Analyzing repeated measures designs in label-free proteomics with MSqRob (MCP 2016 15(2):657-68.)

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Background: In repeated measures designs different observations are obtained on the same experimental unit (EU), which increases statistical power for within subject treatment effects because the between-subject variability can be eliminated from the estimation. Data of the same EU, however, are typically more similar than data between EUs. Most existing workflows cannot address experiments with complex designs and correlation, resulting in a power loss when assessing treatment effects within EU (e.g. compound effects) and improper error rate control for effects between EU (e.g. KO vs WT).

Repeated Measures Design

Baseline control, early and late responses on inflammatory stimuli (IS)

	Wild Type (WT)			Knock Out (KO)		
Time						
0	Con	Con	Con	Con	Con	Con
1	IS1	IS1	IS1	IS1	IS1	IS1
	IS2	IS2	IS2	IS2	IS2	IS2
6	IS1	IS1	IS1	IS1	IS1	IS1
	IS2	IS2	IS2	IS2	IS2	IS2

MSqRob workflow

- Import data (MaxQuant or Mascot)
- Preprocessing (e.g. $\log_2$ transformation, normalization, ...)
- Robust peptide-based regression
- Test contrasts of interest (FDR-corrected p-values)

Model $\log_2$ Intensity $= G \times IS \times T + (1|mouse) + (1|run) + (1|Peptide) + \varepsilon$

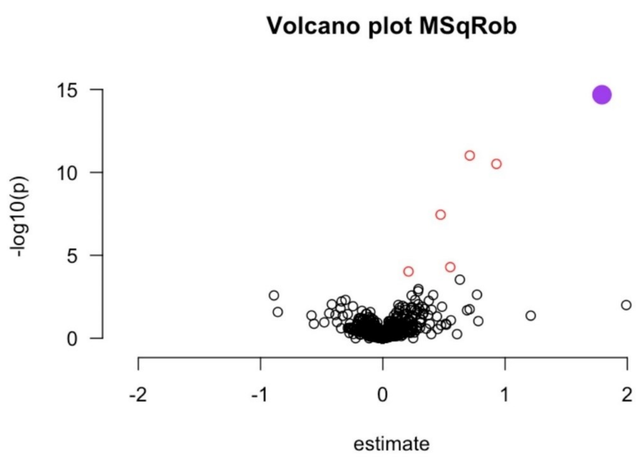
Log_2 peptide intensity modeled by genotype (G), Inflammatory Stimulus (IS) & time (T) main effects & interactions + random effects for mouse, run and peptide to address correlation. Normal error.

Results

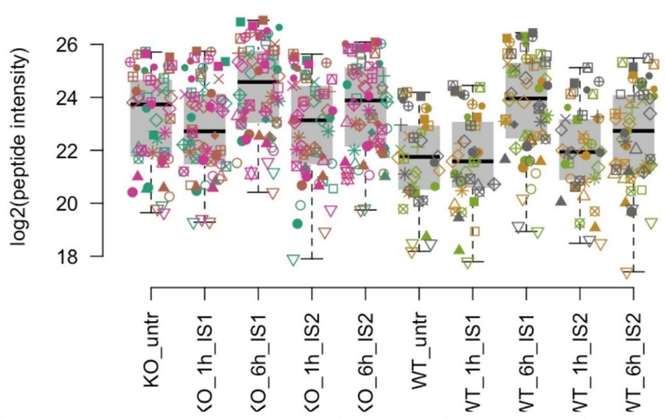
MSqRob GUI Output

```
            Contrast 1
treatKO_untr          1
treatWT_untr         -1
```

Volcano plot

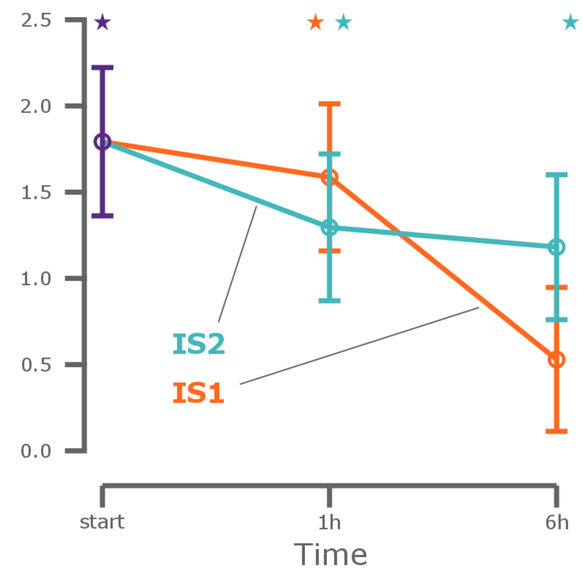


Detail plot



Additional Plot

Log2 FC (KO vs. WT)



Protein with significant upregulation in KO vs WT at baseline, 1h upon treatment with IS1 stimulus and 1h and 6h with IS2, and a significant interaction, i.e. the upregulation in KO decreases over time.

Conclusion

- Powerful analysis of complex designs with fixed and random effects
- Robust estimation and shrinkage of fixed effects
- Data exploration and visualisation
- **Download MSqRob package: https://github.com/ludgergoeminne/MSqRob**

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