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# Sub-project 3

- How does triggering alternate or multiple PRRs affect the outcome?

- What is the impact of endogenous signalling pathways on pathogen sensing?

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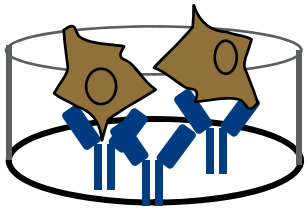
# The experimental system

Mouse bone marrow-derived dendritic cells

8d culture with GM-CSF (X63 sn)

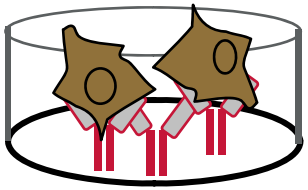


+ Jagged1

(10μg/ml coated o/n)

+/- LPS

- RNA: qRT-PCR, microarray
- Supernatant: ELISA
- Protein: phosphoP analysis



+ control

Analysis over time

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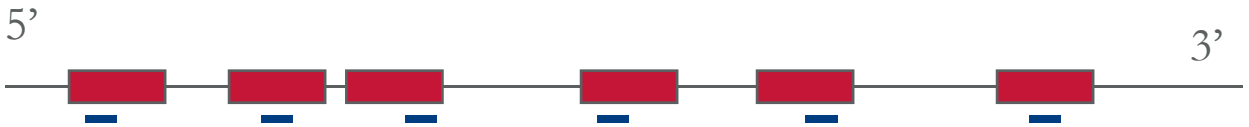
# Microarray analysis: exon arrays




- 4 treatments, time course over 4 hrs with samples takes every 30 mins.
- Triplicates at 0 hrs, 2 hrs and 4 hrs.
- Total of 60 microarrays.

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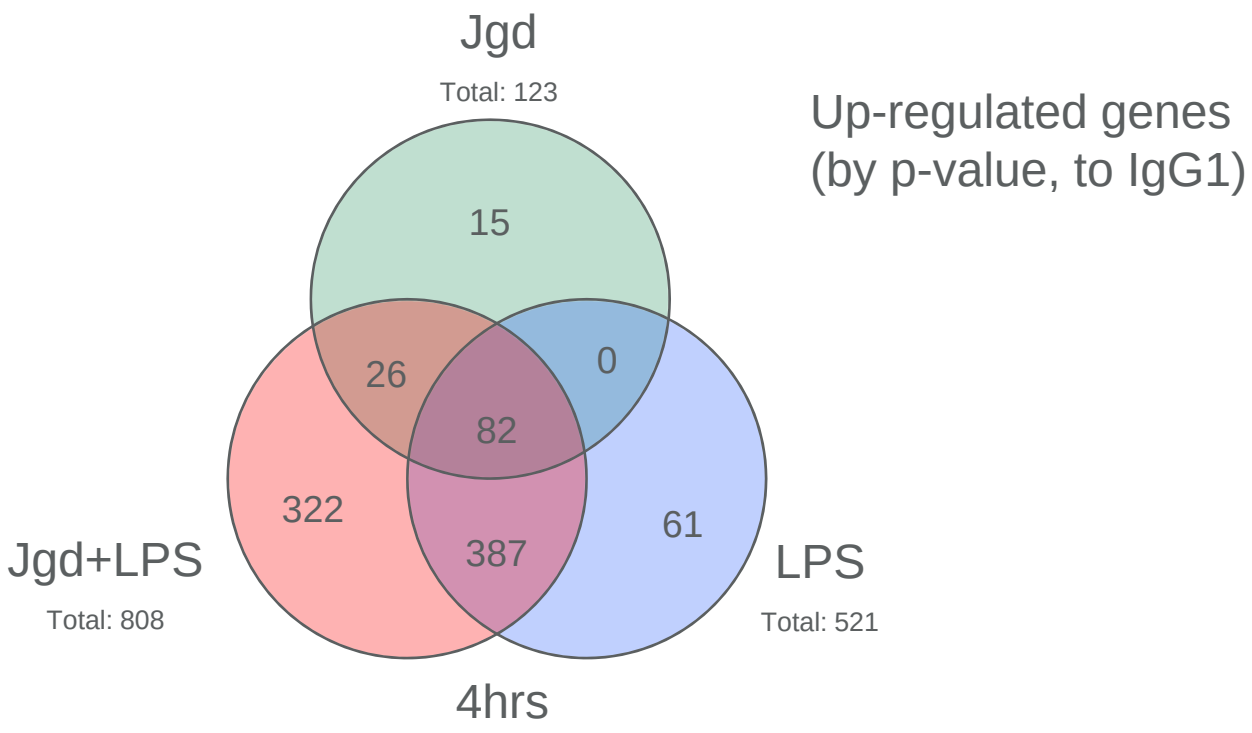
# Microarray

