

University of Groningen

Engineering specificity and activity of thermolysin-like proteases from *Bacillus*

de Kreij, Arno

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2001

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

de Kreij, A. (2001). *Engineering specificity and activity of thermolysin-like proteases from Bacillus*. s.n.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

1. **Dahiyat, B.I., Sarisky, C.A., and Mayo, S.L.** *De novo* protein design: towards fully automated sequence selection (1997) *J.Mol.Biol.* **273**, 789-796
2. **Stemmer, W.P.C.** Rapid evolution of a protein in vitro by DNA shuffling (1994) *Nature* **370**, 389-391
3. **Kuchner, O. and Arnold, F.H.** Directed evolution of enzyme catalysts (1997) *Trends Biotechnol* **15**, 523-530
4. **Priest, F.G.** (1993) Systematics and ecology of *Bacillus*. In: *Bacillus subtilis* and other Gram-positive bacteria., American Society for Microbiology Washington D.C., pp. 3-16
5. **Thorne, C.B.** (1993) *Bacillus anthracis*. In: *Bacillus subtilis* and other Gram-positive bacteria, American Society for Microbiology Washington D.C., pp. 113-124
6. **Aronson, A.I.** (1993) Insecticidal toxins. In: *Bacillus subtilis* and other Gram-positive bacteria, American Society for Microbiology Washington D.C., pp. 953-964
7. **Ferrari, E., Jarnagin, A.S., and Schmidt, B.F.** (1993) Commercial production of extracellular enzymes. In: *Bacillus subtilis* and other Gram-positive bacteria., American Society for Microbiology Washington D.C., pp. 917-938
8. **Sonenshein, A.L., Hoch, J.A., and Losick, R.** *Bacillus subtilis* and other Gram-positive bacteria. Biochemistry, physiology and molecular genetics. (1993) **1st**
9. **Msadek, T., Kunst, F., and Rapoport, G.** (1993) Two component regulatory systems. In: *Bacillus subtilis* and other gram-positive bacteria; biochemistry, physiology, and molecular genetics., American Society for Microbiology Washington, D.C., pp. 729-745
10. **Spizizen, J.** Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate (1958) *Proc.Natl.Acad.Sci.USA* **44**, 1072-1078
11. **Dubnau, D.** (1993) Genetic exchange and homologues recombination. In: *Bacillus subtilis* and other gram-positive bacteria: biochemistry, physiology, and molecular genetics., American Society for Microbiology Washington, D.C., pp. 555-584
12. **Youngman, P.** (1993) Transposons and their applications. In: *Bacillus subtilis* and other Gram-positive bacteria, American Society for Microbiology Washington D.C., pp. 585-596
13. **Perego, M.** (1993) Integrational vectors for genetic manipulation in *Bacillus subtilis*. In: *Bacillus subtilis* and other Gram-positive bacteria, American Society for Microbiology Washington D.C., pp. 625-644
14. **Janni re, L., Gruss, A., and Ehrlich, S.D.** (1993) Plasmids. In: *Bacillus subtilis* and other Gram-positive bacteria, American Society for Microbiology Washington D.C., pp. 625-644
15. **Grossman, A.D.** Genetic networks controlling the initiation of sporulation and the development of genetic competence in *Bacillus subtilis* (1995) *Annu.Rev.Genet.* **29**, 477-508
16. **Chang, S. and Cohen, S.N.** High frequency transformation of *Bacillus subtilis* protoplasts by plasmid DNA (1979) *Mol.Gen.Genet.* **168**, 111-115
17. **Haima, P., Bron, S., and Venema, G.** The effect of restriction on shotgun cloning and plasmid stability in *Bacillus subtilis* Marburg (1987) *Mol.Gen.Genet.* **209**, 335-342
18. **Kunst, F., Ogasawara, N., and Moszer, I.** The complete genome sequence of the gram-positive bacterium *Bacillus subtilis* (1997) *Nature* **390**, 249-256
19. **Harwood, C.R.** (1989) Introduction to the biotechnology of *Bacillus*. In: *Bacillus*, Plenum Press New York, pp. 1-4
20. **Tjalsma, H., Bolhuis, A., Jongbloed, J.D.H., Bron, S., and van Dijl, J.M.** Signal peptide-dependent protein transport in *Bacillus subtilis*: a genome-based survey of the secretome (2000) *Microbiol Mol Biol Rev* **64**, 515-547
21. **Pugsley, A.P.** Translocation of proteins with signal sequences across membranes. (1990) *Curr.Opin.Cell.Biol.* **2**, 609-616
22. **Tjalsma, H., Bolhuis, A., van Roosmalen, M.L., Wiegert, T., Schumann, W., Broekhuizen, C.P., Quax, W.J., Venema, G., Bron, S., and van Dijl, J.M.** Functional analysis of the secretory precursor processing machinery of *Bacillus subtilis*: Identification of a eubacterial homolog of archaeal and eukaryotic signal peptidases (1998) *Genes Dev.* **12**, 2318-2331
23. **Godfrey, T. and West, S.** (1996) Industrial enzymology. In: *Industrial enzymology*, Macmillan London, pp. 3-
24. **Rao, M.B., Tanksale, A.M., Ghatge, M.S., and Deshpande, V.V.** Molecular and biotechnological aspects of microbial proteases (1998) *Microbiol Mol Biol Rev* **62**, 597-635
25. **Gerhartz, W.** Enzymes in industry : production and applications (1990) 1-321
26. **Isowa, Y., Ohmori, M., Ichikawa, T., Mori, K., Nonaka, Y., Kihara, K., Oyama, K., Satoh, H., and Nishimura, S.** The thermolysin-catalyzed condensation reactions of N-substituted aspartic and glutamic acids with phenylalanine alkyl esters (1979) *Tetrahedron lett.* **28**, 2611-2612
27. **Nakanishi, K., Takeuchi, A., and Matsuno, R.** Long-term continuous synthesis of aspartame precursor in a column reactor with an immobilized thermolysin (1990) *Appl.Microbiol.Biotechnol.* **32**, 633-636
28. **He, X.-S., Br uckner, R., and Doi, R.H.** The protease genes of *Bacillus subtilis* (1991) *Res.Microbiol.* **142**, 797-803
29. **Pero, J. and Sloma, A.** (1993) Proteases. In: *Bacillus subtilis* and other Gram-positive bacteria., American Society for Microbiology Washington D.C., pp. 939-952
30. **Yang, M.Y., Ferrari, E., and Henner, D.J.** Cloning of the neutral protease gene of *Bacillus subtilis* and the use of the cloned gene to create an in vitro-derived deletion mutation (1984) *J.Bacteriol.* **21**-
31. **Webb, E.C.** Enzyme nomenclature 1992 (1992) **1st**, 1-862
32. **Hartley, B.S.** (1960) *Annu.Rev.Biochem.* **29**, 45-
33. **Rawlings, N.D. and Barrett, A.J.** Evolutionary families of peptidases (1993) *Biochem.J.* **290**, 205-218
34. **Rawlings, N.D. and Barrett, A.J.** Evolutionary families of metallopeptidases (1995) *Methods Enzymol* **248**, 183-228
35. **O'Donohue, M.J., Roques, B.P., and Beaumont, A.** Cloning and expression in *Bacillus subtilis* of the *npr* gene from *Bacillus thermoproteolyticus* Rokko coding for the thermostable metalloprotease thermolysin (1994) *Biochem.J.* **300**, 599-603
36. **Matsubara, H. and Feder, J.** (1971) Other Bacterial, Mold, and Yeast Proteases. In: *The Enzymes*, Academic Press New York, pp. 721-792
37. **Titani, K., Hermodson, M.A., Ericsson, L.H., Walsh, K.A., and Neurath, H.** Amino Acid Sequence of Thermolysin. Isolation and Characterization of the Fragments Obtained by Cleavage with Cyanogen Bromide (1972) *Biochemistry* **11**, 2427-2435

