hepatitis C virus NS3 helicase domain for application in a chemiluminescent immunoassay.

In WO 2007/077008 chimeric fusion proteins with superior chaperone and folding activities are reported. The conversion of FKBP12 into a powerful catalyst of protein folding by insertion of a chaperone domain is reported by Knappe et al. (Knappe, T.A., et al., J. Mol. Biol. 368 (2007) 1458-1468).

Summary ~~of the Invention~~

The fusion polypeptides as reported herein are fusion polypeptides comprising i) one or more parts derived from one, i.e. the same, or different polypeptides with PPIase activity or belonging to the FKBP family, and ii) an immunogenic polypeptide inserted therein in between.

The fusion polypeptides as reported herein can be used for the immunization of an animal for the generation of antibodies specifically binding to the immunogenic polypeptide inserted into the one or more parts derived from the one or more polypeptides with PPIase activity or belonging to the FKBP family.

There is~~One aspect as~~ reported herein ~~is~~ a fusion polypeptide according to formula I

$$NH_2\text{-}S_2\text{-}X_1\text{-}S_1\text{-}COOH \qquad \text{(formula I)}$$

wherein

X_1 comprises either a random amino acid sequence or an amino acid sequence derived from a first polypeptide,

S_2 and S_1 are non-overlapping amino acid sequences derived from a second polypeptide, and

- denotes a peptide bond,

wherein the second polypeptide is a polypeptide with peptidyl-prolyl cis/trans-isomerase activity (PPIase activity) or is derived from the FKBP domain family.

One aspect as reported herein is a fusion polypeptide comprising a polypeptide according to formula I

$$NH_2\text{-}S_2\text{-}X_1\text{-}S_1\text{-}COOH \qquad \text{(formula I)}$$

wherein

X_1 comprises either a random amino acid sequence or an amino acid sequence derived from a first polypeptide,

S_2 and S_1 are non-overlapping amino acid sequences derived from a second polypeptide, and

- denotes a peptide bond,

wherein the second polypeptide is a polypeptide with peptidyl-prolyl cis/trans-isomerase activity (PPIase activity) or is derived from the FKBP-fold domain family, and

wherein X_1 is inserted in place of the insert-in-flap-domain (IF-domain) of the second polypeptide.

It has been found that with the fusion polypeptides as reported herein antibodies, which specifically bind to internal (so called hidden or buried) epitopes of a (naturally occurring) amino acid sequence, can be obtained. Internal epitopes are not accessible in classical immunization protocols as these are e.g. only accessible

upon activation and concomitant conformational changes of the antigenic polypeptide (such as a receptor). Furthermore, antibodies can be obtained that specifically bind to immunogenic polypeptides, which are derived from structures otherwise difficult to be provided in sufficient amount or quality.

The fusion polypeptides as reported herein are chimeric, recombinant polypeptides that can be used for peptide, secondary and tertiary structure display e.g. in methods for antibody screening/selection or for epitope mapping as well as immunogen for the production of antibodies specifically binding the presented antigenic amino acid sequence or secondary structure. The polypeptides as reported herein can be recombinantly produced, are thermodynamically stable, monomeric and soluble in aqueous solutions.

There is~~One aspect as~~ reported herein ~~is~~ a fusion polypeptide according to formula II

$$NH_2\text{-}S_4\text{-}X_2\text{-}S_3\text{-}S_2\text{-}X_1\text{-}S_1\text{-}S_0\text{-}COOH \qquad \text{(formula II)}$$

wherein

X_1 comprises either a random amino acid sequence or an amino acid sequence derived from a first polypeptide,

S_2 and S_1 are non-overlapping amino acid sequences derived from a second polypeptide,

S_3 and S_0 are either absent or non-overlapping amino acid sequences derived from a third polypeptide,

S_4 is either absent or an amino acid sequence derived from a fourth polypeptide,

X_2 is either absent or a peptidic linker sequence, and

- denotes a peptide bond,

wherein the second polypeptide and the third polypeptide and the fourth polypeptide are different from each other and are polypeptides with peptidyl-prolyl cis/trans-isomerase activity (PPIase activity) or which are derived from the FKBP domain family.