- Previously characterised genes show consistent patterns
- Total of 884 genes altered at 4hr in at least one group

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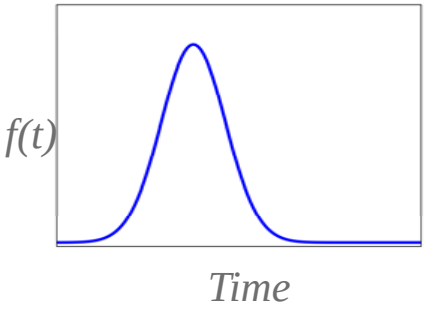
# Modelling microarray data

*Rate of change of expression of a gene*

*Transcription factor activity*




$$\frac{dx_i(t)}{dt} = \beta_i + S_i f_i(t) - \alpha_i x_i(t)$$

*Basal rate*

*Sensitivity*

*Decay rate*

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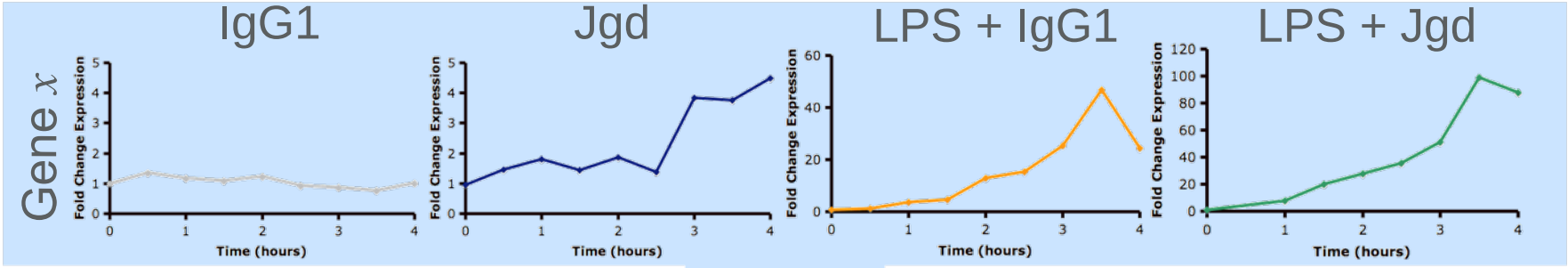
# Analysis of Time Course Data




ODE Model of Gene Expression

Error Function

A putative list of genes whose expression is driven by the same transcriptional activity

