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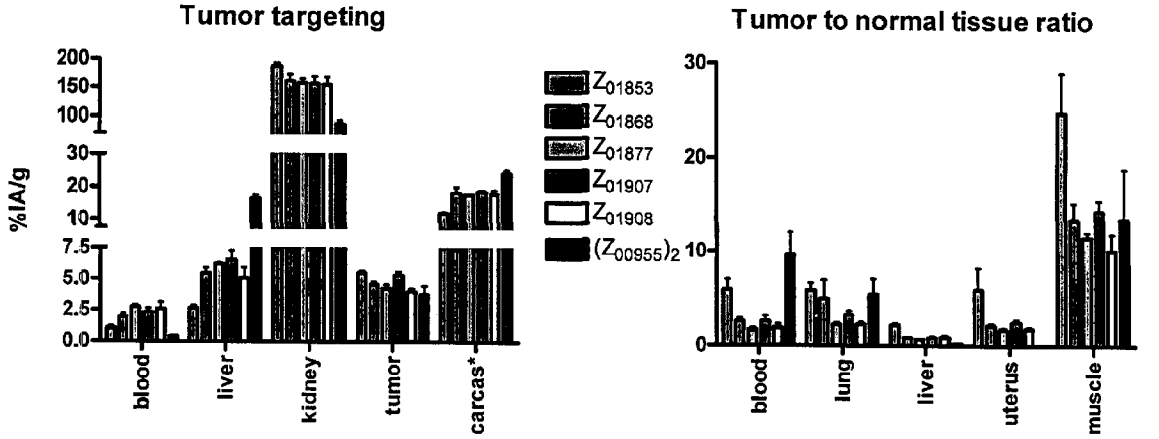
(54) **Titre : POLYPEPTIDES LIANT LE RECEPTEUR DU FACTEUR DE CROISSANCE EPIDERMIQUE**
(54) **Title: EPIDERMAL GROWTH FACTOR RECEPTOR BINDING POLYPEPTIDES**



(57) **Abrégé/Abstract:**
This invention relates to polypetides which bind to EGFR family receptors and to applications of those polypetides in medicine, veterinary medicine, diagnosis diagnostics and imaging. The polypetides comprise an EGFR binding motif consisting of an amino acid sequence selected from i) $EX_2X_3X_4AX_6X_7EIR\ X_{11}LPNLNGWQX_{20}\ TAFIX_{25}SLX_{28}D$ and ii) an amino acid sequence having at least 85% identity to the sequence defined in i).

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Abstract

This invention relates to polypetides which bind to EGFR family receptors and to applications of those polypetides in medicine, veterinary medicine, diagnosis diagnostics and imaging. The polypetides comprise an EGFR binding motif consisting of an amino acid sequence selected from i) $EX_2X_3X_4AX_6X_7EIR\ X_{11}LPNLNGWQX_{20}\ TAFIX_{25}SLX_{28}D$ and ii) an amino acid sequence having at least 85% identity to the sequence defined in i).

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EPIDERMAL GROWTH FACTOR RECEPTOR BINDING POLYPEPTIDES

Field of the Invention

This invention relates to polypeptides which bind to Epidermal Growth Factor Receptor (EGFR). The polypeptides have industrial applications in medicine, veterinary medicine, imaging, separation techniques and diagnostics.

Background

Abnormal expression of receptors in the Epidermal Growth Factor Receptor family, (the EGFR-family; also called the ErbB receptor family), is frequently associated with various malignancies in lung, breast, prostate, colon, ovary, head and neck. It is of interest to study this receptor family to gain a better understanding of the relation of the receptors to patient prognosis and treatment. The family consists of four transmembrane receptors, the epidermal growth factor receptor, EGFR, (ErbB1/HER1), HER2 (ErbB2/neu), HER3 (ErbB3) and HER4 (ErbB4) (Gullick WJ. Endocr Rel Canc 2001; **8**:75-82; Witton CJ. *et al* J Pathol 2003; **200**:290-297). Each receptor comprises an extra-cellular ligand binding domain, a transmembrane domain and an intracellular tyrosine kinase domain (except HER3 which lacks a functional tyrosine kinase domain) (Citri A, *et al*. Exp Cell Res 2003; **284**(1):54-65; Harari D and Yarden Y. Oncogene 2002; **19**:6102-6114). There is one EGFR variant which has almost no ECD- EGFRvIII, Wikstrand CJ *et al* Cancer Res. **55**: 3140-3148, 1995; Huang HS *et al* J Biol. Chem. 272: 2927-2935, 1997; Kuan CT, *et al* Endocr. Relat. Cancer 8:83-96, 2001.

When a ligand binds to a receptor in the EGFR family, the receptor is stimulated to dimerise, either with another identical receptor (homodimerization) or with another receptor in the family (heterodimerization) (Olayioye MA, *et al*. Embo J. 2000; **19**:3159-67; Yarden Y,

Sliwkowski MX. Cell Biol 2001; **2**:127-37). Receptor dimerization activates the intracellular tyrosine kinase domain, leading to proliferation, migration, apoptosis, differentiation or other cellular processes (Yarden Y, Sliwkowski MX. Cell Biol 2001; **2**:127-37; Wells A. Int J Biochem Cell Biol 1999; **31**:637-643; Vermeer PD *et al.* Nature 2003; **422**:322-6). EGFR and HER2 are the most studied receptors of the four in the family and are over-expressed in many malignancies (Nordberg E *et al.* Eur J Nucl Med Mol Imaging. 2005 Jul; **32**(7):771-7). A high expression of these particular receptors is often associated with a poor prognosis (Hendriks BS *et al.* J Biol Chem 2003; **278**:23343-23351; Arteaga CL. Oncologist 2002; **7** Suppl 4:31-9; Earp HS *et al.* Breast Cancer Res Treat 1995; **35**:115-32; Wester K, *et al.* Acta Oncol 2002; **41**:282-8. Lorenzo GD *et al.* Clin Prostate Cancer 2003; **2**(1):50-7).

Several ligands bind to members of the EGFR receptor family. The only receptor that does not have any known natural ligand is HER2. (Citri A, *et al.* Exp Cell Res 2003; **284**(1):54-65; Yarden Y, Sliwkowski MX. Cell Biol 2001; **2**:127-37; Lenferink AEG, *et al.* EMBO J 1998; **17**:3385-3397). The antibody trastuzumab (Herceptin), which binds to the extra-cellular domain, may be used to target the HER2 receptor, especially in HER2 expressed tumors in breast cancer. Binding of trastuzumab can block growth stimulating intracellular signalling, decrease the capacity of cellular repair after chemo- and radiotherapy and possibly also improve the capacity of apoptosis. Bookman MA *et al.* J Clin Oncol 2003; **21**:283-290; Pegram MD *et al.* Cancer Treat Res 2000; **103**:747-75; McKeage K, Perry CM. Drugs 2002; **62**:209-43). Affibody molecules disclosed in W02005/003156 may also be used to target HER2.

EGFR function can be inhibited by blocking ligand binding to the extra-cellular part of the receptor, using antibodies such as cetuximab (Erbitux, ImClone/ Bristol

Myers Squibb) (Baselga J. Eur J Cancer 37: Suppl 4, **S16-22**, 2001, ABX-EGF Ranson M, Curr Opin Mol Ther 5: 541-546, 2003 or mab425/EMD55900 (Merck) or antibody fragments (Boskovitz A *et al*: Expert Opin Biol Ther **4**: 1453-1471, 2004). The receptor function may in some, but not all patients, also be blocked with low molecular weight tyrosine kinase inhibitors such as Iressa (Gefitinib, AstraZeneca) (Sundberg AL *et al*: Eur J Nucl Med Mol Imaging **30**: 1348-1356, 2003; Herbst RS *et al*: Nat Rev Cancer **4**: 956-965, 2004) or Tarceva (Erlotinib, OSI-774) (Krozely P. Clin J Oncol Nurs **8**: 163-168, 2004) that bind the intracellular part of the receptor. In both cases, the aim is to block growth-stimulating signalling, and thereby inhibit tumor cell proliferation (Rich JN, Bigner DD: Nat Rev Drug Discov **3**: 430-446, 2004). There is, however, room for improvement. For example Iressa has proven to be a disappointment, acting in only a fraction of patients over-expressing the EGFR. For cetuximab, it still remains to be seen what will be the best chemotherapy combination treatment modality to increase the therapeutic impact of the treatment. These therapies can be combined with a radionuclide-based approach to kill tumor cells (Carlsson J, *et al*: Radiotherapy and Oncology, **66(2)**, 107-117, 2003), and one interesting example is the recent application of Gefitinib to modify the uptake and therapy effects of radio-labeled (astatinated) EGF (Sundberg AL *et al*: Eur J Nucl Med Mol Imaging **30**: 1348-1356, 2003). Development of polypeptide anti-EGFR targeting agents provides an interesting alternative to the naturally agonistic (tumor-stimulating) biological EGF ligand, for the delivery of radionuclides for both diagnostic (imaging) and therapy purposes, as previously exemplified for HER-2 (Wikman M *et al*. Protein Engineering, Design & Selection (PEDS), **17(5)**, 455-462, 2004; Steffen AC *et al*. Cancer Biotherapy and Radiopharmaceuticals, **20**, 239-248, 2005; Steffen AC *et al*. Eur J Nuclear Medicine, In press, 2005). Such

polypeptides can also have biological effects, even without radioactivity, that are of interest for therapy. Z variants, also called "Affibody® molecules", as disclosed for example in W02005/0003156, are polypeptides which are intermediate in molecular weight (6-15 kDa), and can therefore have better penetration in tumor tissue than antibodies (150 kDa), and at the same time have better systemic circulation properties than low molecular weight substances like Iressa and Tarceva (≈ 1 kDa) which are rapidly eliminated via kidney excretion. In fact, Z variants typically have half-lives in a range suitable for *in vivo* imaging applications, and if needed for therapeutic or other applications, half-lives can be extended dramatically by gene fusion technology (see for example WO 2005/097202A).

Over-expression of EGFR is common in Head and Neck Squamous Cell Carcinomas, (HNSCC) (Rikimaru, K *et al.* Head Neck, 1992. **14(1)**: p. 8-13; Santini, J *et al*, Head Neck, 1991. 13(2): p. 132-9. Ekberg T *et al.* Int J Oncology, 26(5), 1177-1185, 2005). Increased levels of HER2 have been suggested in several studies of HNSCC (Craven, J.M *et al.* Anticancer Res, 1992. **12(6B)**: p. 2273-6), with possible prognostic value in oral Squamous Cell Carcinomas, (SCC) (Werkmeister, *et al.* Oral Oncol, 2000. **36(1)**: p. 100-5; Werkmeister, R. Am J Surg, 1996. **172(6)**: p. 681-3; Xia, W *et al.* Clin Cancer Res, 1997. **3(1)**: p. 3-9; Xia, W *et al.* Clin Cancer Res, 1999. **5(12)**: p. 4164-74). HER3 has been shown to be over expressed in HNSCC cell lines and associated with clinical malignant progression (Xia, W *et al.* Clin Cancer Res, 1999. 5(12): p. 4164-74; Shintani, S *et al.* Cancer Lett, 1995. **95(1-2)**: p. 79-83) and to be over expressed also in other types of malignancies (Gullick, W.J. Cancer Surv, 1996. **27**: p.339-49). Some human mammary carcinoma cell lines have HER4 transcripts (Plowman, G.D *et al.* Proc Natl Acad Sci U S A, 1993. **90(5)**: p.1746-50) but the role of HER4 in cancer is less clear (Srinivasan, R. *et al.* Cancer

Res, 2000. **60(6)**: p.1483-7). It is interesting to study co-expression of the four receptors, since it has been suggested that co-expression patterns may be associated with malignant phenotypes (Xia, W *et al.* Clin Cancer Res, 1999. **5(12)**: p.4164-74; Bei, R. *et al.* J Pathol, 2001. **195(3)**: p.343-8; Krahn, G. *et al.* Eur J Cancer, 2001. **37(2)**: p. 251-9). Immunohistochemical stainings of EGFR and HER2 have shown pronounced membranous staining. In contrast, HER3 and HER4 staining has been mainly cytoplasmic (Plowman, G.D *et al.* Proc Natl Acad Sci U S A, 1993. **90(5)**: p. 1746-50; Srinivasan, R. *et al.* Cancer Res, 2000. **60(6)**: p.1483-7). Furthermore, EGFR and HER2 have been reported to express at high levels in both tumors and metastases. Thus, it seems that EGFR and HER2 are potential targets for macromolecular and peptide-based *in vivo* imaging and therapy applications while this might not be the case with HER3 and HER4.

Increased levels of EGFR-protein have also been found in urinary bladder carcinoma and the over-expression has been related to tumor stage and malignancy grade (Harney, J.V. *et al*, J Urol, **146**, 227-31. (1991); Messing, E.M. Cancer Res, **50**, 2530-7. (1990); Neal, D.E. *et al*, Cancer, **65**, 1619-25. (1990); Sauter, G. *et al.* Int J Cancer, **57**, 508-14. (1994); Gardmark T, *et al.* British Journal of Urology (BJU), **95**, 982-986, 2005).

In Glioblastoma Multiforme (GBM) the most malignant form of the gliomas, which are common primary central nervous system tumors, over-expression of EGFR is detected in at least half of all analyzed tumors (Boskovitz A, *et al.* Expert Opin Biol Ther **4**: 1453-1471, 2004; Shinojima N, *et al.* Cancer Res **63**: 6962-6970, 2003; Ekstrand AJ, *et al.* Cancer Res **51**: 2164-2172, 1991; Rainov NG *et al.* Journal of Neuro-Oncology **35** 13-28 (1997); Carlsson J *et al.* J Neurooncol. 2005 Sep 8; [Epub ahead of print]). The over-expression is due to gene amplification and/or increased transcription rates, and the number of 10^6 receptors per tumor cell has been

reported (Rich JN, Bigner DD: Nat Rev Drug Discov **3**: 430-446, 2004; Bigner SH *et al*. J Neuropathol Exp Neurol **47**, 191-205 (1998); Carpenter G. Ann Rev Biochem **56**, 881-914 (1987); Collins VP. Cancer Biology **4**, 27-32 (1993); Libermann TA *et al*. Nature **313**, 144-147, (1985); Kleihues P, Ohgaki H. Neuro-oncol **1**, 44-51, (1999); Kleihues P, Ohgaki H. Toxicol Pathol **28**, 164-170, (2000); Boskovitz A *et al*. Expert Opin Biol Ther **4**, 1453-1471, (2004)). EGFR over-expression correlates with increased glioma growth rate and decreased survival (Rich JN, Bigner DD: Nat Rev Drug Discov **3**, 430-446, (2004); Carlsson J *et al*. J Neurooncol. 2005 Sep 8; [Epub ahead of print]; Schlegel J *et al*. Int J Cancer **56**, 72-77, (1994); Wikstrand CJ, Bigner DD. J Natl Cancer Inst **90**, 799-801, (1998); Shinojima N *et al*. Cancer Res **63**, 6962-6970, (2003))and it has been indicated that EGFR over-expression is most pronounced at the tumor cell invading edges (Okada Y, *et al*. Cancer Res **63**, 413-416,)(2003)). EFGR-specific binding polypeptides could potentially be employed for therapy applications for glioma therapy, for example, by locoregional administration into the postoperal cavity.

Several other malignancies of epithelial origin, such as those found in lung and breast, are also associated with a high expression of EGFR (Salomon, D.S. *et al*. Crit. Rev. Oncol. Hematol., **19(3)**:183-232, (1995)). EGFR receptors are also distributed among various normal tissues and expressed to rather high levels especially in liver hepatocytes and skin epithelium (Gusterson, B. *et al*. Cell Biol Int Rep, **8**, 649-58. (1984); Damjanov, I. *et al*. Lab Invest, **55**, 588-92. (1986)). This can potentially cause problems in therapy applications, especially radiotherapy, but is probably of less importance in diagnostic and imaging applications where low amounts of diagnostic or imaging markers which bind to EGFR receptors are given. Nevertheless, EGFR-binding polypeptides might find

applications in certain cancers where local administration is to be considered.

It is an object of the invention to provide new EGFR-binding agents, that could be used for diagnostic, *in vitro* or *in vivo* imaging, as well as therapeutic applications. In addition, such EGFR binding polypeptides might find use in staging and as a direct assessment of SME based therapy aimed to down-regulate the target receptor.

In addition to the development of marketed molecular imaging agents, applications include use in the drug development and screening procedure where specific imaging agents are desired to measure outcome of treatment in *in vivo* models and subsequently during clinical development. Molecular Imaging provides a direct read-out of efficacy of a pharmaceutical aimed to down-regulate a growth factor receptor, as well as for assessing the anti-tumor effect.

Summary of the Invention

According to one aspect thereof, the invention provides an epidermal growth factor receptor (EGFR) binding polypeptide, comprising an epidermal growth factor receptor binding motif, EBM, which motif consists of an amino acid sequence selected from:

i) $EX_2X_3X_4AX_6X_7EIX_{10}\ X_{11}LPNLNX_{17}X_{18}QX_{20}\ X_{21}AFIX_{25}SLX_{28}D$,

wherein, independently of each other,
X_2 is selected from M, F, V, L, I and S;
X_3 is selected from W, D, E and L;
X_4 is selected from I, V, G, S, M, L, A, T, N, D and W;
X_6 is selected from W, V, L, I, M and S;
X_7 is selected from D, E, N and K;
X_{10} is selected from R, G, H and K;
X_{11} is selected from D, N, E, Y and S;

X_{17} is selected from G, W and A;
X_{18} is selected from W, G and A;
X_{20} is selected from M, L, F, A and E;
X_{21} is selected from T, D, N, A and Q;
X_{25} is selected from A, S, N, G and L; and
X_{28} is selected from L, W, V, F and A;

and

ii) an amino acid sequence which has at least 85 % identity to the sequence defined in i);

the EGFR-binding polypeptide binding to EGFR such that the K_D value of the interaction is at most 10 µM.

The above definition of a class of sequence related, EGFR-binding polypeptides according to the invention is based on a statistical analysis of a large number of random polypeptide variants of a parent scaffold, that were selected for their interaction with EGFR in several different selection experiments. The identified EGFR-binding motif, or "EBM", corresponds to the target binding region of the parent scaffold, which region constitutes two alpha helices within a three-helical bundle protein domain. In the parent scaffold, the varied amino acid residues of the two EBM helices constitute a binding surface for interaction with the constant Fc part of antibodies. In the present invention, the random variation of binding surface residues and the subsequent selection of variants have replaced the Fc interaction capacity with a capacity for interaction with EGFR.

As the skilled person will realize, the function of any polypeptide, such as the EGFR-binding capacity of the polypeptides according to the invention, is dependent on the tertiary structure of the polypeptide. It is therefore possible to make minor changes to the sequence of amino acids in a polypeptide without affecting the

function thereof. Thus, the invention encompasses modified variants of the EBM of i), which are such that the resulting sequence is at least 85 % identical to a sequence belonging to the class defined by i). For example, it is possible that an amino acid residue belonging to a certain functional grouping of amino acid residues (e.g. hydrophobic, hydrophilic, polar etc) could be exchanged for another amino acid residue from the same functional group.

In one embodiment of the polypeptide according to the invention, X_2 is M.

In one embodiment of the polypeptide according to the invention, X_3 is W.

In one embodiment of the polypeptide according to the invention, X_4 is selected from I, V, G and S.

In one embodiment of the polypeptide according to the invention, X_6 is selected from V and W.

In one embodiment of the polypeptide according to the invention, X_{10} is selected from R and G.

In one embodiment of the polypeptide according to the invention, X_{11} is selected from D, N and E.

In one embodiment of the polypeptide according to the invention, X_{17} is selected from W and G.

In one embodiment of the polypeptide according to the invention, X_{18} is selected from W and G, and may in particular be W.

In one embodiment of the polypeptide according to the invention, X_{20} is M.

In one embodiment of the polypeptide according to the invention, X_{21} is selected from T and D, and may in particular be T.

In one embodiment of the polypeptide according to the invention, X_{25} is selected from A, S and N.

In one embodiment of the polypeptide according to the invention, X_{28} is selected from L and W.

In one embodiment of the polypeptide according to the invention, X_{18} is W and X_{21} is T.

In one embodiment of the polypeptide according to the invention, X_{18} is W and X_{20} is M.

In a more specific definition of a sub-class of the polypeptides according to the invention, the amino acid sequence of i) fulfils at least six, at least seven, at least eight or all nine of the following nine conditions: X_2 is M; X_3 is W; X_6 is W; X_{10} is R; X_{17} is G; X_{18} is W; X_{20} is M; X_{21} is T; X_{28} is L.

In the case where all nine of these conditions are fulfilled, the sequence of i) is

$$EMWX_4AWX_7EIR\ X_{11}LPNLNGWQM\ TAFIX_{25}SLLD.$$

In an alternative specific definition of a sub-class of the polypeptides according to the invention, the amino acid sequence of i) fulfils at least three, at least four or all five of the following five conditions: X_{17} is G; X_{18} is W; X_{20} is M; X_{21} is T; X_{25} is A.

In the case where all five of these conditions are fulfilled, the sequence of i) is

$$EX_2X_3X_4AX_6X_7EIX_{10}\ X_{11}LPNLNGWQM\ TAFIASLX_{28}D.$$

In yet an alternative subclass, the sequence of i) is $EX_2X_3X_4AX_6X_7EIG\ X_{11}LPNLNWGQX_{20}\ X_{21}AFIX_{25}SLWD$, for example $EX_2X_3IAVX_7EIG\ ELPNLNWGQX_{20}\ DAFINSLWD$.

As described in detail in the experimental section to follow, the selection of EGFR-binding variants has led to the identification of a large amount of individual EGFR-binding motif (EBM) sequences. These sequences constitute individual embodiments of the EBM sequence i) in the definition of EGFR-binding polypeptides according to this aspect of the present invention. The sequences of individual EGFR-binding motifs are presented in Figure 1 and as SEQ ID NO:1-163. In embodiments of this aspect of the invention, the EBM sequence i) may in particular be selected from SEQ ID NO:33, SEQ ID NO:48, SEQ ID NO:57, SEQ ID NO:87, SEQ ID NO:88 and SEQ ID NO:147.

In embodiments of the present invention, the EBM may form part of a three-helix bundle protein domain. For example, the EBM may essentially constitute or form part

of two alpha helices with an interconnecting loop, within said three-helix bundle protein domain.

In particular embodiments of the invention, such a three-helix bundle protein domain is selected from domains of bacterial receptor proteins. Non-limiting examples of such domains are the five different three-helical domains of protein A from *Staphylococcus aureus*, and derivatives thereof. Thus, an EGFR-binding polypeptide according to the invention may comprise an amino acid sequence selected from:

ADNNFNK-[EBM]-DPSQSANLLSEAKKLNESQAPK (EBM within domain A of staphylococcal protein A);

ADNKFNK-[EBM]-DPSQSANLLAEAKKLNDAQAPK (EBM within domain B of staphylococcal protein A);

ADNKFNK-[EBM]-DPSVSKEILAEAKKLNDAQAPK (EBM within domain C of staphylococcal protein A);

ADAQQNNFNK-[EBM]-DPSQSTNVLGEAKKLNESQAPK (EBM within domain D of staphylococcal protein A);

AQHDE-[EBM]-DPSQSANVLGEAQKLNDSQAPK (EBM within domain E of staphylococcal protein A); and

VDNKFNK-[EBM]-DPSQSANLLAEAKKLNDAQAPK (EBM within the protein Z derivative of domain B of staphylococcal protein A);

wherein [EBM] is an EGFR-binding motif as defined above.

According to another aspect of the invention, there is provided an EGFR-binding polypeptide comprising an amino acid sequence derived from the amino acid sequence SEQ ID NO:327:

VDNKFNK EQQNAFYEILH LPNLNE EQRNAFIQSLKD DPSQ SANLLAEAKKLNDA QAPK

by comprising amino acid substitutions at any or all of positions 9 to 11, 13 to 14, 17 to 18, 24 to 25, 27 to 28, 32 and 35 of the above sequence, or positions corresponding to those positions, which substitutions

improve binding of the polypeptide to EGFR compared to a polypeptide comprising the unmodified amino acid sequence, and in which the EGFR-binding polypeptide binds to EGFR such that the K_D value of the interaction is at most 10 μM.

According to another alternative aspect thereof, the invention provides an EGFR-binding polypeptide, whose amino acid sequence comprises a sequence which fulfils one definition selected from the following: iii) it is selected from SEQ ID NO:164-326, and iv) it is an amino acid sequence having 85 % or greater identity to a sequence selected from SEQ ID NO:164-326. In embodiments of this aspect of the invention, the EGFR-binding polypeptide may in particular comprise a sequence selected from SEQ ID NO:196, SEQ ID NO:211, SEQ ID NO:220, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:310, and sequences having 85 % or greater identity thereto.

An EGFR-binding polypeptide according to any aspect of the invention may bind to EGFR such that the K_D value of the interaction is at most 1×10^{-6} M, for example at most 1×10^{-7} M.

When reference is made herein to the degree of identity between the amino acid sequences of different polypeptides, the lower limit of 85 % identity to a sequence disclosed herein is given. In some embodiments, the inventive polypeptide may have a sequence which is at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 % or at least 99 % identical to the sequence described herein. The comparison may be performed over a window corresponding to the shortest of the sequences being compared, or over a window corresponding to an EGFR-binding motif in at least one of the sequences being compared.

The polypeptides are advantageous in that they bind well to an EGFR. Typically, the polypeptides can be

relatively short and by virtue of their small size they should have better penetration in tumor tissue than antibodies while at the same time have better systemic circulation properties than conventional low molecular weight EGFR-binding substances (often too short half-lives) and monoclonal antibodies (often too long circulation times).

A polypeptide in accordance with the invention may be about 53-58 amino acids in length. However, the length can be greater or smaller. The length of the polypeptide can for example be reduced at the N terminus by up to four amino acids.

The use of the term "position" is relative. In a polypeptide in accordance with the invention which is also 53 amino acids long like the unmodified polypeptide mentioned above, the positions of amino acids in the polypeptide correspond exactly with those in the unmodified polypeptide when a situation where there is, for example, an N terminal extension compared to the unmodified polypeptide those amino acid residues in the modified peptide corresponding to the unmodified peptide have the same position number. For example if there is a six and amino acid residue extension on the modified polypeptide then amino acid number seven of that modified polypeptide, accounting from the N terminus corresponds to the amino acid in position number one of the unmodified polypeptide.

Accordingly, the polypeptides of the invention may be used as an alternative to conventional antibodies or low molecular weight substances in various medical, veterinary, diagnostic and imaging applications. For example, the EGFR-binding polypeptides of the invention may be used in the treatment of EGFR-related cancers such as those caused by over-expression of EGFR described above, especially when local distribution is applied, e.g. glioma. The EGFR-binding polypeptides of the invention may also be used to inhibit cell signalling by

binding to an EGFR on a cell surface, in the diagnosis of cancer, both *in vivo* and *in vitro* in targeting agents to cells which express EGFR, particularly cells which over-express EGFR, in histochemical methods for the detection of EGFR, in methods of separation and other applications. In addition to the development of molecular imaging agents for the clinic, an application exists for specific preclinical imaging agents to measure outcome of treatment in *in vivo* models and subsequently during clinical development. Molecular Imaging should provide a direct read-out of the efficacy of a pharmaceutical aimed to down-regulate a growth factor receptor e.g. HER2 or EGFR, as well as for assessing the anti-tumor effect. The polypeptides of the invention may be useful in any method which relies on affinity for EGFR of a reagent. Thus, the polypeptides may be used as a detection reagent, a capture reagent or a separation reagent in such methods, but also as a therapeutic agent in their own right or as a means for targeting other therapeutic agents, with direct (e.g. toxic molecules, toxins) or indirect therapeutic effects (e.g. cancer vaccines, immunostimulatory molecules) to the EGFR protein.

Methods that employ the polypeptides in accordance with the invention *in vitro* may be performed in different formats, such as microtitre plates, in protein arrays, on biosensor surfaces, on beads, in flow cytometry, on tissue sections, and so on.

The skilled addressee will appreciate that various modifications and/or additions can be made to a polypeptide according to the invention in order to tailor the polypeptide to a specific application without departing from the scope of the present invention. These modifications and additions are described in more detail below and may include additional amino acids in the same polypeptide chain, or labels and/or therapeutic agents that are chemically conjugated or otherwise bound to the polypeptide of the invention.

Furthermore, the invention also encompasses fragments of EGFR-binding polypeptides derived from protein A that retain EGFR-binding. The possibility of creating fragments of a wild-type SPA domain with retained binding specificity was shown by Braisted AC *et al* in Proc Natl Acad Sci USA **93**:5688-5692 (1996). In the experiments described in that paper, using a structure-based design and phage display methods, the binding domain of a three-helix bundle of 59 residues was reduced to a resulting two-helix derivative of 33 residues. This was achieved by stepwise selection of random mutations from different regions, which caused the stability and binding affinity to be iteratively improved. Following the same reasoning, with the polypeptides of the present invention, the skilled addressee will be able to obtain a "minimized" EGFR-binding polypeptide with the same binding properties as that of the "parent" EGFR-binding polypeptide. Thus, a polypeptide constituting a fragment of a polypeptide according to the invention, is within the scope of the invention.

The terms "EGFR-binding" and "binding affinity for EGFR" as used in this specification refers to a property of a polypeptide which may be tested for example by the use of surface plasmon resonance technology, such as in a Biacore instrument. For example as described in the examples below, EGFR-binding affinity may be tested in an experiment in which EGFR, or a fragment of EGFR such as the extracellular domain thereof, is immobilized on a sensor chip of the instrument, and the sample containing the polypeptide to be tested is passed over the chip. Alternatively, the polypeptide to be tested is immobilized on a sensor chip of the instrument, and a sample containing EGFR, or a fragment of EGFR such as the extracellular domain thereof, is passed over the chip. EGFR may, in this regard, be a polypeptide comprising the amino acid sequence SEQ ID NO:328, and its extracellular domain may be a polypeptide comprising the amino acid

sequence SEQ ID NO:329. The skilled person may then interpret the results obtained by such experiments to establish at least a qualitative measure of the binding affinity of the polypeptide for EGFR. If a qualitative measure is desired, for example to determine a K_D value for the interaction, surface plasmon resonance methods may also be used. Binding values may for example be defined in a Biacore 2000 instrument (Biacore AB). EGFR is immobilized on a sensor chip of the measurement, and samples of the polypeptide whose affinity is to be determined are prepared by serial dilution and injected in random order. K_D values may then be calculated from the results using for example the 1:1 Langmuir binding model of the BIAevaluation 4.1 software provided by the instrument manufacturer (Biacore AB).

Where amino acid substitutions are introduced, these should not affect the basic structure of the polypeptide. For example, the overall folding of the Cα backbone of the polypeptide can be essentially the same as that of a Z "wild-type" domain to which it is related, i.e. having the same elements of secondary structure in the same order. Thus polypeptides having this basic structure will have similar CD spectra to the Z "wild-type" domain. The skilled addressee is aware of other parameters that may be relevant. The requirement of conserving the basic structure, places restrictions on which positions of the amino acid sequence may be subject to substitution. For example, it is preferred that amino acid residues located on the surface of the polypeptide are substituted, whereas amino acid residues buried within the core of the polypeptide "three-helix bundle" should be kept constant in order to preserve the structural properties of the molecule. The same reasoning applies to fragments of polypeptides of the invention.

The invention also covers polypeptides in which the EGFR-binding polypeptide described above is present as an EGFR-binding domain to which additional amino acid

residues have been added at either terminal. These additional amino acid residues may play a role in the binding of EGFR by the polypeptide, but may equally well serve other purposes, related for example to one or more of the production, purification, stabilization, coupling or detection of the polypeptide. Such additional amino acid residues may comprise one or more amino acid residues added for the purpose of chemical coupling. One example of this, is the addition of a cysteine residue at the very first or very last position in the polypeptide chain, i.e. at the N. or C terminus. Such additional amino acid residues may also provide a "tag" for purification or detection of the polypeptide such as a His_6 tag or a "myc" tag or a "flag" tag for interaction with antibodies specific to the tag.

