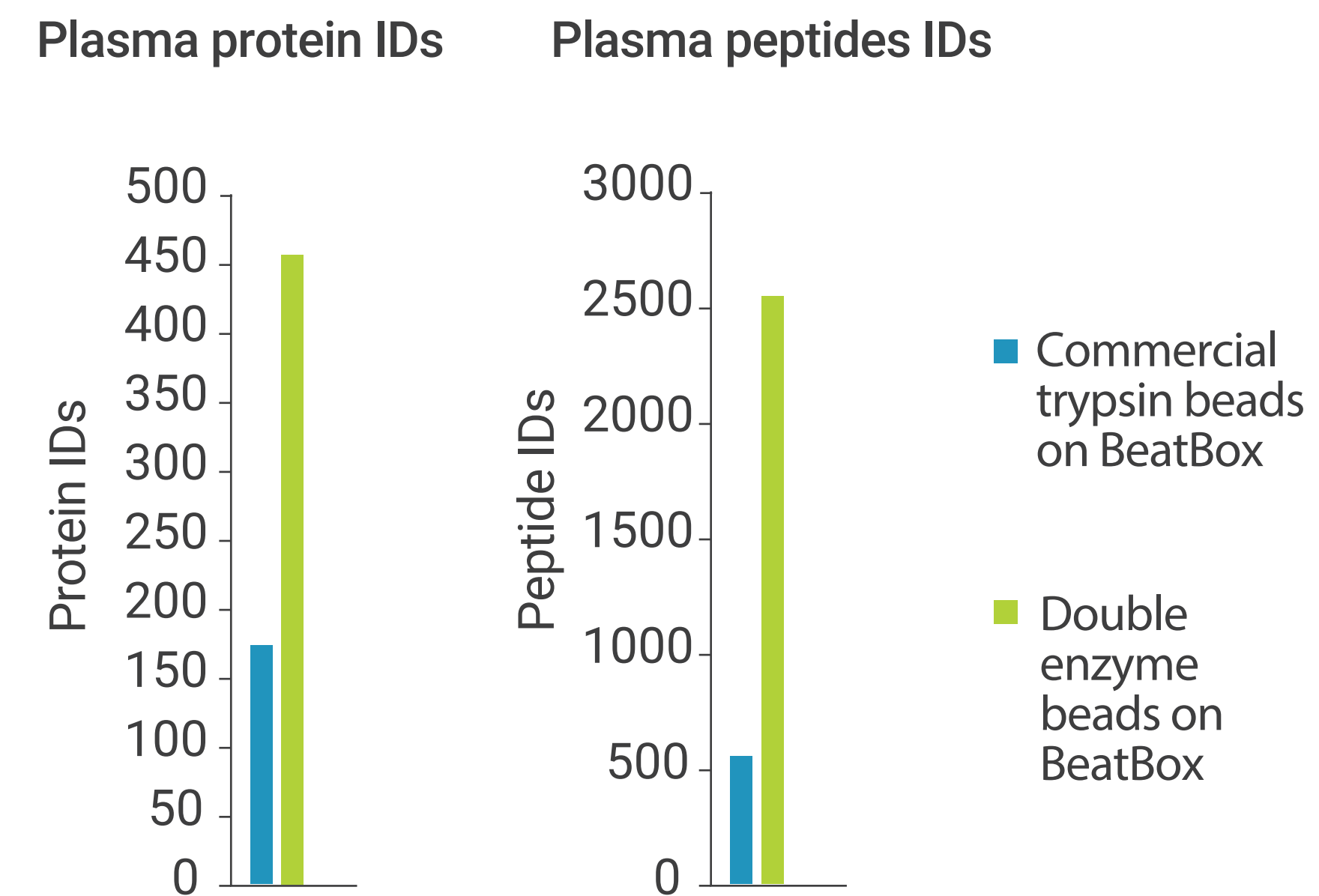


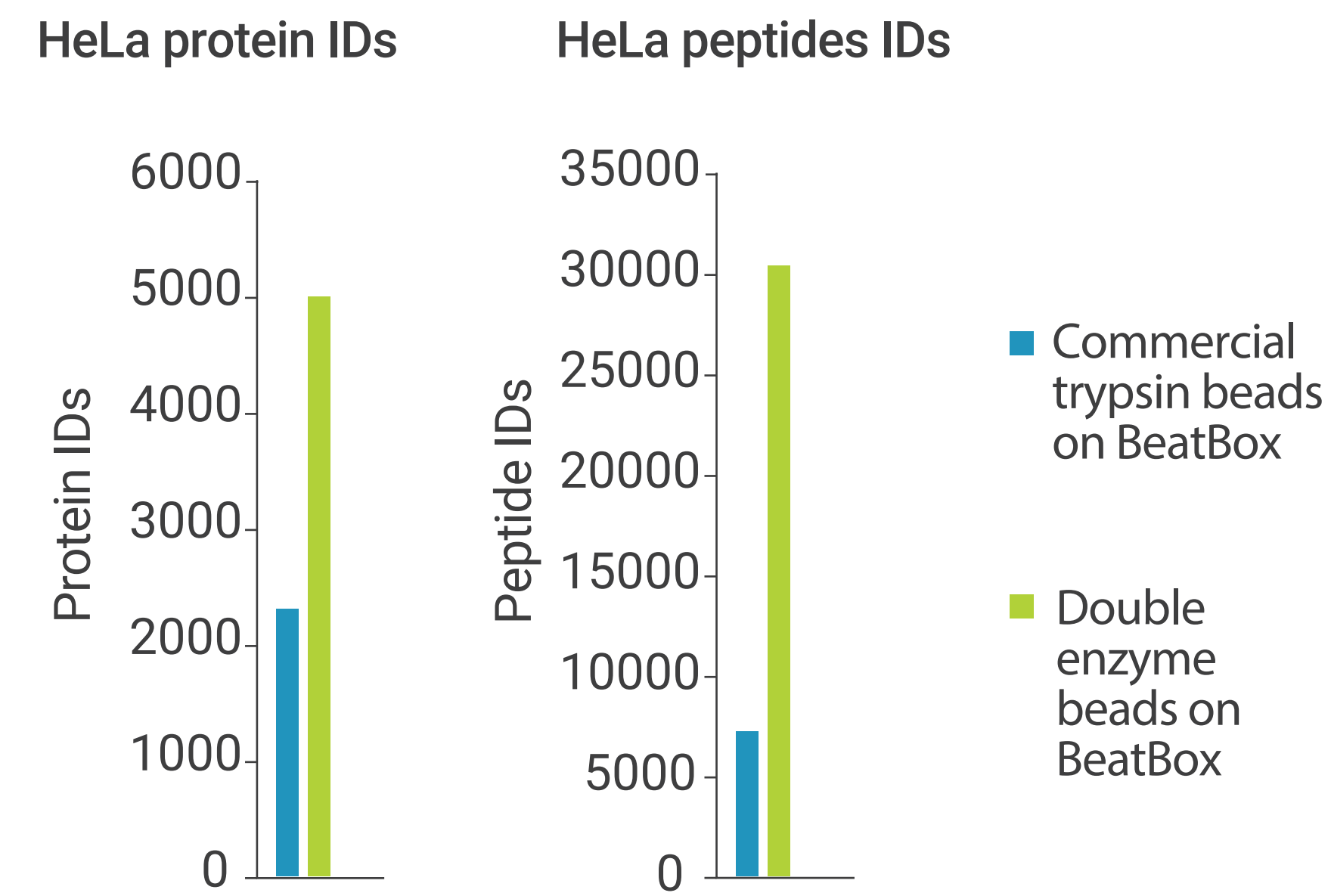
Doubly functionalized magnetic microspheres with immobilized Trypsin and LysC enabling fast, easy and automatable LC-MS sample preparation

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Comparison to commercial trypsin beads



Plasma digested by immobilized enzymes