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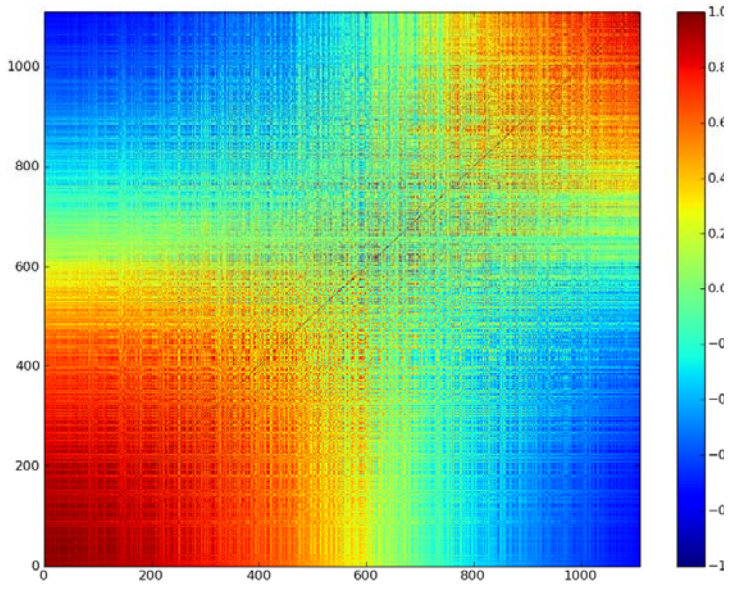
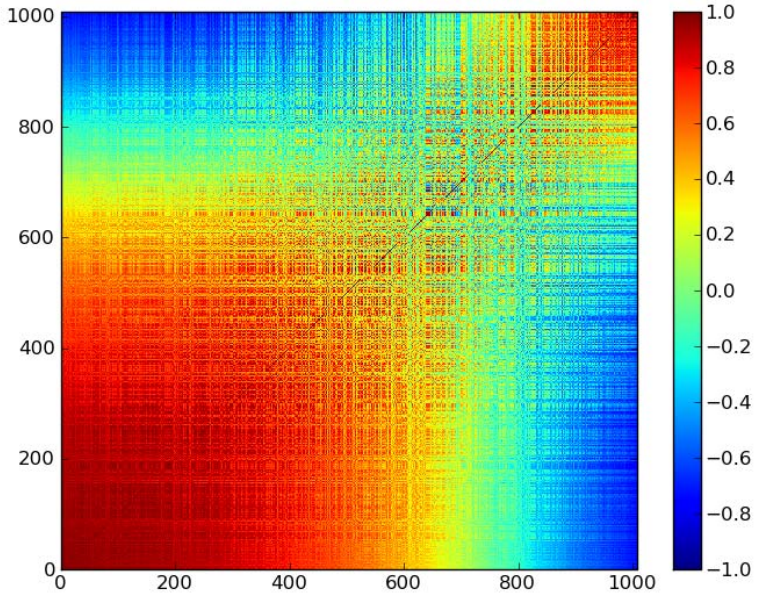
# Two main groups

*Jgd + LPS*




*LPS*

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# Validation

- Model predicts genes that are regulated in a similar fashion.
- Model estimates degradation rates.
- Model can compare genes across treatments.
- Validate predictions by promoter analysis, chromatin immunoprecipitation and use of small molecule inhibitors.