Chapter 8

38. **Colman, P.M., Jansonius, J.N., and Matthews, B.W.** The structure of thermolysin: an electron density map at 2.3Å resolution (1972) *J.Mol.Biol.* **70**, 701-724
39. **Veltman, O.R., Vriend, G., Berendsen, H.J.C., van den Burg, B., Venema, G., and Eijsink, V.G.H.** A single calcium binding site is crucial for the calcium-dependent thermal stability of thermolysin-like proteases (1998) *Biochemistry* **37**, 5312-5319
40. **Rawlings, N.D. and Barrett, A.J.** Families of serine peptidases (1994) *Methods Enzymol* **244**, 19-61
41. **Siezen, R.J. and Leunissen, J.A.M.** Subtilases: the superfamily of subtilisin-like serine proteases (1997) *Protein Sci* **6**, 501-523
42. **Rawlings, N.D.** (1998) Introduction: clan SB containing the subtilisin family. In: Academic Press, pp. 284-288
43. **Veltman, O.R., Vriend, G., van den Burg, B., Hardy, F., Venema, G., and Eijsink, V.G.H.** Engineering thermolysin-like proteases whose stability is largely independent of calcium (1997) *FEBS Lett* **405**, 241-244
44. **Neet, K.E.** Enzyme catalytic power minireview series (1998) *J.Biol.Chem.* **273**, 25527-25528
45. **Pauling, L.** (1946) *Chem.Eng.News.* **24**, 1375-1377
46. **Storm, D.R. and Koshland Jr, D.E.** (1970) *Proc.Natl.Acad.Sci.USA* **66**, 445-457
47. **Mesecar, A.D., Stoddard, B.L., and Koshland Jr, D.E.** Orbital steering in the catalytic power of enzymes: small structural changes with large catalytic consequences (1997) *Science* **277**, 202-206
48. **Cannon, W.R. and Benkovic, S.J.** Solvation, reorganization, energy and biological catalysis (1998) *J.Biol.Chem.* **273**, 26257-26260
49. **Cleland, W.W., Frey, P.A., and Gerlt, J.A.** The low barrier hydrogen bond in enzymatic catalysis (1998) *J.Biol.Chem.* **273**, 25529-25532
50. **Warschel, A.** Electrostatic origin of the catalytic power of enzymes and the role of preorganized active sites (1998) *J.Biol.Chem.* **273**, 27035-27038
51. **Barrett, A.J., Rawlings, N.D., and Woessner, J.F.** (1998) In: Handbook of proteolytic enzymes, Academic Press, pp. 350-369
52. **Weaver, L.H., Kester, W.R., and Matthews, B.W.** A crystallographic study of the complex of phosphoramidon with thermolysin. A model for the presumed catalytic transition state and for the binding of extended substrates (1977) *J.Mol.Biol.* **114**, 119-132
53. **Pauptit, R.A., Karlsson, R., Picot, D., Jenkins, J.A., Niklaus-Reimer, A.-S., and Jansonius, J.N.** Crystal structure of neutral protease from *Bacillus cereus* refined at 3.0 Å resolution and comparison with the homologous but more thermostable enzyme thermolysin. (1987) 525-537
54. **Stark, W., Pauptit, R.A., Wilson, K.S., and Jansonius, J.N.** The structure of neutral protease from *Bacillus cereus* at 0.2-nm resolution (1992) *FEBS Eur.J.Biochem.* **984**, 1-11
55. **Thayer, M.M., Flaherty, K.M., and McKay, D.B.** Three-dimensional structure of the elastase of *Pseudomonas aeruginosa* at 1.5 Å resolution (1991) *J.Biol.Chem.* **266**, 2864-2871
56. **Banbula, A., Potempa, J., Travis, J., Fernandez, C.C., Mann, K., Huber, R., Bode, W., and Medrano, F.J.** Amino-acid sequence and three-dimensional structure of the *Staphylococcus aureus* metalloproteinase at 1.72 Å resolution (1998) *Structure* **6**, 1193
57. **Gaucher, J.F., Selkti, M., Tiraboschi, G., Roques, B.P., Tomas, A., and Fournié-Zaluski, M.-C.** Crystal structures of α -mercaptoacyldipeptides in the thermolysin active site: structural parameters for a Zn monodentation or bidentation in metalloendopeptidases (1999) *Biochemistry* **38**, 12569-12576
