Bifurcation analysis of simple protein reaction networks

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Dit is materiaal voor de cursus Modelleren, Simuleren en Programmeren (MSP), voor tweedejaars natuurkunde studenten, en wel voor het Modelleren gedeelte van de cursus. De bedoeling is om een idee te geven van hoe (stelsels) differentiaalvergelijkingen (DV's) en dynamische systemen een rol spelen bij het modelleren en begrijpen van natuurwetenschappelijke verschijnselen. We gebruiken daarbij het recente artikel *Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell*, van Tyson, Chen en Novak als kapstok, aangevuld met materiaal dat eerder is gebruikt voor master studenten systeem biologie, die vrijwel geen voorkennis hadden over DV's, maar goed bekend waren met alle biologische termen en al wisten wat ATP was etc.

Differentiaalvergelijkingen dateren natuurlijk van lang voor de systeembiologie. DV's en de differentiaalrekening zijn door Newton geintroduceerd en ontwikkeld om de wetten van Kepler te verklaren, en werden pas (veel later) daarna ook voor het modelleren van chemische reacties gebruikt. Denk bijvoorbeeld aan een irreversibele reactie als $A + B \to C$, die in termen van de concentraties a, b, c van de moleculen A, B en C vaak gemodelleerd wordt met een reactiesnelheid v_1 die evenredig is met de concentraties. Dit geeft $v_1 = k_1 ab$ met k_1 een (positieve) reactieconstante, en leidt tot het stelsel

$$\frac{dc}{dt} = k_1 ab = -\frac{da}{dt} = -\frac{db}{dt},$$

waarin k_1 ook gezien kan worden als een parameter, die bijvoorbeeld kan afhangen van de concentratie van een enzym, dat de reactie benvloedt. Maken we de reactie reversibel, dan is er een tweede reactie $C \to A + B$ met reactie snelheid $v_2 = k_2 c$, en wordt het stelsel

$$\frac{dc}{dt} = k_1 ab - k_2 c = -\frac{da}{dt} = -\frac{db}{dt},$$

met twee positieve parameters k_1 en k_2. In de systeembiologie worden reactienetwerken gemodelleerd met stelsels van dit soort DV's.

In het Sniffers artikel staat de zin *"The temptation is irresistible to ask whether physiological regulatory systems can be understood in mathematical terms, in the same way an electrical engineer would model a radio."* In dit gedeelte van de cursus MSP zetten we hiertoe de eerste stappen waar het gaat om uit de *eigenschappen van oplossingen* van door biologen voorgestelde stelsels

DV's te begrijpen en verklaren wat er in levende cellen gebeurt. Dat doen we zoveel mogelijk eerst met pen en papier, zonder Mathematica of andere software, met formules en zelf geschetste grafieken.

Het gaat dus *meer om de eigenschappen van oplossingen dan om oplossingsformules*. Ter illustratie: veel eenvoudige lineaire DV's hebben als oplossingen formules waarin de welbekende transcendente functies voorkomen, in het bijzonder die functies zelf, zoals cos, sin en exp. De eigenschappen die deze functies hebben komen uitgebreid aan de orde in zowel het schoolcurriculum als de calculus vakken in het eerste jaar van universitaire studies, maar over het algemeen niet vanuit het perspectief dat deze functies oplossingen zijn van differentiaalvergelijkingen. Het is instructief om in deze cursus daar bij stil te staan. Dat doen we/jullie met behulp van dit materiaal (over DV's, oscillaties en planeetbanen)

`http://www.few.vu.nl/~jhulshof/nawdec270.pdf`

eerder gebruikt voor een zomercursus voor leraren, en materiaal gemaakt voor een onderwijssymposium over differentiaalvergelijkingen:

`http://www.few.vu.nl/~jhulshof/echtebrrrwiskunde.pdf`

Deze tweede pdf bevat een eerste kennismaking met vergelijkingen als

$$x' = \frac{dx}{dt} = 0, \quad \frac{dx}{dt} = \frac{1}{t}, \quad \frac{dx}{dt} = x, \quad \frac{dx}{dt} = F(x),$$

de laatste voor algemene functies F waarvan de grafiek makkelijk te schetsen is. Hoe bepaalt de grafiek van F het gedrag van oplossingen en hoe verandert dat gedrag als we F veranderen? In het bijzonder, wat zijn de stabiele evenwichten van het systeem? Evenwichten zijn oplossingen van $F(x) = 0$.

Het antwoord op deze vraag helpt ons om Figuur 1 op pagina 222 van het Sniffers verhaal te begrijpen, want deze figuur heeft betrekking op reactienetwerkjes waarvoor het stelsel DV's herleid kan worden tot één DV van het type $x' = F(x)$, met in F nog een parameter, meestal het "signaal", de concentratie van een molecuul dat de reactie beinvloedt (e.g. activation/inhibition).

In het Sniffers verhaal gaan de auteurs ervan uit dat de lezer vertrouwd is met *Michaelis-Menten kinetics*. Google deze term. Hieronder leggen we *niet* uit waar deze kinetica vandaan komt (dat komt wel op het college aan de orde), maar wel hoe deze kinetica leidt tot veel van de zelfstandige naamwoorden in de titel van het Sniffers artikel. Voor een belangrijk deel betreft dit het vinden van evenwichten.

Evenwichtsoplossingen van differentiaalvergelijkingen zijn oplossingen van "gewone" vergelijkingen, in het algemeen vergelijkingen met parameters. De vraag hoe oplossingen van parameters afhangen komt uitgebreid aan de orde. Een veelgebruikte methode hierbij is het verwisselen van de rollen van oplossingen en parameters. Bijvoorbeeld, de vergelijking

$$ax^2 = bx + c$$

voor x heeft drie parameters a, b, c. Hoe oplossingen van a afhangen zien we sneller aan de hand van

$$a = \frac{bx + c}{x^2}$$

dan aan de hand van een oplossingsformule voor x. Merk hierbij op dat we parameters zoveel mogelijk positief kiezen, en dat oplossingen heel vaak positief moeten zijn. Concentraties zijn nu eenmaal nooit negatief.

Vaak zullen we concentraties schalen om het aantal parameters te verminderen. Het veel voorkomende evenwicht bij fosforylatie door ATP betreft zo uiteindelijk oplossingen (x, y) van

$$\frac{ux}{J+x} = \frac{vy}{K+y}, \quad x+y=1.$$

Dit is een stelsel met 4 positieve parameters u, v, J, K, waarin onbekenden en parameters dimensieloos zijn, zie de afleiding (1.10) hieronder. Hoe ziet de grafiek van x versus u eruit, afhankelijk van de andere 3 parameters? Zie Sectie 1.5 en Sectie 3 hieronder voor een stukje praktische wiskunde dat in de calculus vakken wat verborgen zit.

Bij de differentiaalvergelijkingen zelf kunnen we ook de tijdschaal aanpassen. In de DV

$$\frac{dx}{dt} = ax$$

kan een positieve a zo wel in een 1 veranderd kan worden, of in een -1, als a negatief is. Er dus eigenlijk maar drie gevallen: $a = 1$, $a = -1$ en ...?

We besluiten de cursus met een vrije behandeling van Figuur 2 in het Sniffers verhaal en variaties daarop. Figuur 2b en 2c zijn te begrijpen als oplossingen van stelsels van de vorm

$$\frac{dx}{dt} = F(x,y), \quad \frac{dy}{dt} = G(x,y),$$

waarbij in F en G nog een parameter zit. Sectie 2 hieronder, die nog niet uitgewerkt is, bevat vast wat aantekeningen.

Natuurlijk zijn de meeste systemen veel hoger-dimensionaal, maar soms kunnen ze onder de aanname dat een deel van het systeem in evenwicht versimpeld worden. Een mooi voorbeeld daarvan is de afleiding van de Michaelis-Menten reaction rates bij enzymkinetica. Zie Sectie 4.

1 Sniffers, buzzers, toggles, blinkers

In these notes I discuss some of the simple reaction networks in the paper *Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell*, by John Tyson, Katherine Chen and Bela Novak. This paper is online available from

`http://www.mriedel.ece.umn.edu/wiki/images/6/6c/`

Make sure you print it and have it at hand. Throughout this handout SBTB refers to this paper. Essentially this handout explains what is behind Figure 1 and part of Figure 2 in SBTB, introduces some notation which is a bit more systematic, and corrects some of the misprints in the formulas.

The basic math knowledge required is differential calculus. In particular you need to know about the first and the second derivative of (simple) functions and how you use them to find maxima, minima and inflection points. You need to know how to solve a quadratic equation, and how the discriminant of a quadratic tells you if there are solutions. Most important, you need to be able to apply these techniques to functions and equations which involve parameters, and be flexible in interchanging the role of unknowns and parameters.

