BCA-no-more: high throughput protein quantification directly on S-Trap plates

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Introduction
Recent advances in the throughput of analytical proteomics, now often requiring only minutes per sample for identification and quantification, necessitate concomitant advances in bottom-up sample preparation workflows.

Specifically, the simplification and ideally elimination of any extraneous steps in a sample preparation workflow increases both throughput and robustness.

Post lysis, one of the most standard steps preceding sample preparation, is a protein concentration assay, often via BCA due to its compatibility with detergents such as SDS. However, all assays, including BCA, are not instant, are subject to interference and, if performed in a 96-well plate, edge effects.

Here, we demonstrate the new concept of direct quantification of cleaned, surface-bound protein on S-Trap 96-well plates using intrinsic protein fluorescence.

Method Parameters
The optimal z-position (2100 µm) for fluorescence protein concentration quantification on the S-Trap 96-well plate using a Tecan Sparc was experimentally determined on bound protein.

When provided with digestion buffer (e.g. 50 mM TEAB at pH 7.5), fluorescence tracked with protein load at 277 nm excitation and 350 nm emission; fluorescence was quenched as buffer pH became acidic, eventually reaching near-background levels.

As expected, given how the S-Trap (and other solid phases) load, there was a reasonably linear response up to ~100 µg per well. After this, the linearity of response dropped significantly, a phenomenon attributed to fluorescent moieties binding deeper in the trap, which is not UV transparent.

The direct-determination method afforded protein quantification in a significantly reduced time compared to BCA assays with dynamic range and sensitivity compatible with standard bottom-up and top-down proteomics workflows.

Equivalence to BSA
Protein quantification via an on-plate fluorescence reading was of comparable accuracy to in-solution BCA of the same sample without necessitating sample loss, incubation or even further manipulation than loading onto the S-Trap 96-well plate.

Background fluorescence readings of protein-free trapping matrix decreased with increasing emission wavelength. At 277 nm excitation and 410 emission, it was possible to detect as little as 1 µg of protein. Such small quantities are virtually impossible to quantify by colorimetric assays yet are frequently encountered in laser capture micro-dissection.

Equivalence to In-Solution Fluorescence
The S-Trap sample preparation workflow successfully removed matrix contaminants prior to protein concentration determination without the need for additional steps.

Such on-plate protein concentration determination lends itself directly to deployment in high-throughput clinical settings using automated fluid handlers.

Here we demonstrate the equivalence of this technique on-plate compared to in-solution fluorescent measurements on the same sample; both curve fits and CVs are comparable.

Conclusion
This patent-pending “BCA-no-more” direct determination of protein concentration with intrinsic clean-up removes the need for additional costly protein quantification assays and fits directly in automated sample preparation workflows.

A typical BCA kit costs ~$US200 – $US300 and might take an experienced operator 30 - 60 min, depending on the number of samples, replicates, etc. Here, we remove the need for both an additional kit and sample sacrifice, extend sensitivity and quantification to the single µg level out of any kind of lysis buffer and do so in a format amenable to high-throughput automation.