244d Solution Phase Reaction Microarrays for Protease Substrate Specificity Determination

Dhaval N. Gosalia, Cleo Salisbury, Jonathan Ellman, and Scott L. Diamond

Proteases regulate numerous biological processes with a degree of specificity often dictated by the amino acid sequence of the substrate cleavage site. To map protease/substrate interactions, a 722-member library of fluorogenic protease substrates of the general format Ac-Ala-X-X-(Arg/Lys)-coumarin was synthesized (X = all natural amino acids except cysteine) and microarrayed, along with fluorescent calibration standards, in glycerol nanodroplets on glass slides. Specificities of 19 serine proteases (Bovine thrombin, salmon thrombin, human thrombin plasmin, , upa, activated protein C, plasma kallikrein, factor VIIa, factor IXab, factor Xa, factor XIa and factor a XIIa, activated complement C1s, C1r and D, tryptase, trypsin, subtilisin Carlsberg and cathepsin G) and 11 papain-like cysteine proteases (cathepsin B, H, K, L, S and V, rhodesain, papain, chymopapain, ficin and stem bromelain) were obtained from 129,960 separate microarray fluorogenic reactions (722 substrates x 30 different proteases x 6 replicates). This is the first comprehensive study to report the substrate specificity of rhodesain, a papain-like cysteine protease expressed by Trypanasoma brucei rhodesiense, a parasitic protozoa responsible for causing sleeping sickness. Rhodesain displayed a strong P2 preference for Leu, Val, Phe, Tyr in both the P1 = Lys and Arg libraries. Solution phase microarrays facilitate protease/substrate specificity profiling in a rapid manner with minimal peptide library or enzyme usage.