Mapping the residue positions of a protein's post-translational modification

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- **Erisyon introduces the platform technology, fluorosequencing, for single molecule protein sequencing.**
- **The sites of post-translational modifications such as phosphorylation, glycosylation, trimethylation on a protein molecule can be mapped and quantified by the technology.**
- **A number of biomarkers such as Troponin-I, alpha-synuclein etc. have sites of phosphorylation that either needs to be discovered further or needs to be confirmed.**
- **By enriching these biomarkers from bodily fluids (such as saliva, blood etc.) using available antibodies, fluorosequencing can be used to map the positions of post-translational modifications on these proteins.**
- **An example of mapping and confirming sites of phosphorylation on Troponin I, biomarker for dysfunctional heart, is described.**
- **A new dimension of information - digital quantifications of all the modified positions and states of the protein from a single experiment can be made available.**

Erisyon is developing a technology, fluorosequencing, for sequencing a heterogeneous sample of protein molecules and identifying the type and position of amino acids and their modification on individual proteins at a single molecule sensitivity. Spun out of the University of Texas at Austin, Erisyon's concept for single molecule protein sequencing is based on the principle that the positional information of a small number of amino acid types in a peptide (such as x-C-x-x-C-; x = any amino acid; C = Cysteine) may be sufficiently reflective of the peptides' identity, to allow its identification in a known protein sequence database. Fluorosequencing is the process in which one or more amino acids is selectively labeled with fluorophores and then degrading the peptide by Edman sequencing while monitoring the change in fluorescence intensity as it loses one amino acid per cycle.

By extending the technology to selectively and covalently label post-translational modifications on protein molecules, the fluorosequencing technology can be used for precise residue level localization of post-translational modifications (such as phosphorylation, glycosylation, trimethylation etc.) on individual protein molecules.

The case of Troponin-I phosphorylation

Cardiac Troponin I (cTnI) is an important protein biomarker found to be elevated in the bloodstream during heart disorders, such as myocardial infarctions. However, the elevated levels are also observed under non cardiac related conditions such as after severe endurance exercises, renal failures, sepsis and so on[1]. The sensitivity of the diagnostic

assay was improved to 82% when the phosphorylation levels of cTnI in the blood were measured for an acute coronary syndrome study[2]. Multiple studies have revealed more than 14 potential sites of phosphorylation for this protein and some (for example Ser 23/24) have been implicated site-specific roles in various cardiac functions and diseases[3]. It is thus highly likely that mapping the phosphorylated states of all the residues would help discriminate the different causes for elevation in the levels of troponin[4].

The current technologies to measure troponin levels and their phosphorylation states are immunoassays and highly sensitive protein mass spectrometers. While immunoassays are highly selectively for enriching troponins, their lack selectivity to discriminate all the phosphorylation sites makes them less ideal. Mass spectrometry methods for localizing phosphorylated residues, additionally lack the sensitivity to accurately quantify them all on the same protein[3]. Thus a technology to map and measure the heterogeneous sites of phosphorylated troponin, present in low levels in the bloodstream would greatly enable access to the protein for research studies and potentially form the basis of a diagnostic assay with increased sensitivity and specificity for a range of cardiac dysregulations.

