Homo-Oligomers in Signal Transduction: Modelling Approaches, Homeostasis, Ultrasensitivity, Bistability

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Abstract: Homo-oligomerisation of proteins is a ubiquitous phenomenon. Previous research showed that homo-dimerisation can lead to ultrasensitivity and if coupled to positive feedback, to bistability, suggesting that homo-oligomerisation plays important roles in signal transduction. This paper provides a general exploration of dynamical mathematical models of homo-oligomerisation, potential modelling pitfalls and approximative modelling strategies. Simulations and model analyses show that homo-oligomerisation on its own allows for a large variety of complex dynamic and steady-state regulatory behaviour such as short dampened oscillations or homeostatic control of monomer concentration. It furthermore can lead to bistability without feedback by enabling pseudo-multisite modification and kinetic pseudo-cooperativity via multi-enzyme regulation, thereby significantly reducing the requirements previously thought necessary for the occurrence of bistability.

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1 Introduction

Homo-oligomerisation of proteins, i.e. the assembly of supramolecular protein complexes made up from multiple identical subunits, is a ubiquitous phenomenon. In vertebrates, about 30% of all proteins form homo-oligomers consisting of two or more subunits [1]. Oligomerisation may offer several advantages: it represents a way to economically assemble larger molecular structures (thereby reducing genome size) and allows for a higher error-free transcription chance for individual subunits. Moreover, it can provide additional regulatory control via allostery and cooperative binding events (hemoglobin being the classical example). [2, 3]

Dynamical mathematical models based on ordinary differential equations (ODEs) have been used extensively to study many important motifs, mechanisms and phenomena in signal transduction networks. To some extent, ODE models have also been used to study signal transduction processes involving homo-oligomers. Such theoretical studies have shown that in addition to the well known role in the emergence of ultrasensitive responses via cooperative binding, oligomerisation can provide an additional layers of control over such responses.

Bouhaddou and Birtwistle, for example, showed that different dimerisation routes provide an effective means of tuning ultrasensitive cooperative responses [4]. Buchler and Louis showed that homo-oligomerisation itself can lead to modest ultrasensitivity independently from cooperativity [5]. If coupled to positive feedback, the ultrasensitivity generated e.g. by homo-dimerisation is sufficient for the emergence of bistability [6]. For signalling involving dimeric receptors and substrate activation, the presence of a single/dual activation mechanism can lead to complex, non-linear signal dynamics [7]. Taken together, this highlights the importance of homo-oligomerisation and the use of mathematical modelling as a tool to study their roles in signal transduction.

However, apart from above mentioned studies focussing on specific contexts or systems, a general analysis of homo-oligomerisation in terms of assembly dynamics, steady state behaviour and the potential effect of post-translational modifications (PTMs) is neither covered by classical and popular textbooks on mathematical or system's biology (see e.g. [8–12]), nor is the author aware of such an analysis in the recent research literature. It thus seems that an exploration of general dynamical mathematical models of homo-oligomerisation and is still lacking. This paper provides such an exploration. As this study focusses solely on homo-oligomerisation, I will leave out the prefix 'homo-' in the remainder of this article for the sake of brevity.

Starting with simple mass action kinetics based models of dimerisation to tetramerisation we will study assembly dynamics and steady state behaviour numerically. Even though the first presented models are very simple, it is found that they are capable of complex dynamical and steady state behaviour such as undulations, and homeostatic regulation.

As the complexity of potential oligomerisation routes quickly increases with higher numbers of subunits per oligomer, generalised mass action (GMA) rate laws as a simple approximative modelling approach are studied.

Next, PTMs of oligomers are considered. Surprisingly, the application of both conventional mass action and GMA rate laws easily results in thermodynamically inconsistent models due to combinatorial expansion of the oligomerisation routes upon modification. In the case of conventional mass action kinetics the issue can be circumvented by balancing the rate expressions assuming a conservation of total oligomerisation rates.

Finally, I present two novel mechanisms based on oligomerisation which lead to ultrasensitive, bistable PTM responses: pseudo-multisite modification and regulation by multiple enzymes.

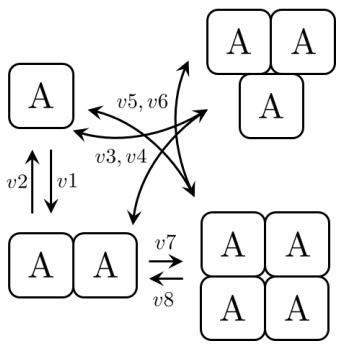
Some of the presented original findings certainly could have been treated in more detail or with more biological examples. My primary intention, however, is to demonstrate that oligomerisation enables more complex regulatory behaviour than previously appreciated. While the broad scope of a general analysis of dynamical mathematical models of oligomerisation does not permit an exhaustive treatment of all aspects within the limit of a single article, some of the most important implications and avenues for future research will be outlined in the discussion.

2 Results

2.1 Simple mass action models of oligomerisation: ultrasensitivity and homeostasis

Let us begin by assuming that a general protein A can form oligomers with a maximum number of n subunits (protomers) per oligomeric complex. We assume furthermore that A can form all intermediate oligomeric species with m subunits (where $m \in \mathbb{N}, 1 < m < n$) and that each oligomeric species is formed through simple one-step, second-order binding reactions described by mass action kinetics. For the remainder of this article, we will study oligomers with a maximum of four protomers or less, i.e.

Reaction scheme



Individual reactions and rate expressions

R1) 2A → AA,	$v1 = k_1[A]^2$
R2) AA → 2A,	$v2 = k_2[AA]$
R3) A + AA → AAA,	$v3 = k_3[A][AA]$
R4) AAA → AA + A,	$v4 = k_4[AAA]$
R5) A + AAA → AAAA,	$v5 = k_5[A][AAA]$
R6) AAAA → A + AAA,	$v6 = k_6[AAAA]$
R7) 2AA → AAAA,	$v7 = k_7[AA]^2$
R8) AAAA → AA + AA,	$v8 = k_8[AAAA]$

Figure 1
Model scheme of homo-tetramerisation based on conventional mass action kinetics assuming that all intermediate species (dimers and trimers) are possible in the reaction pathway. See text for the differential equations describing the system.

tetramers, trimers and dimers. It is likely that many of the presented findings apply to higher-order oligomers as well.

In the case of tetramers we therefore assume that tetramers can be formed by the association of two dimers or, alternatively, of a trimer and a monomer. The reaction scheme and the reaction rates for the individual reactions can then be summarised as in Figure 1. Denoting the monomeric to tetrameric species by $A, ..., AAAA$ we can now formulate the system's equations:

$$\frac{d}{dt}[A] = 2 \cdot v2 + v4 + v6 - 2 \cdot v1 - v3 - v5$$
$$\frac{d}{dt}[AA] = v1 + v4 + 2 \cdot v8 - v2 - v3 - 2 \cdot v7$$
$$\frac{d}{dt}[AAA] = v3 + v6 - v4 - v5$$
$$\frac{d}{dt}[AAAA] = v5 + v7 - v6 - v8$$

The total amount of subunits is conserved by the relation $A_{tot} = [A] + 2 \cdot [AA] + 3 \cdot [AAA] + 4 \cdot [AAAA]$ and can be used to eliminate one of the above equations. Note that models for tri- or dimerisation can be obtained simply by removing reactions R5-R8 or R3-R8, respectively. For the sake of simplicity we will begin by assuming equal rate constants of $10^7 mol^{-1} s^{-1}$ for all association reactions and equal rate constants of $10 s^{-1}$ for all dissociation reactions, thereby yielding a K_d value of 1 μM for all reactions.

Time course simulations of the system with initial conditions $[A]_0 = A_{tot} = 10 \mu M$ show association dynamics typical for binary protein-protein interactions in the case of dimerisation, whereas trimerisation and tetramerisation reactions exhibit a transient overshoot of dimers followed by a slower decrease and increase in trimers and tetramers (Figure 2A-C). More complex dynamics such as dampened oscillations/undulations on different timescales are possible, which could be significant if the individual oligomeric species possess different biological functionality as will be discussed later (Figure 2C, inset).