The invention also covers EGFR-binding polypeptides in which a EGFR-binding polypeptide as described above is present as an EGFR-binding domain to which additional peptides or proteins or other functional groups are coupled N- or C-terminally or to any other residues (specifically or non-specifically) by means of chemical conjugation (using known organic chemistry methods).

The "additional amino acid residues" discussed above may also provide one or more polypeptide domains with any desired function, such as the same binding function as the first, EGFR-binding domain, or another binding function, or an enzymatic function, toxic function (e.g. an immunotoxin), or a fluorescent signalling function, or combinations thereof.

The polypeptide of the invention may be in monomeric or multimeric forms. Multimeric forms of the polypeptide may be advantageous in that they may have enhanced binding properties. Preferred multimeric forms include dimeric, and trimeric forms. Multimeric forms of the polypeptides may comprise a suitable number of polypeptides of the invention. These polypeptides essentially form domains within the multimer. These

domains may all have the same amino acid sequence, but alternatively, they may have different amino acid sequences. The polypeptides may be joined by covalent coupling using known organic chemistry methods, or expressed as one or more fusion polypeptides in a system for recombinant expression of polypeptides, or joined in any other fashion, either directly or via a linker, for example an amino acid linker.

Additionally, fusion polypeptides, in which the EGFR-binding polypeptide of the invention provides a first domain or moiety, and second or further moieties have other functions than binding EGFR are also contemplated and within the scope of the present invention. The second or further moieties of such a fusion polypeptide may comprise a binding domain with an affinity for another target molecule than EGFR. Such a binding domain may be another, similar polypeptide binder. For example, the polypeptide binder may be a Z variant. This makes it possible to create multi-specific reagents that may be used in several types of applications such as medicine, veterinary medicine, diagnosis, separation, and imaging. The preparation of such multi-specific fusion polypeptides may be performed as generally described above.

In other embodiments of the invention, the second or further moieties may comprise an unrelated, naturally occurring or recombinant protein (or a fragment thereof which retains the binding or other ability of the naturally-occurring or recombinant protein) having a binding affinity for a target. For example, an EGFR-binding polypeptide in accordance with the invention may be joined to an albumin-binding domain of streptococcal protein G, or any other protein/peptide with affinity for a serum protein to improve the half-life of the EGFR-binding polypeptide for use in therapeutic applications.

The EGFR-binding polypeptides of the present invention may be provided in the form of other fusion

polypeptides. For example the EGFR-binding polypeptide, or fragment thereof, may be covalently coupled to a second or further moiety or moieties, which in addition to, or instead of target binding, exhibit other functions. One example would be a fusion between one or more EGFR-binding polypeptides and an enzymatically active polypeptide serving as a reporter or effector moiety. Examples of reporter enzymes, which may be coupled to the EGFR-binding polypeptide to form a fusion protein, are well-known to the skilled person and include enzymes such as β-galactosidase, alkaline phosphatase, horseradish peroxidase, carboxypeptidase. Other options for the second and further moiety or moieties of a fusion polypeptide according to the invention include fluorescent polypeptides, such as green fluorescent protein, red fluorescent protein, luciferase and variants thereof.

Other options for the second and further moiety or moieties of a fusion polypeptide according to the invention include a moiety or moieties for therapeutic applications. In therapeutic applications, other molecules can also be coupled, covalently or non-covalently, to the EGFR-binding polypeptide of the invention by other means. For example, other molecules such as enzymes for "ADEPT" (Antibody-Directed Enzyme Prodrug Therapy) applications using the polypeptide of the invention to direct the effector enzyme (e.g. carboxypeptidase) or RNase or DNase fusions; proteins for recruitment of effector cells and other components of the immune system; cytokines, such as IL-2, IL-12, TNFα, IP-10; pro coagulant factors, such as tissue factor, von Willebrand factor; toxins, such as ricin A, *Pseudomonas* exotoxins, calcheamicin, maytansinoid, toxic small molecules, such as auristatin analogues, doxorubicin.

The above-described additional amino acids (particularly hexahistidine, cysteine) can be used to couple chelators for radio-isotopes to the EGFR-binding

polypeptides in order to readily incorporate radionuclides for diagnosis (such as ^{68}Ga, ^{76}Br, ^{111}In, ^{99}Tc, ^{125}I) or therapy (e.g. ^{90}Y, ^{131}I, ^{211}At, ^{177}Lu).

The invention also embraces polypeptides in which the EGFR-binding polypeptide described above has been provided with a label group, such as at least one fluorophore, biotin or radioactive isotope, for example for the purposes of detection of the polypeptide.

The invention as claimed relates to:

- epidermal growth factor receptor (EGFR) binding polypeptide, comprising an epidermal growth factor receptor binding motif, EBM, which motif forms part of a three-helix bundle protein domain and consists of an amino acid sequence selected from: i) $EX_2X_3X_4AX_6X_7EIR\ X_{11}LPNLNGWQX_{20}\ TAFIX_{25}SLX_{28}D$, wherein, independently of each other, X_2 is selected from the group M, V, L and I; X_3 is selected from the group W, D and E; X_4 is selected from the group I, V, G, S, M, L, A, T, N and D; X_6 is selected from the group W, V and I; X_7 is selected from the group D, E, N and K; X_{11} is selected from the group D, N, E, Y and S; X_{20} is selected from the group M, L, and F; X_{25} is selected from the group A, S and G; and X_{28} is selected from the group L, V and F; and ii) an amino acid sequence which has at least 85 % identity to the sequence defined in i); the EGFR-binding polypeptide binding to EGFR such that the K_D value of the interaction is at most 10 µM defined in a Biacore 2000 instrument;

- EGFR-binding polypeptide, whose amino acid sequence comprises a sequence which fulfils one definition selected from the following: i) it is selected from the group SEQ ID NO:164-326; ii) it is an amino acid sequence having 85 % or greater identity to a sequence selected from the group SEQ ID NO:164-326;

- a polynucleotide encoding the polypeptide as described herein;

- method of producing the polypeptide as described herein, the method comprising expressing the polynucleotide as described herein;

- combination of the EGFR-binding polypeptide as described herein and a detectable agent;

- combination of the EGFR-binding polypeptide as described herein and a therapeutic agent;

- method of detection of EGFR, comprising providing a sample suspected to contain an EGFR, contacting the sample with the EGFR-binding polypeptide as described herein, or the combination as described herein and detecting binding of the polypeptide or combination to indicate the presence of an EGFR in the sample;

- method of separating or capturing EGFR from a sample, the method comprising contacting the sample with the EGFR-binding polypeptide as described herein or the combination as described herein, whereby EGFR binds to the polypeptide and can be removed from the sample;

- use of the EGFR-binding polypeptide as described herein or the combination as described herein for determining the presence of an EGFR in a mammalian subject, wherein the EGFR-binding polypeptide or combination is for contact with the subject or a sample derived from the subject;

- use of the EGFR-binding polypeptide as described herein or the combination as described herein for the treatment of an EGFR-related condition in a mammalian subject or in material derived from a mammalian subject;

- use of the EGFR-binding polypeptide as described herein or the combination as described herein for the manufacture of a diagnostic agent for the diagnosis of cancers caused by over-expression of EGFR *in vivo*; and

- use of the EGFR-binding polypeptide as described herein or the combination as described herein for the manufacture of a medicament for the treatment of cancers caused by over-expression of EGFR.

With regard to the description above of fusion polypeptides and proteins incorporating an EGFR-binding polypeptide of the invention, it should be noted that the designation of first, second and further moieties is made for the purposes of clarity to distinguish between the EGFR-binding moiety or moieties on the one hand, and moieties exhibiting other functions on the other hand. These designations are not intended to refer to the actual order of the different domains in the polypeptide chain of the fusion protein or polypeptide. Thus, for example, a first moiety may be appear at the N-

terminal end, in the middle, or at the C-terminal end of the fusion protein or polypeptide.

Further preferred aspects and embodiments of the invention will be apparent from the following listing of embodiments and the appended claims.

Embodiments of the present invention

1. An EGFR-binding polypeptide comprising an amino acid sequence derived from the amino acid sequence:
VDNKFNK EQQNAFYEILH LPNLNE EQRNAFIQSLKD DPSQ SANLLAEAKKLNDA QAPK
by comprising amino acid substitutions at any or all of positions 9 to 11, 13 to 14, 17 to 18, 24 to 25, 27 to 28, 32 and 35 of the above sequence, or positions corresponding to those positions, which substitutions improve binding of the polypeptide to EGFR compared to a polypeptide comprising the unmodified amino acid sequence, in which the EGFR-binding polypeptide binds to EGFR such that the K_D value of the interaction is at most 10 µM.
2. An EGFR-binding polypeptide according to embodiment 1 in which the amino acid substitution at position 9 is a hydrophobic amino acid.
3. An EFGR-binding polypeptide according to embodiment 1 or 2 in which the amino acid substitution at position 9 is a non-polar amino acid.
4. An EGFR-binding polypeptide according to any one of embodiments 1 to 3 in which the amino acid substitution at position 9 has an aliphatic R group.
5. An EGFR-binding polypeptide according to any one of embodiments 1 to 4 in which the amino acid substitution at position 9 has an aromatic R group.
6. An EGFR-binding polypeptide according to any one of embodiments 1 to 2 or 4 or 5 in which the amino acid substitution at position 9 is a polar amino acid.
7. An EGFR-binding polypeptide according to any one of embodiments 1 to 5 in which the amino acid substitution at position 9 is uncharged.
8. An EGFR-binding polypeptide according to any one of embodiments 1 to 7 in which the amino acid substitution at position 9 is a basic amino acid.
9. An EGFR-binding polypeptide according to any one of embodiments 1 to 8 in which the amino acid

substitution at position 9 is selected from W, M, T, F, H, S, L, A and V.

10. An EGFR-binding polypeptide according to embodiment 3 in which the amino acid substitution at position 9 is M, F, L, or V.

11. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 10 is a hydrophobic amino acid.

12. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 10 is a hydrophilic amino acid.

13. An EGFR-binding polypeptide according to any one of embodiments 1 to 11 in which the amino acid substitution at position 10 is neutral.

14. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 10 is a polar amino acid

15. An EGFR-binding polypeptide according to any one of embodiments 1 to 13 in which the amino acid substitution at position 10 is a non-polar amino acid.

16. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 10 is an acidic amino acid.

17. An EGFR-binding polypeptide according to any preceding embodiments in which the amino acid substitution at position 10 is selected from S, L, G, Y, A, E, W, and Q.

18. An EGFR-binding polypeptide according to embodiment 17, in which the amino acid substitution at position 10 is L, Y, E, or Q.

19. An EGFR-binding polypeptide according to any preceding embodiment, in which the amino acid substitution at position 11 is hydrophobic.

20. An EGFR-binding polypeptide according to any one of embodiments 1 to 18, in which the amino acid substitution at position 11 is neutral.
21. And EGFR-binding polypeptide according to any one of embodiments 1 to 19 in which the amino acid substitution at position 11 is hydrophilic.
22. An EGFR-binding polypeptide according to any preceding embodiment, in which the amino acid substitution at position 11 is a non-polar amino acid.
23. An EGFR-binding polypeptide according to embodiment 22 in which the amino acid substitution at position 11 has an aliphatic R group.
24. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 11 has a positively charged R group.
25. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 11 is a basic amino acid.
26. An EGFR-binding polypeptide according to any one of embodiments 1 to 21 in which the amino acid at position 11 is a polar amino acid.
27. An EGFR-binding polypeptide according to any one of embodiments 1 to 23 in which the amino acid at position 11 is uncharged.
28. An EGFR-binding polypeptide according to any one preceding embodiments in which the amino acid substitution at position 11 is selected from A, I, K, P, and N.
29. An EGFR-binding polypeptide according to embodiment 28 in which the amino acid substitution at position 11 is A, I, or K.
30. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 13 is hydrophobic.

31. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 13 is non-polar.
32. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 13 has an aliphatic R group.
33. An EGFR-binding polypeptide according to any one of embodiments 1 to 30 in which the amino acid substitution at position 13 is polar.
34. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 13 is uncharged.
35. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 13 has an aromatic R group.
36. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 13 is selected from A, S, V, M, I, Y, W and T.
37. An EGFR-binding polypeptide according to embodiment 8 in which the amino acid substitution at position 13 is M, I, Y, or V.
38. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid at position 14 is hydrophilic.
39. An EGFR-binding polypeptide according to any one of embodiments 1 to 37 in which the amino acid substitution at position 14 is neutral.
40. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 14 is polar.
41. An EGFR-binding polypeptide according to embodiment 40 in which the amino acid substitution at position 14 is uncharged.
42. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 14 is acidic.

43. An EGFR-binding polypeptide according to any one of embodiments 1 to 41 in which the amino acid substitution at position 14 is basic.
44. An EGFR-binding polypeptide according to any one of embodiments 1 to 39 in which the amino acid substitution at position 14 is non-polar.
45. An EGFR-binding polypeptide according to embodiment 44 in which the amino acid substitution at position 14 has an aliphatic R group.
46. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 14 has a negatively charged R group.
47. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 14 is selected from S, E, R, T, W, V, N, T and A.
48. An EGFR-binding polypeptide according to embodiment 47 in which the amino acid substitution at position 14 is S or T.
49. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 17 is neutral.
50. An EGFR-binding polypeptide according to any one of embodiments 1 to 48 in which the amino acid substitution at position 17 is hydrophilic.
51. An EGFR-binding polypeptide according to any one of embodiments 1 to 48 in which the amino acid substitution at position 17 is hydrophobic.
52. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 17 is polar.
53. An EGFR-binding polypeptide according to embodiment 52 in which the amino acid substitution at position 17 is uncharged.

54. An EGFR-binding polypeptide according to any one of embodiments 1 to 52 in which the amino acid at position 17 is positively charged.
55. An EGFR-binding polypeptide according to any one of embodiments 1 to 51 in which the amino acid substitution at position 17 is non-polar.
56. An EGFR-binding polypeptide according to embodiment 55 in which the amino acid at position 17 has an aliphatic R group.
57. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid at position 17 is basic.
58. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 17 is selected from: S, G, N, and V.
59. An EGFR-binding polypeptide according to embodiment 12 in which the amino acid substitution at position 17 is selected from G, N, and V.
60. An EGFR-binding polypeptide according to embodiment in which the amino acid at position 18 is neutral.
61. An EGFR-binding polypeptide according to any one of embodiments 1 to 59 in which the amino acid substitution at position 18 is hydrophilic.
62. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 18 is non-polar.
63. An EGFR-binding polypeptide according to any one of embodiments 1 to 61 in which the amino acid substitution at position 18 is polar.
64. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 18 is uncharged.
65. An EGFR-binding polypeptide according to any one of embodiments 1 to 63 in which the amino acid substitution at position 18 is positively charged.

66. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 18 is acidic.
67. An EGFR-binding polypeptide according to any one of embodiments 1 to 65 in which the amino acid substitution at position 18 is basic
68. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 18 is selected from G, S, D, R, N, H, E and K.
69. An EGFR-binding polypeptide according to embodiment 68 in which the amino acid substitution at position 18 is R or N.
70. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 24 is hydrophobic.
71. An EGFR-binding polypeptide according to any one of embodiments 1 to 69 in which the amino acid substitution at position 24 is neutral.
72. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 24 is basic.
73. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 24 is non-polar.
74. An EGFR-binding polypeptide according to embodiment 73 in which the amino acid substitution at position 24 has an aliphatic R group.
75. An EGFR-binding polypeptide according to any one of embodiments 1 to 72 in which the amino acid substitution at position 24 is polar.
76. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid at position 24 has an aromatic R group.
77. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid

substitution at position 24 is selected from K, W, N, G, L, R and M.

78. An EGFR-binding polypeptide according to embodiment 77 in which the amino acid substitution at position 24 is V or G.

79. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 25 is neutral.

80. An EGFR-binding polypeptide according to embodiment in which the amino acid substitution at position 25 is hydrophobic.

81. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 25 is non-polar.

82. An EGFR-binding polypeptide according to embodiment 81 in which the amino acid substitution at position 25 has an aliphatic R group.

83. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 35 has an aromatic R group.

84. An EGFR-binding polypeptide according to any one of embodiments 1 to 80 in which the amino acid at position 25 is polar.

85. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 25 is basic.

86. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 25 is selected from L, G, W, V, S , H, and W.

87. An EGFR-binding polypeptide according to embodiment 86 in which the amino acid substitution at position 25 is G or W.

88. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 27 is hydrophilic.

89. An EGFR-binding polypeptide according to any one of embodiments 1 to 88 in which the amino acid substitution at position 27 is hydrophobic.
90. An EGFR-binding polypeptide according to any one of embodiments 1 to 88 in which the amino acid substitution at position 27 is neutral.
91. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 27 is non-polar.
92. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 27 is acidic.
93. An EGFR-binding polypeptide according to any one of embodiments 1 to 90 in which the amino acid substitution at position 27 is polar.
94. An EGFR-binding polypeptide according to embodiment 93 in which the amino acid substitution at position 27 is uncharged.
95. An EGFR-binding polypeptide according to any one of embodiments 1 to 91 in which the amino acid substitution at position 27 is basic.
96. An EGFR-binding polypeptide according to any one of embodiments 1 to 93 in which the amino acid substitution at position 27 has a negatively charged R group.
97. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 27 is selected from A, E, F, M, L, C, K, G, and S.
98. An EGFR-binding polypeptide according to embodiment 97 in which the amino acid substitution at position 27 is E, M, or S.
99. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 28 is neutral.

100. An EGFR-binding polypeptide according to any one of embodiments 1 to 98 in which the amino acid substitution at position 28 is hydrophobic.

101. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 28 is non-polar.

102. An EGFR-binding polypeptide according to embodiment 101 in which the amino acid substitution at position 28 has an aliphatic R group.

103. An EGFR-binding polypeptide according to embodiment in which the amino acid substitution at position 28 is polar.

104. An EGFR-binding polypeptide according to embodiment 103 in which the amino acid substitution at position 28 is uncharged.

105. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 28 is basic.

106. An EGFR-binding polypeptide according to any preceding embodiment in which is the amino acid substitution at position 28 is selected from F, Q, V, A, K, V and T.

107. An EGFR-binding polypeptide according to embodiment 106 in which the amino acid substitution at position 28 is T, Q or V.

108. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 32 is hydrophobic.

109. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 32 is neutral.

110. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 32 is non-polar.

111. An EGFR-binding polypeptide according to embodiment 110 in which the amino acid at position 32 has an aliphatic R group.

112. An EGFR-binding polypeptide according to any one of embodiments 1 to 109 in which the amino acid substitution at position 32 is polar.

113. An EGFR-binding polypeptide according to embodiment 112 in which the amino acid substitution at position 32 is uncharged.

114. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 32 is basic.

115. An EGFR-binding polypeptide substitution according to any preceding embodiment in which the amino acid substitution at position 32 is selected from V,L, S, F, A and R.

116. An EGFR-binding polypeptide according to embodiment 115 in which the amino acid substitution at position 32 is L,S, or A.

117. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 35 is hydrophobic.

118. An EGFR-binding polypeptide according to any one of embodiments 1 to 116 in which the amino acid substitution at position 35 is neutral.

119. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 35 is non-polar.

120. An EGFR-binding polypeptide according to embodiment 119 in which the amino acid substitution at position 35 has an aliphatic R group.

121. An EGFR-binding polypeptide according to any one of embodiments 1 to 118 in which the amino acid substitution at position 35 is polar.

122. An EGFR-binding polypeptide according to embodiment 121 in which the amino acid substitution at position 35 is uncharged.

123. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 35 is basic.

124. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 35 has an aromatic R group.

125. An EGFR-binding polypeptide according to any preceding embodiment in which the amino acid substitution at position 35 is selected from V, W, S, R,M, H, and L.

126. An EGFR-binding polypeptide according to embodiment 125 in which the amino acid substitution at position 35 is W, S or V.

127. An EGFR-binding polypeptide according to any preceding embodiment in which amino acid residues located on the surface of the polypeptide are substituted.

128. An EGFR-binding polypeptide according to any preceding embodiment in which amino acid residues within the core of the polypeptides three-dimensional structure are not substituted.

129. An EGFR-binding polypeptide according to any preceding embodiment which has been extended by C terminal and/or N terminal amino acid extensions.

130. An EGFR-binding polypeptide according to embodiment 129 in which the or each amino acid extension enhances binding of EGFR by the polypeptide.

131. An EGFR-binding polypeptide according to embodiment 129 or 130 in which the or each amino acid extension improves production, purification, stabilization *in vivo* or *in vitro*, coupling, or detection of the polypeptide.

132. An EGFR-binding polypeptide according to embodiment 131 in which the or each amino acid extension includes a cysteine residue at the first or last position in the amino sequence of the polypeptide.

133. An EGFR-binding polypeptide according to embodiment 131 in which the amino acid residue extension comprises a His_6 tag, or a "myc" or a "flag" tag.

134. An EGFR-binding polypeptide according to embodiment 131 in which the extension comprises an albumin-binding domain of streptococcal protein G, or a derivative thereof, which improves the half life of the EGFR-binding polypeptide in therapeutic applications.

135. An EGFR-binding polypeptide according to any preceding embodiment comprising about 53 amino acids.

136. An EGFR-binding polypeptide according to any preceding embodiment which binds to EGFR such that the K_D value of the interaction is at most $1x10^{-6}$ M.

137. An EGFR-binding polypeptide according to embodiment 136 which binds to EGFR such that the K_D value of the interaction is at most $1x10^{-7}$ M.

138. An EGFR-binding polypeptide according to any preceding embodiment which binds to the extra-cellular domain of EGFR.

139. An EGFR-binding polypeptide according to embodiment 138 which binds to a portion of the extra-cellular domain of EGFR (SEQ ID NO:329) corresponding to nucleotides 259-2127 of the mature EGFR (SEQ ID NO:328).

140. An EGFR-binding polypeptide comprising a fragment of an EGFR-binding polypeptide according to any preceding embodiment.

141. An EGFR-binding polypeptide according to embodiment 140 in which the fragment comprises an N terminal reduction of a polypeptide according to any one of embodiments 1 to 139.

142. An EGFR-binding polypeptide according to embodiment 141 in which the N terminal reduction is by up to four amino acids.

143. An EGFR-binding polypeptide according to any preceding embodiment in multimeric form comprising EGFR-binding polypeptide units.

144. An EGFR-binding polypeptide according to embodiment 143 in which the EGFR-binding polypeptide monomer units are covalently coupled together.

145. An EGFR-binding polypeptide according to embodiment 143 in which the EGFR-binding polypeptide monomer units are expressed as a fusion protein.

146. An EGFR-binding polypeptide according to any one of embodiments 143 to 145 in a dimeric form.

147. A nucleotide encoding a polypeptide according to any preceding embodiment.

148. A method of producing a polypeptide according to any one of embodiments 1 to 146 the method comprising expressing a nucleotide according to embodiment 147.

149. A combination of an EGFR-binding polypeptide according to any one of embodiments 1 to 146, and a detectable agent.

150. A combination according to embodiment 149, in which the detectable agent is a radioactive substance for use in radio-imaging.

151. A combination according to embodiment 150 in which the radioactive substance is a radionuclide.

152. A combination according to embodiment 149 in which the detectable agent is an enzyme.

153. A combination according to embodiment 152 in which the enzyme is selected from β-galactosidase, alkaline phosphatase, horseradish peroxidase, and a carboxypeptidase.

154. A combination according to embodiment 149 in which the detectable agent is a fluorescent polypeptide.

155. A combination of an EGFR-binding polypeptide according to any one of embodiments 1 to 146, and a therapeutic agent.

156. A combination according to any one of embodiments 149 to 155 in which the EGFR-binding polypeptide and detectable agent or therapeutic agent are covalently coupled together.

157. A combination according to any one of embodiments 149 to 155 in which the EGFR polypeptide and detectable agent or therapeutic agent are expressed as a fusion protein.

158. A method of radio-imaging in which a combination according to any one of embodiments 150 to 151 is used as a radio-imaging agent.

159. A method of detection of EGFR, comprising providing a sample suspected to contain an EGFR, contacting the sample with an EGFR-binding polypeptide according to any one of embodiments 1 to 146, or a combination according to any one of embodiments 149 to 154 and detecting binding of the polypeptide or combination to indicate the presence of an EGFR in the sample.

160. A method of detection according to embodiment 159 in which more than one EGFR is detected.

161. A method of separating or capturing EGFR from a sample, the method comprising contacting the sample with an EGFR-binding polypeptide according to any one of embodiments 1 to 146 or a combination according to any one of embodiments 149 to 154 whereby EGFR binds to the polypeptide and can be removed from the sample.

162. A diagnostic method, for determining the presence of an EGFR in a subject, the method including contacting the subject, or a sample derived from the subject, with an EGFR-binding polypeptide according to any one of embodiments 1 to 146, or a combination according to any one of 149 to 154 and detecting binding of the polypeptide or combination.

163. A method according to embodiment 162 in which the subject is human or animal.

164. A method according to embodiment 162 in which the method is performed *in vivo*.

165. A method according to embodiment 162 or 163 in which the method is performed on a sample *in vitro*.

166. A method of treatment of an EGFR-related condition in a subject or in material derived from a subject, in which the subject or material is treated with an EGFR -binding polypeptide according to any one of embodiments 1 to 146 or a combination according to any one of embodiments 155 to 157.

167. A method of treatment according to embodiment 166 in which binding of an EGFR-binding polypeptide according to any one of embodiments 1 to 146 or a combination according to any one of embodiments 155 to 157 to an EGFR of the subject, or in the material, inhibits or stimulates activation of the receptor.

168. A method of treatment according to embodiment 166 or 167 in which binding of the EGFR-binding polypeptide to an EGFR of the subject, or in the material, inhibits cell signalling.

169. A method of treatment according to any one of embodiments 166 to 168, in which the EGFR-related condition is a cancer.

170. A method of treatment according to embodiment 169 in which the cancer is selected from lung, breast, prostate, colon, ovary, head and neck cancers.

171. A method according to any one of embodiments 166 to 170 in which subject is human or animal.

Brief description of the drawings

Polypeptides in accordance with the invention and methods for their use will now be described, by way of example only, with reference to the accompanying drawings, Figures 1-12, in which:

Figure 1 is a listing of the amino acid sequences of examples of EGFR binding motifs comprised in EGFR-binding polypeptides of the invention (SEQ ID NO:1-163), examples of EGFR-binding polypeptides according to the invention (SEQ ID NO:164-326), the protein Z derivative of domain B of *Staphylococcus aureus* protein A (SEQ ID NO:327),

entire human EGFR (SEQ ID NO:328) and the extracellular domain of human EGFR (SEQ ID NO:329);

Figure 2A shows the amino acid sequences of different EGFR-binding polypeptides according to the invention selected in Example 1 compared to the protein Z sequence. The figure indicates basic, acidic, non-polar and polar amino acid residues; Figure 2B shows the amino acid sequence of four polypeptides from Figure 2A and indicates hydrophobic, neutral and hydrophilic amino acid residues, Figure 2C shows the amino acid sequences of the polypeptides of Figure 2B with other characteristics highlighted, and Figure 2D illustrates an affinity maturation strategy for producing polypeptides according to the invention;

Figure 3 shows the result of SDS-PAGE analysis of EGFR-binding polypeptides His_6-$Z_{EGFR:942}$ (lane 1), His_6-$Z_{EGFR:948}$ (lane 2), His_6-$Z_{EGFR:955}$ (lane 3), His_6-$(Z_{EGFR:942})_2$ (lane 4), His_6-$(Z_{EGFR:948})_2$ (lane 5), and His_6-$(Z_{EGFR:955})_2$ (lane 6). Lane M contains marker proteins. To the right, molecular mass is given in kilodaltons.

Figure 4 shows the result of biosensor binding studies conducted using various EGFR-binding polypeptides according to the invention;

Figure 5 shows the result of flow cytometric analysis of the affinity for native EGFR of three EGFR-binding polypeptides according to the invention;

Figure 6 is a series of confocal microscopy images of cells exposed to fluorophore-labeled EGFR-binding polypeptides according to the invention;

Figure 7 is a diagram showing the result of cellular binding studies with radio-labeled EGFR-binding polypeptides according to the invention;

Figure 8 is a series of graphs showing the results of saturation and studies with radio-labeled EGFR-binding polypeptides according to the invention;

Figure 9 shows the results of biosensor binding studies conducted using various EGFR-binding polypeptides according to the invention: using Biacore analysis, affinity-matured Z01853, Z01868, Z01877, Z01907 and Z01908 (K_D 10 nM) were compared with a dimeric (K_D 50 nM) form of Z00955 (Figure 9A), and with a monomeric (K_D 185 nM) form of Z00955 (Figure 9B);

Figure 10 is a series of images of cells exposed to EGFR-binding polypeptides according to the invention, using A) fluorescent detection and B) enzymatic detection;

Figure 11 is a diagram showing the results of an *in vitro* specificity test of indium-111 labeled benzyl-DTPA conjugates of EGFR-binding polypeptides according to the invention on A431 cells. All data points are mean values of three measurements, and error bars represent SEM.

Figure 12 is a series of diagrams showing biodistribution of ^{111}In-benzyl-DTPA-EGFR binding conjugates and tumor to normal tissue ratios in mice bearing A431 xenografts. Each data point represents an average from four animals ± standard deviation and is expressed as the percent of injected radioactivity per gram organ or tissue.

In the following experiments, phage display was used to select EGFR-binding variants of protein Z derived from the B domain of *Staphylococcus aureus* protein A. The EGFR-binding Z variants are sometimes collectively denoted Z_{EGFR}. Each individual Z variant has been given a unique identification number #####, and individual variants are interchangeably referred to as Z##### and $Z_{EGFR:\#\#\#\#\#}$.

Example 1
First selection of EGFR binding polypeptides according to the invention

Materials and Methods

Production of polypeptide binders, strains, vectors, and phagemid library

The amber suppressor *Escherichia coli* strain RRIΔM15 (Rüther, U. (1982) Nucleic Acids Res., 10, 5765-5772.) was used as bacterial host for phage production and cloning procedure. The phagemid vector pAffi1, and the construction of the phagemid library, Zlib2002 (3×10^9 members), used in this study are disclosed in Grönwall C, Jonsson A, Lindström S, Gunneriusson E, Ståhl S, Herne N: "Selection and characterization of Affibody ligands binding to Alzheimer amyloid beta peptides", J. Biotechnol. (2006) in press, Epub 27 Sep 2006. Phagemid inserts of selected clones were sub-cloned into the expression vector pAY442 and pAY430, containing a T7 promoter (Studier *et al.*, (1990) Methods Enzymol., 185, 60-89), a DNA fragment encoding a hexahistidyl (His_6) tag and a multiple cloning site, together with a gene conferring resistance to kanamycin, as well as an additional cysteine at the C-terminus for direct labeling for pAY430. The *E.coli* strain BL21(DE3) (Novagen, Madison, WI) was used for protein production from the expression vectors.

Preparation of phage stock

Preparation of phage stocks from the library (a portion of Zlib2002) and between selections was performed according to previously described procedures (Nord, K *et al.*, (1997) Nat. Biotechnol., 15, 772-777; Hansson *et al.*, (1999) Immunotechnology, 4, 237-252) using the helper phage M13K07 (New England Biolabs, Beverly, MA).

PEG/NaCl precipitation yielded phage titres of about 10^{13} pfu/ml.

Phage selections

A ~100 kDa recombinant extra-cellular domain (ECD) of EGFR comprising 623 amino acids, corresponding to nucleotides 259-2127, was used as the target protein during selections (SEQ ID NO:329). The protein was biotinylated *in vitro* using EZ-LinkTM-Sulfo-NHS-LC-Biotin (Pierce, Rockford, IL). A 20-fold molar excess of biotin was added to the EGFR-ECD in phosphate-buffered saline (PBS; 10 mM phosphate, 137 mM NaCl, pH 7.2), and the mixture was incubated at room temperature for 1 h. followed by extensive dialysis against PBS at 4 °C to remove the surplus biotin.

