# Synthetic Molecules as Antibody Replacements

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**ABSTRACT**

Antibodies are by far the most versatile, valuable, and widely used protein-binding agents. They are essential tools in biological research and are increasingly being developed as therapeutic reagents. However, antibodies have a number of practical limitations, and it would be desirable in many applications to replace them with simpler, more robust synthetic molecules. Unfortunately, synthetic protein-binding agents rarely exhibit the high affinity and specificity typical of a good antibody. This article reviews efforts to overcome these limitations and to develop a facile, high-throughput methodology for the isolation of synthetic protein ligands with antibody-like binding characteristics.

## Introduction

Molecules that bind tightly and specifically to proteins are of great utility in biology and medicine. The most common protein-binding biomolecules are antibodies. A good antibody can bind its target protein with an equilibrium dissociation constant ($K_D$) of $10^{-9}$ M, and various optimization protocols can provide picomolar or even femtomolar complexes. Furthermore, extremely high specificities are sometimes obtained. In the best cases, only the protein antigen is recognized in a crude cellular extract containing thousands of different proteins.

For all of these advantages, antibodies have considerable limitations. They are tedious and expensive to produce by the classical route of injecting an animal with a protein or peptide antigen. This process also has a high failure rate and is unpredictable. Furthermore, antibodies, like most proteins, must maintain a relatively delicate three-dimensional structure in order to function, limiting the conditions under which they can be employed. Thus, there is a compelling argument to investigate other classes of molecules as high-affinity and -specificity protein-binding agents. While much work has focused on other classes of biological macromolecules, this article will focus on small synthetic protein-binding agents.

## Protein-Binding Small Molecules

Synthetic molecules have a number of potential advantages over biomolecules as protein-binding agents, including ease of production and purification, robustness, and ready modification with appropriate tags. The problem is that it remains challenging to design or discover synthetic molecules that bind proteins with anything near the affinity and specificity of a good antibody. In the cases where this has been done (either by humans or nature), numerous medicinal chemists or millions of years of evolution were required. An attractive "shortcut" to the development of high-affinity protein-binding molecules is multivalency. Two or more modest-affinity ligands that bind a target protein noncompetitively, when linked appropriately, can cooperate to form a high-affinity bivalent ligand. The trick is to develop a rapid and efficient route to the discovery of noncompetitive "lead molecules" and a linker of appropriate length and geometry to allow for cooperative binding.

## Screening Combinatorial Libraries for Protein-Binding Agents

Most protein-binding compounds are obtained from screening libraries or compound collections since high-resolution structural data are required for ligands to be designed successfully. Combinatorial libraries can be created and screened in a number of ways. Peptide libraries represent a special case, since they can be created and screened using both biological and chemical methods. Of course, there are some applications for which peptides are not ideal ligands. The most obvious is as pharmaceuticals. We will not be concerned here with issues such as oral bioavailability, but in the context of developing antibody substitutes, the sensitivity of peptides to proteases is a major issue.

Large libraries of up to millions of compounds, ranging from peptides to peptidomimetics to more druglike species, can be made using the split and pool technique,[1] which results in a collection of beads, each of which displays many copies of (ideally) a single chemical com-

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pound. Unless these molecules can be sequenced directly, as is the case for peptides and certain peptide-like oligomers, the beads must also be encoded with a tag indicative of the identity of the compound on that bead. While there are many elegant solutions to the encoding problem almost all of these are either very expensive or technically demanding or both (but see ref 2). Thus, if one wishes to employ relatively large libraries, molecules of which the structures can be determined directly from a single bead have significant advantages.

Protein binding assays are most easily done with immobilized compounds. The two most common methods are to screen bead libraries or to first segregate a bead library, release the compounds into solution, and then array them by spotting onto chemically modified glass slides or other suitable supports. Each platform has its strengths and weaknesses. The creation of small molecule microarrays[3−5] requires significant robotics capabilities to segregate beads into the wells of microtiter plates and process them in an automated fashion, and it is not practical to handle libraries of more than a few thousand compounds in this way, whereas much larger libraries can be screened on beads. Bead-bound libraries, on the other hand, do not necessarily require a sophisticated infrastructure to screen. But they introduce some significant technical problems in the screening steps (see below) and generally can only be used once against a single target protein. A major advantage of microarrays however, is that a library synthesis can easily provide enough material for the creation of hundreds of replicate microarrays.

