New Chemical Proteomic Methods To Access Drug-Protein Interactions

Swapan Roy, Ph.D., Matthew Kuruc, Krishna Patel
Biotech Support Group LLC., 1 Deer Park Ave, Suite M, Monmouth Junction NJ 08852 USA
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Functional Proteomics

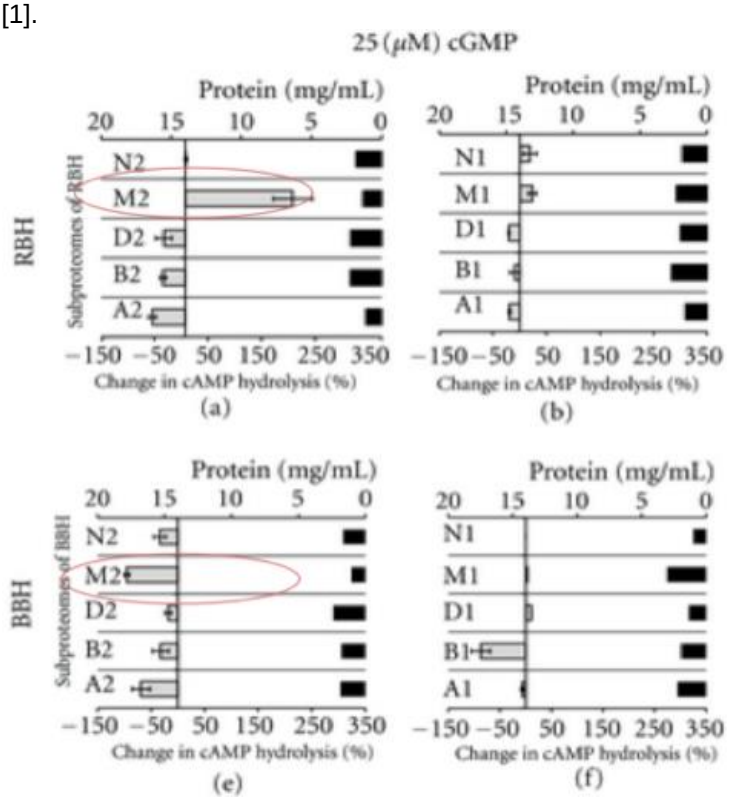
Functional proteomics relies in part, on the functional or structural features of intact, non-denatured proteins. While the terminology can often overlap, chemical and affinity-based proteomic profiles can be considered a subset of functional proteomics. Thus, functional proteomic annotation may complement conventional sequence annotation while supporting the study of mechanism of action and drug promiscuity. Furthermore, the subtleties of protein attributes, when the same or similar underlying sequence can have multiple conformations and functions, and when different sequences sometime perform the same or similar functions, are now open to investigation.

New Functional And Chemical Proteomic Strategies

A new functional proteomics separations toolset based on the **NuGel**™ platform, can be used in unrestricted workflow strategies, so as to sift through these biological complexities. As an example, Oka, et al, describes how it is used to efficiently produce unique subproteomes, with resulting enzyme measurements integrated into molecular profiles. The results demonstrate that the proposed methods provide a means to simplify inter-sample differences, and to enrich protein markers attributable to sample-specific responses [1].

Molecular profiles differ under a competitive small compound challenge. Plotted are the relative changes in in cAMP hydrolysis activity of each derivative subproteome in the presence of the competitive substrate cGMP.

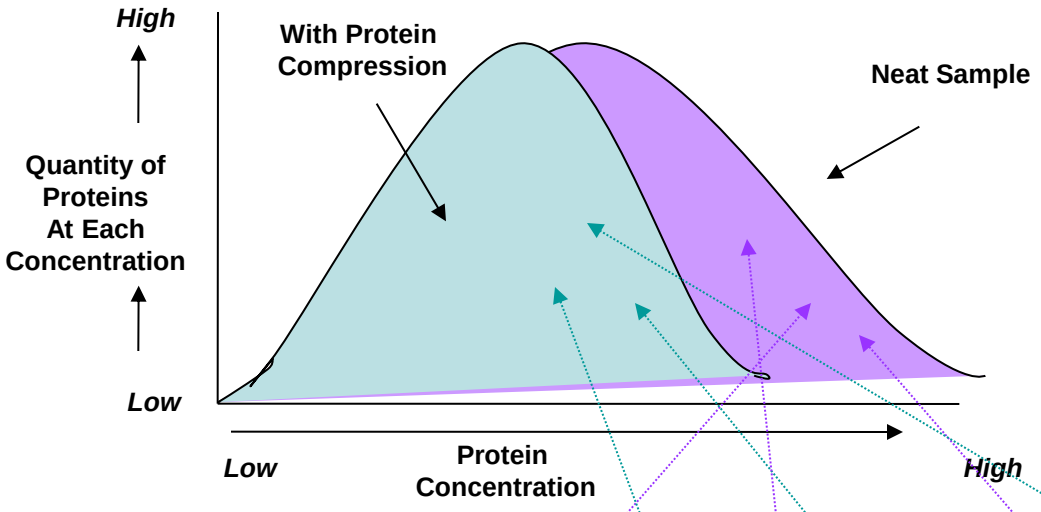
Encircled are the enriched sample-specific catalytic responses that which distinguish the two samples; RBH (rat brain) & and BBH (bovine brain).



