Accurate MALDI-TOF/TOF sequencing of one-bead-one-compound peptide libraries with application to the identification of multiligand protein affinity agents using in situ click chemistry screening.

Lee SS, Lim J, Tan S, Cha J, Yeo SY, Agnew HD, Heath JR.

Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, The Nanos, Singapore 138669. sslee@ibn.a-star.edu.sg

Abstract

Combinatorial one-bead-one-compound (OBOC) peptide libraries are widely used for affinity screening, and the sequencing of peptides from hit beads is a key step in the process. For rapid sequencing, CNBr cleavage of the peptides from the beads, followed by de novo sequencing by MALDI-TOF/TOF, is explored. We report on a semiautomated sequencing algorithm and validate it through comparison against Edman degradation sequencing. The initial 44% sequencing success rate of the standard de novo sequencing software was improved to nearly 100%. The sequencing algorithm incorporates existing knowledge of amino acid chemistry and a new strategy for differentiating isobaric amino acids. We tested the algorithm by using MALDI-TOF/TOF to identify a peptide biligand affinity agent against the protein bovine carbonic anhydrase II, starting from comprehensive one-bead-one-compound peptide libraries comprised of non-natural and artificial amino acid components and using the strategy of in situ click/OBOC library screening.