Your main tools below will first be paper, pen and pencil (PPP). You will draw the relevant diagrams yourself. Print this file one-sided, staple it, so that when you open it, you can draw diagrams on the blank pages on the left. Beginning in Section 1.3 these diagrams will be used to analyse the behaviour of solutions of ODE's, Ordinary Differential Equations. Numerical solution methods (with Mathematica or Maple) complement this PPP-analysis of simple reaction networks. The nouns in the title of SBTB actually correspond to bifurcation diagrams, which you will learn to appreciate and understand. Exercise 5 in Section 1.6 describes the first fundamental example: the one-way switch. Bifurcation diagrams always involve parameters which are varied in the analysis. As a rule we stick to positive parameters, which sometimes are set equal to zero. We avoid negative parameters. At several points you can jump to the appendix for more detailed analysis. Most exercises are PPP-exercises, some of them require a bit of (computer) algebra, that you may take for granted on first reading.

You have seen or will see that (de-)phosphorylation blocks like

$$\begin{array}{ccc} & \mathrm{R} & \\ & \downarrow & \\ \mathrm{E} & \rightleftarrows & \mathrm{EP} \end{array} \tag{1.1}$$

occur frequently in cell regulating reaction networks, and they will be analysed in some detail here . Throughout SBTB the Goldbeter-Koshland function (GK-function for short) is used. It solves equations (1.10) below, and the equilibrium state of (1.1) is defined in terms of the GK-function, a dimensionless function of four dimensionless parameters, see (1.5) below. For a certain parameter regime, discussed in Section 1.5, the GK-function has properties that allow for the phenomena in the title of the STBT paper to occur.

In (1.1) R is some (signal) protein which stimulates the phosporylation of some other protein E, EP being the phosphorylated form of E. Figure 1 in SBTB examines what happens if you combine such a GK-block[1] with a simple signal-response block, which by itself, has a reaction diagram

$$\begin{array}{ccc} \mathrm{S} & & \\ \downarrow & & \\ \rightarrow & \mathrm{R} & \rightarrow \end{array} \tag{1.2}$$

This simple block corresponds to Figure 1a of SBTB. It leads to a linear response curve. The simple combinations are Figures 1e, 1f and 1g. The first combination of (1.2) in Figure 1 with another block however is Figure 1d, where S signals both R and X and X inhibits R. This small reaction network does not involve a GK-block and is discussed in Section 1.10.

[1] I call them GK-blocks after Goldbeter-Koshland.

1.1 Goldbeter-Koshland blocks

In SBTB you will see GK-blocks with different letters. Figure 1c for instance is essentially

$$\begin{array}{ccc} & \mathrm{S} & \\ & \downarrow & \\ \mathrm{R} & \rightleftarrows & \mathrm{RP} \end{array} \tag{1.3}$$

The reaction in (1.3) goes both ways. In SBTB, the reaction rates for phosporylation and dephosporylation are modelled with Michaelis-Menten kinetics[2]

$$\frac{k_1 S R}{K_{m1} + R} \quad \text{(for phosporylation, also called synthesis)}$$

and

$$\frac{k_2 R_P}{K_{m2} + R_P} \quad \text{(for dephosporylation, also called degradation),}$$

in which k_1, K_{m1}, k_2, K_{m2} are the (positive) MM-constants[3], and the concentrations of R and RP are written as R and R_P, under the assumption that the total concentration R_T is constant[4]:

$$R + R_P = R_T. \tag{1.4}$$

Throughout SBTB, the concentration S of the signal S appears only in the phosporylation rate in (de)phosporylation blocks like (1.3). In Figure 1 of SBTB all such GK-blocks[5] are assumed to be in (thermodynamic) equilibrium, that is

$$\frac{k_1 S R}{K_{m1} + R} = \frac{k_2 R_P}{K_{m2} + R_P}. \tag{1.5}$$

The ODE's[6]

$$\frac{dR_P}{dt} = \frac{k_1 S R}{K_{m1} + R} - \frac{k_2 R_P}{K_{m2} + R_P} = -\frac{dR}{dt}, \tag{1.6}$$

are not yet used otherwise.

Given the MM-constants k_1, K_{m1}, k_2, K_{m2}, the signal concentration S and the total concentration R_T, the two equations (1.4,1.5) for the two unknowns R and R_P can be solved to produce one unique positive *steady state*[7]

$$R = R_{ss},\ R_P = R_{P,ss}.$$

If RP drives another reaction, a solution for $R_{P,ss}$ is needed. In SBTB it is written as

$$\frac{R_{P,ss}}{R_T} = G(k_1 S, k_2, \frac{K_{m1}}{R_T}, \frac{K_{m2}}{R_T}) \tag{1.7}$$

where G is the Goldbeter-Koshland function, a function of 4 variables, written as

$$G = G(u, v, J, K) \text{ or } G = G(u_1, u_2, J_1, J_2),$$

[2] As opposed to the reaction rates used in Figure 1b.

[3] From now on MM refers to Michaelis-Menten.

[4] In formula (c) in SBTB the concentration R of R has been replaced by $R_T - R_P$.

[5] This statement actually concerns the blocks with E and EP.

[6] Really only one equation: you can eliminate R or R_P, as you like, using $R + R_P = R_T$.

[7] The subscript *ss* refers to *steady state*.

in which

$$u = u_1 = k_1 S, \quad v = u_2 = k_2, \quad J = J_1 = \frac{K_{m1}}{R_T}, \quad K = J_2 = \frac{K_{m2}}{R_T} \tag{1.8}$$

are *dimensionless* reaction parameters. Early on the SBTB paper uses u, v, J, K, later on, when more GK-blocks are involved, also u_1, u_2, J_1, J_2 and u_3, u_4, J_3, J_4, etc[8]. The last diagram in Figure 1c in SBTB shows the response curve of (vertically) $R_{P,ss}$ versus the signal concentration S, in which it is assumed that J and K are both small[9]. This diagram is to be contrasted with the one in Figure 1b just above, which is based on equilibrium $k_1 S R = k_2 R_P$ for standard linear reaction rates[10].

If it is R that drives or inhibits another reaction, a solution formula for R is needed. Since, as you will see below,

$$G(u, v, J, K) + G(v, u, K, J) = 1, \tag{1.9}$$

we have

$$\frac{R_{ss}}{R_T} = 1 - \frac{R_{P,ss}}{R_T} = 1 - G(k_1 S, k_2, \frac{K_{m1}}{R_T}, \frac{K_{m2}}{R_T}) = G(k_2, k_1 S, \frac{K_{m2}}{R_T}, \frac{K_{m1}}{R_T}).$$

By now you may have some experience with checking physical dimensions. Observe that $G(u, v, J, K)$ is dimensionless. It defines

$$\text{the fraction } y = \frac{R_{P,ss}}{R_T} \text{ of phosphorylated RP,}$$

which, together with

$$\text{the fraction } x = \frac{R_{ss}}{R_T} \text{ of unphosphorylated R,}$$

ads up to 1. The GK-function tells you what y is in terms of the dimensionless reaction parameters, in particular the signal parameter $u = k_1 S$. As you might expect, y runs up from 0 to 1 if you let the signal u go from 0 all the way up to infinity. To get x you simply take $1 - y$, so x runs down from 1 to 0. Note carefully that while above x and y relate to R and RP, with the signal S contained in u, below x and y relate to E and EP, with u containing the signal R. We shall often write x and y as

$$x = x(u) \text{ and } y = y(u),$$

but sometimes it will be convenient to think of u as determined by x or y. We always assume that

$$x \geq 0, \quad y \geq 0, \quad x + y = 1,$$

because x and y are the fractions of respectively the unphosphorylated and phosphorylated form of a certain protein.

[8] I will stick to u, v, J, K as I explain the GK-function.

[9] And that $R_T = 1$, apparently.

[10] Limit case of MM-rates, $k_1, K_{m,1}, k_2, K_{m,2}$ large, bounded ratios $\frac{k_1}{K_{m,1}}, \frac{k_2}{K_{m,2}}$.

1.2 Derivation of the GK-function

To derive the GK-function, consider the reaction balance, written in terms of x and y, as[11]

$$\frac{ux}{J+x} = \frac{vy}{K+y}, \text{ to be solved subject to } x+y=1, \tag{1.10}$$

and the restriction that $x \geq 0$ and $y \geq 0$. You should think of a representation of the GK-block (1.1) in terms of the dimensionless fractions x and y, controlled by the dimensionless signal u. That is, we have put

$$\begin{array}{ccc} & \mathrm{S} & \\ & \downarrow & \\ \mathrm{E} & \rightleftarrows & \mathrm{EP} \end{array} \qquad \text{in dimensionless form} \qquad \begin{array}{ccc} & \mathrm{u} & \\ & \downarrow & \\ \mathrm{x} & \rightleftarrows & \mathrm{y} \end{array} \tag{1.11}$$

The (first) equation in (1.10) corresponds to the steady state equation for

$$\frac{dy}{d\tau} = \frac{ux}{J+x} - \frac{vy}{K+y} = -\frac{dx}{d\tau}, \tag{1.12}$$

in which τ is a scaled[12] time variable.

This first equation in (1.10) looks simpler than (1.5) because we did away with all indices, but we still have 4 parameters: u, v, J, K. Observe the symmetry: interchanging x and y, u and v, J and K, you get the same equations. This explains (1.9). Keep in mind that it is the parameter u which contains the varying signal concentration, as is exhibited in (1.11).

