77. Shotgun scanning the streptavidin−biotin interaction.
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The streptavidin−biotin interaction is among the strongest naturally occurring noncovalent protein−ligand interactions. The combinatorial alanine-scanning method shotgun scanning was used to determine the functional contribution of the 38 C-terminal residues of streptavidin to biotin binding. Two phage-displayed protein libraries were constructed in which amino acids were mutated to alanine or conserved as wild-type. The library pools were subjected to three rounds of selection for functional streptavidin variants that bind biotin. Demonstrating that selection conditions identified streptavidin variants with high affinity for biotin, wild-type streptavidin accounted for approximately 8% of the selected variants. Shotgun scanning results were largely consistent with previous site-directed mutagenesis studies for the few residues probed by conventional mutagenesis. Results from shotgun scanning also demonstrate the importance of previously unreported hydrophobic residues contributing direct contacts with biotin, forming the beta barrel structure of streptavidin, and providing interactions at the tetramer interface.