58. **Hausrath, A.C. and Matthews, B.W.** Redetermination and refinement of the complex of benzylsuccinic acid with thermolysin and its relation to the complex with carboxypeptidase A. (1994) *J.Biol.Chem.* **269**, 18839-18842
59. **Holmes, M.A., Tronrud, D.E., and Matthews, B.W.** Structural analysis of the inhibition of thermolysin by an active-site-directed Irreversible inhibitor (1983) *Biochemistry* **22**, 236-240
60. **Jin, Y. and Kim, D.H.** Inhibition stereochemistry of hydroxamate inhibitors for thermolysin (1998) *Bioorg Med Chem Lett* **8**, 3518
61. **Kester, W.R. and Matthews, B.W.** Crystallographic study of the binding of dipeptide inhibitors to thermolysin: implications for the mechanism of catalysis (1977) *Biochemistry* **16**, 2506-2516
62. **Hangauer, D.G., Monzingo, A.F., and Matthews, B.W.** An interactive computer graphics study of thermolysin-catalyzed peptide cleavage and inhibition by N-carboxymethyl dipeptides (1984) *Biochemistry* **23**, 5730-5741
63. **Mock, W.L. and Aksamawati, M.** Binding to thermolysin of phenolate-containing inhibitors necessitates a revised mechanism of catalysis (1994) *Biochem.J.* **302**, 57-68
64. **Mock, W.L. and Stanford, D.J.** Arazoformyl dipeptide substrates for Thermolysin. Confirmation of a reverse protonation catalytic mechanism. (1996) *Biochemistry* **35**, 7369-7377
65. **Beaumont, A., O'Donohue, M.J., Paredes, N., Rousselet, N., Assicot, M., Bohuon, C., Fournié-Zaluski, M.-C., and Roques, B.P.** The role of Histidine 231 in thermolysin-like enzymes. (1995) *J.Biol.Chem.* **28**, 16803-16808
66. **Toma, S., Campagnoli, S., de Gregoriis, E., Gianna, R., Margarit, I., Zamai, M., and Grandi, G.** Effect of Glu-143 and His-231 substitutions on the catalytic activity and secretion of *Bacillus subtilis* neutral protease (1989) *Protein Eng* **2**, 359-364
67. **Nielsen, J.E.** (2000) pH activity profiles. In: The pH dependence of α -amylase catalysis, Heidelberg, pp. 55-62
68. **Davoodi, J., Wakarchuk, W.W., Campbell, R.L., Carey, P.R., and Surewicz, W.K.** Abnormally high pK_a of an active-site glutamic acid residue in *Bacillus circulans* xylanase (1995) *Eur.J.Biochem.* **232**, 839-843
69. **Fang, T.-Y. and Ford, C.** Protein engineering of *Aspergillus awamori* glucoamylase to increase its pH optimum (1998) *Protein Eng* **11**, 383-388
70. **Joshi, M.D., Sidhu, G., Pot, I., Brayer, G.D., Withers, S.D., and McIntosh, L.** Hydrogen bonding and catalysis: a novel explanation for how a single amino acid substitution can change the pH optimum of a glycosidase (2000) *J.Mol.Biol.* **299**, 255-279
71. **Takase, K.** Effect of mutation of an amino acid residue near the catalytic site on the activity of *Bacillus stearothermophilus* alpha-amylase (1993) *Eur.J.Biochem.* **211**, 899-902
72. **Nielsen, J.E., Beier, L., Otzen, D., Borchert, T.V., Frantzen, H.B., Andersen, K.V., and Svendsen, A.** Electrostatics in the active site of an alpha-amylase (1999) *Eur.J.Biochem.* **264**, 816-824
73. **Bakir, U., Coutinho, P.M., Sullivan, P.A., Ford, C., and Reilly, P.J.** Cassette mutagenesis of *Aspergillus awamori* glucoamylase near its general acid residue to probe its catalytic and pH properties (1993) *Protein Eng* **6**, 939-946
74. **Russell, A.J. and Fersht, A.R.** Rational modification of enzyme catalysis by engineering surface charge (1987) *Nature* **328**, 496-500
75. **Shafferman, A., Ordentlich, A., Barak, D., Kronman, C., Ber, R., Bino, T., Ariel, N., Osman, R., and Velan, B.** Electrostatic attraction by surface charge does not contribute to the catalytic efficiency of acetylcholinesterase (1994) *EMBO* **13**, 3448-3455