Numerical steady state analysis shows that the dose-response curves for the individual species meet in a single intersection point (Figure 2D-F), mirroring the assumption that all

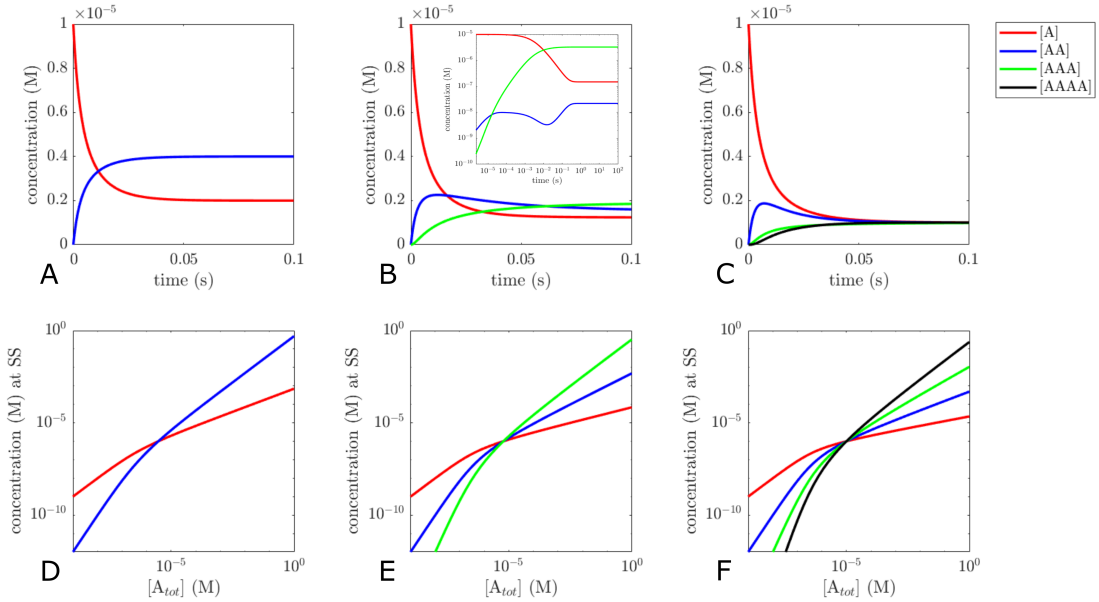


Figure 2
Time course simulations (A-C) and steady state analysis (D-F) for dimers (A,D), trimers (B,E) and tetramers (C,F). Initial conditions for A-C: $[A]_0 = 10\mu M, [AA]_0 = [AAA]_0 = [AAAA]_0 = 0M$. Parameters: (A and D) $k_1 = 10^7 mol^{-1}s^{-1}$, $k_2 = 10s^{-1}$, (B and E) $k_1 = k_3 = 10^7 mol^{-1}s^{-1}$, $k_2 = k_4 = 10s^{-1}$, (C and F) $k_1 = k_3 = k_5 = k_7 = 10^7 mol^{-1}s^{-1}$ (C inset: $k_3 = 10^9 mol^{-1}s^{-1}$), $k_2 = k_4 = k_6 = k_8 = 10s^{-1}$.

reactions have the same K_d value. Local sensitivity analysis at $A_{tot} = 0.1\mu M$ with 2% pertubation yields relative sensitivies $\frac{dS}{dA_{tot}}\frac{A_{tot}}{S}$ (where S is the steady state concentration of either $A, ..., AAAA$) of 0.87 for monomers and 1.76 for dimers in the dimerisation model, 2.58 for trimers in the trimersation model and 3.46 for tetramers in the tetramerisation model. The analysis confirms again that oligomerisation at concentrations below the K_d can lead to modestly ultrasensitive responses to changes in total protein concentration and that ultrasensitivity can increase with higher number of protomers per complex (as can also be seen from the increasing slopes in Figure 2E,F) [5].

For many proteins able to form higher order oligomers, the presence of a single or a small subfraction of possible oligomeric species often dominates over other potential intermediate species, indicating that oligomerisation is often cooperative and that the K_d values for the individual reactions differ. Varying the model parameters in a way to favour formation of the highest order oligomer in the trimerisation and tetramerisation model (e.g. by increasing association rate constants) can reproduce the dominance of the highest order oligomer over many concentration ranges (Figure 3A,B). This also leads to a shift of intersection points, resulting in different ap-

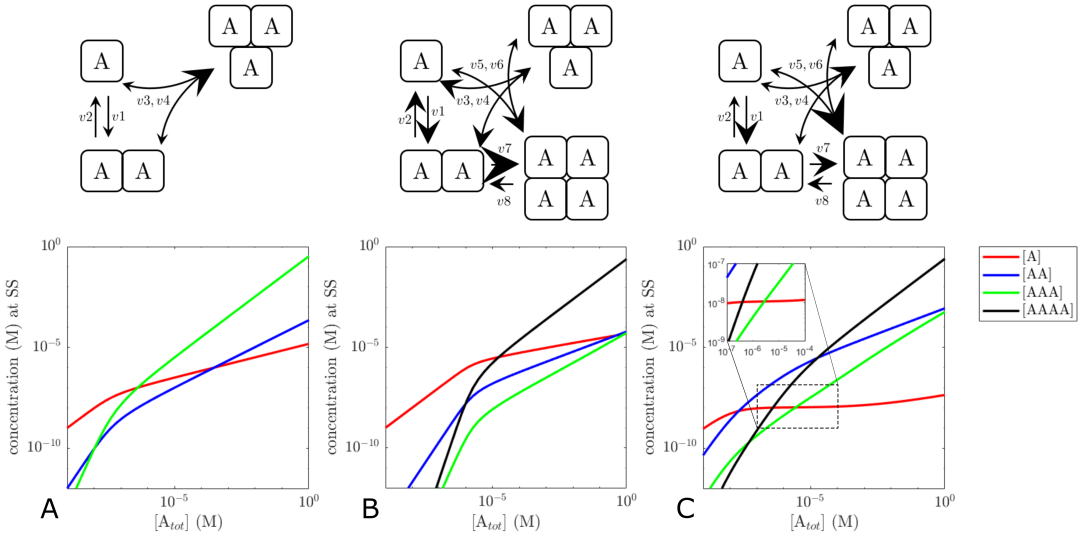


Figure 3
Steady state analysis of trimerisation and tetramerisation models with varied parameters. The relative change of parameters is visualised in the upper reaction schemes. Parameters: (A) $k_1 = 10^7 mol^{-1}s^{-1}$, $k_3 = 10^9 mol^{-1}s^{-1}$, $k_2 = k_4 = 10s^{-1}$, (B) $k_1 = 10^8 mol^{-1}s^{-1}$, $k_3 = 2 \times 10^8 mol^{-1}s^{-1}$, $k_5 = 10^9 mol^{-1}s^{-1}$, $k_7 = 2 \times 10^{11} mol^{-1}s^{-1}$, $k_2 = 5000s^{-1}$, $k_4 = 10^4 s^{-1}$,$k_6 = 10s^{-1}$, $k_4 = 3000s^{-1}$, (C) $k_1 = 5 \times 10^8 mol^{-1}s^{-1}$, $k_3 = 10^9 mol^{-1}s^{-1}$, $k_5 = 10^{11} mol^{-1}s^{-1}$, $k_7 = 10^9 mol^{-1}s^{-1}$, $k_2 = k_6 = 10s^{-1}$, $k_4 = 50s^{-1}$, $k_4 = 3000s^{-1}$.

parent K_d values between the individual intermediate oligomerisation reactions. Tweaking of the parameters allows to shift the curves for each individual species into almost any direction (data not shown). Interestingly, some parameter combinations allow for more complex behaviour than vertical/lateral translation of individual dose-response curves. Modifying the parameters of the tetramerisation model in a way that moderately favours formation of dimers and trimers, but strongly favours tetramerisation via trimers results in a system where the monomer concentration remains essentially constant over more than four orders of magnitude of total protein concentration (Figure 3C). Therefore, if monomers are the biologically active species, oligomerisation could be a very powerful homeostatic mechanism that does not require a complex feedback organisation typically associated with homeostasis [13, 14].