Exercise 1. GK-formula from solving a quadratic: assume all parameters u, v, J, K positive, substitute $x = 1-y$ in the reaction balance in (1.10), and derive a quadratic equation for y. Find a solution formula for y and compare it to the expression in SBTB (page 223). You will probably get frustrated. Actually it seems like they first wrote an equation for $\frac{1}{y}$, and used the solution formula for $\frac{1}{y}$ upside down to get a formula for y. Your result should be the same of course, but it takes some rewriting. Maple or Mathematica may be able to help you. Still, you get 2 solutions, while there is only one physical solution in the relevant window $0 \leq y \leq 1$. You have to pick the right sign[13] to get it right. This has been done for you in SBTB.

Exercise 1 shows that you can solve equations and get complicated solutions formulas[14], from which it is still hard to get the information you need, unless you plot everything, varying all 4 parameters. Below you will learn how you can get by without such formulas, with details discussed in the appendix.

1.3 Bifurcation diagrams: switches?

If the phosphorylated form drives another reaction, it is important *how* the response y varies from 0 to 1 as the signal parameter u is increased: what is the shape of the corresponding graph obtained by plotting the response y

[11] Have to use x and y somewhere: they may relate to R and RP, E and EP, X and XP, etc.
[12] Check that $\tau = \frac{t}{R_T}$.
[13] In front of the square root in the solution formula.
[14] Perhaps earning your name to such a formula.

versus the signal u? What you will observe in the larger system, depends on the shape of this graph. Of importance are questions like: can a line have multiple intersections with this graph? Such intersections appear in Figure 1 of SBTB as equilibria of the small reaction networks in which a GK-block for E and EP is involved[15]. Except for Figure 1d, you see on the right *bifurcation diagrams*, with vertically the possible responses against horizontally a signal. Unstable equilibria are dotted. You need to understand these diagrams: how you get them and what they say.

The first small network with a GK-block, corresponding to Figure 1e in SBTB, is schematically given as

$$\begin{array}{ccc} \mathrm{S} & & \\ \downarrow & & \\ \rightarrow & \mathrm{R} & \rightarrow \\ \uparrow & \downarrow & \\ \mathrm{EP} & \leftrightarrows & \mathrm{E} \end{array} \qquad \text{or, in dimensionless form} \qquad \begin{array}{ccc} \mathrm{s} & & \\ \downarrow & & \\ \rightarrow & \mathrm{u} & \rightarrow \\ \uparrow & \downarrow & \\ \mathrm{y} & \leftrightarrows & \mathrm{x} \end{array} \tag{1.13}$$

In terms of the dimensionless form, s is some other signal (input) which stimulates u. In turn, u stimulates y, and y stimulates u. This is called *mutual activation* between u and x. It is the relation between s as input/signal and u as output/response which is of interest[16]. The remaining arrow to explain in (1.13) is the single arrow to the right of u. This arrow corresponds to the negative term $-ku$ in

$$\frac{du}{d\tau} = s + y(u) - ku, \tag{1.14}$$

the ODE for u. The two positive terms correspond to the signals s and y, and are written without constants, see Exercise 3 below for the appropriate scalings.

There are no ODE's for x and y because of the assumption of equilibrium for the GK-block[17]. Note that here, while still being a signal itself for the GK-block, u is considered as output(response) and s as signal parameter. In s we have absorbed a constant[18] which possibly appears in the synthesis rate of y.

The coefficient k is kept fixed as we vary s. You will see how this small network functions as a *one-way switch*, but only in case of a *sigmoidal* GK-curve $y = y(u)$ for the GK-response fraction y versus the signal u, see (1.22) below. All conclusions follow by looking at the intersections of the graphs of $y(u)$ and $s - ku$ versus u. Equivalently, you may simply set the right hand side of (1.14) equal to zero, and write this as

$$s = ku - y(u), \tag{1.15}$$

which you can plot with u vertically and s horizontally[19]. *This mathematical trick, writing the input in terms of the output*[20] *is very useful: it gives you the bifurcation diagram.* In this bifurcation diagram you can see for a given input

[15] With x as the fraction of E, y as the fraction of EP.
[16] With u as the fraction of R in Figure 1 of SBTB.
[17] In Figure 2 of SBTB you will see examples with ODE's for the GK-blocks.
[18] Not relevant now, from STBT it is not clear this constant is considered to occur.
[19] The diagram you get is essentially the third diagram in Figure 1e.
[20] Not what you would be inclined to do first.

(signal) s what the possible outputs (responses) are. It then remains to figure out which of the possible outputs you will see as you vary initial data and/or the signal.

You observe that in (1.15) the synthesis rate $s + y(u)$ and the degradation rate ku have been blended in the formula. This does not happen if you use the same trick in the second example presented next:

$$\frac{du}{d\tau} = s - ux(u) - ku. \tag{1.16}$$

You can think of (1.16) as the ODE corresponding to

$$\begin{array}{ccc} \mathrm{s} & & \\ \downarrow & & \\ \rightarrow & \mathrm{u} & \rightarrow \\ & \downarrow & \uparrow \\ \mathrm{y} & \leftrightarrows & \mathrm{x} \end{array} \tag{1.17}$$

Can you see why? In this block s is still good for u, but u is bad for x and x is bad for u. This is called *mutual inhibition* between u and x, see Figure 1f in SBTB. For $x = x(u)$ as a function of u you get the same graph as for $y(u)$, but turned upside down[21]: $x(u)$ runs from 1 down to 0 as u is increased from 0 to infinity. The qualitative analysis will show that for $k > 0$ this block functions as a *two-way switch*, provided that $ux(u)$ has a (single) maximum[22], see (1.35) below. In this case the conclusions follow by examining the intersections of the graphs of $ux(u)$ and $s - ku$, starting from the $k = 0$ case, or directly by setting the right hand side of (1.16) equal to zero, and write this as

$$s = ux(u) + ku, \tag{1.18}$$

which you can again plot with u vertically and s horizontally, as above.

These two switch examples contrast this example:

$$\begin{array}{ccc} & & \mathrm{s} \\ & & \downarrow \\ \rightarrow & \mathrm{u} & \rightarrow \\ \uparrow & \downarrow & \\ \mathrm{x} & \rightleftarrows & \mathrm{y} \end{array}$$

See Figure 1g in SBTB. Here s is bad for u, u is bad for x, x is good for u. The corresponding ODE is

$$\frac{du}{d\tau} = k + x(u) - su. \tag{1.19}$$

In this case switch-like behaviour does not occur[23]. A similar example[24] would be

$$\begin{array}{ccc} & & \mathrm{s} \\ & & \downarrow \\ \rightarrow & \mathrm{u} & \rightarrow \\ & \downarrow & \uparrow \\ \mathrm{x} & \rightleftarrows & \mathrm{y} \end{array}$$

[21] I will call this a reversed GK-curve.
[22] $ux(u)$ increases from 0 to a maximum, then decreases to positive limit.
[23] Note that $k = 0$ in Figure 1g of SBTB.
[24] Not included in SBTB.

Here s is bad for u, u is good for y, y is bad for u, and the ODE for u is

$$\frac{du}{d\tau} = k - (y(u) + s)u. \tag{1.20}$$

In both these *nonmutual* cases it is relatively easy[25] to see that the signal-response curve (u versus s) is monotone, once you know that the GK-curve is monotone, and that it has a large flat region if the (sigmoidal) GK-curve has a sharp transition. As we shall see below this is the case for small J and K. The switch-behaviour in the first two examples is then also quickly recognised.

All this requires a solid undertanding of the GK-blocks by themselves. They are called *buzzers* in SBTB, as is explained next.

1.4 The GK-function as a buzzer

Often both K and J in the GK-function are small, at least in the examples in SBTB. The graph of the (fractional) response y versus the (scaled) signal u is then sigmoidal, with, as observed in Exercise 24 below, a rather swift transition near $u = v$ from $y \approx 0$ to $y \approx 1$. Refering to Figure 1c in SBTB, with R and RP signaled by S, this GK-block is called a *buzzer*, in view of the resulting signal-response curve for $R_{P,ss}$ versus S. You have to keep feeding with S (*press the button*) to get and maintain a response. Releasing the button (meaning no more S) you lose the response. The GK-analysis below, which explains the dependence of the equilibrium state of (1.6) on the signal concentration S, given the total concentration R_T, implies that, whatever the initial data are, the solution of the ODE for R_P,

$$\frac{dR_P}{dt} = \frac{k_1 S(R_T - R_P)}{K_{m1} + R_T - R_P} - \frac{k_2 R_P}{K_{m2} + R_P}, \tag{1.21}$$

quickly converges to the unique steady state. Although what you see eventually[26] is independent of the initial concentration of R, this does of course depend on S, through the GK-formula (1.7), but there are no surprises: turning the signal up, you turn up the response, turning the signal down again, you turn down the response again.

