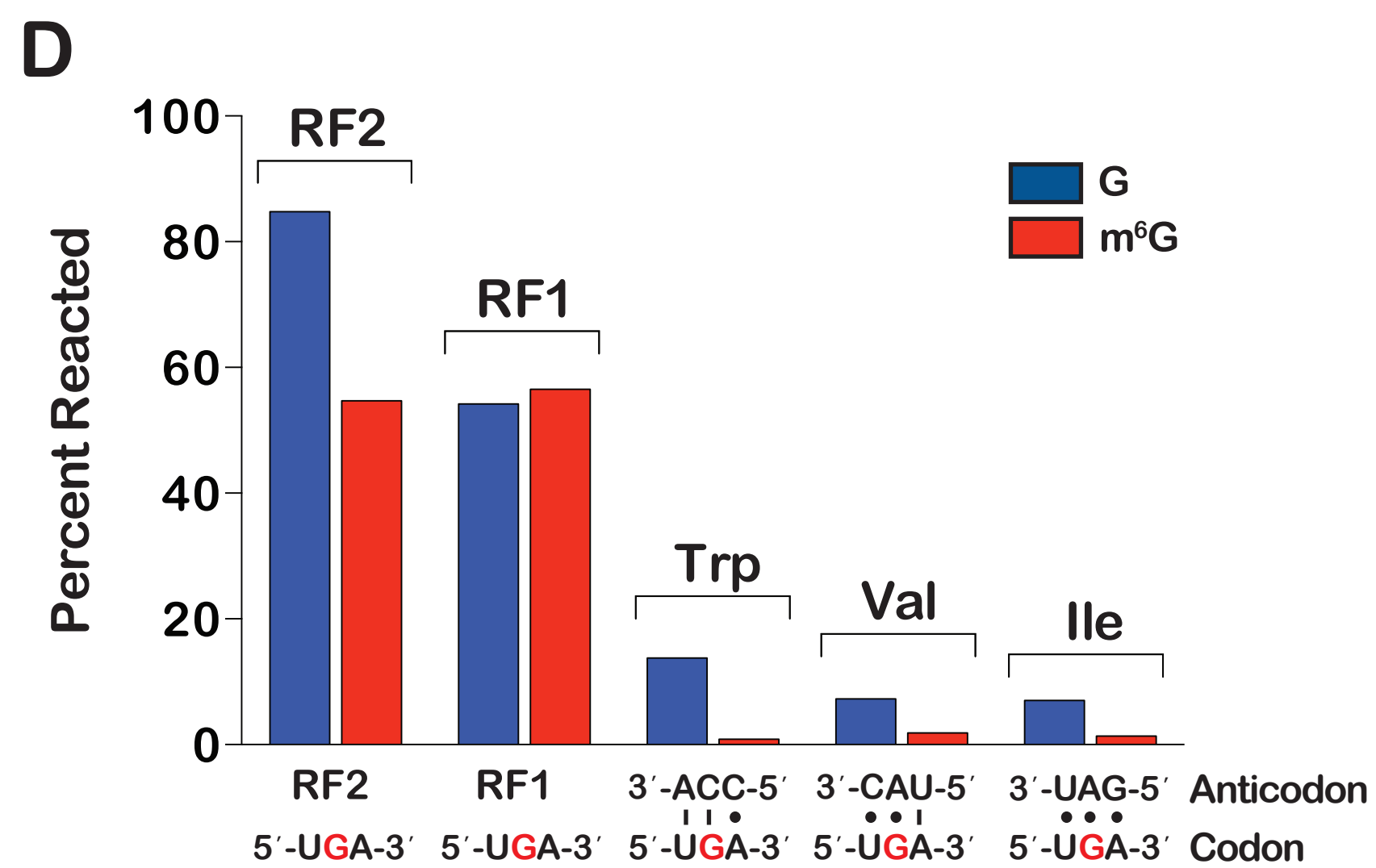
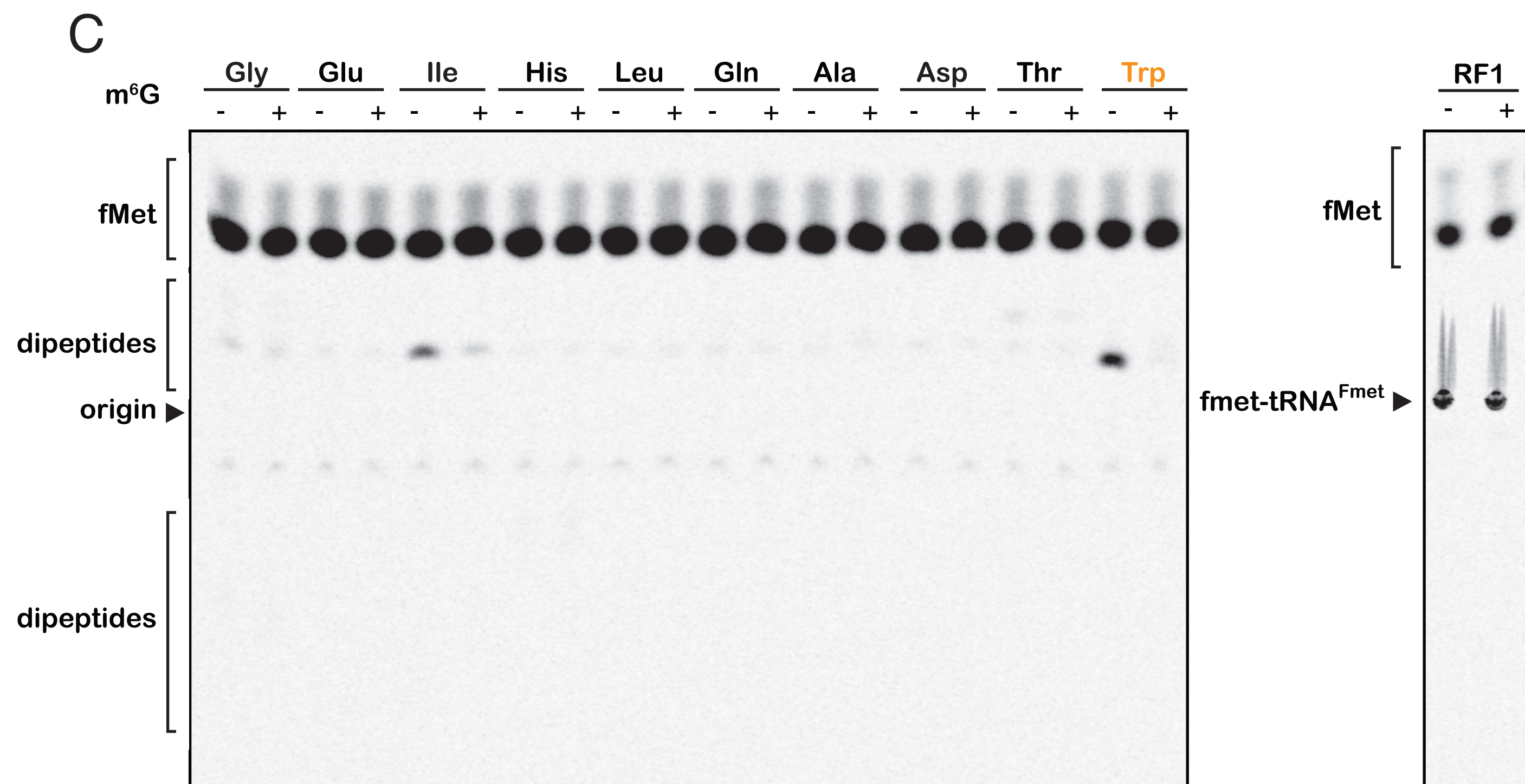
