method using paper sheets is a low cost methodology that has gained success notably within the area of proteomics and epitope mapping due to simple synthesis equipment and standard SPPS conditions. It allows for the rapid synthesis of several hundred to a few thousand peptides and a simple screening procedure that can identify the ones with highest binding affinity. The latter chip-based method utilizes photolithographic techniques and noncommercially available building blocks to construct the arrays. Due to this large number of peptides, both technologies offer the opportunity to use simple positional scanning or even combinatorial design strategies (Figure 9.6).

9.3.3 De Novo Design

The term "de novo design" essentially describes a design process leading to a biologically active, novel, peptide or protein without an active structural template to begin from. While most biopharmaceuticals (excluding antibodies) to date are modified versions of nature's repertoire of peptides and proteins, the erythropoietin mimetic peginesatide (hematide) from Affymax, and a peptide-Fc fusion thrombopoietin mimetic, romiplostim by Amgen, were the first de novo designed protein mimetics to be approved by FDA. Hematide binds and activates the erythropoietin receptor leading to red blood cell production, and romiplostin stimulates the formation of blood platelets with a prolonged effect due to FcRn recycling. Despite the subsequent withdrawal from market of hematide, both cases are hallmarks of what can be accomplished by combining de novo *design* using large combinatorial libraries to identify an active peptide, and using peptide or protein drug design to make a suitable drug based on the peptide.

9.3.3.1 Biological Approaches

In the very early 1990s, the random peptide phage display approach appeared and literally revolutionized de novo design. It is a technology where hundreds of millions to billions of random (or semi-random) peptides are displayed on the surface of bacteriophages. A simple screening scheme then allows for the identification of peptides binding to the immobilized protein target. Various formats have since appeared that do not employ phages but are done directly on ribozymes as an example. In addition to peptides, larger proteins may be expressed. Indeed, antibody-based phage display has revolutionized the way pharmaceutical companies identify protein–protein interaction inhibitors using various protein scaffolds. Serving as vast source of potential drug molecules, these huge libraries are screened against the target of interest. Notably, within the area of cancer and inflammation, a number of blockbuster antibody drugs have reached the market demonstrating the success of this approach. Less attention has been given to using peptide display technology as the direct drug. One reason for this is that protein scaffolds often offer the identification of binders with a higher affinity than linear peptide-based binders. However, cyclic peptides as well as bicyclic variants may also offer ligands that can bind to protein surfaces with good affinity. Another reason is that many receptors of drug interest are G-protein-coupled receptors (GPCR or 7TM-type), and those specific receptors are not readily screened in these phage libraries. Finally, it is believed that although high-affinity peptides may be found against a target receptor, the half-life is much shorter than the corresponding half-life of, e.g., a humanized antibody.

9.3.3.2 Synthesis Approach

One approach first published in 1991 that is similar to the phage method was termed one-bead-one-peptide library or one-bead-one-compound (OBOC), since the random peptides were displayed on small beads. The method relies on the synthesis of millions of random (or semi-random) compounds synthesized by a method termed split-and-mix synthesis. This ensures that each and every bead in the library only displays one unique sequence. The whole set of beads comprising the library can then be screened against a biotin or fluorescent-labeled target receptor and only the active beads are retrieved from the library and analyzed. As opposed to the phage technology the synthetic bead library approach only allows the synthesis of shorter peptides, and not larger proteins. On the other