Review

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Chemical kinetics for drug discovery to combat protein aggregation diseases

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Protein misfolding diseases are becoming increasingly prevalent, yet there are very few effective pharmacological treatments. The onset and progression of these diseases is associated with the aberrant aggregation of normally soluble proteins and peptides into amyloid fibrils. Because genetic and physiological findings suggest that protein aggregation is a key event in pathogenesis, an attractive therapeutic strategy against this class of disorders is the search for compounds able to interfere with this process, in particular by suppressing the formation of soluble toxic oligomeric aggregates. In this review, we discuss how chemical kinetics can contribute to the fundamental understanding of the molecular mechanism of aggregation, and speculate on the implications for the development of therapeutic molecules that inhibit specific steps in the aggregation pathway that are crucial for preventing toxicity.

Chemical kinetics and neurodegenerative disorders

Chemical kinetics (i.e., the measurement and analysis of the rates of chemical reactions) is widely applied in the physical and chemical sciences to study reaction mechanisms and their engineering at the molecular level. Such mechanistic information is particularly valuable in the context of the development of therapeutic strategies to combat diseases. Indeed, information about the microscopic processes underlying changes in macroscopic variables are crucial for understanding the mechanism of action of a given drug as well as for identifying strategies to change both the thermodynamics and the kinetics of disease-associated processes. This general strategy has found widespread applications in enzymology, where chemical kinetics has become a standard tool for testing inhibition mechanisms, including identifying competitive, uncompetitive, and noncompetitive inhibition mechanisms. This approach has already resulted in significant progress in our understanding of the mechanisms of action of enzymes and has led, for example, to the development of anticancer drugs targeting kinase activity [1,2]. However, the power of this approach in the area of protein aggregation disorders largely remains unexploited. The purpose of this review is to highlight the opportunities and challenges that emerge from the application of chemical kinetics to the study of protein aggregation phenomena and their biological consequences. In particular, we discuss the key role that chemical kinetics can play in the search for measure of combatting neurodegenerative disorders, including Alzheimer's disease (AD) and Parkinson's disease (PD), which collectively represent one of the leading causes of death in the modern world [3].

With increasingly aging populations, the number of people affected by these diseases is predicted to grow further in the coming years. Currently, few effective disease modifying pharmacological therapies are available for these disorders, and indeed the commonly available therapies mainly focus on ameliorating symptoms [4]. The absence of effective drugs originates in large part from our lack of understanding of the molecular mechanisms underlying the generation of pathogenic species. One common aspect shared by this family of disorders is the connection between the progression of the disease and the aberrant aggregation of normally soluble proteins and peptides into insoluble fibrillar aggregates known as amyloid fibrils [5]. Genetic, physiological, and biophysical evidence has led to a picture where the formation of toxic aggregates is an upstream process that triggers a pathological response, often called the amyloid cascade hypothesis [6,7]. For instance, simply an increase in the concentration of aggregating proteins (the Aβ peptide in AD or α-synuclein in PD) through genomic duplication leads to aggressive early onset forms of these neurodegenerative diseases in individuals with an otherwise normal genetic background [8].

The exact causative relationship between amyloid formation and organ dysfunction remains unclear, but the inhibition of protein aggregation is an attractive candidate for generating disease modifying therapeutic strategies against AD and other neurodegenerative disorders because toxicity has been associated with the soluble forms of the aggregating peptides and proteins. In this context, kinetics is not only a tool to investigate the inhibition mechanism but can also represent a therapeutic target *per se*, because the delay of the aggregation process for a significant number of years can be practically considered as effective as the overall (i.e., thermodynamic) inhibition of the process. However, strategies for inhibition should not simply aim at a complete arrest or delay of the formation of the final fibril load, but

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rather at a specific intervention that targets the molecular species that mediate cellular toxicity. Indeed, an uncontrolled disassembly of higher order aggregates, thought to be relatively inert in a biological context, could lead to the increase in the concentration of soluble toxic oligomers and hence have a negative outcome in terms of suppressing pathogenicity. Furthermore, kinetic intermediates are currently considered to be among the most toxic species [9–12]. To develop effective therapeutic strategies, therefore, an understanding is required not only of the protein aggregation process and its connection to pathogenicity but also of how potential drugs interfere with these processes.

In the past two decades, many biophysical studies have shed light on protein aggregation mechanisms [13–20]. In parallel with these advances, many groups have started to search for compounds able to interfere with the protein self-assembly. This research has led to the discovery of molecular species able to inhibit the aggregation process both *in vitro* and *in vivo*, and some drugs have entered clinical trials [21]. However, the development of these drugs is still limited by incomplete knowledge of the molecular inhibition of these compounds and, typically, the identification of the molecular processes affected by the presence of extrinsic factors – a key requirement for rational drug design – remains elusive.

