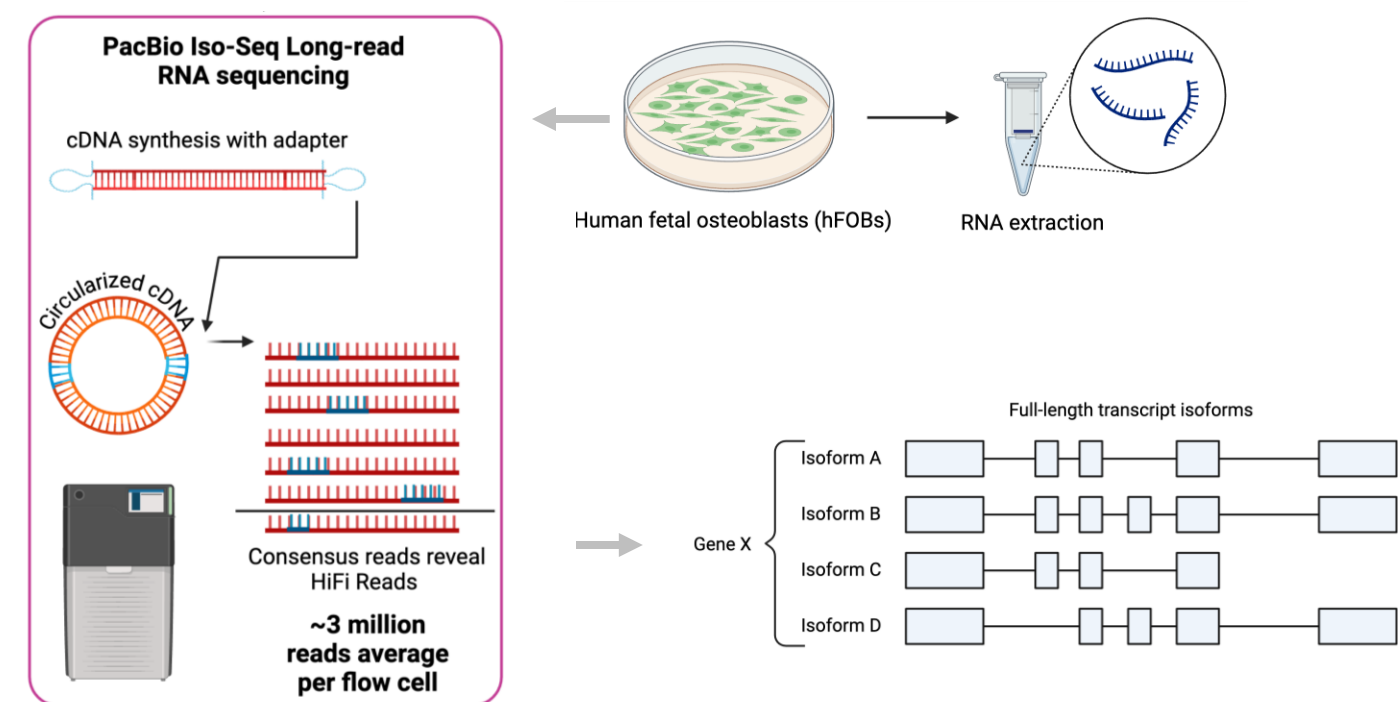


## Proteoform characterization – critical for biomedical applications

- Aberrant RNA splicing generates proteoforms that underlie a spectrum of human diseases
- Molecular detection of paralogous and alternatively spliced proteoforms could represent a new source of critical biomarkers or drug targets
- To annotate protein-coding genes, particularly those with high sequence homology, using genomics data to track altered amino acid variants is useful for protein-level validation
- LR-RNA-Seq revealed isoforms of vimentin and TPM1/2 (87% identity). Protein sequencing distinguished isoforms for filament proteins involved in cancer and skeletal muscle diseases.

