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Designing transient binding drugs: A new concept for drug discovery

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A multitude of weak, or transient, biological interactions (dissociation constant: $K_d > \mu M$), either working alone or in concert, occur frequently throughout biological systems. We are starting to appreciate their importance in complex biological networks. This realization has important implications to drug discovery as we can question the current paradigm of drug design to find the highest possible binders (drugs) to a given target (receptor). Development of transient drugs, defined by their binding to target, can be based on high-off-rates, multivalent approaches or multiple targets. Now, techniques are available to discover such drug candidates. The greatest problem yet to overcome is probably the mind-set of the individual researcher that weak binders are undesired and therefore of no benefit.

The interactome: a universe of transient/weak biological interactions

Interplay of biological molecules is of vital importance for the life of biological entities encompassing a whole world of biological interactions (the interactome) where myriads of binding events take place every second. Almost all molecules are involved in these complex networks from nucleic acids, to peptides/proteins, to carbohydrates, to small organic molecules. The nature of interactions determines the biological outcome whether it is catalysis, signaling, transmission, movement, sequestration, assembly, sensing or other such events. The size of the interactome is probably far beyond our imagination, involving all types of binding from subtle van der Waals interactions to strong covalent bonds. The size of the human interactome in terms of protein–protein interactions has been estimated [1] and involves at least several hundred thousand simultaneous interactions.

Features of interacting biological pairs (affinity and kinetics; see Box 1) provide useful information on the strength and kinetics of binding. The interactome displays binding strengths ranging from very weak, involving small organic molecules with K_d values exceeding millimolar levels, to extremely tight-binding transition states, to enzymes with K_d values of femtomolar or less. There is great diversity in binding constants where, for example, drug–receptor interactions show mean K_d of $10^{-7.3}$ M [2]. Even though the occurrence of transient or weak biological interactions, defined as $K_d > 1$ μM, is indeed a common event (see Box 1), they have, for a long time, been regarded as rather non-specific and, in some cases, irrelevant.

Transient interactions exert their biological effects mainly through parallel- or serial binding. In parallel binding [3], simultaneous binding occurs between multiple sites on a biological molecule and sites on another molecule. With these polyvalent interactions, total binding strength is much stronger than corresponding monovalent interactions. There are many examples illustrating this type of recognition; two of which are presented as follows. First, during each infection cycle, influenza virus attaches to target cells by multiple binding of hemagglutinin (HA), densely located on the viral surface, to carbohydrate sialic acid moieties [4] on the cell surface. The monovalent bond of HA to an individual receptor sialic acid residue is itself weak (in the mM range), but with many parallel-binding events, a firm attachment of virus is achieved. A second example is dynamic binding of cells to cells, such as white blood cells rolling over the surface of endothelial cells during inflammation [5]. Simultaneous transient binding of selectin molecules on the surface of one type of cell with sialyl Lewis X structures on the other cell mediates this interaction. These interactions lead to penetration of white blood cells at the inflammation site.

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BOX 1

Affinity and kinetics of a transient (weak) interaction

For simplicity, consider a second-order bimolecular binding:

$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

where A is the target (host) molecule (usually a protein receptor) and B is the ligand (guest). The k_a [M^{-1} s^{-1}] and k_d [s^{-1}] are the association rate- and dissociation rate constants, respectively.

At equilibrium, $K_d = 1/K_a = [A][B]/[AB] = k_d/k_a$ where K_d [M] and K_a [M^{-1}] are the dissociation and association constants, respectively. [A], [B] and [AB] are the concentrations of target, ligand and complex, respectively.

For a transient (weak) non-covalent interaction, K_d is > approximately 10^{-6} M (usually in the range of 10^{-5} to 10^{-3} M). For many transient interactions, k_d is higher than 0.1 s^{-1}. This means that dissociation of the complex ([AB]) is more rapid than seconds.

With serial binding, by contrast, there are repeated weak-binding events that have various biological effects. One example of serial binding is the weak binding of substrates and inhibitors in various enzyme systems. Another intriguing example of serial binding is activation of T cells during immune recognition. Small peptide fragments, in conjunction with the major histocompatibilty complex (MHC), are recognized by T cell receptors through weak affinity and rapid kinetics [6]. Affinity ranges in these and similar serial-binding examples have been demonstrated in the range of K_d = 1–50 μm with off rates (k_d) above 0.1 s^{-1}. Signal transduction seems to be as a result of repeated serial binding (knocking on receptor) between peptide–MHC complexes and multiple T cell receptors. It is, therefore, reasonable to assume that transient interactions play a fundamental role in the interactome of any cellular network, whether related to intracellular activities or to cells interacting with their environment [7]. It could well be that strong biological recognition is more an exception than a rule.