Here, we discuss the insights provided by chemical kinetics towards a detailed understanding of the inhibition of fibril formation at the molecular level. After summarizing the currently identified potential molecular targets for intervention against protein aggregation and the different classes of inhibitors that have been proposed recently, we highlight the power of kinetic analysis and its implications for the rational design of new drugs.

Potential therapeutic targets and proposed inhibitors

Increasing experimental evidence indicates that under *in vitro* conditions the formation of amyloid fibrils from soluble monomers is the consequence of a range of microscopic aggregation processes which involve the formation of a variety of molecular species, as outlined in Figure 1A [10,22]. The appearance of mature fibrils is accompanied by the formation of low molecular weight oligomers and protofibrils, which can be both on-pathway and off-pathway to the fibril formation. Potentially, each of these species may represent a toxic agent whose formation needs to be suppressed.

Many compounds have been suggested in the literature to interact with one or more of such species and to interfere with the aggregation process and/or inhibit the formation of mature fibrils. Examples are shown in Figure 1B and include peptides [23,24] and proteins [25], in particular molecular chaperones [26] and antibodies [27]; small chemical compounds such as polyphenols [28–30], anti-inflammatory agents [31], metal chelators [32], and tetracyclines [33], and inorganic [34] and organic nanoparticles [35]. For a more complete overview of compounds proposed as therapeutic drugs, we refer the reader to recent reviews and references therein [4,36–39].

Some of the proposed inhibitors appear to interact with the monomer to stabilize the native structure and there-

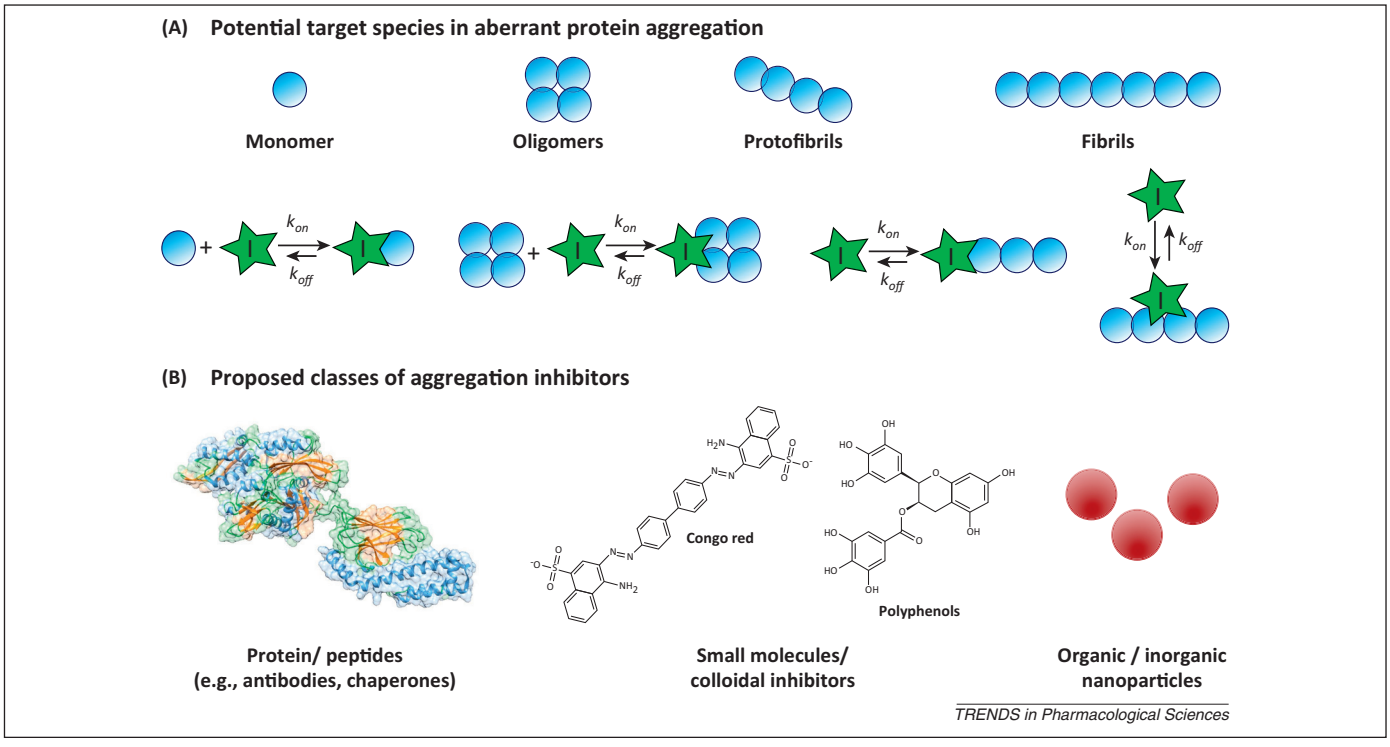

Figure 1. Potential therapeutic targets and proposed inhibitors. **(A)** In many neurodegenerative disorders the onset and progression of the disease is connected with the aggregation of normally soluble proteins and peptides into insoluble fibrils. The kinetic or thermodynamic inhibition of the aggregation process is therefore an attractive therapeutic strategy. This inhibition can be achieved by targeting different protein species involved in the aggregation process, including the soluble monomers, the fibrils, and several intermediate species, such as oligomers and protofibrils, which accompany the formation of mature fibrils. **(B)** Classes of inhibitors which are able to interfere with the aggregation process by interacting reversibly or irreversibly with the different species in the system. Examples include proteins and peptides, small chemical compounds, and organic and inorganic nanoparticles.

