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Design of experiments and automation for the efficient protein LC-MS method development

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16th EBF Open Symposium

17th November 2023, Barcelona

Protein LC-MS

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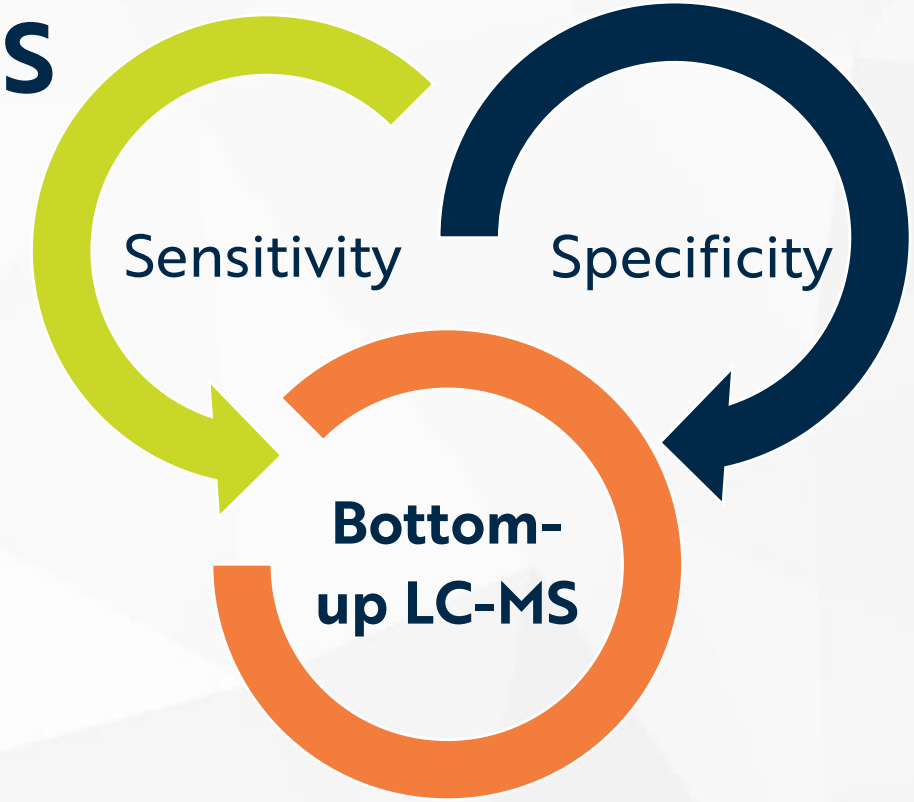

Goal: Streamline Protein LC-MS Sample Preparation

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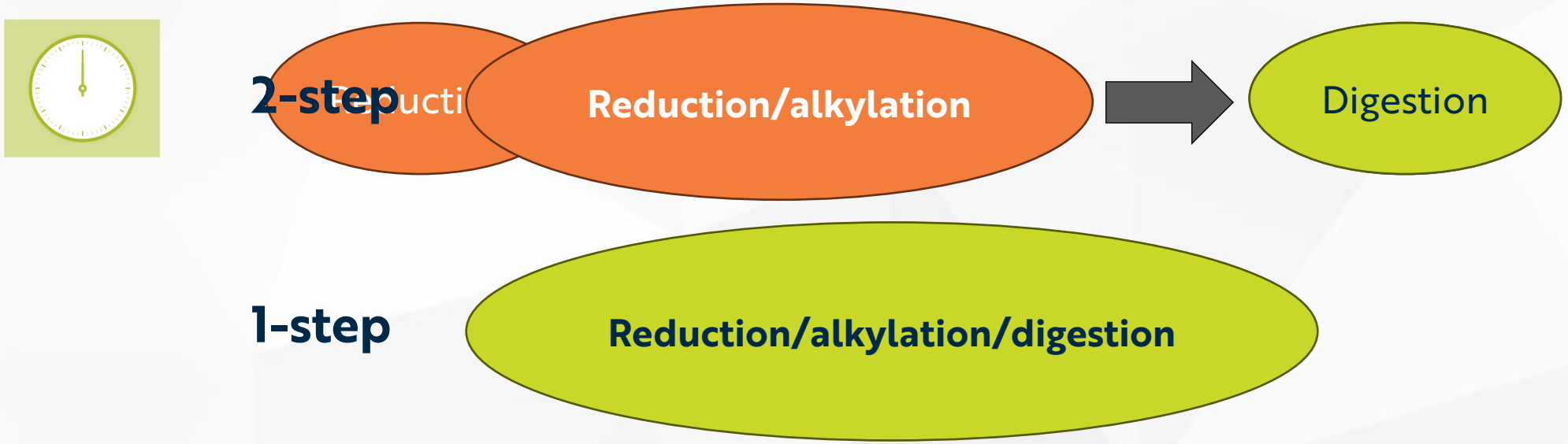
Bottom-up Sample Preparation Workflow



