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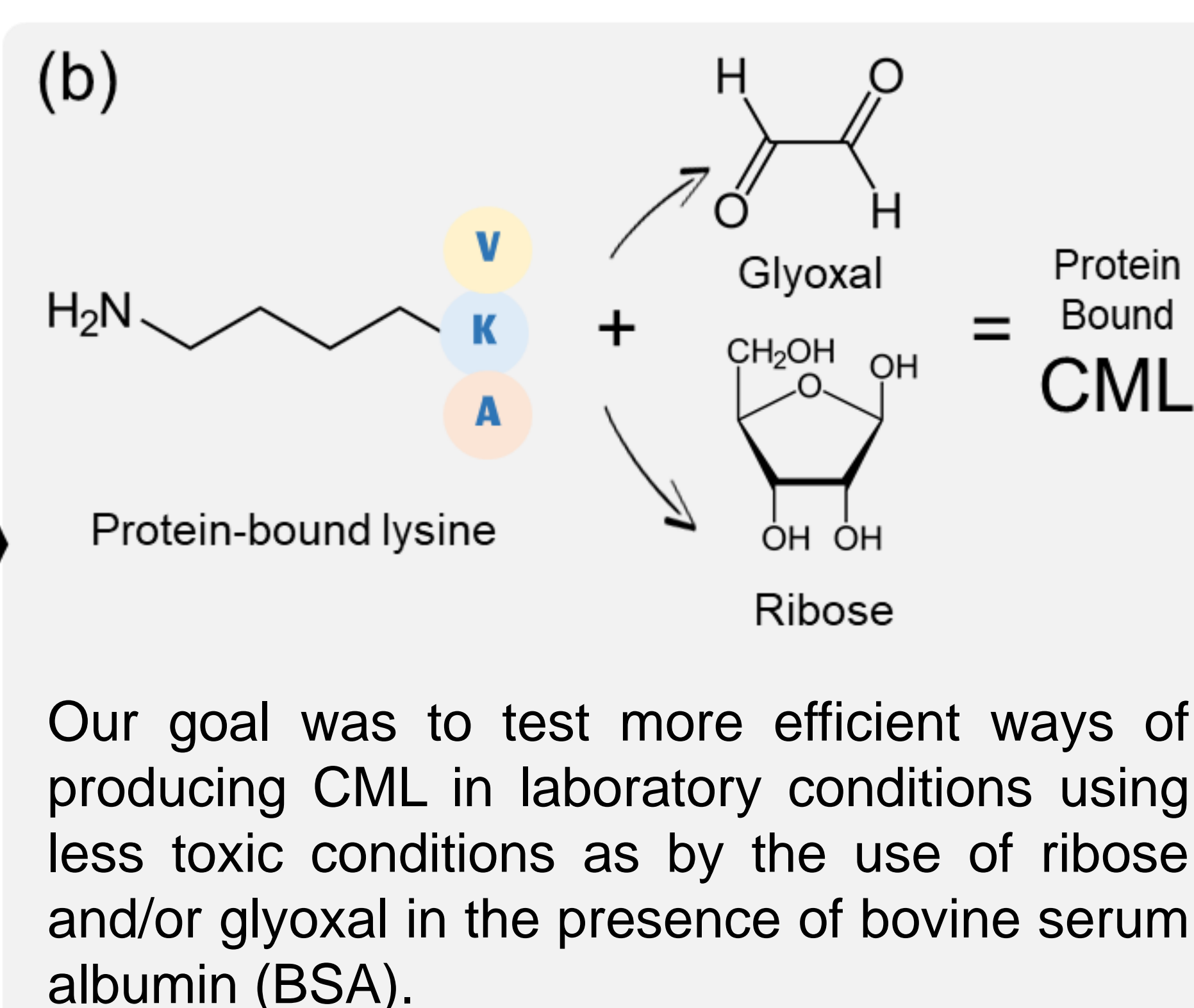
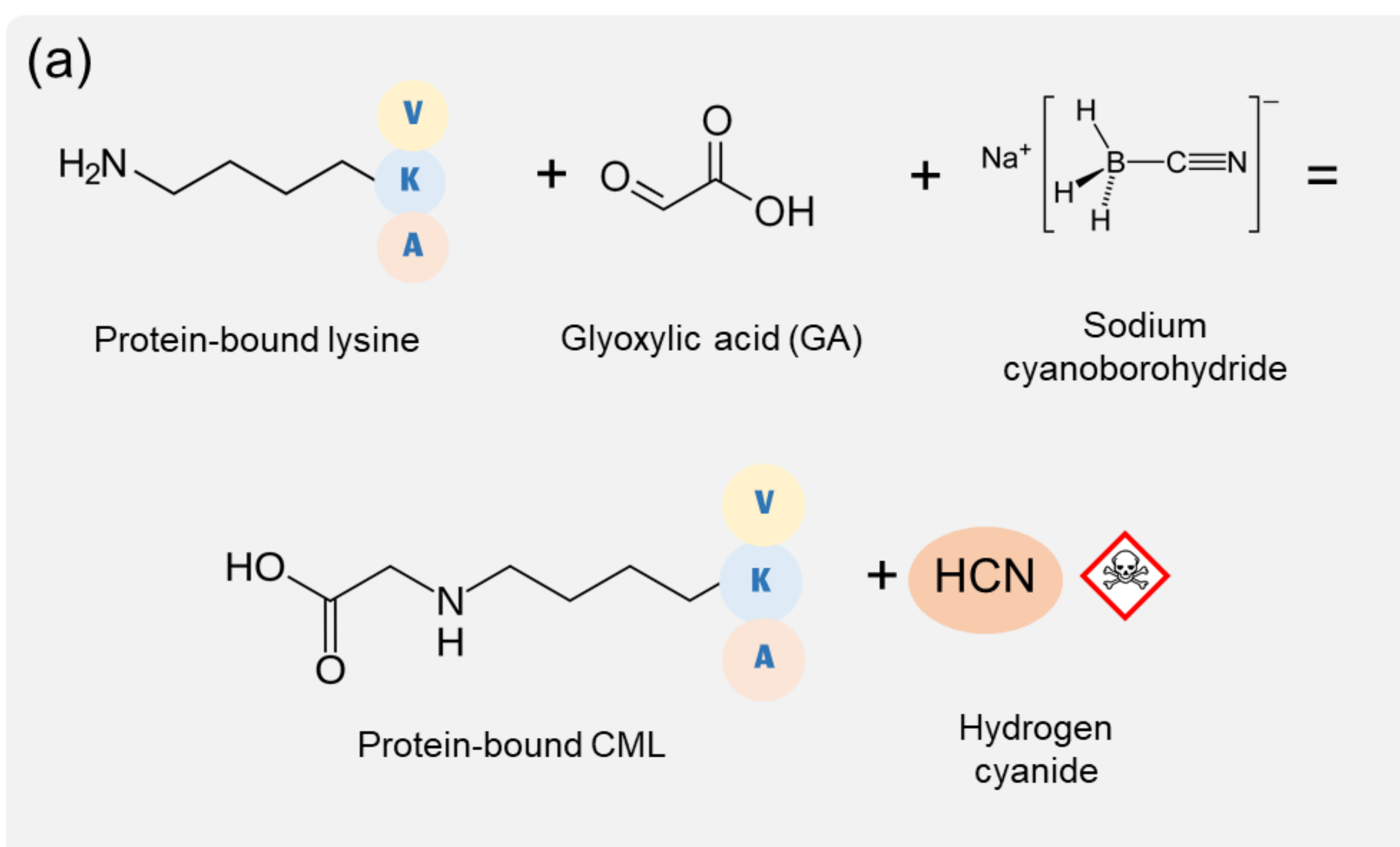
# In vitro fortification of N $\epsilon$ -carboxymethyl-lysine in bovine serum albumin model systems