HeLa cell lysate digested by immobilized enzymes

Fig. 1 | Comparison of protein and peptide IDs of plasma and HeLa lysate digested by immobilized enzymes.

Workflow

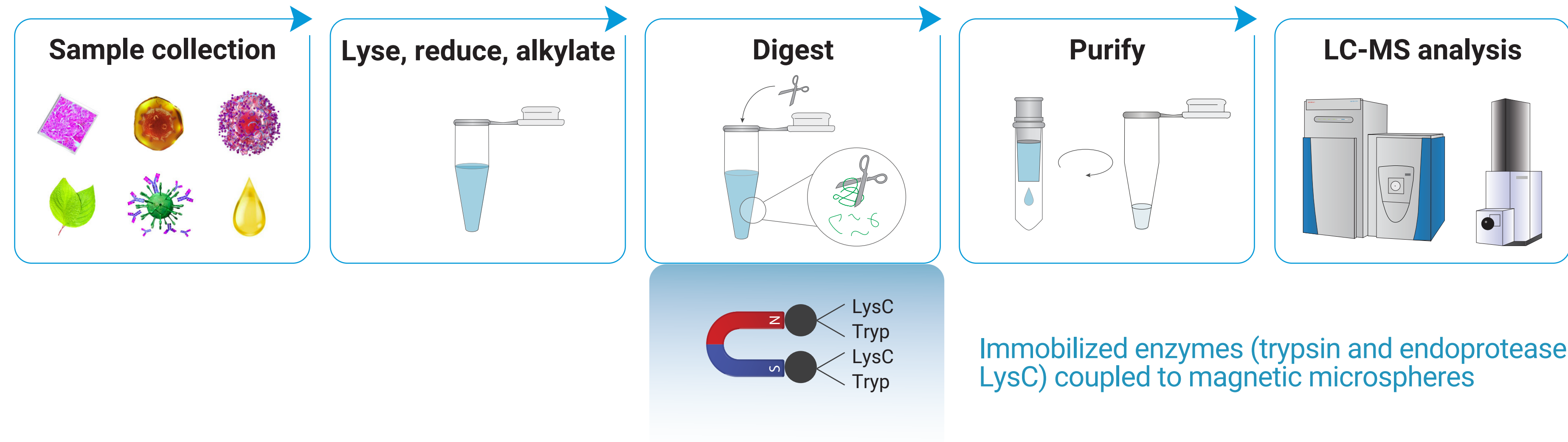


Fig. 2 | Graphical representation of the iST workflow with digestion by doubly functionalized magnetic microspheres.

