

# A complete and automated sample preparation strategy for high throughput and standardized proteomics

Dorte B. Bekker-Jensen<sup>1</sup>, Florian S. Harking<sup>2</sup>, Angela McArdle<sup>1</sup>, Lasse Falkenby<sup>1</sup>, Jakob Bunkenborg<sup>1</sup>, Ole B. Hørrning<sup>1</sup>, Jesper V. Olsen<sup>2</sup>, Nicolai Bache<sup>1</sup>

<sup>1</sup> Evosep Biosystems, Denmark

<sup>2</sup> Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Denmark

## A need for standardization

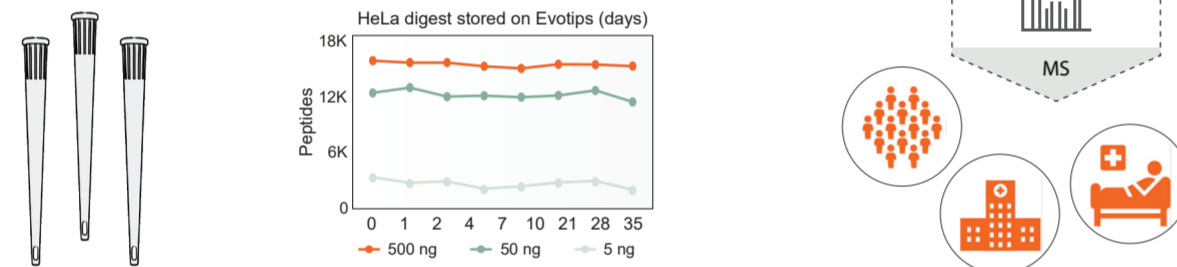
### Simple workflows

Standardized methods and protocols with few steps are needed to analyze large clinical sample cohorts in clinical proteomics and ultimately for diagnostics.

### Integration with the Evotip

Evotips are disposable trap columns, which integrates elution with liquid chromatography. It serves as a reproducible and sensitive storage device for peptides over time.

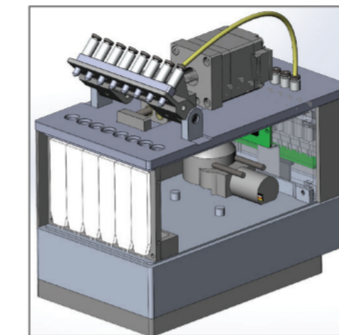
Can we simplify sample preparation by integration of the Evotip?



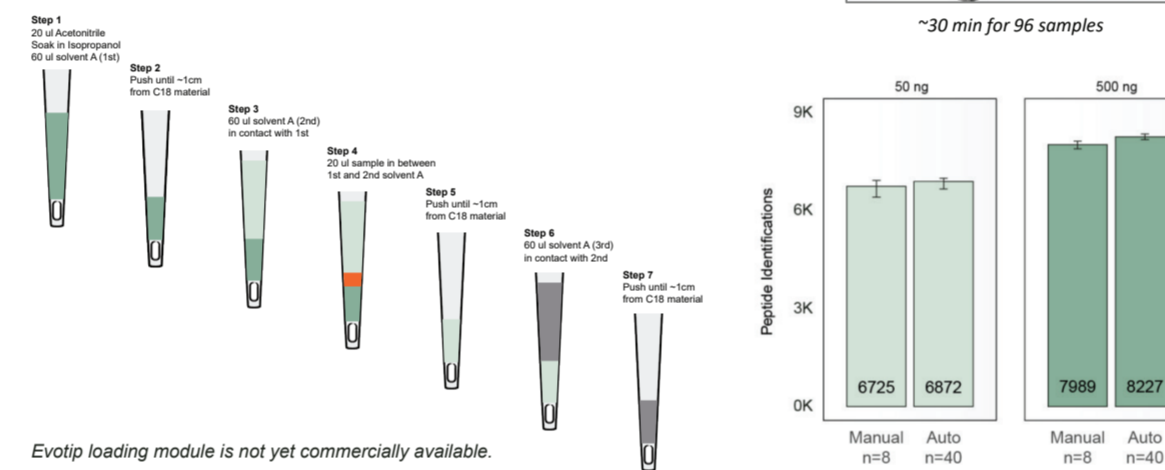
## Automated loading of peptides on Evotips

### Evotip loading module

Specially designed pneumatic 8-channel module with MTP footprint. The device uses positive air pressure to move liquid through the Evotip. Each tip is monitored individually with vision software. Modified loading protocol for automation were benchmarked against standard manual protocol.