The biotinylated target protein was then immobilized on streptavidin-coated paramagnetic beads (Dynabeads M-280 Streptavidin; Dynal A.S., Oslo, Norway). For each round of selection, beads were washed twice with PBS supplemented with 0.1 % Tween-20 (PBST). To avoid unspecific binders, all tubes used in this procedure were pre-treated with PBST supplemented with 0.1 % gelatin. To further avoid binders against the streptavidin present on the paramagnetic beads, the phage stock in PBST supplemented with 0.1 % gelatin was pre-incubated with 0.2 mg of the beads (previously washed twice with PBST) for round 1 and 2. The unbound phage stock was then subjected to biopanning against the EGFR-ECD target protein for 1 h 45 min at room temperature under continuous end-over-end rotation, followed by incubation with the streptavidin-coated paramagnetic beads for 15 min (room temperature, continuous end-over-end rotation). Two separate selections, with each two different decreasing target concentrations in each panning round were performed as follows. For round 1; 12 and 1.2 µg of target protein were incubated with 6 and 0.6 mg of beads, respectively, for round 2; 5, 2.5, 0.5, and 0.35 µg of

target protein were incubated with 2.5, 1.25, 0.25, 0.125 mg of beads, respectively, and for rounds 3 and 4; 5, 1, 0.5, and 0.1µg of target protein were incubated with 1, 0.5, 0.1, 0.05 mg of beads, respectively. This procedure resulted in an immobilization of ~2 µg of the target protein per mg of beads, as determined by SDS-PAGE analysis. The four rounds of biopanning were performed as follows. The beads were washed twice with PBST in round 1, five times in round 2, seven times in round 3 and 10 times in round 4. The bound phages were subsequently eluted with 500 µl of 50 mM glycine-HCl, pH 2.1, for 10 min at room temperature, followed by immediate neutralization with 50 µl of 1 M Tris-HCl, pH 8.0 and 450 µl PBS.

The eluted phages were used to infect log phase RRIΔM15 cells for 30 min at 37 °C. The infected cell suspensions were spread on TYE agar plates (15 g/l agar, 8 g/l NaCl, 10 g/l tryptone and 6 g/l yeast extract), supplemented with 2 % glucose and 100 mg/l ampicillin, and followed by overnight incubation at 37 °C. The grown colonies were collected by re-suspension in tryptic soy broth (TSB, 30 g/l; Merck, Darmstadt, Germany), supplemented with 5g/l yeast extract, 2 % glucose and 100 µg/ml ampicillin, and a fraction (~500 times excess of cells compared to the phage titre after elution) was used for inoculation, leading to the next generation of phage stock. The selection process was monitored by titrating the phage stocks before selection and after elution. A serial dilution of phages was allowed to infect log phase RRIΔM15 cells for 5 min at room temperature, followed by plating on TYE agar plates, supplemented with 2 % glucose and 100 µg/ml ampicillin, and overnight incubation at 37 °C.

Streptavidin ELISA

After four rounds of biopanning, an ELISA was performed on 372 randomly picked colonies from all four selections, to exclude phagemid (pAffi1) inserts with streptavidin binding capacity. Cell lysates from the randomly picked colonies were incubated in pre-blocked (PBST supplemented with 2 % dry milk) 96 well streptavidin coated plate (Nunc transparent, c96, 236001) for 1.5 hours at room temperature. As a primary antibody a rabbit IgG pan-anti-polypeptide-specific binder (1.5 hours, room temperature, continuous shake) and as secondary antibody a rabbit immunoglobulin-HRP were used (P0448 Daco Cytomatation; 1 hour, room temperature, continuous shake). The A_{405nm} absorbency was measured with a Tecan Sunrise spectrophotometer after the addition of the substrate solution (Immunopure TMB; Pierce).

DNA sequencing

DNA sequencing of phagemid (pAffi1) inserts was performed on non-streptavidin binding clones from the fourth round of panning, where 64 clones were from selection 1 and 2, and 32 from selection 3 and 4. Specific primers and Big Dye terminators (Amersham Biosciences, Uppsala, Sweden) were used and the Sanger fragments analyzed on a DNA sequencer ABI prism 3700 Analyzer (Applied Biosystems, Foster City, CA). Sub-cloned DNA fragments were verified by the same procedure.

After excluding sequences with amber stop codons (three), more than one cysteine (one), and sequences that have been found in selections to other targets (three), ten sequences were chosen to be further investigated. The respective amino acid sequences of these polypeptide binders is shown in Figure 1 and disclosed in the sequence listing as SEQ ID NO:164-173. The deduced EGFR binding motif of these variants are presented as SEQ ID NO:1-10. The sequences of the selected variants are also presented in Figure 2A. Specifically, in Figure 2A, the

amino acid sequence corresponding to the "wild-type" Z domain is aligned to the deduced amino acid sequences of the 10 different polypeptide binders selected against EGFR-ECD, the dashes used in that Figure, and elsewhere in this specification, represent an amino acid which is the same as the corresponding amino acid in the "wild-type" sequence. The 13 randomized amino acid residues (Q9, Q10, N11, F13, Y14, L17, H18, E24, E25, R27, N28, Q32, K35) are presented. Amino acid residues that occur at the same position in more than one of the variants are presented in bold. Horizontal bars indicate amino acid identities. Figures to the right represent the number of times each polypeptide binder was detected upon DNA sequencing of 372 colonies. The three α-helices in the wild-type Z domain are boxed.

Figure 2B and Figure 2C give further characteristics of the amino acid substitutions in the polypeptide binders of the invention. In the context of hydrophobicity/hydrophilicity, "neutral" means an amino acid which is relatively neither hydrophobic nor hydrophilic.

Figure 2D illustrates a maturation strategy for improving the initially-determined polypeptide binders. In this connection, the residues at positions 9, 10, 11, 13, and 14 may be less important and subjected to substitutions, whereas for positions 17 and 18, asparagine and arginine are especially preferred although serine and histidine, which may be preferred for technical reasons, may also be produced and used for binding as a result of codon similarity. At position 35, valine and serine are preferred although for technical reasons, leucine and alanine may be particularly selected as well. For positions 24, 25, 27, 28 and 32, amino acids G, W, M, T, and A are contemplated respectively, although single substitutions at any of these sites may occur with retained binding EGFR-capacity of the molecules.

DNA constructs

DNA fragments encoding different EGFR polypeptide binders were sub-cloned into the expression vectors pAY442 and pAY430. The fragments were amplified from the pAffi1 vector with specific primers introducing an AccI site both 3′ and 5′, and ligated into the pAY442 and pAY430 vectors, previously restricted with the same enzyme, and dephosphorylated using Calf Intestine Alkaline Phosphatase (CIAP; Fermentas). The amplified DNA fragments were purified with QIAuick PCR Purification Kit (Qiagen GmbH, Hilden, Germany) and hybridized prior to ligation with T4 DNA Ligase (New England Biolabs). The ligations resulted in expression vectors denoted pAY442-$Z_{EGFR:no}$ and pAY430-$Z_{EGFR:no}$, encoding the different polypeptide binders fused to an N-terminus His_6 tag, allowing purification by immobilized metal ion affinity chromatography (IMAC). All plasmid preparations were, after cultivation of transformed *E. coli* cells overnight, performed using QIAprep Spin Miniprep Kit (Qiagen GmbH) according to the manufacturer's instructions.

Protein production and purification

Selected polypeptide binders were expressed as His_6-tagged fusion proteins from the pAY442 and pAY430 plasmids in *E.coli* strain BL21(DE3).

Cells were inoculated in 5 ml of TSB medium (30 g/l Tryptic Soy Broth), containing 50 mg/l kanamycin, and grown in deep well plate overnight at 37°C at ~150 rpm. Fresh TSB (5 ml), supplemented with 5 g/l yeast extract and 50 mg/l kanamycin, was inoculated with 20 µl of the overnight cultures and the cells were grown at 37 °C for 4 hours, when gene expression was induced by addition of isopropyl β-D-thiogalactoside (IPTG) to a final concentration of 1 mM. After overnight cultivation at 25 °C, the cells were harvested by centrifugation (10000 *g*, 10 min) and lysated by freeze thawing (-80 °C, 40 min). The cell pellets were subsequently re-suspended in urea

buffer (8 M, pH 8.0). The His_6-Z_{EGFR} fusion proteins were recovered by IMAC purification on Ni-NTA Superflow columns under denaturing conditions (Qiagen) using BR3000 robot. The bound proteins were eluted with low pH urea buffer (8 M, pH 4.5) and renaturation of the purified fusion protein was performed by changing the buffer to HBS (10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.005 % surfactant P20, pH 7.4) on NAP™-5 size exclusion chromatography columns (Amersham Biosciences). Protein concentration for the polypeptides was calculated from absorbance measurements at 280 nm, using the appropriate extinction coefficient for each protein. The purified polypeptides were further analyzed by SDS-PAGE on Phastgel™ Homogenous 20 % gels using a Phast system (Amersham Biosciences, Uppsala, Sweden). Protein concentrations for selected Z_{EGFR} variants were also determined by amino acid analysis (Aminosyraanalyscentralen, Uppsala, Sweden).

Figure 3 shows SDS-PAGE analysis of the expressed and IMAC-purified EGFR-binding polypeptides His_6-$Z_{EGFR:942}$ (lane 1), His_6-$Z_{EGFR:948}$ (lane 2), His_6-$Z_{EGFR:955}$ (lane 3), His_6-$(Z_{EGFR:942})_2$ (lane 4), His_6-$(Z_{EGFR:948})_2$ (lane 5), and His_6-$(Z_{EGFR:955})_2$ (lane 6). Lane M, marker proteins with molecular masses in kilodaltons.

Biosensor analyses

A BIAcore® 2000 instrument (Biacore AB, Uppsala, Sweden) was used for real-time biospecific interaction (BIA) between selected polypeptide binders and the target protein. EGFR-ECD (diluted in 10 mM NaAc, pH 4.5) was immobilized (~2600 RU) on the carboxylated dextran layer of one flow-cell surface of a CM5 sensor chip (Biacore) by amine coupling, according to the manufacturer's instructions. Another flow-cell surface was activated and deactivated to be used as a reference surface, and HER2-ECD and human IgG (Amersham Biosciences, Uppsala, Sweden) were immobilized on separate flow-cell surfaces on the

CM5 sensor chip, to serve as negative controls. Samples of all polypeptide binders under test were diluted in the running buffer HBS (10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.005 % surfactant P20, pH 7.4) and filtrated (0.45 µm; Millipore, Billerica, MA) before binding analysis were performed at 25 °C. In a first experiment, ~1 µM of each polypeptide binder under test (diluted in HBS) was injected over all surfaces with a flow rate of 20 µl/min. An unrelated 53 amino acid polypeptide binder, having no affinity for EGFR, was used as negative control, and the natural ligand hEGF (Chemicon International, Temecula, CA, USA) and commercial monoclonal antibody cetuximab (MERCK Darmstadt, Germany) as positive controls, were also injected. In a second experiment, the monomeric His_6-Z_{EGFR} and dimeric His_6-$(Z_{EGFR})_2$ polypeptide binders were subjected to kinetic analysis, in which the proteins were injected over an EGFR-ECD surface at concentrations ranging from 0.00625 µM to 12.8 µM with a flow rate of 30 µl/min. The dissociation equilibrium constant (K_D), the association rate constant (k_a), and the dissociation rate constant (k_D) were calculated using BIAevaluation 3.2 software (Biacore), assuming a one-to-one binding. For the second experiment the samples were run in duplicates in random order, and after each injection the flow cells were regenerated by the injection of 10 mM HCl.

The results of the biosensor ranking analyses are depicted in Table 1 and Figure 4. Table 1 gives a comparison of kinetic parameters of the monovalent and bivalent EGFR-ECD binding polypeptide binders from biosensor analysis on BIAcore. The dimeric EFGR-binding polypeptide constructs were generated through a gene duplication strategy, produced and affinity purified as previously described in Steffen *et al* Cancer Biother. & Radiopharmaceuticals, 20, 239-248. An additional polypeptide, $Z_{EGFR:1239}$ (identified as a sequence-relative to $Z_{EGFR:955}$), was included after sequencing of additional clones, and data on its performance as monomer are

disclosed. The dissociation equilibrium constant gives the following affinity ranking of the four His_6-Z_{EGFR} polypeptide binders: His_6-$Z_{EGFR:1239}$ < His_6-$Z_{EGFR:955}$ < His_6-$Z_{EGFR:948}$ < His_6-$Z_{EGFR:942}$.

Table 1

EFGR-binding polypeptide	K_D[a] (nM)	k_a[b] ($M^{-1}s^{-1}$)	k_d[c] (s^{-1})
His_6-$Z_{EGFR:942}$	~130	~3.0 x 10^5	~4.0 x 10^{-2}
His_6-$(Z_{EGFR:942})_2$	~30	~6.0 x 10^5	~1.6 x 10^{-2}
His_6-$Z_{EGFR:948}$	~180	~4.2 x 10^5	~7.7 x 10^{-2}
His_6-$(Z_{EGFR:948})_2$	~40	~1.9 x 10^5	~8.1 x 10^{-3}
His_6-$Z_{EGFR:955}$	~190	~6.2 x 10^4	~1.2 x 10^{-2}
His_6-$(Z_{EGFR:955})_2$	~50	~4.8 x 10^4	~2.4 x 10^{-3}
His_6-$Z_{EGFR:1239}$	~490	~1,9 x 10^5	~9,2 x 10^{-2}

[a] Dissociation equilibrium constant
[b] Association rate constant
[c] Dissociation rate constant

It can be seen that from this *in vitro* binding analysis, all four EFGR-binding polypeptides bound EFGR with rather high affinity and that they differed somewhat in their binding kinetics characteristics.

Figure 4A shows the results of sensorgrams obtained after injection of the purified His_6-$(Z_{EGFR:942})_2$ (squares), His_6-$(Z_{EGFR:948})_2$ (triangles), and $His_6(Z_{EGFR:955})_2$ (circles) variants over sensor chip flow-cell surfaces containing amine-coupled EGFR-ECD (filled square/triangles/circles) or HER2-ECD (open squares/triangles/circles). This demonstrates a specific binding of the three His_6-Z_{EGFR} variants (His_6-$Z_{EGFR:942}$, His_6-$Z_{EGFR:948}$, and His_6-$Z_{EGFR:955}$) to the EGFR-ECD immobilized flow-cell surfaces, whereas no binding to the HER2-ECD immobilized flow-cell surface is seen.

Figure 4B shows the results of sensorgrams obtained after the injection of monovalent (lighter line) and bivalent (darker line) EGFR-binding polypeptides over an EGFR-ECD flow-cell surface. The diagram shows the three candidate binders, where the difference in off-rate between monovalent and bivalent EGFR-binding polypeptides is demonstrated, proving that the improvement of apparent affinity by avidity effect was achieved by primarily obtaining a slower off-rate in the second generation clones.

Cell culture

For the Fluorophore Labeling FACS, and Confocal Microscope studies below, Human epithelial cancer cells A431 (European Collection of Cell Cultures, Wiltshire, UK), known to express ~2 x 10^6 EGFR per cell, were cultured in complemented medium, containing EMEM medium supplemented with 10 % foetal calf serum, 2 mM L-glutamine, 1 % non-essential amino acids, and 1 % antibiotic-antimycotic, all from Gibco (Invitrogen AB). The cells were cultured at 37 °C in humidified air containing 5 % CO_2.

Fluorophore labeling

His_6-$(Z_{EGFR:942})_2$, His_6-$(Z_{EGFR:948})_2$, and His_6-$(Z_{EGFR:955})_2$ polypeptide binders were labeled directly to the introduced cysteine (at C-terminus) with Oregon Green® 488 maleimide (Molecular Probes). Approximately 1 mg of His_6-$(Z_{EGFR})_2$ polypeptide binder was re-suspended in PBS and reduced with 20 mM DTT for 45 min at 37 °C. Surplus DTT was removed on a NAP™-5 size exclusion column (Amersham Biosciences) equilibrated with PBS. A 10 mM solution of Oregon Green 488 maleimide was added at 20-fold molar excess and kept dark for 2 hours at room temperature with continuous shaking. Extensive dialysis against PBS was performed to remove excess fluorophore. The concentration and labeling performance of the

fluorophore-labeled polypeptide binders under test were done by calculations according to manufacturer's protocol using absorbance measurements at 280 and 496 nm. The labeled polypeptide binders were also analyzed on an SDS-PAGE Phastgel™ Homogenous 20 % gel using a Phast system (Amersham Biosciences).

FACS

The flow cytometric analyses were performed on a FACS Vantage SE stream-in-air flow cytometry instrument (BD Biosciences, San Jose, CA, USA). The laser was aligned using flow cytometry alignment beads for 488 nm (Molecular Probes, Leiden, The Netherlands). Samples were illuminated with an air-cooled argon laser (488 nm). The fluorescence, the forward scattered and side scattered light from 10000 cells were detected at a rate of approximately 300 events s^{-1}. Flow cytometric data were analyzed with CellQuest software (BD Biosciences). Prior to flow cytometric analyses, cells seeded in Petri dishes ~3 days before experiment were trypsinated (0.25 % Trypsin, 37 °C, 10 min). The cells were centrifuged (582 g, 3 min) and the pellet re-suspended in PBS+1 % BSA, and aliquoted at ~300000 cells per well in a 96 well plate. The cells were incubated with 10 µg/ml fluorophore-labeled $His_6-(Z_{EGFR})_2$ polypeptide binder for ~30 min on ice. After centrifugation and washing with PBS+1 % BSA the cell pellet was re-suspended in 300 µl PBS+1 % BSA and subjected to flow cytometric analysis. A similar (His_6-tagged dimeric construct) polypeptide having no binding capacity for EGFR was used as negative control.

The results of these studies are shown in Figure 5. Specifically, Figure 5 shows a flow cytometric analysis demonstrating a ranking of affinity for the three candidate binders ($His_6-(Z_{EGFR:942})_2$, $His_6-(Z_{EGFR:948})_2$, $His_6-(Z_{EGFR:955})_2$) towards native EGFR on A431 cells. An unrelated Z variant molecule, used as a negative control (white), is positioned to the far left in the histogram.

The three Z_{EGFR} binders are then positioned in the order His_6-$(Z_{EGFR:942})_2$ (light grey)<His_6-$(Z_{EGFR:948})_2$ (grey)<His_6-$(Z_{EGFR:955})_2$ (black). These data suggest that $Z_{EGFR:955}$ may be the best candidate of the three, in spite of its somewhat poorer affinity in BIAcore, since the assay is based on binding of native EGFR on cells.

Confocal microscopy

Approximately 300000 A431 cells were seeded per 30 mm Petri dish the day before the experiment. The His_6-$(Z_{EGFR:942})_2$, His_6-$(Z_{EGFR:948})_2$, and His_6-$(Z_{EGFR:955})_2$ polypeptide binders under test were diluted to approximately 10 µg/ml in complete EMEM medium, added to separate Petri dishes and incubated in the dark for 2 hours at 37 °C. The three polypeptide binders under test were also diluted as above in serum-free EMEM medium, added to separate Petri dishes and incubated in the dark 1 hour on ice. Following the incubation the cells were washed once with normal medium and some medium was added for image analysis in a confocal microscope (LSM 5 Pascal; Zeiss). Consecutive scans were performed to cover the thickness of the cell and a scan representing the middle of the cell was chosen. As a negative control, a similar polypeptide having no affinity for EGFR was analyzed in the same way.

The results of the confocal microscopy are shown in Figure 6. Specifically, Figure 6 shows confocal microscopy images of A431 cells exposed to Oregon Green labeled His_6-Z_{EGFR} polypeptide for A) 1 hour on ice and B) 2 hours in 37 °C. From left to right, His_6-$(Z_{EGFR:942})_2$, His_6-$(Z_{EGFR:948})_2$, and His_6-$(Z_{EFGR:955})_2$ are seen cell membrane bound in (A) and internalized in (B). The results demonstrate that the three EFGR-binding polypeptides seem, as expected, to bind to the cellular membrane, and that internalization seems to occur at incubation at 37 °C.

Cell culture

For the radio labeling, specificity and saturation studies below, cells were cultured in 75 cm^2 culture bottles and in 24-well plates (Nunclon surface, Denmark). For the labeling method, ^{125}I (Amersham Biosciences, Uppsala, Sweden), acetic acid (Merck Darmstadt, Germany), chloramine-T (Sigma, USA), sodium metabisulphite (Aldrich, USA) and N-succinimidyl-4-[tri-methylstannyl] benzoate (synthesized at our laboratory) were used. NAP-5 column (Sephadex G-25, Amersham Biosciences, Uppsala, Sweden) was applied for gel filtration. The cells were detached with Trypsin-EDTA (0.25/0.02 %) (Biochrom Kg) and counted in a cell counter (Beckman Coulter Z2, Fullerton, CA, USA). Radioactivity was measured with a gamma counter (1480 Wizard, Wallac Oy, Turku, Finland). The EGFR-rich squamous carcinoma cell line A431 (ATCC, CLR 1555, Rocksville, MD, USA) was used. The cells were cultured in Ham's F-10 medium supplemented with L-glutamine (2 mM Biochrom Kg, Berlin, Germany), PEST (penicillin 100 IU/ml and streptomycin 100 µg/ml) and 10 % foetal calf serum (Biochrom Kg) ("complete medium"). The cells were grown at 37 °C in an incubator with humidified air equilibrated with 5 % CO_2.

Radio-labeling

Dimers of the polypeptide binders $Z_{EGFR:942}$, $Z_{EGFR:948}$ and $Z_{EFGR:955}$ were indirectly labeled with ^{125}I via N-succinimidyl groups. Acetic acid (2 µl, 0.1 % acetic acid in milli-Q) and N-succinimidyl-4-[tri-methylstannyl] benzoate (5 µl, 5 % acetic acid in methanol) was added to the ^{125}I (15 MBq). The iodine was coupled to the N-succinimidyl-4-[tri-methylstannyl] benzoate by adding 10 µl cloramine-T. The solution was then re-suspended for 30 seconds and further incubated at room temperature for 5 minutes. To stop the reaction, 15 µl sodium metabisulphite was added. The polypeptide binders were diluted in borate-buffer and added to the iodine solution

and additional borate-buffer was added to a total volume of 150 µl, whereupon the solution was incubated for 30 minutes. To separate labeled polypeptide binders from low molecular weight compounds, a NAP-5 column equilibrated with PBS was used.

Specificity test

A431 cells were cultured in 24-well plates and washed once with serum free Ham's F-10 medium. The three dimeric polypeptide binders being tested were labeled with ^{125}I and added to the cells with a molar excess of approximately 10:1 in relation to the number of available receptors and incubated in 37 °C for 4 hours. In some wells unlabeled polypeptide binders (molar excess of approx. 500:1) were added together with [^{125}I]polypeptide binders to determine the unspecific binding. EGF (molar excess of approx. 200:1) and cetuximab (molar excess of 500:1) were used in the same way, but to investigate if the polypeptide binders have the same binding site as EGF and cetuximab. The cells were then washed 6 times with serum free Ham's F-10 medium and detached by adding 0.5 ml Trypsin-EDTA and incubated at 37 °C for 30 min or until the cells were detached. 1 ml of Ham's F-10 complete medium was added and the cells were re-suspended. In some wells a 0.5 ml suspension was used to count the cells. The radioactivity (1.5 ml and 1 ml, respectively, for the cells that were counted) was measured with a gamma counter.

The results are presented in Figure 7. Specifically, in Figure 7, cellular binding of [^{125}I](Z00942)$_2$ (42*), [^{125}I](Z00948)$_2$ (48*) and [^{125}I](Z00955)$_2$ (55*) is shown. The data support the unexpected results from the previous FACS-ranking of the binders which indicate that $Z_{EFGR:955}$ seem to be the best binder of native EFGR on cells, followed by $Z_{EGFR:948}$ and $Z_{EGFR:942}$ in spite of the fact that $(Z_{EGFR:942})_2$ displayed the highest affinity in the BIAcore analysis. In addition, the three EFGR-binding polypeptide

constructs seem to bind overlapping epitopes. Furthermore, they seem to all compete for the same binding site as the natural ligand EFG and the monoclonal antibody cetuximab.

Saturation assay

To determine the affinity constant, the saturation of polypeptide binder binding was determined. The EGFR-rich cell line A431 was cultured in 24-well plates. Cells were kept on ice and washed once in cold serum free Ham's F-10 medium. A dilution series of the ^{125}I labeled-polypeptide dimeric binders was prepared and added to the cells with a molar excess of approximately 10:1. The cells were incubated for 4 hours, during slow movement, on ice in an environment where air from an incubator was trapped within a plastic bag together with the cell plate. For every concentration there was also a blocked control containing unlabeled polypeptide binders with a molar excess of approximately 300:1 for estimation of unspecific binding. The cells were then washed 6 times in cold Ham's F10 serum free medium and the cells were detached by adding 0.5 ml Trypsin-EDTA and incubated in 37 °C for 30 min or until the cells were detached. 1 ml of Ham's F-10 complete medium was added and the cells were re-suspended. In some wells 0.5 ml suspension was used to count the cells. The radioactivity was measured with a gamma counter. The data was analyzed by GraphPad Prism 4.

The results are shown in Figure 8. Specifically, in Figure 8, the results of saturation studies of [^{125}I]Z00942 (A), [^{125}I]Z00948 (B) and [^{125}I]Z00955 (C) are shown. Mean values and standard deviations from three values are shown.

Example 2
Second selection of EGFR-binding polypeptides according to the invention

Materials and Methods

Strains and vectors

The amber suppressor *Escherichia coli* strain RRIΔM15 (Rüther, U. (1982) *Nucleic Acids Res.* 10, 5765-72) was used for library construction, as bacterial host for phage production and for the cloning procedure. The phagemid vector pAffi1 was used for library construction and is described elsewhere (Grönwall C, Jonsson A, Lindström S, Gunneriusson E, Ståhl S, Herne N: "Selection and characterization of Affibody ligands binding to Alzheimer amyloid beta peptides", J. Biotechnol. (2006) in press, Epub 27 Sep 2006). Phagemid inserts of selected clones were subcloned into the expression vectors pAY442, containing a T7 promoter (Studier et al., (1990) *Methods Enzymol.* 185, 60-89), a DNA fragment encoding a hexahistidyl (His_6) tag and a multiple cloning site, together with a gene conferring resistance to kanamycin. The *E.coli* strain BL21(DE3) (Novagen, Madison, WI) was used for protein production from the expression vectors.

Construction of a secondary phagemid library

A strategy for affinity maturation was decided based upon the alignment of four sequences from the first selection of EGFR-binding molecules (Example 1, Figure 2). The secondary library was created by PCR amplification from a single 129-nucleotide template oligonucleotide with certain degenerated codons (5′ ctc gag gta gac aac aaa ttc aac aaa gaa nnk nnk nnk gcg nnk nnk gag atc mry mry tta cct aac tta aac ggt tgg caa atg acc gcc ttc atc gcg agt tta kyt gat gac cca agc caa agc 3′), encoding helices 1 and 2 of protein Z. The gene fragment was amplified using the forward primer 5′-

ccccccccctcgaggtagacaacaaattcaa-3′ (*Xho*I site underlined) and the reverse primer 5′-ccccccctgctagcaagttagcgctttggcttgggtcatc-3′ (*Nhe*I site underlined), with 1 pmol template oligonucleotide for each of 95 parallel reactions. The amplification was done using AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA) for 15 cycles (15 seconds at 96 °C, 15 seconds at 60 °C, and 1 minute at 72 °C), pooled, purified using QIAquick PCR purification kit (Qiagen, Hilden, Germany), *Xho*I/*Nhe*I digested and ligated to *Xho*I/*Nhe*I digested phagemid vector pAffi1 encoding the third nonvariegated α helix of protein Z. The ligated library vector was fenol:chloroform:isoamyl alcohol (25:24:21 v/v) (Invitrogen) extracted . Electrocompetent *Escherichia coli* RRIΔM15 cells were transformed with 30 aliquots of ligated material using 0.2-cm gap size cuvettes in an ECM 630 set (BTX, Genetronics) at 2500 V, 125 Ω and 50 µF. Cells were grown in SOC medium (tryptone soy broth (TSB) + yeast extract (YE) supplemented with 1 % glucose, 10 mmol/l $MgCl_2$, 10 mmol/l $MgSO_4$, 10 mmol/l NaCl and 2.5 mmol/l KCl) for ~1 h at 37 °C and transferred to six Erlenmeyer flasks, each containing 1 l of TSB supplemented with 2 % glucose and 25 µg/ml carbenicillin and grown overnight at 37 °C. The cells were centrifugated at 6000 *g* (15 min, 4 °C), following resuspension in PBS/glycerol solution to a final approximate concentration of 20 % glycerol, aliquoted and stored at -80 °C.

Phage selection procedures

A ~100 kDa recombinant extracellular domain of EGFR (denoted EGFR-ECD) was used as target protein during selections (1095-ER; R&D Systems). The EGFR-ECD was biotinylated *in vitro* using EZ-LinkTM-Sulfo-NHS-LC-LC-Biotin (Pierce, Rockford, IL, USA). A 20-fold molar excess of biotin was added to EGFR-ECD in phosphate-buffered saline (PBS; 10 mM phosphate, 137 mM NaCl, pH

7.2), and the mixture was incubated at room temperature (RT) for 1 h followed by extensive dialysis against PBS over night (ON) at 4 °C to remove the surplus of biotin.

Preparation of phage stocks from the library and between selections was performed according to previously described procedures (Nord, K *et al.*, (1997) Nat. Biotechnol., 15, 772-777; Hansson *et al.*, (1999) Immunotechnology, 4, 237-252) using the helper phage M13K07 (New England Biolabs, Beverly, MA, USA). PEG/NaCl precipitation yielded phage titers of about 10^{13} phage forming units (pfu) per ml. The selection was performed in solution and the bound phages were captured on streptavidin-coated paramagnetic beads (Dynabeads M-280 Streptavidin; Dynal, Oslo, Norway). To avoid unspecific binders all tubes were pretreated with PBST (0.1 % Tween-20 in PBS) supplemented with 5 % bovine serum albumin (PBST-5%BSA). To further avoid binders against the streptavidin present on the streptavidin-coated paramagnetic beads ~1 ml of the phage stock in PBST-3%BSA was pre-incubated (30 min, end-over-end rotation) with 0.2 mg of the beads for the first two rounds of selection.

Four rounds of biopanning starting at target concentrations of 100 nM were performed as follows. In round 1, an aliquot of the library containing approximately 10^{12} pfu was incubated in 1 ml of 100 nM of biotinylated EGFR-ECD in PBST-3%BSA for 1 h at RT with continuous rotation, followed by ~72 h at 4 °C. For round 2, 50 nM and for round 3, 1 nM of biotinylated EGFR-ECD in 1 ml PBST-3%BSA, respectively, was incubated (1 h, RT, continuous end-over-end rotation) with a portion of the phage stock from previous round. The bound phages were captured by incubation with streptavidin-coated M-280 Dynabeads for 15 min (RT, continuous end-over-end rotation). The amount of beads was added allowing an immobilization of ~2 µg of the target protein per mg of beads, as previously determined by SDS-PAGE analysis

(data not shown). For round 4, six slightly different selection protocols were performed, as detailed below in Table 2. In protocol 4-A and 4-B, 0.01 nM and 0.1 nM of biotinylated EGFR-ECD, respectively, was incubated for 2 h at RT with a portion of the phage stock from previous round, followed by incubation with a 100-fold excess of EGFR-ECD for 1 h at RT, capturing of bound phages by incubation with streptavidin-coated beads for 15 min, washing 18 times, incubation with a 100-fold excess of the first generation EGFR-binders Z00942, Z00948 and Z00955 (Example 1) for 1 h at RT, and finally washed twice. In protocol 4-C, 0.5 nM of biotinylated EGFR-ECD was incubated for 2 h at RT with a portion of the phage stock from previous round, followed by capturing of bound phages by incubation with streptavidin-coated beads for 15 min, washing 18 times, incubation with a 100-fold excess of first generation EGFR-binders for 1 h at RT, and finally washed twice. In protocol 4-D and 4-E, 0.1 and 0.5 nM of biotinylated EGFR-ECD, respectively, was incubated for 2 h at 37 °C with a portion of the phage stock from previous round, followed by incubation with a 100-fold excess of EGFR-ECD for 1 h at 37 °C, capturing of bound phages by incubation with streptavidin-coated beads for 15 min, washing 18 times, incubation with a 100-fold excess of first generation EGFR-binders for 1 h at 37 °C, and finally washed twice. In protocol 4-F, 0.1 nM of biotinylated EGFR-ECD was incubated for 2 h at RT with a portion of the phage stock from previous round, followed by capturing of bound phages by incubation with streptavidin-coated beads for 15 min and 20 washes. The number of washing steps was kept constant at 20 washes during the selection procedure and was performed in PBST-3%BSA in all washing steps except for the last wash where PBST was used. The phages were eluted with 500 µl of 50 mM glycine-HCl (pH 2.1) for 10 min, followed by immediate neutralization by adding 50 µl of 1 M Tris-HCl, pH 8.0 and 450 µl PBS. The eluted phages were used to infect log

phase RRIΔM15 cells for 30 min at 37 °C. The infected cell suspensions were spread on TYE agar plates (15 g/l agar, 3 g/l NaCl, 10 g/l tryptone and 5 g/l yeast extract), supplemented with 2 % glucose and 100 mg/l ampicillin, and incubated over night at 37 °C. The grown colonies were collected by resuspension in tryptic soy broth (TSB, 30 g/l; Merck, Darmstadt, Germany), supplemented with 5 g/l yeast extract, 2 % glucose and 100 mg/l ampicillin, and a fraction (~500 times excess of cells compared to the phage titer after elution) was used for inoculation, leading to the next generation of phage stock. Phagemid particles were rescued from infected cells using helper phage M13K07, purified and concentrated with PEG precipitation. The selection process was monitored by titrating the phage stocks before each selection and after elution. A serial dilution of phages was allowed to infect log phase RRIΔM15 cells for 5 min at RT, followed by plating on TYE agar plates, supplemented with 2 % glucose and 100 mg/l ampicillin, and ON at 37 °C.