- Model analyte: IgG1 mAb – 4 HC surrogate peptides monitored
- Matrix: rat plasma
- LC-MS: Acquity UPLC and Xevo TSQ (Waters)

-**DTLM**ISR-
-**TPEV**TCVVVDVSHEDPEVK-
-**FNWY**VDGVEVHNAK-
-**VVSV**LTVLHQDWLNGK-

Design of Experiments (DoE)

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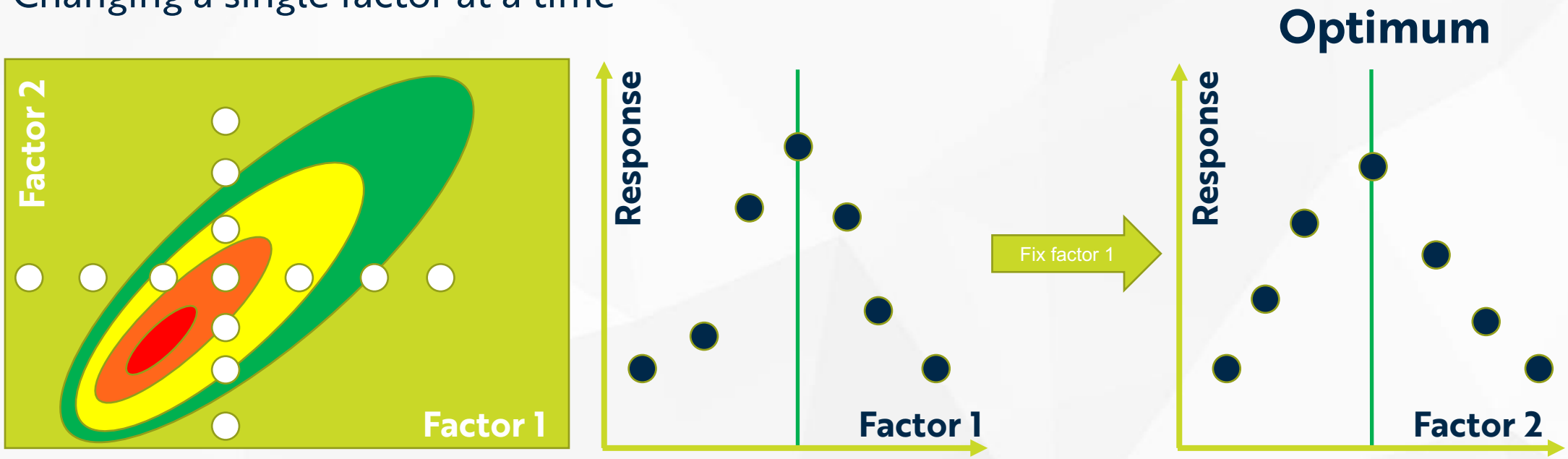
Changing a single factor at a time



- Does not always lead to real optimum
- Limited information
- Many experiments

Design of Experiments (DoE)