Clearly, transient binding plays a significant role in different cellular networks such as the cytoskeleton, gene transcription and metabolic or signaling events. It is interesting in this context that chaperones (heat shock proteins) are taking part in cellular networks by modulating, sequestering and repairing proteins by utilizing transient binding to their targets [8].

Aging of a cellular (both *intra* and *inter*) networks may result in decreased binding affinities between enzymes and their substrates because of, for example, mitochondrial decay [9]. This process, which can be a significant contributor to major diseases, such as cancer and neurodegenerative conditions, could involve a whole new array of subtle binding interactions virtually unknown to us at this point. Cell-system damage caused by age-related events will tremendously affect the nature and frequency of transient binding events [9].

Where is drug discovery going?

The central dogma of drug discovery and development has been to find a single target that is clinically relevant and amenable to new-drug development. The target is usually a protein that plays a major role in the disease process. The next step in the discovery process is to find the best possible binder (high-affinity hit) by screening possible candidates from molecular libraries containing molecules of interest, or by rational drug design based on detailed knowledge of the target (such as three-dimensional structures). These high-affinity hits then become lead compounds (potential new drugs) as they are optimized to improve efficiency, to show desirable pharmacokinetic properties and to minimize side-reactions. If relevant lead compounds can determined by this process, they are tested in preclinical (animal) and in clinical (human) trials. If the candidate compound passes all these barriers, it is manufactured and marketed as a drug.

Even though the pharmaceutical industry has recently experienced a number of success stories in the treatment of infectious, gastrointestinal and cardiovascular diseases, the output of new drugs has been relatively meager, considering demand and the industry has faced ever-increasing hurdles to find efficacious, safe and profitable new drugs. Expenditures for R&D have grown significantly, roughly doubling in 10 years (1995–2005) to approximately $40 billion in the US alone [10]. During the same period, the number of approved new molecular entities (NMEs) has declined, with only 17 approved by the FDA in 2006. Of special interest is the increased number of failures in human phase III trials, the most costly part of the drug-development process, where in recent years approximately half of the trials failed whereas historically the success rate has been as high as 85% [11]. Despite the introduction of new potent techniques, as well as different approaches to streamline the drug-discovery process, including high-throughput screening, multi-array analysis and structure based design; disappointments have been many during early-phase drug discovery. So, what is the problem? There are, of course, many factors contributing to difficulties and failures in the drug-discovery process, and since the process is complex and multi-faceted, it is not surprising that we are facing a number of bottlenecks. Even though high-throughput screening procedures were able to identify potential candidates against many targets, failure was common, because of inconsistency with desired ADMET (absorption, distribution, metabolism, excretion and toxicity) properties such as low solubility and instability of drug candidates [12]. Problems relating to toxicity and adverse reactions have been difficult to foresee and were thus discovered late in the drug-development process. More than 80% of drug candidates fail in human clinical trials because of toxicity and efficacy problems [11]. It would be valuable for the drug-discovery process if selection for ADMET properties could be carried out earlier, ideally during initial screening phases.

In our quest to discover more and more drugs to aid humankind, it is probable that we have gone too far in our reductionist approach to drug discovery and are thus lacking in a proper understanding of the effects of new drugs on the whole biological network. It may be that pharmaceuticals on the market today have been the 'easy access' drugs, whereas future compounds will be much harder to discover and develop, because they are directed toward more complicated diseases that have many complex and interactive targets. Clearly, a major objective in the drug-discovery process needs to be proper selection of target binding and ADMET properties as early as possible in the process, in order to avoid late and costly failure for drug candidates that are inherently unsuitable.

