Solid phase sample preparation method: Concept, Features & Benefits

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- **Erisyon introduces a novel sample preparation method for general mass-spectrometry based proteomics and specifically for its single molecule fluorosequencing technology**
- **Fundamental concept: A method that allows for the binding of peptides on resin support in a covalent and reversible manner enabling complex manipulations of peptides and**
- **Architecture: The solid phase support is functionalized with Pyridinecarboxaldehyde for selective N-terminal reactions and the resin material optimized for low non-specific binding**
- **Technical features: (a) Selective and covalent reaction to N-termini of peptides (b) Does not cross-react with any other biomolecules (c) Can work at high detergent concentrations and (d) prevents loss of peptides during washing and fluorescent labeling steps**
- **Benefits: (a) Proteins from low abundant samples, such as single cells or tumor samples can be losslessly captured enabling new proteomic applications and (b) Amenable to rapid workflow due to the ability to be automated**

Concept of the sample preparation method

Briefly, as described in **figure 1**, the sample preparation method, as applicable to the fluorosequencing, involves the imidazolinone formation from the N-terminus of peptides using *2-pyridinyl carboxaldehyde (2PCA)* as the very first step to immobilize the peptide onto the bead. Following immobilizing of peptides, which enables multiple rounds of labeling reactions and incorporating fluorophores and other reactive reagents, the peptides are released in a scarless fashion using an optimized dimethylaminoethyl hydrazine reaction. **Figure 2** illustrates the fluorosequencing results of this sample preparation method for a test peptide and demonstrates the proof-of-concept for the effectiveness of the sample preparation method [1,2].

In the design of the PCA resin, we utilized the water-swellable PEG beads to prevent non-specific peptide binding and functionalized with a number of modular groups to enable efficient cleavage and appropriate spacer to increase the coupling efficiency to >90% and cleavage to >85%. In addition, we measured for any bias in the N-terminal peptide capture. We found only minor bias for N-terminal Ala (~2.5×) and against Met (~3×) (Figure 4). Additionally, we found no peptides with a Pro at the second position due to the fact that Pro contains a tertiary amide and is incapable of undergoing imidazolinone formation. The design of the functional groups enables a number of interesting proteomic applications from multiplexing samples by introducing bar-coded peptides to low amount protein capture in scenarios where protein samples are limited such as tissue biopsies.

Technical features and benefits

This solid phase sample preparation method offers the following features/benefits:

1. **Solid phase peptide capture:** Capturing peptides on solid phase prevents sample losses due to the vagaries of peptide solubility in different solutions. By transferring the peptides on a solid phase, the peptides are now handled and transferred losslessly without any precipitation or non-specific binding to microcentrifuge tubes or vials. The benefits allow the ability to capture proteins from low abundant samples such as tumor biopsies or single cells.

2. **Covalent peptide immobilization:** The covalent and selective attachment of the peptides enables enriching for proteins and peptides in samples with other biomolecules such as carbohydrates, lipids or small molecules. This benefits the adoption in the proteomics for use in difficult biological samples such as plant tissues or dense bones, where large amounts of contaminants are present or detergents would be required at high concentrations.

3. **Modular bead construction and functionalization:** The solid phase beads are modular in construction, where different sets of linkers are stitched together using commonly used peptide synthesis techniques. This enables the beads to be adopted to different needs, such as including TMT isobaric tags for mass-spectrometry or with radioactive linkers for protein sample storage.

4. **Scarless release of peptides:** With the ability to release peptides without any protecting N-terminal group, the work-flow is easily adaptable for multiple proteomics technologies including single molecule sequencing methods such as fluorosequencing.

Conclusions

Erisyon's solid-phase sample preparation technology, where peptides are covalently bound through their N-termini and reversibly released, enables integration into downstream proteomic technologies including fluorosequencing. Working with smaller protein concentrations will be important for these single-molecule techniques and helps advance proteomic techniques to be better able to analyze limiting samples, as for clinical biopsies.