1.5 Sigmoidal GK-functions

Switch behaviour is different in that it does come with a surprise: turning the signal up, you turn up the response, turning the signal down again, you may keep the response. To understand how this works, you need to know more of the GK-response curve of $y(u)$ versus u than just its monotonicity. The GK-response curves in SBTB are convex (curved upwards) for small u and then concave (curved downwards), with only one inflection point. It is important to know for sure when this is the case, because although the GK-response curve of y versus u can very well have a sigmoidal convex-concave shape[27], it may also be globally concave[28]. It is instructive to spend some time on the precise shape

[25] Maybe not by writing s in terms of u.
[26] Almost immediately if the time scale is fast.
[27] The curve in the last diagram in Figure 1c of SBTB.
[28] Like the hyperbolic curve in the last diagram in Figure 1b of SBTB.

of the GK-curve. The simple criterium you will find or believe is:

$$JK < 1 + J \quad \Leftrightarrow \quad y \text{ versus } u \text{ is a convex-concave sigmoidal graph} \tag{1.22}$$

Thus, if J and K are small, the GK-curve is certainly sigmoidal, and quite steep in fact, see Exercise 24 in the appendix (Section 3).

To discover this criterium (1.22) from the GK-formula is not so easy. Solution formulas are rarely transparent. Understanding the graph of a solution formula versus a control parameter is not impossible[29], but often a qualitative analysis of the original equations[30], here

$$\frac{ux}{J+x} = \frac{vy}{K+y}; \quad x + y = 1; \quad x \geq 0; \quad y \geq 0, \tag{1.23}$$

turns out to be easier and more clarifying. This kind of analysis, and the way of thinking it introduces, is a stepping stone to understanding the SBTB paper, in particular some of its bifurcation diagrams. This is done in a series of exercises in the appendix (Section 3).

1.6 The one-way switch in qualitative detail

An important message of the SBTB paper is that combining a GK-block with a simple signal-response block, this signal-response may change completely. Below you will see how a short injection with S can account for turning on a response which can persist after the signal S is gone. Such systems are called switches. They have in common that they exhibit, depending on S, a varying number of multiple steady states.

You will now examine in detail what happens if you combine the GK-block (1.1),

$$\begin{array}{ccc} & \mathrm{R} & \\ & \downarrow & \\ \mathrm{E} & \rightleftarrows & \mathrm{EP} \end{array}$$

with a simple signal-response block (1.2),

$$\begin{array}{ccc} \mathrm{S} & & \\ \downarrow & & \\ \rightarrow & \mathrm{R} & \rightarrow \end{array}$$

with only one synthesis rate and one degradation rate. The presentation below includes again how to nondimensionalise the equations.

Look at the signal-response block (1.2) first. Except for the MM-reaction rates in GK-blocks, all degradation rates in SBTB are proportional to concentration. The degradation rate of R in (1.2) is simply

$$v_{degradation} = v_d = k_2 R,$$

and the synthesis rate of R is a linear function

$$v_{synthesis} = v_s = k_0 + k_1 S.$$

[29] Begin by picking explicit numerical values of the parameters and (computer)plot.
[30] Completely ignoring the solution formula.

of signal strength. Without any other signals, the time dependent concentration $R = R(t)$ of R satisfies

$$\frac{dR}{dt} = v_s - v_d = k_0 + k_1 S - k_2 R, \tag{1.24}$$

and a stationary balance is given by

$$R = R_{ss} = \frac{k_0 + k_1 S}{k_2}.$$

Thus, the signal response of (1.2) by itself is just a linear function of S.

Exercise 2. Easy but instructive (PPP): sketch the graph of (vertically) the linear response R_{ss} versus (horizontally) the signal concentration S, with both k_0 and k_1 positive, and with $k_0 = 0 < k_1$. Compare with Figure 1a in SBTB.

One possibility to combine a signal-response block with a GK-block is to let the response R in (1.2) act a signal in the GK-block (1.1), and, simultaneously, have EP enhance[31] the synthesis of R by changing v_s to

$$v_s = k_0 + k_0' E_P + k_1 S.$$

The new term in v_s is proportional to the concentration E_P of EP. Now both S and EP stimulate synthesis (in a similar fashion). In nondimensional form, this case of mutual activation[32] is the first example in Section 1.3 above. The reaction diagram is

$$\begin{array}{ccc} \mathrm{S} & & \\ \downarrow & & \\ \rightarrow & \mathrm{R} & \rightarrow \\ \uparrow & \downarrow & \\ \mathrm{EP} & \leftrightarrows & \mathrm{E} \end{array} \qquad \text{or, in dimensionless form} \qquad \begin{array}{ccc} \mathrm{s} & & \\ \downarrow & & \\ \rightarrow & \mathrm{u} & \rightarrow \\ \uparrow & \downarrow & \\ \mathrm{y} & \leftrightarrows & \mathrm{x} \end{array} \tag{1.25}$$

For the sake of presentation[33] EP appears on the left in (1.25). Assuming the time scale for E$\rightleftarrows$EP to be fast, E_P is set equal to its GK-equilibrium, so that the synthesis rate of R becomes

$$v_s = k_0 + k_0' E_{P,ss}(R) + k_1 S.$$

In view the GK-analysis the steady state concentration of EP is

$$E_{P,ss}(R) = E_T G(k_3 R, k_4, J_3, J_4), \tag{1.26}$$

with variables defined as in (1.8), which, as we recall, corresponds to a balance

$$\frac{k_3 R E}{K_{m3} + E} = \frac{k_4 E_P}{K_{m4} + E_P}.$$

[31] Positive feedback between R and EP.
[32] Figure 1e in STBT.
[33] Drawing transparent diagrams combining the simple components.

The ODE (1.24) is modified accordingly as

$$\frac{dR}{dt} = v_s - v_d = k_0 + k_0' E_{P,ss}(R) + k_1 S - k_2 R \tag{1.27}$$

$$= k_0 + k_0' E_T G(k_3 R, k_4, J_3, J_4) + k_1 S - k_2 R,$$

analysed in the SBTB paper with $k_0 = 0$. Apparently[34] $k_0' E_T$ is written as k_0 in Box 1 of SBTB (page 224).

Exercise 3. Equation (1.27) can be rewritten as (1.14): verify that you get the ODE

$$\frac{du}{d\tau} = s + y(u) - ku,$$

for $u = k_3 R$ with

$$s = \frac{k_0 + k_1 S}{k_0' E_T}, \quad k = \frac{k_2}{k_3 k_0' E_T}, \quad \tau = k_3 k_0' E_T t.$$

Note that, unlike x and y, u is not a fraction, it can be (much) larger than one.

The analysis of this small block may be done in terms of either (1.14) or (1.27), that is, either in terms of u and s, or in terms R and S. I was already unhappy with (1.24) and now I prefer (1.14), for which the steady states must solve the equation

$$s + y(u) = ku. \tag{1.28}$$

The left and right sides in (1.28) are the *normalised synthesis and degradation rates* of u. Observe that k must be positive to have steady states.

Exercise 4. Steady states of (1.14) with $s = 0$: suppose $y = y(u)$ versus u is a sigmoidal GK-curve as above. Make a sketch with $u \geq 0$ and $0 \leq y \leq 1$ and draw a straight line through the origin with slope $k > 0$. The u-coordinates of the intersection points of the GK-curve and this line are the solutions of $y(u) = ku$, the steady states of the ODE with $s = 0$. Convince yourself that beside the trivial solution $u = 0$ there are no positive solutions if k is large, while there are 2 positive solutions if k is small. Also explain that for precisely one (positive) value of k inbetween there is exactly one positive solution. For this $k = k_{touch}$ the two graphs touch in one point. See Exercise 28 in the appendix.

Exercise 5. Steady states when $s > 0$, continued from Exercise 4: starting from some $k > 0$ for which there are two positive solutions, show, using a sketch again, that $s + y(u) = ku$ has 3 positive solutions for $s > 0$ small and only one positive solution for large $s > 0$. Also explain that for precisely one (positive) treshhold value of s inbetween there is exactly one positive solution. Make a sketch in which you vary s horizontally and put the corresponding solution(s) u vertically. Which of the 3 solutions survives as s crosses the treshhold? Compare your sketch to the last diagram in Figure 1e of SBTB.

Exercise 6. Stabilisation when $s = 0$, continued from Exercise 4: assume that the parameters k, J and K make for a convex-concave $y(u)$ which is intersected 3 times by ku, beginning in $u = 0$. Explain from a sketch there are 3 stationary

[34] As you will noted, there are many typo's in the equations in the SBTB paper.

states for (1.14), by inspecting the ordering of $y(u)$ and ku in your sketch. Explain why $u = 0$ is (locally) stable: starting with no or a little u, you will quickly see u disappear. Explain that, starting with a large u, the concentration u stabilizes to the third intersection, which is also (locally) stable. The second, middle, intersection is unstable. Explain that, starting with u below it, all u disappears[35], and starting with u above it, $u(\tau)$ stabilizes to the larger (third) intersection point.

This last two exercises tell you that, in terms of S and R, without the signal S, any small (or zero) initial concentration R of R leads to eventual death[36]: R will be gone quickly[37] and *the larger stable steady state is unreachable*. However, the signal S changes everything, as you can see next, in terms of s and u again.

Exercise 7. Switching on: bringing in the signal amounts to increasing the synthesis rate in the left hand side of (1.28). Explain from your sketch in Exercise 5 that the intersection points vary nicely with s until 2 of them come together and disappear as the concentration s crosses a certain treshhold value $s_c = s_{critical}$. Draw another sketch in which you put (vertically) the steady state(s) for the concentration of the response u versus (horizontally) signal concentration s. Explain that for $s > s_c$ there is only one (large[38]) stable steady state. Raising s above the threshold value s_c switches u on.