Table 2
Protocols for Round 4 of selection

	4-A	4-B	4-C	4-D	4-E	4-F
Incubation with bio-EGFR	2 h, RT	2 h, RT	2 h, RT	2 h, 37°C	2 h, 37°C	2 h, RT
Incubation with EGFR (100-fold excess)	1 h, RT	1 h, RT	-	1 h, 37° C	1 h, 37°C	-
Capturing of bound phages on streptavidin-coated beads	15 min	15 min	15 min	15 min	15 min	15 min
Wash	1-18	1-18	1-18	1-18	1-18	1-20
Incubation with first generation binders (100-fold excess)	1 h, RT	1 h, RT	1 h, RT	1 h, 37°C	1 h, 37°C	-
Wash	19-20	19-20	19-20	19-20	19-20	-

ELISA-based ranking of second generation binders

Single colonies were inoculated in 1 ml TSB-YE medium supplemented with 100 µmol/l isopropyl-L-thio-β-D-galactopyranoside (IPTG) and 100 µg/ml ampicillin in deep well plates (Nunc, Roskilde, Denmark), and grown over night at 37 °C. Cells were pelleted by centrifugation at 3000 *g* for 10 minutes. The pellets were resuspended in 300 µl PBST and frozen over night at -80 °C. The samples were thawed and centrifuged at 3500 *g* for 20 minutes. The supernatants (100 µl), containing ABD-tagged Z variant molecules were loaded in microtiter wells, which had been previously coated with 6 µg/ml HAS (A-3782; Sigma) in 15 mmol/l Na_2CO_3 and 35 mmol/l $NaHCO_3$ (pH 9.6) ON at 4 °C and blocked with 2 % skimmed milk powder in PBST for 1 h at RT (continuous shaking). The plates were washed four times with PBST prior to the addition of 50 µl of 8.4 µg/ml biotinylated EGFR-ECD per well and incubated for 1.5 h. After washing the wells four times with PBST, 50 µl of streptavidin-horseradish peroxidase (1:5000, DAKO Cytomation, Denmark) per well was added and incubated for 1 h. The wells were washed four times and 50 µl developing solution ImmunoPure TMB substrate kit (Pierce) was added to each well. After 30 min, 100 µl stop solution (2 M H_2SO_4) was added to each well. The absorbance at 450 nm was measured with a Tecan Sunrise spectrophotometer.

DNA sequencing and sequence clustering

DNA sequencing of phagemid (pAffi1) inserts was performed on 187 EGFR-binding clones from the fourth round of panning. Specific primers and Big Dye terminator (Amersham Biosciences, Uppsala, Sweden) was used and the Sanger fragments analyzed on a DNA sequencer ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Subcloned DNA fragments were verified by the same procedure. The sequences of the EGFR-binding polypeptides were clustered using the so-called average-

link hierarchical clustering method described in more detail by Orlova *et al.* (*Cancer Res.* 66, 4339-48 (2006)).

The deduced amino acid sequences of candidate polypeptides exhibiting binding to EGFR in the ELISA screen described in the previous section are examples of EGFR-binding polypeptides according to the invention. They are presented in Figure 1 and in the sequence listing as SEQ ID NO:174-309. The sequences of the corresponding EGFR-binding motif of each such binding polypeptide are presented in Figure 1 and in the sequence listing as SEQ ID NO:11-146.

Screening of EGFR-binding polypeptides with Biacore

The cell supernatants containing ABD-tagged Z variants produced from the phage pAffi-vector prepared for ELISA was also subjected to a biosensor analysis. Supernatants from 54 clones demonstrating good binding from the ELISA were analyzed with real-time biospecific interaction on a Biacore® 2000 instrument. The target protein EGFR-ECD (diluted in 10 mM NaAc, pH 4.5) was immobilized (~1200 RU) on the carboxylated dextran layer of one flow-cell surface of a CM5 sensor chip (Biacore) by amine coupling, according to the manufacturer's instructions. Another flow-cell surface was activated and deactivated to be used as a reference surface and HSA was immobilized on a separate flow-cell surface on the CM5 sensor chip, to serve as a control of the amount of ABD-tagged Z variant that was expressed. A first generation EGFR-binder, $(Z00955)_2$ of Example 1, was also run as a control.

DNA constructs

DNA fragments encoding different variants of second generation EGFR-binding Z variants (Z_{EGFR}) were subcloned into the expression vectors pAY442. The fragments were amplified from the pAffi1 vector with specific primers introducing an *AccI* overhang both 3′ and 5′, and ligated

into the pAY442 vector, previously restricted with the same enzyme and dephosphorylated using calf intestine alkaline phosphatase (CIAP; Fermentas, Ontario, Canada). The amplified DNA fragments were purified with QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany) and hybridized prior to ligation with T4 DNA Ligase (New England Biolabs, Ipswich, MA, USA). The ligations resulted in expression vectors encoding, under the control of the T7 promoter, the different Z variants fused to an N-terminus His_6 tag, allowing purification by immobilized metal ion affinity chromatography (IMAC). Dimer constructs of the EGFR-binding Z variants from both vectors were constructed, where a second Z variant gene fragment was introduced head-to-tail, giving rise to His_6-$(Z_{EGFR})_2$ variants. All plasmid preparations were, after cultivation of transformed *E. coli* cells overnight, performed using QIAprep Spin Miniprep Kit (Qiagen GmbH) according to manufacturer's instructions.

Protein expression and purification

Selected EGFR-binding Z variants were expressed as His_6-tagged fusion proteins from the pAY442 plasmid in *E. coli* strain BL21(DE3). Cells were inoculated in 25 ml of TSB medium (30 g/l Tryptic Soy Broth) supplemented with 5 g/l yeast (TSB+YE) and 50 mg/l kanamycin and grown at 37 °C in shake flasks. Fresh TSB+YE containing 50 mg/l kanamycin was inoculated with preculture to OD_{600} ~0.06 and grown 3 h at 37 °C in a batch fermentor, when gene expression was induced by addition of isopropyl-L-thio-β-D-galactopyranoside (IPTG; Apollo Scientific Ltd, Bradbury, UK) to a final concentration of 0.5 mM. After 5 h cultivation the cells were harvested by centrifugation (15000 *g*, 20 min). The cell pellets were frozen over night, thawed and resuspended in denaturing buffer (7 M urea, 100 mM NaH_2PO_4, 10 mM Tris-HCl, pH 8.0). After incubation at RT for 30 min the cells were centrifuged at 25000 *g* for 15 min and the denatured protein from the

supernatant was diluted in denaturing buffer (7 M urea, 100 mM NaH_2PO_4, 10 mM Tris-HCl, pH 6.3) and applied to a Ni-NTA Superflow Column (Qiagen). The bound protein was eluted with urea buffer (8 M urea, 100 mM NaH_2PO_4, 10 mM Tris-HCl, pH 4.5). The proteins were applied to a PD-10 column (GE Healthcare) and eluted with PBS (pH 7.4). The monomeric proteins are hereafter referred to as $Z_{EGFR:no}$ (pAY442 vector) and the dimeric proteins referred to as $(Z_{EGFR:no})_2$ (pAY442 vector). Protein concentrations were calculated from absorbance measurements at 280 nm, using the appropriate extinction coefficient for each protein. To confirm the purity and correct molecular mass of the protein they were run on a SDS-PAGE gel (NuPAGE 4-12 % Bis-Tris Gel; Invitrogen), and on HPLC-MS (HPLC-MS 1100; Agilent Technologies). The purified proteins were further analyzed by CD, where CD spectra of 16 EGFR-binding Z variants were recorded using a Jasco-810 spectropolarimeter. All constructs were diluted with PBS to a final concentration of 0.5 mg/ml and 200 µl of each sample was placed in a 1 mm cuvette and scanned from 195 to 250 nm at 20 °C. The thermal stability was examined by applying a temperature gradient from 20 to 90 °C at a fixed wavelength of 220 nm. The melting point, defined as the temperature at which 50 % of the protein is unfolded, was interpreted from thermal unfolding spectra. Protein concentrations for selected Z_{EGFR} variants were also determined by amino acid analysis (Aminosyraanalyscentralen, Uppsala, Sweden).

Biosensor analyses

A Biacore® 2000 instrument (Biacore AB, Uppsala, Sweden) was used for real-time biospecific interaction analysis (BIA) between selected Z variants and the target protein. EGFR-ECD (diluted in 10 mM NaAc, pH 4.5) was immobilized (~2400 RU) on the carboxylated dextran layer of one flow-cell surface of a CM5 sensor chip (Biacore) by amine coupling, according to the manufacturer's

instructions. Another flow-cell surface was activated and deactivated to be used as a reference surface and HER2-ECD (Horak *et al*, (2005) *Cancer Biother Radiopharm*. 20, 603-13) (kindly supplied by Greg Adams, Fox Chase Cancer Center, PA) and ErbB3/Fc (R&D Systems, 348-RB) were immobilized on separate flow-cell surfaces on the CM5 sensor chip, to serve as negative controls. All Z variant samples were diluted in the running buffer HBS (10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.005 % surfactant P20, pH 7.4) before binding analysis was performed at 25 °C. In a first experiment, 500 nM of each Z variant (diluted in HBS) was injected over all surfaces with a flow rate of 30 µl/min. A first generation EGFR-binding molecule ($(Z_{EGFR:955})_2$; Example 1) was also injected as a control. After each injection the flow cells were regenerated by the injection of 10 µl of 10 mM HCl.

In a second experiment, five selected monomeric Z_{EGFR} variants were more subjected to kinetic analysis, in which the proteins were injected over an EGFR-ECD surface at concentrations ranging from 6.25 nM to 500 nM with a flow rate of 50 µl/min. The dissociation equilibrium constant (K_D), the association rate constant (k_a), and the dissociation rate constant (k_d) were calculated using BIAevaluation 3.2 software (Biacore). The samples were run in duplicates and after each injection the flow cells were regenerated by the injection of 10 µl of 10 mM HCl.

Immunofluorescence staining

The cell line A431, obtained from European collection of cell cultures (www.ecacc.org.uk), was grown at 37 °C in 5 % CO_2 environment in medium suggested by the provider. Media contained Fetal bovine serum (FBS) at concentrations suggested by the cell line providers (from Sigma-Aldrich). Sub-confluent cells were washed once with PBS, detached with a Trypsin/EDTA solution (Cambrex), and were resuspended in complete growth medium. Approximately 10000 cells in 20 µl were added per well of an 8 well,

multi-well slide (Histolab) and were incubated overnight. On the following morning the cells were fixed with freshly prepared 3 % formaldehyde in PBS for 15 minutes and washed twice with PBS. The cells were stained with 20 µl/well of the Z variants His_6-Z01859, His_6-Z01865, His_6-Z01864, His_6-Z01877, His_6-Z01868, His_6-Z01913, His_6-Z01836, His_6-$(Z01907)_2$-Cys and His_6-$(Z01953)_2$-Cys (2-10 µg/ml) for one hour, or with 1 µg/ml mouse anti-EGFR antibody (Abcam, no. ab30). Slides stained with Z variants were washed in PBS, incubated with goat antibody against Z (prepared in house) mixed with 5 µg/ml anti-goat IgG Alexa Fluor 488 (Molecular Probes) for one hour. The slide stained with antibody was washed in PBS and incubated with goat anti-mouse IgG-Alexa Fluor 488 (Molecular Probes) for one hour. After this second incubation step, the slides were washed again with PBS. The antibody slide was counterstained with 20 µl DAPI (Molecular Probes) at a concentration of 1 µg/ml for 10-20 seconds and washed again. All slides were dried and mounted with anti-fading reagent (Vector Laboratories) and membrane fluorescence was analyzed using a DM-LA microscope, equipped with a Leica DC camera (Leica Microsystems). Images were acquired using the IM1000 software (Leica Microsystems).

Immunohistochemical staining

A431 xenograft tissues were obtained from biodistribution studies described below. The tumors were snap-frozen in liquid nitrogen and 6 µm thick cryosections were made using a Ljung CM3000 automated cryostat (Leica Microsystems). The sections were fixed with freshly prepared 3 % formaldehyde in PBS for 15 minutes and washes twice with PBS. The sections were stained with His_6-$(Z01864)_2$-Cys or His_6-Z01877 at a concentration of 5 µg/ml, with His_6-$(Z01907)_2$-HRP or His_6-$(Z01853)_2$-HRP at a dilution of 1/40, approximately 6 µg/ml, for 1 hour. His_6-$(Z01864)_2$-Cys and His_6-Z01877 were

detected with goat antibody against Z (prepared in-house) followed by 5 µg/ml rabbit anti-goat HRP. As a positive control, one slide was stained with 3 µg/ml anti EGFR antibody (Abcam, no. ab2430), washed and detected with rabbit Envision HRP (Dako, no. K4002) The HRP stained sample was washed once with PBS followed by incubation with DAB chromogen substrate (Dako Cytomation) for 7 minutes, followed by washes with PBS and counterstaining with Mayers HTX (Histolab) for 20 seconds. Slides were mounted with Mount-quick (Histolab). The slides were analyzed in a DMLA microscope, equipped with a Leica DC camera (Leica Microsystems). Images were acquired and saved using the IM1000 software (Leica Microsystems).

Binding specificity and biodistribution of ^{111}In-labeled EGFR-binding Z variants

Radioactivity measurements

Radioactivity was measured using an automated gamma-counter with 3-inch NaI(Tl) detector (1480 WIZARD, Wallac Oy, Turku, Finland). Distribution of radioactivity along ITLC strips was measured on the Cyclone™ Storage Phosphor System and analyzed using the OptiQuant™ image analysis software.

Coupling of p-SCN-benzyl-DTPA to Z variants and labeling of conjugates with ^{111}In

Conjugation of isothiocyanate-benzyl-DTPA to Z_{EGFR} variants was performed according to the method described by Mirzadeh et al. (Bioconjug Chem. 1990;1:59-65), using a chelator-to-protein molar ratio of 1:1. Briefly, 300 µl of Z variant solution in PBS was mixed with 43 µl of freshly prepared solution (1 mg/ml) of isothiocyanate-benzyl-DTPA in 0.07 M sodium borate buffer, pH 9.2. The total volume was adjusted to 500 µl with 0.07 M borate buffer (pH 8.5-9.0), after which the mixture was vortexed for about 30 s and then incubated overnight at 37 °C.

After incubation, the reaction mixture was purified on a NAP-5 size exclusion column, pre-equilibrated with 0.2 M acetate buffer, pH 5.3 according to the manufacturer's instructions (high molecular weight fraction was 0.9 ml). The eluate was vortexed, whereafter the fraction containing 50 µg of Z variant conjugate was taken for further labeling and the rests of the solutions were frozen.

For labeling, 50 µg conjugate was mixed with a pre-determined amount of ^{111}In (18 MBq) and incubated at room temperature for 60 minutes. To benzyl-DTPA-Z01908 conjugate, 37 µl of acetate buffer was added, to balance a high concentration of this Z variant.

For quality control of the labeling, ITLC eluted with 0.2 M citric acid was used. In this system, radiolabeled Z variants remain at the origin, free indium migrates with the front of solvent, and ^{111}In-isothiocyanate-DTPA complex has a R_f of 0.4. Labeled conjugates were purified on NAP-5 columns (high molecular fraction was 0.9 ml), and products were checked for purity on ITLC.

Binding specificity of ^{111}In-labeled conjugates to EGFR-expressing A431 cells

Labeled conjugates were added to two groups of Petri dishes (3 dishes per group) with a calculated ratio of one labeled conjugate per one EGFR receptor (1.5 x 10^6 receptors per A431 cell). One group of dishes was pre-saturated with a 100-fold excess of non-labeled Z variant 10 min before the labeled conjugate was added. Cells were incubated for 1 hour at 37 °C and incubation medium was collected. Cell dishes were washed 6 times with cold serum-free medium and treated with 0.5 ml trypsin-EDTA for 10 min at 37 °C. When cells were detached, 0.5 ml complete medium was added to every dish and cells were re-suspended. Cell suspension was collected for radioactivity measurements. Cell-associated radioactivity

(C) was measured on an automated gamma-counter in parallel with 1 ml corresponding incubation medium (M). The fraction of added radioactivity bound to cells was calculated as % bound radioactivity = C x 100 % / (C + M).

Animal tumor models

The animal study was approved by the local Ethics Committee for Animal Research. Female outbred Balb/c nu/nu mice (10-12 weeks old at arrival) were used in the *in vivo* experiments. The animals were acclimatized for one week at the Rudbeck laboratory animal facility using standard diet, bedding and environment before tumor implantation. Mice had free access to food and drinking water. A431 tumors were grafted by subcutaneous (s.c.) injection of ~10^7 cells in the right hind leg. Xenografts were allowed to develop during 2 weeks.

Biodistribution studies

Biodistribution of EGFR-binding polypeptides was evaluated in A431 tumor-bearing mice of the Balb/c *(nu/nu)* strain 4 h pi of indium-111 labeled EGFR Z variant conjugates (sc). Mice were anesthetized by an intraperitoneal injection of ketamine HCl (Ketalar, Pfizer) and xylazine HCl (Rompun; Bayer) mixture (20 µl of solution per gram of body weight; Ketalar- 10 mg/ml, Rompun- 1 mg/ml) 4 hours post-injection (pi) in all biodistribution experiments. Thereafter, the mice were euthanized through heart puncture with 1 ml syringe rinsed with diluted heparin (5000 IE/ml, from Leo Pharma, Copenhagen, Denmark). Organ samples of blood, lung, liver, spleen, colon, kidney, uterus, salivary glands, muscle, skin, bone, and tumor were collected, weighed and measured for radioactivity with a gamma-counter. Intestines (with content) were measured as whole organs and were not weighed. Organ uptake values were calculated as percent injected activity per gram tissue (% IA/g). In

all experiments, the mice were randomly divided into groups with 4 animals in each group.

Results

Affinity maturation of the first generation EGFR-binding Z variants

An affinity maturation library based on a primary set of EGFR-binding molecules (Example 1) was designed and constructed. The sequences of the three best binders and a fourth sequence from further sequences analysis in Example 1 were aligned. It was considered reasonable to fix 5 positions (24, 25, 27, 28, and 32), and allow a certain bias for N and R in position 17 and 18 and for S and V in position 35 (Figure 2D). Thus, positions 9, 10, 11, 13, and 14 were targeted for randomization using NNG/T degenerated codons (Figure 2D). Due to the small size of protein Z, it was possible to use a single 129 nucleotide oligonucleotide with degenerated codons, encoding helices 1 and 2 of the Z-domain, to create a secondary library. The oligonucleotide was PCR-amplified and subsequently ligated into a phagemid vector encoding the third α-helix of protein Z. The resulting library consisted of ~1 x 10^9 members, which should well include a majority of the theoretical variants. Phage stocks were prepared and selections performed essentially as previously described, using decreasing concentrations of target protein and intensive washing, as well as blocking of rebinding of binders with fast off-rate with an excess of non-biotinylated target protein and competition of first generation binders (Example 1) with second generation binders generated, to select for the strongest EGFR-binding variants in the library.

Clones obtained after four rounds of selection were cultivated in 96-well plates, freeze-thawed to release periplasmic content, and subjected to an ELISA screening procedure for EGFR-binding activity. When subjecting 372

randomly picked clones to the ELISA screening a majority of the clones demonstrated high absorbance values, indicating good binding to the target protein. From the clones with highest absorbance value, 186 clones were subjected to DNA sequencing and upon clustering of the sequenced clones the relationship between selected clones was visualized.

Additionally, a biosensor analysis screening was performed on periplasmic content containing ABD-tagged Z variants on 54 clones in order to select for clones with the best binding to EGFR and the slowest off-rate (data not shown).

Based on the values in the ELISA screening, the clustering results from the DNA sequencing and the biosensor analysis screening, 16 clones were selected for further characterization, namely Z01836, Z01848, Z01853, Z01859, Z01864, Z01865, Z01868, Z01877, Z01887, Z01888, Z01905, Z01907, Z01908, Z01913, Z01917 and Z01960 (see Figure 1 and sequence listing). Virtually all binders were shown to be soluble at concentrations ≥1.0 mg/ml and showed a characteristic α-helix shaped CD spectrum in the far-UV spectral region (190-250 nm), with absorption maximum at 207 and 220 nm. The melting point was interpreted from thermal unfolding spectra and was determined to 50 °C or higher for virtually all binders. Spectra recorded after thermal denaturation showed a complete refolding into α-helix structure.

Biosensor screening

To obtain an initial ranking of binding affinities, the 16 selected Z variants as well as the monomeric and dimeric $Z_{EGFR:955}$ (Example 1) were expressed and analyzed for their EGFR binding using a Biacore instrument. The different Z_{EGFR} variants were separately injected over sensor chip flow-cell surfaces containing the immobilized target protein EGFR-ECD and control proteins HER2-ECD and Fc-fused HER3, respectively. Binding affinities in low

nanomolar range was observed for all 16 binders (data not shown). Most binders did not show any unspecific binding to HER2-ECD and Fc-fused HER3. Five binders with the best affinity and off-range from biosensor analysis were selected for further characterization, namely Z01853, Z01868, Z01877, Z01907 and Z01908.

Comparing first and second generation binders in vitro

The affinity-matured Z01853, Z01868, Z01877, Z01907 and Z01908 (K_D ~10 nM) were compared with a monomeric (K_D ~185 nM) and dimeric (K_D ~50 nM) form of Z00955 using Biacore analysis (Figure 9). The association rate for the affinity matured Z variants are about the same as the monomeric and dimeric first generation binders. The dissociation rate, however, was improved ~20-fold.

Fluorescense and immunohistochemical analysis

The results are shown in Figure 10. Figure 10A shows A431 cells stained with the following Z variants specific for EGFR; a) His_6-Z01859, b) His_6-Z01865, c) His_6-Z01864, d), His_6-Z01913, e) His_6-Z01877, f) His_6-Z01868, g) His_6-Z01836, h) His_6-$(Z01853)_2$-cys and i) His_6-$(Z01907)_2$-cys. The monomeric Z variants were detected with goat antibody against Z, followed by detection with Alexa 488 conjugated anti-goat antibodies. The dimeric Z variants were labeled with Oregon Green. As a positive control, A431 were stained with an anti-EGFR antibody (j).

Figure 10B shows cryosections of A431 xenografts stained with a) His_6-$(Z01864)_2$-Cys, b) His_6-Z01877, c) His_6-$(Z01853)_2$-Cys and d) His_6-$(Z01907)_2$-Cys. His_6-$(Z01864)_2$-Cys, and His_6-Z01877 (a and b) were detected with goat antibody against Z followed by detection with HRP conjugated anti-goat antibodies. The His_6-$(Z01853)_2$-Cys (c)and His_6-$(Z01907)_2$-Cys (d) molecules were directly conjugated to HPR. As a positive control, A431 were stained with an anti-EGFR antibody (e).

Specificity and biodistribution of ^{111}In-labeled EGFR-binding Z variants

All Z variant conjugates were successfully labeled with indium-111 with labeling yields higher than 90 %, and after NAP-5 purification, all conjugates had a purity of over 95 %.

The binding specificity of the labeled conjugates was evaluated in the EGFR expressing epidermoid carcinoma cell line A431. The results are shown in Figure 11. In the figure, all data points are mean values of three measurements, and the error bars represent SEM. The binding of all conjugates was found to be EGFR-specific (see Figure 11), since it was possible to block the uptake by addition of 100-fold excess of non-labeled Z_{EGFR} ($p<0.0001$).

The biodistribution results for indium-111 labeled Z variant conjugates 4 h pi in A431 tumor bearing mice are summarized in Figure 12. In the figure, each data point represents an average from four animals ± standard deviation and is expressed as the percent of injected radioactivity per gram organ or tissue. Data for ^{111}In-CHX-DTPA-$(Z_{EGFR:955})_2$ were obtained by Erika Nordberg (Biomedical radiation Sciences, Uppsala University) in collaboration with Affibody AB (VINNOVA) and included for comparison.

Tumor targeting *in vivo* was successful, with all five new Z variants on the level of 4-6 %IA/g, but was not improved in comparison to non-maturated dimer (4 %IA/g).

The main differences between the first-generation dimer $(Z00955)_2$ and all maturated monomers could be observed in the blood clearance, liver uptake and kidney accumulation: for the new monomers selected in the maturation experiment, the blood concentration of radioactivity was higher, the liver uptake was lower and the kidney uptake was higher than for $(Z00955)_2$. Most likely, these observations are related: the new monomers

have a weaker binding to EGFR receptors in the liver, due to lower cross-reactivity to murine receptors and/or due to monovalent binding to the receptor, which does not trigger internalization and binding is reversible.

Example 3
Third selection of EGFR-binding polypeptides according to the invention

Based on a statistical analysis of the selection results from Example 2, a third library of putative EGFR binding polypeptides was prepared essentially as described above. Following phage display selection using EGFR as target and ELISA screening of the selected variants, 17 additional sequences of EGFR binding Z variants were identified. Their amino acid sequences are presented in Figure 1 and in the sequence listing as SEQ ID NO:310-326. The deduced EGFR binding motifs of these EGFR binding Z variants are presented in Figure 1 and in the sequence listing as SEQ ID NO:147-163.

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 22819-624 Seq 20-05-08 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

SEQUENCE TABLE

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<151> 2005-12-05

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 68
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<400> 68
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 69
<211> 29
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<400> 69
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 70
<211> 29
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 71
<211> 29
<212> PRT
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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<400> 74
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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<220>
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 77
<211> 29
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<220>
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<400> 77
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 78
<211> 29
<212> PRT
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<220>
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<400> 78
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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<211> 29
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<220>
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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<220>
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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<212> PRT
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<220>
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<400> 81
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Ala Asp
20 25

<210> 82
<211> 29
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<400> 82
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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<220>
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<400> 83
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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<220>
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 85
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 86
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 87
<211> 29
<212> PRT
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<220>
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 88
<211> 29
<212> PRT
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 89
<211> 29
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<220>
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 90
<211> 29
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 91
<211> 29
<212> PRT
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 92
<211> 29
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<220>
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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<211> 29
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 94
<211> 29
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Ala Asp
20 25

<210> 95
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 96
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 98
<211> 29
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 99
<211> 29
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 100
<211> 29
<212> PRT
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 101
<211> 29
<212> PRT
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 102
<211> 29
<212> PRT
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<400> 102
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 103
<211> 29
<212> PRT
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<400> 103
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 104
<211> 29
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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<400> 106
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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<211> 29
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<220>
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<400> 107
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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<400> 109
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 111
<211> 29
<212> PRT
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<220>
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 112
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<212> PRT
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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<211> 29
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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<400> 114
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 116
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Ala Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 120
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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<400> 131
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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<400> 132
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

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<400> 133
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 134
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<400> 134
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1 5 10 15

Gly Gly Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 135
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<400> 135
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 137
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Ala Asp
20 25

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<400> 138
Glu His Trp Asn Ala Leu His Glu Ile Arg Ser Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
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<210> 139
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<400> 139
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 140
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 142
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<212> PRT
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<220>
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<400> 142
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 143
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<400> 143
Glu Arg Asp Glu Ala Ile Gln Glu Ile His Ser Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 144
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<400> 144
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1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 145
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<400> 145
Glu Met Glu Lys Ala Trp Val Glu Ile Arg Asn Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 146
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 146
Glu Leu Asp Asn Ala Ile Asp Glu Ile Arg Asn Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Phe Asp
20 25

<210> 147
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 147
Glu Met Trp Ile Ala Trp Glu Glu Ile Arg Asp Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Leu Asp
20 25

<210> 148
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 148
Glu Met Trp Leu Ala Trp Glu Glu Ile Arg Asn Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Leu Thr Ala Phe Ile Ala Ser Leu Leu Asp
20 25

<210> 149
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 149
Glu Met Trp Ser Ala Trp Asp Glu Ile Arg Ala Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ser Ser Leu Leu Asp
20 25

<210> 150
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 150
Glu Met Trp Asn Ala Trp Asn Glu Ile Arg Asp Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Leu Asp
20 25

<210> 151
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 151
Glu Met Trp Gly Ala Trp Asn Glu Ile Arg Asp Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ser Ser Leu Leu Asp
20 25

<210> 152
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 152
Glu Met Trp Ile Ala Trp Asp Glu Ile Arg Asp Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Phe Thr Ala Phe Ile Ala Ser Leu Leu Asp
20 25

<210> 153
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 153
Glu Leu Trp Ile Ala Trp Asp Glu Ile Arg Tyr Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Leu Asp
20 25

<210> 154
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 154
Glu Met Trp Lys Ala Trp Glu Glu Ile Arg Ser Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Leu Asp
20 25

<210> 155
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 155
Glu Met Trp Asp Ala Trp Gly Glu Ile Arg Asn Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Leu Asp
20 25

<210> 156
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 156
Glu Val Trp Val Ala Trp Glu Glu Ile Arg Asp Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Leu Asp
20 25

<210> 157
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 157
Glu Met Trp Gly Ala Trp Glu Glu Ile Arg Asn Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Val Asp
20 25

<210> 158
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 158
Glu Met Trp Met Ala Trp Asp Glu Ile Arg Tyr Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Leu Thr Ala Phe Ile Ser Ser Leu Leu Asp
20 25

<210> 159
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 159
Glu Met Trp Val Ala Trp Glu Glu Ile Arg Asn Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Gly Ser Leu Leu Asp
20 25

<210> 160
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 160
Glu Met Trp Asp Ala Trp Asp Glu Ile Arg Tyr Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Phe Thr Ala Phe Ile Ala Ser Leu Leu Asp
20 25

<210> 161
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 161
Glu Leu Trp Gly Ala Trp Asp Glu Ile Arg Tyr Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Met Thr Ala Phe Ile Ala Ser Leu Leu Asp
20 25

<210> 162
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 162
Glu Ser Trp Asn Ala Val Lys Glu Ile Gly Glu Leu Pro Asn Leu Asn
1 5 10 15

Trp Gly Gln Ala Asp Ala Phe Ile Asn Ser Leu Trp Asp
20 25

<210> 163
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 163
Glu Ser His Glu Val Trp Gln Glu Ile Arg Ser Leu Pro Asn Leu Asn
1 5 10 15

Gly Trp Gln Leu Thr Ala Phe Ile Asn Ser Leu Leu Asp
20 25

<210> 164
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 164
Val Asp Asn Lys Phe Asn Lys Glu Trp Ser Ala Ala Ala Ser Glu Ile
1 5 10 15

Ser Gly Leu Pro Asn Leu Asn Lys Leu Gln Ala Phe Ala Phe Ile Val
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 165
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 165
Val Asp Asn Lys Phe Asn Lys Glu Met Leu Ile Ala Met Glu Glu Ile
1 5 10 15