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide with peptidyl-prolyl cis/trans-isomerase activity or derived from the FKBP domain family is SlyD.

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is a polypeptide from a thermophile.

In one embodiment the thermophile is a thermophile bacterium. In one embodiment the thermophile bacterium is from the family of Thermaceae. In one embodiment the thermophile is Thermus thermophilus.

In one embodiment the thermophile is a thermophile Archaea. In one embodiment the thermophile Archaea is a hyperthermophilic Archaea. In one embodiment the thermophile is from the class of Thermococci. In one embodiment the thermophile is Thermococcus gammatolerans

In one embodiment the thermophile has an optimal growth temperature of at least 60 °C.

In one embodiment ~~of all aspects~~ as reported herein the immunogenic sequence is comprised in the X_1 amino acid sequence. In one embodiment the X_1 amino acid sequences comprises the immunogenic sequence and one or more parts derived from a further polypeptide with peptidyl-prolyl cis/trans-isomerase activity (PPIase activity) or derived from a further polypeptide from the FKBP-fold domain family, whereby the further polypeptide is different from the second polypeptide.

The amino acid sequence of X_1 is inserted in place of the insert-in-flap-domain (IF-domain) of the second polypeptide. Thus, if X_1 is identical to the IF-domain, i.e. has the amino acid sequence of the IF-domain, the fusion polypeptide S_2-X_1-S_1 is identical to the corresponding part of the naturally occurring second polypeptide.

In one embodiment ~~of all aspects~~ as reported herein the S_2 and S_1 amino acid sequences derived from a second polypeptide are connected (directly) to each other by the IF-domain in the wild-type (naturally occurring) second polypeptide.

In one embodiment ~~of all aspects~~ as reported herein X_1 is inserted in place of the insert-in-flap-domain (IF-domain) of the second polypeptide.

One aspect as reported herein is a polypeptide that has at least 70 % amino acid sequence identity to the polypeptide of formula I when determined excluding X_1, or

formula II when determined excluding X_1, X_2 and the absent sequences. In one embodiment the polypeptide has at least 80 % amino acid sequence identity. In one embodiment the polypeptide has at least 90 % amino acid sequence identity. In one embodiment the polypeptide has at least 95 % amino acid sequence identity. In one embodiment the polypeptide has at least 98 % amino acid sequence identity.

In one embodiment ~~of all aspects~~ as reported herein X_1 comprises an amino acid sequence that corresponds to a hidden epitope.

In one embodiment ~~of all aspects~~ as reported herein X_1 has an amino acid sequence length from 4 to about 500 amino acid residues. In one embodiment X_1 has an amino acid sequence length from 5 to about 100 amino acid residues. In one embodiment X_1 has an amino acid sequence length of about 7 to about 60 amino acid residues.

In one embodiment ~~of all aspects~~ as reported herein at least one amino acid residue of X_1 comprises a post-translational modification. In one embodiment one, or two, or three, or four, or five, or six, or seven, or eight, or nine, or ten amino acid residues of X_1 comprise a post-translational modification.

In one embodiment the fusion polypeptide is according to the following formula

$$NH_2\text{-}S_3\text{-}S_2\text{-}X_1\text{-}S_1\text{-}S_0\text{-}COOH$$

wherein

X_1 comprises either a random amino acid sequence or an amino acid sequence derived from a first polypeptide,

S_2 and S_1 are non-overlapping amino acid sequences derived from a second polypeptide,

S_3 and S_0 are either absent or non-overlapping amino acid sequences derived from a third polypeptide, and

- denotes a peptide bond,

wherein the second polypeptide and the third polypeptide are different from each other and are polypeptides with peptidyl-prolyl cis/trans-isomerase activity (PPIase activity) or which are derived from the FKBP domain family.