Excellent performance under harsh conditions: Stress tested enzyme beads

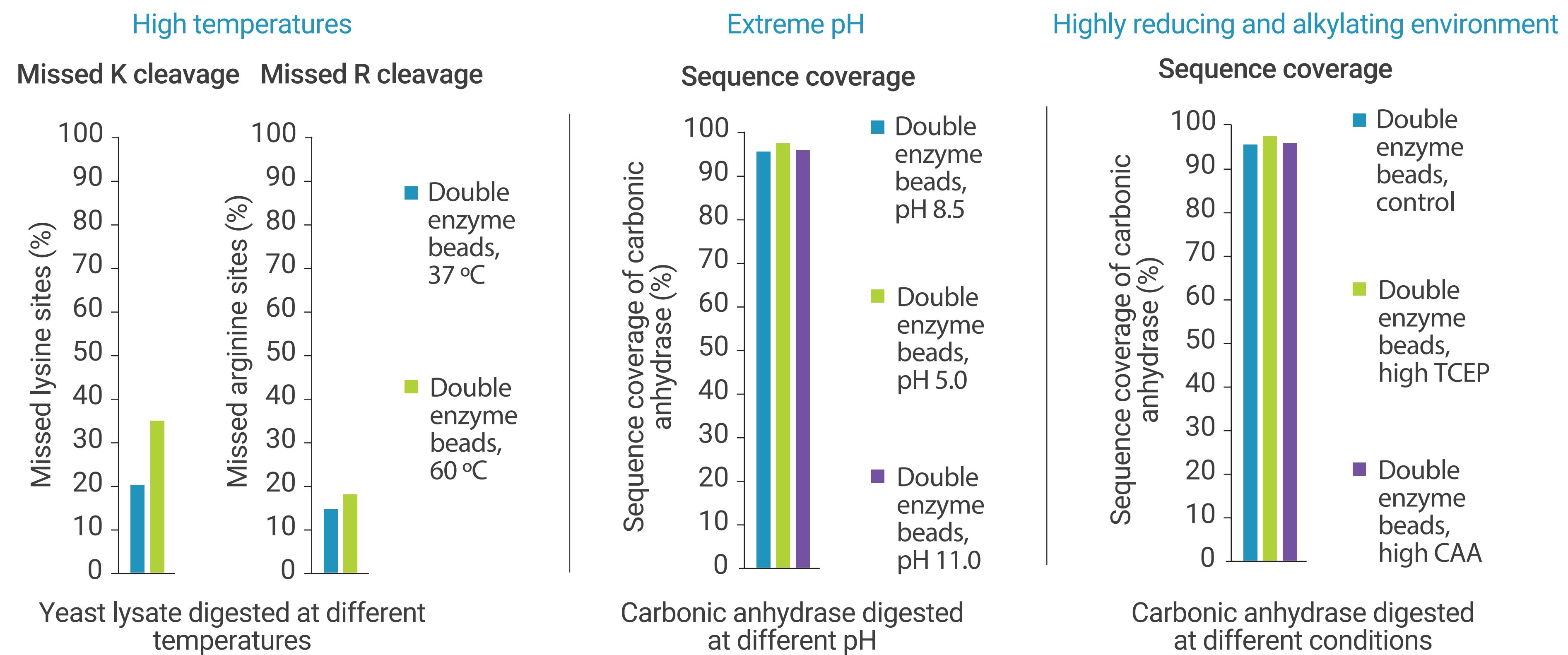


Fig. 3 | Performance of doubly functionalized enzyme beads at high temperature, extreme pH and in highly reducing or alkylating environments.

Key features

- Excellent digestion efficiency and high-quality LC-MS results within 3 hrs
- Excellent performance under harsh conditions: High temperature, extreme pH, aggressive chemicals
- Beads handling possible by hand, on automation platforms or on PreOmics' BeatBox



Fig. 4 | PreOmics' BeatBox

Materials and methods

- **Input:** 100 µg protein lysate from *S.cerevisiae* or HeLa cell line, 2 µl human blood plasma or 25 µg carbonic anhydrase
- **Workflow:** Samples were prepared as described in PreOmics' iST protocol. For the digestion step, 100 µg doubly functionalized magnetic microspheres were used. Samples were incubated on a shaker at 37 °C (or 60 °C for stress test) for one hour while shaking at 1400 rpm or on PreOmics' BeatBox at low settings at room temperature. Subsequent clean up was done following the iST workflow. If indicated, samples were also digested by commercially available trypsin beads applying identical digestion conditions
- **LC-MS analysis:** Peptide loads of 300 ng were analyzed on an Easy nLC 1200 coupled to a timsTOF Pro; DDA mode; 45 minutes total acquisition time. Data analysis was done with MaxQuant software 2.0.1.0