



ProFACT Proteomics, Inc.

Technology Centre of NJ, CCIT
 675 U.S. Highway One
 North Brunswick, NJ 08902
 732-246-1190 · f 732-246-3118
 www.profactproteomics.com

Proteins are the principal active engines of biology. Since proteins are subject to post-translational modifications, protein-protein interactions, and changes in expression patterns, proteomics applications are more comprehensive in scope than genomics. For these reasons, protein science may shed more light on cellular responses to environmental changes and stress conditions than DNA-based applications.

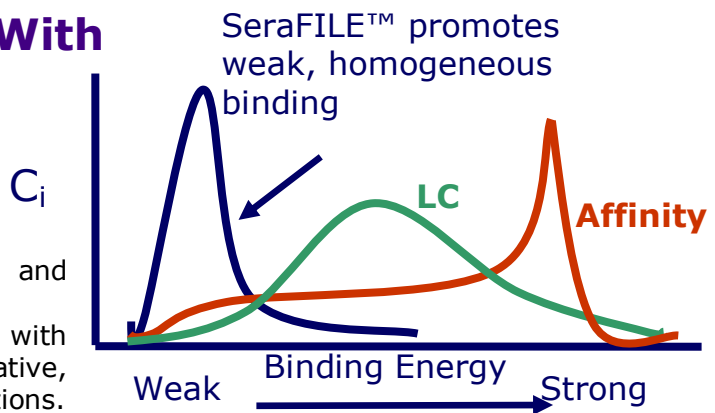
Global protein analysis can be problematic using traditional electrophoretic profiling techniques. High abundance proteins may hamper the resolution and detection of those present at lower concentration. Because of this, many researchers employ a sample fractionation method prior to profiling efforts. A successful protein pre-fractionation scheme separates proteins based on their physio-chemical properties and is amenable to a number of downstream analysis applications. Various chromatographic methods can be used in combination with two dimensional gel electrophoresis (2DGE) or mass spectrometry^{3,6}.

ProFACT Proteomics' multi-disciplinary team of scientists have invented and characterized a novel proteomic profiling tool. SeraFILE™ (patent pending) is a surface library for sorting proteins for differential analysis. The SeraFILE™ surface library is not based on conventional liquid chromatography stationary phases. Whereas conventional LC suffers from a heterogeneous mix of binding energies, and Affinity has exceedingly high binding energy, SeraFILE™ promotes weak, homogeneous binding - ideal for proteomic investigation. Selectivity modulation comes from the structural morphology and spatial distribution of immobilized electrolytes, thus generating protein pools with both differential constituents and activity states.

Molecular Profiling With SeraFILE™

The SeraFILE™ surface library provides:

- A modality that is open-ended and industrially productive.
- Reduced protein complexity with subsequent maintenance of native, biologically functional conformations.



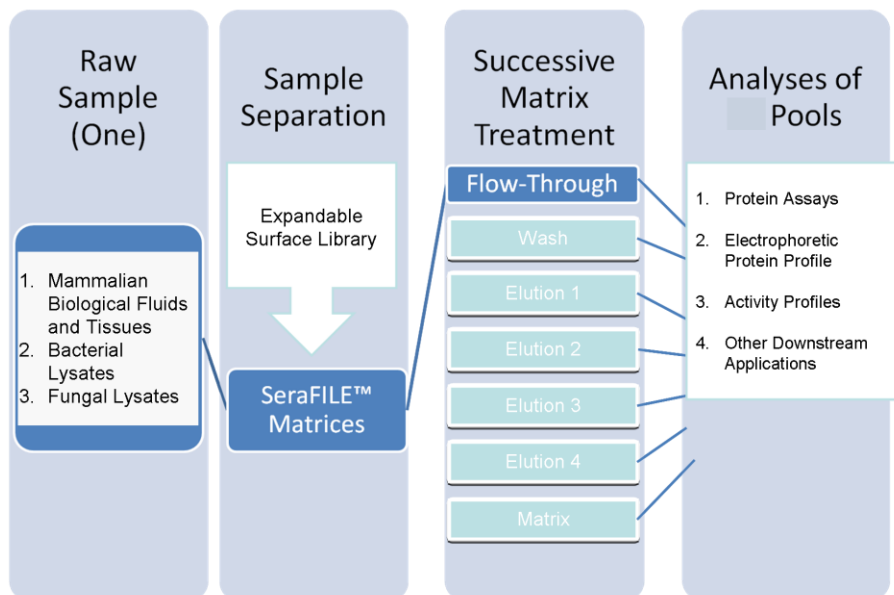
- New profiling techniques which generate signatures across a multiplicity of sub-proteomes and interrogations.