76. **Eijsink, V.G.H., van den Burg, B., and Vriend, G.** Engineering a hyperstable enzyme by manipulation of early steps in the unfolding process. (2001) *Biocatalysis and Biotransformation in press*.
77. **Veltman, O.R., Vriend, G., Hardy, F., Mansfeld, J., van den Burg, B., Venema, G., and Eijsink, V.G.H.** Mutational analysis of a surface area that is critical for the thermal stability of thermolysin-like proteases. (1997) *Eur.J.Biochem.* **248**, 433-440
78. **Vriend, G. and Eijsink, V.G.H.** Prediction and analysis of structure, stability and unfolding of thermolysin-like proteases (1993) *J Comput Aided Mol Des* **7**, 367-396
79. **Eijsink, V.G.H., Veltman, O.R., Aukema, W., Vriend, G., and Venema, G.** Structural determinants of the stability of thermolysin-like proteinases (1995) *Nat Struct Biol* **2**, 374-379
80. **Eijsink, V.G.H., Vriend, G., van der Vinne, B., Hazes, B., van den Burg, B., and Venema, G.** Effects of changing the interaction between subdomains on the thermostability of *Bacillus* neutral proteases (1992) *Proteins* **14**, 224-236
81. **Vriend, G., Berendsen, H.J.C., van den Burg, B., Venema, G., and Eijsink, V.G.H.** Early steps in the unfolding of thermolysin-like proteases (1998) *J.Biol.Chem.* **273**, 35074-35077
82. **Mansfeld, J., Vriend, G., Dijkstra, B.W., Veltman, O.R., van den Burg, B., Venema, G., Ulbrich-Hofmann, R., and Eijsink, V.G.H.** Extreme stabilization of a thermolysin-like protease by an engineered disulfide bond (1997) *J.Biol.Chem.* **272**, 11152-11156
83. **Mitchinson, C. and Wells, J.A.** Protein Engineering of Disulfide Bonds in Subtilisin BPN' (1989) *Biochemistry* **28**, 4807-4815
84. **van den Burg, B., Vriend, G., Veltman, O.R., Venema, G., and Eijsink, V.G.H.** Engineering an enzyme to resist boiling (1998) *Proc.Natl.Acad.Sci.USA* **95**, 2056-2060
85. **Arnold, F.H.** Enzyme engineering reaches the boiling point (1998) *Proc.Natl.Acad.Sci.USA* **95**, 2035-2036
86. **Holland, D.R., Tronrud, D.E., Pley, H.W., Flaherty, K.M., Stark, W., Jansonius, J.N., McKay, D.B., and Matthews, B.W.** Structural comparison suggests that thermolysin and related neutral proteases undergo hinge-bending motion during catalysis. (1992) *Biochemistry* **31**, 11310-11316
87. **Faber, H.R. and Matthews, B.W.** A mutant T4 lysozyme displays five different crystal conformations. (1990) *Nature* **348**, 263-266
88. **Zhang, X.-J., Wozniak, J.A., and Matthews, B.W.** Protein flexibility and adaptability seen in 25 crystal forms of T4 lysozyme. (1995) *J.Mol.Biol.* **250**, 527-525
89. **van Aalten, D.M.F., Amadei, A., Linssen, A.B.M., Eijsink, V.G.H., Vriend, G., and Berendsen, H.J.C.** The essential dynamics of thermolysin: confirmation of the hinge-bending motion and comparison of simulations in vacuum and water (1995) *Proteins* **22**, 45-54
90. **van Aalten, D.M.F., Findlay, J.B.C., Amadei, A., and Berendsen, H.J.C.** Essential dynamics of the cellular retinol-binding protein- evidence for ligand-induced conformational changes (1995) *Prot.Engng* **8**, 1129-1135
91. **van Aalten, D.M.F., Jones, P.C., de Sousa, M., and Findlay, J.B.C.** Engineering protein mechanics: inhibition of concerted motions of the cellular retinol binding protein by site-directed mutagenesis. (1997) *Prot.Engng.* **10**, 31-37
92. **Matthews, B.W., Colman, P.M., Jansonius, J.N., Titani, K., Walsh, K.A., and Neurath, H.** Three dimensional structure of thermolysin (1972) *Nature* **238**, 41-43
93. **Holmes, M.A. and Matthews, B.W.** Structure of thermolysin refined at 1.6 Å resolution (1982) *J.Mol.Biol.* **160**, 623-639