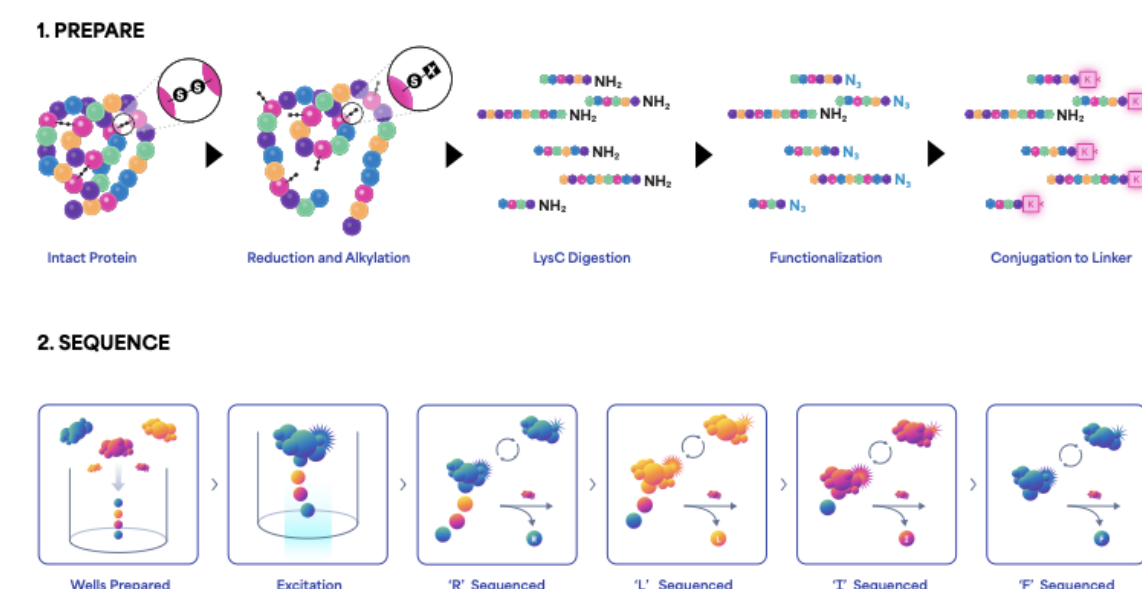
This study represents the first integration of proteogenomics with with single molecule protein sequencing technology to detect proteoform-specific peptides.

## Full-length transcript sequencing and proteoform prediction



Long read RNA sequencing (PacBio) Assesses presence & quantity of isoforms at mRNA level; however, does not confirm protein expression.

## Next generation protein sequencing platform



## Platinum™ benchtop protein sequencer



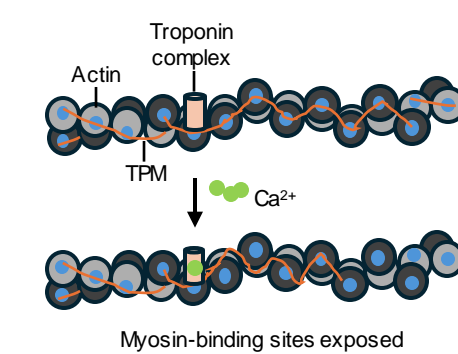
### Kinetic signatures:

**Coverage:** Frequency of amino acid detection  
**Pulse Duration (PD):** Statistical distribution of kinetic data for all traces

Single molecule protein sequencing (Quantum-Si) Sequences peptides by single amino acid (AA) recognition of a polypeptide. Fluorophore-labeled N-terminal AA recognizers generate kinetic signatures that are converted to AA calls to identify peptides and proteins.

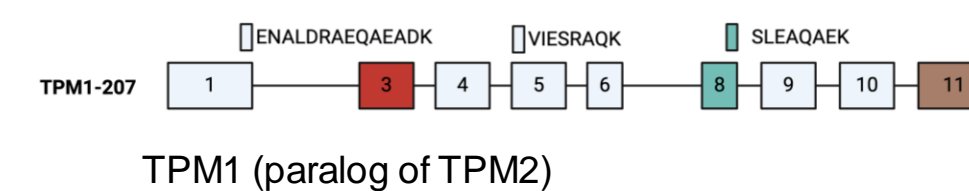
## Molecular diversity of filamentous proteins

- Filament structural proteins are highly diverse: >40 human genes code for intermediate filament (IF) proteins and homologous tropomyosins (TPMs).
- TPM isoforms regulate distinct actin filament populations to mediate cell-specific structural properties.
- Paralogs and isoforms with similar physicochemical properties are difficult to distinguish with antibodies, and, sometimes, mass spectrometry.
- Antibodies don't exist for all TPM isoforms; synthetic peptide immunogens are often unknown.



