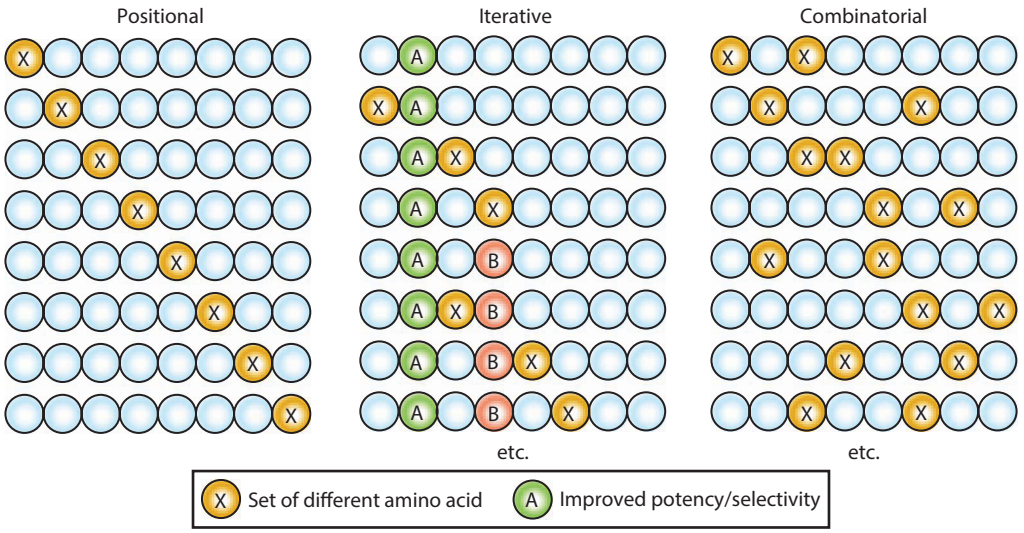

FIGURE 9.6 Schematic representation of different approaches for replacement analysis. In positional scanning (e.g., Ala-scan) each position is replaced in the context of the native sequence. In the iterative approach positions are replaced in the context of a new optimized sequence and in the combinatorial approach replacement analysis is done in a random approach allowing structural changes from more than one residue.

the binding activity. It is however challenging to unambiguously determine if a particular residue is responsible for the direct interaction with the receptor, or if it is more important for maintaining the correct fold and positioning of other residues. In situations where the peptide or protein is an agonist which is most often the case when investigating endogenous peptides and proteins, it is important to determine both the potency (agonist efficacy) and the binding affinity. Although these tend to follow the same trend, some amino acid replacements may affect the binding very little, but may have a dramatic impact on potency, whereas changes that have a hugely negative effect on the affinity also negatively affect potency as well. The in vitro data acquired from an Ala-scan can point to the regions of the peptide or protein where the interaction with the receptor occurs, however, it is important to realize that the Ala-scan only reveals the effect when the side chain is absent and not *per se* the type of surroundings that the side chains are engaged in. However, once the positions of critical residues are known in the peptide or protein, then more dedicated efforts can be initiated in order to enhance potency or selectivity by the introduction of other residues by an iterative approach (Figure 9.6). Peptide chemists have hundreds of amino acids that are commercially available to work with. But it is also important to mention that backbone modification, including the introduction of rigidity to the backbone, can be an important part of this molecular toolbox.

9.3.2.2 Multi-Parallel Synthesis and Peptide Arrays

The SPPS approach in plate format is an attractive tool for the parallel synthesis of hundreds or even thousands of different peptide analogs. It is much less labor intensive to synthesize many analogs in parallel as compared to recombinant expressions of proteins, and by integrating the SPPS format with in vitro pharmacology screening assays vast in vitro biological data sets can be obtained that facilitate the drug design process. Since residues in close proximity can affect each other, combinatorial replacement analysis may reveal particular combinations that are not readily identified by other means (Figure 9.6). For certain ligand–receptor interactions, even more complex synthesis and screening methods can be used such as paper (SPOT array) and chip-based arrays. In situations where the target receptor exists as a free soluble and stable receptor, peptide arrays offer the opportunity to screen thousands of peptide analogs with respect to binding affinity. The former spot array