In terms of S and R this says that a sufficiently large signal concentration S takes the system to the larger steady positive concentration R. Moreover, there is no way back to the original state:

Exercise 8. The punchline: starting from the system with u switched on, decrease s to $s = 0$ (i.e. take the signal away), and explain why the system stays switched on[39]. This is why this example is called a one-way switch. It is obtained from a GK-block and a signal-response block through mutual activation. Do you see how this conclusion follows just from looking at s as a function of u? Why can the system not be switched off?

The second example in Section 1.3 above, *mutual inhibition*, is studied next.

1.7 The two-way switch in qualitative detail

Whereas Section 1.6 and Figure 1e in SBTB combine a GK-block (1.1) and a signal-response block (1.2) in which EP and R are mutual activating, Figure 1f in SBTB has mutual inhibiting[40] R and E. This amounts to one single change in the diagrams in (1.25). In terms of S, R, E and EP the difference is clear if

[35] $u(\tau) \to 0$.
[36] Or whatever you want to call it.
[37] With exponential decay in time.
[38] Large means: large compared to the small steady state we had for $s < s_c$.
[39] This must have scary real life examples.
[40] Note that: R activates EP $\Leftrightarrow$ R inhibits E.

we line up the diagrams in Figures 1e and 1f of SBTB:

$$\underbrace{\begin{array}{ccc} \text{S} & & \\ \downarrow & & \\ \rightarrow & \text{R} & \rightarrow \\ \uparrow & \downarrow & \\ \text{EP} & \leftrightarrows & \text{E} \end{array}}_{\text{mutual activation}} \qquad \underbrace{\begin{array}{ccc} \text{S} & & \\ \downarrow & & \\ \rightarrow & \text{R} & \rightarrow \\ & \downarrow & \uparrow \\ \text{EP} & \leftrightarrows & \text{E} \end{array}}_{\text{mutual inhibition}} \tag{1.29}$$

In dimensionless form, with x, y, u and s:

$$\underbrace{\begin{array}{ccc} s & & \\ \downarrow & & \\ \rightarrow & u & \rightarrow \\ \uparrow & \downarrow & \\ y & \leftrightarrows & x \end{array}}_{\text{mutual activation}} \qquad \underbrace{\begin{array}{ccc} s & & \\ \downarrow & & \\ \rightarrow & u & \rightarrow \\ & \downarrow & \uparrow \\ y & \leftrightarrows & x \end{array}}_{\text{mutual inhibition}} \tag{1.30}$$

Compared to the single signal-response block (1.2), which has

$$v_s = k_0 + k_1 S, \quad v_d = k_2 R,$$

the change is in v_d. This degradation rate becomes

$$v_d = k_2 R + k_2' E_{ss}(R) R = \left(k_2 + k_2' E_{ss}(R)\right) R,$$

in which the new term is $k_2' E R$, with E set equal to its equilibrium state $E_{ss}(R)$. Drawing v_s en v_d in one diagram, with varying values of S, you see that the equilibria of

$$\frac{dR}{dt} = v_s - v_d = k_0 + k_1 S - k_2 R - k_2' E_{ss}(R) R \tag{1.31}$$

are given by

$$k_2 + k_2' E_{ss}(R) = R\left(k_0 + k_1 S\right), \tag{1.32}$$

but again I prefer Equation (1.27) rewritten, as (1.16) this time:

Exercise 9. Verify that, with

$$s = k_3 \frac{k_0 + k_1 S}{k_2' E_T}, \quad k = \frac{k_2}{k_2' E_T}, \quad \tau = k_2' E_T t.$$

you get (1.16),

$$\frac{du}{d\tau} = s - u x(u) - k u,$$

as the ODE for $u = k_3 R$. Conclude that the bifurcation diagram is given by

$$s = u x(u) + k u. \tag{1.33}$$

In the next exercises you can again do most of the analysis of (1.16) by looking at (1.33) and see why this example is called a *two-way switch*. Just as in the case of (1.14), this bifurcation diagram shows, for a given input (signal) s, what the possible outputs (responses) are, and it remains to figure out which of the possible outputs you will see as you vary initial data and/or the signal. In the end the main conclusion is Exercise 12 below, to which you might want to jump without going through the details below, but then you are likely to miss a first difference between the two-way and the one-way switch, as the case $k = 0$ shows next.

Exercise 10. Consider a reversed GK-curve $x = x(u)$. You can multiply $x(u)$ by u and plot $ux(u)$ vertically against u horizontally. Clearly[41] your plot must start from the origin with slope 1 because $x(0) = 1$. Verify that u can be written as a function of x in the window $0 \leq x \leq 1$, since

$$\frac{u}{v} = \frac{1-x}{K+1-x}\frac{J+x}{x}, \tag{1.34}$$

in which $u \to \infty$ corresponds to $x \to 0$, and show that in this limit

$$ux = v\frac{1-x}{K+1-x}(J+x) \to v\frac{J}{K+1}.$$

Use this to plot two possible graphs of $ux(u)$ versus u, one with a single maximum and one which is monotone.

You can now show or believe that

$$JK < 1 + K \quad \Longrightarrow \quad ux(u) \text{ has a (single) maximum} \tag{1.35}$$

If $JK \geq 1 + K$ then $ux(u)$ increases monotonically from 0 to a positive limit[42]. If $JK < 1 + K$ then $ux(u)$ first increases monotonically from 0 to a maximum and *decreases* to a positive limit afterwards.

Exercise 11. Verify (1.35) for small J and K, using the steepness of the GK curve near $u = v$.

If you don't believe (1.35) then do the exercises in the appendix.

Exercise 12. Let $k > 0$. If $JK \geq 1 + K$, then $ku + ux(u)$ is increasing in u. If $JK < 1 + K$ then $ux(u)$ has a maximum. You can then verify, using a sketch, that $ku + ux(u)$ must have a maximum and a minimum if $k > 0$ is small, and that $ku + ux(u)$ is monotone increasing if $k > 0$ is large. In fact, there is precisely one $k = k_{hip} > 0$ inbetween for which there is a horizontal inflection point. For $k \geq k_{hip}$ the graph is monotone, for $0 < k < k_{hip}$ there are precisely 2 extrema, a maximum and a minimum. You can infer this from Exercise 31 which implies that (also) the graph of $ku + ux(u)$ has only one point of inflection. Make a sketch.

Exercise 13. If $JK < 1 + K$ then as $k \uparrow k_{hip}$, the maximum and minimum of $ux(u) + ku$, as well as the inflection point, move to the horizontal inflection

[41] Why?
[42] As u runs from 0 to ∞.

point of the graph of $ux(u) + k_{hip}u$. It is a fact that for $JK < 1 + K$ and $0 < k < k_{hip}$, as u runs up from 0 to infinity, the graph of $ku + ux(u)$ increases from 0 to a maximum, then falls down to a positive minimum[43] and finally increases to infinity with a asymptotic slope k. Draw the solution set of

$$ku + ux(u) = s$$

in a diagram with u versus s and compare to the last diagram in Figure 1f in SBTB. Now explain in detail what you expect to happen if you start the reaction with $u = 0$, turn up s slowly. Explain how the system switches on if you cross the maximum of $ku+ux(u)$. Then let s decrease slowly, what happens if s crosses the minimum of $ku + ux(u)$. Explain why this is called a two-way switch. The hysteretic effect should be noted. Switching off is possible, but it is not like you play back the movie.

To have a two-way-switch, this mutual inhibition block requires $JK < 1+K$ and $0 < k < k_{hip}$. This k_{hip} may be computed implicitly, see again the appendix.

1.8 Homeostasis

The third example in Section 1.3 concerns Figure 1g in SBTB.

$$\begin{array}{ccc} & & \mathrm{S} \\ & & \downarrow \\ \rightarrow & \mathrm{R} & \rightarrow \\ \uparrow & \downarrow & \\ \mathrm{E} & \rightleftarrows & \mathrm{EP} \end{array} \tag{1.36}$$

The equation is

$$\frac{dR}{dt} = k_0 E_{ss} - k_2 SR, \tag{1.37}$$

and can be rewritten as (1.16):

Exercise 14. Verify that you get

$$\frac{du}{d\tau} = x(u) - su,$$

corresponding to

$$\begin{array}{ccc} & & \mathrm{s} \\ & & \downarrow \\ \rightarrow & \mathrm{u} & \rightarrow \\ \uparrow & \downarrow & \\ \mathrm{x} & \rightleftarrows & \mathrm{y} \end{array}$$

for $u = k_3 R$ with

$$s = \frac{k_2 S}{k_0 k_3 E_T}, \quad \tau = k_0 k_3 E_T t.$$

Exercise 15. Do the qualitative analysis of the bifurcation diagram and compare to Figure 1g in SBTB.

[43] For all other positive values of J, K, k the graph of $ku + ux(u)$ is monotone.