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide and the third polypeptide and the fourth polypeptide are from different species.

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is a human polypeptide, or a plant polypeptide, or a bacterial polypeptide, or an archaic polypeptide.

In one embodiment ~~of all aspects~~ as reported herein the third polypeptide is a human polypeptide, or a bacterial polypeptide, or an archaic polypeptide.

In one embodiment ~~of all aspects~~ as reported herein the fourth polypeptide is a bacterial polypeptide, or an archaic polypeptide.

In one embodiment ~~of all aspects~~ as reported herein the fourth polypeptide is a bacterial polypeptide. In one embodiment the bacterial polypeptide is a polypeptide from a thermophilic bacterium. In one embodiment the thermophile is from the family of Thermaceae. In one embodiment the thermophile is Thermus thermophilus.

In one embodiment ~~of all aspects~~ as reported herein the fourth polypeptide is an archaic polypeptide. In one embodiment the archaic polypeptide is a polypeptide from a hyperthermophilic Archaea. In one embodiment the thermophile is from the class of Thermococci. In one embodiment the archaic organism is Thermococcus gammatolerans.

In one embodiment ~~of all aspects~~ as reported herein the thermophile has an optimal growth temperature of at least 60 °C.

In one embodiment ~~of all aspects~~ as reported herein the first polypeptide is a human polypeptide.

In one embodiment ~~of all aspects~~ as reported herein X_1 is either a random amino acid sequence or an amino acid sequence derived from a first polypeptide wherein the dipeptide GS is added at the N-terminus and the tripeptide GSS is added at the C-terminus.

In one embodiment ~~of all aspects~~ as reported herein X_1 is a polypeptide of formula III

$$X_aX_bX_cX_d\text{-}X_0\text{-}X_eX_fX_gX_h \qquad \text{(formula III)}$$

wherein X_0 is either a random amino acid sequence or an amino acid sequence of a first polypeptide, and

wherein each of X_a to X_h denotes an (naturally occurring) amino acid residue and any of X_{a-h} may be individually present or absent.

In one embodiment ~~of all aspects~~ as reported herein X_1 is a polypeptide selected from the group of formula IV to formula XIII, with

GS-X_0-GSS	(formula IV),
AGS-X_0-GSS	(formula V),
CG-X_0-GC	(formula VI),
C-X_0-GC	(formula VII),
G-X_0-G	(formula VIII),
S-X_0-GSS	(formula IX),
GG-X_0-GG	(formula X),
G-X_0-TGG	(formula XI),
GGGS-X_0-GGGS	(formula XII),
GGNP-X_0-GPT	(formula XIII),

wherein X_0 is either a random amino acid sequence or an amino acid sequence derived from a first polypeptide.

In one embodiment X_0 is flanked at its N- and C-terminus by individual (single) cysteine residues.

In one embodiment X_1 comprises a cysteine residue within the N-terminal amino acid residues and a cysteine residue within the C-terminal amino acid residues. In one embodiment the N- or C-terminal amino acid residues are the eight terminal residues. In one embodiment X_1 comprises one cysteine residue at its N-terminus and one cysteine residue at its C-terminus.

In one embodiment X_1 is a circularly constrained polypeptide.

In one embodiment X_1 is a circular polypeptide.

In one embodiment the cysteine residues of X_1 have an alpha carbon atom distance of 4.3 Angstroms to 6.5 Angstroms. In one embodiment the cysteine residues of X_1 have an alpha carbon distance of 4.5 Angstroms. In one embodiment the cysteine residues of X_1 have a mean alpha carbon atoms distance of 5.6 Angstroms.

In one embodiment ~~of all aspects~~ as reported herein X_1 or X_0 has a length from 4 to about 500 amino acid residues.

In one embodiment ~~of all aspects~~ as reported herein X_2 is a linker amino acid sequence of from about 10 to about 30 amino acid residues.