fore to decrease the overall aggregation propensity [25,38,40–42]. Other inhibitory compounds attempt to deplete the oligomers and other protofibrillar species on-pathway to amyloid fibrils, or to redirect the amyloid aggregation pathway into alternative and less harmful states [29,30,43–45]. Several small aromatic molecules or peptides, often called β breakers, have been designed to disrupt formation of the β-sheet structure of the fibrils and eliminate the addition of soluble monomers to the growing aggregates [24,46]. Naturally occurring chaperones have also been shown to interact with prefibrillar species and also to inhibit the elongation of different amyloidogenic protein fibrils [47–50].

All the compounds mentioned above potentially inhibit the formation of mature amyloid fibrils. However, effective therapeutic strategies require targeting to specific species and not a nonspecific arrest of the high molecular weight aggregation process. In particular, low molecular weight oligomers are thought to be the most toxic species for impairing neuronal activity through a series of possible mechanisms [9,12,51,52]. According to this view, therefore, an attractive therapeutic strategy against neurodegenerative disorders is to focus on preventing the formation of such soluble oligomers rather than mature fibrils. Compounds that prevent toxic oligomers from forming or redirect the aggregation to alternative nontoxic oligomers are likely to be promising drug candidates.

Another possible approach is the search for compounds able to accelerate the aggregation of oligomers to less toxic mature fibrils [53]. However, the pathological consequences related to the accumulation of amyloid fibrils in the tissues, such as inflammatory reactions, cannot be excluded, and in certain conditions, such as systemic amyloidosis, there is evidence that the accumulating mass of fibrils can also be intrinsically damaging [54]. Furthermore, in the case of the Aβ peptide, it has been recently shown that the presence of amyloid fibrils catalyzes the formation of toxic oligomeric species [10]. Even once such a target has been identified, due to the large number of microscopic steps underlying protein aggregation and the broad range of species present in the system, it is clear that the understanding of the inhibition mechanism of any potential drug at a molecular level is a key requirement to achieve the desired specific targeting.

The inhibitory efficiency of different compounds for inhibiting protein aggregation has currently been evaluated by monitoring the kinetics of aggregation *in vitro*, in particular by means of thioflavin T (ThT) fluorescence based assays. Potential drugs are selected based on their capacity to delay or arrest fibril formation as monitored in this way. This approach has the advantage of rapidly providing potential information on specific compounds. However, the detailed mechanism by which fibril formation is inhibited remains *a priori* unknown.

Challenges in understanding aggregation inhibition mechanisms at the molecular level

In the past 20 years, a picture has emerged in which protein aggregation occurs via a nucleation step which generates small oligomers from a pool of monomeric proteins. Such oligomers are likely to be very reactive and metastable, and to elongate rapidly into protofibrils and fibrils by monomer addition [55,56]. In most cases, however, the aggregation rate is significantly accelerated by secondary nucleation processes, such as fibril fragmentation and surface catalyzed nucleation, which form new reactive nuclei and result in a high level of apparent cooperativity in the aggregation process [56,57]. The corresponding macroscopic kinetics of fibril formation follow a characteristic sigmoidal profile, in which a lag-phase precedes fast growth until a final plateau results from monomer depletion [13,22]. An increase of the length of the lag-phase is commonly, but often incorrectly, associated only with a reduction of the primary nucleation rate due to sequestration of monomers or oligomers by inhibitors. Indeed, kinetic analysis of the aggregation process shows that lag-phases and the kinetic profiles are significantly affected by elongation and secondary processes [17,19,22,56,57] and therefore identifying the molecular level origin of the inhibitory action is in general challenging. In the following sections, we discuss how the *in vitro* kinetic data can be combined with additional experimental and theoretical analysis to shed light on which protein species are targeted by a specific compound and which microscopic processes are inhibited by such an interaction.

Most of the inhibitors proposed in the literature interfere with the aggregation process by binding covalently or noncovalently to one or more of the species on the aggregation pathway. Many aromatic compounds in solution form supramolecular assemblies ranging from oligomers to colloidal aggregates [58,59], which are able to sequester protein monomers and/or oligomers in a nonspecific manner. A similar mechanism is observed with other classes of inhibitors such as nanoparticles [35] and some naturally occurring chaperones [26]. Such observations suggest that in at least many of the cases where this mechanism applies, the inhibitory effect can be attributed to the physical binding between the protein and the inhibitor, although the chemical nature of the inhibitor plays a key role in determining the affinity of the binding and the strength of the intermolecular interactions, including electrostatic, van der Waals, and hydrophobic interactions, hydrogen bonding, and aromatic stacking. In the case of nanoparticles, the surface chemistry [35], the total available surface [60], and the protein structure upon binding [61] affect the binding affinity and the inhibitory effect.