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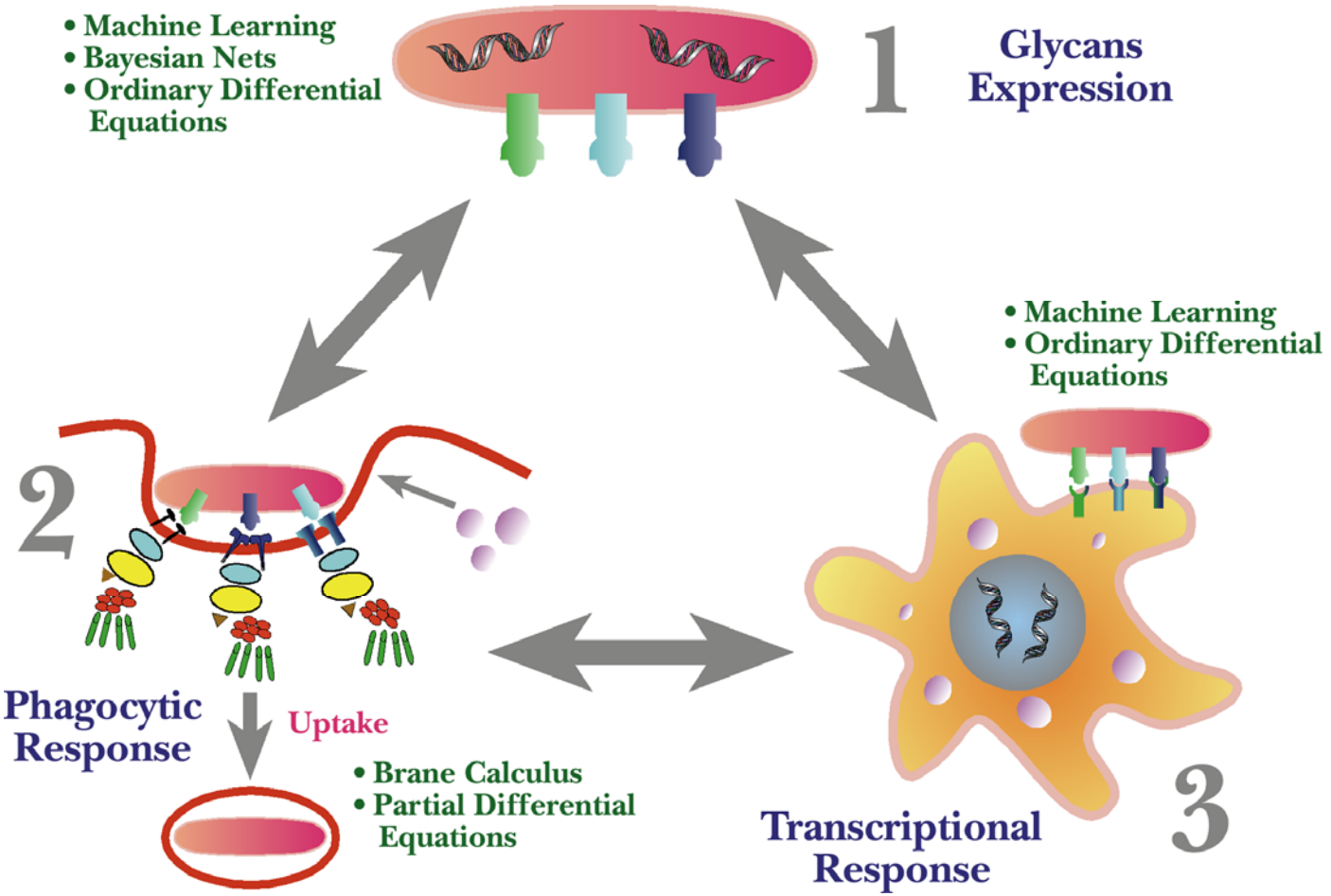
# Integration of Sub-projects