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Arabidopsis thaliana FKBP13 (SEQ ID NO: 01), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and S_4, X_2, S_3, and S_0 are absent. In one embodiment S_2 has the amino acid sequence SEQ ID NO: 02 (DRGAGC), and S_1 has the amino acid sequence SEQ ID NO: 03 (CLIPPASV), and S_4, X_2, S_3, and S_0 are absent. In one embodiment S_2 has the amino acid sequence SEQ ID NO: 04, and S_1 has the amino acid sequence SEQ ID NO: 05, S_4, X_2, S_3, and S_0 are absent, and X_1 is a polypeptide of formula IV (GS-X_0-GSS).

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is human FKBP12 (SEQ ID NO: 06), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and S_4, X_2, S_3, and S_0 are absent. In one embodiment S_2 has the amino acid sequence SEQ ID NO: 07, and S_1 has the amino acid sequence SEQ ID NO: 08 (LVFDVELLKLE), and S_4, X_2, S_3, and S_0 are absent, and X_1 is a polypeptide of formula III (GS-X_0-GSS) or formula VI ((P)CG-X_0-GC).

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Thermus thermophilus SlyD (SEQ ID NO: 09), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and S_4, X_2, S_3, and S_0 are absent. In one embodiment S_2 has the amino acid sequence SEQ ID NO: 10, and S_1 has the amino acid sequence SEQ ID NO: 11, and S_4, X_2, S_3, and S_0 are absent, and X_1 is a polypeptide of formula V (AGS-X_0-GSS) or formula VI ((P)CG-X_0-GC).

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Arabidopsis thaliana FKBP13 (SEQ ID NO: 01), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and the third polypeptide is human FKBP12 (SEQ ID NO: 06), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and S_4 and X_2 are absent. In one embodiment S_3 has the amino acid sequence of SEQ ID NO: 07, S_2 has the

amino acid sequence SEQ ID NO: 02 (DRGAGC), S_1 has the amino acid sequence SEQ ID NO: 03 (CLIPPASV), S_0 has the amino acid sequence SEQ ID NO: 08 (LVFDVELLKLE), S_4 and X_2 are absent, and X_1 is a polypeptide of formula IV (GS-X_0-GSS) or formula VI ((P)CG-X_0-GC).

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Arabidopsis thaliana FKBP13 (SEQ ID NO: 01), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and the third polypeptide is Thermus thermophilus SlyD (SEQ ID NO: 09), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and S_4 and X_2 are absent. In one embodiment S_3 has the amino acid sequence of SEQ ID NO: 10, S_2 has the amino acid sequence SEQ ID NO: 02 (DRGAGC), S_1 has the amino acid sequence SEQ ID NO: 03 (CLIPPASV), S_0 has the amino acid sequence SEQ ID NO: 11 (LVFDVELLKLE), S_4 and X_2 are absent, and X_1 is a polypeptide of formula IV (GS-X_0-GSS) or formula VI ((P)CG-X_0-GC).

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Arabidopsis thaliana FKBP13 (SEQ ID NO: 01), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and the third polypeptide is human FKBP12 (SEQ ID NO: 06), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and S_4 and X_2 are absent. In one embodiment S_3 has the amino acid sequence of SEQ ID NO: 07, S_2 has the amino acid sequence SEQ ID NO: 02 (DRGAGC), S_1 has the amino acid sequence SEQ ID NO: 03 (CLIPPASV), S_0 has the amino acid sequence SEQ ID NO: 08 (LVFDVELLKLE), S_4 and X_2 are absent, and X_1 is a polypeptide of formula IV (GS-X_0-GSS) or formula VI ((P)CG-X_0-GC).