2.2 Using generalised mass action rate laws to approximate complex oligomerisation pathways

As can already be seen from the tetramerisation model, the number of potential oligomerisation pathways through various intermediate species rapidly increases with higher numbers of subunits per oligomeric complex. Mass action kinetics based models of hexamers or even higher oligomers could quickly become intractable both experimentally and with respect to model formulation. It would thus

be desirable if oligomerisation reaction pathways could be approximated appropriately by simpler models. Using reversible Hill-equations or 'convenience' rate laws to approximate enzyme-catalysed reactions with potentially complex reaction mechanisms is a well established strategy [15, 16] and often makes it easier to fit model parameters to experimental data. Another way to approximate reaction rates are generalised mass action (GMA) rate laws, in which the law of mass action is applied to *activities* rather than concentrations; activities can be considered as *apparent kinetic orders* (in contrast to mechanistic orders) [12]. Activities are obtained from concentrations of a molecular species S by applying a power-law of the form $[S]^\gamma$ with the exponent γ being a parameter describing A's activity. GMA rate laws such as $v = k[S]^\gamma$ might then be used to describe the reaction rate [12].

We will use such GMA rate laws to approximate whole oligomerisation pathways rather than modelling each individual oligomerisation step. Let the formation of a general n-tamer A_n of A be described by the GMA rate law $v = k([A]^n)^\gamma = k[A]^{n\gamma}$. In contrast, we describe the dissociation rate of the n-tamer simply by $v = k'[A_n]$. As mentioned above, a single oligomeric species is sometimes the most abundant oligomeric species present at physiological protein concentration ranges. Such a situation is likely particularly well suited for approximative modelling and the whole system might be described simply by:

$$\frac{d}{dt}[A] = nk'[A_n] - nk[A]^{n\gamma}$$
$$\frac{d}{dt}[A_n] = k[A]^{n\gamma} - k'[A_n],$$

where one equation, again, can be eliminated by the conservation of mass. In the remainder of this section, we will focus on approximating tetramerisation by GMA modelling. The system behaviour is therefore governed by:

$$\frac{d}{dt}[A] = 4k'[AAAA] - 4k[A]^{4\gamma}$$
$$\frac{d}{dt}[AAAA] = k[A]^{4\gamma} - k'[AAAA]$$

In order to find out how appropriate this approximation is, we will attempt to fit the GMA model to data generated by the full model by using non-linear regression algorithms. We will restrict this approach to a total concentration range in which monomers and tetramers are the predominant molecular species (cf. Figure 3B).

We will begin the exploration by merely considering the steady state data and by limiting γ to either taking the value 1 or 0.5. The latter effectively reduces the rate to a second order mass action rate. As can be seen from Figure 4A, the GMA model with $\gamma = 1$ perfectly reproduces the steady state behaviour of the full model. For $\gamma = 0.5$ the GMA model shows a reasonable approximation for tetramers, but significant deviations for monomers. Restricting the range of data markedly improves fitting of the GMA model for $\gamma = 0.5$. Note that the fitted values for the association rate constant k and dissociation rate constant k' are separated by over a dozen orders of magnitude when fitting the model for $\gamma = 1$, but only six orders of magnitude for $\gamma = 0.5$. To find out how the choice of γ affects the kinetic behaviour of the GMA model we will add time-course simulations generated by the full model.

Fitting the GMA model to the steady state

data together with three oligomerisation time courses from the full model leads to essentially identical steady state behaviour (Figure 4B,C upper left subgraph). In contrast, the kinetic behaviour of the full model is not captured GMA model for $\gamma = 1$, whereas for $\gamma = 0.5$ the oligomerisation dynamics appear to be similar apart from the deviation in steady state concentrations (Figure 4B,C). Using alternative parameter estimation algorithms yields similar results, indicating that this result does not stem from a failure of the algorithm to converge (data not shown). Further restricting the range of total concentration improves the fit for $\gamma = 1$ with respect to the oligomerisation kinetics, but slightly worsens the reproduction of the steady state behaviour (Figure 4D). For $\gamma = 0.5$, restricting the range of total concentration allows the GMA model to capture both steady state behaviour and oligomerisation kinetics to a degree that might be appropriate for many applications (Figure 4E). Finally, the GMA model was fitted to the same data as in Figure 4D and E with also γ being a free parameter. Interestingly, the resulting parameter values were $\gamma = 0.500228, k = 5.06 \times 10^7$ and $k' = 21.02$, which are in close agreement to the values shown in Figure 4E where γ is fixed to 0.5, indicating that a pseudo second-order mass action rate resulting from the GMA rate law might be optimal for approximating both steady state and oligomerisation kinetics.

In practice, the purpose of the model will determine how appropriate the GMA approximation of oligomerisation is in a given situation. If only the steady state concentration of monomers and oligomers is of interest, a GMA model with $\gamma = 1$ can be appropriate. However, the huge separation of k and k' by many orders of magnitudes could lead to stiffness in large models. If there is evidence that the timescale of oligomerisation is faster than the rest of the model, it might therefore be appropriate to reduce the GMA model with $\gamma = 1$ e.g. by performing a QSSA to substitute the ODE for the oligomer by an algebraic expression. If both steady state and oligomerisation dynamics are relevant, fitting a GMA model with $\gamma = 0.5$ or γ as free parameter can be a successful approximation strategy if the relevant range of total concentration is sufficiently limited.

2.3 Considering PTMs: ensuring thermodynamical consistency

Just like non-oligomeric proteins, oligomeric proteins can, unsurprisingly, be modified by various post-translational modifications such as phosphorylation, ubquitinylation, lipidation and others. Sometimes these modifications can regulate the equilibria between monomeric and oligomeric species via conformational changes or sterical hindrance. Other times these modifications are irrelevant to the protein's oligomerisation behaviour. As we will see shortly, even accounting merely for a single PTM makes model formulation of anything higher than dimers unlikely more difficult due to the combinatorial expansion of potential oligomerisation routes, making appropriate approximation approaches even more desirable.