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## Introduction

The heat treatment of foods results in the production of a heterogeneous group of glycated molecules related to the flavor and aroma of foods, but associated with potential negative health effects. Carboxymethyl-lysine (CML), result of the glycation of lysine residues, is a major dietary advanced glycation end-products (AGE) for the investigation of the effects of AGE consumption on human health (1).



## Methods

Traditional reducing agents (2) > NaBH<sub>4</sub>  
> NaNH<sub>3</sub>CN  
> NaBH(OAc)<sub>3</sub>

Alternatives (3) > Ribose  
> Glyoxal

Western blot  
LC-MS/MS (Acid hydrolysis)  
Proteomics (Enz. hydrolysis)

Reaction conditions: phosphate buffer (0.2M, pH 8.0) - 37°C for different periods.

**Figure 1.** Traditional (a) and alternative (b) methods on the production of protein-bound CML.

## Results

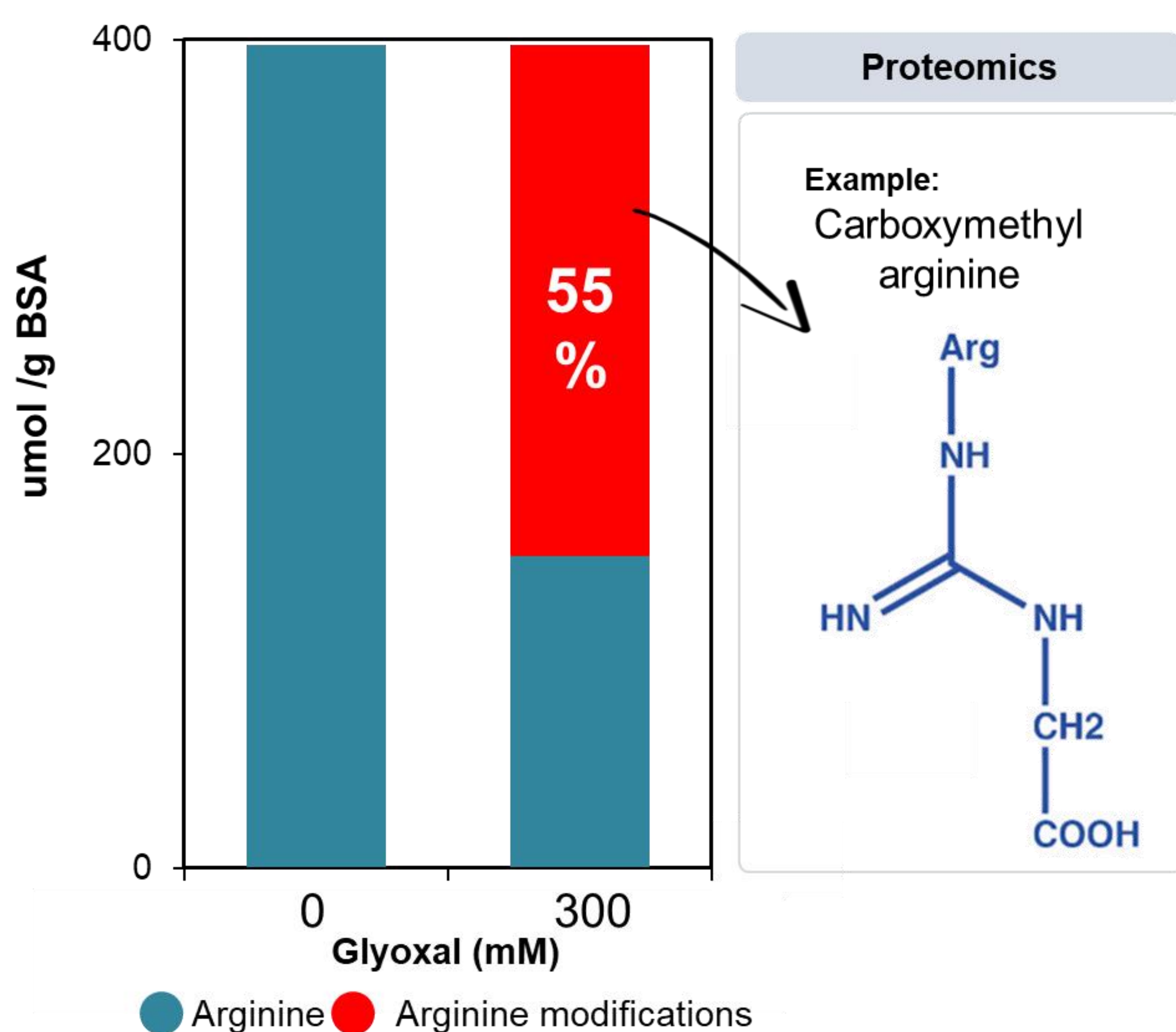
Degree of lysine modification into CML(%) in BSA using traditional methods:

Reagent	CML formation (% of lysine)
NaNH <sub>3</sub> CN	37.0
NaBH <sub>4</sub>	3.5
NaBH(OAc)	1.6

Ribose with or without glyoxal induced a loss of 35 to 57% of lysine, but low CML formation (1-2%).