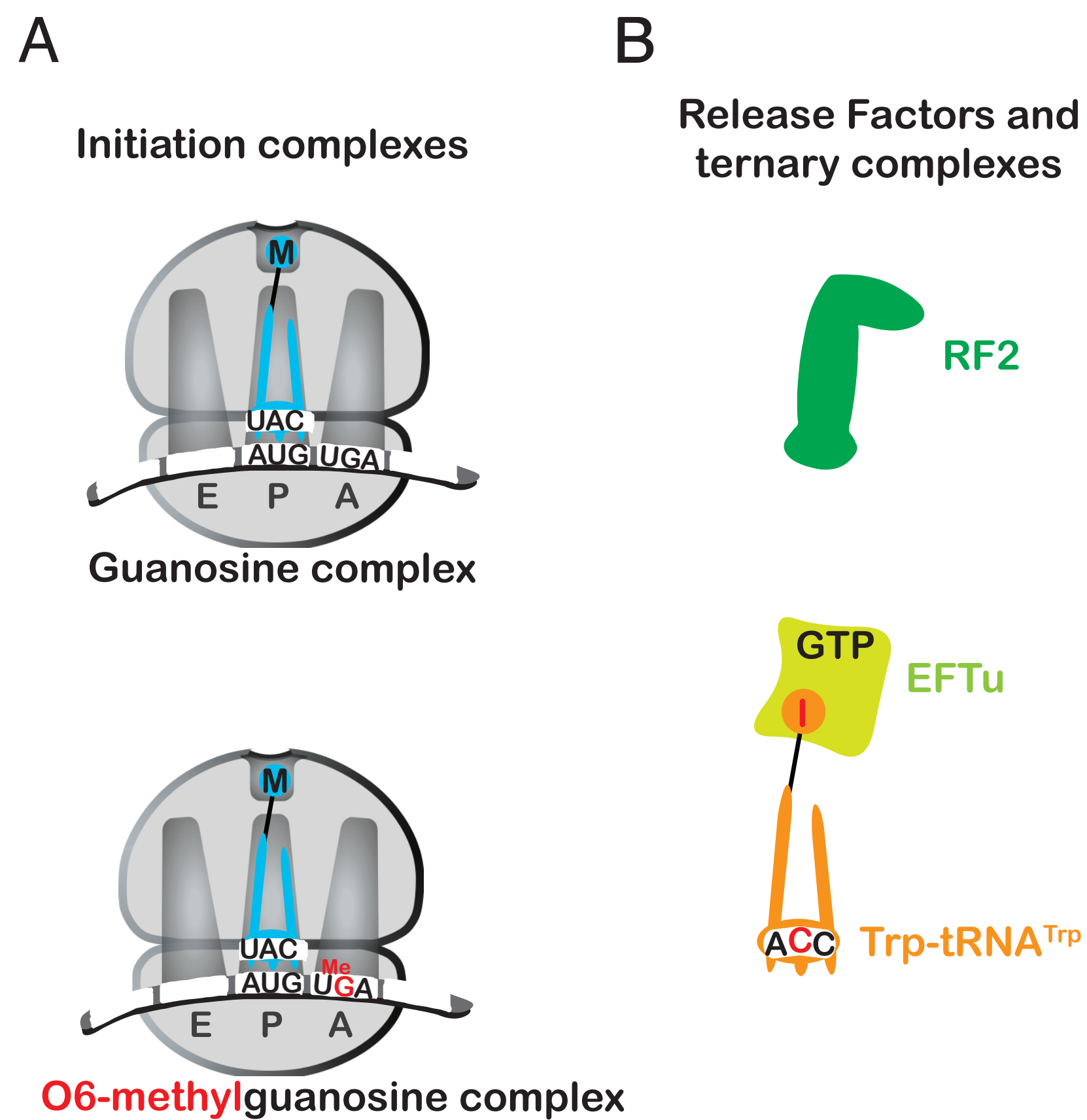
**3rd position**