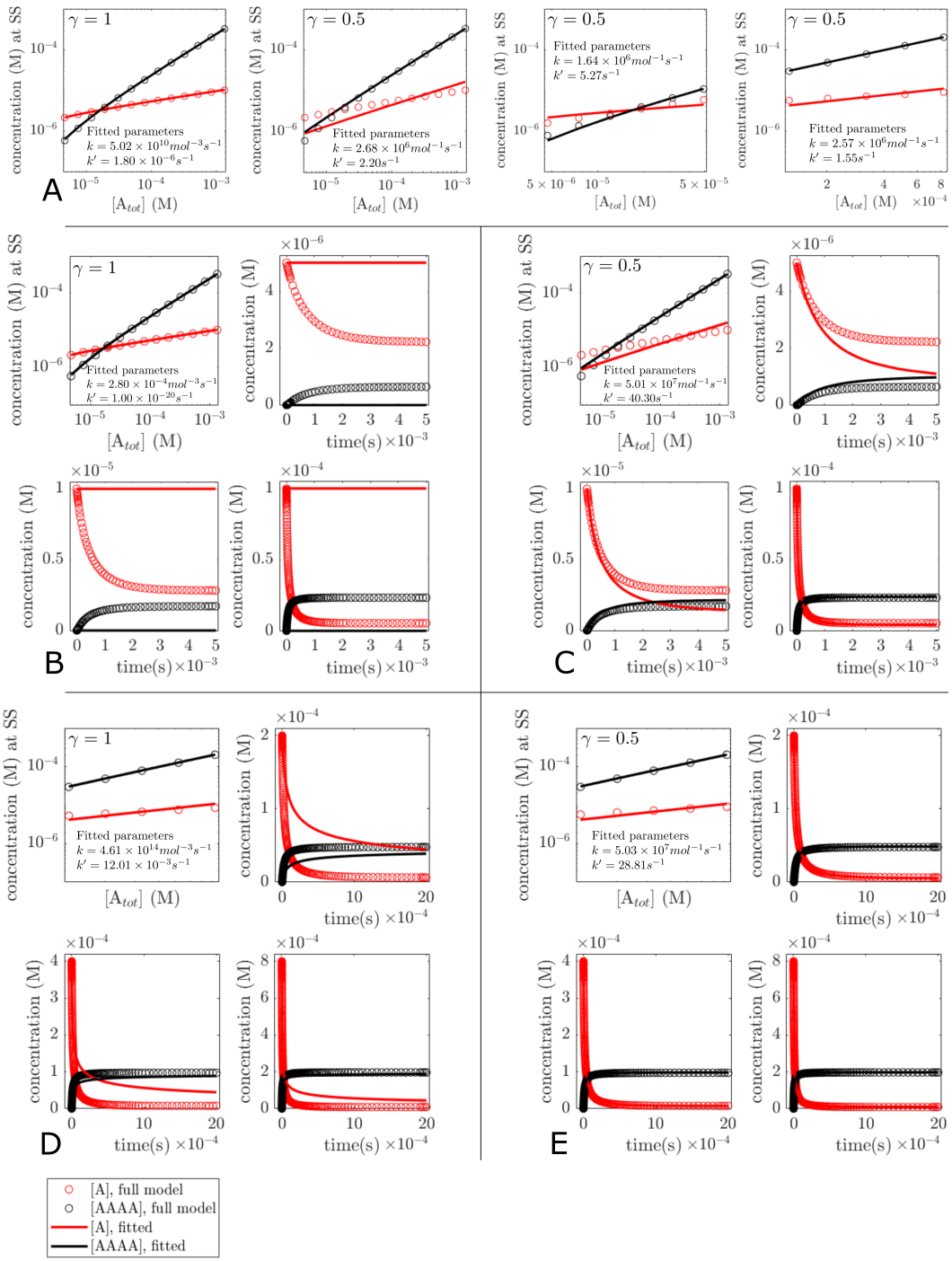


Figure 4
Fitting of the GMA model (straight lines) to the data generated by the full tetramerisation model (circles). (A) GMA model fitted to steady state data only. (B-E) GMA model fitted to steady state and time course data.

In the following, we will therefore restrict mass action models to a maximum of trimerisation and use GMA approximations for higher order oligomerisation. Unfortunately, combinatorial expansion is not the only challenge when PTMs of oligomers are considered: it is surprisingly easy to slip into thermodynamical inconsistency even with models based purely on conventional mass action kinetics. Before we incorporate PTMs into our models we will therefore formulate some biochemical intuitions and expectations which will later guide us to avoid thermodynamic inconsistency.

Let us suppose an oligomeric protein can be modified by a PTM at a single site. For the sake of simplicity we assume that the site lies remote from the oligomerisation interface and does not alter any of the reaction parameters. Intuitively, we would then expect the following to be true:

(a) The equilibrium and dissociation constants for the oligomerisation of only unmodified protein, completely modified protein and for the oligomerisation of unmodified protein with modified protein are identical.

(b) The association rate constants between only unmodified, only modified and between unmodified and modified protein are identical.

(c) The dissociation rate constants of oligomers with the same number of protomers are identical regardless of how many protomers carry the PTM.

(d) If the modifying enzyme is added to an equilibrated mixture of completely unmodified monomers and oligomers, both monomers and oligomers will be modified over time, yet the total concentrations of monomers and oligomers remains constant.

(e) If equal parts of modified and unmodified monomers are mixed to form n-tamers, there are $\binom{i}{2}$ possibilities to combine monomers to n-tamers with $0 \leq i \leq n$ modified subunits, i.e. the distribution of oligomers with respect to their number of modified protomers will be binomial.

To proceed with model formulation, let us indicate the PTM by an asterisk such that $A^*, AA^*, AA^{**}, ...$ denotes modified monomers, dimers with one and dimers with two modified protomers and so forth. Keeping the assumption that each oligomeric species is formed through binary association reactions, it follows from (a)-(c) that each pair of molecular species X,Y which is able to associate in the absence of any PTMs is also able to associate with identical reaction parameters regardless of how many protomers of X or Y are modified. We will assume that there is a modifying enzyme $E1$ and a demodifying enzyme $E2$ which operate by a non-cooperative, irreversible and distributive mechanism and that all molecular species, regardless the number of their protomers, are (de-)modified with the same kinetic parameters, i.e. the oligomeric state does not influence the (de-)modification reactions. We therefore expect the individual monomeric and oligomeric species to compete for enzymes $E1$ and $E2$. In such situations

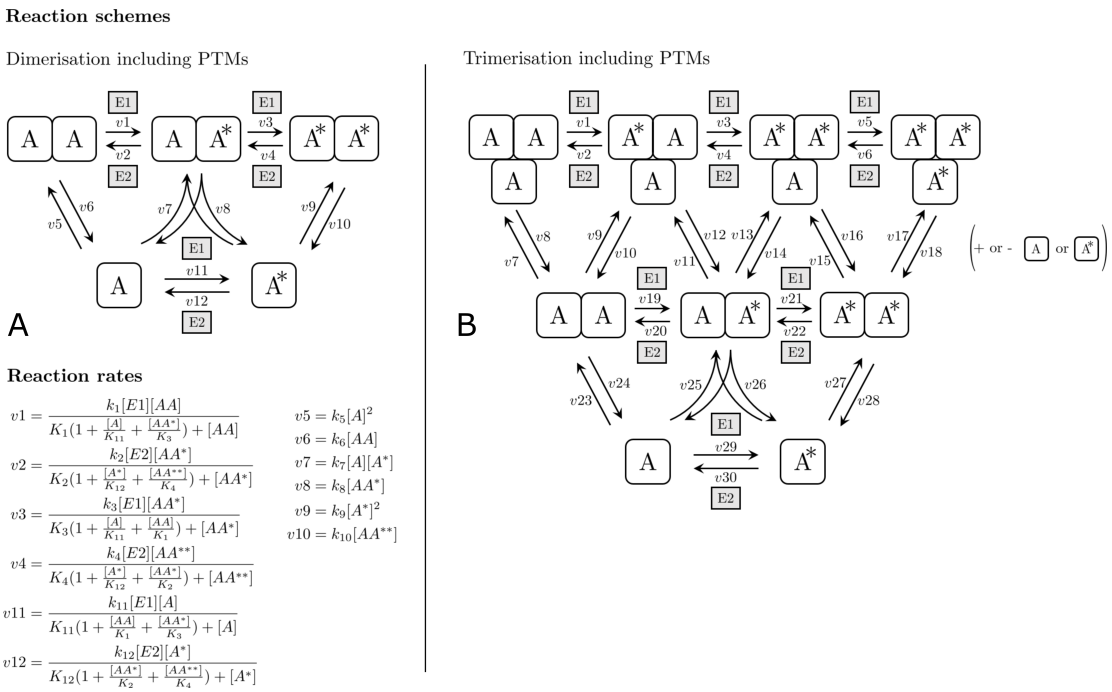


Figure 5
Reaction schemes for the mass action kinetics models of dimerisation (A) and trimerisation (B) including reversible post-translational modifications. See supplementary information for reaction rates and ODEs of the trimersation model.

with multiple competing substrates $S_1, ..., S_n$ a irreversible Michaelis-Menten type rate law of the form

$$v_i = \frac{V_{max} S_i}{K_{m_i}(1 + \sum_{j \in J \setminus \{i\}} \frac{S_j}{K_{m_j}}) + S_i},$$

where $J = \{1, ..., n\}$, can be employed to describe the rate of consumption v_i of substrate S_i, i.e. the individual substrates act as competitive inhibitors for each other [17]. We are now able to formulate reaction schemes, reaction rates and model equations.