## Small Molecule Microarrays

Schreiber and co-workers have used small molecule microarrays to great effect in chemical genetics experiments.[6,7] For example, fluorescently labeled Ure2 protein, a yeast transcription factor, was employed as the target in a microarray-based screen of 3780 dioxin-containing small molecules. A compound, called uretupamine A, was isolated and a closely related derivative (uretupamine B) with enhanced solubility was created subsequently, and the uretupamine B−Ure2p complex was found to have a $K_D$ of 7.5 $\mu$M. When yeast were treated with this compound, DNA microarray-based expression analysis showed that only Ure2p-regulated genes (and only a subset of these) were effected substantially.[6] This implies a high level of specificity of the small molecule for the Ure2 target protein, at least in terms of functional effects, if not binding. It also represents yet another example of the general finding that molecules selected in simple binding assays generally affect the function of the target protein.[8]

A fundamentally different approach to the creation of spatially addressable small molecule microarrays is to carry out the synthesis on the array itself.[9] This has been limited mostly to the synthesis of peptide arrays on cellulose membranes (but see refs 10 and 11 for the synthesis of other compounds). This so-called SPOT synthesis employs spotting of standard peptide synthesis intermediates to build up the chain on the cellulose matrix. Arrays of a few thousand spots can be created in this fashion. A different, and potentially powerful approach to in situ synthesis is photolithography. In this approach to oligomer synthesis, the reactive group at the end of a chain, for example, the 5′-OH of an oligonucleotide, is protected with a photo-unmaskable group. To create an array, UV light is directed only to certain spots on the chip by the use of physical masks or, more recently, by digital masks created with computer-controlled micromirror arrays.[12,13] The precise application of light in a spatially controlled fashion allows for the next monomeric unit to be added at one feature while the same group at another feature remains protected. The advantage of photolithographic synthesis is that high-density arrays with micrometer-sized features containing millions of compounds can be created. This chemistry is practiced on an industrial scale for the creation of DNA oligonucleotide arrays[14] but has not been used extensively to create arrays of potential protein-binding compounds. Fodor and co-workers demonstrated in 1991 that by using photo-unmaskable amino acid derivatives arrays of peptides can be made photolithographically,[15] but this chemistry is not practical because it is logistically difficult and expensive to maintain stocks of many photo-unmaskable amino acid derivatives. Our laboratory, in collaboration with Skip Garner and co-workers, has developed a photolithographic synthesis of peptoids[16] (Figure 1A) that avoids this problem, requiring only a single photounmaskable monomer (Figure 1C).[17] While several technical issues remain to be solved before peptoid arrays with hundreds of thousands of features are readily available, simple arrays have been produced (Figure 1D), providing proof-of-principle.

## Protein-Binding Compounds from Bead-Based Screens

As mentioned above, screening on beads is an alternative to the creation of microarrays. For a variety of reasons, including a lack of robotic infrastructure and limited funds, we decided to commit to bead-based screens when we entered this area approximately 4 years ago. The major issue in bead-based screens is to find a support with all of the appropriate properties to make both synthesis and screening convenient and effective. The resin must be mechanically stable and have good swelling properties in both organic (for synthesis) and aqueous (for screening) solvents. After some experimentation, we settled on Tentagel beads (Rappe Polymere) as the support of choice. These are comprised of a polystyrene core but are coated with long poly(ethylene glycol) (PEG) chains that terminate in an amine functionality. In addition to improving the swelling properties of the beads in water, the PEG chains drastically reduce the level of nonspecific protein binding to the beads, a critical issue in screening experiments. The only drawback of these beads is that they exhibit a significant autofluorescence background that complicates binding assays using fluorescent tags.