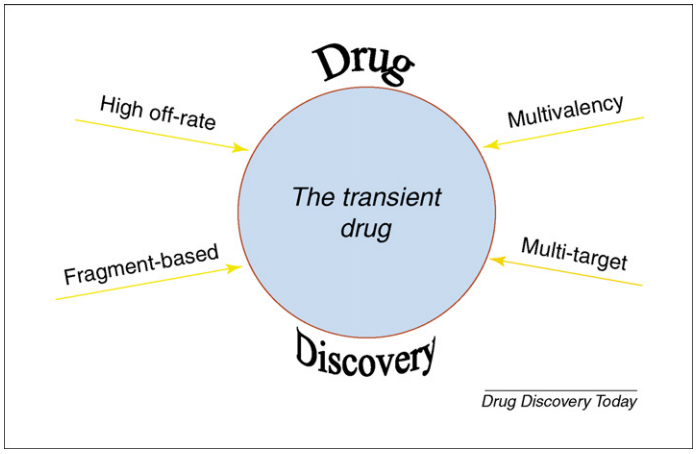

FIGURE 1
Types of transient drugs.

The transient drug

With these challenges in mind, the purpose of this article is to draw attention to the potential discovery of a whole new range of drug candidates that bind to their targets in a more transient manner, that is, characterized by weaker binding and/or faster kinetic profiles. For example, weak-affinity molecules can bind monovalently with rapid off-rates, can bind polyvalently with individual weak binding, or can bind transiently to many targets (the multi-target drug; see Figure 1). The paradigm in drug discovery is that the tightest drug binder (highest affinity) shows the best potency (efficacy) and selectivity. By doing so, as the paradigm goes, the new drug will minimize undesirable side reactions. The goal has therefore been to find candidates that bind in the submicromolar range (often nM) to assure close binding to the target. Unfortunately, the role of transient interactions (weak affinity) has been neglected for a long time as a tool for drug discovery (and elsewhere) in biological sciences for a number of reasons: First, it has been envisioned that a weak binder is not specific to its target, and typically shows high cross reactivity to other binding sites. This statement is not always true, since specificity is a relative concept considering relevant binding in the light of other non-relevant binding events. Specificity can be defined as the ratio of affinities between a desired interaction versus other non-desired interaction, that is, specificity = K(desired)/K (non-desired). Ratios can be higher for a weaker binder against a given target than a stronger-binding molecule depending quantitatively on affinity of cross-binding, therefore showing improved specificity for the target. Confusion seems to arise from the fact that many non-relevant binding reactions are weak and, therefore, considered to be non-specific. Second, the amount of binding to the target can be perceived as a problem for a weak binder, as it is much lower and, thus, may not be sufficient to propel a response. However, if local concentrations of the weak binder are high enough, it can drive the equilibrium, resulting in considerable bound ligand. For example, assuming a K_d of 1 μM, a concentration of 1 μM is sufficient to fill half of the receptor sites. By compartmentalization in biological systems, the concentration of binding pairs can be rather high, while net concentration for the whole cell is quite low (protein concentration based on the whole volume of a cell is less than 0.1 μM [13]). Third, weak natural interactions can be efficient if the partners are potent enough or if they are multivalent [14] Fourth, weakly binding drugs/weak biological interactions are not studied because of difficulties in screening or analyzing them; that is, 'if you cannot see them, they do not exist.' Fortunately, there are a number of emerging techniques to study and screen transient interactions (see below). It is important to emphasize that transient (weak) binders typically demonstrate a dynamic binding profile possibly with high k_d rates. For example, weak affinity antibodies towards carbohydrate antigens exhibit k_d rates in the range of 1–100 s^{-1} [15] which means the antibody-antigen pair dissociates within a fraction of a second. Estimates of the duration of drug–receptor residence time can be indicative of drug performance [16].

Examples of drugs that can be considered as transiently binding are alcohol (ethanol) [17] and nonsteroidal anti-inflammatory drugs (NSAIDs) [18], such as aspirin, naproxen and ibuprofen. In a complex way, alcohol affects synapses of the central nervous system (CNS) and can be considered a transient binder, because of its perceived low affinity for different receptors in the brain where rather high concentrations are needed to produce biological effect. Many NSAIDs bind receptors and enzymes, such as cyclooxygenases (COX) at affinities higher than 1 μM K_d. For a complete list, see the National Institutes of Mental Health Psychoactive Drug Screening Program (PDSP K_d database at http://pdsp.med.unc.edu). Other examples of weak enzyme inhibitors are valproic acid and butyric acid, which affect histone deacetylases.