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Arabidopsis thaliana FKBP13 (SEQ ID NO: 01), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, the third polypeptide is human FKBP12 (SEQ ID NO: 06), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and the fourth polypeptide is E.coli SlyD (SEQ ID NO: 12), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity. In one embodiment S_4 has the amino acid sequence SEQ ID NO:12, X_2 has the amino

acid sequence SEQ ID NO: 13, S_3 has the amino acid sequence of SEQ ID NO: 07, S_2 has the amino acid sequence SEQ ID NO: 02 (DRGAGC), S_1 has the amino acid sequence SEQ ID NO: 03 (CLIPPASV), S_0 has the amino acid sequence SEQ ID NO: 08 (LVFDVELLKLE), and X_1 is a polypeptide of formula IV (GS-X_0-GSS) or formula VI ((P)CG-X_0-GC).

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Arabidopsis thaliana FKBP13 (SEQ ID NO: 01), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, the third polypeptide is Thermus thermophilus SlyD (SEQ ID NO: 09), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and the fourth polypeptide is E.coli SlyD (SEQ ID NO: 12), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity. In one embodiment S_4 has the amino acid sequence SEQ ID NO:12, X_2 has the amino acid sequence SEQ ID NO: 13, S_3 has the amino acid sequence of SEQ ID NO: 10, S_2 has the amino acid sequence SEQ ID NO: 02 (DRGAGC), S_1 has the amino acid sequence SEQ ID NO: 03 (CLIPPASV), S_0 has the amino acid sequence SEQ ID NO: 11 (LVFDVELLKLE), and X_1 is a polypeptide of formula IV (GS-X_0-GSS) or formula VI ((P)CG-X_0-GC).

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Thermus thermophilus SlyD (SEQ ID NO: 09), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and the fourth polypeptide is E.coli SlyD (SEQ ID NO: 12), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and S_3 and S_0 are absent. In one embodiment S_4 has the amino acid sequence SEQ ID NO: 12, X_2 has the amino acid sequence SEQ ID NO: 13, S_2 has the amino acid sequence SEQ ID NO: 10, S_1 has the amino acid sequence SEQ ID NO: 11, S_3 and S_0 are absent, and X_1 is a polypeptide of formula V (AGS-X_0-GSS) or formula VI ((P)CG-X_0-GC).

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Arabidopsis thaliana FKBP13 (SEQ ID NO: 01), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and the fourth polypeptide is E.coli SlyD (SEQ ID NO: 12), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and S_3 and S_0 are absent. In one

embodiment S_4 has the amino acid sequence SEQ ID NO: 12, X_2 has the amino acid sequence SEQ ID NO: 13, S_2 has the amino acid sequence SEQ ID NO: 04, S_1 has the amino acid sequence SEQ ID NO: 05, S_3 and S_0 are absent, and X_1 is a polypeptide of formula IV (GS-X_0-GSS) or formula VI ((P)CG-X_0-GC).

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Thermococcus gammatolerans SlyD (SEQ ID NO: 106), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and S_4, X_2, S_3, and S_0 are absent. In one embodiment S_2 has the amino acid sequence SEQ ID NO: 107, S_1 has the amino acid sequence SEQ ID NO: 108, S_4, X_2, S_3, and S_0 are absent, and X_1 is a polypeptide selected from formula IV to formula XIII.

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Arabidopsis thaliana FKBP13 (SEQ ID NO: 01), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and the third polypeptide is Thermococcus gammatolerans SlyD (SEQ ID NO: 106), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and S_4 and X_2 are absent. In one embodiment S_3 has the amino acid sequence of SEQ ID NO: 107, S_2 has the amino acid sequence SEQ ID NO: 02 (DRGAGC), S_1 has the amino acid sequence SEQ ID NO: 03 (CLIPPASV), S_0 has the amino acid sequence SEQ ID NO: 108, S_4 and X_2 are absent, and X_1 is a polypeptide selected from formula IV to formula XIII.