Figure 5A shows the reaction scheme and rate expressions for the dimerisation model based on mass action kinetics for oligomerisation and mentioned Michaelis-Menten type rate law for addition and removal of PTMs. The equations are:

$$\frac{d}{dt}A = 2 \cdot v6 + v8 + v12 - 2 \cdot v5 - v7 - v11$$
$$\frac{d}{dt}A^* = v8 + 2 \cdot v10 + v11 - v7 - 2 \cdot v9 - v12$$
$$\frac{d}{dt}AA = v2 + v5 - v1 - v6$$
$$\frac{d}{dt}AA^* = v1 + v4 + v7 - v2 - v3 - v8$$
$$\frac{d}{dt}AA^{**} = v3 + v9 - v4 - v10$$

Figure 5B shows the reaction scheme for the trimerisation model including PTMs. See supplementary section 1 for reaction rates and

model equations. Note that the reaction scheme for trimerisation and a single PTM is already more complex than the tetramerisation scheme without PTMs. In addition, we will consider GMA models of trimerisation and tetramerisation which include a single PTM (see supplementary section 2 for reaction schemes, rate expressions and model equations). For dimerisation, we will use the same parameter values as in Figure 2A. For trimerisation, we will use the same parameter values as in Figure 3A (the GMA model was fitted to the data from the full model), and for tetramerisation, the same parameter values as in Figure 3B. We chose catalytic rate constants to be $k_i = 1s^{-1}$ and Michaelis constants to be $K_{m_i} = 1\mu M$ for all (de-)modification reactions i in all models.

Let us what happens if we add catalytic concentrations of $E1$ to an equilibrated mixture of monomers and oligomers. Interestingly, as the modification reaction proceeds, transient changes in the total concentrations of monomeric and oligomeric species occur in all models (Figure 6A; the total concentrations being the sum of concentrations of all modified isoforms for a given monomeric or oligomeric species). The amplitude and direction of these transients appear to be both influenced by the maximum number of protomers per oligomer and the modelling approach. The underlying cause for this phenomenon is an expansion of oligomerisation routes which become accessible in the models during the modification reaction. Consider for example the dimerisation reaction scheme (Figure 5A). If A is either completely unmodified or modified, the reaction occurs between a single monomeric and a single dimeric species. If a mixture of unmodified and modified A is present, the reaction occurs between two monomeric and three dimeric species. We would therefore expect that decreasing the velocity of oligomerisation/dissociation or increasing the speed of modification would diminish the effect, as both changes would limit A's capacity to change its oligomerisation state during the transition time from completely unmodified protein to complete modification. Indeed, while decreasing the velocity of oligomerisation/dissociation reduces the amplitude, increasing the enzyme concentration limits the peak width of the amplitude (Figure 6B).

Clearly, these transient changes contradict expectation (d). I am also unaware of any experimental data which would suggest such transient alterations of the oligomerisation state upon post-translation without affecting oligomerisation/dissociation rates. Might these transient changes be a modelling artefact? To answer this question, let us see what happens if equimolar mixtures of modified and unmodified A are simulated to steady state. According to (a) we would expect K_d values to be identical to that of completely unmodified and completely modified A and we would futhermore expect a binomial distribution of modified oligomers according to (e). With the exception of the GMA trimerisation model, all models show altered K_d values and all models without exception show a uniform distribution of modified oligomers (Figure 6C, suppl. Figure S1). Taken together this suggests that the transient changes are indeed a modelling artefact resulting from thermodynamic inconsistency.

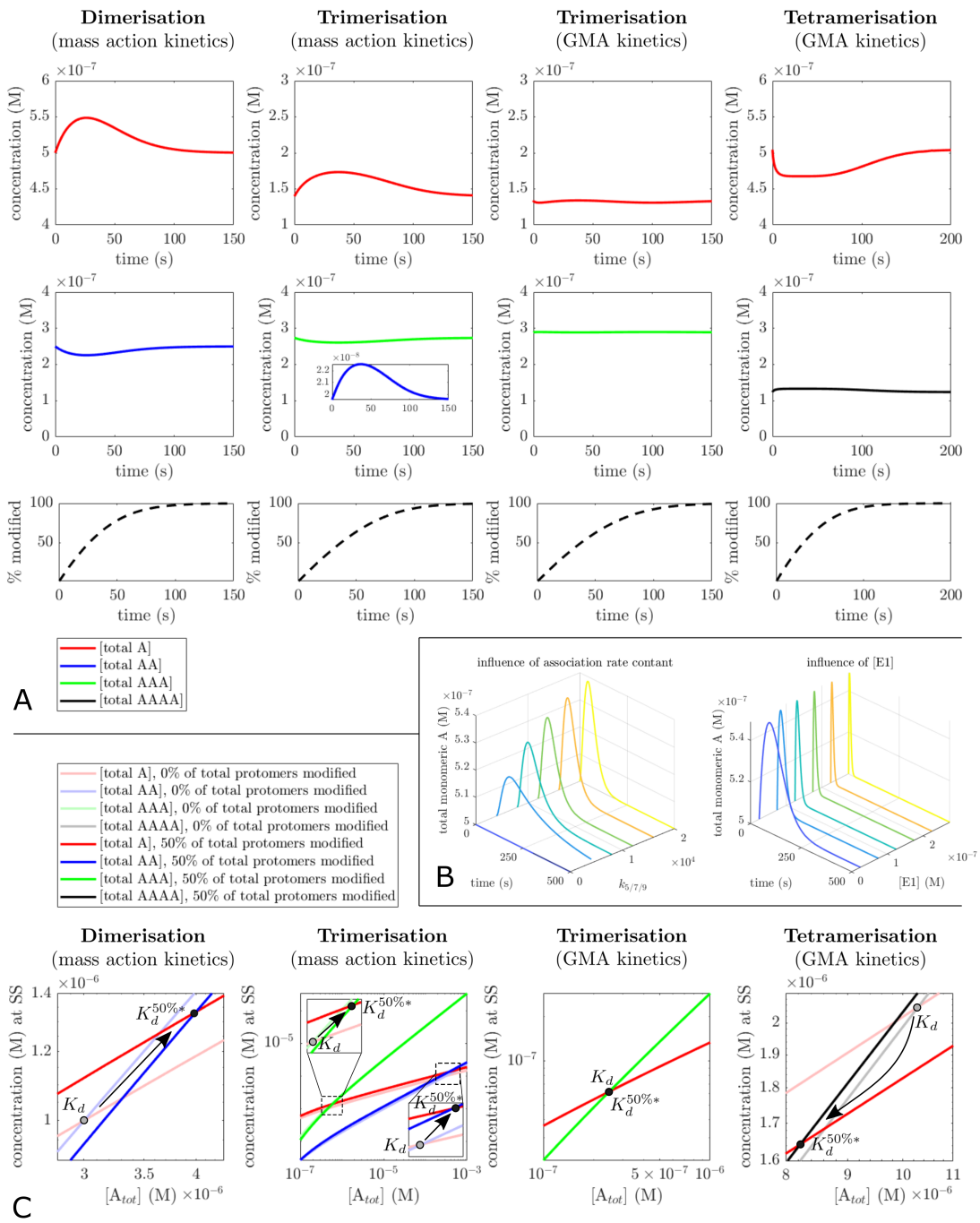


Figure 6
(A) simulated transient changes in total monomer and total oligomer concentrations after addition of $50nM$ $[E1]$ starting with $[A_{tot}]$ = $10\mu M$ at equilibrium. (B) dependence of transient changes in the mass action dimerisation model on association rate constant and modifying enzyme concentration. (C) apparent shift in dissociation constants in equimolar mixtures of modified and unmodified protein.

Clearly, this phenomenon is not a result of the GMA approximation. But where did we go astray if even using simple mass action kinetics, arguably the most established deterministic modelling approach for chemical reactions, leads to thermodynamic inconsistency? The answer is: naively deriving ordinary differential equations by applying mass action kinetics and stoichiometrical balancing to reaction schemes such as in Figure 5.

