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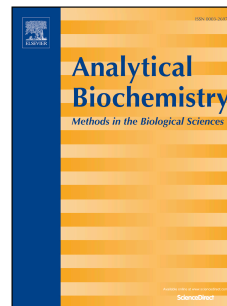

Adapting the chemical unfolding assay for high-throughput protein screening using experimental and spectroscopic corrections

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ACCEPTED MANUSCRIPT

Adapting the Chemical Unfolding Assay for High-Throughput Protein Screening Using Experimental and Spectroscopic Corrections

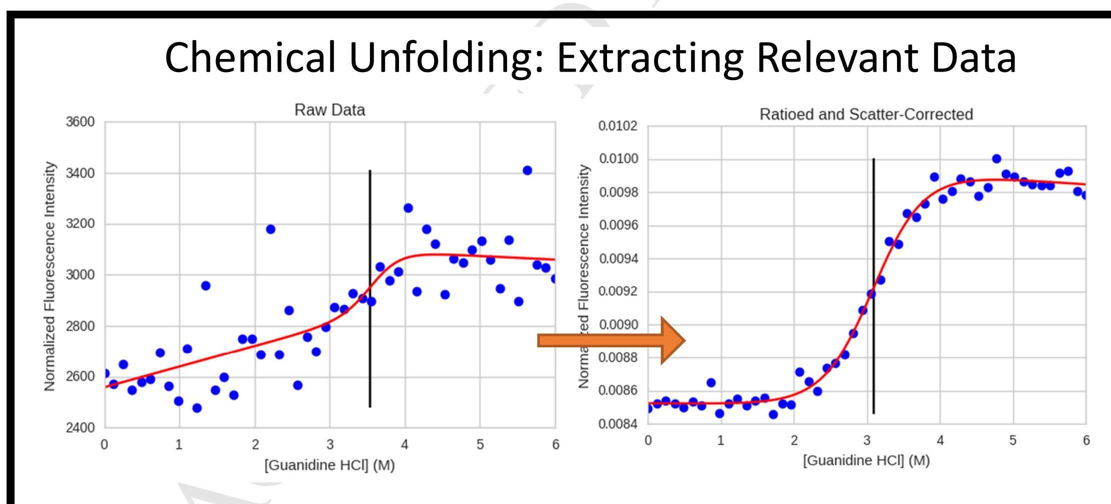
J. Alaina Floyd[a*], Christine Siska[a], Rutilio H. Clark[a], Bruce A. Kerwin[a], and Jeremy M. Shaver[a,b*]

[a]Just Biotherapeutics, Inc. 401 Terry Ave N., Seattle, WA, USA 98109
[b]Corresponding author, jeremy.shaver@just.bio
[*]These authors contributed equally to this work

Abstract

The chemical unfolding (denaturation) assay can be used to calculate the change in the Gibbs free energy of unfolding, ΔG, and inflection point of unfolding, to collectively inform on molecule stability. Here, we evaluated methods for calculating the ΔG across 23 monoclonal antibody sequence variants. These methods are based on how the measured output (intrinsic fluorescence intensity) is treated, including utilizing (a) a single wavelength, (b) a ratio of two wavelengths, (c) a ratio of a single wavelength to an area, and (d) a scatter correction plus a ratio of a single wavelength to an area. When applied to the variants, the three ratio methods showed comparable results, with a similar pooled standard deviation for the ΔG calculation, while the single-wavelength method is shown as inadequate for the data in this study. However, when light scattering is introduced to simulated data, only the scatter-correction area normalization method proves robust. Using this method, common plate-based spectrophotometers found in many laboratories can be used for high-throughput screening of mAb variants and formulation stability studies.

Graphical Abstract



Keywords

Chemical unfolding; high-throughput; antibody; denaturation; fluorescence; scatter correction; equilibration time

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