An Automated Sample Preparation Workflow for Proteomic Profiling of Membrane-Bound Vesicles from Urine

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INTRODUCTION

- Urine is a diverse source of information related to a patient's health and is becoming a common biofluid for clinical proteomics
- • The benefits of MagReSyn® for potential sample processing of urine include
  - High capacity and porosity beads allows for low volume for sample processing
  - Strong magnetic moment for rapid handling and ease of automation
- Mag-Net applied to urine can potentially provide additional insights into pathophysiology of diseases
- We evaluated various methods and strategies for the preparation of urine samples using a range of magnetic bead solutions, including Mag-Net™ (Wu *et al*. DOI 10.1101/2023.06.10.544439), PAC (Batth *et al*., DOI: 10.1074/mcp.TIR118.001270, and uHLC (DOI:10.3390/proteomes11040029), and a filter-based approach MStern (DOI: 10.1074/mcp.O115.049650)
- We compare performance by applying an in-house R-script for the automated performance assessment of Mag-Net™

METHODS

- All workflows performed in automated or semi-automated manner using King Fisher Duo magnetic handling station (.bdz method files available on request)
- 25% of digests prepared using the various workflows were loaded on Evotip, analysis using Evosep One 60SPD, diaPASEF short gradient method on Bruker timsTOF HT
- DIA-NN (version 1.8.1) in library-free mode. Conditions were searched separately. Data collated using r-studio scripts.

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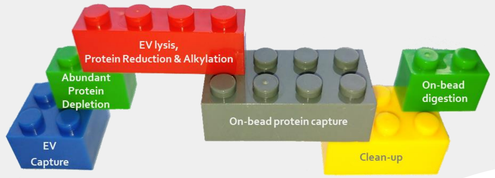
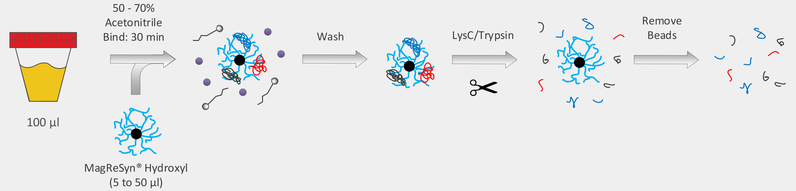
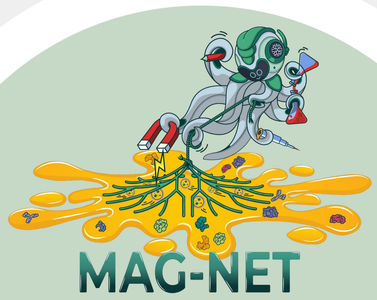
MAG-NET™ WORKFLOW







Adaptation of Mag-Net™ for urine proteome profiling increased coverage by 65%

MagReSyn® HILIC & SAX complementarity can be used to increase proteome depth in urine

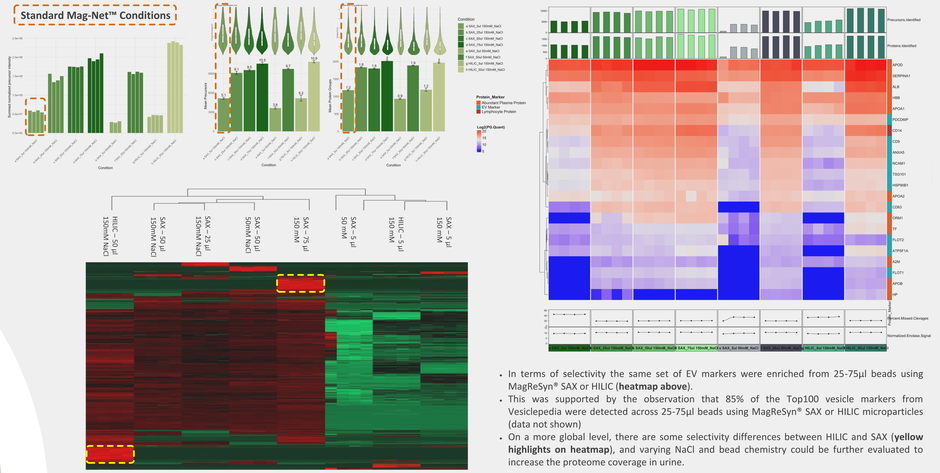
Mag-Net™ & uHLC enrich vesicles, while PAC and MStern enrich extracellular proteins

Stoyan Stoychev, Justin Jordaan, Ireshyn Govender, Adele Nel, Andrea Ellero are employed by ReSyn Biosciences, proprietors of MagReSyn® technology.

