Understanding Stability of Cys^{34}-Adducts from Intraclass Correlation Coefficient

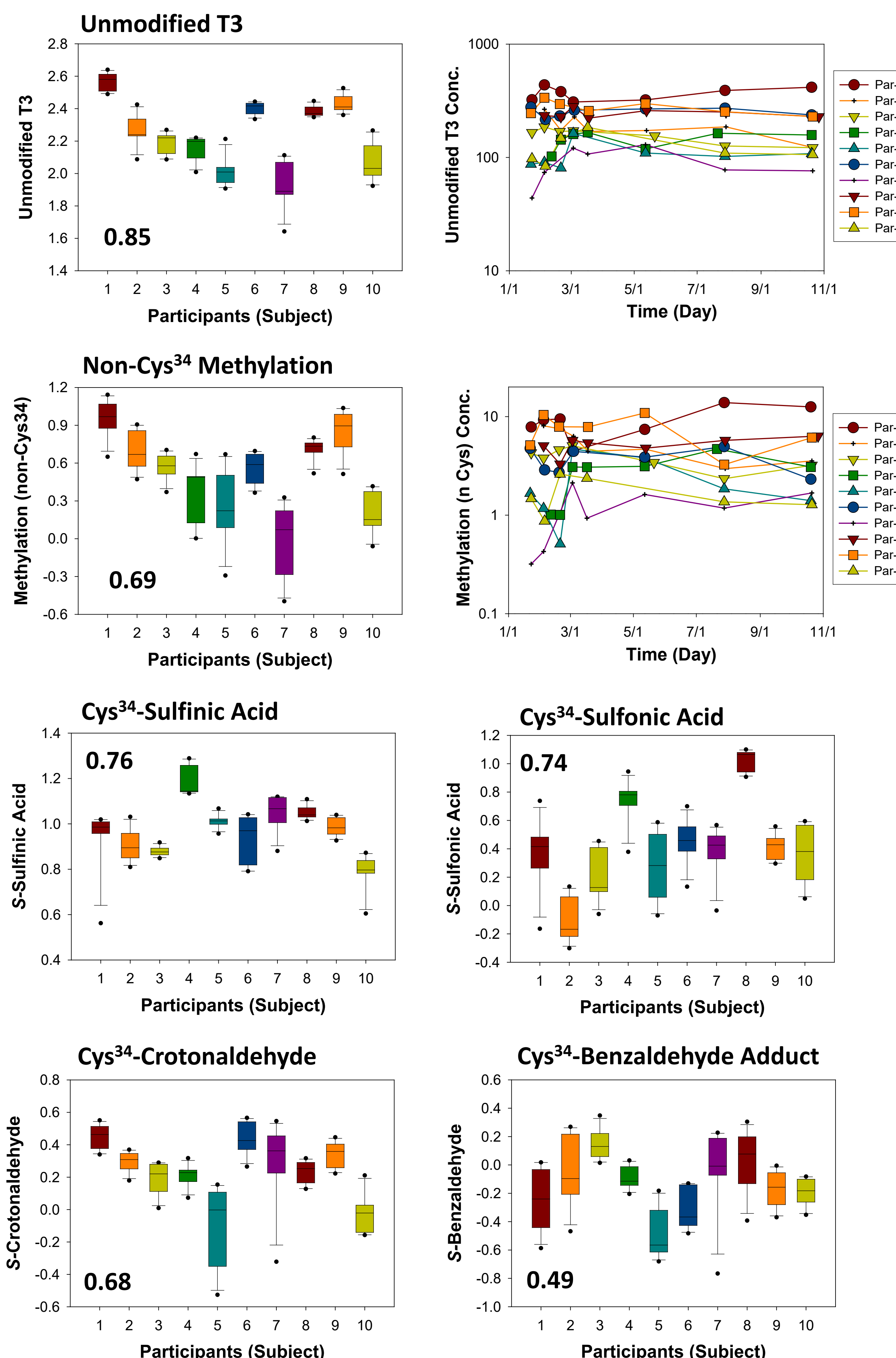*Yeunook Bae[1], Nathan T. Montgomery[1], and William E. Funk[1]*