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Arabidopsis thaliana FKBP13 (SEQ ID NO: 01), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, the third polypeptide is human FKBP12 (SEQ ID NO: 06), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and the fourth polypeptide is Thermococcus gammatolerans SlyD (SEQ ID NO: 106), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity. In one embodiment S_4 has the amino acid sequence SEQ ID NO: 106, X_2 has the amino acid sequence SEQ ID NO: 13, S_3 has the amino acid sequence of SEQ ID NO: 07, S_2 has the amino acid sequence SEQ ID NO: 02 (DRGAGC), S_1 has the amino acid sequence SEQ ID NO: 03

(CLIPPASV), S_0 has the amino acid sequence SEQ ID NO: 08 (LVFDVELLKLE), and X_1 is a polypeptide selected from formula IV to formula XIII.

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Arabidopsis thaliana FKBP13 (SEQ ID NO: 01), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, the third polypeptide is Thermococcus gammatolerans SlyD (SEQ ID NO: 106), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and the fourth polypeptide is E.coli SlyD (SEQ ID NO: 12), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity. In a specific embodiment S_4 has the amino acid sequence SEQ ID NO: 12, X_2 has the amino acid sequence SEQ ID NO: 13, S_3 has the amino acid sequence of SEQ ID NO: 107, S_2 has the amino acid sequence SEQ ID NO: 02 (DRGAGC), S_1 has the amino acid sequence SEQ ID NO: 03 (CLIPPASV), S_0 has the amino acid sequence SEQ ID NO: 108, and X_1 is a polypeptide selected from formula IV to formula XIII.

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Thermococcus gammatolerans SlyD (SEQ ID NO: 106), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and the fourth polypeptide is E.coli SlyD (SEQ ID NO: 12), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and S_3 and S_0 are absent. In one embodiment S_4 has the amino acid sequence SEQ ID NO: 12, X_2 has the amino acid sequence SEQ ID NO: 13, S_2 has the amino acid sequence SEQ ID NO: 107, S_1 has the amino acid sequence SEQ ID NO: 108, S_3 and S_0 are absent, and X_1 is a polypeptide selected from formula IV to formula XIII.

In one embodiment ~~of all aspects~~ as reported herein the second polypeptide is Arabidopsis thaliana FKBP13 (SEQ ID NO: 01), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and the fourth polypeptide is Thermococcus gammatolerans SlyD (SEQ ID NO: 106), or a polypeptide with at least 70 %, or at least 80 %, or at least 90 %, or at least 95 %, or at least 98 % amino acid sequence identity, and S_3 and S_0 are absent. In one embodiment S_4 has the amino acid sequence SEQ ID NO: 107, X_2 has the amino acid sequence SEQ ID NO: 13, S_2 has the amino acid

sequence SEQ ID NO: 04, S_1 has the amino acid sequence SEQ ID NO: 05, S_3 and S_0 are absent, and X_1 is a polypeptide selected from formula IV to formula XIII.

The fusion polypeptides ~~of the aspects~~ as reported herein have many applications because they can be produced recombinantly in good yields e.g. in E.coli. For example the fusion polypeptides can be used for presenting amino acid sequence for immunization, antibody generation, antibody screening, antibody epitope mapping, or immunohistochemistry screening.

In one embodiment ~~of all aspects~~ as reported herein X_2 has the amino acid sequence GGGSGGGSGGGSGGGSGGGSGGG (SEQ ID NO: 14).

In one embodiment ~~of all aspects~~ as reported herein X_1 has the amino acid sequence GGGSGGNPX$_0$GPTGGGS (SEQ ID NO: 32), wherein X_0 is an amino acid sequence from 4 to 85 amino acid residues.

In one embodiment a fusion polypeptide comprising E.coli SlyD and human FKBP12 amino acid sequences is used for the presentation of non-stable conformational polypeptides.

In one embodiment a fusion polypeptide comprising human FKBP12 and Arabidopsis thaliana FKBP13, or solely Thermus thermophilus SlyD, or Thermus thermophilus SlyD and Arabidopsis thaliana FKBP13, or solely Thermococcus gammatolerans SlyD, or Thermococcus gammatolerans SlyD and Arabidopsis thaliana FKBP13 amino acid sequences is used for the presentation of stable secondary structures.