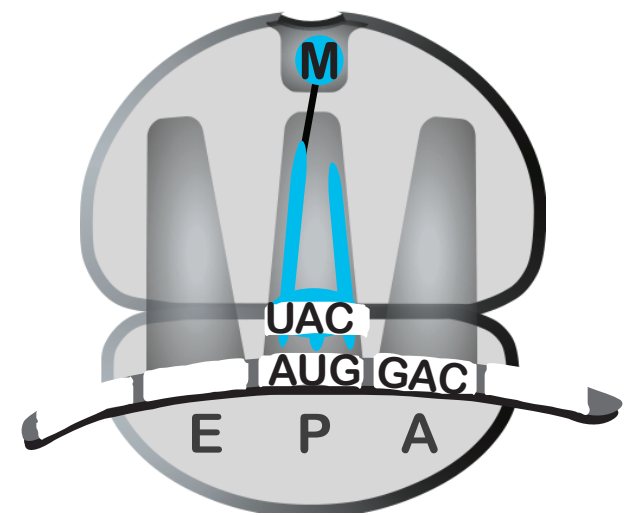




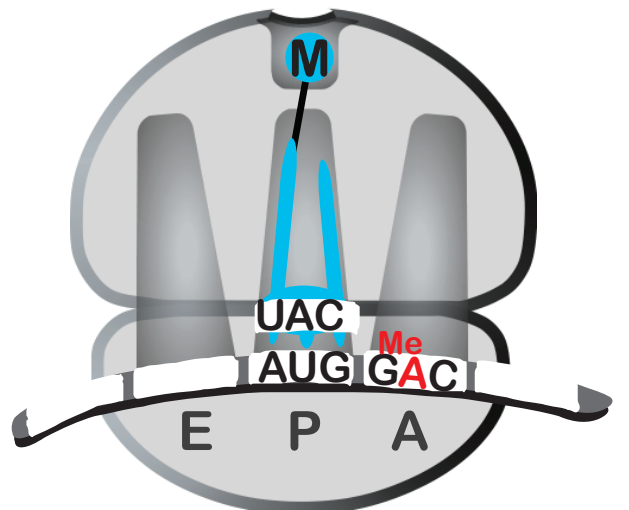


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Initiation complexes