In some systems, the inhibitory effect of a given compound is mediated by specific interactions [62]. For example, the analysis of several different small polyphenolic compounds has led to the identification of key structural features required for the inhibitory effect to be observed [28]. Inhibitors designed to bind specifically to lysine residues and inhibit aromatic and electrostatic interactions promoting aggregation have recently been proposed [45]. Specific structural features of the inhibitors are required, in particular, when the mechanism of action is the stabilization of native secondary, tertiary, or quaternary structures, as in the case for the native tetramer of transthyretin, a protein responsible for a systemic amyloidosis, by the drug tafamidis [40]. In some studies, covalent binding and subsequent chemical modification of the

proteins have been reported, as in the case of quinone inhibitors with α-synuclein [43] and lysozyme [63].

The examples mentioned above indicate the complexity of the problem and the many potential scenarios which need to be considered. From an experimental point of view, the characterization of the binding of small molecules, protein monomers, and fibrils can be achieved by combining assays of aggregation kinetics with a variety of biophysical methods, as recently reviewed by Buell *et al.* [64]. Common techniques are calorimetric [65] and imaging [47] techniques, surface plasmon resonance [35] and quartz crystal microbalance biosensors [66], linear dichroism [64] and nuclear magnetic resonance spectroscopy [30], immunolabeling [67], and mass spectrometry [62]. These techniques can provide direct information about the soluble or aggregated species which can interact with the inhibitor, the stoichiometry of the binding, the interactions and the structural regions of the protein involved in the binding, and the corresponding binding affinity.

It is also important to note that the ThT fluorescence signal can be biased in the presence of other compounds [66,68]. When this happens, it is preferable to monitor the fibril formation kinetics by alternative experimental techniques, such as nuclear magnetic resonance [69] and circular dichroism spectroscopy [70], size exclusion chromatography [71], capillary electrophoresis [72], or light scattering [73].

Kinetic analysis of inhibition mechanisms can play a key role in the development and the evaluation of drug-like small molecules

Because of the low binding affinity and the low enthalpy of binding often observed for inhibitor–protein interactions, the conventional experimental methods described above, which have been developed in the context of enzyme inhibition, remain challenging to apply in the study of the inhibition of amyloid formation.

Chemical kinetics, by contrast, offers the possibility of detecting and analyzing even very weak binding events and their effects. In enzymology, kinetics are routinely applied to investigate the inhibition mechanism and to test the relative efficiency of different inhibitors. In this area, kinetic data are commonly visualized in linear plots of the Michaelis–Menten equation, such as the Lineweaver–Burk plot, which allow discrimination between competitive and noncompetitive inhibition and the quantification of relevant kinetic parameters.

The main challenge of applying the full power of chemical kinetics to protein aggregation phenomena originates from the difficulty of finding integrated rate laws for a process that is the combination of a large number of microscopic reactions. In the absence of inhibitors it has been shown that approximate analytical solutions of conservative mass balance equations provide information on the microscopic aggregation processes by considering the macroscopic observed kinetic profile of fibril formation (Box 1) [13,22]. The same strategy can be applied to elucidate the aggregation inhibition mechanism by analyzing the kinetic profiles of fibril formation in the presence of an inhibitor when the aggregation is partially arrested, a situation which is commonly reported in the literature [26,34,35,74].

A potential drug molecule could sequester monomers or oligomers, or bind to fibril surface and fibril ends (Figure 1A). Each of these events can change the concentration of one or more species in the system. In the case of noncovalent binding, the inhibitor decreases the concentration of the free species but acts as a reservoir: the bound species are released according to the law of mass action into the system when their unbound counterparts are consumed by the aggregation reaction in accordance with the binding equilibrium with the inhibitor. The various binding events affect the microscopic steps constituting the overall process differently, as shown in Figure 2A. Sequestration of monomers results in a decrease in the rate of essentially all the microscopic steps (i.e., primary and secondary nucleation and elongation). Binding to the oligomers reduces primary and secondary nucleation rates, whereas binding to the fibril ends primarily slows down the elongation process, and binding to the fibrils can decrease the surface catalyzed secondary nucleation rate.