There is ~~One aspect as~~ reported herein ~~is~~ the use of a fusion polypeptide as reported herein for eliciting an immune response against X_1 or X_0 in an animal. One aspect as reported herein is the use of a fusion polypeptide as defined above for eliciting an immune response against X_1 in an experimental animal.

There is ~~One aspect as~~ reported herein ~~is~~ a method for eliciting an immune response in an animal against a polypeptide comprising the step of administering to the animal a fusion polypeptide as reported herein for at least one time, whereby X_0 is the immunogenic amino acid sequence.

One aspect as reported herein is a method for eliciting an immune response in an experimental animal against a polypeptide comprising the step of administering to the experimental animal a fusion polypeptide as defined above for at least one time, whereby X_1 is the amino acid sequence of the polypeptide.

There is ~~One aspect as~~ reported herein ~~is~~ a method for obtaining a nucleic acid encoding an antibody specifically binding to a target antigen comprising the following steps:

a) administering to an animal a fusion polypeptide as reported herein for at least one time, whereby the amino acid sequence of X_1 comprises the amino acid sequence of the target antigen,

b) recovering from the animal three to ten days after the last administration of the polypeptide B-cells that produce the antibody specifically binding to the target antigen, and

c) obtaining from the B-cells the nucleic acid encoding an antibody specifically binding to a target antigen.

There is~~One aspect as~~ reported herein ~~is~~ a method for the production of an antibody specifically binding to a target antigen comprising the following steps:

a) administering to an animal a fusion polypeptide as reported herein for at least one time, whereby the amino acid sequence of X_1 comprises the amino acid sequence of the target antigen,

b) recovering from the animal three to ten days after the last administration of the polypeptide B-cells that produce the antibody specifically binding to the target antigen,

c) optionally obtaining from the B-cells the nucleic acid encoding the antibody specifically binding to the target antigen, and

d) cultivating a cell comprising a nucleic acid encoding the antibody specifically binding to the target antigen and recovering the antibody from the cell or the cultivation medium and thereby producing an antibody specifically binding to a target antigen.

One aspect as reported herein is a method for producing an antibody specifically binding to a target antigen comprising the following steps:

a) administering to an experimental animal a fusion polypeptide as defined above for at least one time, whereby the amino acid sequence of X_1 comprises the amino acid sequence of the target antigen, b) recovering from the experimental animal three to ten days after the last administration of the polypeptide B-cells that produce the antibody specifically binding to the target antigen, and c) cultivating a cell comprising a nucleic acid encoding the antibody specifically binding to the target antigen and recovering the antibody from the cell or the cultivation medium and thereby producing an antibody specifically binding to a target antigen.

There is~~One aspect as~~ reported herein ~~is~~ a method for the production of an antibody specifically binding to a target antigen comprising the following steps:

a) recovering from an experimental animal after the administration of a fusion polypeptide as reported herein B-cells that produce the antibody specifically binding to the target antigen that has the amino acid sequence of X_0, and

b) cultivating a cell comprising a nucleic acid encoding the antibody specifically binding to the amino acid sequence of X_0 and recovering the antibody from the cell or the cultivation medium and thereby producing an antibody specifically binding to a target antigen.

One aspect as reported herein is a method for producing an antibody specifically binding to a target antigen comprising the following steps: a) recovering from an experimental animal after the administration of a fusion polypeptide as defined above B-cells that produce the antibody specifically binding to the amino acid sequence of X_1, and b) cultivating a cell comprising a nucleic acid encoding the antibody specifically binding to the amino acid sequence of X_1 and recovering the antibody from the cell or the cultivation medium and thereby producing an antibody specifically binding to a target antigen.

One aspect as reported herein is the use of a fusion polypeptide as reported herein for epitope mapping, whereby the amino acid sequence of X_1 comprises the epitope.

