

Claims

1. A method of increasing the intestinal protease stability of a polypeptide consisting of a heavy chain variable domain, wherein the heavy chain variable domain comprises three complementarity determining regions (CDR1-CDR3) and four framework regions, wherein the method comprises the step of substituting:
 - (a) at least one lysine residue in CDR1, CDR2 and/or CDR3 with at least one histidine residue,
and/or
 - (b) at least one arginine residue in CDR1, CDR2 and/or CDR3 with at least one histidine residue.
2. The method according to claim 1, wherein the substitutions increase the protease stability of the polypeptide in the intestinal tract, such as in the small and/or large intestine, such as in the duodenum, jejunum, ileum cecum, colon, rectum and/or anal canal, relative to a corresponding polypeptide not having said histidine substitutions.
3. The method according to either claim 1 or 2, wherein the substitutions increase the protease stability of the polypeptide in a model of the intestinal tract, such as in the small and/or large intestine, such as in the duodenum, jejunum, ileum cecum, colon, rectum and/or anal canal, relative to a corresponding polypeptide not having said histidine substitutions.
4. The method according to claim 3 wherein the model of the intestinal tract is the Standard Human Faecal Supernatant Intestinal Tract Model.
5. The method according to any one of claims 1 to 4, wherein the protease stability of the polypeptide is increased by at least 5%, more suitably 30%, more suitably 50%, relative to a corresponding polypeptide not having said histidine substitutions, after 1 hour incubation in the Standard Human Faecal Supernatant Intestinal Tract Model.
6. The method according to any one of claims 1 to 5, wherein the one or more proteases are selected from the group consisting of trypsin, chymotrypsin, MMPs, cathepsin, enteropeptidase, host inflammatory proteases, proteases originating from gut commensal microflora and/or pathogenic bacteria actively secreted and/or released by lysis of microbial cells, and proteases originating from pathogenic bacteria, such as *C. difficile*-specific proteases.
7. The method according to any one of claims 1 to 6 wherein the potency of the polypeptide is substantially the same as the potency of a corresponding polypeptide not having said histidine substitutions.

8. The method according to any one of claims 1 to 6 wherein the EC50 of the polypeptide is increased by no more than 400% relative to a corresponding polypeptide not having said histidine substitutions.
- 5 9. The method according to any one of claims 1 to 8, wherein the substitutions are introduced synthetically.
10. The method according to claim 9, wherein the substitutions are introduced by a method selected from the group consisting of: error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, Gene Site Saturation Mutagenesis (GSSM), synthetic ligation reassembly (SLR), recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, ensemble mutagenesis, chimeric nucleic acid multimer creation, or a combination thereof.
- 10 15 20 11. The method according to any one of claims 1 to 10, wherein the substitutions are not introduced by V(D)J recombination or somatic mutation.
- 25 12. The method according to any one of claims 1 to 11, wherein each lysine and/or arginine residue in CDR1, CDR2 and/or CDR3 has been substituted with at least one histidine residue each.
- 30 13. The method according to any one of claims 1 to 12, wherein the polypeptide is a VHH or a VH..
14. The method according to any one of claims 1 to 13, wherein the polypeptide binds to a target accessible via the intestinal tract.
- 35 15. The method according to any one of claims 1 to 14, wherein the polypeptide binds to a target within the intestinal tract.