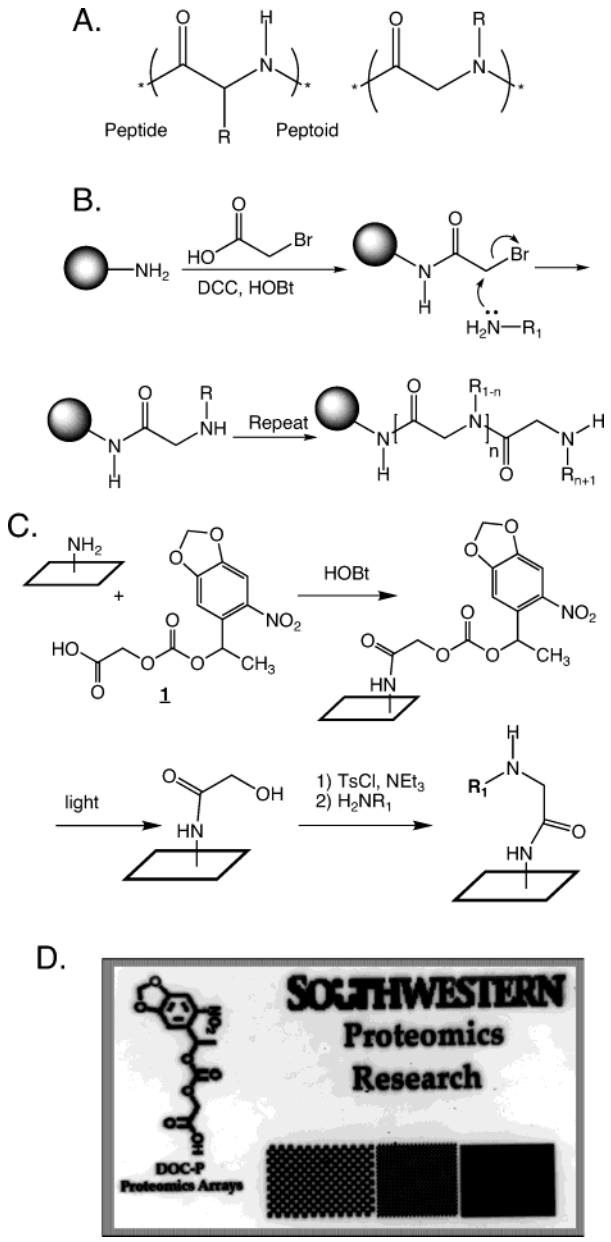


**FIGURE 1.** Peptoids are ideal molecules for combinatorial chemistry: (A) structural comparison of a peptide and peptoid; (B) the submonomer synthesis of peptoids; (C) an adaptation of the submonomer synthesis that employs a protecting group that is unmaskable by UV irradiation;[17] (D) construction of a simple peptoid array by digital photolithography. The pattern seen was created by synthesizing peptoid monomers containing fluorescent donors or acceptors at the appropriate positions on the array. See ref 17 for details.

We have focused on peptoid libraries[18] (Figure 1A) as a source of protein ligands. Peptoids are almost ideal molecules for combinatorial chemistry. They are easy to make using the submonomer chemistry described by Zuckermann and colleagues[19] (Figure 1B). This approach is particularly well suited to split-pool synthesis because the diversity-generating step is the displacement of a bromide ion by a primary amine, hundreds of which are commercially available. We have made chemically diverse libraries of over 500 000 peptoids.[20] Another advantage is that encoding is unnecessary. A 160 $\mu$m Tentagel bead has more than enough peptoid on it to allow its sequence to be determined directly by Edman sequencing or MS/MS.[20]

The screening protocol that was developed after considerable labor-intensive research differs from most in the following ways. First, the beads are blocked and the binding assays are conducted in the presence of a 1000−10 000-fold excess of proteins derived from a bacterial extract. This large excess of diverse competitor proteins minimizes the isolation of nonspecific, "sticky" protein-binding reagents (L. Troitskaya, M. M. Reddy, and T. Kodadek, manuscript in preparation). In addition, the binding assays employ relatively low concentrations (100−500 nM) of a fluorescently labeled protein and are carried out in a buffer containing 0.5−1 M NaCl and 0.5−1% Tween-20, a nonionic detergent. Peptoids that bind their protein target with modest affinity ($K_D$'s in the 1−50 $\mu$M range) and high specificity are obtained. The major advantage of using these extremely demanding conditions is that false positives or generally sticky, nonspecific ligands are rarely obtained. It is difficult to overemphasize this issue of binding specificity if one wishes to employ hits, or derivatives thereof, as antibody substitutes. Most studies of synthetic protein-binding molecules, either designed or isolated from a library, do not address the issue of whether they can recognize their target in the presence of a large excess of diverse competitors, which we demand in the screen.

A significant problem in developing an effective screening protocol was that Tentagel resin has a significant autofluorescence.[20,21] This provided an annoyingly high level of background when attempting to see fluorescently labeled protein bound to beads.[20] This represents another disadvantage of beads relative to glass microarrays since the latter have much lower levels of background signal (Figure 2). Unfortunately, other assays commonly employed to observe protein binding to bead-displayed compounds, such as dye deposition,[22] were ineffective in our hands under the conditions described above.

The first generation solution was to label the protein with a lysine-reactive derivative of Texas Red that emitted in a region where the bead fluorescence was less intense. This allowed us to visually scan through fields of beads using a fluorescence microscope and pick out the brightest beads, which usually proved to be true hits.[20] While this approach worked, the microscopic screening of the bead library was quite tedious. The level of contrast between the true hits and false positives was not huge (Figure 2B). This situation could be improved tremendously by prescreening the library to remove the most intensely autofluorescent beads. Tentagel beads are quite heterogeneous with regard to their level of autofluorescence. We found that the COPAS sorter (Union Biometrica), marketed as a fluorescence-activated sorter for large cells or embryos worked nicely with beads up to ~200 $\mu$m in diameter. Thus, we were able to use the sorter to eliminate the brightest beads from the population (7−10%), greatly improving the contrast in subsequent screens.

