

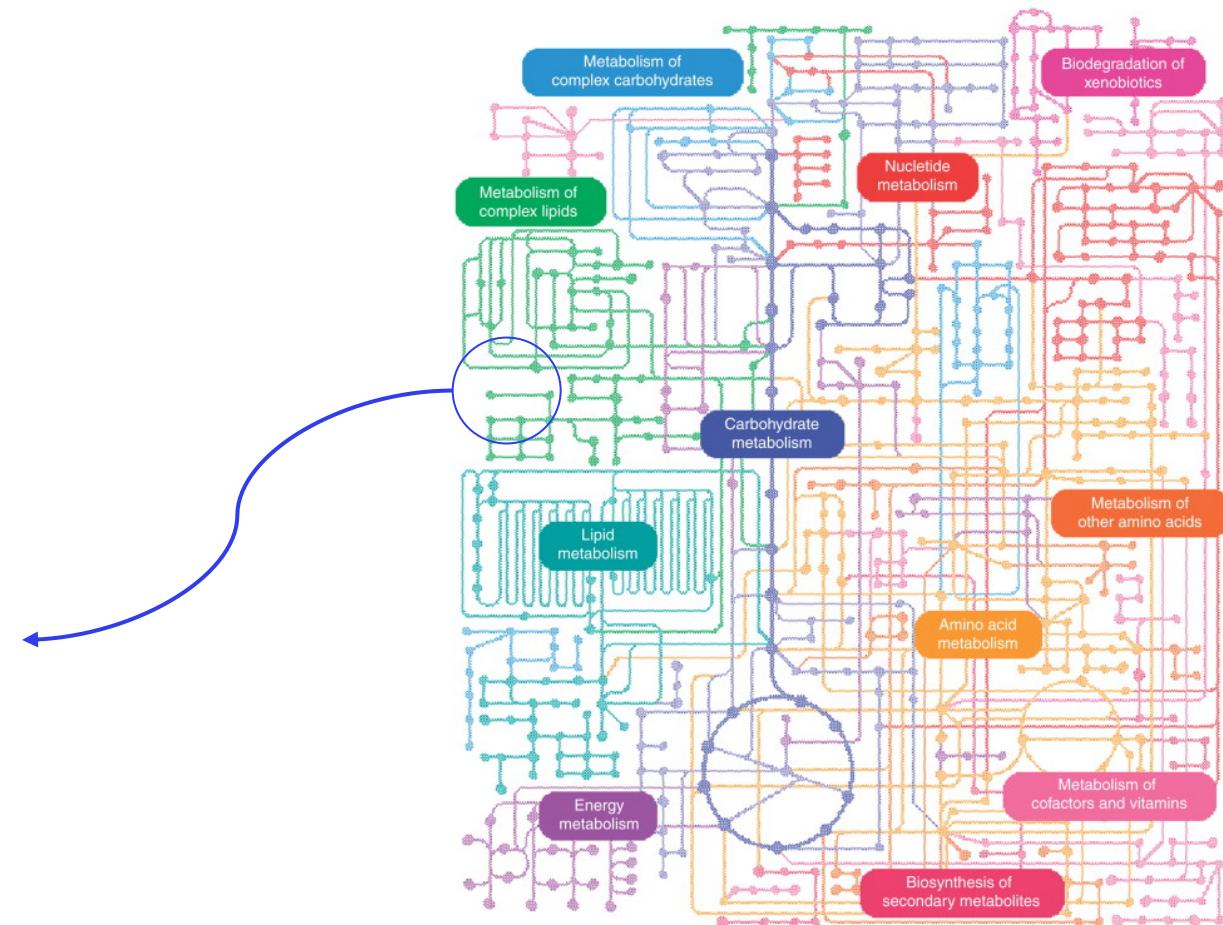
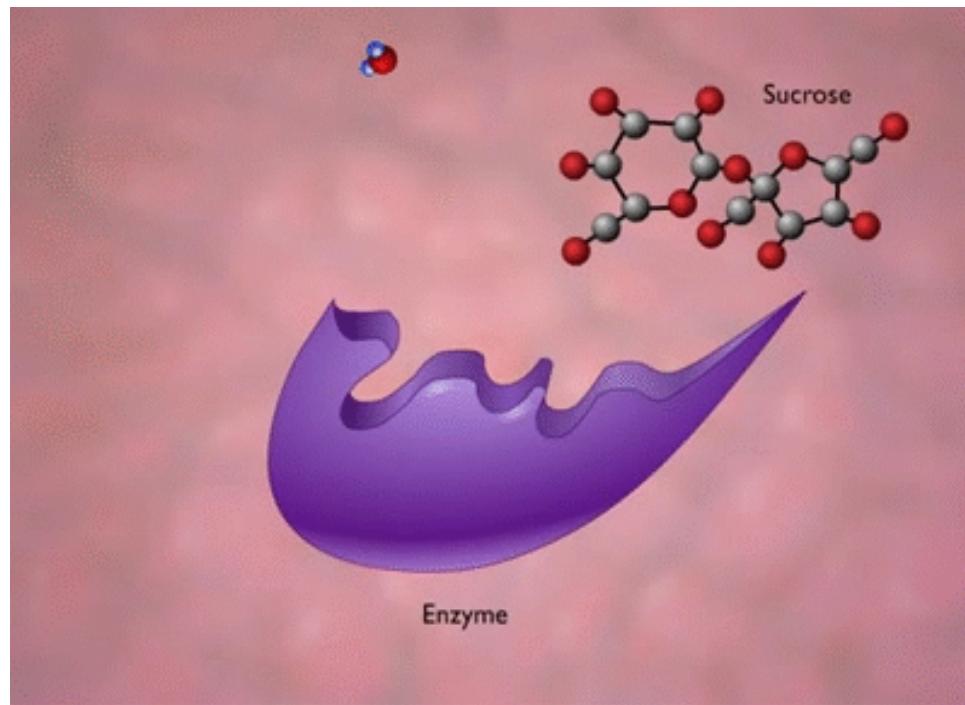
Teddy Groves