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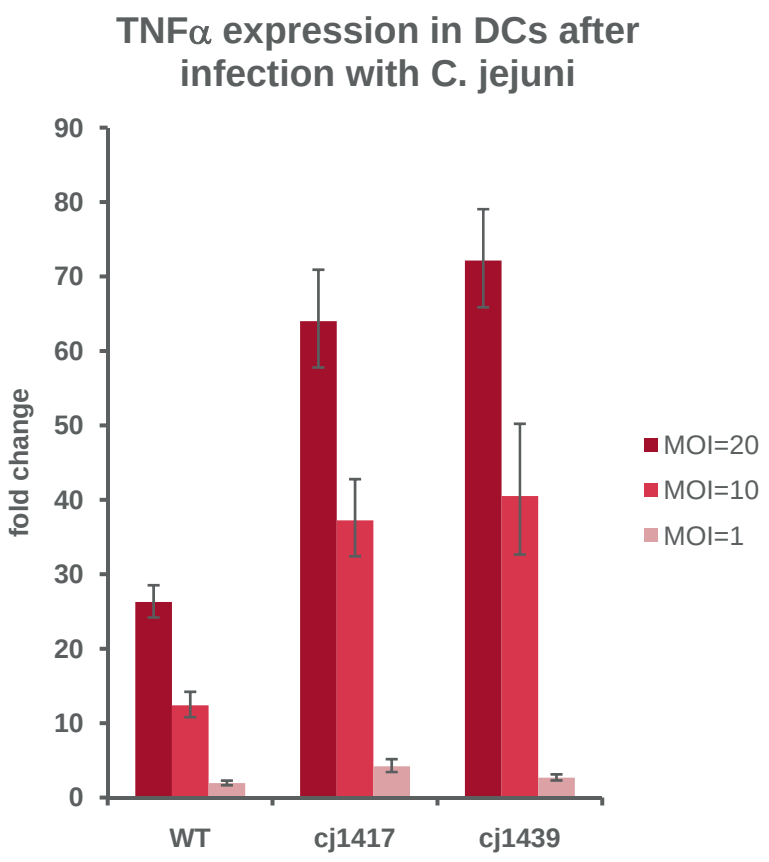
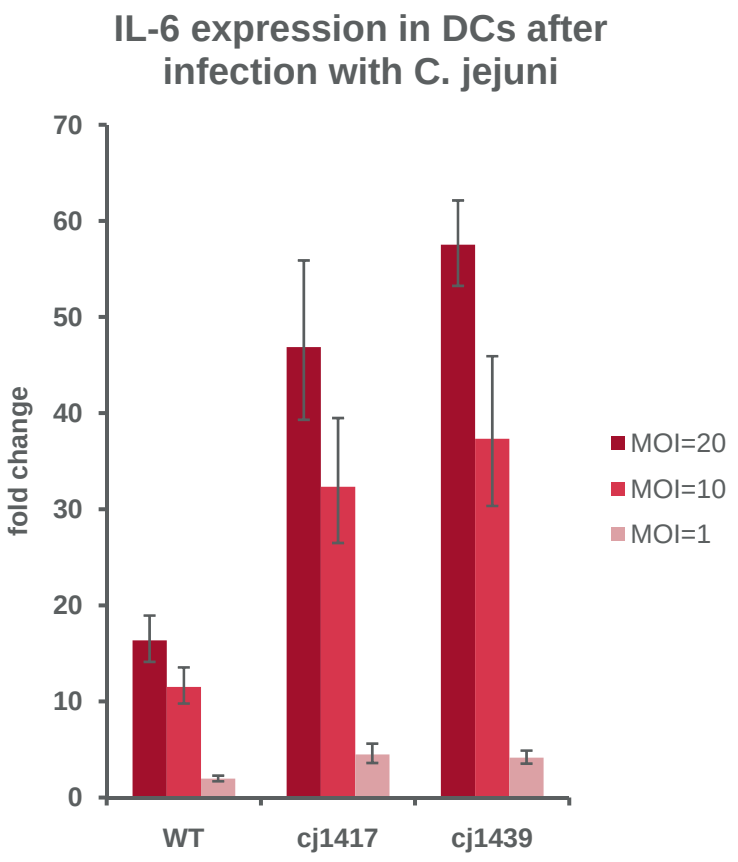
# Triggering of cytokine expression by *C. jejuni* mutants




WT: Wild type *C. jejuni*
cj1417: side branch of capsule missing
cj1439: acapsular