- A means to characterize enzyme regulation and related functional sub-states from disease.
- Discovery strategies that enrich catalytic activity and directly couple to drug development.

The SeraFILE™ Technique

Our high throughput SeraFILE™ protocol generates biologically active sub-proteome pools with a lesser degree of complexity than the parent protein sample. Thus, dissecting the proteome prior to downstream analyses allows researchers to profile and study proteins in new ways. ProFACT's SeraFILE™ surface library affords high-resolution partitioning of complex protein extracts into differential sub-proteomes that can be further characterized using a variety of downstream applications. Collectively, SeraFILE™-derived protein profiles offer a more comprehensive signature of the starting sample. SeraFILE™ can fractionate proteins and thus signatures may reveal discrete differences in low abundance proteins across samples. It is important to note that no high abundance proteins are removed from the sample prior to fractionation; they are distributed across sub-proteomes. Therefore, SeraFILE™ facilitates detection of disease-specific differences in clinical proteomics applications. As an example, preliminary molecular profiling data support clear protein degradation pattern differences in the Ubiquitin Proteasome Pathway of patient matched cancer samples.

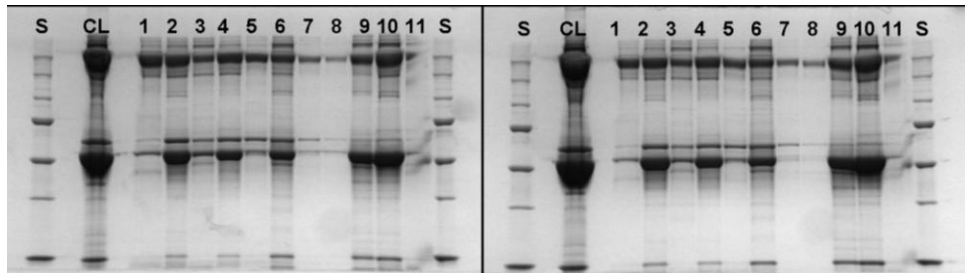
SeraFILE™ is a rapid, environmentally-friendly method that reduces protein pool complexity while maintaining native proteins that are still functionally active. These protein pools can then be profiled by traditional electrophoretic or bioassay methods.



SeraFILE™ is a proteomic fractionation process that results in numerous native protein pools per sample. Sample preparation using SeraFILE™ is minimal. Protein lysates or serum samples are applied and bound to the SeraFILE™ surfaces and subjected to a mild, consecutive, multiple elution scheme. Any unbound protein is collected in the first fraction (FT) and the matrices are washed (fraction W) prior to elution. The resultant eluates, designated E1, E2, E3, and E4, are then analyzed by a variety of methods. Any protein remaining on the matrix, designated B, is also biologically active. A 'fingerprint' of the sample, comprised of profiles of all native protein pools, can be a predictive tool for further enzyme enrichment or purification applications.