The key to applying this approach to analyze inhibition of aggregation is to note that the changes in different microscopic events result in distinct characteristic macroscopic aggregation profiles, as shown qualitatively in the model simulations in Figure 2B. For instance, decrease of the primary nucleation rate increases the lag-time preceding the growth phase. Elongation and secondary nucleation events have different effects on both lag-phases and growth rates, and therefore we can discriminate the inhibition of these two microscopic processes by considering the macroscopic profile of the kinetics of aggregation at different protein and inhibitor concentrations. Figure 2B shows the qualitative change of the global aggregation profile

Box 1. Kinetic analysis of amyloid formation

Kinetic models represent a powerful tool for connecting macroscopic measurements with microscopic mechanisms [19,20]. They allow a mechanistic understanding of the filamentous assemblies of proteins involved in Alzheimer's and Parkinson's diseases to be developed. The time evolution of the fibril distribution can be described by a master equation, such as for example: $\frac{\partial f_j(t)}{\partial t} = 2k_+ m(t)\, f_{j-1}(t) - 2k_+ m(t)\, f_j(t) + k_n m(t)^{n_C} \delta_{j,n_C} + k_2 M(t) m(t)^{n_2} \delta_{j,n_2}$, where $m(t)$ is the monomer concentration and f_j is the concentration of fibrils with length j. The terms on the right-hand side of the equation represent the elementary microscopic aggregation processes: the first term accounts for the generation of filaments of length j by monomer addition to a shorter fibril; the second term describes the disappearance of fibrils of length j which grow further to length $j + 1$; the last two terms refer to the generation of new nuclei of size n_C and n_2 by primary and secondary nucleation events, respectively [13,22,56,79]. Recently available analytical solutions of this type of master equation have led to closed expressions of the time evolution of the principal moments of the fibril distributions, that is, the fibril number concentration, P, and the fibril mass concentration, M, and have provided key insights into the relationship between relevant physical quantities of the system [13,22,56]. In the presence of the inhibitor, I, the reversible binding reaction $I + S \rightleftarrows SI$ is introduced in the reaction scheme, where S can be the soluble monomer, a fibril end or a monomeric unit located on the fibril surface. The additional terms $-k_{on} \cdot I \cdot f_j + k_{off} \cdot \left(I f_j^*\right)$ are introduced into the master equation, where k_{on} and k_{off} are the binding and unbinding rate constants, respectively, and If_j^* is the inhibitor–protein complex. The set of mass balance equations can be solved numerically providing a simulation of the macroscopic time evolution of fibril formation.