Another improvement was to employ biotinylated target protein and then visualize the bound factor using

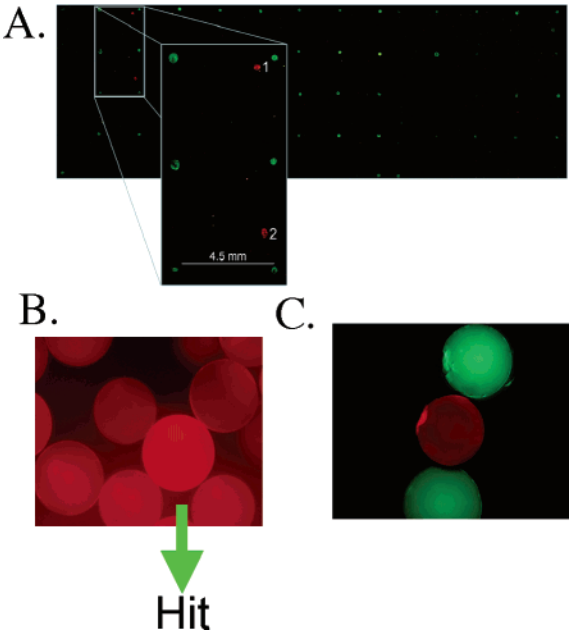


**FIGURE 2.** Images of the binding of fluorescent proteins to synthetic molecules immobilized on different platforms. Panel A shows hybridization of GST−Hap3 fusion protein to a small molecule microarray comprised of 6912 compounds printed on a chemically modified glass slide. Rhodamine was printed as a marker (false-colored green) in the upper right-hand corner of each 12 × 12 subarray. Binding of the protein was detected using a Cy5-labeled anti-GST antibody (false-colored red)[7] (image provided by Drs. Angela Koehler and Stuart Schreiber. Reprinted from ref 7. Copyright 2003 American Chemical Society). Panel B shows a photomicrograph of a field from a screening experiment in which a Tentagel-displayed library of ~78 000 peptoids was screened against Texas Red-labeled Mdm2. Panel C shows a two-color assay based on red quantum dots. In the top micrograph, biotinylated ubiquitin was incubated with Tentagel beads displaying a ubiquitin-binding peptide or beads displaying a control peptide. Binding was visualized by incubating the beads with streptavidin-coated red-emitting quantum dots followed by photography under 390−410 nm light. The micrograph shows two control beads, which fluoresce green, and one ubiquitin-binding peptide displaying bead, which fluoresces red.

red-emitting, streptavidin-conjugated quantum dots.[21] When irradiated with UV light, these nanoparticle semiconductors exhibit a tremendous Stokes shift and emit intensely in the red region. The advantage of this protocol is that the beads fluoresce green under UV irradiation with an intensity well below that of the quantum dot's red emission. This provides a "two color" assay that allows one to visually distinguish beads that have picked up the target protein (Figure 2C). The only limitation of this assay is the "stickiness" of streptavidin, which can give rise to false positives if great care is not taken. It may be that other coatings of the dots will improve the utility of this approach in the future.

Using the techniques described above, we can now routinely isolate protein-binding peptoids (or peptides) of high quality. For example, a peptoid was isolated from a library of approximately 78 000 compounds in a screen using a Texas Red-labeled domain of the Mdm2 protein[20] (Figure 2B). The peptoid−Mdm2 complex was found to have a $K_D$ of 37 $\mu$M. "Pull-down" experiments using the peptoid immobilized on beads showed that unlabeled Mdm2 protein could be retained from a crude extract, as determined by Western blotting.

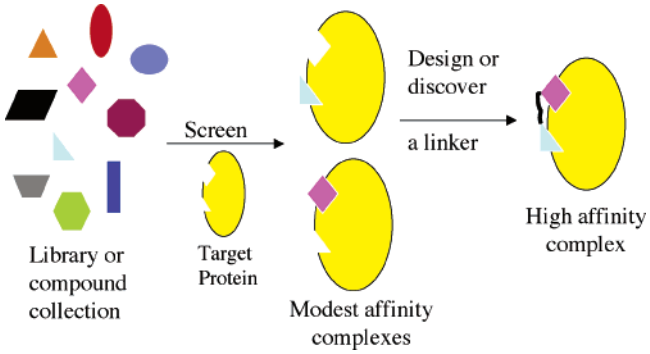


**FIGURE 3.** Classic route to a bivalent protein-binding molecule. A library is screened to identify two molecules that bind the target protein noncompetitively. An appropriate linker must then be designed or discovered that allows the two molecules to bind in a cooperative fashion.

