

# Brief summary of Masquant data analysis

## Experimental design:

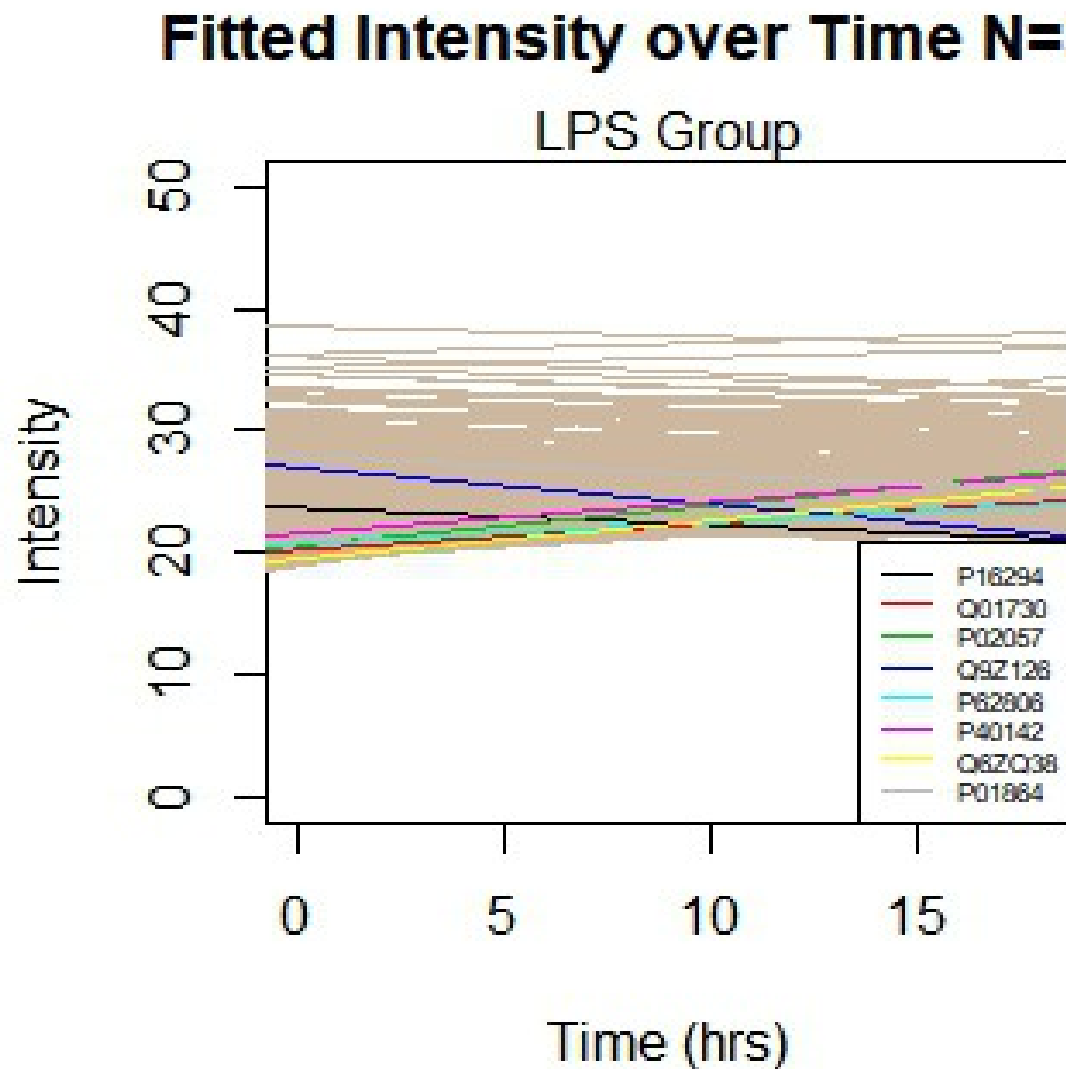
Treatment	PBS	LPS	LPS+TLR-/-
Size 5/group			
Time points:			
0	↓	↓	↓
6	↓	↓	↓
12	↓	↓	↓
18	↓	↓	↓
Hours	↓	↓	↓
Technical rep: 2			