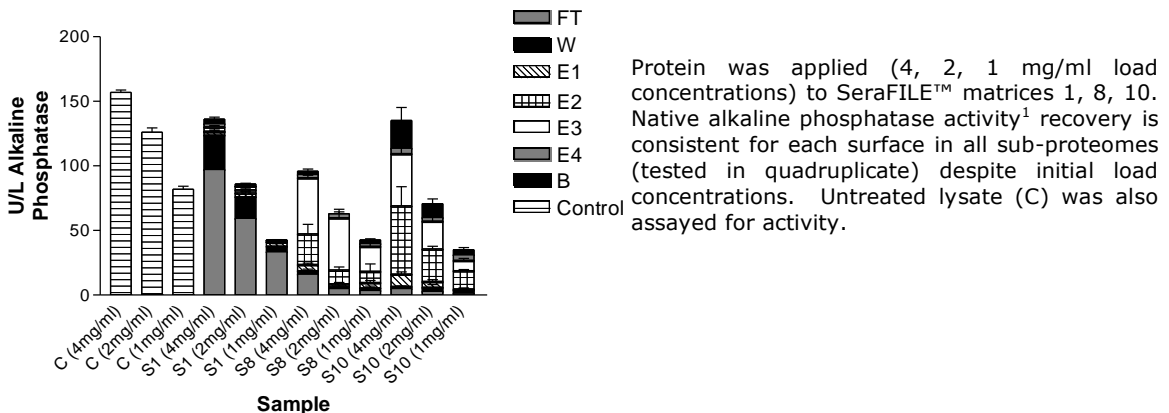
SeraFILE™ may be used to profile proteins derived from any source. Samples of particular interest at ProFACT are biofluids that may provide insight into the diagnosis of human disease. For instance, serum and specific tissues are a source of overexpressed and/or modified proteins unique to the disease state. Thus, these biofluids are desirable targets for biomarker discovery and therapeutic applications. However, current serum proteomics applications have a suite of limitations, including the following 1) many protocols used in serum biomarker research purposely discard abundant serum proteins 2) discarded proteins may bind diagnostically relevant proteins in a given sample and 3) finding a single protein biomarker representative of the diseased state has proven difficult^{2,4,5}.

SeraFILE™ may overcome these challenges in numerous ways. Certain surfaces remove albumin while others leave it intact, so all proteins in a serum sample can be resolved in the presence/absence of abundant serum proteins. Since proteins profiled by SeraFILE™ are still functionally active, they can be analyzed by a variety of downstream methods. The utility of SeraFILE™ allows for a more comprehensive understanding of the serum proteome in both the normal and diseased state.



SDS-PAGE gels of separate SeraFILE™ treatments using sheep serum demonstrate the high degree of reproducibility afforded by SeraFILE™ in a 96 well format. Replicate E2 samples for all eleven surfaces are shown. Note that several fractions are albumin-depleted compared to the control lysate. Using a combinatorial approach, surfaces may be mixed and matched to easily enrich for a protein of interest.

Sample heterogeneity is a widely recognized problem in clinical proteomics. SeraFILE™ is well suited to address the complex normalization issues associated with tissue/tumor proteomics applications. SeraFILE™ may circumvent an intrinsic standardization problem in clinical proteomics; relative differences across samples can be interpreted despite any disparities in the starting material (i.e. total tissue weight and/or total protein analyzed). In serum studies, it was experimentally determined that the total protein applied to the SeraFILE™ surface library can vary by up to 4X, while enzymatic activity profile patterns of native alkaline phosphatase are consistently observed.



The SeraFILE™ Difference

- SeraFILE™ is an automated proteomic profiling system that generates protein pools that retain biological activity.
- Different protein profiles are observed after treatment with each individual matrix. When all matrices are used in combination, numerous sub-proteomes are generated. These protein pools are amenable to a variety of downstream analyses.
- Low abundance proteins can be unmasked using SeraFILE™ profiling technology.
- High abundance proteins, like albumin, can be removed in certain SeraFILE™ sub-proteomes. It is important to note that SeraFILE™ technology does not discard any proteins in a given sample; all protein can be accounted for in either the FT, W, E1-E4, or bound to the matrix.
- Native enzyme activity can be retained and effectively profiled in SeraFILE™ sub-fractions. Despite initial protein load concentration, the distribution of protein and enzyme activity recovered from the matrices in each fraction is the similar for each surface tested. Load concentrations may vary by up to 75%, and a reproducible pattern of total protein and activity recovery is observed in each matrix.
- Clinical proteomics applications are encumbered by standardization protocol difficulties, so samples may not be adequately compared. For example, biopsies of tumor cells often include some portion of the normal adjacent tissue or necrotic portions with no cellular activity. These problems, inherent in tissue based proteomics, may be by-passed by profiling with SeraFILE™ because within a given range, the amount of protein applied to the SeraFILE™ matrices does not appear to affect the proteomic profile generated. This would imply that normalizing to tissue weight or total soluble protein would not be a factor in the experimental outcome.

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Authors: Meghan Tierney, PhD., Senior Scientist, and Swapan Roy, PhD., Chief Scientific Office, may be contacted at the offices of ProFACT Proteomics, 732-246-1190 or mtierney@profactproteomics.com.