In order to circumvent this issue, let us formulate a further expectation. Let A_n^m be an oligomeric complex with n protomers of which $0 \leq m \leq n$ carry a PTM at site x. From (b) and (c) it follows that all association reactions i leading to an oligomeric complex A_n have the same rate constant $k = k_i$ and all dissociation reactions j of A_n $(i \neq j)$ have the same rate constant $k' = k_j$. We would then expect:

> If a PTM does not influence the parameters of a reaction producing an oligomeric species A_n, the sum of all rates v_i of reactions leading to A_n of any modification status is the association rate based on the total concentrations (i.e. all modification isoforms) of A_n's educts. Conversely, the sum of all rates v'_j of reactions dissociating A_n of any modification status is equal to the dissociation rate based on A_n's total concentration. That is
>
> $$\sum v_i = k \cdot \sum_{m=0}^{r} [A_r^m] \cdot \sum_{m=0}^{s} [A_s^m],$$
>
> where $r + s = n$,
>
> $$\text{and} \sum v'_j = k' \cdot \sum_{m=0}^{n} [A_n^m].$$

We will call this principle the *conservation of rates* (in analogy to the conservation of mass) as it conserves the reaction rates at given total concentrations of educts regardless the distribution of PTM isoforms. Although the formulation might appear complicated, it is straightforward to illustrate the principle using dimerisation as an example. Applying it to Figure 5A yields

$$v5 + v7 + v9 = k([A] + [A^*])^2 \tag{1}$$

and

$$v6 + v8 + v10 = k'([AA] + [AA^*] + [AA^{**}]). \tag{2}$$

How does this principle help us to avoid thermodynamic inconsistency? Instead of assigning *a priori* reaction rates based on mass action kinetics we will first expand the right-hand side of the rate conservation identity. In a second step, we will substitute the rate expression on the left-hand side with their mass action kinetics expression. Finally, we will compare both sites: if they are equal, all rates can readily be identified with their mass action kinetics expression. If they are not equal, we balance the terms on the left-hand side where the discrepancy occurs to ensure the validity of the equality and subsequently identify the respective rates with the balanced terms. Let us illustrate this using equations (1) and (2) derived from the dimerisation scheme:

(1) → expanding RHS, substituting LHS:

$$k[A]^2 + k[A][A^*] + k[A^*]^2$$
$$= k[A]^2 + 2k[A][A^*] + k[A^*]^2$$

→ *balancing deviating terms in LHS:*

$$k[A]^2 + \underline{\mathbf{2}} \cdot k[A][A^*] + k[A^*]^2$$
$$= k[A]^2 + 2k[A][A^*] + k[A^*]^2$$

→ *assigning reaction rates:*

$$v5 = k[A]^2, v7 = 2k[A][A^*]$$
$$v9 = k[A^*]^2$$

(2) → *expanding RHS, substituting LHS:*

$$k'[AA] + k'[AA^*] + k'[AA^{**}]$$
$$= k'[AA] + k'[AA^*] + k'[AA^{**}]$$

→ *no balancing necessary*
→ *assigning reaction rates:*

$$v6 = k'[AA], v8 = k'[AA^*]$$
$$v10 = k'[AA^{**}]$$

It is straightforward to apply the same procedure to the trimerisation model (cf. supplementary section 3). Other things being equal, the dimerisation and trimerisation model updated with the balanced rate expressions exhibit neither transient changes in the oligomerisation, nor shifts in the apparent K_d of equimolar mixtures of unmodified/modified A, whereas the distribution of PTM isoforms at steady state is binomial (Figure 7), indicating that the balanced models are indeed thermodynamically consistent. In fact, the postulated conservation principle can readily be derived if we consider mixtures of unmodified/modified A at equilibrium. If a PTM is irrelevant to the oligomerisation reaction, we would treat different PTM isoforms of monomers and oligomers as a single monomeric and a single oligomeric species from the perspective of the oligomerisation reaction. In the dimerisation case, for example, we would therefore define the dissociation constant as

$$K_d = \frac{([A] + [A^*])^2}{([AA] + [AA^*] + [AA^{**}])} = \frac{k'}{k}$$
$$\Leftrightarrow k([A] + [A^*])^2 = k'([AA] + [AA^*] + [AA^{**}]).$$

As at equilibrium the rate of association equals the rate of dissociation, the latter equation must denote association rates $v5 + v7 + v9$ and dissociation rates $v6 + v8 + v10$, leading directly to equations (1) and (2), supporting the validity of the principle.

Unfortunately, the conservation of rates can not readily be applied in the same way to the GMA models as it does not lead to a binomial PTM isoform distribution (Figure 7B). Efficient and thermodynamically consistent approximation of higher order oligomers models which also take into account PTMs therefore remain an open challenge which we will leave for future research.

2.4 Considering PTMs: ultrasensitivity and bistability

Now that we have thermodynamically consistent models of oligomers which can be post-translationally (de-)modified, we will explore the steady state behaviour in the presence of (de-)modification enzymes $E1, E2$ using the dimer model as an example. The relative fraction of modified dimer and monomer shows pronounced ultrasensitivity in response to increasing concentrations of modifying enzyme $E1$ (Figure 8A).

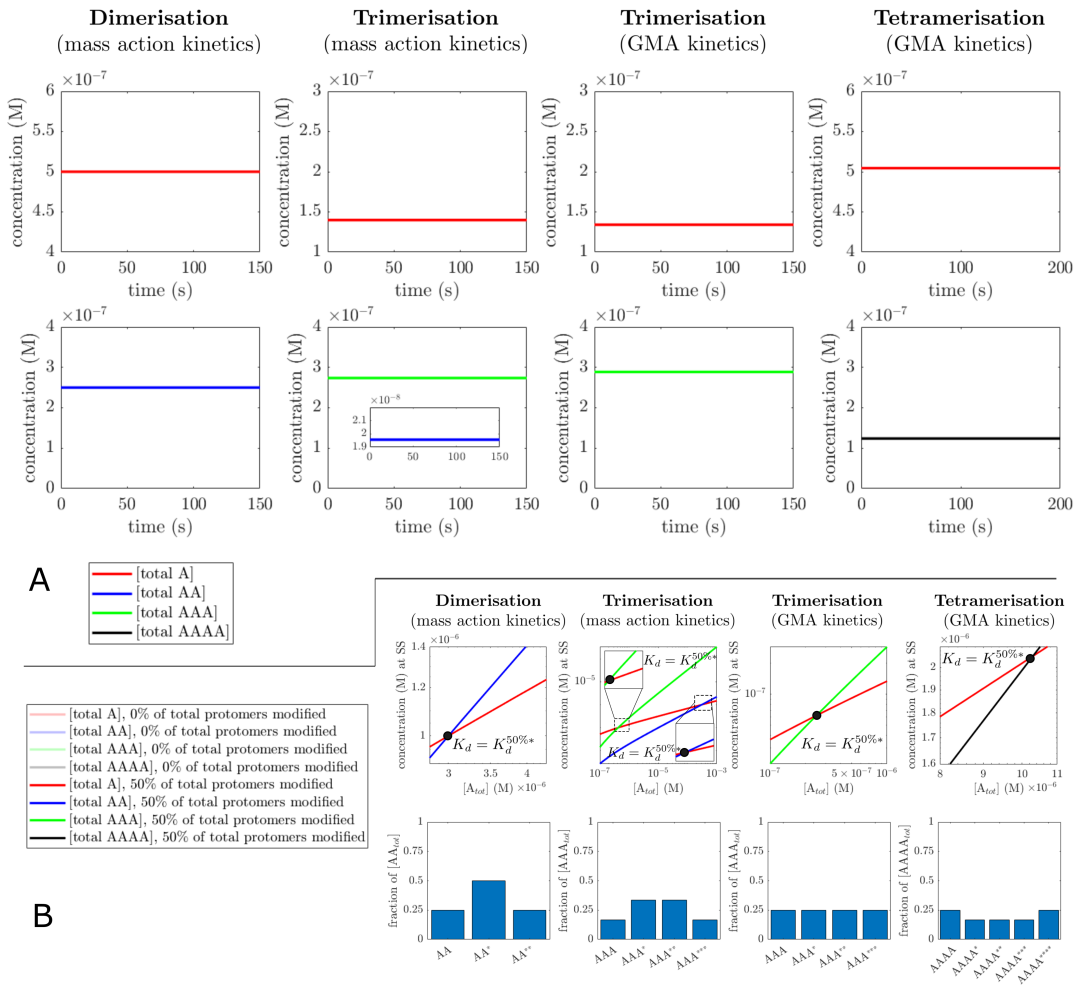


Figure 7
Behaviour of the balanced models. (A) total monomer and total oligomer concentrations after addition of $50nM$ $[E1]$ starting with $[A_{tot}] = 10\mu M$ at equilibrium. (B, upper panel) dissociation constants in equimolar mixtures of modified and unmodified protein, (B, lower panel) simulated PTM isoform distribution of highest order oligomer in mixtures of $5\mu M$ modified and $5\mu M$ unmodified protein at equilibrium.