1.9 A missing figure?

Figure 1h in SBTB could have been

$$\begin{array}{ccc} & & \mathrm{S} \\ & & \downarrow \\ \rightarrow & \mathrm{R} & \rightarrow \\ & \downarrow & \uparrow \\ \mathrm{E} & \rightleftarrows & \mathrm{EP} \end{array}$$

Exercise 16. Verify that you get

$$\frac{du}{d\tau} = k - (s + y(u))u,$$

corresponding to

$$\begin{array}{ccc} & & \mathrm{s} \\ & & \downarrow \\ \rightarrow & \mathrm{u} & \rightarrow \\ & \downarrow & \uparrow \\ \mathrm{x} & \rightleftarrows & \mathrm{y} \end{array}$$

Exercise 17. Do the qualitative analysis of the bifurcation diagram for the fourth example in Section 1.3 and prepare Figure 1h for SBTB.

1.10 Sniffers

The first combination of (1.2) in Figure 1a with another block is Figure 1d, where S signals both R and X. By itself this would be:

$$\begin{array}{ccc} \rightarrow & \mathrm{X} & \rightarrow \\ \uparrow & & \\ \mathrm{S} & & \\ \downarrow & & \\ \rightarrow & \mathrm{R} & \rightarrow \end{array} \tag{1.38}$$

For this block you get a system of ODE's for the concentrations,

$$\frac{dR}{dt} = k_1 S - k_2 R; \quad \frac{dX}{dt} = k_3 S - k_4 X,$$

which would give linear response curves for both X and R, because this system is decoupled. You can independently solve for R and X. But if X inhibits[44] R, the system reads

$$\frac{dR}{dt} = k_1 S - k_2 X R; \quad \frac{dX}{dt} = k_3 S - k_4 X. \tag{1.39}$$

We have not yet discussed systems of coupled ODE's. But the steady state is easily computed by setting the right hand sides equal to zero:

Exercise 18. Compute the steady state of this system for $S > 0$ and note that R_{ss} is independent of S. This steady state is stable, can you guess why? Explain the term sniffer.

[44]Draw the corresponding arrow yourself, in tex I can't.

2 Figure 2 in SBTB

2.1 Figure 2a

The example in Figure 2a is deceptive. Let us first consider

$$\begin{array}{ccc}
\mathrm{s} & & \\
\downarrow & & \\
\rightarrow & \mathrm{u} & \rightarrow \\
 & \downarrow & \uparrow \\
\mathrm{x} & \rightleftarrows & \mathrm{y}
\end{array}$$

This has ODE's

$$\frac{du}{d\tau} = s - (k+y)u, \quad \epsilon\frac{dy}{d\tau} = \frac{ux}{J+x} - \frac{vy}{K+y}, \quad x+y=1.$$

I have put a parameter ϵ to have the quasi-stationary case

$$\frac{ux}{J+x} = \frac{vy}{K+y}, \quad x+y=1$$

with ϵ. The bifurcation diagram is given by

$$s = (k + y(u))u,$$

as before, which is a sigmoidal function if $JK < K+1$ (the same condition as for $ux(u)$ to have a maximum). For $y(u)$ close to a step function, it has a very steep part, corresponding to an almost flat part in the bifurcation diagram. The phase plane for u and y has null clines $(k+y)u = s$ and $y = y(u)$. You will find that the steady state is always stable. But with 2 GK-blocks the story changes, as the numerics in STBT show. We'll come back to this in the next version of these notes.

2.2 Figure 2b

$$\begin{array}{ccccc}
\mathrm{s} & & \rightarrow & \mathrm{r} & \rightarrow \\
\downarrow & & \uparrow & \downarrow & \\
\longrightarrow & & \mathrm{u} & \rightarrow & \\
 & \uparrow & \downarrow & & \\
 & \mathrm{y} & \leftrightarrows & \mathrm{x} &
\end{array} \tag{2.40}$$

$$\frac{dr}{d\tau} = k_u u - k_r r, \quad \frac{du}{d\tau} = s + y(u) - (k+r)u.$$

The bifurcation diagram is given by

$$s = (k + \frac{k_u}{k_r}u)u - y(u), \quad r = \frac{k_u}{k_r}u$$

We introduce new parameters

$$\epsilon = k_r, \quad \mu = \frac{k_u}{k_r},$$

which changes the equations into

$$\dot{r} = \frac{dr}{d\tau} = \epsilon(\mu u - r), \quad \dot{u} = \frac{du}{d\tau} = s + y - (k+r)u$$

and the bifurcation diagram into

$$s = (k + \mu u)u - y(u), \quad r = \mu u.$$

You have seen this diagram for $\mu = 0$. For a sigmoidal GK-function $y = y(u)$ increasing from 0 to 1 as u runs from 0 to ∞ any k for which $ku = y(u)$ has 3 solutions (one being $u = 0$), produced a diagram $s = ku - y(u)$ which was a one-way switch. For small μ the diagram looks almost the same, but what about stability? If (u_s, r_s) is steady state then the matrix

$$A = \begin{pmatrix} a & b \\ c & d \end{pmatrix} = \begin{pmatrix} \frac{\partial \dot{r}}{\partial r} & \frac{\partial \dot{r}}{\partial u} \\ \frac{\partial \dot{u}}{\partial r} & \frac{\partial \dot{u}}{\partial u} \end{pmatrix} = \begin{pmatrix} -\epsilon & \epsilon\mu \\ u_s & y'(u_s) - k - r_s \end{pmatrix}$$

in (u_s, r_s) is needed. Recall

$$D = \det(A) = ad - bc = \lambda_1\lambda_2, \quad T = \text{trace}(A) = a + d = \lambda_1 + \lambda_2,$$

in which λ_1, λ_2 are the eigenvalues of A. If $T^2 < 4D$ the eigenvalues are complex ($\lambda = \alpha \pm \beta i$) and (u_s, r_s) is a spiral point. The sign of $T = 2\alpha$ then decides the stability: a stable spiral point for $T < 0$, an unstable spiral point for $T > 0$. A spiral point requires a positive D, but a positive D also allows real nonzero eigenvalues λ_1, λ_2 of the same sign: $0 < 4D < T^2$ makes the point a node, a stable node for $T < 0$, an unstable node for $T > 0$.

The bifurcation curve

$$s = (k + \mu u)u - y(u)$$

has turning point in the zero's of

$$s'(u) = \frac{ds}{du} = k + 2\mu u - y'(u)$$

Show that

$$A = \begin{pmatrix} -\epsilon & \epsilon\mu \\ u_s & \mu u_s - s'(u_s) \end{pmatrix}$$

and that

$$D = \det(A) = \epsilon s'(u_s)$$

This tells you that part of the bifurcation curve corresponds to saddle points (these are unstable stationary states). Indicate which part this in your figure.

Now the remaining parts are not necessarily stable. Show that

$$T = \mu u_s - \epsilon - s'(u_s)$$

and explain how a stable steady state may become unstable by increasing μ.

2.3 Figure 2c

$$
\begin{array}{ccccccc}
\text{s} & & & & & & \\
\downarrow & & & & & & \\
\rightarrow & r & \rightarrow & \text{u} & \rightarrow & & \\
 & & \uparrow & \downarrow & & & \\
 & & \text{y} & \leftrightarrows & \text{x} & &
\end{array}
\tag{2.41}
$$

with the system of equations

$$\frac{dr}{d\tau} = s - (k_1 + y(u))r, \quad \frac{du}{d\tau} = (k_1 + y(u))r - k_2 u.$$

Note that the 3 horizontal arrows correspond to 3 reactions (only the middle one appears twice in the equations). This network looks similar to the one-way switch, but does it behave similarly? You will find out below.

Determine the steady states for each $s > 0$ and sketch the bifurcation diagram in terms of s and the u-component of the steady states. Compute the matrix

$$A = \begin{pmatrix} a & b \\ c & d \end{pmatrix} = \begin{pmatrix} \frac{\partial \dot{r}}{\partial r} & \frac{\partial \dot{r}}{\partial u} \\ \frac{\partial \dot{u}}{\partial r} & \frac{\partial \dot{u}}{\partial u} \end{pmatrix}$$

in the steady state and show that the trace and determinant are given by

$$D = k_2(k_1 + y(u)), \quad T = ry'(u) - y(u) - k_1 - k_2$$

(the matrix A will contain $k_1, k_2, s, y(u), y'(u), r$). Discuss the possibility that the steady state is an unstable spiral point or unstable node. For which value of s do you expect this to be certainly the case? Assume that $y = y(u)$ has a sigmoidal graph with a steep transition from $y \approx 0$ to $y \approx 1$. Sketch the null clines, draw the arrows, and argue that orbits starting on the positive r-axis will spiral inwards to a periodic orbit.

3 Sigmoidal GK-functions: details

In the next exercises you will see and learn how simple algebra and PPP-plots quickly lead to insight in the GK-balance (1.10), beginning with explaining why $y(u)$ runs monotonically from 0 to 1 as u runs from 0 to large values and eventually ∞. If you like you may skip the first few simple exercises which concern the MM-rates and jump directly to Exercise 21 to continue from there. Just before Exercise 23 you will see a trick you have already seen before. Exercises 23 and 24 explain the shape of the GK-curve for J and K small.