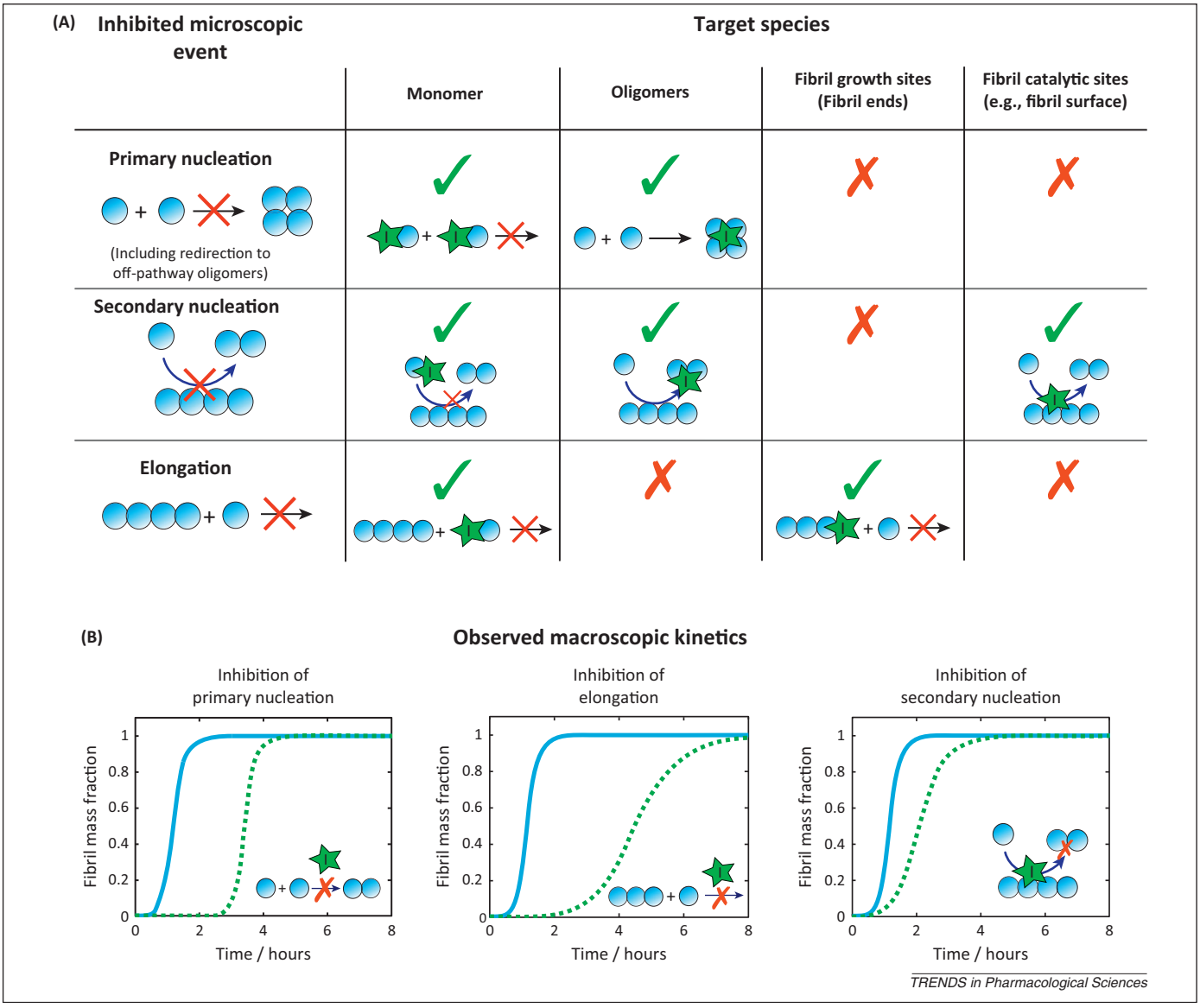

Figure 2. Kinetic analysis of aggregation inhibition mechanisms. **(A)** The binding between the inhibitor, *I*, and the different species in the system leads to specific changes in the microscopic steps of the aggregation process, such as primary nucleation, elongation, or heterogeneous secondary processes. Redirection of the aggregation pathway into alternative off-pathway oligomers can be considered in this scheme as an effective reduction of the nucleation rates. **(B)** Model simulations of the macroscopic time evolution of fibril formation show how changes in individual specific microscopic aggregation events affect the observed kinetic macroscopic profile in a characteristic way (see main text and Box 1 for details). Reference simulations (blue lines) are performed at a protein concentration of 4 μM with model parameters $k_n = 1 \times 10^{-5}$ $M^{-1}s^{-1}$, $k_+ = 3 \times 10^6$ $M^{-1}s^{-1}$, and $k_2 = 1 \times 10^4$ $M^{-2}s^{-1}$. Simulations including perturbations (dotted green lines) are obtained with $k_n = 1 \times 10^{-12}$ $M^{-1}s^{-1}$, $k_+ = 2 \times 10^5$ $M^{-1}s^{-1}$, and $k_2 = 2 \times 10^3$ $M^{-2}s^{-1}$ for specific inhibition of primary nucleation, elongation, and secondary nucleation, respectively. The kinetic analysis of the global kinetic profiles at different protein and inhibitor concentrations can therefore lead to the identification of the specific process that is being inhibited and of the corresponding consequences on the generation of soluble toxic species.

induced by the specific modification of a single microscopic event. However, the different microscopic processes have significantly different quantitative impacts on the lag-phase. For instance, to achieve comparable changes in the lag-phase in the simulations of Figure 2, the primary nucleation rate has been reduced by six orders of magnitude more than has the secondary nucleation rate.

It is interesting to note that inhibitors may suppress heterogeneous secondary processes via several distinct microscopic mechanisms which differ from binding to aggregates, for instance, by interfering with the nucleation reaction itself. In addition, several different types of binding sites can be present along the fibril surface. To this effect, complementary experimental analysis is required to identify the specific mechanism of action, although kinetic analysis still plays a crucial role in quantifying the relative effects of the inhibition of secondary processes on the global kinetic profile and on the generation of oligomers.

It is also important to observe that the specific inhibition of heterogeneous processes delays fibril formation but does not affect the final fibril load, because monomers are still consumed by elongation events. To affect the final quantity of fibrils thermodynamic inhibitions, which prevents primary nucleation and elongation processes, is required. However, the kinetic inhibition of secondary processes can suppress the generation of toxic intermediates, in contrast to the kinetic inhibition of primary nucleation and elongation processes, which delay but do not suppress

the formation of soluble oligomers. For a specific system under consideration, calculations based on a kinetic model can quantify the qualitative aspects described above, leading to the identification of the inhibition strategy likely to have the best potential therapeutic benefits.

A limiting situation occurs when the inhibitor binds irreversibly to the monomer, to prefibrillar species, or to reactive fibril ends. In this case, the inhibition affects the final quantities of fibrils and the kinetic profile of fibril formation flattens at the time point when the inhibitor is introduced in the system [75]. In other cases commonly reported in the literature [29,30,43,62,76], the interaction between an inhibitor and the aggregating protein species redirects the aggregation pathway to less harmful off-pathway oligomers. In the framework of the previous analysis, we can describe this mechanism as an effective decrease of the primary and secondary nucleation rates which generate oligomers that are on-pathway to fibrils.