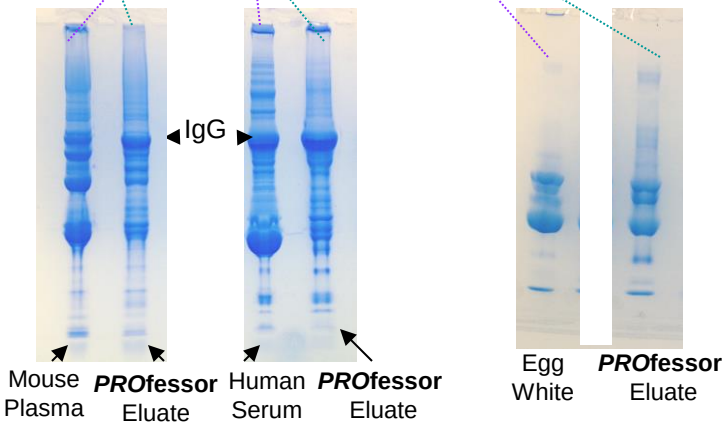
The **NuGel**™ ***PRO*spector**™ kit can be applied to functional proteomics applications. Included in the kit are 6 mixed mode surface chemistries to efficiently produce up to 12 differentiated sub-proteomes (6 eluates & 6 flowthroughs) with uncompromised functional and structural integrity. No specialized instruments or HPLC is required and its derivative sub-proteomes seamlessly integrate with virtually all functional, structural or enzymatic interrogation reporting methods.

The **NuGel**™ ***PRO*fessor**™ is a composite mixture of the same 6 mixed mode architectures supplied with ***PRO*spector**™, designed to both compress protein concentrations from highly complex proteomes, and to immobilize protein content with weak affinity. Both of these unique features are described in the investigations that follow.

Many methods have been commercialized to reduce the dynamic range of protein concentrations within complex mixtures [2]. A new method is proposed here, based on the protein compression features of ***PRO*fessor™**. The theoretical basis for protein compression in the composite ***PRO*fessor™** material is that each singular surface has its own complement bias. The highest abundance proteins will complement to some but not all surface chemistries. Under saturation overload conditions, the highest protein concentrations will be reduced due to competitive displacement effects. Each of the 6 surface ligand architectures has sufficiently different bias in its complement proteome so as to compress the overall protein concentrations relative to their starting concentrations. This is illustrated by the graph below.



Protein Compression
Methods are described on page 3. These SDS-Page profiles show that major high abundance bands are greatly reduced. The low abundance bands are enriched relative to their high abundance bands, visually estimated to be 5-10X for most bands. Because of its heterogeneity, the IgG band remains in high abundance. The protein compression appears to be both tissue and species agnostic



Chemical Proteomics

Chemical proteomics is a term encompassing a range of methods to derive profiles that can identify and characterize proteins that interact with small molecules of synthetic or natural origin. These methods are used to elucidate drug mechanism of action, as well as gauging on-target/off-target specificity [3-9]. The field to date has primarily relied on two methods: activity-based protein profiling (ABPP) and compound-centric chemical proteomics (CCCP) [3-9]. ABPP employs specially designed small molecule probes that covalently attach to active sites of their targeted protein. Such probes require conserved active sites and detailed structural information. CCCP is an affinity chromatography method whereby the small molecule compound is covalently immobilized to a solid support, and incubated with a protein lysate to pull down the interacting proteins. However, as therapeutic compounds exhibit their specificity due to their unique spatial orientation within the protein complex, surface immobilization can compromise efficiency, leading to false specificity and high background binding. High background can also come from low-affinity interactions with high abundance proteins [7,8]. Finally, some compounds are simply not easily immobilized.