Modelling metabolic networks with Stan

Cell factories

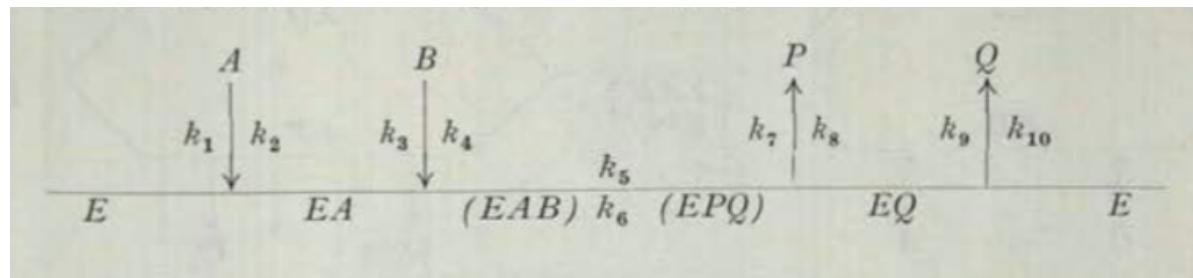


Metabolic networks



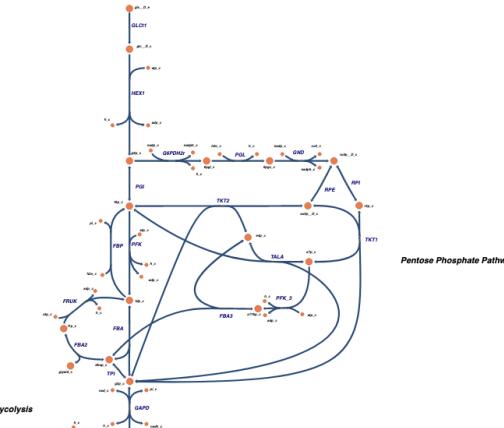
Structural information

Mechanism

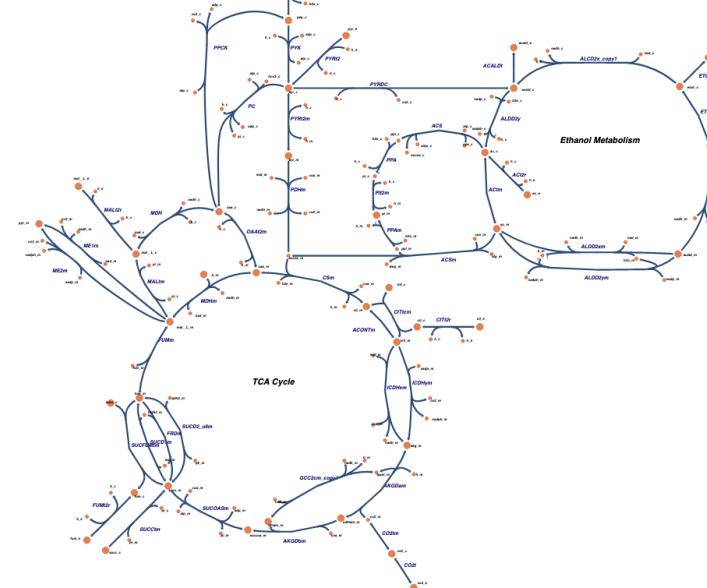


Rate equation

$$v = \frac{V_1 V_2 \left(AB - \frac{PQ}{K_{\text{eq}}} \right)}{K_{\text{fa}} K_{\text{fb}} V_2 + K_{\text{fb}} V_2 A + K_{\text{fa}} V_2 B + V_2 A B + \frac{K_{\text{q}} V_1 P}{K_{\text{eq}}} + \frac{K_{\text{p}} V_1 