Gly Ser Leu Pro Asn Leu Asn Trp Gly Gln Glu Gln Ala Phe Ile Leu
20 25 30

Ser Leu Trp Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 166
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 166
Val Asp Asn Lys Phe Asn Lys Glu Thr Gly Ala Ala Met Arg Glu Ile
1 5 10 15

Asn Asp Leu Pro Asn Leu Asn Asn Leu Gln Phe Phe Ala Phe Ile Val
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 167
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 167
Val Asp Asn Lys Phe Asn Lys Glu Phe Tyr Ala Ala Ile Thr Glu Ile
1 5 10 15

Asn Arg Leu Pro Asn Leu Asn Gly Trp Gln Met Val Ala Phe Ile Ser
20 25 30

Ser Leu Ser Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 168
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 168
Val Asp Asn Lys Phe Asn Lys Glu His Ala Lys Ala Met Trp Glu Ile
1 5 10 15

Gly Asn Leu Pro Asn Leu Asn Leu Val Gln Leu Ala Ala Phe Ile Phe
20 25 30

Ser Leu Arg Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 169
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 169
Val Asp Asn Lys Phe Asn Lys Glu Ser Leu Ala Ala Ser Val Glu Ile
1 5 10 15

Ser His Leu Pro Asn Leu Asn Gly Ser Gln Cys Lys Ala Phe Ile Arg
20 25 30

Ser Leu Met Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 170
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 170
Val Asp Asn Lys Phe Asn Lys Glu Leu Glu Lys Ala Tyr Asn Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 171
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 171
Val Asp Asn Lys Phe Asn Lys Glu Ala Ala Pro Ala Trp Thr Glu Ile
1 5 10 15

Val Arg Leu Pro Asn Leu Asn Arg Gly Gln Lys Gln Ala Phe Ile Val
20 25 30

Ser Leu His Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 172
<211> 58

<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 172
Val Asp Asn Lys Phe Asn Lys Glu Leu Trp Ile Ala Thr Ser Glu Ile
1 5 10 15

Val Glu Leu Pro Asn Leu Asn Met His Gln Gly Val Ala Phe Ile Arg
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 173
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 173
Val Asp Asn Lys Phe Asn Lys Glu Val Gln Asn Ala Val Ala Glu Ile
1 5 10 15

Val Lys Leu Pro Asn Leu Asn Gly Trp Gln Ser Thr Ala Phe Ile Ala
20 25 30

Ser Leu Ser Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 174
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 174
Val Asp Asn Lys Phe Asn Lys Glu Tyr Glu Glu Ala Trp Asn Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 175
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 175
Val Asp Asn Lys Phe Asn Lys Glu Ile Glu Arg Ala Met Gln Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 176
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 176
Val Asp Asn Lys Phe Asn Lys Glu Val Glu Thr Ala Trp Met Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 177
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 177
Val Asp Asn Lys Phe Asn Lys Glu Thr Glu Thr Ala Ile Gln Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 178
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 178
Val Asp Asn Lys Phe Asn Lys Glu Thr Asp Arg Ala Val Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 179
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 179
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Arg Ala Trp Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 180
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 180
Val Asp Asn Lys Phe Asn Lys Glu Ser Gln Asp Ala Trp Glu Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 181
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 181
Val Asp Asn Lys Phe Asn Lys Glu Arg Glu Glu Ala Ile Lys Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 182
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 182
Val Asp Asn Lys Phe Asn Lys Glu Ser Trp Glu Ala Trp His Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 183
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 183
Val Asp Asn Lys Phe Asn Lys Glu Leu Tyr Asp Ala Met Ile Glu Ile
1 5 10 15

Asn His Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 184
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 184
Val Asp Asn Lys Phe Asn Lys Glu Thr Asp Lys Ala Val Gln Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 185
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 185
Val Asp Asn Lys Phe Asn Lys Glu Gln Val Arg Ala Trp Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 186
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 186
Val Asp Asn Lys Phe Asn Lys Glu Leu Trp Gly Ala Trp Glu Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 187
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 187
Val Asp Asn Lys Phe Asn Lys Glu Arg Asp Ala Ala Trp Glu Glu Ile
1 5 10 15

Arg His Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 188
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 188
Val Asp Asn Lys Phe Asn Lys Glu Val Phe Pro Ala Leu Gln Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 189
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 189
Val Asp Asn Lys Phe Asn Lys Glu Val Glu Met Ala Thr Gln Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 190
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 190
Val Asp Asn Lys Phe Asn Lys Glu Leu Tyr Gln Ala Met Asp Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 191
<211> 58

<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 191
Val Asp Asn Lys Phe Asn Lys Glu Ala Thr Glu Ala Trp Asp Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 192
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 192
Val Asp Asn Lys Phe Asn Lys Glu Val Glu Trp Ala Leu Gln Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 193
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 193
Val Asp Asn Lys Phe Asn Lys Glu Val Ser Pro Ala Leu Glu Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 194
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 194
Val Asp Asn Lys Phe Asn Lys Glu Arg Glu Arg Ala Ile Glu Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 195
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 195
Val Asp Asn Lys Phe Asn Lys Glu Ala Glu Ser Ala Trp Asn Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 196
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 196
Val Asp Asn Lys Phe Asn Lys Glu Phe Trp Trp Ala Ser Asp Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Ala Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 197
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 197
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Ser Ala Trp Glu Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 198
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 198
Val Asp Asn Lys Phe Asn Lys Glu His Trp Asn Ala Met His Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 199
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 199
Val Asp Asn Lys Phe Asn Lys Glu Val Glu Lys Ala Trp Ser Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 200
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 200
Val Asp Asn Lys Phe Asn Lys Glu Arg Glu Lys Ala Trp Met Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 201
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 201
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Ser Ala Trp Ser Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 202
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 202
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Ser Ala Trp Ala Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 203
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 203
Val Asp Asn Lys Phe Asn Lys Glu Arg Ser Leu Ala Ile Arg Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 204
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 204
Val Asp Asn Lys Phe Asn Lys Glu Arg Asp Thr Ala Ile Ser Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 205
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 205
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Ala Ala Trp Gly Glu Ile
1 5 10 15

His Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 206
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 206
Val Asp Asn Lys Phe Asn Lys Glu Arg Asp Thr Ala Ile Tyr Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 207
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 207
Val Asp Asn Lys Phe Asn Lys Glu Pro Trp Leu Ala Trp Ala Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 208
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 208
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Asp Ala Trp Glu Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 209
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 209
Val Asp Asn Lys Phe Asn Lys Glu Asp Met Glu Ala Val Asp Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 210
<211> 58

<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 210
Val Asp Asn Lys Phe Asn Lys Glu Ala Glu His Ala Trp Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 211
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 211
Val Asp Asn Lys Phe Asn Lys Glu Leu Trp Ile Ala Trp Asp Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 212
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 212
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Asn Ala Trp Ser Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 213
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 213
Val Asp Asn Lys Phe Asn Lys Glu Ile Asn Ser Ala Ile Gly Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 214
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 214
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Arg Ala Trp Glu Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 215
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 215
Val Asp Asn Lys Phe Asn Lys Glu Ser Trp Lys Ala Trp Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 216
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 216
Val Asp Asn Lys Phe Asn Lys Glu Thr Glu Trp Ala Ile Gln Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 217
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 217
Val Asp Asn Lys Phe Asn Lys Glu Ala Glu Phe Ala Trp Thr Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 218
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 218
Val Asp Asn Lys Phe Asn Lys Glu Leu Leu Val Ala Met Leu Glu Ile
1 5 10 15

Asn His Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 219
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 219
Val Asp Asn Lys Phe Asn Lys Glu Arg Asp Phe Ala Ile Asp Glu Ile
1 5 10 15

His Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 220
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 220
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Ile Ala Trp Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 221
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 221
Val Asp Asn Lys Phe Asn Lys Glu Ser Asn Ser Ala Trp Gln Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 222
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 222
Val Asp Asn Lys Phe Asn Lys Glu Val Trp Thr Ala Trp Glu Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 223
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 223
Val Asp Asn Lys Phe Asn Lys Glu Pro Trp Met Ala Trp Asp Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 224
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 224
Val Asp Asn Lys Phe Asn Lys Glu Arg Asp Gly Ala Ile Gln Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 225
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 225
Val Asp Asn Lys Phe Asn Lys Glu Lys Trp Thr Ala Trp Glu Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 226
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 226
Val Asp Asn Lys Phe Asn Lys Glu Met Trp His Ala Trp Asp Glu Ile
1 5 10 15

Arg His Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 227
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 227
Val Asp Asn Lys Phe Asn Lys Glu Val Asp Gln Ala Val Ala Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 228
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 228
Val Asp Asn Lys Phe Asn Lys Glu Arg Tyr Trp Ala Ile Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 229
<211> 58

<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 229
Val Asp Asn Lys Phe Asn Lys Glu Arg Glu Glu Ala Ile Ser Glu Ile
1 5 10 15

His Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 230
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 230
Val Asp Asn Lys Phe Asn Lys Glu Met Glu Trp Ala Trp Gln Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 231
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 231
Val Asp Asn Lys Phe Asn Lys Glu Val Glu Pro Ala Ile Arg Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 232
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 232
Val Asp Asn Lys Phe Asn Lys Glu Gln Asp Glu Ala Val Lys Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 233
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 233
Val Asp Asn Lys Phe Asn Lys Glu Ala Asp Ser Ala Trp Thr Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 234
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 234
Val Asp Asn Lys Phe Asn Lys Glu Thr Asp Tyr Ala Ile Gly Glu Ile
1 5 10 15

His Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 235
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 235
Val Asp Asn Lys Phe Asn Lys Glu Ala Asp Lys Ala Val Gln Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 236
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 236
Val Asp Asn Lys Phe Asn Lys Glu Thr Asp Lys Ala Val Gln Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 237
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 237
Val Asp Asn Lys Phe Asn Lys Glu Leu Trp Ala Ala Trp Ser Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 238
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 238
Val Asp Asn Lys Phe Asn Lys Glu Ala Trp Ala Ala Trp Ser Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 239
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 239
Val Asp Asn Lys Phe Asn Lys Glu Val Asp Arg Ala Val Val Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 240
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 240
Val Asp Asn Lys Phe Asn Lys Glu Ala Glu Ser Ala Ile Glu Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 241
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 241
Val Asp Asn Lys Phe Asn Lys Glu Leu Gly Gly Ala Val Asn Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 242
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 242
Val Asp Asn Lys Phe Asn Lys Glu Val Asp Thr Ala Ile Trp Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 243
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 243
Val Asp Asn Lys Phe Asn Lys Glu Leu Ala Asn Ala Phe Asp Glu Ile
1 5 10 15

His Arg Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 244
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 244
Val Asp Asn Lys Phe Asn Lys Glu Phe Arg Arg Ala Ser Asp Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Ala Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 245
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 245
Val Asp Asn Lys Phe Asn Lys Glu Ile Glu Lys Ala Ile Arg Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 246
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 246
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Glu Ala Trp Asp Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 247
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 247
Val Asp Asn Lys Phe Asn Lys Glu Ser Lys Trp Ala Trp Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 248
<211> 58

<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 248
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Arg Ala Trp Glu Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 249
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 249
Val Asp Asn Lys Phe Asn Lys Glu Ile Asp Pro Ala Leu Gln Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 250
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 250
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Ala Ala Trp Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 251
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 251
Val Asp Asn Lys Phe Asn Lys Glu Lys Tyr Trp Ala Val Asp Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 252
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 252
Val Asp Asn Lys Phe Asn Lys Glu His Trp Ala Ala Trp His Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 253
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 253
Val Asp Asn Lys Phe Asn Lys Glu Tyr Gln Thr Ala Trp Lys Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 254
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 254
Val Asp Asn Lys Phe Asn Lys Glu Thr Asp Arg Ala Ile Lys Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 255
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 255
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Asn Ala Trp His Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 256
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 256
Val Asp Asn Lys Phe Asn Lys Glu Pro Trp Val Ala Trp Asn Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 257
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 257
Val Asp Asn Lys Phe Asn Lys Glu Leu Ile Gly Ala Tyr Asp Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Ala Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 258
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 258
Val Asp Asn Lys Phe Asn Lys Glu Arg Asp Tyr Ala Leu Trp Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 259
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 259
Val Asp Asn Lys Phe Asn Lys Glu Thr Gln Asp Ala Trp Asp Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 260
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 260
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Glu Ala Trp Gly Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 261
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 261
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Ser Ala Trp His Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 262
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 262
Val Asp Asn Lys Phe Asn Lys Glu Leu Trp Gln Ala Trp Gly Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 263
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 263
Val Asp Asn Lys Phe Asn Lys Glu Val Glu Arg Ala Trp Asn Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 264
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 264
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Glu Ala Trp Gly Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 265
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 265
Val Asp Asn Lys Phe Asn Lys Glu Arg Thr Gln Ala Ile Arg Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 266
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 266
Val Asp Asn Lys Phe Asn Lys Glu Thr Glu Glu Ala Trp Glu Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 267
<211> 58

<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 267
Val Asp Asn Lys Phe Asn Lys Glu Ala Glu Thr Ala Trp Ser Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 268
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 268
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Cys Ala Trp Asn Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 269
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 269
Val Asp Asn Lys Phe Asn Lys Glu Arg Asp Tyr Ala Ile Glu Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 270
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 270
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Ser Ala Trp Asp Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 271
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 271
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Thr Ala Trp His Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 272
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 272
Val Asp Asn Lys Phe Asn Lys Glu Thr Asp Arg Ala Val Arg Glu Ile
1 5 10 15

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Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
            20                  25                  30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
        35                  40                  45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
    50                  55
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<210> 273
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 273

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Val Asp Asn Lys Phe Asn Lys Glu Thr Trp Arg Ala Trp His Glu Ile
1               5                   10                  15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
            20                  25                  30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
        35                  40                  45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
    50                  55
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<210> 274
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 274

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Val Asp Asn Lys Phe Asn Lys Glu Met Trp Leu Ala Trp Gln Glu Ile
1               5                   10                  15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
            20                  25                  30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
        35                  40                  45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
    50                  55
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<210> 275
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 275
Val Asp Asn Lys Phe Asn Lys Glu Val Asp Tyr Ala Ile Gln Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 276
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 276
Val Asp Asn Lys Phe Asn Lys Glu Met Glu Ser Ala Trp Ile Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 277
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 277
Val Asp Asn Lys Phe Asn Lys Glu Thr Glu Glu Ala Trp Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 278
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 278
Val Asp Asn Lys Phe Asn Lys Glu Ser Glu Ala Ala Leu Gln Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 279
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 279
Val Asp Asn Lys Phe Asn Lys Glu Phe Arg Lys Ala Ser Asn Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Ala Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 280
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 280
Val Asp Asn Lys Phe Asn Lys Glu Val Gln Leu Ala Trp Asp Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 281
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 281
Val Asp Asn Lys Phe Asn Lys Glu Ala Asp Arg Ala Trp Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 282
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 282
Val Asp Asn Lys Phe Asn Lys Glu Ile Lys Pro Ala Ile Arg Glu Ile
1 5 10 15

His Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 283
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 283
Val Asp Asn Lys Phe Asn Lys Glu Leu Asp Gln Ala Ile Leu Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 284
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 284
Val Asp Asn Lys Phe Asn Lys Glu Pro Trp Ile Ala Trp His Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 285
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 285
Val Asp Asn Lys Phe Asn Lys Glu Arg Asp Val Ala Ile Thr Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 286
<211> 58

<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 286
Val Asp Asn Lys Phe Asn Lys Glu Phe Asp Lys Ala Val Ser Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 287
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 287
Val Asp Asn Lys Phe Asn Lys Glu Val Asp Val Ala Met Gln Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 288
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 288
Val Asp Asn Lys Phe Asn Lys Glu Thr Asn Ala Ala Leu Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 289
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 289
Val Asp Asn Lys Phe Asn Lys Glu Ala Glu Lys Ala Trp Glu Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 290
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 290
Val Asp Asn Lys Phe Asn Lys Glu Pro Trp Leu Ala Trp Ser Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 291
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 291
Val Asp Asn Lys Phe Asn Lys Glu Gly Leu Asn Ala Val Asn Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 292
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 292
Val Asp Asn Lys Phe Asn Lys Glu Trp Glu Val Ala Met Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 293
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 293
Val Asp Asn Lys Phe Asn Lys Glu Val Glu Ser Ala Trp Thr Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 294
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 294
Val Asp Asn Lys Phe Asn Lys Glu Thr Asp Arg Ala Trp Asp Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 295
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 295
Val Asp Asn Lys Phe Asn Lys Glu Arg Glu Gln Ala Thr Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 296
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 296
Val Asp Asn Lys Phe Asn Lys Glu Met Glu His Ala Trp Glu Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 297
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 297
Val Asp Asn Lys Phe Asn Lys Glu His Trp Asn Ala Leu His Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Gly Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 298
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 298
Val Asp Asn Lys Phe Asn Lys Glu Tyr Glu Ala Ala Trp Asp Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 299
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 299
Val Asp Asn Lys Phe Asn Lys Glu Gly Glu Met Ala Leu Gln Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 300
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 300
Val Asp Asn Lys Phe Asn Lys Glu Phe Arg Trp Ala Ser Asp Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Ala Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 301
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 301
Val Asp Asn Lys Phe Asn Lys Glu His Trp Asn Ala Leu His Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 302
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 302
Val Asp Asn Lys Phe Asn Lys Glu Ile Asp Tyr Ala Ile Arg Glu Ile
1 5 10 15

His Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 303
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 303
Val Asp Asn Lys Phe Asn Lys Glu Leu Leu Gln Ala Met Leu Glu Ile
1 5 10 15

Asn His Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 304
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 304
Val Asp Asn Lys Phe Asn Lys Glu Val Asn Pro Ala Leu Gln Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 305
<211> 58

<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 305
Val Asp Asn Lys Phe Asn Lys Glu Leu Leu Ser Ala Met Leu Glu Ile
1 5 10 15

Asn His Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 306
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 306
Val Asp Asn Lys Phe Asn Lys Glu Arg Asp Glu Ala Ile Gln Glu Ile
1 5 10 15

His Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 307
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 307
Val Asp Asn Lys Phe Asn Lys Glu Thr Asp Trp Ala Ile Gln Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 308
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 308
Val Asp Asn Lys Phe Asn Lys Glu Met Glu Lys Ala Trp Val Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 309
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 309
Val Asp Asn Lys Phe Asn Lys Glu Leu Asp Asn Ala Ile Asp Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Phe Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 310
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 310
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Ile Ala Trp Glu Glu Ile
1 5 10 15

Arg Asp Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 311
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 311
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Leu Ala Trp Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Leu Thr Ala Phe Ile Ala
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 312
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 312
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Ser Ala Trp Asp Glu Ile
1 5 10 15

Arg Ala Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ser
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 313
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 313
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Asn Ala Trp Asn Glu Ile
1 5 10 15

Arg Asp Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 314
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 314
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Gly Ala Trp Asn Glu Ile
1 5 10 15

Arg Asp Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ser
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 315
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 315
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Ile Ala Trp Asp Glu Ile
1 5 10 15

Arg Asp Leu Pro Asn Leu Asn Gly Trp Gln Phe Thr Ala Phe Ile Ala
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 316
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 316
Val Asp Asn Lys Phe Asn Lys Glu Leu Trp Ile Ala Trp Asp Glu Ile
1 5 10 15

Arg Tyr Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 317
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 317
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Lys Ala Trp Glu Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 318
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 318
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Asp Ala Trp Gly Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 319
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 319
Val Asp Asn Lys Phe Asn Lys Glu Val Trp Val Ala Trp Glu Glu Ile
1 5 10 15

Arg Asp Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 320
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 320
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Gly Ala Trp Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 321
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 321
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Met Ala Trp Asp Glu Ile
1 5 10 15

Arg Tyr Leu Pro Asn Leu Asn Gly Trp Gln Leu Thr Ala Phe Ile Ser
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 322
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 322
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Val Ala Trp Glu Glu Ile
1 5 10 15

Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Gly
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 323
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 323
Val Asp Asn Lys Phe Asn Lys Glu Met Trp Asp Ala Trp Asp Glu Ile
1 5 10 15

Arg Tyr Leu Pro Asn Leu Asn Gly Trp Gln Phe Thr Ala Phe Ile Ala
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 324
<211> 58

<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 324
Val Asp Asn Lys Phe Asn Lys Glu Leu Trp Gly Ala Trp Asp Glu Ile
1 5 10 15

Arg Tyr Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile Ala
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 325
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 325
Val Asp Asn Lys Phe Asn Lys Glu Ser Trp Asn Ala Val Lys Glu Ile
1 5 10 15

Gly Glu Leu Pro Asn Leu Asn Trp Gly Gln Ala Asp Ala Phe Ile Asn
20 25 30

Ser Leu Trp Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 326
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 326
Val Asp Asn Lys Phe Asn Lys Glu Ser His Glu Val Trp Gln Glu Ile
1 5 10 15

Arg Ser Leu Pro Asn Leu Asn Gly Trp Gln Leu Thr Ala Phe Ile Asn
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 327
<211> 58
<212> PRT
<213> Artificial sequence

<220>
<223> Engineered EGFR binding polypeptide

<400> 327
Val Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile
1 5 10 15

Leu His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Ala Phe Ile Gln
20 25 30

Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 328
<211> 1210
<212> PRT
<213> Homo sapiens

<400> 328
Met Arg Pro Ser Gly Thr Ala Gly Ala Ala Leu Leu Ala Leu Leu Ala
1 5 10 15

Ala Leu Cys Pro Ala Ser Arg Ala Leu Glu Glu Lys Lys Val Cys Gln
20 25 30

Gly Thr Ser Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe
35 40 45

Leu Ser Leu Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly Asn
50 55 60

Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys
65 70 75 80

Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val
85 90 95

Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr
100 105 110

Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn
115 120 125

Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu
130 135 140

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His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val Glu
145                 150                 155                 160

Ser Ile Gln Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Met
                165                 170                 175

Ser Met Asp Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp Pro
            180                 185                 190

Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln
        195                 200                 205

Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys Arg
    210                 215                 220

Gly Lys Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys
225                 230                 235                 240

Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg Asp
                245                 250                 255

Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro
            260                 265                 270

Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly
        275                 280                 285

Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His
    290                 295                 300

Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu Glu
305                 310                 315                 320

Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys Val
                325                 330                 335

Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn
            340                 345                 350

Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp
        355                 360                 365

Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Thr
    370                 375                 380

Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys Glu
385                 390                 395                 400

Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp
                405                 410                 415

Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln
            420                 425                 430

His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu
        435                 440                 445

Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser
    450                 455                 460
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Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu
465 470 475 480

Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly Glu
485 490 495

Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser Pro
500 505 510

Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg Asn
515 520 525

Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu Gly
530 535 540

Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro
545 550 555 560

Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro
565 570 575

Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val
580 585 590

Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val Trp
595 600 605

Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn Cys
610 615 620

Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn Gly
625 630 635 640

Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu Leu
645 650 655

Leu Leu Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg Arg His
660 665 670

Ile Val Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg Glu Leu
675 680 685

Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala Leu Leu
690 695 700

Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu Gly Ser
705 710 715 720

Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu Gly Glu
725 730 735

Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala Thr Ser
740 745 750

Pro Lys Ala Asn Lys Glu Ile Leu Asp Glu Ala Tyr Val Met Ala Ser
755 760 765

Val Asp Asn Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu Thr Ser
770 775 780

```
Thr Val Gln Leu Ile Thr Gln Leu Met Pro Phe Gly Cys Leu Leu Asp
785                 790                 795                 800

Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu Leu Asn
                805                 810                 815

Trp Cys Val Gln Ile Ala Lys Gly Met Asn Tyr Leu Glu Asp Arg Arg
            820                 825                 830

Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Thr Pro
        835                 840                 845

Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu Gly Ala
    850                 855                 860

Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile Lys Trp
865                 870                 875                 880

Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln Ser Asp
                885                 890                 895

Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Ser
            900                 905                 910

Lys Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile Ser Ser Ile Leu Glu
        915                 920                 925

Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr
    930                 935                 940

Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys
945                 950                 955                 960

Phe Arg Glu Leu Ile Ile Glu Phe Ser Lys Met Ala Arg Asp Pro Gln
                965                 970                 975

Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser Pro
            980                 985                 990

Thr Asp Ser Asn Phe Tyr Arg Ala  Leu Met Asp Glu Glu  Asp Met Asp
        995                 1000                1005

Asp Val  Val Asp Ala Asp Glu  Tyr Leu Ile Pro Gln  Gln Gly Phe
    1010                1015                1020

Phe Ser  Ser Pro Ser Thr Ser  Arg Thr Pro Leu Leu  Ser Ser Leu
    1025                1030                1035

Ser Ala  Thr Ser Asn Asn Ser  Thr Val Ala Cys Ile  Asp Arg Asn
    1040                1045                1050

Gly Leu  Gln Ser Cys Pro Ile  Lys Glu Asp Ser Phe  Leu Gln Arg
    1055                1060                1065

Tyr Ser  Ser Asp Pro Thr Gly  Ala Leu Thr Glu Asp  Ser Ile Asp
    1070                1075                1080

Asp Thr  Phe Leu Pro Val Pro  Glu Tyr Ile Asn Gln  Ser Val Pro
    1085                1090                1095
```

Lys Arg Pro Ala Gly Ser Val Gln Asn Pro Val Tyr His Asn Gln
1100 1105 1110

Pro Leu Asn Pro Ala Pro Ser Arg Asp Pro His Tyr Gln Asp Pro
1115 1120 1125

His Ser Thr Ala Val Gly Asn Pro Glu Tyr Leu Asn Thr Val Gln
1130 1135 1140

Pro Thr Cys Val Asn Ser Thr Phe Asp Ser Pro Ala His Trp Ala
1145 1150 1155

Gln Lys Gly Ser His Gln Ile Ser Leu Asp Asn Pro Asp Tyr Gln
1160 1165 1170

Gln Asp Phe Phe Pro Lys Glu Ala Lys Pro Asn Gly Ile Phe Lys
1175 1180 1185

Gly Ser Thr Ala Glu Asn Ala Glu Tyr Leu Arg Val Ala Pro Gln
1190 1195 1200

Ser Ser Glu Phe Ile Gly Ala
1205 1210

<210> 329
<211> 621
<212> PRT
<213> Homo sapiens

<400> 329
Leu Glu Glu Lys Lys Val Cys Gln Gly Thr Ser Asn Lys Leu Thr Gln
1 5 10 15

Leu Gly Thr Phe Glu Asp His Phe Leu Ser Leu Gln Arg Met Phe Asn
20 25 30

Asn Cys Glu Val Val Leu Gly Asn Leu Glu Ile Thr Tyr Val Gln Arg
35 40 45

Asn Tyr Asp Leu Ser Phe Leu Lys Thr Ile Gln Glu Val Ala Gly Tyr
50 55 60

Val Leu Ile Ala Leu Asn Thr Val Glu Arg Ile Pro Leu Glu Asn Leu
65 70 75 80

Gln Ile Ile Arg Gly Asn Met Tyr Tyr Glu Asn Ser Tyr Ala Leu Ala
85 90 95

Val Leu Ser Asn Tyr Asp Ala Asn Lys Thr Gly Leu Lys Glu Leu Pro
100 105 110

Met Arg Asn Leu Gln Glu Ile Leu His Gly Ala Val Arg Phe Ser Asn
115 120 125

Asn Pro Ala Leu Cys Asn Val Glu Ser Ile Gln Trp Arg Asp Ile Val
130 135 140

Ser Ser Asp Phe Leu Ser Asn Met Ser Met Asp Phe Gln Asn His Leu
145 150 155 160

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Gly Ser Cys Gln Lys Cys Asp Pro Ser Cys Pro Asn Gly Ser Cys Trp
                165                 170                 175

Gly Ala Gly Glu Glu Asn Cys Gln Lys Leu Thr Lys Ile Ile Cys Ala
            180                 185                 190

Gln Gln Cys Ser Gly Arg Cys Arg Gly Lys Ser Pro Ser Asp Cys Cys
        195                 200                 205

His Asn Gln Cys Ala Ala Gly Cys Thr Gly Pro Arg Glu Ser Asp Cys
    210                 215                 220

Leu Val Cys Arg Lys Phe Arg Asp Glu Ala Thr Cys Lys Asp Thr Cys
225                 230                 235                 240

Pro Pro Leu Met Leu Tyr Asn Pro Thr Thr Tyr Gln Met Asp Val Asn
                245                 250                 255

Pro Glu Gly Lys Tyr Ser Phe Gly Ala Thr Cys Val Lys Lys Cys Pro
            260                 265                 270

Arg Asn Tyr Val Val Thr Asp His Gly Ser Cys Val Arg Ala Cys Gly
        275                 280                 285

Ala Asp Ser Tyr Glu Met Glu Glu Asp Gly Val Arg Lys Cys Lys Lys
    290                 295                 300

Cys Glu Gly Pro Cys Arg Lys Val Cys Asn Gly Ile Gly Ile Gly Glu
305                 310                 315                 320

Phe Lys Asp Ser Leu Ser Ile Asn Ala Thr Asn Ile Lys His Phe Lys
                325                 330                 335

Asn Cys Thr Ser Ile Ser Gly Asp Leu His Ile Leu Pro Val Ala Phe
            340                 345                 350

Arg Gly Asp Ser Phe Thr His Thr Pro Pro Leu Asp Pro Gln Glu Leu
        355                 360                 365

Asp Ile Leu Lys Thr Val Lys Glu Ile Thr Gly Phe Leu Leu Ile Gln
    370                 375                 380

Ala Trp Pro Glu Asn Arg Thr Asp Leu His Ala Phe Glu Asn Leu Glu
385                 390                 395                 400

Ile Ile Arg Gly Arg Thr Lys Gln His Gly Gln Phe Ser Leu Ala Val
                405                 410                 415

Val Ser Leu Asn Ile Thr Ser Leu Gly Leu Arg Ser Leu Lys Glu Ile
            420                 425                 430

Ser Asp Gly Asp Val Ile Ile Ser Gly Asn Lys Asn Leu Cys Tyr Ala
        435                 440                 445

Asn Thr Ile Asn Trp Lys Lys Leu Phe Gly Thr Ser Gly Gln Lys Thr
    450                 455                 460

Lys Ile Ile Ser Asn Arg Gly Glu Asn Ser Cys Lys Ala Thr Gly Gln
465                 470                 475                 480
```

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Val Cys His Ala Leu Cys Ser Pro Glu Gly Cys Trp Gly Pro Glu Pro
                485                 490                 495

Arg Asp Cys Val Ser Cys Arg Asn Val Ser Arg Gly Arg Glu Cys Val
            500                 505                 510

Asp Lys Cys Asn Leu Leu Glu Gly Glu Pro Arg Glu Phe Val Glu Asn
        515                 520                 525

Ser Glu Cys Ile Gln Cys His Pro Glu Cys Leu Pro Gln Ala Met Asn
    530                 535                 540

Ile Thr Cys Thr Gly Arg Gly Pro Asp Asn Cys Ile Gln Cys Ala His
545                 550                 555                 560

Tyr Ile Asp Gly Pro His Cys Val Lys Thr Cys Pro Ala Gly Val Met
                565                 570                 575

Gly Glu Asn Asn Thr Leu Val Trp Lys Tyr Ala Asp Ala Gly His Val
            580                 585                 590

Cys His Leu Cys His Pro Asn Cys Thr Tyr Gly Cys Thr Gly Pro Gly
        595                 600                 605

Leu Glu Gly Cys Pro Thr Asn Gly Pro Lys Ile Pro Ser
    610                 615                 620
```

CLAIMS:

1. Epidermal growth factor receptor (EGFR)-binding polypeptide, comprising an epidermal growth factor receptor binding motif, EBM, which motif forms part of a three-helix bundle protein domain and consists of an amino acid sequence selected from:

i) $EX_2X_3X_4AX_6X_7EIR\ X_{11}LPNLNGWQX_{20}\ TAFIX_{25}SLX_{28}D$,

wherein, independently of each other,

X_2 is selected from the group M, V, L and I;

X_3 is selected from the group W, D and E;

X_4 is selected from the group I, V, G, S, M, L, A, T, N and D;

X_6 is selected from the group W, V and I;

X_7 is selected from the group D, E, N and K;

X_{11} is selected from the group D, N, E, Y and S;

X_{20} is selected from the group M, L, and F;

X_{25} is selected from the group A, S and G; and

X_{28} is selected from the group L, V and F;

and

ii) an amino acid sequence which has at least 85 % identity to the sequence defined in i);

the EGFR-binding polypeptide binding to EGFR such that the K_D value of the interaction is at most 10 µM defined in a Biacore 2000 instrument.