Exercise 19. Simple but important: consider the synthesis rate

$$\frac{ux}{J + x} \tag{3.42}$$

in (1.10). You need to specify the parameters u and J in order to (computer)plot this rate as a function of x. Since both parameters u and J are positive, an obvious first choice is $u = J = 1$. Sketch the graph of the function

$$f(x) = \frac{x}{1 + x}$$

using PPP with x running from 0 to a value far beyond its contextual maximal value 1. What is the dominating term in the denominator when x is small? And when x is large? In both cases examine the expression you get by keeping only this dominating term in the denominator. These can be plotted in the same diagram as $f(x)$. The two lines you get have a meaning in relation to the graph of f. Explain. Convince yourself that the graph of f is concave, starts of with slope 1, and asymptotes from below to 1 as x gets large. Never in your life forget the shape of this graph.

Exercise 20. All MM-rates are the same: starting from $u = J = 1$ examine how the graph of

$$\frac{ux}{J+x}$$

changes if you vary u and J, one at the time first. Pay special attention to the slope in the origin and to the asymptote. Discuss the (near) limit cases: u small and $u = 0$, J small and $J = 0$, u large and $u \to \infty$, J large and $J \to \infty$. Also examine variations in which the ratio $\frac{u}{J}$ is kept fixed. All this should also convince you that all these graphs look the same if you scale the units appropriately. In the GK-context you will want to restrict your attention to x between 0 and 1.

Exercise 21. Balancing both MM-rates in (1.23): now that the synthesis rate is well understood, the same holds for the degradation rate

$$\frac{vy}{K+y}, \tag{3.43}$$

as a function of y with parameters v and K. You can sketch the graphs of both rates, and combine them in one picture, remembering that $x+y=1$. Start with the diagram with the degradation rate plotted versus y. On the horizontal axis you put $x = 0$ where $y = 1$ and $x = 1$ where $y = 0$, and draw the graph of the x-dependent synthesis rate from right to left. Note that in the resulting picture the windows $0 < x < 1$ and $0 < y < 1$ coincide. You can vary u and have various synthesis rate curves in the picture. This should give you a diagram which resembles the second diagram in Figure 1c of SBTB. Explain that for each choice of positive values u, v, J, K, the two graphs intersect in one point only. *This identifies the GK-equilibrium.*

Exercise 22. Monotone GK-curves: we are interested is the dependence of the response y on the signal u. Varying u, you should be able to figure out that y runs monotonically from 0 to 1 as u runs from 0 to ∞. Just look at what happens in the diagram of Exercise 21 in which you drew both rates. As you vary u, one curve changes, the other remains where it is, so you can see how the intersection point moves.

The conclusion in Exercise 22 is rather immediate from examining the graphs, while getting the GK-formula for y right *and recognising it is monotone* is not so easy. Now here is *the nice trick again*: if you want to know the shape[45] of the graph of y versus u, you can also examine the graph of u versus y:

[45]For which parameter values is the graph of y versus u convex-concave?

Exercise 23. Inverted GK-curves: eliminate x by plugging $x = 1 - y$ in (1.10) and show that

$$w = \frac{u}{v} = \frac{y}{K+y}\frac{J+1-y}{1-y} = g_{JK}(y).$$

I have introduced w as a name for $\frac{u}{v}$, and denoted the right hand side in Exercise 23 by $g_{JK}(y)$, a function of y with (positive) parameters J and K. It looks complicated, but with some experience you may wish to see immediately what its graph $w = g_{JK}(y)$ must look like. *First we look at a (near) limit case which shows what you can expect:*

Exercise 24. Steep sigmoidal GK-curves: In most examples in SBTB both K and J are small. For $J = K = 0$, you get $w = 1$ in the formula in Exercise 23, meaning that $u = v$. In the graph of y versus u, with a vertical window $0 \leq y \leq 1$, this is the vertical line segment with $u = v$. With the segment $0 \leq u \leq v$ on the u-axis and the segment $v \leq u < \infty$ on $y = 1$, you get a broken curve of three line segments. Choose some $v > 0$ and make a drawing. For small positive K and J the graph of y versus u must be close to this broken curve, with a rather swift transition from $y \approx 0$ to $y \approx 1$. It is a difficult to imagine such a smooth curve with a steep transitional part to be anything else but sigmoidal in this *near limit case*.

To see how far into the parameter domain the sigmoidal shape of the GK-curve persists we look at y in terms of u:

Exercise 25. Highschool math revisited with PPP: With y horizontally (and $w = \frac{u}{v}$ vertically) guess from a sketch, while keeping track of the signs of the factors in $g_{JK}(y)$, that, varying y from $-\infty$ to $+\infty$, that the graph of $g_{JK}(y)$ starts from the horizontal asymptote $w = 1$, runs up to a vertical asymptote in $y = -K$, where it flips over from $w = +\infty$ to $w = -\infty$, runs up again from $-\infty$ to intersect the horizontal axis in $y = 0$ and run up to $+\infty$ along other vertical asymptote in $y = 1$, flipping over a second time, running up from $-\infty$ again to intersect the horizontal axis a second time in $y = J + 1$, finally asymptoting back to $w = 1$ from below.

You will improve your hands on math skills greatly by spending some PPP-time on the previous exercise and convincing yourself of the scenario it sketches. Note that all 3 branches of the graph are monotone and that g_{JK} is really *the simplest function you can think of that has such a graph*. You may have learnt how to do draw such graphs, using the second derivative to find the inflection points, of which there must be at least one here[46], as you can infer from a first PPP-sketch. Judiciously guessing that there is probably only one inflection point[47], the question is really if this point lies in the window $0 < y < 1$ of interest.

In this window of interest, the graph $w = g_{JK}(y)$ is a curve which starts of in the origin and runs up to the asymptote in $y = 1$. You already knew this from Exercise 22. Does the graph start off convex or concave from $y = 0$? An easy Maple/Mathematica calculation, or a more tedious one by hand, shows that the second derivative of $g_{JK}(y)$ in $y = 0$ is

$$g''_{JK}(0) = \frac{2(JK - 1 - J)}{K^2},$$

[46] Between the two vertical asymptotes.

[47] Graphs of simple functions can only do so much and they usually don't.

which tells you that the graph starts off convex if

$$JK > 1 + J. \tag{3.44}$$

The next exercise explains why this is the boring case, apparently avoided by nature and the authors of SBTB.

Exercise 26. Concave GK-curves: assume (3.44) so that $g''_{JK}(0) \geq 0$. Guess that, as it approaches the asymptote $y = 1$, the graph must be convex. Take notice of the following line of reasoning. Inbetween it might change to concave and then back to convex again, but you can show that the graph has only one inflection point[48]. In case of (3.44), the inflection point lies to the left of $y = 0$, implying the graph is convex in the window $0 < y < 1$ for all positive J and K satisfying (3.44). Translated to y as a function of w or u, it is a fact that the graph with (vertically) the response y as a function of (horizontally) the (nonnegative) signal u is boringly concave if (3.44) holds[49].

On the other hand, if

$$JK < 1 + J,$$

the graph of y versus u starts of convex, and must turn concave eventually as it asymptotes from below to $y = 1$. For simple graphs like the one under consideration, there is only one inflection point. The important conclusion is (1.22). This conclusion carries over to all phosporylation blocks appearing in the SBTB paper. This last exercise may help convince you the GK-graphs in SBTB are correct.

Exercise 27. Sigmoidal GK-curves for small K (or small J): examine the limit case $K = 0$ (or $J = 0$) to convince yourself of the conclusions above. Draw diagrams (y versus u and u versus y) for $K = 0$ (or $J = 0$) and $K > 0$ (or $J > 0$) small.

Exercise 28. If you like, the critical $k = k_{touch}$ for which the graphs of $y(u)$ and ku touch may be computed: explain why the 2 equations

$$k = \frac{y(u)}{u} = y'(u) \tag{3.45}$$

are equivalent to saying that the graphs of $y(u)$ and ku touch in u. Writing

$$u = v\frac{y}{K+y}\frac{J+1-y}{1-y}, \quad u' = \frac{du}{dy}, \tag{3.46}$$

see Exercise 23, use the fact that

$$u'y' = \frac{du}{dy}\frac{dy}{du} = 1$$

allows u-derivatives to be expressed in y. Thus, *avoiding the GK-formula*, you can show that the second equality in (3.45) is equivalent to

$$\frac{u(y)}{u'(y)} - y = 0,$$

[48] A nice math exercise.