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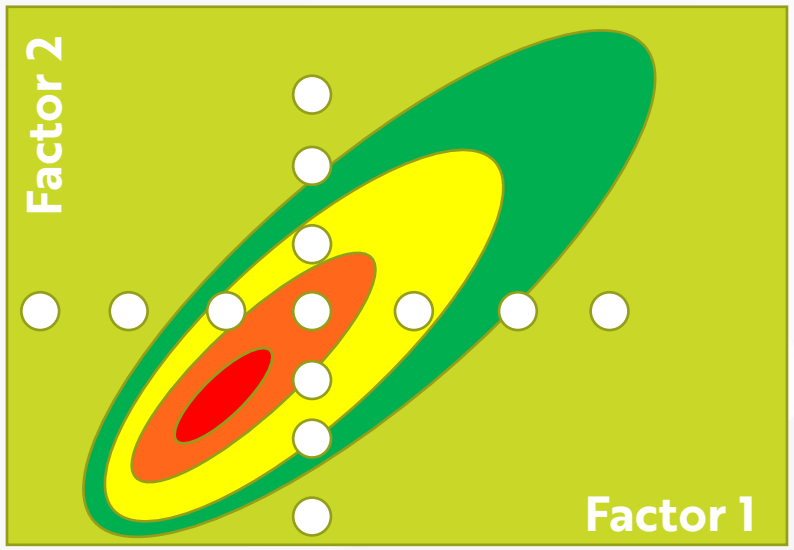
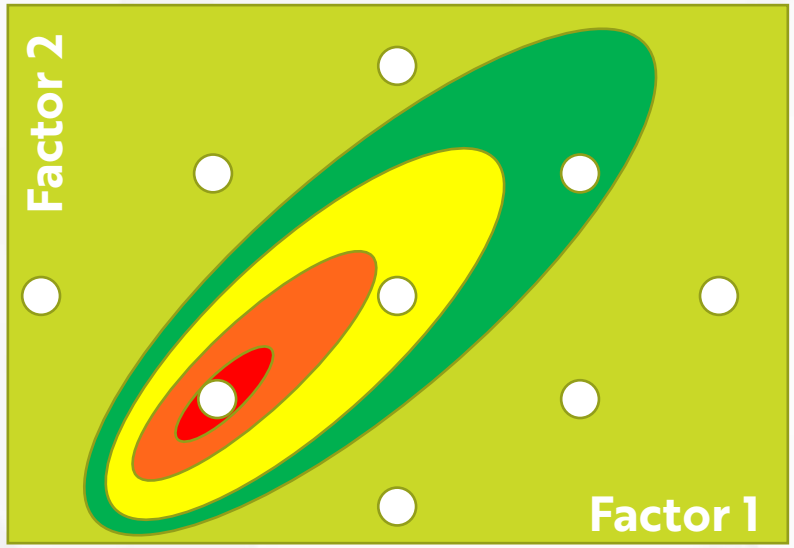
Conventional



VS

DoE



- A strategically planned and executed series of experiments
- All factors (e.g. pH, solvent, temperature) are changed simultaneously
- Allows to investigate multiple factors at the same time
- More information, model setup and predictive power
- Fewer experiments

Screening DoE – 2-step prep.

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Objective: find the important factors

Variables

- Reduction reagent: ~~DTT~~, TCEP, THPP
- Alkylation reagent: IAA, CAA
- Reduction reagent concentration: 1-50 mM
- Alkylation reagent concentration: 2-100 mM
- Incubation temperature: 22-94°C
- Red/alk incubation time: 10-30 min
- Digestion incubation time: 1-3 hours

974 samples

DoE: 19 samples

Screening DoE Results

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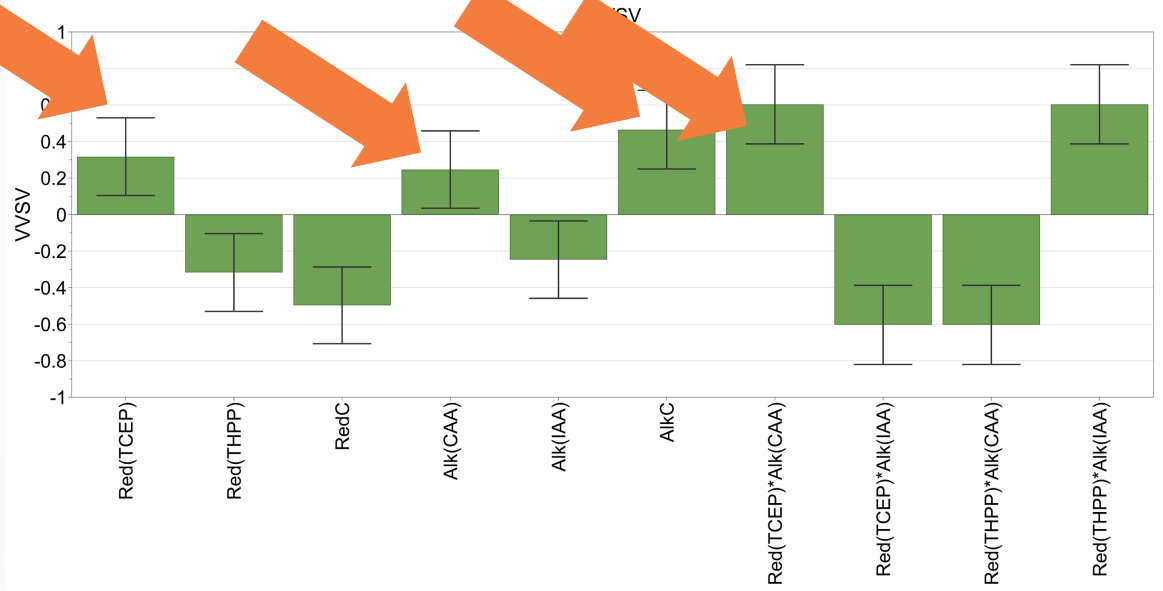
Coefficient plot for VVSV surrogate peptide