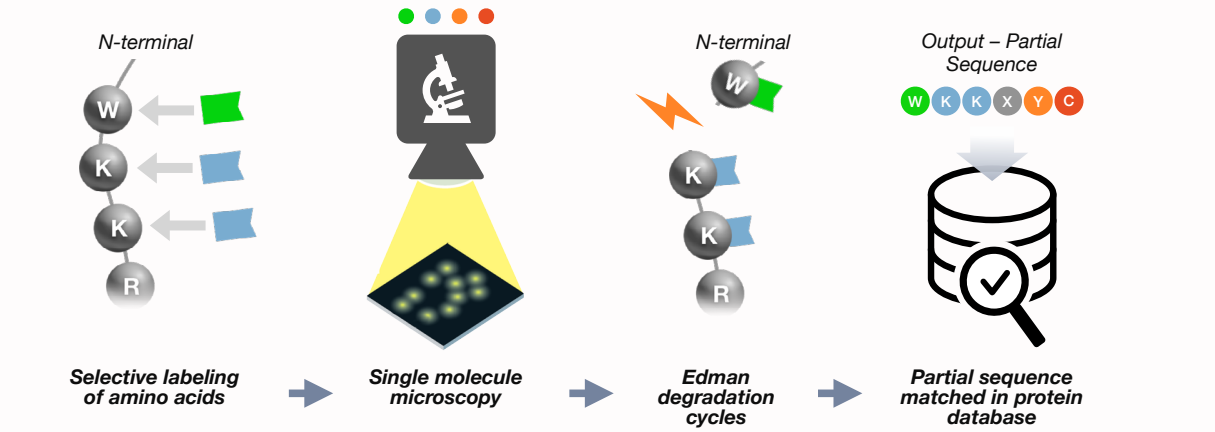

Figure 1: Principle of fluorosequencing *– A) Every bright spot shown in the image from the microscope is a single protein molecule. The change in the intensity of a spot after a cycle of chemistry correlates to a specific protein signature. By tracking intensity changes for many spots across multiple chemistry cycles, we can accurately identify billions of different proteins in a sample. B) Using samples supplied by customers, peptides are isolated and labeled for fluorosequencing. Using our patented technology, we sequence them at the single molecule level to identify and digitally quantify the proteins in the sample.*

Erisyon's technology for mapping all phosphorylated residues on troponin-I

Fluorosequencing can identify the positions of phosphorylated residues on every individual peptide molecules. Each individual sequence observations can be counted to quantify "digitally" the levels of phosphorylation for each of the potential sites on the protein molecule. This highly sensitive method for mapping and measuring all the phosphosites on protein molecules is an unmet need for proteomics research.

Similar to the application of mapping phosphorylation sites on proteins, fluorosequencing is capable of localizing other post-translational modification such as glycosylation, trimethylation of lysines, citrullination and many other modifications on clinically important protein samples.

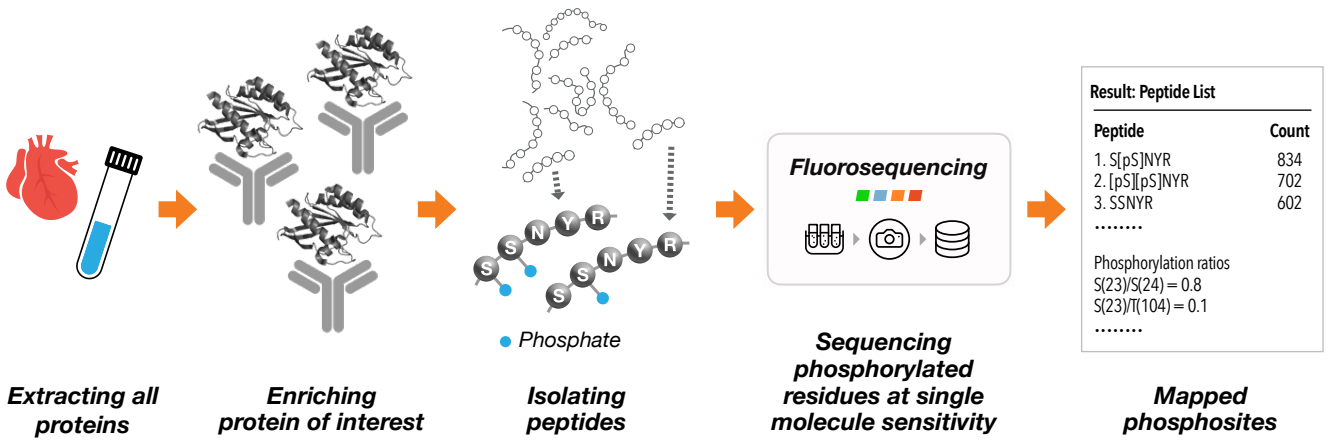

Figure 2. Fluorosequencing technology is used to map positions of phosphorylated residues on peptides *– By enriching protein of interest (such as cTnI) from the tissue samples or bloodstream using a known antibody, protease digestion and covalently labeling all the phosphorylated serine and/or threonine residues on the peptides with fluorophores, the technology can provide the positions of phosphorylated residues on individual protein molecules.*

References

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3. Zhang, P. et al. *Circulation* 126, 1828–1837 (2012).
4. Feng, J. et al. *Circulation* 103, 2035–7 (2001).