Q}{K_{\text{eq}}} + \frac{V_1 PQ}{K_{\text{eq}}} + \frac{K_{\text{q}} V_1 AP}{K_{\text{fa}} K_{\text{eq}}} + \frac{K_{\text{a}} V_2 B Q}{K_{\text{fqb}}} + \frac{V_2 A B P}{K_{\text{fp}}} + \frac{V_1 B P Q}{K_{\text{fb}} K_{\text{eq}}}}$$



Network topology

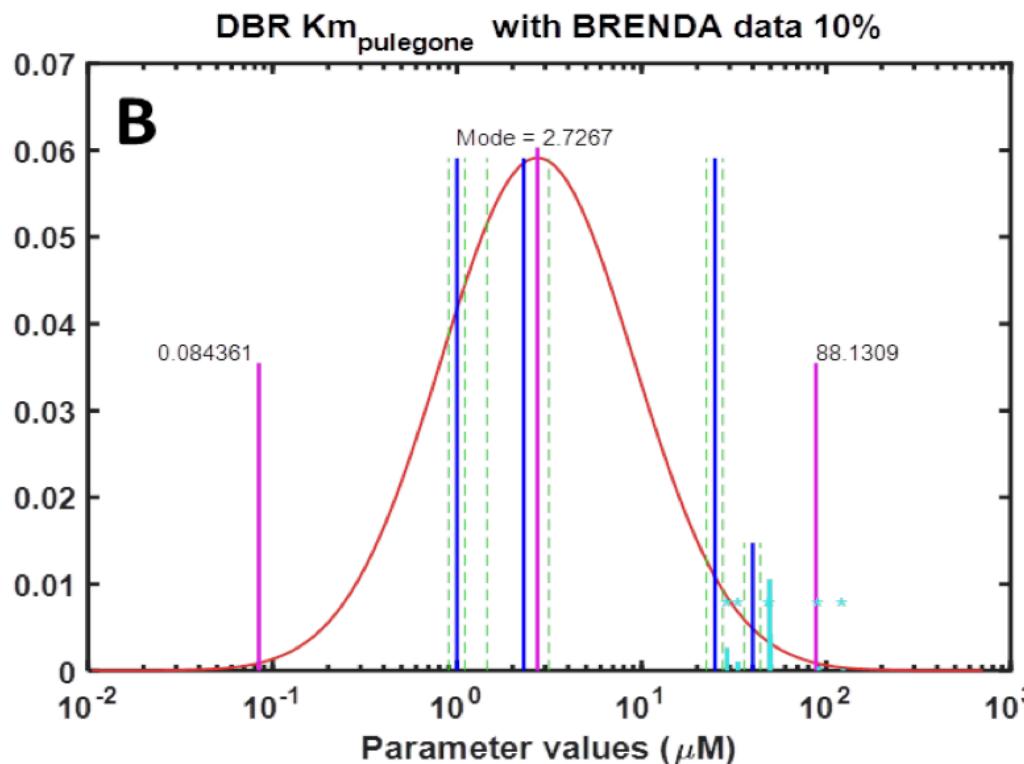


Experimental data

- Metabolomics
(metabolite concentrations)
- Proteomics
(Enzyme concentrations)
- Fluxomics
(Steady state fluxes)