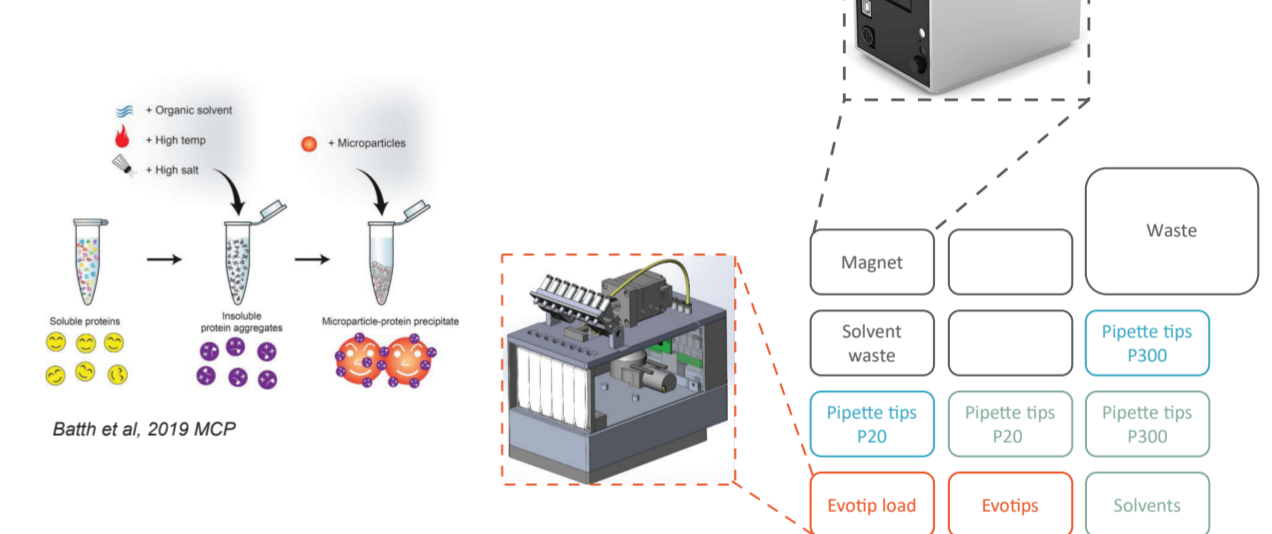
~30 min for 96 samples



## Opentrons end-to-end workflow

### Layout for PAC digestion

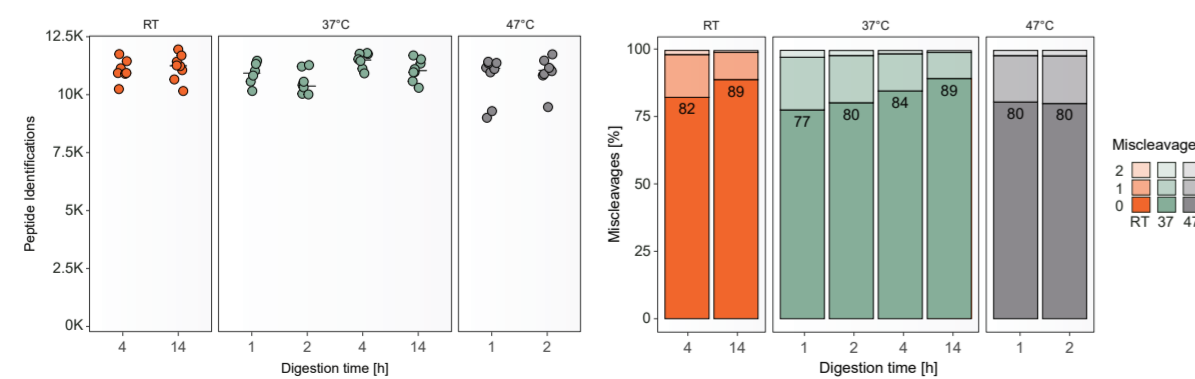
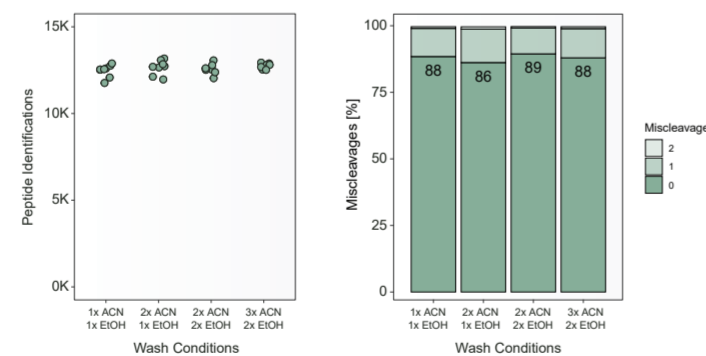
A magnetic rack and the Evotip loading module were used as special labware on the Opentrons for the end-to-end workflow based on PAC digestion. Hydroxyl beads (Resyn Biosciences) were used for aggregation.