For a particular inhibitor–protein system, in the case where the inhibitory mechanism can be described by binding, the changes of the kinetics of aggregation can be quantified by introducing in the reaction scheme the binding and dissociation reactions between the inhibitor and the different target species. By solving the corresponding mass balance equations (Box 1), the time evolution of fibril formation can be simulated and compared with experimental data acquired at different inhibitor concentrations. If the set of kinetic rate constants in the absence of inhibitor is known, the binding and dissociation rate constants and the corresponding binding free energy can be quantified by fitting the model simulations to experimental data. In this approach, all the chemical features of the inhibitor–protein binding interactions are contained in the kinetic parameters. In addition, comparison between model and experimental data provides validation and quantification of the stoichiometry of the binding.

Insights into the inhibition of microscopic reactions by chemical kinetic analysis

An example of the potential offered by this approach is shown in Figure 3, where the *in vitro* kinetics of fibril formation by the prion protein Ure2p in the presence of different concentrations of a molecular chaperone belonging to the Hsp70 family are shown [77]. The chaperone delays fibril formation in a concentration-dependent manner. By applying the chemical kinetic analysis described above, we are able to identify the specific microscopic event inhibited by the molecular chaperone, in this case the elongation rate (Figure 3) [77]. In addition, we can try to describe the inhibitory effect by considering in the reaction scheme the reversible binding to both fibril ends and the monomer, and comparing the corresponding simulations to experimental data. The analysis shows that the molecular chaperone preferentially binds to fibril ends, whereas binding to the monomer can be considered negligible. The model analysis provides information on the molecular inhibition mechanism of the molecular chaperone, which cannot be achieved by qualitative analysis of the experimental data only.

The example described above shows the potential of combining chemical kinetics with experimental data to analyze the inhibitory mechanism of anti-aggregation compounds, opening an avenue to the rational evaluation of potential new drugs. A scheme showing the proposed strategy is represented in Figure 4. First, the kinetic analysis is applied to elucidate the aggregation mechanism of the system under consideration in the absence of any compounds. An example of this step has recently been provided by the study of the mechanism of aggregation of the Aβ42 peptide [10]. Then, the analysis is repeated in the presence of selected compounds to identify any specific inhibited microscopic aggregation events and thereby test the compound efficiency. An example of this step has recently been provided by using a molecular chaperone to inhibit the aggregation of Ure2p [77]. This powerful approach, which has the advantage of possessing a rigorous theoretical foundation, can guide the selection of the best potential drug from different proposed candidates. In addition, this strategy allows the rational engineering of the aggregation pathway, which can be modified at the microscopic level by the addition of suitable identified compounds.

The proposed approach is flexible with respect to the introduction of additional reactions, such as structural changes of the protein upon binding [78], covalent reactions between the protein and the inhibitor [43], and off-pathway mechanisms. The latter may be of particular interest because toxic soluble oligomers may also be

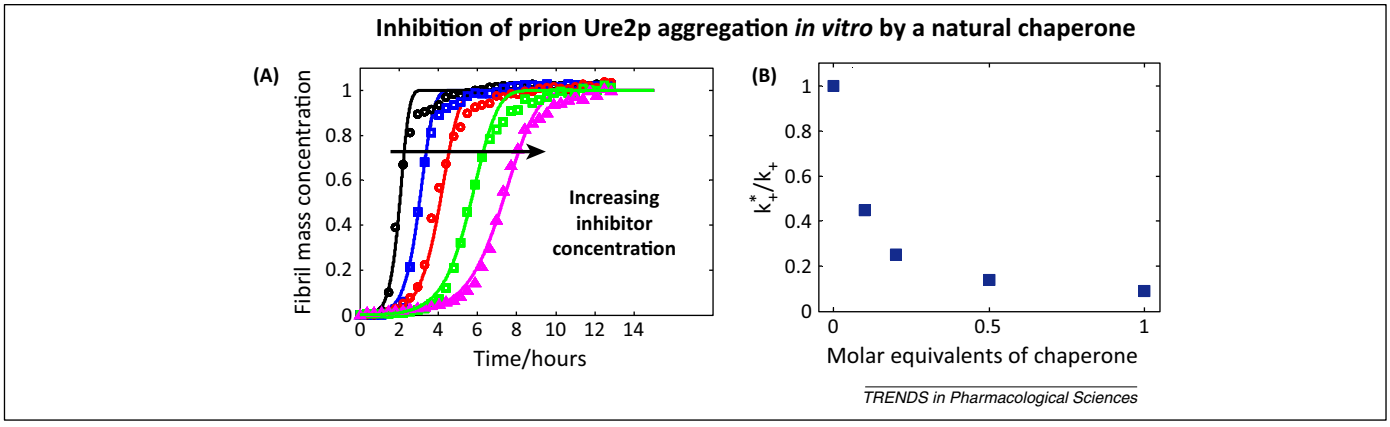

Figure 3. Example of the proposed kinetic analysis. Inhibition of the aggregation of the prion protein Ure2p aggregation by a natural chaperone. **(A)** The global kinetic profiles of aggregation at different chaperone concentrations (represented by different colors) are well described by a kinetic model that considers a specific inhibition of the elongation rate constant, k_+. The decrease of the apparent reaction rate constants at different chaperone concentrations is reported in **(B)** [77].