Transient binding could also be of great value to screen for weak interactions of drug candidates to targets that produce side effects, since these effects can be subtle. Cytochrome P450 (CYP) enzymes are a group of approximately 50 enzymes that metabolize, and are largely responsible for clearance of, many drug compounds [19]. It is estimated that approximately half of all drugs have challenges related to CYP metabolism. It may be of interest to study the upregulation of CYP enzymes because of weak interactions of drug substances during a longer period of time. Negative screening for weak binding to CYP enzymes has potential for selection of drug candidates with minimal CYP interference.

Another important issue in drug discovery is receptor desensitization. It may be situations where weakly binding substances are sufficient to cause signal transduction but not receptor desensitization.

The transient drug: high off rate/low on rate

Recent animal and human trials have demonstrated that memantine, a weakly binding drug, is of potential therapeutic value in treating neurodegenerative diseases such as dementia [20,21]. This is indeed of great interest, as the action of memantine opposes the central dogma of finding high-affinity leads to target molecules. Many neurological disorders, including Alzheimer's disease, Parkinson's disease and multiple sclerosis might be related to a common mechanism of injury in neurons. In this case, overstimulation of glutamate receptors, most typically the *N*-methyl-D-aspartate glutamate (NMDA) receptors in the brain [22], leads to, among other things, excessive Ca^{2+} influx into the cell through ion channels. This can produce significant adverse effects, such as cell degeneration, because of toxic free radicals. Neuroprotective agents that bind with high affinity to these receptors will block all activity, leading to severe side-effects, such

as hallucinations, drowsiness and coma, and are therefore undesirable as therapeutic drugs. However, by finding a more-gentle binder, such as memantine that has an appreciable off-rate, neuronal transmission will normalize and receptor shutdown side-reactions are minimized. Memantine, which was recently approved for the treatment of Alzheimer's disease, shows binding to the NMDA receptor in the μM range with an off-rate of approximately 0.4 s^{-1} [22]. It is clear that this approach, using weakly binding drugs with fast off-rates, could be a key factor in designing effective ion-channel blockers, and that this principle can apply to a number of neurological and other targets. Interestingly enough, a transient drug can also be designed by lowering the on-rates even though this can be considered to be a lesser contributing factor than increasing off-rates. The works by Foser *et al.* [23] and Bailon *et al.* [24] showed that PEGylated interferons for treatment of chronic hepatitis C showed decreased affinities (near to μM) compared to the parent interferon, because of partly lower on-rates.

The transient drug: multivalency

Multivalent interactions result from simultaneous binding of several sites on the same molecule with multiple receptor sites. As a result, apparent affinity (avidity) can be raised several orders of magnitude (e.g. from mM to μM) compared to individual binding between two binding entities. The exact enhancement in affinity is because of spatial organization and number of binding sites on the ligand–receptor complex, and to inherent monovalent affinity of individual binding. The antigen-antibody complex with the immunoglobulin M (IgM) is an illustrative example of the natural existence of a multivalent assembly that can be composed of individual weak binding sites.

Considerable effort has been focused on developing procedures and drugs based on a polyvalent approach, especially in the area of new inhibitors of bacterial- and viral adhesion to host cells (see reviews by Pieters [25] and Mammen *et al.* [3]), but also for regulating inflammatory processes in autoimmune conditions and for vaccine development (see short overview by Kiick [26]). Of special interest is the recent work on targeting tumor cells with multivalent weak affinity interactions [27]. However, there is an apparent contradiction as multivalent drugs are usually of high avidity. I discuss them here albeit these drugs are based on multiple simultaneous binding they can be built from structural elements carrying weak affinities and the resulting multivalent drug can be transient.

Most efforts so far have been addressed to drugs that can inhibit bacterial adhesion as a viable alternative to antibiotics. This strategy seems to be very attractive, since it will most likely not face resistance problems as is often the case with antibiotics. Bacterial adhesion is frequently based on weak binding (typical individual binding is in the range of K_d = 1 mM) of proteins on the bacterial surface to carbohydrate moieties on the host-cell surface. It is, therefore, essential to design polymers or conjugates that carry multiple carbohydrates, thus, enhancing binding strength sufficiently to the target. Even though many interesting conjugates have been produced in the laboratory that can act on pathogens such as *Escherichia coli*, *Streptococcus suis* and *Helicobacter pylori*, there are still major obstacles to overcome relating to insufficient avidity, administration, immunological and toxic responses, all of which have to be solved before clinical applications can be realized. There is, however, a strong driving force to find alternative remedies to classical antibiotics for bacterial infections, and this will certainly fuel an even greater interest in the field of weak-affinity research.