Information from online databases

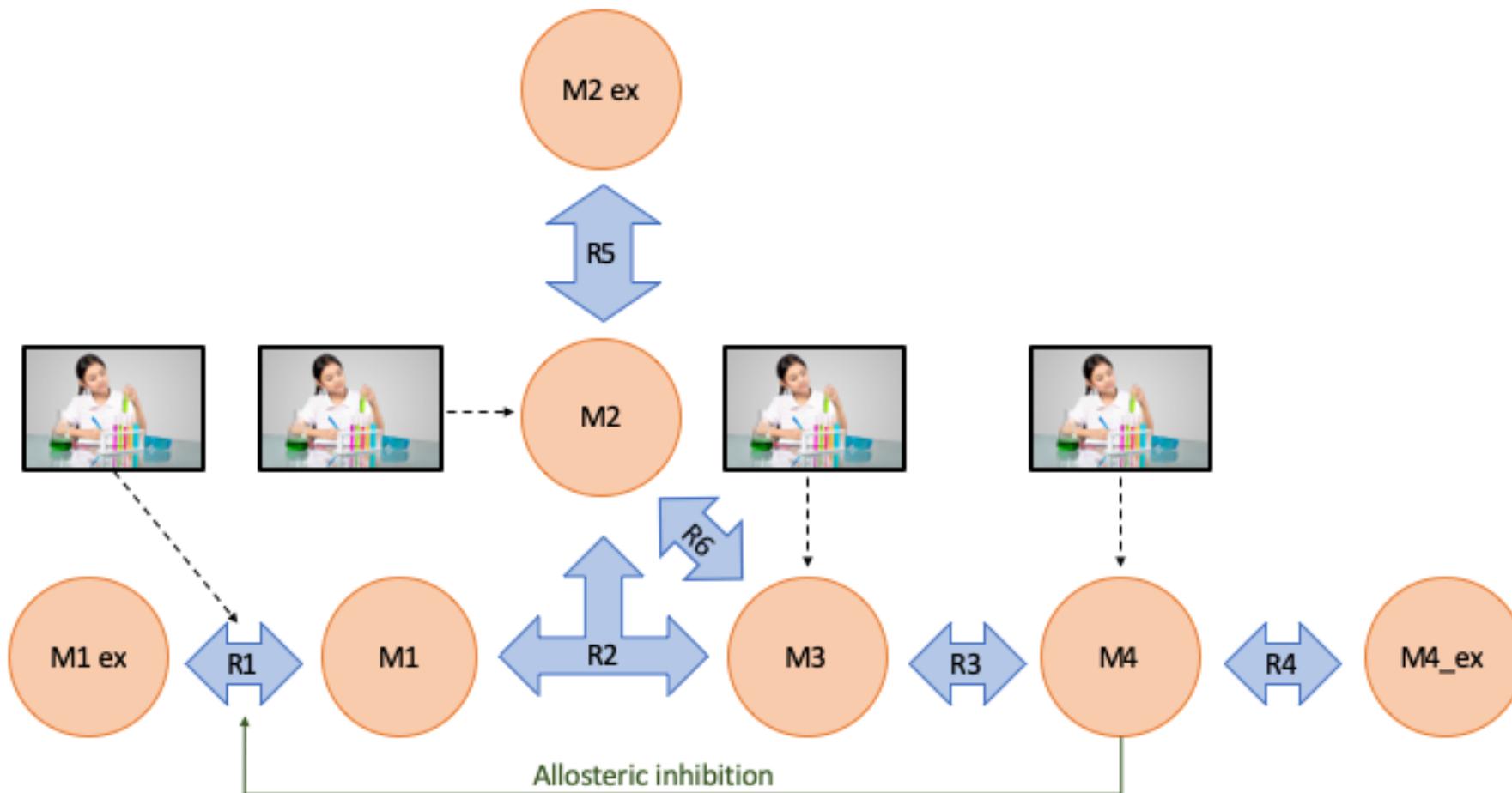


B10NUMB3R5
THE DATABASE OF USEFUL BIOLOGICAL NUMBERS

The plan



A simple reaction network



Challenges

- Some enzymes bind to molecules that change their behaviour
(allosteric regulation)
- Some parameter configurations imply perpetual motion machines
(thermodynamic feasibility)