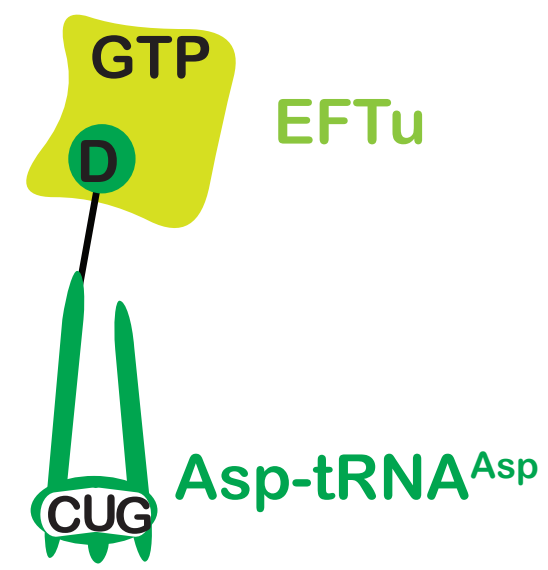
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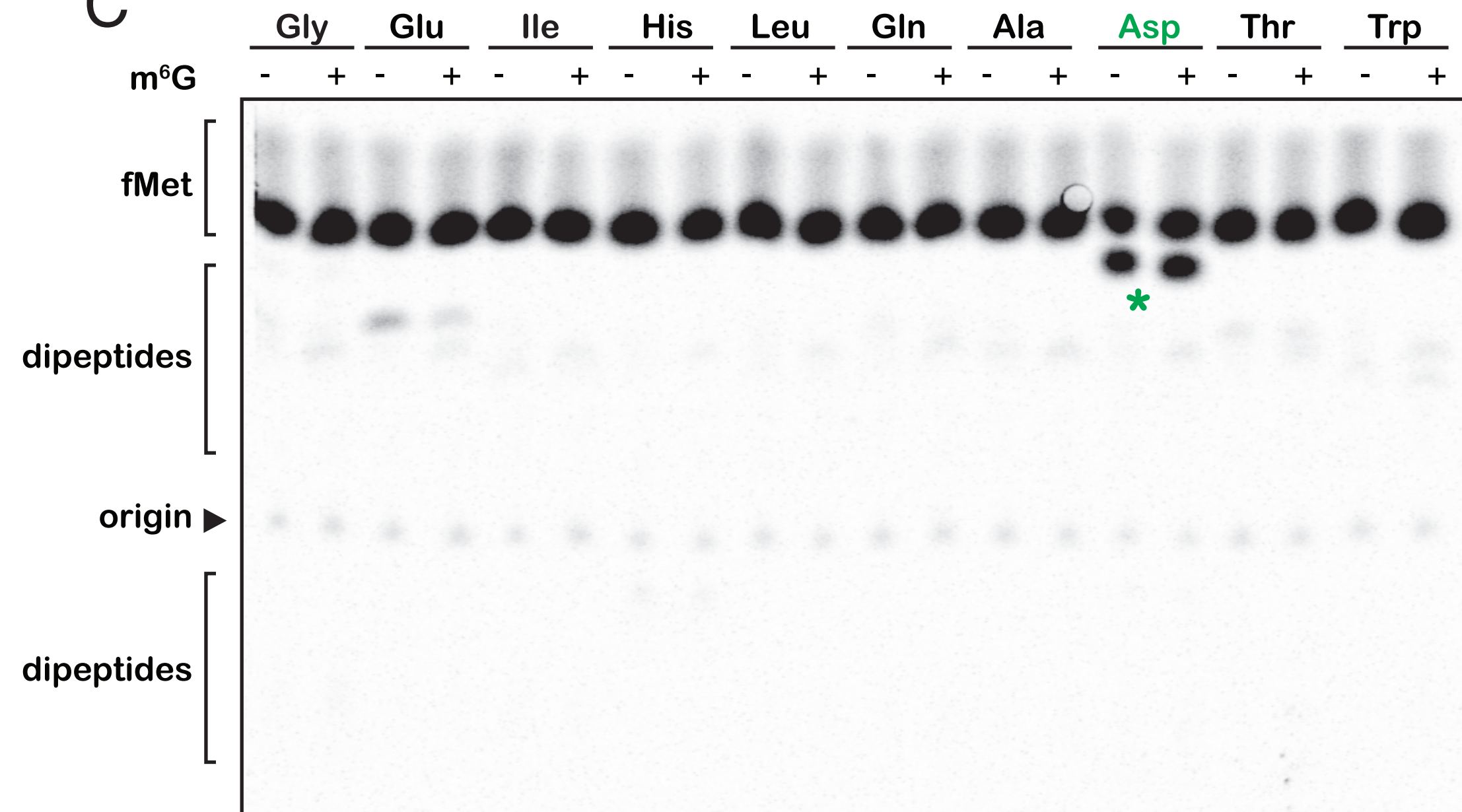
O6-methylguanosine complex