The transient drug: multi-target

Finding drug candidates that selectively (at high affinity) bind single targets has recently been questioned by a new hypothesis suggesting it is far more productive to aim for several targets at the same time (the multi-target drug approach) [28–31]. The reasons for this interest in 'promiscuous' or 'dirty' drugs come from the fact that complex conditions, such as cancer, inflammation, depression and cardiovascular diseases, are not caused by a single molecular defect, but are rather the result of a combination of molecular dysfunctions. An illustrative example of a 'promiscuous' drug is the anti-cancer agent Gleevec® which shows promise in treatment of leukemia [28]. Although it was originally designed to hit a particular target, it was soon realized that this drug was a multiple-target kinase inhibitor. Another illustrative group of drug compounds that show a broad binding spectrum are anti-psychotic agents where many of these bind to a plethora of neuronal receptors [32]. It is clear that the 'magic bullet' strategy to solve complex diseases has not been as successful as anticipated, suggesting instead that a 'magic shotgun' strategy may be a viable alternative for a variety of disorders [33]. A multi-target drug will frequently be a transient binder, since it can interact with a number of disparate targets. In other words, cross reactivity of the drug should be substantial so that it can theoretically interact with multiple targets for maximum efficiency. By including transient multi-target drugs, the size of drug-amenable targets will increase significantly in terms of potential druggable proteins [32]. Databases on cellular and protein networks show potential to define new targets for drug design. However, much work remains to give a more complete picture of the different networks and yet-to-be discovered areas. For example, databases on networks involving protein–protein interactions contain numerous false-positives making extraction/assessment of weak-affinity interactions troublesome [32]. In addition, many relevant weak-affinity interactions are, to a large extent, unknown and databases lack such information.

The transient fragment for drug design

The rationale behind fragment-based drug discovery is to find small-sized fragment molecules that bind to drug targets [34,35]. These scaffold-like structures are simple organic molecules that bind weakly (with a K_d of mM to μM) to the target. Once identified, they are further linked and grown into larger drug-like molecules with much increased affinity (generally significantly better than μM). It should also be possible to construct a transient drug with affinities above the μM level from fragments with very weak affinities in the mM range. One major problem that has slowed down development of this technique has been the difficulty in detecting weak binding of fragments. This technique holds great promise, but it remains to be seen how successful fragment-based approaches will be.

Tools to screen for transient drug candidates

Current tools available to the pharmaceutical industry to help define new drugs rely heavily on high throughput screening (HTS)

procedures, where large molecular libraries (in the range of hundreds of thousands) are tested for their binding to specific targets [12,36]. Other approaches to drug design have also played significant roles in pharmaceutical research, such as modifications of already existing drugs/natural products, and rational drug design based on detailed structural knowledge of target molecules determined by X-ray crystallography and NMR. Even though HTS has been a success in some cases, it is now abundantly clear that it has been a disappointment in many drug discovery projects. The reason for these disappointments have been frequently debated in the past few years [12,36], but it is clear that the causes of attrition in later phases of drug discovery have been attributed to poor solubility and permeability, metabolic instability, high interference with irrelevant proteins and cytotoxicity.

Screening for weak binders, while very promising, is a challenge for a number of reasons. They are difficult to detect, since only minor amounts are bound at equilibrium and because molecular libraries are typically screened at μm concentrations or lower. Furthermore, they are easily washed out in heterogeneous procedures. Most HTS assays rely on indirect detection methods, such as fluorescence, absorbance or radioactivity that could be a barrier for estimating weak-binding events. Because of limitations in assay design, HTS procedures can produce false positives and negatives, especially when estimating the presence of weak binders. Nevertheless, HTS based on inhibition assays of enzyme activities, for example, can, if properly designed, detect weak binding of compounds with IC_{50}s of $<10^{-4}$ M. There are a number of potential methods for screening weak binders to protein targets; they are briefly reviewed below and are compared in Table 1.