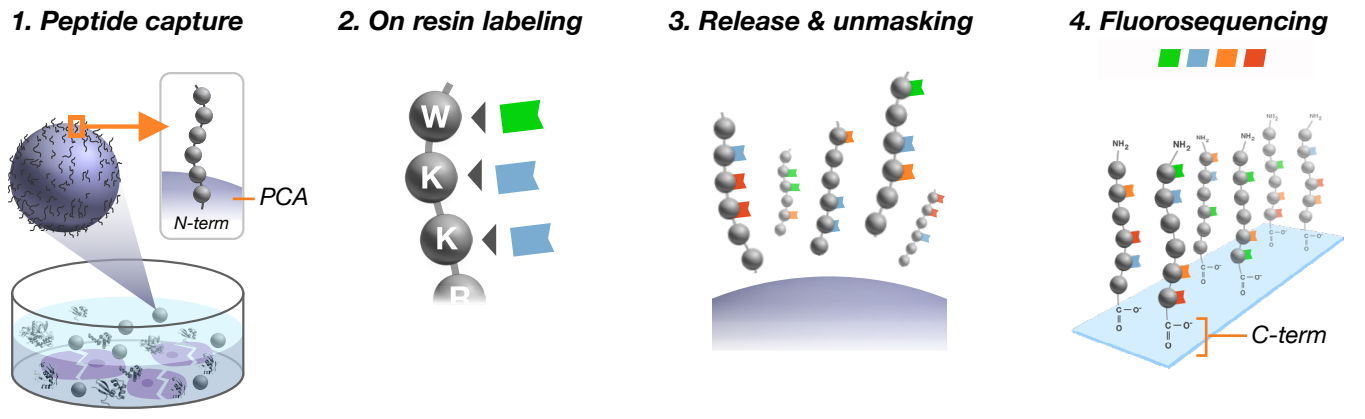

Fig 1. Overall scheme of using an N-Terminal capture resin for fluorosequencing. *– (1) N-terminal capture using a pyridinyl carboxaldehyde derivative attached to a resin. (2) Chemical labelling of lysine. (3) Release from resin and (4) Attachment to surface via C-terminus for sequencing.*

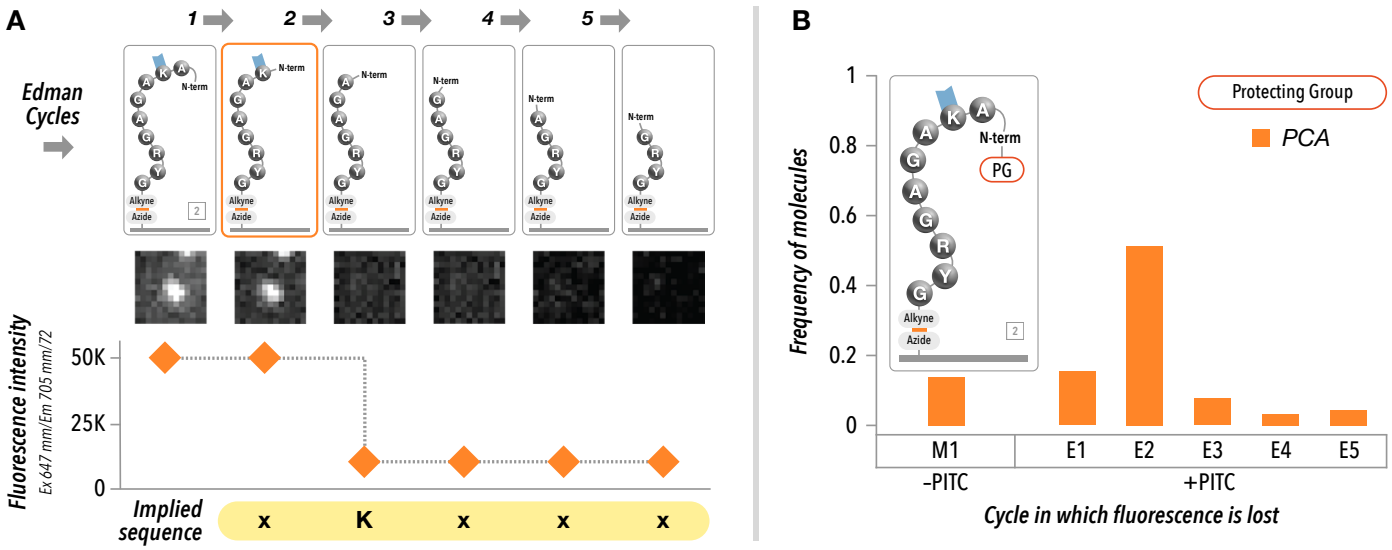

Figure 2. *– (A) A field of view observed in the fluorosequencing experiments with individual fluorescent peptides (Seq: AKAGAGRYG) obtained through multiple steps of the sample preparation method. (B) The image of a single peptide through rounds of fluorosequencing. (C) Light emitted from one peptide molecule across Edman cycles and its measured fluorescence intensities at each cycle. (C) The frequency of molecules exhibiting a loss of fluorescence after each Edman cycle is plotted for both a PCA or Fmoc protected peptide, showing data from 103 671 and 59 720 molecules, respectively. M1 is a "mock" cycle where the slide is washed with all reagents used in fluorosequencing without the phenyl isothiocyantate (PITC) being present to remove nonspecifically bound peptides or free fluorophores. E1–E5 represent consecutive Edman cycles.*

References

1. Howard, Cecil J., *et al.* "Solid-Phase Peptide Capture and Release for Bulk and Single-Molecule Proteomics." *ACS Chemical Biology* (2020).PMID: 32363853

2. Swaminathan, J. *et al.* Highly parallel single-molecule identification of proteins in zeptomole-scale mixtures. *Nat. Biotechnol.* (2019).