[49] Like the hyperbolic curve in the last diagram in Figure 1b of SBTB.

which Maple reduces to

$$y^2\,\frac{y^2-2\,(J+1)\,y-JK+J+1}{(J+K)\,y^2-2Ky+K(1+J)}=0. \tag{3.47}$$

The quadratic numerator has negative discriminant and is positive for $0<y<1$. Show that, provided $JK<1+J$, there is a unique $0<y<1$ for which (3.47) holds. For this

$$y=y_{touch}=J+1-\sqrt{J(J+1+K)},$$

which through (3.46) corresponds to a unique $u=u_{touch}$, we must have, solving (3.47) for K rather than y, that

$$K=\frac{J+1-2(J+1)y_{touch}+y_{touch}^2}{J},$$

which implies that, looking at the first equation in (3.45) and using Maple again,

$$k=k_{touch}=\frac{(1-y_{touch})^2}{Jv}.$$

To see why (1.35) holds you need to differentiate. Using the fact that

$$x'=x'(u)=\frac{dx}{du}=\frac{1}{\frac{du}{dx}}=\frac{1}{u'(x)}=\frac{1}{u'},$$

and Leibniz' product rule, you can check that (using Maple)

$$\frac{d}{du}\,(ux(u))=x(u)+ux'(u)=x+\frac{u}{u'}=-\frac{x^2Q(x)}{(K+J)\,x^2-2\,Jx+J(1+K)},$$

with

$$Q(x)=x^2-2\,(K+1)\,x-JK+K+1.$$

Now u-derivatives are expressed in x, again this trick[50] *avoids the GK-formula.* The window $0<x<1$ corresponds to $\infty>u>0$. The denominator is positive since it is positive in $x=0$. Its discriminant is $-(4(J+K+1))JK<0$, so you only need to look at the quadratic $Q(x)$. Since $Q(1)=-K-JK<0$ and $Q'(x)=2(K+1-x)<0$ for $0\le x\le 1$, it depends on $Q(0)=1+K-JK$ whether $ux(u)$ has a maximum.

Exercise 29. Verify that $ux(u)$ has a maximum only if $JK<1+K$, and sketch its graph for $JK<1+K$ and $JK>1+K$.

Exercise 30. Inflection points: if $JK<1+K$ then for $u>0$ there is in fact precisely one $u>0$ for which the second derivative of $ux(u)$ is 0. Believe this. The graph of $ux(u)$ has only inflection point. Verify directly that the second order derivative of $ux(u)$ is negative in $u=0$ because the first order derivative of $x(u)$ is negative in $u=0$.

If you don't believe do the last exercise in the appendix (Section 3).
read on, otherwise jump to Exercise 12. Since

$$\frac{d^2}{du^2}\,(ux(u))=\frac{d}{du}\,(x(u)+ux'(u))=\frac{dx}{du}\frac{d}{dx}\,(x(u)+ux'(u))=\frac{dx}{du}\frac{d}{dx}\left(x+\frac{u}{u'}\right),$$

[50]These calculation details are remarkably similar to (3.47).

and (with Maple again)

$$\frac{d}{dx}\left(x+\frac{u}{u'}\right)=\left(x+\frac{u}{u'}\right)'=2-\frac{uu''}{u'^2}=\frac{2x(K+1-x)C(x)}{\left((K+J)\,x^2-2\,Jx+J(1+K)\right)^2},$$

in which the cubic $C(x)$ is given by

$$C(x)=(J+K)\,x^3-3\,Jx^2+3\,J\,(K+1)\,x+J\,(-1+JK-K)\,. \tag{3.48}$$

The inflection points of the graph of $ux(u)$ versus u correspond to solutions of $C(x)=0$ with $0<x<1$. The cubic $C(x)$ turns out be increasing for $0\le x\le 1$. You can see this because

$$C'(x)=(3(J+K))x^2-6Jx+3J(K+1),\quad C''(x)=6((K+J)x-J),$$

$$C'(0)=3J(K+1)>0,\quad C'(1)=3K(J+1)>0,$$

$$C''(0)=-6J<0,\quad C'(1)=6K>0,$$

$$C''(\frac{J}{J+K})=0,\quad C'(\frac{J}{J+K})=\frac{3JK(J+K+1)}{J+K}>0,$$

the latter being the positive minimum of $C'(x)$ in the window $0<x<1$. Since

$$C(0)=J(-1+JK-K),\quad C(1)=K(J+1)^2>0,$$

there is an inflection point if and only if $JK<1+K$. This point is then unique because $C'(x)>0$ for $0<x<1$. It corresponds to the unique solution $x=x_{inflection}$ in the window $0<x<1$ for which $C(x)=0$.

Exercise 31. Inflection points for $k>0$: knowing that the graph of $ux(u)$ has an inflection point if and only if $JK<1+K$, explain why the same holds for the graph of $ux(u)+ku$, and that the inflection point, if it does occur, occurs for the same $u=u_{inflection}$, independent of k. This is the u value which through (1.34) corresponds to the x-value for which $C(x)=0$ above.

Exercise 32. Implicit calculation of $k=k_{hip}$, see Exercise 28: solve $C(x)=0$ simultaneously with

$$\frac{d}{du}\left(ku+ux(u)\right)=k+x+\frac{u}{u'}=k-\frac{x^2Q(x)}{(K+J)\,x^2-2\,Jx+J(1+K)}=0. \tag{3.49}$$

Note that $C(x)=0$ defines $x=x_{inflection}$. Solving with respect to K show that

$$K=\frac{J(1-x)^3}{x^3+3Jx+J^2-J},\quad x=x_{inflection},$$

Plug K into (3.49). Maple quickly shows that this reduces (3.49) to

$$\frac{kJ-x^3}{J}=0,$$

so that, since $x=x_{inflection}$, again with Maple,

$$k_{hip}=\frac{x^3_{inflection}}{J}.$$

Of course, you still need to find $x=x_{inflection}$ from the cubic equation $C(x)=0$.

4 Afleiding Michaelis-Menten reaction rate

In de reactie

$$S + E \underset{v_2}{\overset{v_1}{\rightleftarrows}} SE \underset{v_4}{\overset{v_3}{\rightleftarrows}} PE \underset{v_6}{\overset{v_5}{\rightleftarrows}} E + P$$

zijn S en P het substraat en het product, E het enzym, SE het enzym met gebonden substraat, PE het enzym met gebonden product, met concentraties

$$s = [S], c_0 = [E], c_1 = [SE], c_2 = [PE], p = [P],$$

en reactiesnelheden

$$v_1 = k_1 s c_0, v_2 = k_2 c_0, v_3 = k_3 c_1, v_4 = k_4 c_2, v_5 = k_5 c_2, v_6 = k_6 c_0 p, \tag{4.50}$$

met reactieconstanten $k_1, k_2, k_3, k_4, k_5, k_6$. Dit heeft aanleiding tot een stelsel gekoppelde differentiaalvergelijkingen van de vorm

$$\begin{aligned} \dot{s} &= -v_1 + v_2 \\ \dot{c}_0 &= -v_1 + v_2 + \ldots \\ \dot{c}_1 &= v_1 + \ldots \\ \dot{c}_2 &= \ldots \\ \dot{p} &= \ldots \end{aligned}$$

voor de concentraties, waarin de reactie rates in (4.50) moeten worden ingevuld. De kolom 3-vector c heeft als entries c_0, c_1, c_2.

Exercise 33. Schrijf het stelsel dat je zo krijgt in de vorm

$$\begin{aligned} \dot{s} &= -k_1 c_0 s + k_2 c_1 \\ \dot{c} &= A(s,p)c \\ \dot{p} &= -k_6 c_0 p + k_5 c_2 \end{aligned}$$

met $A(s,p)$ een 3×3-matrix waarin s en p lineair voorkomen.

Exercise 34. Verifieer dat $c_0 + c_1 + c_2$ constant is en noem die constante $\epsilon = e_T$. Introduceer $\gamma_0, \gamma_1, \gamma_2$ door $c = \epsilon\gamma$. Dan verandert het stelsel in Opgave 33 in

$$\begin{aligned} \dot{s} &= \epsilon(-k_1 \gamma_0 s + k_2 \gamma_1) \\ \dot{\gamma} &= A(s,p)\gamma \\ \dot{p} &= \epsilon(-k_6 \gamma_0 p + k_5 \gamma_2) \end{aligned}$$

en dus is de tijdschaal voor de verandering van s en p veel groter dan die van γ als ϵ klein is. De aanname dat $\gamma(t)$ dus veel sneller in evenwicht komt dan $s(t)$ en $p(t)$ leidt tot $A(s,p)\gamma = 0$, drie vergelijkingen voor $\gamma_0, \gamma_1, \gamma_2$ waarvan de derde volgt uit de eerste twee (waarom?). Combineer dit met $\gamma_0 + \gamma_1 + \gamma_2 = 1$ en bepaal $\gamma_0, \gamma_1, \gamma_2$ in termen van s en p. Gebruik de uitdrukking die je vindt in de expressies voor $\dot{s}$ en $\dot{p}$ en laat zien dat dit leidt tot differentiaalvergelijkingen van de vorm

$$\dot{s} = \frac{\epsilon(s - Kp)}{a_0 + a_1 s + a_2 p} = -\dot{p}$$

en druk K, a_0, a_1, a_2 uit in $k_1, k_2, k_3, k_4, k_5, k_6$.

Exercise 35. Een andere manier om het stelsel in Opgave 33 te vereenvoudigen is de aanname dat reacties 1,2,5,6 heel snel zijn waardoor de reactie $S+E \rightleftarrows SE$ en $PE \rightleftarrows E+P$ heel snel in evenwicht raken en $k_1 s c_0 = k_2 c$ en $k_5 c_2 = k_6 c_0 p$ met $c_0 + c_1 + c_2 = e_T$ de concentraties c_0, c_1, c_3 geven in termen van s en p. Dit leidt tot dezelfde vergelijkingen als in Opgave 34 maar met andere coëfficiënten. Druk die weer uit in $k_1, k_2, k_3, k_4, k_5, k_6$.