94. **Schechter, I. and Berger, A.** On the size of the active site in proteases (1967) *Biochem.Biophys.Res.Commun.* **27**, 157-162
95. **Mei, H.-C., Liaw, Y.-C., Li, Y.-C., Wang, D.-C., Takagi, H., and Tsai, Y.C.** Engineering subtilisin YaB: restriction of substrate specificity by the substitution of Gly124 and Gly151 with Ala (1998) *Protein Eng* **11**, 109-117
96. **Bech, L.M. and Breddam, K.** Significance of hydrophobic S4-P4 interactions in Subtilisin 309 from *Bacillus lentus* (1993) *Biochemistry* **32**, 2845-2852
97. **Rheinacker, M., Baker, G., Eder, J., and Fersht, A.R.** Engineering a novel specificity in Subtilisin BPN' (1993) *Biochemistry* **32**, 1199-1203
98. **Bech, L.M., Sørensen, S.B., and Breddam, K.** Mutational replacements in subtilisin 309: Val104 has a modulating effect on the P₄ substrate preference (1992) *Eur.J.Biochem.* **209**, 869-874
99. **Perona, J.J., Hedstrom, L., Rutter, W.J., and Fletterick, R.J.** Structural origins of substrate determination in trypsin and chymotrypsin (1995) *Biochemistry* **34**, 1489-1499
100. **Perona, J.J. and Craik, C.S.** Structural basis of substrate specificity in the serine proteases (1995) *Protein Sci* **4**, 337-360
101. **Perona, J.J. and Craik, C.S.** Evolutionary divergence of substrate specificity within the chymotrypsin-like serine protease fold (1997) *J.Biol.Chem.* **272**, 29987-29990
102. **Matthews, B.W., Sigler, P.B., Henderson, R., and Blow, D.M.** Three-dimensional structure of tosyl-alpha-chymotrypsin. (1967) *Nature* **214**, 652-656
103. **Ruhlmann, A., Kukla, D., Schwager, P., Bartels, K., and Huber, R.** Structure of the complex formed by bovine trypsin and bovine pancreatic trypsin inhibitor. Crystal structure determination and stereochemistry of the contact region. (1973) *J.Mol.Biol.* **77**, 417-437
104. **Graf, L., Jansco, A., Szilagyi, L., Hegyi, G., Pinter, K., Naray-Szabo, G., Hepp, J., Medzihradzky, K., and Rutter, W.J.** Electrostatic complementarity within the substrate-binding pocket of trypsin. (1988) *Proc.Natl.Acad.Sci.USA* **85**, 4961-4965
105. **Hedstrom, L., Szilagyi, L., and Rutter, W.J.** Converting trypsin to chymotrypsin: the role of surface loops. (1992) *Science* **255**, 1249-1253
106. **Bone, R., Silen, J.L., and Agard, D.A.** Structural plasticity broadens the specificity of an engineered protease. (1989) *Nature* **339**, 191-195
107. **Bone, R., Fujishige, A., Kettner, C.A., and Agard, D.A.** Structural basis for broad specificity in alpha-lytic protease mutants. (1991) *Biochemistry* **30**, 10388-10398
108. **Fierke, C.A. and Jencks, W.P.** Two functional domains of coenzyme A activate catalysis by coenzyme A transferase. Pantetheine and adenosine 3'-phosphate 5'-diphosphate. (1986) *J.Biol.Chem.* **261**, 7603-7606
109. **Olesen, K. and Breddam, K.** Increase of the P₁ Lys/Leu substrate preference of carboxypeptidase Y by rational design based on known primary and tertiary structures of serine carboxypeptidases (1995) *Biochemistry* **34**, 15689-15699
110. **Olesen, K., Mortensen, U.H., Aasmul-Olesen, S., Kielland-Brandt, M.C., Remington, S.J., and Breddam, K.** The activity of carboxypeptidase Y toward substrates with basic P₁ amino acid residues is drastically increased by mutational replacement of leucine 178 (1994) *Biochemistry* **33**, 11121-11126
111. **Sørensen, S.B. and Breddam, K.** The specificity of carboxypeptidase Y may be altered by changing the hydrophobicity of the S'₁ binding pocket (1997) *Protein Sci* **6**, 2227-2232