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Motivation

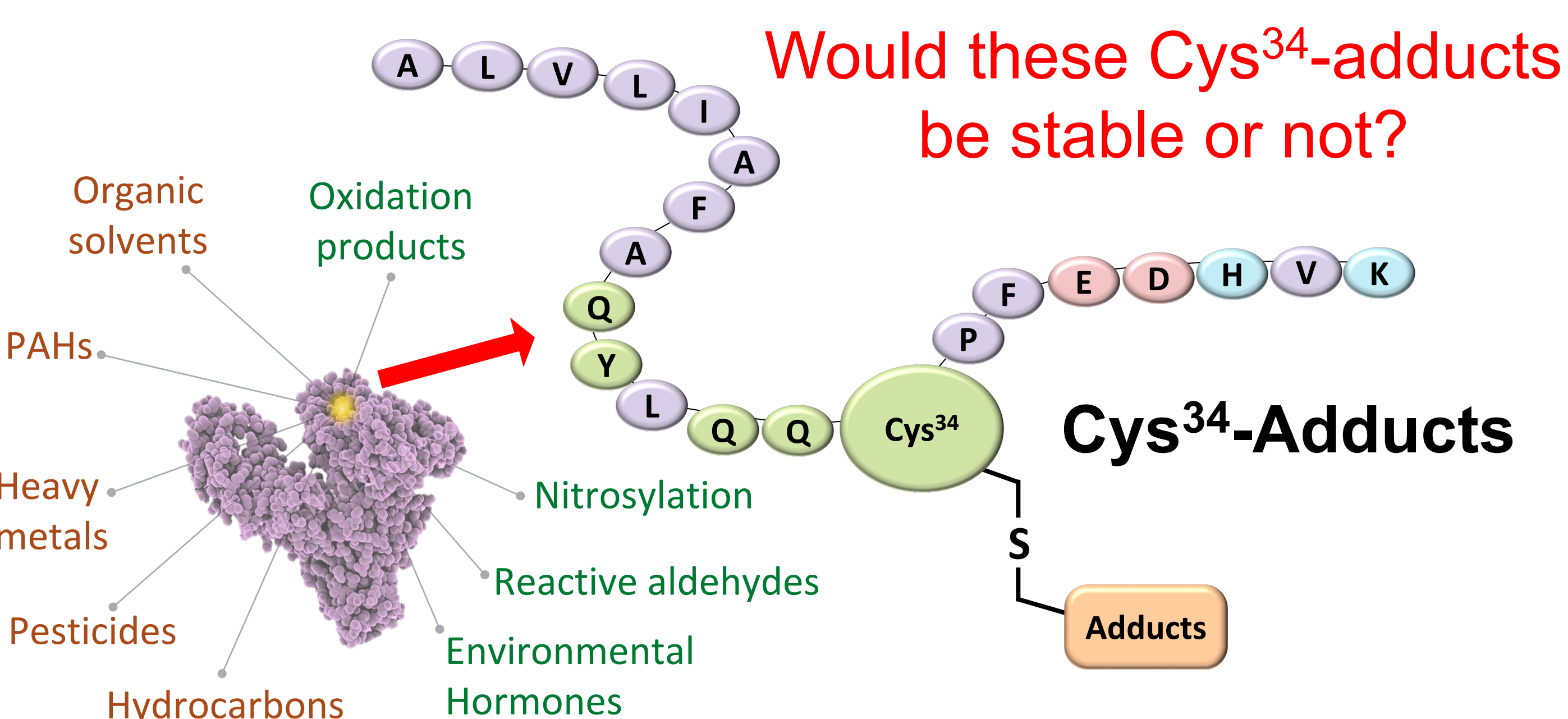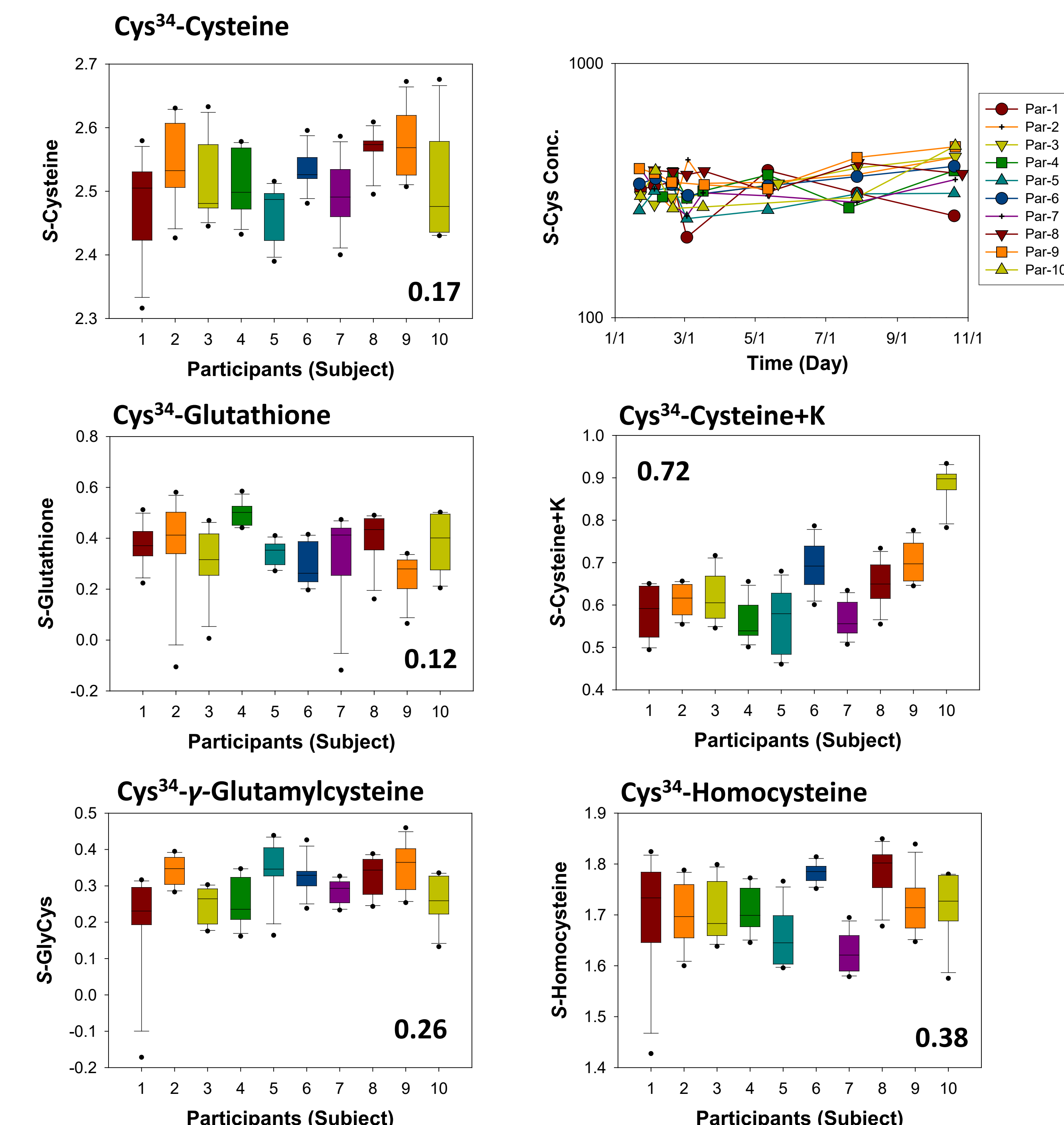- Cys^{34}-adducts in the blood can be used as biomarkers for testing potential adverse health effects and environmental exposures.