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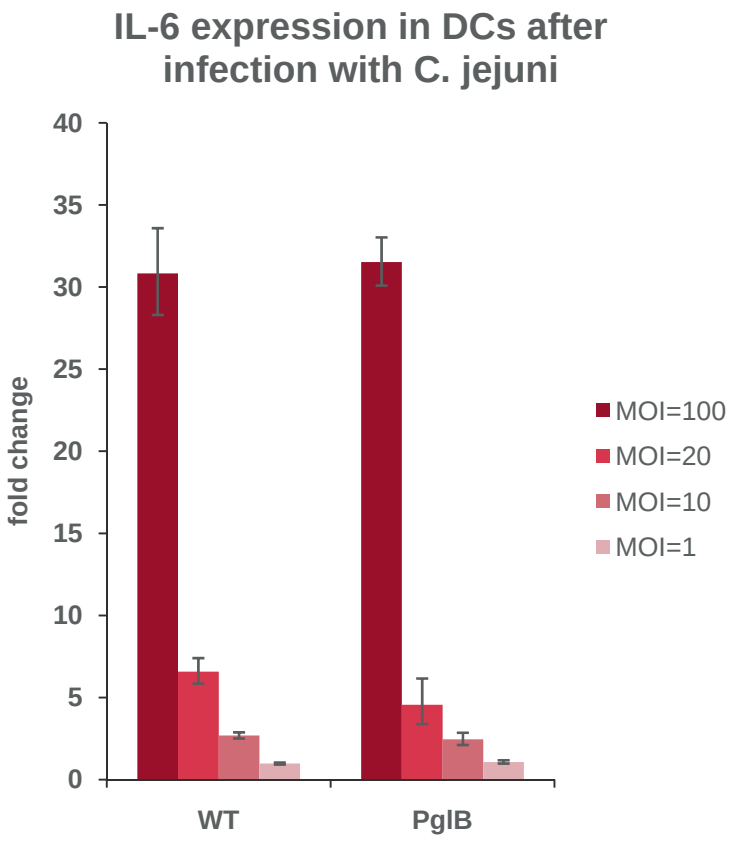
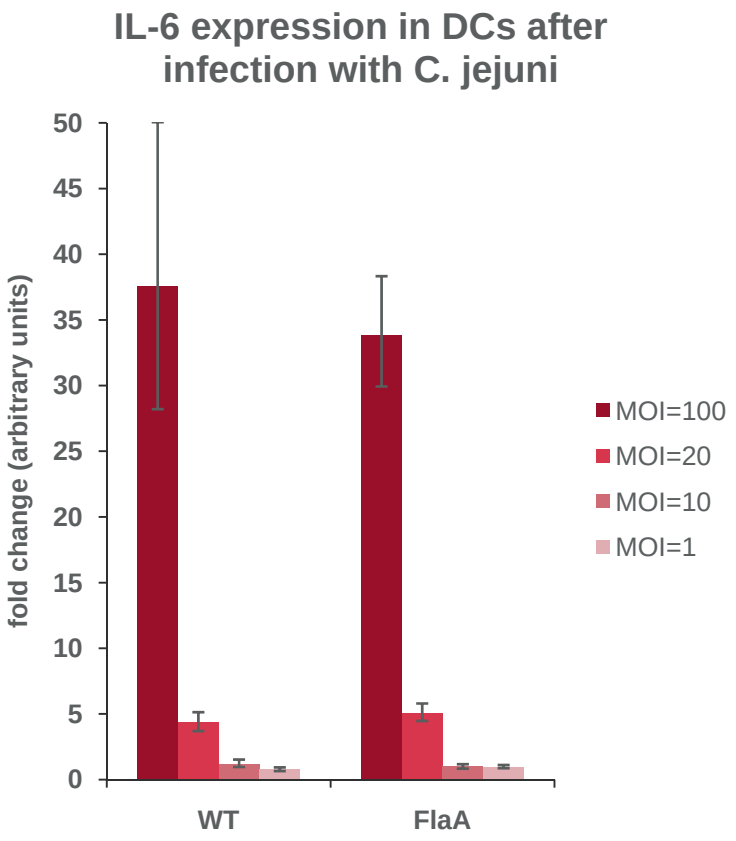
# Cytokine expression in some mutants is similar to WT *C. jejuni*