Factors to improve peptide abundance

- TCEP
- CAA
- High alkylation reagent concentration
- Combination of TCEP + CAA

No impact

- Incubation time and temperature

Conclusions

- Use TCEP and CAA
- Alkylation reagent concentration > reduction reagent concentration

DoE Optimisation

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Objective: optimise important factors

Variables

- Reduction reagent concentration: 1-50 mM
- Incubation temperature: 22-94°C
- Red/alk incubation time: 10-30 min
- Digestion incubation time: 1-3 hours

83 samples

DoE: 27 samples

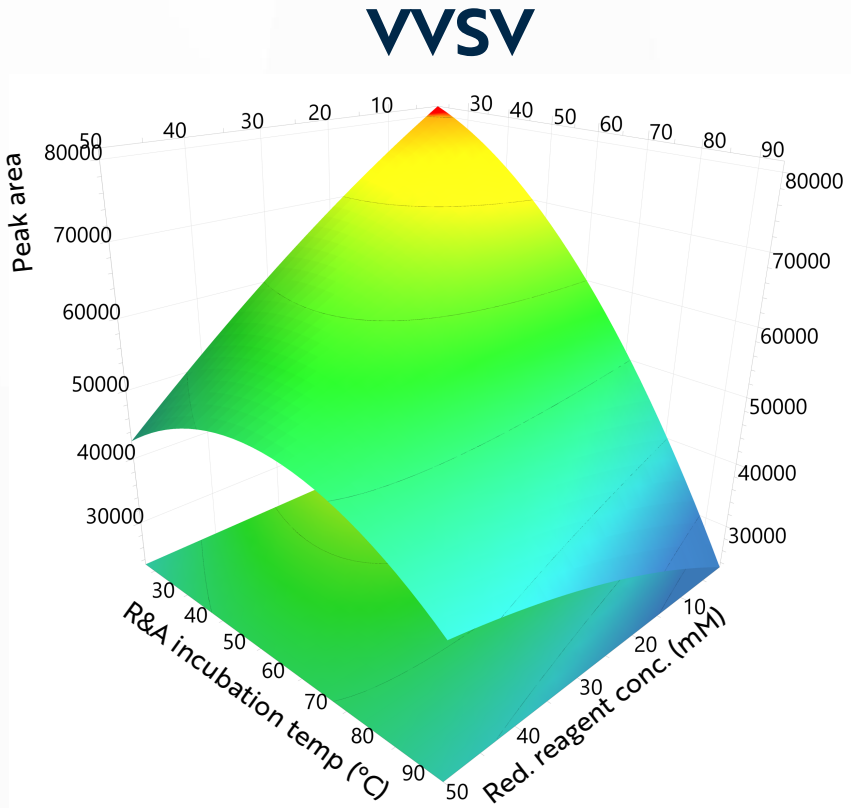
DoE Optimisation Results

LC-MS data

Model fitted for each peptide

Optimal simultaneous R&A:

- 6.6 mM TCEP
- 19.8 mM CAA
- R&A incubation at 48°C for 30 min
- 1.5-hours trypsin digestion