2. EGFR-binding polypeptide according to claim 1, wherein in the amino acid sequence i), X_2 is M.

3. EGFR-binding polypeptide according to claim 1 or 2, wherein in the amino acid sequence i), X_3 is W.

4. EGFR-binding polypeptide according to any one of claims 1 to 3, wherein in the amino acid sequence i), X_4 is selected from the group I, V, G and S.

5. EGFR-binding polypeptide according to any one of claims 1 to 4, wherein in the amino acid sequence i), X_6 is selected from the group V and W.

6. EGFR-binding polypeptide according to any one of claims 1 to 5, wherein in the amino acid sequence i), X_{11} is selected from the group D, N and E.

7. EGFR-binding polypeptide according to any one of claims 1 to 6, wherein in the amino acid sequence i), X_{20} is M.

8. EGFR-binding polypeptide according to any one of claims 1 to 7, wherein in the amino acid sequence i), X_{25} is selected from the group A and S.

9. EGFR-binding polypeptide according to any one of claims 1 to 8, wherein in the amino acid sequence i), X_{28} is L.

10. EGFR-binding polypeptide according to any one of claims 1 to 9, wherein the amino acid sequence i) fulfils at least two of the following four conditions I-IV:

I) X_2 is M;

II) X_6 is W;

III) X_{20} is M; and

IV) X_{28} is L.

11. EGFR-binding polypeptide according to claim 10, wherein the amino acid sequence i) fulfils at least three of the four conditions I-IV.

12. EGFR-binding polypeptide according to claim 11, wherein the amino acid sequence i) is

$EMWX_4AWX_7EIR\ X_{11}LPNLNGWQM\ TAFIX_{25}SLLD$.

13. EGFR-binding polypeptide according to claim 12, wherein in the amino acid sequence i), X_{25} is A.

14. EGFR-binding polypeptide according to any one of claims 1 to 11, wherein the amino acid sequence i) is selected from SEQ ID NOs:48, 57, 87, 146-148, 150-153 and 156-161.

15. EGFR-binding polypeptide according to claim 14, wherein the amino acid sequence i) is selected from the group, SEQ ID NO:48, SEQ ID NO:57, SEQ ID NO:87 and SEQ ID NO:147.

16. EGFR-binding polypeptide according to any one of claims 1 to 15, in which said EGFR-binding motif forms part of

two alpha helices and a loop connecting them, within said three-helix bundle protein domain.

17. EGFR-binding polypeptide according to claim 16, in which said three-helix bundle protein domain is a domain of a bacterial receptor protein.

18. EGFR-binding polypeptide according to claim 17, in which said three-helix bundle protein domain is a domain of protein A from *Staphylococcus aureus* or a derivative thereof.

19. EGFR-binding polypeptide according to claim 18, which comprises an amino acid sequence selected from the group:

ADNNFNK-[EBM]-DPSQSANLLSEAKKLNESQAPK;

ADNKFNK-[EBM]-DPSQSANLLAEAKKLNDAQAPK;

ADNKFNK-[EBM]-DPSVSKEILAEAKKLNDAQAPK;

ADAQQNNFNK-[EBM]-DPSQSTNVLGEAKKLNESQAPK;

AQHDE-[EBM]-DPSQSANVLGEAQKLNDSQAPK; and

VDNKFNK-[EBM]-DPSQSANLLAEAKKLNDAQAPK;

wherein [EBM] is an EGFR-binding motif as defined in any one of claims 1 to 15.

20. Epidermal growth factor receptor (EGFR)-binding polypeptide, whose amino acid sequence comprises a sequence which fulfils one definition selected from the following:

i) it is selected from the group consisting of SEQ ID NOs:164-326;

ii) it is an amino acid sequence having 85 % or greater identity to a sequence selected from the group consisting of SEQ ID NOs:164-326.

21. EGFR-binding polypeptide according to claim 20, whose amino acid sequence comprises a sequence which fulfils one definition selected from the following:

i) it is selected from the group consisting of SEQ ID NO:196, SEQ ID NO:211, SEQ ID NO:220, SEQ ID NO:250, SEQ ID NO:251 and SEQ ID NO:310;

ii) it is an amino acid sequence having 85 % or greater identity to a sequence selected from the group consisting of SEQ ID NO:196, SEQ ID NO:211, SEQ ID NO:220, SEQ ID NO:250, SEQ ID NO:251 and SEQ ID NO:310.

22. EGFR-binding polypeptide according to any one of claims 1 to 21 which has been extended by an albumin-binding domain of streptococcal protein G, or a derivative thereof, which improves the half life of the EGFR-binding polypeptide in treatment of cancer.

23. EGFR-binding polypeptide according to any one of claims 1 to 22, which binds to EGFR such that the K_D value of the interaction is at most 1 μM defined in a Biacore 2000 instrument.

24. EGFR-binding polypeptide according to claim 23, which is a dimer and binds to EGFR such that the K_D value of the interaction is at most 0.1 μM defined in a Biacore 2000 instrument.

25. EGFR-binding polypeptide according to any one of claims 1 to 24 which binds to the extra-cellular domain of EGFR.

26. EGFR-binding polypeptide according to claim 25 which binds to a portion of the extra-cellular domain of EGFR corresponding to SEQ ID NO:329.

27. EGFR-binding polypeptide according to any one of claims 1 to 26 in multimeric form, comprising at least two EGFR-binding polypeptide monomer units, whose amino acid sequences may be the same or different.

28. EGFR-binding polypeptide according to claim 27, in which the EGFR-binding polypeptide monomer units are covalently coupled together.

29. EGFR-binding polypeptide according to claim 28, in which the EGFR-binding polypeptide monomer units are expressed as a fusion protein.

30. EGFR-binding polypeptide according to any one of claims 27 to 29 in a dimeric form.

31. A polynucleotide encoding the polypeptide according to any one of claims 1 to 30.

32. Method of producing the polypeptide according to any one of claims 1 to 30, the method comprising expressing the polynucleotide according to claim 31.

33. Combination of the EGFR-binding polypeptide according to any one of claims 1 to 30 and a detectable agent.

34. Combination according to claim 33, in which the detectable agent is a radioactive substance for use in radio-imaging.

35. Combination according to claim 34, in which the radioactive substance is a radionuclide.

36. Combination according to claim 33, in which the detectable agent is an enzyme.

37. Combination of the EGFR-binding polypeptide according to any one of claims 1 to 30 and a therapeutic agent.

38. Combination according to any one of claims 33 to 36, in which the EGFR-binding polypeptide and detectable agent are covalently coupled together.

39. Combination according to claim 37, in which the EGFR-binding polypeptide and therapeutic agent are covalently coupled together.

40. Combination according to any one of claims 33 to 36 and 38, in which the EGFR-binding polypeptide and detectable agent are expressed as a fusion protein.

41. Combination according to any one of claims 37 and 39, in which the EGFR-binding polypeptide and therapeutic agent are expressed as a fusion protein.

42. Method of detection of EGFR, comprising providing a sample suspected to contain an EGFR, contacting the sample with the EGFR-binding polypeptide according to any one of claims 1 to 30, or the combination according to any one of claims 33

to 36, 38 and 40 and detecting binding of the polypeptide or combination to indicate the presence of an EGFR in the sample.

43. Method according to claim 42, in which the combination according to any one of claims 33 to 36, 38 and 40 is used as a radio-imaging agent.

44. Method of separating or capturing EGFR from a sample, the method comprising contacting the sample with the EGFR-binding polypeptide according to any one of claims 1 to 30 or the combination according to any one of claims 33 to 36, 38 and 40, whereby EGFR binds to the polypeptide and can be removed from the sample.

45. Use of the EGFR-binding polypeptide according to any one of claims 1 to 30 or the combination according to any one of claims 33 to 36, 38 and 40 for determining the presence of an EGFR in a mammalian subject, wherein the EGFR-binding polypeptide or combination is for contact with the subject or a sample derived from the subject.

46. Use according to claim 45, in which the subject is human.

47. Use according to claim 45 or 46 which is *in vivo*.

48. Use according to claim 45 or 46 which is *in vitro*.

49. Use of the EGFR-binding polypeptide according to any one of claims 1 to 30 or the combination according to any one of claims 37, 39 and 41 for the treatment of an EGFR-related condition in a mammalian subject or in material derived from a mammalian subject.

50. Use according to claim 49, in which binding of said EGFR-binding polypeptide or said combination to an EGFR of the subject, or in the material, modulates activation of the receptor.

51. Use according to claim 49 or 50, in which binding of the EGFR-binding polypeptide to an EGFR of the subject, or in the material, inhibits cell signaling.

52. Use according to any one of claims 49 to 51, in which the EGFR-related condition is a cancer.

53. Use according to claim 52, in which the cancer is selected from the group lung, breast, prostate, colon, ovary, head and neck cancers.

54. Use according to any one of claims 49 to 53, in which said subject is human.

55. Use of the EGFR-binding polypeptide according to any one of claims 1 to 30 or the combination according to any one of claims 33 to 36, 38 and 40 for the manufacture of a diagnostic agent for the diagnosis of cancers caused by over-expression of EGFR *in vivo*.

56. Use of the EGFR-binding polypeptide according to any one of claims 1 to 30 or the combination according to any one of claims 37, 39 and 41 for the manufacture of a medicament for the treatment of cancers caused by over-expression of EGFR.

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
EBM00940	EWSAAASEISGLPNLNKLQAFAFIVSLVD	1
EBM00942	EMLIAMEEIGSLPNLNWGQEQAFILSLWD	2
EBM00947	ETGAAMREINDLPNLNNLQFFAFIVSLVD	3
EBM00948	EFYAAITEINRLPNLNGWQMVAFISSLSD	4
EBM00949	EHAKAMWEIGNLPNLNLVQLAAFIFSLRD	5
EBM00951	ESLAASVEISHLPNLNGSQCKAFIRSLMD	6
EBM00955	ELEKAYNEIRNLPNLNGWQMTAFIASLVD	7
EBM00956	EAAPAWTEIVRLPNLNRGQKQAFIVSLHD	8
EBM00957	ELWIATSEIVELPNLNMHQGVAFIRSLLD	9
EBM01239	EVQNAVAEIVKLPNLNGWQSTAFIASLSD	10
EBM01831	EYEEAWNEIRNLPNLNGWQMTAFIASLVD	11
EBM01832	EIERAMQEIRNLPNLNGWQMTAFIASLVD	12
EBM01833	EVETAWMEIRNLPNLNGWQMTAFIASLVD	13
EBM01834	ETETAIQEIRSLPNLNGWQMTAFIASLFD	14
EBM01835	ETDRAVEEIRNLPNLNGWQMTAFIASLFD	15
EBM01836	EMWRAWEEIRNLPNLNGWQMTAFIASLVD	16
EBM01837	ESQDAWEEIRSLPNLNGWQMTAFIASLVD	17
EBM01838	EREEAIKEIHNLPNLNGWQMTAFIASLFD	18
EBM01839	ESWEAWHEIRNLPNLNGWQMTAFIASLVD	19
EBM01840	ELYDAMIEINHLPNLNGWQMTAFIASLVD	20
EBM01841	ETDKAVQEIHNLPNLNGWQMTAFIASLFD	21
EBM01842	EQVRAWEEIRNLPNLNGWQMTAFIASLVD	22
EBM01843	ELWGAWEEIHNLPNLNGWQMTAFIASLVD	23
EBM01844	ERDAAWEEIRHLPNLNGWQMTAFIASLVD	24
EBM01845	EVFPALQEIRNLPNLNGWQMTAFIASLFD	25
EBM01846	EVEMATQEIRNLPNLNGWQMTAFIASLFD	26
EBM01847	ELYQAMDEIRSLPNLNGWQMTAFIASLVD	27
EBM01848	EATEAWDEIRNLPNLNGWQMTAFIASLVD	28
EBM01849	EVEWALQEIRNLPNLNGWQMTAFIASLFD	29
EBM01850	EVSPALEEIRSLPNLNGWQMTAFIASLFD	30
EBM01851	ERERAIEEIHNLPNLNGWQMTAFIASLFD	31
EBM01852	EAESAWNEIHNLPNLNGWQMTAFIASLVD	32

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
EBM01853	EFWWASDEIRNLPNLNGWQMTAFIASLAD	33
EBM01854	EMWSAWEEIHNLPNLNGWQMTAFIASLVD	34
EBM01855	EHWNAMHEIRSLPNLNGWQMTAFIASLFD	35
EBM01856	EVEKAWSEIRSLPNLNGWQMTAFIASLVD	36
EBM01857	EREKAWMEIRNLPNLNGWQMTAFIASLVD	37
EBM01858	EMWSAWSEIHNLPNLNGWQMTAFIASLVD	38
EBM01859	EMWSAWAEIRNLPNLNGWQMTAFIASLVD	39
EBM01860	ERSLAIREIHNLPNLNGWQMTAFIASLFD	40
EBM01861	ERDTAISEIRNLPNLNGWQMTAFIASLFD	41
EBM01862	EMWAAWGEIHSLPNLNGWQMTAFIASLVD	42
EBM01863	ERDTAIYEIRNLPNLNGWQMTAFIASLFD	43
EBM01864	EPWLAWAEIRNLPNLNGWQMTAFIASLVD	44
EBM01865	EMWDAWEEIHNLPNLNGWQMTAFIASLVD	45
EBM01866	EDMEAVDEIRNLPNLNGWQMTAFIASLFD	46
EBM01867	EAEHAWEEIRNLPNLNGWQMTAFIASLVD	47
EBM01868	ELWIAWDEIRNLPNLNGWQMTAFIASLVD	48
EBM01869	EMWNAWSEIRNLPNLNGWQMTAFIASLVD	49
EBM01870	EINSAIGEIHNLPNLNGWQMTAFIASLFD	50
EBM01871	EMWRAWEEIHNLPNLNGWQMTAFIASLVD	51
EBM01872	ESWKAWEEIRNLPNLNGWQMTAFIASLVD	52
EBM01873	ETEWAIQEIRNLPNLNGWQMTAFIASLFD	53
EBM01874	EAEFAWTEIRNLPNLNGWQMTAFIASLVD	54
EBM01875	ELLVAMLEINHLPNLNGWQMTAFIASLVD	55
EBM01876	ERDFAIDEIHSLPNLNGWQMTAFIASLFD	56
EBM01877	EMWIAWEEIRNLPNLNGWQMTAFIASLVD	57
EBM01878	ESNSAWQEIRNLPNLNGWQMTAFIASLVD	58
EBM01879	EVWTAWEEIHNLPNLNGWQMTAFIASLVD	59
EBM01880	EPWMAWDEIRSLPNLNGWQMTAFIASLVD	60
EBM01881	ERDGAIQEIRNLPNLNGWQMTAFIASLFD	61
EBM01882	EKWTAWEEIRSLPNLNGWQMTAFIASLVD	62
EBM01883	EMWHAWDEIRHLPNLNGWQMTAFIASLVD	63
EBM01884	EVDQAVAEIRNLPNLNGWQMTAFIASLFD	64

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
EBM01885	ERYWAIEEIRNLPNLNGWQMTAFIASLFD	65
EBM01886	EREEAISEIHSLPNLNGWQMTAFIASLFD	66
EBM01887	EMEWAWQEIRNLPNLNGWQMTAFIASLVD	67
EBM01888	EVEPAIREIHNLPNLNGWQMTAFIASLFD	68
EBM01889	EQDEAVKEIRNLPNLNGWQMTAFIASLFD	69
EBM01890	EADSAWTEIRNLPNLNGWQMTAFIASLVD	70
EBM01891	ETDYAIGEIHSLPNLNGWQMTAFIASLFD	71
EBM01892	EADKAVQEIRNLPNLNGWQMTAFIASLFD	72
EBM01893	ETDKAVQEIRNLPNLNGWQMTAFIASLFD	73
EBM01894	ELWAAWSEIRNLPNLNGWQMTAFIASLVD	74
EBM01895	EAWAAWSEIRNLPNLNGWQMTAFIASLVD	75
EBM01896	EVDRAVVEIRSLPNLNGWQMTAFIASLFD	76
EBM01897	EAESAIEEIHNLPNLNGWQMTAFIASLFD	77
EBM01898	ELGGAVNEIRNLPNLNGWQMTAFIASLFD	78
EBM01899	EVDTAIWEIRNLPNLNGWQMTAFIASLFD	79
EBM01900	ELANAFDEIHRLPNLNGWQMTAFIASLVD	80
EBM01901	EFRRASDEIRNLPNLNGWQMTAFIASLAD	81
EBM01902	EIEKAIREIHNLPNLNGWQMTAFIASLVD	82
EBM01903	EMWEAWDEIHNLPNLNGWQMTAFIASLVD	83
EBM01904	ESKWAWEEIRNLPNLNGWQMTAFIASLVD	84
EBM01905	EMWRAWEEIHNLPNLNGWQMTAFIASLVD	85
EBM01906	EIDPALQEIRNLPNLNGWQMTAFIASLFD	86
EBM01907	EMWAAWEEIRNLPNLNGWQMTAFIASLVD	87
EBM01908	EKYWAVDEIRNLPNLNGWQMTAFIASLFD	88
EBM01909	EHWAAWHEIRSLPNLNGWQMTAFIASLVD	89
EBM01910	EYQTAWKEIRNLPNLNGWQMTAFIASLVD	90
EBM01911	ETDRAIKEIHNLPNLNGWQMTAFIASLFD	91
EBM01912	EMWNAWHEIRNLPNLNGWQMTAFIASLVD	92
EBM01913	EPWVAWNEIRNLPNLNGWQMTAFIASLVD	93
EBM01914	ELIGAYDEIRSLPNLNGWQMTAFIASLAD	94
EBM01915	ERDYALWEIRNLPNLNGWQMTAFIASLFD	95
EBM01916	ETQDAWDEIRNLPNLNGWQMTAFIASLVD	96

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
EBM01917	EMWEAWGEIHNLPNLNGWQMTAFIASLVD	97
EBM01918	EMWSAWHEIRSLPNLNGWQMTAFIASLVD	98
EBM01919	ELWQAWGEIRNLPNLNGWQMTAFIASLVD	99
EBM01920	EVERAWNEIRNLPNLNGWQMTAFIASLVD	100
EBM01921	EMWEAWGEIRSLPNLNGWQMTAFIASLVD	101
EBM01922	ERTQAIREIHNLPNLNGWQMTAFIASLFD	102
EBM01923	ETEEAWEEIHNLPNLNGWQMTAFIASLVD	103
EBM01924	EAETAWSEIRNLPNLNGWQMTAFIASLVD	104
EBM01925	EMWCAWNEIRNLPNLNGWQMTAFIASLVD	105
EBM01926	ERDYAIEEIHNLPNLNGWQMTAFIASLFD	106
EBM01927	EMWSAWDEIHNLPNLNGWQMTAFIASLVD	107
EBM01928	EMWTAWHEIHNLPNLNGWQMTAFIASLVD	108
EBM01929	ETDRAVREIRNLPNLNGWQMTAFIASLFD	109
EBM01930	ETWRAWHEIRSLPNLNGWQMTAFIASLVD	110
EBM01931	EMWLAWQEIRNLPNLNGWQMTAFIASLVD	111
EBM01932	EVDYAIQEIHNLPNLNGWQMTAFIASLFD	112
EBM01933	EMESAWIEIRNLPNLNGWQMTAFIASLVD	113
EBM01934	ETEEAWEEIRNLPNLNGWQMTAFIASLVD	114
EBM01935	ESEAALQEIRNLPNLNGWQMTAFIASLFD	115
EBM01936	EFRKASNEIRSLPNLNGWQMTAFIASLAD	116
EBM01937	EVQLAWDEIRSLPNLNGWQMTAFIASLVD	117
EBM01938	EADRAWEEIRNLPNLNGWQMTAFIASLVD	118
EBM01939	EIKPAIREIHSLPNLNGWQMTAFIASLFD	119
EBM01940	ELDQAILEIHNLPNLNGWQMTAFIASLFD	120
EBM01941	EPWIAWHEIRNLPNLNGWQMTAFIASLVD	121
EBM01942	ERDVAITEIHNLPNLNGWQMTAFIASLFD	122
EBM01943	EFDKAVSEIRNLPNLNGWQMTAFIASLFD	123
EBM01944	EVDVAMQEIRNLPNLNGWQMTAFIASLFD	124
EBM01945	ETNAALEEIRNLPNLNGWQMTAFIASLFD	125
EBM01946	EAEKAWEEIHNLPNLNGWQMTAFIASLVD	126
EBM01947	EPWLAWSEIRNLPNLNGWQMTAFIASLVD	127
EBM01948	EGLNAVNEIRNLPNLNGWQMTAFIASLFD	128

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
EBM01949	EWEVAMEEIRNLPNLNGWQMTAFIASLFD	129
EBM01950	EVESAWTEIRNLPNLNGWQMTAFIASLVD	130
EBM01951	ETDRAWDEIRNLPNLNGWQMTAFIASLVD	131
EBM02268	EREQATEEIRNLPNLNGWQMTAFIASLFD	132
EBM02269	EMEHAWEEIRSLPNLNGWQMTAFIASLVD	133
EBM02270	EHWNALHEIRSLPNLNGGQMTAFIASLFD	134
EBM02271	EYEAAWDEIRNLPNLNGWQMTAFIASLVD	135
EBM02272	EGEMALQEIRNLPNLNGWQMTAFIASLFD	136
EBM02273	EFRWASDEIRNLPNLNGWQMTAFIASLAD	137
EBM02274	EHWNALHEIRSLPNLNGWQMTAFIASLFD	138
EBM02275	EIDYAIREIHNLPNLNGWQMTAFIASLFD	139
EBM02276	ELLQAMLEINHLPNLNGWQMTAFIASLVD	140
EBM02277	EVNPALQEIRSLPNLNGWQMTAFIASLFD	141
EBM02278	ELLSAMLEINHLPNLNGWQMTAFIASLVD	142
EBM02279	ERDEAIQEIHSLPNLNGWQMTAFIASLFD	143
EBM02280	ETDWAIQEIRSLPNLNGWQMTAFIASLFD	144
EBM02281	EMEKAWVEIRNLPNLNGWQMTAFIASLVD	145
EBM02282	ELDNAIDEIRNLPNLNGWQMTAFIASLFD	146
EBM02377	EMWIAWEEIRDLPNLNGWQMTAFIASLLD	147
EBM02378	EMWLAWEEIRNLPNLNGWQLTAFIASLLD	148
EBM02379	EMWSAWDEIRALPNLNGWQMTAFISSLLD	149
EBM02380	EMWNAWNEIRDLPNLNGWQMTAFIASLLD	150
EBM02381	EMWGAWNEIRDLPNLNGWQMTAFISSLLD	151
EBM02382	EMWIAWDEIRDLPNLNGWQFTAFIASLLD	152
EBM02383	ELWIAWDEIRYLPNLNGWQMTAFIASLLD	153
EBM02384	EMWKAWEEIRSLPNLNGWQMTAFIASLLD	154
EBM02385	EMWDAWGEIRNLPNLNGWQMTAFIASLLD	155
EBM02386	EVWVAWEEIRDLPNLNGWQMTAFIASLLD	156
EBM02387	EMWGAWEEIRNLPNLNGWQMTAFIASLVD	157
EBM02388	EMWMAWDEIRYLPNLNGWQLTAFISSLLD	158
EBM02389	EMWVAWEEIRNLPNLNGWQMTAFIGSLLD	159
EBM02390	EMWDAWDEIRYLPNLNGWQFTAFIASLLD	160

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
EBM02391	ELWGAWDEIRYLPNLNGWQMTAFIASLLD	161
EBM02392	ESWNAVKEIGELPNLNWGQADAFINSLWD	162
EBM02393	ESHEVWQEIRSLPNLNGWQLTAFINSLLD	163
Z00940	VDNKFNKEWSAAASEISGLPNLNKLQAFAFIVSLVDDPSQSANLLAEAKKLNDAQAPK	164
Z00942	VDNKFNKEMLIAMEEIGSLPNLNWGQEQAFILSLWDDPSQSANLLAEAKKLNDAQAPK	165
Z00947	VDNKFNKETGAAMREINDLPNLNNLQFFAFIVSLVDDPSQSANLLAEAKKLNDAQAPK	166
Z00948	VDNKFNKEFYAAITEINRLPNLNGWQMVAFISSLSDDPSQSANLLAEAKKLNDAQAPK	167
Z00949	VDNKFNKEHAKAMWEIGNLPNLNLVQLAAAFIFSLRDDPSQSANLLAEAKKLNDAQAPK	168
Z00951	VDNKFNKESLAASVEISHLPNLNGSQCKAFIRSLMDDPSQSANLLAEAKKLNDAQAPK	169
Z00955	VDNKFNKELEKAYNEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	170
Z00956	VDNKFNKEAAPAWTEIVRLPNLNRGQKQAFIVSLHDDPSQSANLLAEAKKLNDAQAPK	171
Z00957	VDNKFNKELWIATSEIVELPNLNMHQGVAFIRSLLDDPSQSANLLAEAKKLNDAQAPK	172
Z01239	VDNKFNKEVQNAVAEIVKLPNLNGWQSTAFIASLSDDPSQSANLLAEAKKLNDAQAPK	173
Z01831	VDNKFNKEYEEAWNEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	174
Z01832	VDNKFNKEIERAMQEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	175
Z01833	VDNKFNKEVETAWMEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	176
Z01834	VDNKFNKETETAIQEIRSLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	177
Z01835	VDNKFNKETDRAVEEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	178
Z01836	VDNKFNKEMWRAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	179
Z01837	VDNKFNKESQDAWEEIRSLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	180
Z01838	VDNKFNKEREEAIKEIHNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	181
Z01839	VDNKFNKESWEAWHEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	182
Z01840	VDNKFNKELYDAMIEINHLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	183
Z01841	VDNKFNKETDKAVQEIHNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	184
Z01842	VDNKFNKEQVRAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	185
Z01843	VDNKFNKELWGAWEEIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	186
Z01844	VDNKFNKERDAAWEEIRHLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	187
Z01845	VDNKFNKEVFPALQEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	188
Z01846	VDNKFNKEVEMATQEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	189
Z01847	VDNKFNKELYQAMDEIRSLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	190
Z01848	VDNKFNKEATEAWDEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	191
Z01849	VDNKFNKEVEWALQEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	192

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
Z01850	VDNKFNKEVSPALEEIRSLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	193
Z01851	VDNKFNKERERAIEEIHNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	194
Z01852	VDNKFNKEAESAWNEIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	195
Z01853	VDNKFNKEFWWASDEIRNLPNLNGWQMTAFIASLADDPSQSANLLAEAKKLNDAQAPK	196
Z01854	VDNKFNKEMWSAWEEIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	197
Z01855	VDNKFNKEHWNAMHEIRSLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	198
Z01856	VDNKFNKEVEKAWSEIRSLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	199
Z01857	VDNKFNKEREKAWMEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	200
Z01858	VDNKFNKEMWSAWSEIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	201
Z01859	VDNKFNKEMWSAWAEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	202
Z01860	VDNKFNKERSLAIREIHNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	203
Z01861	VDNKFNKERDTAISEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	204
Z01862	VDNKFNKEMWAAWGEIHSLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	205
Z01863	VDNKFNKERDTAIYEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	206
Z01864	VDNKFNKEPWLAWAEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	207
Z01865	VDNKFNKEMWDAWEEIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	208
Z01866	VDNKFNKEDMEAVDEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	209
Z01867	VDNKFNKEAEHAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	210
Z01868	VDNKFNKELWIAWDEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	211
Z01869	VDNKFNKEMWNAWSEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	212
Z01870	VDNKFNKEINSAIGEIHNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	213
Z01871	VDNKFNKEMWRAWEEIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	214
Z01872	VDNKFNKESWKAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	215
Z01873	VDNKFNKETEWAIQEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	216
Z01874	VDNKFNKEAEFAWTEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	217
Z01875	VDNKFNKELLVAMLEINHLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	218
Z01876	VDNKFNKERDFAIDEIHSLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	219
Z01877	VDNKFNKEMWIAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	220
Z01878	VDNKFNKESNSAWQEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	221
Z01879	VDNKFNKEVWTAWEEIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	222
Z01880	VDNKFNKEPWMAWDEIRSLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	223
Z01881	VDNKFNKERDGAIQEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	224

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
Z01882	VDNKFNKEKWTAWEEIRSLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	225
Z01883	VDNKFNKEMWHAWDEIRHLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	226
Z01884	VDNKFNKEVDQAVAEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	227
Z01885	VDNKFNKERYWAIEEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	228
Z01886	VDNKFNKEREEAISEIHSLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	229
Z01887	VDNKFNKEMEWAWQEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	230
Z01888	VDNKFNKEVEPAIREIHNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	231
Z01889	VDNKFNKEQDEAVKEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	232
Z01890	VDNKFNKEADSAWTEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	233
Z01891	VDNKFNKETDYAIGEIHSLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	234
Z01892	VDNKFNKEADKAVQEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	235
Z01893	VDNKFNKETDKAVQEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	236
Z01894	VDNKFNKELWAAWSEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	237
Z01895	VDNKFNKEAWAAWSEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	238
Z01896	VDNKFNKEVDRAVVEIRSLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	239
Z01897	VDNKFNKEAESAIEEIHNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	240
Z01898	VDNKFNKELGGAVNEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	241
Z01899	VDNKFNKEVDTAIWEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	242
Z01900	VDNKFNKELANAFDEIHRLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	243
Z01901	VDNKFNKEFRRASDEIRNLPNLNGWQMTAFIASLADDPSQSANLLAEAKKLNDAQAPK	244
Z01902	VDNKFNKEIEKAIREIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	245
Z01903	VDNKFNKEMWEAWDEIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	246
Z01904	VDNKFNKESKWAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	247
Z01905	VDNKFNKEMWRAWEEIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	248
Z01906	VDNKFNKEIDPALQEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	249
Z01907	VDNKFNKEMWAAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	250
Z01908	VDNKFNKEKYWAVDEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	251
Z01909	VDNKFNKEHWAAWHEIRSLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	252
Z01910	VDNKFNKEYQTAWKEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	253
Z01911	VDNKFNKETDRAIKEIHNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	254
Z01912	VDNKFNKEMWNAWHEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	255
Z01913	VDNKFNKEPWVAWNEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	256

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
Z01914	VDNKFNKEL-GAYDEIRSLPNLNGWQMTAFIASLADDPSQSANLLAEAKKLNDAQAPK	257
Z01915	VDNKFNKERDYALWEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	258
Z01916	VDNKFNKETQDAWDEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	259
Z01917	VDNKFNKEMWEAWGEIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	260
Z01918	VDNKFNKEMWSAWHEIRSLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	261
Z01919	VDNKFNKELWQAWGEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	262
Z01920	VDNKFNKEVERAWNEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	263
Z01921	VDNKFNKEMWEAWGEIRSLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	264
Z01922	VDNKFNKER-QAIREIHNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	265
Z01923	VDNKFNKETEEAWEEIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	266
Z01924	VDNKFNKEAETAWSEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	267
Z01925	VDNKFNKEMWCAWNEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	268
Z01926	VDNKFNKERDYAIEEIHNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	269
Z01927	VDNKFNKEMWSAWDEIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	270
Z01928	VDNKFNKEMWTAWHEIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	271
Z01929	VDNKFNKETDRAVREIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	272
Z01930	VDNKFNKETWRAWHEIRSLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	273
Z01931	VDNKFNKEMWLAWQEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	274
Z01932	VDNKFNKEVDYAIQEIHNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	275
Z01933	VDNKFNKEMESAWIEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	276
Z01934	VDNKFNKETEEAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	277
Z01935	VDNKFNKESEAALQEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	278
Z01936	VDNKFNKEFRKASNEIRSLPNLNGWQMTAFIASLADDPSQSANLLAEAKKLNDAQAPK	279
Z01937	VDNKFNKEVQLAWDEIRSLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	280
Z01938	VDNKFNKEADRAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	281
Z01939	VDNKFNKEIKPAIREIHSLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	282
Z01940	VDNKFNKELDQAILEIHNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	283
Z01941	VDNKFNKEPWIAWHEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	284
Z01942	VDNKFNKERDVAITEIHNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	285
Z01943	VDNKFNKEFDKAVSEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	286
Z01944	VDNKFNKEVDVAMQEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	287
Z01945	VDNKFNKETNAALEEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	288