NMR is a powerful tool to detect weak-binding events [13] and is useful for screening, especially in fragment-based approaches. Sensitivity, throughput and consumption of target can still be a problem with NMR, but significant improvements have been made in recent years [37]. Mass spectrometry (MS) is an emerging technique for HTS that can be used to detect the binding of a wide range of ligands, including lipids and steroids, which are not easily accomplished with traditional screening techniques. A major challenge in this approach is to integrate, in a high-throughput manner, MS with purification steps (the so-called 'hyphenated' MS approach), such as HPLC. For example, weakly binding inhibitors can be indirectly detected by studying the substrate/product mass ratios of enzyme conversions [38]. Another interesting approach to discover and study weakly binding ligands has been electrospray ionization MS [39].

X-ray crystallography is a powerful method to give a full picture of ligand-binding to target molecules, including weak binders. Originally there was concern that weak-binding events could not be seen in electron density maps of crystals, but reports (see for example [40]) have shown that it is possible to detect weak binding with X-ray crystallography. While this technique has been considered rather slow, recent developments in computing and automation have made the technique more amenable to higher throughput [41].

Affinity chromatography is of considerable interest for screening weak-affinity binders, since it is a robust and simple procedure, and offers a plethora of detection techniques, including MS. Chromatography can be performed in two ways: Frontal- [42] or zonal-affinity chromatography [43]. In frontal analysis, drug candidates are continuously infused over an immobilized target until equilibrium is reached. In zonal analysis, discrete samples are injected onto a column having immobilized target and compound retention is then followed on-line. These techniques can be adapted to high throughput. For example, zonal-affinity analysis was performed with 24 parallel capillary microcolumns to detect albumin binders and yielded an output of 3000–4000 runs per day (S. Ohlson *et al.*, unpublished).

Other techniques, based on capillary electrophoresis (CE) [44] and surface plasmon resonance (SPR) [45], are also available for screening and evaluation of binding and kinetics (in the case of SPR). They hold promise as high throughput screening tools for transiently binding drug candidates.

In addition, cell based or *in-vivo* procedures offer alternative methods for screening of transient drug candidates [46]. For

TABLE 1

Comparison of screening methods for binding of transient binders to a drug target: strengths and weaknesses

Parameter	*Functional biochem. screening*	NMR	MS	Crystallography	Affinity chromatography, frontal analysis	Affinity chromatography, zonal analysis	CE	SPR
Throughput[a]	High	Medium	Low–medium	Low	Medium	Medium–high	Low	Medium
Protein required[b]	Medium	High	Medium	Medium	Medium	Medium	Low–medium	Low
Affinity data	IC_{50}	Yes	No	Yes	Yes	Yes	Yes	Yes
Kinetic data	No	No	No		No	Yes	No	Yes
Need of target immobilization	No	No	No	No	Yes	Yes	No	Yes
Ranking of compounds	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
False positives	High	Low	Low–medium	Low	Low	Low	Low	Medium
Knowledge of protein structure	No	Yes	Medium	Yes	No	No	No	No
Knowledge of protein function	Yes	No	No	No	No	No	No	No

[a] Low = <100/week, medium = 100–10,000/week and high = >10,000/week.
[b] Low = <1 mg, medium = 1–100 mg and high = >100 mg.

example, screening for activation on the millisecond time scale of G protein-coupled receptor can be performed in living cells [47].

As seen from the above discussion, there is an abundant supply of techniques available for measuring weak binding of analytes to targets. They both have their own strengths and limitations, and no single method may yet be pinpointed as the ultimate choice. Apart from laboratory procedures, virtual-screening (VS) methods have become a widespread and complementary tool which, in the early stages of drug discovery, can give information on possible leads [48]. It is clear that VS can work in concert with experimental high throughput screening procedures and can also be of value to identify hits with low affinity profiles.

Conclusions

The purpose of this review has been to draw your attention to the fact that drug design does not necessarily have to involve high-affinity screening of target molecules. On the contrary, transiently binding drugs have real opportunities for maximum efficiency and, at the same time, they may offer the potential of reduced adverse side reactions. There are three major areas for development of transient drugs when high-off rates, multivalency and multi-target are key parameters for drug design. It could even be useful to revisit older drug discovery programs to examine for discarded or yet undiscovered weakly binding lead compounds.

However, there are still two major barriers for increased interest in and growth of this area of drug research. First, there is still a paradigm in the minds of many scientists stating that effect and specificity only come from drugs that bind tightly to a target molecule. Second, there is still need for continued development of screening platforms for transient binders. If these problems can be overcome, I am confident that transient drugs can offer a bright and promising future for new-drug discovery.

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