- Because repeat blood sampling is not possible, blood samplings can be taken only limited numbers from patients.

- It is important to understand the stability of the Cys^{34}-adducts' levels in repeat measurements from patents during a long-term period.

- To understand the stability, intraclass correlation coefficient (ICC) value for each Cys^{34}-adduct was obtained.

Would these Cys^{34}-adducts be stable or not?

Organic solvents, Oxidation products, PAHs, Heavy metals, Pesticides, Hydrocarbons, Nitrosylation, Reactive aldehydes, Environmental Hormones

Cys^{34}-Adducts

ICC Calculation & Experimental Methods

What is ICC and How to Calculate?

- Between-subject variance (σ_b^2) and within-subject variance (σ_w^2)

  ➤ ICC close to 0: Repeated measurements from a certain subject tends to take any values across the range of distribution.

  ➤ ICC close to 1: Repeated measurements tend to stay similar values in particular subjects.

- ICC calculation: Analysis of variance (ANOVA) by Microsoft Excel.

Plasma Samples → Tryptic Digestion → QQQ-MS Analysis

HSA → Cys^{34}-Adducts → Data
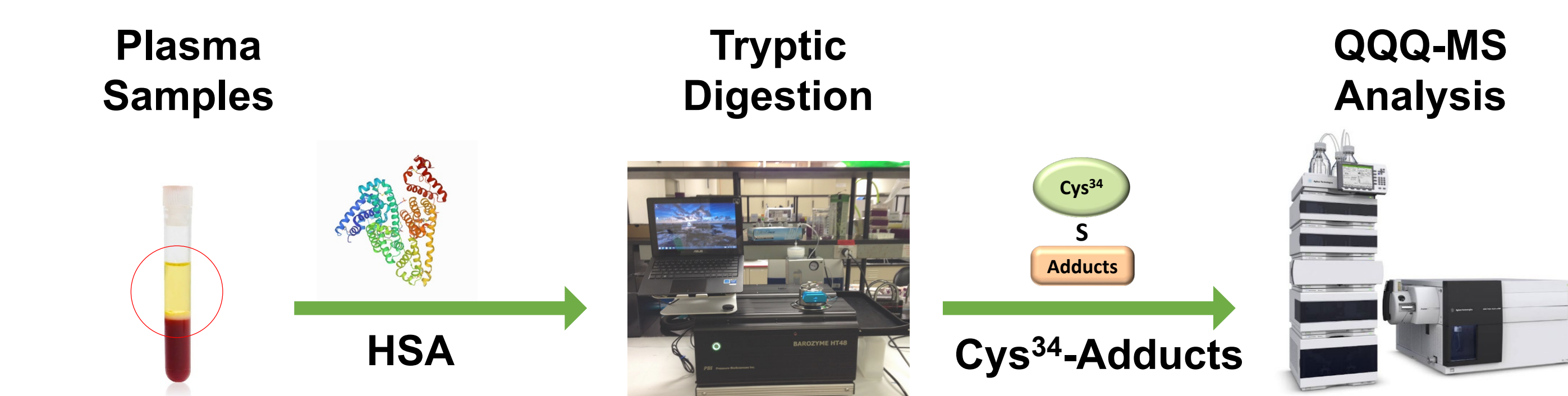
Sample Collection

- Plasma extracted from venous blood samples.

- Samples taken from 10 female volunteers: from each volunteer over a year during 2018.

$$ICC = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_w^2}$$

Sample Pretreatment & Analysis

- Pretreat plasma with methanol to extract human serum albumin (HSA).

- Digest the HSA with trypsin.

- Analyze Cys^{34}-adducts using triple-quadrupole mass spectrometry (QQQ-MS).

Results & Discussion

Adducts with High ICC

- Unmodified T3 and methylated adducts on non-Cys^{34} peptide, direct oxidation products (i.e., Cys^{34}-SO$_2$H and Cys^{34}-SO$_3$H), and aldehyde adducts (i.e., crotonaldehyde) showed high ICC.

- Single-time point measurements are robust: Represent participants' long-term exposure of toxic matters.

What is Cys^{34}-Adducts?

- ✓ Cys^{34} is one of the amino acids which are the parts of protein (peptide bond) in the human blood.

- ✓ Due to the thiol side chain, Cys^{34} is strongly nucleophilic, which is highly reactive with the toxic electrophiles, such as environmental contaminants.

- ✓ By reacting with electrophiles, Cys^{34} can transform into adducts, which is Cys^{34}-adducts!

Adducts with Low ICC

- Cysteine-containing small thiol Cys^{34}-adducts (e.g., adducts of cysteine, γ-glutamylcysteine, or glutathione) or Cys^{34}-homocysteine have low ICC values.

- Adducts with low ICC have a narrow range of variation.

- Since the average among participants is similar, the samples would become good control group in terms of these adducts.

- e.g., High adduct levels from certain subjects can clearly distinguish from those of other subjects.

Conclusion

- This study used repeat sampling over a year to evaluate within- vs between-person variance of individual protein adducts using ICC.

- Several important Cys^{34}-adducts had high ICC values, suggesting single-spot measure provide robust estimates of exposure when repeat sampling is not possible.

- To understand average and population variability of certain adduct level of control group, low ICC can become a valuable tool.

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