DoE Optimisation Results

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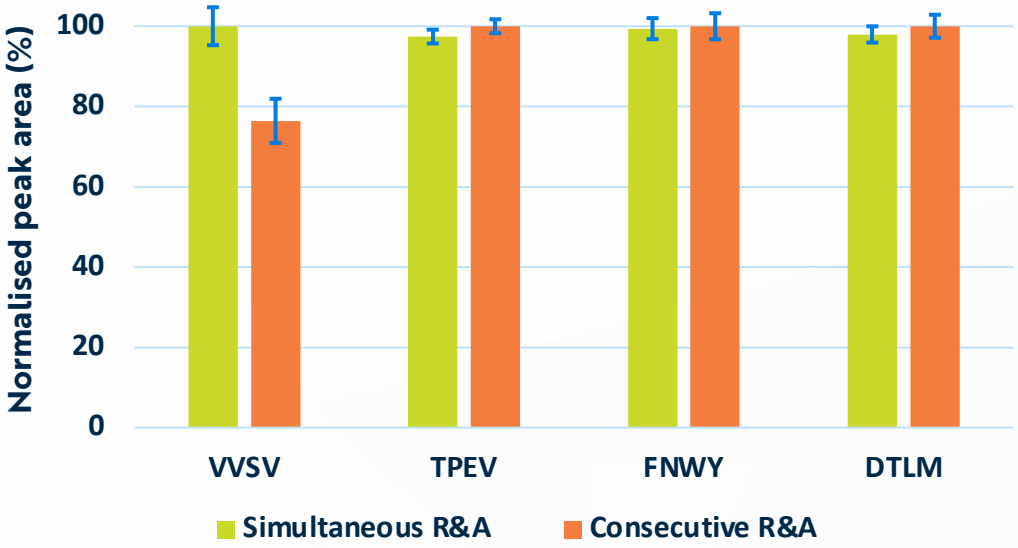
Simultaneous R&A **vs** sequential R&A



Conclusions

- Simultaneous R&A yields high and consistent peptide responses
- 30-60-min shorter preparation time
- combination with rapid digestion > single day preparation

Challenge

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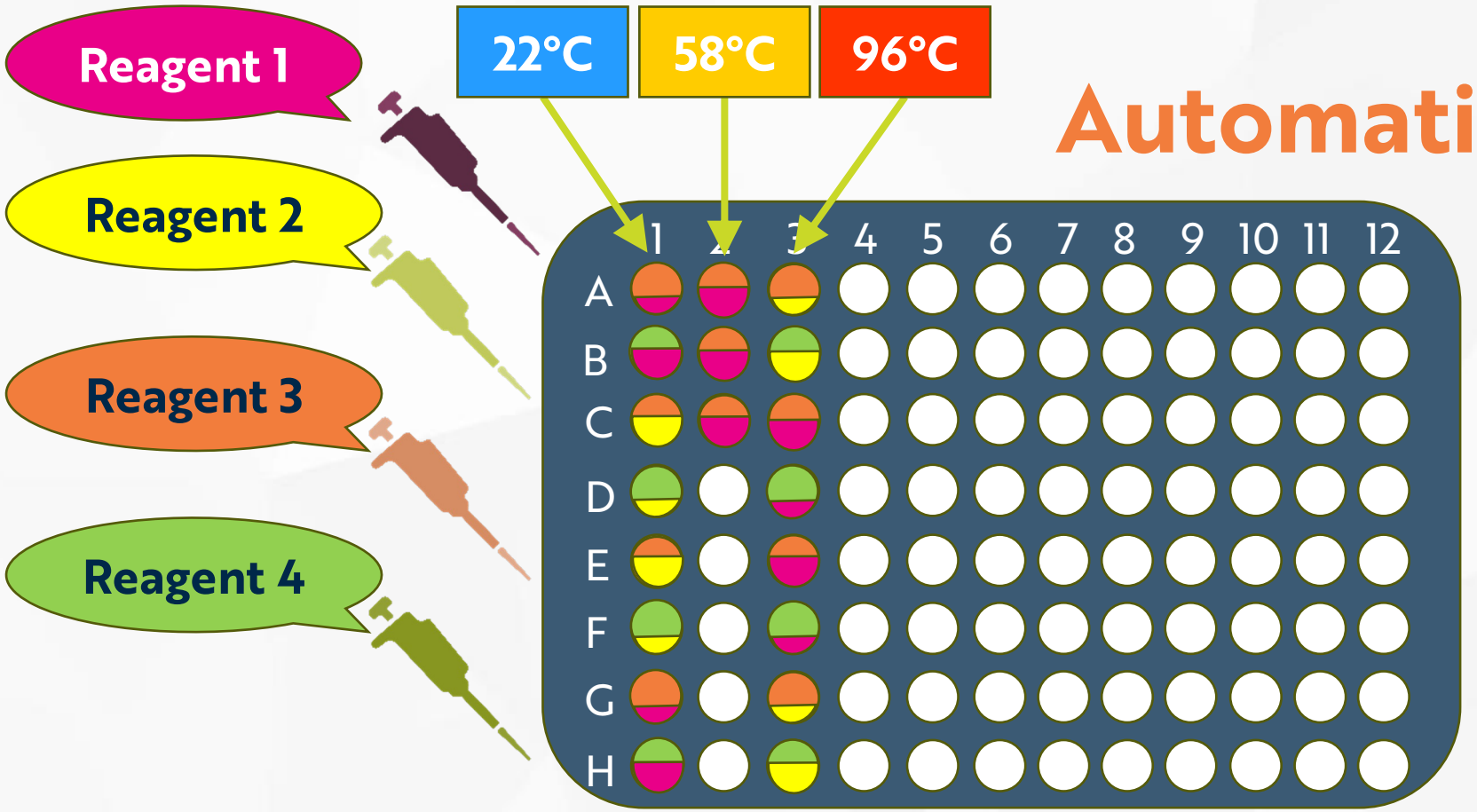
DoE screening **only 19** samples, but...