The major remaining challenge in the isolation of protein-binding peptoids is to increase the throughput of the screening efforts greatly. Fortunately, all of the steps in the protocol are amenable to automation, including the screening step itself, which can be done using the COPAS instrument (see below). These efforts are ongoing. In addition, because of the significant advantages of microarrays, we are also in the process of creating medium density (~20 000 compounds) spotted peptoid microarrays.

## Bivalent Binding Agents Provide a Potential Shortcut to High Affinity

As mentioned above, library-derived synthetic protein ligands rarely, if ever, exhibit the binding properties of a good antibody and classical procedures for optimization are far too tedious for proteomics-scale projects. Therefore, no matter how efficient lead compound discovery becomes, there remains the significant hurdle of gaining an extra 3−4 orders of magnitude in binding affinity without sacrificing specificity or limiting throughput. An approach that has attracted many investigators is to use bivalency as a shortcut to high affinity. Two or more lead compounds are isolated and linked together to form a high-affinity ligand (Figure 3). For instance, Fesik and colleagues screened a collection of small molecules against FK506 binding protein (FKBP) using NMR and isolated several ligands for the protein.[23] The structures of these protein−small molecule complexes were then solved by NMR, and the structural data was used to design an appropriate spacer to link two noncompeting ligands. This approach generated a chimeric molecule that bound to FKBP with a low nanomolar $K_D$.

Another interesting and higher-throughput approach was reported by Ellman and colleagues.[24] They screened a small molecule collection of aldehyde-derived methyloxime derivatives against the cSrc kinase using an enzyme inhibition assay. Several weak inhibitors were identified. Rather than relying on structural models, they employed

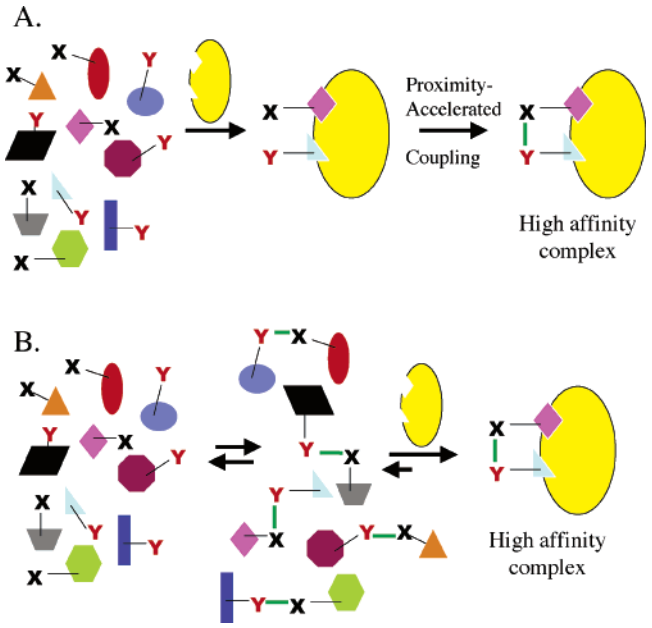


**FIGURE 4.** Novel routes to bivalent protein-binding molecules. In panel A, library components are provided with functional groups (X and Y) the reaction of which is favorable thermodynamically but slow kinetically. Binding to proximal surfaces on the protein will accelerate the coupling of suitable molecules to form a bivalent ligand in situ. Shown in panel B, in dynamic combinatorial chemistry, a kinetically facile coupling reaction is employed. Binding of a subset of possible products will shift the equilibrium in favor of the best ligands.

a combinatorial approach to screen all possible pairs of inhibitors linked by methylene chains of various lengths. This was again done using an enzyme inhibition assay but with lower levels of the linked molecules, thus demanding more potent inhibitors. A 90 nM ($K_I$) Src inhibitor was discovered. An elegant feature of the Ellman approach is that the set of "monomers" that was screened initially was derived from diverse aldehydes, all of which were then transformed into methyloximes, the same functional group that was employed to link the lead compounds to create potential bivalent inhibitors. This strategy reduced the possibility that the linkage itself would interfere with binding of the lead molecules to Src.