How we represent rate equations

```
real ordered_unibi(real A, real P, real Q, real V1, real V2, real Ka,  
                    real Kp, real Kq, real Kia, real Kip, real Kiq, real Keq){  
  
    real num = V1*V2*(A-P*Q/Keq);  
  
    real denom =  
  
        Ka*V2  
  
        + V2*A  
  
        + Kq*V1*P/Keq  
  
        + Kp*V1*Q/Keq  
  
        + V1*P*Q/Keq  
  
        + V2*A*P/Kip;  
  
    return num / denom;  
}
```

$$v = \frac{V_1 V_2 \left(A - \frac{PQ}{K_{eq}} \right)}{K_a V_2 + V_2 A + \frac{K_q V_1 P}{K_{eq}} + \frac{K_p V_1 Q}{K_{eq}} + \frac{V_1 PQ}{K_{eq}} + \frac{V_2 AP}{K_{ip}}}$$

How we represent thermodynamic constraints

$$K_{\text{eq}} = \frac{V_1 K_{\text{ip}} K_{\text{q}}}{V_2 K_{\text{ia}}} = \frac{V_1 K_{\text{p}} K_{\text{iq}}}{V_2 K_{\text{a}}}$$

```
real get_Kip_ordered_unibi(real Keq, real Kia, real Kq, real V1, real V2){  
    return Keq*Kia*V2 / (Kq*V1);  
}  
  
real get_Kiq_ordered_unibi(real Keq, real Ka, real Kp, real V1, real V2){  
    return Keq*V2*Ka / (V1*Kp);  
}
```

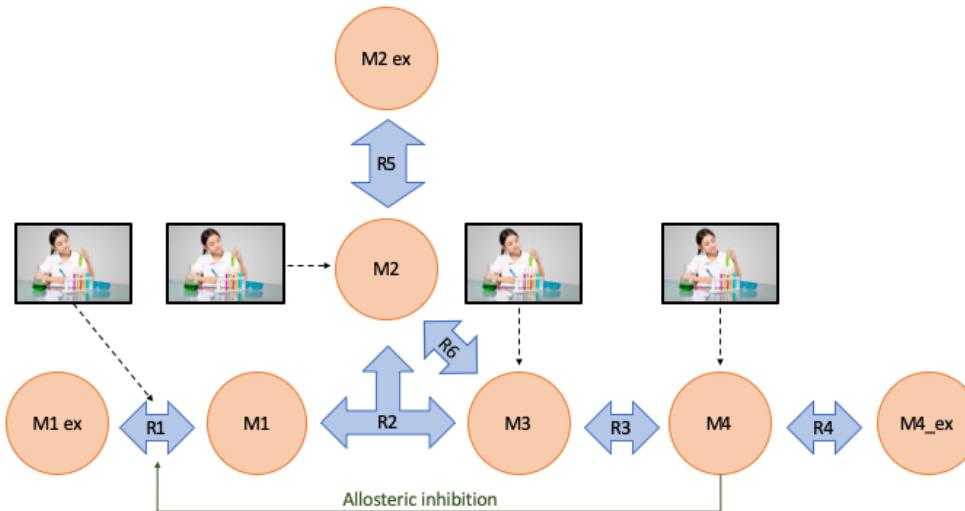
How we represent allosteric regulation

```
real get_regulatory_effect(real[] activator_concentration,
                           real[] inhibitor_concentration,
                           real free_enzyme_ratio,
                           real[] dissociation_constant_r,
                           real[] dissociation_constant_t,
                           real transfer_constant){

    real Q_num = size(inhibitor_concentration) == 0 ? 1 :
        1 + sum(to_vector(inhibitor_concentration) ./ to_vector(dissociation_constant_t));
    real Q_denom = size(activator_concentration) == 0 ? 1 :
        1 + sum(to_vector(activator_concentration) ./ to_vector(dissociation_constant_r));
    real Q = transfer_constant * free_enzyme_ratio * Q_num / Q_denom;

    return inv(1 + Q);
}
```