Dataset: average of the 2 reps for 3 animals per group in text file

## Key points in analysis of this data set:

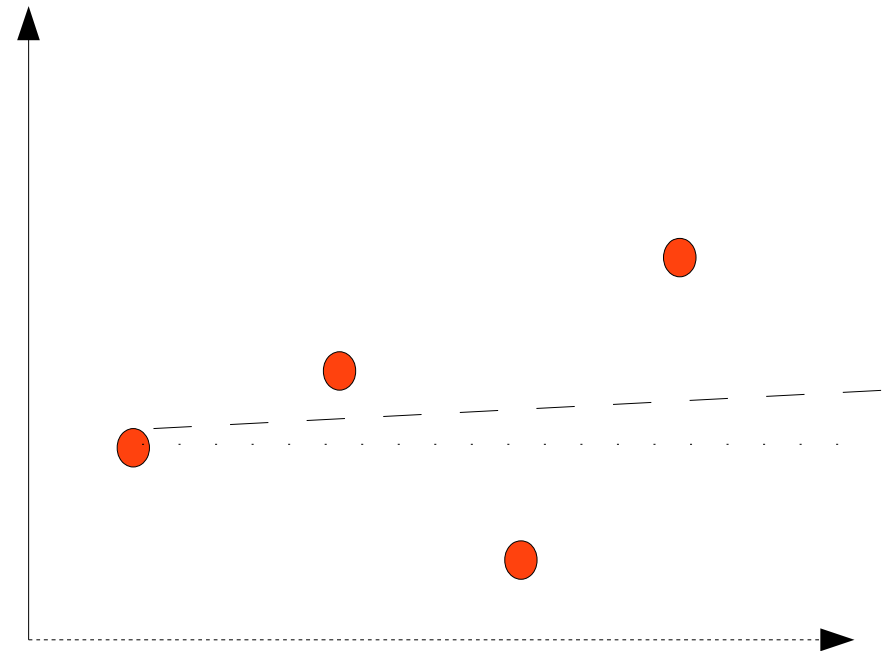
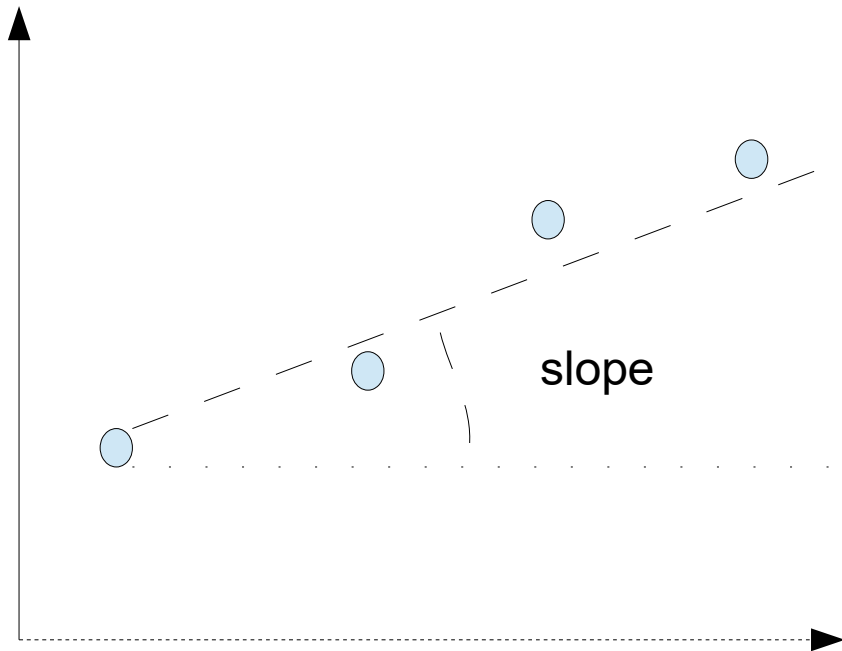
1. Multiple comparison: when making large number of comparisons from one experiment, the false positive rate, or the  $\alpha$  value at 0.05, may lead to many false positive discoveries. e.g.,  $400 \times 0.05 = 20$  (expected)
2. Time series sampling: repeated sampling of the same study object invalidates the independence assumption, i.e., simple one-way ANOVA becomes unsuitable.
3. Program: R with dplyr and broom packages

Time series approach I: estimate the change by slope



	Downregulated				
Protein ID	Name	Gene name	slope	adj P	
P16294	Coagulation factor IX	F9	-0.125	0.001	
Q9Z126	Platelet factor 4	pf4	-0.306	0.041	
P01864	Ig gamma-2A chain C region secreted form		-0.142	0.095	
	Upregulated				
Protein ID	Name	Gene name	slope	adj P	
Q01730	Ras suppressor protein 1	Rsu1	0.225	0.012	
P02057	Hemoglobin subunit beta	HBB	0.333	0.017	
P62806	Histone H4	Hist1h4a	0.188	0.045	
P40142	Transketolase	Tkt	0.259	0.069	
Q6ZQ38	Cullin-associated NEDD8-dissociated protein 1	Cand1	0.312	0.073	

## Time series approach I: potential issues



## Time series approach II: using time points as factors

- \* retaining repeated measure property but not counting each time point as a continuous variable
- \* allowing comparisons between time points

	Hour 6 upregulated			
Protein ID	Name	Gene name	intensity +	adj P
P10810	Monocyte differentiation antigen CD14	Cd14	6.004	0.000
P51437	Cathelin-related antimicrobial peptide	Camp	4.343	0.017
P07901	Heat shock protein HSP 90-alpha	HSP90AA1	2.950	0.002
P08071	Lactotransferrin	Ltf	5.600	0.047
Q61646	Haptoglobin	Hp	5.293	0.054
	No significant downregulated at Hour 6			