Another notable advance in the area of bivalent compounds is the use of the protein target as a template for linking together two binding moieties. The general idea behind this approach is to screen pools of molecules that have functionality suitable to allow them to link covalently to one another if they are brought into close proximity by binding to a protein target. There are two versions of this scheme. One is to employ functional groups that participate in a reaction that is highly favorable thermodynamically, but sluggish kinetically, thus demanding an accelerating effect of protein templating (Figure 4A). A striking proof of principle for this approach was reported by Sharpless and his colleagues, who employed "click chemistry" to discover a subpicomolar inhibitor of acetylcholine esterase (AchE).[25] AchE is known to have two binding sites in close proximity. Two known site-specific inhibitors, one for each site, were modified with alkyl azides and alkyl acetylenes of varying chain lengths. Azides and acetylenes undergo 1,3-dipolar cycloaddition to yield 1,2,3-triazoles in a reaction that is highly favorable thermodynamically but very slow. The binary mixtures were incubated in the presence of the enzyme and assayed for the formation of the triazole product by mass spectrometry. No product was formed in the absence of the enzyme at room temperature.

A fundamentally different strategy that employs protein templating is dynamic combinatorial chemistry.[26,27] In this approach, a kinetically facile but highly reversible reaction between library components is employed, and the protein target is then employed to "bleed" the complex set of equilibrium processes toward the most stable bivalent molecule−protein complexes (Figure 4B). An illustrative example of this type is the isolation of a nanomolar inhibitor of AchE starting from a small collection of building blocks containing hydrazide and aldehyde functionality.[28]

## Mixed Element Capture Agents (MECAs): A Simple Class of Synthetic, High-Affinity Protein Capture Agents

We have developed two approaches to the isolation of high-affinity bivalent ligands that are quite different from those described above. The first is designed to provide compounds that, when displayed on a suitable surface, would capture target proteins from a complex mixture with high affinity and specificity, allowing the synthetic analogue of an immunoprecipitation. As mentioned above, standard routes to the creation of bivalent ligands must deal with the issue of finding or designing a relatively optimized linker for two binding elements, which is as difficult as finding a good binder in the first place. For the special case of an immobilized protein capture agent, we suspected that it might be possible to ignore linker optimization if two noncompetitive ligands were co-immobilized at high density on the surface. Statistically, some fraction of all possible pairs of the molecules on the surface should be positioned appropriately to function as a high-affinity bivalent ligand (see Figure 5). This idea was supported by the observation that peptides that bind homodimeric target proteins with only micromolar dissociation constants in solution act as subnanomolar capture agents when immobilized on high-capacity beads.[29]

To test this idea on a monomeric protein, a fusion was made between maltose-binding protein and a fragment of Mdm2. This was a convenient model system since peptides were available in our laboratory that bind each protein with $K_D$s of about 30 $\mu$M. We initially attempted to co-immobilize the two binding peptides on a various surfaces, but this was complicated by the different reactivities and solubilities of the molecules, resulting in a nonstatistical distribution of molecules on the surface (unpublished results). Therefore, we settled on the simpler strategy of synthesizing the peptides as a linear fusion with a single serine arbitrarily chosen to link them (Figure 5).

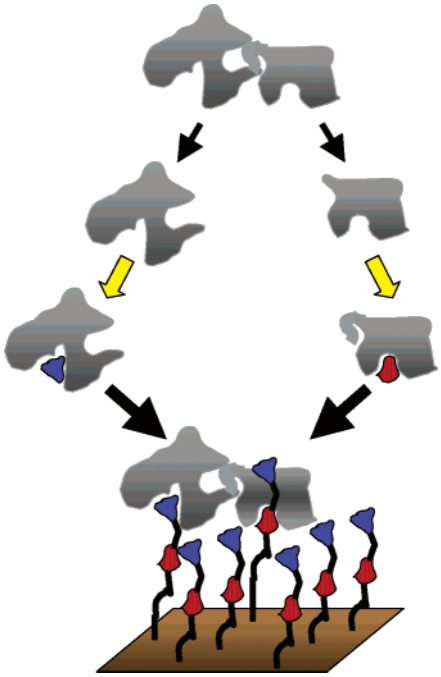

**FIGURE 5.** A potential high-throughput strategy for the development of mixed element capture agents (MECAs). Individual domains of a multidomain protein would be expressed separately and screened against combinatorial libraries or compound collections to identify modest-affinity ligands. These compounds (the blue and red shapes) would then be coupled and immobilized at high density to create a high-affinity MECA.

As expected, this was a poor choice for an optimal linker and the solution $K_D$ of the fusion peptide for the MBP−Mdm2 protein was similar to that of the individual peptides.[30] But when the fusion peptide was displayed on Tentagel, very high-affinity binding to the fusion protein was observed. "Pull-down" revealed that even at subnanomolar protein concentrations MBP−Mdm2 were retained efficiently by the fusion peptide (Figure 5).[30] As will be reported elsewhere, we have subsequently demonstrated that mixed element capture agents (MECAs) can also be made to native proteins (H. Olivos, K. Bachhawat-Sikder and T. Kodadek, manuscript in preparation). It is noteworthy that in all of our experiments, the peptides have been affixed to high-capacity surfaces via a long PEG linker, and this flexibility may be important in allowing collaboration between immobilized molecules.

