

High Abundance Proteins: Proteomer's Thorns in the Flesh?

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Editorial

Identification of low-abundance proteins is one of the major challenges in Proteomics because of the high dynamic range of the protein concentration in the biological samples [1]. Therefore, one of the prime objectives of the proteomers is to reduce the dynamic protein concentration range to shed a light on the "low-abundance proteome" or sometimes referred as "Hidden Proteome" [2]. The development and utilization of latest mass spectrometers have increased the sensitivity of the protein identification, fostering the identification of proteins present in extremely small amounts (up to attomoles, in isolation) [3]. However, the identification of low-abundance proteins during the analysis of whole cell/tissue proteome is still not achievable without any prefractionation of the samples [4]. One of the major reasons of this limited resolution, during total proteome analysis, is the presence of high-abundance proteins which occupies a major portion of the cell/tissue proteome. Albumin in blood [1], RuBisCO in green leaves [5], and storage proteins in seeds, tubers, and roots [6] are some of the common examples of the high-abundance proteins in the biological samples. These high abundance proteins are products of the genes which are present in large copy numbers and inevitably impede the identification and characterization of low abundance proteins. Biomarkers or signaling/regulatory proteins are generally low-abundance in nature and are masked by the presence of these abundant proteins which are present in numbers 10^5 - 10^6 copies per cell [2,7,8]. As an example, 22 most abundant proteins, in the human blood plasma, constitute approximately 99% of the total plasma proteome with low-abundance proteins present as only 1% [9]. Because of the presence of these high-abundance proteins, proteomics studies majorly ends up with the repeated identification of different components of these abundant or house-keeping proteins and thus actual information of the biological phenomenon remains concealed. The possibility to look beyond these abundant proteins or go further lies on the successful enrichment and identification of the low-abundance proteins which are present less than 100 copies per cell [7].

Several methods have been developed in the last two decades to enrich and identify the low-abundance proteome [2,8]. Most of these methods, if not all, depend on the specific depletion of high-abundance proteins, thus enriching the low-abundance proteins in the remaining fraction [10,11]. These depletion based methods utilize different chemicals [12,13], solvents [14] or antibodies against the target proteins [15] to efficiently remove the high-abundance proteins from the total protein samples. Previous reports have shown that depletion of albumin from plasma [15], RuBisCO from leaf [5,12,13] and storage proteins from seed [16,17] and tuber [14] samples lead to the significant increase in the proteome coverage and identification of low-abundance proteins. However, although the precipitation based methods are rapid and cost-effective, there is always a risk of loss of some non-target proteins that might precipitate along with the depleted target proteins. Previously, it was shown that depletion of albumin also removes low-abundance cytokines from the human plasma [18]. As these low-abundance proteins are of biological interest, non-specific depletion of these could result in potential loss of biological information.

In addition to these depletion based methods, hexapeptide ligand

library (available as a trade name of "ProteoMiner", BioRad), has also been proved to be highly efficient in reducing the dynamic protein concentration range of different biological extracts from both plants and animals [19-21]. This technique utilizes hexapeptide ligand libraries for capturing proteins where different ligands are capable of binding different proteins. As the library consists of the equal amount of each ligand, it can lead to the enrichment of low-abundance proteins when total protein extracts are applied on the columns under overloading conditions [19,22].

However, as these prefractionation techniques involve an additional step, these could potentially affect the reproducibility of the obtained data, leading to the preanalytical bias [23,24]. Increasing the number of biological and technical replicates per sample can reduce the preanalytical bias. Moreover, analysis of both the high-abundance protein depleted and enriched fractions can further help in reducing the preanalytical bias. Overall, these methods have been proved fruitful in the removal of the high-abundance proteins, which are the major hurdle in the identification of low-abundance proteins and utilization of these techniques, are, therefore, highly recommended for increasing the proteome coverage of the biological samples.

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