Chapter 8

112. Olesen, K. and Breddam, K. Substrates with charged P1 residues are efficiently hydrolyzed by serine carboxypeptidases when S3-P1 interactions are facilitated (1997) *Biochemistry* **36**, 12235-12241
113. Sørensen, S.B., Meldal, M., Bech, L.M., and Breddam, K. Mutational replacements of the amino acid residues forming the hydrophobic S4 binding pocket of subtilisin 309 from *Bacillus lentus*. (1993) *Biochemistry* **32**, 8994-8999
114. Feder, J. Studies on the specificity of *Bacillus subtilis* neutral protease with synthetic substrates (1968) *Biochemistry* **6**, 2088-2093
115. Morihara, K. and Tsuzuki, H. Thermolysin: kinetic study with oligopeptides (1970) *Eur.J.Biochem.* **15**, 374-380
116. DeSantis, G. and Jones, J.B. Chemical modification of enzymes for enhanced functionality (1999) *Curr.Opin.Biotechnol* **10**, 324-330
117. DeSantis, G., Berglund, P., Stabile, M.R., Gold, M., and Jones, J.B. Site-directed mutagenesis combined with chemical modification as a strategy for altering the specificity of the S₁ and S₁' pockets of Subtilisin *Bacillus lentus* (1998) *Biochemistry* **37**, 5968-5973
118. DeSantis, G., Shang, X., and Jones, J.B. Toward tailoring the specificity of the S₁ pocket of subtilisin *B.lentus*: chemical modification of mutant enzymes as a strategy for removing specificity limitations (1999) *Biochemistry* **38**, 13391-13397
119. Davis, B.G., Shang, X., DeSantis, G., Bott, R.R., and Jones, J.B. The controlled introduction of multiple negative charge at single amino acid sites in subtilisin *Bacillus lentus* (1999) *Bioorg Med Chem Lett* **7**, 2293-2301
120. Davis, B.G., Lloyd, R.C., and Jones, J.B. Controlled site-selective protein glycosylation for precise glycan structure-catalytic activity relationships. (2000) *Bioorg Med Chem* **8**, 1527-1535
121. Davis, B.G., Lloyd, R.C., and Jones, J.B. Controlled site-selective glycosylation of proteins by a combined site-directed mutagenesis chemical modification approach. (1998) *J.Org.Chem.* **63**, 9614-9615
122. Barrett, A.J., Rawlings, N.D., and Woessner, J.F. Handbook of proteolytic enzymes (1998) **1**, 1-1666
123. Wetmore, D.R., Wong, S.-L., and Roche, R.S. The efficiency of processing and secretion of the thermolysin-like neutral protease from *Bacillus cereus* does not require the whole prosequence, but does depend on the nature of the amino acid sequence in the region of the cleavage site (1994) *Mol.Microbiol.* **12**, 747-759
124. Wetmore, D.R., Wong, S.-L., and Roche, R.S. The role of the pro-sequence in the processing and secretion of ther thermolysin-like neutral protease from *Bacillus cereus* (1992) *Mol.Microbiol.* **6**, 1593-1604
125. O'Donohue, M.J. and Beaumont, A. The roles of the prosequence of thermolysin in enzyme inhibition and folding *in Vitro* (1996) *J.Biol.Chem.* **271**, 26477-26481
126. Feder, J., Keay, L., Garrett, L.R., Cirulis, N., Moseley, M.H., and Wildi, B.S. *Bacillus cereus* neutral protease (1971) *Biochim.Biophys.Acta* **251**, 74-78
127. Thayer, M.M., Flaherty, K.M., and McKay, D.B. Three-Dimensional Structure of the Elastase of *Pseudomonas aeruginosa* at 1.5-Å Resolution (1991) *J.Biol.Chem.* **266**, 2864-2871
128. Morihara, K. Pseudolysin and other pathogen endopeptidases of thermolysin family (1995) *Methods Enzymol* **248**, 242-253
129. Domann, E., Leimeister-Wachter, M., Goebel, W., and Chakraborty, T. Molecular cloning, sequencing, and identification of a metalloprotease gene from *Listeria monocytogenes* that is species specific and physically linked to the listeriolysin gene. (1991) *Infect Immun* **59**, 65-72
130. Dhanaraj, V., Ye, Q.-Z., Johnson, L.L., Hupe, D.J., Ortwine, D.F., Dunbar, J.B.jr., Rubin, J.R., Pavlovsky, A., Humblet, C., and Blundell, T.L. Designing inhibitors of the metalloproteinase superfamily: comparative analysis of representative structures (1996) *Drug Des Discov* **13**, 3-14
131. Lennarz, W.J. and Strittmatter, W.J. Cellular functions of metallo-endoproteinases (1991) *Biochim.Biophys.Acta* **1071**, 149-158
132. Marie-Claire, C., Ruffet, E., Antoniczak, S., Beaumont, A., O'Donohue, M.J., Roques, B.P., and Fournié-Zaluski, M.-C. Evidence by site-directed mutagenesis that arginine 203 of thermolysin and arginine 717 of neprilysin (Neutral Endopeptidase) play equivalent critical roles in substrate hydrolysis and inhibitor binding (1997) *Biochemistry* **36**, 13984-13945
133. Feder, J. and Schuck, J.M. Studies on the *Bacillus subtilis* Neutral-Protease- and *Bacillus thermoproteolyticus* thermolysin-catalyzed hydrolysis of dipeptide substrates (1970) *Biochemistry* **14**, 2784-2791
134. Kunugi, S., Koyasu, A., Kitayaki, M., Takahashi, S., and Oda, K. Kinetic characterization of the neutral protease vimelysin from *Vibrio* sp. T1800 (1996) *Eur.J.Biochem.* **241**, 368-373
135. Kubo, M. and Imanaka, T. Cloning and nucleotide sequence of the highly thermostable neutral protease gene from *Bacillus stearothermophilus* (1988) *J.Gen.Microbiol.* **134**, 1883-1892
136. Fujii, M., Takagi, M., Imanaka, T., and Aiba, S. Molecular Cloning of a Thermostable Neutral Protease Gene from *Bacillus stearothermophilus* in a vector plasmid and its expression in *Bacillus stearothermophilus* and *Bacillus subtilis* (1983) *Journal of Bacteriology* **154**, 831-837
137. Tran, L., Wu, X., and Wong, S. Cloning and Expression of a Novel Protease Gene Encoding an Extracellular Neutral Protease from *Bacillus subtilis* (1991) *J.Bacteriol.* **173**, 6364-6372
138. Sabat, A., Kosowska, K., Poulsen, K., Kasproicz, A., Sekowska, A., van den Burg, B., Travis, J., and Potempa, J. Two allelic forms of the aureolysin gene (aur) within staphylococcus aureus. (2000) *Infect Immun* **68**, 973-976
139. Sarkar, G. and Sommer, S.S. The "megaprimer" method of site directed mutagenesis (1990) *BioTechniques* **8**, 404-407
140. Vriend, G. WHAT IF: A Molecular modeling and drug design program (1990) *J.Mol.Graph.* **8**, 52-56
141. Mosimann, S., Meleshko, R., and James, M.N.G. A critical assessment of comparative molecular modeling of tertiary structures of proteins. (1995) *Proteins* **23**, 301-317
142. Veltman, O.R., Vriend, G., Hardy, F., Mansfeld, J., van den Burg, B., Venema, G., and Eijsink, V.G.H. Analysis of a calcium binding surface loop critical for the stability of the thermolysin-like protease of *Bacillus stearothermophilus*. (1997) *Eur.J.Biochem.* **248**, 433-440
143. Hardy, F., Vriend, G., van der Vinne, B., Frigerio, F., Grandi, G., Venema, G., and Eijsink, V.G.H. The effect of engineering surface loops on the thermal stability of *Bacillus subtilis* neutral protease (1994) *Protein Eng* **7**, 425-430
144. van den Burg, B., Eijsink, V.G.H., Stulp, B.K., and Venema, G. One-step affinity purification of *Bacillus* neutral proteases using Bacitracin-silica (1989) *J.Biochem.Biophys.Meth.* **18**, 209-220
145. Hunter, A. and Downs, C.E. (1945) *J.Biol.Chem.* **157**, 427-446
146. Holt, C. and Sawyer, L. Primary and predicted secondary structures of the caseins in relation to their biological functions (1988) *Protein Eng* **2**, 251-259