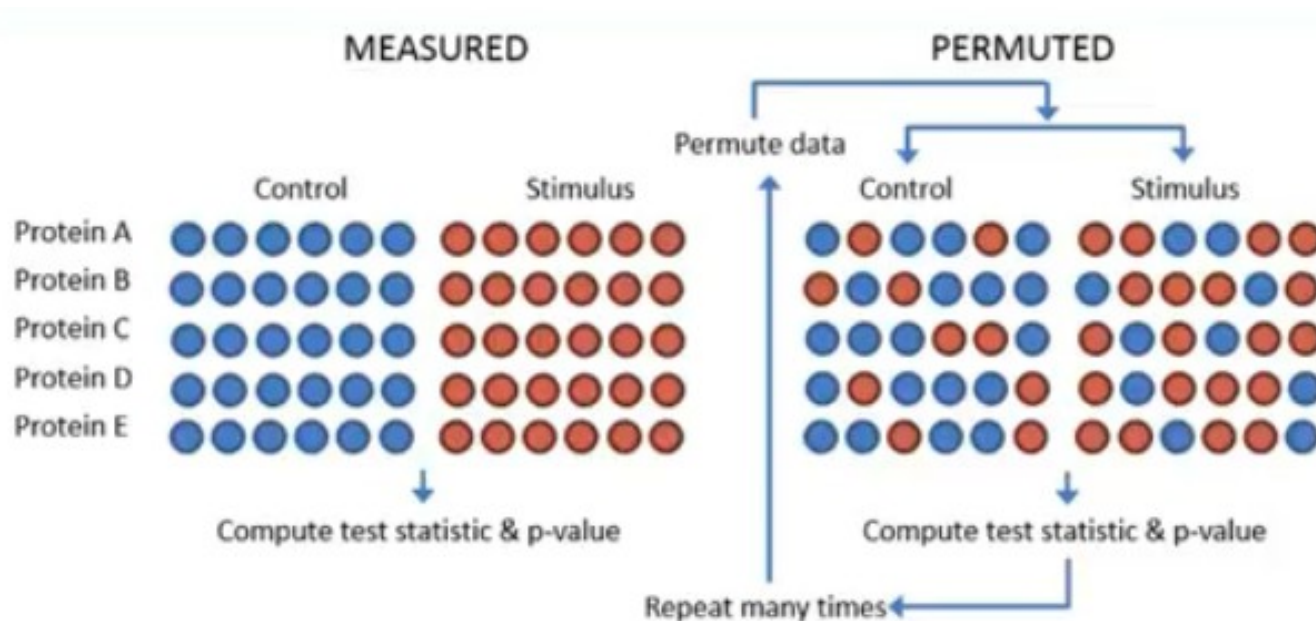
	<b>Hour 12 Upregulated</b>			
<b>Protein ID</b>	<b>Name</b>	<b>Gene name</b>	<b>intensity+</b>	<b>adj P</b>
P10810	Monocyte differentiation antigen CD14	Cd14	7.565	0.000
P40142	Transketolase	Tkt	5.267	0.001
P07901	Heat shock protein HSP 90-alpha	HSP90AA1	3.502	0.003
Q61646	Haptoglobin	Hp	6.515	0.070
P05367	Serum amyloid A-2 protein	Saa2	8.587	0.079
P08071	Lactotransferrin	Ltf	6.128	0.087
P51437	Cathelin-related antimicrobial peptide	Camp	4.647	0.088
	<b>Hour 12 Downregulated</b>			
<b>Protein ID</b>	<b>Name</b>	<b>Gene name</b>	<b>intensity-</b>	<b>adj P</b>
P16294	Coagulation factor IX	F9	-1.941	0.001



	<b>Hour 18 Upregulated</b>			
<b>Protein ID</b>	<b>Name</b>	<b>Gene name</b>	<b>intensity+</b>	<b>adj P</b>
P10810	Monocyte differentiation antigen CD14	Cd14	6.782	0.000
P07901	Heat shock protein HSP 90-alpha	HSP90AA1	3.447	0.005
P40142	Transketolase	Tkt	4.663	0.010
Q01730	Ras suppressor protein 1	Rsu1	4.242	0.055
	<b>Hour 18 Downregulated</b>			
<b>Protein ID</b>	<b>Name</b>	<b>Gene name</b>	<b>intensity-</b>	<b>adj P</b>
P16294	Coagulation factor IX	F9	-1.910	0.002
P32261	Antithrombin-III	Serpinc1	-2.038	0.010
P01864	Ig gamma-2A chain C region secreted form		-2.576	0.046
P23953	Carboxylesterase 1C	Ces1c	-1.691	0.060
O08677	Kininogen-1	Kng1	-2.192	0.071

## Notes:

1. Adjusted P values are not really p values, could be used as a ranked indicator.
2. Discrepancy with Perseus analysis:
  - a. validity of one-way ANOVA
  - b. permutation approach by Perseus could be a little too liberal for this settingPowerful tool for small sample size and unknown population distribution  
Nonparametric, prone to potential issues in data quality.



❖ False discovery rate estimated by counting hits on permuted data