How we represent network structure



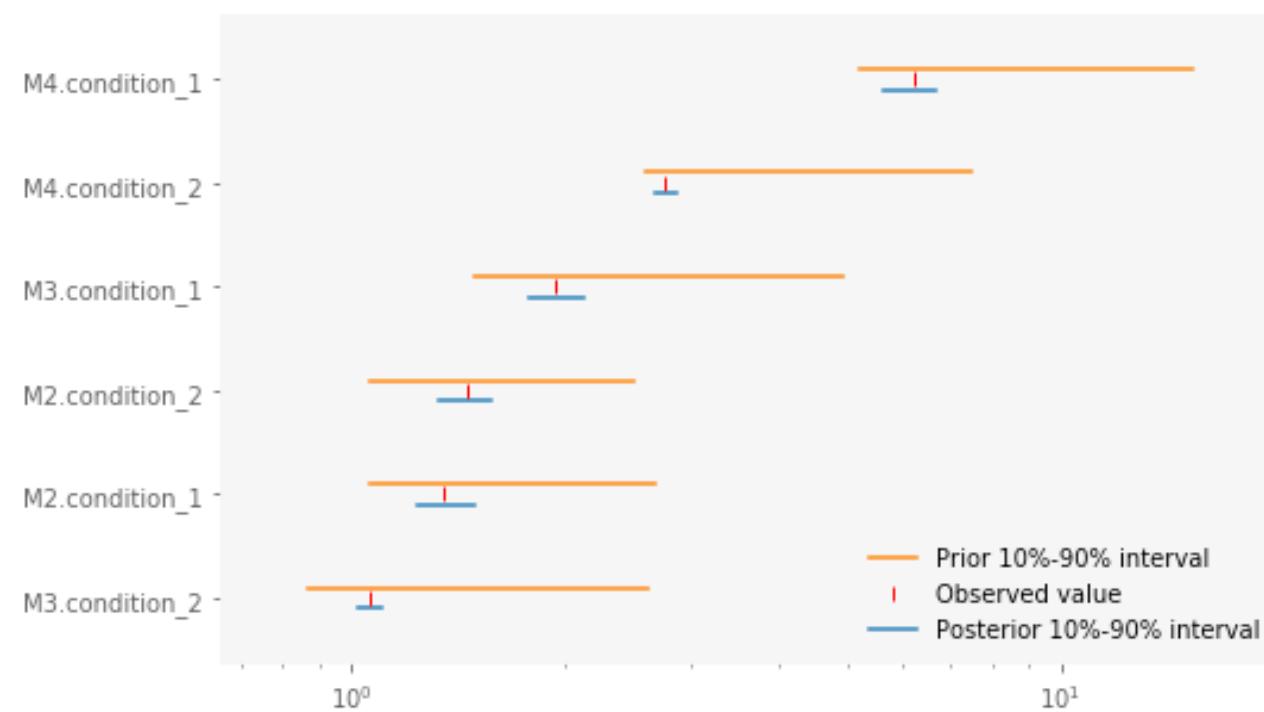
```
real[] get_odes(real[] fluxes){  
    return {  
        1*fluxes[1]-1*fluxes[2],  
        0,  
        1*fluxes[2]-1*fluxes[5]-1*fluxes[6],  
        1*fluxes[2]-1*fluxes[3]+1*fluxes[6],  
        1*fluxes[3]-1*fluxes[4],  
        0,  
        0  
    };  
}
```