The MECA concept is extremely simple but has important implications for the creation of high-affinity synthetic capture agents. Most proteins are comprised of separable domains. Thus, one could imagine expressing two or more of these domains and screening each independently against a combinatorial library using the methods described above, which reliably produce hits with micromolar dissociation constants. These can then be combined to form a high-affinity MECA against the native protein (Figure 5). If this strategy proves general, it would dramatically simplify efforts to obtain large numbers of high-affinity capture ligands for the creation of protein-detecting microarrays and other proteomics tools.

## Rapid Elaboration of Lead Compounds into Higher-Affinity Binding Agents

MECAs are obviously limited to the special case of immobilized protein capture agents. An alternative approach that could provide high-affinity solution ligands from a single lead is shown schematically in Figure 6A. A library of oligomers is capped with the lead compound. This conjugated library is then screened against the target protein under conditions determined empirically to be too challenging for the lead alone to provide a clean hit. The concept behind this approach is that there will be a binding element within the oligomer library capable of recognizing a second site on the target protein, which could be proximal or distal to the lead compound binding site (Figure 6A). We imagined that in most cases not all of the units that comprise the oligomer would be required to make this putative second contact. For example, suppose that in a library of octameric peptides capped by a lead molecule a three amino acid unit provided the second binding element. In a comprehensive library, this unit would be represented many times in the library with different numbers and types of amino acids between it and the lead compound (Figure 6A). Since the screen is done under conditions designed to register only very high-affinity hits, the hope is that only molecules with the putative second binding element linked to the lead with residues that provided a linker of appropriate length and geometry would come through the screen. In other words, we imagined that this procedure would provide high-affinity bivalent protein-binding compounds by combining a screen for a second binding element and an optimal linker into a single step. Whereas this "extension" library approach has been employed previously in cases where structural information revealed the presence of a binding pocket immediately adjacent to the lead molecule binding site on the protein,[31] it seemed to us that the approach could be employed more generally even in the absence of structural information.

We have recently reported a test of this concept using a low-affinity ($K_D$ = 220 $\mu$M) Mdm2 protein-binding chalcone as a lead[32] (Figure 6B). The chalcone was appended to the end of a library of approximately 78 000 octameric peptoids displayed on Tentagel resin through coupling to a lysine monomer. This library was prescreened using the COPAS instrument to remove the most intensely autofluorescent beads. The remaining 66 862 beads were then screened against fluorescein-labeled Mdm2 protein under harsh buffer conditions (a 10 000-fold excess of unlabeled *Escherichia coli* proteins in a buffer containing 1 M NaCl and 1% Tween-20) that were far too stringent for the chalcone alone to retain Mdm2. After being washed, the beads were poured into the sorter. Only four beads (0.006% of the total) displayed fluorescence intensity well above that of the bulk population, demonstrating the stringency of the screen.[32] Edman sequencing showed that two of the isolated compounds were identical (Figure 6B) and the other two were closely related, highly basic sequences. Subsequent isothermal