One aspect as reported herein is a method for selecting an antibody specifically binding to a target antigen comprising the following steps:

a) determining the binding affinity of a plurality of antibodies to a target antigen, which is immobilized, whereby the amino acid sequence of X_1 of a fusion polypeptide as reported herein comprises the target antigen amino acid sequence,
b) selecting the antibody having an apparent complex stability above a pre-defined threshold level.

There is~~One aspect as~~ reported herein ~~is~~ a method for selecting an antibody suitable for immunohistochemical analysis of a target polypeptide comprising the following steps:
a) determining the binding kinetics of a plurality of antibodies,
c) selecting the antibody having an apparent complex stability above a pre-defined threshold level.

There is~~One aspect as~~ reported herein ~~is~~ a method for mapping a binding site of an antibody to a target amino acid sequence comprising the following steps:
a) contacting a solid support to which a fusion polypeptide as reported herein is immobilized, whereby the amino acid sequence of X_1 comprises the target amino acid sequence, with an antibody,
b) determining the kinetic properties of the antibody with the fusion polypeptide as reported herein,
c) selecting the antibody having an apparent complex stability above a pre-defined threshold level.

There is~~One aspect as~~ reported herein ~~is~~ the use of a fusion polypeptide as reported herein for determining structure-function-relationships, whereby the amino acid sequence of X_1 comprises the polypeptide for which the structure-function-relationship shall be determined.

There is~~One aspect as~~ reported herein ~~is~~ the use of a fusion polypeptide as reported herein for presenting a polypeptide with its correct secondary and/or tertiary structure, whereby the amino acid sequence of X_1 comprises the polypeptide.

There is~~One aspect as~~ reported herein ~~is~~ the use of a fusion polypeptide as reported herein in a screening method.

In one embodiment the screening method is a screening method for identifying or selecting molecules that specifically bind to X_1. In one embodiment the molecule is a small molecule or a polypeptide. In one embodiment the polypeptide is an antibody, or antibody-fragment, or antibody-fusion polypeptide.

There is~~One aspect as~~ reported herein ~~is~~ the use of a fusion polypeptide as reported herein in ribosome display.

There is~~One aspect as~~ reported herein ~~is~~ the use of a fusion polypeptide as reported herein in phage display.

There is~~One aspect as~~ reported herein ~~is~~ the use of a polypeptide as reported herein in cell surface display. In one embodiment the cell is a prokaryotic cell. In one embodiment the prokaryotic cell is a bacterial cell. In one embodiment the cell is a eukaryotic cell. In one embodiment the eukaryotic cell is a CHO cell, or a HEK cell, or a BHK cell, or a Sp2/0 cell, or a NS0 cell, or a yeast cell.

There is~~One aspect as~~ reported herein ~~is~~ the antibody produced by a method as reported herein.

There is~~One aspect as~~ reported herein ~~is~~ a pharmaceutical formulation comprising the fusion polypeptide as reported herein and a pharmaceutically acceptable carrier.

There is~~One aspect as~~ reported herein ~~is~~ a diagnostic formulation comprising the fusion polypeptide as reported herein conjugated to a detectable label.

There is~~One aspect as~~ reported herein ~~is~~ the use of a fusion polypeptide as reported herein for the manufacture of a medicament.

There is~~One aspect as~~ reported herein ~~is~~ the use of a fusion polypeptide as reported herein for the treatment of a disease.

There is~~One aspect as~~ reported herein ~~is~~ a method of treating an individual comprising administering to the individual an effective amount of the fusion polypeptide as reported herein.

Detailed Description of the Invention

In general, the antigen or antigenic or immunogenic amino acid sequence, e.g. comprised in the amino acid sequence of X_1 is inserted in place of the insert-in-flap-domain (IF-domain) of the SlyD part of the second polypeptide in the fusion polypeptide as reported herein.

In one embodiment of all aspects as reported herein X_0 is selected from a fragment of a naturally occurring polypeptide or is a randomized amino acid sequence. In one embodiment the naturally occurring polypeptide is a human polypeptide.