Images of Evosep one and timsTOF were created with BioRender.com

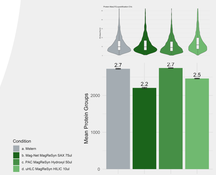
- We evaluated a range of binding conditions to establish which would provide the deepest urine proteome coverage and highest selectivity for membrane vesicles
- These included varying the bead amount (5-75µl), salt concentration in the binding buffer (50 and 150mM) and the bead type (MagReSyn® SAX and HILIC)
- Increased bead volume and salt concentration in the binding buffer increased recovery by Mag-Net™ (based on precursor normalised intensity)
- This result in ~65% increase in the depth of coverage of the urinary proteome, over the standard conditions for Mag-Net™ in plasma

ADAPTING MAG-NET™ FOR EV ENRICHMENT FROM URINE

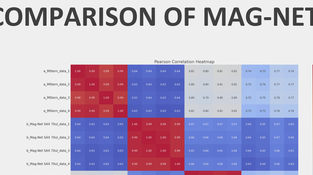


- In terms of selectivity the same set of EV markers were enriched from 25-75µl beads using MagReSyn® SAX or HILIC (**heatmap above**).
- This was supported by the observation that 85% of the Top100 vesicle markers from Vesiclepedia were detected across 25-75µl beads using MagReSyn® SAX or HILIC microparticles (data not shown)
- On a more global level, there are some selectivity differences between HILIC and SAX (**yellow highlights on heatmap**), and varying NaCl and bead chemistry could be further evaluated to increase the proteome coverage in urine.

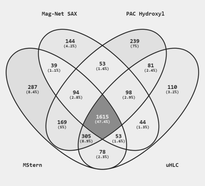
COMPARISON OF MAG-NET™ TO PAC, uHLC & MSTERN

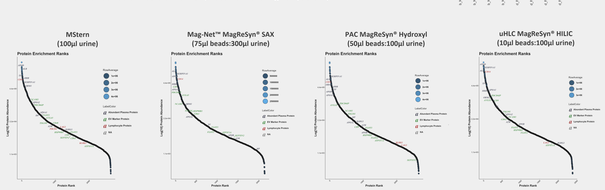


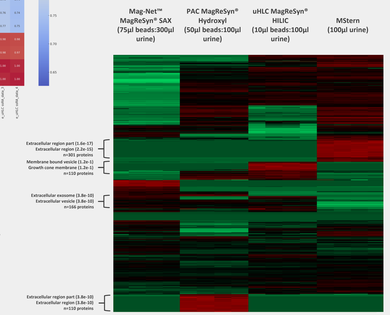
- The optimal Mag-Net urine workflow was compared to three published methods: PAC, uHLC and MStern (**above**)
- The four workflows showed a high level of orthogonality based on low Pearson correlation across the methods (**right**)
- This resulted in over 3,500 protein groups identified with <50% of these being common across the methods (**Venn diagram bottom left**)
- MStern and PAC generated similar urinary proteome profiles
- Mag-Net™ Urine and uHLC workflows identified fewer proteins, but the relevant abundance of vesicle marker proteins were higher (**below**)



- All methods achieved high quantitative precision with median protein-level CVs <15%, with the highest urinary proteome coverage achieved using MStern and PAC
- GO compartment enrichment analysis on unique clusters detected from unsupervised hierarchical clustering (**heatmap below**) and unique proteins identified from each workflow (**below left**) where Mag-Net™ and uHLC enriched for proteins associated with extracellular exosomes, while MStern and PAC primarily enriched for extracellular proteins.







CONFLICT OF INTEREST DISCLOSURE