How we determine steady state quantities

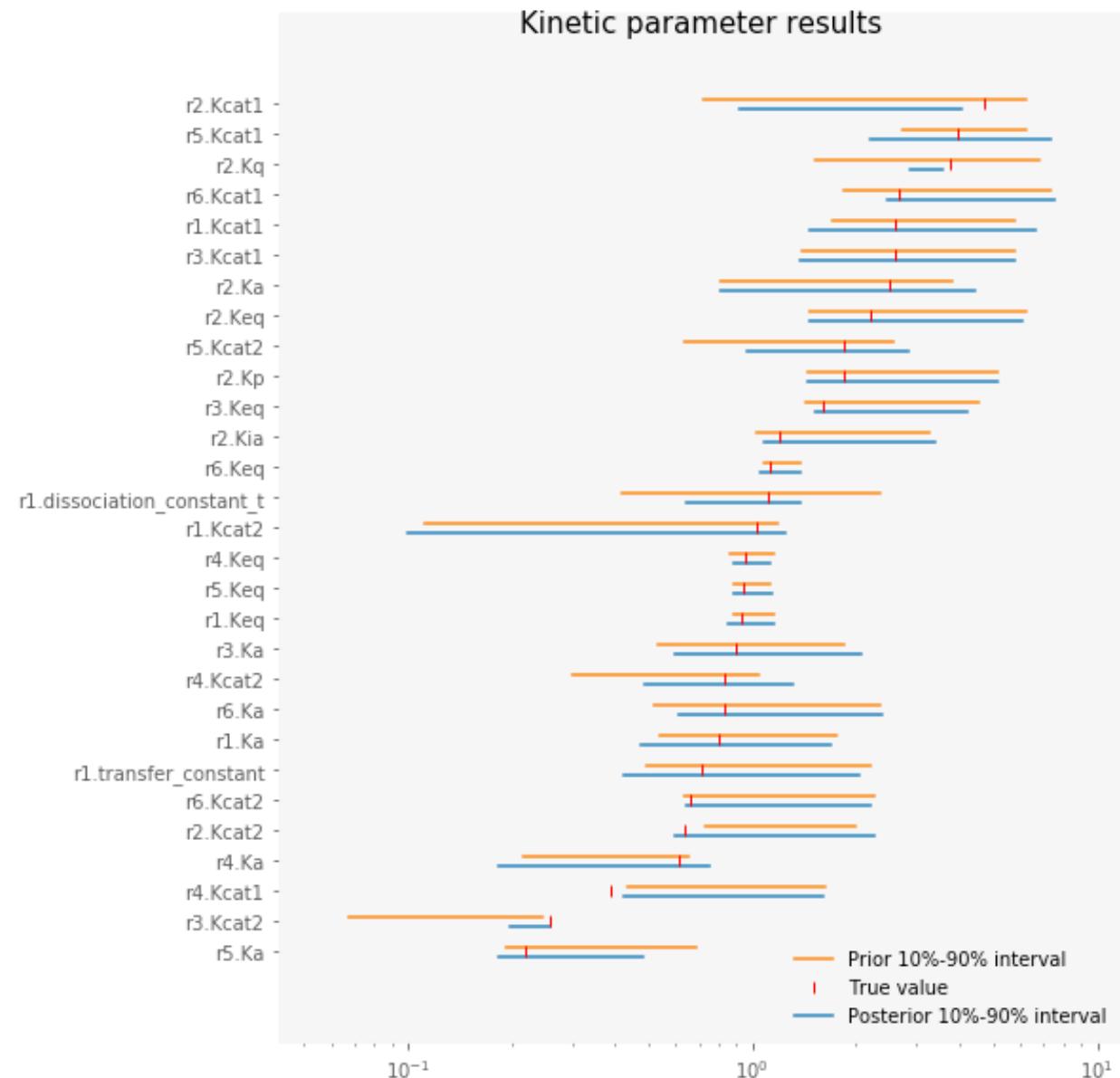
```
transformed parameters {  
    ...  
    concentration[,e] = integrate_ode_bdf(steady_state_equation,  
                                            initial_concentration,  
                                            initial_time,  
                                            {steady_time},  
                                            kinetic_parameter,  
                                            known_reals[,e],  
                                            known_ints,  
                                            rel_tol, abs_tol, max_steps)[1];  
    flux[,e] = get_fluxes(concentration[,e], kinetic_parameter, known_reals[,e]);  
    ...  
}
```