On closer examination, this is not very surprising. Apart from some degree of zero-order ultrasensitivity arising from enzyme saturation [18], oligomerisation additionally creates a substrate competition situation between monomeric and oligomeric species for (de-)modification and provides pseudo-multisites for PTMs (i.e. multiple protomers with identical PTM sites). Both motifs are capable of generating ultrasensitivity [19, 20]. Moreover, multisite modification can in principle generate bistability if there is a sufficient asymmetry in the sequential modification cycles, i.e. if either the demodification and/or

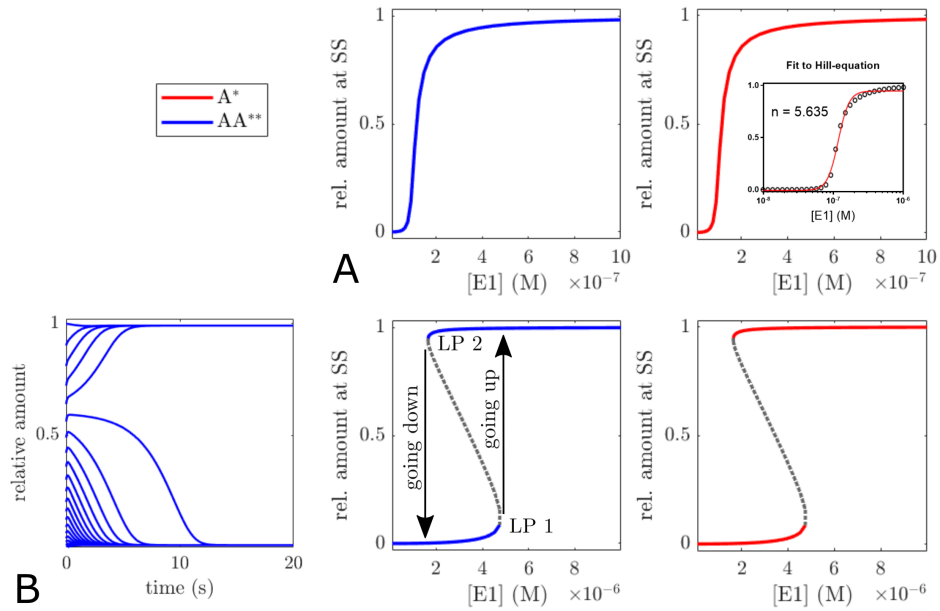


Figure 8
Ultrasensitivity and Bistability of modification response. (A) fractional modification of both monomers and dimers in response to increasing concentrations of modifying enzyme $E1$ is notably ultrasensitive. (B) left, time course simulations demonstrate that the approached steady state is determined by the initial conditions if demodification is assumed to be cooperative. Parameters: $k2 = 100s^{-1}, k4 = 10s^{-1}, [E1] = 3\mu M, [E2] = 0.1\mu M, A_{tot} = 10\mu M$ with different fractional modification at t = 0), other parameters as specified for Figure 2A. (B) middle and right, bifurcation diagrams show identical positions of limit point bifurcations for dimers and monomers. Unstable steady states are indicated by dotted lines.

the modification steps exhibit kinetic cooperativity [21, 22]. For dual-site modification of monomeric proteins, Conradi and Mincheva have proven that in general, bistability must occur for some concentrations of demodifying and modifying enzyme if the product of the rate constants for the first modification and demodification steps is smaller than the product of the rate constants for the second modification and demodification steps. Without considering the oligomeric nature, introducing positive kinetic cooperativity for the demodification of the dimer, i.e. assuming $k_2 > k_4$, would fulfill this requirement. Indeed, implementing this assumption leads to bistability with respect to the modification status in the dimer model (Figure 8B). As the bistable range increases with the number of cooperative modification steps [22], the likelihood for a bistable PTM status will increase with higher order oligomers.

Interestingly, not only the dimer, but also the monomer exhibits bistability even without multiple sites for PTMs. This becomes less surprising if one considers that the dimer is in equilibrium with the monomer allowing modified dimers to dissociate into monomers. Furthermore, when dimers are completely (de-)modified, substrate competition for (de-)modification of the monomer abates, allowing

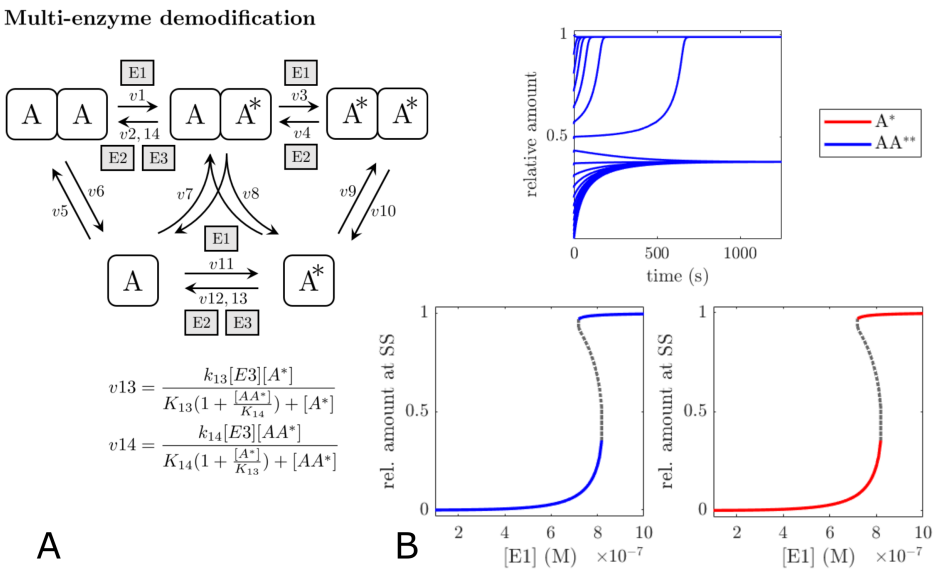


Figure 9
Bistability through multi-enzyme regulation of modification status of oligomers. (A) scheme of (balanced) dimer model with additional demodification enzyme $E3$ which can not catalyse the first step of the dimer demodification. (B) time course simulations and bifurcation plots demonstrating bistability in the dimer model. Parameters: $[E2] = 10nM, [E3] = 0.1\mu M, k13 = 0.1s^{-1}, k14 = 100s^{-1}, K13 = 10\mu M, , K14 = 1\mu M$, other parameters as specified for Figure 2A.

for more monomer (de-)modification.