147. van den Burg, B., de Kreijl, A., van der Veek, P., Mansfeld, J., and Venema, G. Characterization of a novel stable biocatalyst obtained by protein engineering (1999) *Biotechnol Appl Biochem* **30**, 35-40
148. Veltman, O.R., Eijnsink, V.G.H., Vriend, G., de Kreijl, A., Venema, G., and van den Burg, B. Probing catalytic hinge bending motions in thermolysin-like proteases by glycine → alanine mutations (1998) *Biochemistry* **37**, 5305-5311
149. Matthews, B.W. Structural and genetic analysis of protein stability (1993) *Annu.Rev.Biochem.* **62**, 139-160
150. Holland, D.R., Hausrath, A.C., Juers, D., and Matthews, B.W. Structural analysis of zinc substitutions in the active site of thermolysin. (1995) *Protein Sci* **4**, 1955-1965
151. Amadei, A., Linssen, A.B.M., and Berendsen, H.J.C. Essential dynamics of proteins (1993) *Proteins* **17**, 412-425
152. Takagi, M., Imanaka, T., and Aiba, S. Nucleotide sequence and promoter region for the neutral protease gene from *Bacillus stearothermophilus* (1985) *J.Bacteriol.* **163**, 824-831
153. Veltman, O.R., Vriend, G., Middelhoven, P.J., van den Burg, B., Venema, G., and Eijnsink, V.G.H. Analysis of structural determinants of the stability of thermolysin-like proteases by molecular modelling and site-directed mutagenesis. (1996) *Protein Eng* **9**, 1181-1189
154. Takagi, M. and Imanaka, T. Addition of a methyl group changes both the catalytic velocity and thermostability of the neutral protease from *Bacillus stearothermophilus* (1989) *FEBS Lett* **254**, 43-46
155. Matthews, B.W. Structural Basis of the Action of thermolysin and related zinc peptidases (1988) *Acc.Chem.Res.* **21**, 333-340
156. Argos, P., Rossmann, M.G., Grau, U.M., Zuber, H., Frank, G., and Tratschin, J.D. Thermal Stability and Protein Structure (1979) *Biochemistry* **18**, 5698-5703
157. Menéndez-Arias, L. and Argos, P. Engineering Protein Thermal Stability Sequence Statistics Point to Residue Substitutions in α -Helices (1989) *J.Mol.Biol.* **206**, 397-406
158. Matthews, B.W. Genetic and structural analysis of the protein stability problem (1987) *Biochemistry* **26**, 6885-6888
159. Matthews, B.W., Nicholson, H., and Becktel, W.J. Enhanced protein thermostability from site-directed mutations that decrease the entropy of unfolding (1987) *Proc.Natl.Acad.Sci.USA* **84**, 6663-6667
160. Ganter, C. and Pluckthun, A. Glycine to alanine substitutions in helices of glyceraldehyde-3-phosphate dehydrogenase: effects on stability (1990) *Biochemistry* **29**, 9395-9402
161. Margarit, I., Campagnoli, S., Frigerio, F., Grandi, G., De Fillippis, V., and Fontana, A. Cumulative stabilizing effects of glycine to alanine substitutions in *Bacillus subtilis* neutral protease (1992) *Protein Eng* **5**, 543-550
162. Serrano, L., Neira, J., Sancho, J., and Fersht, A.R. Effect of alanine versus glycine in α -helices on protein stability (1992) *Nature* **356**, 453-455
163. Blaber, M., Zhang, X.-J., and Matthews, B.W. Structural basis of amino acid α helix propensity (1993) *Science* **260**, 1637-1640
164. Gerstein, M., Lesk, A.M., and Chothia, C. Structural mechanisms for domain movements in proteins. (1994) *Biochemistry* **33**, 6739-6749
165. Dixon, M.M. and Matthews, B.W. Is gamma-Chymotrypsin a Tetrapeptide Acyl-Enzyme Adduct of α -Chymotrypsin? (1989) *Biochemistry* **28**, 7033-7038
166. Mchaurab, H.S., Joon Oh, K., Fang, C.J., and Hubbell, W.L. Conformation of T4 lysozyme in solution. Hinge-bending motion and the substrate-induced conformational transition studied by site-directed spin labeling. (1997) *Biochemistry* **36**, 307-316
167. Arnold, G.E. and Ornstein, R.L. Protein hinge bending as seen in molecular dynamics simulations of native and M6I mutant T4 lysozymes (1997) *Biopolymers* **41**, 533-544
168. Johnson, K.A. Conformational coupling in DNA polymerase fidelity. (1993) *Annu.Rev.Biochem.* **62**, 685-713
169. Yan, B.X. and Sun, Y.Q. Glycine residues provide flexibility for enzyme active sites (1997) *J.Biol.Chem.* **272**, 3190-3194
170. Dahlquist, F.W., Long, J.W., and Bigbee, W.L. Role of calcium in the thermal stability of thermolysin (1976) *Biochemistry* **15**, 1103-1111
171. Kidokoro, S., Miki, Y., Endo, K., Wada, A., Nagao, H., Miyake, T., Aoyama, A., Yoneya, T., Kai, K., and Ooe, S. Remarkable activity enhancement of thermolysin mutants. (1995) *FEBS Lett* **367**, 73-76
172. Imanaka, T., Shibasaki, M., and Takagi, M. A new way of enhancing the thermostability of proteases (1986) *Nature* **324**, 695-697
173. Jencks, W.P. Binding energy, specificity, and enzymic catalysis: the circe effect. (1975) *Adv.Enzymol.Relat.Areas.Mol.Biol.* **43**, 219-241
174. Schechter, I. and Berger, A. (1967) *Biochem.Biophys.Res.Comm.* **27**, 157-162
175. Izquierdo, M.C. and Stein, R.L. Mechanistic studies of thermolysin (1990) *J.Am.Chem.Soc.* **112**, 6054-6062
176. de Kreijl, A., Venema, G., and van den Burg, B. Substrate specificity in the highly heterogeneous M4 peptidase family is determined by a small subset of amino acids (2000) *J.Biol.Chem.* **275**, 31115-31120
177. Hardy, F., Vriend, G., Veltman, O.R., van der Vinne, B., Venema, G., and Eijnsink, V.G.H. Stabilization of *Bacillus stearothermophilus* neutral protease by introduction of prolines (1993) *FEBS Lett* **317**, 89-92
178. Eijnsink, V.G.H., Vriend, G., van den Burg, B., Venema, G., and Stulp, B.K. Contribution of the C-terminal amino acid to the stability of *Bacillus subtilis* neutral protease. (1990) *Protein Eng* **4**, 99-104
179. Feder, J. A spectrophotometric assay for neutral protease (1968) *Biochem.Biophys.Res.Comm.* **32**, 326-332
180. Chinae, G., Padron, G., Hooft, R.W.W., Sander, C., and Vriend, G. The use of position specific rotamers in model building by homology (1995) *Proteins* **23**, 301-317
181. Venclovas, C., Zemla, A., Fidelis, K., and Moulton, J. Some measures of comparative performance in the three CASPs (1999) *Proteins* **S3**, 231-237
182. Tronrud, D.E., Monzingo, A.F., and Matthews, B.W. Crystallographic structural analysis of phosphoramidates as inhibitors and transition-state analogs of thermolysin (1986) *Eur.J.Biochem.* **157**, 261-268
183. Holden, H.M., Tronrud, D.E., Monzingo, A.F., Weaver, L.H., and Matthews, B.W. Slow and fast binding inhibitors of thermolysin display different modes of binding. (1987) *Biochemistry* **26**, 8542-
184. Tronrud, D.E., Holden, H.M., and Matthews, B.W. Structures of two thermolysin-inhibitor complexes that differ by a single hydrogen bond (1987) *Science* **235**, 571-574
185. Takagi, H., Ohtsu, I., and Nakamoris, S. Construction of novel subtilisin E with high specificity, activity and productivity through multiple amino acid substitutions. (1997) *Prot.Engng* **10**, 985-989