Results

Metabolite concentration results

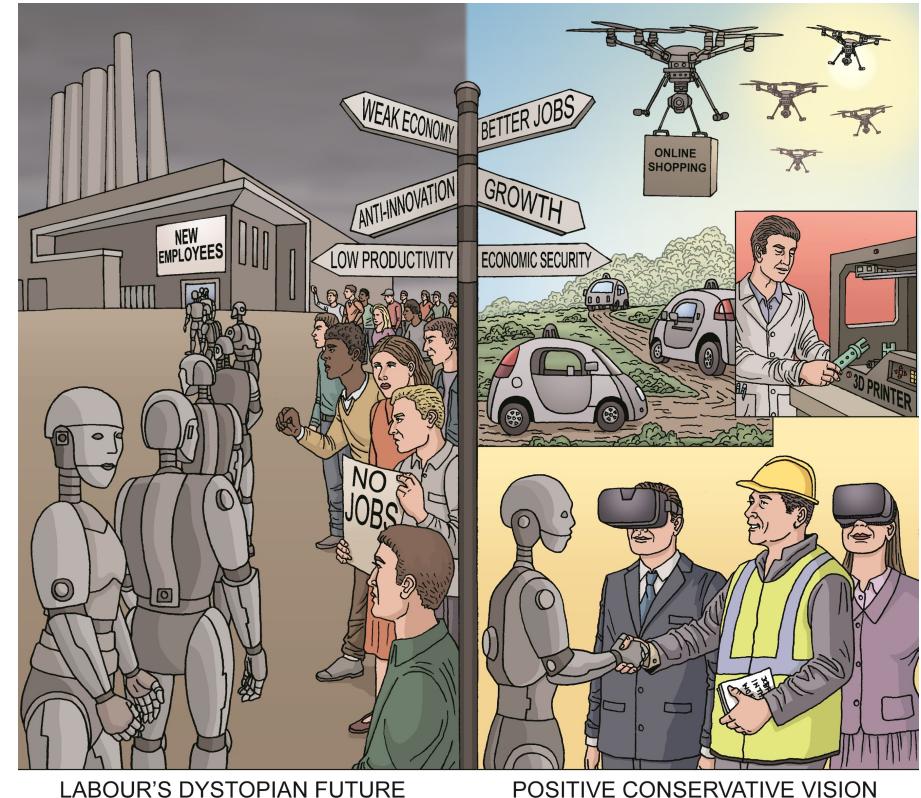


Kinetic parameter results



The future

- Networks with 50-100 reactions
- Support for custom reaction mechanisms
- Realistic measurement model
- Integration with popular tools
 - [Escher](#)
 - [COBRA](#)
 - [DD-Decaf](#)



LABOUR'S DYSTOPIAN FUTURE

POSITIVE CONSERVATIVE VISION

Challenges (please help!)

- Is there a better way to solve for steady state?
- How to exploit sparse ODE Jacobians?
- How to minimise posterior density evaluations?



References

Rate equations

- Cleland, W. W. (1963). The kinetics of enzyme-catalyzed reactions with two or more substrates or products: I. Nomenclature and rate equations. *Biochimica et Biophysica Acta (BBA) - Specialized Section on Enzymological Subjects*, 67(), 104–137. [http://dx.doi.org/10.1016/0926-6569\(63\)90211-63](http://dx.doi.org/10.1016/0926-6569(63)90211-63)

Enzyme kinetics in general

- Saa, P. A., & Nielsen, L. K. (2017). Formulation, construction and analysis of kinetic models of metabolism: A review of modelling frameworks. *Biotechnology Advances*, 35(8), 981–1003.
<http://dx.doi.org/10.1016/j.biotechadv.2017.09.005>

Allosteric regulation

- Popova, S. V., & Sel'kov, E. E. (1975). Generalization of the model by monod, wyman and changeux for the case of a reversible monosubstrate reaction. *FEBS Letters*, 53(3), 269–273. [http://dx.doi.org/10.1016/0014-5793\(75\)80034-2](http://dx.doi.org/10.1016/0014-5793(75)80034-2)
- Saa, P., & Nielsen, L. K. (2015). A General Framework for Thermodynamically Consistent Parameterization and Efficient Sampling of Enzymatic Reactions. *PLOS Computational Biology*, 11(4), 1004195.
<http://dx.doi.org/10.1371/journal.pcbi.1004195>