While perhaps not uncommon, kinetic cooperativity might not be the only way to realise bistability in (pseudo-)multisite PTM systems. From a biochemical point of view, asymmetry in the (de-)modification rate of a multi-site PTM protein could effectively be realised, too, if one of the (de-)modification steps would also be catalysed by another enzyme $E3$. Let us, for instance, assume that in a dually modified dimer, each PTM mutually prevents (e.g. due to sterical reasons) access to the other PTM for demodifying enzyme $E3$. Only when one of the PTMs has already been removed by demodifying enzyme $E2$ (which we assume to catalyse PTM removal from the singly and dually modified dimer equally well), can $E3$ bind to the singly modified substrate and catalyse the last demodification step. Assuming that $E3$ can catalyse demodification of the modified monomer, the scheme for updated dimer model is shown in Figure 9A. Using the updated dimer model, it is not difficult to find parameter values that lead to bistability (Figure 9B), showing that multi-enzyme regulation can be an effective alternative for realising the asymmetry required for bistability in multisite PTM systems.

3 Discussion

Complex dynamics and steady state behaviour

Even simple oligomerisation systems, as we have seen, can in principle be capable of surprisingly complex behaviour. Dynamical phe-

nomena such as transient overshoots of dimers followed by a slower increase in higher order oligomers will be relevant to proteins which are not in constitutive monomer/oligomer equilibrium. Examples include membrane receptors which oligomerise upon ligand binding [24] or proteins that oligomerise upon recruitment to a membrane. If dimers and higher order oligomers have different downstream signalling functions, such transients could be an effective way to encode the duration of the input signal (e.g. ligand presence or membrane recruitment) and thereby lead to different cellular responses for short and prolonged stimuli.

In addition to the previously described but modest ultrasensitivity through oligomerisation [5, 6], we have seen that oligomerisation could also be an effective homeostatic regulatory mechanism to keep a constant monomer concentration. In contrast to the dynamical phenomena, this more likely applies to proteins which are in a constitutive monomer/oligomer equilibrium. For many tetrameric proteins, however, dimers are the only observed intermediate species between monomers and tetramers. Powers and Powers used a mathematical model similar to the presented mass action tetramerisation model here to explain this observation [25]. Their analysis suggested that the tetramerisation pathway via trimers might 'trap' many molecules in intermediate forms leading to incomplete tetramerisation, which, so the authors argue, might lead to unfavourable consequences such as proteolysis, misfolding or aggregation.

At first, this may speak against oligomerisation as a homeostatic mechanism at least for tetramers, suggesting that the typical behaviour of tetramers would be better described by Figure 3B rather than C. However, more recent studies provided evidence that some proteins such as the EGF-receptor can form dimers, tetramers and trimers, too [26, 27]. In addition, few oligomeric proteins, including tetramers, have been studied experimentally extensively enough to exclude scenarios such as depicted in Figure 3C. Since individual species concentrations in the homeostatic scenario often differ by ≥ 2 orders of magnitude, experimental testing of such behaviour would at least require to determine the equilibrium distribution of monomeric and oligomeric species over several of magnitude of total protein concentration but ideally also kinetic data on oligomer (dis-)assembly which can be technically challenging [28, 29]. Secondly, the possibility of monomer homeostasis via oligomerisation is not restricted to tetramers and could be realised by any higher order oligomersation, provided enough reaction routes allow to buffer changes in the monomer concentration. An interesting question for future research is whether homeostatic regulation could also apply to dimers or trimers if there are enough higher order oligomeric species.

Modelling approaches

As the order of oligomers increases and PTMs are taken into account, the number of species and possible reactions quickly grows. This is a typical situation of 'combinatorial explosion' which poses a significant challenge for many signal transduction models [30, 31]. GMA rate laws can be a viable alternative, both

with respect to kinetics and steady state behaviour, to reduce combinatorial complexity by approximating oligomerisation pathways provided only two species are of interest and the relevant range of total protein concentration is sufficiently restricted. If PTMs are to be considered, another unanticipated challenge arises when creating models from reaction schemes: ensuring thermodynamic consistency of the model. If the PTM does not affect the oligomerisation parameters, thermodynamically consistent models can be derived by balancing rate equations based on a rate conservation principle in a similar fashion to stoichiometric balancing of chemical equations. While the procedure is straightforward to apply to mass action kinetics models, it is not applicable to GMA models of oligomerisation yet.

An alternative strategy for simplified but at steady state thermodynamically consistent oligomerisation models including PTMs would be to derive the full mass action kinetic model, balance it, and to apply a QSSA reduction to irrelevant intermediate species. Such an approach would at least reduce computational complexity, although, unfortunately, not the work for the modeller. Another open question is whether the procedure is also sufficient to deal with situations where the PTM does affect the oligomerisation. I conjecture that once the balancing coefficient has been introduced into the rate equations, changing parameter values for individual reactions will not affect thermodynamic consistency.

A different alternative to tackle combinatorial explosion resulting both from higher order oligomerisation and PTMs would be to not explicitly specify all species and equations but to use implicit modelling approaches such as rule based modelling. Rule based modelling has already been successfully used to model EGF-receptor oligomerisation [32] and will generally be necessary for higher order oligomers with multiple PTM sites.

Bistability through oligomerisation

Ultrasensitivity and bistability are important properties in signal transduction networks for cellular decision making, allowing to respond in a switch-like, binary and sometimes irreversible fashion. Oligomerisation can also lead to ultrasensitivity and bistability by providing pseudo-multisite complexes (i.e. complexes with multiple identical PTM sites). Given previous work on ultrasensitivity and bistability arising through multisite modification from the Kholodenko Lab and others [19, 21–23], this possibility seems obvious from a biochemical point of view, yet, to the best of my knowledge, has not been appreciated before. An interesting and unique twist of this motif is that the bistability resulting from modification of the oligomer extends to the monomer due to intrinsic substrate competition and because both species are in equilibrium with each other. We also demonstrated that kinetic cooperativity of multisite modification systems is not a requirement for bistability if there are multiple enzymes regulating the modification steps and if some can only catalyse a subset of the individual modification steps, resulting effectively in the same kinetic asymmetry [22, 23] as kinetic cooperativity. While oligomers might be particularly

suited for this mechanism due their symmetrical structure, bistability through multi-enzyme regulation could in principle arise in any multisite PTM system.

The relevance of these findings is that they significantly expand the range of contexts in which one should look for biochemical 'switches' as both homo-oligomerisation and multi-enzyme regulation are extremely common. Phosphatases and GTPase activating proteins (GAPs), just two name two examples, are known to often act promiscuously on multiple substrates [33–35]. As a consequence many phosphorylation sites can be dephosphosphorylated by multiple phosphatases and many small GTPases (some of which can also dimerise), can be inactivated by multiple GAPs, creating potential situations in which bistability could occur. Alternatively, multi-enzyme regulation of the modification rather than demodification steps is also conceivable. Phosphorylating a protomer within a dimer, for example, could lead to a new binding site for a second kinase facilitating phosphorylation of the same residue in the other protomer. The combination of both mechanisms, oligomerisation and multi-enzyme regulation, therefore represents an interesting novel signalling motif that does not require feedback or kinetic cooperativity to generate bistable responses.

Conclusion

We have demonstrated that homo-oligomers, making up approximately 30% of the proteome [1], offer an even greater variety of regulatory mechanisms than previously appreciated and it is likely that some of these will be relevant to many cellular signalling pathways. I hope the presented findings will be helpful to modellers interested in homo-oligomeric signalling proteins and stimulate experimental research into signalling processes to which the presented findings might apply.

4 Computational Methods

Presented models have been implemented as MATLAB® scripts for numerical simulation and model analysis. All simulations have been performed using the ode23s integrator. Sensitivity analysis has been performed as described in [12]. Parameter estimation and model fitting of GMA models has been performed in COPASI using the evolutionary programming algorithm (400 generations, population size 40) or the Levenberg-Marquardt algorithm using COPASI's standard settings [36]. Bifurcation diagrams have been generated using a custom algorithm that iteratively identifies the unstable steady state. If the algorithm does not converge within the specified number of iterations, it approximates the unstable steady state from the distribution of concentrations from all time courses taking advantage of the fact that simulations very close to the unstable steady state take a long time until they eventually tip to either of the stable steady states.

The model code will be released on a public database upon final publication.

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