*WT: wild type*
*PglB: no N-linked glycosylation*
*FlaA: no flagella*

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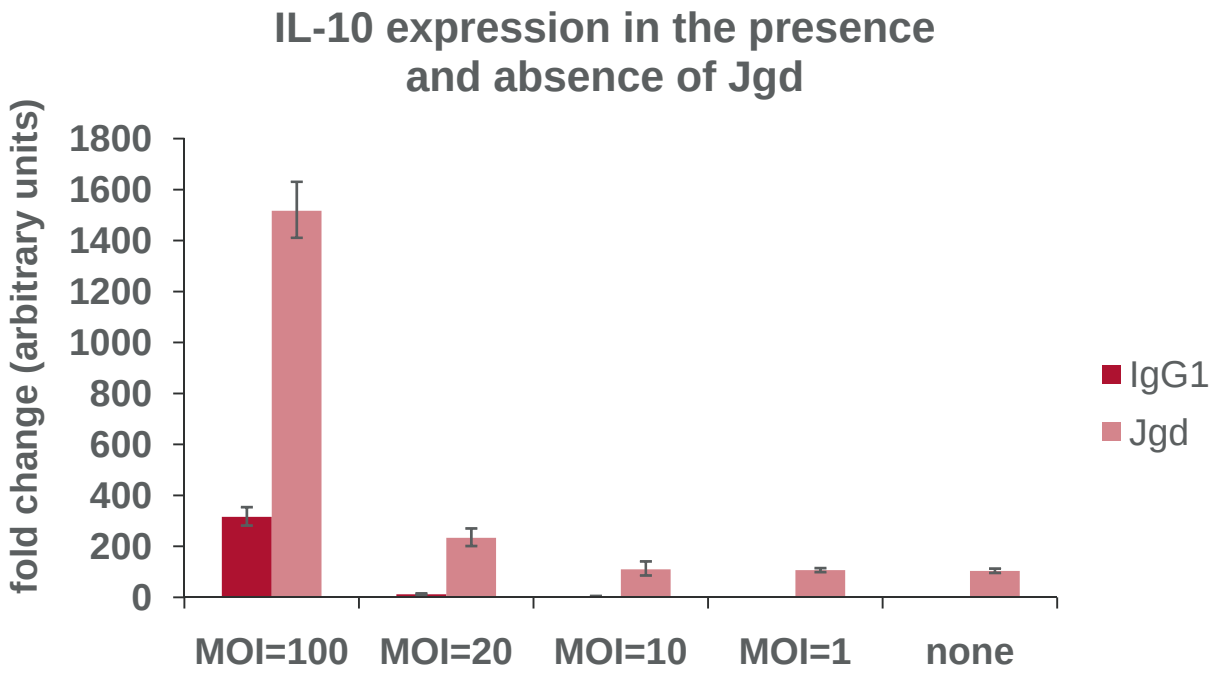
# Notch signalling can enhance IL-10 production from C. jejuni

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# Summary

- Notch modulates TLR signalling and vice versa in DCs
- Model has generated some predictions of genes co-regulated with IL-10
- Work on sub-project integration has started, investigating the cytokine response of DCs to different *C. jejuni* mutants.

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# Achievements

- Detailed biological exploration of the system including
  - Time course microarray analysis
  - Measurement of degradation rates
  - Investigation of the role of a number of signalling pathway components.
- Modelling of global transcriptional response

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# Future plans

- Predictions generate groups of genes that are similarly regulated.
- Validation of those predictions will initially focus on promoter analysis, chromatin immunoprecipitation, measurement of degradation rates and use of small molecule inhibitors.
- Integration with sub-project 1 will continue. Areas to investigate include the role of TLR4 and LOS in the innate immune response to *C. jejuni* and whether different WT strains elicit different levels of cytokine response.
- Initial experiments for integration with sub-project 2 are planned.

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# Acknowledgements

Thank you

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