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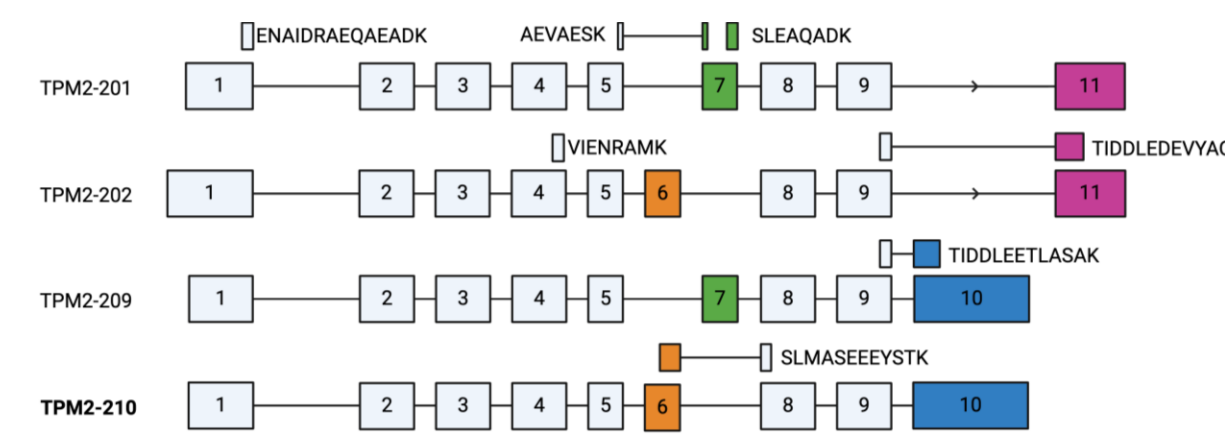
## TPM1



TPM1 (paralog of TPM2)

- Proteomic analyses indicate TPM1 is involved in bone matrix stiffness and osteogenesis.

