Measurement of number of low molecular weight compounds labeled to proteins

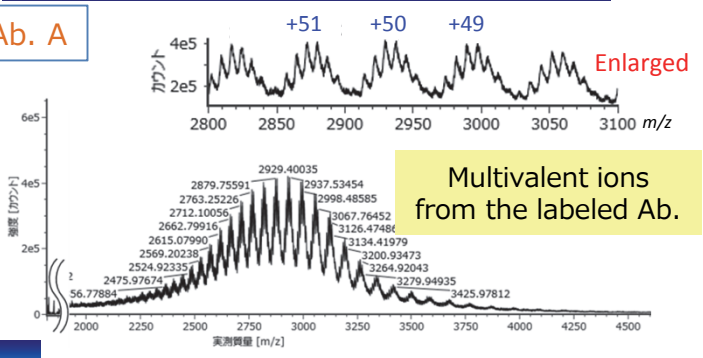
In the field of diagnosis, etc., it is often required to evaluate how many compounds are covalently labeled per molecule of the protein. Here we introduce examples of how many FITC dyes were labeled in the antibody. This measurement is also applied to analysis of antibody–drug conjugates (ADCs).

Preparation of FITC labeled sample

1. FITC labeling reagents were added with a molar ratio of 50-500 times to two commercially available antibodies
2. Labeled at room temperature for 1 hour
3. Removal unreacted reagents by ultrafiltration
4. PNGase F processing
5. Molecular weight measurement by LC-MS (Q-TOF MS)

Mass spectrum of FITC–labeled antibody

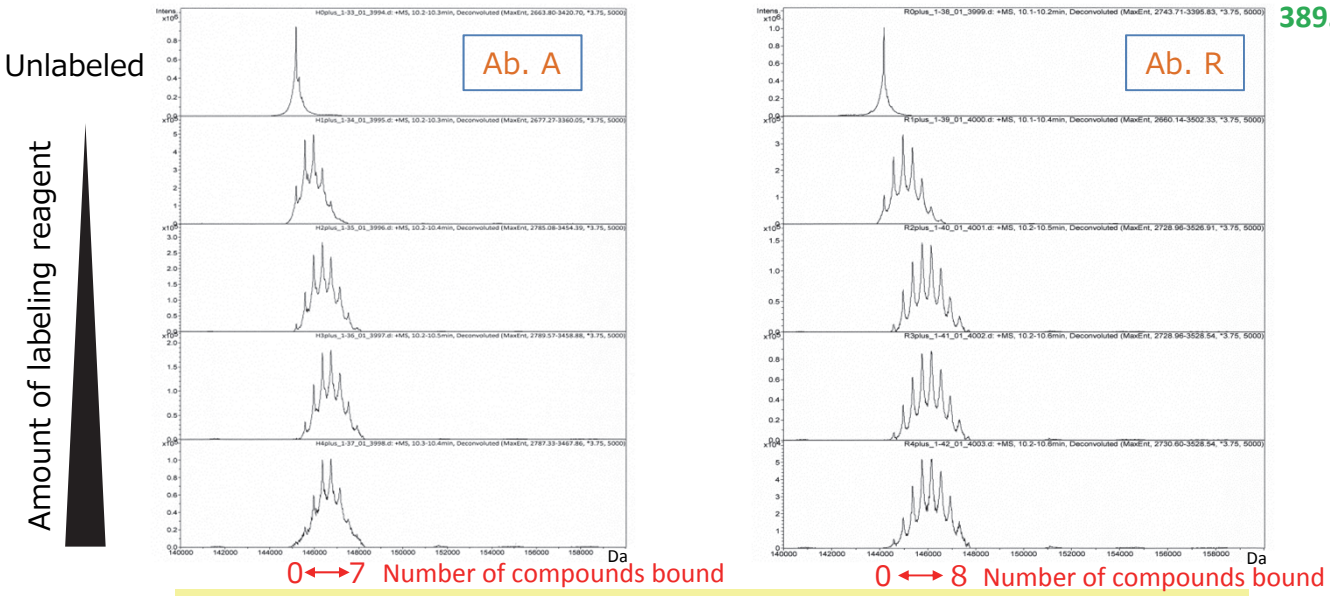
Ab. A



Multivalent ions from the labeled Ab.

Result of molecular weight analysis by deconvolution

Molecular weight changed by FITC labeling: 389.4 Da



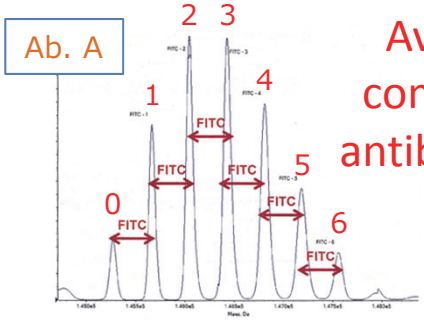
0⟷7 Number of compounds bound

0 ⟷ 8 Number of compounds bound

Labeled antibodies were clearly observed for each labeled number

Calculation of the compound/antibody rate

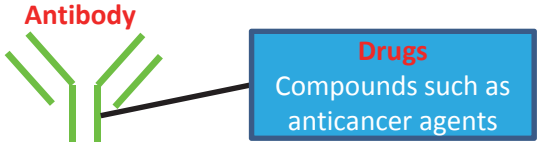
Calculation of the averaged compound/antibody rate from the each peak area after background subtraction



Averaged compound/ antibody rate: 2.7

Antibody–Drug Conjugate (ADC)

- One of the next generation antibody-pharmaceuticals
- Having binding specificity by antibody and high activity by drug



This measurement system can also be applied to analysis of ADC