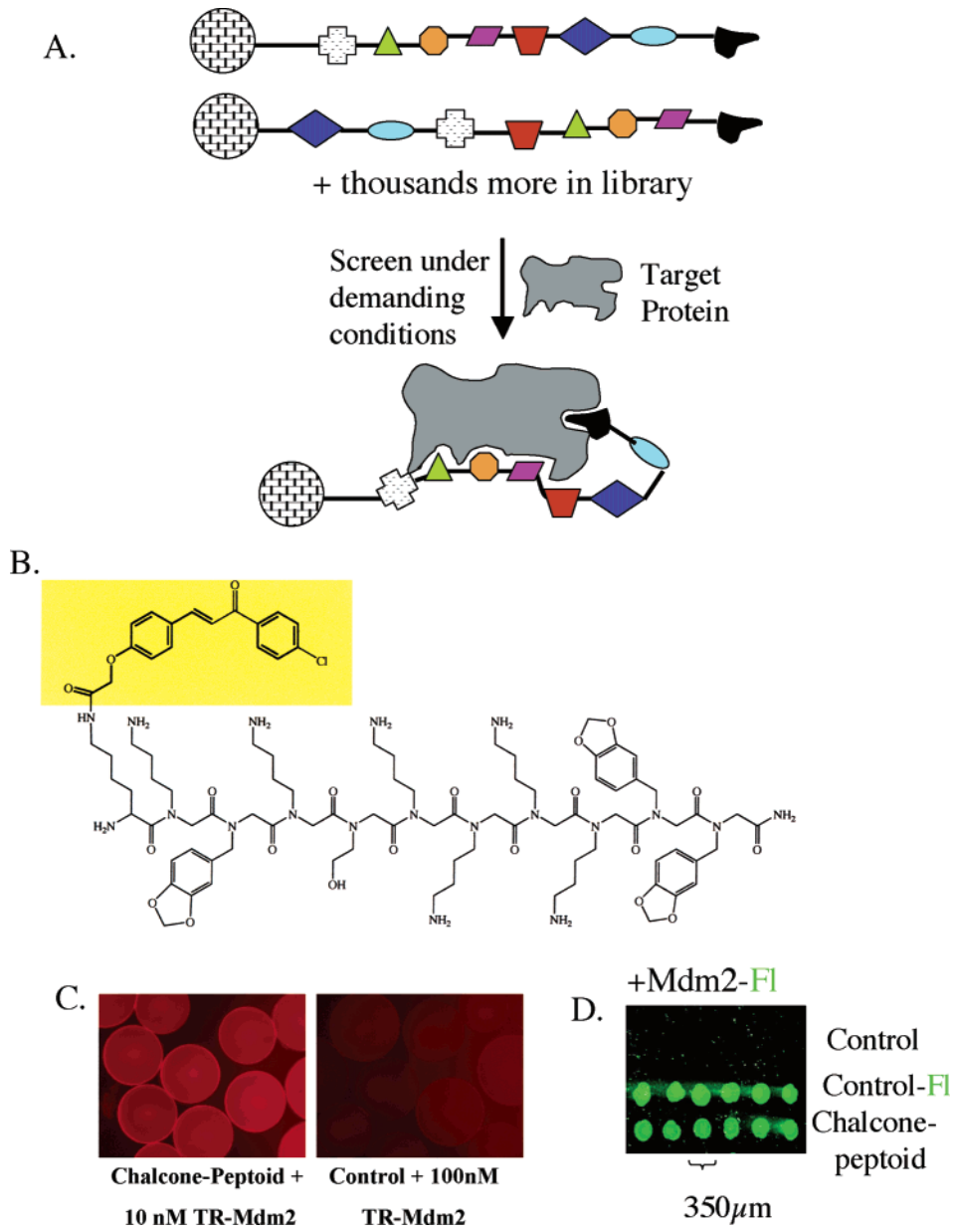


**FIGURE 6.** Rapid identification of high-affinity derivatives of a lead compound: (A) schematic representation of a scheme for the discovery of high-affinity ligands based on a single lead molecule; (B) a chalcone−peptoid chimera resulting from a stringent screen of ~67 000 peptoids capped with the chalcone−lysine residue shown; (C) photomicrograph of a binding assay in which 10 nM Texas Red-labeled Mdm2 protein was mixed with a 100-fold excess of unlabeled bacterial proteins, followed by incubation with Tentagel beads displaying either the chalcone−peptoid chimera shown in panel B or a control peptoid; (D) photomicrograph of a peptoid microarray experiment in which labeled MBP−Mdm2 protein was retained from a crude extract by the immobilized chalcone−peptoid chimera shown in panel B.[32]

calorimetry studies with one of the resynthesized hits showed that it formed an Mdm2 complex with a $K_D$ of 1.3 $\mu$M, a 170-fold improvement over the lead chalcone.[32] When immobilized on Tentagel beads (Figure 6C) or on chemically modified glass slides (Figure 6D), the chalcone−peptoid conjugate acted as a specific and efficient Mdm2 capture agent.

Another example of this approach was reported that provided a high-affinity ubiquitin capture agent derived by capping a library of seven amino acid peptides with a lead peptide derived from a primary screen.[32] An ELISA-like assay showed that the apparent affinity for the Tentagel-immobilized peptide for ubiquitin was approximately 6 nM.[32] While this approach to bivalent ligands remains to be explored extensively, the early results (also see ref 33) are quite promising.

## Summary

The goal of creating synthetic molecules capable of substituting for antibodies in many biological applications is challenging. As reviewed here, we and others have provided data suggesting that bivalent ligands may provide the most effective general strategy to access these compounds. However, we are in early days. Very few of the molecules derived from such studies bind their tar-

get with a $K_D$ of 10 nM or below. Furthermore, the specificity of binding remains to be determined in the majority of cases. Also, the generality of any specific screening technique must be determined through the analysis of many more protein targets. In the course of these efforts, the critical issue of increasing throughput by 100- to 1000-fold must also be addressed if this work is to have a serious impact on proteomics science. Nonetheless, while the challenges are formidable, the utility of "synthetic antibody equivalents" would be so great that we anticipate that this field will be the focus of intense activity for years to come.

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