Automation

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Formulatrix Mantis liquid dispenser

- **Objective:** compare automation vs manual

1-step DoE optimisation

- Reduction reagent concentration: 0.1-4 mM
- Incubation time: 1-3 hours
- E/P ratio: 1:25-1:100

DoE

- **17** samples

Automated vs Manual DoE

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Predicted optimal conditions

	Reduction reagent c (mM) Auto./manual	Incubation t (min) Auto./manual	E/P Auto./manual
VVSV	0.1/0.1	30/30	1:25/1:25
DTLM	2.0/0.1	105/180 [a]	1:63/1:25
TPEV	3.6/3.9	165/145	1:33/1:25
FNWY	0.5/0.1	45/180 [a]	1:33/1:25

[a] Incubation time has no significant impact

- Same predicted conditions for the most abundant peptide
- Differences > may be experimental artifact > automation: fixed conc. and different volume; manual: different conc. and fixed volume > under investigation

Summary

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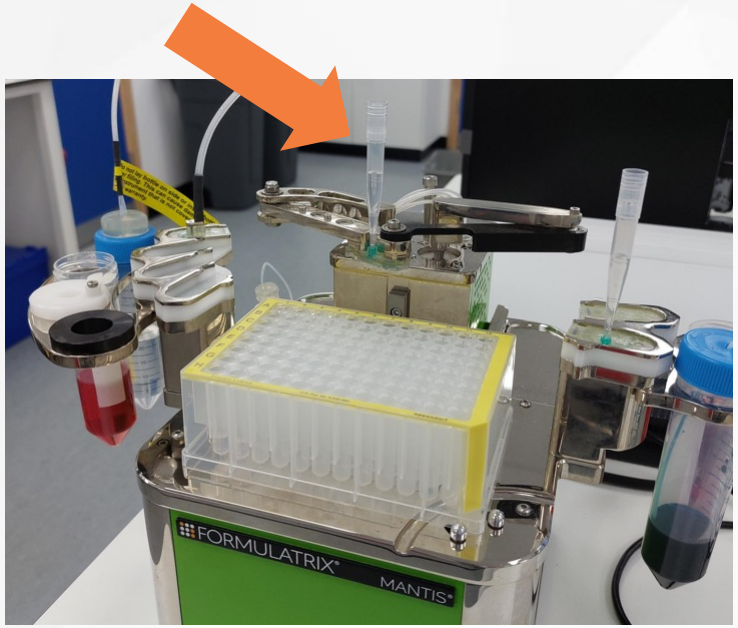
Process improvement

- DoE > comprehensive optimisation in 2 experiments > only **~50** vs ~1000 samples
- Unexpected interaction > combination of TCEP and CAA
- 2-step (R&A) – time saving and simplified process > to be implemented



Automation - Mantis

- Automated vs manual comparable results
- Small footprint, compact design
- Can hold reagents in a pipette tip
- Very low dead volumes

} useful when reagent is limited

- Easy, user-friendly programming

Acknowledgement

Matthijs Van De Waal

Thank you for your attention

Any questions?

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