**Biomarker Discovery with the Megaplex Platform: Application in Chronic Kidney Disease**

Leonidas Alexopoulos, PhD

ProtATonce Ltd & National Technical University of Athens

[www.protATonce.com](http://www.protATonce.com)

**Abstract**

Biomarkers are cornerstones of healthcare spanning a wide range of applications from disease diagnosis to stratification of patient populations. The translational of a biomarker to clinical practice remains poor, averaging 1.5 approvals per year across all diseases. This inefficiency arises from limitations of current proteomic technologies as well as the single-biomarker-to-single-disease approach that does not capture the multifactorial nature of complex diseases. Diseases are multifactorial and can originate from diverse pathological mechanisms, while a pathological mechanism can lead to diverse diseases. Chronic kidney disease (CKD) is a major public health problem that affects approximately to 14% of the general population that requires asymptomatic, early-stage, and disease-specific biomarkers to deliver more precise diagnostic and predictive information. Here, we present a biomarker discovery scheme that combines experimental and computational tools. On the computational front, CKD gene expression data and knowledge-bases are employed to generate a CKD-specific model. This model is then analyzed with state-of-the-art network analysis and pathway analysis tools which results in a molecular profile that captures disease biology. This profile is next optimized using an iterative active machine learning approach by means of plasma samples which identifies relevant features that connect disease mechanism with endpoint candidate biomarkers. Approximately 100 targets were suggested that standard Luminex sandwich-ELISA assays cannot measure simultaneously due to detection antibody cross reactivity. On this front, a new antibody based scheme termed Megaplex has been developed that allows the screening of hundreds of antibodies. Megaplex is based on a modified dual-capture single-antibody or single-somamer scheme [Gold et al. PlosOne 2010, Ayoglu et al. Proteomics 2016, Zhou et al. Anal Biochem. 2010] offering improved enrichment through antibody-captured beads followed by on-bead biotinylation, elution, and transfer to Luminex beads. The new scheme bypass the cross-reactivity limitation of multiplexed secondary antibodies and allows the screening of hundreds of proteins simultaneously. When applied to CKD, our platform was able to identify a panel of novel biomarkers with ROC analysis that reach 95%. Further analysis of those biomarkers show very good correlation with the estimated Glomerular Filtration Rate (eGFR), a well-known biomarker in kidney function. Our network analysis approach was able to suggest biomarkers originated from underlying molecular mechanisms.