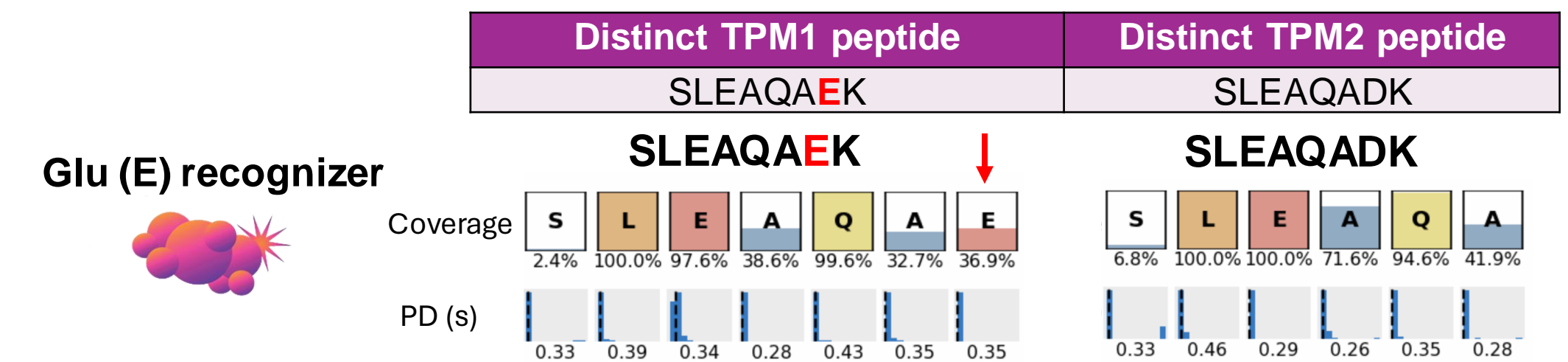
## TPM2



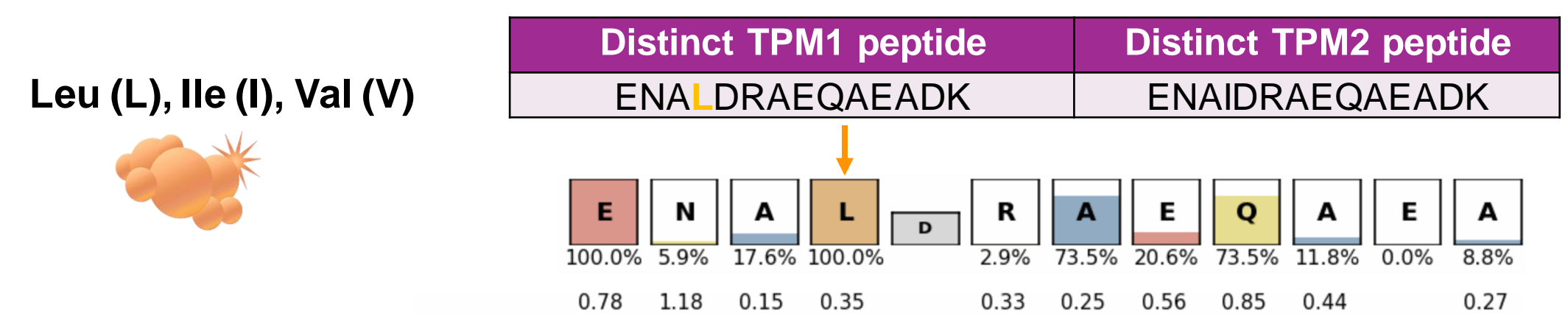
TPM2 spliced isoforms

- TPM isoforms regulate distinct actin filament populations to mediate cell-specific structural properties.
- TPM isoforms are detectable in muscle, the cytoskeleton, and in biofluids such as urine.
- Shown here are TPM2 isoform-informative peptides

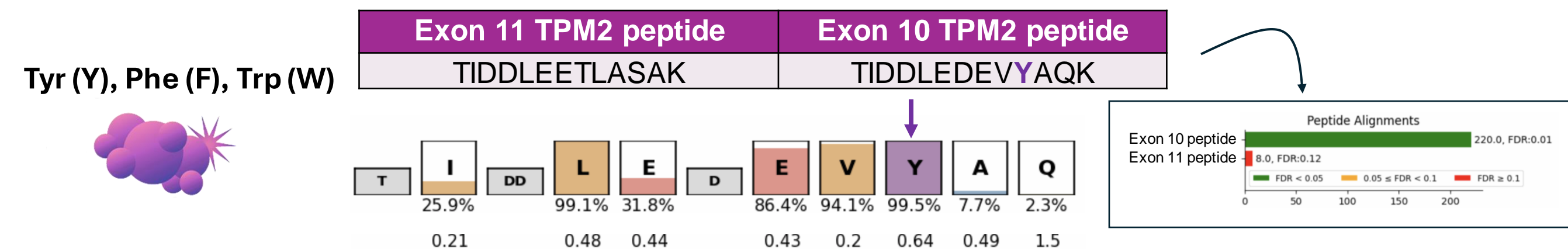
## Amino acid recognizers distinguish TPM1/2 paralogs and splice proteoforms



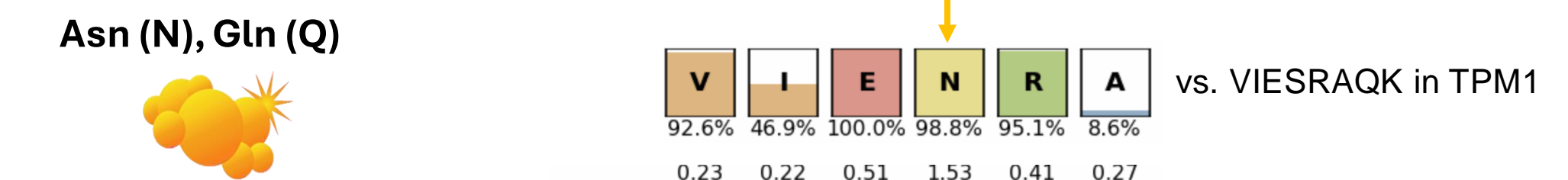
## Differential pulse durations distinguish isobaric TPM1/2 peptides differing by Leu/Ile



## Y recognizer distinguishes TPM2 isoform-informative peptides



## Detecting TPM2 spliceforms within mixture of 16 TPM1/2 peptides



## Acknowledgments

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