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
Z01946	VDNKFNKEAEKAWEEIHNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	289
Z01947	VDNKFNKEPWLAWSEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	290
Z01948	VDNKFNKEGLNAVNEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	291
Z01949	VDNKFNKEWEVAMEEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	292
Z01950	VDNKFNKEVESAWTEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	293
Z01951	VDNKFNKETDRAWDEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	294
Z02268	VDNKFNKEREQATEEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	295
Z02269	VDNKFNKEMEHAWEEIRSLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	296
Z02270	VDNKFNKEHWNALHEIRSLPNLNGGQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	297
Z02271	VDNKFNKEYEAAWDEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	298
Z02272	VDNKFNKEGEMALQEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	299
Z02273	VDNKFNKEFRWASDEIRNLPNLNGWQMTAFIASLADDPSQSANLLAEAKKLNDAQAPK	300
Z02274	VDNKFNKEHWNALHEIRSLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	301
Z02275	VDNKFNKEIDYAIREIHNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	302
Z02276	VDNKFNKELLQAMLEINHLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	303
Z02277	VDNKFNKEVNPALQEIRSLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	304
Z02278	VDNKFNKELLSAMLEINHLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	305
Z02279	VDNKFNKERDEAIQEIHSLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	306
Z02280	VDNKFNKETDWAIQEIRSLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	307
Z02281	VDNKFNKEMEKAWVEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	308
Z02282	VDNKFNKELDNAIDEIRNLPNLNGWQMTAFIASLFDDPSQSANLLAEAKKLNDAQAPK	309
Z02377	VDNKFNKEMWIAWEEIRDLPNLNGWQMTAFIASLLDDPSQSANLLAEAKKLNDAQAPK	310
Z02378	VDNKFNKEMWLAWEEIRNLPNLNGWQLTAFIASLLDDPSQSANLLAEAKKLNDAQAPK	311
Z02379	VDNKFNKEMWSAWDEIRALPNLNGWQMTAFISSLLDDPSQSANLLAEAKKLNDAQAPK	312
Z02380	VDNKFNKEMWNAWNEIRDLPNLNGWQMTAFIASLLDDPSQSANLLAEAKKLNDAQAPK	313
Z02381	VDNKFNKEMWGAWNEIRDLPNLNGWQMTAFISSLLDDPSQSANLLAEAKKLNDAQAPK	314
Z02382	VDNKFNKEMWIAWDEIRDLPNLNGWQFTAFIASLLDDPSQSANLLAEAKKLNDAQAPK	315
Z02383	VDNKFNKELWIAWDEIRYLPNLNGWQMTAFIASLLDDPSQSANLLAEAKKLNDAQAPK	316
Z02384	VDNKFNKEMWKAWEEIRSLPNLNGWQMTAFIASLLDDPSQSANLLAEAKKLNDAQAPK	317
Z02385	VDNKFNKEMWDAWGEIRNLPNLNGWQMTAFIASLLDDPSQSANLLAEAKKLNDAQAPK	318
Z02386	VDNKFNKEVWVAWEEIRDLPNLNGWQMTAFIASLLDDPSQSANLLAEAKKLNDAQAPK	319
Z02387	VDNKFNKEMWGAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPK	320

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
Z02388	VDNKFNKEMWMAWDEIRYLPNLNGWQLTAFISSLLDDPSQSANLLAEAKKLNDAQAPK	321
Z02389	VDNKFNKEMWVAWEEIRNLPNLNGWQMTAFIGSLLDDPSQSANLLAEAKKLNDAQAPK	322
Z02390	VDNKFNKEMWDAWDEIRYLPNLNGWQFTAFIASLLDDPSQSANLLAEAKKLNDAQAPK	323
Z02391	VDNKFNKELWGAWDEIRYLPNLNGWQMTAFIASLLDDPSQSANLLAEAKKLNDAQAPK	324
Z02392	VDNKFNKESWNAVKEIGELPNLNWGQADAFINSLWDDPSQSANLLAEAKKLNDAQAPK	325
Z02393	VDNKFNKESHEVWQEIRSLPNLNGWQLTAFINSLLDDPSQSANLLAEAKKLNDAQAPK	326
Z00000	VDNKFNKEQQNAFYEILHLPNLNEEQRNAFIQSLKDDPSQSANLLAEAKKLNDAQAPK	327
EGFR	MRPSGTAGAALLALLAALCPASRALEEKKVCQGTSNKLTQLGTFEDHFLSLQRMFNNCEVVLGNLEITYVQRNYD LSFLKTIQEVAGYVLIALNTVERIPLENLQIIRGNMYYENSYALAVLSNYDANKTGLKELPMRNLQEILHGAVRF SNNPALCNVESIQWRDIVSSDFLSNMSMDFQNHLGSCQKCDPSCPNGSCWGAGEENCQKLTKIICAQQCSGRCRG KSPSDCCHNQCAAGCTGPRESDCLVCRKFRDEATCKDTCPPLMLYNPTTYQMDVNPEGKYSFGATCVKKCPRNYV VTDHGSCVRACGADSYEMEEDGVRKCKKCEGPCRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVA FRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGL RSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVS CRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVM GENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFMRRRHIVR KRTLRRLLQERELVEPLTPSGEAPNQALLRILKETEFKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREA TSPKANKEILDEAYVMASVDNPHVCRLLGICLTSTVQLITQLMPFGCLLDYVREHKDNIGSQYLLNWCVQIAKGM NYLEDRRLVHRDLAARNVLVKTPQHVKITDFGLAKLLGAEEKEYHAEGGKVPIKWMALESILHRIYTHQSDVWSY GVTVWELMTFGSKPYDGIPASEISSILEKGERLPQPPICTIDVYMIMVKCWMIDADSRPKFRELIIEFSKMARDP QRYLVIQGDERMHLPSPTDSNFYRALMDEEDMDDVVDADEYLIPQQGFFSSPSTSRTPLLSSLSATSNNSTVACI DRNGLQSCPIKEDSFLQRYSSDPTGALTEDSIDDTFLPVPEYINQSVPKRPAGSVQNPVYHNQPLNPAPSRDPHY QDPHSTAVGNPEYLNTVQPTCVNSTFDSPAHWAQKGSHQISLDNPDYQQDFFPKEAKPNGIFKGSTAENAEYLRV APQSSEFIGA	328
EGFR ECD	LEEKKVCQGTSNKLTQLGTFEDHFLSLQRMFNNCEVVLGNLEITYVQRNYDLSFLKTIQEVAGYVLIALNTVERI PLENLQIIRGNMYYENSYALAVLSNYDANKTGLKELPMRNLQEILHGAVRFSNNPALCNVESIQWRDIVSSDFLS NMSMDFQNHLGSCQKCDPSCPNGSCWGAGEENCQKLTKIICAQQCSGRCRGKSPSDCCHNQCAAGCTGPRESDCL VCRKFRDEATCKDTCPPLMLYNPTTYQMDVNPEGKYSFGATCVKKCPRNYVVTDHGSCVRACGADSYEMEEDGVR KCKKCEGPCRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVK EITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANT INWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREF VENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNC TYGCTGPGLEGCPTNGPKIPS	329

	VDNKFNK	*Helix 1* EQQNAFYEILH	LPNLNE	*Helix 2* EQRNAFIQSLKD	DPSQ	*Helix 3* SANLLAEAKKLNDA	QAPK	
Z_{wt}	-------	-WSA-AS--SG	-----K	L-AF---V--V-	----	--------------	----	1
$Z_{EGFR:940}$	-------	-MLI-ME--GS	-----W	G-EQ---L--W-	----	--------------	----	45
$Z_{EGFR:942}$	-------	-TGA-MR--ND	-----N	L-FF---V--V-	----	--------------	----	1
$Z_{EGFR:947}$	-------	-FYA-IT--NR	-----G	W-MV---S--S-	----	--------------	----	1
$Z_{EGFR:948}$	-------	-HAK-MW--GN	-----L	V-LA---F--R-	----	--------------	----	1
$Z_{EGFR:949}$	-------	-SLA-SV--SH	-----G	S-CK---R--M-	----	--------------	----	1
$Z_{EGFR:951}$	-------	-LEK-YN--RN	-----G	W-MT---A--V-	----	--------------	----	1
$Z_{EGFR:955}$	-------	-AAP-WT--VR	-----R	G-KQ---V--H-	----	--------------	----	1
$Z_{EGFR:956}$	-------	-LWI-TS--VE	-----M	H-GV---R--L-	----	--------------	----	1
$Z_{EGFR:957}$	-------	-VQN-VA--VK	-----G	W-ST---A--S-	----	--------------	----	1
$Z_{EGFR:1239}$								

Basic:
H, R, K

Acidic:
D, E

Non-Polar:
G, I, F, A, L, M, W, P, V

Polar:
C, Q, N, S, Y, T, S

FIGURE 2A

	Helix 1		Helix 2		Helix 3	
Zwt	VDNKFNK	EQQNAFYEILH	LPNLNE	EQRNAFIQSLKD	DPSQ	SANLLAEAKKLNDA	QAPK
$Z_{EGFR:942}$	-------	-*MLI*-M**E**--**GS**	-----*W*	G-**E**Q---*L*--*W*-	----	----------	----45
$Z_{EGFR:948}$	-------	-*FYA*-*IT*--**NR**	-----G	*W*-*MV*---**S**--**S**-	----	----------	---- 1
$Z_{EGFR:955}$	-------	-*LEK*-*YN*--**RN**	-----G	*W*-*MT*---**A**--*V*-	----	----------	---- 1
$Z_{EGFR:1239}$	-------	-*VQN*-*VA*--*VK*	-----G	*W*-**ST**---**A**--**S**-	----	----------	---- 1

Hydrophobic
Neutral
Hydrophilic

FIGURE 2B

	Helix 1		Helix 2		Helix 3	
Zwt	VDNKFNK	EQQNAFYEILH	LPNLNE	EQRNAFIQSLKD	DPSQ	SANLLAEAKKLNDA	QAPK
$Z_{EGFR:942}$	-------	-**MLI**-Me--**GS**	-----*W*	G-eQ---**L**--*W*-	----	----------	----45
$Z_{EGFR:948}$	-------	-*FYA*-*IT*--**NR**	-----G	*W*-*MV*---**S**--**S**-	----	----------	---- 1
$Z_{EGFR:955}$	-------	-LeK-*YN*--**RN**	-----G	*W*-*MT*---**A**--**V**-	----	----------	---- 1
$Z_{EGFR:1239}$	-------	-**VQN**-**VA**--**VK**	-----G	*W*-**ST**---**A**--**S**-	----	----------	---- 1

Aromatic R groups (italic)
Nonpolar, aliphatic R groups (bold)
Polar, uncharged, amino acids (underline)
negatively charged r groups (lower case)
Positively charged R groups (double underline)

FIGURE 2C

Affinity maturation strategy

9	10	11	13	14	17	18	24	25	27	28	32	35
X	X	X	X	X	N/R	N/R	G	W	M	T	A	S/V

FIGURE 2D

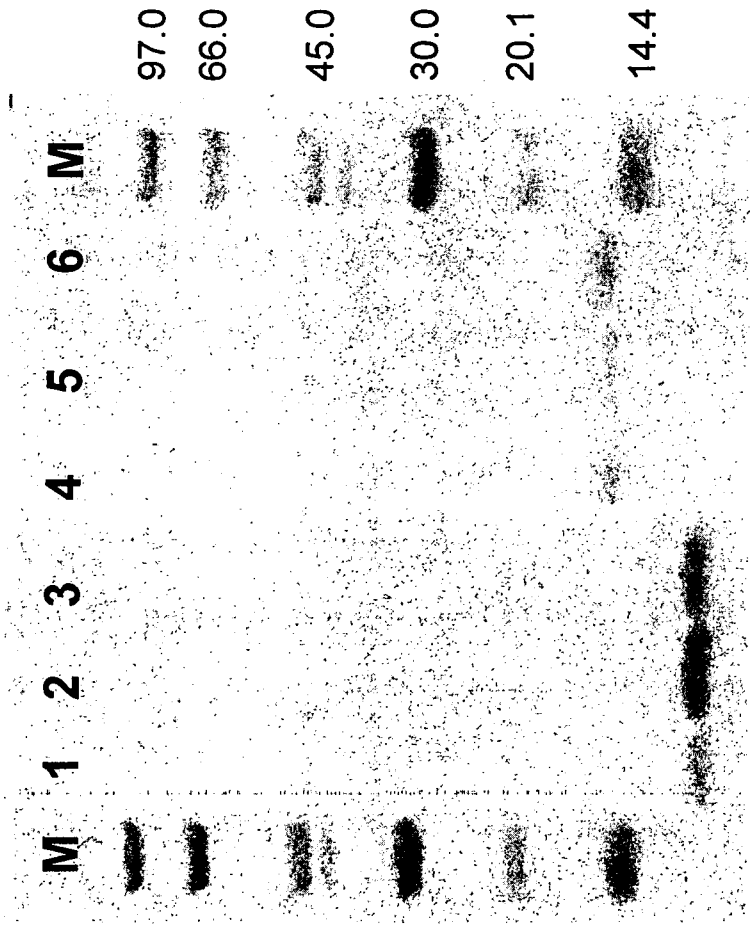


FIGURE 3

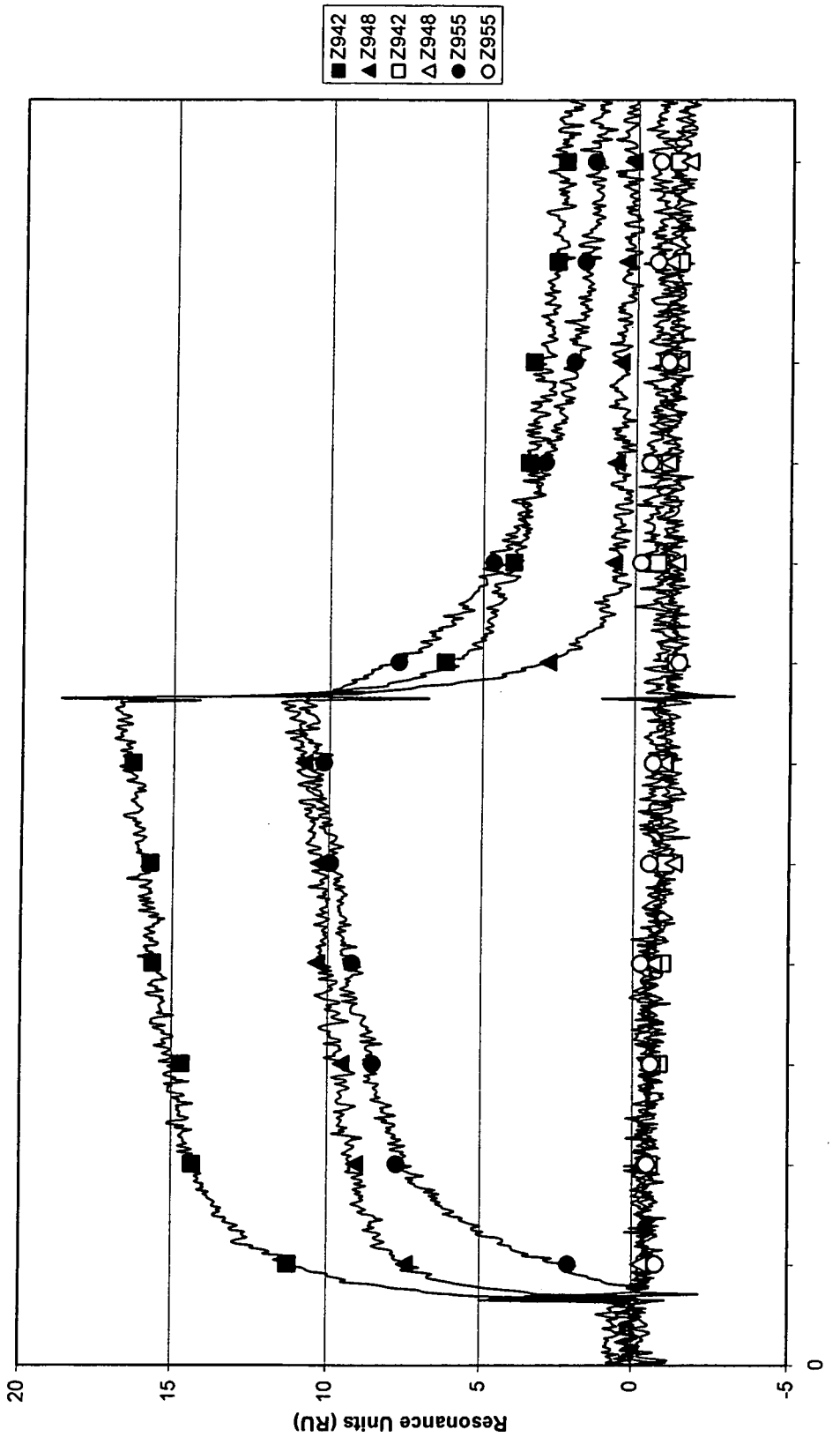

Z942
Z948
Z942
Z948
Z955
Z955
Resonance Units (RU)
20
15
10
5
0
-5
0
Time (s)

FIGURE 4A

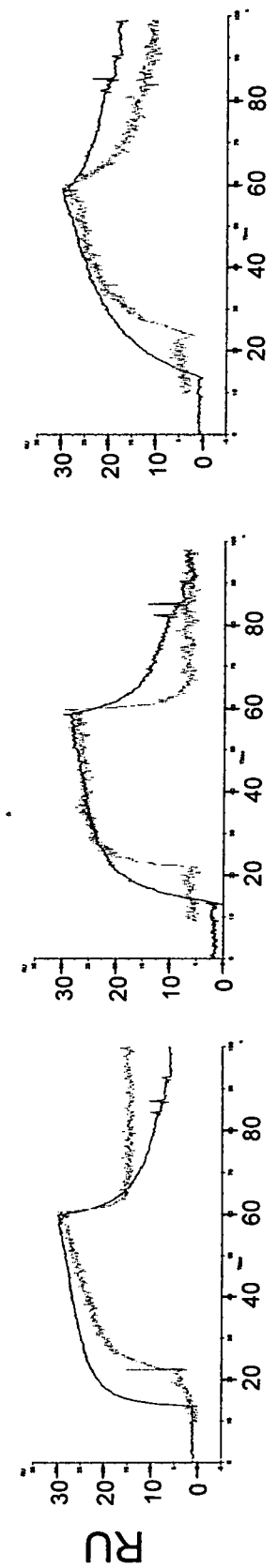


FIGURE 4B

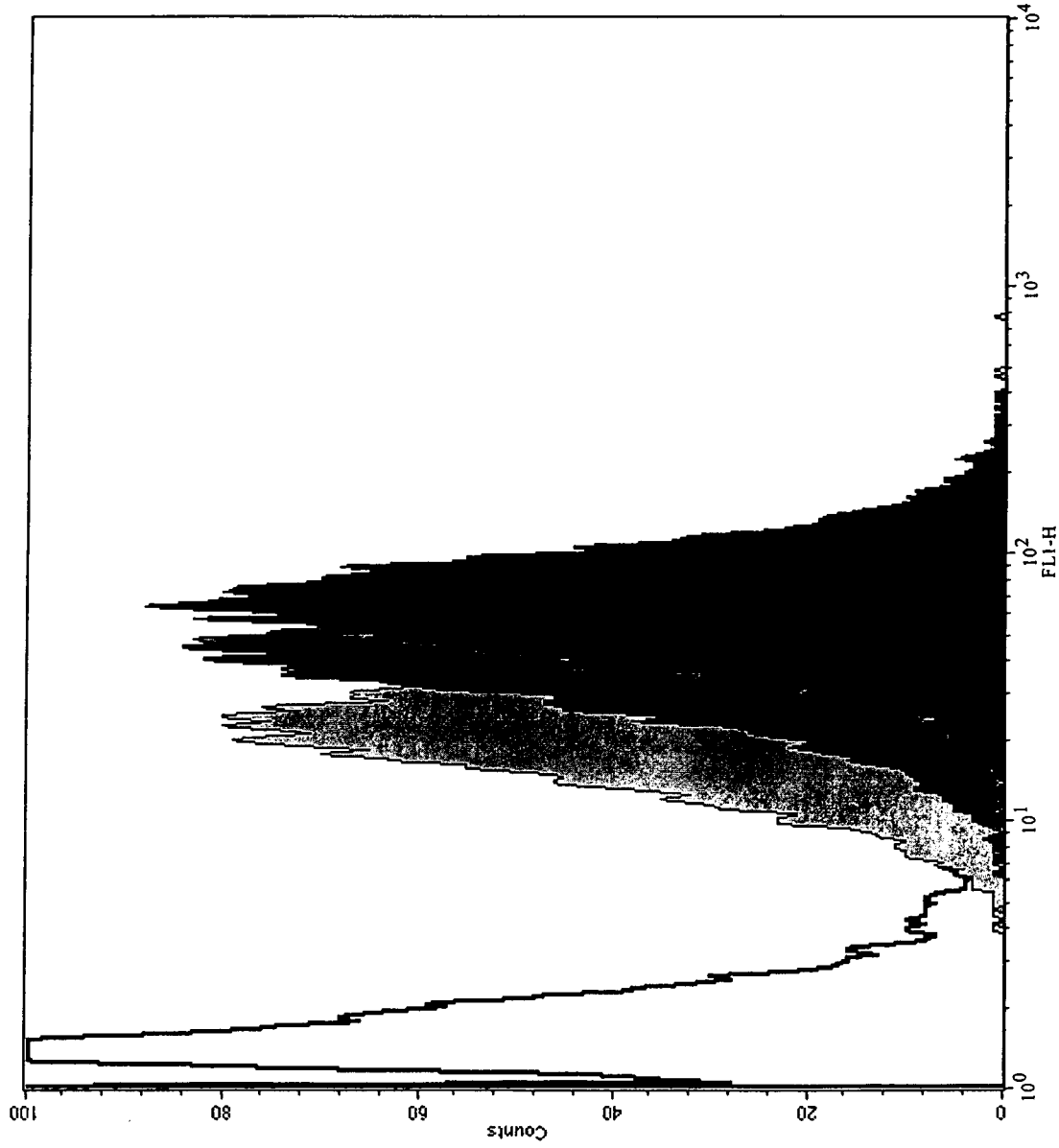


FIGURE 5

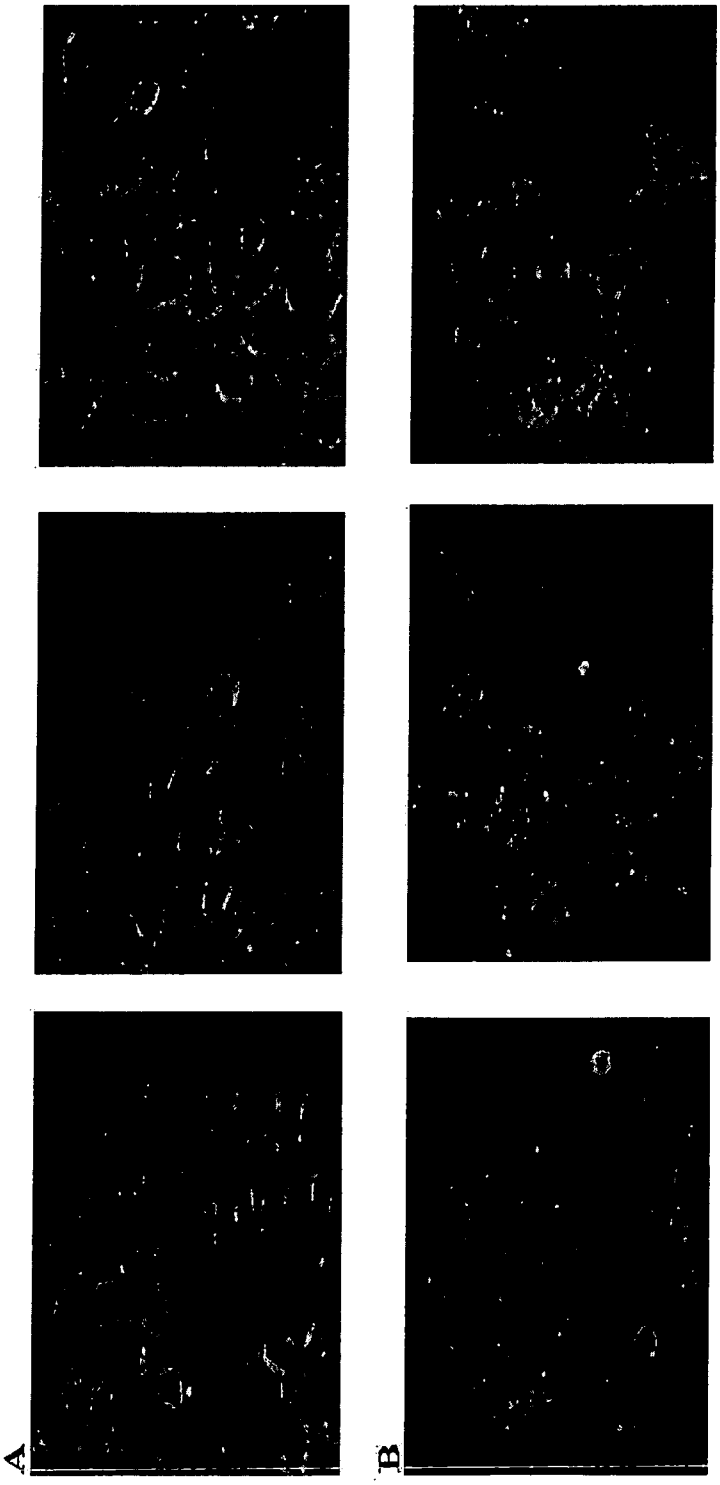


FIGURE 6

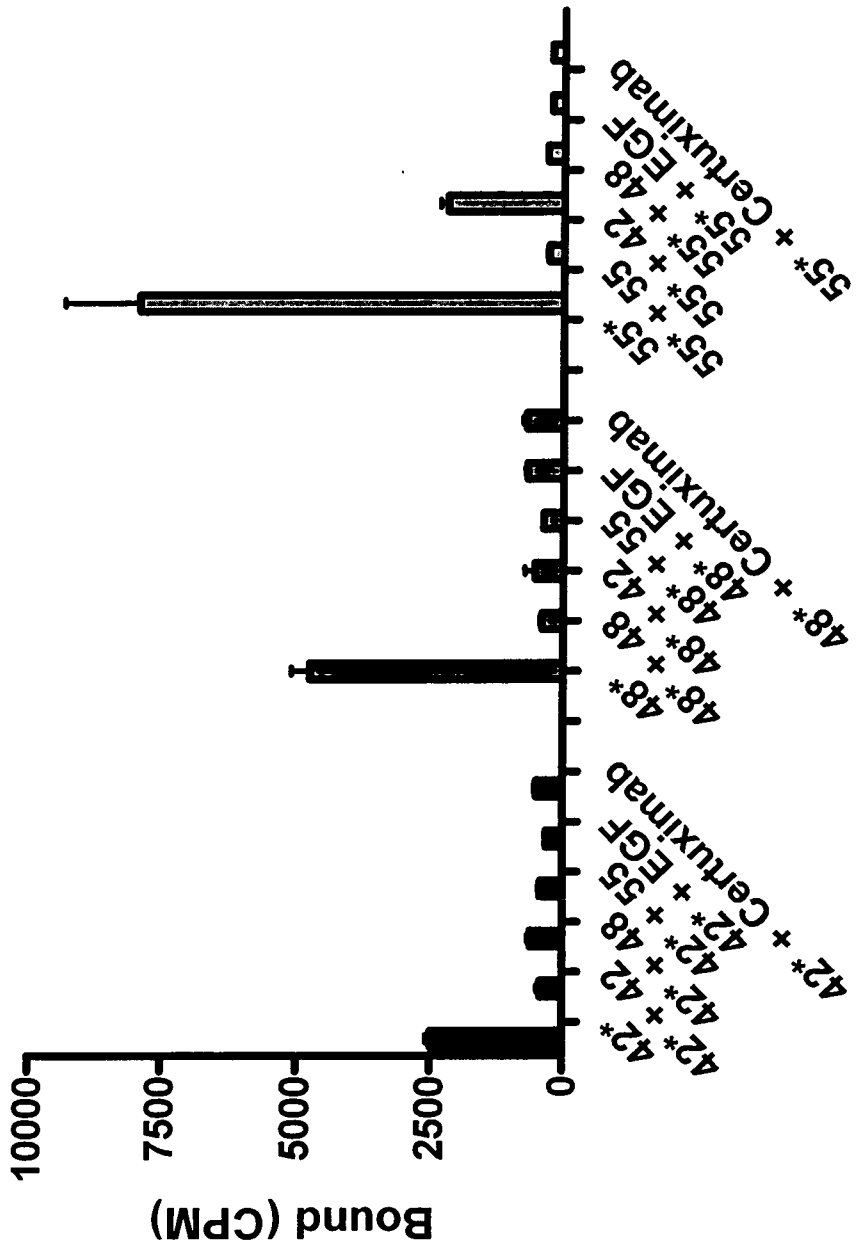

Bound (CPM)
10000
7500
5000
2500
0
42*
42* + 42
42* + 48
42* + 55
42* + EGF
42* + Cetuximab
48*
48* + 48
48* + 42
48* + 55
48* + EGF
48* + Cetuximab
55*
55* + 55
55* + 42
55* + 48
55* + EGF
55* + Cetuximab

FIGURE 7

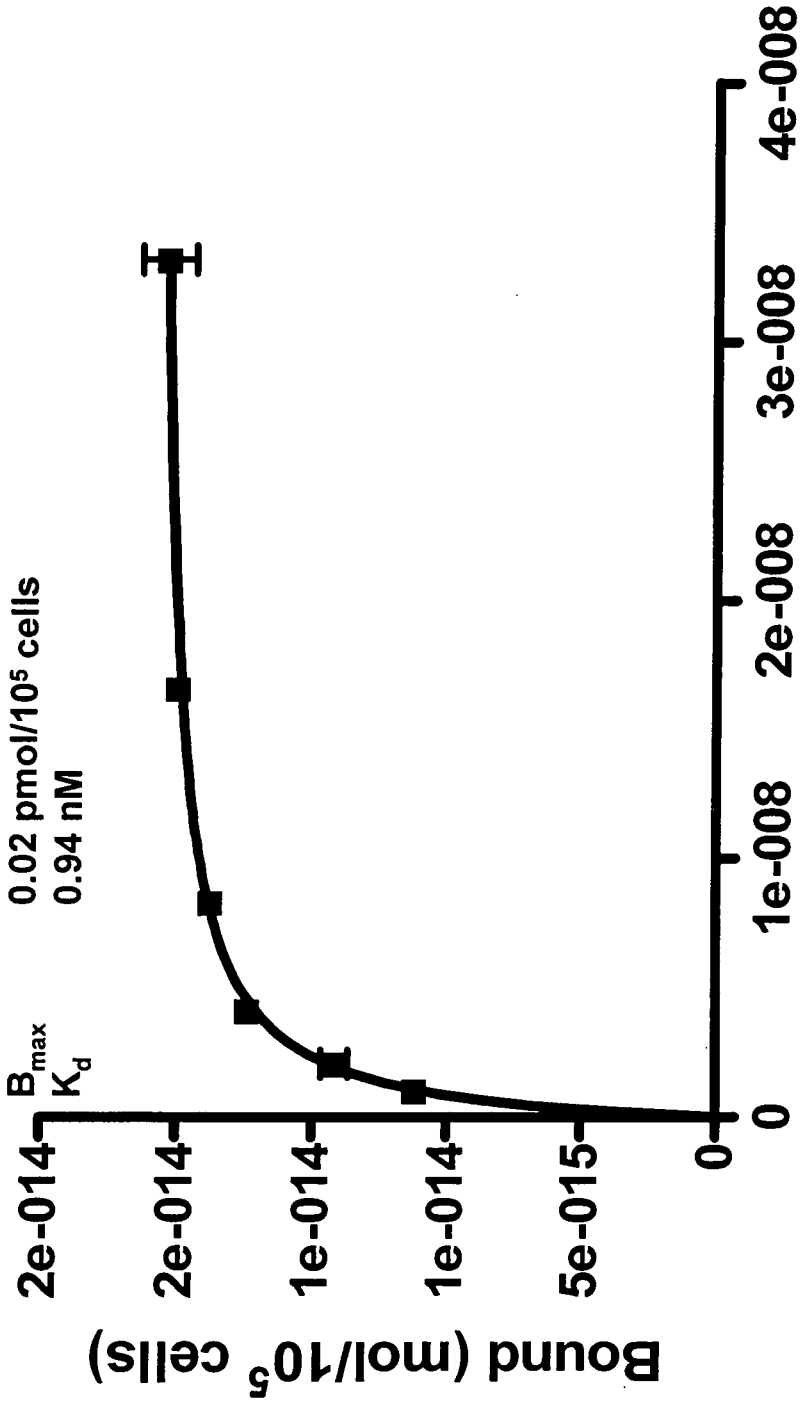

Bound (mol/10^5 cells)
2e-014
2e-014
1e-014
1e-014
5e-015
0
B_max
K_d
0.02 pmol/10^5 cells
0.94 nM
0
1e-008
2e-008
3e-008
4e-008
[125I]Z00942 (M)