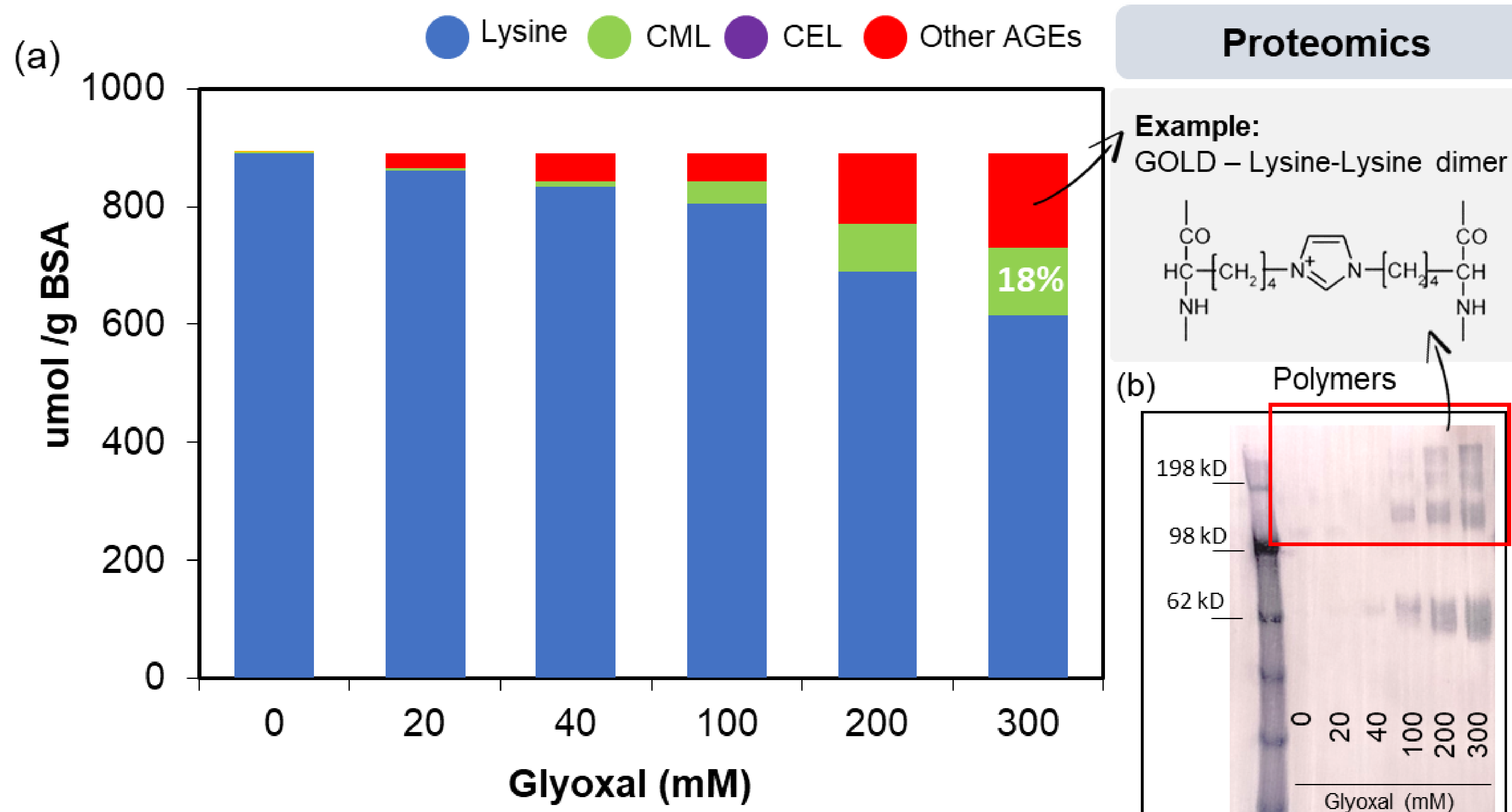
The CML fortification of BSA with increasing concentrations of glyoxal alone formed CML in proportion to the glyoxal concentration: 0.5, 1.0, 4.5, 9.8 and 18% of lysine with 20, 40, 100, 200 and 300 mM glyoxal, respectively (Fig. 2a).

Further characterization:



**Figure 3.** Glycation between BSA and glyoxal. Assessment of arginine modifications by LC-MS/MS.

Proteomic analysis identified arginine modifications as carboxymethyl-arginine in this preparation (Fig. 3).



**Figure 2.** Glycation between BSA and increasing concentrations of glyoxal (0 to 300 mM). (a) LC-MS/MS quantification of CML, carboxyethyl-lysine (CEL), lysine, and estimation of other AGEs. (b) CML detection on a western blot. Antibody Cell Signaling 4764S (1/5000).

## Conclusion

**Alternative:**

**BSA + 300mM glyoxal alone is a suitable alternative to GA with NaBH<sub>3</sub>CN**

Pros: > No HCN production

> Satisfactory lysine modification into CML (18%) compared to other methods

Perspectives:

> Finding a model protein selectively fortified only with CML remains to be discovered;  
> Characterize the crosslinks between proteins.

## References

- (1) Nguyen et al. (2014). *F Reviews Inte*, 30, 36.
- (2) Andersen et al. (2012). *Synlett*, 4, 531.
- (3) Glomb et al. (1995). *J Bio Chem*, 270, 10017.

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