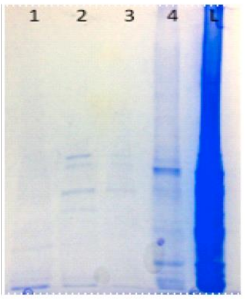
Weak affinity chromatography has recently been proposed as an efficient approach to access the relative binding interactions of compound libraries and drug fragments (from fragment-based drug design), towards an immobilized protein target [10,11]. As proteins are in constant transition between conformationally distinct states [12], ligand induction to a more energetically favorable conformational state can displace weakly bound proteins after compound interaction. We report here that by applying a similar strategy as was reported for pure proteins weakly immobilized, drug interactomes from complex proteomes using the composite ***PRO*spector™** material can be isolated. We call this method compound-centric displacement proteomics (CCDP).

In our work here, we report how compound-centric displacement proteomics (CCDP) can be used to efficiently profile small molecule compounds as protein displacing agents from surface architectures that support weak affinities of complex proteomes. The advantage of this method is that solution phase compounds are not subject to orientation biases. Furthermore, it can be applied towards any compound as it does not require any chemical or tag modifications. As compound concentrations can be varied, some measure of relative binding affinities between compounds can be characterized. Finally, when sufficient protein quantity is available, protein compression may help minimize bias towards the high abundance protein content and uncover low abundance interactions that otherwise would have been hidden.

Methods

Serum and Plasma were applied neat. Egg White was prepared at 4x dilution with PBS. The protocol outlined in the ***PRO*spector™** [13] was followed with the following modifications. Briefly, 100 µls of sample is conditioned 1:1 v/v by a binding buffer (0.1 M HEPES, pH 6), and applied to 25 mg of the dry powder composite of the 6 ***PRO*spector™** surfaces, in 500 µl volume micro-spin-filter tubes. After binding the composite material, ***PRO*fessor™**, was washed twice with 500 µl Wash buffer (0.001 M HEPES pH 7).

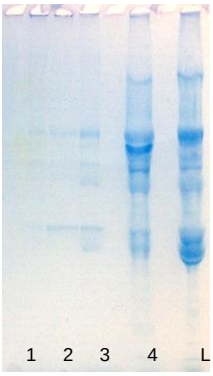
Lane 1 Imatinib
Lane 2 Caffeine
Lane 3 Wash
Lane 4 Eluate
Lane L Load (Rat Brain Homogenate)



For Protein Compression experiments (page 2), elution was with 100 Elution Buffer (0.2 M Tris, 0.5 M NaCl, pH 9.5). For Compound-centric displacement experiments (on right), 'Wash' elution was the same as Wash buffer, displacement challenge compounds Imatinib or Caffeine-Benzoate were at 10 mM, in the same Wash buffer; the Eluate buffer was as before.

Sample:
Clarified Mouse Plasma

Lane 1 Wash
Lane 2 Imatinib
Lane 3 Caffeine
Lane 4 Eluate
Lane L Load



Results

Compound-centric Displacement Proteomics (CCDP).

In the two examples on right, complex proteomes bind with weak affinities and are readily displaced in a compound specific manner. Different banding profiles in all 4 eluate fractions demonstrate that there are different displaced proteins attributable to the challenging compounds, in this case Imatinib (a kinase inhibitor) and Caffeine. The compound-centric sub-proteomes have greatly reduced complexity and differ in their band profiles. We expect that these same compound-centric sub-proteomes can be furthered characterized by LC-MS to identify and quantify protein content.

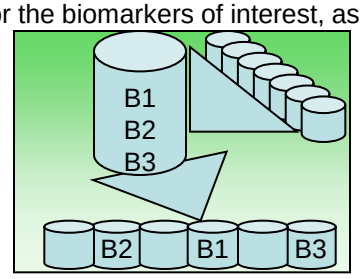
Discussion

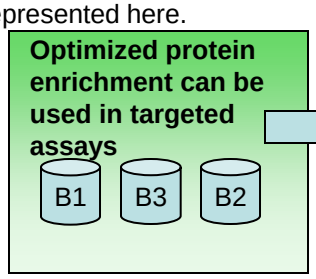
As consumables, both ***PRO*spector™** and ***PRO*fessor™** products feature:

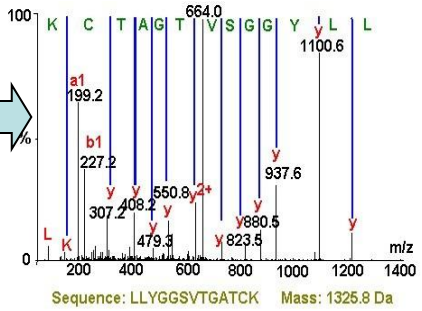
- Separations readily compatible with virtually all proteomic interrogations
- Microtube kit formats, simple bind/wash/elute protocols
- No specialized instruments, or HPLC required
- Disposable, no column regeneration
- Tryptic digestion or enzyme assay can be 'on-bead'
- Universal, species and tissue type agnostic

Once biomarkers are discovered, the composite materials of ***PRO*fessor™** can be deconstructed to ***PRO*spector™**, and optimized to enrich for the biomarkers of interest, as represented here.

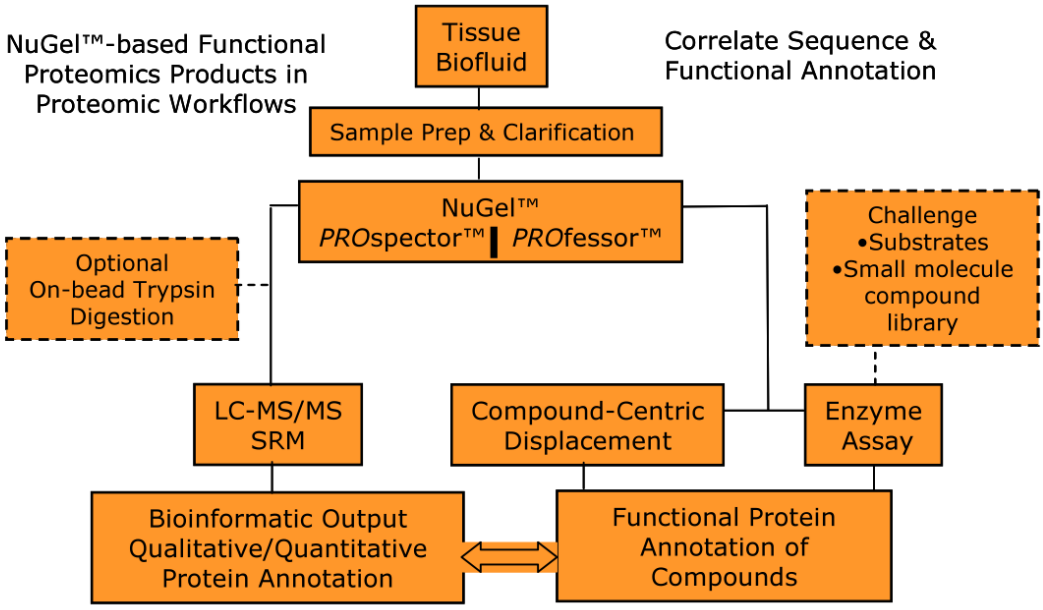
Biomarkers (B#) discovered in the composite materials, can be deconstructed/ optimized for target peptides/proteins







In the schematic below, we show how the two functional proteomics products, ***PRO*spector™** and ***PRO*fessor™** can be used in proteomic workflows to correlate sequence and functional annotation.



Functional proteomic profiles can help optimize drug candidates to tissue-specific expression of isoforms, gauge promiscuity, elucidate mechanism of action and identify biomarkers

Conclusions and Future Directions

We have shown that using unique combinations of **NuGel™**-based mixed mode architectures, two different sub-proteome content strategies can support functional and chemical proteomic comparisons. ***PRO*spector™** can:

•Efficiently produce up to 12 differentiated subproteomes with uncompromised functional and structural integrity
•Generate characteristic molecular profiles for comparison and discovery
•Enrich functional biomarkers for sequence and structural annotation

And ***PRO*fessor™** can:

•compress protein concentrations and enrich low abundance proteins, under saturation/overload conditions
•perform compound-centric displacement proteomics (CCDP)
•through deconstruction strategies, optimize biomarker enrichment

In the future, we look to consider ***PRO*fessor™** and protein compression in LC-MS workflows for discovery and SRM/MRM applications. Furthermore, we look to consider these same strategies using singular proteins as the displacing agent, for characterizing protein-protein interactions.

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