Chapter 8

186. **Rheinnecker, M., Eder, J., Pandey, P., and Fersht, A.R.** Variants of Subtilisin BPN' with altered specificity profiles (1994) *Biochemistry* **33**, 221-225
187. **Takagi, H., Maeda, T., Ohtsu, I., Tsai, Y.C., and Nakamoris, S.** Restriction of substrate specificity of subtilisin E by introduction of a side chain into a conserved glycine residue. (1996) *FEBS Lett* **395**, 127-132
188. **Hudaky, P., Kaslik, G., Venekei, I., and Graf, L.** The differential specificity of chymotrypsin A and B is determined by amino acid 226. (1999) *Eur.J.Biochem.* **259**, 528-533
189. **Holmquist, B., Blumberg, S., and Vallee, B.L.** Superactivation of neutral proteases: acylation with N-Hydroxysuccinimide esters. (1976) *Biochemistry* **15**, 4675-4680
190. **Tanaka, T., Matsuzawa, H., and Ohta, T.** Engineering of S₂ site of Aqualysin I; alteration of P₂ specificity by excluding P₂ side chain (1998) *Biochemistry* **37**, 17402-17407
191. **Jackson, S.E. and Fersht, A.R.** Contribution of long-range electrostatic interactions to the stabilization of the catalytic transition state of the serine protease Subtilisin BPN' (1993) *Biochemistry* **32**, 13909-13916
192. **Nielsen, J.E., Andersen, K.V., Honig, B., Hooft, R.W.W., Klebe, G., Vriend, G., and Wade, R.C.** Improving macromolecular electrostatics calculations. (1999) *Protein Eng* **12**, 657-662
193. **Carter, P.J., Winter, G., Wilkinson, A.J., and Fersht, A.R.** The use of double mutants to detect structural changes in the active site of the tyrosyl-tRNA synthetase (*Bacillus stearothermophilus*) (1984) *Cell* **38**, 835-840
194. **Albeck, S. and Schreiber, G.** Biophysical characterization of the interaction of the beta-lactamase TEM-1 with its protein inhibitor BLIP. (1999) *Biochemistry* **38**, 11-21
195. **Albeck, S., Unger, R., and Schreiber, G.** Evaluation of direct and cooperative contributions towards the strength of buried hydrogen bonds and salt bridges. (2000) *J.Mol.Biol.* **298**, 503-520
196. **Wilkinson, A.J., Fersht, A.R., Blow, D.M., and Winter, G.** Site-directed mutagenesis as a probe of enzyme structure and catalysis: tyrosyl-tRNA synthetase cysteine-35 to glycine-35 mutation. (1983) *Biochemistry* **22**, 3581-3586
197. **Fersht, A.R., Matouschek, A., and Serrano, L.** The folding of an enzyme I. Theory of protein engineering analysis of stability and pathway of protein folding (1992) *J.Mol.Biol.* **224**, 771-782
198. **Fersht, A.R.** (1998) The basic equations of enzyme kinetics. In: *Structure and mechanism in protein science*, W.H. Freeman and Company New York, pp. 103-131
199. **Nicholls, A. and Honig, B.** A Rapid Finite Difference Algorithm, Utilizing Successive Over-Relaxation to Solve the Poisson-Boltzmann Equation., (1991) *J.Comp.Chem.* **12**, 435-445
200. **Honig, B. and Nicholls, A.** Classical electrostatics in biology and chemistry. (1995) *Science* **268**, 1144-1149
201. **Russell, A.J., Thomas, P.G., and Fersht, A.R.** Electrostatic effects on modification of charged groups in the active site cleft of subtilisin by protein engineering (1987) *J.Mol.Biol.* **193**, 803-813