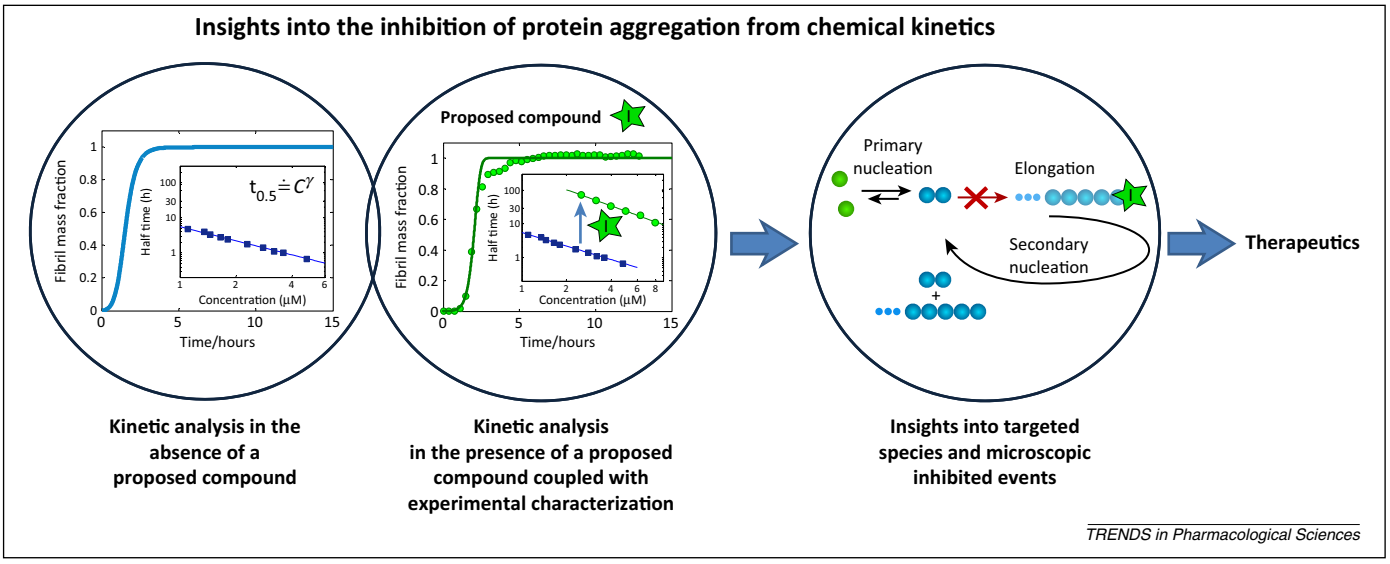

Figure 4. Chemical kinetic analysis for drug discovery. The kinetic analysis of the aggregation mechanism in the presence and in the absence of a proposed inhibitor, together with its experimental characterization of the reaction rates, provides information about the microscopic inhibited events and targeted species. These insights, which are not achievable by qualitative analysis because of the high nonlinear nature of the microscopic processes underlying aggregation (Box 1), represent valuable information for predicting the potential therapeutic benefits of strategies which aim at the kinetic or the thermodynamic inhibition of the aggregation process.

produced by mechanisms that are different from the amyloidogenic pathway [67]. Moreover, many compounds have been shown to exert anti-amyloidogenic activity by coordinating complexes with the proteins and redirecting the aggregation process to alternative off-pathway species [29,30,43,62,76]. All these additional reactions can be described in the general framework of the kinetic master equation approach described above provided that sufficient experimental information is available to define the microscopic aggregation processes and constrain the fitting.

Concluding remarks

AD and many other neurodegenerative disorders are fatal conditions currently lacking effective pharmaceutical treatment. Therapeutic strategies to target these diseases can be based on the identification of compounds capable of perturbing the multistep aggregation process of peptides and proteins involved in the molecular and cellular pathogenicity of the diseases. Such inhibitors can act via different mechanisms and target different species produced during the aggregation process. For the successful development of a therapeutic strategy, a controlled intervention targeted at specific toxic species is likely to be required, focusing in particular on soluble oligomers, which are currently thought to be central species in the development of disease. From this perspective, an understanding of the molecular inhibition mechanism of the potential drugs is of fundamental importance but remains challenging to achieve. To address this issue, we have discussed a strategy which complements the experimental characterization of the binding obtained by a series of biophysical techniques with an analytical approach based on mass balance laws. This approach allows information to be obtained about the molecular target species and the microscopic processes inhibited by the drug from the analysis of the macroscopic experimental observation of fibril formation, providing a framework for the rational design and evaluation of new drugs against neurodegenerative diseases.

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