FIGURE 8A

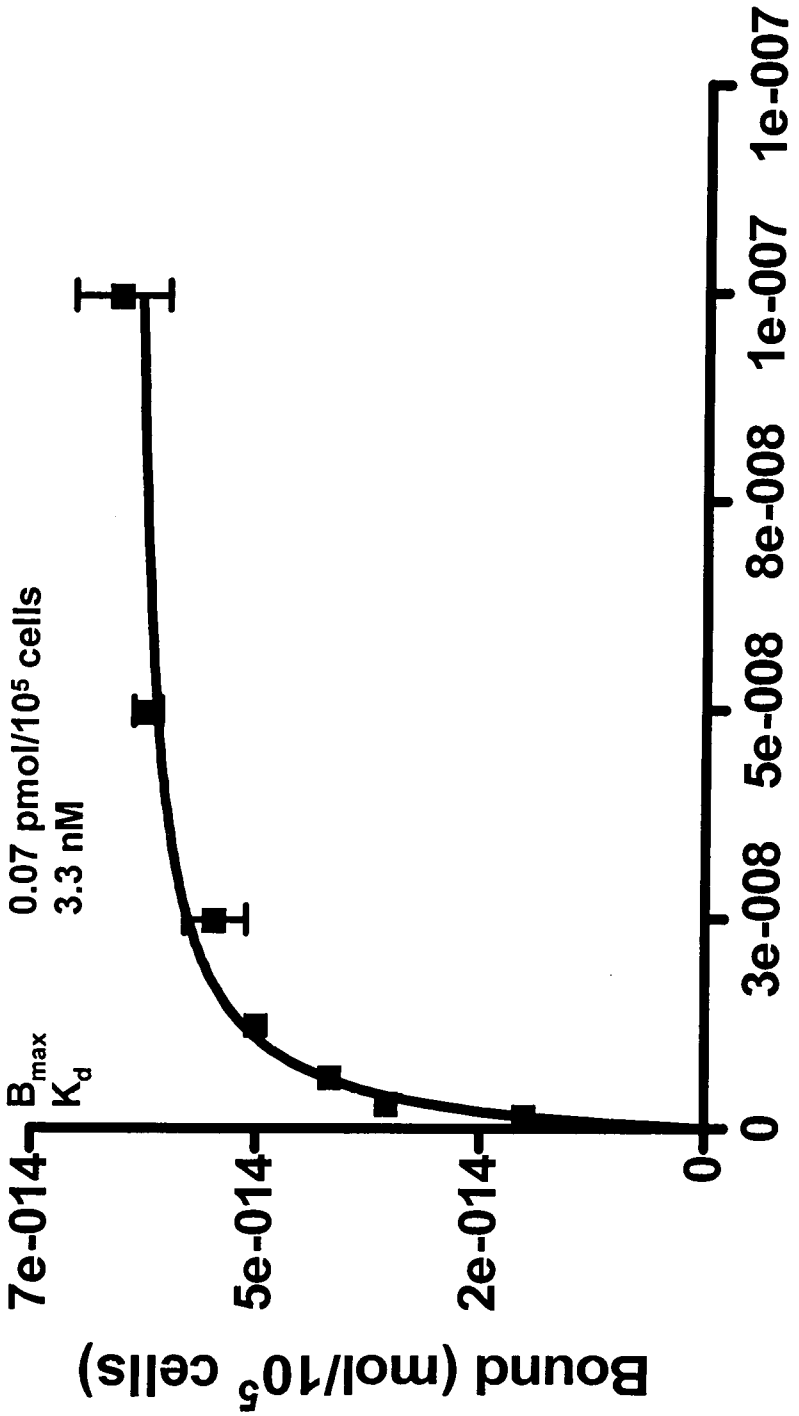

Bound (mol/10⁵ cells)
7e-014
5e-014
2e-014
0
B_max
K_d
0.07 pmol/10⁵ cells
3.3 nM
0
3e-008
5e-008
8e-008
1e-007
1e-007
[125I]Z00948 (M)

FIGURE 8B

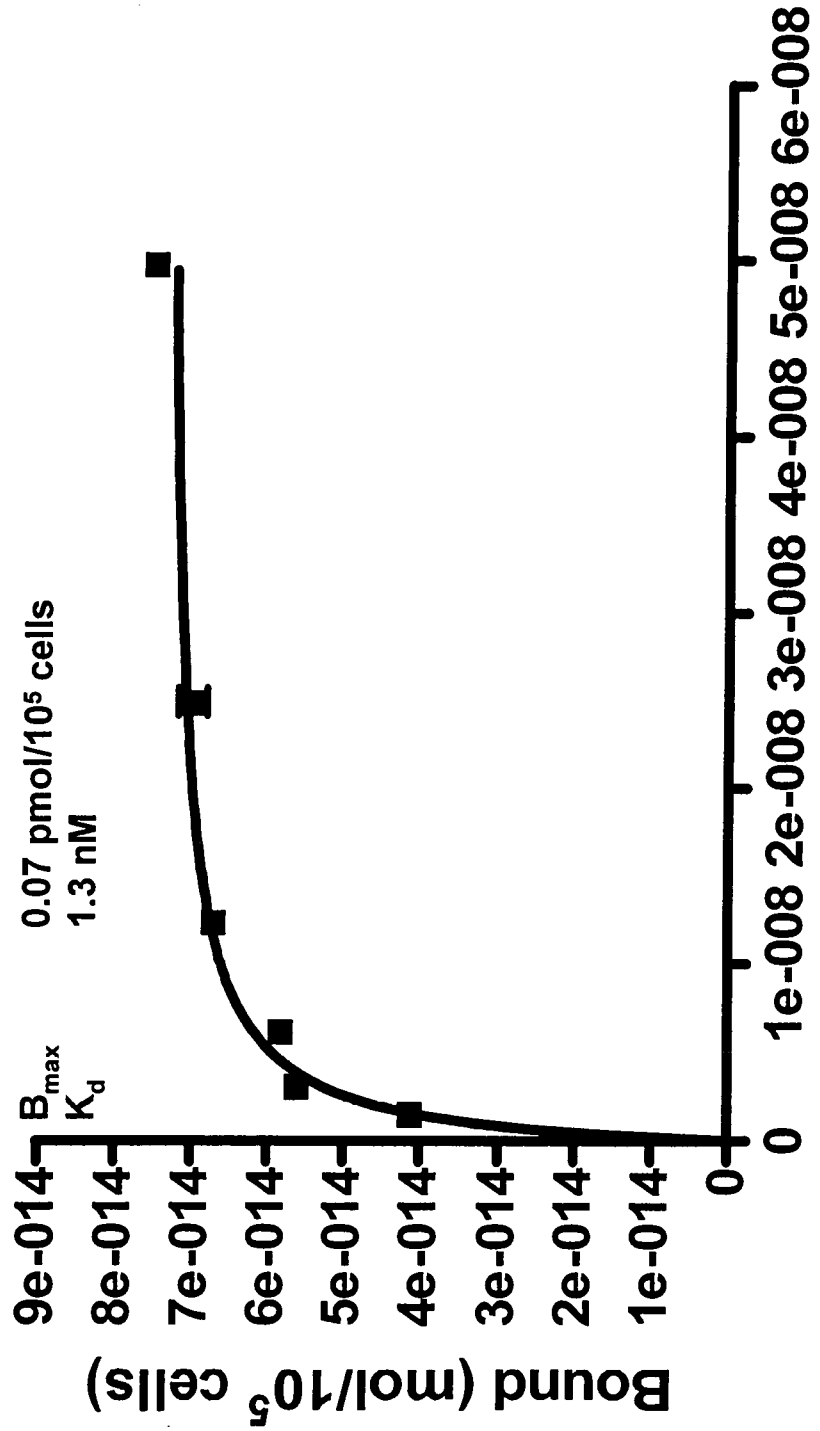

Bound (mol/10^5 cells)
9e-014
8e-014
7e-014
6e-014
5e-014
4e-014
3e-014
2e-014
1e-014
0
B_max
K_d
0.07 pmol/10^5 cells
1.3 nM
0
1e-008
2e-008
3e-008
4e-008
5e-008
6e-008
[125I]Z00955 (M)

FIGURE 8C

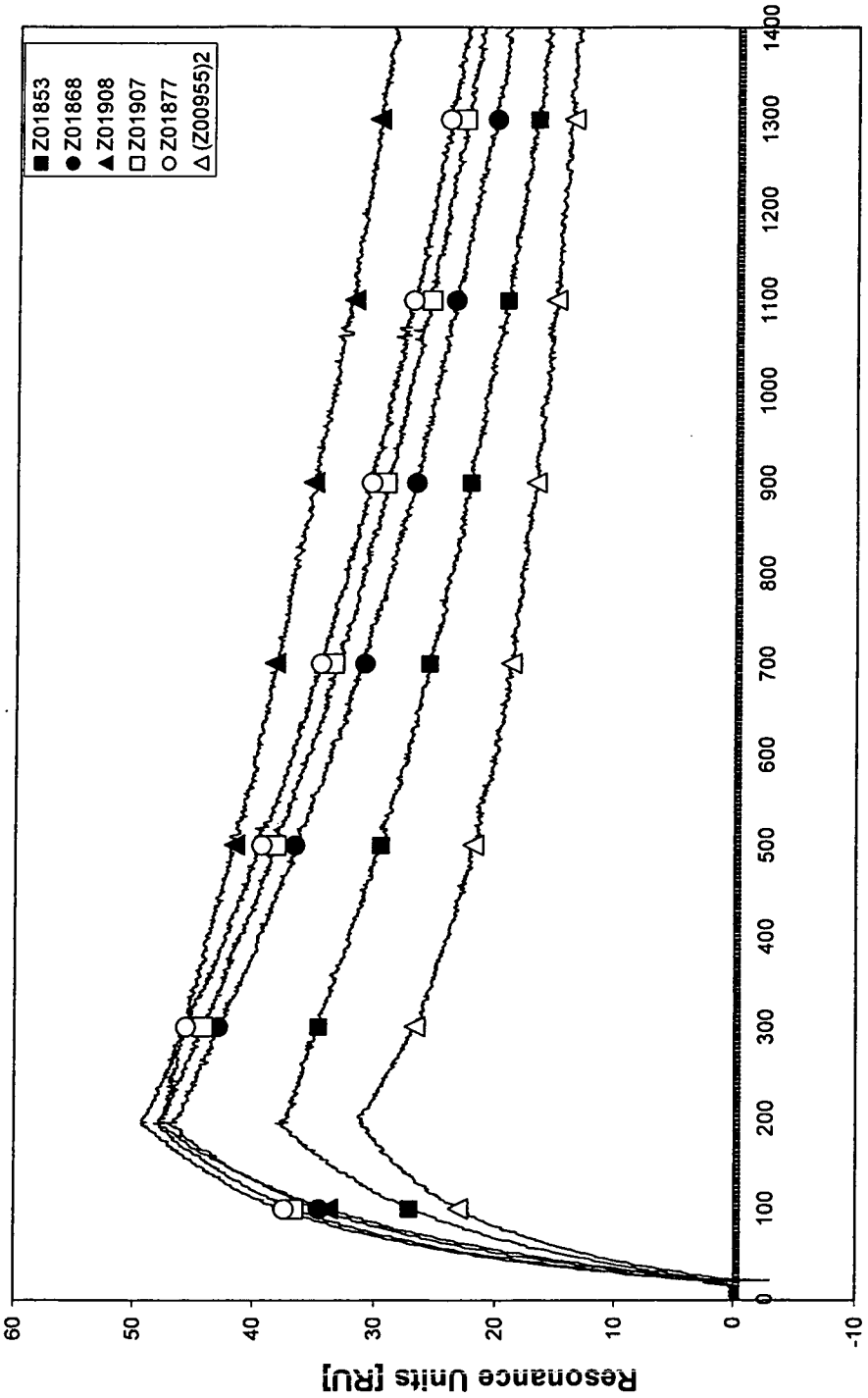

Z01853
Z01868
Z01908
Z01907
Z01877
(Z00955)2
Resonance Units [RU]
60
50
40
30
20
10
0
-10
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
Time [s]

FIGURE 9A

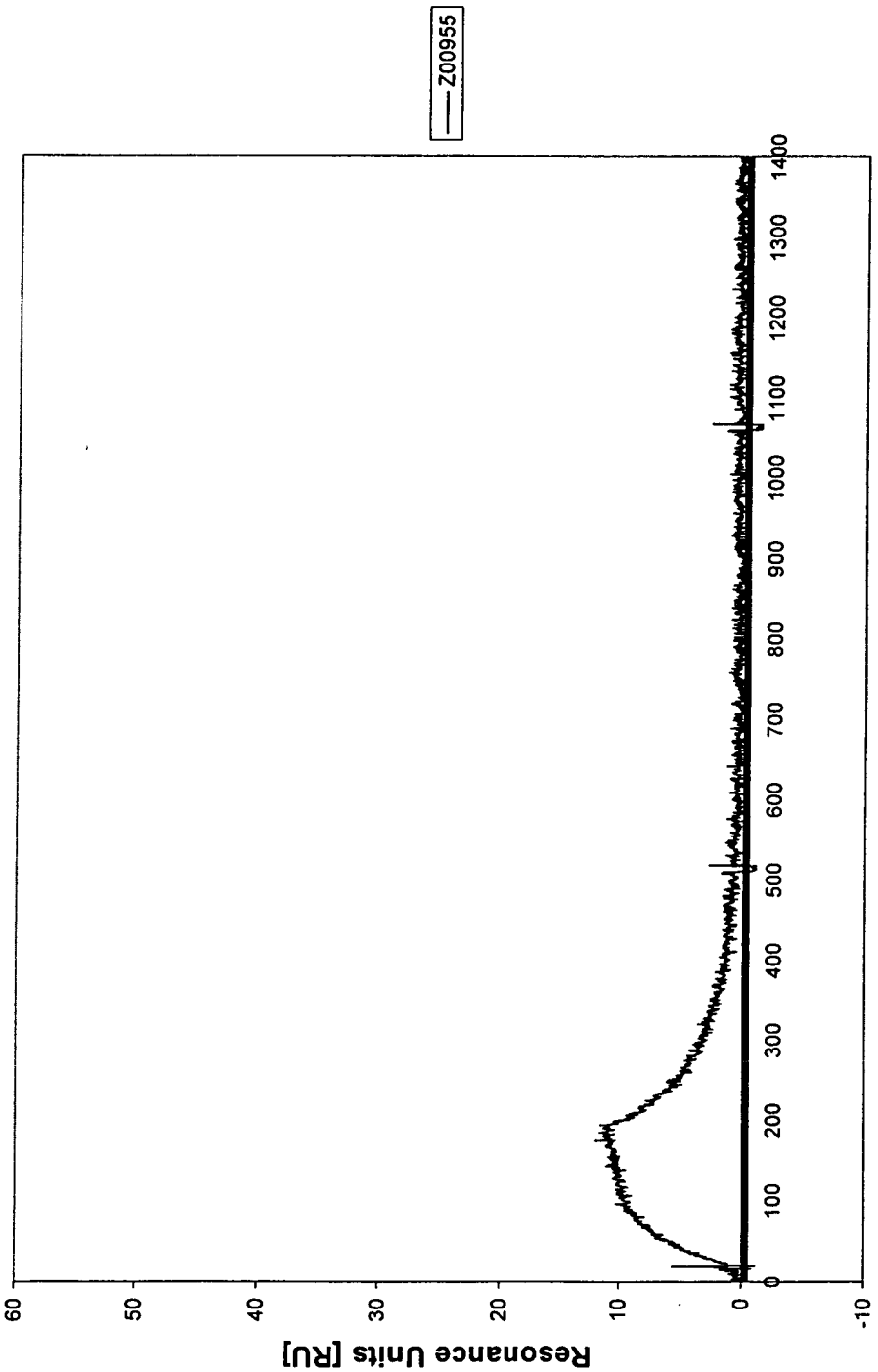

Z00955
Resonance Units [RU]
60
50
40
30
20
10
0
-10
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
Time [s]

FIGURE 9B

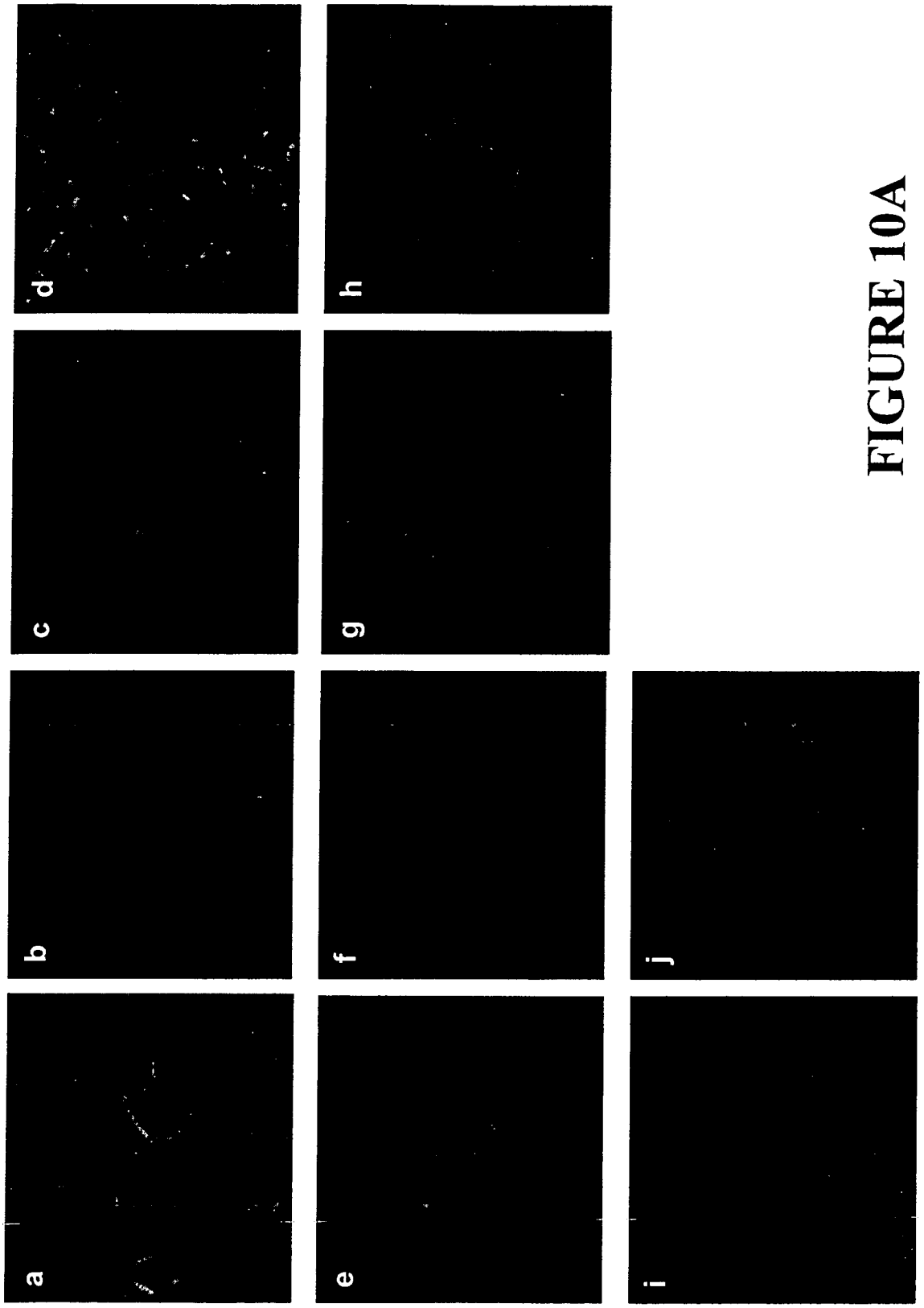


FIGURE 10A

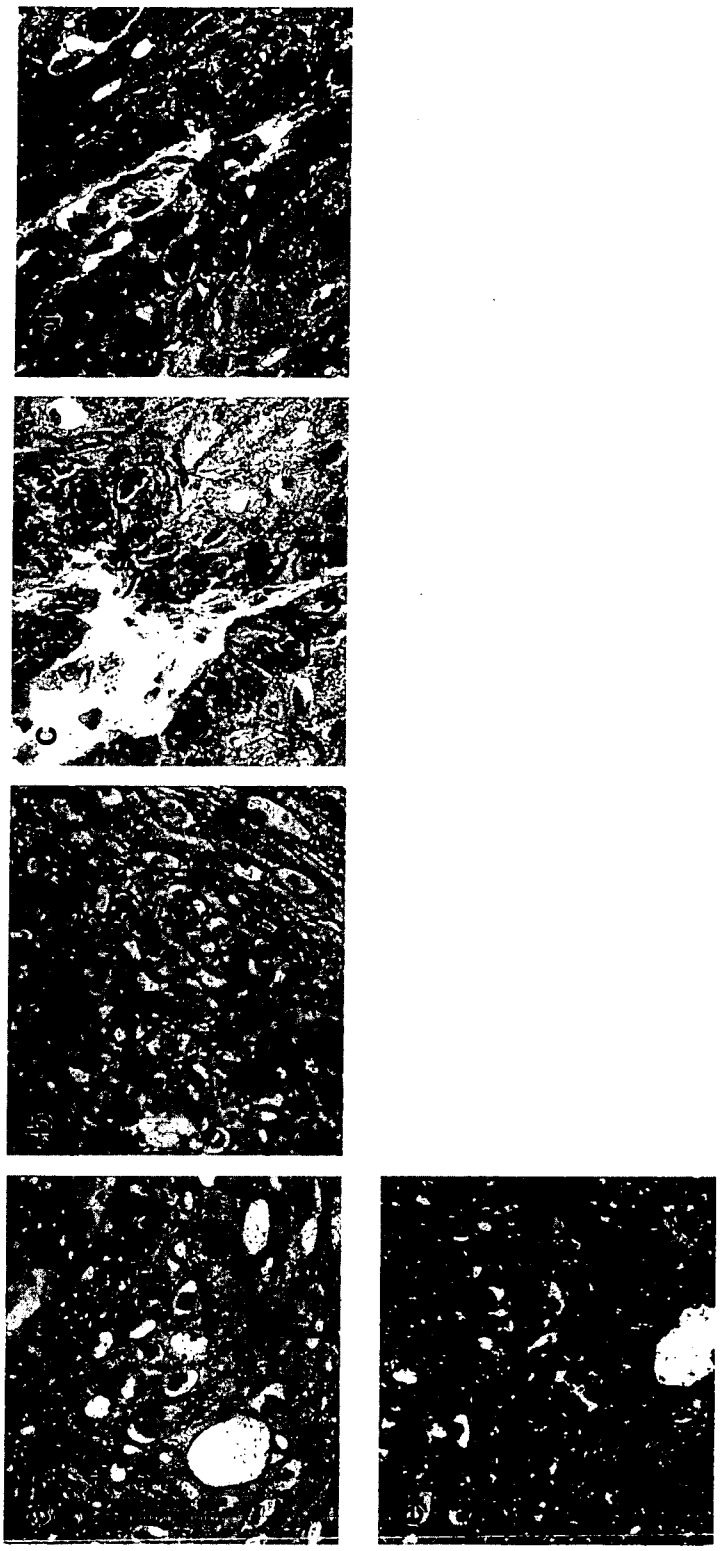


FIGURE 10B

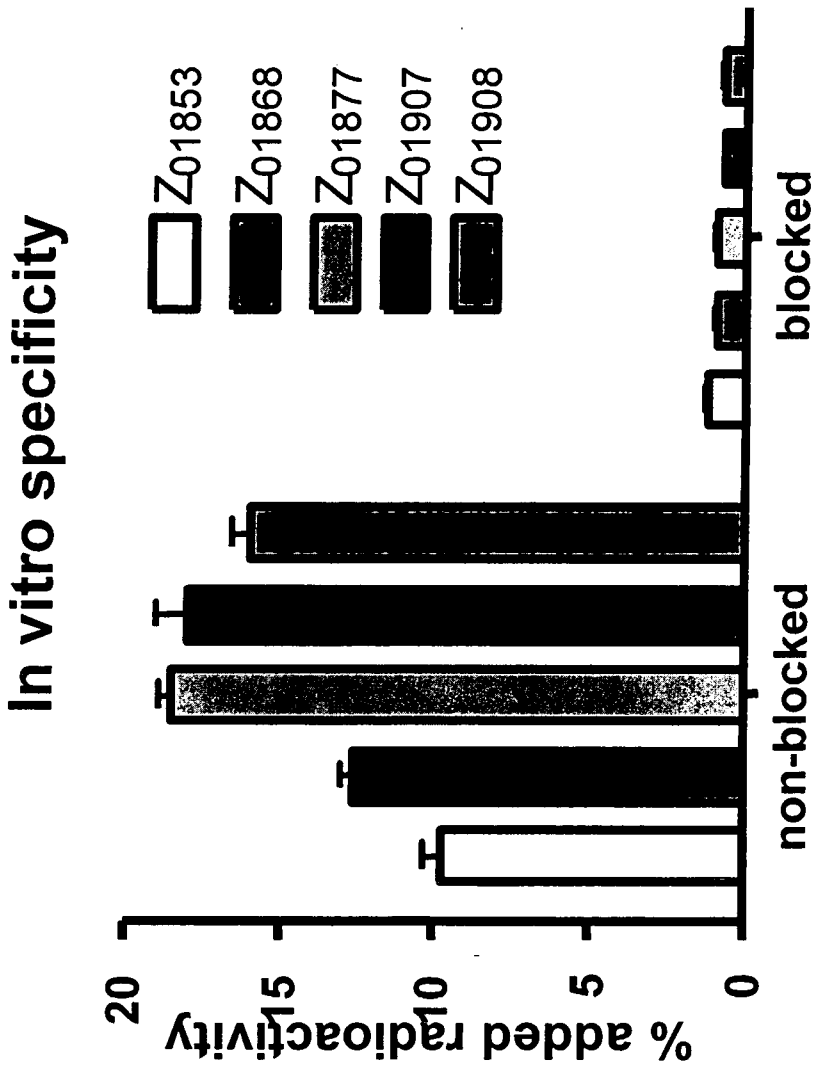

In vitro specificity
Z01853
Z01868
Z01877
Z01907
Z01908
% added radioactivity
20
15
10
5
0
non-blocked
blocked

FIGURE 11

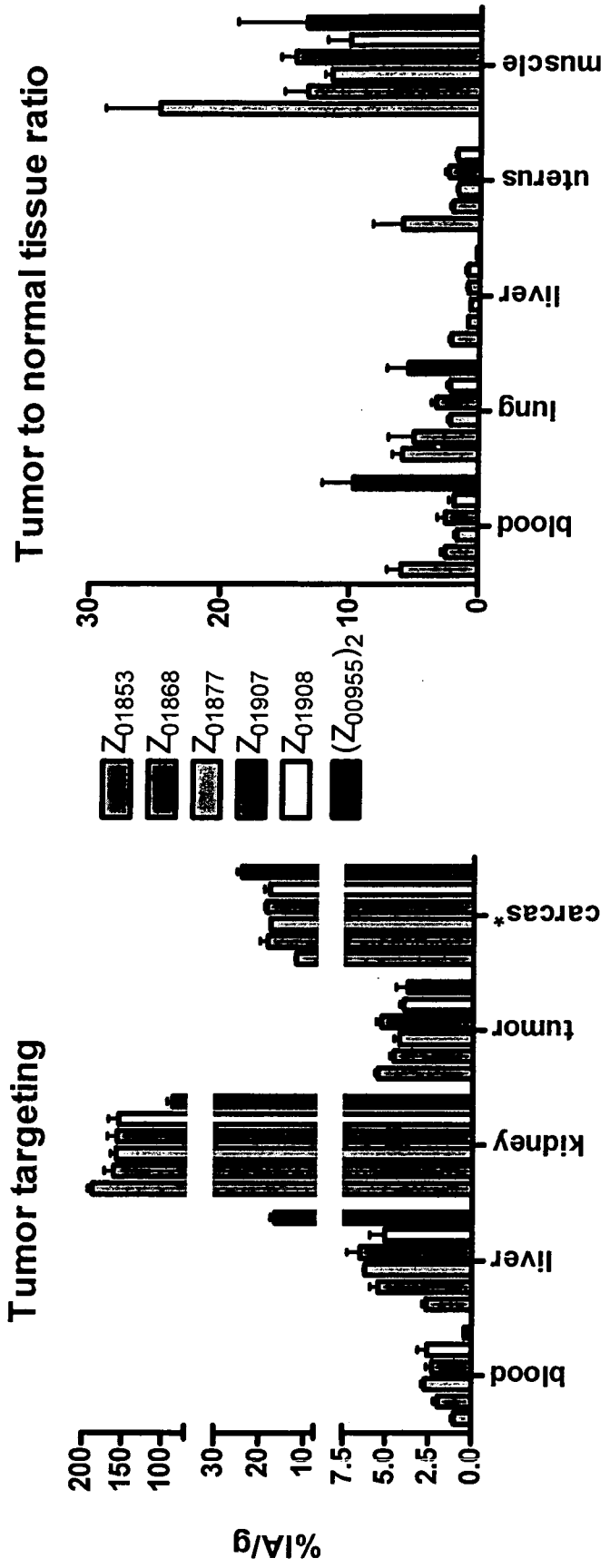
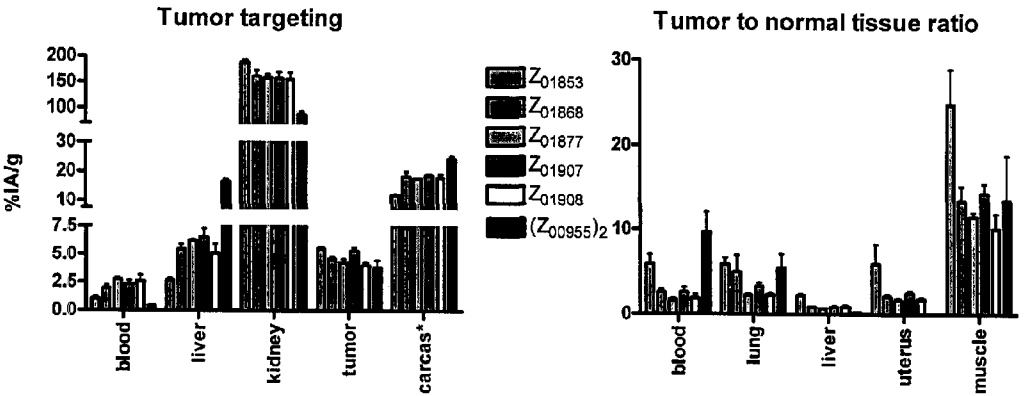

Tumor to normal tissue ratio
30
20
10
0
blood
lung
liver
uterus
muscle
Z_{01853}
Z_{01868}
Z_{01877}
Z_{01907}
Z_{01908}
$(Z_{00955})_2$
Tumor targeting
200
150
100
30
20
10
7.5
5.0
2.5
0.0
blood
liver
kidney
tumor
carcas*
%IA/g

FIGURE 12


Tumor targeting
%IA/g
200
150
100
30
20
10
7.5
5.0
2.5
0.0
blood
liver
kidney
tumor
carcas*
Z_{01853}
Z_{01868}
Z_{01877}
Z_{01907}
Z_{01908}
$(Z_{00955})_2$
Tumor to normal tissue ratio
30
20
10
0
blood
lung
liver
uterus
muscle