## Optimization of the workflow

### Digestion efficiency

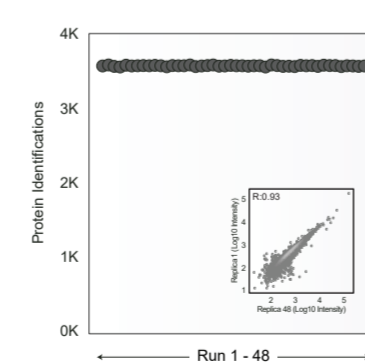
The protocol was optimized for speed and reproducibility in terms of number of washes and digestion time, using peptide IDs and digestion efficiency as a read-out. Protein amount input was 50ug. Samples were measured with 60 SPD on a Thermo Fusion Lumos.



## Scaling up the workflow

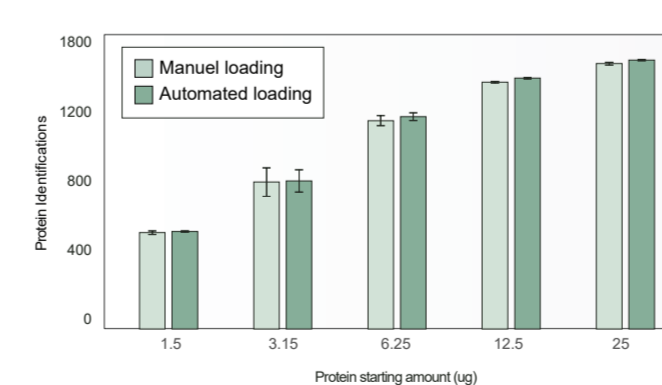
### Excellent reproducibility

The workflow was tested in terms of reproducibility by digesting 50ug of HeLa lysate in 48 technical replicates. Samples were measured with 100 SPD on a Thermo Orbitrap Exploris 480 with DIA.



### High sensitivity

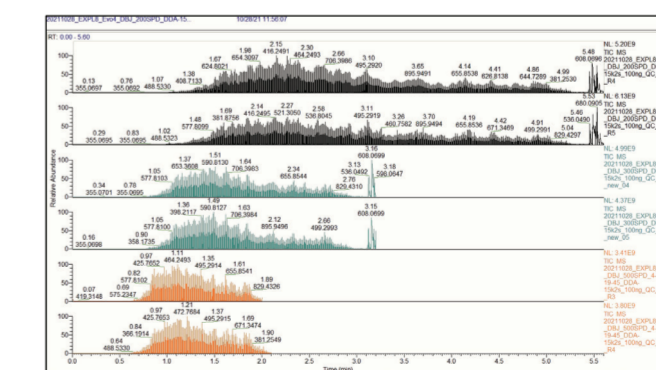
The sensitivity was challenged by a protein dilution series in technical triplicates, where the peptides were loaded by the automatic loading module and by manual loading. Samples were measured with 200 SPD on a Thermo Orbitrap Exploris 480 with DIA.



## Faster chromatography

### 500 samples per day

Automated sample prep workflows require faster chromatography. We are working on a robust method with 500 samples analyzed per day with two minutes gradient time.



100 ng HeLa peptides analyzed with 200, 300 and 500 SPD.

### Highest peak capacity

For high throughput analysis of complex samples, highest peak capacity in the shortest time is a relevant measure, which is achieved by the fastest method.

