

Claims

1. A method of increasing the intestinal stability of a polypeptide comprising an immunoglobulin chain variable domain, wherein the immunoglobulin 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. A method of making a polypeptide comprising an immunoglobulin chain variable domain, wherein the immunoglobulin 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

wherein the polypeptide has increased intestinal stability 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 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.
4. The method according to any one of claims 1 to 3, wherein the substitutions increase the 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.
5. The method according to any one of claims 1 to 4 wherein the model of the intestinal tract is the Standard Human Faecal Supernatant Intestinal Tract Model.
6. The method according to any one of claims 1 to 5, wherein the 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.

7. The method according to any one of claims 1 to 6, wherein the substitutions increase the stability of the polypeptide to one or more proteases produced in the small or large intestine, relative to a corresponding polypeptide not having said histidine substitutions.
- 5 8. The method according to any one of claims 1 to 7, 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.
- 10 9. The method according to any one of claims 1 to 8 wherein the potency of the polypeptide is substantially the same as the potency of a corresponding polypeptide not having said histidine substitutions.
- 15 10. The method according to any one of claims 1 to 9 wherein the EC50 of the polypeptide is increased by no more than 400% relative to a corresponding polypeptide not having said histidine substitutions.
- 20 11. The method according to any one of claims 1 to 10, wherein the substitutions are introduced synthetically.
- 25 12. The method according to claim 11, 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.
- 30 13. The method according to any one of claims 1 to 12, wherein the substitutions are not introduced by V(D)J recombination or somatic mutation.
- 35 14. The method according to any one of claims 1 to 13, wherein the at least one lysine residue is present in a window defined as the second third of CDR1 and/or the second third of CDR2 and/or the second third of CDR3.
- 40 15. The method according to any one of claims 1 to 14, wherein the at least one arginine residue is present in a window defined as the second third of CDR1 and/or the second third of CDR2 and/or the second third of CDR3.
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16. The method according to any one of claims 1 to 15, wherein each lysine and/or arginine residue in CDR1, CDR2 and/or CDR3 has been substituted with at least one histidine residue each.
- 5 17. The method according to any one of claims 1 to 16, wherein the polypeptide consists of an immunoglobulin chain variable domain.
- 10 18. The method according to any one of claims 1 to 17, wherein the polypeptide is an antibody, a modified antibody containing additional antibody binding regions, an antibody fragment such as an scFv, a Fab fragment, a F(ab')₂ fragment or an immunoglobulin chain variable domain such as a VHH, a VH, a VL, a V-NAR.
- 15 19. The method according to any one of claims 1 to 18, wherein the polypeptide binds to a target accessible via the intestinal tract.
20. The method according to any one of claims 1 to 19, wherein the polypeptide binds to a target within the intestinal tract.