B

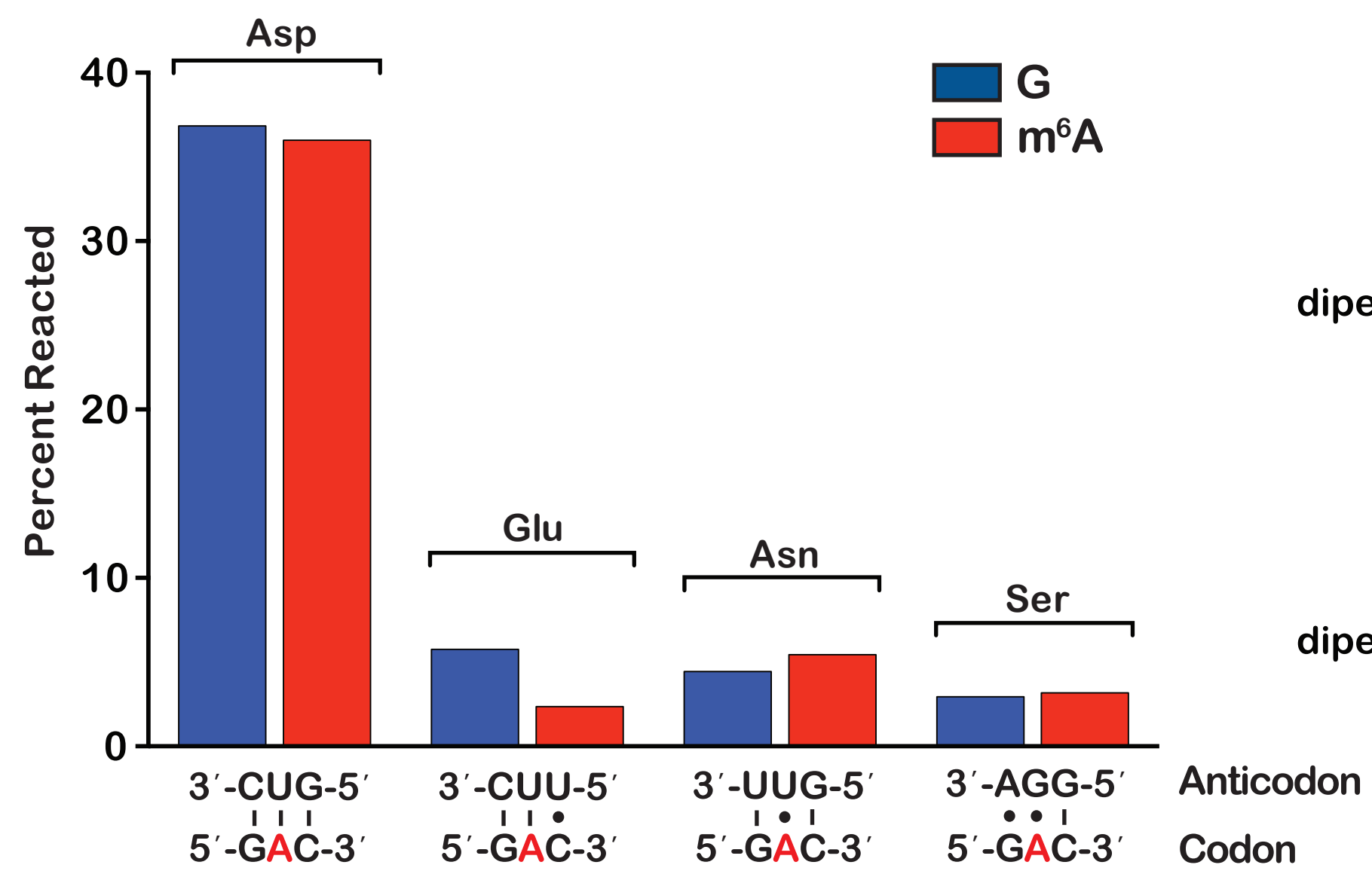
Ternary complexes



C



D



m<sup>6</sup>G

